

ISSN: 2141-3819

CENTREPOINT JOURNAL

(Science Edition)

**A Journal of Intellectual,
Scientific
and Cultural Interest**

A Peer-Reviewed Journal

Published by
The Library and Publications Committee
University of Ilorin, Ilorin, Nigeria

Founded by



Vol. 25. No. 1

2019

This Journal is funded by



EDITORIAL BOARD

Chairman, Editorial Board

S.F. Ambali

University of Ilorin, Ilorin, Kwara State, Nigeria.

Email: fambali2001@gmail.com; ambali.sf@unilorin.edu.ng

Editor-In-Chief

J.A. Obaleye

University of Ilorin, Ilorin, Kwara State, Nigeria.

Email: jobaleye@yahoo.com; jobaleye@unilorin.edu.ng

Associate Editor-In-Chief

I.O. Adimula

University of Ilorin, Ilorin, Kwara State, Nigeria.

Email: adimula@unilorin.edu.ng

Managing Editor

L.O. Aina

University of Ilorin, Ilorin, Kwara State, Nigeria.

Email: ainalo2000@yahoo.com

Business Manager

Noah Bisi Oyediji

University of Ilorin, Ilorin, Kwara State, Nigeria.

Email: oyedejinb@gmail.com

Editors

A.A. Adeloye	(University of Ilorin, Ilorin, Kwara State, Nigeria)
K.A. Asamoah	(University of Cape Coast, Ghana)
M.R. Caira	(University of Cape Town, South Africa)
O. Fatoki	(Cape Peninsula University of Technology, South Africa)
A.O. Ogunlela	(University of Ilorin, Ilorin, Kwara State, Nigeria)
A.B.O. Omotoso	(University of Ilorin, Ilorin, Kwara State, Nigeria)

Segmental Phoneme Articulation as a Means of Identifying Nigerian Speakers

Consulting Editors

K.O. Adebawale	(University of Ibadan, Oyo State, Nigeria)
A.J. Afolayan	(University of Fort Hare, Alice 5700, South Africa)
A. Akinola	(Obafemi Awolowo University, Ile-Ife, Nigeria)
B. Everitt	(University of York, YO10, 50DD England)
J.F. Frielingsdorf	(Shell Petroleum Development Company, Port Harcourt, Nigeria)
O. Gureje	(University College Hospital, Ibadan, Oyo State, Nigeria)
B.W. Reinisch	(University of Massachusetts-Lowell, MA, U.S.A.)
I.H. Krause	(United State Air Force Academic Space Physics and Atmospheric Research Colorado (U.S.A))
O.A. Odunola	(Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria)

NOTES TO CONTRIBUTORS

CentrepoinT is an interdisciplinary journal of intellectual, scientific and cultural interest with particular reference to Africa.

Contributions written in English should be submitted in three copies typed double space with the following margins; Top: 1.25"; Bottom: 2.3", Left: 1.85"; Right: 1.4" Articles should not exceed twenty double space pages on A4 paper.

Notes and references should be carefully listed at the end of each article.

References should be arranged alphabetically.

References within the text should be cited in Harvard style, by author and year.

Two papers published in the same year should be given identifying lower case letters, both in the text and in the reference list.

Authors should submit a printed copy and computer disk following these guidelines:

- Use rewritable compact disc (CD)
- Specify computer used (either IBM compatible PC not apple Macintosh)
- State software used, WordPerfect, Microsoft word etc.
- Use New Times Roman font size twelve (12).
- State the file name
- Keep a back up CD for reference and safety.

The views expressed in this journal are those of the contributors and not necessarily those of the Editorial Board.

Segmental Phoneme Articulation as a Means of Identifying Nigerian Speakers

SUBSCRIPTION

Nigeria:	Individual Subscribers – N500 per copy (Excluding Postage) Institution or Library – N100 per copy (Excluding Postage)
Africa:	\$ 25 per copy (Including Postage)
Europe:	\$ 25 per copy (Including Postage)
North America:	\$ 30 per copy (Including Postage)
Other Continents:	\$ 30 per copy (Including Postage)

All correspondence should be addressed to:

PROF. J. A. OBALEYE

The Editor-in-Chief

Centrepoint: Science Edition,

Faculty of Physical Science, Department of Chemistry

University of Ilorin, Ilorin, Kwara State, Nigeria.

Email: jobaleye@yahoo.com, centrepoinsciunilorin@gmail.com

OR

PROF. L. A. AINA

Managing Editor

Faculty of Information and Communication Science,

University of Ilorin, Ilorin, Kwara State, Nigeria.

Email: ainalo2000@yahoo.com

OR

PROF. NAOH BISI OYEDEJI

Business Manager

Department of Education Management,

University of Ilorin, Ilorin, Kwara State, Nigeria

Email: sadekunlet@unilorin.edu.ng; setokade@yahoo.com OR

PROF. S.F. AMBALI

Chairman, Library and Publication Committee,

University of Ilorin, Ilorin, Kwara State, Nigeria.

Email: fambali2001@gmail.com; ambali.sf@unilorin.edu.ng

CONTENTS

	Page
CPJ 2019001/25101 Leaf Anatomy of <i>Letestudoxa Bella Pellegr</i> (Annonaceae) <i>Adeniran, S.A., Kadiri, A. B. and Olowokudejo J.D.</i>	1-10
CPJ 2019002/25102 Preliminary investigation of petrographic and geochemical characteristics of crystalline rocks in jebba, central Nigeria <i>O. A. Omotoso, O. J. Ojo, A. I. Aina, J. I. Udeafor, M. T. Alebiosu</i>	11-28
CPJ 2019003/25103 Antibacterial activity OF <i>Mangifera Indica</i> leaves on multidrug resistant clinical isolates <i>I. I. Anibijuwon, I. D. Gbala, J. A. Abioye, M.O. Bamigboye, I. O. Salawu</i>	29-41
CPJ 2019004/25104 chemical and functional properties of raw and cooked underutilized <i>Blighia Sapida</i> seed flour <i>Abiodun O.A., Adepeju, A. B., Olosunde, O.O. and Dauda, A.O.</i>	42-54
CPJ 2019005/25105 Steady blood flow through vascular stenosis under the influence of magnetic field <i>Abubakar J. U., Gbadeyan J.A. and Ojo J.B.</i>	55-75
CPJ 2019006/25106 Plants in the diet of African forest elephants in Okomu National Park, Nigeria <i>Bamidele, O.A, Akinsoji, A. and Adeniran, S.A</i>	76-95
CPJ 2019007/25107 Synthesis, characterization and catalytic activity study of Mn(II) metal complexes of Schiff base ligands derived from amino acids and vanillin <i>Samson O. Owalude, Adedibu C. Tella, Olubunmi S. Oguntoye, Olatayo T. Adekeye, Uche B. Eke and Ezekiel O. Odebunmi</i>	96-105

Segmental Phoneme Articulation as a Means of Identifying Nigerian Speakers

CPJ 2019008/25108

Plasma DPP-4 levels In prediabetics selected from three communities in Ilorin, Nigeria

Olarinoye, J. K, Biliaminu, A. S, AbdulAzeez, I. M, Olarinoye, A. O, Olagbaye, B. A, Sanya E. O, Olatunji L. A, Soladoye A. O.

106-121

CPJ 2019009/25109

Status of Some Metal Ions Pollution in Selected Groundwater within Iloffa District Using Multivariate Statistical Techniques

Hussein K. Okoro, Tunbosun T. Popoola, Benjamin O Orimolade, Clement O. Ogunkunle

122-140

CPJ 2019010/25110

Alterations in the levels of female sex hormones following administration of nicotine to Wistar rats

Gabriel Olaiya Omotoso, R Eniola Kadir, Ade Stephen Alabi, Luqman Aribidesi Olayaki, Omowumi Femi-Akinlosotu

141-147

CPJ 2019011/25111

Plasmodium falciparum Resistance in Selected Areas of Niger State, Nigeria: Chloroquine and Sulphadoxine/Pyrimethamine Treatment Outcome and Predictive Value of Molecular Markers

Nyamngee, A., Edungbola, L. D., Edogun H. A., Ikpe, R. T., Agbendeh L. N. and Ighodalo, M.

148-164

CPJ 2019022/25112

Sedimentological, Depositional environment and Sequence stratigraphic studies of OML AB field, Southeastern Niger Delta, Nigeria

Ibrahim, O.I, Adekeye, O.A, Bale R.B and Babatunde, W.S

165-181

LEAF ANATOMY OF LETESTUDOXA BELLA PELLEGR (ANNONACEAE)

Adeniran, S.A.¹, Kadiri, A. B.² and Olowokudejo J.D.³

¹*Department of Plant Biology, University of Ilorin, Ilorin Nigeria*

^{2,3}*Department Botany, University of Lagos, Akoka, Lagos State*

Corresponding author: Email- debunm11@gmail.com

Abstract

A study of leaf anatomy (epidermis, architecture and midrib) of *Letestudoxa bella* was carried out using light microscope in order to provide useful anatomical features that will aid its identification. Hence, both qualitative and quantitative data of the leaf were assessed for their taxonomic significance. The shape of epidermal cells and its cell wall, type of wax depositions, type and number of stomata, areola formation, nerves ending, vein category, venation, type, shape and nature of vascular bundle, presence or absence of parenchyma and sclerenchyma are reliable criteria useful for identifying the plant. Outstandingly, the record of stephanocytic stomata in the genus is novel in the family Annonaceae. Other important findings in this study are the epidermal cell and stomatal number, length and width, and stomatal index which varied reasonably across the individuals of the species studied. The stomata number was recorded and its index calculated. A combination of these features proved can be reliably used to identify the species even when the leaf samples are fragmentary.

Keyword: Annonaceae, architecture, epidermis, stephanocytic stomata

Introduction

The Annonaceae Juss. is a pantropical family consisting of about 130 genera and 2300 species (Fries, 1959). Most of the species occur primarily in tropical lowland rainforest. The family is often represented by high numbers of individuals and species. The Annonaceae are clearly delimited (Fries 1959) and are regarded monophyletic in all studies including those based on molecular data (Qiu *et al.*, 2000). The genus *Letestudoxa* Pellegr. is a member of the family Annonaceae in the major group Angiosperms (Flowering plants). It is represented by three species worldwide, namely *L. glabrifolia* Chatrou & Repetur and *L. lanuginosa* Le Thomas (Chatrou, *et al.*, 2012) and is restricted to central Africa (Couvreur *et al.*, 2012).

They are most trees, sometimes lianas, with spiral phyllotaxis; indument of lepidote, stellate and/or simple hairs; bracts present; inflorescences rhipidiate, terminal; flowers bisexual; carpels free in flower, in fruit usually fused; placentation basal; stipe absent; aril rudimentary, rarely absent; ruminations lamelliform; pollen inaperturate, sometimes sulcate (Chatrou & Saunders, 2012). It occurs as one of the plant species in the flora of Cameroon.

Taxonomically, *Letestudoxa bella* is in the tribe *Duguetieae*, other genera include *Duguetia*, *Fusea*, and *Pseudartabotrys*. This tribe featured genera with both apocarpous (*Uvaria*, *Cleistopholis* Pierre ex Engl.) and syncarpous [*Letestudoxa*, *Pachypodanthium* Engl. & Diels (now *Duguetia*)] fruits. Parsimony analysis resulted in two strongly supported clades: a *Fusaea* clade, comprising *Fusaea*, *Duckeanthus*, *Letestudoxa*, and *Pseudartabotrys*, and a *Duguetia* clade, comprising all Neotropical and African species of *Duguetia*. Characters used to resolve relationships between and within the two clades are difficult to separate by outgroup comparison (Chatrou, et al., 2000).

Anatomical features are valuable to scientists who need to identify small fragments of plant materials and the taxonomic significance of epidermal morphology is well documented in botanical literature (Dehgan 1980). Though, a series of publications have contributed to a better understanding of the taxonomy of African *Annonaceae* (Couvreur, 2009; Botermans et al., 2011) but there has not been any information on the anatomical characters of genus *Letestudoxa*. Therefore, the present study is carried out in order to present and document leaf anatomical characters of *Letestudoxa bella* Pellagr. so as to complement the existing identification criteria upon which the recognition and delimitation of the plant could be based.

Materials and Methods

Leaf Epidermal Preparation

Dry materials of leaf were used for the study. Nomenclatural authentication was carried out in the National Herbarium Yaounde, Cameroon (YA). Herbarium abbreviation follows Holmgren et al., 1990. All existing data sources (e. g. Metcalfe and Chalk, 1950; Olowokudejo, 1993; Kadiri et al., 2009) were also consulted for further information.

Cross sections of the samples were obtained with a razor blade by free-hand method. For foliar epidermis, leaf samples were examined using the standard method established for tropical plant taxa including *Annonaceae*, e.g. Ogundipe and Kadiri (2012) on *Amaranthaceae*, Olowokudejo (1993) on *Jatropha*, Kadiri et al. (2009) on *Acalypha*, Yang and Lin (2005) on *Schisandraceae*. However, modifications were introduced where necessary for optimal result. The method involved cutting 1- 5cm portions from the standard median portion of the leaf lamina i.e. near the mid-rib

and then boiled in water for 20-30 minutes. The leaf pieces were later soaked in concentrated Trioxonitrate (v) acid (HNO_3) in capped specimen bottles for about 8-24 hours to macerate the mesophyll. Tissue disintegration was indicated by bubbles, and the epidermises were transferred into Petri dishes containing water for cleansing and then, epidermises were separated with forceps and mounting needles. Tissue debris was cleared off the epidermises with fine hair brush and washed in several changes of water.

For examination of the petiole and midrib, this was done using hand free sectioning to make semi-permanent slides for microscopic studies. Drops of different grades of ethanol- 50%, 75% up to 100% were added in turn to harden the cells. Preparations were later stained with Safranin O in 50% alcohol before mounting in glycerine on a glass slide. The epidermises were mounted on a glass slide with upper surfaces facing up and then covered with cover-slips and ringed with nail varnish to prevent dehydration. Scraped epidermis of the seed was obtained and stained with Safranin O and then examined under the microscope. Slides were examined with light microscopes at x100 and x400.

For quantitative analysis of epidermal characters, 20 epidermal cells and 10 stomata were randomly selected for measurement per microscope field. Stomata index was calculated using the formula reported by Stace (1965).

$$\text{StomataIndex} = 100 \left(\frac{S}{S + E} \right)$$

Where S= No of stomata present per unit area

E= No of Epidermal cell per unit area

Leaf Architecture

The areolation patterns and higher order venation in the leaf lamina were studied. Dried leaf samples were boiled in water for about five minutes and later transferred into commercial bleach solution for diaphanization (approximately 5% sodium hypochlorite), for 30 to 120 minutes. Thereafter, the samples were transferred into Petri dishes containing water for cleansing and then, drops of different grades of ethanol were added in turn to harden the cells after mounting on the slides. The adopted method follows Foster (1952), Hickey (1973) and Dilcher (1974), with some modifications. Slides were examined with light microscope at x100 only while photomicrographs were taken using Toupview 3.2 Image Camera attached to a computer.

Midrib Anatomy

Dried specimens were used through free hand sectioning. The sections were obtained transversely and boiled for about five minutes then drop of Sodium hypochlorite was added; washed in several changes of water and then stained with few drops aqueous Safranin. Samples were mounted in glycerin, covered with cover-slips and ringed with nail polish to prevent dehydration. Specimens were observed at x40 magnification and hand drawings were taken.

Results

Table 1: Leaf anatomical characteristics of *Letestudoxa bella*

S/N	CHARACTERS	UPPER SURFACE	LOWER SURFACE
1	Epidermal cell shape	Polygonal	Polygonal
2	Epidermal cell crystal	Absent	Absent
3	Anticlinal wall pattern	Straight	Straight
4	Stomata type	-	Stephanocytics
5	Trichome type	Absent	Present
6	Epidermal cell number	251(261.9±1.42)272	168(174.1±0.55)177
7	Epidermal cell length	5(6.4±0.16)7	4(5.55±0.32)7
8	Epidermal width	4(4.7±0.23)7	2(2.75±0.17)4
9	Epidermal cell thickness	0.5(0.5±0)0.5	0.5(0.5±0)0.5
10	Stomata number/mm	-	15(15.9±0.12)17
11	Stomata length	-	3(3.35±0.10)4
12	Stomata width	-	1.5(1.5±0)1.5
13	Stomata Index	-	8.4%
	Leaf Architecture		
14	Angle of Divergence	82-100	
15	Nerves Ending	1-2	
16	Areola Formation	Well developed	
17	Areola shape	4	
18	Vein Category	Alternate	
19	Vein course	Straight	
20	Venation	Eucamptodromous	
21	Leaf Shape	Elliptic	
22	Leaf base	Round	
23	Leaf Margin	Entire	
24	Leaf Apex	Acute	
25	Leaf Type	Simple	
26	Leaf Arrangement	Alternate	
27	Leaf Surface	Glabrous	
	Midrib		
28	Collenchyma layers	2-3	
29	Pith shape	Oval	
30	Parenchyma layers	3-4	
31	Parenchyma shape	Polygonal	

32	Outer surface	Slightly uneven
33	Trichome	Present
34	AB Shape	Slightly crescentiform
35	AD shape	Slightly crescentiform
36	Vascular Bundle type	Collateral
37	Vascular Bundle Shape	Oval
38	Vascular Bundle Nature	Continuous
39	Sclerenchyma	Present

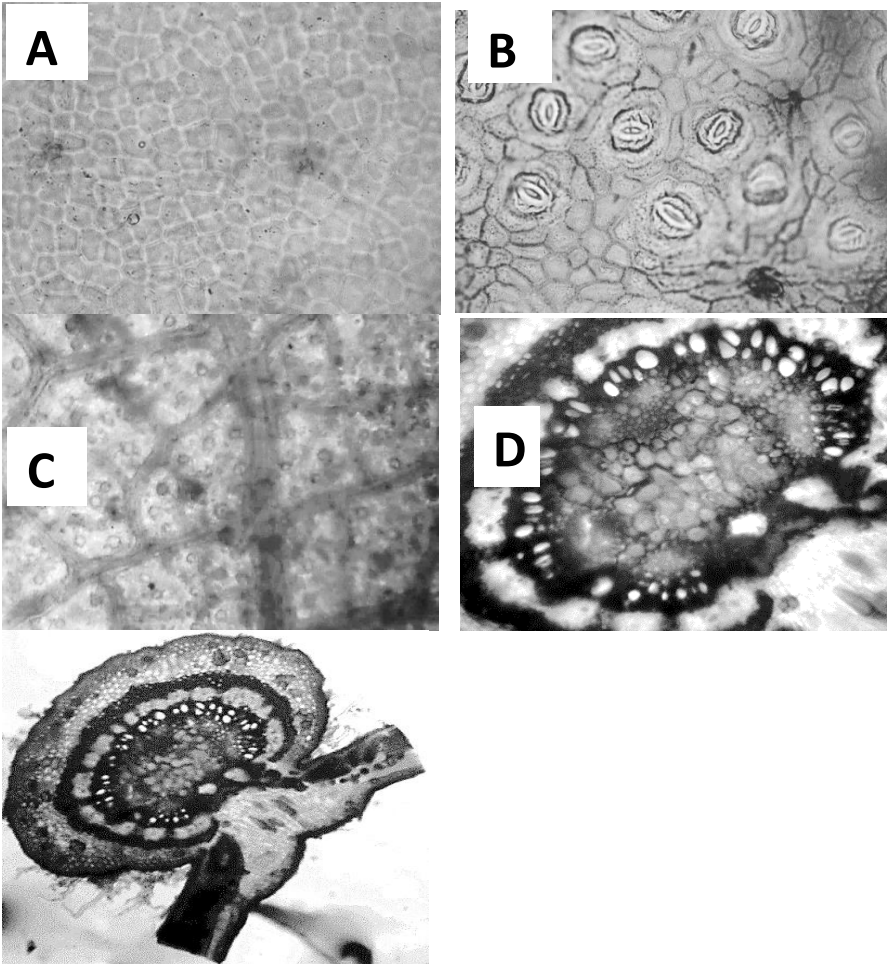


Figure 1: Character of leaf anatomy under light Microscopy: A- Adaxial surface, B- Abaxial surface, C- lamina architecture, D&E- Midrib of *Letestudoxa bella* Scale bar- 25 μ m.

The summary of the result in Table 1 and Figs. 1 & 2

Leaf Epidermal

The leaf is hypostomatic, with stephanocytics stomata at the abaxial surface. The stomata are sparse. Epidermal cell shape and anticlinal wall pattern are polygonal and straight on both surfaces. Crystal can be found only on the abaxial. Quantitatively, the mean epidermal cells number is about 261.9 ± 1.42 on the adaxial and 174.1 ± 0.55 on the abaxial (Table 1). The range of epidermal cell size is $6.4 \pm 0.16 \mu\text{m} \times 4.7 \pm 0.23 \mu\text{m}$ on the upper surface and $5.55 \pm 0.32 \mu\text{m} \times 2.75 \pm 0.17 \mu\text{m}$ on the lower surface (Table 1). Mean epidermal cell number per square millimeter is 15.7 ± 0.09 and stomatal size is $3.35 \pm 0.10 \times 1.5 \pm 0$ on the abaxial.

Lamina Architecture

Leaves alternate, simple leaf with petiole. Leaf shape elliptical, leaf apex and base is acute and round or acute. Leaf margin entire, venation is eucamptodromous. Areoles well developed polygonal. Angle of divergence is between 82-100 degrees, Nerves ending mostly between 1 to 2, well developed areola, four shaped areola more like rectangular, vein category possesses alternate, the vein course straight.

Midrib

The midrib is slightly uneven on the surface with the presence of trichomes on both surfaces. Parenchyma cells are polygonal and most abundant with about 3-4 layers while collenchyma cell is about 2-3 layers. The upper and lower surfaces are slightly crescentiform. The vascular bundle is oval-shaped and its type is collateral. There is continuous ring of the vascular bundle. There are some of crystals in the vascular bundle. Pith shows deposit of sclerenchyma cell (Table 1).

Discussion

The Foliar epidermis is one of the most noteworthy taxonomic characters from the biosystematic point of view and the taxonomic studies of a number of families are made on the basis of leaf epidermis (Stace, 1984; Baranova, 1972). In contrast to the paracytic stomata of the family Annonaceae reported by different authors (Metcalf & Chalk, 1950; Olowokudejo 1990; Pereira-sheteolu 1994 and Michael & James, 2012), this investigation reveals stephanocytics stomata at the abaxial surface of *Letestudoxa bella*. This type is a diagnostic feature for the identification of the genus *Letestudoxa*. Metcalfe & Chalk, (1950) recorded the presence of clustered or solitary crystals in Annonaceae but were not observed in the epidermis.

Davis and Heywood (1963) and Radford *et al.* (1974) have enunciated the taxonomic significance of anatomical characters as an aid to establishing the interrelationships of taxa at and above the specific level. The midrib anatomy revealed useful taxonomic information for the recognition of the species in this genus. Van sulen *et al.*, 1994 reported simple hair in some of the species of

Duguetia, which is a sister genus to *Letestudoxa* in the tribe *Duguetieae* (Chatrou *et al.*, 2012). These simple hairs are also present in abaxial surface of *Letestudoxa bella*. Furthermore, Metcalfe and Chalk (1950) also reported sclereid elements of various kinds in the parenchymatous tissue of the leaf and axis. These are also present in the vascular bundles and crystals in the pith of midrib. Stace, (1965); Inamdhar & Gangadhara (1977) noted that trichome distribution can be under the influence of environmental factors. Presence of trichomes was reported by Metcalfe & Chalk (1950, 1979) in the family Annonaceae. Likewise in this study, trichomes are restricted to the lower surface of the epidermis and found on both surfaces of the midrib.

Vein architecture has been confirmed to be a taxonomic option to solving some problems of identification. The principal characteristics of the leaf venation pattern of a species are genetically fixed. This provides the basis for using the leaf venation as a taxonomic tool (Hickey, 1973). Doyle & Le Thomas (1996) asserted that Annonaceae vary more than the families in spacing, angle and course. Angle of divergence on the secondary vein is highest in between the angles of 82-100. Nerve ending is between 1 and 2. He further revealed that many members of Annonaceae have higher secondary angles, recorded brochidodromous to eucamptodromous in their works. This study revealed eucamptodromous venation in the studied species which falls within the conclusion of the previous (Hickey, 1973; Radford *et al.*, 1974). With all the characters described in this study, it will be easy to identify the quest to document leaf anatomy of *Letestudoxa bella* with its leaf samples even when they are in fragments.

References

- Baranova, M. (1972). Systematic anatomy of the leaf epidermis in the Magnoliaceae and so Bayer C and Kubitzki K (2003). Malvaceae. In: Kubitzki K and Bayer C [eds.], The families and genera of vascular plants, vol. 5, Malvales, Capparales and non-betalain Caryophyllales, pp.225-310. Springer-Verlag, Berlin, Germany related families. Taxon, 21: 447-469.
- Botermans, M.Sosef, M.S. M, Chatrou, L.W., and Couvreur, T.L.P. (2011). A revision of the African genus *Hexalobus* (Annonaceae). *Systematic Botany* 36: 33-48.
- Chatrou, L., Koek-Noorman, J., & Maas, P. J. (2000). Studies in Annonaceae, XXXVI: the *Duguetia* alliance: where the ways part. *Annals of the Missouri Botanical garden*, 87(2), 234-245.

- Chatrou, L.W., Pirie, M.D, Erkens R.H.J, Couvreur, T.L.P., Neubig, K.M., Abbott, J.R, Mols, J.B Maas, J.W., Saunders, R.M.K. and Chase, M.W., (2012). A new higher-level classification of the pantropical flowering plant family Annonaceae informed by molecular phylogenetics. *Botanical Journal of the Linnean Society*.169: 5–40.
- Chatrou and Saunder, (2012).
<http://annonaceae.myspecies.info/taxonomy/term/2308>. World Annonaceae 13p
- Couvreur, T.L.P. (2009). Monograph of the syncarpous African genera *Isolona* and *Monodora* (Annonaceae). *Systematic Botany Monographs*.87: 1–150.
- Couvreur, T.L.P., Maas, P.J.M., Meinke, S., Johnson, D.M., Kebler, P.J.A., (2012). Keys to the genera of Annonaceae. *Botanical Journal of the Linnean Society* 169: 74–83.
- Davis, P.H. and Heywood, V.H. (1963). *Principle of Angiosperm Taxonomy*. Oliver & Boyd. Edinburgh. 556pp.
- Dehgan, B. (1980). Application of epidermal morphology to taxonomic delimitations in the genus *Jatropha* L. (Euphobiaceae). *Botanical Journal of the Linnean Society*, 80 (3) 257- 278
- Dilcher, D. L. (1974). Approaches to the Identification of Angiosperm leaf remains. *Botanical Review*.40: 1-157.
- Doyle, J. A and Le Thomas, A. (1996). Phylogenetic analysis and character evolution in Annonaceae. Bulletin duMuséum National d'Histoire Naturelle, Paris, 4e série, Section B, *Adansonia*.18: 279–334
- Fries, R.E. (1959). Annonaceae. In: Engler A, Prantl K, eds. Die natürlichen Pflanzenfamilien, 2nd edn, Vol. 17aII. Berlin: Duncker & Humblot, 1–171.
- Hickey, L. F. (1973).Classification of the Architecture of Dicotyledonous Leaves, *Amer. J. Bot.* 60(1): 17-33.
- Holmgren, P.K.; Keuken, W. and Schofield, E.K.1990.*Index Herbariorum: Part 1: The Herbaria of the World*. Reg. Veg. 120.
- Inamdar, J. A. and Gangadhara, M. (1977). Studies on the trichomes of some Euphorbiaceae. *Feddes Repertorium*. 88: 103-111.

- Kadiri, A.B.Olowokudejo, J.D. and Ogundipe, O.T. (2009). Leaf epidermis morphology of West African species of the genus *Acalypha* (Euphorbiaceae). *Bot. Lith.* 15(2), 65-78.
- Metcalf, C.R and Chalk, L. (1950). Anatomy of the Dicotyledons. Oxford University Press, Oxford. 724 pp.
- Metcalf, C.R and Chalk (1979). Anatomy of the Dicotyledons (2nd ed.). Vol. 1. Oxford University Press, Oxford. 276 pp.
- Michael, D. P and James, A.D. (2012). Dating clades with fossils and molecules: the case of Annonaceae. *The Linnean Society of London*. 169, 84–116.
- Ogundipe, O.T. and Kadiri, A.B. (2012) Comparative Foliar epidermal morphology of the West African Species of Amaranthaceae Juss. *Feddes Repert.* 123, 97–116.
- Olowokudejo, J.D. (1990). Comparative morphology of leaf epidermal of Genus *Annona* (Annonaceae) in West Africa. *Phytomorphology*. 40(3&4): 407-422
- Olowokudejo, J.D. (1993). Comparative epidermal morphology of West African species of *Jatropha* L. (Euphorbiaceae). *Botanical Journal of the Linnean Society*. 111:139-154.
- Pereira-Sheteolu, O. A. (1992). Taxonomy of medicinal plants: Foliar epidermal characters in the genus *Monodora* (Annonaceae). *Feddes Repertorium*. 103(5-6).
- Qiu, Y. L. & Lee, J. (2000) Transition to a land flora: a molecular phylogenetic perspective. *J. Phycol.* 36: 799-802
- Radford, A. E., Dickson, W. C., Massey, J. R. and Bell, C. R. (eds.) (1974). Vascular Plant Systematics. Harper & Row Publishers, New York. 886pp.
- Stace, C.A. (1965). "Cuticular studies as an aid to plant taxonomy". *Bull. Brit. Mus. (Nat. Hist.) Botany*. 4:3-37
- Stace C.A. (1984). The taxonomic importance of the leaf surface. In: Heywood, V.H, Moore, D.M, (editors) Current concepts in Plant Taxonomy. London Academic Press, 67-94

- Van Zuilen, C. M., Koek Noorman, J. and Maas, J.M.P. (1994). A phylogenetic analysis of *Duguetia* (Annonaceae) based on morphological data. *Plant systematic Evolution* 194(3): 173-188
- Yang, Z.R. and Lin, Q. (2005). Comparative morphology of the leaf epidermis in *Schisandra* (Schisandraceae). *Bot. J. Linn. Soc.* 148: 39–56.

Preliminary Investigation of Petrographic and Geochemical Characteristics of Crystalline Rocks in Jebba, Central Nigeria

O. A. Omotoso^{1*}, O. J. Ojo², A. I. Aina¹, J. I. Udeafor¹, M. T. Alebiosu¹

¹Department of Geology and Mineral Sciences, University of Ilorin, Ilorin, Nigeria

²Department of Geology, University of Oye-Ekiti, Nigeria

Corresponding Author: *Dr. O. A. Omotoso; deletoso2002@yahoo.co.uk;
+2348067780267

Abstract

The preliminary investigation of petrographic and geochemical characteristics of crystalline rocks in Jebba, central Nigeria was carried out in order to establish the various rock types in the area. Petrographic studies were carried out using thin sections of the rock samples and geochemical analysis was carried out using X-ray Fluorescence (XRF) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Field mapping indicated that the area is underlain by granite (porphyritic granite), granite gneiss, quartzite and schist. The petrographic studies indicated the main mineral assemblages in the crystalline rocks as: quartz, plagioclase, microcline and biotite. Results of geochemical analysis established SiO₂ and Al₂O₃ as dominant major oxides in the investigated rocks ranging from 46.66 to 97.6 wt. % and 0.25 to 17.48 wt. % respectively. Average values of CIA (Chemical Index of Alteration), PIA (Plagioclase Index of Alteration) and CIW (Chemical Index of Weathering) of the rocks are 59.2, 65.0 and 72.8 (granite gneiss); 58.4, 62.9 and 70.6 (granite); 67.7, 79.9 and 83.3 (quartzite); 57.9, 61.4 and 66.4 (schist) respectively. The intensive weathering of the rocks would have contributed to the concentration of metals in the water phase of the study area. Most of the rare earth elements in granite gneiss are highly enriched with respect to abundance in the earth crust having values greater than 1 ppm while granite and quartzite are depleted in these elements.

Keywords: rock types, petrography, geochemistry, weathering indications and enrichment.

Introduction

Rocks are considered as aggregates of one or more minerals formed from cooling of molten magmas, consolidation of sediments, and/or alteration of pre-existing rock materials under high pressures and temperatures (Pelig-Ba, 2012; Hatch *et al.*, 1979). As reported by Pelig-Ba 2012, minerals may be either amorphous or crystalline and also vary in grain size and morphology. However, it may be very difficult to trace the origin of minerals in rocks to the original magma since the

original composition may have changed due to the loss of volatile substances during exposure leaving behind residual materials (Pelig-Ba, 2012; Hatch *et al.*, 1979). The type of crystalline rock formed and its mineralogical content depends on the mode of formation and the host environment as a whole. Hence, the chemical make-up of rocks depends on the original chemical composition of the magma as well as the processes occurring during or after the cooling, duration and geochemical environments in which the crystalline rock was initially deposited. This is the reason why various rock types are found with varying compositions ranging from minimal chemical differences to very different rock types. There are many different types of rocks with varying mineralogical and geochemical compositions and these determine their respective suitability for man's uses. Hence, there is the need to critically examine their mineralogical and geochemical compositions.

The study area forms part of the Nigerian basement complex (Fig. 1). The Nigerian basement complex is a part of a late Proterozoic to early Paleozoic mobile belt east of the West Africa craton. This basement complex comprises gneisses, migmatites psammitic rocks, schists, amphibolites and intrusive igneous rocks. It is reported that the basement complex is polycyclic and bears the imprint of four thermo-tectonic events (Liberian-ca 2700 Ma, Eburnean- ca 2000 Ma, Kibaran- ca 1100 Ma, and Pan African- ca 600 Ma (Van Breemen, *et al.*, 1977; Grant, 1978; Fitches *et al.*, 1985; Ekwueme, 1987; Rahaman, 1988; Okonkwo, 1992). Jebba area lies at the northern margin of the south-western sector of the basement complex and is separated from the north-western sector by the Cretaceous to Tertiary sedimentary rock of the Bida Basin. Okonkwo (1992) discussed the lithostratigraphic units of Jebba area as migmatitic gneiss, granitic gneiss, metagreywacke, quartzite, quartz-biotite schist and post-tectonic granitic rocks.

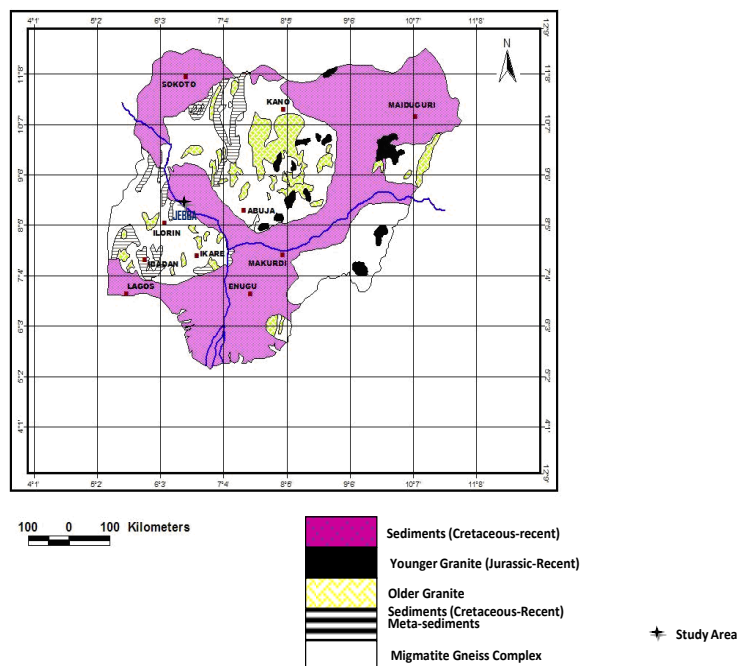


Figure 1: Generalized geological map of Nigeria showing study location (*Geological Survey of Nigeria, 2004*)

Methodology

The mapped area is situated within longitude $4^{\circ} 48'E$; $4^{\circ} 52'E$ and latitude $9^{\circ} 07'N$; $9^{\circ} 11'29''N$ (Fig. 2). Geological mapping was undertaken to determine the rock types, their distribution and lithological boundaries. Accessibility was facilitated by roads and foot paths. At each location, rock samples were examined and located on the base map using Global Positioning System (GPS). *Field measurement* of strikes, dips, joint directions and faults were taken for structural study with compass clinometers. Foliations, joints, mineral lineation and intrusions were some of the observed features. Twenty three samples were selected from representative rock types in the study area and subjected to geochemical analysis to determine the concentrations of the major oxides, trace elements and the rare earth elements and to assess their weathering history. The rock samples were pulverized in the Geology laboratory of University of Ilorin, Nigeria prior to geochemical analysis in ACME Laboratory, Canada using Inductively Coupled Plasma- Mass Spectrometry (ICP-MS). The petrographic preparation of the rock slides was carried out in Geology Laboratory of University of Ibadan, Nigeria. It involved cutting the respective rock

sample into small rectangular pellet of 3 mm, mounted on glass slide using resin araldite. The slide was then lapped on a glass plate using medium-grain carborundum as abrasive in order to create friction between the rock pellet and the glass. This automatically reduced the slide thickness to a thickness of 0.3 mm. The thinned rock was sealed using Canada balsam.

Results and Discussion

The main rock types mapped in the study area consist of granite, granite-gneiss, quartzite, biotite schist and schist (Fig. 2).

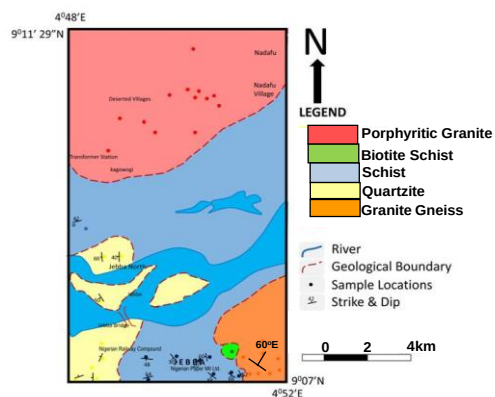


Figure 2: Geological map of the study area

Granite-Gneiss

Granite-gneiss is not well exposed in the area and generally occurs in the south eastern part of the study area (Fig. 3). They are generally low lying, weakly foliated and strongly mineralogical lineated. Occasionally, there are minor intercalations of hornblende-biotite gneiss which are generally enclosed in the granite gneisses. The granite gneisses are intruded by granite and pegmatite while the contact between granite gneiss and quartzite are gradational. Augen structures (eye-like structure) and asymmetry folds are prominent on the granite gneiss (Fig.3). Generally, joints trend NW-SE direction. The planar surfaces mainly dip towards the east. Quartz vein and quartzo-feldsparitic intrusions (average width of 4 cm) are observed on the granite-gneiss. In hand specimen, the rocks are medium to coarse grained and in some places dissected with quartzo-feldspathic veins. In thin section, modal analysis (Table 1) shows that the granite-gneiss is chiefly comprised of quartz (24 %), plagioclase (31 %), biotite (35 %) and chlorite (7 %). Similarly, the light and dark-coloured minerals representing quartz and biotite respectively are clearly seen in the photomicrographs of the granite-gneiss (Fig.8). The quartz is colourless under plane polarized light and occurs as euhedral prismatic crystals. The plagioclase is characterized with its distinguished polysynthetic twinning according to Albite law.

Biotite is brownish in thin section and exhibits pleochroism. It occurs as plates and laths which show preferential alignment with the foliation plane.

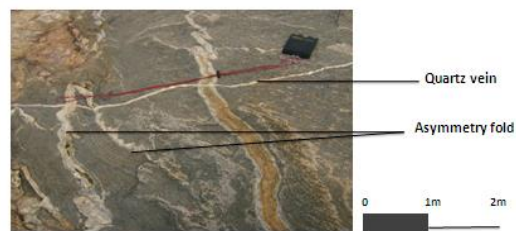


Figure 3: Field photograph showing Asymmetry folds and, augen structures defined by quartz veins and foliation planes (9°8'30''N; 4°49'30''E)

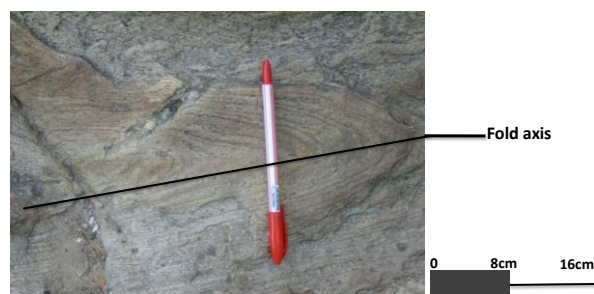


Figure 4: Field photograph showing Open folds in granite gneiss showing fold axis (9°8'0''N; 4°49'0''E)

The SiO₂ content of the granite gneiss ranges from 48.82-73.47 wt % (average, 65 wt %) which corresponds to an intermediate to felsic composition. Al₂O₃ ranges from 11.85-15.85 wt % (average, 13.7 wt %) while Fe₂O₃ ranges from 3.22 – 15.35 wt % (average, 7.1 wt %). MgO varies from 0.17 – 5.62 % (average, 1.9 wt %), Na₂O content ranges from 1.99 – 3.68 wt % (average, 2.6 wt %), K₂O from 1.09 – 6.25 % (average, 4.1 wt %) and CaO from 0.53 – 8.94 wt % (2.9 wt %). MnO ranges from 0.003 - 0.21 wt % (average, 0.08 wt %) while TiO₂ ranges from 0.26 - 2.02 wt % (average, 0.8 %). P₂O₅ ranged from 0.04 – 0.58 wt % (average, 0.2 wt %), Cr₂O₃ varied from 0.002 – 0.04 wt % (average, 0.02 wt %), Table 2. The TiO₂ values were generally less than 1 wt % in the granite gneiss but some samples showed values up to 2.02 wt %. This confirmed the presence of mica and feldspar groups of minerals in the rock (Pelig-Ba, 2012). The Na₂O concentration in the rocks is consistently lower than K₂O. K₂O/Na₂O ratios are consistently higher than 1 which reflects abundance of potassium bearing rock forming silicate e.g. biotite (Oyinloye, 2011). The concentration of SiO₂, Al₂O₃, Na₂O and K₂O in granite-gneiss indicates abundance of felsic silicates.

The trace elements concentrations are presented in Table 3. The level of Rb ranges from 26.2 to 260 ppm (average=146.76 ppm) while Sr and Ba range from 89.1 to 3317 ppm (average=173.6 ppm) and 310 to 1148 ppm (average=714 ppm)

respectively. In the granite gneiss, the moderately high average value of Rb indicates high plagioclase mineral concentration as observed in the thin section study (Oyinloye, 2011). The ratio of trace element concentration of the rock samples with the corresponding average value in the earth crust (Krauskopf and Bird, 1995) was obtained to determine the enrichment value. The mean ratios of Rb for all the samples selected for granite-gneiss was 2.4 which was more than unity suggesting high enrichment value of Rb in the rock. The range of the enrichment value was from 0.4 to 4.3 for Rb, 0.73 to 2.7 for Ba and 0.2 to 0.9 for Sr. The average enrichment value for Ba and Sr were 1.7 and 0.5 respectively depicting that Ba is enriched and Sr is less enriched (< 1). Table 5 presents the average enrichment values for trace metals in the samples analyzed. There is significant enrichment of Au in the granite-gneiss in the study area. Pb shows a significant enrichment of more than 1. This may enhance its contributions to its concentration in the water phase during weathering and dissolution. Fig. 9 illustrates the UCC-normalized averages in trace element concentrations in the granite gneiss of the study area. Rare earth elements analyzed in the rock samples of the study area include Th, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu (Table 4). Th, Y and Ce ranged from 1.6 to 93.4 ppm, 15.2 to 50.4 ppm and 66.1 to 162.7 ppm respectively. It has been reported that REEs most especially Dy, Ho, Er, Tm, Yb and Lu reside in refractory minerals such as zircon, sphene and garnet phases and their levels could be attributed to the presence of these minerals in the granite gneiss (Sahoo *et al.*, 2011). Their enrichment values were determined with respect to Upper Continental average Concentrations (UCC) (Krauskopf and Bird, 1995). The enrichment value for the rare elements were more than 1 except Dy and Eu which shows unity value respectively (Table 5). This implies that granite gneiss in the study area is highly enriched in most of the rare earth elements. The enrichment values of these rare earth elements are highest in granite-gneiss compared to granite, quartzite, and schist/biotite schist. The high enrichment value recorded for the elements in granite-gneiss could contribute to their concentrations in the water phase during weathering and dissolution. Fig. 10 illustrates the UCC-normalized averages in rare earth element concentrations in the granite gneiss of the study area.

Quartzite

Quartzite occurs in well exposed outcrops in the area where its forms prominent ridge trending N –S which extends west of Jebba town, very close to the River Niger. It varies between massive to foliated quartzite (Fig. 5). The massive types of quartzite are not well enriched in muscovite compared with the foliated quartzite. They are probably thought to be produced by regional metamorphism of sandstones during Orogenic activities (Okonkwo, 1992). The foliation planes dip toward the west. Micro-folds are observed on the massive quartzite and the joints trend NW-SE direction.



Figure 5: Field photograph showing Foliation of quartzite in south west Jebba, joint cuts across the foliation trend. ($9^{\circ}7'30''\text{N}$; $4^{\circ}49'0''\text{E}$)

In thin section, the rock contains quartz (90 %) which is colourless under plane polarized light and also exhibits itself as euhedral prismatic crystals. Trace of muscovite is also observed with modal composition of 9 %. The photomicrograph is presented in Fig. 8. The SiO_2 content ranges from 78.22 – 97.6 %, Al_2O_3 ranges from 0.25 – 11.75 %, Fe_2O_3 ranges from 1.18 – 2.06 % and MgO varies from 0.02 – 0.71 %. Others major oxides like CaO varies from 0.04 to 0.33 %, Na_2O varies from 0.02 to 4.67 %, K_2O varies from 0.06 to 2.4 %, TiO_2 ranges from 0.01 to 0.18 %, P_2O_5 ranges from 0.02 to 0.08 % and Cr_2O_3 ranges from 0.02 to 0.003 %. Only MnO is below detection limit of 0.01 %. The relatively high values of silica in the rock samples are characteristic of quartzite. The geochemical data of the analyzed rock samples in the study area is presented in Table 2. The descriptive statistics of the trace elements is presented in Table 3. Ba, Rb, Sr and Pb range from 30 to 645 ppm, 2.2 to 52.9 ppm, 3.8 to 148.1 ppm and 0.5 to 1.4 ppm respectively. The values obtained are less than those obtained for granitic rocks. In terms of relative enrichment Pb, Rb and Sr indicate low enrichment except Ba that has an average value of unity and may probably decrease their contributions to the water phase during weathering and dissolution. Fig. 9 illustrates the UCC-normalized averages in trace element concentrations in the quartzite of the study area. The concentrations of the rare elements are presented in Table 4. The average values of almost all the elements in quartzite are relatively less than their corresponding elements in the granitic rocks. Consequently, they have low enrichment values. The relative loss of the less mobile elements such as, Nb and Y suggested that samples had undergone some early metamorphism or deformation to some extent. Fig. 9 illustrates the UCC-normalized averages in rare earth element concentrations in the quartzite of the study area.

Schist

The schist in the study area is a highly massive weathered outcrop with different joints. Schist is formed in the area due to constant high temperature of rock during metamorphism. They composed of visible platy pattern with mineral alignments

trending NW – SE direction. The schists are leucocratic quartz mica schist in which white bands is alternating the grayish ones. The schist also contains thin, more siliceous bands possibly to be a reflection of original sedimentary grading (Okonkwo, 1992). Biotite schists are also identified on the field. Pinch and swell structures were observed on the quartz-mica schist which cut cross joint and asymmetric folds (Fig. 6). Joints trend NW-SE and the dip of foliation planes is toward the west.

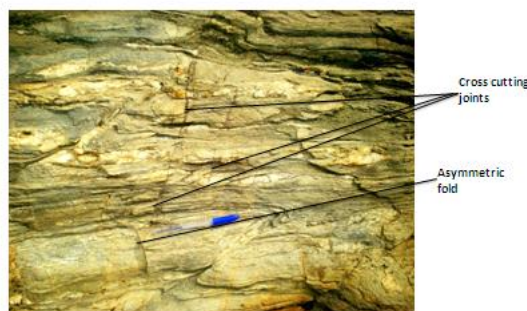


Figure 6: Field photograph of Pinch and swell structure on quartz-mica schist. Note also the cross-cutting joint as well as an asymmetric fold. (9°7'28''N; 4°50'12''E)

Thin sections were prepared and examined under the petrographic microscope to determine the mineralogical compositions of the biotite schist/schist. The following minerals were observed: Quartz (32 %), plagioclase (26 %), microcline (27 %) and biotite (12 %). The biotite schist (sample BL10) is impoverished in silica (53.75 wt %) but enriched in Al_2O_3 , Fe_2O_3 , MgO and CaO which is consistent with mafic characteristics. The low value in silica content is compensated by its enrichment in TiO_2 (2.02 wt %). The TiO_2 content is low in all the samples, generally < 1 wt %. However, some samples in granite-gneiss, schists and biotite-schist are relatively richer in TiO_2 with levels > 1 wt %. Titanium is believed to be associated with mica and feldspar groups of minerals and its level could be attributed to the presence of these minerals (mica and feldspar groups of minerals) in the rocks. Other sources of Ti are from minerals where it can substitute for Al, Mg or Fe (Deer et al, 1996; Pelig-Ba, 2012). Some of the schist and biotite schist samples have higher percentage of CaO and MgO contents up to 4.88 and 2.5 wt % respectively suggesting the availability of mafic minerals.

The results of geochemical analysis and the statistics of the trace elements are presented in Table 4.3. Ba, Co, Cs, Ga, Hf, Nb, Sn, Sr, Ta, U, V, Zr, Mo, Cu, Pb, Zn, Ni, As, Au and Tl were detected in the geochemical analysis of the meta-sedimentary rocks. However, Be, W, Cd, Sb, Bi, Ag, Hg and Se are below detection limits. The relatively high value of Rb reflects an evidence of plagioclase minerals in the samples. On the basis of enrichment value, Zr, Sn, Rb, Hf and Ba are enriched in

relation to their respective average compositions in the earth crust. Others are less enriched. The enrichment of Ba, Zr, Sn, Rb and Hf may constitute their solubility in the water phase during weathering of the rocks thereby increasing their natural composition in water phase. Fig. 9 illustrates the UCC-normalized averages in trace element concentrations in the schist of the study area. The Rare elements detected in Biotite schist/schist are: Th, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu. Their respective concentrations and statistics are presented in Table 4. Based on the enrichment values, Dy, Ho, Pr and Th are depleted and others are enriched. They could therefore contribute to their respective concentration in the water phase during weathering and dissolution. Fig. 10 illustrates the UCC-normalized averages in rare earth element concentrations in the schist of the study area.

Granite

Granite occurs in the northeastern and northwestern part of the study area (Jebba North). It occurs as high-rising exfoliated and rounded bodies usually called inselbergs or small low-lying exposures. The rock is medium to coarse grained and mesoporphyritic in texture (Fig. 7). The outcrops have sharp contacts with the surrounding metamorphic rocks and the pegmatitic and quartz veins found within them. The width of the veins ranges from about 4 cm to 7 cm and they mainly trend NE-SW. In outcrops, the rock is leucocratic to mesocratic, depending on the ferromagnesian contents.



Figure7: Field photograph showing Porphyritic granite and pegmatitic vein (9°8'30"N; 4°49'30"E)

The following minerals were identified: quartz, plagioclase, microcline, biotite, chlorite, scolecite and epidote. The modal composition of the minerals under the petrographic microscope indicates the percentages of the minerals as follows: quartz (25 %), plagioclase (10 %), microcline (40 %), biotite (14 %), chlorite (5 %), scolecite (2) and epidote (3). The photomicrograph and modal compositions of the minerals observed are presented in Figure 8 and Table 1 respectively.

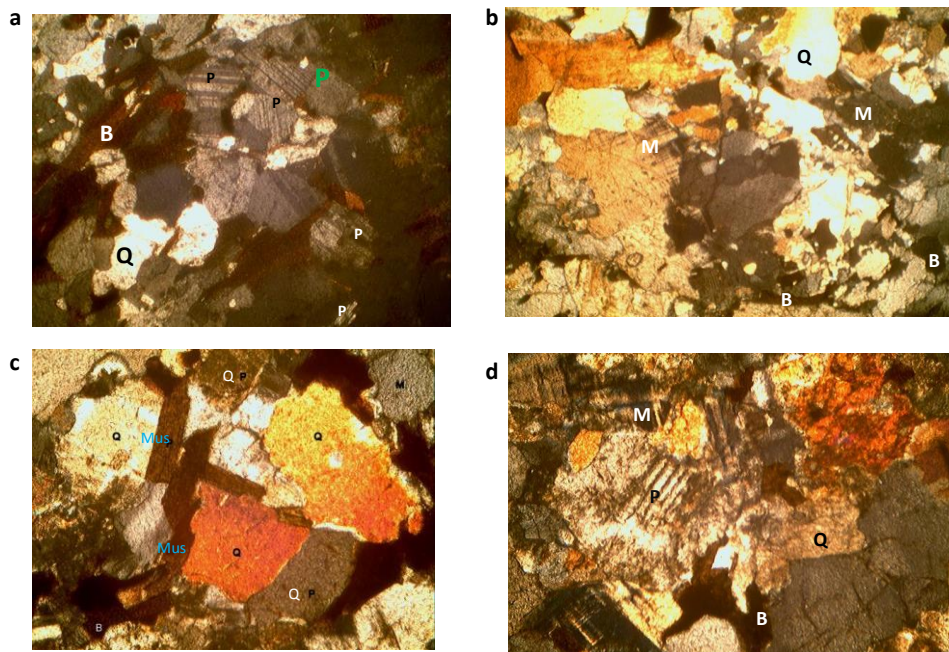
The concentrations and the statistics summary of the major oxides in the granite rock samples of the study area are presented in Table 2. The SiO₂ content of granite varies from 60.07 – 72.58 wt % (Average, 67 wt %). This denotes that majority of the granite rocks may be classified as felsic igneous rock. Al₂O₃ ranges from 14.76 – 17.48 wt %, Fe₂O₃ from 0.04 – 2.95 wt %, CaO from 1.02 - 5.2 wt %, Na₂O from 3.22 – 5.27 wt % and K₂O from 3.4 – 6 wt %. Others like TiO₂ vary from 0.03 – 0.74 wt %, P₂O₅ from 0.02 – 0.31 wt % MnO from 0.04 to 0.09 wt % and Cr₂O₃ from 0.02 to 0.03 wt %. K₂O/Na₂O ratio ranges from 0.6 – 1.5 which reflects almost the same compositions of potassium bearing rock forming silicate (Alkali feldspar – microcline) and sodium rock forming silicate (plagioclase feldspar – Albite) in the granite (Oyinloye, 2011). The trace elements in the granite samples are presented in Table 3. Ba, Rb and Sr ranged from 130 to 3056 ppm, 48.2 to 238 ppm and 145.7 to 1635.8 ppm respectively. The average values of Ba and Sr in granite is higher than those of granite-gneiss but the concentration of Rb is less in granite than in granite-gneiss. This is due to the fact that the rocks have undergone some early metamorphism or deformation to some extent. In terms of relative enrichment values, Ba, Rb and Sr are highly enriched having values greater than unity (> 1). Certain degrees of these elements are dissociated into the water phase thereby increasing their respective concentration. More important is lead, having a concentration of range of 2.2 to 41 ppm and enrichment of 1.3. The granitic rock could contribute part of the Pb concentrate into water phase during weathering. In general, about 50% of the elements show high enrichment in granite elements. Fig.9 illustrates the UCC-normalized averages of trace element concentrations in the granite of the study area. The statistics of the rare elements are presented in Table 4. Th, Y and Ce ranged from 0.5 to 12.1 ppm, 4.7 to 17.4 ppm and 6.4 to 39.4 ppm respectively. The average values obtained are lower than those obtained in granite-gneiss. On the basis of enrichment value, the rare elements are less enriched in granite (Table 5). Fig. 10 illustrates the UCC-normalized averages of rear earth element concentrations in the granite of the study area.

