Chemical analysis of *Dalbergia sissoo* (Roxb.) pod oil by (GC-MS) / GC-FID and evaluation of antioxidant potential

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Abstract: *Dalbergia sissoo* (Roxb.) is one of the important plant species having extensive commercial and medicinal uses. The current study aims to assess the chemical constituents in pod oil of *Dalbergia sissoo* (Roxb.) by using two spectroscopic techniques i.e. GC-FID (Gas Chromatography Flame Ionization Detection) and GC-MS (Gas Chromatography Mass Spectroscopy). In GC-FID technique, nine fatty acids were identified with their respective composition, capric acid (1) (1.496%) lauric acid (2) (5.695%), myristic acid (3) (4.925%), palmitic acid (4) (10.130%), palmitoleic acid (5) (2.166%), stearic acid (6) (2.862%), oleic acid (7) (10.232%), linoleic acid (8) (22.350%) and behenic acid (9) (9.283%). In second technique, i.e. GC-MS, a series of hydrocarbons (10-37) along with two triterpenoids (38-39) were found in pod oil of the plant used. Important structure indices such as Iodine value and Saponification values were also determined. These findings can be helpful to understand the important medicinal and commercial aspects of seeds oil of the plant, like fuel value, degree of unsaturation and oxidative stability. Antioxidant testing (DPPH-Radical Scavenging Assay) was also performed on pods oil but no any significant activity was found.

Keywords: *Dalbergia sissoo*, antioxidant activity, gas chromatography, mass spectroscopy.

INTRODUCTION

The medicines are being discovered primarily from natural sources, the Kingdom Plantae has been serving as a potential source for medicinal compounds since long ago. According to a report by WHO (World Health Organization) in 2008, greater than 80% of the world's population consumes traditional medicines as a primary approach for therapy. In general, the medicinal plants have been widely used throughout the world for prevention or treatment of health conditions, therefore historically, the herbal medicines are considered harmless due to their natural origin (Lanini, Duarte-Almeida et al. 2012).

Dalbergia is a genus comprising of plants specifically lianas, shrubs and trees from family *pappilionacea*; its 300 species are distributed worldwide. It is found nurturing in the tropical region of Southern Asia, Madagascar, and Central as well as in Southern parts of America (India 1972). About 25 species from the *Dalbergia* genus grows in south eastern part of Asia that includes Bangladesh and near Himalayas of Pakistan, Nepal and India. *Dalbergia sissoo* is considered one of the valuable timber species both in India and Pakistan.

Its *Sissoo* specie has been known for its various medicinal and commercial benefits. Its commercial benefits include plantation for landscaping, windbreak for weak and feeble plants. It is also used as a soils fertilizer due to its decomposed shredded matter that enriches soil with nitrogen, organic matter and phosphorus. It is also used as a cattle feed. (Hunter 2001). Timbers of *Sissoo* tree is considered valuable due to the hardness and a specific gravity of 0.62-0.82 and its heartwood which makes it resistant to termites (Meena 2012). Heartwood also yields non-drying oil (5.35%) a lubricant for machines (Ansari, Razdan et al. 2000). Its methanolic and aqueous extracts are used worldwide as pesticides (Kharkwal, Kharkwal et al. 2014) and insecticides for species such as *Spodotera litura* (Loganathan 2004). Flowers are the source of dark ambered colored honey (Marwat, Khan et al. 2013). Its wood pulp has major utilization in printing and paper industries.

Among its medicinal benefits *Sissoo* plant is used as a popular herbal remedy for some skin ailments like gonorrhea, excoriation and other ailments of the dermis (Duke and Wain 1981). Its leaf extracts is an Ayurveda treatment for eye ailments (Shah, Mukhtar et al. 2010). Bark and wood of *Sissoo* possesses properties like aphrodisiac, expectorant, antipyretic, abortifacient and anthelmintic (Shah, Mukhtar et al. 2010). Some studies

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published its uses in disorders of anal region, in sensation of burns, leukoderma, dermal ailments, dyspepsia, dysentery, blood disorders (Duke and Wain 1981), scabies, syphilis and gastric issues (Lal and Sanjay 2012). Indians use the wood for eruptions, nausea, boils and leprosy (Kirtikar and Basu 1975).

