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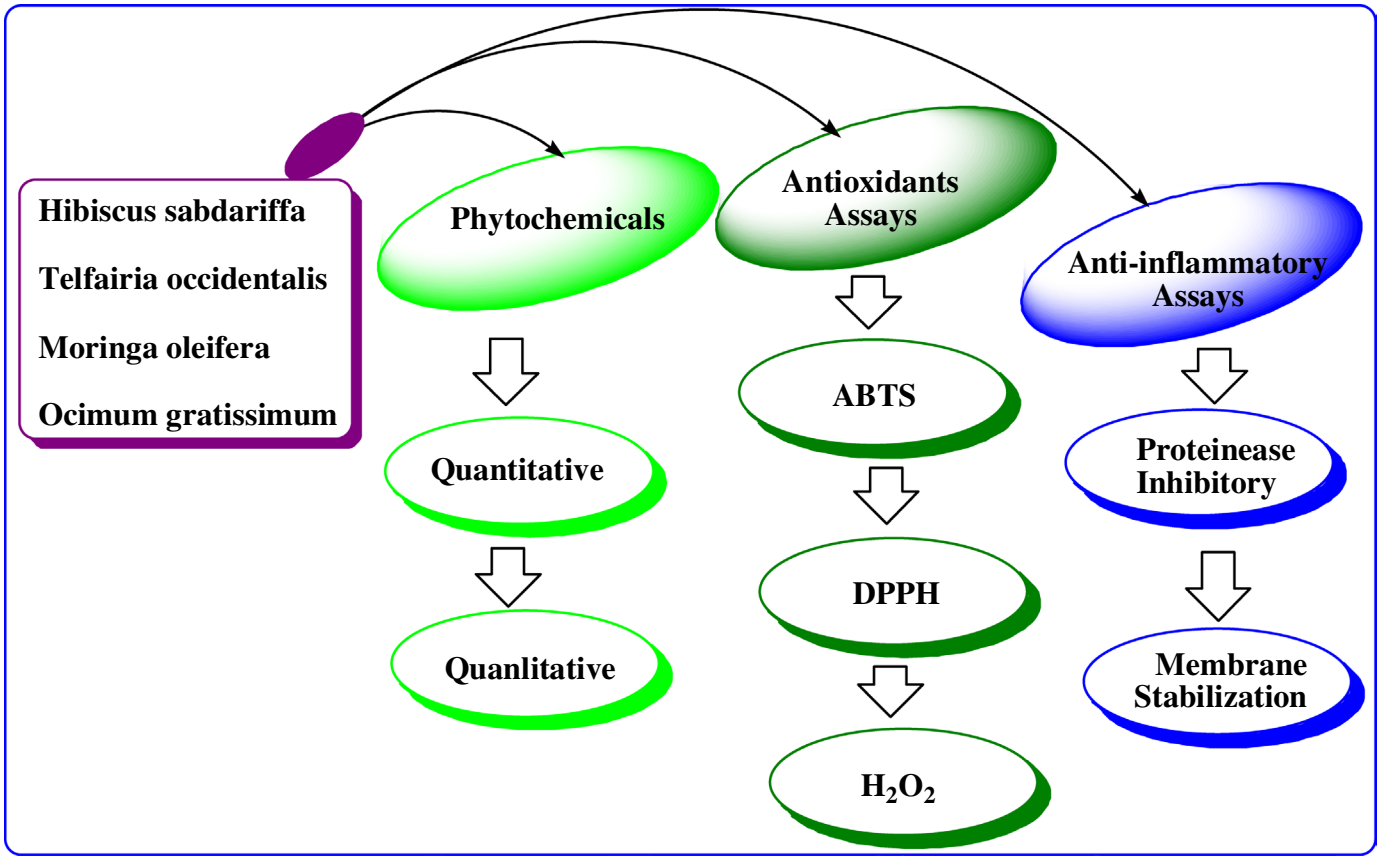

MEDICINAL CHEMISTRY | RESEARCH ARTICLE

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Cogent Chemistry (2017), 3: 1314064

cogent • oa

Kambizi et al., *Cogent Chemistry* (2017), 3: 1314064
http://dx.doi.org/10.1080/23312009.2017.1314064

cogent chemistry

Received: 19 February 2017
Accepted: 28 March 2017
First Published: 07 April 2017

*Corresponding author: O. Atolani, African Centre for Herbal Research Ilorin (ACHRI), Central Research Laboratory, University of Ilorin, Ilorin, Nigeria; Department of Chemistry, University of Ilorin, Ilorin, Nigeria
E-mail: atolani.o@unilorin.edu.ng

Reviewing editor:
Youcef Mehellou, Cardiff University, UK

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MEDICINAL CHEMISTRY | RESEARCH ARTICLE

Proteinease inhibition, membrane stabilization, antioxidant and phytochemical evaluations of leaves, seeds and calyces of four selected edible medicinal plants

L. Kambizi[1,2], M.T. Bakare-Odunola[1,3], A.T. Oladiji[1,4], A.T. Kola-Mustapha[1,5], T.O. Amusa[1,6], O. Atolani[1,7*], N.S. Njinga[1,3] and A.L. Quadri[1,4]

Abstract: The aqueous extracts of the edible parts of four common medicinal plants (*Hibiscus sabdariffa, Telfairia occidentalis, Moringa oleifera* and *Ocimum gratissimum*) readily consumed as food or supplements in many African settings are examined for some of their acclaimed pharmacological activities and possible potential associated risks. The classes of phytochemicals present are examined qualitatively and quantitatively, while the antioxidant activities were determined using three assays which include 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) and hydrogen peroxide (H_2O_2) assays. The anti-inflammatory activities were also examined using proteinase inhibitory assays and red blood cell membrane stabilisation assays. Both the antioxidant and anti-inflammatory activities of the plants indicated that the plants have potentials for applications in foods and nutraceuticals that can be used for the management of various ailments

ABOUT THE AUTHORS

ACHRI is an acronym for African Centre for Herbal Research Ilorin. The centre is domiciled at the Central Research Laboratory building of the University of Ilorin, Ilorin, Nigeria. It was established by the U6 initiative for development comprising six funding African Universities namely; Cape Peninsula University of Technology, South Africa; Jaramogi Oginga Odinga University of Science and Technology, Kenya; Kwara State University, Malete, Nigeria; University of Cape Coast Ghana; University of the Gambia, Banjul, Gambia; and University of Ilorin, Ilorin, Nigeria. The centre is envisioned to conduct relevant research on African flora, organise workshops, collate research proposals for funding, advice Government on issues related to exploitation of African herbal resources and maintain the research laboratory. Scientists from these Universities form the basic network of researchers involved in ACHRI research. However, the centre is committed to regional and international research collaborations. The Director, Prof. Kambizi L. and Coordinator, Prof. Bakare-Odunola M.T. currently guide the affairs and research activities of ACHRI.

