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Fatty acids analysis of *Ficus religiosa* stem bark by gas chromatography-mass spectrometry

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SHORT COMMUNICATION

ABSTRACT

The *Ficus religiosa* Linn. (Moraceae) stem bark is used traditionally to treat burns, dysentery, diarrhoea, gastrohelcosis, gonorrhoea, hiccup, piles, glandular swellings of the neck, skin diseases, scabies, urinogenital disorders, anxiety, vomiting and to improve the complexion.The objective of this study was to describe the systematic fatty acid composition of the petroleum ether extract of *F. religiosa stem* bark. Petroleum ether extract of the *F. religiosa* stem bark was prepared by cold extraction. Fatty acid composition of the extract was identified by gas chromatography-mass spectrometry with their relative concentrations. The petroleum ether extract of *F. religiosa* stem bark showed the presence of thirty five compounds including eight unsaturated fatty acids (59.24%), thirteen saturated fatty acids (31.19%), cyclic fatty acid ester (0.82%), fatty alcohols (0.97%), alkane (6.02%) and alkene (1.76%) hydrocarbons. Most of the saturated and unsaturated fatty acids were identified as their methyl esters. The present study enriched the chemical profile of *F. religiosa* stem bark and designated the fatty acids composition of petroleum ether extract. *F. religiosa* stem bark has potential to be exploited further for the number of applications or as a source of poly and monounsaturated fatty acids.

Keywords: *Ficus religiosa*, Moraceae, Petroleum ether extract, GC-MS analysis, Fatty acid methyl esters.

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INTRODUCTION

Ficus religiosa Linn. (Moraceae) is commonly known as 'sacred fig'. It is a popular member of the genus *Ficus* and widely cultivated in south-eastern Asia especially in vicinity of temples throughout India (Galil, 1984). The specific epithet "religiosa" shows the religious importance of the *F. religiosa*, as it is the most holy tree to both Buddhists and Hindus in southern Asia (Kala et al., 2006; Sitaramam et al., 2009). Its description and benefits are mentioned in various ancient holy transcripts like, Arthasastra, Bhagavadgita, Mahabharata, Puranas, Ramayana, Upanisads and Buddhistic literature (Prasad, 2006). It is a branched, large deciduous tree with few aerial roots, long-tipped leaves and purple fruits growing in pairs. The bark is grey, slightly curved or flat, smooth inner surface, yellowish to orange brown

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and fibrous (Anonymous, 2005; Warrier et al., 1995; Babu et al., 2010). The bark exhibits anti-inflammatory, antiseptic, astringent, aphrodisiac and refrigerant properties and is used to treat diarrhoea, dysentery, gastrohelcosis, anxiety, burns, glandular swellings of the neck, hiccup, piles, scabies and other skin diseases, gonorrhoea, urinogenital disorders and vomiting (Warrier et al., 1995; Kaur et al., 2011; Kapoor, 1990). Ayurvedic formulations such as *Chanda-nasavam, Nalpamaradi tailam, Nyagrodhadi churna* and *Saribadyasavam* contain *F. religiosa* bark as an important ingredient (Sivarajan and Balachandran, 1994; Simha and Laxminarayana, 2007). The bark contains tannin, wax, saponin, vitamin k1, bergapten, bergaptol, lanosterol, β-sitosterol and its glucoside, stigmasterol, lupen-3-one, *n*-octacosanol, methyl oleonate, lupeol and its acetate, anthocyanidins and their glycosides, cerylbehenate, α-amyrin acetate ((Swami and Bisht, 1996; Singh et al., 2011), naphthyl-1',3'-diol-1'-(3β-lanostan-19-oic acid-yl)-3'-*n*-octadec-9",12"-dienoate, naphthyl-1',3'-diol-1'-(3β-lanost-5,24-dienyl)-3'-*n*-octadec-9",12",15"-trienoate, lanostan-19-oic acid-3β-olyl-*n*-octadec-9',12',15'-trienoate and β-sitosteryl oleate (Ali et al., 2014). Fatty acids such as hexanoic acid pentadecayl ester, cyclopropane tetra decanoic acid, octadecanoic acid, oleic acid (Saravanan et al., 2014), palmitic acid and its methyl ester and stearic acid (Manorenjitha et al., 2013) have been reported from the alcoholic extract of stem bark by GC-MS analysis.

Owing to traditional uses of *F. religiosa* bark and its use as important ingredient in various Ayurvedic formulations, it was thought worthwhile to study its fatty acids composition. The objective of this study was to describe the systematic fatty acid composition of the petroleum ether extract of *F. religiosa stem* bark.

