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RESEARCH ARTICLE :

Analysis of chemical compounds from the withered brown leaves of *Tectona grandis*

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SUMMARY : Plant metabolites play a special role in the maintenance of good health. India is rich in natural wealth and there is an ample scope to explore phytochemicals from the plant kingdom. Trees are also one of the important sources of secondary metabolites. *Tectona grandis* (Teak) one of the best-known tropical timbers, is native to the Indian subcontinent which extends to areas like Myanmar, Thailand and Laos. The whole plant is also medicinally important as it contains enormous number of phytoconstituents which helps in curing the ailments. The GC - MS analysis of withered brown leaves of *Tectona grandis* showed 26 compounds of which squalene, dibutyl phthalate, geranyl-p-cymene and caryophyllene oxide showed the greatest contribution to the percentage of the total area.

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KEY WORDS :
Tectona grandis, Brown leaf, Methanol, GC-MS, Metabolites, Squalene

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BACKGROUND AND OBJECTIVES

Plant metabolites play a special role in the maintenance of good health. The bases of many modern cosmetic and pharmaceutical products used today are plants and plant based products. Natural products, such as plant extracts, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Koperuncholan and Manogaran, 2015). The plants have health enhancing factors owing to their secondary metabolites such as terpenes, flavonoids, saponins, anthraquinones, glycosides, *etc*., which are found in leaves, flowers, stem, seeds and roots (Zippel *et al*., 2009). Thus, trees are also one of the important sources of secondary metabolites.

Tectona grandis (Teak) one of the best-known tropical timbers, is native to the Indian subcontinent which extends to areas like Myanmar, Thailand and Laos. Genetic differences are known to occur between various provenances. In addition, teak tree is a source for a multitude of known and yet unknown commercial properties, such as medicinal drugs, natural insecticides and non timber products, whereas no literatures are available regarding tchemical constituents of withered leaves of *Tectona grandis* and hence this present investigation is undertaken.

Resources and Methods

Plant collection :

The withered brown leaves of *Tectona grandis* were collected from plantations across Tamil Nadu during 2015 - 2016 and crushed to obtain a coarse powder to ease chemical compound extraction using soxhlet apparatus.

Preparation of methanol leaf extract :

Exactly 5.0 grams of the crushed leaves of *Tectona grandis* were extracted with 25 mL of methanol in an automated soxhlet apparatus (SOXTEC 2043 FOSS). The extraction was performed at 60^0 C for 2 hours and 30 minutes completing three cycles. All the phytoconstituents were extracted from the leaves at the end of the third cycle. The drying of the extract took place at room temperature after which they were stored and refrigerated in air tight sterile vials at 4^0 C.

GC - MS analysis :

The chemical composition of the leaf extract was analysed using Thermo GC - Trace Ultra Ver: 5.0 and Thermo MS DSQ II fitted with a DB 35 - MS capillary standard non - polar column (30 m, ID: 0.25 mm and film thickness of 0.25 µm). 0.5 µl of methanol extract was injected for analysis and Helium was used as a carrier gas at 1 ml/ min. The instrument was set as follows, Injector port temperature set to 250^0 C, source kept at 220^0 C. The oven temperature was programmed from 70^0 C to 260^0 C at the 6^0 C/ min rate. The MS was set to scan from 50 - 650 Da. The MS also had inbuilt pre - filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum, NIST4 and WILEY9 containing more than five million references.

Identification of compounds :

Interpretation of mass spectrum of GC - MS was done using the database of National Institute Standard and Technology (NIST4) and WILEY9 (Dool and Kratz, 1963).

Observations and Analysis

Twenty nine compounds were identified from the leaf extract of *Tectona grandis*. Thus, among the 29 compounds, the most prevailing major components were found to be squalene (19.40%), dibutyl phthalate (16.45%), geranyl-p-cymene (7.01%), caryophyllene oxide (5.78%), melatonin (5.73%), geranylgeraniol (4.19%), preg-4-en-3-one, 12,17- dihydroxy-20-nitrilo- (3.77%), (-)-α-selinene (3.58%), farnesane (2.90%) and abietic acid (2.85%). The quantitative results were presented in Table 1 and chromatograph of methanolic extract from *Tectona grandis* leaves by GC - MS was given in Fig. 1.

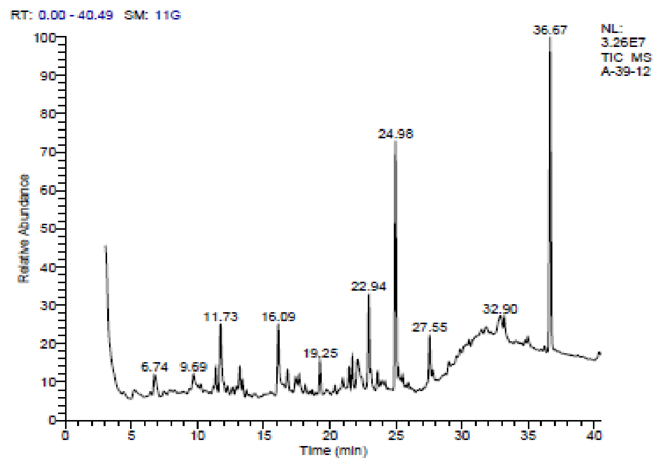

Fig. 1 : The chromatograph of withered brown leaf of *Tectona grandis* by GCMS

The compound, dibutyl phthalate is considered to be a contaminant in the leaf extract as the solvent leaches out the plastic material from the vials in which the extract was stored (Shubhangi, 2016). Guil – Guerrero *et al.* (2000) reported the possible role for squalene content in the leaves of plant species can be a photoprotective function to a part of epicuticular structures. Katarzyna *et al.* (2013) reported that the plant based squalene may be used in cosmetics and personal care products as squalene is slowly absorbed through the skin. The toxicity of squalene by all routes is low. At 100% concentrations, the compound is a non irritant to the skin and eyes. Gregory (1999) observed that squalene was similar to beta-carotene. In humans, about 60% of dietary squalene is distributed to the skin and protecting it from UV radiation. Supplementation of the diet with squalene can reduce cholesterol and triglyceride levels. Hence the dried leaves of teak can be used as a viable option to extract the squalene.