PUBLIC INTEREST STATEMENT

The global burden of cardiovascular disease conditions remains a paramount issue. Many epidemiological studies have established strong nexus between antioxidant and anti-inflammatory mechanism. Many antioxidant compounds have been indicated to possess important anti-inflammatory activity because reactive oxygen species known to react with cellular components and damaging the DNA and proteins causing cellular and tissue injury are forestalled at the onset. The excessive generation of the reactive oxygen species can overwhelm the biological system and induce other conditions such as premature aging, cancer, diabetes and atherosclerosis. On the basis of this, the antioxidant and anti-inflammatory studies were conducted on the common edible plants. This study revealed that *Hibiscus sabdariffa, Telfairia occidentalis, Moringa oleifera* and *Ocimum gratissimum* are potential natural antioxidant sources with high anti-inflammatory activities. The incorporation of such natural antioxidants in foods, drinks and nutraceuticals affords a green and sustainable means of health management especially in the developing nations.

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and diseases. However, the consumption of *H. sabdariffa* leaf should be highly regulated as the extract exhibited reduced cell membrane stabilisation activities at increased concentrations.

Subjects: Biochemistry; Pharmaceutical Science; Biology; Food Chemistry

Keywords: antioxidant; membrane-stabilisation; proteinase inhibition; *Telfairia occidentalis*; *Moringa oleifera*; *Hibiscus sabdariffa*

1. Introduction

Nature has endowed mankind with numerous plants of important medicinal values that have reportedly been used for the management of various ailments and diseases in human history. The pharmacological relevance of these plants is believed to rely on their phytoconstituents which are predominantly secondary metabolites. World Health Organisation reported that large proportion of the world population, especially those in developing countries, relies on plant-derived drugs for their primary health care (Modi, Patel, Shah, & Nayak, 2010).

Recently, scientific attention has been re-directed to the fact that these bioactive agents are crucial for the direct or indirect management of human health today. This is owing to the fact that plants containing these agents are relatively safe candidates for health care management in preference to synthetic drugs (Dubey, Kumar, & Tripathi, 2004). Pharmacological potentials of medicinal plants are due to the presence of plant-derived secondary metabolites such as flavonoids, saponins, alkaloids, tannins, phenolics and glycosides all of which use various mechanisms in the cure or management of diseases. The presence of these metabolites in fruits and vegetables is responsible for the antioxidants, anti-inflammatory and anti-cancer among other activities which prevents the onset of many diseases attributed to oxidative stress (Adeosun, Olaseinde, Opeifa, & Atolani, 2013; Aruoma, 1998). Some of the medicinal plants mainly applied as food or nutraceuticals for various pharmacological activities in Nigeria includes *Hibiscus sabdariffa*, *Telfairia occidentalis, Moringa oleifera* and *Ocimum gratissimum.*

H. sabdariffa L. (Hs, roselle; Malvaceae) is an important medicinal plant of Asia and tropical Africa origin. The calyces are used to make drinks in Africa where it is served either cold or hot. *H. sabdariffa* is well reported for its medicinal values such as antioxidant, anti-hypertensive and hypocholesterolemic activities (Hirunpanich et al., 2006; Inuwa et al., 2012; Onyenekwe, Ajani, Ameh, & Gamaniel, 1999). It is used for the treatment of fever, high blood pressure, external wound, cough, restiveness, sleeping disorder and cardiac conditions (Herrera-Arellano et al., 2007; Meyer-Buchtela, 2004; Mozaffari-Khosravi, Jalali-Khanabadi, Afkhami-Ardekani, Fatehi, & Noori-Shadkam, 2008). *T. occidentalis* is a prominent member of the Cucurbitaceae family. It is indigenous to southern Nigeria where it is cultivated for its culinary purpose (Kayode & Kayode, 2011). The leaves have been used in the treatment of anaemia, fatigue and diabetes (Alada, 2000; Dina, Adedapo, Oyinloye, & Saba, 2006; Oboh, Nwanna, & Elusiyan, 2006). It is also reported for its potent antioxidant, anti-inflammatory and anti-microbial activity (Oluwole, Falode, & Ogundipe, 2003; Oyewole & Abalaka, 2012).

M. oleifera Lam., of the family Moringaceae is a multipurpose tropical medicinal plant reported for activities such as antifungal, anti-inflammatory, anti-tumour, anti-ulcer, treatment of sexually transmitted infections, stimulant, aphrodisiac, abortifacient, cholagogue, malnutrition and diarrhoea (Farooq, Sajid, Muhammad, & Anwarul, 2007; Mehta, Balaraman, Amin, Bafna, & Gulati, 2003; Siddhuraju & Becker, 2003). All parts of the plant which include leaves, seed, pod, stem bark and gum are used in folkloric medicine and cosmetic application (Anwar, Ashraf, & Bhanger, 2005; Anwar & Bhanger, 2003; Atolani et al., 2016). Different parts of the plant are known to contain important minerals, nutrients and phytochemicals such as protein, vitamins, carotenoids, amino acids, alkaloids, glycosides and phenolics (Farooq et al., 2007; Sharma, Kumari, Srivastava, & Srivastava, 2006; Yaeesh, Jamal, Khan, & Gilani, 2006). *O. gratissimum* L. belonging to Lamiaceae family is a perennial shrub grown as vegetable and spice Asia, tropical Africa and South America (Gupta, Singh, Kumar, &

Bhanot, 2011). A decoction of *O. gratissimum* roots is used as a sedative for children while the leaves are used for the treatment of blocked nostrils, abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, tooth gargle, regulation of menstruation, headache, influenza, diarrhoea, haemorrhoids, pneumonia, fever, skin infections and inflammation (Adebolu & Salau, 2005; Kabir, Olukayode, Chidi, Christopher, & Kehinde, 2005; Ngassoum et al., 2003; Orafidiya, Oyedele, Shittu, & Elujoba, 2001).