MATERIAL AND METHODS

Plant materials

The Fresh *F. religiosa* stem bark was collected from the campus of Jamia Hamdard, New Delhi. The sample was authenticated by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen of the plant sample was deposited in the herbarium of NISCAIR with reference number NISCAIR/RHMD/Consult/-2010-11/1665/263.

Preparation of extracts

Finely grounded powder (100 g) of *F. religiosa* stem bark was soaked in petroleum ether (250 ml × 3) for eight hours and then filtered through a whatmann filter paper No.1. Sodium sulphate was used to remove traces of moisture in the filtrates. The filtrates were concentrated under reduced pressure using rotary evaporator at 45°C to get a yellowish white waxy mass. The percentage yield of petroleum ether extract was 6.82% v/w.

Fatty acid methyl ester (FAME) preparation

A one step extraction-methylation procedure of Browse et al (1986) was applied with slight modification to the petroleum ether extract. The extract (1 g) was mixed with 3 ml of the methanolic sulphuric acid which was prepared by diluting a 3 M solution of sulphuric acid to 1 M with methanol. After cooling, 0.3 ml of hexane and 1 ml of 0.9% sodium chloride were added and the fatty acid methyl esters (FAMEs) were vigorously extracted by shaking. The sample was then centrifuged (1000 × g × 30 s) and the hexane layer were used for the fatty acids analysis.

GC-MS analysis

The analysis of the petroleum ether extract was carried out on a GC-MS system (Shimadzu QP-2010) with AB-Innowax 7031428 WCOT column (60 m x 0.25 mm x 0.25 µm). Helium was the carrier gas with a flow rate of 1.21 mL/min. The temperature of oven was 80 °C for 1 min and subsequently controlled isothermally for 2 min. Injector port: 270 °C, detector: 280 °C, split ratio 1:50, injected volume of the sample: 1 µL. The recording was executed at 70 eV, scan duration 1.5 s; mass range 40-750 amu. Software implemented to handle mass spectra and chromatograph was a Chem station (Figure 1).

Identification of components

All the constituents were identified by comparison of their retention indices (R.I.) either with those of standard compounds available in author's library or with those of literature in close agreement to R.I (Babushok et al., 2011; Ali, 2001; McLaerty, 1989).

Further identification of compounds was done by comparison of mass spectra and their fragmentation pattern obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K.L, WILEY8 libraries and published literature. Retention indices of the components were determined relative to the retention times of a series of *n*-alkanes relative to C_9-C_{20} on HPS and HP-20M columns.

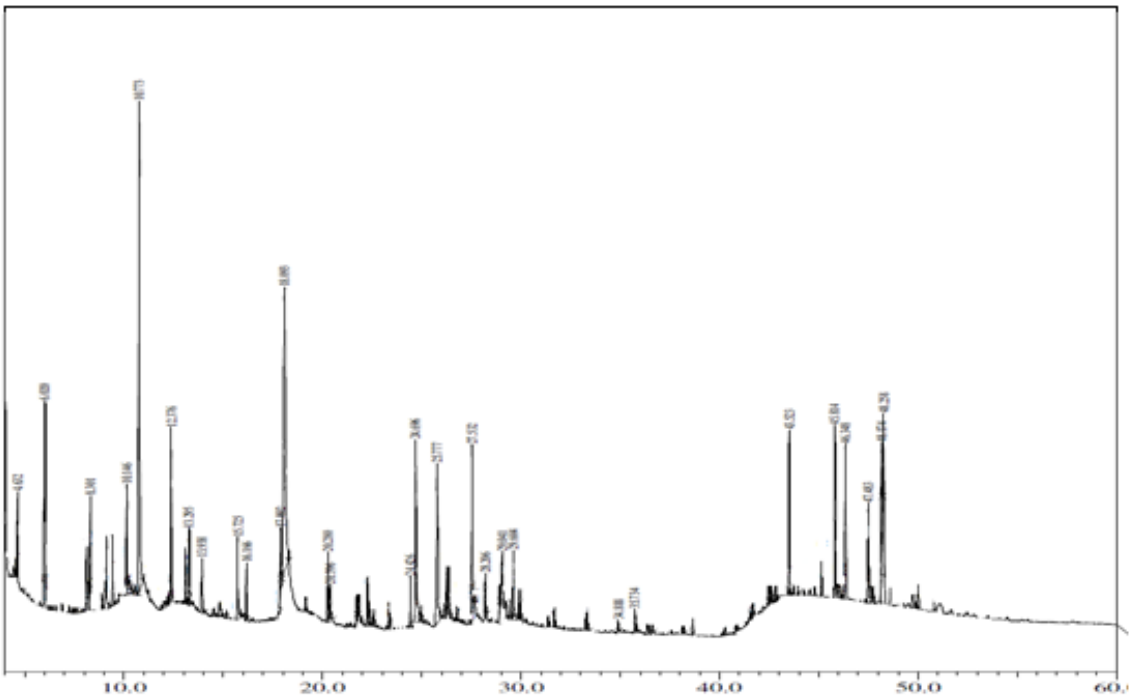

Figure 1. GC-MS spectrum of the petroleum ether extract of stem bark of *F. religiosa.*