Weathering Indications

These are determined in this research using Chemical Index of Alteration (CIA), Plagioclase Index of Alteration (PIA) and Chemical Index of Weathering (CIW) as suggested by Nesson and Young (1982). The CIA, PIA and CIW are calculated using the molecular proportions as follows; $CIA = [Al_2O_3 * 100 / (Al_2O_3 + CaO + Na_2O + K_2O)]$, $PIA = (Al_2O_3 - K_2O) / (Al_2O_3 + CaO + Na_2O - K_2O) * 100$ and $CIW = (Al_2O_3 / (Al_2O_3 + CaO + Na_2O)) * 100$. The CaO content was assumed to be derived from silicate fraction since carbonate and apatite were negligible. Fresh rocks or minerals such as albite, anorthite or K-feldspars have CIA up to 50%, fresh basalts have between 30 and 45% while granite and granodiorites range from 45 to 55% and fresh muscovite having values up to 75%. Clay minerals which are weathered products of fresh rocks have higher values, illites and

montmorillonites have a range from 75 to 85%, kaolinites have up to 100% (Nessbitt and Young, 1982). These values suggest that the higher the CIA value the more weathered the rock. The CIA values obtained in this study for granite-gneiss, granite, quartzite and biotite schist/schist range from 52.7 to 70 % (average=59.2 %), 57 to 59.3 % (average, 58.4 %), 61.4 to 74.1 % (average, 61.36 %) and 51 to 63.7 % (average, 57.9 %) respectively (Table 4.6). The PIA values computed for granite-gneiss, granite, quartzite and biotite schist/schist range from 52.9 – 76.4 % (average, 65 %), 61.9 – 64.6 % (average, 62.9 %), 65.2 – 98.6 % (average, 79.9 %) and 51.6 – 68.3 % (average, 61.4 %) respectively Table 4.2. Similarly, the CIW values calculated for granite-gneiss, granite, quartzite and biotite schist/schist varied from 55 – 79.6 % (average, 72.8 %), 67.6 – 72.5 % (average, 70.6 %), 70.1 – 99.1 % (average, 83.3 %) and 52.6 – 76.2 % (average, 66.4 %) respectively.

Quartzite has the highest average value of CIA, PIA and CIW while Schist/biotite schist has the lowest. This shows that quartzite has highest degree of weathering. Hence, there is contribution of weathering of some minerals in the water phase of the study area.



Q=quartz, P=plagioclase, M=microcline, B=biotite, Mus=muscovite, C=chlorite, S=schist, E=epidote

Figure 8: Photomicrograph of (a) granite gneiss, (b) granite (c) quartzite and (d) schist

2µm

Table 1: Average modal analysis of mineralogical compositions of the crystalline rocks in the study area

Minerals (%)	Granite-gneiss	Granite	Quartzite	Schist
Quartz	24	25	90	32
Plagioclase	31	10	-	26
Microcline	-	40	-	27
Biotite	35	14	-	12
Muscovite	-	-	9	-
Chlorite	7	5	-	-
Scolecite	-	2	-	-
Epidote	-	3	-	-
Total	97	99	99	97

Table 2: Chemical Compositions of Major Oxides in wt % of analyzed Rock samples in Jebba environs and their weathering indications

Granite-Gneiss														
Sample ID	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MgO	CaO	Na ₂ O	K ₂ O	TiO ₂	P ₂ O ₅	MnO	Cr ₂ O ₃	CIA	PIA	CIW
3L3X	63.8	15.85	8.24	2.35	1.81	2.24	2.75	1.11	0.17	0.12	0.04	69.98	76.38	79.65
KJ12	68.8	14.6	3.22	0.74	1.96	2.57	6.02	0.46	0.12	0.003	0.03	58.05	65.45	76.32
BL4	71.68	12.68	4.19	0.54	1.32	1.99	6.25	0.37	0.09	0.04	0.002	57.01	66.02	79.30
BL9	73.47	11.85	4.66	0.17	0.53	3.68	4.28	0.26	0.04	0.03	<0.002	58.26	64.26	73.79
LP1	48.82	13.73	15.35	5.62	8.94	2.31	1.09	2.02	0.58	0.21	0.011	52.67	52.91	54.96
average	65.314	13.742	7.132	1.884	2.912	2.558	4.078	0.844	0.2	0.0806	0.02075	59.2	65.0	72.8
min	48.82	11.85	3.22	0.17	0.53	1.99	1.09	0.26	0.04	0.003	0.002	52.7	52.9	55.0
max	73.47	15.85	15.35	5.62	8.94	3.68	6.25	2.02	0.58	0.21	0.04	70.0	76.4	79.6
stdev	9.92	1.57	4.97	2.25	3.42	0.66	2.19	0.74	0.22	0.08	0.02	6.4	8.3	10.3
Granite														
Sample ID	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MgO	CaO	Na ₂ O	K ₂ O	TiO ₂	P ₂ O ₅	MnO	Cr ₂ O ₃	CIA	PIA	CIW
JN11	60.07	17.48	4.09	0.9	5.2	5.06	5.43	0.27	0.23	0.09	<0.002	52.70	54.01	63.01
JN2	65.81	15.61	4.1	1.22	3.01	4.93	3.73	0.34	0.11	0.07	0.005	57.22	59.94	66.28
JN6	68.93	15.61	2.41	0.52	1.9	4.77	4.76	0.2	0.06	0.04	0.002	57.73	61.93	70.06
JN8	68.03	15.42	3.04	0.65	2.11	4.53	4.82	0.22	0.06	0.06	0.002	57.37	61.48	69.90
JN13	70.21	15.32	2.42	0.59	2.2	5.13	3.4	0.18	0.03	0.05	0.002	58.81	61.92	67.64
KR5	68.82	15.29	2.69	0.64	1.93	4.53	4.8	0.24	0.05	0.06	0.002	57.59	61.89	70.30
R1	66.91	16.97	2.43	0.27	1.58	4.87	6	0.15	0.08	0.06	<0.002	57.68	62.97	72.46
R2	72.56	14.76	1.95	0.04	1.02	5.27	4.07	0.03	0.02	0.04	<0.002	58.76	62.96	70.12
2L19X	61.2	16.35	5.66	2.95	4.62	3.22	3.63	0.74	0.31	0.09	0.03	58.77	61.87	67.59
1L7X	65.7	15.55	3.72	1.6	2.66	3.26	4.74	0.39	0.15	0.07	0.03	59.33	64.61	72.43
average	66.824	15.836	3.251	0.938	2.623	4.557	4.538	0.276	0.11	0.063	0.01	58.4	62.9	70.6
min	60.07	14.76	1.95	0.04	1.02	3.22	3.4	0.03	0.02	0.04	0.002	57.6	61.9	67.6
max	72.56	17.48	5.66	2.95	5.2	5.27	6	0.74	0.31	0.09	0.03	59.3	64.6	72.5
stdev	3.86	0.84	1.13	0.84	1.33	0.73	0.83	0.19	0.09	0.02	0.01	0.8	1.1	2.0
Quartzite														
Sample ID	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MgO	CaO	Na ₂ O	K ₂ O	TiO ₂	P ₂ O ₅	MnO	Cr ₂ O ₃	CIA	PIA	CIW
JR35	89.01	5.37	2.06	0.36		0.05	1.83	0.18	0.04	<0.01	0.002	74.07	98.61	99.08
JR3Q	97.6	0.25	1.77	0.02	0.04	0.02	0.06	0.01	0.02	<0.01	<0.002	67.57	76.00	80.65
R4	78.22	11.75	1.18	0.71	0.33	4.67	2.4	0.14	0.08	<0.01	0.003	61.36	65.16	70.15
average	88.277	5.790	1.670	0.363	0.185	1.580	1.430	0.110	0.047	BDL	0.003	67.7	79.9	83.3
min	78.22	0.25	1.18	0.02	0.04	0.02	0.06	0.01	0.02		0.002	61.4	65.2	70.1
max	97.6	11.75	2.06	0.71	0.33	4.67	2.4	0.18	0.08		0.003	74.1	98.6	99.1
stdev	9.711	5.761	0.448	0.345	0.205	2.676	1.220	0.089	0.031		0.001	6.4	17.1	14.6
Schist and Biotite Schist														
Sample ID	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MgO	CaO	Na ₂ O	K ₂ O	TiO ₂	P ₂ O ₅	MnO	Cr ₂ O ₃	CIA	PIA	CIW
AL2	75.52	11.44	3.18	0.78	1.24	2.34	4.46	0.29	0.09	0.03	0.005	58.73	66.10	76.17
AL7	46.66	13.74	17.28	4.55	9.85	2.53	0.52	3.15	0.26	0.004	0.004	51.58	51.64	52.60
AL8	77.27	11.23	2.74	0.93	1.01	3.18	2.21	0.33	0.13	0.02	0.005	63.70	68.28	72.83
BL10	53.75	14.46	14.4	2.5	4.88	3.22	2.5	2.02	0.75	0.15	0.003	57.70	59.62	64.10
average	63.3	12.7175	9.4	2.19	4.245	2.8175	2.4225	1.4475	0.3075	0.115	0.00425	57.9	61.4	66.4
min	46.66	11.23	2.74	0.78	1.01	2.34	0.52	0.29	0.09	0.02	0.003	51.6	51.6	52.6
max	77.27	14.46	17.28	4.55	9.85	3.22	4.46	3.15	0.75	0.26	0.005	63.7	68.3	76.2
stdev	15.412	1.625	7.531	1.755	4.136	0.449	1.615	1.392	0.304	0.113	0.001	5.0	7.5	10.5

Table 3: Geochemical Results of Trace elements in the crystalline rock of the study area in ppm

Sample ID	Granite-Gneiss																				
	Ba	Be	Co	Cs	Ga	Hf	Nb	Rb	Sn	Sr	Ta	U	V	W	Zr	Mo	Cu	Pb	Zn	Ni	As
3L3X	426		42.3	7.55	20.9	6.4		117	3	126		2.18	154	146	249	<2	122	24	115	60	
K12	964	17	1.34	20.6	9.6		260	1	231		1.93	24	91	393	<2	5	30	63	8		<0.1
BL4	722	4	5.5	4.1	11.3	9.9	17.8	210.2	4	89.1	0.7	2.8	20	0.6	362.3	1.4	29.7	12.6	34	7.5	<0.5
BL9	1148	4	1.5	1.2	18.7	9.4	13.2	120.4	4	90.2	0.8	1.7	<8	<0.5	376.7	1.6	16.2	1.5	30	5.8	2.7
LP1	310	3	46	2.1	15.3	3.4	7.4	26.2	2	331.7	0.4	0.4	256	<0.5	129.9	0.3	103.4	1.6	51	42.7	<0.5
average	714.0	3.7	22.5	3.3	17.4	7.7	12.8	146.8	2.8	173.6	0.6	1.8	113.5	79.2	302.2	1.1	55.3	13.9	58.6	24.8	2.7
min	310.0	3.0	1.5	1.2	11.3	3.4	7.4	26.2	1.0	89.1	0.4	0.4	20.0	0.6	129.9	0.3	5.0	1.5	30.0	5.8	2.7
max	1148.0	4.0	46.0	7.6	20.9	9.9	17.8	260.0	4.0	331.7	0.8	2.8	256.0	146.0	393.0	1.6	122.0	30.0	115.0	60.0	2.7
stdev	352.5	0.6	20.6	2.7	4.1	2.8	5.2	90.8	1.3	105.7	0.2	0.9	113.6	73.4	111.7	0.7	53.6	12.9	34.2	25.0	BDL
	Granite																				
	Ba	Be	Co	Cs	Ga	Hf	Nb	Rb	Sn	Sr	Ta	U	V	W	Zr	Mo	Cu	Pb	Zn	Ni	As
JN11	2600	2	4.4	0.7	14	4	11	83.7	3	1635.8	0.6	2	47	<0.5	171.4	0.5	6.6	5.5	6	2	<0.5
JN2	2560	4	6.8	0.2	15.1	2.9	6.6	48.2	2	1498.8	0.4	0.3	37	<0.5	121.2	0.8	9.1	2.2	13	5.3	<0.1
JN6	2712	3	2.9	1.1	13.3	3.7	5.1	79.7	4	1358.7	0.3	0.3	18	<0.5	150.4	0.5	7.1	4	10	3.5	<0.5
JN8	2758	1	5.8	1	15.2	3.5	6.8	95.4	2	1190.1	0.4	0.7	23	<0.5	138.2	0.8	21.7	5.6	13	12.6	<0.5
JN13	1831	<1	3.2	0.5	15	2.8	4.3	56.4	2	1352.3	0.2	2	17	<0.5	108.9	0.6	7.3	3.8	12	3.1	<0.5
KR5	3056	3	4.6	0.8	15.6	3.6	7.6	86.9	1	1394	0.5	0.5	24	<0.5	132	0.4	4.4	4.2	8	3.1	<0.5
R1	1476	4	2.4	1.5	17.5	3	9.3	140.1	<1	826.5	0.6	1.2	16	<0.5	98.7	0.5	7.1	16.7	16	3.2	<0.5
R2	130	9	0.6	2.3	22.9	4	6.1	173.9	1	145.7	0.4	1.7	<8	<0.5	74.1	0.9	9.3	15.4	23	3.3	<0.5
2L19X	2070		30.9	13.7	20.5	6.2		124	2	922		1.26	101	104	266	<2	8	28	78	24	BDL
1L7X	1795		20.8	18.7	19.8	5.4		238	4	633		1.82	58	103	197	<2	6	41	36	24	BDL
average	2098.8	3.7	8.2	4.1	16.9	3.9	7.1	112.6	2.3	1095.7	0.4	1.2	37.9	103.5	145.8	0.6	8.7	12.6	21.5	8.4	BDL
min	130.0	1.0	0.6	0.2	13.3	2.8	4.3	48.2	1.0	145.7	0.2	0.3	16.0	103.0	74.1	0.4	4.4	2.2	6.0	2.0	BDL
max	3056.0	9.0	30.9	18.7	22.9	6.2	11.0	238.0	4.0	1635.8	0.6	2.0	101.0	104.0	266.0	0.9	21.7	41.0	78.0	24.0	BDL
stdev	857.6	2.6	9.7	6.5	3.2	1.1	2.2	58.3	1.1	460.2	0.1	0.7	27.8	0.7	55.1	0.2	4.8	12.9	21.7	8.7	BDL
	Quartzite																				
	Ba	Be	Co	Cs	Ga	Hf	Nb	Rb	Sn	Sr	Ta	U	V	W	Zr	Mo	Cu	Pb	Zn	Ni	As
JR3S	547	2	1.5	1.1	8.5	4.3	3.9	52.9	<1	21.8	0.4	1	15	2.1	168.2	0.6	6.8	1.4	1	2.2	<0.5
JR3Q	30	<1	0.6	<0.1	3.4	0.4	0.6	2.2	<1	3.8	<0.1	0.2	<8	<0.5	14.7	1.1	11.5	0.7	2	3.5	0.7
R4	645	2	3.9	0.5	7.4	2.4	2.8	52.5	<1	148.1	0.2	0.7	<8	<0.5	88.5	0.5	5.5	0.5	5	9.8	<0.5
average	407.3	2.0	2.0	0.8	6.4	2.4	2.4	35.9	BDL	57.9	0.3	0.6	15.0	2.1	90.5	0.7	7.9	0.9	2.7	5.2	0.7
min	30.0	2.0	0.6	0.5	3.4	0.4	0.6	2.2		3.8	0.2	0.2	15.0	2.1	14.7	0.5	5.5	0.5	1.0	2.2	0.7
max	645.0	2.0	3.9	1.1	8.5	4.3	3.9	52.9		148.1	0.4	1.0	15.0	2.1	168.2	1.1	11.5	1.4	5.0	9.8	0.7
stdev	330.4	0.0	1.7	0.4	2.7	2.0	1.7	29.2		78.6	0.1	0.4			76.8	0.3	3.2	0.5	2.1	4.1	BDL
	Schist																				
	Ba	Be	Co	Cs	Ga	Hf	Nb	Rb	Sn	Sr	Ta	U	V	W	Zr	Mo	Cu	Pb	Zn	Ni	As
AL2	748	<1	4.5	1.5	10.6	3.3	5.6	108.2	2	187	0.4	0.9	30	<0.5	124.5	1.2	13.2	0.9	21	12.6	0.6
AL7	105	<1	47.9	0.2	22.8	4.8	15.3	5.6	3	248.7	0.8	0.3	384	<0.5	188.8	0.7	235.5	0.9	31	9.9	<0.5
AL8	672	<1	5	1.6	10.5	5.1	5.5	67.9	8	148.7	0.5	1.4	19	<0.5	212.6	0.6	6.5	0.8	22	9.8	0.7
BL10	895	<1	28.8	3.2	20.4	8.5	13	90.4	4	463.5	0.7	1	226	<0.5	348.6	0.5	19.6	3.3	127	20.9	0.6
average	605	BDL	21.55	1.625	16.075	5.425	9.85	68.025	4.25	261.975	0.6	0.9	164.75	BDL	218.625	0.75	68.7	1.475	50.25	13.3	0.63333
min	105	BDL	4.5	0.2	10.5	3.3	5.5	5.6	2	148.7	0.4	0.3	19	BDL	124.5	0.5	6.5	0.8	21	9.8	0.6
max	895	BDL	47.9	3.2	22.8	8.5	15.3	108.2	8	463.5	0.8	1.4	384	BDL	348.6	1.2	235.5	3.3	127	20.9	0.7
stdev	345.947	BDL	20.9085	1.228	6.45465	2.19602	5.053	44.7644	2.63	140.524	0.18257	0.45461	174.378	BDL	94.3024	0.31091	111.329	1.21758	51.3639	5.23004	0.05774

Table 4: Geochemical Results of the rare elements in the Rock samples of the study area in ppm

Granite-Gneiss																
	Th	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
3L3X	13.2	34.7	-	-	-	-	-	-	-	-	-	-	-	-	3.89	-
KJ12	93.4	15.2	-	-	-	-	-	-	-	-	-	-	-	-	1.31	-
BL4	48.8	30.3	85.5	162.7	15.03	47.2	7.25	1.05	6.41	0.97	5.48	1.16	3.2	0.45	2.75	0.39
BL9	11.6	50.4	60.3	118.5	14.49	54.4	9.85	2.19	9.24	1.46	8.18	1.92	5.32	0.82	5.3	0.82
LP1	1.6	28	30	66.1	8.02	34.7	6.53	2.02	6.45	0.97	5.59	1.09	3.05	0.43	2.75	0.44
average	33.72	31.72	58.60	115.77	12.51	45.43	7.88	1.75	7.37	1.13	6.42	1.39	3.86	0.57	3.20	0.55
min	1.60	15.20	30.00	66.10	8.02	34.70	6.53	1.05	6.41	0.97	5.48	1.09	3.05	0.43	1.31	0.39
max	93.40	50.40	85.50	162.70	15.03	54.40	9.85	2.19	9.24	1.46	8.18	1.92	5.32	0.82	5.30	0.82
stdev	37.85	12.71	27.79	48.36	3.90	9.97	1.75	0.62	1.62	0.28	1.53	0.46	1.27	0.22	1.49	0.24
Granite																
	Th	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
JNL1	4.1	9.1	21	39.4	4.64	17.9	3.13	1.05	2.52	0.34	1.68	0.3	0.84	0.12	0.71	0.12
JN2	3	10.2	19.8	36.2	4.14	15.2	2.84	0.83	2.29	0.34	1.86	0.38	1.03	0.15	1	0.14
JN6	3.1	8.8	17.1	32.3	3.4	12.7	1.99	0.62	1.83	0.26	1.45	0.3	0.9	0.13	0.81	0.12
JN8	4.1	8.7	21.3	37	4.02	13.7	2.21	0.66	1.82	0.27	1.65	0.34	0.88	0.16	1	0.17
JN13	1.7	5.5	10.7	19.4	2.12	8.1	1.27	0.47	1.22	0.18	0.98	0.2	0.65	0.09	0.66	0.1
KR5	2.9	8.8	17.3	35.5	3.48	11.5	2.05	0.67	1.96	0.27	1.69	0.3	0.92	0.14	0.95	0.14
R1	2.5	4.9	9.6	18.9	2.19	8.2	1.39	0.34	1.13	0.16	0.82	0.16	0.41	0.06	0.41	0.07
R2	0.5	4.7	4.2	6.4	0.86	3.4	0.53	0.11	0.46	0.09	0.47	0.11	0.37	0.06	0.43	0.07
2L19X	11.65	17.4	-	-	-	-	-	-	-	-	-	-	-	-	1.79	-
1L7X	12.1	14.8	-	-	-	-	-	-	-	-	-	-	-	-	1.55	-
average	4.57	9.29	15.13	28.14	3.11	11.34	1.93	0.59	1.65	0.24	1.33	0.26	0.75	0.11	0.93	0.12
min	0.50	4.70	4.20	6.40	0.86	3.40	0.53	0.11	0.46	0.09	0.47	0.11	0.37	0.06	0.41	0.07
max	12.10	17.40	21.30	39.40	4.64	17.90	3.13	1.05	2.52	0.34	1.86	0.38	1.03	0.16	1.79	0.17
stdev	4.00	4.12	6.24	11.81	1.27	4.61	0.85	0.29	0.68	0.09	0.50	0.09	0.25	0.04	0.45	0.04
Quartzite																
	Th	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
JR3S	4.4	12.8	19.1	71.9	3.82	13.4	2.57	0.53	2.17	0.32	2	0.39	1.27	0.17	1.16	0.18
JR3Q	<0.2	3.2	2	6.4	0.4	1.8	0.4	0.07	0.43	0.07	0.45	0.12	0.37	0.06	0.37	0.07
R4	2.4	7.1	16.2	30.4	3.46	13.6	2.37	0.54	1.89	0.25	1.24	0.25	0.67	0.1	0.7	0.11
average	3.40	7.70	12.43	36.23	2.56	9.60	1.78	0.38	1.50	0.21	1.23	0.25	0.77	0.11	0.74	0.12
min	2.40	3.20	2.00	6.40	0.40	1.80	0.40	0.07	0.43	0.07	0.45	0.12	0.37	0.06	0.37	0.07
max	4.40	12.80	19.10	71.90	3.82	13.60	2.57	0.54	2.17	0.32	2.00	0.39	1.27	0.17	1.16	0.18
stdev	1.41	4.83	9.15	33.14	1.88	6.76	1.20	0.27	0.93	0.13	0.78	0.14	0.46	0.06	0.40	0.06
Schist/biotite schist																
	Th	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
AL2	4.4	13.4	24.5	46.8	5.46	19.2	3.27	0.88	3.11	0.46	2.71	0.56	1.39	0.21	1.24	0.19
AL7	1.3	37.1	12.1	32.7	4.54	22.5	6.16	2.26	7.37	1.24	7.39	1.51	4.33	0.61	3.8	0.57
AL8	7	16	29.5	54.7	6.28	23.3	4.07	0.86	3.34	0.49	2.8	0.55	1.72	0.25	1.75	0.26
BL10	4.8	51.6	73.4	147.1	17.53	71.7	12.86	3.21	11.99	1.71	9.23	1.89	5.38	0.73	4.74	0.69
average	4.38	29.53	34.88	70.33	8.45	34.18	6.59	1.80	6.45	0.98	5.53	1.13	3.21	0.45	2.88	0.43
min	1.30	13.40	12.10	32.70	4.54	19.20	3.27	0.86	3.11	0.46	2.71	0.55	1.39	0.21	1.24	0.19
max	7.00	51.60	73.40	147.10	17.53	71.70	12.86	3.21	11.99	1.71	9.23	1.89	5.38	0.73	4.74	0.69
stdev	2.35	18.14	26.70	51.99	6.09	25.08	4.35	1.14	4.18	0.61	3.29	0.68	1.96	0.26	1.66	0.24

Table 5: Enrichment values of trace and rare elements in rock samples of the study area

Trace Elements					
Element	Abundance in Earth Crust (ppm)	Enrichment			
		Granite-Gneiss	Granite	Quartzite	Schist/Biotite-schist
Ag		BDL	BDL	BDL	BDL
As	2.1	1.3	BDL	0.3	0.3
Au	0.0031	387.1	301.1	290.3	1346.8
Ba	425	1.7	4.9	1.0	1.4
Be	1.9	1.4	BDL	BDL	BDL
Bi	0.025	4.0	BDL	BDL	BDL
Cd	-	BDL	BDL	BDL	BDL
Co	30	0.7	0.3	0.1	0.7
Cs	1.9	1.7	2.1	0.4	0.9
Cu	68	0.8	0.1	0.1	1.0
Ga	19	0.9	0.9	0.3	0.8
Hf	3.3	2.3	1.2	0.7	1.6
Hg	-	BDL	BDL	BDL	BDL
Mo	1.1	1.0	0.6	0.7	0.7
Nb	17	0.8	0.3	0.1	0.6
Ni	90	0.3	0.1	0.1	0.1
Pb	10	1.4	1.3	0.1	0.1
Rb	60	2.4	1.9	0.6	1.1
Sb	0.2	1.0	BDL	BDL	0.5
Se	-	BDL	BDL	BDL	BDL
Sn	2.2	1.3	1.1	BDL	1.9
Sr	360	0.5	3.0	0.2	0.7
Ta	1.7	0.4	0.3	0.2	0.4
Tl	0.53	0.3	BDL	0.2	0.4
U	1.8	1.0	0.7	0.4	0.5
V	190	0.6	0.2	0.1	0.9
W	1.1	72.0	94.1	1.9	BDL
Zn	79	0.7	0.3	0.0	0.6
Zr	130	2.3	1.1	0.7	1.7

BDL= below detection limit

Rare Elements					
Element	Abundance in Earth Crust (ppm)	Enrichment			
		Granite-Gneiss	Granite	Quartzite	Schist/biotite schist
Ce	60	1.9	0.5	0.6	1.2
Dy	6.2	1.0	0.2	0.2	0.9
Er	3	1.3	0.3	0.3	1.1
Eu	1.8	1.0	0.3	0.2	1.0
Gd	5.2	1.4	0.3	0.3	1.2
Ho	1.2	1.2	0.2	0.2	0.9
La	34	1.7	0.4	0.4	1.0
Nd	33	1.4	0.3	0.3	1.0
Pr	8.7	1.4	0.4	0.3	1.0
Sm	6	1.3	0.3	0.3	1.1
Tb	0.94	1.2	0.3	0.2	1.0
Th	6	5.6	0.8	0.6	0.7
Tm	0.45	1.3	0.3	0.2	1.0
Y	29	1.1	0.3	0.3	1.0
Yb	2.8	1.1	0.3	0.3	1.0

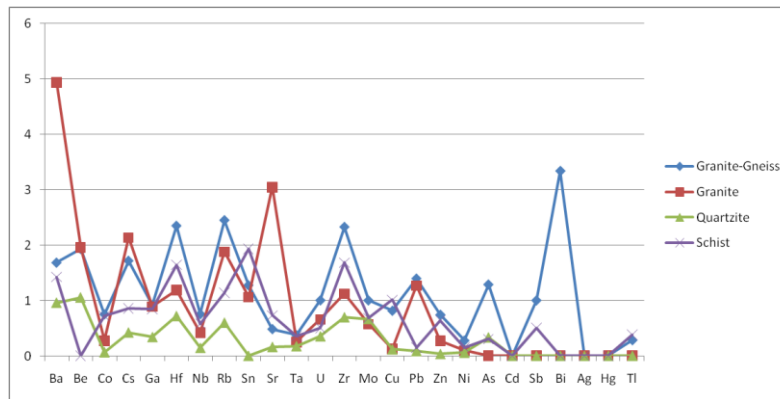


Figure 9: UCC-normalized averages of trace element concentrations for crystalline rocks in the study area (after Taylor and McLennan, 1985)

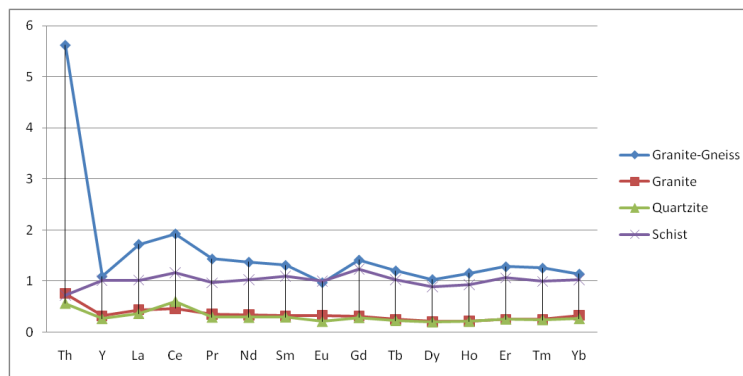


Figure 10: UCC-normalized averages of rare earth element concentrations for crystalline rocks in the study area (after Taylor and McLennan, 1985)

Conclusions

The petrological and geochemical characteristics of rocks in Jebba area were investigated in this study. Porphyritic granite, granite gneiss, quartzite and schist are the main rock types in the study area. The petrographic analysis of the rock samples revealed the main minerals constituent common to the rocks as quartz (modal composition=24% for granite-gneiss, 25% for granite, 90% for quartzite and 32% for schist), plagioclase (modal composition=31% for granite-gneiss, 10% for granite and 26% for schist), microcline (modal composition=40% for granite and 27% for schist) and biotite (modal composition=35% for granite-gneiss, 14% for granite and 12% for schist). Others include: muscovite, chlorite, scapolite and epidote. The rocks have undergone moderate to high degree of chemical weathering as revealed by the values computed for chemical index of alteration, plagioclase index of alteration and chemical index of weathering. This has direct influence on the water found around

the vicinity of the country rocks. While Au, Ba and W are enriched in granite others trace elements are depleted. Also, Zr, Sn, Rb, Hf, Cu, Ba and Au are enriched in biotite schist/schist. The rare earth elements are enriched in granite-gneiss and schist/biotite schist but are generally depleted in granite and quartzite.

Acknowledgment

The authors acknowledged the effort of Dr. A.D. Adedoyin of the Department of Geology and Mineral Sciences, University of Ilorin, Ilorin, Nigeria for his active participation in the geological field mapping of the study area.

References

- Deer, W. A., Howie, R. A. and Zussman, J. (1996). An introduction to the rock forming minerals. Pearson. Prentice hall, Harlow.
- Ekwueme, B.N. (1987). Structural orientations and Precambrian deformational episodes of Uwet area, Oban Massif, S.E. Nigeria. *Prec. Res.*, 34:269-289.
- Fitches, W.R., Ajibade, C.A., Egbuniwe, I.G., Holt, R.W. and Wright, J.B. (1985). Late proterozoic schist belt and plutonism in NW Nigeria. *Journal of mining and geological society*, 124:319-337.
- Geological Map of Nigeria, (2004). Nigerian Geological Survey Agency.
- Grant, N.K. (1978). Structural distinction between a meta-sedimentary cover and an underlying basement in the 600my old Pan African domain in the North Western Nigeria. *Bulletin of the United State Geological Survey*. No.88.
- Hatch, F. H., Wells, A. K. and Wells, M. K. (1979). Textbook of Petrology. Petrology of the Igneous Rocks. 13th Edn., Thomas Nurbey and CO. London. Vol:1.
- Krauskopf, K.B. and Bird, D. K. (1995). Introduction to Geochemistry. 3rd Edition, McGraw-Hill, New York.
- Nesbitt, H. W. and Young, G. M. (1982). Early proterozoic climates and plate motions inferred from major element chemistry of lutites. *Nature*, 299: 715-717.
- Okonkwo, C.T. (1992). Structural geology of basement rocks of Jebba area, Nigeria. *Journal of Mining and Geology*. 28(2):203-209.
- Oyinloye, A. O. (2011). Geology and geotectonic setting of the basement complex rocks in south western Nigeria: implications on provenance and evolution. *Environmental Sciences*, 5:98-118.

- Pelig-Ba, K. B. (2012). Geochemistry of Crystalline rocks from the East of the upper East region of Ghana. *Research journal of Environmental and Earth Sciences*. 4(5): 534-545.
- Rahaman, M.A. (1988). Recent advances in the study of the basement complex of Nigeria. In: Oluyide, P.O., Mbonu, W.C., Ogezi, A.E., Egbuniwe, I.G., Ajibade, A.C. & Umeji, A.C. (eds.), *Precambrian Geology of Nigeria*, p. 11-41.
- Taylor, S.R. and S.M. McLennan, (1985). *The Continental Crust: Its Composition and Evolution*. Blackwell, Oxford, ISBN-13: 978-0632011483, pp: 312.
- Van-Breeman, O., Pidgeon, R.T. and Bowden, P. (1977). Age and isotopic studies of some Pan-African granite from North Central Nigeria. *Precambrian Research*. 4: 307-319.

ANTIBACTERIAL ACTIVITY OF *Mangifera Indica* LEAVES ON MULTIDRUG RESISTANT CLINICAL ISOLATES

I. I. Anibijuwon^{*1}, I. D. Gbala¹, J. A. Abioye¹, M.O. Bamigboye², I. O. Salawu¹

¹*Public Health Laboratory Unit, Department of Microbiology, University of Ilorin, Kwara State, Nigeria.*

²*Department of Industrial Chemistry, University of Ilorin, Kwara State, Nigeria.*

Corresponding author's E-mail: kunledoexploit@gmail.com,
kunledoexploit@unilorin.edu.ng
Phone number: +234(0)8036115296

Abstract

Antibacterial activity of methanolic, ethanolic and hot water extracts of *Mangifera indica* leaves was investigated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*. The assay was carried out using the agar well diffusion method with varying concentrations (5, 25, 50, 75, 100, 150mg/ml of the extracts and the minimum inhibitory concentration was determined by broth dilution method. The results obtained showed that the ethanolic extracts of *M. indica* leaf had higher antibacterial activities than the methanolic and aqueous extracts. All bacteria used in this study were susceptible to ethanolic extract of *M. indica* leaf but none was susceptible to the aqueous extract. The methanolic extract was also effective against the organisms with its greatest activity on *P. aeruginosa* at a concentration of 150mg/ml. Minimum inhibitory concentration (MIC) of the ethanol extract of *M. indica* leaf was obtained at a concentration of 75 mg/ml for *Salmonella typhi*, 100 mg/ml for *Escherichia coli* and 200 mg/ml for *Staphylococcus aureus*. No cidal effect was observed for the extracts against any of the test organisms. This study has shown that *M. indica* leaves possess phytochemicals with pharmacological properties and justifies its use in Nigerian traditional medicine.

Introduction

Plants have been a valuable source of natural products for maintaining well-being in the last decade, with more increasing studies for natural therapies. Medicinal plants might be the best source to obtain a variety of drugs. According to WHO, a medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Junaaid *et al.*, 2006). The primary benefits of using plant-derived medicines are that they are relatively cheaper than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments. Many of the plant materials used in traditional medicines are readily available in rural areas and this has made traditional system of medicine relatively cheaper than modern medicine in this part of the world although the use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community (Sharif and Banik, 2006, Kubmarawa *et al.*, 2007). Ethnobotanical surveys carried out in Nigeria and elsewhere in the world have reported a number of plants that are used in the treatment of infectious diseases, including *Mangifera indica* (Bukanya *et al.*, 2003; Mustapha *et al.*, 2013). *Mangifera indica* have been used traditionally to treat diseases. The leaves decoction is taken as a remedy for fever, chest complaints, diabetes and other illnesses (Aderibigbe *et al.*, 2001). Investigations on the biological activities of *Mangifera indica* showed that these plants have a potential to be used as antioxidant agents (Khan *et al.*, 2003). This objective of this study is to test for the presence of phytochemicals in extracts of *M. indica* leaves, evaluate the antibacterial activity of leaf extracts against selected clinical pathogens and assess the bacteriostatic/ bactericidal efficacy of the extracts.

Materials and Methods

Collection of Plant Materials

The fresh leaves of *Mangifera indica* were collected in a clean polythene bag at Tanke, Ilorin, Kwara State. It was thereafter taken to the Plant Biology Department of the University of Ilorin for identification and authentication.

Sources and Maintenance of Test Organisms

Bacterial isolates were obtained from Wesley Guild Hospital, Ilesha, Osun State and the Medical Laboratory of the Microbiology and Parasitology unit of the University of Ilorin Teaching Hospital (UITH), Ilorin, Kwara State. The test organisms were inoculated on nutrient agar slant and stored in a working refrigerator at 4 °C as a stock culture. Bacteria were subcultured onto fresh media at intervals to maintain the viability of the test organisms. The bacterial isolates collected were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*.

Preparation of Extract

The leaves were properly washed with distilled water and air dried. The dried leaves were then blended using a household electrical blender into fine powder. The leaf powder was stored sealed in five labeled reagent bottles for further use. The total weight of the powdered plant sample was taken and then stored until it is required.

Extraction

One hundred gram (100g) of the fine powder of the grounded *Mangifera indica* leaves was immersed in 500ml of ethanol, methanol and hot water respectively to get a concentration of 200mg/ml. The extraction was done by subjecting it to agitation on a rotator shaker at 200 revolution per minute (rpm) for 3days. The resulting suspension was then filtered using muslin cloth and Whatman No.1 filter paper and the filtrate was allowed to evaporate to dryness at 45°C in a water bath

Phytochemical Screening

The leaves extract of *Mangifera indica* (Edward variety) were analyzed for the presence of alkaloid, saponins, anthraquinone, steroids, tannin, flavonoid, reducing sugars and cardiac glycosides according to standard methods (Odebiyi and Sofowora, 1978; Sofowora, 1982).

Screening for Alkaloids: Three gram of extract was stirred with ethanol containing 3% tartaric acid. The filtrate was shared into 3 beakers and tested for alkaloids as follows: into the first beaker, Hagar's reagent was added, into the second beaker, Mayer's reagent was added and into third beaker, Marquin's reagent was added. Precipitation in any of the 3 test indicates the presence of alkaloids.

Screening for saponin: About 0.5g of the plant extract was shaken with water in a test tube. Frothing, which persist on warming was taking as a preliminary evidence for the presence of saponin. Few drops of olive oils was added to 0.5g of the extract and vigorously shaken. Formation of soluble emulsion in the extract indicates the presence of saponin (Odebiyi and Sofowora, 1978).

Screening for tannin: Water extract of the sample was treated with 15% ferric chloride test solution. The resultant color was noted. A blue color indicated the presence of hydrolysable tannin. Or into 10ml of freshly prepared potassium hydroxide (KOH) in a beaker, 0.5g of the extract was added and shaken to dissolve. A dirty precipitate observed indicates the presence of tannin (Odebiyi and Sofowora, 1978; Sofowora, 1982).

Screening for steroids (Salkowski's test): About 100mg of *Mangifera indica* extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a

lower layer. A reddish brown color at the interface is indicative of the presence of steroidal ring (Sofowora, 1982).

Screening for flavonoid: About 2g of the powered leaves was completely detained with acetone. The residue was extracted in warm water after evaporating the acetone in a water bath. The mixture was filtered while, still hot. The filtrate was cooled and used. Five millimeters of 20% NaOH was added to equal volume of the detained water extract. A yellow solution indicates the presence of flavonoid.

Screening for anthraquinone (Borntrager's test): About 0.5g of the extract was taken into a dry test tube and 5ml of chloroform was added and shaken for 5min. the extract was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) indicates the presence of anthraquinone.

Screening for cardiac glycosides (Keller Killiani's test): About 100mg of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayer with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a de-oxy sugar characteristic of cardenolides.

Screening for reducing sugar (in glycosides): The residue was re-dissolved in water in the water bath. Two millimeters of the solution in a test tube was added 1ml of Fehling's solution A and B. the mixture was shaken and heated in a water bath for 10min. A brick-red precipitate indicates a reducing sugar.

Antimicrobial Assay of *Mangifera indica* Leaf Using Agar Well Diffusion

The antimicrobial screening was carried out using the agar well diffusion method as described by Lino and Deogracious (2006) with slight modifications. The test bacteria were first inoculated into tubes of nutrient broth separately and incubated at 37°C for 18 hours. Each of the cultures was then adjusted to 0.5 McFarland turbidity standards and inoculated (0.2 ml each) onto Muller Hinton agar (MHA, Oxoid) plates . A sterile cork borer was then used to make seven wells (6 mm diameter) for different concentrations of the extract on each of the plates containing cultures of the different test organisms. The dried aqueous and organic extracts were separately re-dissolved in sterile glycerol at concentrations of 50,100, 150, 200 and 250mg/ml and 0.5 ml of each of the extracts was then introduced into the wells using sterile Pasteur pipettes. The culture plates were allowed to stand on the working bench for 30 minutes for pre diffusion (for the extract to diffuse into the agar) and were then incubated at 37°C for 24 hours. After 24 hours, antimicrobial activity was determined by measurement of diameter zones of inhibition (mm) (against the test organisms) around each of the extracts (Lino and Deogracious, 2006).

Antibiotic Susceptibility Testing

Each of the organisms was inoculated into sterile nutrient broth and the media were incubated for 24 hours. Pour plate method was adopted for the inoculation of organisms. Positive antibiotic disc was pressed down on the media containing *Staphylococcus aureus* and the negative discs for *Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*. The plates were incubated 37 °C for 24 hours. Zones of inhibition around each disc were measured. The antibiotics used and their corresponding concentrations are as follows:

For Gram negative: Ceftazidime (Caz) 30µg, Cefuroxime (Crx) 30µg, Gentamicin (Gen) 10µg, Ceftriaxone (Ctr) 30µg, Erythromycin (Ery) 5µg, Cloxacilin (Cxc) 5µg, Ofloxacin (Ofl) 5µg, Augmentin (Aug) 30µg.

For Gram positive: Amoxicillin (Amx) 25µg, Cotrimoxazole (Cot) 25µg, Nitrofurantoin (Nit) 300µg, Gentamicin (Gen) 10µg, Nalidix acid (Nal) 30µg, Ofloxacin (Ofl) 30µg,

Augmentin (Aug) 30µg, Tetracycline (Tet) 30µg.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Only the organisms that were found susceptible to the extracts of *Mangifera indica* were inoculated into sterile nutrient broth and incubated at 32 °C for about 16 hours. 1ml of each of the varying concentrations of extracts of *M. indica* that were found potent (150mg/ml, 100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml and 5mg/ml) were thereafter inoculated with 0.3ml of the overnight broth culture of the test organisms. A negative control was setup to contain only nutrient broth and the extract. The inoculated and control tubes were then incubated at their proper growth temperatures for 16 hours for bacterial cultures. The tubes were observed for turbidity after incubation. The lowest concentration that shows no turbidity (indicating no growth of the test organism) was taken as the MIC (Adetitun *et al.*, 2013). The minimum bactericidal concentration (MBC) of the plant extract on the clinical bacterial isolates was carried out according to Ajaiyeoba *et al.*, (2003). Briefly, 1 ml bacterial culture was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and subcultured on to MHA and incubated at 37°C for 24 hours. After incubation the concentration at which there was no single colony of bacteria was taken as MBC (Ajaiyeoba *et al.*, 2003).

Results

The pH value results of methanolic, hot water and ethanolic extracts in this study as shown in Figure 1 are 3.9, 2.9 and 3.8 respectively indicating all extracts to be acidic. Table 1 shows the phytochemical screening of leaf extract of *Mangifera*

indica indicating the presence of saponin, tannin, glycosides, alkaloids, anthraquinone and flavonoids in the ethanolic extract. The methanolic extract shows the presence of tannin, glycosides, alkaloids, anthraquinone and flavonoid except saponin while in hot water extract all the phytochemical constituents are present as in ethanolic extract except alkaloids. Table 2 shows the results of preliminary screening of methanolic, hot water and ethanolic extracts of *Mangifera indica* leaves. The result indicates that none of the extracts shows effect on *E. coli* and *Klebsiella pneumoniae*. Hot water and methanolic extracts of the leaves have no effect on *S. aureus* and *Pseudomonas aeruginosa* except the ethanolic extract. *Salmonella typhi* shows zone of inhibition of 11mm and 10mm on hot water and ethanolic extracts respectively except methanolic extract with no inhibition. In Figure 2, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* showed sensitivity to varying concentrations of the extract. *Staphylococcus aureus* was the most susceptible organism with sensitivity to all concentrations of the Ethanolic extract, while *Klebsiella pneumoniae* is resistant.

According to Figure 3, *Pseudomonas aeruginosa* showed sensitivity to varying concentrations of the extract and it's the most susceptible organism with sensitivity to all concentrations of the methanolic extract, while *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* were resistant.

Table 3 shows the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of the different leaf extract (ethanol, methanol and hot water) of *M. indica* on the clinical isolates. There is no Minimum Bactericidal Concentration (MBC) because there was bacterial growth in the entire surface of the agar medium. Generally the ethanolic extract showed greater antibacterial activity compared to the methanolic extract while Hot water extract displayed no antibacterial activity as it was unable to inhibit the clinical isolates at all concentrations. The ethanolic extract concentration that were used and found to inhibit *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* were 200, 75, 100mg/ml.

Table 4 shows the Antimicrobial activity of standard Gram positive antibiotic disk and Gram negative antibiotic disk on the test organisms.

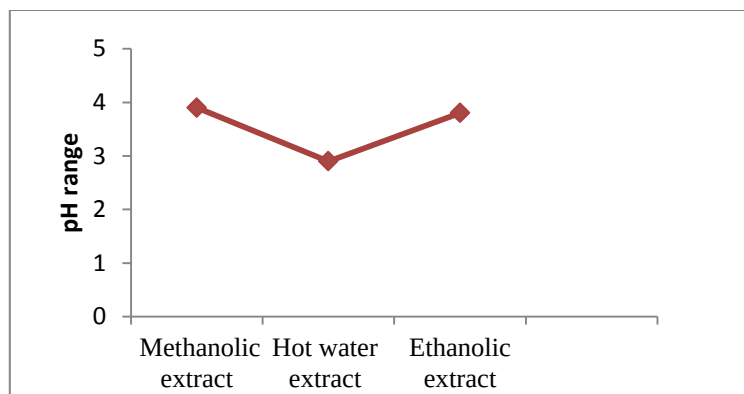


Figure 1: pH value of extracts of *Mangifera indica*

Table 1: Phytochemical Screening of The Leaf Extracts of *M. Indica*

Phytochemical parameters	Methanolic extract	Ethanolic Extract	Hot water extract
Saponin	-	+	+
Tannin	+	+	+
Glycosides	+	+	+
Alkaloids	+	+	-
Anthraquinone	+	+	+
Flavonoids	+	+	+
(-):Absent (+):Present			

Table 2: Preliminary Screening of Leaf Extracts of *Mangifera indica*

S/N	Organisms	Hot water extract	Ethanolic extract	Methanolic extract
1	<i>Staphylococcus aureus</i>	0.0	15	0.0
2	<i>Salmonella typhi</i>	11	10	0.0
3	<i>Pseudomonas aeruginosa</i>	0.0	15	0.0
4	<i>Escherichia coli</i>	0.0	0.0	0.0
5	<i>Klebsiella pneumoniae</i>	0.0	0.0	0.0

Values represent the diameter of zone of inhibition in mm

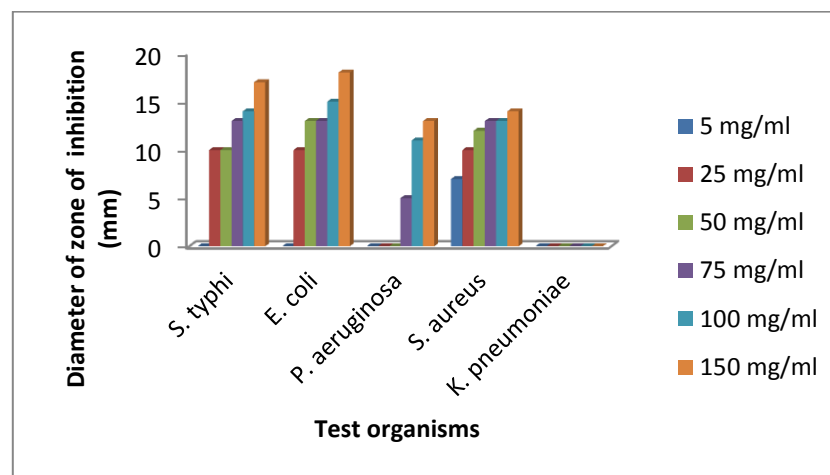


Figure 2: Effect of varying concentrations of the Ethanolic leaf extract of *M. indica*.

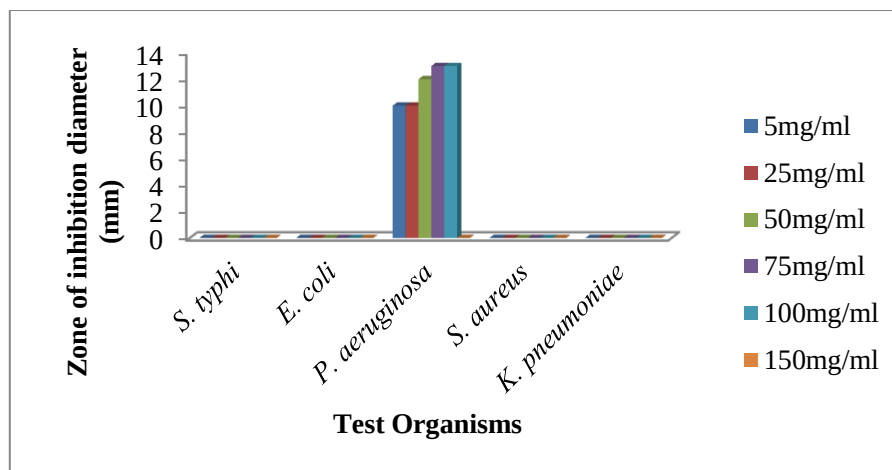


Figure 3: Effect of varying concentrations of the Methanolic leaf extract of *M. indica*.

Table 3: Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal of the leaf extracts of *Mangifera indica*

SN	Organisms	MIC (mg/ml)			MBC (mg/ml)		
		Ethanollic	Methanolic	HWE	Ethanollic	Methanolic	HWE
1	<i>S. aureus</i>	200	0.0	0.0	0.0	0.0	0.0
2	<i>S. typhi</i>	75	0.0	0.0	0.0	0.0	0.0
3	<i>P. aeruginosa</i>	0.0	0.0	0.0	0.0	0.0	0.0
4	<i>K. pneumoniae</i>	0.0	0.0	0.0	0.0	0.0	0.0
5	<i>E. coli</i>	100	0.0	0.0	0.0	0.0	0.0

HWE: Hot water extract.

Table 4: Antimicrobial Activity of standard antibiotic discs on the test organisms

	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella Typhi</i>
Ceftazidime	0.0	0.0	0.0	0.0
Cefuroxime	0.0	0.0	0.0	0.0
Gentamycin	14.0	0.0	0.0	23.0
Ceftriaxone	0.0	0.0	0.0	0.0
Erythromycin	23.5	0.0	0.0	26.5
Cloxacillin	0.0	0.0	0.0	0.0
Ofloxacin	17.5	20.5	0.0	29.5
Augmentin	0.0	0.0	0.0	0.0

Discussion

Mangifera indica has been used in most rural communities for the treatment of a handful of infectious diseases due to its apparent medicinal properties that stands it out from other herbal plants. In this study, we evaluated as a preliminary examination the possible antibacterial activity of *M. indica* leaves as a way to give credence to its wide usage in most rural communities. Figure 1 and 2 presents the results of the effect of varying concentration of the ethanolic and methanolic leaf extract of *M. indica* on clinically important bacterial pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella typhi*. It can be inferred from the results that both the ethanol and methanol leaf extracts of *M. indica* had a broader spectrum of activity against the test isolates. However, better antimicrobial activity was recorded with the ethanolic extracts at different zones of inhibition. It could therefore be deduced from this study that ethanol is a better extractor of the bioactive constituents of the *M. indica* leaves than the methanol solvents used. The hot water extract did not exert antimicrobial effect on the isolates due to failure of the active ingredient to dissolve in it.

Antibiotics	Zones of Inhibition (mm)
	<i>Staphylococcus aureus</i>
Amoxicillin	0.0
Cotrimoxazole	0.0
Nitrofurantoin	0.0
Gentamicin	0.0
Nalidixic acid	0.0
Ofloxacin	33.5
Augmentin	0.0
Tetracycline	0.0

The result of this work showed that Methanol extract of *M. indica* had activity on *Pseudomonas aeruginosa* (16 mm). Ethanolic extracts of *M. indica* also showed varying levels of activity on *S. aureus* (14 mm), *S. typhi* (17mm), *E. coli* (18mm), and *P. aeruginosa* (13mm) while *K. pneumoniae* did not inhibit any growth. The notable antimicrobial activities of *M. indica* on bacterial pathogens have been previously reported and these are also in line with the results obtained in this study (Aderibigbe *et al.*, 1999; Aderibigbe *et al.*, 2001; Garrido *et al.*, 2001; Khan *et al.*, 2003; Amrita *et al.*, 2009). The MIC of the ethanol extracts of *M. indica* on the test isolates was determined at 75mg/ml for *Salmonella typhi*, 100mg/ml for *Escherichia coli* and 200mg/ml for *Staphylococcus aureus*. Extract of *M. indica* has been found in this study to be acidic with a pH value 2.9, 3.8, and 3.9 for hot water, ethanol and methanol respectively. The antimicrobial activity of *M. indica* leaf extracts which obviously tells their bioactivity on the test isolates is encouraging owing to the fact that these organisms are of hospital origin.

In the Antimicrobial Susceptibility Test using the standard antibiotics the Gram negative clinical isolates were more susceptible compared to the susceptibility of the Gram positive organism. *Staphylococcus aureus* was susceptible only to Ofloxacin with a zone of inhibition (33.5 mm) and resistant to the other antibiotics. Antibiotics works in three forms in mode of action, range of activity and its chemical components. Ofloxacin is a broad spectrum antibiotic which is used against both Gram negative and Gram positive organisms. The resistance of *Staphylococcus aureus* could be due to it multiple drug resistance and also the mode of action of the antibiotics against the organism. The Gram negative organisms showed susceptibility to one or more antibiotics except *Pseudomonas aeruginosa* which was resistant to all the antibiotics. The resistance of *Pseudomonas aeruginosa* and the other Gram negative isolate used in this study could be due to the cell wall of Gram negative organisms which is complex having inner and outer membranes. However with the use the plant extracts, *Staphylococcus aureus* was the most susceptible at lower concentration which gives the use of medicinal plants more advantage as antimicrobial agents

The ability of the crude extracts of *M. indica* leafs to inhibit the growth of pathogenic bacteria (as obtainable in this study) is an indication that *M. indica* is a medicinal plant and could serve as a source of lead bioactive compounds for the development of potent antimicrobial agents. This further validates its use in traditional herbal medicine in most rural Nigerian communities to treat a variety of bacterial related infections including those associated with respiratory tract infections.

