



## Chemical constituents, toxicity and antimicrobial activities of the essential oil from the leaves of *tectona grandis*

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### ABSTRACT

The leaves of *Tectona grandis* (Verbanaceae) was subjected to hydrodistillation in order to obtain the essential oil from the plant. The pale yellow essential oil gave a percentage yield of 0.184%. Relative percentages of individual component were analyzed by GC/GC-MS. A total of fifty-four (54) constituents were identified representing 86.5% of the total essential oil fraction. Oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, apocarotenoids, phenylpropanoids and non-terpene derivatives were the various classes of compounds identified. The LC<sub>50</sub> value from the brine shrimp toxicity assay was 183.29µg/ml. The oil extract was also subjected to antibacterial assay and it showed significant activities against all the clinical test organisms used except *Pseudomonas aeruginosa*.

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### Introduction

*Tectona grandis* Linn., commonly known as teak tree or sagwan (Hindi) is known in the world for its dimensional stability, extreme durability and hardness in timber production (10,11). It is classified in the division Magnoliophyta, class Magnoliopsida, order, Fumiales, and the family Verbenaceae /Lamiaceae. *Tectona grandis* is one of three species in the genus *Tectona*. The other two species, *Tectona hamiltoniana* and *Tectona philippinensis*, are relatively found in particular areas with relatively small native distributions in Myanmar and the Philippines, respectively (20). *Tectona grandis* is an excellent timber for bridge building and many other constructions while some other species in the family are notable ornamentals, such as *Clerodendrum*, *Callicarpa*, *Vitex*, *Lantana*, and *Verbena* (22).

*Tectona grandis* wood is found useful in the treatment of headache, constipation, biliousness, burning sensation and pain, liver-related troubles, worms, cough, microbial, fungal, piles, leucoderma and dysentery infections. The oil of the nuts and flowers promotes hair growth and also useful in the treatment of scabies while the roots are useful in the treatment of urinary system-related troubles (16).

Compounds such as Lapachol and its derivatives, methyl quinizarin and squalene isolated from the heart wood were found to have cytotoxic (17), antiulcer, wound healing and anaemia activities in experimental animals (6,12). The antibacterial and cytotoxic potential of the chloroform extract and the antioxidant potential of the ethyl acetate extract from the plant have also been reported (12). The alcoholic and aqueous extracts of the stem-barks showed significant and dose-dependent analgesic and anti-inflammatory effects (2).

As reported by Purushotham *et al.* (18) in a study aimed at formulating new cost-effective antimicrobial agent for multi-drug resistant organisms, the antibacterial activity of methanol extract from the leaves with tetracycline showed maximum

synergistic activity against different bacteria both gram-positive and gram-negative species. This present work is however aimed at extracting, characterizing and carrying out bioassays on the leaf essential oil of the plant as a source of bioactive substances.

### Materials and methods

#### Plant Materials:

Fresh leaves sample was collected and identified from the Botanical garden of the University of Ibadan, Oyo State, Nigeria. The samples were air dried and later crushed into smaller sizes. 250 g of the sample was subsequently subjected to hydrodistillation process for 3½ hours in an all glass Clevenger distillation apparatus designed according to the British Pharmacopoeia (BP) specifications. The essential oil obtained was stored in a refrigerator until further analysis was carried out.

#### Identification of Volatile Oil Constituents by GC/GC-MS Analysis:

Gas Chromatographic analysis was done with a HP-5890 Series II instrument required with a HP-Wax and HP-5 capillary columns (both 30 m x 0.25 mm, 0.25 µm film thickness), working with the following temperature program: 60°C for 10 min, rising at 5°C/min to 220°C. The injector and detector temperatures were maintained at 250°C; carrier gas, N<sub>2</sub> (2 mL/min); detector dual, FID; split ratio, 1:30. The volume injected was 0.5 µL. The identification of components was carried out by comparing the retention times of the constituents with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of n-hydrocarbons. The relative proportions of the oil constituents were percentages obtained by FID peak-area normalization without the use of response factors. GC-EIMS analysis was carried out with a Varian CP-3800 gas chromatograph equipped with a HP-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were: injector and transfer line

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temperature, 220°C and 240°C, respectively; oven temperature programmed from 60–240 °C at 3°C/min; carrier gas, He at 1 mL/min; injection of 0.2 µL (10% hexane solution); and split ratio, 1:30. Mass spectra were recorded at 70 eV. The acquisition mass range was 30–30 *m/z* at a scan rate of 1 scan/s