Geranyl-p-cymene is known to be the most important hydrophobic antibacterial agent in thyme (Eaton, 1997).

Table 1 : Chemical compounds identified in withered brown leaf of *Tectona grandis*

Sr. No.	RT (min)	Compound name	Molecular formula	Molecular weight	Area %
1.	3.08	7 – hydroxyl 8- prenylflavanone	$C_{20}H_{20}O_3$	308	0.99
2.	5.23	2-[9-(10-phenylanthryl)]ethanol	$C_{22}H_{18}O$	298	1.75
3.	6.74	Farnesane	$C_{15}H_{32}$	212	2.90
4.	9.69	Cyclotridecane	$C_{13}H_{26}$	182	2.04
5.	11.37	Caryophyllene	$C_{15}H_{24}$	204	1.91
6.	11.73	Melatonin	$C_8H_7N_3$	145	5.73
7.	13.18	(-)-α-Selinene	$C_{15}H_{24}$	204	3.58
8.	16.09	Caryophyllene oxide	$C_{15}H_{24}O$	220	5.78
9.	16.80	5,6-dihydroxy-2- indolecarboxylic acid	$C_9H_7NO_4$	193	1.29
10.	17.40	3-tert-Butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	180	1.22
11.	17.67	10-Heneicosene (c,t)	$C_{21}H_{42}$	294	1.54
12.	19.25	4,4,5,8-Tetramethylchroman-2-ol	$C_{13}H_{18}O_2$	206	2.08
13.	20.97	Bergamotol, Z-α-trans-	$C_{15}H_{24}O$	220	1.17
14.	21.46	geranyl-α-terpinene	$C_{20}H_{32}$	272	1.12
15.	21.71	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.75
16.	22.12	Preg-4-en-3-one, 12,17-dihydroxy-20-nitrilo-	$C_{20}H_{27}NO_3$	329	3.77
17.	22.94	geranyl-p-cymene	$C_{20}H_{30}$	270	7.01
18.	23.62	2,2-Difluoro-2-(2-naphthyl)acetonitrile	$C_{12}H_7F_2N$	203	1.15
19.	23.91	Ferulaldehyde	$C_{10}H_{10}O_3$	178	0.94
20.	24.98	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	16.45
21.	25.52	Methyl stearate	$C_{19}H_{38}O_2$	298	0.75
22.	27.55	Geranylgeraniol	$C_{20}H_{34}O$	290	4.19
23.	30.51	2-Chloro-1,4-bis(dibromomethyl)benzene	$C_8H_5Br_4Cl$	452	1.08
24.	31.47	8,8'-Dihydroxy-4,4'-dimethoxy-3',6-dimethyl-2,2'-bi naphthyl-1,1'-quinone	$C_{24}H_{20}O_6$	404	2.64
25.	31.79	1-[bis(trimethylsilyl)amino]-3,3-di-t-butyl-1-fluoro- 3-[(fluorodiisopropyl)amino]-1-methyldisiloxane	$C_{21}H_{54}F_2N_2OSi_5$	528	1.68
26.	32.90	Abietic acid	$C_{20}H_{30}O_2$	302	2.85
27.	33.20	Di-(2-ethylhexyl)phthalate 15.29	$C_{24}H_{38}O_4$	390	1.33
28.	34.99	Caryophyllene oxide	$C_{15}H_{24}O$	220	1.03
29.	36.67	Squalene	$C_{30}H_{50}$	410	19.40

The compound was also found in *Eucalyptus camaldulensis* and *E. urophylla* which is known to exhibit strong inhibiting effects on *Staphylococcus aureus* and *Escherichia coli* (Bachir and Benali, 2008). Caryophyllene oxide is known to be an important component of many essential oils which imparts antibacterial activities (Vania *et al.,* 2016). It is a constituent of many essential oils of traditionally used folk medicinal plants and spices (Heymann *et al.,* 1994) and also been used as a flavouring agent.

Geranylgeraniol was found to be the antileishmanial component in *Bixa orellana* (Lopes *et al.,* 2012). It has also shown antiparasitic activity against *Trypanosoma cruzi* (Menna-Barreto *et al.,* 2008). The compound α-selinene has been reported in the leaf essential oils of various other species like amla (Acharya, 2016), *Citrus aurantium* (Fatemeh and Mohammad, 2016), *Syzygium aromaticum* (Gaylor *et al.*, 2016), *Zanthoxylum armatum* (Hebind Pun *et al.,* 2016). *Juglans regia* (Ikram *et al.*, 2016), *Lantana camara* (Murugesan *et al.*, 2016), *Pinus wallichiana* (Joshi *et al.,* 2016), *Geranium robertianum* (Vania *et al.,* 2016) and *Tectona grandis* (Sherifat *et al.,* 2013).

Conclusion :

Tectona grandis apart from possessing valuable heartwood is also the unique source of secondary metabolites with many pharmacological utilities.

Therefore, the leaves considered as a waste in the teak plantation can also be utilised for extracting the metabolites.

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