Conclusion

This study has shown that the ethanolic and methanolic extracts of *M. indica* leaf possess antimicrobial activities. *Mangifera indica* contain bioactive compounds that are effective against the test organisms, thus the crude extracts could be used directly for chemotherapy or treatment of infections caused by these organisms. Further research and characterization is therefore required to identify the specific phytochemicals responsible for the activity and also the quantitation of the concentration at which cidal effects can be exerted on the test organisms

References

- Aderibigbe, A.O., Emudianughe, T.S., Lawal, B.A. (1999). Antihyperglycemic effect of *Mangifera Indica* in rats. *Phytotherapy Research*. (13):504-507.
- Aderibigbe, A.O., Emudianughe, T.S., Lawal, B.A. (2001). Evaluation of antidiabetic action of *Mangifera indica* in mice. *Phytotherapy Research* (15):456-458.
- Ajaiyeoba, E.O., Onocha, P.A., Nwozo, S.O., Sama, W. (2003). Antimicrobial and cytotoxicity evaluation of *Buchholzia coriacea* stem bark. *Fitoterapia* 74: 706-709.
- Amrita, B., Liakot, A., Masfida, A., Begum, R. (2009). Studies on the antidiabetic effects of *Mangifera indica* stem bark and leaves on non diabetic, type 1 and 2 diabetic model Rats. *Phytotherapy Research*. (4):110-114.
- Bukenya, R.Z., Kamoga, D. (2003). An Inventory of Medicinal Plants used against Fowl Diseases in Budondo Subcounty, Jinja District. Department of Botany. Makerere University, Kampala, Uganda.
- Falodun, A, Okunrobo, L.O., Uzoamaka, N. (2006). Phytochemical Screening and Inflammatory evaluation of methanolic and aqueous extracts of *Euphorbia Heterophyllalinn* (*Euphorbiaceae*). *African Journal of Biotechnology*.5(6):529-531.
- Garrido, G, Gonzalez, D, Delporte, C. (2001). Analgesic and anti-inflammatory effects of *Mangifera indica* extract (Vimang). *Phytotherapy Research*. (15):18-21.

- Jigna, P, Nehal, K, Sumitra, C. (2006). Evaluation of antibacterial and phytochemical analysis of *Bauhinia variegata* L. bark. *African Journal of Biomedical Research*. 9(1): 53-56.
- Junaid, S.A., Olabode A.O., Onwuliri, F.C., Okworiu, A.E.J, Agina, S.E. (2006). The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. *African Journal of Biotechnology*. 5 (22): 2315-2321.
- Khan, M.N., Nizami, S.S., Khan, M.A., Ahmed, Z. (2003). New saponins from *Mangifera indica*. *Nutritional Production*. (56):767-770.
- Kubmarawa, D, Ajoku, G.A., Enwerem, N.M., Okorie, D.A. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of Biotechnology*. 6 (14): 90-1696, (<http://www.academicjournals.org/AJB>).
- Lino, A, Deogracious, O. (2006). The in-vitro antibacterial activity of *Annona senegalensis*, *Securidacalangi pendiculata* and *Steanotaenia araliacea*, Ugandan Medicinal Plants. *African Health Science*. 6(1): 31-35.
- Mustapha A.A., Owuna G, Uthman I.I., (2013). Plant Remedies Practiced by Keffi People in the Management of Dermatitis. *Journal of Medicinal Plants Studies*. 1:112-118.
- Sharif, M. D. M, and Banik, G..R. (2006). Status and Utilization of Medicinal Plants in Rangamati of Bangladesh. *Research Journal of Agricultural and Biological Science*. 2(6): 268-273.
- Vaghasiya, Y., Patel, H., Chanda, S. (2000). Antibacterial activity of *Mangifera indica* L seeds against some human pathogens, *Pharmacology Research*. 4(2):514-602.

Chemical and Functional Properties of Raw and Cooked Under Utilized *Blighia Sapida* Seed Flour

Abiodun O.A.^{1*}, Adepeju, A. B.², Olosunde, O.O.³ and Dauda, A.O.¹

¹Department of Home Economics and Food Science, University of Ilorin, Kwara State, Nigeria

²Department of Food Science and Technology, Joseph Ayo Babalola University Ikeji Arakeji, Osun State, Nigeria.

³Department of Food Science and Technology, Osun State Polytechnic, Iree, Nigeria

*Corresponding author email: funmiabiodun2003@yahoo.com +2348030701354

Abstract

Chemical and functional properties of raw and cooked under-utilized *Blighia sapida* (Ackee) seeds flour were determined. The seeds were divided into two portions: portion A was peeled and dried while portion B was cooked, peeled, dried and milled. The flours were subjected to chemical, physico-chemical and anti-nutritional analyses. Higher protein content (14.97 %) was observed in the raw flour. Calcium and potassium contents were the major mineral in the seed flour. Amino acids in the flour include glutamic acid, aspartic acid, leucine, arginine and lysine in decreasing order. Raw flour had higher peak viscosity (78.25 RVU), holding strength (53.66 RVU), breakdown (24.59 RVU), final viscosity (66.79 RVU), setback (13.13 RVU) and pasting temperature (82.43 °C). The swelling power of raw flour increased drastically at 80-90 °C than the cooked flour. Swelling power of the flours increased with increasing temperature. Cooked *Blighia sapida* flour had low tannin, oxalate and alkaloid contents but high in phytate content.

Keywords: Amino acids, Anti-nutritional factor, *Blighia sapida* seed, Cooked flour, Pasting properties

Introduction

Blighia sapida, commonly known as *Ackee* (English), *Gwanja Kusa* (Hausa; Northern Nigeria), *Isin* (Yoruba; Western Nigeria), *Okpu* (Igbo; Eastern Nigeria) and *Yila* (Nupe; North Central Nigeria) is a soap berry plant of the family *Sapindaceae*. It is a perennial herbaceous plant that is prominently found in Western

Tropical Africa (Onuekwusi *et al.*, 2014). *Blighia sapida* is a woody perennial multipurpose fruit tree species native to the Guinean forests of West Africa. The fleshy arils of the ripened fruits are edible (Ekueet *et al.*, 2010). At maturity, arils are consumed directly fresh, added to sauce to replace sesame (*Sesamum indicum* L.). Arils are also fried in peanut (*A. hypogaea*) or oil palm (*Elaeis guineensis* Jacq.) oil (Ekue *et al.*, 2010). Ackee arils could serve as an appreciable source of natural antioxidants which could play a key role in health maintenance and prevention of the chronic and degenerative diseases (Dossou *et al.*, 2014). The fruits splits open while still on the tree to reveal three glassy black seeds surrounded by a thick, yellow and oily aril (Nwoso *et al.*, 2014). The physical properties, fatty acid composition and phytochemical contents of *Blighia sapida* aril and seed oil have been determined (Omobuwajoet *et al.*, 2000; Omosuli, 2013; Onuekwusi *et al.*, 2014). The arils are eaten or used to make sauce while the seeds are discarded and under-exploited. According to Blake *et al.* (2006) the seed contains a natural toxin, which exists as the cyclic amino acid, hypoglycin A, and its gamma-glutamyl derivative, hypoglycin B which causes vomiting and death. Presence of hypoglycin in the seed of *Blighia sapida* limits the use of the seed in food production. The seed had been used in feeding rat and the acute dose had been established (Blake *et al.*, (2006). Due to this, there is a dearth of information on the properties and usefulness of the seed. Therefore, this paper studied the chemical and functional properties of raw and cooked flours obtained from the under-utilized *Blighia sapida* seed.

MATERIALS AND METHODS

Sample Collection and Preparation

Blighia sapida seeds were obtained at a farm in Iree, Osun State, Nigeria. The seeds were peeled manually with knives and the cotyledons were separated. The peeled seed was dried in the oven at 60 °C to obtain the raw flour while whole seed was cooked at 100±2 °C for 20 min, peeled, dried in hot air oven at 60 °C and milled into flour to obtain cooked flour. The dried seeds were milled and packaged.

Proximate, mineral and anti-nutritional composition

Proximate composition (Crude protein, fat, ash and crude fibre contents) analyses were done following AOAC methods (AOAC, 1990). The phytate contents of *Blighia sapida* seeds were determined using the method of Dairo (2008). Alkaloid, oxalate and tannin were quantified using the method of Alexis and Georges (2012).

Determination of amino acid profile

The Amino Acid profile in the known sample was determined using methods described by Benitez (1989). The sample was defatted using chloroform/methanol mixture of ratio 2:1. Sample (4g) was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus (AOAC, 2006).

Nitrogen determination

Sample (200 mg) was weighed, wrapped in Whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10mL) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate (CuSO_4) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added. The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100mL in standard volumetric flask. Aliquot (10mL) of the diluted solution with 10mL of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10mL 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70mL of distillate was collected. The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured. The amount of nitrogen present was determined following the equation below

$$\text{Percentage Nitrogen} = \frac{(a - b) \times 0.01 \times 14 \times v \times 100}{W \times C}$$

Where: a=Titre value of the digested sample, b=Titre value of blank sample, v.=Volume after dilution (100 mL), W=Weight of dried sample (mg), C.=Aliquot of the sample used (10mL), 14=Nitrogen constant in mg.

Hydrolysis of the sample

Sample (30 mg) was weighed into glass ampoule. 7 ml of 6NHCl was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^\circ\text{C} \pm 5^\circ\text{C}$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered. The filtrate was then evaporated to dryness at 40°C under vacuum using a rotary evaporator. The residue was dissolved with 5 mL of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into Amino acid analyzer (TSM)

Ten microlitre of the hydrolysate was loaded and dispensed into the cartridge of the analyzer. The TSM analyzer was designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis was 76 minutes.

$$\% N = \frac{(a - b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Determination of Amino Acid Values from the Chromatogram Peaks.

An integrator attached to the Analyzer auto-calculates the peak area proportional to the concentration of each of the amino acids. The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula.

$$NE = \frac{\text{Area of Norleucine peak}}{\text{Area of each amino acid}}$$

A constant S was calculated for each amino acid in the standard mixture:

Where $S_{\text{std}} = NE_{\text{std}} \times \text{Molecular weight} \times \mu\text{MAA}_{\text{std}}$

Finally, the amount of each amino acid present in the sample was calculated

Concentration (g/100g protein) = $NH \times W \times NH/2 \times S_{\text{std}} \times C$

Where

$$C = \frac{\text{Dilution} \times 16}{\text{Sample weight (g)} \times N(\%) \times 10 \times \text{vol. loaded}} / NH \times W (nleu)$$

Where: NH = Net height

W = Width @ half height

nleu = Norleucine

Determination of swelling power

This was determined as described by Appiah *et al.* (2011). One gram of the flour sample was mixed with 10 mL distilled water in a centrifuge tube and heated in a hot water bath at 60, 70, 80 and 90°C for 30 min while continuously shaking the tube. After heating, the suspension was centrifuged at 1000 g for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as:

Swelling power = Weight of the paste/weight of dry flour

Determination of Pasting Properties

Pasting profile of the flour sample was studied using a Rapid Visco-Analyzer (RVA) (Newport Scientific Pty. Ltd) with the aid of a thermocline for windows version 1.1 software. The RVA was connected to a PC where the pasting properties and curve were displayed directly. Flour suspension was prepared by addition of the equivalent weight of 3.0 g flour to distilled water to make a total of 28.0 g suspension in the

RVA sample canister. The flour suspension temperature was held at 50 °C for 1 min and later heated to 95 °C for 3 min. It was held at 95 °C for 3 min before the sample was subsequently cooled to 50 °C over a 4 min period. This was followed by a period of 1 min where the temperature was kept at constant temperature of 50 °C.

Statistical analyses

The data obtained were in triplicate and the mean and standard deviation were calculated.

RESULTS AND DISCUSSION

Chemical composition of *Blighia sapida* flour

Chemical compositions of *Blighia sapida* flour are shown in Table 1. Moisture contents of the raw and cooked *Blighia sapida* seed flour were 8.29 and 8.57 % respectively. Higher protein content (14.97 %) was observed in the raw flour but there was slight reduction in the protein value (14.68 %) of the cooked flour. The protein contents of *Blighia sapida* seed flours were comparable to protein contents of *Monodora myristica* (12.83 %) and *Afzelia Africana* seed (9.56 %) (Ojiako et al., 2010). Boiling and blanching methods were reported to reduce the protein contents of *Sphenostylis stenocarpa* (African yam bean), *Sesamum indicum* (Sesame seed), *Monodora myristica* (Calabash nutmeg), *Afzelia Africana* (African oak) and black bean flour (Ojiako et al., 2010; Vongsumran et al., 2014). These may be due to denaturation and leaching of the protein in the seed during cooking. Raw *Blighia sapida* seed flour also had higher ash, fat and crude fibre contents of 2.70, 13.14 and 1.71% respectively. Carbohydrate content of cooked *Blighia sapida* seed flour was 60.01 % which was higher than raw flour. Energy compositions of the raw and cooked flour were 414.91 and 412.93 kcal/100g. Energy compositions observed in the raw and cooked flour were higher than that of yam (210.5 kcal/100g), cornstarch (376.87 kcal/100g) and sweet potato flour (344.56 kcal/100g) (Akaninwor and Okechukwu, 2006; Adenuga, 2010). The high energy values of *Blighia sapida* seed flours signified its potential to meet the energy requirements of both infants and adults. Calcium and potassium contents were the major mineral in the seed flour. Raw flour contained higher calcium (834.32 mg/kg), sodium (64.84 mg/kg) and iron (0.05 mg/kg) contents while the cooked flour had higher magnesium (23.78 mg/kg) and potassium (641.38 mg/kg) contents. Increase in magnesium content of the cooked flour may be due to migration of the nutrient from the peel into the seed endosperm during cooking. Likewise, high potassium content observed in cooked flour may be due to reduction in the anti-nutrients which bind with the mineral. Flour high in calcium are useful in the diet for formation of strong bone and teeth, while the presence of high potassium makes it valuable due to its ability to aid digestion.

Composition	Raw seed flour	Cooked seed flour
Moisture (%)	8.29±0.04	8.57±0.05
Protein (%)	14.97±0.21	14.68±0.15
Ash (%)	2.70±0.02	2.43±0.04
Fat (%)	13.14±0.06	12.69±0.03
Fibre (%)	1.71±0.02	1.64±0.01
Carbohydrate (%)	59.21±0.09	60.01±0.02
Energy (kcal/100g)	414.91±0.15	412.93±0.14
Ca (mg/kg)	834.32±0.56	811.31±0.52
Mg (mg/kg)	23.64±0.17	23.78±0.20
K (mg/kg)	615.59±0.10	641.38±0.04
Na (mg/kg)	64.84±0.07	42.14±0.02
Fe (mg/kg)	0.05±0.08	0.04±0.10

Mean± SD

Amino acid compositions of *Blighia sapida* seed flour are presented in Table 2. Major amino acids in the flour include glutamic acid, aspartic acid, leucine, arginine and lysine. Boiling of the seeds led to increase in the amino acid values of the cooked flour. This was contrary to the result obtained for protein content of cooked seed flour. Slight reduction in protein content was observed in the cooked seed flour. Limiting amino acids in the raw flour were cysteine and methionine. All the essential amino acids in the cooked flour were greater than approved standards by FAO/WHO (1991). This showed that cooked *Blighia sapida* seed flour meet the amino acid requirement for proper growth and development. Amino acids content obtained in *Blighia sapida* seed flour were higher than those of *Bosqueia angolensis* but compares with *Luffacyl indrica* seed flour (Nwosu et al., 2008, Oyetayo and Ojo, 2012). Total essential amino acid in the cooked flour was higher than the raw flour and the FAO/WHO (1991) reference pattern. This was also higher than the total amino acid in the *Luffacyl indrica* seed flour (38.76g/100g) (Oyetayo and Ojo, 2012) but comparable to *Pisumsativum* (Achat pea) (40.96 g/16g N) (Amarakoon, 2012).

Table 2: Amino acid compositions of raw and cooked flour of *Blighiasapida* seed

Amino Acid	Raw (g/100g protein)	Cooked (g/100g protein)	FAO/WHO (1991)
Lysine	4.59	6.00	5.8
Histidine	2.02	2.59	1.9
Arginine	5.18	6.82	1.1
Aspartic acid	9.37	10.17	
Threonine	3.37	4.03	3.4
Serine	3.47	3.58	
Glutamic acid	11.66	12.57	
Proline	2.64	3.46	
Glycine	3.70	3.70	
Alanine	3.57	4.03	
Cysteine	0.93	1.32	
Valine	3.53	4.23	3.5
Methionine	0.99	1.20	2.5+cysteine
Isoleucine	2.66	3.17	2.8
Leucine	6.88	7.54	6.6
Tyrosine	3.17	3.25	
Phenylalanine	4.13	4.47	6.3+tyrosine
Tryptophan	ND	ND	1.1
Total EAA	33.35	40.05	35
Total NEAA	38.51	42.08	
Total amino acid	71.86	82.13	

ND=Not determined

EAA= Essential amino acid, NEAA= Non-essential amino acid

Pasting properties of *Blighia sapida* flour

Pasting properties of *Blighia sapida* seed flour are shown in Table 3. Raw flour had higher peak viscosity (78.25 RVU), holding strength (53.66 RVU), breakdown (24.59 RVU), final viscosity (66.79 RVU), setback (13.13 RVU) and pasting temperature (82.43 °C) than the cooked flour. Peak and final viscosities observed in the seed flours were lower than the values reported for legume starches (Sandhu *et al.*, 2008) and maize flour (Bolade, 2009). Low peak viscosities are desirable for high-calorie food formulations.

Longer pasting time (6.86 min) was required for cooked flour but at low pasting temperature (49.83 °C). Breakdown value (0.52 RVU) was low in the cooked flour. Yadav *et al.* (2011) explained the causes of breakdown in flour as disintegration of gelatinized starch granules structure during continued stirring and heating leading to shear thinning property of starch. A low breakdown value suggests higher stability of the flour under hot conditions. There was increase in the final viscosity of the cooked flour which may be due to the aggregation of the amylose molecules according to Bhupender *et al.* (2013). Cooked *Bligha sapida* seed flour showed higher stability ratio (0.91) than raw flour. Stability ratio indicates the ability of the cooked flour to retain its viscous properties after cooking and cooling. Pasting properties of the cooked flour suggests the feasible production and utilization as weaning food which require low viscosities.

Table 3: Pasting properties of *Blighia sapida* and flour

Parameter	Raw flour	Cooked flour
Peak viscosity (RVU)	78.25±0.35	5.51±0.02
Holding strength (RVU)	53.66±0.37	5.00±0.00
Breakdown (RVU)	24.59±0.01	0.52±0.02
Final viscosity (RVU)	66.79±0.54	8.41±0.01
Setback (RVU)	13.13±0.18	3.41±0.01
Pasting time (min)	4.72±0.02	6.86±0.01
Pasting Temperature (°C)	82.43±0.04	49.83±0.04
Stability ratio	0.69	0.91

Mean± SD

Swelling properties of *Blighia sapida* flour

Swelling properties of *Bligha sapida* seed flour are shown in Fig. 1. At low temperature (60-70 °C), cooked flour swelled than the raw flour. This may be as a result of the treatment given to the flour which led to rupturing and gelatinization of the starch thereby facilitating its ability to imbibe water at low temperatures. At 80-90 °C, the raw flour increased significantly than the cooked flour. Raw flour had higher swelling power (8.3) at 90 °C. Swelling power of the flours increased with temperature. Swelling power observed in *Bligha sapida* seed flour was lower than that of cassava and potato at 90 °C (Nuwamanya *et al.*, 2011). According to Iwuoha

(2004) and Nuwamanya *et al.* (2011) swelling power depends on amylose/amylopectin ratio, presence of lipid and starch granule size. This corroborates the finding of Kauret *et al.* (2007) that starches with lower amylose content swell more than those with higher amylose content. Swelling takes place when the amylose content had been leached into the cooking medium during processing. Low swelling power observed in cooked seed flour at high temperatures resulted into low breakdown viscosity as confirmed by Yuan *et al.* (2007).

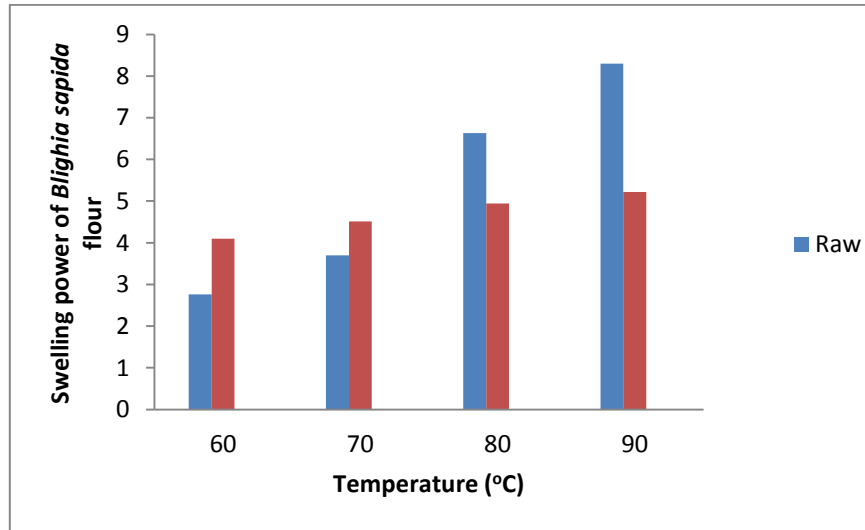


Fig. 1: Swelling power of raw and cooked *Blighia sapida* seed flour

Anti-nutritional factors in *Blighia sapida* flour

Anti-nutritional contents of *Blighia sapida* flour are shown in Table 4. *Blighia sapida* flour had low tannin (0.22-0.51 mg/kg), oxalate (0.43-0.57 mg/kg) and alkaloid (0.15-0.27 mg/kg) contents but high in phytate content (5.23-6.18 mg/kg). Cooked flour had lower values in all the anti-nutrients determined. Cooking caused reductions in the anti-nutritional contents of the *Blighia sapida* flour. Oxalate and alkaloid had been reported to be water soluble (Eka, 1998) and according to Albihn and Savage (2001) boiling may cause considerable skin (epidermal) rupture and facilitate the leakage of soluble oxalate into cooking water. Kumar *et al.* (2010) also reported partial phytate dephosphorylation of the food during processing. Reduction in the anti-nutritional factors may be as a result of leaching into the cooking medium.

Anti-nutritional factor	Raw flour	Cooked flour
Tannin (mg/g)	0.51±0.01	0.22±0.01
Phytate (mg/g)	6.18±0.04	5.23±0.02
Oxalate (mg/g)	0.57±0.03	0.43±0.10
Alkaloid (mg/g)	0.27±0.02	0.15±0.11

Table 4: Anti-nutritional factors in *Blighia sapida* flour

Mean± SD

Conclusions

Cooking for twenty minutes caused reduction in the chemical, anti-nutrients and pasting properties of the flour while the amino acids contents were improved. Cooking also increased the swelling power of the flour at lower temperature. The flour exhibited properties that are useful in the food industry. *Blighia sapida* seed flour could be extensively researched using *in vitro* and *in vivo* methods in order to ascertain its potential toxicity in animal model.

References

- Abdoulaye, S., Wei-Rong, Y., Issoufou, A., Heya, W., He, Q. and Moustapha, S., "Influence of process conditions on digestibility of African locust bean (*Parkia biglobosa*) Starch". American Journal of Food Technology, 7: 552-561, 2012.
- Adenuga, W., "Nutritional and sensory profiles of sweet potato based infant weaning food fortified with cowpea and peanut". Journal of Food Technology, 8 (5): 223-228, 2010
- Akaninwor, J.O. and Okechukwu, P.N., "Evaluation of processed sweet potato-crayfish – soya bean and sweet potato-crayfish-bambara groundnut weaning mixtures". Journal of Applied Science and Environmental Management 10 (1): 55 – 61, 2006.
- Albihn, P.B.E. and Savage, G. P., "The effect of cooking on the location and concentration of oxalate in three cultivars of New Zealand grown oca (*Oxalis tuberosa* Mol)". Journal of the Science of Food and Agriculture 81:1027–1033, 2001.

- Alexis, S.D. and Georges, A.N., “Classification of some wild yam species tubers of Ivory Coast Forest zone”. International Journal of Biochemistry Research and Review, 2(4):137-151, 2012
- Amarakoon, R., Study on Amino Acid Content in Selected Varieties of *Pisumsativum* (peas) by Ion Exchange Chromatography. International Conference on Nutrition and Food Sciences *IPCBEE* 39: 211-214, 2012.
- AOAC. Association of Official Analytical Chemists Official methods of Analysis 15th edition, Washington, DC, USA. 1990
- AOAC (Association of Official Analytical Chemicals), Official Method of Analysis of the AOAC (W. Horwitz Editor Eighteen Edition, Washington; D. C., 2006
- Benitez, L. V., “Amino Acid and fatty acid profiles in aquaculture nutrition studies, p. 23- 35. in S.S. De Silva (ed.) Fish Nutrition Research in Asia” Physicochemical, functional, thermal and pasting properties of starches isolated from pearl millet cultivars. International Food Research Journal 20(4): 1555-1561, 2013.
- Blake, O.A., Bennink, M.R. and Jackson, J.C. (2006). Ackee (*Blighia sapida*) hypoglycin A toxicity: Dose response assessment in laboratory rats. Food and Chemical Toxicology 44: 207–213
- Bolade, M.K. (2009). Effect of flour production methods on the yield, physicochemical properties of maize flour and rheological characteristics of a maize-based non-fermented food dumpling. African Journal of Food Science 3(10): 288-298.
- Dairo, F.A.S. (2008). Performance and haematological evaluation of weaner rabbits fed loofah gourd seed meal (*Luffa cylindrical* m. j. roem)”. African Journal of Food Agriculture and Nutritional Development, 8(4):451-463
- Dossou, V.M., Agbenorhevi, J.K., Combey, S., Afi-Koryoe S. (2014), Ackee (*Blighia sapida*) fruit arils: nutritional, phytochemicals and antioxidant properties. International Journal of Nutrition and Food Sciences, 3(6): 534-537
- Eka, O.U. (1998). Root and Tubers. In: Osagie, A.U. and Eka, O.U. (eds). “Nutritional Quality of Plant Foods”. Macmillian press, London. Pp 1 – 31.
- Ekué, M.R.M., Sinsin, B., Eyog-Matig, O. and Finkeldey, R. (2010). Uses, traditional management, perception of variation and preferences in ackee (*Blighia sapida* K.D. Koenig) fruit traits in Benin: Implications for

- domestication and conservation. Journal of Ethnobiology and Ethnomedicine, 6 (12): 1-14
- FAO/WHO (1991). Protein quality evaluation". Report of a Joint FAO/WHO Expert Consultation Food and Agriculture Organization of the United Nations. FAO, Rome, Food and Nutrition paper 51: 1-66
- Kumar, V., Sinha, A.K. and Makkar, H.P.S., Becker, K. (2010). Dietary roles of phytate and phytase in human nutrition: A review". Food Chemistry 120: 945–959.
- Nuwamanya, E., Baguma, Y., Wembabazi, E. and Rubaihayo, P. (2011). A comparative study of the physicochemical properties of starches from root, tuber and cereal crops. African Journal of Biotechnology 10(56): 12018-12030.
- Nwoso, S.O., Iwuoha, E.I., Waryo, T., Kgarebe, B., (2014). Isolation, partial purification and characterization of antifungal trypsin inhibitor protease from the seed of *Blighia sapida* K.D. Koenig (Ackee)". African Journal of Biotechnology, 13(29): 2996-3007
- Nwosu, J.N., Ubbaonu, C.N., Banigo, E.O.I., Uzomah, A. (2008). The effects of processing on the amino acid profile of Oze (*Bosqueia angolensis*) seed flour". Life Science Journal, 5(4): 69–74.
- Ojiako, O.A, Igwe, C.U., Agha, N.C., Ogbuji, C.A. and Onwuliri, V.A. (2010). Protein and Amino Acid Compositions of *Sphenostylis stenocarpa*, *Sesamum indicum*, *Monodora myristica* and *Azelia Africana* Seeds from Nigeria". Pakistan Journal of Nutrition, 9 (4): 368-372
- Omobuwajo, T.O., Sanni, L.A. and Olajide, J.O. (2000). Physical properties of ackee apple (*Blighia sapida*) seeds". Journal of Food Engineering 45(1): 43-48
- Omosuli, S.V. (2013). Physicochemical properties and fatty acid composition of oil extracted from akee apple (*Blighia sapida*) Seeds. Research and reviews": Journal of Food and Dairy Technology, 2(1): 5-8
- Onuekwusi, E.C., Akanya, H.O. and Evans, E.C. (2014). Phytochemical constituents of seeds of ripe and unripe *Blighia Sapida* (K. Koenig) and physicochemical properties of the seed oil". International Journal of Pharmaceutical Science Invention, 3(9): 31-40

- Oyetayo, F. L. and Ojo, B.A., (2012). Food Value and Phytochemical Composition of *Luffacyl indrica* Seed Flour”. American Journal of Biochemistry 2(6): 98-103
- Vongsumran, K., Ratphitagsanti, W. Chompreeda, P. and Haruthaitanasan, V. (2014). Effect of cooking conditions on black bean flour properties and its utilization in donut cake”. Kasetsart J. (Nat. Sci.) 48: 970 - 979
- Yadav, B.S. Yadav, R.B. and Kumar, M. (2011). Suitability of pigeon pea and rice starches and their blends for noodle making”. LWT Food Science and Technology 44: 1415-1421
- Yuan, Y., Zang, L., Dai, Y. and Yu, J., (2007). Physicochemical properties of starch obtained from *Dioscoreanipponica* Makino comparison with other tuber starches”. Journal of Food Engineering 82.4: 436-442.

Steady Blood Flow through Vascular Stenosis under the Influence of Magnetic Field

Abubakar J. U., Gbadeyan J.A. and Ojo J.B.

Department of Mathematics, University of Ilorin, Ilorin, Nigeria.

Corresponding author: abubakar.ju@unilorin.edu.ng

Abstract

The influence of magnetic field on a steady blood flow through vascular stenosed artery was investigated by assuming blood to be a Newtonian fluid. The Navier-Stokes equations governing the flow through the axial directions were solved using integral momentum method. The velocity profile graphs obtained shows that as magnetic field increases on the stenosis area the velocity decreases.

Keywords: Magnetic field, Newtonian fluid and stenosis.

NOMENCLATURE:

B_0 Magnetic field	σ Electrical conductivity
L_0 Length of the stenosis	μ Viscous coefficient of blood
M Magnetic parameter	τ_w Wall shear stress
P Pressure	τ_{w0} Wall shear stress in unobstructed tube
$\frac{\partial p}{\partial x}$ Pressure gradient	ρ Density,
Q flow flux,	Re Reynold's number,
R_0 Radius of normal tube,	$R_{(x)}$ Radius of stenosed tube,
$\frac{dR}{dx}$ Slope of the stenosed wall,	(u, w, v) Velocity components in (r, θ, x) ,
U Centerline velocity,	$\bar{U}_0$ Average velocity,
δ Maximum height of the stenosis,	(r, θ, x) Cylindrical polar coordinate,

Introduction

The vitality of the human body depends on a continuous supply of oxygen and nutrients delivered by the blood via the circulatory system. As such, the structural integrity and function of the circulatory system is the keystone of health. Diseases that impede blood flow through the circulatory system can cause serious, potentially

life-threatening effects throughout the body.

Vascular Stenosis (Atherosclerosis) is defined as the localized narrowing or constriction of the blood vessels due to abnormal growth of tissues, which causes resistance to blood flow through the artery. Once constriction is formed, the blood flow is significantly altered and vascular fluid dynamics factors play important role in the development and progression of arterial stenosis as the stenosis continues to enlarge leading to the development of cardiovascular diseases such as angina pectoris, heart attack, cardiac arrest, stroke and so on.

Many authors have reported that the rheologic and fluid dynamics properties of blood and its flow behavior through non-uniform cross-section of the tube could play an important role in the fundamental understanding, diagnosis and treatment of many cardiovascular diseases. Forrester and Young (1970) have studied blood flow in arteries with stenosis by considering blood as a Newtonian fluid. Results of experimental work on models of arterial stenoses were presented by Young and Tsai (1973). They observed that at Reynolds number 1000, the dimensionless pressure drop for severely constricted axisymmetric model is approximately 23 times the corresponding value of moderately constricted model. MacDonald (1979) studied the steady flow through a mild axisymmetric stenosed artery. The pressure drop and wall shear stress distribution for different Reynolds numbers and axial distance were discussed. El-Shahed (2003) showed that in the arterial system of human or animals, it is quite common to find localized narrowing, commonly called stenosis. In that study it was further described that these narrowing are caused by intravascular plaques. These stenosis disturb the normal pattern of blood flow through the artery. Perkkio and Keskinen (1983) studied the effects of concentrated flow of blood through a vessel with stenosis. They found out its important aspect from psychological point of view. It was observed that apparent viscosity increases as the stenosis grows but the same is not true in the absence of stenosis. The viscosity of blood flow has been found to vary with the radius of the artery in blood flow.

Recently, Mistra and Shit (2007), Biswas and Chak Raborty (2010) and Verma et al. (2011) developed mathematical models for blood flows through arterial segment by taking a velocity slip condition at the constricted wall. Biswas and Laskas (2011) studied the influence of magnetic field and slip velocity on pulsatile blood flow through a constricted artery.

Furthermore, Hazarika and Sharma (2014), investigated two-layered model of blood flow through composite stenosed artery in porous medium under the effect of applied magnetic field by considering blood to be a Newtonian fluid of variable viscosity in the central region and plasma fluid of constant viscosity in the peripheral region of the stenotic tube.

Thus in this present study, we present the mathematical modeling formulated to study the effect of magnetic field on a stenosed vascular artery.

Mathematical Formulation

A mathematical model of the axisymmetric flow of blood in an uniform circular tube with an axisymmetric mild stenosis under the influence of magnetic field was considered by treating blood as a Newtonian fluid. We use cylindrical polar coordinates $(\bar{r}, \bar{\theta}, \bar{x})$, with $\bar{x}$ as the central axis of the artery and $(\bar{u}, \bar{w}, \bar{v})$ denoting the velocity component of blood along the axial, tangential and radial direction respectively. Due to the axisymmetric flow of blood being considered, the flow variation is independent of $\bar{\theta}$. Figure 1 illustrates the geometry of the stenosis together with a standard cylindrical polar coordinate system used for theoretical analysis.

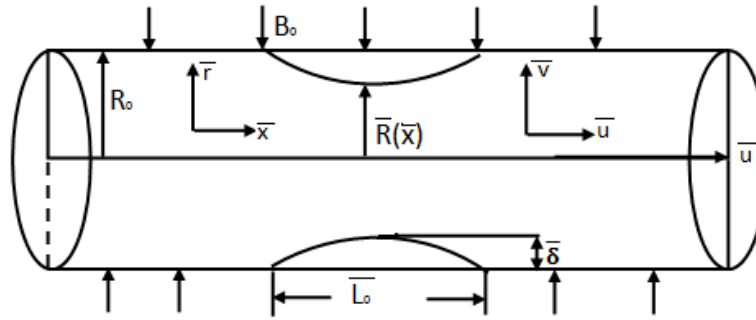


Figure 1: Geometry of Senosis

The axial coordinate and velocity are $\bar{x}$ and $\bar{u}$ respectively. The radial coordinate and velocity are $\bar{r}$ and $\bar{v}$ respectively. The geometry of the stenosis assumed to be axisymmetric is described by Young (1968) as;

$$\bar{R}(\bar{x}) = R_0 - \frac{\bar{\delta}}{2} \left(1 + \cos \frac{2\pi}{\bar{L}_0} \bar{x} \right) \quad (1)$$

Where $\bar{R}(\bar{x})$ is the radius of the stenosed tube, R_0 is the radius of normal tube, $\bar{\delta}$ is the maximum height of the stenosis, $\bar{x}$ is the axial coordinate and $\bar{L}_0$ is the length of the stenosis. Some dimensional variables are designated by the notation(-) to differentiate them from corresponding dimensionless variables.

Flow Analysis

With this given model, a two-dimensional steady, incompressible blood flow under the influence of externally applied magnetic field through the stenosis vascular is governed by Navier-Stokes equation and the continuity equation given below respectively;

$$\bar{u} \frac{\partial \bar{u}}{\partial \bar{x}} + \bar{v} \frac{\partial \bar{u}}{\partial \bar{r}} = -\frac{1}{\rho} \left(\frac{\partial \bar{p}}{\partial \bar{x}} \right) + \frac{\mu}{\rho} \left(\frac{\partial^2 \bar{u}}{\partial \bar{r}^2} + \frac{1}{\bar{r}} \frac{\partial \bar{u}}{\partial \bar{r}} + \frac{\partial^2 \bar{u}}{\partial \bar{x}^2} \right) - \frac{\sigma B_0^2 \bar{u}}{\rho} \quad (2)$$

in the axial direction and

$$\bar{u} \frac{\partial \bar{v}}{\partial \bar{x}} + \bar{v} \frac{\partial \bar{v}}{\partial \bar{r}} = -\frac{1}{\rho} \left(\frac{\partial \bar{p}}{\partial \bar{r}} \right) + \frac{\mu}{\rho} \left(\frac{\partial^2 \bar{v}}{\partial \bar{r}^2} + \frac{1}{\bar{r}} \frac{\partial \bar{v}}{\partial \bar{r}} + \frac{\partial^2 \bar{v}}{\partial \bar{x}^2} - \frac{\bar{v}}{\bar{r}^2} \right) \quad (3)$$

in the radial direction.

The continuity equation can be written as

$$\frac{\partial \bar{u}}{\partial \bar{x}} + \frac{\partial \bar{v}}{\partial \bar{r}} + \frac{\bar{v}}{\bar{r}} = 0 \quad (4)$$

where $\bar{u}$ represents the axial velocity, $\bar{v}$ the radial velocity, ρ the density, $\bar{p}$ the pressure, σ the electrical conductivity, B_0 the applied magnetic field, μ the viscosity coefficient of blood, $\bar{x}$ and $\bar{r}$ the axial and radial coordinates respectively.

The above equations can be rewritten in dimensionless form using the following dimensionless transformations

$$\begin{aligned} r &= \frac{\bar{r}}{R_0}, & x &= \frac{\bar{x}}{R_0}, & u &= \frac{\bar{u}}{\bar{U}_0}, & v &= \frac{\bar{v}}{\bar{U}_0}, \\ p &= \frac{\bar{p}}{\rho \bar{U}_0^2}, & R &= \frac{\bar{R}}{R_0}, & U &= \frac{\bar{U}}{\bar{U}_0}, & \delta &= \frac{\bar{\delta}}{R_0}, & L_0 &= \frac{\bar{L}_0}{R_0} \end{aligned} \quad (5)$$

where $\bar{U}$ is the centerline velocity and $\bar{U}_0$ is the average velocity in the unobstructed tube. Using these transformations in equation (1) we derived the geometry of the stenosis in dimensionless form as;

$$R(x) = 1 - \frac{\delta}{2} \left(1 + \cos \frac{2\pi}{L_0} x \right) \quad (6)$$

Similarly, the Navier-Stokes equation are derived as

$$u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial r} = -\frac{\partial p}{\partial x} + \frac{2}{Re} \left[\frac{\partial^2 u}{\partial r^2} + \frac{1}{r} \frac{\partial u}{\partial r} + \frac{\partial^2 u}{\partial x^2} \right] - \frac{2M^2}{Re} u \quad (7)$$

in the axial direction; and

$$u \frac{\partial v}{\partial x} + v \frac{\partial v}{\partial r} = -\frac{\partial p}{\partial r} + \frac{2}{Re} \left[\frac{\partial^2 v}{\partial r^2} + \frac{1}{r} \frac{\partial v}{\partial r} + \frac{\partial^2 v}{\partial x^2} - \frac{v}{r^2} \right] \quad (8)$$

in the radial direction, where

$$Re = \frac{2R_0 \bar{U}_0 \rho}{\mu} \text{ is Reynolds number and } M = B_0 R_0 \sqrt{\frac{\sigma}{\mu}} \text{ is Magnetic parameter.}$$

The continuity equation then becomes

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial r} + \frac{v}{r} = 0 \quad (9)$$

It is assumed that the term due to viscous component of the normal stress in the axial direction ($\partial^2 u / \partial x^2$) are negligible. This assumption has been used in the analysis of non-uniform flow. Therefore equation (7) becomes

$$u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial r} = -\frac{\partial p}{\partial x} + \frac{2}{Re} \left[\frac{\partial^2 u}{\partial r^2} + \frac{1}{r} \frac{\partial u}{\partial r} \right] - \frac{2M^2}{Re} u \quad (10)$$

Method of Solution

We employed the momentum integral technique to resolve equation (10) over the cross-section of the tube with boundary condition, $v = 0$, (that is, no slip in the radial velocity at the stenosed wall) and equation (10) was multiply by ∂r to obtain the integral-momentum equation

$$u \frac{\partial u}{\partial x} \partial r + v \frac{\partial u}{\partial r} \partial r = -\frac{\partial p}{\partial x} \partial r + \frac{2}{Re} \left[\frac{\partial^2 u}{\partial r^2} + \frac{1}{r} \frac{\partial u}{\partial r} \right] \partial r - \frac{2M^2}{Re} u \partial r \quad (11)$$

From the continuity equation (9),

$$\frac{\partial u}{\partial x} = -\left(\frac{\partial v}{\partial r} + \frac{v}{r} \right) \quad (12)$$

Substitute equation (12) into equation (11) and multiply through by r we to obtain

$$-ru \frac{\partial v}{\partial r} \partial r - uv \partial r + rv \frac{\partial u}{\partial r} \partial r = -r \frac{\partial p}{\partial x} \partial r + \frac{2r}{Re} \left[\frac{\partial^2 u}{\partial r^2} + \frac{1}{r} \frac{\partial u}{\partial r} \right] \partial r - \frac{2M^2}{Re} ru \partial r \quad (13)$$

From equation (9),

$$\frac{\partial v}{\partial r} = -\left(\frac{\partial u}{\partial x} + \frac{v}{r} \right) \quad (14)$$

Substituting equation (14) into equation (13) we obtain

$$ru \left(\frac{\partial u}{\partial x} + \frac{v}{r} \right) \partial r - uv \partial r + rv \frac{\partial u}{\partial r} \partial r = -r \frac{\partial p}{\partial x} \partial r + \frac{2r}{Re} \left[\frac{\partial^2 u}{\partial r^2} + \frac{1}{r} \frac{\partial u}{\partial r} \right] \partial r - \frac{2M^2}{Re} ru \partial r \quad (15)$$

Since $v = 0$,

$$ru \frac{\partial u}{\partial x} \partial r = -r \frac{\partial p}{\partial x} \partial r + \frac{2r}{Re} \left[\frac{\partial^2 u}{\partial r^2} + \frac{1}{r} \frac{\partial u}{\partial r} \right] \partial r - \frac{2M^2}{Re} ru \partial r \quad (16)$$

Integrating equation (16) over the cross section of the tube at $r = R$

$$\frac{\partial}{\partial x} \int_0^R r u^2 dr = -\frac{\partial p}{\partial x} \int_0^R r dr + \frac{2}{R_e} \int_0^R r \left[\frac{\partial^2 u}{\partial r^2} + \frac{1}{r} \frac{\partial u}{\partial r} \right] dr - \frac{2M^2}{R_e} u \int_0^R r dr \quad (17)$$

$$\frac{\partial}{\partial x} \int_0^R r u^2 dr = -\frac{R^2}{2} \frac{\partial p}{\partial x} + \frac{2}{R_e} \left[\left(r \frac{\partial u}{\partial r} \right) \Big|_0^R - \int_0^R \frac{\partial u}{\partial r} dr \right] + \int_0^R \frac{\partial u}{\partial r} dr - \frac{M^2 R^2}{R_e} u \quad (18)$$

$$\frac{\partial}{\partial x} \int_0^R r u^2 dr = -\frac{R^2}{2} \frac{\partial p}{\partial x} + \frac{2R}{R_e} \left(\frac{\partial u}{\partial r} \right)_R - \frac{M^2 R^2}{R_e} u \quad (19)$$

Applying the velocity-profile constraints for axisymmetric tube flow as stated by Verma (2009)

$$u = U \quad \text{at} \quad r = 0 \quad (20)$$

$$u = 0 \quad \text{at} \quad r = R \quad (21)$$

$$\frac{\partial u}{\partial r} = 0 \quad \text{at} \quad r = 0 \quad (22)$$

$$\frac{\partial^3 u}{\partial r^3} = 0 \quad \text{at} \quad r = 0 \quad (23)$$

$$\frac{\partial p}{\partial x} = \frac{2}{R_e} \left[\frac{\partial^2 u}{\partial r^2} + \frac{1}{r} \frac{\partial u}{\partial r} \right] \quad \text{at} \quad r = R \quad (24)$$

Equation (20) is defining the center line velocity, equation (21) is stating the no-slip condition at the wall. While equation (22) defines the regularity condition and it is deduce by considering the forces on a cylindrical element as if the pressure and the inertial forces are to be infinity as the radius of the element tends to zero, the viscous force that is proportional to $\partial u / \partial r$ must tends to zero.

Equation (23) was obtained by eliminating the pressure between equation (2) and equation (3) and considering the resulting equation as r tends to zero.

Equation (24) represents the validity of equation (5) at $r = R$.

In obtaining the velocity profile, the fourth degree polynomial used by Verma (2009) was used. That is,

$$\frac{u}{U} = A + B \left(\frac{r}{R} \right) + C \left(\frac{r}{R} \right)^2 + D \left(\frac{r}{R} \right)^3 + E \left(\frac{r}{R} \right)^4 \quad (25)$$

The velocity constraints (20) – (24) together with (25) give the values A, B, C, D and E as $A = 1$, $B = 0$, $C = -\frac{1}{3}(4 - \lambda)$, $D = 0$, $E = \frac{1}{3}(1 - \lambda)$, respectively.

Substituting these values into equation (25) we obtain,

$$\frac{u}{U} = 1 - \frac{1}{3}(4 - \lambda) \left(\frac{r}{R}\right)^2 + \frac{1}{3}(1 - \lambda) \left(\frac{r}{R}\right)^4 \quad (26)$$

where

$$\lambda = -\frac{R_e R^2}{8U} \frac{\partial p}{\partial x} \quad (27)$$

It is observed that if $\lambda = 1$ it is a parabolic profile corresponding to the Poiseuille profile

$$\frac{u}{U} = 1 - \left(\frac{r}{R}\right)^2 \quad (28)$$

The flow flux Q is given by

$$Q = \pi R^2 U_a = 2\pi \int_0^R r u dr \quad (29)$$

where $U_a = \bar{\bar{U}}/\bar{U}_0$ is non-dimensional average velocity at any cross section.

Substituting equation (26) into the equation (29) we obtain

$$Q = \frac{1}{18} \pi U R^2 (8 + \lambda) \quad (30)$$

Substituting the value of λ from (27) in (30) we obtain the expression for centerline velocity as

$$U = \frac{9}{4\pi} \frac{1}{R^2} \left[Q + \frac{\pi R_e}{144} R^4 \frac{\partial p}{\partial x} \right] \quad (31)$$

The parameter λ is determined from equation (19) using (25) and (27) hence from (19)

$$\left(\frac{\partial u}{\partial r}\right)_R = \frac{\partial}{\partial r} \left(U \left[1 - \frac{1}{3}(4 - \lambda) \left(\frac{r}{R}\right)^2 + \frac{1}{3}(1 - \lambda) \left(\frac{r}{R}\right)^4 \right] \right)_R \quad (32)$$

$$\left(\frac{\partial u}{\partial r}\right)_R = -\frac{2U}{3R} (2 + \lambda) \quad (33)$$

now putting (33) into (19) we have

$$\frac{\partial}{\partial x} \int_0^R ru^2 dr = -\frac{R^2}{2} \frac{\partial p}{\partial x} - \frac{4U}{3R_e} (2 + \lambda) - \frac{M^2 R^2}{R_e} u \quad (34)$$

also from (26)

$$-\frac{R^2}{2} \frac{\partial p}{\partial x} = \frac{4U\lambda}{R_e} \quad (35)$$

substituting (35) in (34) to obtain

$$\frac{\partial}{\partial x} \int_0^R ru^2 dr = \frac{4U\lambda}{R_e} - \frac{4U}{3R_e} (2 + \lambda) - \frac{M^2 R^2}{R_e} u \quad (36)$$

simplifying (36) to get

$$\lambda = 1 + \frac{3}{8U} \left[M^2 R^2 u + R_e \frac{\partial}{\partial x} \int_0^R ru^2 dr \right] \quad (37)$$

In the velocity profile equation (26) neglecting terms with degree higher than two in r , and $\lambda = 1$, we have the Poiseuille profile

$$u = 2U_a \left[1 - \left(\frac{r}{R} \right)^2 \right] \quad (38)$$

where

$$U_a = -\frac{R_e R^2}{16} \frac{\partial p}{\partial x} \quad (39)$$

is the non-dimensional average velocity at any given cross-section and $\partial p / \partial x < 0$.

Using equation (27), (29) and (31) in (19) we obtain the expression for pressure gradient. From (19)

$$\left(\frac{\partial u}{\partial r} \right)_R = \frac{\partial}{\partial r} \left[2U_a \left[1 - \left(\frac{r}{R} \right)^2 \right] \right]_R \quad (40)$$

$$= -\frac{4U_a}{R} \quad (41)$$

and

$$\frac{\partial}{\partial x} \int_0^R ru^2 dr = \frac{\partial}{\partial x} \int_0^R r \left[2U_a \left[1 - \left(\frac{r}{R} \right)^2 \right] \right]^2 dr \quad (42)$$

on simplifying we have

$$\frac{\partial}{\partial x} \int_0^R r u^2 dr = \frac{2U_a^2}{3} R \frac{dR}{dx} \quad (43)$$

substituting the expressions of $\left(\frac{\partial u}{\partial r}\right)_R$ and $\frac{\partial}{\partial x} \int_0^R r u^2 dr$ into (19) we have

$$\frac{2U_a^2}{3} R \frac{dR}{dx} = -\frac{R^2}{2} \frac{\partial p}{\partial x} - \frac{2R}{R_e} \left(\frac{4U_a}{R}\right) - \frac{M^2 R^2}{R_e} \left[2U_a \left[1 - \left(\frac{r}{R}\right)^2\right]\right] \quad (44)$$

simplifying (44) further to obtain

$$\frac{4U_a^2}{3R} \frac{dR}{dx} = \frac{8U}{R_e R^2} - \frac{16U_a}{R_e R^2} - \frac{4U_a M^2}{R_e} \left[1 - \left(\frac{r}{R}\right)^2\right] \quad (45)$$

substituting for U in (45) above to get

$$\frac{4U_a^2}{3R} \frac{dR}{dx} = \frac{8}{R_e R^2} \left[\frac{9}{4\pi} \frac{1}{R^2} \left(Q + \frac{\pi R_e}{144} R^4 \frac{\partial p}{\partial x}\right)\right] - \frac{16U_a}{R_e R^2} - \frac{4U_a M^2}{R_e} \left[1 - \left(\frac{r}{R}\right)^2\right] \quad (46)$$

simplifying (46) and using $U_a = \frac{Q}{\pi R^2}$ and $U_a^2 = \frac{Q^2}{\pi^2 R^4}$ we obtain

$$\frac{4Q^2}{3\pi^2 R^5} \frac{dR}{dx} = \frac{18Q}{\pi R_e R^4} + \frac{1}{8} \frac{\partial p}{\partial x} - \frac{16Q}{\pi R_e R^4} - \frac{4QM^2}{\pi R_e R^2} \left[1 - \left(\frac{r}{R}\right)^2\right] \quad (47)$$

simplifying (47) further to obtain the required pressure gradient. From (19)

$$\frac{\partial p}{\partial x} = \frac{32Q^2}{3\pi^2 R^5} \frac{dR}{dx} - \frac{16Q}{\pi R_e R^4} + \frac{32QM^2}{\pi R_e R^2} \left[1 - \left(\frac{r}{R}\right)^2\right] \quad (48)$$

Further using equation (27) and (31) in (26) we obtain the expression for velocity profile as follows:

from (26) we have

$$u = U \left[3 - 4 \left(\frac{r}{R}\right)^2 + \left(\frac{r}{R}\right)^4\right] + \lambda U \left[\left(\frac{r}{R}\right)^2 - \left(\frac{r}{R}\right)^4\right] \quad (49)$$

substituting (27) and (31), for U and λ respectively we have (50)

$$u = \frac{9}{4\pi} \frac{1}{R^2} \left[Q + \frac{\pi R_e}{144} R^4 \frac{\partial p}{\partial x}\right] \left[3 - 4 \left(\frac{r}{R}\right)^2 + \left(\frac{r}{R}\right)^4\right] - \frac{R_e R^2}{8} \frac{\partial p}{\partial x} \left[\left(\frac{r}{R}\right)^2 - \left(\frac{r}{R}\right)^4\right] \quad (50)$$

simplifying (50) and substituting (48) for $\frac{\partial p}{\partial x}$ we have

$$u = \frac{R_e R^2}{192} \left\{ \frac{32Q^2}{3\pi^2 R^5} \frac{dR}{dx} - \frac{16Q}{\pi R_e R^4} + \frac{32QM^2}{\pi R_e R^2} \left[1 - \left(\frac{r}{R}\right)^2\right] \right\}$$

$$\left[3 - 12 \left(\frac{r}{R}\right)^2 + 9 \left(\frac{r}{R}\right)^4\right] + \frac{3}{4\pi R^2} \left[3 - 4 \left(\frac{r}{R}\right)^2 + \left(\frac{r}{R}\right)^4\right] \quad (51)$$

The flow flux in normal unobstructed blood vessel, $\bar{Q} = \pi R_0^2 \bar{U}_0$. This gives non-dimensional flow flux $Q_0 = \bar{Q}_0 / R_0^2 \bar{U}_0 = \pi$, which is same for unobstructed blood vessel. Therefore replacing Q by Q_0 i.e. Q by π in equation (48) and (51) we obtain the expression for pressure gradient and velocity profile, respectively, as

$$\frac{\partial p}{\partial x} = \frac{32}{3} \frac{1}{R^5} \frac{dR}{dx} - \frac{16}{R_e R^4} + \frac{32M^2}{R_e R^2} \left[1 - \left(\frac{r}{R}\right)^2\right] \quad (52)$$

and

$$u = \frac{2}{R^2} \left[1 - \left(\frac{r}{R}\right)^2\right] + \frac{1}{18} \frac{R_e}{R^3} \frac{dR}{dx} \left[3 - 12 \left(\frac{r}{R}\right)^2 + 9 \left(\frac{r}{R}\right)^4\right] + \frac{M^2}{6} \left[3 - 15 \left(\frac{r}{R}\right)^2 + 21 \left(\frac{r}{R}\right)^4 - 9 \left(\frac{r}{R}\right)^6\right] \quad (53)$$

The wall shearing stress is expressed as

$$\bar{\tau}_w = \mu \left(\frac{\partial \bar{u}}{\partial \bar{r}}\right)_{\bar{R}} \left[1 + \left(\frac{d\bar{R}}{d\bar{x}}\right)^2\right] \quad (54)$$

where $d\bar{R}/d\bar{x}$ is the slope of the wall. The dimensionless wall shearing stress is defined as

$$\tau_w = \frac{\bar{\tau}_w}{\tau_{w0}}$$

where τ_{w0} is the corresponding shearing stress due to Poiseuille flow in the unobstructed tube. Thus, in terms of dimensionless variables,

$$\tau_w = -\frac{1}{4} \left(\frac{\partial u}{\partial r}\right)_R \left[1 + \left(\frac{dR}{dx}\right)^2\right] \quad (55)$$

Now, from (53) we get

$$\begin{aligned} \left(\frac{\partial u}{\partial r}\right)_R &= -\frac{2}{R^2} \left(\frac{2R}{R^2}\right) - \frac{1}{18} \frac{R_e}{R^3} \frac{dR}{dx} \left(\frac{24R}{R^2} - \frac{36R^3}{R^4}\right) - \frac{M^2}{6} \left(\frac{30R}{R^2} - \frac{84R^3}{R^4} + \frac{54R^5}{R^6}\right) \\ &= -\frac{4}{R^3} - \frac{1}{18} \frac{R_e}{R^3} \frac{dR}{dx} \left(-\frac{12}{R}\right) - \frac{M^2}{6} (0) \end{aligned}$$

$$= -\frac{4}{R^3} + \frac{12 R_e}{18 R^4} \frac{dR}{dx}$$

$$\left(\frac{\partial u}{\partial r}\right)_R = -\frac{4}{R^3} \left[1 - \frac{1 R_e}{6 R} \frac{dR}{dx}\right] \quad (56)$$

Thus, (55) becomes

$$\tau_w = -\frac{1}{4} \left\{ -\frac{4}{R^3} \left[1 - \frac{1 R_e}{6 R} \frac{dR}{dx}\right] \right\} \left[1 + \left(\frac{dR}{dx}\right)^2\right]$$

$$\tau_w = \frac{1}{R^3} \left[1 - \frac{1 R_e}{6 R} \frac{dR}{dx}\right] \left[1 + \left(\frac{dR}{dx}\right)^2\right] \quad (57)$$

At points along the wall where $\tau_w = 0$, flow separation and reattachment will occur. From equation (57), the condition of zero wall shear stress is given as

$$\frac{dR}{dx} = \frac{6R}{R_e} \quad (58)$$

when $\frac{dR}{dx} > \frac{6R}{R_e}$, τ_w is negative, indicating back-flow and the separated region is clearly described.