Identification of the constituents was based on comparison of the retention times with those of the authentic samples, comparing their linear indices relative to the series of n-hydrocarbons, and on computer matching against commercially available spectra (NIST 98 and Adams) (1,19). Further identifications were also made possible by the use of homemade library mass spectra built up from pure substances and components of known oils and MS literature data (5,9,15). Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

#### Toxicity Assay (Brine Shrimp Lethality Test):

To evaluate the level of toxicity in the essential oils using *Artemia salina* eggs hatched in sea water, solution of the extracts were made in DMSO, at varying concentrations (1000, 100, 10) ppm in triplicate vials. 10 brine shrimp larvae were then placed in each of the triplicate vials in a total volume of 5 ml. A solution as reference standard was also made consisting of DMSO and 10 brine shrimps in sea water. This serves as negative control. After 24 hours, the total number of dead shrimps was counted and the lethal concentration at 50% level was determined by the Finney probit computer programme.

#### Antibacterial Assay

The agar diffusion methods were employed to determine the bacterial susceptibility of the oil extract using six clinical test organisms. The agar well diffusion method was used for the antibacterial assay on the oil extract while standard antibiotic discs of Chloramphenicol (30 µg), Augmentin (30 µg) and Gentamycin (5 µg) were used as positive reference standards.

#### Results And Discussion

The essential oil from *Tectona grandis* leaves gave a pale yellow colour with percentage yield of 0.184. Table 1 shows the chemical constituents and relative percentages of individual components of oil analyzed by GC/GC-MS. These constituents were identified on the basis of their linear retention values, co-injection with the available authentic samples and by comparison of their mass spectra with those reported in the literature. A total of fifty-four (54) constituents were identified representing 86.5% of the total oil fraction.

The essential oil consists of the following class of compounds: oxygenated monoterpenes (14.1%), sesquiterpene hydrocarbons (7.3%), oxygenated sesquiterpenes (15.8%), apocarotenoids (20.5%), phenylpropanoids (1.2%) and non-terpene derivatives (27.6%). The major constituents are linalool (8.7%), β-eudesmol (8.5%), (E)-β-ionone (7.8%), mesitylene (6.0%) and (E)-geranylacetate (5.1%). Other significant constituents include n-decane (4.2%), edulan I (3.6%), dodecanal (3.1%), isocaryophyllene (2.9%), nonanal (2.7%), β-caryophyllene (2.6%), caryophyllene oxide (2.5%) and hexahydrofarnesylacetone (2.3%). The most abundant constituent was linalool (8.7%).

Of the classes of terpenes/terpenoids present in the essential oil of *Tectona grandis* is the apocarotenoids (20.5% of the total oil fraction). These are organic compounds derived from carotenoids by oxidative cleavage (specific cleavage of the polyene chain double bonds) (14). They are isoprenoid molecules which display key health-related roles in humans and animals and are important for primary and secondary metabolisms of plants and other living organisms (4). Products

of these oxidative cleavage can act as hormones (example, abscisic acid), signaling compounds (such as the visual and signaling molecules; retinal and retinoic acid), chromophores and scent/aroma constituents (such as the aromatic volatile Ionones and safranal) etc. (21).

The brine shrimp lethality assay was used as a tool for the preliminary assessment of toxicity of the essential oil extract. This is carried out to determine the lethal concentration at 50% level of toxicity (LC<sub>50</sub>). LC<sub>50</sub> above 1000 µg/ml implies a non-toxic property; LC<sub>50</sub> between 500 – 1000 µg/ml implies a less toxic property while LC<sub>50</sub> between 100 – 500 µg/ml implies a moderately toxic property. LC<sub>50</sub> less than 100 µg/ml imply a high toxic property. From this study, the LC<sub>50</sub> of the essential oil extract on table 2 was calculated to be 183.29 µg/ml. This is an indication that there are active component(s) present in the essential oil extract, making it toxic.

The result of the antibacterial assay is presented in table 3. The essential oil extract shows significant activity against all the clinical test organisms except *Pseudomonas aeruginosa* which is known to show natural resistance to many antibiotics as reported in previous works done (3,7,8,23). The highest zone of inhibition was observed for *Escherichia coli* to be 29 mm while the least was observed for *Staphylococcus epidermidis* at 20 mm.

Mahesh and Jayakumar (12), reported that the chloroform extract from the leaves of the plant showed inhibition to growth of *Staphylococcus aureus* (14 mm).

#### Conclusion

The plant essential oil extract shows promising activities which also suggests that there is correlation in its use in treating ailments as recorded in various reports. Likewise the antibacterial and toxicity potential of the oil extract corroborate with some reports earlier carried out.

