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PHYTOCHEMICAL ANALYSIS OF LEAVES OF TEAK (*TECTONA GRANDIS* L.F.) BY GC MS

Greeshma, M., G.S. Manoj and K. Murugan*
Plant Biochemistry and Molecular Biology Laboratory, Department of Botany, University College, Trivandrum-695 034, Kerala, India.
*E mail: harimurukan@gmail.com

ABSTRACT

The timber value of *Tectona grandis*has been established from decades. Teak is an exotic species cultivated along most parts of tropical regions. Anthocyanins are natural colourants which have raised a growing demand due to their extensive range of colours, innocuous and beneficial health effects. Anthocyanins belong to large group of polyphenolics - flavonoids, which are secondary metabolites synthesized by higher plants. Despite the remarkable application of anthocyaninsin food, pharmaceutical and cosmetic industries, still it is not properly exploited. In the present study, the bioactive components of *Tectonagrandis*young leaves have been evaluated using Perkin-Elmer Gas chromatography-Mass spectrometry. GC-MS analysis revealed the existence of eleven compounds. 5,9-Methanobenzocycloocten-1(2H)-one,3,4,5,6,7,8,9,10-octahydro-5,10-dihydroxy-3,3,7,7,9-pentamethyl(76.02%) and 1-naphthalene carboxylic acid, 5-[2-(3-furanyl)ethyl]decahydro-1,4a-dimethyl-6-methylene-[1R-(1.alpha.,4a.)] (13.95%). Other compounds present in minor quantities were ledol(0.92%), 3-Buten-2-one,4-(2,6,6-trimethyl-1-cyclohexen-1-yl)orionone(0.49%),9,12,15 Octadecatrienoic acid, methyl ester, (Z,Z,Z)- or Linolenic acid methyl ester (0.82%), Phytol(0.69%), Cedran-diol, 8S,14- (0.60%), Lupeol (0.71%), 3-Methoxymethyl-2,5,5,8a-tetramethyl-6,7,8a-tetrahydro-5H-chromene (3.45%) and Retinol(1.27%). This is the first report of identification of active constituents from the young leaves of *Tectonagrandis.*

Keywords: GC-MS analysis, *Tectonagrandis*, Anthocyanin, Biological potentialities.

1. INTRODUCTION

Teak belongs to Verbenaceaeis known for the valuable timber and cultivated around the world. Teak is an exotic species dominated along the tropical regions like India and other South-East Asian countries.*Tectona grandis* is found adapted to variety of habitats and climatic conditions from arid areas with only 500 mm of rain per year to moist forests with up to 5,000 mm. Leaves of the teak are used for making Pellakaigatti, where batter is poured into the teak leaf and is steamed.This type of usage is found in the coastal district of Udupi in the Tulunadu region in South India. Teak leaves are used by the larvae of moths of the genus Endoclita and other Lepidoptera including Turnip moth for its development.

Anthocyanins are members of the flavonoid group of phytochemicals, which is predominant in most of the plants. Anthocyanins are exceptional natural pigments with range of colours from red to blue and medicinally potential health benefits via antioxidants. Apart from the antioxidant property they also possess anti-inflammatory, cardioprotective, hepatoprotective and anti-cancerous role. They are also found to be a perfect alternative of the synthetic food colourant and dye(Sujata and Bhaskar 2013). Anthocyanins are water-soluble vacuolar pigments that may appear as red, purple, or blue depending on pH. They also form glycosidated derivatives. The main anthocyanidin structure comprises an aromatic ring that contains oxygen, which is also bonded by carbon-carbon bond to third aromatic ring (Ananthaswamy *et al*., 2004). Diverse types of anthocyanins are reported in nature.

Extraction is an important step in the isolation, identification and use of anthocyanin compounds.There is no single and standard extraction method available universally. Fruits, vegetables and herbs can be ground, dried, or lyophilized, and some fresh plants can be soaked with subsequent solvent extraction to extract phenolic compounds (Merken and Beecher 2000). In the case of teak, anthocyanin can be extracted from the fresh leaves using methanol:HCl mixture. No literature available regarding the GC-MS analysis of young leaves of *Tectona grandis* and hence the present investigation is undertaken. The main objective of the present study is the extraction and quantification of anthocyanin from the young leaves

of teak and to analyze the various phytochemical constituents from the extract by GC MS analysis.