Discussion of Result

The behaviour of the flow of blood through a compressed vessel with mild stenosis under the influence of magnetic field have been investigated by modeling of blood as a Newtonian fluid. This Study is concerned with the approximate solution for velocity profile, wall shearing stress, pressure gradient and separation phenomena for specific geometry of the stenosis. It involves integral method for the solution of the problem.

Analytic expressions have been computed numerically for different Reynolds number. It has been suggested by Forrester and Young (1970) in the theoretical and experimental studies, the stenosis parameter R_0 , $\bar{L}_0$ and δ are taken to be related as $\bar{L}_0 = 8R_0 = 24\delta$. The computation has been carried out for the location of stenosis defined by $-4 \leq x \leq 4$ for $\delta = 1/3$ and $L_0 = 8$ for different Reynolds number between 10-100.

Figures 1 to 15 are the non-dimensional axial velocity profiles of blood flow in the mild stenosed vessel segment ($\delta = 1/3$) with the influence of magnetic field under

the consideration for Reynolds number 10, 50 and 100 respectively. It is observed that in converging region near the throat of the stenosis, velocity increases for each value of x as magnetic field increases. It is also observed that in converging region near the throat of the stenosis, velocity decreases but increases away from the throat as Reynolds number increases for r/R up to 0.5 and then in reverse order for $r/R > 0.5$, while in diverging region, all above observations are reversed as Reynolds number increases.

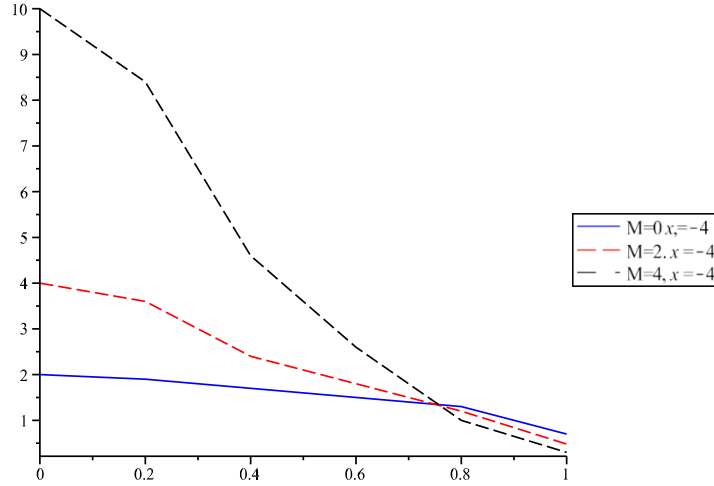


Figure 1: Velocity Profile for $Re=10$ and $\delta=1/3$

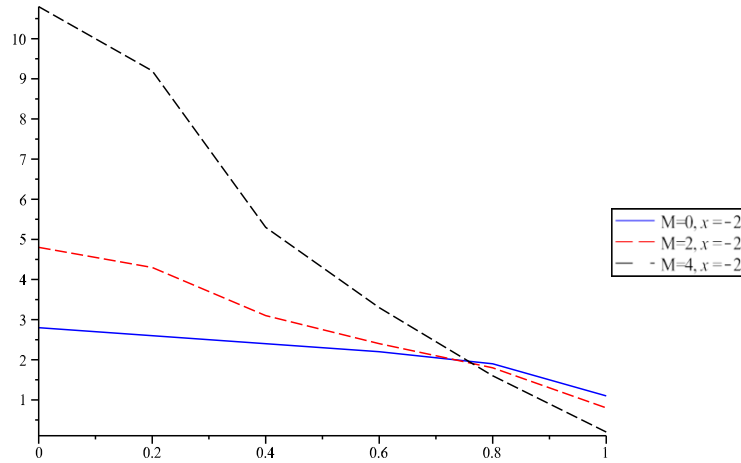


Figure 2: Velocity Profile for $Re=10$ and $\delta=1/3$

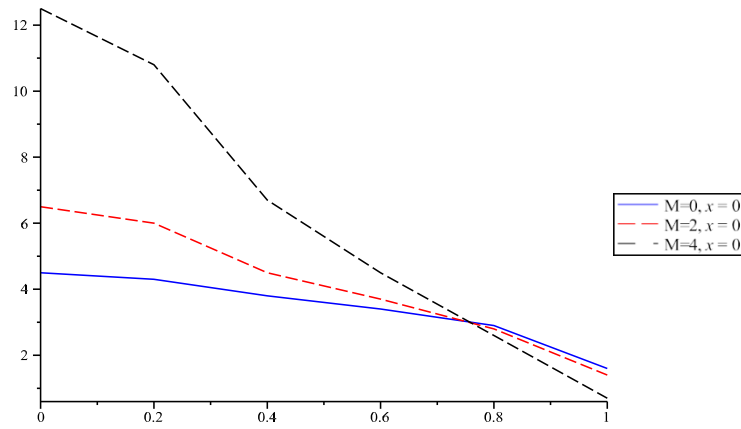


Figure 3: Velocity Profile for $Re=10$ and $\delta=1/3$

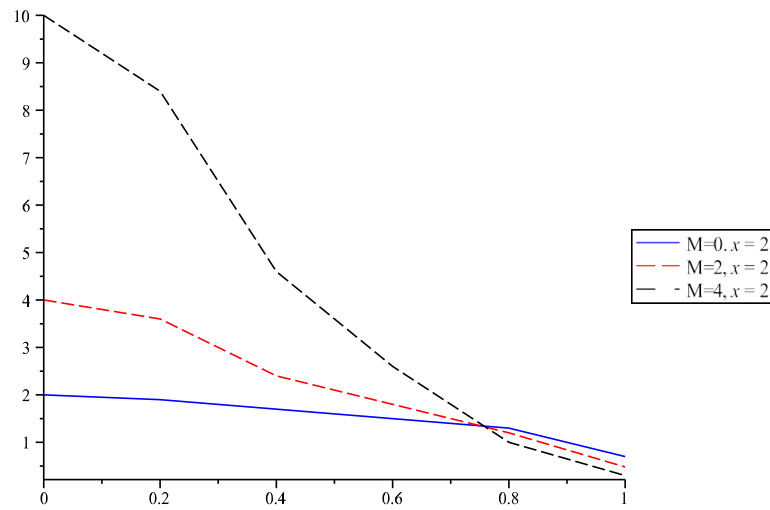


Figure 4: Velocity Profile for $Re=10$ and $\delta=1/3$

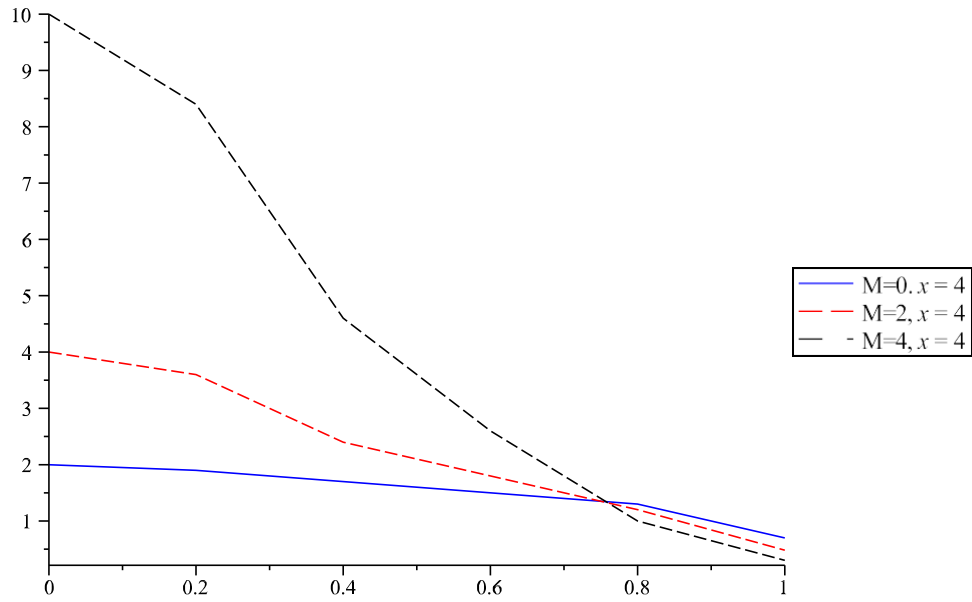


Figure 5: Velocity Profile for Re=10 and $\delta=1/3$

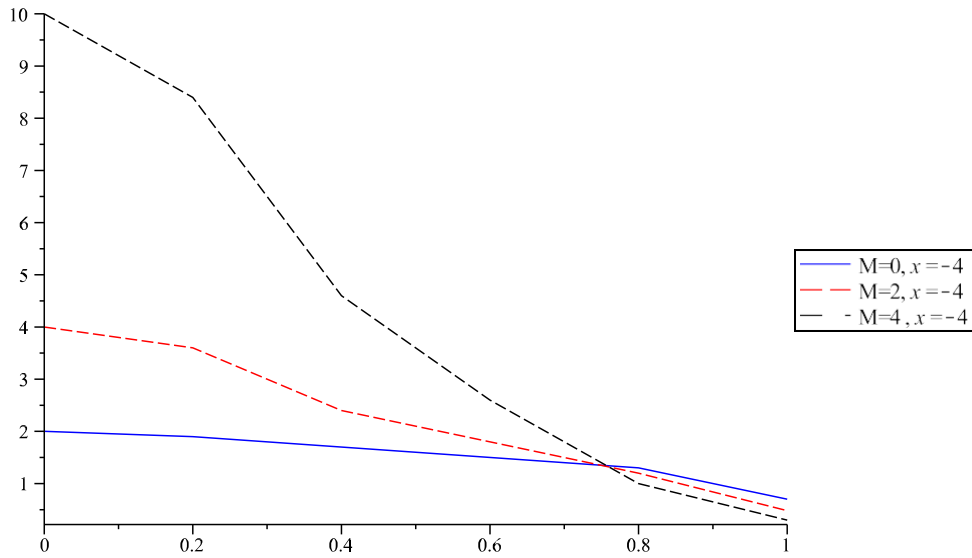


Figure 6: Velocity Profile for Re=50 and $\delta=1/3$

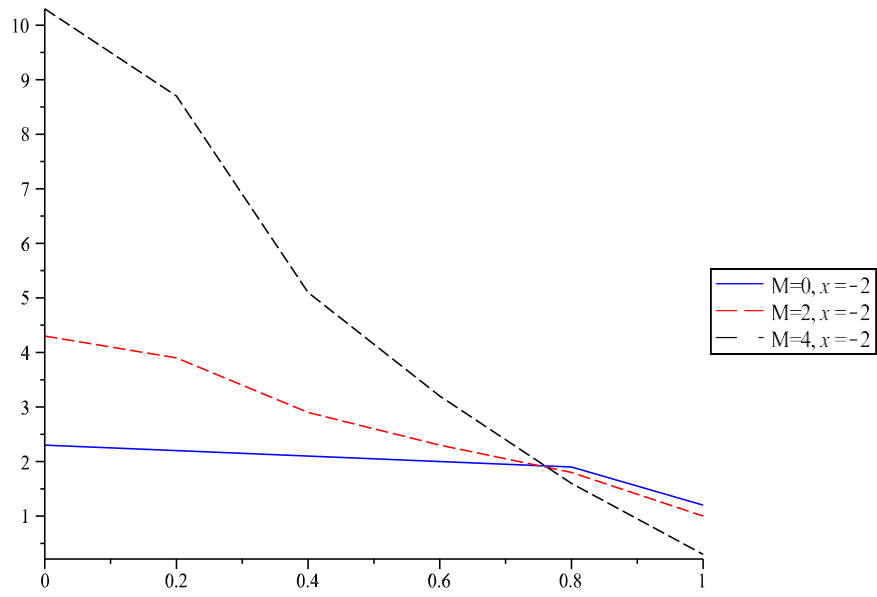


Figure 7: Velocity Profile for $Re=50$ and $\delta=1/3$

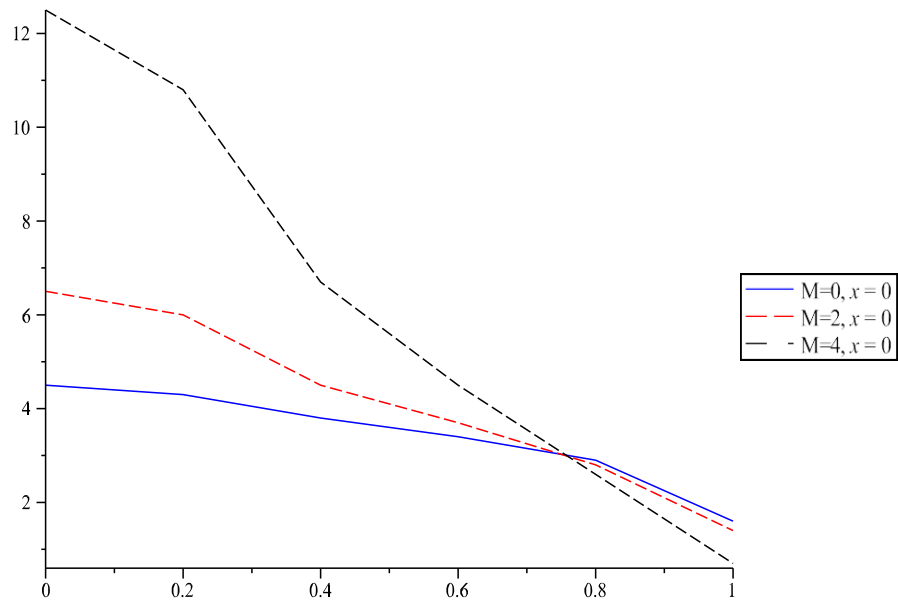


Figure 8: Velocity Profile for $Re=50$ and $\delta=1/3$

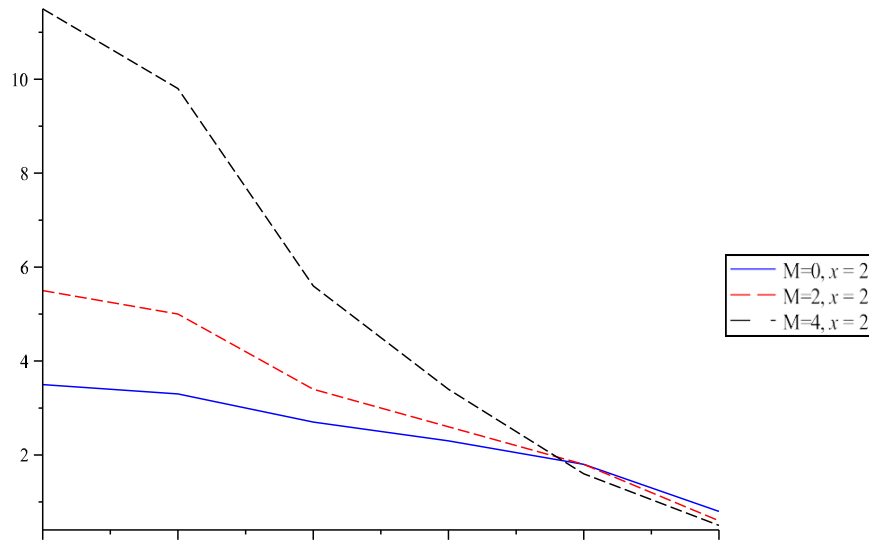


Figure 9: Velocity Profile for $Re=50$ and $\delta=1/3$

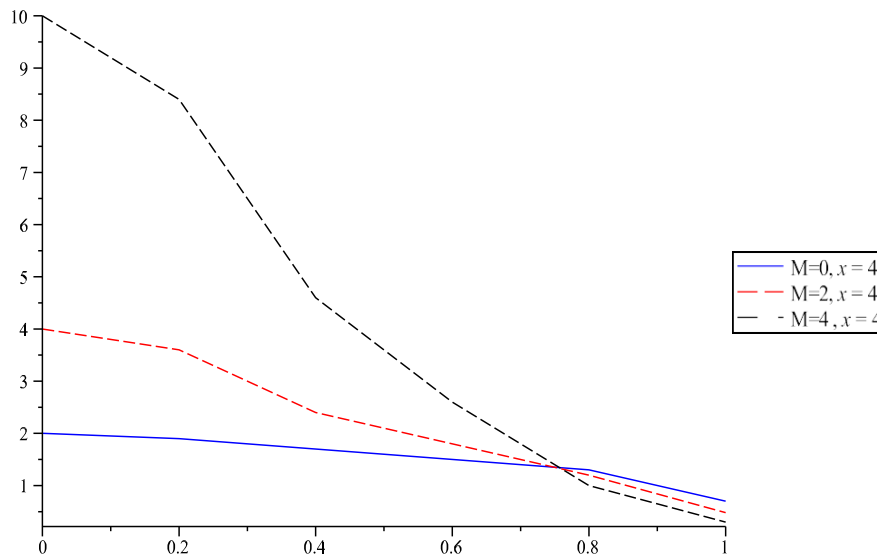


Figure 10: Velocity Profile for $Re=50$ and $\delta=1/3$

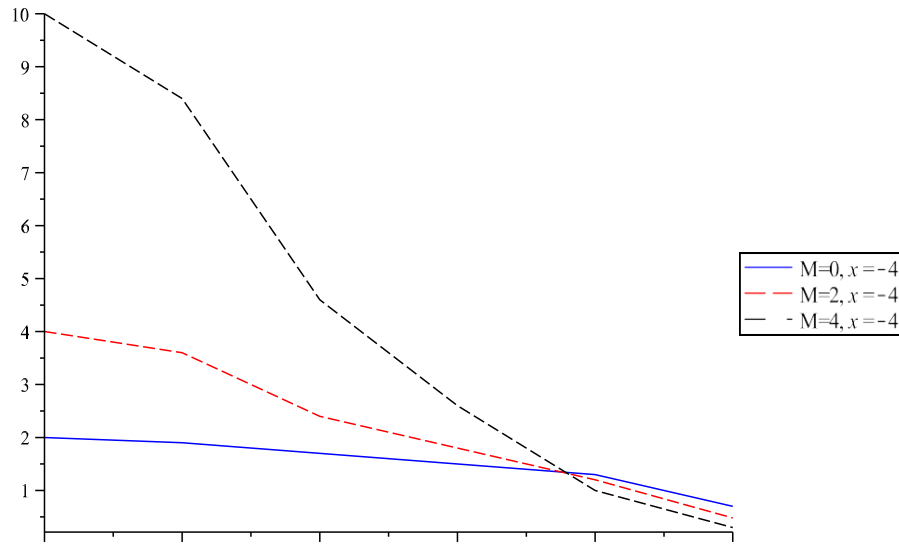


Figure 11: Velocity Profile for $Re=100$ and $\delta=1/3$

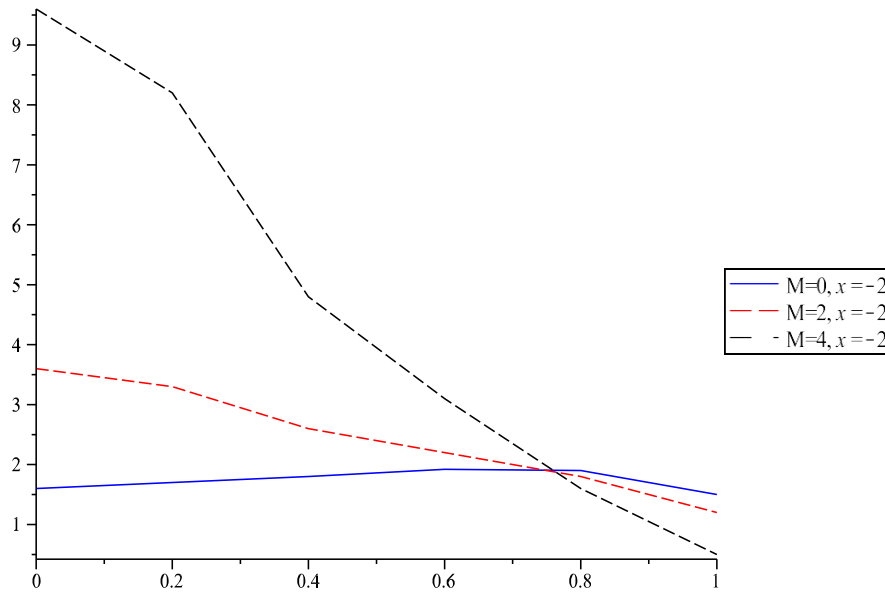


Figure 12: Velocity Profile for $Re=100$ and $\delta=1/3$

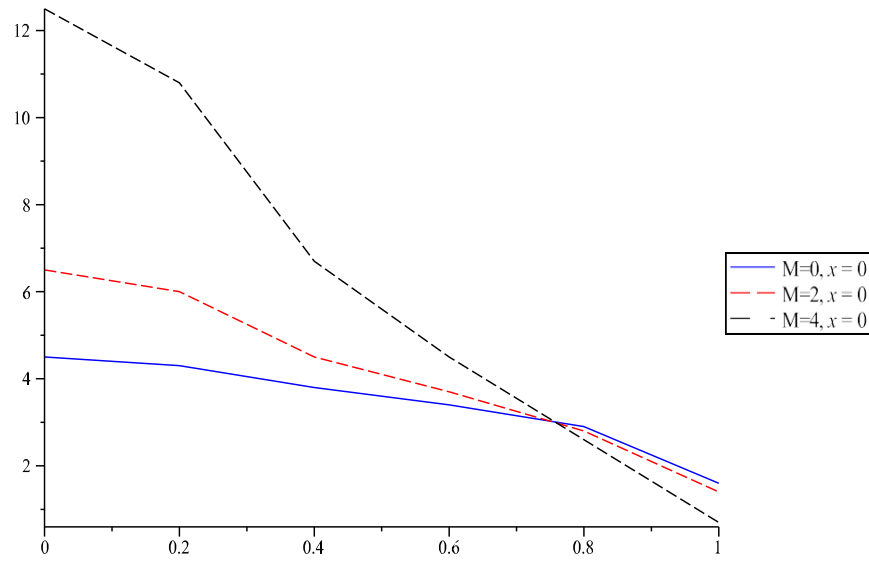


Figure 13: Velocity Profile for $Re=100$ and $\delta=1/3$

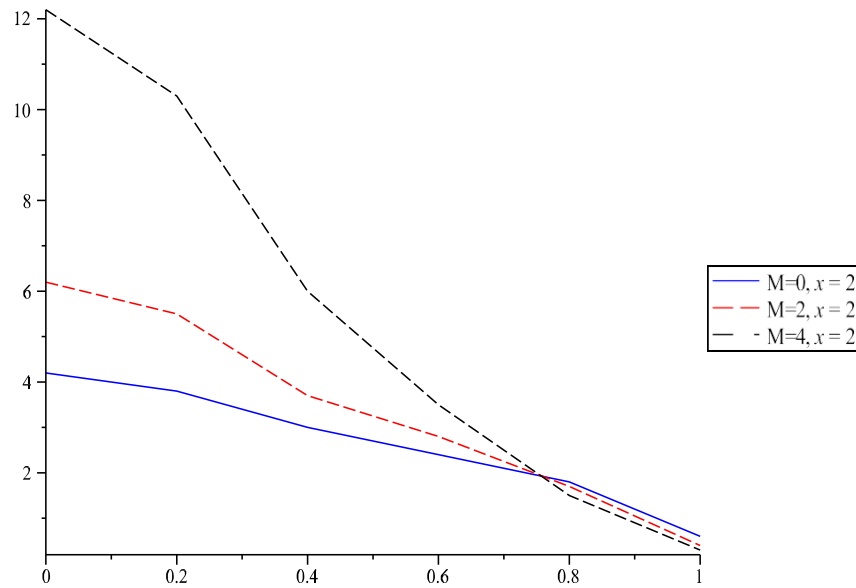


Figure 14: Velocity Profile for $Re=100$ and $\delta=1/3$

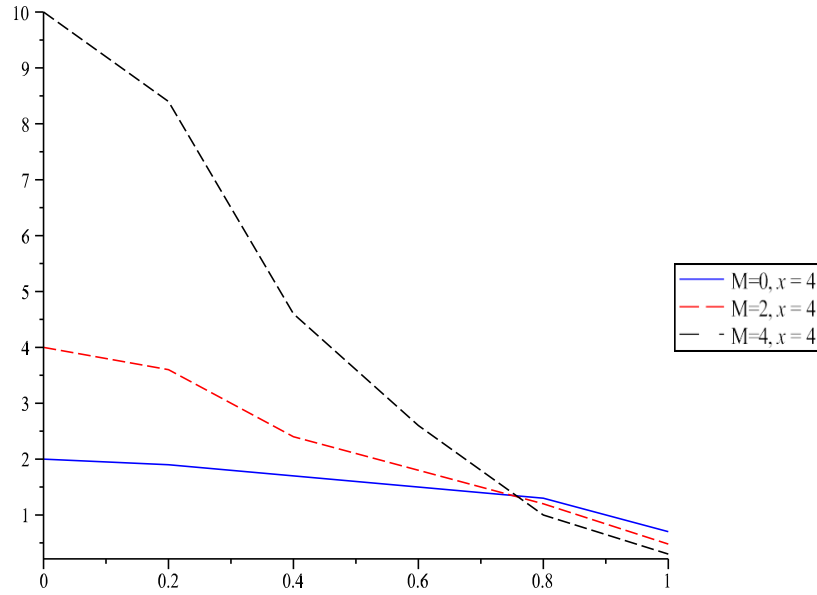


Figure 15: Velocity Profile for $Re=100$ and $\delta=1/3$

Conclusion

In this study, the continuity equation and Navier-Stokes equation have been converted from Cartesian form to cylindrical form. Considering the continuity and Navier-Stokes equation which were derived from the conservation of mass and Cauchy's equation respectively. These equations were then solved using the integral-momentum technique and the obtained results were used to plot the graphs showing the behaviour of the blood at the stenosed part. The effect of mild stenosis in an artery under the influence of magnetic field by considering the blood as a Newtonian fluid. It has been concluded that the velocity of the blood decreases as the size of the stenosis increases for a given Newtonian model of the blood.

References

- Alfio Q. and Luca F. (2002), Mathematical modelling and numerical simulation of the cardiovascular system. *Journal of Eng. Math.* 47, 175-183.
- Devajyoti B. (2000), Blood Flow Model: A Comparative Study, Naurang Rai for Mattal New Delhi.
- Biswas, D. and Chakraborty, U. S., (2010), Pulsatile Blood Flow through a Catheterized Artery with an Axially Nonsymmetrical Stenosis. *Applied Mathematical Sciences*, 4, no. 58, 2865 – 2880
- Biswas, D. Laskar, R. B., (2011) Influence of magnetic field and slip velocity on pulsatile blood flow through a constricted artery. *Int J Pharm Biomed Res*, 2(4), 227-232.
- Dintenfass L. (1977), Viscosity factors in hypertensive and cardiovascular diseases. *Cardiovascular Medicine*, 2, 337-349.
- Youngab, D. F. and Tsai, F. Y., (1973), Flow characteristics in models of arterial stenoses Steady flow. *Journal of Biomechanics*, 6(4), 395-402.
- El-Shahed M., (2003), Pulsatile flow of blood through a stenosed porous medium under periodic body acceleration, *Appl. Math. Comput.* 138, 479–488.
- Forrester J.H. and Young D. F. (1970): Å–Flow through a converging diverging tube and its Implications in occlusive vascular disease. *J. Biomech.*,3, 297-316.
- Gupta A.K. and Gupta G.D. (2001), Unsteady blood flow in an artery through a non-symmetrical stenoses. *Acta Ciencia Indica.* 2, 137-142.
- Hazarika G.C. and Sharma, B. (2014), Two Layered Model of Blood Flow through Composite Stenosed Artery in Porous Medium under the Effect of Applied Magnetic Field *Int. Journal of Mathematics Trends and Technology.* 15(1) 11 - 22.
- McDonald D.A. (1979), On steady flow through modelled vascular stenosis. *J. Biomechanics* 12, 13-30.
- Misra J.C. and Shit G.C. (2006), Blood flow through arteries in a pathological state: A theoretical study. *International Journal of Eng. Sci.* 44, 662-671.
- Misra J.C. and Shit G.C. (2007), Effect of magnetic field on blood flow through an artery: A numerical model. *Center for theoretical studies*, 3 - 16.

- Mwapinga A., (2012), Computational modelling of arterial blood flow in the presence of body exercise. *Journal of Applied Mathematics and computational*, 138, 479 - 488.
- Verma, N. K., Mishra, S., Siddiqui, S. U. and Gupta, R. S., (2011), Effect of Slip Velocity on Blood Flow through a Catheterized Artery. *Applied Mathematics*, 2, 764-770.
- Perkkio, J. and Keskinen, R., (1983). On the effect of the concentration profile of red cells on blood flow in the artery with stenosis, *Bull. Math. Biol.*, Vol. 45, 259-267.
- Shukla J.B., Parihar R.S. and Rao B.R. (1980), Effect of stenosis on non-Newtonian flow of blood in an artery. *Bulletin of Mathematical Biology*, 42, 283-294.
- Singh M.P., Rashid A., Ramindar K. and Koitiyar (2009), Mathematical modelling of blood flow through vertebral Artery with stenoses. *Indian Journal of Biomechanics*, Special Issue (NCBM 7-8 March 2009) 151 - 158.
- Verma S.R. (2008), Study of blood flow through modelled vascular stenosis. *Indian Journal of Biomechanics*, Special Issue (NCBM 7-8 March 2009) 22 - 25.
- Young D.F. (1968), Effect of time-dependent stenosis on flow through a tube. *J. Engng. Ind.* 90, 248-254.
- Yunus A.C. and John M.C. (2006), Fluid Mechanics: Fundamental and Applications, 426-431.

Plants in the Diet of African Forest Elephants in Okomu National Park, Nigeria

Bamidele, O.A ¹, Akinsoji ², A and Adeniran, S.A ³

^{1,2}*Department Botany, University of Lagos, Akoka Lagos*

³*Department Plant Biology, University of Ilorin, Ilorin Nigeria*

¹Correseponding Author: olumidebamidele.a@gmail.com

Abstract

The study was carried out to assess the phytosociological and proximate properties of vegetation foraged by elephant in Okomu National Park, Edo State Nigeria. Five (5) of the compartments with foraged plants were randomly sampled with two quadrats each measuring 20m x 20m in dimension at breast height of the trees. A total number of forty-seven (47) species belonging to thirty (30) families were identified of which seventeen (17) plant species were fed upon by the elephants in the area studied. The most dominant species on the basis of Importance Value ranking were *Ceiba pentandra*, *Terminalia superba* and *Strombosia pustulata*. The life form pattern of the foraged plants consists of 71% tree, 18% herb, 6% shrub and 6% vine. The part fed upon by elephants consists of tree barks, leaves, roots, tubers, fresh young fronds and fruits. The proximate composition of selected plant species eaten by the elephants showed highest percentage of moisture in the root of *Thaumatococcus daniellii* and highest percentage of carbohydrate in the tuber of *Dioscorea praehensilis*. The high moisture content in these species suggests a daring need for the survival of the elephants in the forest. However, few plant species did not meet the elephants' nutrient requirements, hence their dependence on many species. The pattern of results obtained indicates reduction of tree densities which may lead to increase in herbaceous species because elephants feed on wide variety of plants which may alter the vertical structure of plants community. This kind of information will enhance management decision for sustainable management of the Okomu National Park.

Keywords: Phytosociological and Proximate properties, African elephants, Okomu Park.

Introduction

Plant-Animal Interaction (PAI) is the relationship between organisms in the kingdoms Animalia and Plantae. This relationship conceals an enormous number and diversity of ecological relationships and fundamental processes ranging from general to those that are highly specific which involve elaborate evolutionary adaptations (Herrera *et al.*, 2002). Plant-Animal Interaction has been categorized to be commensal, in which one partner benefits while the other is unaffected. Similarly, antagonistic is the interaction leading to the detriment of at least one partner whilst mutualistic is when both the plant and animal partners both benefit. Such, is the interaction between elephants and plants which is largely symbiotic. Elephants are classified as mega herbivores and consume up to 150kg of plant per day (Samansiri and Weerakoon, 2007). Being mega-herbivores, elephants need to take sufficient amount of forage and water per day (Jackson and Erasmus 2005; Stephenson 2007). Numerous studies on feeding habits of African and Asian elephants have shown that proportion of various plant categories in diet vary widely from one region to another (Sukumar, 1985). Grasses, shrubs, tree leaves, aquatic plants and occasionally fruits make up the component of their diet (Borah and Deka, 2008). Although elephants harvest food from a range of plant life forms, it is their conspicuous impact on woodlands that has the greatest potential to cause long-term vegetation change (Morrison *et al.*, 2016). Extensive conversion of woodlands to shrubland by elephants (Spinage, 1994) and the potential associated loss of biodiversity (Cumming *et al.*, 1997; Herremans, 1995; Kerley and Landman, 2006) may require management intervention (O'Connor *et al.*, 2007), but such action should be based on the understanding of why elephants choose to utilize woody plants in a destructive manner. Impact on woody vegetation is greatest when harvesting methods such as breaking branches, debarking stems, or toppling, pollarding or uprooting whole plants are used, and less when trunkloads of leaves are stripped without breaking branches (O'Connor *et al.*, 2007).

Elephants' utilization of trees entails switching to bark stripping as tree height increases (Smallie and O'Connor, 2000). Tree breaking is consistent with the elephant's diet (Ben-Shahar, 1993). Elephants consume considerable amounts of bark and roots of the felled trees to supplement their grass intake (Barnes, 1982). Large *Acacia* spp. trees are uprooted by 5 bull elephants for nutritional rather than social reasons as it is the younger low ranking bulls that are often involved (Croze, 1974). Elephant are constantly on the move looking for the necessary supply of food and sources of water (Davies, 2008). The survival of African as well as Asian elephants largely depends upon availability of extensive habitations for adequate protection and food for their healthiness and growth. The African forest elephant *Loxodonta cyclotis* (Matschie, 1900) is one of the world's largest terrestrial mammals.

Presently, very few forest areas in South Western Nigeria still contain elephants. One of such forest area is Okomu National Park. Amusa *et al.* (2017) carried out population status and distribution of forest elephants (*Loxodonta cyclotis*) in Okomu National Park and Omo Forest Reserve, South-western Nigeria. Okomu area has well marked rainy and dry seasons. The dry season which lasts from November to March is usually accompanied by northeast trade wind that causes harmattan (Azeez *et al.*, 2010). Okomu soil is acidic, nutrient-poor sandy loam. The soil pH ranged from very strongly acid (4.88) in the sub soil to neutral (6.75) in the top soil of Okomu forest (Agboola *et al.*, 2017). Vegetation is Guinea–Congolia lowland rain forest, which includes areas of Swamp-forest, high forest, secondary forest, and open scrub (Okomu National Park, 2010). In Nigeria, forest elephants have become extinct in many areas while the remnant fragile populations remain at high risk of total extirpation. As human populations increase rapidly, the elephant range is constantly being broken up into small fragments traversed by roads, human settlements and infrastructures thereby bringing elephants into conflict with humans. The aim of the present study is to describe the vegetation and forest structure of vegetation where the African elephants foraged using phytosociological and nutritional characteristics of the prominent plants fed upon by the elephants in Okomu National Park, Edo state Nigeria and to examine the impacts of continuous elephant activities on the structure and composition of the foraged vegetation.

Materials and Methods

Study area description

Okomu National Park is located in Ovia South-West Local Government Area of Edo State. It occupies an area of 202.24 km² within the lowland forest of Nigeria. It lies between Longitude 5°00'E – 5°30'E and latitude 6°00'N - 6°30'N (Figure 1) between the River Osse and Siluko to the East and West respectively and about 40km from Benin city, the Edo State Capital. Okomu National Park has seventy-nine compartments out of which five compartments (45, 52, 53, 61, 64) have been observed by Park rangers to be foraged by elephants. The Park holds a remnant of the Nigerian lowland forests that once formed a continuous 50 - 100 km wide belt from the Niger River west to the Dahomey Gap in Benin. To the South and Southeast the forest was separated from the coast by mangrove and swamp forests, while to the north it merged into the Guinean Forest-Savanna Mosaic eco-region (Okomu National Park, 2010).

Data Collection

Five out of the seventy-nine compartments foraged by the elephants were randomly sampled. These compartments are compartments 45, 52, 53, 61, 64. In each compartment, two quadrats measuring 20m x 20m in dimension were established and the diameter at breast height of tree within the quadrats was recorded. The

Global Position System location of each quadrat was recorded during plot sampling in Table 1

Table 1: GPS Location of the Sampled Compartments

SITE	COMPARTMENT	QUADRATS	LATITUDE	LONGITUDE
			(N)	(E)
A	45	Plot 1	06° 22.307	005° 20.897
		Plot 2	06° 22.315	005° 20.848
B	52	Plot 1	06° 22.615	005° 21.609
		Plot 2	06° 21.165	005° 19.567
C	53	Plot 1	06° 21.408	005° 19.822
		Plot 2	06° 21.199	005° 20.347
D	61	Plot 1	06° 20.179	005° 18.943
		Plot 2	06° 20.164	005° 18.990
E	64	Plot 1	06° 20.495	005° 18.107
		Plot 2	06° 20.522	005° 18.110

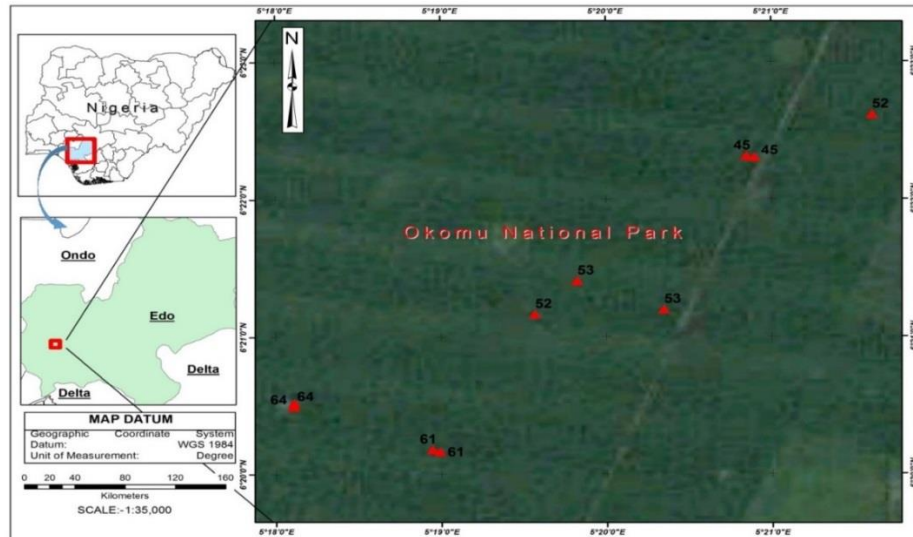


Figure 1: Map showing sample points in the study area in the Okomu National Park

Data Analysis

Data of the trees species in all quadrats were analyzed to determine the values of density, dominance and frequency (Sevda and Emire, 2018). The relative values of density, dominance and frequency values were determined for each species (Akinsoji, 2013). They were used to calculate the Importance Values of each species.

$$\text{Density} = \frac{\text{Number of individuals}}{\text{Area sampled}}$$

$$\text{Relative Density} = \frac{\text{Density for a species}}{\text{Total density for all species}} \times 100$$

$$\text{Dominance} = \frac{\text{Total of basal area or areal coverage values}}{\text{Area sampled}}$$

$$\text{Relative Dominance} = \frac{\text{Dominance for a species}}{\text{Total dominance for all species}} \times 100$$

$$\text{Frequency} = \frac{\text{Number of plots in which species occurs}}{\text{Total number of plots sampled}} \times 100$$

$$\text{Relative Frequency} = \frac{\text{Frequency value for a species}}{\text{Total of frequency values for all species}} \times 100$$

$$\text{Importance Value} = \text{Relative density} + \text{Relative dominance} + \text{Relative Frequency}$$

Collections of Plant Materials

The plant parts of *Desplatsia subericarpa* (fruit), *Dioscorea praehensilis* (tuber), *Thaumatococcus daniellii* (root), *Anthonotha macrophylla* and *Trichilia monadelph*a (bark) were collected from study area. These plants were collected from their natural habitat identified by rangers in the Park.

Proximate Analysis

Proximate analysis of fruits, herbs and tree barks played a crucial role in assessing their nutritional significance. Proximate analyses of five selected species of plant parts fed upon by elephants and collected from the study site were carried out. They were subjected to a series of relatively simple chemical tests so as to determine the percentage content of moisture, crude protein, crude fiber, ash, fat and digestible carbohydrate.

Sample treatment

The leaves were ground into fine powder using pestle and mortar, and sieved through 20mm-mesh sieve. The dried powdered sample was used for the analysis while for the moisture content however, fresh leaves were used.

Ash content was determined by incineration of 10g sample in a furnace at 600°C for 8hours and was ashed to constant weight. Each ash sample was dissolved in 10cm³ 6M nitric acid in 100cm³ volumetric flask and made up to mark with water. The sample solution was kept in plastic labeled containers for analysis. The recommended methods of the Association of Official Analytical Chemist (AOAC, 1990) were used for the determination of moisture, ash, crude lipid, crude fiber and nitrogen contents.

For **moisture content**, fresh leaves of the sample in triplicate were weighed in Petri dishes and dried in an oven at 105°C for 24 hours. The percentage loss in weight was expressed as percentage moisture content. A similar determination was carried out on dried sample so as to evaluate the residual moisture content. The nitrogen content of the powdered leaves was used as an index of the protein termed ‘crude protein’ as distinct from true protein. The crude protein (CP) content was calculated from the nitrogen determined by Kjeldahl method (Pearson, 1999).

To extract **crude protein**, all the nitrogen present in the sample was converted to ammonia by digesting the sample with sulphuric acid, (H₂SO₄). Sodium hydroxide, (NaOH) was then added to the digest to liberate the ammonia, which was distilled off, and collected in standard acid. The quantity so collected was determined by titration. It was assumed that the nitrogen was derived from protein containing the percent nitrogen, and multiplying the nitrogen figure by 6.25; an approximate crude protein value was obtained.

Fat content is followed by continuous extraction of known weight of the powdered sample with petroleum ether (40 – 60°C) in a soxhlet extractor. The residue after evaporation of the solvent was then weighed and the lipid content determined.

Digestible carbohydrate content was determined by what is known as ‘estimation by difference’, where the sum of the moisture, ash, protein and lipid contents were subtracted from one hundred (100).

The **crude fiber** was determined by subjecting the residual powdered sample from ether extraction to successive treatment with boiling using 0.25M H₂SO₄ acid and 0.312M NaOH, alkaline. The insoluble residue was washed with hot water until it is base free. It was then dried to constant weight in an oven at 105°C, cooled in desiccators and weighed. The weighed samples were incinerated in a furnace at 550°C for 2hrs until constant weight was obtained. The crude fiber was calculated as the loss in weight on ashing. The organic residue was the crude fiber.

Results

A total of forty-seven (47) plant species belonging to 30 families were encountered in the study compartments (Table 2). The Family Meliaceae is represented by 5 species (Figure 3); Annonaceae and Apocynaceae with 4 species each; Fabaceae and Rubiaceae have 3 species each; Cannabaceae, Myristicaceae and Sterculiaceae are represented by 2 species each while Bombacaceae, Burseraceae, Caesalpiniaceae, Capparaceae, Combretaceae, Ebenaceae, Euphorbiaceae, Irvingiaceae, Lecythidaceae, Loganiaceae, Malvaceae, Moraceae, Ochnaceae, Olacaceae, Passifloraceae, Putranjivaceae, Rhizophoraceae, Rutaceae, Sapotaceae, Tiliaceae, Urticaceae and Violaceae have 1 species. The species with highest number of trees occurrence are *Strombosia pustulata* represented by twenty-four (24) tree plants while trees species, *Monodora myristica*, *Canarium schweinfurthii*, *Hylodendron gabunense*, *Cylicodiscus gabunensis*, *Irvingia wombolu*, *Petersianthus macrocarpus*, *Strychnos innocua*, *Khaya ivorensis*, *Milicia excelsa*, *Pycnanthus angolensis*, *Massularia acuminata* and *Chrysophyllum albidum* were represented by one species each (Table 2).

Table 2: List of Okomu National Park elephant-foraged Species and their individual number with respect to Family

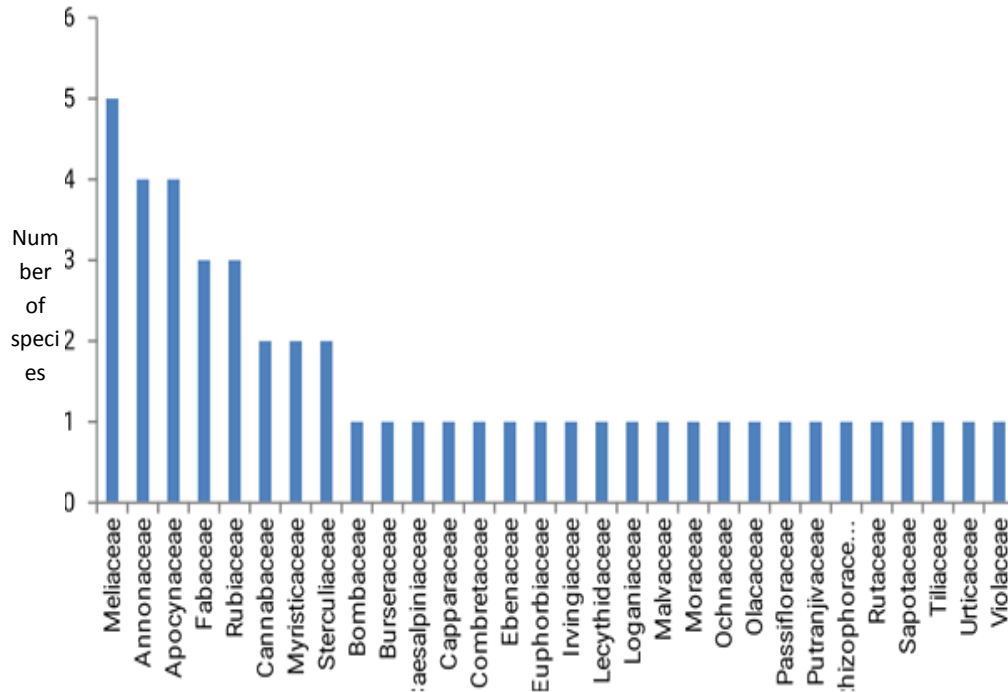
S/N	Families	Tree species	Number of trees	IUCN Red List status
1	Annonaceae	<i>Enantia chlorantha</i> (Oliver) van Setten & Maas.	4	NE
		<i>Annonidium mannii</i> Engl. & Diels	4	NE
		<i>Cleistopholis patens</i> (Benth.) Engl. & Diels	8	NE
		<i>Monodora myristica</i> (Gaertn.) Dunal	1	NE
2	Apocynaceae	<i>Alstonia boonei</i> De Wild.	3	NE
		<i>Funtumia elastica</i> (Preuss) Stapf	12	NE
		<i>Rauvolfia vomitoria</i> Afzel.	2	NE
		<i>Voacanga africana</i> Stapf	5	NE
3	Bombacaceae	<i>Ceiba pentandra</i> (Linn.) Gaertn.	12	LC
4	Burseraceae	<i>Canarium schweinfurthii</i> Engl.	1	NE
5	Caesalpiniaceae	<i>Hylodendron gabunense</i> Taub.	1	NE
6	Cannabaceae	<i>Celtis biondii</i> Pamp.	17	NE
		<i>Celtis zenkeri</i> Engl.	4	LC
7	Capparaceae	<i>Buchholzia coriacea</i> Engler	14	NE
8	Combretaceae	<i>Terminalia superba</i> Engl. & Diels	3	NE

9	Ebenaceae	<i>Diospyros gabunensis</i> L.	11	NE
10	Euphorbiaceae	<i>Ricinodendron heudelotii</i> (Baill.) Pierre ex Pax	2	VU
11	Fabaceae	<i>Anthonothea macrophylla</i> P. Beauv. Fl. Oware1	23	NE
		<i>Cylicodiscus gabunensis</i> Harms	1	NE
		<i>Piptadeniastrum africanum</i> (Hook. f.) Brenan	3	NE
12	Irvingiaceae	<i>Irvingia wombolu</i> Vermoesen	1	NE
13	Lecythidaceae	<i>Petersianthus macrocarpus</i> (P Beauv.) Liben	1	LC
14	Loganiaceae	<i>Strychnos innocua</i> subsp. <i>innocua</i> var. <i>innocua</i>	1	NE
15	Malvaceae	<i>Cola smithii</i> Schott & Endl.	11	NE
16	Meliaceae	<i>Lovoa trichilioides</i> Harms	12	LC
		<i>Entandrophragma cylindricum</i> (Sprague)	3	VU
		<i>Guarea cedrata</i> De Wild. C.A Chev.) pellegr.	5	VU
		<i>Khaya ivorensis</i> A. Chev.	1	VU
		<i>Trichilia monadelpha</i> (Thonning) de Wildeman	5	LC
17	Moraceae	<i>Milicia excelsa</i> (Welw.) C.C. Berg	1	NT
18	Myristicaceae	<i>Pycnanthus angolensis</i> (Welw.) Warb.	1	NE
		<i>Staudtia stipitata</i> Warburg	3	NE
19	Ochnaceae	<i>Lophira alata</i> Banks ex Gaertn. F	4	VU
20	Olacaceae	<i>Strombosia pustulata</i> Oliv. var. <i>lucida</i>	24	LC
21	Passifloraceae	<i>Barteria fistulosa</i> (Mast.) Sleumer	3	NE
22	Putranjivaceae	<i>Drypetes gossweileri</i> S.Moore.	2	NE
23	Rhizophoraceae	<i>Anopyxis klaineana</i> (Pierre) Engl.	2	VU
24	Rubiaceae	<i>Canthium glabriflorum</i> Lam	2	VU
		<i>Massularia acuminata</i> (G.Don) Bullock ex Hoyle	1	LC
		<i>Rothmannia hispida</i> (K.Schum.) Fagerl.	23	NE
25	Rutaceae	<i>Zanthoxylum zanthoxyloides</i> (Lam.) Zepern. & Timler	3	NE
26	Sapotaceae	<i>Chrysophyllum albidum</i> G. Don.	1	NE

27	Sterculiaceae	<i>Sterculia oblonga</i> MAST. and S.	10	VU
		<i>Sterculia tragacantha</i> Lindl.	5	LC
28	Tiliaceae	<i>Desplatsia subericarpa</i> Bocq.	3	NE
29	Urticaceae	<i>Myrianthus arboreus</i> P. Beauv	6	NE
30	Violaceae	<i>Rinorea dentata</i> (P. Beauv.) O. Ktze.	10	NE

KEY: NE: Not Evaluated, NT: Near Threatened, LC: Least Concern, VU: Vulnerable.

Figure 2: Family Distribution of Species encountered in Sampled Compartments



Plants fed upon by elephants are listed in Table 3. It consists of tree barks, leaves, roots, tubers, fresh young fronds and fruits. Most of the plants and part eaten were commonly the leaves and stems which formed the bulks of the elephant diet. In the area studied, Elephants fed on thirty- six percent (36%) of the 47 plants species which consist of various life forms. Elephant fed on the tree species like *Anthonotha macrophylla*, *Rothmannia hispida*, *Myrianthus arboreus*, *Ceiba pentandra*, *Trichilia monadelpha*, *Canarium schweinfurthii*, *Elaeis guineensis* and *Macaranga barteri*. Besides the elephants also fed on various shrubs, herbs and vine of *Laccosperma secundiflora*, *Aframomum melegueta*, *Thaumatococcus daniellii*, and *Dioscorea praehensilis* as their food resources. Fruits of *Desplatsia subericarpa*, *Anonidium*

mannii, *Irvingia wombolu*, *Myrianthus arboreus*, *Strychnos innocua* and *Sarcocephalus diderrichi* were consumed by the elephants. They often uprooted the plant with the help of their trunk and sometimes forefoot. The seed of *Myrianthus arboreus* was observed in the dung piles of elephants collected in the study area. The composition of different plant growth forms, family and species name eaten by elephant in study area was revealed and recorded (Table 3). This was obvious both from the examination of elephant dung which consist of 100% woody plants debris and from the collection of plants utilized by elephants. The percentage frequencies of the five plant parts consumed is as follow fruit eating (40%), bark peeling (27%), leaf eating (14%), root uprooting (14%) and tuber uprooting (5%) (Figure 3). The elephants preferred to feed on soft part of plant mostly the fruits and leaves of the plant. The bark of woody plant is often ripped off, since it is rich in calcium, which is essential for the favorable growth of their bone and for the tusks in males.