RESULTS AND DISCUSSION

GC-MS analysis of the petroleum ether extract of the stem bark of *F. religiosa* showed the presence of thirty five compounds. Components with their retention times (RT), molecular formulae (MF), molecular weights (MW) and relative concentrations (%) are tabulated in Table 1. It mainly showed the presence of unsaturated fatty acids (59.24%), saturated fatty acids (31.19%), alkane (6.02%) and alkene (1.76%) hydrocarbons, and a cyclic fatty acid (0.82%). Fatty acids were determined as their respective methyl esters. The predominant unsaturated fatty acids identified were methyl esters of a monounsaturated fatty acids erucic acid (*cis*-13-docosenoic acid) (25.83%), oleic acid (11.86%), 11-ecosenoic acid (10.16%) and a polyunsaturated fatty acid, linoleic acid (8.22%) along with low percentage of 5-octadecenoic (1.55%), palmitoleic (0.63%), vaccenic (0.51%) and *cis*-10-heptadecenoic (0.48%) acids (Table 1).

Thirteen saturated fatty acids were determined positively to constitute 31.19% of fixed oil as palmitic (15.85%), stearic (3.63%), docosanoic (2.96%), ecosanoic (1.85%), lignoceric (1.56%) and myristic (1.40%) acids along with the traces of margaric (0.85%), hexacosanoic (0.72%), tricosanoic (0.58%), pentadecanoic (0.52%), lauric (0.49%), pentacosanoic (0.41%) and heneicosanoic (0.37%) acids. A cyclic fatty acid ester was partially identified as 2-hexyl-cyclopropaneoctanoic acid (0.82%) along with two positively determined fatty alcohols as behenic alcohol (0.60%) and *n*-tetracosanol (0.37%) (Table 1).

There were eight alkane hydrocarbons (6.02%), the predominant one was tetradecane (1.44%). The hydrocarbons present in the trace amounts were recognized as pentadecane (0.84%), heneicosane (0.79%), hexadecane (0.78%), 2,6,10,14-tetramethyl pentadecane (0.70%), nonadecane (0.55%), eicosane (0.54%) and 2,6,10-trimethyl pentadecane (0.38%). The three alkene hydrocarbons (1.76%) were distinguished as 1-heptadecene (0.70%), 1-nonadecene (0.56%) and

1-tridecene (0.50%), Table 1. The fatty acids were present both in saturated and unsaturated forms with similar number of carbon atoms but varies in their percentage and kovats retention indices as palmitic acid ($C_{16:0}$) and palmitoleic acid ($C_{16:1(9)}$); margaric acid ($C_{17:0}$) and *cis*-10-heptadecenoic acid ($C_{17:1(10)}$); stearic acid ($C_{18:0}$), 5-octadecenoic acid ($C_{18:1(5)}$), oleic acid ($C_{18:1(9)}$) and vaccenic acid ($C_{18:1(11)}$); ecosanoic acid ($C_{20:0}$) and 11-ecosenoic acid ($C_{20:1(11)}$). All the identified chemical components with their retention index (RI), molecular formula, molecular weight (MW), and Relative composition (RC) are presented in Table 1.

Table 1. Chemical composition of the petroleum ether extract of stem bark of *F. religiosa.*