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Table 1: Chemical Constituents of the Essential Oil of *Tectona grandis* leaves from GC-MS Analysis

Constituents	l.r.i.	% composition
6-methyl-5-hepten-2-one	986	1.1
2-pentyl furan	994	0.4
Mesitylene	996	6.0
<i>n</i> -decane	1000	4.2
Pentyl propanoate	1016	1.0
Dehydrolinalool	1091	0.2
Linalool	1099	8.7
Nonanal	1103	2.7
( <i>E,Z</i> )-2,6-nonadienal	1156	1.1
( <i>E</i> )-2-nonenal	1158	0.7
<i>cis</i> -chrysanthenol	1162	0.2
1-nonanol	1172	0.4
4-terpineol	1178	0.2
Naphthalene	1180	0.2
$\alpha$ -terpineol	1190	0.4
Methyl salicylate	1192	0.3
<i>n</i> -dodecane	1200	0.8
Decanal	1204	0.7
<i>trans</i> -pulegol	1215	1.8
$\beta$ -cyclocitral	1217	2.2
<i>neo</i> -iso-dihydrocarveol	1229	0.2
3-methyl-3-hexen-1-yl-butanoate	1237	0.2
<i>n</i> -tridecane	1300	0.5
Edulan I	1313	3.6
Eugenol	1358	1.2
$\alpha$ -copaene	1376	0.6
<i>n</i> -tetradecane	1400	1.1
Isocaryophyllene	1405	2.9
Dodecanal	1408	3.1
$\beta$ -caryophyllene	1418	2.6
( <i>E</i> )- $\alpha$ -ionone	1427	1.2
<i>cis</i> - $\alpha$ -ambrinol	1438	0.5
( <i>E</i> )-geranyl acetone	1454	5.1
$\gamma$ -muurolene	1477	0.4
Germacrene D	1480	0.1
( <i>E</i> )- $\beta$ -ionone	1485	7.8
$\alpha$ -selinene	1494	0.7
<i>n</i> -pentadecane	1500	0.7
Tridecanal	1510	Tr
$\delta$ -cadinene	1524	Tr
Citronellyl <i>n</i> -butyrate	1532	0.2
( <i>E</i> )-nerolidol	1565	1.2
( <i>Z</i> )-3-hexenyl benzoate	1570	Tr
Caryophyllene oxide	1581	2.5
1-hexadecene	1593	0.5
<i>n</i> -hexadecane	1600	0.6
Eremoligenol	1630	1.8
Hinesol	1638	1.4
$\beta$ -eudesmol	1649	8.5
$\alpha$ -eudesmol	1652	0.4
<i>n</i> -heptadecane	1700	0.5
Pentadecanal	1717	0.2
<i>n</i> -octadecane	1800	0.6
Hexahydrofarnesylacetone	1845	2.3
Class of Terpene/Terpenoid Present		
Monoterpene hydrocarbons		0.0
Oxygenated monoterpenes		14.1
Sesquiterpene hydrocarbons		7.3
Oxygenated sesquiterpenes		15.8
Apocarotenoids		20.5
Phenylpropanoids		1.2
Non-terpene derivatives		27.6
Total identified		86.5

Tr = Trace quantity (&lt; 0.1%)

l.r.i. = Linear Retention Indices

% = Percentage

**Table 2: Brine Shrimp lethality test of *Tectona grandis* leaves essential oil**

Sample	LC <sub>50</sub> µg/ml	UCL	LCL
TGLEO	183.29	285.84	124.03

**TGLEO:** *Tectona grandis* leaves essential oil.

**UCL:** Upper Confidence Limit.

**LCL:** Lower Confidence Limit.

**Table 3: Antibacterial analysis on the Essential oil of the leaves of *Tectona grandis***

Test organisms with zone of inhibition (mm)

PLANT ESSENTIAL OIL	SA	SE	EC	PA	PS	BS
TGLEO	21.0	20.0	29.0	-	25.5	22.5
STANDARD CONTROL						
Augmentin (30 µg)	-	-	-	-	-	-
Chloramphenicol (30 µg)	7.0	10.0	20.0	-	-	28
Gentamycin (5 µg)	-	-	14.0	-	-	16.0
Hexane (100%)	-	-	-	-	-	-

**TGLEO:** *Tectona grandis* leaves essential oil.

**(SA):** *Staphylococcus aureus*

**(SE):** *Staphylococcus epidermidis*

**(EC):** *Escherichia coli*

**(PA):** *Pseudomonas aeruginosa*

**(PS):** *Proteus* sp.

**(BS):** *Bacillus subtilis*

**(-):** No inhibition

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