2. MATERIALS AND METHODS

2.1. Plant material

For the study fresh tender leaves of*Tectona grandis* were collected from the natural habitat of Thiruvananthapuram district, Kerala.

2.2. Estimation of anthocyanin content

1g leaf sample homogenized in 3ml methanol with 1% HCl and vortexed for 30 sec and kept in water bath at 60° C for 20 min. The samples were vortexed twice during incubation. Subsequently, the sample was centrifuged at 10000 rpm for 10 min. The supernatant was transferred to 10 ml volumetric flask. The residue was again mixed with 3 ml of methanol. The supernatant was again centrifuged and combined with the previous supernatant and made up to 10ml. The final extract solution was kept at 0°C for further analysis.

1ml of extract was taken and transferred to 10ml volumetric flask for preparing two dilutions of sample, one adjusted with KCl buffer, pH 1.0 and the other with sodium acetate buffer pH 4.5. These dilutions were equilibrated for 15 min. The absorbance of each dilutions was read at 510 and 700 nm against blank distilled water (Sutharut and Sudarat, 2012).

2.3. Plant sample extraction

The leaf powder (100 g) was extracted with methanol using Soxhlethot continuous extraction method. The extract was collected and evaporated to dryness by using rotary vacuo unit. The final residue thus obtained was then subjected to GC-MS analysis.

2.4. Gas chromatography – Mass spectrum (GC-MS) analysis

GC–MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (330mm x 0.25mm ID x 1µm df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5µl was employed (split ratio of 10:1) injector temperature 250°C; ionsource temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da.

2.5. Identification of compounds

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. RESULTS AND DISCUSSION

3.1. Anthocyanin content

The anthocyanin was quantified according to the method of Sutharut and Sudarat, (2012). 35.2 mg/g anthocyanin was present in the fresh tender leaf tissue.

3.2. GC MS Analysis

The compounds present in the methanolic extract of *Tectona grandis* identified by GC MS analysis are shown in the Fig.1. A total of 11 compounds were obtained from the analysis and the active principles with their retention time (RT), molecular formula, and concentration (%) in the ethanol extract of *T. grandis* are presented in Table - 1.Of the 11 compounds obtained the most prevailing compounds were 5,9-Methanobenzocycloocten-1(2H)-one,3,4,5,6,7,8,9,10-octahydro-5,10-dihydroxy-3,3,7,7,9-pentamethyl(76.02%) and 1-naphthalenecarboxylic acid,5-[2-(3-furanyl)ethyl]decahydro-1,4a-dimethyl-6-methylene-[1R-(1.alpha.,4a.)] (13.95%). Other compounds present in minor quantities were Ledol(0.92%), 3-Buten-2-one,4-(2,6,6-trimethyl-1-cyclohexen-1-yl) or ionone (0.49%),9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- or Linolenic acid methyl ester (0.82%), Phytol(0.69%), Cedran-diol, 8S,14- (0.60%), Lupeol (0.71%), 3-Methoxymethyl-2,5,5,8a-tetramethyl-6,7,8a-tetrahydro-5H-chromene (3.45%) and Retinol (1.27%).

Among the phytochemicals identified, ledol is a poisonous sesquiterpene that can cause cramps, paralysis and delirium. Rita *et al*. (2008) reported the variation based on age and localities in the content of ledol in the shoot oil from Ledumpalustre. Ionone derivatives occur mainly in plants containing beta-carotene. They have been detected in a variety

of foods including raspberries, carrots, roasted almonds, fruits and herbs.Linolenic acid methyl ester is the precursor of jasmonic acid. Sermakkani and Thangapandian (2012) reported the biological activities of octadecatrienoic acid methyl ester as anti-inflammatory, insectifuge hypocholesterolemic, Cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, and anticoronaryfrom the methanol leaf extract of *Cassia italica*.