Table 3: List of foraged plants their family, life form and plant parts eaten by the elephants of Okomu National Park

S/N	Species	Family	Local name (Benin)	Life form	Parts eaten	IUCN Red List Status
1	<i>Aframomum melegueta</i>	Zingiberaceae	Oseghe	H	r, f	NE
2	<i>Annonidium mannii</i>	Annonaceae	Oghede-egbo	T	f	NE
3	<i>Anthonotha macrophylla</i>	Fabaceae	Oghaba	T	b, f	NE
4	<i>Canarium schweinfurthii</i>	Burseraceae	Ekpakpogho	T	b, f	NE
5	<i>Ceiba pentandra</i>	Bombacaceae	kapok	T	b	LC
6	<i>Desplatsia subericarpa</i>	Tiliaceae	Ighiwogha	T	f	NE
7	<i>Dioscorea praehensilis</i>	Dioscoreaceae	Ogigba	H	t	NE
8	<i>Elaeis guineensis</i>	Arecaceae	Ivi-oromila	T	f y f	LC
9	<i>Irvingia wombolu</i>	Irvingiaceae	Ogwi/Ogui.	T	f	NE
10	<i>Laccosperma secundiflora</i>	Arecaceae	Ikan, okakan	V	l	LC
11	<i>Macaranga barteri</i>	Euphorbiaceae	Ohaha	T	r	NE
12	<i>Myrianthus arboreus</i>	Urticaceae	ayon udin	T	f	NE
13	<i>Sarcocephalus</i>	Euphorbiaceae	Obhiakhe	T	b, f	VU

	<i>diderrichii</i>					
14	<i>Rothmannia</i>	Rubiaceae	Asun-	T	b	NE
	<i>hispida</i>		leghere			
15	<i>Strychnos</i>	Loganiaceae	UN	S	f	NE
	<i>innocua</i>					
16	<i>Thaumatococcus</i>	Marantaceae	Ebe-eba	H	l, r	NE
	<i>daniellii</i>					
17	<i>Trichilia</i>	Meliaceae	Ovallo	T	b	LC
	<i>monadelpha</i>					

KEY (T – tree, S – shrub, H – herb, V – vine, l – leaves, b – bark, r – root, f – fruits, fyf –fresh young frond, t- tuber, UN- Unknown., NE: Not evaluated, LC: Least concern, VU: Vulnerable.)

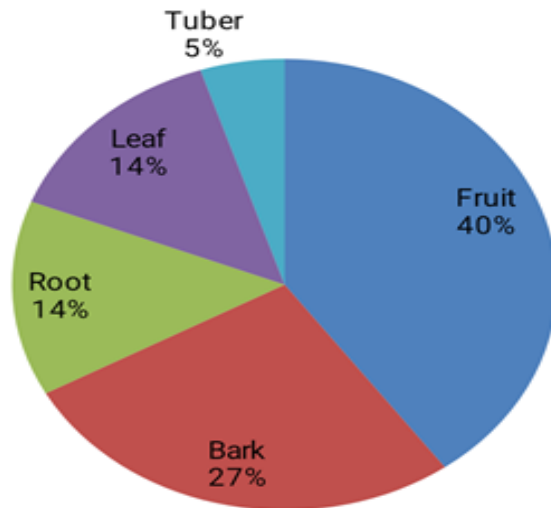


Figure 3: Relative frequencies of plant parts foraged by elephants in Okomu National Park.

The plant density of species in area studied within Okomu National Park ranged from 0.00025m² - 0.006m² with the species of *Strombosia pustulata* having the highest density while species of *Canarium schweinfurthii*, *Chrysophyllum albidum*, *Cylicodiscus gabunensis*, *Hylodendron gabunense*, *Irvingia wombolu*, *Khaya ivorensis*, *Massularia acuminata*, *Milicia excelsa*, *Monodora myristica*, *Petersianthus macrocarpus*, *Pycnanthus angolensis* and *Strychnos innoca* were recorded with the lowest density using the quadrat method. *Strombosia pustulata* has the highest Frequency (90%) followed by *Celtis biondii* (80%) and *Cola smithii* (60%). 50% Frequency was recorded for five species namely *Anthonotha macrophylla*, *Diospyros gabunensis*, *Funtumia elastica*, *Lovoa trichilioides*, *Rothmannia hispida* species. However, the species with the lowest Frequency were

Drypetes gossweileri, *Canarium schweinfurthii*, *Chrysophyllum albidum*, *Cylicodiscus gabunensis*, *Hylocodendron gabunense*, *Irvingia wombolu*, *Khaya ivorensis*, *Massularia acuminata*, *Milicia excelsa*, *Monodora myristica*, *Petersianthus macrocarpus*, *Pycnanthus angolensis* and *Strychnos innoca*. The plant dominance in the area varied from 1.32cm² - 8886.03cm² with *Ceiba pentandra* having the highest dominance species however, the lowest dominance species was recorded for *Chrysophyllum albidum*. The maximum Relative Frequency (RF= 7.14%) and the highest Relative Density (RD= 8.73%) were documented for *Strombosia pustulata*. The species with highest Importance Value rank is *Ceiba pentandra* (SIV=50.4608) while the species with the lowest Importance Value rank is *Chrysophyllum albidum* (SIV=1.1639) (Table 4).

Proximate analysis of five different selected plant part fed upon by elephant collected from the area studied are subjected to a series of relatively simple chemical tests known as nutritional analysis. These species with their part mostly consume by elephants include *Anthonotha macrophylla* (bark), *Trichilia monadelphpha* (bark), *Thaumatococcus daniellii* (root), *Dioscorea praehensilis* (tuber) and *Desplatsia subericarpa* (fruit). All the samples were analyzed for moisture, crude protein, crude fiber, crude fat and total ash content. Highest amount of moisture (89.42%) was observed in *Thaumatococcus daniellii* whereas *Dioscorea prehensilis* (38.11%) showed lowest amount. In case of crude protein high amount (11.42%) was in *Anthonotha macrophylla*, low amount (0.98%) in *Thaumatococcus daniellii*. High amount (10.09%) of crude fibre was observed in *Dioscorea praehensilis* whereas *Anthonotha macrophylla* showed low (4.28%) fiber content. *Desplatsia subericarpa* showed high amount (3.98%) of crude fat and *Dioscorea praehensilis* showed low amount (0.98%). The total ash content of *Dioscorea* was high (8.47%), while *Anthonotha macrophylla* showed low amount (3.76%). The crude carbohydrate was high (41.28%) in *Dioscorea praehensilis*, however, *Thaumatococcus daniellii* showed low amount (1.92%) (Table 5).

Discussion

International Union for Conservation Nature (2007) presented the result in the study of conservation of Asian elephant in Bangladesh where the elephants foraged about 12% of the total local plant species. Although there were about 143 plant species recorded in the Chunati Wild Life Sanctuary in the report but about 17 species being utilized by the Asian elephants. This is in agreement with 17 species eaten by the African elephant in Okomu National Park. The nutrition of the elephant in the West African forest zone consists almost entirely of woody plant part and fruit (Short, 1981) which is similar to plant sampled in the five compartments in Okomu National Park where the larger percentage of plants that elephants feed on were woody species which is about 71 percent and the rest plant parts shared the remaining percentage. Joshi and Singh (2008) specifically stated that the elephants fed on 74% tree, 14% grass, 8% shrub and 4% of climber out of two hundred and sixty two

(262) flora plant species and this resonate with observations from this study. Elephants have evolved to be generally herbivores consuming a wide variety of plants of over a hundred species (Sukumar, 1989, Samansiri and Weerakoon, 2007). The compartments where the elephant foraged in Okomu National Park has lower number of plants compared to the 57 plant species reported by Raj *et al.* (2016), but the families are diverse in Okomu National Park. In a similar report Sukumar (1985) listed 33 plants species fed upon by the elephant, belonging to Malvaceae, Leguminosae, Palmae and Bamboo. There were 17 families foraged by African elephant in this study, this is far diverse than the 4 families reported by Sukumar (1985). The proximate analysis of fruits, herbs and tree barks plays a crucial role in assessing their nutritional significance. The study revealed that plant parts fed upon by elephants consists of tree barks, shrubs, fruits and vines. These observations are in agreement with the findings on food and feeding behaviour of Asiatic elephant (*Elephas Maximus* Linn.) in Kuldiha Wild Life Sanctuary, Odisha, in India by Mohapatra *et al.* (2013). The nutritional analysis result showed that most plants fed upon by elephants contained high amount of moisture and carbohydrate respectively. Borah and Deka, (2008) pointed out that highest percentage of carbohydrate found in *Anthrocephalus* sp. (62.33%) and high content of moisture and digestible carbohydrate could be supplementary sources of moisture to the elephant's diet. In like manner, supplementary moisture content resulted to highest percentage moisture content in the root of *Thaumatococcus daniellii* while the highest percentage of carbohydrate was observed in the tuber of *Dioscorea praehensilis*. The location of Okomu national park might be a consequence of high moisture content in the root which is between River Osse and Siluko. In addition, the study was carried out during raining season. The availability of these plant species in the compartments provides a suitable habitat for elephants in Okomu National Park. The distribution of preferred plants close to the lake shore influences the elephant's preference to use most of their time utilizing the habitats. While elephant signs such as elephant trails, damaged trees, and areas in forest are conspicuous, elephant themselves are at low density and their overall impact on the forest is little. Among the seventeen identified plant species seed of *Myrianthus arboreus*

Table 4: Phytosociological features of the sampled quadrats in Okomu National Park

S/N	Tree species	No of individual species	No of plots in which species occurred	Total number of plots sampled	Frequency %	Density m ²	Dominance Cm ²	Relative frequency %	Relative density %	Relative dominance %	Importance Value %
1	<i>Ceiba pentandra</i>	12	2	10	0.2	0.003	8886.029	1.587302	4.363636	44.5098	50.4608
2	<i>Terminalia superb</i>	3	2	10	0.2	0.00075	4216.714	1.587302	1.090909	21.1214	23.7996
3	<i>Strombosia pustulata</i>	24	9	10	0.9	0.006	46.156	7.142857	8.727273	0.2312	16.1013
4	<i>Celtis biondii</i>	17	8	10	0.8	0.00425	25.978	6.349206	6.181818	0.1301	12.6611
5	<i>Anthanathia macrophylla</i>	23	5	10	0.5	0.00575	58.855	3.968254	8.363636	0.2948	12.6267
6	<i>Rothmannia hispida</i>	23	5	10	0.5	0.00575	23.853	3.968254	8.363636	0.1195	12.4514
7	<i>Cola smithii</i>	11	6	10	0.6	0.00275	516.348	4.761905	4	2.5864	11.3483
8	<i>Euntunia elastic</i>	12	5	10	0.5	0.003	102.266	3.968254	4.363636	0.5122	8.8441
9	<i>Lecoa trichilioides</i>	12	5	10	0.5	0.003	77.677	3.968254	4.363636	0.3891	8.7210
10	<i>Diospyros gahumensis</i>	11	5	10	0.5	0.00275	33.066	3.968254	4	0.1656	8.1339
11	<i>Cleistopholis patens</i>	8	3	10	0.3	0.002	429.443	2.380952	2.909091	2.1511	7.4411
12	<i>Rinorea dentate</i>	10	4	10	0.4	0.0025	33.123	3.174603	3.636364	0.1659	6.9769
13	<i>Buchholzia coriacea</i>	14	2	10	0.2	0.0035	15.239	1.587302	5.090909	0.0763	6.7545
14	<i>Khaya ivorensis</i>	1	1	10	0.1	0.00025	1109.327	0.793651	0.363636	5.5566	6.7139
15	<i>Pistadeniastrum africanum</i>	3	3	10	0.3	0.00075	445.861	2.380952	1.090909	2.2333	5.7052
16	<i>Alstonia boonei</i>	3	3	10	0.3	0.00075	443.143	2.380952	1.090909	2.2197	5.6915
17	<i>Celtis zenkeri</i>	4	2	10	0.2	0.001	499.363	1.587302	1.454545	2.5013	5.5431
18	<i>Sterculia tragacantha</i>	5	4	10	0.4	0.00125	46.27	3.174603	1.818182	0.2318	5.2245
19	<i>Myrianthus arboreus</i>	6	3	10	0.3	0.0015	100.354	2.380952	2.181818	0.5027	5.0654
20	<i>Sterculia oblonga</i>	10	1	10	0.1	0.0025	104.414	0.793651	3.636364	0.5230	4.9530
21	<i>Bicnodendron heudelotii</i>	2	2	10	0.2	0.0005	475.006	1.587302	0.727273	2.3793	4.6939
22	<i>Trichilia monadelphica</i>	5	3	10	0.3	0.00125	62.572	2.380952	1.818182	0.3134	4.5126
23	<i>Lophira alata</i>	4	2	10	0.2	0.001	273.839	1.587302	1.454545	1.3717	4.4135
24	<i>Guarsa cedrata</i>	5	3	10	0.3	0.00125	20.015	2.380952	1.818182	0.1003	4.2994
25	<i>Voacanga Africana</i>	5	3	10	0.3	0.00125	16.17	2.380952	1.818182	0.0810	4.2801
26	<i>Desplatia subericarpa</i>	3	3	10	0.3	0.00075	137.028	2.380952	1.090909	0.6864	4.1582
27	<i>Ammonidium mannii</i>	4	3	10	0.3	0.001	25.783	2.380952	1.454545	0.1291	3.9646
28	<i>Staudtia stipitata</i>	3	2	10	0.2	0.00075	177.054	1.587302	1.090909	0.8869	3.5651
29	<i>Barteria fistulosa</i>	3	3	10	0.3	0.00075	10.462	2.380952	1.090909	0.0524	3.5243
30	<i>Enantia chlorantha</i>	4	2	10	0.2	0.001	30.605	1.587302	1.454545	0.1533	3.1951
31	<i>Zanthoxylum zanthoxylodes</i>	3	2	10	0.2	0.00075	82.069	1.587302	1.090909	0.4111	3.0893
32	<i>Casarium schweinfurthii</i>	1	1	10	0.1	0.00025	379.145	0.793651	0.363636	1.8991	3.0564
33	<i>Canthium glabriflorum</i>	2	2	10	0.2	0.0005	117.959	1.587302	0.727273	0.5909	2.9054
34	<i>Anopyxis klaineana</i>	2	2	10	0.2	0.0005	117.714	1.587302	0.727273	0.5896	2.9042
35	<i>Entandrophragma cylindricum</i>	3	2	10	0.2	0.00075	21.816	1.587302	1.090909	0.1093	2.7875

36	<i>Irvingia wombolu</i>	1	1	10	0.1	0.00025	277.331	0.793651	0.363636	1.3891	2.5464
37	<i>Rauvolfia vomitoria</i>	2	2	10	0.2	0.0005	7.207	1.587302	0.727273	0.0361	2.3507
38	<i>Pycnanthus angolensis</i>	1	1	10	0.1	0.00025	183.637	0.793651	0.363636	0.9198	2.0771
39	<i>Pterisanthus macrocarpus</i>	1	1	10	0.1	0.00025	111.983	0.793651	0.363636	0.5609	1.7182
40	<i>Drypetes gossweileri</i>	2	1	10	0.1	0.0005	6.119	0.793651	0.727273	0.0306	1.5516
41	<i>Strychnos imoca</i>	1	1	10	0.1	0.00025	76.52	0.793651	0.363636	0.3833	1.5406
42	<i>Millettia excels</i>	1	1	10	0.1	0.00025	60.277	0.793651	0.363636	0.3019	1.4592
43	<i>Cylicodiscus gabunensis</i>	1	1	10	0.1	0.00025	28.751	0.793651	0.363636	0.1440	1.3013
44	<i>Monodora myristica</i>	1	1	10	0.1	0.00025	27.251	0.793651	0.363636	0.1365	1.2938
45	<i>Massularia acuminata</i>	1	1	10	0.1	0.00025	13.463	0.793651	0.363636	0.0674	1.2247
46	<i>Hylocladon gabunense</i>	1	1	10	0.1	0.00025	9.622	0.793651	0.363636	0.0482	1.2055
47	<i>Chrysophyllum albidum</i>	1	1	10	0.1	0.00025	1.32	0.793651	0.363636	0.0066	1.1639

Table 5: Proximate composition of parts of selected plant species foraged by elephant in Okomu National Park

S/N	Name of Species	Plant Part	Carbohydrate %	Protein %	Fibre %	Lipid %	Moisture %	Ash %
1	<i>Anthonotha macrophylla</i>	Bark	31.43	11.42	4.28	1.79	47.26	3.76
2	<i>Trichilia monadelpha</i>	Bark	30.57	1.08	9.42	1.34	49.16	8.41
3	<i>Thaumatococcus daniellii</i>	Root	1.92	0.98	7.44	1.39	89.42	7.85
4	<i>Dioscorea praehensilis</i>	Tuber	41.28	1.04	10.09	0.98	38.11	8.47
5	<i>Desplatsia subericarpa</i>	Fruit	11.85	2.98	7.04	3.98	67.81	6.32

was observed in the dung piles of elephants which was also listed by Short (1981) in addition to two other seeds types namely: *Strychnos aculeatu* and *Klainedoxa gabonensis*. Elephant's droppings contain high percentage of woody material and seeds hence transport considerable dropping material from one place to another serving as an agent of seed dispersal thus contributing to the complexity and diversity of the Park. Elephant feeding behaviour is very important in its effects on the maintenance and functioning of the Okomu National Park. **Elephant browsing and movement opens dense vegetation, creating suitable establishment sites for dispersed seeds (Yumoto et al., 1995). As a result, seed dispersal by elephants favors seedling survival and growth (Nchanji and Plumptre, 2003; Jothish 2013; Spanbauer and Adler, 2015) and promotes forest species diversity (Campos-Arceiz and Blake, 2011).** The study revealed that the total amount of plant matter mouthed by the elephants was not fully consumed (this may be regarded as a wasteful mode of feeding). A relatively large part usually dropped to the ground sometimes utilized by other herbivores thus representing associational behavior between animal inhabitants of the park. Perhaps, the non-eaten vegetation could form a secondary food source to other herbivores in such cases thus; it is not a waste after all. An abundance of alternative food items such as regenerating trees is

perhaps the reason for the low incidence of pushing over. Elephant consumption can alter the vertical structure of the woody plant community, commonly manifested as reduced tree density and increased shrub density (Guy, 1989) and continuous browsing may result in more accessibility in the classes plants size (Jachmann and Bell, 1985; Mapaire and Mhlanga, 2000). The consequences of elephants' presence in the ecosystem such as Okomu National Park may therefore have implications, both positive and negative, for biodiversity. For example, Anderson and Coe's (1974) report on elephant dung demonstrates its use as food for baboons and birds but mainly for both adult and larval beetles (and thus also as an oviposition site), with an estimated 16000 beetle individuals removing 1.5kg of dung in two hour, elephant dominance has conservation implication because of their impact on other species, especially when occurring at relatively high densities (Cumming *et al.*, 1997; Western and Maitumo, 2004; Wiseman, *et al.*, 2004). Under such conditions, their foraging and feeding habits may reduce tree densities and transform forests and intact woodlands into mixed woodlands and even grassland (Dublin *et al.*, 1990; Ben-Shahar, 1993; Barnes *et al.*, 1994; Leuthold, 1996; Trollope *et al.*, 1998; Van De Vijver *et al.*, 1999; Eckhardt *et al.*, 2000; Mosugelo *et al.*, 2002). Elephants have thus been implicated in increased impacts on herbaceous and woody vegetation (Van Wyk and Fairall, 1969; Ben-Shahar, 1993), and declines in plant species composition, density and diversity (Conybeare, 1991) in areas close to water such as lake area. However few species may not be able to satisfy the nutrient requirement hence dependence of the many species. The study will enhance management decision that will ensure sustainable management of the Okomu National Park.

In conclusion, this study revealed the browsing activities of Africa elephant at Okomu National Park and how it's altered the structure and species composition of the foraged vegetation. It is therefore, pertinent to collect and conserve at the gene bank for posterity to prevent the imminent loss of biodiversity at the park.

References

- Agboola, O. O, Akinsoji, A. and Oyedele, S. (2017). Changes in Nutrient contents of soil different land uses in a Forest Reserve. *Notulae Scientia Biologicae*, **9** (3): 414 - 421.
- Akinsoji, A. (2013). Quantitative Analysis of a Forest Fragment in Olokemeji Forest Reserved, Nigeria. *Journal of Natural Sciences Research*, **13** (3): 111- 114.
- Amusa, T. O., Omonu, C., Olabode, E. and Newton, N. J. (2017). Population status and distribution of forest elephants (*Loxodonta Cyclotis*) (Matschie, 1900) in okomu national park and omo forest reserve, South-western Nigeria. *Journal of Research in Forestry, Wildlife & Environment*, **9** (2): 44 – 56.

- Anderson, M. J. and M.J. Coe (1974). Decomposition of elephant dung in an arid, tropical environment. *Oecologia* 14 (1-2):111-125
- AOAC, (1990). Official Methods of analysis, 14th edition, Association of official analytical chemists, Washington DC.
- Azeez, I. O., Ikponmwonba, O. S., Popoola, L. and Amusa, T. O. (2010). Land use activities among forest environments' dwellers in Edo State, Nigeria: Implications for livelihood and sustainable forest management. *International Journal of Social Forestry* 3(2):164-187.
- Barnes, R.F.W. (1982) Elephant feeding behavior in Ruaha National Park, Tanzania. *Afr. J. Ecol.* 20, 123-136.
- Barnes, R. F. W., Barnes K. L. and Kapela. E. B. (1994). The long term impact of elephant browsing on baobab trees at Msembe, Ruaha National Park, Tanzania. *African Journal of Ecology*, 32: 177-184.
- Ben-Shahar, R. (1993). Patterns of elephant damage to vegetation in northern Botswana. *Biological Conservation*, 65: 249-256.
- Borah, J. and Deka, K. (2008). Nutritional Evaluation of forage preferred by wild elephants in the Rani Range Forest, Assam, India. *Gajaha*, 28: 41-43.
- Campos-Arceiz, A. and Blake, S. (2011). Megagardeners of the forest - the role of elephants in seed dispersal. 37:542–553. Elsevier Masson SAS.
- Conybeare, A. M. (1991). Elephant occupancy and vegetation change in relation to artificial water points in a Kalahari sand area of Hwange National Park. DPhil Thesis, University of Zimbabwe, Harare.
- Cumming, D.H.M., Fenton, M.B., Rautenbach, I.L., Taylor, R.D., Cumming, G.S., Cumming, M.S., Dunlop, J.M., Ford, G.A., Hovorka, M.D., Johnston, D.S., Kalcounis, M., Mahlangu, Z. and Portfors, C.V.R. (1997). Elephants, woodlands and biodiversity in southern Africa. *South African Journal of Science*, 93: 231– 236.
- Croze (2008). The Seonera bull problem. The elephant. *African Journal of Ecology* 12 (1):1-27
- Curtis, J. T. and McIntosh, R. P. (1950). The Interrelations of Certain Analytic and Synthetic Phyto-Sociological Characters. *Ecology*, 31: 434-455.
- Davies, G. (2008). Spirit of the elephant. Parragon, Queen Street house, 4 Queen Street Bath BAI, IHE, UK, 72-126.

- Dublin, H. T., Sinclair, A. R. E. and Mcglade J. (1990). Elephants and fire as causes of multiple stable states in the Serengeti–Mara woodlands. *Journal of Animal Ecology*, 59:1147–1164.
- Eckhardt, H. C., Van Wilgen, B. W. and Biggs, H. C. (2000). Trends in woody vegetation cover in the Kruger National Park, South Africa, between 1940 and 1998. *African Journal of Ecology*, 38: 108–115.
- Guy, P. R. (1989). The Influence of Elephants and Fire on a *Brachystegia-Julbernardia* Woodland in Zimbabwe. *Journal of Tropical Ecology*. 5(2): 215-22
- Herremans, M. (1995). Effects of woodland modification by African elephant *Loxodonta africana* on bird diversity in northern Botswana. *Ecography*, 18(4):440–45
- Herrera, Carlos M. and Pellmyr, O. (Eds.). (2002). Plant-animal interactions: An evolutionary approach. Oxford, UK: Wiley-Blackwell.
- IUCN, (2007). Asian elephants (Phase-I), Action research for conservation of Asian elephants in Bangladesh: Phase–I Ministry of Environment and forest. Government of the People’s Republic of Bangladesh.
- Jachmann, H. and Bell, R.H.V. (1985). Utilization by elephants of the *Brachystegia* woodlands of the Kasungu National park, Malawi. *African Journal of Ecology*, 23: 245-258.
- Jackson, T. and Erasmus D. G. (2005). *Assessment of seasonal home-range use by elephants across southern Africa’s seven elephant clusters*. Conservation Ecology Research Unit, University of Pretoria, South Africa.
- Joshi, R. and Singh, R. (2008). Feeding behaviour of wild Asian Elephants (*Elephas maximus*) in the Rajaji National Park. *The Journal of American Science*. 4(2): 34-48
- Jothish P. S. (2013). Frugivory and seed dispersal of woody species by the Asian elephant (*Elephas maximus*) in a mid-elevation tropical evergreen forest in India. *Journal of Tropical Ecology*, 29:181–185. Cambridge University Press.
- Kerley, G.I. H. and Landman, M. (2006). The impacts of elephants on biodiversity in the Eastern Cape Subtropical Thickets. *South African Journal of Science*. 102(9):395–402.

- Leuthold, W., (1996).Vegetation changes in queen Elizabeth National Park, Uganda. *African Journal of Ecology*. **31**:106–117.
- Mapaure, I. and Lindah Mhlanga, L. (2000). Patterns of elephant damage to *Colophospermum mopane* on selected islands in Lake Kariba, Zimbabwe . *Kirkia* 17:89-198
- Mohapatra K. K, Patra A. K and Paramanik D. S, (2013). Food and feeding behaviour of Asiatic elephant (*Elephas maximus* Linn.) in Kuldina Wildlife Sanctuary, Odisha, India. *Journal of Environmental Biology*, **34**:87-92.
- Mosugelo, D. K., Moe, S. R. Ringrose, S. and Nelleman, C. (2002). Vegetation changes during a 36-year period in northern Chobe National Park, Botswana. *African Journal of Ecology* 40:232–240
- Nchanji, A. C. and Plumptre, A. J. (2003). Seed germination and early seedling establishment of some elephant-dispersed species in Banyang-Mbo Wildlife Sanctuary, South-Western Cameroon. **19**: 229-237
- O’Connor,T.G., Goodman, P.S. and Clegg, B.A (2007). Functional hypothesis of the threat of local extirpation of woody plant species by elephant in Africa. *Biological Conservation*.136(3):329–345.
- Okomu National Park, (2010). Nigeria National Park Service. Retrieved 4 November 2010.
- Raj, K. K., Weihong, J.I., Achyut, A., Rothman, J. and Raubenheimer, D (2016). Dispersal and ranging patterns of the Asian Elephant (*Elephas maximus*) in relation to their interactions with humans in Nepal. *Ethology ecology and evolution* 28(2):221-231
- Samansiri, A. K. P. and Weerakoon, K. D. (2007). Feeding behavior of Asian elephants in the northwestern region of Srilanka Gajah, **27**: 27-34
- Short, J. (1981). Diet and feeding behaviour of the forest elephant. *Mammalia*, **45**(2): 177- 185.
- Smallie, J. J. and O’Connor, T. G. (2000). Elephant utilization of *Colophospermum mopane*: possible benefits of hedging. *African Journal of Ecology*. 38(4):352–359.
- Spanbauer, B. R. and Adler, G. H.(2015). Seed protection through dispersal by African savannah elephants (*Loxodonta africana africana*) in northern Tanzania. *African Journal of Ecology*. **53**(4) 389-618

- Stephenson, P. J. (2007). WWF Species Action Plan: African elephants, 2007-2011, WWF, Gland, Switzerland.
- Sukumar, R (1985). Ecology of the Asian Elephant (*Elephas maximus*) and its interaction with Man in South India. Unpublished Ph.D. Thesis, Indian institute of Science, Bangalore.
- Sukumar, R, (1989). The Asian elephant: ecology and management. Cambridge University press, Cambridge.
- Trollope, W. S. W., Trollope, L. A. Biggs, H. C., Pienaar, D. and Potgieter, A. L. F. (1998). Long-term changes in the woody vegetation of the Kruger National Park, with special reference to the effects of elephants and fire. *Koedoe*, **41**: 103- 112.
- Van de Vijver, C. A.D.M., van de Vijver, Foley, C. A. and Olff, H. (1999). Changes in the woody component of an East African savanna during 25 years. *Journal of Tropical Ecology*. **15**:545-564.
- Van Wyk, P. and Fairrall. N. (1969). The influence of the African elephant on the vegetation of the Kruger National Park. *Koedoe*, **12**: 57-89
- Western, D. and Maitumo, D. (2004). Woodland loss and restoration in a savanna park: a 20-year experiment. *African Journal of Ecology*, **42**: 111-121.
- Wiseman, R., Page, B. R. and O'Connor, T. G. (2004). Woody vegetation change in response to browsing in Ithala Game Reserve, South Africa. *South African Journal of Wildlife Research*, **34**: 25-37.
- Yumoto, T., Maruhashi, T., Yamagiwa, J. and Mwanza, N. (1995). Seed-dispersal by elephants in a tropical rain forest in Kahuzi-Biega National Park, Zaire. *Biotropica*: 526 –530.

Synthesis, characterization and catalytic activity study of Mn(II) metal complexes of Schiff base ligands derived from amino acids and vanillin

Samson O. Owalude*, Adedibu C. Tella, Olubunmi S. Oguntoye, Olatayo T. Adekeye, Uche B. Eke and Ezekiel O. Odebunmi

Department of Chemistry, University of Ilorin, P. M. B. 1515, Ilorin, Nigeria

Corresponding author's E-mail: owalude@unilorin.edu.ng

Abstract

Mn(II) complexes were prepared from Schiff base ligands obtained by condensation reaction of three amino acids (alanine, glycine and methionine) and *o*-vanillin. The ligands and their metal complexes were characterized structurally by means of analytical and spectroscopic techniques. The FT-IR spectral data indicates that the binding of the ligands to the metal involved oxygen atom of the OH group of the amino acid moiety and the azomethine nitrogen of the Schiff base ligand. The observed low conductivity values for the complexes indicate that they are non-electrolytes. The three complexes showed moderate activity in the catalytic conversion of phenol into industrially useful products, catechol or hydroquinone. The reaction in the presence of the manganese(II) complexes gave a total phenol conversion of 67.7% compared to 51.5% for the uncatalysed reaction.

Keywords: Manganese(II), Schiff base, amino acids, vanillin, phenol

Introduction

Schiff bases are prepared through the condensation reaction of a primary amine with an active carbonyl group. They are characterized by the presence of the azomethine group (-RC=N-) in their structure. Schiff bases continue to attract the attention of synthetic chemists over the years because they are easy to prepare and readily form complexes with most metals. Ettinger has discovered several metal Schiff base complexes in the mid nineteenth century, even before the report of their general preparation protocol by Schiff and co-workers (Hobday and Smith, 1973). Interesting new results in the field of coordination chemistry now exist based on the research into physicochemical properties and molecular structure of Schiff base metal complexes. Schiff bases form complexes with p- and d-block metals readily and these complexes have been reported as efficient catalysts in syntheses and general transformation of organic functional groups (Canali and Sherrington, 1999). Series of Schiff base complexes of ruthenium and other transition metals have been

reported as active catalyst in this regard (Prakash and Viswanathamurthi, 2014; Turan and Buldurun, 2018; Dalia *et al.*, 2018).

A review on unique asymmetric catalytic activities of metal complexes of salen type and related Schiff base ligands have been documented (Katsuki, 2004). Effective oxidation of olefins using Mn(II) amino acid Schiff base complexes has also been reported (Wang *et al.*, 1999). Other important reactions such as oxidation, hydrogenation, polymerization, various coupling reactions, ring opening and closures catalyzed by Schiff base complexes have been reported (Gupta and Sutar, 2008; Gupta *et al.*, 2009; Che and Huang, 2003). For better selectivity and recyclability, heterogenization of homogenous catalysts has become a subject of attraction to synthetic chemists in recent times (Yang *et al.*, 2010; Rayati and Abdolalian, 2013). This has resulted into an exponential increase in the number of publications in catalysis by supported Schiff base complexes. However, homogenous catalysis still remain relevant since the mechanism of such processes can be obtained more easily.

In the present study, we report the synthesis and characterization of Mn(II) complexes of new Schiff base ligands {sodium (E)-2-((4-hydroxy-3-methoxybenzylidene)amino)-4-(methylthio) butanoate (**HL**₁), sodium (Z)-2-((4-hydroxy-3-methoxybenzylidene)amino)propanoate acid (**HL**₂) and sodium (E)-2-((4-hydroxy-3-methoxybenzylidene)amino)acetate (**HL**₃)} derived from amino acids and *o*-vanillin. Catalytic potential of these complexes in the hydroxylation of phenol were investigated and reported.

Materials and methods

Materials

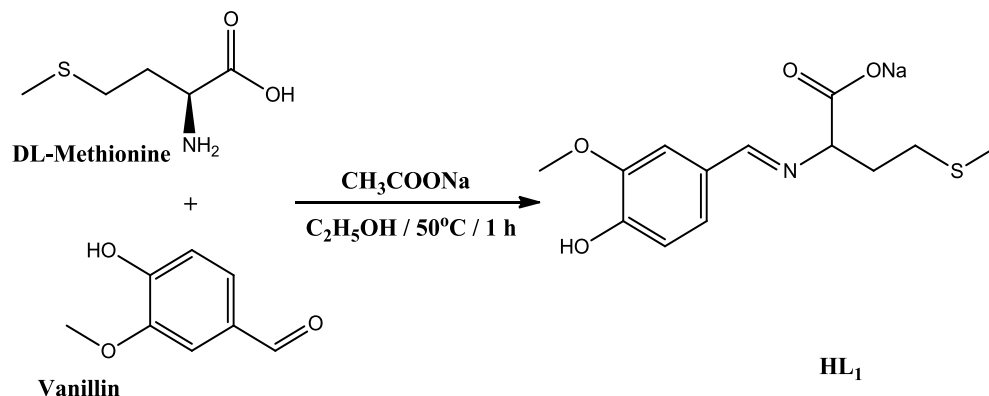
The starting materials, manganese(II) chloride, vanillin, methionine, glycine and alanine were all AR grade and commercially sourced. The solvents were freshly distilled before use.

Analysis and physical measurements

Melting points were obtained on a Kofler microhotstage apparatus and were adequately calibrated. Infra-red spectra were recorded on a SCHIMADZU Scientific FTIR 8400s spectrophotometer in the form of KBr pellets. The elemental analyses were determined on Perkin-Elmer 240C series elemental analyzer (USA) and the GCMS analyses were carried out on Agilent 5975 Series MSD.

General Procedures for the Synthesis of the Schiff base ligands

The Schiff base ligands were synthesized according to a procedure described in the literature (Al-Shaheen and Al-Mula, 2014). The synthesis involves a condensation reaction between vanillin and the amino acids glycine, alanine and methionine respectively. This procedure is outlined in Scheme 1 for **HL**₁.

Scheme 1: Synthesis of Schiff base derived from vanillin and DL-methionine (**HL₁**)

sodium (E)-2-((4-hydroxy-3-methoxybenzylidene)amino)-4-(methylthio)butanoate (HL₁**):** A solution of the amino acid DL-methionine (0.746 g, 5 mmol) in 10 ml (25% distilled water + 75% ethanol) was added to a solution of vanillin (0.76 g, 5 mmol) in 10 ml absolute ethanol in the presence of sodium acetate (0.410 g, 5 mmol) under magnetic stirring. The mixture was slightly acidified with 0.1M dilute hydrochloric acid and heated at 50°C on a water bath for an hour and then allowed to cool at room temperature. The resulting solution was then evaporated to about half its volume and left overnight for complete precipitation. The precipitates were collected by filtration, washed with 1:1 ethanol-water mixture and diethylether and dried over anhydrous CaCl₂. The same procedure was applied for the synthesis of **HL₂** and **HL₃** by using alanine and glycine respectively. White powder; Yield 2.36 g, 61 %; m.p. (°C) 288-292; IR (cm⁻¹, KBr): 3500 ν(O-H), 1658 ν(C=N), 1581 ν_{asy}(COO⁻), 1413 ν_{sym}(COO⁻), 1276 ν(C-O). Conductivity (Ω⁻¹cm²mol⁻¹) 19; Anal. Calc. (%) for C₁₃H₁₆NNaO₄S C, 51.14; H, 5.28; N, 4.59 Found: C, 50.98, H, 5.14, N, 4.65.

sodium (Z)-2-((4-hydroxy-3-methoxybenzylidene)amino)propanoate (HL₂**):** The procedure was the same as for ligand **HL₁** using L-alanine (0.375 g, 5 mmol) in place of methionine. White powder; Yield 1.86 g, 54 %; m.p. (°C) 176-179; IR (cm⁻¹, KBr): 3427 ν(OH), 1624 ν(C=N), 1583 ν_{asy}(COO⁻), 1410 ν_{sym}(COO⁻) 1236 ν(C-O). Conductivity (Ω⁻¹cm²mol⁻¹) 15; Anal. Calc. (%) for C₁₁H₁₂NNaO₄ C, 53.86; H, 4.36; N, 5.71. Found: C, 53.55; H, 4.95; N, 5.86.

sodium (E)-2-((4-hydroxy-3-methoxybenzylidene)amino)acetate (HL₃**):** The procedure was the same as for ligand **HL₁** using L-glycine (0.375 g, 5 mmol) in place of methionine. White powder; Yield 1.44 g, 53 %; m.p. (°C) 267-273; IR (cm⁻¹, KBr): 3497 ν(O-H), 1627 ν(C=N), 1597 ν_{asy}(COO⁻), 1437 ν_{sym}(COO⁻), 1155 ν(C-O). Conductivity (Ω⁻¹cm²mol⁻¹) 12; Anal. Calc. (%) for C₁₀H₁₀NNaO₄ C, 51.95; H, 4.36; N, 6.06. Found: C, 51.01; H, 4.21; N, 6.15.

Synthesis of the Mn(II) Complexes

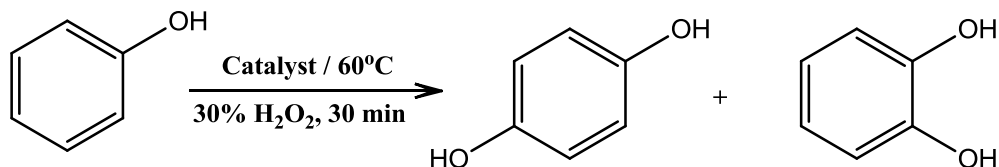
Complex 1: DL-methionine (0.746 g, 5 mmol) was dissolved in 10 ml solvent (25% distilled water + 75% ethanol) and mixed with vanillin (0.76 g, 5 mmol) in 10 ml ethanol in the presence of sodium acetate (0.410 g, 5 mmol). The mixture was refluxed at 50°C for an hour followed by the addition of manganese(II) chloride (0.630 g, 5 mmol) in 10 ml hot ethanol, then aqueous solution of sodium acetate (0.820 g, 1 mmol) was slowly added. The mixture was further refluxed for 1 hour at 50°C under magnetic stirring and then allowed to cool at room temperature. The resulting solution was further left for five days to complete the precipitation. The precipitate was collected through filtration, washed with ether and dried over anhydrous CaCl_2 . Yellow powder; Yield 1.32 g, 32 %; m.p. (°C) 219-224; IR (cm^{-1} , KBr): 3383 $\nu(\text{O-H})$, 1622 $\nu(\text{C=N})$, 1581 $\nu_{\text{asy}}(\text{COO})$, 1413 $\nu_{\text{sym}}(\text{COO})$, 1276 $\nu(\text{C-O})$, 553 $\nu(\text{Mn-O})$, 439 $\nu(\text{Mn-N})$. Conductivity ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$) 17; Anal. Calc. (%) for $\text{C}_{26}\text{H}_{36}\text{Cl}_2\text{MnN}_2\text{O}_{10}\text{S}_2$ C, 42.98; H, 4.99; N, 3.86. Found: C, 43.55; H, 5.27; N, 1.53.

Complex 2: The procedure was the same as for **1** using L-alanine in place of DL-methionine. Brown powder; Yield 1.26 g, 32 %; m.p. (°C) Decomp > 310; IR (cm^{-1} , KBr): 3389 $\nu(\text{O-H})$, 1666 $\nu(\text{C=N})$, 1464 $\nu_{\text{asy}}(\text{COO}^-)$, 1315 $\nu_{\text{sym}}(\text{COO}^-)$, 1240 $\nu(\text{C-O})$, 599 $\nu(\text{Mn-O})$, 495 $\nu(\text{Mn-N})$. Conductivity ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$) 18; Anal. Calc. (%) for $\text{C}_{22}\text{H}_{28}\text{ClMnN}_2\text{O}_{10}$ C, 46.29; H, 4.94; N, 4.91. Found: C, 46.01; H, 3.95; N, 2.22.

Complex 3: The procedure was the same as for **1** using L-glycine in place of DL-methionine. Brown crystals; Yield: 1.72 g, 36 %; m.p. (°C) 248-252; IR (cm^{-1} , KBr): 3417 $\nu(\text{O-H})$, 1627 $\nu(\text{C=N})$, 1597 $\nu_{\text{asy}}(\text{COO})$, 1437 $\nu_{\text{sym}}(\text{COO})$, 1240 $\nu(\text{C-O})$, 509 $\nu(\text{Mn-O})$, 472 $\nu(\text{Mn-N})$. Conductivity ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$) 13; Anal. Calc. (%) for $\text{C}_{20}\text{H}_{24}\text{Cl}_2\text{MnN}_2\text{O}_{10}$ C, 41.54; H, 4.18; N, 4.84. Found: C, 39.97; H, 5.01; N, 5.22.

Catalytic hydroxylation of phenol

The hydroxylation reaction was carried out in a 50 mL two necked round bottom flask fitted with a water cooled condenser using acetone as solvent. The equation for the reaction is as represented in scheme 2. For the control reaction in which the catalyst was absent, phenol (1 g, 10 mmol) was dissolved in 10 ml acetone and heated to reflux under continuous stirring for 30 minutes after which 2 ml of aqueous solution of 30% H_2O_2 was added. The reflux was continued for further 3 hours under regulated temperature of 343 K. The products were analyzed using a gas chromatography-coupled with mass spectroscopy (GC-MS). The same procedure was followed for the catalyzed hydroxylation reactions using the three manganese(II) complexes as catalyst. Phenol (1 g, 10 mmol) was dissolved in 10 ml acetone and 50 mg of the desired manganese(II) complex was added. The solution was heated under reflux for 30 minutes, 2 ml of an aqueous solution of 30% H_2O_2 was then added and the reflux was continued for further 3 hours at 343 K. After the reaction the catalyst was separated through filtration and the products were analyzed using GC-MS.

Scheme 2: Catalytic hydroxylation of phenol with H_2O_2

Results and discussion

The results of the C, H and N analysis are in good agreement with the proposed stoichiometry for both the ligands and their corresponding complexes. The only exceptions are the non-closeness of the theoretical to the experimental values for the nitrogen in complex **1** and **2**. This does not necessarily imply an off-base synthesized compounds, there were every indication that new compounds were formed based on the results from other analysis such as, melting point determination, infrared spectroscopic analysis and solubility studies. The exact orientation of their constituent elements were unable to be fully ascertained at the present level of the work. These results however indicate a successful synthesis of the ligands and their corresponding manganese complexes. The three manganese complexes were stable under the atmospheric conditions and soluble in DMSO and most other organic solvents. The observed low conductivity values for the complexes indicate that they are all non-electrolytes in solution ([Ejidike, 2018](#)).

FT-IR spectra analysis

The important infrared spectral data of ligand and their manganese complexes are summarized in the experimental section. There are two basic groups with potent capability to coordinate with the manganese metal ions in the ligands, the azomethine $\text{C}=\text{N}$ and the OH of the carboxylate groups of the amino acids. The band attributable to $\text{C}=\text{N}$ between 1658 cm^{-1} and 1624 cm^{-1} in the spectra of the ligands is slightly shifted to lower frequency at 1622 cm^{-1} in **1** and to higher frequencies at 1666 cm^{-1} and 1627 cm^{-1} in **2** and **3** respectively, an indication that coordination of the Schiff bases occurs through azomethine nitrogen atom (Joseph and Janaki, 2014). The infrared spectra of the ligands contain broad band at $3500 - 3427\text{ cm}^{-1}$ assigned to the stretching frequency of phenolic OH of the vanillin moiety and a second band at $1392 - 1354\text{ cm}^{-1}$ due to bending phenolic OH group (Olajire *et al.*, 2017). These two bands are still present in the spectra of the complexes indicating non-coordination of this group. Stretching vibration of $\text{O}-\text{CH}_3$ of vanillin was observed as band of medium intensity at 1080 , 1016 and 1043 cm^{-1} respectively in **HL**₁, **HL**₂ and **HL**₃ (Balachandran and Parimala, 2012). These bands appeared in the three complexes with no significant changes in the wavenumber values, an indication that the $\text{O}-\text{CH}_3$ of vanillin did not coordinate to the manganese metal ions. Not too strong band observed in the spectrum of the free ligands at $1153-1236\text{ cm}^{-1}$ attributable to the amino acids carboxylate $\text{C}-\text{O}$ stretching were shifted to

higher wavenumbers in the complexes (Al-Shaheen and Al-Mula, 2014). This is an indication that the amino acid Schiff bases coordinated through the COO^- with the manganese metal ions. In the spectra of the ligands, intense bands at $1597 - 1581 \text{ cm}^{-1}$ and $1437 - 1410$ were assigned to asymmetric and symmetric stretching frequencies of COOH group of the amino acid moiety in the ligands respectively. The symmetric bands were not significantly shifted in the complexes while asymmetric bands were shifted to lower frequencies. The difference between the symmetric and asymmetric stretching vibration of COO^- groups ($\Delta\nu$) were in the range between $149 - 168 \text{ cm}^{-1}$. These values indicate that the ligands coordinated through the COO^- group of the amino acid moieties of the Schiff bases in monodentate fashion (Al-Shaheen and Al-Mula, 2014). The COOH groups are therefore deprotonated during coordination to the manganese metal ions. The complexes were characterized by weak to medium broad band at $3417 - 3383 \text{ cm}^{-1}$ attributable to stretching vibrations of the OH group of water molecules (Bala *et al.*, 2018). A sharp shoulder between 1508 and 1516 cm^{-1} were assigned to bending vibration of water, while evidence for existence of coordinated water molecules were obtained by the occurrence of additional sharp bands at $813 - 815 \text{ cm}^{-1}$ in the spectra of the complexes (Vashi and Shelat, 2010). In the spectra of all the complexes, new bands at $439 - 495$ and $509 - 599 \text{ cm}^{-1}$ due to the stretching modes of Mn-N and Mn-O respectively were observed (Bala *et al.*, 2018). The existence of these bands further support the formation of the complexes as represented in Fig. 1-3.

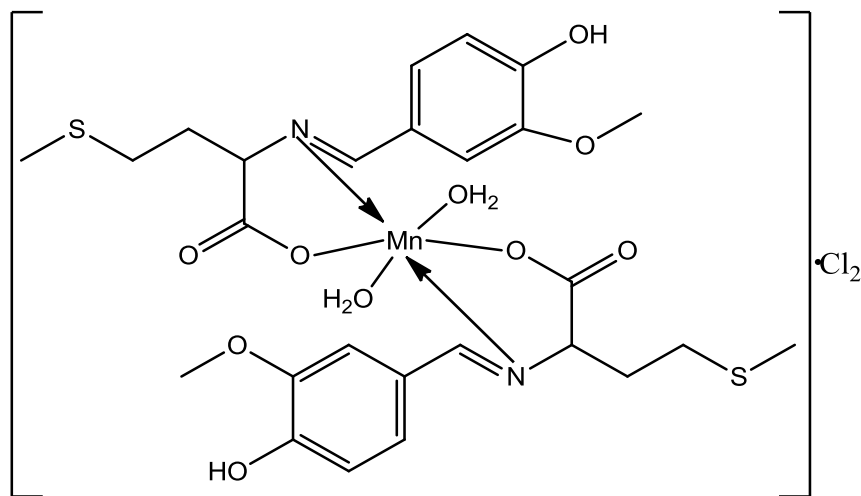


Fig. 1: Proposed Structure for Complex 1

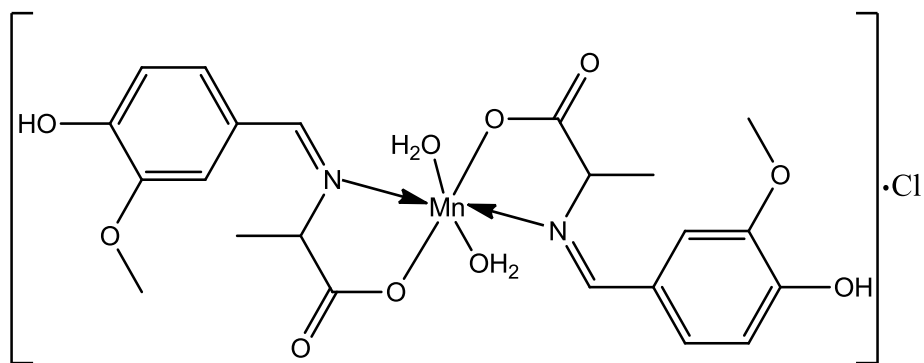


Fig. 2: Proposed Structure for Complex 2

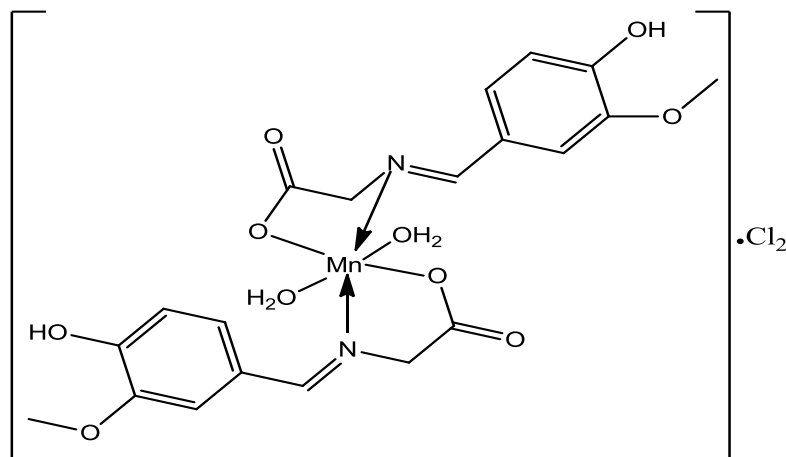


Fig. 3: Proposed Structure for Complex 3

Catalytic hydroxylation of phenol

The hydroxylation reactions were carried out with 50 mg of each manganese complex for 3 h at 60°C and the overall phenol conversions after this period are recorded in Table 1. The results showed that hydroquinone and catechol are the main products of the reaction. Control experiments without the catalyst indicates a 55.50% phenol conversion with 44% and 7.6% selective for catechol and hydroquinone. There was appreciable increase in percentage phenol conversion with the introduction of the manganese complexes and the highest conversion of 67.65% was obtained with the alanine complex 2. The least effective is complex 1 though with percentage conversion greater than that of complex 3 but with a poorer selectivity (Table 1). In terms of selectivity therefore, the best performance was obtained with complex 3 that gave 55.7% conversion and selectivity of 55.57% for hydroquinone and 41.79% selective for catechol.

Table 1: Summary of activity of Mn(II) catalysts in the hydroxylation of phenol

Catalyst	X_{phenol} (%)	Selectivity (%)			
		CAT	HYD	BZQ	%H ₂ O ₂ _{eff}
Blank	51.5	44.04	7.46	0.00	25.73
1	57.88	20.86	37.02	0.00	28.93
2	67.65	36.33	31.32	0.00	33.80
3	55.70	41.79	55.57	0.00	29.85

X_{phenol} (%) = percentage of converted phenol, CAT = catechol, HYD = hydroquinone, BZQ = benzoquinone, %H₂O₂_{eff} = efficiency of H₂O₂

Conclusion

The overall results show a successful preparation of manganese(II) metal complexes of some selected amino acids (alanine, glycine and methionine). The three complexes are effective catalyst in the hydroxylation of phenol to catechol and hydroquinone, however the efficiency is not so pronounced as the average phenol conversion with catalyst relative to the uncatalyzed reaction is rather low. Work is ongoing on the structural modifications of these complexes for better performance and their catalytic applications in other reactions.

References

- Al-Shaheen J.A., Al-Mula A.M. (2014): Schiff base complexes of Fe(III) derived from amino acids. *Res. J. Chem. Sci.* 4(8): 25-32.
- Bala M., Kumar S., Devi R., Taxak V.B., Boora P., Khatkar S.P. (2018): Synthesis and photoluminescence properties of europium(III) complexes sensitized with β -diketonato and N, N-donors ancillary ligands. *Spectrochim. Acta A.* 196: 67-75.
- Balachandran V., Parimala K. (2012): Vanillin and isovanillin: Comparative vibrational spectroscopic studies, conformational stability and NLO properties by density functional theory calculations. *Spectrochim. Acta, A* 95: 354-368.
- Canali L., Sherrington D.C. (1999): Utilization of homogeneous and supported chiral metal (salen) complexes in asymmetric catalysis. *Chem. Soc. Rev.* 28: 85-93.

- Che C-M, Huang J-S. (2003): Metal complexes of chiral binaphthyl Schiff base ligands and their application in stereoselective organic transformations. *Coord. Chem. Rev.*, 242(1-2): 97-113.
- Dalia SA, Afsan F, Hossain S, Khan N, Zakaria CM, Zahan K-E, Ali M. (2018): A short review on chemistry of Schiff base metal complexes and their catalytic application, *Inter. J. Chem. Studies*. 6(3): 2859-2866.
- Ejidike PI. (2018): Cu(II) complexes of 4-[(1*E*)-*N*-{2-[(*Z*)-benzylidene-amino]ethyl}ethanimidoyl]benzene-1,3-diol Schiff base: Synthesis, spectroscopic, In-Vitro antioxidant, antifungal and antibacterial studies. *Molecules*, 23(7): 1581-1599.
- Gupta K.C., Sutar A.K. (2008): Catalytic activities of Schiff base transition metal complexes. *Coord. Chem. Rev.* 252: 1420-1450.
- Gupta K.C., Sutar A.K., Lin C.C. (2009): Catalytic activity of polymer anchored *N,N'*-bis(*o*-hydroxy acetophenone) ethylene diamine Schiff base complexes of Fe(III), Cu(II) and Zn(II) ions in oxidation of phenol. *Coord. Chem. Rev.* 253: 1926-1946.
- Hobday M.D., Smith T.D. (1973): *N,N'*-ethylenebis (salicylideneiminato) transition metal ion chelates. *Coord. Chem. Rev.* 9(3-4): 311-337.
- Joseph J, Janaki G.B. (2014): Synthesis, structural characterization and biological studies of copper complexes with 2-aminobenzothiazole derivatives. *J. Mater. Environ. Sci.* 5(3): 693-704.
- Katsuki T. (2004): Unique asymmetric catalysis of cis-beta metal complexes of salen and its related Schiff-base ligands. *Chem. Soc. Rev.* 33: 437-444.
- Olajire, A.A., Kareem A. Olaleke A. (2017): Green synthesis of bimetallic Pt@Cu nanostructures for catalytic oxidative desulfurization of model oil. *J. Nanostruct. Chem.*, 7(2) 159-170.
- Prakash G, Viswanathamurthi P. (2014): New ruthenium(II) carbonyl complexes bearing disulfide Schiff base ligands and their applications as catalyst for some organic transformations. *Spectrochim. Acta A.* 129: 352-358.
- Rayati S, Abdolalian P. (2013): Heterogenization of a molybdenum Schiff base complex as a magnetic nanocatalyst: An eco-friendly, efficient, selective and recyclable nanocatalyst for the oxidation of alkenes. *Comptes Rendus Chimie.* 16(9): 814-820.