Some of the factors that affect the pharmacological activities of medicinal plants include chemical composition, geographical origin, ages and some others. It is therefore important to provide baseline data on the phytochemicals, antioxidants and nutritional facts of the various parts of medicinal plants in gross use by the people. Hence, this study was conceived to investigate the antioxidant, anti-inflammatory and membrane stabilisation potentials of leaves, seeds and calyces of *H. sabdariffa* as well as the leaves of *T. occidentalis, M. oleifera* and *O. gratissimum* cultivated in the farm of University of Ilorin, Ilorin, Nigeria.

2. Materials and methods

2.1. Collection of plant materials

Plant materials (edible parts) which include *T. occidentalis* leaves, *M. oleifera* leaves, *O. gratissimum* leaves and *H. sabdariffa* leaves, seed and calyx were collected from the plantation of African Centre for Herbal Research Ilorin (ACHRI) University of Ilorin, Ilorin, Nigeria and authenticated at the herbarium section of the Centre. Voucher specimen numbers ACHRI/H16/101, ACHRI/H16/102, ACHRI/H16/103 and ACHRI/H16/104 were obtained for *T. occidentalis*, *M. oleifera, O. gratissimum* (Efirin) and *H. sabdariffa* (Zobo), respectively.

2.2. Solvents and reagents

All chemical reagents for phytochemical screening and evaluation were analytical grade, and where necessary, solvents were redistilled before use. Antioxidant and anti-inflammatory assay reagents were obtained from Santa Cruz Biotechnology, US.

2.3. Extraction of plant material

T. occidentalis (381 g leaves and 393 g stem), *H. sabdariffa* (840 g leaves, 808 g calyx and 265 g seed), *M. oleifera* leaves (849 g) and *O. gratissimum* (240 g) aerial parts were collected. Plant parts were selected based on ethno-botanical applications and consistent recommendation by the local users. The various plant parts were removed and air dried under shade. The dried samples were pulverised and extracted using cold maceration method with distilled water for 24 h. The extracts were concentrated on water bath at 40°C, freeze dried and stored in a cool place for further analyses.

2.4. Phytochemical analyses

The aqueous extracts and pulverised plant samples were subjected to qualitative analysis for the determination of their phytochemical constituents using standard procedures (Edeoga, Okwu, & Mbaebie, 2005; Harborne, 1998; Sofowora, 2006). Quantitative estimation of the phytoconstituents which include phenols and tannins was carried out using the spectrophotometric method (Krishnaiah, Devi, Bano, & Sarbatly, 2009), while quantification of saponins, alkaloids, flavonoids and glycosides was carried out using procedure described by Harborne (1998).

2.5. Antioxidant evaluations

In order to evaluate the antioxidant capacity of the extracts, three established complimentary antioxidant assays which includes DPPH, ABTS and hydrogen peroxide scavenging activities were adopted.

2.6. Determination of antioxidant activity using the DPPH free radical scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric assay was carried out following standard procedure with slight modifications (Atolani & Olatunji, 2016; Atolani, Omere, Otuechere, & Adewuyi, 2012). The DPPH free radical reagent was freshly prepared and kept in a dark bottle in the refrigerator overnight. The standard (Ascorbic acid) and extracts were prepared in triplicate in 0.02–0.10 mg/mL concentrations. 1 mL DPPH solution was added to all samples shaken together and immediately incubated in the dark for 30 min. The absorbance value was measured at 517 nm. Blank experiment was also carried out to determine the absorbance of DPPH before interacting with the sample. The decreasing absorbance of the DPPH solution was used as an indication of the DPPH radical scavenging activity of the samples. The radical scavenging activity was calculated using the equation:

$$\% \text{AA} = 100 \times [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]/\text{Abs}_{\text{control}}$$

where % AA indicates percentage antioxidant activity, while $\text{Abs}_{\text{control}}$ and $\text{Abs}_{\text{sample}}$ were the absorbance of the control and sample at 517 nm, respectively. Results were expressed as mean values ± standard error of mean (SEM) of triplicate determinations.

2.7. Determination of antioxidant activity using the abts free radical scavenging assay

The 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonate, ABTS radical cation decolorisation assay based on the scavenging of $\text{ABTS}^{\cdot+}$ radicals by antioxidants component of the extracts was used. The assay follows the procedure of Atolani, Olatunji, Fabiyi, Adeniji, and Ogbole (2013), with slight modifications. The reaction mechanism involves the donation of electron by the samples which results into the decolorisation of the solution. ABTS reagent was first dissolved in deionised water to afford a concentration 7 mM and the solution of 2.45 mM potassium persulfate freshly prepared was mixed with it at ratio of 1:1 and kept in the dark for 24–48 h. The ABTS solution was then diluted in aqueous methanol with a ratio of 1:25. A volume of 20 µL (diluted 1:10) of aqueous samples was added to 2 mL of $\text{ABTS}^{\cdot+}$ solution, and the mixture was kept at a standard temperature of 30°C. The absorbance was measured at 734 nm at 10 min after initial mixing. All analysis was determined in triplicate. The ABTS antioxidant activity (AA) was calculated and compared with ascorbic acid using the expression:

$$\text{AA} = 100 \times [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]/\text{Abs}_{\text{control}}$$

where $\text{Abs}_{\text{control}}$ and $\text{Abs}_{\text{sample}}$ are the absorbances of the control and the samples, respectively.

2.8. Determination of antioxidant activity using the hydrogen peroxide radical scavenging assay

The hydrogen peroxide (H_2O_2) radical scavenging ability of the extracts was measured using a modified method (Keser, Celik, Turkoglu, Yilmaz, & Turkoglu, 2012). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). 0.6 mL of the solution was added to 1.0 mL sample (100 µg/mL). The absorbance of the hydrogen peroxide was read at 230 nm after 10 min at 28.4°C temperature against a blank solution containing phosphate buffer solution without hydrogen peroxide. Ascorbic acid served as positive control. The ability of the extract to scavenging hydrogen peroxide as an equivalence of the ascorbic acid was determined using the formula:

$$\% \text{AA} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]/(\text{Abs}_{\text{control}}) \times 100$$

where % AA means percentage antioxidant activity, $\text{Abs}_{\text{control}}$ and $\text{Abs}_{\text{sample}}$ means the absorbances of the control and the samples, respectively.