Lupeol is a pharmacologically active triterpenoid. The compound could possess potential antiporotzoal, anti-inflammatory, anti-tumour, cardioprotective, hepatoprotective, antimicrobial activity (Subban *et al*., 2011). Saratha *et al*. (2011) found out the potent bioactive compound, lupeol, from the latex of *Calotropis gigantean,* which is vastly used as ananti-inflammatory compound. The present study suggests the possible use of this plant for lupeol that helps and supports the pharmaceutical industry in drug formulation. In the review of Gallo and Sarachine (2009) demonstrates lupeol have shown to possess a range of folk and proven biological activities such as anti-neoplastic, anti-inflammatory, anti-hypertensive and anti-urolithiatic drugs.Cedran-diol,8S,14- is a sequiterpene alcohol which possess both anti-microbial and anti-inflammatory activities (Thanga *et al*., 2012).

Table 1. Components detected from *Tectona grandis* ethanolic leaf extract

S. No.	Compounds	Chemical formula	Retention time	Area (%)
1.	Ledol	$C_{15}H_{26}O$	22.803	0.92
2.	3-Buten-2-one,4-(2,6,6-trimethyl-1-cyclohexen-1-yl) or ionone	$C_{13}H_{20}O$	40.597	0.49
3.	Linolenic acid methyl ester 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	41.447	0.82
4.	Phytol	$C_{20}H_{40}O$	41.819	0.69
5.	Cedran-diol, 8S,14-	$C_{15}H_{26}O_2$	46.538	0.60
6.	Lupeol	$C_{30}H_{50}O$	47.705	0.71
7.	5,9-Methanobenzocycloocten-1(2H)-one,3,4,5,6,7,8,9,10-octahydro-5,10-dihydroxy-3,3,7,7,9-pentamethyl	$C_{18}H_{28}O$	49.063	76.02
8.	1-Naphthalenecarboxylic acid,5-[2-(3-furanyl)ethyl]decahydro-1,4a-dimethyl-6-methylene-[1R-(1.alpha.,4a.)]	$C_{20}H_{28}O_3$	50.585	13.95
.	3-Methoxymethyl-2,5,5,8a-tetramethyl-6,7,8a-tetrahydro-5H-chromene	$C_{15}H_{24}O_2$	51.004	3.45
10.	Retinol	$C_{20}H_{30}O$	53.625	1.27

Phytol is diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin k and also in fragrance industry and used in cosmetics, shampoos, soaps, cleaners and detergents. Thanga *et al*. (2012) detectedphytol from *Canscora perfoliata* is also found to be effective at different stages of arthritis. It is found to be effective as well as preventive against arthritis. The results show that reactive oxygen species scavengers such as phytol constitute another promising novel class of pharmaceutical for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases.3-buten-2-one,4-(2,6,6-trimethyl-1-cyclohexen-1-yl) or iononederivatives occur mainly in plants containing beta-carotene. They have been detected in a variety of food including raspberries, carrots, roasted almonds, fruits and herbs. Keith *et al*. (2004) analysed the aroma ofcultivar Meeker red raspberry from Oregon and Washington by aroma extraction dilution analysis. From their analysis, they isolated 21 compounds had an equivalent odour impact and the major odour compound is ionone.Subban *et al*. (2011) identified phytol from the chloroform, petroleum ether and ethanol extract of the leaves of the *Memecylon umbellatum* and showed that the compound posses many medicinal values and can be used for various human ailments.

The biological potentialities and functional role of these major components present in the methanolic leaf extract of teak i.e.5,9-methanobenzocycloocten-1(2H)-one,3,4,5,6,7,8,9,10-octahydro-5,10-dihydroxy-3,3,7,7,9-pentamethyl and 1-naphthalenecarboxylic acid,5-[2-(3-

furanyl)ethyl]decahydro-1,4a-dimethyl-6-methylene-[1R-(1.alpha.,4a.)] are still unknown and unexploited.

4. CONCLUSION

This study confirms that the tender leaves of teak act as good source of anthocyanin components which possesses many pharmoacological potentialities. 11compounds have been identified from the ethanolic extract of leaves by Gas Chromatography and Mass Spectrometry analysis. Since the biological potentiality of the lead molecule in this plant extract is unknown further studies are warranted to isolate, purify the lead molecule and its therapeutic evaluation.

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