Pods contain 2% tannin and used as dye stuff (Al-Snafi 2017). In favorable conditions, there is extensive production of pods containing two to three seeds per pod. There are approximately 45,000-55,000 seeds/kg. *Dalbergia sisso* is reported to contain several important chemical constituents in its different parts, which are very valuable with respect to their medicinal and commercial uses. Constituent reported to date in *Dalbergia sisso* included chalcone, 2,3-dimethoxy4-ɣ,ɣ-dimethylallyloxy-2-hydroxychalcone and an isoflavone,7-ɣ,ɣ-demethlyallyloxy-5-hydroxy-4-methoxyisoflavone together, flavones,7-hydroxy-6-methoxyflavone, biochanin A and a retinoid, dehydroamophogenin. 3-hydroxycarbonyl-2,5- diethylpyrrolidine (7.83%) and formic acid, 1-methylethyl ester (7.38%) (Lomarat, Chancharunee et al. 2015). In present study, pod oil of plant *Dalbergia sissoo* was used for the identification of chemical constituents and evaluation of antioxidant potential. Extraction of pod oil was done to estimate the extent of antioxidant properties by mass spectroscopy in chemical constituents identified by gas chromatographic method.

MATERIALS AND METHODS

Chemicals

Ethanol, dimethyl sulfoxide-2,2-diphenyl-1-picrylhydazyl DPPH), n-hexane, Gallic acid and n-acetyl cysteine were used. All the chemicals used were of analytical grade.

Collection of plant material

Fresh Pods of *Dalbergia Sissoo* (Roxb.) were collected from University of Karachi. Collected plant materials were identified and authenticated by Department of Pharmacognosy and Department of Botany, University of Karachi.

Isolation of Oil/ Oil extraction

After the collection of plant, first the pods were powdered then 70g of aliquots were taken from the powdered sample and placed in a Soxhlet Extractor with laboratory grade n-hexane as solvent, for 4 hours. The product obtained was dried in a rotary evaporator to remove extraction oil then freeze dried at 20ºC and stored at 4ºC before subjecting it to further analysis.

Gas chromatography mass spectroscopy (GCMS) analysis of Dalbergia sissoo pods oil

Initial profiling of pods oil was performed using Gas Chromatography Flame Ionization Detection (GC-FID) on a Shimadzu GC-17 A (Shimadzu Corp., Kyoto, Japan), equipped with a SPB-5VR capillary column (30 m x 0.25mm ID and 0.25µm df). Carrier gas was helium with a flow rate of 1mL/min. The oven was kept initially at 70ºC for 5 min, programmed to 260ºC at a rate of 5 ºC/min and kept isothermal for 20 min. Temperature and splitting ratio of injector was set to 280ºC and 1:30 respectively. For GCMS, Hewlett-Packard 5890 (Bunker Lake Blvd, Ramsey, MN) Gas Chromatograph coupled to Jeol JMS-600 mass spectrometer and equipped with ZB-5MSVR capillary column (30 m x 0.25mm ID and 0.25 µm df) was used, analytical conditions were kept similar as described for GC-FID. EI ion source was kept at 250ºC and 70 eV. For the identification of compounds, electronic mass spectral survey (NIST 2005) and EIMS data reported in literature were compared with the experimental mass spectra (Moir and Thomson 1973). The order of elution of compounds was confirmed through reported retention indices (RI) data (Kovats 1958). Area normalization method was used to quantify constituents.

Antioxidant activity

DPPH-Radical scavenging assay

The free-radical scavenging activity of the pods extract of *Dalbergia sisso* was determined using DPPH (2,2-diphenyl picryl hydrazyl) (Mensor, Menezes et al. 2001). The 300µ M solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was prepared by dissolving it in ethanol (100%). The test samples were dissolved in DMSO (Dimethyl sulfoxide .100%). To the 96-Well Plate, 5µL of sample was added and pre-reading was taken at 515 nm. Then 95 µL of DPPH was added to each well. The 96-Well plates were then incubated for 30minutes at 37˚C after covering with para film to avoid evaporation of the solvent. Final absorbance was then recorded on microplate reader at 515 nm. DMSO (100%) was used as controlled. Gallic acid and N-acetyl cysteine is used as standard for DPPH-RSA. Percent radical scavenging activity (% RSA) was calculated by using following equation.

% RSA = 100- (O.D of sample/ O.D of control×100).