2.9. In vitro anti-inflammatory activity

The anti-inflammatory activities of the plant extracts were investigated using standard procedures of the proteinease inhibitory activity and the red blood cell (RBC) membrane stabilisation assays.

2.10. Protein inhibitory assay

The protein inhibitory assay was determined using standard procedure (Govindappa, Sadananda, Channabasava, & Raghavendra, 2011; Sakat, Juvekar, & Gambhire, 2010). The reaction mixture (2 mL) containing 0.06 mg trypsin, 1 mL of 20 mM Tris HCl buffer (pH 7.4) and 1 mL test samples of different concentrations (5–250 µg/mL) was prepared. The reaction mixture was incubated at 37°C for 5 min and then 1 mL of 0.8% (w/v) casein was added. The reaction was inhibited for additional 20 min after which 2-mL 70% perchloric acid was added to terminate the reaction. The cloudy suspension which was obtained was centrifuged and the absorbance of the supernatant was read at 210 nm using the buffer as blank on the absorbance reader. The experiment was performed in triplicate and the percentage inhibition of proteinase was calculated using the expression:

$$\% \text{ Inhibition} = 100 \times (1 - \text{Vt/Vc}).$$

where Vt = absorbance of test sample; Vc = absorbance of control.

2.11. Red blood cell (RBC) membrane stabilisation assay

The red blood cell membrane stabilisation activities of the extracts were determined using standard procedure (Govindappa et al., 2011; Kardile, Mahajan, Shaikh, Goyal, & Patil, 2016; Sakat et al., 2010). Blood sample was collected from mice that have not been given any nonsteroidal anti-inflammatory drugs (NSAIDs) for 2 weeks prior to the experiment. The blood sample was mixed with equal volume of Alsever solution comprising 2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was constituted with normal saline. 50 and 100 µg/mL of the samples was prepared in distilled water and 1-mL phosphate buffer, 2-mL hyposaline and 0.5 mL of RBC suspension were added. The mixture was incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. The hemoglobin content of the supernatant solution was measured at 560 nm on the multi-scan spectrophotometer. Diclofenac (100 µg/mL) was used as reference while a control that has none of the test sample was used to monitor the experiment. The experiment was performed in triplicates and mean values determined. The percentage of RBC membrane stabilisation or protection was determined using the expression:

$$\% \text{ Protection} = 100 - (\text{Absorbance of treated sample/Absorbance of control}) \times 100.$$

2.12. Data analysis

Data were analysed using analysis of variance (ANOVA) on Graphpad Prism 3.0 software (USA). Differences among the mean values were evaluated using the Dunnett Test. Differences at $p < 0.05$ are considered significant. The IC_{50} was determined on GraphPad Prism 3 software (San Diego, USA) through a non-regression analysis. The IC_{50} was taken as the concentration of sample that scavenged 50% of the radicals. Results are presented as mean ± standard error of the mean.

3. Results and discussion

3.1. Extraction of plant materials

The percentage yield of the leaves and stem of *T. occidentalis* extracts (Figure 1) were 26.94 and 15.45%, respectively. Those of the leaves, calyx and seed of *H. sabdariffa* were 26.19, 23.27 and 47.15%, respectively. The yield of the leaves of *M. oleifera* and *O. gratissimum* was 37.15 and 8.33%, respectively. The calyx of *H. sabdariffa* showed the highest aqueous extractives followed by *M. oleifera,* while *O. gratissimum* showed the least indicating it contained less polar constituents.

3.2. Qualitative phytochemical analysis

The phytoconstituents in aqueous extracts of leaves, seeds and calyces of *H. sabdariffa* as well as leaves of *M. oleifera* and *O. gratissimum* are as shown (Table 1). Leaves of *H. sabdariffa* and *M. oleifera* as well as calyx and seeds of *H. sabdariffa* contained tannins, saponins, phenols, glycosides, alkaloids and flavonoids. However, leaf of *O. gratissimum* contained all these secondary metabolites except for glycosides which were absent within the limit of the detection of the method used (Table 1).

Figure 1. Percentage yield of the extracts.

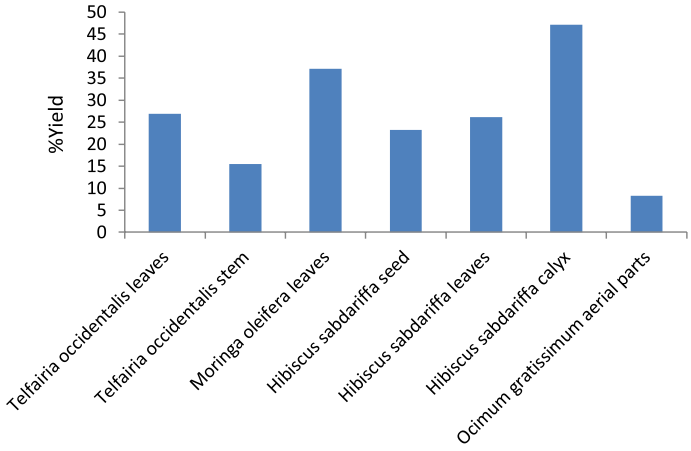

Table 1. Qualitative phytochemical constituents of aqueous extracts of the selected parts of *H. sabdariffa*, *M. oleifera* and *O. gratissimum*

Phytochemicals	HL	ML	OL	HS	HC
Tannins	+	+	+	+	+
Saponins	+	+	+	+	+
Phenols	+	+	+	+	+
Glycosides	+	+	−	+	+
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	+

Notes: + = present; − = absent.

HL = *H. sabdariffa* leaf extract; ML = *M. oleifera* leaf extract; OL = *O. gratissimum* leaf extract; HS = *H. sabdariffa* seed extract; HC = *H. sabdariffa* calyx extract.

