



FEDERAL COLLEGE OF ANIMAL HEALTH AND
PRODUCTION TECHNOLOGY, IBADAN.

Maiden Conference AND CALL FOR PAPER



THEME:
**SCIENCE, TECHNOLOGY
AND INNOVATION
FOR SUSTAINABLE ECONOMIC
AND AGRICULTURAL RESEARCH
DEVELOPMENT IN NIGERIA**

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TECHNOLOGY, IBADAN

BOOK OF PROCEEDINGS

Edited by: Dr Popoola M. A., Dr. Banjo O. T., Mrs Awodola-Peters O. O., Mrs Osunlaja N. A.
Mr Sodeeq A. E., Mrs Adedeji O. Y., Mr Shittu S.M.A.



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FEDERAL COLLEGE OF ANIMAL HEALTH AND PRODUCTION TECHNOLOGY, IBADAN

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ECONOMIC AND AGRICULTURAL
RESEARCH DEVELOPMENT IN NIGERIA

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31st August and 1st September, 2020

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**Biological, Life and
Natural Sciences**

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *Tectona grandis* Linn

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Abstract

Tectona grandis Linn (commonly known as teak) is a large, deciduous tree reaching over 30m in height known for decades for its timber value. This plant's wood is renowned for its extreme durability, dimensional stability, and hardness, which also resist decay even when unprotected by paints and preservatives. The present study analyzed the phytochemical & pharmacological potential of the methanolic extracts from leaf, stem-bark, and roots of *Tectona grandis*. *Tectona grandis* extracts revealed the presence of six phytochemicals with Saponins present in all extracts. Antibacterial activity of all extracts from *Tectona grandis* was checked against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Streptococcus mutans*, and *Staphylococcus aureus* by disc diffusion assay. The methanol extract of stem bark showed inhibition of the growth of *Escherichia coli* and *Staphylococcus aureus*. Antifungal activity of all extracts from *Tectona grandis* was checked against *Aspergillus flavus*, *Aspergillus fumigatus*, and *Candida albicans*. The methanol extract of leaves showed inhibition of the growth of *Candida albicans*. The study results provide a scientific basis for the use of this plant extract in future development as antibacterial and antifungal agents.

Keywords: Teak; Phytochemicals; Infections; Dysentery; *Candida albicans*

Introduction

Plants have been an indispensable source of medicine since time immemorial, 25% of the medicinal drugs prepared in developed countries are based on plants and their derivatives (Ramesh et al., 2013). These plant extracts have been extensively investigated as sources of medicinal agents as they contain secondary plant metabolites (phytochemicals), which are pharmacologically active. *Tectona grandis* Linn. commonly known as Teak is one of the most famous timber in the world, belonging to the Verbenaceae family. It is a large, deciduous tree reaching over 30m in height in favourable conditions (Neha and Sangeeta, 2013). Teak is a tropical tree species distributed naturally in countries including India, Myanmar, Thailand, and Nigeria. (Mahesh et al., 2016). It is confirmed that the accumulation of certain quinones and quinone derivatives are mainly responsible for its quality (Kokutse et al., 2006). Extracts from various parts of Teak are widely used in folklore; the leaves are used for the treatment of haemorrhages, skin diseases, bronchitis; the barks used for burning sensation, dysentery; the wood for biliousness, headache; and the roots are useful in anuria and retention of urine (Neha and Sangeeta, 2013). It has been established that naturally derived substances are safer and more desirable than synthetic chemical products (Opara et al., 2014). Therefore, this study was aimed at investigating the phytochemical constituents and antimicrobial activity of *Tectona grandis* extracts.

Materials and Methods

Collection and Preparation of Plant Sample: The Stem barks, leaves, and roots of *Tectona grandis* were collected around the University of Ilorin's campus. The plant was identified at the species level and deposited in the Department of Plant Biology Herbarium, University of Ilorin. Samples were powdered and stored in airtight containers at 4°C for further use.

Extraction of Powdered Plant Sample: Extraction was done using the modified methods of Gbadamosi et al., (2012) and Adeyemi et al., (2014). The crude extracts were dispensed into labelled sample vials and stored at 4°C prior to further use. The table below shows the enumeration of the various recipes analyzed in this study Table 1.

