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Analysis of n-alkanes in the cuticular wax of leaves of *Ficus glomerata* Roxb.

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Abstract: Leaf cuticle was covered by epicuticular wax consisting mainly of straight chain aliphatic hydrocarbons with a variety of substituted groups. The studies of cuticular characters of leaves had played an important role in chemotaxonomy. An *n*-hexane extract of fresh and mature leaves of *Ficus glomerata*, containing a thin layer of epicuticular waxes was analyzed for the first time by Thin Layer Chromatography, Infrared Spectroscopy, Gas Chromatography and Scanning Electron Microscopy using standard hydrocarbons. The leaves contained 18 identified long chain (C_{15}–C_{33}) n-alkanes except C_{23} accounting for 68.82% of the hydrocarbons, and an unknown number of unidentified branched chain alkanes. The predominant *n*-alkanes were C_{16} (5.92%), C_{17} (6.18%), C_{27} (5.11%), C_{29} (5.29%), C_{31} (5.47%), whilst C_{15} (4.21%), C_{18} (4.57%), C_{26} (3.88%), C_{28} (3.53%), C_{30} (3.43%) *n*-alkanes were moderately abundant. The C_{19} (2.53%), C_{20} (2.52%) and C_{22} (2.16%) homologues were present only in minor amounts. SEM views were also taken for epicuticular layers and hydrocarbons of the leaves. Qualitative and Quantitative characterization of n-alkanes present in the epicuticular wax can be used as an effective tool in chemotaxonomical work.

Keywords: Epicuticular leaf waxes, *Ficus glomerata* Roxb., GC, *n*-Alkanes, SEM

INTRODUCTION

Ficus glomerata Roxb. syn. *F. racemosa* Linn. (Family: Moraceae), commonly known as Gular in Hindi and Cluster fig in English (Chopra *et al.,* 2002). It is medium sized to large evergreen or occasionally deciduous tree and found all over India and Southeast Asia, New Guinea, and Queensland in Australia. It grows in secondary forests, open lands, and riverbanks, up to 1200 m in altitude (Ripu *et al.,* 2006; Paarakh, 2009). The decline in the world supplies of hydrocarbons necessitates a search for alternate sources of fuel and chemicals. Plant species are potential sources of hydrocarbons. An exhaustive survey of the literature reveals that no systematic research work has been reported on the leaves hydrocarbon of *F. glomerata*. But different phytochemicals, essential minerals have been found from leaves, fruits, roots and bark of *F. glomerata*. Its fruits are mixed with rice for making bread and used in several dishes. It has been reported to have many medicinal properties. Traditionally the bark, fruits and latex are used to treat anaemia and gastrointestinal disorders like constipation, dysentery (Chopra *et al.,* 2002). The alcoholic extract of the fruit also possessed anti-filarial activity against Set aria cervi (Mishra *et al.,* 2005). Fruits of *F. glomerata* contain glauanol, glauanol acetate, β-sitosterol, lupeol acetate (Baruah and Gohain, 1992). The aerial part of plant contains β-sitosterol, lupeol and quercetin as major active constituents (Khan and Sultana, 2005). Fruits of *F. glomerata* showed significant gastroprotective activity on physically and chemically induced gastric ulceration in rats (Rao *et al*., 2008). Fig fruits of this plant contain numerous amino acids like asparagine and tyrosine in fruit edible part; alanine, threonine, tyrosine, and valine in seeds (Ali and Quadry, 1987). Essential minerals such as calcium, iron, copper, manganese, zinc, nickel, cobalt, chromium are found in considerable amount from the fruits and they may be directly or indirectly helpful in the management of many diseases (Ramasamy *et al.,* 2011).

Plant cuticles are a protective waxy covering produced only by the epidermal cells of leaves (Kolattukudy, 1996). Surface, hydrocarbons present on all plants cause the leaf cuticle to become waxy and shiny (Baker, 1982), but neither the chemical composition nor the concentration of aliphatic compounds present in the thin layer of epicuticular waxes on the leaves of *F. glomerata* have been determined. The aim of the present study was to characterize the n-alkanes profile of the epicuticular waxes by TLC, IR, GC and SEM techniques.

MATERIALS AND METHODS

All solvents employed were of analytical grade and purchased from E. Merck (Mumbai, India). Fresh and mature leaves of *F. glomerata* were collected in the month of December, 2011 from garden adjacent to the University of Burdwan, Golapbag. Leaves were initially rinsed with distilled water and dried on paper toweling, and a sample of dried leaves (100g) was dipped in 2 L of n-hexane for

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45 min at room temperature. The hexane extract was passed through Whatman (Maidstone, UK) number 41 filter paper, and the solvent was removed under reduced pressure. The crude extract thus obtained was purified by preparative TLC on silica gel G (Merck) layers (thickness 0.5 mm), which had been prepared using a Unoplan (Shandon, London) coating apparatus, with carbon tetrachloride as the mobile phase. The wax band obtained was eluted with chloroform. The eluted single band showed no absorption for any detectable functional group in infrared region (IR).