3.3. Quantitative phytochemical estimation

The aqueous extract of *H. sabdariffa* leaves had the highest quantity of flavonoids (20.09 mg/g) of the total phytoconstituents present, while glycosides (0.84 mg/g) were the lowest (Table 2). Similarly, flavonoids (5.68 mg/g) presence was the highest while the tannins (0.49 mg/g) were lowest for aqueous extract of calyces of *H. sabdariffa.* Aqueous extract of leaves of *M. oleifera* had the highest value (29.28 mg/g) of flavonoids, while tannins (0.69 mg/g) were the lowest. The seeds of *H. sabdariffa* contained 34 folds of flavonoids (17.34 mg/g) as compared to glycosides (0.52 mg/g) which was the lowest. In the aqueous extract of leaf of *O. gratissimum*, flavonoids (11.10 mg/g) were the highest of all the constituents while phenols were the lowest (0.52 mg/g) (Table 2).

Table 2. Quantitative phytochemical constituents of aqueous extracts of selected parts of *H. sabdariffa*, *M. oleifera* and *O. gratissimum*

Phytochemicals (mg/g)	HL	ML	OL	HS	HC
Tannins	16.10 ± 0.4	0.69 ± 0.1	4.72 ± 0.2	15.42 ± 0.2	0.49 ± 0.0
Saponins	1.84 ± 0.1	1.18 ± 0.2	6.11 ± 0.2	1.30 ± 0.0	1.20 ± 0.1
Phenols	1.00 ± 0.0	0.96 ± 0.1	0.52 ± 0.0	4.68 ± 0.1	1.12 ± 0.1
Glycosides	0.84 ± 0.1	0.14 ± 0.0	0.00 ± 0.0	0.52 ± 0.0	0.66 ± 0.0
Alkaloids	2.18 ± 0.2	6.47 ± 0.5	2.15 ± 0.1	3.10 ± 0.0	3.42 ± 0.0
Flavonoids	20.09 ± 0.3	29.28 ± 0.8	11.10 ± 0.2	17.34 ± 0.3	5.68 ± 0.1

Notes: Values are expressed as Mean ± Standard Error of Mean (SEM) of three replicates.

HL = *H. sabdariffa* leaf extract; ML = *M. oleifera* leaf extract; OL = *O. gratissimum* leaf extract; HS = *H. sabdariffa* seed extract; HC = *H. sabdariffa* calyx extract.

Pharmacological potencies of medicinal plants are primarily as a result of the presence of bioactive compounds like secondary metabolites such saponins, flavonoids, alkaloids, phenols, tannins and glycosides. These phytochemicals have been employed for the treatments of diseases such as malaria, cancer, diabetes, typhoid, diarrhoea, ulcer, haemorrhage and heart-related diseases (Hossain & Nagooru, 2011). Saponins have been found to be significant as antihypercholesterol, hypotensive, antimicrobial and cardiac depressant agents (Ayoola et al., 2008; Price, Johnson, & Fenwick, 1987; Watt & Breyer-Brandwyk, 1984). Tannins are regarded as important anti-diarrhoea, anti-haemorrhagic and antioxidant agents (Harborne, 1973). Phenols have been reported as important secondary metabolites in plants because of their antioxidant and anti-microbial activities (Cowan, 1999; Paganga, Miller, & Rice-Evans, 1999). Flavonoid compounds exhibit antioxidant, anti-inflammatory, anti-microbial, anti-angionic, anticancer and anti-allergic properties (Anyasor, Ogunwenmo, Oyelana, & Akpofunure, 2010). Alkaloids are present in low concentration in all the tested extracts. Alkaloids are known as compounds that affect the central nervous system, they also reduce appetite and behave as diuretic (USDA, 2010). Glycosides perform important role on immune system by increasing body strength and possess antimicrobial and anti-inflammatory effects (Theis & Lerdau, 2003).

3.4. Antioxidant activities: DPPH, ABTS and hydrogen peroxide radical scavenging activities

Plants of medicinal values are usually characterised by their antioxidants and anti-inflammatory potentials. The antioxidant and anti-inflammatory activity of *H. sabdariffa* (leaves, seeds and calyx), *M. oleifera* leaves, *O. gratissimum* and *T. occidentalis* leaves have been evaluated using established *in vitro* protocols.

Free radical species have been implicated in the pathogenesis of many disease conditions including inflammation, cancer, type-II diabetes, rheumatoid arthritis, acute respiratory disease, vascular disease including stroke and more recently periodontal disease (Chapple, 1997). Antioxidants which scavenge the free radicals in the body are important in preventing the onset of these disease conditions. Natural antioxidants which include carotenoids, vitamins A, B, C and E are critical for maintaining optimum health and wellbeing. Fruits and leaves of medicinal plants are important sources of these antioxidants. Due to the side effects of many synthetic antioxidants, attention has been shifted to natural sources of antioxidants. Hence, the consumption of herbs, fruits and medicinal plants has been encouraged as a chemopreventive measure for many disease conditions (Shetti & Patil, 2011). The free radical usually exists as reactive oxygen species such as hydroxyl radical ($^{\cdot}OH$), superoxide anion ($^{\cdot}O_2$), hydrogen peroxide (H_2O_2) and hypochlorous acid ($HClO^{\cdot}$) and numerous assay systems have been used for the comprehensive investigation of the radical species and antioxidants that scavenges them (Luximon-Ramma, Bahorun, Soobrattee, & Aruoma, 2002; Shetti & Patil, 2011). Despite the numerous antioxidant assay systems that exist, the DPPH, ABTS and hydrogen peroxide antioxidant assays which are adopted in this study are widely acceptable antioxidant indices for plants and food species (Huang, Ou, & Prior, 2005; Ozgen, Reese, Tulio, Scheerens, & Miller, 2006). The three widely acceptable antioxidant assay systems (DPPH, ABTS and hydrogen peroxide assays) were adopted in this study because the different types of radicals and reactive oxygen species generated by normal cellular processes, environmental stresses, and UV irradiation have different mechanisms.