Table 1: Enumeration of recipes

S/No	Recipes	Constituents
1	Recipe I (RI)	Stem barks
2	Recipe II (RII)	Leaves
3	Recipe III (RIII)	Roots
4	Recipe IV (RIV)	Stem barks + Roots
5	Recipe V (RV)	Leaves + Stem barks + Roots

Phytochemical Screening: The samples' phytochemical composition was screened using standard procedures (Harbone, 1984; Sofowora, 1993; Raaman, 2006; Tiwari et al., 2011; Gbadamosi et al., 2012).

Collection and Maintenance of Test organisms: The bacterial isolates used in this study are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mutans*, *Salmonella typhi*, and *Escherichia coli* obtained from the Microbiology Department, University of Ilorin.

Preparation of Culture Media and Standardization of Inoculums: Mueller Hinton Agar, Nutrient Agar, and Nutrient Broth were all prepared according to the manufacturer's specifications. The McFarland nephelometer method was used to standardize the test organisms, as described by Albert et al., (1991).

Tests for antibacterial activity of the plant Extracts and Minimum Inhibitory Concentration (MIC) determination: The agar well method was used to determine the antibacterial activity of the plant extracts. Sterile Mueller Hinton agar plates were prepared (Sani and Bansa, 2003). The broth dilution method of Albert et al. (1991) was used for MIC.

Tests for antifungal activity of the plant Extracts: The Agar tube dilution method was carried out for antifungal activity. Three (3) fungal strains, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Candida albicans* were used Aida et al., (2001).

Analysis of variance (ANOVA) and comparison of means was carried out on all data of proximate analysis of the plant samples using Statistical Package for Social Science (SPSS). Differences between means were assessed for significance at $p < 0.05$ by Duncan's Multiple Range Test (DMRT).

Results

Phytochemical Activity: The result of the qualitative phytochemical analysis of methanol extract of *Tectona grandis* recipes revealed the presence of six classes of secondary metabolites shown in Table 2. Saponin and flavonoids were detected in all the extracts. Six out of the seven phytochemicals investigated were detected in the leaves, while four were present in the stem barks and roots.

Table 2: Phytochemical evaluation of the recipes of *Tectona grandis*

Phytochemicals	RI	RII	RIII
Alkaloids	-	+	-
Flavonoids	+	+	+
Phenols	+	-	+
Saponins	+	+	+
Steroids	+	+	-
Tannins	-	+	+
Terpenoids	-	+	-

Note: - = Absent; + = Present

Antibacterial Activity: The antibacterial activities of the methanol extract of *T. grandis* are given in Table 3. All extracts showed different inhibitory potentials. Methanol extracts show excellent activities (100-300mg/ml) against all bacterial strains. The extract from the stem bark at 50mg/ml exhibited the lowest antibacterial activity against all the test organisms, and the highest activity was recorded at 300mg/ml (Table 3). The activity exhibited against *S. aureus* at 50mg/ml was significantly different from that of other graded concentrations. The stem bark extract at 100mg/ml, 200mg/ml and 300mg/ml had significantly the same activity against *E. coli*. The antibacterial activity of the stem bark extract against *P. aeruginosa* was also significant, especially at 300mg/ml, which was the highest concentration to exhibit inhibition. There is was no significant difference at concentrations of 100mg/ml and 200mg/ml against the test organism. The antibacterial activity of the stem bark extract against *S. aureus* was also remarkable. Stem bark extract at 50mg/ml exhibited the lowest inhibition against the test organisms, while the highest inhibitory activity was 300mg/ml.

Though the extracts from leaves exhibited some antimicrobial activity against the test organisms, they were not as remarkable as that of the stem bark. Inhibitory activity against the test organisms increased with increasing concentration of the extract. At 50mg/ml, *T. grandis* leaves showed little inhibitory activity against all the test organisms. For *S. typhi*, there were no significant differences in the activity of 100, 200, 300mg/ml, but 50mg/ml showed a remarkably significant difference compared to other graded concentrations. The inhibition exhibited by *T. grandis* leaves against *S. typhi* at 50mg/ml and 100mg/ml was significantly different. The activities exhibited against *E. coli*, *P. aeruginosa*, and *S. mutans* are presented in Table 4.