Note: DPPH-Radical Scavenging Assay was carried out at (ICCBS) International Center of Chemical and Biological Sciences, H.E.J Research Institute of Chemistry and (PCMD) Dr. Panjwani Center for Molecular Medicine and Drug Research, Third World Center, University of Karachi.

Analysis of acid, saponification and iodine value

Methyl esters of fatty acids were prepared according to the standard IUPAC Method 2.301 (Paquot and Hautfenne 1987). The chemical composition of fatty acid methyl esters were determined with a Perkin Elmer gas chromatograph model Clarus 500 fitted with a polar capillary column Rtx-2330 (60m, 0.25mm 0.20 lµm film thickness) and equipped with flame ionization detector

Table 1: Fatty acids identified in oil of Dalbergia sisso by GC-FID

Peak#	Name	RT[min]	Area	(Area x Mass) 100	Medicinal Use
1	C10:0 Capric Acid ($C_{10}H_{20}O_2$) (1)	17.102	2.398	4.130	Used to prepare organic solvents (Zhao, Lu et al. 2007)
2	C12:0 Lauric Acid ($C_{12}H_{24}O$) (2)	21.500	5.695	11.407	Antimicrobial (Nakatsuji, Kao et al. 2009)
3	C14:0 Myristic Acid ($C_{14}H_{28}O_2$) (3)	24.926	4.925	11.229	Antimicrobial (Huang, Alimova et al. 2011)
4	C16:0 Palmitic Acid ($C_{16}H_{32}O_2$) (4)	28.051	10.130	13.33	Enhance lung surface activity (Cockshutt, Absolom et al. 1991)
5	C16:1 Palmitoleic Acid ($C_{16}H_{30}O_2$) (5)	29.262	2.166	2.850	Antibacterial in skin (Wille and Kydonieus 2003)
6	C18:0 Stearic Acid ($C_{18}H_{36}O_2$) (6)	30.770	2.862	3.770	In Skin Cream Preparation (Frosch and Kurte 1994)
7	C18:1 Oleic Acid ($C_{18}H_{34}O_2$) (7)	31.420	10.232	13.47	Skin Papilloma (Gustafsson, Leijonhufvud et al. 2004)
8	C18:2 Linoleic Acid ($C_{18}H_{32}O_2$) (8)	32.492	22.350	29.42	Coronary Heart Disease (Ramsden, Zamora et al. 2013)
9	C22:0 Behenic Acid ($C_{22}H_{44}O_2$) (9)	35.429	9.283	12.22	Increases Cholesterol Level (Cater and Denke 2001)
			Total	100.00	

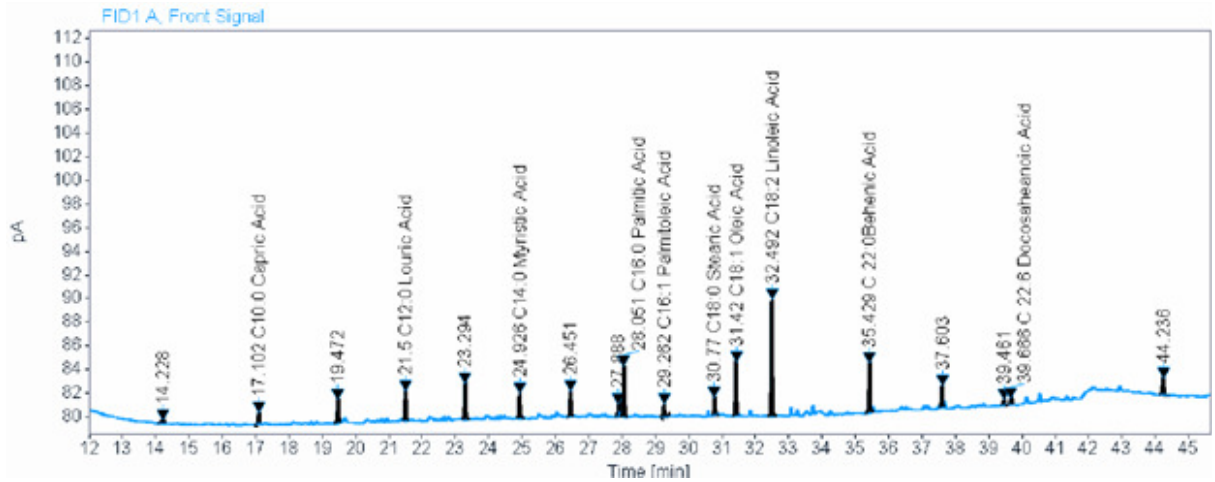

Fig. 1: GC-FID chromatogram of *Dalbergia sisso* pods oil.