All the tested extracts had higher DPPH antioxidant activity than the standard, ascorbic acid (Figure 2). The extracts had dose-dependent activities which range from approximately 67 to 72%, while the ascorbic acid had activity which ranges from 13 to 68%. The ascorbic acid had IC_{50} value (0.02 ± 1.75 mg/mL) which was more significant ($p < 0.05$) than all the extracts which had IC_{50} value ranging from 0.14 to 0.17 mg/mL (Table 3). At 0.1 mg/mL, *T. occidentalis* exhibited the highest DPPH antioxidant activity (71.66%) followed by *H. sabdariffa* calyx extract (70.95%), *M. oleifera* leaf extract (70.44%), *H. sabdariffa* leaf extract (69.71%), *O. gratissimum* leaf extract (69.34%), *H. sabdariffa* leaf extract (68.47) and in the order. From the ABTS assay result (Figure 3), all the extract had high antioxidant activities slightly below the standard, ascorbic acid (98.88%) at 100 mg/mL. *H. sabdariffa*

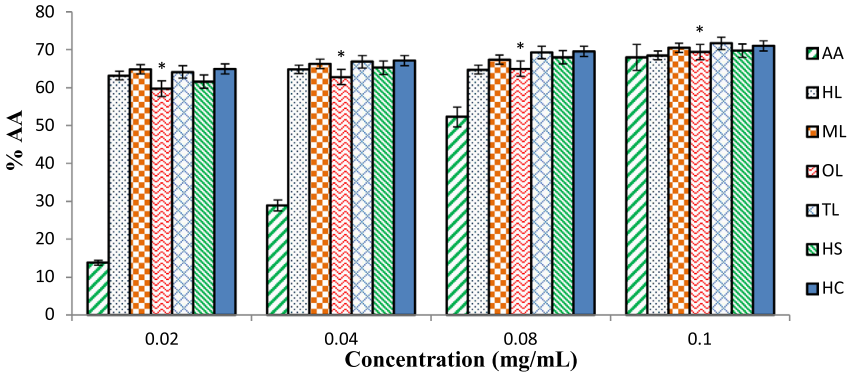

Figure 2. DPPH antioxidant activities of the extracts.

Notes: AA = Ascorbic acid; HL = *H. sabdariffa* leaf extract; ML = *M. oleifera* leaf extract; OL = *Ocimum gratissimum* leaf extract; TL = *T. occidentalis* leaf extract; HS = *H. sabdariffa* seed extract; HC = *H. sabdariffa* calyx extract.
*Significantly different at $p < 0.05$ compared to the control.

Table 3. IC_{50} Values Determined for the Extracts (DPPH Antioxidant)

S No	Sample	IC_{50} ± SEM (mg/mL)
1	Ascorbic acid	0.02 ± 1.75
2	HL	0.14 ± 0.86
3	ML	0.16 ± 0.81
4	OL	0.14 ± 0.86*
5	TL	0.16 ± 0.77
6	HS	0.16 ± 0.16
7	HC	0.17 ± 0.77

Notes: AA = Ascorbic acid; HL = *H. sabdariffa* leaf extract; ML = *M. oleifera* leaf extract; OL = *O. gratissimum* leaf extract; TL = *T. occidentalis* leaf extract; HS = *H. sabdariffa* seed extract; HC = *H. sabdariffa* calyx extract.
*Data are not significantly different at $p < 0.05$ compare to the control.

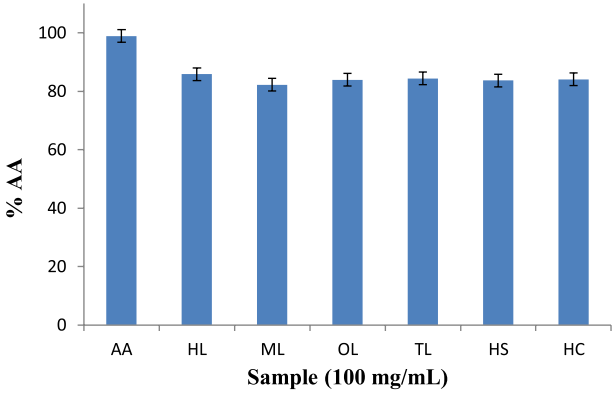

Figure 3. ABTS antioxidant activities of the extracts.

Notes: AA = Ascorbic acid; HL = *H. sabdariffa* leaf extract; ML = *M. oleifera* leaf extract; OL = *O. gratissimum* leaf extract; TL = *T. occidentalis* leaf extract; HS = *H. sabdariffa* seed extract; HC = *H. sabdariffa* calyx extract.

leaf extract had the highest ABTS activity (85.82%) with an ascorbic acid equivalence of 825.09 ± 2.10 mg/g (Table 4) followed by *T. occidentalis* leaf extract (84.40%) and *H. sabdariffa* calyx extract (84.03%). *M. oleifera* leaf extract had the least activity (82.22%) with an ascorbic acid equivalence of 777.69 ± 0.90 mg/g, while *O. gratissimum* leaf extract and *H. sabdariffa* seed extract exhibited moderate activity of 83.92 and 83.71%, respectively. The result is consistent with reported (Chetia, Upadhyaya, & Saikia, 2014). There was significant difference ($p < 0.05$) in the DPPH and ABTS free radical scavenging activities of the extracts as depicted in Figure 2 and Table 4. The high antioxidant potential (*in vitro* and *in vivo*) of *H. sabdariffa* has been confirmed by previous report as well (EcoCrop, 2007; Ekor et al., 2010; Mossalam, Aty, Morgan, Youssaf, & Mackawy, 2011).

Table 4. Equivalent (mg) of Ascorbic acid/g of Extract in ABTS Assay

S.No.	Samples	*Equivalent (mg) of Ascorbic acid/g (SEM)
1	HL	825.0955 ± 2.1**
2	ML	777.69315 ± 0.9
3	OL	806.28945 ± 3.6**
4	TL	810.99095 ± 2.2**
5	HS	804.1171 ± 5.4**
6	HC	807.3606 ± 1.8**

Notes: HL = *H. sabdariffa* leaf extract; ML = *M. oleifera* leaf extract; OL = *O. gratissimum* leaf extract; TL = *T. occidentalis* leaf extract; HS = *H. sabdariffa* seed extract; HC = *H. sabdariffa* calyx extract.

*Values are average of triplicate samples; SEM-Standard error of mean.