For the root extracts, the lowest antibacterial activity was recorded at 50mg/ml against all the test organisms, and the highest activity was recorded at 300mg/ml. Also, 100, 200, and 300mg/ml concentrations had significantly the same activity against *E. coli*. In the case of *S. typhi*, there were no significant differences in the activity of 100, 200, 300mg/ml, but 50mg/ml showed a remarkable significant difference when compared against other graded concentrations (Table 3).

Table 3: Antibacterial Activity of Crude Methanolic Extract of *Tectona grandis* recipes

Test organism	Diameter of zone of inhibition (mm) $\pm$ S.E.M				
	Concentration of extracts (mg/ml)				
	Recipes	50	100	200	300
<i>Escherichia coli</i>	RI	10.00 $\pm$ 0.50 ^a	19.50 $\pm$ 1.00 ^b	23.25 $\pm$ 0.75 ^c	26.25 $\pm$ 1.25 ^d
	RII	10.25 $\pm$ 0.75 ^a	22.00 $\pm$ 3.00 ^b	24.50 $\pm$ 0.50 ^b	26.25 $\pm$ 0.25 ^b
	RIII	2.75 $\pm$ 0.75 ^a	20.25 $\pm$ 3.75 ^b	23.00 $\pm$ 1.50 ^b	24.75 $\pm$ 0.75 ^b
	RIV	13.50 $\pm$ 1.32 ^a	15.50 $\pm$ 1.32 ^a	17.67 $\pm$ 2.91 ^a	11.83 $\pm$ 0.88 ^{a*}
	RV	4.00 $\pm$ 0.00 ^b	5.00 $\pm$ 0.00 ^b	7.00 $\pm$ 0.29 ^b	8.17 $\pm$ 0.44 ^b
<i>Pseudomonas aeruginosa</i>	RI	12.00 $\pm$ 1.00 ^a	25.00 $\pm$ 1.00 ^b	25.50 $\pm$ 0.00 ^b	25.50 $\pm$ 0.00 ^b
	RII	10.50 $\pm$ 1.00 ^a	22.50 $\pm$ 0.50 ^b	25.75 $\pm$ 0.75 ^b	25.75 $\pm$ 0.75 ^b
	RIII	5.00 $\pm$ 0.50 ^a	19.00 $\pm$ 0.00 ^b	21.50 $\pm$ 2.50 ^b	21.50 $\pm$ 2.50 ^b
	RIV	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
	RV	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
<i>Salmonella typhi</i>	RI	11.00 $\pm$ 2.00 ^a	24.00 $\pm$ 1.50 ^b	25.75 $\pm$ 0.25 ^b	25.25 $\pm$ 0.25 ^b
	RII	2.00 $\pm$ 0.50 ^a	20.00 $\pm$ 1.00 ^b	21.00 $\pm$ 1.50 ^{bc}	25.00 $\pm$ 1.00 ^c
	RIII	2.00 $\pm$ 0.50 ^a	20.00 $\pm$ 1.00 ^b	21.00 $\pm$ 2.50 ^{bc}	25.00 $\pm$ 1.00 ^c
	RIV	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
	RV	6.83 $\pm$ 0.93 ^a	5.00 $\pm$ 0.29 ^a	7.50 $\pm$ 0.58 ^a	13.00 $\pm$ 0.58 ^a
<i>Streptococcus mutans</i>	RI	11.25 $\pm$ 0.75 ^a	21.50 $\pm$ 0.50 ^b	25.50 $\pm$ 0.50 ^b	26.00 $\pm$ 1.00 ^b
	RII	11.50 $\pm$ 2.00 ^a	18.50 $\pm$ 0.00 ^b	24.75 $\pm$ 0.75 ^c	23.50 $\pm$ 0.50 ^{cd}
	RIII	3.25 $\pm$ 1.25 ^a	18.75 $\pm$ 0.25 ^b	25.00 $\pm$ 2.50 ^b	24.50 $\pm$ 2.00 ^b
	RIV	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	1.33 $\pm$ 1.33 ^a	2.67 $\pm$ 2.67 ^a
	RV	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
<i>Staphylococcus aureus</i>	RI	8.75 $\pm$ 6.25 ^a	23.25 $\pm$ 0.75 ^b	26.75 $\pm$ 0.25 ^{bc}	29.00 $\pm$ 2.50 ^c
	RII	9.50 $\pm$ 0.50 ^a	19.75 $\pm$ 0.25 ^b	23.25 $\pm$ 1.25 ^c	24.50 $\pm$ 0.00 ^{cd}
	RIII	4.25 $\pm$ 0.75 ^a	18.75 $\pm$ 0.75 ^b	20.25 $\pm$ 0.25 ^{bc}	22.25 $\pm$ 0.25 ^c
	RIV	8.17 $\pm$ 0.73 ^a	9.33 $\pm$ 1.17 ^a	10.50 $\pm$ 0.87 ^a	9.67 $\pm$ 1.01 ^a
	RV	9.67 $\pm$ 1.01 ^a	9.67 $\pm$ 1.76 ^a	9.00 $\pm$ 1.61 ^a	10.50 $\pm$ 1.54 ^a