- Turan N, Buldurun K. (2018): Synthesis, characterization and antioxidant activity of Schiff base and its metal complexes with Fe(II), Mn(II), Zn(II), and Ru(II) ions: Catalytic activity of ruthenium(II) complex. *Eur. J. Chem.* 9(1): 22-29.
- Vashi R.T., Shelat C.D. (2010): Transition metal complexation studies on heterocyclic ligands, *Asian J. Chem.* 22(3): 1745-1750.
- Wang R-M., Hao C-J., Wang Y-P., Li S-B. (1999): Amino acid Schiff base complex for effective oxidation of olefins with molecular oxygen. *J. Mol. Catal. A: Chem.* 147: 173-178.
- Yang Y., Zhang Y., Hao S., Guan J., Ding H., Shang F., Qiu P., bin Kan Q. (2010): Heterogenization of functionalized Cu(II) and VO(IV) Schiff base complexes by direct immobilization onto amino-modified SBA-15: Styrene oxidation catalysts with enhanced reactivity. *Appl. Catal. A: General* 381(1-2): 274-281.

Plasma DPP-4 Levels in Prediabetics Selected from Three Communities in Ilorin, Nigeria

Olarinoye, J. K¹, Biliaminu, A. S², AbdulAzeez, I. M², Olarinoye, A. O³, Olagbaye, B. A⁴, Sanya E. O¹, Olatunji L. A⁵, Soladoye A. O⁵.

¹*Department of Medicine, University of Ilorin, Ilorin, Nigeria*

²*Department of Chemical Pathology and Immunology, University of Ilorin, Ilorin, Nigeria*

³*Department of Obstetrics and Gynecology, University of Ilorin, Ilorin, Nigeria*

⁴*Department of Anatomy, University of Ilorin, Ilorin, Nigeria.*

⁵*Department of Physiology, University of Ilorin, Ilorin, Nigeria*

Corresponding Email: kolaolarinoye@yahoo.com

Abstract

Early detection and prompt treatment of people with prediabetes is an important step towards the control and prevention of diabetes. The objective of this study was to determine plasma DPP-4 levels in prediabetics and compare with those with normoglycemic subjects. In this cross-sectional study, 118 apparently healthy adult Nigerians were randomly recruited from Ayetoro, Tanke and Ganmo communities in Ilorin. They were divided into 3 glycemic categories (normoglycemic, prediabetic and diabetic) following 75g-OGTT (Oral glucose tolerance test). Plasma DPP-4 levels were measured by ELISA and compared in all groups. Fasting plasma insulin, lipid profiles and anthropometric measurements were also compared. Categorical variables were compared using the chi-square statistics while t-test and ANOVA were used for continuous variables. Correlations between plasma DPP-4 and certain parameters were tested using Pearson's correlation coefficients. The result showed that 67 (58.8%) of the subjects had normal glucose tolerance, 28 (23.7%) had prediabetes while 24 (20.3%) had diabetes. Of the prediabetic subjects, 2 (1.7%) had impaired fasting glycemia (IFG), 19 (16.1%) had impaired glucose tolerance (IGT) while the remaining 7 (5.9%) had IFG/IGT. Mean plasma DPP-4 level was 0.5 ± 0.3 U/L in all subjects, 0.51 ± 0.33 in normoglycemic, 0.53 ± 0.55 in prediabetic and 0.41 ± 0.22 U/L in diabetic subjects. No significant difference in the mean plasma DPP-4 levels among the three glycemic groups. However, there is a positive association between prediabetes and dyslipidemia. These findings established that

plasma DPP-4 level is neither a sensitive diagnostic marker for prediabetes in Nigerians nor a plausible target for therapeutic intervention. Abnormal lipid profiles correlate better with prediabetes than plasma DPP-4 levels.

Keywords: Diagnostic marker, Prediabetes; Plasma DPP-4 levels, lipid profile.

Introduction

Prediabetes is an intermediate state of dysglycaemia with plasma glucose levels above normal but below the diabetes threshold in individuals with impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) (Bansal, 2015). It is a high risk state for future development of diabetes with about 20-34% of people with IFG or IGT evolving into Type 2 Diabetes within 4-5 years; while those with combined IGT and IFG have a cumulative diabetes incidence of 38-65% (Papaetis, 2014).

With the global prevalence of diabetes rising at an alarming rate (WHO, 2016) and its attendant disease burden and economic impact, prediabetic population provides a natural window of opportunity for primary prevention. Several trials have demonstrated that early recognition and prompt treatment of prediabetes will revert the subjects to normoglycemic and prevent progression to Type 2 diabetes, thus making it a useful tool in diabetes primary prevention (DPPRG, 2002).

Lifestyle modification is the cornerstone for primary prevention of diabetes reducing diabetes incidence by 58% among prediabetics population, but lifestyle intervention is usually inadequate to prevent diabetes in a large percentage of individuals. This is because sustaining a healthy lifestyle is often challenging thereby making pharmacologic intervention desirable most of the time (DPPRG, 2002).

Dipeptidyl peptidase-4 (DPP-4) is a trans-membrane glycoprotein and exo-protease cleaving N-terminal dipeptides from various substrates. This enzyme, equally described as a lymphocyte cell surface protein CD-26, exists also as a soluble circulating protein with proteolytic activity. The soluble form (sDPP-4) accounts for a significant power of DPP-4 activity in human serum (Chyan and Chuang, 2007; Yazbeck *et al.*, 2009). By far the most important proteolytic action of DPP-4 enzyme is its ability to also cleave and inactivate the incretin hormones like Glucagon-like peptide-1 (GLP-1) and Glucose-dependent insulintropic peptide (GIP). One of the most important properties of GLP-1 and GIP is their capacity to promote insulin secretion and biosynthesis in a glucose-dependent manner, thus maintaining post-prandial glucose homeostasis without causing hypoglycemia. Shortly after their secretion however, GIP and GLP-1 are rapidly deactivated by DPP-4 (Baggio and Drucker, 2007).

Several studies have shown that the incretin effect is impaired in patients with Type 2 Diabetes Mellitus (Nauck *et al.*, 1986). Furthermore, increased serum sDPP-4

levels and activity have been demonstrated in the same group of individuals but this finding is not consensual as other researchers reported decreased activity (Mannucci *et al.*, 2005; Godinho *et al.*, 2015). Since incretin hormones are rapidly degraded by DPP-4 enzyme, it is logical to presume that elevated DPP-4 levels may be contributory to the impaired incretin effect observed in patients with type 2 diabetes mellitus (T2DM). Studies assessing DPP-4 activity in T2DM patients have shown contradictory results. While some researchers demonstrated significantly higher plasma sDPP-4 levels in diabetic patients and prediabetic individuals compared with healthy control (Mannucci *et al.*, 2005; Ryskjaer *et al.*, 2006), others did not agree, showing that DPP-4 activities in patients with Type 1 DM, Prediabetes or early onset Type 2 DM were not significantly different from that in non-diabetic control subjects (Mannucci *et al.*, 2005; McKillop *et al.*, 2008). In addition to this controversy is the fact that there is virtually no documented data regarding the levels and activities of sDPP-4 in diabetic and non-diabetic Nigerians.

This study therefore aimed to assess the plasma levels of soluble DPP-4 in prediabetics selected from three communities in Ilorin, Nigeria and to compare them with non-diabetic control. It was also to evaluate the possible determinants of DPP-4 levels among apparently healthy Nigerians.

Methodology: - It was a cross-sectional comparative study that involved + apparently healthy consenting adult from Ayetoro, Tanke and Ganmo communities in Ilorin, Nigeria, who were neither diabetic nor taking any medications that could affect glucose tolerance. Recruitment was carried out in the three communities within Ilorin metropolis using convenient sampling method.

All consenting individuals were interviewed with a researcher-generated questionnaire to obtain the socio-demographic information and to identify presence of specific risk factors for glucose intolerance. Anthropometric measurements like weight, height, waist circumference and blood pressure were taken and recorded for each subject.

Oral Glucose Tolerance Test

The glycaemic status of the subjects were determined by subjecting each participant to a 75g Oral Glucose Tolerance Test (OGTT) for 2 hours which is a standard WHO protocol (WHO, 2006). The results were interpreted using the WHO (2006) diagnostic criteria (WHO, 2006), following which they were grouped into four definite categories of glycemic state:

- i. Normoglycemia
- ii. Impaired Fasting Glycemia (IFG)
- iii. Impaired Glucose Tolerance (IGT)
- iv. Diabetes Mellitus (DM)

Prediabetes was diagnosed in any individual with IFG, IGT or IFG/IGT.

Laboratory Procedure: - Six (6) mls of venous blood was taken from each subject (in the fasting state) for the estimation of plasma circulating DPP-4 level, plasma insulin level and plasma lipid level. Samples were sent to the University of Ilorin Chemical Pathology Research Laboratory where plasma sDPP-4 levels were estimated using the Human DPP-4 Elisa kit (Bioassay Technology Laboratory, Shanghai, China).

The Human DPP-4 was measured using Bioassay Technology Laboratory Elisa assay kit (Korain Biotech Co., Shanghai, China). The plate has been pre-coated with human DPP-4 antibody. DPP-4 present in the sample got bound to antibodies coated on the wells. And then biotinylated human DPP-4 Antibody was added and got bound to DPP-4 in the sample. Then streptavidin-HRP was added and got bound to the biotinylated DPP-4 antibody. After incubation unbound streptavidin-HRP was washed away during a washing step. Substrate solution was then added and pink colour developed in proportion to the amount of human DPP-4. The reaction was terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Plasma insulin levels were measured using the Human Insulin Assay Elisa kit (Calbiotech Inc, Chicago, USA). Laboratory procedures were carried out by two chemical pathologists from the University of Ilorin Teaching Hospital.

Statistical Analysis: - Continuous variables were recorded as (means $\pm$ S.D) while the categorical variables were presented as frequencies and proportions. Association between categorical variables was tested using the chi-square (χ^2) while the student-t test was used for the continuous variables. A *p* value of <0.05 was taken to be statistically significant.

Ethical Consideration: - Ethical approval was obtained from the Investigation Review Board of the Kwara State Ministry of Health, Ilorin.

Results

A total of 118 subjects were recruited for the study. Eighty-six (72.9%) were female while the remaining were male. Majority of the subjects, 103(87.3%) belonged to the Yoruba ethnic group. Their mean age was 50.6 ± 1.03 years. Twenty-two (18.6%) were below 40 years, 25(21.2%) were above 60 years, while the others were in between. Forty-two (35.6%) were civil servants, 36(30.5%) were traders, 22 (18.6%) were artisans, 8 (6.8%) were retired while the remaining 10(8.5%) were unemployed. Five subjects (4.2%) had no education at all, 28(23.7%) had primary education, 30 (25.4) had secondary and 55 (46.6%) had tertiary education.

Medical History: - Twenty-three (19.5%) respondents had positive family history of hypertension while 14 (11.9%) had positive family history of diabetes. Twenty-eight (23.7%) were on medications for high blood pressure. Only 20 (16.9%) subjects engaged in regular active exercise, ninety (76.3%) of them exercised just slightly while the remaining 8 people (6.8%) were essentially sedentary. Only 3 (2.5%) took alcoholic beverages and one person smoked cigarettes.

Anthropometric Parameters: - The anthropometric measurements of the subjects are shown in Table 1 below. Sixty subjects (50.8%) had normal weight, 32(27.1%) were overweight while the remaining 26 (22%) were obese. Forty-four (37.3%) had truncal obesity. The mean systolic blood pressure (SBP), diastolic blood pressure (DBP) and the mean arterial pressure (MAP) are displayed in Table 1. Fifty-eight people (49.2%) had normal BP, 37(31.4%) had mild hypertension, 15(12.7%) had moderate hypertension while 8 (6.6%) had severe hypertension.

Laboratory Values

The mean Fasting Plasma Glucose, 2hr post-glucose and lipids levels; the plasma levels of Insulin, DPP-4 and calculated HOMA-IR and HOMA- β are shown in Table 2.

Glycaemic Status

All study subjects were categorized into 3 glycaemic groups using 2hr-75g OGTT interpreted by the WHO (2006) diagnostic criteria (Figure 1). Sixty-seven (58.6%) had normal glucose tolerance; 28 (23.7%) had prediabetes while 24 (20.3%) had undiagnosed diabetes. Of the prediabetic subjects, 2 (1.7%) had Impaired Fasting Glycaemia (IFG), 19 (16.1%) had Impaired Glucose Tolerance (IGT) while the remaining 7 (5.9%) had IFG/IGT.

Table 3 shows the comparison of the clinical and laboratory values in the different glycaemic groups using ANOVA. HOMA- β is the only parameter that was significantly associated with development of prediabetes and diabetes. BMI had a positive but weak association with hyperglycaemia. Plasma sDPP-4 levels were not in any way associated with development of prediabetes or diabetes.

Serum s-DPP4 Levels: - The mean sDPP-4 levels of the entire study population were 0.50 ± 0.38 U/L while the value in normoglycaemic, prediabetic and diabetic groups were 0.51 ± 0.33 , 0.53 ± 0.55 and 0.41 ± 0.22 U/L. There were no significant differences between the glycaemic groups (Table 3). The fasting plasma insulin levels and calculated HOMA-IR were not significantly different ($p > 0.05$) in any of the glycaemic groups. However, mean HOMA- β steadily declined from normoglycaemic group (92.7 ± 128.6), through to the prediabetic group (51.5 ± 92.3) and then to the fully diabetic individuals (24.0 ± 31.7).

Table 4 shows the comparison of some clinical and laboratory parameters between prediabetic subjects and normoglycemic controls using the Student t-test. There was no significant association between serum DPP-4 levels and prediabetes. Plasma insulin levels, HOMA-IR are weakly but positively associated with prediabetes. Prediabetic subjects have significantly higher plasma levels of LDL and lower levels of HDL than their non-diabetic counterparts.

Correlates of Serum DPP-4: - Some clinical and laboratory parameters were correlated against serum DPP-4 levels using the Pearson's coefficient of correlation in Table 5. None of the parameters correlated significantly with serum DPP-4.

DISCUSSION: - The prevalence of prediabetes in this study was 23.7% according to WHO diagnostic criteria (WHO, 2019). This is well outside the global range of 4.8-11.9% but less than the US prevalence of 37% among the adults and 39% among the blacks (NDSR, 2017). Uganda has a prevalence of 20.2%, while Tanzania 13.8% (Chiwanga *et al.*, 2016).

In Nigeria, prevalence of prediabetes varies depending on the study location and the diagnostic criteria used. A Calabar-based study (Enang *et al.*, 2014) found IGT in 20% of their subjects while in Sokoto, its prevalence was 31.5% among the urban Fulanis (Sabir *et al.*, 2013). In Ekiti state however, the prevalence of prediabetes was just 6% (Shittu *et al.*, 2017). Another study from the Eastern part of the country, Enugu, estimated prevalence of prediabetes to be 21.5%, 9.2% for IFG and 15.8% for IGT (Nwatu *et al.*, 2015).

Predictors of Prediabetes

The only two parameters that showed significant association with prediabetes are the HDL and LDL. Prediabetic subjects have higher LDL values and lower HDL levels. Other parameters like the age, BMI and waist circumference showed positive but weak association with prediabetes. HOMA-B which is a measure of beta cell function is associated with diabetes incidence but not with prediabetes. The association between abnormal lipid profiles and prediabetes has been well documented in several studies. In India, total cholesterol, low density lipoprotein (LDL), triglyceride (TG), very low density lipoprotein, TG/HDL ratio and LDL/HDL ratio were significantly raised in prediabetic individuals as compared to normal healthy subjects, whereas high density lipoprotein (HDL) was significantly lower in prediabetic individuals as compared to normal healthy subjects (Kansal and Kamble, 2016). The predictors of prediabetes in an American study were hypertension, obesity and hypercholesterolaemia as well as presence of arthritis (Li *et al.*, 2009). In an earlier referenced Nigerian study (Nwatu *et al.*, 2015), hypertension was found to be the strongest predictor of prediabetes followed by obesity.

Plasma DPP-4 Level: - The mean plasma DPP-4 level in this study was 0.50 ± 0.38 . This value is low compared to the values in other documented series. However, it is within the reference range specified in the manual of the test kit used (0.15 – 2.4U/L). The mean plasma DPP4 activity among healthy Americans subjects was 25.9 ± 12.3 U/L while among the Asians it was 52.6 ± 27.8 ng/ml (Neidert *et al.*, 2016).

The difference may be due to difference in the assay technique and unit of measurement. But it may also be due to unique metabolic profile of the native Africans which have been known to be different from those of the Caucasians and even their counterparts in diaspora.

Plasma DPP-4 Levels and Glycaemic Status: - Table 4 shows that there is no consistency in the gradient of serum DPP4 level along the continuum of glycaemia. DPP-4 level is slightly higher in the prediabetic subjects than in normoglycaemic subjects (0.51 versus 0.53 U/L). It is however lowest in the diabetic subjects (0.41U/L). The differences were however not significant. These findings suggest that there was no association between DPP-4 activity and glycemic status in our study subjects. Similar observations were made by Mannucci *et al.*, 2005 who found that serum DPP-4 levels were not different in diabetic patients from non-diabetic subjects.

Diminished incretin effect in patients with diabetes has been described several years back (Nauck *et al.*, 1986), but its role in pathogenesis of the disease is still a subject of debate. A couple of studies concluded that abnormal incretin secretion and action are consequences of hyperglycaemia rather than being the primary cause of diabetes (Knopp, 2010). This has also been corroborated by the finding that insulin treatment of hyperglycaemia improves incretin effect in diabetic subjects (Meier *et al.*, 2001). In addition to this, DPP-4 activity has not been studied in native Africans and it is well noted that native Africans possess different glucose homeostasis from Africans in diaspora and Caucasians. Studies have shown that glucose-tolerant American blacks manifest significantly higher insulin concentrations and lower insulin sensitivity than native African blacks who reside in their respective countries (Osei *et al.*, 1997). All these may account for the seeming disparity in the plasma incretin levels observed in this study.

Determinants of DPP-4 Levels: - There is no significant association seen between serum DPP-4 level and several clinical and laboratory parameters tested. This finding is in contrast with most studies reviewed. In the American study, DPP-4 activity did not have a significant correlation with age, BMI or visceral tissue but was negatively correlated with the absolute lean mass (Neidert *et al.*, 2016). In the Asian series, serum DPP-4 levels were positively correlated with the waist/hip ratio, plasma insulin, LDL and presence of Type 2 DM.

CONCLUSION: - Serum DPP-4 level is not a significant determinant of glycaemic status among the adults Nigerians resident in Ilorin. Therefore, it cannot be used as a marker for detecting prediabetes in that population. Beta cell secretory dysfunction as measured by HOMA- β is a better determinant of diabetes incidence than incretin dysfunction. Low HDL and high LDL correlated better with prediabetes than serum DPP-4 levels among the study population.

The major limitation of this study is its relatively small sample size. A more extensive survey would be recommended to validate these findings.

References

- Baggio, L., and Drucker, D. J. (2007). Biology of Incretins: GLP-1 and GIP. *Gastroenterology*, 132, 2131–2157
- Bansal, N. (2015). Prediabetes diagnosis and treatment: A review. *World J Diabetes*, 6(2), 296-303
- Chiwanga, F.S., Njelekela, M.A., Diamond, M.B., Bajunirwe, F., Guwatudde, D., Nankya-Mutyoba, J., Kalyesubula, R., Adebamowo, C., Ajayi, I., Reid, T.G. and Volmink, J. (2016). Urban and rural prevalence of diabetes and pre-diabetes and risk factors associated with diabetes in Tanzania and Uganda. *Global health action*, 9(1), 31440.
- Chyan, Y., and Chuang, L. (2007). Dipeptidyl Peptidase-IV Inhibitors: An Evolving Treatment for Type 2 Diabetes from the Incretin Concept. *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery*, 11, 15-24
- Diabetes Prevention Program Research Group (DPPRG), (2002). Reduction in the incidence of Type 2 Diabetes with lifestyle intervention or metformin. *N Engl J Med*, 346(6), 393–403.
- Enang, O. E., Otu, A. A., Essien, O. E., Okpara, H., Fasanmade, O. A., Ohwovoriole, A. E., & Searle, J. (2014). Prevalence of dysglycemia in Calabar: a cross-sectional observational study among residents of Calabar, Nigeria. *BMJ Open Diabetes Research and Care*, 2(1), e000032.
- Godinho, R., Mega, C., Teixeira-de-Lemos, E., Carvalho, E., Teixeira, F., Fernandes, R., & Reis, F. (2015). The place of dipeptidyl peptidase-4 inhibitors in type 2 diabetes therapeutics: a “me too” or “the special one” antidiabetic class?. *Journal of diabetes research*, 2015.

- Kansal, S. and Kamble, T. K. (2016). Lipid Profile in Prediabetes. *J Assoc Physicians India*, 64(3), 18-21.
- Knopp, F. K. (2010). Incretin hormones and beta cell function in chronic pancreatitis. *Dan Med Bull*, 57(7), B4163.
- Li, C., Ford, E. S., Zhao, G. and Mokdad, A. H. (2009). Prevalence of Pre-Diabetes and its association with clustering of cardiometabolic risk factors and hyperinsulinemia among U.S. adolescents. National Health and Nutrition Examination Survey 2005–2006. *Diabetes Care*, 32(2), 342-347.
- Mannucci, E., Pala, L., Ciani, S., Bardini, G., Pezzatini, A., Sposato, I., Cremasco, F., Ognibene, A. and Rotella, C.M. (2005). Hyperglycaemia increases dipeptidyl peptidase IV activity in diabetes mellitus. *Diabetologia*, 48(6), 1168-1172.
- McKillop, A. M., Duffy, N. A., Lindsay, J. R., MO'Harte, F. P., Bell, P. M., and Flatt, P. R. (2008). Decreased dipeptidyl peptidase-IV activity and glucagon-like peptide-1(7-36) amide degradation in type 2 diabetic subjects. *Diabetes Research and Clinical Practice*. 79(1), 79–85.
- Meier, J., Huking, K., Holst, J., Deacon C., Schmiegel W. and Nauck M. (2001). Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. *Diabetes*, 50, 2497-2504.
- National Diabetes Statistics Reports (NDSR), (.2017). Estimates of Diabetes and its burden in the United States. Available on internet: <https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf>. Accessed 2018/07/30.
- Nauck, M. A., Homberger, E., Siegel, E. G., Allen, R. C., Eaton, R. P., Ebert, R., & Creutzfeldt, W. (1986). Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *The Journal of Clinical Endocrinology & Metabolism*, 63(2), 492-498.
- Neidert, L. E., Wainright, K. S., Zheng, C., Babu, J. R. and Kluess, H. A. (2016). Plasma dipeptidylpeptidase IV activity and measures of body composition in apparently healthy people. *Heliyon*, 2, e000097.
- Nwatu, C. B., Ofoegbu, E. N., Unachukwu, C. N., Young, E. E., Okafor, C. I. and Okoli, C. E. (2015). Prevalence of prediabetes and associated risk factors in a rural Nigerian community. *International Journal of Diabetes in Developing Countries*, 36(2), 197-203.

- Osei, K., Schuster, D. P., Owusu-Albert, K. S. and Amoah, G. B. (1997). Race and ethnicity determine serum insulin and C-peptide concentrations and hepatic insulin extraction and insulin clearance: Comparative studies of three populations of West African Ancestry and White Americans. *Metabolism*, 46(1), 53-8
- Papaetis, G. S. (2014). Incretin-based therapies in prediabetes: Current evidence and future perspectives. *World J Diabetes*, 5(6), 817-834.
- Ryskjær, J., Deacon, C. F. and Carr, R. D. (2006). Plasma dipeptidyl peptidase-IV activity in patients with type-2 diabetes mellitus correlates positively with HbA_{1c} levels, but is not acutely affected by food intake. *European Journal of Endocrinology*, 155(3), 485–493.
- Sabir, A., Ohwovoriole, A. E., Isezuo, S., Fasanmade, O., Abubakar, S. and Iwuala, S. (2013). Type 2 Diabetes and its risk factors among the rural Fulanis of Northern Nigeria. *Annals of African Medicine*, 12, 217-222.
- Shittu, R. O., Kasali, F. O., Biliaminu, S. A., Odeigha, L. O., Sule, A.G and Musa, Y. (2017). Prevalence of Diabetes and Pre-Diabetes in Oke- Ogun Region of Oyo State, Nigeria. *J Med Res Health Educ*.1, 1.
- World Health Organization (WHO), Global Reports on Diabetes (2016). In internet: <http://www.who.int>. Retrieved 2018/02/21.
- World Health Organization (WHO). (2006). Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Available on internet: https://www.who.int/diabetes/publications/Definition%20and%20diagnosis%20of%20diabetes_new.pdf. Accessed 2019/03/21.
- Yazbeck, R., Howarth, G. S. and Abbott, C. A. (2009). Dipeptidyl peptidase inhibitors, an emerging drug class for inflammatory disease? *Trends Pharmacol Sci*, 30, 600-607

TABLE 1. MEAN ANTHROPOMETRIC PARAMETERS OF THE STUDY SUBJECTS

Anthropometric Parameter	Mean	SD
Weight (kg)	66	13.5
Height (m)	1.61	0.08
BMI (kg/m²)	25.5	5.3
Waist circumference (cm)	86.4	11.7
Hip circumference (cm)	96.8	10.7
Waist/Hip Ratio	0.89	0.72
Systolic BP (mmHg)	135.7	23.8
Diastolic BP (mmHg)	84.8	15.7
Mean Arterial Pressure (mmHg)	192.3	32.4

BMI = Body Mass Index; BP = Blood Pressure

Total no of subjects = 118

TABLE 2. LABORATORY VALUES OF THE STUDY SUBJECTS

	Mean	SD	Min	Max
FPG (mmol/l)	5.09	1.3	1.6	11.0
2HPG (mmol/l)	7.8	3.0	2.8	21.6
T.Chol (mmol/l)	4.7	0.9	2.9	6.5
HDL (mmol/l)	1.6	0.6	0.7	3.6
LDL (mmol/l)	1.4	0.5	0.7	3.4
TG (mmol/l)	1.7	0.4	1.0	3.0
DPP-4 (U/L)	0.5	0.38	0.05	2.4
INSULIN (μIU/ml)	3.37	3.38	0.01	18.0
HOMA-IR	0.74	0.76	0	3.61
HOMA-β	69.5	110.7	-185	666.7

FPG = Fasting Plasma Glucose; 2HPG = 2 hours post glucose load; HDL = high density lipoprotein; LDL = Low density lipoprotein; TG = Triglyceride; T.chol = Total Cholesterol.

TABLE 3. CLINICAL AND LABORATORY VALUES COMPARED IN THE DIFFERENT GLYCEMIC GROUPS OF STUDY SUBJECTS

	Normal	Prediabetes	Diabetes	Total	F	P value
Age(yrs)	49.9±12.5	52.1±9.5	50.7±9.2	50.6±11.2	0.383	0.68
BMI (kg/m ²)	25.5±12.4	25.6±5.2	25.7±4.9	25.5±5.3	0.016	0.98
Waist(cm)	86.6±12.4	86.0±11.4	86.1±10.4	86.4±11.7	0.041	0.96
MAP(mmHg)	194.7±33.1	187.8±33.8	190.8±29.9	192.3±33.4	0.480	0.62
Insulin(μIU/ml)	3.9±4.0	2.9±2.3	2.4±2.2	3.4±3.4	2.227	0.11
DPP4 (U/L)	0.51±0.33	0.53±0.55	0.41±0.22	0.50±.38	0.78	0.46
HOMA-IR	0.77±7.8	0.66±0.60	0.74±0.91	0.74±0.76	0.216	0.81
HOMA-β	92.7±128.6	51.5±92.3	24.0±31.7	69.5±110.7	3.936	0.02**

BMI = Body Mass Index; MAP = Mean arterial Pressure; DPP-4 = Dipeptidyl Peptidase-4; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; HOMA-β = Homeostatic Model Assessment for beta cell secretion

TABLE 4: COMPARISON BETWEEN PREDIABETIC AND NORMOGLYCEMIC SUBJECTS

	Normoglycemia	Prediabetes	t Test	p Value
Age (yrs)	49.9±12.5	52.1±9.5	-0.84	0.40
Weight(kg)	66.9±13.7	65.6±13.0	0.43	0.67
Height(m)	1.62±0.08	1.60±0.06	1.17	0.24
BMI(kg/m²)	25.5±5.5	25.5±5.2	-0.06	0.95
Waist(cm)	86.6±12.4	86.0±11.4	0.24	0.81
Hip (cm)	97.6±10.1	95.6±12.0	0.81	0.42
W/H ratio	0.89±0.07	0.90±0.09	-0.90	0.36
SBP(mmHg)	137.6±24.8	132.2±24.6	0.98	0.33
DBP(mmHg)	85.5±14.2	83.4±14.9	0.65	0.52
MAP (mmHg)	194.8±33.1	187.8±33.1	0.93	0.35
Insulin (U/L)	5.15±11.1	4.7±9.7	0.16	0.87
DPP4(μIU/ml)	0.51±1.2	0.53±0.55	0.14	0.89
T.chol(mmol/l)	4.7±0.9	4.8±0.9	-0.45	0.66
TG (mmol/l)	1.72±0.4	1.77±0.4	-0.43	0.67
HDL (mmol/l)	1.77±0.6	1.2±0.4	4.7	0.00**
LDL (mmol/l)	1.2±0.4	1.7±0.6	-4.93	0.00**
HOMA-IR	0.9±1.5	1.1±2.6	-0.55	0.59
HOMA-β	45.9±34.1	65.5±16.8	-0.30	0.77

BMI = Body Mass Index; W/H = Waist/Hip; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; MAP = Mean Arterial Pressure; DPP-4 = Dipeptidyl Peptidase-4; T.chol = Total Cholesterol; TG = Triglyceride; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; HOMA-IR = Homeostatic Model Assessment for Insulin Resistance; HOMA-β = Homeostatic Model Assessment for beta cell secretion.

TABLE 5. SOME CORRELATES OF PLASMA sDPP-4 LEVELS IN PREDIABETIC STUDY SUBJECTS

	Mean	SD	R	P value
Age	50.6	11.2	0.04	0.66
Weight	66.0	13.5	-0.03	0.69
Height	1.61	0.08	-0.01	0.91
BMI	25.5	5.3	-0.03	0.76
Waist	86.4	11.7	0.01	0.93
W/H Ratio	0.89	0.72	-0.05	0.57
Systolic BP	135.7	23.8	0.07	0.46
Diastolic BP	84.8	15.7	0.14	0.13
MAP	192.3	32.4	0.10	0.30
FPG	5.1	1.3	-0.04	0.66
2HPP	7.8	3.0	-0.11	0.25
Insulin	3.37	3.39	-0.01	0.92
T.CHOL	4.7	0.91	0.15	0.11
TG	1.7	0.40	-0.04	0.70
HDL	1.5	0.6	0.05	0.61
LDL	1.42	0.51	0.18	0.06
HOMA-IR	0.92	1.71	-0.02	0.88
HOMA-B	65.2	103.1	0.01	0.96

BMI = Body Mass Index; W?H = Waist/Hip; BP = Blood Pressure; MAP = Mean Arterial Pressure; FPG = Fasting Plasma Glucose; 2HPP = 2 Hours Post Prandial; T.chol = Total Cholesterol; TG = Triglyceride; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; HOMA-IR = Homeostatic Model for Insulin Resistance; HOMA- β = Homeostatic Model Assessment for beta cell secretion.

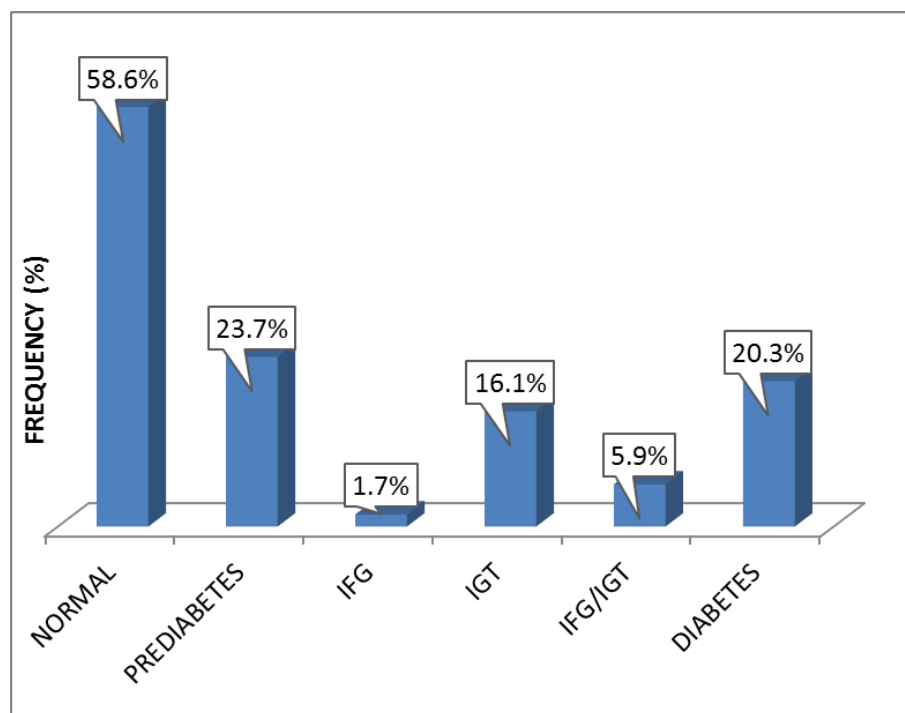


Figure 1. GLYCEMIC STATUS OF THE SUBJECTS FOLLOWING Oral Glucose Tolerance Test

IFG = Impaired Fasting Glycemia; IGT = Impaired Glucose Tolerance.

Status of Some Metal Ions Pollution in Selected Groundwater within Iloffa District Using Multivariate Statistical Techniques

Hussein K. Okoro^{1*}, Tunbosun T. Popoola¹ Benjamin O Orimolade^{1, 2} Clement O. Ogunkunle³

¹*Analytical-Environmental & Material Science Research Group, Department of Industrial Chemistry, Faculty of Physical Sciences, P.M.B. 1515, University of Ilorin, Postal code +234000, Ilorin, Nigeria.*

²*Analytical-Environmental and Membrane Nanotechnology Research Group, Department of Chemical Science, Faculty of Science, University of Johannesburg, P.O. Box 17011, Doornfontein 2028, Johannesburg, Republic of South Africa.*

³*Environmental Biology Unit, Department of Plant Biology, Faculty of Life Sciences, P.M.B. 1515, University of Ilorin, Postal code +234000, Ilorin, Nigeria*

**Corresponding author email's: okoro.hk@unilorin.edu.ng or hkoadeola@gmail.com*

Abstract

The assessment of underground water in Iloffa community, North-central region, in Nigeria for heavy metals was carried out within the months of December, February and April. Samples were analysed for heavy metal contents using Atomic Absorption Spectrometer (AAS). Statistical analyses of the data were done using the Pearson's correlation and the extent of pollution was determined using pollution indices. The results of the assessment carried out on selected boreholes water and well water showed that majority of the sampling locations are not polluted with heavy metals. In some locations, some metals ion are within the minimum contamination level while others are above the minimum contamination level (> 1). Relatively, the metal concentration in the borehole was found to be greater than in the well water. The order of concentration of the metals in all location is given as Fe>Mn> Zn> Cu>Pb> Cd. The contamination factor and pollution load index indicated that the pollution levels in the sampling areas were low while the enrichment factor showed that the metal concentrations present in the locations are largely from anthropogenic sources than from the crustal material. It was observed that most of the polluted waters are those located close to dumpsites. Therefore, it is

necessary to consider the industrial activities and other activities in areas to dig wells or boreholes.

Keywords: Heavy metals, boreholes, wells, pollution indices, multivariate

Introduction

Water is an essential support for plants and animals. It is generally obtained from two principal natural sources: surface water such as fresh water, lakes, rivers and stream and ground water such as borehole water and well water (Vanloon, and Duffy, 2005; McMurry and Fay, 2004; Mendie, 2005). Water is an essential resource for industrial processes, agricultural production and domestic use (Itodo and Itodo, 2010).

The two major sources of water supply for rural dwellers in Nigeria are hand-dug well and borehole. One of the most important environmental issues today is ground water contamination (Vodola et al., 1997) and between the wide diversity of contaminant affecting water resources, heavy metals receive particular concern considering their strong toxicity even at low concentrations (Marcovecchio et al., 2007). Heavy metals are poisonous and serious groups of pollutants due to their high toxicity, abundance and ease of accumulation in various plants and animals (Thambavani and Mageswari, 2013; Okoro et al., 2013, Okoro et al., 2016). Some of the metals are essential to sustain life, calcium, magnesium, potassium and sodium must be present for normal body functions (Adepoju-Bello et al., 2009). Also, cobalt, copper, iron, manganese molybdenum and zinc are needed at low levels as catalyst for enzyme activities, however excess exposure to heavy metals can result in toxicity (Adepoju-Bello et al., 2009). Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs (Kayeet al., 2002). Long-term exposure may result in slow and progressive physical, muscular, and neurological degenerative processes such as muscular dystrophy and multiple sclerosis (IOSHC, 1999).

There are various ways in which groundwater is contaminated, amongst which are the use of fertilizer in farming (Altman and Parizek, 1995), runoff from road and disposal of industrial waste water. It is difficult to state what concentration of heavy metal that is safe. Different agencies state different levels for a particular metal (Duruibe et al., 2007). In the recent times, several studies within Nigeria have reported the presence of heavy metals in groundwater above the recommended limits (Edet et al., 2011; Nwankwoala and Udom, 2011; Akunobi and Chibuzor, 2012; Adamu et al., 2013). Therefore, it is important to assess the concentration of the heavy metal in ground water around the country and its health risk so that appropriate measures can be taken. In view of this, the aim of this research was to

assess the level of some metals in selected boreholes and wells within Iloffa community, Kwara State, North-Central. Nigeria

Materials and methods

Study Area

Iloffa, the capital of Oke-Ero Local Government in Kwara State, Nigeria is situated approximately between the latitude $8^{\circ}05'36.3''$ and longitude $5^{\circ}08'32.4''$ E (**Figure 1**). It is sparingly populated, their major activities are farming, industrial activities such as quarry and local production of soap. Iloffa is marked by two climatic seasons, the dry and the raining season with an interfering harmattan from December to January. Underground water (borehole and well water) is the major source of drinking water and for domestic activities. The coordinates of the sampling locations is presented in **Table 1**

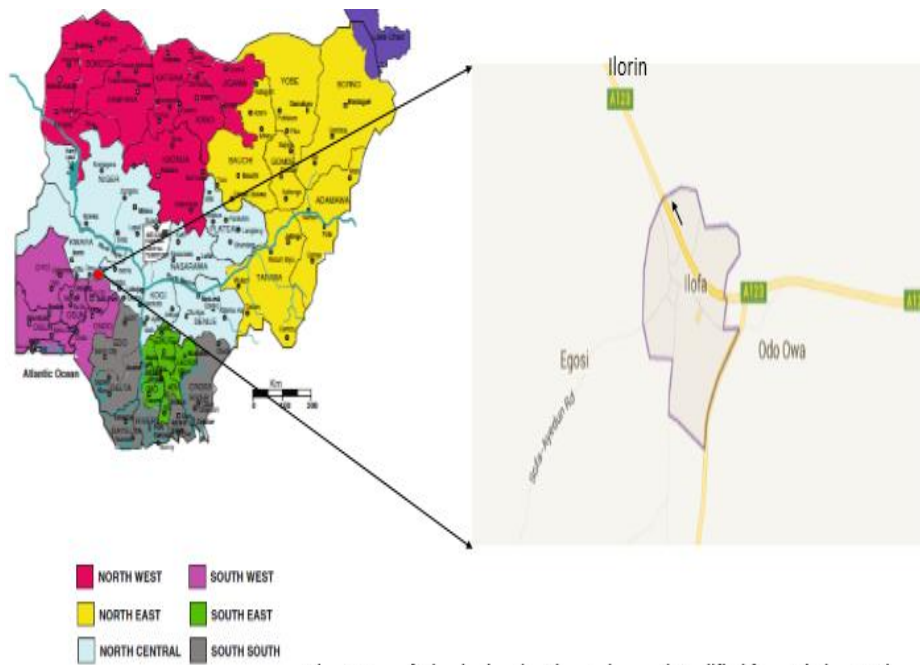


Figure 1: Map showing Kwara State and sampling locations

Table 1: Description of sampling points referenced with global position system (GPS)

Locations	Description	Water type	Coordinate
L1	EjiwumiQuary	Well	N08°06'44.2" E005°07'43.7"
L2	Nomadic school	Borehole	N08°06'23.7" E005°08'16.9"
L3	Muftrol petrol station	Well	N08°05'46.2" E005°08'36.1"
L4	Oke-EroAbattior	Borehole	N08°05'43.1" E005°08'27.5"
L5	Honey moon area	Borehole	N08°05'37.8" E005°08'46.2"
L6	NUT Farm	Well	N08°05'22.2" E005°08'06.5"
L7	Egosi	Borehole	N08°05'09.0" E005°07'29.2"
L8	Garage square	Borehole	N08°05'31.9" E005°08'32.6"
L9	Adi Industry	Well	N08°05'56.7" E005°08'33.5"
L10	Olorunsogo	Well	N08°07'02.8" E005°07'31.5"

Preparation of Standard Solution

Standard solutions of metals (100 ppm) were prepared from the soluble salt of the metals of highest purity (analytical grade reagent available). Metals were dissolved in concentrated HNO₃ and made up to 1L with deionized water in a volumetric flask. Different concentrations were prepared from this stock solution for plotting calibration graph for AAS analysis (Adeniyi, 2009).

Sampling and Sample Digestion

Groundwater samples that were used for metal ions determination were collected from ten (10) different locations within Ilofa community, North-Central, Nigeria. The samples were collected in high density polyethylene containers and transported on an ice chest to the laboratory. Samples were acidified with 2 ml of 2 M nitric acid and stored cool at 4 °C in the refrigerator before further analysis. Physico-chemical parameters such as pH and temperature were taken at in-situ. 10 ml of aqua regia (HCl/HNO₃ 3:1) was added to the sample. Then the mixture was digested on a hot plate until it concentrated to about 20 ml. The mixture was cooled, filtered and made up to mark with deionized water in a 100 ml volumetric flask. 25ml was

poured into the sample bottle and analyzed for heavy metal contents using Atomic Absorption Spectrometer (AAS) (Momodu and Anyakora, 2010).

Instrumentation

Atomic Absorption Spectrophotometer (AAS) model 210 was used for the analysis of the water samples. AAS analytical instrument is based on the principle of atomic absorption spectroscopy and is very useful to detect the metal ion concentration present in drinking water samples (Bhavtosh and Shweta, 2013). AAS method follows the Beer's law, which states that absorbance is directly proportional to concentration.

Quality Control

Analysis were carried out in triplicates and the average of the data was used for data analysis. Results were compared with blank and at intervals. The standard and the blank were re-run to validate the working conditions of the instrument. Quantification of metals was based on calibration curves of standard solutions of the metals (Abdus-Salam and Oyede, 2013).

Data Analysis

In order to assess heavy metal enrichment and degree of contamination in the water samples, analytical data were subjected to pollution calculation methods such as Enrichment Factor (EF), Contamination Factor (CF) and Pollution Load Index (PLI), with the aim of deriving realistic estimates for the amount of contamination that has impacted soils around the sample locations.

The enrichment factor was calculated using the formula originally introduced by Buat-Menard and Chesselet (Taylor and McLennan, 1995) as shown in equation 1.

$$EF = \frac{(C_n/C_{ref})}{(B_n/B_{ref})} \quad 1$$

Where C_n (sample) is the concentration of the examined chemical element in the examined environment, C_{re} (sample) is the concentration of the examined chemical element in the reference environment, B_n is the concentration of the reference chemical element in the examined environment and B_{re} is the concentration of the reference element in the reference environment. The analysis of EF data could provide information on the status of environmental contamination. In the calculation of EF, Al or Fe is often used as normalizers, in this case Fe will be used since Al was not analyzed. Because we do not have the background value of heavy metals of interest in the studied area, we adopted the crustal values for the metals as shown in the **Table 2** (Abdus-Salam and Oyede, 2013).

Table 2: Average Crustal Value of Heavy Metal

Metals	Mn	Fe	Cu	Zn	Cd	Pb
Average crustal value (µg/g)	85	47200	45	95	29	20

The contamination factor can be calculated from the relation shown in equation 2.

$$CF = \frac{C_{sample}}{C_{background}} \quad 2$$

Where C_{sample} and $C_{background}$ refer to the measured concentrations of pollutant and the average shale respectively (Thambavani and Mageswari, 2013).

The pollution load index, proposed by (Tomlinson et al., 1980) was calculated using the expression in equation 3.

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \dots \times CF_n)^{1/n} \quad 3$$

Where n is the number of metals studied and CF is the contamination factor calculated as described above. The PLI is able to give an estimate of the metal contamination status and the necessary action that should be taken.

Results and Discussion

The results of the physicochemical parameters are shown in Table 3

Table 3: pH and temperature of water sample

Locations	1	2	3	4	5	6	7	8	9	10
pH	6.8	5.6	4.8	6.8	6.5	5.2	8.3	6.8	5.2	6.2
Temperature°C	27.0	28.3	27.1	28.5	30.0	28.8	27.0	28.0	27.2	27.0

pH is the measure of the acidity or alkalinity of water. From the pH measurement, some of the water samples have low pH value, which implies the water is acidic. Most of the water sample falls within the (WHO, 2008) limits of natural water for domestic use. The average pH range is between 4.8 and 8.3 with location L3 having the lowest value and location L7 the highest value. The temperature of all the water samples falls within the WHO limits for domestic uses (**Table 3**). The temperature ranges from 27 °C to 30°C. This is similar to the result obtained by Abdus-Salam et al. 2013.

The results for the heavy metal concentrations in the samples are presented in Table 4

Tables 4: Average concentration of the six heavy metals studied in Iloffa borehole and well waters in the months of December, February and April.

Sample	Months	Mn (mg/L)	Fe (mg/L)	Cu (mg/L)	Zn (mg/L)	Cd (mg/L)	Pb (mg/L)
L1	December	BDL	0.56±0.01	0.04±0.01	0.06±0.01	BDL	BDL
	February	0.11±0.01	2.55±0.01	BDL	0.05±0.00	BDL	BDL
	April	BDL	2.78±0.01	0.01±0.00	0.14±0.02	BDL	BDL
L2	December	0.52±0.01	1.73±0.01	0.06±0.01	0.06±0.01	BDL	BDL
	February	0.51±0.01	5.05±0.01	0.03±0.01	0.05±0.01	BDL	BDL
	April	0.54±0.01	6.52±0.25	0.02±0.01	0.06±0.01	BDL	0.01±0.00
L3	December	0.10±0.01	0.31±0.01	0.04±0.00	0.05±0.01	BDL	BDL
	February	0.11±0.01	0.41±0.01	BDL	0.02±0.01	BDL	BDL
	April	0.11±0.01	0.72±0.00	0.01±0.01	0.04±0.01	BDL	BDL
L4	December	BDL	4.11±0.01	0.07±0.01	0.09±0.01	BDL	BDL
	February	0.10±0.01	9.26±0.01	0.13±0.01	0.12±0.01	BDL	BDL
	April	0.01±0.00	2.68±0.01	0.00±0.01	0.23±0.02	0.01±0.01	0.17±0.00
L5	December	BDL	2.74±0.01	0.04±0.01	0.40±0.01	BDL	BDL
	February	BDL	0.63±0.01	BDL	0.03±0.01	BDL	BDL
	April	BDL	3.09±0.01	0.13±0.15	0.14±0.01	BDL	0.01±0.00
L6	December	0.10±0.01	0.97±0.01	0.02±0.01	0.03±0.01	BDL	BDL
	February	0.21±0.01	2.76±0.01	0.02±0.01	0.10±0.00	BDL	BDL
	April	0.33±0.02	0.73±0.01	BDL	0.07±0.03	BDL	BDL
L7	December	BDL	0.10±0.00	BDL	0.05±0.00	BDL	BDL
	February	BDL	BDL	BDL	BDL	BDL	BDL
	April	0.33±0.01	22.65±0.02	0.03±0.01	0.18±0.01	BDL	BDL

L8	December	0.10±0.00	0.20±0.01	BDL	0.03±0.01	BDL	BDL
	February	BDL	0.43±0.02	0.01±0.00	0.01±0.00	BDL	BDL
	April	0.00±0.01	2.15±0.02	0.00±0.01	0.04±0.00	BDL	BDL
L9	December	BDL	1.72±0.01	0.02±0.00	0.43±0.01	BDL	BDL
	February	0.11±0.02	0.10±0.00	0.01±0.01	0.01±0.00	BDL	BDL
	April	0.11±0.01	0.43±0.02	BDL	0.21±0.00	0.01±0.01	0.01±0.01
L10	December	BDL	0.64±0.01	0.03±0.00	0.04±0.00	BDL	BDL
	February	BDL	0.32±0.01	0.02±0.01	0.01±0.00	BDL	BDL
	April	0.00±0.01	4.19±0.02	0.02±0.01	0.30±0.00	0.00±0.01	0.00±0.01

BDL: Below Detection Limit

The result of the analysis showed that in the month of December manganese was below detection level in six sampling locations; L1, L4, L5, L7, L9 and L10 but present at a detectable level in locations L2, L3, L6 and L8 within the range 0.10 mg/L to 0.52 mg/L (**Table 4**). Manganese in L3, L6 and L8 are within the maximum contamination level (MCL) given by World Health Organization (WHO) and Federal Environment Protection Agency (FEPA) which is 0.1 mg/L and 5 mg/L respectively but above the USEPA limit of metal contamination. L2 has the highest concentration of Mn, 0.52 mg/L. In the observations made, the metal concentration falls within the FEPA limit but above WHO and United States Environmental Protection Agency (WHO, 2008; FEPA, 2001; USEPA, 2011). The result obtained is comparable with that of Abdus-Salam *et al.*, 2013 except for location L2. The presence of manganese in high concentration in L2 can be as a result of the farm activities which involve the use of fertilizers and other chemicals and probably erosion of manganese containing soil as well as the high pH of the sample. Manganese concentration above acceptable limit may cause objectionable and tenacious stain to laundry and plumbing fixtures (Okoro *et al.*, 2012).

The concentration of Fe observed in the sampling locations for the month of December ranges from 0.010 mg/L to 4.11 mg/L with wide variation. Locations L1, L3, L6 L7, L8 and L10 are within the WHO/USEPA limits, while locations L2, L4, L5 and L9 exceeds the limits but falls below FEPA limit. The concentration of iron was found to be above the Maximum Contamination Limit (MCL) of WHO and USEPA but below the limit of FEPA except L7 in the month of April (WHO, 2008; FEPA, 2001; USEPA, 2011). The result from this study is similar to that of Abdus-Salam *et al.* (2013) and that of Momodu and Anyakora (2009). The high concentration of Fe in L2, L4, L5, and L7 could be as a result of the dumpsite, clay soil deposit in these areas. Most groundwater contains some iron because it is

common in many aquifers and it is found in trace amounts in practically all sediments and rocks formations.

The concentration of copper observed in the ten locations was within the range 0.02 – 0.07 mg/L. All the results obtained from this study are below the MCL of WHO and USEPA but within the range of FEPA (WHO, 2008; FEPA,2001; USEPA, 2011). In February, the concentrations dropped to concentration range of 0.01 mg/L to 0.13 mg/L except location L4. The low concentration or the absence of Cu in some locations may be due to their low pH and some geological factors (Elinge, 2011).

The concentration of Zn was found to be between the range 0.03 mg/L and 0.43 mg/L in the ten locations for the month of December which is far below the limit of WHO, USEPA and FEPA (WHO, 2008; FEPA, 2001; USEPA, 2011). The result obtained from this study can be related to that of Abdus-Salam et al. (2013) and Momodu and Anyakora (2009). The presence of Zn in drinking water impact an undesirable astringent taste to water at a taste threshold concentration at about 4mg/L (as Zinc sulfate).

The samples showed the absence of Cd and Pb in the ten locations for the month of December and February. In April Cd was detected in locations L4, L9, L10 between the range 0.003 mg/L to 0.01 mg/L. locations L4 and L9 showed high level of contamination above MCL of WHO and USEPA (Elinge, 2011). The high level of Cd in L4 (Oke-Ero Abattoir) could be as a result of leaching from the dumpsite and the activities of the Abattoir around the borehole. It could also be as a result of the deterioration of the galvanized pipe used for the borehole. The possible long term effects of chronic exposure to Pb present in drinking water are subjects to considerable public concern (Zietz and Suchenwirth, 2007). In April, lead was detected in locations L2, L4, L5, L9 and L10 in the range 0.003 mg/L to 0.17 mg/L. The lead concentration in these locations is within the MCL of WHO (WHO, 2008) except in location L4 with the concentration 0.17 mg/L. The presence of Pb in high concentration level can be related to pollution from the dumpsite and abattoir's activities in the location.

Statistical Analysis of Data

Pearson Correlation

Pearson's correlation is used to compare the relationship between two variables. There are two types of correlations, positive and negative correlation. Two variables are said to have positive correlation when the Pearson's correlation tends to +1, while we have negative correlation when the value tends to -1.

Tables 5: Correlation of the metals in the ten locations in for the two seasons.

		Mn	Fe	Cu	Zn	Cd	Pb
Correlation	Mn	1.000	0.364	0.077	-0.147	0-.097	-0.105
	Fe	0.364	1.000	0.136	0.213	-0.028	0.005
	Cu	0.077	0.136	1.000	0.142	-0.112	-0.075
	Zn	-0.147	0.213	0.142	1.000	.212	0.256
	Cd	-0.097	-0.028	-0.112	0.212	1.000	0.519
	Pb	-0.105	0.005	-0.075	.0256	.519	1.000

The correlation matrix helps to explain the level of correlation between the heavy metals. **Table 5** shows that manganese has a positive but weak correlation with iron and copper and a negative correlation with zinc, cadmium and lead. This implies manganese has weak relationship with copper and iron and no relationship with other metals in the water samples.

Iron was found to have positive correlation with all the metals except cadmium. The correlation with other metals is weak because it tends to 0, but the correlation is significant at the 0.05 level which means that the correlation is insignificant with Pb. Copper has positive correlation with all the metals. Its correlation with other metals is significant but weak because the value is tending towards 0. Cadmium has negative correlation with Mn, Fe and Cu, and positive correlation Zn and Pb. This means that there is a relationship which between Cd, Zn and Pb, which may suggest a common pollution sources or a similar geochemical behavior for these metals (Ahdy and Khaled, 2009). Lead has negative correlation with manganese and copper but positive correlation with iron, zinc and cadmium. Its relationship with iron is negligible because the value is very close to 0. The strong association of Pb, Zn, and Cd indicates common sources, and these metals might have been derived from anthropogenic sources (Syed et al., 2012).

Principal Component Analysis

Principal component analysis is based on the correlation matrix of the variables involved, and this requires a large sample size before they stabilize. It is used to categorize variables into components. Principal component analysis helps us to know which of the metals are most likely responsible for the contamination of the water sample. For any metal present to be active in a component, its communality value has to be close to 1.

Table 6 Communalities of heavy metal under study.