**Data are not significantly different at $p < 0.05$ compare to the control.

T. occidentalis leaf extract displayed the highest hydrogen peroxide scavenging potential of 57.85% (Figure 4) followed by *M. oleifera* leaf extract (55.91%). The hydrogen peroxide scavenging activities were significantly different ($p < 0.05$) for *H. sabdariffa* seed extract and *H. sabdariffa* calyx extract. *H. sabdariffa* leaf extract, *H. sabdariffa* calyx extract, *H. sabdariffa* seed extract and *O. gratissimum* leaf extract had 53.27, 53.02, 52.91 and 51.81% activities, respectively. In the antioxidant assays results, *T. occidentalis* had the most significant activity in both the DPPH and hydrogen peroxide radical scavenging assays than other extracts investigated. This high antioxidant potential of the *T. occidentalis* is corroborated by previous reports (Aminu et al., 2012; Nwanna & Oboh, 2007). It is pertinent to mention that while hydrogen peroxide is not very reactive by itself, its decomposition products including the hydroxyl radicals are toxic to the cells (Halliwell, 1991, 1996). Therefore, removing hydrogen peroxide in the biological system is desirable for a good heath (Halliwell, 1991; Keser et al., 2012).

3.5. Anti-inflammatory activities

In vitro anti-inflammatory activities using the red blood cell (RBC) membrane stabilisation assay and proteinase inhibition assay were evaluated. *H. sabdariffa* calyx extract had the most significant protection (61.10%) of the red blood cell at 150 mg/mL (Figure 5). The activities were most significantly different ($p < 0.05$) at concentrations 100 mg/mL for all the extracts. At 100 mg/mL, the *H. sabdariffa* calyx extract also had the highest activity (54.28%) apart from the standard; diclofenac which had 60.78% cell protection. All the extracts had dose-dependent activity. However, apart from *H. sabdariffa* leaf extract which had decreased activities with increasing concentrations, all other extracts exhibited increased protection with increasing concentration. Hence, lower concentrations of the *H. sabdariffa* leaf extract possess a higher stabilisation capacity thereby indicating a note of caution to indiscriminate consumers of the *H. sabdariffa* leaf extract. It is therefore pertinent to note that increased or uncontrolled consumption of the *H. sabdariffa* leaf extract may impair the cell and lead to other complication as a result of lack of protection for the membrane. The dose-dependent

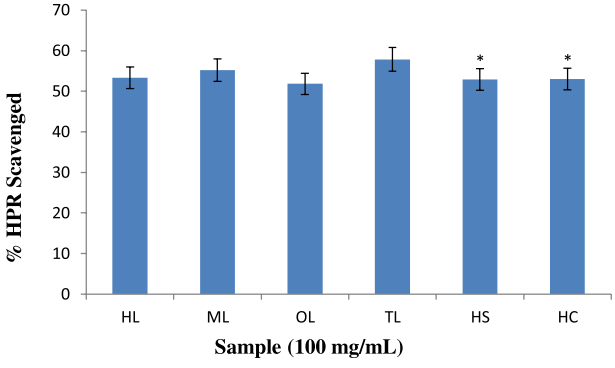

Figure 4. Hydrogen peroxide antioxidant activities of the extracts.

Notes: HPR = Hydrogen peroxide radical; HL = *H. sabdariffa* leaf extract; ML = *M. oleifera* leaf extract; OL = *O. gratissimum* leaf extract; TL = *T. occidentalis* leaf extract; HS = *H. sabdariffa* seed extract; HC = *H. sabdariffa* calyx extract. *Data are significantly different at $p > 0.05$.

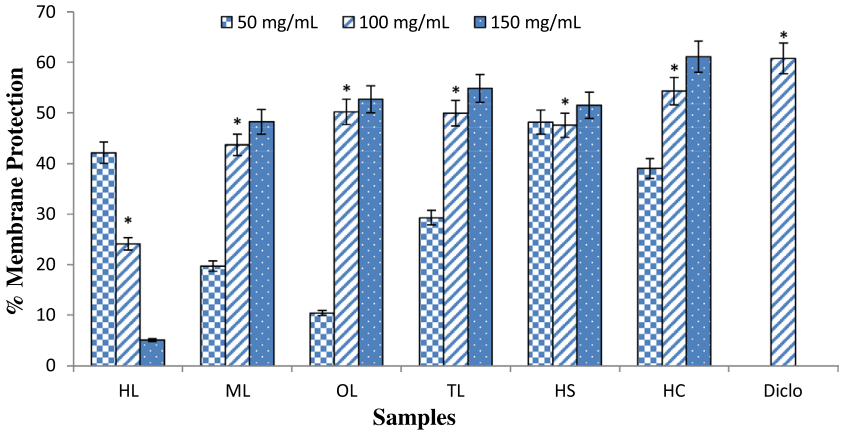

Figure 5. Cell membrane stabilization activities.

Notes: HL = *H. sabdariffa* leaf extract; ML = *M. oleifera* leaf extract; OL = *O. gratissimum* leaf extract; TL = *T. occidentalis* leaf extract; HS = *H. sabdariffa* seed extract; HC = *H. sabdariffa* calyx extract; Diclo = Diclofenac.
*Data are significantly different at $p < 0.01$.

anti-inflammatory activity of *T. occidentalis* leaf is in agreement with previous research outcome (Okokon, Dar, & Choudhary, 2012; Oluwole, Falode, & Ogundipe, 2005).

The ability of the extract to inhibit proteinase was investigated. The proteinase inhibition activities were significantly different ($p < 0.05$) as depicted (Figure 6). The result indicated that at 100 µg/mL, *H. sabdariffa* calyx extract had the highest proteinase inhibitory activity (38.33%) with *H. sabdariffa* seed extract possessing the least observable proteinase inhibitory potential (6.18%). Other extracts exhibited activity in the order: *M. oleifera* leaf extract (29.35), *O. gratissimum* leaf extract (22.57%), *T. occidentalis* leaf extract (19.65%) and *H. sabdariffa* leaf extract (13.58%). Interestingly, *H. sabdariffa* seed extract lost all observable activity at 100 µg/mL.