(FID). Nitrogen was used as a carrier gas at a flow rate of 2.5 mL min^{-1}. Other conditions were as follows: Initial oven temperature, 70ºC for 5 min. then ramped at 10ºC/min to 180ºC, then ramped at 3ºC/min to a final temperature 220ºC where it was hold for 8 min; injector temperature 250ºC; detector temperature 275ºC. A sample volume (0.3µL) was injected; (split less). Fatty acid methyl esters were identified by comparing their relative and absolute retention times to those of standards of fatty acid methyl esters purchased from Supelco Sigma Aldrich Co. The quantification was done by a built-in data handling program, provided by the manufacturer of the gas chromatograph. The analyses were performed in triplicate. GCMS analysis was performed on HP 5890 gas chromatograph equipped with a HP-5 capillary column (30 m, 0.22 mm, 0.25µ; film thickness) and a Jeol Mass Spectrometer HX-110 from Tokyo, Japan. The analytical conditions were as follows: Injector temperature 270ºC; oven temperature programmed from 70 to 260ºC at 8 ºC/min, initial hold time was 2 min; carrier gas helium at a flow rate of 1.98mL min^{-1}; injection volume 0.3µL; split ratio, 1:50. Identification of the constituents was based on computer matching against the commercial mass spectral reference library of the National Institute of Standards and Technology (NIST 2005).

Iodine value and saponification value of the pods oil were also determined in order to determine number of acid group present in the molecule and the degree of unsaturation of the molecule. Factor was calculated by taking KI solution (0.1N) as standard (table 3).

Table 2: Chemical constituents identified in Pods oil of *Dalbergia sisso* by GCMS

S. No	Compound name	RT (Min)	Medicinal Importance/ Reference
1	Undecane (10)	12.578	Straight Chain Hydrocarbons as Substitutes for the Oil in Freund's Adjutants in the Production of Experimental "Allergic" Encephalomyelitis in the Guinea Pig (Shaw, Alvord et al. 1964). Sex attractant in moths and cockroaches (Hölldobler and Wilson 1990).
2	Dodecane (11)	14.614	It is used as a solvent, distillation chaser and scintillator component. It is used as a diluent for tributyl phosphate (TBP) in reprocessing plants (Rydberg 2004).
3	Trimethyl decane or dimethyl undecane (12)	15.814	
4	Tridecane (13)	16.462	Nymphs of the southern green stink bug produce *n*-tridecane as a dispersion/aggregation pheromone which possibly serves also as a defense against predators (Todd 1989). It is also the main component of the defensive fluid produced by the stink bug *Cosmopepla bimaculata* (Krall, Bartelt et al. 1999).
5	Tetradecane (14)	18.172	
6	Pentadecane (15)	19.759	
7	Chained Pentadecane (16)	20.843	
8	Hexadecane (17)	21.273	With water it Decrease bioavailability of allicin, Formation of small spherical micelle (Miron, Rabinkov et al. 2000).
9	2-methyl hexadecane (18)	22.105	
10	3-methyl hexadecane (19)	22.290	
11	Heptadecane (20)	22.726	Antifungal (Abubacker and Devi 2015)
12	4-methyl octadecane (21)	22.805	
13	Octadecane (22)	24.386	Antifungal (Abubacker and Devi 1779)
14	Branched Nonadecane (23)	24.564	
15	Nonadecane (24)	26.593	Pesticide (Çakir, Özer et al. 2016).
16	Eicosane (25)	29.629	Antifungal activity (Ahsan, Chen et al. 2017).
17	Heneicosane (26)	33.875	Antioxidant, antimicrobial (Albouchi, Hassen et al. 2013).
18	Docosane (27)	39.917	Antimicrobial (Karabay Yavasoglu, Sukatar et al. 2007).
19	Tricosane (28)	45.829	Allergenic (Givianrad 2012)
20	Tetracosane (29)	48.552	Adoption process enhancer in *Phengaris,Alzheimer's disease* potent inhibitor of β-amyloid aggregation and acetyl choline esterase (Lomarat, Chancharunee et al. 2015).
21	Pentacosane (30)	50.411	Antifungal (Marrufo, Nazzaro et al. 2013).
22	Plasticizer	51.144	
23	Hexacosane (31)	51.896	Antimicrobial (Rukaiyat, Garba et al. 2015).
24	Heptacosane (32)	53.158	Insecticidal activity (Hammami, Khoja et al. 2006).
25	Octacosane (33)	54.243	Mosqitosidal (Rajkumar and Jebanesan 2004).
26	Nonacosane (34)	55.315	Antimutagenic (Lawson, Nunnally et al. 1989).
27	Triacontane (35)	66.323	Anti-inflammatory (Abatan and Mogbojuri 2015).
28	Untriacontane (36)	57.572	It is main component of candelilla wax which is used in food and cosmetic industry(Toro-Vazquez, Morales-Rueda et al. 2007).
29	Unidentified	59.837	
30	Tritriacontane (37)	60.576	Anti-carcinogenic (Takaba, Hirose et al. 1997).
31	Lupenone (38)	62.661	Antiprotozoal activity (Na, Kim et al. 2009).
32	Hop-22(29)-en-3β-ol (39)	63.391	