Table 4: Bacterial Minimum Inhibitory Concentration (MIC)

S/N	TEST ORGANISM	RIV (mg/ml)	RII (mg/ml)	RIII (mg/ml)	RIV (mg/ml)	RV (mg/ml)
1	<i>Escherichia coli</i>	10	10	10	50	30
2	<i>Pseudomonas aeruginosa</i>	10	10	10	NA	NA
3	<i>S. typhi</i>	10	10	10	NA	10
4	<i>Staphylococcus aureus</i>	10	10	10	50	50
5	<i>Streptococcus mutans</i>	10	10	10	200	NA

The Methanolic extracts of recipe IV (Stem bark + Roots) also showed antibacterial activities. However, the extract exhibited weak antibacterial activity. There was no inhibition on the growth of *P. aeruginosa* and *S. typhi*, but exhibited very low inhibition against *S. mutans* at 200 and 300mg/ml. For *S. aureus* at 50mg/ml, the lowest inhibition was recorded, while the highest inhibitory activity was exhibited at 200mg/ml. For *E. coli*, there were no significant differences in the activity of 50, 100, 300 mg/ml, but 200mg/ml showed a remarkable significant difference when compared against other graded concentrations (Table 4). The combined effects of the leaves, roots, and stem bark extracts also exhibited weak antibacterial activities. There were no inhibitions against *P. aeruginosa* and *S. mutans*.

The methanolic extracts of *T. grandis* plant parts also showed antifungal activities. All the recipes were active against *A. flavus* and *C. albicans*. The inhibitory activity against the test organisms increased with increasing concentration of the extract.

The results showed that the extracts exhibited weak antifungal activities as compared with antibacterial activities. The stem bark extracts at 50mg/ml exhibited lowest antifungal activity against all the test organisms and highest activity was recorded at 300mg/ml (Table 5). There were no inhibitions against *A. fumigatus* exhibited by all the recipes except Recipe III which exhibited remarkable antifungal activity at 300mg/ml. The antifungal activity of *T. grandis* stem bark extract against *C. albicans* was significant at 300mg/ml. The minimum inhibitory concentration (MIC) of all plant extracts are recorded (Table 6).