	Initial	Extraction
Mn	1.000	0.784
Fe	1.000	0.723
Cu	1.000	0.653
Zn	1.000	0.682
Cd	1.000	0.704
Pb	1.000	0.703

Form **Table 6** the metals under consideration are close to 1, making it possible to run principal component analysis on the data gotten from the metal concentrations. Three component matrix and total variance of heavy metal contamination are shown in **Table 7 & Table 8**

Table 7.: Three component matrix for the heavy metals.

	Component		
	1	2	3
Pb	0.808	0.113	0.193
Cd	0.794	0.051	0.265
Zn	0.526	0.446	-0.454
Fe	-0.090	0.827	0.178
Mn	-0.383	0.574	0.554
Cu	-0.157	0.464	-0.642

Table 8 Total Variance of Heavy Metal concentration.

Component	Initial Eigenvalues			Extraction Sums of Squared			Rotation Sums of Squared		
	Total	%	ofCumulative %	Total	%	ofCumulative %	Total	%	ofCumulative %
	Variance			Variance			Variance		
1	1.740	29.007	29.007	1.740	29.007	29.007	1.680	27.993	27.993
2	1.443	24.046	53.053	1.443	24.046	53.053	1.362	22.695	50.688
3	1.065	17.756	70.809	1.065	17.756	70.809	1.207	20.122	70.809
4	0.780	12.998	83.808						
5	0.494	8.241	92.049						
6	0.477	7.951	100.000						

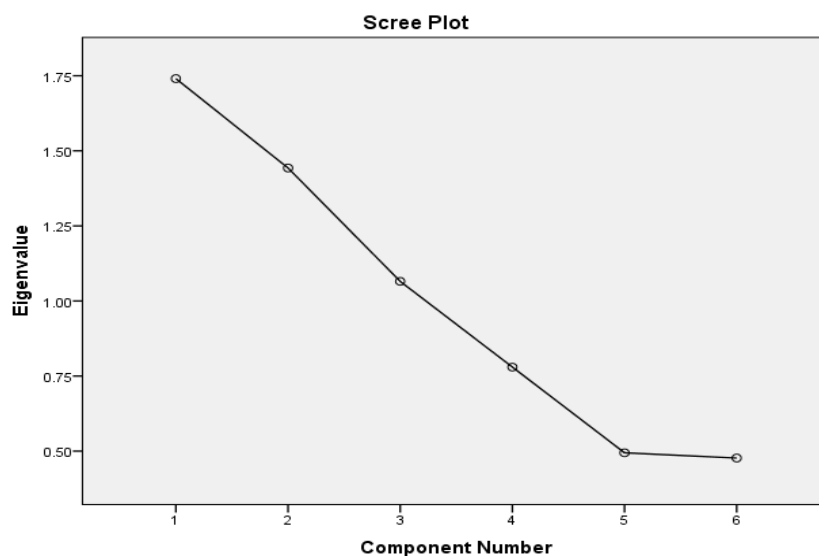


Figure 2:. Screen Plot for Principal Component Analysis

From **Figure 2**, it can be seen that three components are formed in the samples investigated. The first component is formed by Lead, Cadmium and Zinc, the second component is formed by Iron and the third component by manganese. Copper tends not to form any component.

Enrichment Factor (EF)

The application of the established pollution indices to the data gave an insight to the degrees and various sources of the trace metals input into the wells and boreholes

(Abdus-Salam et al., 2013). Enrichment factor categories proposed by Sutherland et al., 2000 were used as follows: $EF < 2$ = deficiently to minimal enrichment, $2 \leq EF < 5$ = moderate enrichment, $5 \leq EF < 20$ = significant enrichment, $20 \leq EF < 40$ = very high enrichment and $EF \geq 40$ = extremely high enrichment. The EF values for the trace metals are presented in **Table 9**.

Table 9 EF values of heavy metals in the sample areas in Iloffa.

Sample location	Mn	Cu	Zn	Cd	Pb
1	0.000	3.773	25.020	0.000	0.000
2	86.871	3.218	4.572	0.000	3.619
3	84.837	14.568	27.602	0.000	0.000
4	2.072	1.174	42.639	6.073	149.702
5	0	44.128	22.511	0	7.638
6	251.023	7.087	47.642	0	0
7	8.090	1.391	3.952	0	0
8	0.775	1.464	9.244	0	0
9	142.052	0	242.640	37.851	54.884
10	0.398	5.007	35.573	1.165	1.689

From the result presented above, it is evident that the enrichment factor of manganese, cadmium and lead had were very low. This implies that it does not have anthropogenic enrichment of these metals. Therefore, the presence of this metals were entirely from crustal materials or natural weathering but copper falls in the category of moderate enrichment while zinc has very high enrichment, the major source of this metal is anthropogenic. The enrichment of manganese in locations L2 and L6 are extremely high, copper, zinc and lead have moderate enrichment factors except for some locations where there is variations. Cadmium has low enrichment factors except in location 9. Majority of the metals in this location are enriched from anthropogenic source.

In location 3 manganese showed a similar pattern of enrichment as in location L2. Copper has a significant enrichment; zinc has a very high enrichment. This result indicates that the source of the metals present is anthropogenic. Cadmium and lead

are deficient of enrichment from the crustal materials and no enrichment from anthropogenic activities. In location L4 manganese and copper have low enrichment, which shows it has no enrichment from anthropogenic source. Zinc and lead are highly enriched by anthropogenic source while cadmium has moderate enrichment from anthropogenic sources.

Lead has a significant enrichment in location L4, zinc has very high enrichment factor while copper has extremely high enrichment factor. This results show that lead zinc and copper have anthropogenic source of enrichment in this location but manganese and cadmium are basically enriched from the crustal materials. The enrichment factor of manganese and zinc in location L6 is extremely high, this means the concentration of manganese in this well is largely as a result of the anthropogenic activities such as the farming activities like dungs from pig farm. copper also have a significant enrichment. Enrichment factor of heavy metals in location L7 is relatively low, this means it is enriched by anthropogenic activities. Manganese is within the contamination category of significant enrichment, while copper and zinc have moderate enrichment factor. The enrichment factor for the metals in location L8 and L10 is also relatively low except for zinc in location L8 which has a very high enrichment factor. The enrichment factor of the metals present in location L9 is extremely high compared to the other locations except zinc. This could have resulted from human activities, such as the local soap production and farming around the well

Contamination Factor

The assessment of water contamination was carried out using the contamination factor based on the four classification (**Table 10**) categories as given by Hakanson, (1980).

Table 10: Contamination factor based on Hakanson classification

Contamination (CF)	Factor	Classification
<1		Low contamination factor
1-3		Moderate contamination factor
3-6		Considerable contamination factor
>6		Very high contamination factor

Table 11 Contamination factor and pollution load index for selected sampling location.

Sampling	Mn	Fe	Cu	Zn	Cd	Pd	PLI
Location	10^{-3}		10^{-3}	10^{-3}	10^{-4}	10^{-3}	10^{-2}
1	0.00	0.59	0.20	1.50	0.00	0.00	7.49
2	6.40	1.38	0.40	0.60	0.00	0.50	1.01
3	1.30	0.15	0.20	0.40	0.00	0.00	1.59
4	0.10	0.58	0.00	2.40	3.00	8.50	0.08
5	0.00	0.65	2.90	1.50	0.00	0.50	3.35
6	3.90	0.15	0.00	0.70	0.00	0.00	8.66
7	3.90	4.79	0.70	1.90	0.00	0.00	5.40
8	0.00	0.46	0.00	0.40	0.00	0.00	23.81
9	1.30	0.09	0.00	2.20	3.00	0.50	0.58
10	0.00	1.04	0.40	3.20	0.00	0.00	1.65

The contamination factor from the **Table 11** indicates that the water from the various sampling location has low contamination degree of heavy metals because their values are far less than 1, except iron in locations L2, L7 and L10 which falls between moderate and considerable contamination factor.

Pollution Load Index

The result obtained for pollution load index shows that the pollution from the anthropogenic sources are tolerable. The $PLI > 1$ is polluted whereas the $PLI < 1$ indicates no pollution (Thambavani and Mageswari, 2013). The PLI values obtained for all the locations indicated practically uncontaminated condition. This result is as a result of the fact that the values of the heavy metals are below the MCL by (WHO, 2008) and other authorized agencies except for Mn in most locations, Cd and Pb in some locations. The PLI value range from 0.0058 to 0.2381 which confirms that the underground water in this area are in unpolluted condition. The PLI for the locations in decreasing order is $L8 > L6 > L1 > L7 > L5 > L10 > L3 > L2 > L4 > L9$.

Conclusions

The results of the assessment carried out on selected borehole and well water showed that majority of the sampling locations are not polluted with heavy metals. In some locations some metals are within the minimum contamination level (< 1) while others are above the minimum contamination level. Relatively the metal concentrations in the borehole were found to be greater than that in well water. The concentration of the metals in decreasing order in all location is given as $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Pb} > \text{Cd}$. Heavy metals are harmful both at high concentration and low concentration with long term exposure. Though some metals are essential in life processes, in the cases of iron, zinc, magnesium and calcium, at moderate concentrations, quantities extremely low can still pose danger especially when exposed for extended period of time. The seasonal variation showed that there was an increase in the heavy metal concentration except zinc which has a lower concentration during the raining season. The impact of the anthropogenic heavy metal pollution in the sampling area was evaluated using Enrichment Factor, (EF) Contamination Factor, (CF) and Pollution Load Index (PLI). The contamination factor and pollution load index indicated that the sampling areas are not very polluted while the enrichment factor showed that the metal concentrations present in the locations are largely from anthropogenic sources than from the crustal materials. From the research, it was observed that most of the polluted waters are located close to dump sites, considerations of activities such as farming activities and industrial activities in areas to dig wells or boreholes should be made. Regular analysis should be done on of the borehole and wells water used for drinking purpose and other domestic uses. The pipes used in boreholes should be checked regularly and replaced when needed. Appropriate measures should be taking to for the remediation of contaminated water.

References

- Abdus-Salam N. and Oyede F. A (2013). The Impact of a River on the Water Quality of Nearby Wells: A Case Study of Aluko River, Ilorin, Nigeria. *Chem. Search J* 4(2): 10-20.
- Adamu G.K, Tukur R. and Kankara A.I (2013). Groundwater assessment in the basement complex of Kano state, Nigeria. *Amer . of Enginee Res* 2(7): 171-175.
- Adeniyi A.A (2009). Heavy metals, traces elements distribution and sediments analysis of selected groundwater in Ilorin, BSC Thesis, University of Ilorin, Faculty of Science, Ilorin, Kwara State.

- Ahdy H.H, and Khaled A (2009). Heavy metals contamination in sediments of the western part of Egyptian Mediterranean Sea. *Austral J of Basic and Appl Sci* 3 (4): 3330-3336.
- Adepoju-Bello A.A, Ojomolade O.O, Ayoola G. A, and Coker H. A. B (2009). Quantitative analysis of some toxic metals in domestic water obtained from Lagos metropolis. *The Nig. J. of Pharmacol* 42(1): 57-60.
- Altman, S. J, and Parizek, R. R (1995). Dilution of nonpoint-source nitrate in groundwater. *J. of Environ. Quality* 24(4): 707-718.
- Akunobi B.E.B. and Chibuzor N. (2012). Hydrochemical evaluation of water sources in Warrimetropolis, Delta State, Nigeria. *J. of Basic Physical Res* 3: 64-72.
- Bhavtosh S. and Shweta T. (2013). Simplification of Metal Ion Analysis in Fresh Water Samples by Atomic Absorption Spectroscopy for Laboratory Students. *J. of Lab. Chem. Edu* 1(3): 54-58.
- Duruibe J.O, Ogwuegbu M. O. C, and Egwurugwu J. N. (2007). Heavy metal pollution and human biotoxic effects. *Int.l J. of Phy. Sci* 2(5):112-118.
- Edet A.E, T.N. Nganje A.J. Ukpong and A.S. Ekwere (2011). Groundwater chemistry and quality of Nigeria: A status review. *Afri. J. of Environ. Sci. and Technol* 5(13): 1152-1169.
- Elinge C.M, Itodo A. U, Peni I. J, Birnin-Yauri U. A and Mbongo A. N. (2011). Assessment of heavy metals concentrations in bore-hole waters in Aliero community of Kebbi State. *Adv. in Appl. Sci. Res* 2(4): 279-282.
- FEPA, (2001). Guidelines and Standards for Environmental Pollution Control in Nigeria. Fed. Environ. Prot. Agen., Lagos.
- Kaye P, Young H., and O'sullivan I (2002). Metal fume fever: a case report and review of the literature. *Emerg. Med. JI: EMJ*, 19(3): 268.
- Hakanson L.(1980). An ecological risk index for aquatic pollution control, a sedimentological approach. *Water Research*,14: 975–1001.
- International Occupational Safety and Health Information Centre (1999). Basics of chemical safety, International Labour Organization Conference, Geneva. 52(4): 592-603.

- Itodo U. A, and Itodo U. H. (2010). Estimation of Toxic metals in canned milk products from unplaquered tin plate cans. *Journal of American science*, 6 (5): 173178.
- Marcovecchio, J.E., Botte, S.E. and Freije, R.H. (2007). Heavy Metals, Major Metals, Trace Elements. In: Handbook of Water Analysis. L.M. Nollet, (Ed.). 2ndEdn. London: CRC Press, 275-311.
- McMurry J. and Fay R.C (2004). Hydrogen, Oxygen and Water. In: McMurry Fay Chemistry. K.P. Hamann, (Ed.). 4th Edn. New Jersey: Pearson Education, 575-599.
- Mendie U (2005). The Nature of Water. In: The Theory and Practice of Clean Water Production for Domestic and Industrial Use. Lagos: Lacto-Medals Publishers, 1-21.
- Momodu, M. A., and Anyakora, C. A. (2010). Heavy metal contamination of groundwater: the Surulere case study. *Research Journal of Environmental Earth Science*, 2(1) 39-43.
- Nwankwoala, H.O., and Udom G.J. (2011). Hydrochemical evaluation of groundwater in parts of eastern Niger Delta, Nigeria. *Journal of Academic and Applied Sciences*, 1 (2): 33-58.
- Okoro H. K. Adeyinka A, Jondiko O. E and Ximba, B. J (2012). Physico-chemical analysis of selected groundwater samples of Ilorin town in Kwara State, Nigeria. *Scientific Research and Essays*, 7 (23): 2063-2069.
- Okoro H.K., Fatoki O.S, Adekola F.A, Ximba B.J and Snyman R.G (2013): Physicochemical characteristics and One-year monitoring of heavy metal pollution in Seawater from Cape Town Harbour and their Seasonal Variation, *Fresenius Environmental Bulletin, Germany*. 22(10): Pp. 2855-2866.
- Okoro, H.K. Ximba B.J., Tamba O, Fatoki, O.S, Adekola F.A, Snyman R.G. Waheed BY. (2016): Distribution and Seasonal Variations of Selected Heavy Metals in Seawater from Cape Town Harbour of Western Cape Province, Republic of South Africa. *Zimbabwe J. of Sci. and Technol.*
- Syed H.R, Dilara K, Tanveer M.A, Mohammad S.I, Mohammad A.A and Mohammad A.A (2012). Assessment of Heavy Metal Contamination of Agricultural Soil round Dhaka Export Processing Zone (DEPZ),

Bangladesh: Implication of Seasonal Variation and Indices. *Appl. Sci* ,2: 584-601.

- Sutherland R. A, Tolosa C. A, Tack F. and Verloo M. G (2000). Characterization of selected element concentration and enrichment ratios in background and anthropogenically impacted roadside areas, *Archives of Environmental Contamination and Toxicology*, 38, 428–438.
- Taylor S. R. and McLennan S. M. (1995). The geochemical evolution of the continental crust. *Reviews of Geophysics* 33: 241–265.
- Thambavani S.D. and Mageswari T.S.R (2013). Metal Pollution Assessment in Ground Water. *Bulletin of Environment, Pharmacol and Life Sci* 2(12): 122-129.
- Tomlinson D. L, Wilson J. G., Harris C. R and Jeffrey D. W. (1980) Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. *Helgoländer Meeresuntersuchungen*, 33(1): 566.
- USEPA, United States Environmental Protection Agency (2011). National Primary Drinking Water Regulations. Retrieved from <http://water.epa.gov/drink/contaminants/index.cfm#List>.
- Vanloon G.W. and Duffy S.J (2005). The Hydrosphere. In: *Environmental Chemistry: A Global Perspective*. 2nd Edn. New York: Oxford University Press, 197-211.
- Vodala J.K, Renden J.A, Lenz S.D, MchellHenney W.H. and Kemppainen B.W (1997). Drinking water contaminants. *Poll. Sci*, 76: 1474-1492.
- WHO, World Health Organization (2008). Guidelines for drinking-water quality [electronic resource]: incorporating 1st and 2nd addende, 3rd Ed., Recommendations. Geneva. 1, 515.
- Zietz B.P, Lap J. and Suchenwirth R (2007). Assessment and management of tap water Lead contamination in Lower Saxon, Germany. *International Journal of Environmental Health Research* 17(6): 407-418.

Alterations in the levels of female sex hormones following administration of nicotine to Wistar rats

Gabriel Olaiya Omotoso^{1*}, R Eniola Kadir¹, Ade Stephen Alabi¹, Luqman Aribidesi Olayaki², Omowumi Femi-Akinlosotu³

^{1*}*Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria.*

²*Department of Physiology, College of Health Sciences, University of Ilorin;*

³*Department of Anatomy, College of Medicine, University of Ibadan, Nigeria*

Corresponding Email: gabrielolaiya@yahoo.com, omotoso.go@unilorin.edu.ng
Tel: +2347030505707

Abstract

The indiscriminate use of nicotine is a serious public health challenge worldwide. In this work, the effect of nicotine was studied on female sex hormones using adult female Wistar rats. Fifteen (15) animals were grouped into three equally as, Group A (Control, given 0.1 ml of normal saline), Group B (given 0.03 mg/kg nicotine), and Group C (received 0.06 mg/kg nicotine). All administration was via the intraperitoneal route, once daily for five (5) consecutive days. All the animals were sacrificed by cervical dislocation about 24 hours after the last treatment, and intracardial blood samples obtained. Using appropriate biochemical kits, the serum levels of follicle-stimulating hormone (FSH), luteinising hormone (LH), prolactin, oestrogen and progesterone were determined. The results showed a dose-dependent significant increase in serum levels of the gonadotropins and progesterone, and a marked increase in serum levels of oestrogen and prolactin in animals treated with low dose nicotine, but significantly low levels of these hormones were recorded in animals treated with higher dose of nicotine. Administration of nicotine affects the female sex hormones adversely, and this by extension could affect female reproductive activities.

Keywords: female rats, nicotine, sex hormones, gonadotropin, progesteron

Introduction

A very common source of exposure to nicotine is through cigarette smoking, which is a major risk factor for the development of many clinical conditions, including cardiovascular diseases, metabolic disorders, and the induction of a variety of

neoplasias (1-3). With increasing percentage of women smokers, the deleterious effects of tobacco smoking on female reproductive functions have been reported by various authors (2, 4, 5). Women smokers have a risk of impaired fertility (4-6), apart from other effects smoking has on other body organs and structures.

Tobacco contains thousands of constituents, out of which nicotine is a major component, and is responsible for many of the toxic effects of tobacco products. The possible mechanisms of the effect of cigarette smoking on fertility include its effects on angiogenic processes (7) and apoptosis (2). A direct inhibition of aromatase by nicotine and its alkaloids could also result in decreased oestrogen levels as seen in women who smoke (8).

Administration of nicotine induces oxidative stress, significantly promotes lipid peroxidation in ovarian tissue and significantly reduces ovarian antioxidant enzymes activities (9, 10).

Liu *et al.*, (11) studied *in vitro* maturation of bovine oocytes and observed that nicotine affects bovine oocyte cumulus cell expansion, maturation rate, and chromosomal complement in a dose-dependent and an oocyte-stage-dependent manner. Similarly, according to Li *et al.*, (12) nicotine affects oocyte maturation, and subsequently results in poor blastocyst development.

Follicular growth is a crucial step in normal ovulation and fertilization (2); nicotine induces granulosa cell apoptosis, which probably underlies some of the fertility disorders associated with cigarette smoking (2). Furthermore, ovarian steroidogenesis is altered by administration of nicotine, resulting in increased serum progesterone and decreased oestrogen levels (5, 13). In addition, nicotine also causes inhibition of androgen production by the *theca interna* (13). Studies by Patil *et al.*, (14) recorded reduction in number of follicles, uterine weight and diameter, thickness of myometrium and endometrium, and height of epithelium in albino rats treated with nicotine.

Aside its direct effect on adult women, the effect following prenatal exposures has also been studied in the offspring. Prenatal and postnatal exposure to nicotine has been observed by Holloway *et al.*, (5) to result in delayed ovarian dysfunction in female offspring. Studies in male offspring showed that if nicotine is given during pregnancy or lactation, it induces transient structural changes in the testis and epididymis of male offspring (15).

The current study looked at the effects of intraperitoneal nicotine administration on female sex hormones.

Materials and Methods

Experimental Animals

Fifteen (15) adult female Wistar rats of mean weight 188.00 ± 5.77 g were divided into three groups. The animals were housed in different cages and fed on standard rat feeds and water. Group A animals were given 0.1 ml of normal saline, serving as Control; Group B had 0.03 mg/kg nicotine in 0.05 ml vehicle; while Group C received 0.06 mg/kg nicotine in 0.1 ml vehicle. All administration was intraperitoneal, once daily for 5 consecutive days.

Specimen Collection

The animals were sacrificed by cervical dislocation about 24 hours after the last intraperitoneal administration, and blood samples were collected intra-cardially into plain specimen bottles (16) for onward hormonal assay, using sex hormonal assay kit from Monobind Inc[®], USA. A spectrophotometric method was adopted in determining the levels of follicle-stimulating hormone, luteinising hormone, prolactin, oestrogen and progesterone in the serum of experimental rats used.

Statistical Analysis of Data

Using SPSS software version 15.0, the data obtained were expressed as means $\pm$ SEM, and subjected to Student's t-test. The level of significance was taken at p values < 0.05 .

Results

The level of luteinising hormone (LH) significantly increased in the treatment groups compared with the Control ($p < 0.05$), with Group C which received a higher dose of intraperitoneal nicotine (0.06 mg/kg) having the higher level than Group B (0.03 mg/kg), and the difference between these two groups were statistically significant ($p < 0.05$) (Table 1).

The serum level of follicle-stimulating hormone (FSH) also increased in the treatment groups, but not as marked as serum LH; the increase was also statistically significant ($p < 0.05$) in the high dose group compared with the Control. However, only a marginal difference existed between the Control and Group B that received a lower dose of nicotine, and this was not statistically significant ($p > 0.05$). FSH level was higher in the high dose Group C than in the low dose Group B, with statistically significant difference ($p < 0.05$) (Table 1).

Serum prolactin decreased significantly ($p < 0.05$) in the higher dose Group C, but increased significantly ($p < 0.05$) in the low dose treatment Group B, compared with

the Control Group A. The difference in prolactin levels between Treatment Groups B and C was statistically significant ($p < 0.05$) (Table 1).

There was also increase in the serum level of progesterone in the treatment groups, which was significant when compared with the progesterone level in the Control animals ($p < 0.05$). Although the higher dose group had a higher level of the Progesterone hormone when compared with the low dose group, the difference was not statistically significant ($p > 0.05$) (Table 1).

Serum level of oestrogen significantly reduced in Group C, which received a higher dose of nicotine compared with Control Group A, while Group B treated with a lower dose had a very high oestrogen level compared with the Control ($p < 0.05$). The difference between the treatment groups was also significant ($p < 0.05$) (Table 1).

Discussion

A very common source of nicotine is tobacco, and its consumption has increased in some population, in form of cigarette smoking. Of concern is the increase in the percentage of women who smoke. The adverse effects of nicotine and other components of tobacco have been demonstrated in various organs and systems of the body. The current study however examined the effects on female sex hormones in relation to the reproductive functions of women. This work further confirmed earlier studies that showed a decrease in the level of oestrogen in women smokers (5, 8, 13). The higher dose of nicotine used in this study led to a significantly lower serum level of oestrogen compared with the Control, and surprisingly, the animals that received a lower dose had a higher level of oestrogen compared to the Control ($p < 0.05$).

Cigarette smoking adversely affects ovarian function. It is anti-oestrogenic, causing a direct inhibitory effect on granulosa cell aromatase activity, and also, less specific cytotoxic effects (8, 17). In the current study, the serum levels of progesterone were significantly raised in animals treated with nicotine in a dose-dependent fashion, contrary to a previous report where administration of nicotine caused a dose-dependent inhibition of progesterone synthesis (18). Even though the reason for the differences might not be very clear, studies by Holloway *et al* (5) on postnatal ovarian function following foetal exposure to nicotine, reported increased serum progesterone levels in the female offspring.

The gonadotropins and prolactin are peptide hormones, whose serum levels were altered in animals treated with nicotine. A dose-dependent increase in serum levels of LH was noted in this study, with a very marked increase in the high dose group, signifying the function of the hormone in stimulating the synthesis of oestrogen to increase the level of the latter. Usually, the control of hormone secretion is via a

negative feedback mechanism that ensures a proper level of hormone activity at the target tissue. However, in a few instances, positive feedback occurs when the biological action of the hormone causes additional secretion of the hormone. The stimulatory effect of oestrogen on the anterior pituitary before ovulation results in a surge of LH, and the latter further stimulates the ovaries to secrete more oestrogen, which in turn causes more secretion of LH. Eventually, LH reaches an appropriate concentration, and typical negative feedback control of hormone secretion is then exerted (19).

Given the various forms of hormonal alterations observed in female animals given intraperitoneal nicotine administration, there is the possibility of a resultant adverse implication on female reproductive function.

Reference

- Omotoso GO, Ibitolu JO, Femi-Akinlosotu OM, Akinola OB, Enaibe BU: Morphological and neurohistological changes in adolescent rats administered with nicotine during intrauterine life. *Niger. J. Physiol. Sci.* 28(2):147-151. 2013.
- [Bordel](#) R, [Laschke](#) MW, [Menger](#) M D, [Vollmar](#) B: Nicotine does not affect vascularization but inhibits growth of freely transplanted ovarian follicles by inducing granulosa cell apoptosis. [Human Reproduction](#) 21(3):610-617. 2005.
- Ueta E, Tadokoro Y, Yamamoto T, Yamane C, Suzuki E, Nanba E, *et al.* The effects of cigarette smoke exposure and ascorbic acid intake on gene expression of antioxidant enzymes and other related enzymes in the livers and lungs of Shionogi rats with osteogenic disorders. *Toxicological Sciences* 73:339-47. 2003.
- Iranloye BO, Bolarinwa AF: Effect of nicotine administration on weight and histology of some vital visceral organs in female albino rats. *Niger. J. Physiol. Sci* 24(1):7–12. 2009.
- Holloway A, Kellenberger L, Petrik J: Foetal and neonatal exposure to nicotine disrupts ovarian function and fertility in adult female rats. [Endocrine](#) 30(2):213-216. 2006.
- Hughes EG, Brennan BG: Does cigarette smoking impair natural or assisted fecundity? [Fertility and Sterility](#) 66:679-689. 1996.

Shiverick KT, Salafia C: *Cigarette smoking and pregnancy I: ovarian, uterine and placental effects. Placenta* 20:265–272. 1999.

[Barbieri RL](#), [Gochberg J](#), [Ryan KJ](#): [Nicotine, cotinine, and anabasine inhibit aromatase in human trophoblast in vitro. Fertility and Sterility](#) 92(2):798–805. 2009.

Chattopadhyay K, Chattopadhyay BD: Effect of nicotine on lipid profile, peroxidation and antioxidant enzymes in female rats with restricted dietary protein. *Indian J Med Res* 127:571-576. 2008.

[Sudheer AR](#), [Muthukumaran S](#), [Devipriya N](#), [Devaraj H](#), [Menon VP](#): Influence of ferulic acid on nicotine-induced lipid peroxidation, DNA damage and inflammation in experimental rats as compared to N-acetylcysteine. [Toxicology](#) 243(3):317-29. 2008.

Liu Y, Li G, Rickords LF, White KL, Sessions BR, Astin KI, *et al.* Effect of nicotine on *in vitro* maturation of bovine oocytes. [Animal Reproduction Science](#) 103(1–2):13–24. 2008.

Li G, Yang S, Liu Y, Sessions BR, White KL, Bunch TD: Nicotine combined with okadaic acid or taxol adversely affects bovine oocyte maturation and subsequent embryo development. [Fertility and Sterility](#) 92(2):798–805. 2009.

Sanders SR, Cuneo SP, Turzillo AM: Effects of nicotine and cotinine on bovine theca interna and granulosa cells. [Reproductive Toxicology](#) 16(6):795–800. 2002.

[Patil S](#), [Patil S](#), [Bhaktaraj B](#), [Patil SB](#): Effect of graded doses of nicotine on ovarian and uterine activities in albino rats. [Indian J Exp Biol.](#) 37(2):184-6. 1999.

Lagunov A, Anzar M, Sadeu JC, Khan MI, Bruin JE, Woynillowicz AK, *et al.* Effect of *in utero* and lactational nicotine exposure on the male reproductive tract in peripubertal and adult rats. [Reproductive Toxicology](#) 31(4):418–423. 2011.

Kadir RE, Omotoso GO, Enaibe BU, Caxton-Martins EA: Some Effects of *Cyperus Articulatus* on the Pituitary and Gonadotropic Hormones in Adult Female Wistar Rats. *Asian J Exp Biol Sci* 2(3):462-466. 2011.

Tanko LB, Christiansen C: An update on the anti-estrogenic effect of smoking: a literature review with implications for researchers and practitioners. *Menopause* 11:104–109. 2004.

Gocze PM, Freeman DA: Cytotoxic effects of cigarette smoke alkaloids inhibit the progesterone production and cell growth of cultured MA-10 Leydig tumor cells. *Eur J Obstet Gynaecol Reprod Biol* 93:77–83. 2000.

Guyton AC, Hall JE (1996): Textbook of Medical Physiology. 9th Edition. W.B. Saunders Company, Philadelphia.

Result

Table 1: Results of Serum Hormonal Assay (n=5 per group)

Groups		LH (mIU/ml)	FSH (mIU/ml)	Prolactin (muU/ml)	Progesterone (ng/ml)	Oestrogen (ng/ml)
A: Control		0.26±0.02	0.29±0.01	0.42±0.01	20.41±0.7	333.72±30.7
B: 0.03 mg/kg Nicotine	0.03	0.36±0.02*‡	0.29±0.01‡	0.63±0.02*‡	31.98±0.8*	720.56±20.9*‡
C: 0.06 mg/kg Nicotine	0.06	0.89±0.03*‡	0.37±0.01*‡	0.23±0.02*‡	33.09±0.5*	237.80±3.7*‡

*Statistically significant difference compared with the Control Group;

‡ Statistically significant difference between treatment groups (p< 0.05).

LH- luteinising hormone, FSH- follicle-stimulating hormone

***Plasmodium falciparum* Resistance in Selected Areas of Niger State, Nigeria: Chloroquine and Sulphadoxine/Pyrimethamine Treatment Outcome and Predictive Value of Molecular Markers**

¹*Nyamngee, A., ¹Edungbola, L. D., ¹Edogun H. A., ¹Ikpe, R. T., ²Agbendeh L. N. and ³Ighodalo, M.

¹*Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria.*

²*James P Grant School of Public Health, BRAC University, Dhaka, Bangladesh.*

³*Department of Pharmacology and Therapeutic, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria*

*Corresponding Author Email: [anyamngee2010@yahoo.com](mailto:nyamngee2010@yahoo.com);
nyamnge.a@unilorin.edu.ng

Abstract

Malaria is a public health problem affecting two third of the world population with high mortality among children and pregnant women. A study of the determination of drug-resistance molecular markers in *Plasmodium falciparum* infection was carried out in three malaria endemic local government areas of Niger State, Nigeria between October 2018 and September 2019. The objective of the study was to determine the prevalence of molecular markers of resistance to chloroquine and sulphadoxine/pyrimethamine. *P. falciparum* infected red blood cells (RBC) were confirmed using microscopy, and deoxyribonucleic acid extraction from *P. falciparum* positive RBC specimen was done using chelex extraction method. Nested polymerase chain reaction followed by Restriction Fragment Length Polymorphisms (PCR/RFLP) were used for the detection of *Plasmodium falciparum* chloroquine resistance transporter (*pfcr*t), *P. falciparum* multidrug resistance 1 (*pfmdr* 1), *P. falciparum* dihydrofolatereductase (*pfdhfr*) and *P.falciparum* dihydropteroate synthase (*pfdhps*). The results revealed well characterized molecular markers of *P. falciparum* resistance to the 4-aminoquinolines ($p<0.05$) while the antifolate drugs revealed high prevalence($p<0.05$) of resistance: 41%, 60%, 51% and 47% of *P. falciparum* isolates at codons N86Y, K76T, S108N, N51I and A437G respectively. The prevalence of resistance of isolates to these antimalarial drugs were comparatively high. Therefore,

strategies that reduce multiple-strain of malaria infections should be implemented in order to improve antimalarial drug efficacy and reduce rate of spread of drug resistance.

Key words: Molecular Markers, *Plasmodium falciparum*, Chloroquine and sulphadoxine/pyrimethamine resistance, Nigeria

Introduction

Malaria which occurs in over 106 countries is one of the most severe public health problems affecting about 200 million people globally and it is a leading cause of avoidable death especially among children and pregnant women in tropical countries (CDC, 2012).

Malaria used to be rife all over the world but since the 1950's it been eliminated from many regions including Europe, North America and Carribean (Sarah, 2011). However, it continues to be a huge social, economic and health problem particularly in the tropical countries, especially in Nigeria according to Demographic Health Surveys (DHS,2011). Malaria fever certainly arose in Africa and spread within Africa as nomadic lifestyle was abandoned for agriculture which accidentally created favourable habitats for mosquitoes to breed (Weiming *et al.*,2014). Trading and migration eventually brought malaria to China, India and Europe. By the medieval era, malaria was endemic throughout Africa, Europe, the Balkans to Southern Sweden and Southern Asia and parts of Russia (Sarah, 2011)

The classic symptom of malaria is cyclical occurrence of sudden coldness followed by rigor and then fever and sweating lasting for four to six hours, occurring every two days in *P.vivax* and *P.ovale* infections, while every three days for *P.malariae* and *P. falciparum*(*ref*). There could be recurrent fever every 36-48 hours or a less pronounced and almost continuous fever. Malaria has been found to cause cognitive impairments, especially in children(*ref*). It causes widespread anaemia during a period of rapid brain development and direct brain damage. This neurologic damage results from cerebral malaria to which children are more vulnerable. Cerebral malaria is associated with retina whitening, which may be a useful clinical sign in distinguishing malaria from other causes of fever (MacCormick *et al.*, 2014).

Severe malaria is almost exclusively caused by *P.falciparum* infection, and usually arise 6-14 days after infection. If untreated severe malaria can result to coma and death among young children and pregnant women. Splenomegaly, severe headache, cerebral ischemia, hepatomegaly (enlarged liver), hypoglycaemia, and hemoglobinuria with renal failure may occur. Renal failure is a feature of black water fever, where haemoglobin from lysed red blood cells leaks into the urine. Severe malaria can progress extremely rapidly and cause death within hours or days (WHO, 2013). In the most severe cases of the disease, fatality rates can exceed 20%,

even with intensive care and treatment. In endemic areas, treatment is often less satisfactory and the overall fatality rate for all cases of malaria can be as high as one in ten. Over the long term, developmental impairments have been documented in children who have suffered episodes of severe malaria (WHO, 2014).

Chloroquine was discovered by a German, Hans Andersag, in 1934 which he named resochin. In 1945, chloroquine replaced quinine as the first-line treatment for malaria due to low cost, straightforward synthesis and low toxicity. The first resistance to chloroquine was documented in 1957. The onset of chloroquine resistance marked the beginning of a new chapter in the history of malaria (Akshawa *et al.*, 2012). The emergence of drug resistance, widespread resistance to available insecticides, wars and massive population movements, difficulties in obtaining sustained funding from donor countries and lack of community participation made the long-term maintenance of the effort to eradicate malaria untenable. Completion of eradication campaign was eventually abandoned. The goal of most current National Malaria Prevention and Control Programs and most malaria activities conducted in endemic countries is to reduce the number of malaria-related cases and deaths. To reduce malaria transmission to a level where it is no longer a public health problem is the goal of what is called malaria control (CDC, 2016). According to Prugnolle and Yalcindag (2012), drug resistance is the reduction in the effectiveness of a medication in treating a disease or a condition. This is very rampant in the management of malaria including preventive therapy in its target population.

The genetic complexity of *P. falciparum*, particularly its ability to crossmate and generate mutant variants, makes it a successful pathogen. Genetic variants are involved in pathogenicity and in immune responses and have led to the emergence of resistance against virtually all available anti-malaria drugs including chloroquine and sulphadoxine/pyrimethamine. Such variants are under strong selective pressure, but analyses of sequence variation in gene segments that are not directly subjected to selection permit the study of inter population diversity of the parasite and its evolutionary origin (Pameta *et al.*, 2013).

Microsatellites (MS) are molecular markers that can provide valuable information on population structure in malaria parasite and are common in the *P. falciparum* genome. They can be defined as nucleotide repeats ranging between 2 and 6 base pairs (bp), one MS can be found in every kilo-base genome-wide. MS are highly polymorphic due to variation of length of these multiple alleles, and more neutral than single nucleotide polymorphisms (SNPs) among parasite isolates. Fortunately, mutations that are associated with drug resistance in *P. falciparum* provide a unique window to identify allelic associations because these must have occurred within the past 50 years (Daniel *et al.*, 2012). These drug resistant genotypes are characterized by reduced diversity around the major resistance alleles. In Asia and some African countries, microsatellite markers with multiple alleles flanking genes associated with drug resistance such as *dhf* and *pfcr* were investigated and allowed typing of

specific haplotypes flanking these regions thus enabling identification of drug resistance origin as well as tracing spread of these mutations (Okombo *et al.*, 2014). However, there have been no supporting literature of such study in Nigeria despite the high mortality, morbidity and public health concern of malaria in the country. Therefore, this study was aimed to determine the prevalence of molecular markers associated with *P. falciparum* resistance to chloroquine and sulphadoxine/pyrimethamine in three malaria endemic local government areas of Niger state, Nigeria.

Materials and Methods

This study was carried out in three Local Government Areas of Niger State, North Central Nigeria, located between latitude 8°00'-11°30'N and longitude 4°00'-8°00'E while sharing international boundary with the Republic of Benin to the West and inter-state boundaries with six Nigerian states. A descriptive cross sectional study was designed for this research work while subjects were selected randomly. The study population were Kambaris of Niger State who are adversely rural, uneducated, and underdeveloped. The target population were school pupils and community members in selected local government areas where 1,264 samples were screened for malaria and only 100 confirmed *Plasmodium falciparum* positive samples were used for molecular detection of mutations in drug targets.

Polymerase Chain Reaction for Detection of *Pfcr*t and *Pfmdr*1 Genes

The detection of mutations responsible for chloroquine resistance was performed by amplifying sequences marking the *Plasmodium falciparum* chloroquine resistance transporter (*pfcr*t) and *Plasmodium falciparum* multidrug resistance 1 (*pfmdr*1) genes using nested polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) according to previously described procedures (WHO, 2013). Primers used for *pfcr*t (K76T) and *pfmdr*1 (N86Y) primary amplification included curiously procuring template pattern (Crtp) 1 & 2, Mdr1 and Mdr2 and their sequences. The primary PCR components in a final volume of 15µl was buffered by 25mM MgCl₂ and 50mM deoxynucleotide triphosphate (dNTPs), 10µm of each of the primer (Crtp1, Crtp2, Mdr1 and Mdr2), 5µl mM deoxynucleotide triphosphate (dNTPs), 10µm of Taq polymerase and 2µl of DNA samples were then added. The cycling protocol for *pfcr*t was as follows: initial denaturation of 94°C for 3 minutes followed by 45 cycles of 94°C for 30 seconds, 60°C for 1 minute and 72°C for 15 minutes. For *pfmdr* 1 the cycling protocol was initial denaturation at 95°C for 5 minutes followed by 45 cycles of 95°C for 30 seconds, 45°C for 30 seconds, 65°C for 45 seconds and 72°C for 15 minutes.

Nested Polymerase Chain Reaction and Restriction (PCR) Fragment Length Polymorphism (RFLP) for *pfcr* and *Mdr* 1 mutation-specific detection

Secondary PCR was conducted by using the forward primer Crtd 1 and the reverse primer Crtd2. Two (2) µl of 10x dilution of primary PCR was used in a follow-up, nested, allele specific PCR amplification to detect the codon for *pfcr* K76T. The PCR stages for these diagnostic amplifications were initial denaturation at 94°C for 3 minutes followed by 45 cycles at 94°C for 30 seconds, 56°C for 30 seconds and 60°C for 1 minute and a final extension at 72°C for 15 minutes.

Purified genomic DNA from *P. falciparum* clones 3D7 (Chloroquine sensitive) and Dd2 (Chloroquine Resistant) were used as positive controls and water and uninfected blood spots on filter paper were used as negative controls. After amplifications, 20µl of the amplicons was incubated overnight at 50°C with mutation-specific restriction enzyme *Apo* I. In the PCR products, the DNA sequence was cleaved at the wild type (76K) codon site (if present) into two fragments, while the mutant alleles (76T) codon found in chloroquine-resistant *P.falciparum* were not cut. The digested products were separated by electrophoresis in a 2% agarose gel containing ethidium bromide and DNA was visualised by ultraviolet trans illumination. Similarly, amplification of codon 86 of the *pfmdr* 1 gene was carried out using the following primers: *Mdr* 1 and *Mdr* 2 for the primary PCR reactions and *Mdr* 3 and *Mdr* 4 for the secondary reactions after which restrictions with *Apo* 1 or *Afl*III was done. DNA fragments were compared by size and with the PCR products generated from genomic DNA of the 3D7 and Dd2 strains (used as references for susceptible and resistant genotypes respectively). Thermocycling conditions for *pfmdr*1 secondary reaction were initial denaturation at 95°C for 3 minutes, followed by 25 cycles at 95°C for 30 seconds, 45°C for 30 seconds and 65°C for 45 seconds and a final extension at 72°C for 15 minutes.

Polymerase Chain Reaction (PCR) Assay for *dhfr* and *dhps* genes

A nested PCR described by Pearce *et al*(2003) was used to amplify fragments of the *dhfr* and *dhps*. The detection of mutations responsible for sulphadoxine and pyrimethamine resistance was performed by amplifying sequences marking the *dhfr* and *dhps* genes using nested PCR followed by restriction fragment length polymorphism. The 15µl *dhfr/dhps* final PCR component consisted of buffer 10x, 50mM dNTPs, 25mM MgCl₂, 10µM of each of the primer, 5µ/µl of Taq polymerase and 2µl of template DNA. The cycling protocol was as follows: an initial denaturation of 95°C for 5 minutes, 40 cycles of 30 seconds at 95°C, 40 seconds at 52°C, 1 minute at 72°C and elongation at a final cycle of 10 minutes at 72°C.

Subsequently, a nested PCR was performed to increase the yields of the specific amplicons, 3µl of the primary PCR product was used in a reaction volume of 15µl with corresponding primers, the PCR mixture was the same as the primary reaction.

The cycling condition was previously described (Pearce *et al.*, 2003). The nested PCR products were confirmed by electrophoresis on a 2% agarose gel stained with ethidium bromide along with a set of controls.

The RFLP was performed by incubating the PCR products according to specification with corresponding restriction enzymes (*Alu* 1 for *dhfr* S108N/T; *Ava* II for *dhps* A437G; TSP 5091 for and *dhfr* N51I) and buffers obtained from the biopolymer factory, Soflinger Str. 100 D-89077 Ulm, Germany. The digested products were visualised by electrophoresis on 2% agarose gels and visualised by ultraviolet trans illumination.

Structured questionnaires were used to harvest demographic data (age, sex etc) from the pupils who participated in this study.

Data were finally analysed using the Statistical Package for Social Sciences (SPSS) version 21.

Ethical Considerations

Ethical clearance was sought and obtained from the Niger State Ministry of Health Ethical Review Committee (Ref. MOH/NS/EC/777/88/32). A letter of introduction which spelt out the purpose of the research work and benefits to be derived was taken to the Local Government Education Board, the Education Secretary, Area supervisors and Head Teachers of the various schools.

Results

Prevalence of molecular markers of *plasmodium falciparum* resistance to chloroquine and sulphadoxine/pyrimethamine

One hundred (100) selected *Plasmodium falciparum* isolates were typed with markers of Chloroquine (pfcrt and pfmdr 1) and Sulphadoxine/Pyrimethaxine (pfdhfr and pfdhps) resistance at five codons namely **K76T**, **N86Y**, **S108N**, **N51I** and **A437G**. Whereas the prevalence of Wild (sensitive) type alleles **N86** of pfmdr1 (59.0%) was higher than the mutant alleles **Y86** (41.0%), prevalence of mutant alleles **T76** (40.0%) of pfcrt was considerably higher than wild type alleles **K76**. At codons 51 and 108, the prevalence of mutant alleles **I51** (55.0%) and **N108** (51.0%) was higher than wild type alleles at the two codons. However, the differences were not statistically significant. At codon 437, the Wild type alleles **A437** (53%), was more represented and prevalent than the mutant alleles **G437** (47%). Twenty-nine percent and 16% of the isolates recorded double mutation and triple mutation at codons (**N108/ I51**) and (**N108/ I51/ G437**) respectively (Table 1).

Prevalence of molecular markers associated with *plasmodium falciparum* resistance to chloroquine and sulphadoxine-pyrimethamine in study population according to age

The ages of 100 participants for drug resistance typing ranges from 1year to above 30 and were grouped into four: 1-10years, 11-20, 21-30 and greater than 30. The age groups 1-10 and 11-20 were more represented being 62% and 24% respectively. The least representation was observed in the age groups 21-30 and > 30 being 9% and 5% respectively.

Of the five codons examined for the presence of mutant alleles, T76 and I51 of pfcr1 and pfdr1 had the highest overall prevalence of mutant alleles, followed by N108 and G437 of pfdr1. The least overall prevalence of mutant alleles was observed among Y86 of pfmdr1. The prevalence of mutant alleles by age showed an increasing rate of mutation with age in most of the codons but analysis of data by age categories did not discover any statistical difference ($p < 0.05$). (Table 2).

Prevalence of drug resistance marker by sex

Of the 100 isolates that were successfully typed by drug resistance markers, 44 were males while 55 were females. Thus females were more represented. While higher prevalences of mutant alleles was observed in both sexes at codons 76 and 51 of pfcr1 and dhfr respectively, lower prevalence of mutant alleles was observed in both sexes at codon 86 of pfmdr1. At codons 437 and 108 of dhps and dhfr respectively, males had higher prevalence of mutant alleles than females ($p > 0.05$). However, there is no significant difference in the distribution of mutant alleles in both sexes (Table 3).

Prevalence of drug resistant markers in relation to the local government areas of the participants

Of the three Local Government Areas where participants who consented to the study were drawn for participation, Borgu LGA had the highest representation, followed by Magama while Mashegu had the least representation. Both mutant and wild type alleles were discovered at all codons examined in all the L.G.A.S. In Mashegu, except for codon 86 of pfmdr1 in which the Wild type alleles was more prevalent (53.0%), analysis of other codons showed higher prevalence ($p < 0.05$) of mutant alleles whereas at Magama, higher prevalence of Wild type genotypes was observed at all codons except for codon 76 of pfcr1 in which mutant alleles had higher prevalence. However, the differences were not statistically significant ($p < 0.05$). At Borgu, prevalence of Wild type alleles was higher at codons 437 and 86 of dhps and mdr1 respectively whereas equal prevalences of both Mutant and Wild type alleles was observed at codon 108 of dhfr but at codons 51 and 86 of dhfr and pfcr1 respectively, higher prevalence of mutant alleles was observed (Table 4).

Table 1: Prevalence of drug resistant markers among confirmed *Plasmodium falciparum* infected Population

Drug Resistance Markers	% of Mutant Alleles	% of Wild-type Alleles
Pfcr (K76T)	60	40
Pfmdr 1 (N86Y)	41	59
Pfdhfr (S108N)	51	49
Pfdhfr (N511)	55	45
PfdhfrN108/I51	29	71
dhps(A437G)	47	53
dhfr/dhps N/108/I51/G437	16	84

Table 2: Prevalence of Molecular Markers Associated with *P. falciparum* Resistance to Chloroquine and Sulphadoxine-Pyrimethamine by Age

Age	% Examined (100)	No (%) of Mutant		Alleles Examined		
Group		T76	Y86	N108	I51	G437
1-10	62	36(58.1)	25(40.3)	29(46.8)	34(54.8)	30(48.4)
11-20	24	15(62.5)	10(41.7)	13(54.2)	13(54.2)	9(37.5)
21-30	9	6(66.7)	4(44.4)	5(55.6)	5(55.6)	4(44.4)
>30	5	3(60.0)	2(40.0)	4(80.0)	3(60.0)	4(80.0)
Total	100	60(60.0)	41(41.0)	51(51.0)	55(55.0)	47(47.0)

Table 3: Prevalence of Drug-Resistance Markers by Sex

Drug Resistance Markers	Males (44)	FEMALES (56)
	No.(%) of Mutant	No.(%) of Mutant
pfcr (T76)	26(59.1)	34(60.7)
pfmdr1(Y86)	15(34.1)	26(46.4)
pfdhfr(N108)	28(63.6)	23(41.1)
pfdhfr(I51)	24(54.5)	31(55.4)
pfdhfrN108/I51	13(29.5)	16(28.6)
pfdhps(G437)	23(52.3)	24(42.9)
dhfr/dhps N108/I51/G437	7(15.9)	9(16.1)

Table 4: Prevalence of Drug-Resistant Markers by LGA

Number examined by L.G.As	Pfcr 76		Pfmdr1 86		Pfdhfr 108		pfdhrt 51		Dhps437	
	Mutant (%)	Wild (%)	Mutant (%)	Wild (%)	Mutant (%)	Wild (%)	Mutant (%)	Wild (%)	Mutant (%)	Wild (%)
<u>Mashegu</u> (13)	10 (76.9)	3 (23.1)	6 (46.2)	7 (53.8)	8 (61.5)	5 (38.5)	9 (69.2)	4 (30.8)	8 (61.5)	5 (38.5)
<u>Magama</u> (29)	16 (55.2)	13 (44.8)	13 (44.8)	16 (55.2)	14 (48.3)	15 (51.7)	14 (48.3)	15 (51.7)	13 (44.8)	16 (55.2)
<u>Borgu</u> (58)	34 (58.6)	24 (41.4)	22 (37.9)	36 (62.1)	29 (50.0)	29 (50.0)	32 (55.2)	26 (44.8)	26 (44.8)	32 (55.2)
Total	60 (60.0)	40 (40.0)	41 (41.0)	59 (59.0)	51 (51.0)	49 (49.0)	55 (55.0)	45 (45.0)	47 (47.0)	53 (53.0)

Discussion

Resistance to Chloroquine

One of the factors to be considered in the prophylaxis, treatment and control of *P. falciparum* malaria is the resistance of parasite strains that may arise against every drug available with time. Chloroquine-resistant *P. falciparum* malaria was a major health problem, particularly in Sub-Saharan Africa that led to change in treatment policy (FMH, 2005). According to Atroosh *et al.* (2012), chloroquine resistance has been associated *in vitro* with point mutations in two genes, *pfcr* and *pfmdrl*, which encodes the *P. falciparum* digestive-vacuole transmembrane proteins and *p*-glycoprotein-mediated multidrug resistance (*Pgh1*), respectively. Identification of *pfcr* as the central determinant of chloroquine-resistant *P. falciparum* malaria provides a molecular marker that can be used for surveillance of resistance to evaluate drug treatment and prophylaxis policies. Analysis of well-characterized molecular markers of *P. falciparum* resistance to the 4-amino-quinolines and the antifolates revealed a high prevalence of resistant genotypes.

The T76 mutation which is associated with chloroquine resistant i.e the substitution of threonine (T76) for lysine (K76) at position 76 (K76T) of the amino acid sequence in *pfcr* which encodes a transporter protein of the *P. falciparum* digestive vacuole (ref) was found in 60.0% of samples with *P. falciparum* malaria infection and Y86 mutation (associated with resistance to amodiaquine, mefloquine, halofantrine and lumefantrine) that is the substitution of tyrosine (Y86) for asparagine (N86) at position 86 (N86Y) was found in 41.0%.

The high prevalence of resistance to chloroquine observed in this study indicates a persistence of resistance to chloroquine 10 years after the change of malaria treatment policy in Nigeria (FMH, 2005). The result is consistent with the findings from other parts of Nigeria and most regions of high drug resistance (; Micheal *et al.*, 2011; Ojurongbe *et al.*, 2011; Olasehinde *et al.*, 2014). This result is however in sharp contrast to findings from Malawi where plasmodium became susceptible to chloroquine after cessation of usage(ref). Also in Tanzania, chloroquine regained its sensitivity after two and half years of withdrawal of drug pressure(ref).

Chloroquine (CQ) and Sulphadoxine (SP) were replaced with artemisinin in 2005 due to widespread resistance and high level clinical failure across the country. It is expected that the sensitivity to CQ will be restored about 10 years after withdrawal as it happened in Malawi and Tanzania. Persistence of CQ resistance witnessed in this study might not be unconnected with poor drug policy in the country which allows the drug to be freely available for use outside government hospitals. Cross-resistance between CQ and amodiaquine(AQ) may also be a contributory factor due to the similarities in their modes of action. Amodiaquine is currently a partner drug with artemisinin in the treatment of malaria in Nigeria. Although this drug remains

effective in areas of substantial CQ resistance, the two drugs are chemically related and several clinical and *in vitro* reports have shown cross resistance between CQ and AQ or active metabolite of AQ (Micheal *et al.*, 2011).

Adagu and Warshurst (1999) observed that *pfcr*t K76 and the *pfmdr*1Y86 alleles are closely associated in chloroquine resistant strains. A similar observation was found in this study as all isolates carrying *pfmdr*1 mutated allele were also positive for *pfcr*t mutated allele. However, studies comparing the associations of the *pfmdr*1 variant and the *pfcr*tK76 variant have also shown that the impact of the *pfcr*t gene was stronger than that of the *pfmdr* gene (Ojurongbe *et al.*, 2007). It has been suggested that the degree of chloroquine resistance is further modulated by factors linked to genes other than *pfcr*t or *pfmdr* (Chen *et al.*, 2001). Consistent with other studies, one can assume that the prevalence of *pfcr*tT76 is a function of the actual chloroquine level and influence of the acquired immunity and natural resistance factors of the host. Furthermore, one can also suggest that chloroquine intake contributes essentially to the selection of the *pfcr*tT76 allele.

Regarding *pfmdr*1, the present study showed a predominance of wild type of *pfmdr*1 N86 (59%). The role of *pfmdr*1 gene mutations in anti-malarial drug resistance is still controversial. Previous *in vivo* studies reported a strong association between *pfmdr*1 86 in the chloroquine resistant infections (Von *et al.*, 1997). Similarly, a previous study by Zakeri *et al.* (2008) reported a strong association between *pfcr*tK76T but not *pfmdr*1 N86Y mutation *in vivo* chloroquine resistance. In contrast, a recent study reported an association with *pfcr*t gene (Andriantsoanirina *et al.*, 2010). Moreover, an *in vitro* study showed that *pfmdr*1 mutations in *P.falciparum* can confer resistance to high levels of chloroquine and that these *pfmdr*1 mutations have an important role in the resistance of *P.falciparum* to mefloquine and quinine (Reed *et al.*, 2000).