Table 3: Iodine Value of *Dalbergia sisso* pods oil.

Composition	Name	Factor	Area	FAC x Area
C16:1	Palmitoleic acid	0.956	2.166	2.070
C18:1	Oleic Acid	0.859	10.232	8.789
C18:2	Linoleic Acid	1.371	22.350	38.687
C18:3	Linolenic Acid	2.616	0.000	0
C20:4		3.201		0
C20:5		4.027		0
C22:1		0.723	0.000	0
C22:5		3.697		0
C22:6		4.463	1.491	6.654
Total				56.202

Table 4: % RSA of *Dalbergia sisso* pods oil.

Sample Code	IC50 ± SEM	% RSA
Pods oil (Ijaz sample)	Inactive	2.584
Gallic acid	23.436±0.43(µM)	93.93
N-Acetyl Cysteine	111.44±0.7(µM)	95.95

RESULTS

Chemical composition of oil and antioxidant activity

The yield of the reddish colored oil, extracted from the pods of *Dalbergia sisso* was 0.15% (v/w). GCMS analysis of the extracted pods oil directed to the identification of 32 compounds accounting for 100% of the total oil. Identified compounds of the oil from GC-FID are listed in table 1 and fig. 1, with their respective retention times (RT) and percent compositions (peak area %). Principle compounds identified in the oil were capric acid (1.496%), lauric acid (5.695%), myristic acid (4.925%), palmitic acid (10.130%), palmitoleic acid (2.166%), stearic acid (2.862%), oleic acid (10.232%), linoleic acid (22.350%) and behenic acid (9.283%). The major compounds identified in the pods oil by GCMS are listed in table 2. DPPH scavenging assay of pods oil of *Dalbergia sissoo* showed no any insignificant activity table 4.

DISCUSSIONS

Pods oil was subjected to GC-FID, GC-MS and antioxidant analysis using established protocols. 70.014 % of chemical constituents were identified and quantified by area normalization method on the basis of GC-FID responses. Identification was done on the basis of electronic mass spectral analysis using NIST 2005, further confirmation was done on the basis of Kovats retention indices data. Total thirty nine constituents (1-39) including fatty acids, hydrocarbons and triterpenoids were identified for the first time from *Dalbergia sisso*. Some of these hydrocarbons have already been reported in genus *Dalbergia.* Results of these qualitative and quantitative aspect of selected plant species has been summarized in tables (1-3).

Fatty acid constituents identified in this study exhibit antimicrobial (Wille and Kydonieus 2003), skin protecting (Frosch and Kurte 1994), and beneficial cardiovascular effects (Cater and Denke 2001). Identified hydrocarbons possess various commercial as well as medicinal uses. Most prominent among them include diluents, manufacturing of cosmaceuticals, micelle formation, antifungal, antibacterial, insecticidal and pesticide actions. Whereas triterpenoids (Lupenone) possess antiprotozoal effects (Miron, Rabinkov et al. 2000). These findings can serve as guideline for further exploration of pods oil of this important plant species. Further experimentations are required to assess the actual potential of pods oil of *Dalbergia sisso*. Regarding antioxidant potential Pods oil was found to be inactive against DPPH free radical scavenging assay.

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