Table 5: Antifungal activity of recipes of *Tectona grandis*

Test organism	Diameter of zone of inhibition (mm) $\pm$ S.E.M				
	Concentration of extracts (mg/ml)				
	Recipes	50	100	200	300
<i>Aspergillus flavus</i>	RI	8.67 $\pm$ 0.88	13.33 $\pm$ 3.51	16.83 $\pm$ 1.59	18.33 $\pm$ 1.20
	RII	12.83 $\pm$ 1.04	13.00 $\pm$ 1.16	16.50 $\pm$ 0.87	20.00 $\pm$ 1.16
	RIII	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	16.00 $\pm$ 1.15	16.33 $\pm$ 1.20
	RIV	0.00 $\pm$ 0.00	19.17 $\pm$ 0.60	19.50 $\pm$ 1.04	25.17 $\pm$ 1.01
	RV	18.83 $\pm$ 1.16	20.00 $\pm$ 0.76	21.33 $\pm$ 0.73	24.50 $\pm$ 0.29
<i>Aspergillus fumigatus</i>	RI	-	-	-	-
	RII	-	-	-	-
	RIII	-	-	-	32.00 $\pm$ 1.04
	RIV	-	-	-	-
	RV	-	-	-	-
<i>Candida albicans</i>	RI	8.58 $\pm$ 1.17	14.25 $\pm$ 0.87	19.92 $\pm$ 0.60	21.42 $\pm$ 0.88
	RII	18.58 $\pm$ 0.60	20.25 $\pm$ 0.87	23.75 $\pm$ 1.15	25.42 $\pm$ 0.88
	RIII	17.25 $\pm$ 0.76	19.08 $\pm$ 1.30	19.26 $\pm$ 1.45	20.42 $\pm$ 0.85
	RIV	15.65 $\pm$ 1.15	17.92 $\pm$ 1.48	20.92 $\pm$ 1.09	22.81 $\pm$ 0.58
	RV	5.25 $\pm$ 1.04	5.14 $\pm$ 0.20	8.92 $\pm$ 0.92	13.90 $\pm$ 2.25

Table 6: Fungal Minimum Inhibitory Concentration (MIC)

S/N	Test Organisms	RIV (mg/ml)	RII (mg/ml)	RIII (mg/ml)	RIV (mg/ml)	RV (mg/ml)
1	<i>A. flavus</i>	100	50	200	100	50
2	<i>A. fumigates</i>	-	-	300	-	-
3	<i>C. albicans</i>	50	30	50	50	50

Discussion

Phytochemical analysis of the methanolic extract of *T. grandis* revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins and terpenoids which agrees with the findings of Ogunmefun et al. (2017) as well as Neha & Sangeeta, (2003). The phytochemicals present in the study plant have been identified to possess antimicrobial activity (Reddy et al, 2008). The presence of alkaloids, flavonoids, tannins and terpenoids in plants extracts has also been shown to enhance antimicrobial properties as reported by Singh and Bhat (2003). The presence of numerous phytochemicals in *T. grandis* leaves (RII) is an evidence that that plant part is a known site for the synthesis of different classes of compounds, hence the chance of finding bioactive novel compounds is high (Shruthi et al, 2012). These findings concur with that of Ashvin and Rajaram, (2014).

The antibacterial activity of plant extracts is believed to be due to the presence of flavonoids, tannins, and alkaloids (Draughon, 2004). Among the extracts from the different parts of the tree tested for antibacterial activities, methanolic extracts from the stem bark had the most outstanding activity against all the bacterial strains. It especially showed good activity against *E. coli* and *S. aureus*. The activity against *E. coli* by the plant extracts justifies the use in the treatment of dysentery and diarrhea which is sometimes caused by *E. coli* (Adams and Moss, 1999). Another study showed that the crude extracts from the leaf, bark and stem showed good activity against *S. aureus* (Krishna and Nair, 2009) which is in agreement with our findings.

The extracts from the leaves showed remarkable activity against the fungal strains on which they were tested on. Flavonoids and tannins in leaves have been reported to confer antimicrobial activity on them. The antimicrobial activity of flavonoids may be due to their ability to complex with extracellular, soluble proteins and to complex with fungal cell walls while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelope proteins (Elisee et al., 2016). There is some concern about the growing resistance of *C. albicans* to some common antimicrobials (Zaidi et al, 2018). This study however has shown *C. albicans*'s susceptibility to the *T. grandis* extracts.

Conclusion

Tectona grandis L. was investigated in this study for its phytochemical constituents and antimicrobial activities. The presence of various phytochemicals was revealed, and the plant also exhibited significant antibacterial and antifungal activities. The results obtained from this study against the various micro-organisms shows the therapeutic potential of the plant *T. grandis*. Therefore, it validates the ethnomedicinal use of the extracts from *T. grandis* in treating infections caused by the susceptible bacteria and fungi.

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