S. No.	Components	RI	Molecular formula	Molecular weight	RC
1.	*1-Tridecene*	1304	$C_{13}H_{26}$	182	0.50
2.	*Tetradecane*	1421	$C_{14}H_{30}$	198	1.44
3.	*Pentadecane*	1529	$C_{15}H_{32}$	212	0.84
4.	*Lauric acid, methyl ester*	1571	$C_{13}H_{26}O_2$	214	0.49
5.	*Hexadecane*	1612	$C_{16}H_{34}$	226	0.78
6.	*2,6,10-Trimethyl pentadecane*	1618	$C_{18}H_{38}$	254	0.38
7.	*1-Heptadecene*	1701	$C_{17}H_{34}$	238	0.70
8.	*2,6,10,14-Tetramethyl pentadecane*	1711	$C_{17}H_{36}$	240	0.70
9.	*Myristic acid, , methyl ester*	1778	$C_{15}H_{30}O_2$	242	1.40
10.	*Palmitic acid, methyl ester*	1797	$C_{17}H_{34}O_2$	270	15.85
11.	*Pentadecanoic acid, methyl ester*	1853	$C_{16}H_{32}O_2$	256	0.52
12.	*Nonadecane*	1908	$C_{19}H_{40}$	268	0.55
13.	*1-Nonadecene*	1937	$C_{19}H_{38}$	266	0.56
14.	*2-Hexyl-cyclopropaneoctanoic acid, methyl ester*	1941	$C_{18}H_{34}O_2$	282	0.82
15.	*Palmitoleic acid, methyl ester*	1953	$C_{17}H_{32}O_2$	268	0.63
16.	*Margaric acid, methyl ester*	1978	$C_{18}H_{36}O_2$	284	0.85
17.	*cis-10-Heptadecenoic acid, methyl ester*	1986	$C_{18}H_{34}O_2$	282	0.48
18.	*Eicosane*	2022	$C_{20}H_{42}$	282	0.54
19.	*Linoleic acid, methyl ester*	2033	$C_{19}H_{34}O_2$	294	8.22
20.	*5-Octadecenoic acid, methyl ester*	2081	$C_{19}H_{36}O_2$	296	1.55
21.	*Vaccenic acid, methyl ester*	2085	$C_{19}H_{36}O_2$	296	0.51
22.	*Oleic acid, methyl ester*	2091	$C_{19}H_{36}O_2$	296	11.86
23.	*Heneicosane*	2109	$C_{21}H_{44}$	296	0.79
24.	*Stearic acid, methyl ester*	2158	$C_{19}H_{38}O_2$	298	3.63
25.	*cis-11-Eicosenoic acid, methyl ester*	2284	$C_{21}H_{40}O_2$	324	10.16
26.	*Eicosanoic acid, methyl ester*	2299	$C_{21}H_{42}O_2$	326	1.85
27.	*Heneicosanoic acid, methyl ester*	2375	$C_{22}H_{44}O_2$	340	0.37
28.	*Behenic alcohol*	2451	$C_{22}H_{46}O$	326	0.60
29.	*Docosanoic acid, methyl ester*	2475	$C_{23}H_{46}O_2$	354	2.96
30.	*Erucic acid, methyl ester*	2483	$C_{23}H_{44}O_2$	352	25.83
31.	*Tricosanoic acid, methyl ester*	2574	$C_{24}H_{48}O_2$	368	0.58
32.	*n-Tetracosanol*	2650	$C_{24}H_{50}O$	354	0.37
33.	*Lignoceric acid, methyl ester*	2674	$C_{25}H_{50}O_2$	382	1.56
34.	*Pentacosanoic acid, methyl ester*	2773	$C_{26}H_{52}O_2$	396	0.41
35.	*Hexacosanoic acid, methyl ester*	2872	$C_{27}H_{54}O_2$	410	0.72

RI:-Retention index, RC:- Relative composition.

The present study exhibited that the presence of monounsaturated omega-9 fatty acids, erucic (25.83%) and cis-11-eicosenoic (10.16%) acids; and a polyunsaturated omega-6 fatty acid, linoleic acid (8.22%) have been reported for the first time. Earlier reports exhibited that the high intakes of linoleic acid protect from the cancer development, possibly through the generation of 13-hydroxyoctadecadienoic acid as it prevents cell adhesion to endothelial cells and can inhibit cancer metastasis (Horrobin and Ziboh, 1997). Further, linoleic acid is an essential fatty acid and important for human survival. Linoleic acid and its metabolites may function as anti-hypertensives, anti-atherosclerotic, nitric oxide enhancers, endogenous angiotensin converting enzyme and HMG-CoA reductase inhibitors (Das, 2006a; Das, 2006b). Fatty acids such as, palmitic acid and its methyl ester and stearic acid (Manorenjitha et al., 2013), hexanoic acid pentadecayl ester, cyclopropane tetra decanoic acid, octadecanoic acid and oleic acid (Saravanan et al., 2014) have been reported from the alcoholic extracts of *F. religiosa* bark by GC-MS analysis.

CONCLUSION

The present study enriched the chemical profile of *F. religiosa* stem bark and designated the fatty acids composition of petroleum ether extract. The number of components (35) and percentage of palmitic acid methyl ester (15.85%), stearic acid (3.63%) (Manorenjitha et al., 2013) and oleic acid methyl ester (11.86%) (Saravanan et al., 2014) were higher than earlier reports. However, the presence of monounsaturated omega-9 fatty acids, erucic (25.83%) and cis-11-eicosenoic (10.16%) acids; and a polyunsaturated omega-6 fatty acid, linoleic acid (8.22%) have been reported for the first time. As per earlier reports, linoleic acid protects against the cancer development and may acts as cardio-protective, anti-atherosclerotic and nitric oxide enhancers. In conclusion, *F. religiosa* stem bark has potential to be exploited further for number of applications or as a source for poly and monounsaturated fatty acids.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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