The search for natural antioxidants and anti-inflammatory agents from plants with bioactive principles has increased due to the reported toxicity and side effects of synthetic counterparts. Therefore, more attention has been diverted to edible natural products with such potential. In this study, our result indicated that all the plant extracts have high antioxidant potentials. Plants which are known to possess high quantity of tannin and alkaloids are reported to have both antioxidant and anti-inflammatory activities (Luo, Basile, & Kennelly, 2002; Okoli & Akah, 2004). Hence, the presence of tannin in the leaves of *H.sabdariffa* may be responsible for the high antioxidant exhibited in the ABTS assay system.

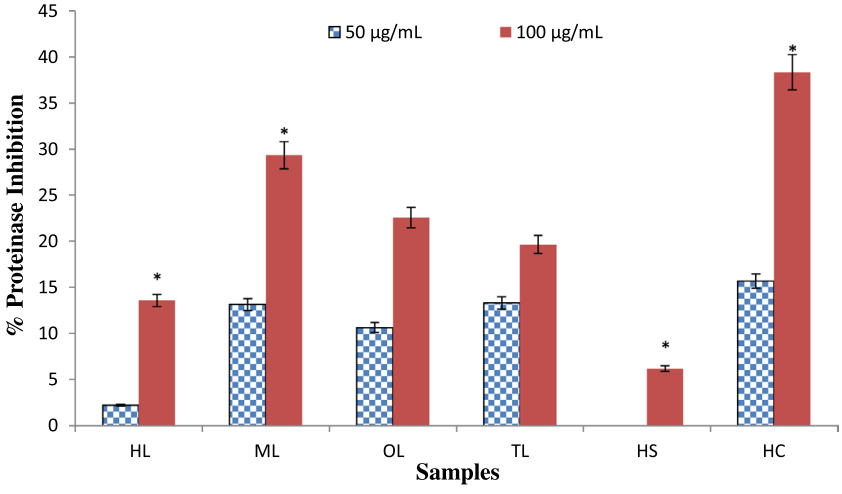

Figure 6. Proteinase inhibition.

Notes: HL = *H. sabdariffa* leaf extract; ML = *M. oleifera* leaf extract; OL = *O. gratissimum* leaf extract; TL = *T. occidentalis* leaf extract; HS = *H. sabdariffa* seed extract; HC = *H. sabdariffa* calyx extract. Values obtained are average of triplicate determinations.
*Data are significantly different at $p < 0.01$.

Neutrophils are known to be a source of proteinase which carries several serine proteinases in their lysosomal granules. It has been reported that leukocytes proteinase play important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das & Chatterjee, 1995). In this study, *H. sabdariffa* calyx extract at 100 µg/mL was able to inhibit proteinase significantly ($p < 0.05$) compared to other extract.

The protection of the red cell membrane is imperative to the integrity and safety of the cell as alteration to it exposes the cells to secondary damage through free radical-induced lipid peroxidation. Membrane stabilisation could lead to the maintenance of the cell integrity and prevention of serum leakage and fluids into the tissues during a period of increased permeability initiated by inflammatory mediators (Chaitanya, Sandhya, David, Vinod, & Murali, 2011). Therefore, the ability of phytochemicals to protect the cell membrane at such instance may not be overemphasised. All tested extracts with the exclusion of *H. sabdariffa* leaf extract exhibited potential to reduce the susceptibility of the cell to oxidative damage. Further purification of the crude extract may lead to the characterisation of important "lead" compound with antioxidant and anti-inflammatory potential.

4. Conclusion

This research establishes that aqueous extracts of leaves, seeds and calyces of *H. sabdariffa*, *M. oleifera* and *O. gratissimum* are rich in bioactive principles such as saponins, tannins, phenols, alkaloids, flavonoids and glycosides which apparently important contributors to the overall medicinal values observed in the plants. *T. occidentalis* leaf extract and *H. sabdariffa* leaf extract exhibited highest antioxidant activities which are comparable to standard compounds used. On the contrary, the *H. sabdariffa* also had the least cell protection activity at increased concentration. It is noteworthy that *H. sabdariffa* calyx extract had the highest proteinase inhibitory activity. Therefore, the indiscriminate consumption of *H. sabdariffa* extract should be avoided as high concentration is observed to lack cell protection ability in the cell membrane stabilisation assay. The results of this study shows that the plant extracts can be used as a renewable source of natural safe antioxidants in food supplements or nutraceutical preparations.

Funding
The research work was supported by the University of Ilorin, Ilorin, Senate Research Grant [grant number UIL/CREDIT/30] offered to African Centre for Herbal Research Ilorin (ACHRI), University of Ilorin, Ilorin, Nigeria.

Author details
L. Kambizi[1,2]
E-mail: KambiziL@cput.ac.za
M.T. Bakare-Odunola[1,3]
E-mail: odunola.moji1@gmail.com
A.T. Oladiji[1,4]
E-mail: toladijii@gmail.com
A.T. Kola-Mustapha[1,5]
E-mail: atkmust@yahoo.com
T.O. Amusa[1,6]
E-mail: teejayui@gmail.com
O. Atolani[1,7]
E-mail: atolani.o@unilorin.edu.ng
ORCID ID: http://orcid.org/0000-0002-3580-5904
N.S. Njinga[1,3]
E-mail: olous202@gmail.com
A.L. Quadri[1,4]
E-mail: quadriluqman@gmail.com
[1] African Centre for Herbal Research Ilorin (ACHRI), Central Research Laboratory, University of Ilorin, Ilorin, Nigeria.
[2] Department of Horticulture, Cape Peninsula University of Technology, South Africa.
[3] Department of Pharmaceutical and Medicinal Chemistry, University of Ilorin, Ilorin, Nigeria.
[4] Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.
[5] Department of Pharmaceutics and Industrial Pharmacy, University of Ilorin, Ilorin, Nigeria.
[6] Department of Forest Resources Management, University of Ilorin, Ilorin, Nigeria.
[7] Department of Chemistry, University of Ilorin, Ilorin, Nigeria.

Citation information
Cite this article as: Proteinease inhibition, membrane stabilization, antioxidant and phytochemical evaluations of leaves, seeds and calyces of four selected edible medicinal plants, L. Kambizi, M.T. Bakare-Odunola, A.T. Oladiji, A.T. Kola-Mustapha, T.O. Amusa, O. Atolani, N.S. Njinga & A.L. Quadri, *Cogent Chemistry* (2017), 3: 1314064.

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