The high prevalence of wild alleles of *pfmdr*1 N86 could be considered as an indicator for low susceptibility of *P.falciparum* isolates to mefloquine, amodiaquine and quinine. On the other hand, previous studies have found increased sensitivity to the anti-malarials mefloquine and artemisinin in *P. falciparum* isolates with mutations in the *pfmdr*1 gene (Sidhu *et al.*, 2005). However, a previous *in vivo* study found that the treatment failure with mefloquine is significantly associated with increased *pfmdr*1 (Price *et al.*, 2004).

This current study did not discover any positive association in the prevalence of mutant alleles in relation to either sex or age of participants, although lower age had lower prevalence of mutant alleles T76 (58.1%) and Y86 (40.3%). However, the differences were not statistically significant, similar to the findings of Atroosh *et al.* (2012).

Resistance to sulfadoxine and pyrimethamine

Resistance to sulfadoxine and pyrimethamine was observed in this study among the isolates analysed for resistance markers, *pf dhfr*S108N/N51I and *pf dhps*A437G genes coding for pyrimethamine and sulfadoxine respectively. It has been earlier observed that resistance to pyrimethamine is primarily conferred by a non-synonymous point mutation at codon 108 and is consecutively enhanced by mutations at codons 51 and 59 of the *P.falciparum* *pf dhfr* gene located on chromosome 4 while point mutations at codon 437 and 540 of the *pf dhps* gene located on chromosome 8 of *P.falciparum* are considered responsible for sulfadoxine resistance (Sharma *et al.*2015).

Selection for sulfadoxine/pyrimethamine(SP) resistance markers in all the study areas remained high even after the replacement of SP as second-line treatment of uncomplicated malaria in 2005. A similar high prevalence of mutant *dhfr*and *dhps* alleles was recorded by Ojurongbe *et al.* (2011). in a study conducted at Osogbo, Oyo State, Nigeria. The result is also comparable to that of Agomo *et al.*(2016) and Matondo *et al.*(2014).

High prevalence of *dhfr* and *dhps* mutant alleles recorded in this study especially with the presence of double and triple mutant alleles is an indication that there is widespread sulfadoxine/pyrimethamine resistance and this is likely due to continued use of sulfadoxine/pyrimethamine for intermittent preventive therapy in pregnancy (IPTp). Sulfadoxine/pyrimethamine is readily available in both public and the private sector making its restriction to only IPTp impossible. In the current situation it is unlikely that self-medication with sulfadoxine/pyrimethamine can be prevented especially due to its low cost compared to artemisinin-based combination therapy (ACT), which may also explain the reason for the observed high prevalence of SP resistance markers despite its replacement with ACT. Use of artesunate/sulfadoxine/pyrimethamine combination is also another selection factor for sulfadoxine/pyrimethamine resistance markers. The continued use of pyrimethamine has been based partly on the belief that the drug retains activity against the liver stages of *P.falciparum* (Nahlen *et al*, 1989) . Several studies have also shown that although implementation of SP-IPTp does not prevent malaria infection during pregnancy, especially in the presence of high prevalence of SP-resistance markers (Gutman *et al.*, 2013), there is a significant protection against severe outcomes of pregnancy in malaria, such as low birth weight, maternal and fetal outcomes (Eisle *et al.*,2012) . This is the reason behind WHO's continued recommendation of SP for IPTp (WHO-MPAC 2013).

It is concluded that the high prevalence of molecular marker of chloroquine resistance (*pf crt*76) is suggestive of persistent drug pressure and continuing non efficacy of chloroquine for malaria treatment in Nigeria. And that the high prevalence of *dhfr* and *dhps* gene mutations recorded in this study is an indication that an alternative drug would be necessary in the near future for successful

implementation of IPTp in Nigeria. Therefore we recommend an urgent re-evaluation of the malaria treatment policy in Nigeria, and ensure effective regulation of the manufacture, importation, sale and use of chloroquine if the purpose behind its withdrawal would be realized. Finally, Regular assessment of the effectiveness of sulfadoxine and pyrimethamine among children and pregnant women is desirable.

Acknowledgements

We wish to acknowledge the Nigerian Institute for Medical Research (NIMR) for making available, the laboratory used for this study. We also acknowledge the school pupils, the teachers, parents/guardians, community members and all those who freely consented to this study. We thank the Local Government Chairmen, Community Leaders and the Education Secretaries of the Local Governments, for granting us permissions to carry out the study in the various study areas.

References

- Adagu, I.S and Warhurst, D.C. (1999). Association of *Cg2* and *Pfmdr1* Genotype with Chloquine Resistance in Field Samples of *Plasmodium falciparum* from Nigeria. *Parasiotology*199:343-348.
- Agomo, C.O., Oyibo, W.A., Sutherland, C., Hallet, R and Oguike, M. (2016). Assessment of Markers of Antimalarial Drug Resistance in *Plasmodium falciparum* Isolates from Pregnant Women in Lagos Nigeria. *Plos one*11(1). E0146908
- Akshaya, B.S., Nesrin, M.A and Chandra, B.S. (2012). Chloroquine-Resistance Malaria. *Journal of Scientific Research*3 (3): 11-14.
- Andriantsoanirina , V., Ratsimbaoa, A., Bouchier, C., Tichit, M., Jahevitra, M., Rabearimanana, S., Raherinjafy, R., Mercereau-Puijalon, O., Durand, R and Mernard, D.(2010). Chloroquine Clinical Failures in *Plasmodium falciparum* Malaria are Associated with Mutant *pfmdr-1*, not *pfcr1* in Madagascar. *Plos one*5: e13281. Doi: 10.1371.
- Atroosh, W.M., Al-Mekhlafi, M., Mohammed, A.M., and Johari, S. (2012). The Detection of *pfcr1* and *pfmdr 1* Point Mutations as Molecular Markers of Chloroquine Resistance in Pahang, Malaysia. *Malaria Journal*11: 251 doi: 10.1186/1475-2875-11- 257.
- CDC (2016). The history of Malaria, an Ancient Disease. Available at <https://www.cdc.gov/malaria/about/history.html>. Accessed on 22/05/2019.

- CDC (2012). Malaria Worldwide-Impact of Malaria. Available at www.cdc.gov/malaria/worldwide/impact.htm Accessed on 2/3/2019.
- Chen, Q., Schlichtherle, M and Wahlgren, M. (2001). Molecular Aspects of Severe Malaria. *Clinical Microbiology Reviews* 13 (3): 439-450.
- Daniel, J.P., Amanda, K.L., Daniel, E.N., Stephen, F.S., Chang, H.H., Clarissa, V., Ribiaek, U., Diara, V.T., kelvin, G., Meghan, G., Justin, S.B., Daouda, N., Souleymane, M., Roger, C.W., Daniel, L.H., Pardis, C.S., Dyann, F.W and Sarah,
- K.V. (2012). Sequenced-Based Association and Section Scans Identify Resistance Loci in the *Plasmodium falciparum* Malaria Parasite. *Proceedings of the National Academy of Sciences of the United States of America* 102(32): 13052-13057.
- DHS. (2011). Demographic and Health Survey. Malaria Situation in Nigeria USAID President's Malaria Initiatives FY 2012 Malaria Operational Plan 2011.
- Eisele, T.P., Larsen, D.A., Anglewicz, P.A., Keating, J., Yukich, J., Bennett, A., Gutman, J., Mwandama, D., WEigand, R.E., Ali, D., Mathanga, D.P. and Skarbinski, J. (2013). Effectiveness of Intermittent Preventive Treatment with Sulfadoxine-Pyrimethamine during Pregnancy on Maternal and Birth Outcomes in Machinga District, Malawi. *Journal of Infectious Diseases*. 208: 907-916.
- FMH (2005). Federal Ministry of Health. National Antimalarial Treatment Policy. Available at [#http://app.who.int/medicinedocs/documents/s18401en/s18401en.pdf](http://app.who.int/medicinedocs/documents/s18401en/s18401en.pdf). Accessed on 23/04/2018.
- Hutchinson, P and Steketee, R.W. (2012). Malaria prevention in Pregnancy, Birth Weight and Neonatal Mortality: A Meta-analysis of 32 National Cross-Sectional Datasets in Africa. *Lancet of Infectious Diseases*. 12 (12): 28-33.
- MacCormick, J.C., Nicholas, A.V., Terrie, E.T., Valentine, B., Valerie, A., Paul, H., Malcom, E.M., Baljean, D and Simon, P.H. (2014). Cerebral Malaria in Children Using Retina to Study the Brain. *Journal of Neurology*. 10 (226). Doi <http://dx.doi.org/10.1093/brain/awu001>.
- Matondo, S.I., Godfrey, S.T., Adelaida, A.K., Julius, S.K., Akili, K., Marco, Z., Hugh, R and Reginald, A.K. (2014). High Levels of Sulphadoxine-Pyrimethamine Resistance *pf dhfr-pf dhps* Quintuple Mutations: A cross

Sectional Survey of Six Regions in Tanzania. *Malaria Journal*.10:276.doi: 10.1186/1475-2875-10-276.

- Michael, E., Tamramat, R.a., Beniam, G., Wolfgang, K and Brigitte, K. (2011). Prevalence of Molecular Marker of Chloroquine Resistance (*Pfcr* 76) in Nigeria 5 Years After Withdrawal of the Drug as a First-line Antimalarial: A Cross sectional study. *South-African Journal of Child Health* 5(2):39-42.
- Nahlen, B.L., Alakija, T., Ogunbode, O., Adetoro, O., Akintunde, A., Nguyen-Dinh, P., Edungbola L.D. and Breman, J.G. (1989). Lack of Efficacy of Pyrimethamine Prophylaxis in Pregnant Women. *The lancet*.7:830-834.
- Ojurongbe, O., Fagbenro-Beyioku, A.F., Deyaba, O.A., Kun, J.F. (2011). Allelic Diversity of Merozoite Surface Protein 2 Gene of *Plasmodium falciparum* among Children in Osogbo, Nigeria. *West Indian Medical Journal*.60(1).
- Ojurongbe, O., Ogungbamigbe, T.O., Fagbenro-Beyioku, A.F., Fendel, R., Kremsner, P.G and Kun J.F. (2007). Rapid Detection of *Pfcr* and *Pfmdr* 1 Mutations in *Plasmodium falciparum* Isolates by FRET and *in-vivo* Response to Chloroquine among Children from Osogbo, Nigeria. *Malaria Journal*6:41.
- Okombo, J., Kamau, A.W., Kelvin, M., Sutherland, C.J and Ochola-Oyier, L. (2014). Temporal Trends in Prevalence of *Plasmodium falciparum* Drug Resistance Alleles Over Two Decades of Changing Antimalarial Policy in Coasta Kenya. *International Journal of Parasitology: Drugs and Drug Resistance*.4(3):152-163.
- Olasehinde, G.I., ojurongbe, O.O., Fagade, E.O., Ruchi, S., Egwari, L.O., Ajayi, A.A and Adeyaba, O.A. (2014). Detection of Molecular Markers of Antimalarial Drug Resistance in *Plasmodium falciparum* From South-western Nigeria. *Covenant Journal of Physical and Life Sciences (CJPL)*.1 (2):1-15.
- Pameta, O.S., Michelle, C.B and Marcelo, U.F. (2013). Microsatellite Analysis of Malaria Parasites. *Methods in Molecular Biology*.1006:247-258.
- Pearce, R., Drakeley, C., Chandramohan, D., Mosha, F and Roper, C. (2003). Molecular Determination of Point Mutation Haplotypes in the Dihydrofolate Reductase and Dihydropteroate Synthase of *Plasmodium falciparum* in Three Districts of Northern Tanzania. *Antimicrobial Agents and Chemotherapy*.47:1347-1354.

- Price, R.N., Uhlemann, A.C., Brockman, A., McGready, R., Ashley, E., Phaipun, L., Patel, R., Laing, K., Looareesuwan, S., White, N.J., Nosten, F and Krishna, S. (2004). Mefloquine Resistance in *Plasmodium falciparum* and Increased *Pfmdr 1* Gene Copy Number. *Lancet*.364: 438-447.
- Prugnolle, F and Yalcindag,, E. (2012). Multiple Independent Introductions of *Plasmodium falciparum* in South America: How Did it Reach The America. *Proceedings of the National Academy of Sciences*. 109 (2):511-516.
- Reed, M.B., Saliba, K.J., Caruana, S.R., Kirk, K and Cowman, A.F. (2000). *Pgh 1* Modulates Sensitivity and Resistance to Multiple Antimalarials in *Plasmodium falciparum*. *Nature* 403:906-909.
- Sarah, K (2011). A year Round Resource to Count Malaria Out. World Malaria Day 2012. Available at <http://www.worldmaliaday.org/worldmaliaday-live> Accessed on 11/11/2018
- Sharma, D., Manila, L., Prashant, K.M., Tridibes, A., Amita, S.D., Neena, V and Singh, O.P. (2015). Polymorphism in Drug Resistance Genes Dihydrofolate Reductase and Dihydropterase Synthase in *Plasmodium falciparum* in Some States of India. *Parasites and Vectors*8: 471 doi.10.1186/13071.
- Sidhu, A.B., Valderramos, S.G and Fidock, D.A. (2005). *Pfmdr 1* Mutations Contribute To Quinine Resistance and Enhance Mefloquine and Artemisinin Sensitivity in *Plasmodium falciparum*. *Journal of Molecular Microbiology*.5 (57): 913-926.
- Von, S., Duraisingh, M.T., Drakeley, C.J., Bailey, R., Greenwood, B.M and Pinder, M. (1997). Polymorphism of the *Pfmdr 1* Gene and Chloroquine Resistance in *Plasmodium falciparum* in the Gambia. *Transaction of Royal Society of Tropical Medicine and Hygiene*.91:450-453.
- Weiming, L., Yingying, L., Katharina, S., Gerald, H., Lindsey, J., Plenderleith, J., Malenke, S., Sundararaman, M., Patricia, A and Crystal, A. (2014). African Origin of Malaria Parasite. *Nature Communications*. 5;doi 10.1038/ncoms 4346.
- W.H.O-MPAC (2013). Malaria Policy Advisory Committee to The W.H.O: Conclusions And Recommendations of March 2013 Meeting. *Malaria Journal*.12:213.

- W.H.O. (2013). World Malaria Report 2013. Available at http://www.who.int/malaria/publications/world_malaria_report_2013/report/en. Accessed 13/05/2018.
- W.H.O. (2014). Insecticide-Treated Nets to Reduce the Risk of Malaria in Pregnant Women. Available at http://www.who.int/elena/titles/bednets_malariapregnancy/en/ Accessed on 15/05/2019.
- Zakeri, S., Afshar, M., Kazemzadeh, T., Mehdizadeh, K., Shabani, A and Djadid, N.D. (2008). Association of *Pfcr* But not *Pfmdr* 1 Alleles with Chloroquine Resistance In Human Isolates of *Plasmodium falciparum*. *American Journal of Tropical Medicine and Hygiene*. 78:633-640.

Sedimentological, Depositional environment and Sequence stratigraphic studies of OML AB field, Southeastern Niger Delta, Nigeria

Ibrahim, O.I.¹, Adekeye, O.A.², Bale R.B.² and Babatunde, W.S.²

¹*Lower Niger River Basin Development Authority, P.M.B 1529, Ilorin, Kwara State.*

²*Department of Geology and Mineral Sciences, University of Ilorin, Ilorin, Kwara State.*

Corresponding author is ibroibrahim72@gmail.com Phone no 08030445019

Abstract

Geoscientific studies of these wells were done to reveal the sediment types, sediment geometry, depositional environment, depositional sub-environments, tracts delineation, sequence boundaries, maximum flooding surfaces, parasequence sets and stacking patterns of the wells deposit. OML AB field is in southeastern Niger Delta, Nigeria. It covers approximately 568 km² in area. It is located around Latitude 4° 18' N and 4° 42' N to Longitude 6° 6' and 7° 36' E. From the base to the top of the section, it consists essentially of paralic sequence overlain by clastic deposit. Lithologies penetrated by the wells were determined by the gamma ray log to map the sedimentary facies, while the resistivity well log allowed the fluid content study of the wells. Gamma ray and resistivity logs were generated in the study area from the well and the data analysis revealed the sequence boundaries and maximum flooding surfaces to be SB1_BPS, SB2_BMS, SB3_BQC, MFS1 and MFS2. Lithologies of the wells were determined and found to be more of sandstone facies, with interbeds of minor shale and mudstone facies. 14 HST's and 9 TST's were recorded across the 5 wells of study and ranges from 3000-8403 and 3450-7093 feet respectively. 24 Parasequence sets were determined across the study wells with 4-6 set recorded per well. 2 depositional systems of Prograding and Backstepping shorelines were identified in the study with other sub-environments of tidal coastal plain, upper shoreface, lower shoreface and proximal offshore of the block well sediments. The tidal coastal plain consist of barrier bar, mouth bar and beach ridge sediments of coarse grained sandstone bodies. Geology of the area revealed more sandstone facies with interbed of shale lithology, the sedimentary facie geometry types studied include coarse, medium and fine grained sandstone of the tidal coastal to the upper and lower shoreface sub-environments that serve as good reservoir targets.

Keywords: *Parasequence, Highstand System Tract, Transgressive System Tract and shoreface*

Introduction

Geoscientists have shown with various evidences that Niger Delta basin has spectacular thick, extensive sedimentary apron and salient geological and geophysical features favorable for petroleum generation, expulsion and trapping from the onshore through the continental shelf to the deep and ultradeep offshore terrains. With Geological and Geophysical techniques becoming viable tools conventionally adopted for the search of hydrocarbon, underground water and solid minerals to meet up with the current trend of increasing global demand, then one of these sedimentary basins (Niger Delta basin) that constitute the habitat for these resources should be explored for the natural resources that will be sustainable for mankind. The underlying tenet of the sequence stratigraphy is that a change in base level (usually relative sea level) results in a change in the patterns of sedimentation in almost all depositional environments of geologic deposit that harbor the fluid content as proposed by Kendal (2003,2004 and 2008).

With the recognition of the sediment pattern in the deposits produced by, say, a rise in sea level in a coastal plain, a beach, a shallow shelf and the deep sea, then it should be possible to correlate using these patterns across the study wells as in the OML AB field using geophysical well logs and other models for the basic sequence stratigraphic components of system tracts, sediment types, geometry, eustatic sea level changes, accommodation space, shoreline trajectory, facies pattern dynamics, parasequence sets and many more as proposed by Ibrahim et al (2019). For this study, data from 5 wells of KTM-1, ADN-1, EB-1, EB-2 and MB-2 wells were made available.

Location of the Delta and study areas

The Niger Delta occupies the southern end of Nigeria bordering the Atlantic Ocean. In the West, this basin is separated from the Dahomey Basin by the Okitipupa basement high, and in the east, it is bounded by the Cameroon volcanic line. Its northern margin transects several older (Cretaceous) tectonic elements such as the Anambra basin, Afikpo Syncline and the Abakaliki Anticlinorium (Fig 1). It is believed that the modern Niger Delta stratigraphy was formed in the early Tertiary and that sediments began to accumulate in this region during the Mesozoic rifting associated with the separation of the African and South American continents. Weber and Daukoru (1975). From the Eocene to the present, the delta has prograded extensively. This has led to the formation of fracture elements like chain, charcourt and romanche fracture systems that extends to Dahomey basin in southwestern Nigeria (Fig. 1).

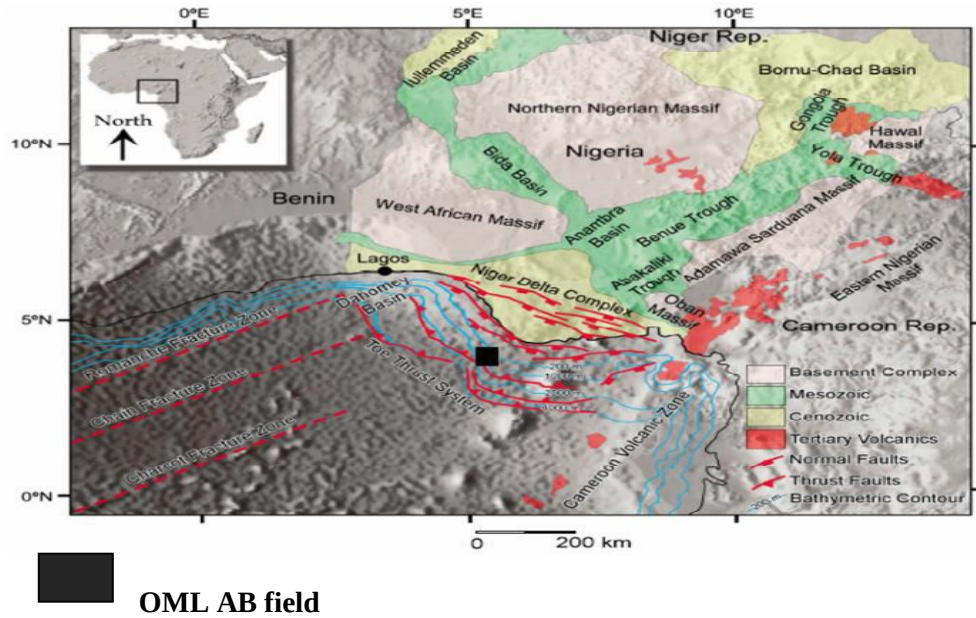


Fig. 1: Geological map of Niger Delta showing fracture trends (Modified from Wiener et al., 2010).

Geology of the basin and study areas

The growth of the Niger Delta started during the Eocene in response to the positive epeirogenic movements along the Benin and Calabar flanks. In the mid-Eocene, a major sea regression started which accelerated the expansion of the Niger Delta, leading to regression that gave rise to the formation of the present Niger Delta in Mid-Eocene time. From the initial 3 depocenters (Fig. 2 and Table 1) that are 30-60 kilometers wide and prograded southwestward 250 kilometers over oceanic crust into the Gulf of Guinea, as published by Stacher (1995) and Ejedawe (1981, 1984 and 1989), the delta has grown to consist of 6 depocenters as sites of great accumulation for deposits of varying geologic ages (Table 1).

Table 1: Identified depobelts of geologic deposit in Niger Delta

DEPOBELTS	AGE OF PARALLIC SEQUENCE	AGE OF ALLUVIAL SANDS
Northern Delta	Late Eocene to Early Miocene	Early Miocene
Greater Ughelli	Oligocene to Early Miocene	Early Miocene
Central Swamp I	Early to Middle Miocene	Middle Miocene
Central Swamp II	Middle Miocene	Middle Miocene
Coastal Swamp I and II	Middle to late Miocene	Late Miocene
Offshore	Late Miocene	Late Miocene

Each depobelt is a separate unit that corresponds to a break in regional dip of the delta and is bounded landward by growth faults and seaward by large counter-regional faults or the growth fault of the next seaward belt. Evamy et. al (1978). Six depobelts were later recognized in Tertiary Niger Delta, each with its own sedimentation, deformation, and petroleum play history (Fig. 2 and Table 1). The six depocenters include the Northern Delta Depobelt, Greater Ughelli depobelt, Central Swamp I and II depobelts, Coastal Swamp I and II depobelts and offshore depobelt, parts of which are illustrated in (Fig. 1) Doust and Omatsola (1990). On the other hand, the depobelts of OML AB field include Afam, Opuoma and Qua-iboe channels dated late Miocene to early Pliocene (Fig. 2 and Table 2). They represent the sites of accumulation of the investigated deposits of the wells in the OML AB field.

Table 2: Identified depobelt of geologic deposit in OML AB field

S/N	Depobelts	Age
1	Afam channel	Early Pliocene
2	Opuama channel	Early Pliocene
3	Qua-Iboe	Late Miocene

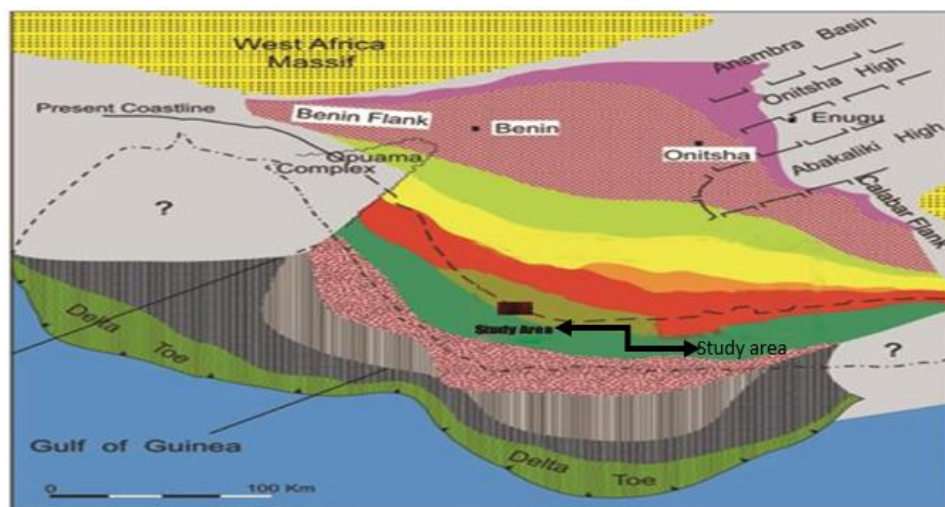


Fig. 2: Depobelt map of Niger Delta deposit showing the study area of OML AB field (Modified from Ekweozor and Daukoru, 1994).

Materials and Methodology

The materials utilized in this study include the generated gamma ray and resistivity logs of 5 wells in the study area. These data were loaded into the petrel software for further geologic interpretations. Developed models were constructed based on sedimentological principle of the study area to explain the different geologic and geophysical characteristics at play. The technique of making geophysical measurements in the subsurface earth formations through the drilled wells can be done better with the use of well logs, in order to determine both the physical properties of the rocks and the fluid they contain. The properties were interpreted in terms of flooding surfaces, system tracts analyses, parasequence sets and others based on the log patterns.

Data interpretation and Discussions:

Sequence boundaries, maximum flooding surfaces and System tracts

Marine Flooding surfaces were the best markers or datum on which the correlation cross sections were adopted. Correlation was done to determine lateral continuity or discontinuity of facies, hence aiding facies geometry and reservoir studies in the wells of the block. Correlation was done using the recognized and identified constrained chronostratigraphic surfaces typified by Maximum Flooding Surfaces (MFSs) and Sequence Boundaries as advanced by Van Wagoner et al (1990) and Reijers et al (1997). Worthy of note is that in the KTM-1 well, its continuous and discontinuous sequence boundaries are BB, SB3_BQC, SB2_BMS and SB1_BPS at

1670, 2006, 3148 and 5927 feet respectively. The well's gamma ray and resistivity log sequences revealed interbeds of shale and sandstone facies. The gamma ray log showed more deflections to the right of the baseline than the left side, with maximum peaks recorded at 2008, 3353 and 6453 feet to show good reservoir sand units of the well and low to moderate peaks noticed at most intervals in the log pattern. With more sandstone reservoirs delineated across the sequence than the shale lithology. KTM-1 well has 3 HST and 2 TST in its well. HST 1 has a depth ranges from 3001 to 3453 feet, HST 2 ranges from 3699-5650 feet, HST 3 ranges from 5949-7850 feet, TST 1 and TST 2 ranges from 3447-3801 and 5651-5953 feet respectively. It is dominated by funnel gamma ray log shape (Table 1).

Table 1: System tracts and other key sequence stratigraphic structure of KTM-1 well.

KTM-1 WELL				
SYSTEM TRACTS	DEPTH INTERVAL(FT)	KEY SURFACES	AGE	DOMINANT GAMMA-RAY LOG SHAPE
HST 1	3001-3453	SB3_BQC	Early Pliocene	SERRATED
HST 2	3700-5650	SB2_BMS	Early Pliocene	
HST 3	5950-7850	SB1_BPS	Late Miocene	
TST 1	3447-3801	MFS2	Early Pliocene	
TST 2	5650-5953	MFS1	Early Pliocene	

ADN-1 well revealed its sequence boundaries are SB3_BQC, SB2_BMS and SB1_BPS at 3321, 5261 and 7291 feet respectively. Gamma and resistivity logs data were available for a better picture in this well and the gamma ray response was interpreted to be having more sandstone with deflections to the left with less radioactive elements. Moderate clay and shale mapping with deflections to the right where more radioactive elements are present. The deflection pattern of this well showed increase in mud, shale content of the well down the depositional sequence. The well showed a highly faulted block, as such, the sequence boundaries are at different depths of occurrence. Its gamma ray log showed deflections to the right and left side of the baseline, almost on equal basis, signifying the more dominance of shale, mudstone lithologies than sandstone facie. Of more importance is the low deflection at 3350 feet that coincides with a high resistivity spike and SB3_BQC surface, this shows the presence of reservoir fluid of interest ie hydrocarbon at the depth. At 5150 feet, it was noted that the SB2_BMS surface passed through the

deposit to enhance the porosity and permeability of the well's deposit better. ADN-1 well has 2 HST and 1 TST in its deposit. HST 1 has a thickness ranging from 3350-4807 feet, HST 2 has a depth thickness ranging from 5150-6900 feet and TST 1 ranging from 4900 to 5150 feet (Table 2).

Table 2: System tracts and other key sequence stratigraphic structure of ADN-1 well.

ADN-1 WELL				
SYSTEM TRACTS	DEPTH INTERVAL(FT)	KEY SURFACES	AGE	DOMINANT GAMMA-RAY LOG SHAPE
HST 1	3350-4807	SB3_BQC	Early Pliocene	FUNNEL
HST 2	5150-6900	SB2_BMS	Early Pliocene	
TST 1	4807-5150	MFS2	Early Pliocene	

EB-1 well is just 8721 meter to the ADN-1 well (Fig. 3 and Table 3) with its sequence boundaries are SB3_BQC, SB2_BMS and SB1_BPS at 4129, 5126 and 7021 feet respectively. The well showed a highly faulted block, as such, the sequence boundaries are at different depths of occurrence (Table 3). Its gamma ray log showed deflections to the right and left side of the baseline, almost on equal basis, signifying the more dominant of shale, mudstone lithologies than sandstone facie. Of more importance is the low deflection at 3350 feet that coincides with a high resistivity spike and SB3_BQC surface, this shows the presence of reservoir fluid of interest ie hydrocarbon at the depth interval. At 5150 feet it was noted that the SB2_BMS surface passed through the deposit to enhance the porosity and permeability of the well deposit better. EB-1 well has 3 HST and 2 TST. HST 1 ranges from 4100-5400 feet, HST 2 ranges from 5550 to 6801 feet, HST 3 ranges from 7011 to 8403 feet. TST 1 ranges from 5400 to 5550 feet and TST 2 ranges from 6801 to 7011 feet. It is mostly cylindrical in gamma ray log shape pattern (Table 3).

Table 3: System tracts and other key sequence stratigraphic structure of EB-1 well.

EB-1 WELL				
SYSTEM TRACTS	DEPTH INTERVAL(FT)	KEY SURFACES	AGE	DOMINANT GAMMA-RAY LOG SHAPE
HST 1	4100-5400	SB3_BQC	Early Pliocene	CYLINDRICAL
HST 2	5550-6801	SB2_BMS	Early Pliocene	
HST 3	7011-8403	SB1_BPS	Late Miocene	
TST 1	5400-5550	MFS 2	Early Pliocene	
TST 2	6801-7011	MFS 1	Early Pliocene	

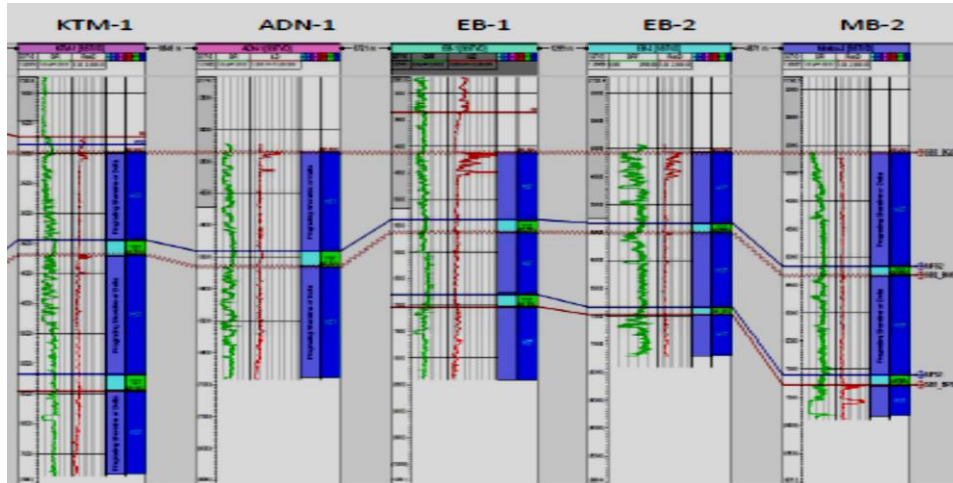
EB-2 well has its Maximum flooding surface of SB1_BPS at a depth of 6950 feet dated to be Late Miocene and sequence boundaries are SB3_BQC, SB2_BMS and SB1_BPS at 4211, 5473 and 7033 feet respectively. The gamma ray log available in the EB-2 showed a consistent deflection of the spikes to the left than the right side of the baseline, interpreted to be of more shale and mudstone units than the interbeds of sandstone lithology with high amount of radioactive elements. Of great interest is deflection at 4200 feet with low to moderate intensity of gamma ray deflection coinciding with high resistivity value at that depth and its fracture better modified with SB3_BQC surface (Fig. 4.22). This area serve as a potential reservoir position in the well of study that could host commercial accumulations of hydrocarbon in a possible dominant mudstone or shale sediment. Of another interest is moderate gamma ray value delineating sandstone lithology at 5500 feet with SB2_BMS surface noticed at that depth. Shale and mudstone are more in this well. Worthy of note is the last point at 7033 feet where SB1_BPS was evaluated to have a low gamma ray and resistivity values and could also serve as a good point reservoir of interest.

EB-2 well has 3 HST and 2 TST. HST 1, HST 2 and HST 3 have depth ranging from 4100-5600, 5450-6850 and 7029-7700 feet respectively. TST 1 and TST 2 have depths ranging from 5350-5450 and 6850-7029 feet respectively. MB-2 has 3 HST and 2 TST. HST 1, HST 2 and HST 3 have depth ranging from 3150-5151, 5300-

7093 and 7250-7903 feet respectively. TST 1 and TST 2 have depths ranging from 5151-5300 and 7093-7250 feet respectively (Table 4).

Table 4: System tracts and other key sequence stratigraphic structure of EB-2 well.

EB-2 WELL				
SYSTEM TRACTS	DEPTH INTERVAL(FT)	KEY SURFACES	AGE	DOMINANT GAMMA-RAY LOG SHAPE
HST 1	4100-5600	SB3_BQC	Early Pliocene	SERRATED
HST 2	5450-6850	SB2_BMS	Early Pliocene	
HST 3	7029-7700	SB1_BPS	Late Miocene	
TST 1	5350-5450	MFS 1	Early Pliocene	
TST 2	6850-7029	MFS 2	Early Pliocene	



- Highstand System Tract (HST)
- Transgressive System Tract (TST)

Fig. 3: Tract delineation and surface correlation of the investigated wells in OML AB field.

MB-2 well is the last well laterally correlated and studied in this field of deposit (Fig. 3) with sequence boundaries of SB3_BQC, SB2_BMS and SB1_BPS at 3326, 5363 and 7338 feet respectively. It is worthy of note that the gamma ray log showed a very consistent mudstone or shale lithology that acts as reservoir in the well with its low to moderate intensity of deflections to the right of the baseline. Of notable interest is at 3326 feet with SB3_BQC surface delineated. Also, at 5350 feet shows a moderate deflection of the gamma ray. With a moderate gamma ray deflection and very high resistivity deflection at 7338 feet shows the occurrence of thick reservoir mudstone units that could be a good fracked reservoir at the depth. MB-2 is the last well studied for the tract analysis. It consists of 3 HST and 2 TST, with the HST 1 bounded at the top and bottom by SB3_BQC and MFS2 respectively with sediment thickness ranging from 3150 to 5151 feet. It is succeeded by the TST that is having a thickness that ranges in value from 5151 to 5300 feet. HST 2 with a depth thickness ranging from 5300 to 7093 feet is bounded at the top by SB2_BMS and MFS1 respectively. It is in turn succeeded by the TST 2 with sediment thickness that ranges from 7093 to 7250 feet.

The HST 3 which is the last and the oldest at the bottom of the depositional sequence of this well showed a thickness from 7250 to 7903 feet. Gamma ray shape in this well include serrated and funnel, with funnel being dominant (Table 5).

Table 5: System tracts and other key sequence stratigraphic structure of MB-2 well.

MB-2 WELL				
SYSTEM TRACTS	DEPTH INTERVAL(FT)	KEY SURFACES	AGE	DOMINANT GAMMA-RAY LOG SHAPE
HST 1	3150-5151	SB3_BQC	Early Pliocene	FUNNEL
HST 2	5300-7093	SB2_BMS	Early Pliocene	
HST 3	7250-7903	SB1_BPS	Late Miocene	
TST 1	5151-5300	MFS 2	Early Pliocene	
TST 2	7093-7250	MFS 1	Early Pliocene	

Parasequence sets:

Each of the system tracts (Figs. 4 and 5) of OML AB field consists of several separate packages of strata, describing its facies (Figs. 6 and 7). In detail, each package is itself a cycle of beds showing progradational and retrogradational patterns and these depositional cycles are known as parasequences of the field

deposit (Fig. 4). The system tracts are made up of 1 or more parasequence sets while the parasequence sets are made up of 1 or more tracts. All sets in the same tract showed same trend. The packages of its beds revealed they developed as a consequence of the variation of the accommodation space that was then filled up by variation of high and low amount of sediments supply during the transgression and regression episodes of eustatic sea level changes. As such, progradational parasequence and retrogradational parasequence stacking patterns developed with coarsening and finning upward depositional sequences respectively (Figs. 4, 5, 6 and 7).

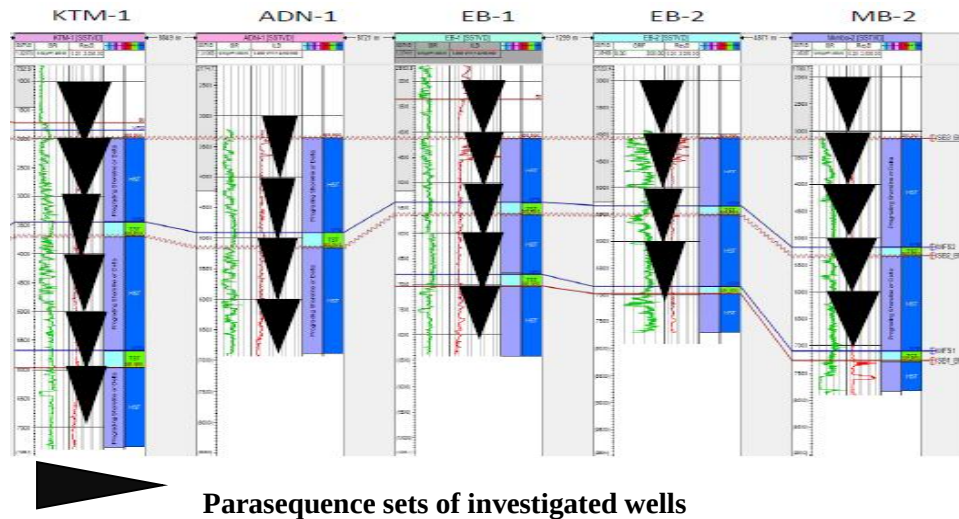


Fig. 4: Parasequence sets of OML AB field showing other sequence stratigraphic structures

KTM-1 well was studied to consist of 6 parasequence sets and its boundaries well defined (Fig. 4). The sand units of this well revealed better sorting, but reduced in quantity and wave action as the facie was higher than the previous wells, as such, mud and marine shale were more deciphered in its depositional sequence on the gamma ray log. The facie is still more of sandstone, but with a higher percentage of mud in its component matrix. Thus, this well showed coastal to proximal offshore deposit with a low energy sub-environment in the depositional sequence. ADN-1 well was studied to consist of 4 parasequence sets and its boundaries well defined as intervals of each of the parasequences (Fig. 4). The sand units of this well showed a peculiar depositional facies of almost equal sandstone and mud content. EB-1 well was studied to consist of 5 parasequence sets and its boundaries well defined (Fig. 4). The sand units of this well were well defined, with the mud streaks and marine shales being deposited. EB-2 well was studied to consist of 4 parasequence sets and its boundaries well defined. The sand units of this well showed almost depleted

section with the mud streaks and marine shales becoming dominant. Thus, this well showed migration from onshore to proximal offshore to offshore proper of the marine terrain of the field deposit. This is evident in the marine shale and mud deposit. MB-2 well was studied to consist of 5 parasequence sets and its boundaries well defined (Fig. 4). The sand units of this well showed almost depleted sequence, with the mud streaks and marine shales becoming more dominant. Thus, this well revealed migration from onshore to proximal offshore of the marine terrain of the field. This is evident in the marine shale and mud deposit of the well. In a nutshell, 24 parasequences have been recorded in these investigated wells with distinct depositional facies of the field to be fine, medium and coarse grained sandstone and mud/shale. At most intervals of the sequence in the field of study, the gamma ray log showed a coarsening upward trend, typical of Agbada formation in this field deposit across all the wells (Fig. 5) and showed period of sea regression that culminated in the depositional environmental sequence within the depositional episode of the field deposit.

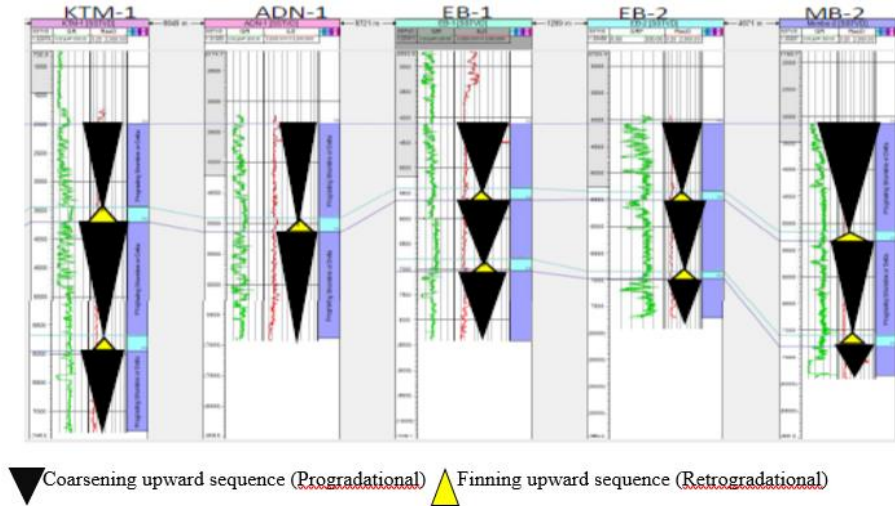


Fig. 5: Sequence stratigraphic structure of the studied wells

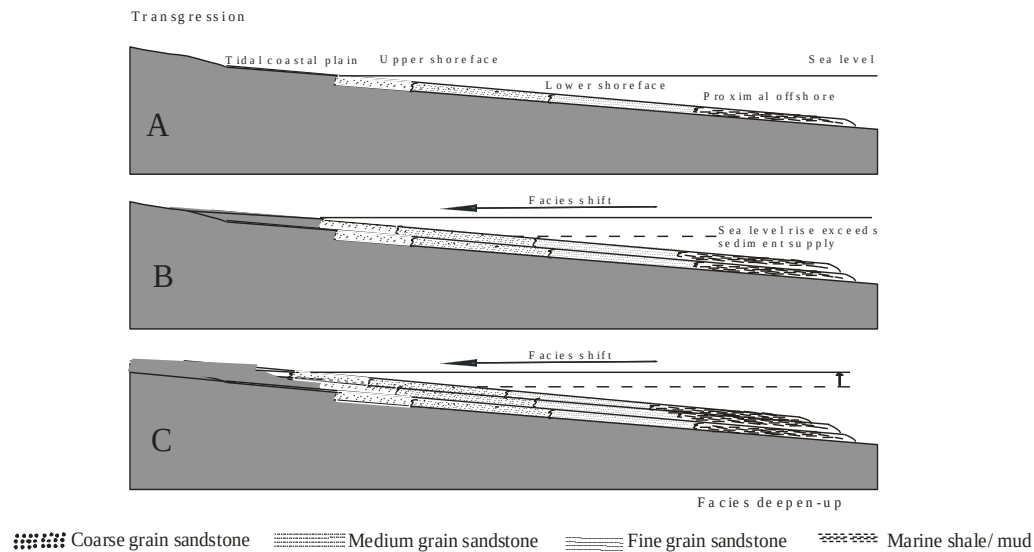


Fig. 6: Depositional and facies model for the retrogradational deposit of OML AB field.

With eustatic sea level fall (reduced accommodation space) and more sediment supplied to the area, a progradational parasequence set formed, marked by a facies shift from shallower to deeper water deposition ie from coastal plain to shoreface to proximal offshore environments, which signaled a sudden increase in relative sea level of deposition of the block sediment again (Figs. 7 and 8). This consequentially led to the deposition of the coarse grained sandstone of the tidal coastal area on the medium grained sandstone and fine grained sandstone of the upper and lower shoreface respectively (Figs. 7 and 8). This in-turn was deposited in the proximal offshore sub-environment as mud/shale lithological facies. This setup gave rise to the coarsening upward sequence on the gamma ray log and as exemplified in fig. 5. The study of the parasequence set in OML AB deposit using gamma ray log pattern showed the deposit is very rich in its sets deposit with 3 to 6 parasequence in each studied well (Fig. 4).

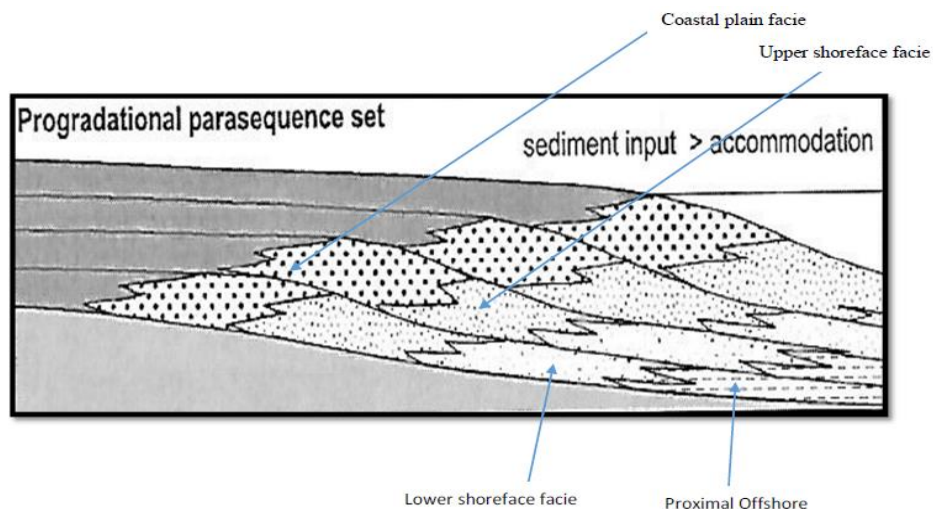


Fig. 7: Progradational parasequence of the facies in OML XB field

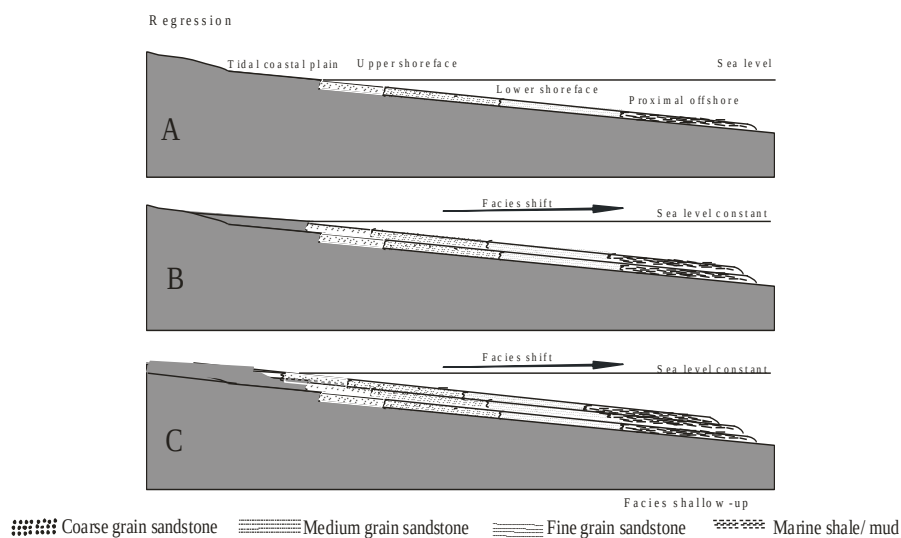


Fig. 8: Depositional and facies model for the progradational deposit of OML AB field.

The sea level rose faster than the rate of sediment supplied in the study area and this gave rise to the formation of all TST ie Transgressive system tracts with retrogradational stacking pattern of its beds, as such, the shoreline and facie patterns moved landward and led to finning upward depositional sequence (Figs. 5 and 9). Finally, the tidal coastal plain deposit was submerged as was overlain by the

medium grained sandstone of the upper shoreface sub-environment, which in turn was deposited on the fine grained sandstone and in turn on the mud/shale lithological facies of the proximal offshore (Fig. 9). This set-up equally gave rise to a fining upward arrangement in the depositional sequence of the field deposit (Fig. 5).

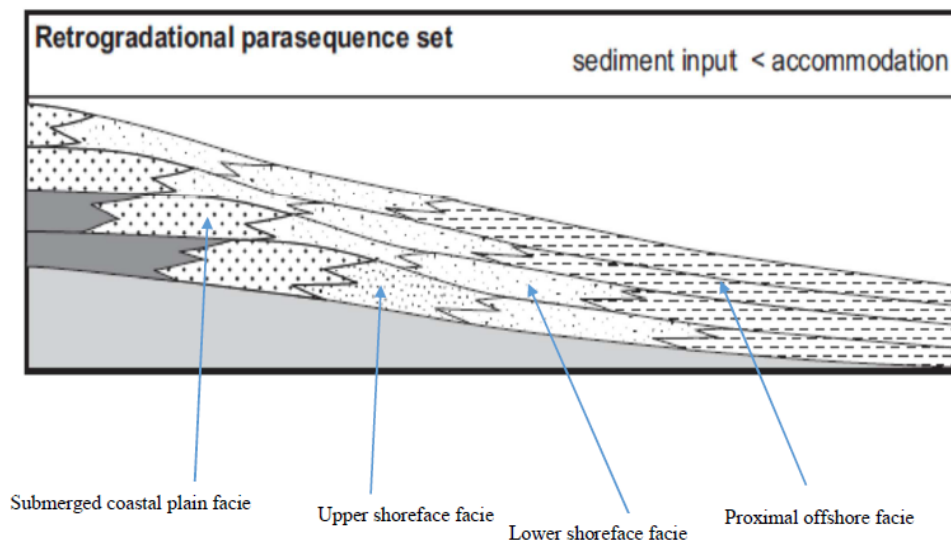


Fig. 9: Retrogradational parasequence of the facies in OML AB field

6.0 Conclusion:

KTM-1, ADN-1, EB-1, EB-2 and MB-2 wells were investigated for their sequence stratigraphic and depositional environments of the deposit and 24 parasequence sets were recorded in the wells and they represent packages of beds of the deposit formed with variation of sediment supply and created accommodation space during the eustatic sea level changes. 19 flooding surfaces that served as the parasequence boundaries were also enumerated in the study. SB1_BPS, SB2_BMS, SB3_BQC, MFS1 and MFS2 are the recorded sequence stratigraphic structure that have led to the modification of the porosity and permeability of the well's deposit. 14 HST's and 9 TST's were recorded across the 5 wells of study and ranges from 3000-8403 and 3450-7093 feet respectively. Tidal coastal plain, upper shoreface, lower shoreface and proximal offshore of the block well sediments were mapped out as sub-depositional environments studied. The tidal coastal plain consist of barrier bar, mouth bar and beach ridge sediments of coarse grained sandstone bodies for reservoir hydrocarbon production.

References

- Doust, H. and Omatsola, E. (1990). Petroleum Geology of the Niger Delta. Geochemical Society, London, Special Publications. Vol. 50, pp. 365.
- Ejedawe, J. E. (1981). Patterns of incidence of oil reserves in Niger delta basin: American Association of Petroleum Geologists Bulletin, Vol. 65, pp. 1574-1585.
- Ejedawe, J.E., Coker, S.J.L., Lambert-Aikhionbare, D.O., Alofe, K.B., and Adoh, F.O. (1984). Evolution of oil-generative window and oil and gas occurrence in Tertiary Niger Delta 251 Basin: American Association of Petroleum Geologists, Vol. 68, pp.1744 -1751.
- Ejedawe J.E. (1989). The Eastern Niger Delta: geological evolution and hydrocarbon occurrences. SPDC Internal Report, Exploration Note 89.
- Ekweozor, C. M., and Daukoru, E. M. (1994). Northern delta depobelt portion of the Akata– Agbada petroleum system, Niger Delta, Nigeria, in L. B. Magoon and W. G. Dow, eds, The petroleum system—from source to trap: AAPG Memoir 60, pp. 599–613.
- Evamy, B.D., Haremboure, J., Kamerling, P., Knaap, W.A., Molloy, F.A., and Rowlands, P.H. (1978). Hydrocarbon habitat of Tertiary Niger Delta: American Association of Petroleum Geologists Bulletin, Vol. 62, pp. 1-39.
- Reijers, T. J. A., Petters, S. W., and Nwajide, C. S. (1997). The Niger Delta basin. In: Selley, R. C. (Ed), African basins, Amsterdam, Elsevier science, sedimentary basins of the world; Vol. 3, pp. 151–172.
- Kendall, C. (2003). Use of well logs for sequence stratigraphic interpretation of the subsurface. USC Sequence Stratigraphy Web. <http://strata.geol.sc.edu/index.html>, University of South Carolina.
- Kendall, C.G. (2004). Critical accidents in paleo-geography and oceanography induced by abrupt changes in base level, signaled by hard or firm grounds in shallow water clastics and carbonates: <http://strata.geol.sc.edu/index.html>. University of south carolina.
- Kendall, C. G. (2008). Template for “conceptual models” used to interpret depositional systems USC Sequence Stratigraphy Web. <http://strata.geol.sc.edu/index.html>, University of South Carolina.

- Stacher, P. (1995). Present understanding of the Niger Delta hydrocarbon habitat; In: M.N. Oti and G.Postma (Eds.), *Geology of deltas*; A. A. Balkema, Rotterdam; pp. 257– 267.
- Van Wagoner, J.C., Mitchum, R.M., Campion, K.M., and Rahmanian, V.D. (1990). *Siliciclastic Sequence Stratigraphy in Well Logs, Cores, and Outcrops*. American Association of Petroleum Geologists, Tulsa, pp. 55-63.
- Weber, K. J., and Daukoru, E. M. (1975). *Petroleum geology of the Niger Delta: Proceedings of the 9th World Petroleum Congress, Vol. 2, Geology*: London, Applied Science Publishers, Ltd., pp. 210–221.