The purified hydrocarbon fraction obtained from crude extract was analyzed directly by GC on a Hewlett Packard (HP; Palo Alto, CA, USA) Agilent 6890 Plus instrument fitted with an HP-5 capillary column (30m x o.32 mm i.d.; film thickness 0.25µm) and a flame ionization detector (FID). The temperature of the injection and detector ports was set at 300°C. The oven temperature program was initially at 170^{0}C, held for 1 min., then raised at 5^{0}C/min to 280^{0}C, and finally held for 15 min. The carrier gas was nitrogen at a flow rate of 1.5 mL/min. Components were characterized by co-elution with authentic n-alkane standards obtained from Sigma (USA). Standard Error was calculated following Singh and Chaudhary (1985).

Scanning electron microscopy (SEM): SEM of surface waxes (epicuticular wax) of different portions of a fresh and mature leaf of *F. glomerata* and isolated pure hydrocarbons had been carried out in the month of December, 2011. Pure hydrocarbon sample and portions of leaf samples of *F. glomerata* were mounted on circular aluminium stubs with double sticky tape, and coated with 20 nm of gold using IB2 ion coater. Samples were examined and photographed in a Hitachi Scanning Electron Microscope (Model: Hitachi S-530 Scanning Electron Microscope, Hitachi Ltd., Tokyo, Japan) at an accelerating potential of 25 kV. Photographic views of the results of SEM are cited in Figs. 2 - 4.

RESULTS AND DISCUSSION

From 96.50 mg crude extract (from hexane) of the leaves, 8.0 mg pure surface wax (hydrocarbons) was obtained. Fig. 1 showed the GC separation of the hydrocarbons in an n-hexane extract of the epicuticular wax of mature leaves of *F. glomerata*. Eighteen long chain alkanes (n-C_{15} to n-C_{33}, except n-C_{23}) were identified and quantified (Table 1) representing 68.82% of the hydrocarbons. The balance was made up of an unknown number of unidentified branched chain alkanes (31.18%). Hexadecane (5.92%), heptadecane (6.18%) hentriacontane (5.47%), nonacosane (5.29%) and heptacosane (5.11%) were the more abundant n-alkanes, whilst docosane was the least abundant (2.16%).The main component of epicuticular waxes of plant leaves were higher alkanes where the alkanes having odd number of carbons predominate (Saber *et al.*, 2010; Chowdhury *et al.*, 2010). There was a significant difference in the individual and total n-alkane concentrations in tree leaves (Piasentier *et al*., 2000; Jetter and Schaffer, 2001). Further in the present investigation, hexadecane and heptadecane were clearly the most predominant alkanes, although pentadacane, octadecane and hexacosane were moderately abundant in the thin epicuticular layer of the fresh and mature leaves of this species. Moreover, the ratio of odd and even numbered n-alkanes is 1.19:1 (Table 1). Considering all these above facts, it may be concluded that this is the characteristics feature of a higher plant (Castillo *et al*., 1987; Dyson and Harbin, 1970). Distribution of surface wax and characterization of n-alkane profile might be used in chemo-taxonomy (Hegnauer *et al*., 1986).

SEM pictures of epicuticular wax on the upper surface

Table 1. Hydrocarbon constituents (mol %) of surface wax of leaves of *Ficus glomerata* Roxb*.

Peak No.*	(Carbon number)	n-alkane	Amount (mol%)
1.	C_{14}	Tetradecane	----
2.	C_{15}	Pentadecane	4.21 ±0.093
3.	C_{16}	Hexadecane	5.92 ±0.110
4.	C_{17}	Heptadecane	6.18 ±0.112
5.	C_{18}	Octadecane	4.57 ±0.096
6.	C_{19}	Nonadecane	2.53 ±0.072
7.	C_{20}	Eicosane	2.52 ±0.072
8.	C_{21}	Heneicosane	2.81 ±0.076
9.	C_{22}	Docosane	2.16 ±0.006
10.	C_{23}	Tricosane	----
11.	C_{24}	Tetracosane	2.65 ±0.073
12.	C_{25}	Pentacosane	3.20 ±0.081
13.	C_{26}	Hexacosane	3.88 ±0.089
14.	C_{27}	Heptacosane	5.11 ±0.102
15.	C_{28}	Octacosane	3.53 ±0.085
16.	C_{29}	Nonacosane	5.29 ±0.104
17.	C_{30}	Triacontane	3.43 ±0.084
18.	C_{31}	Hentriacontane	5.47 ±0.106
19.	C_{32}	Dotriacontane	2.70 ±0.074
20.	C_{33}	Tritriacontane	2.66 ±0.073
21.	C_{34}	Tetratriacontane	----
22.	C_{35}	Pentatriacontane	----
23.	C_{36}	Hexatriacontane	----
24.	C_{37}	Heptatriacontane	----
25.	C_{38}	Octatriacontane	----
Total n-alkane			68.82
Branched chain alkane			31.18
Composition ratio of odd numbers to total alkane			37.46
Ratio of odd and even numbered alkanes			1.19 : 1
Odd			37.46
Even			31.36

*Values are means of three determinations (n=3, ±SE)

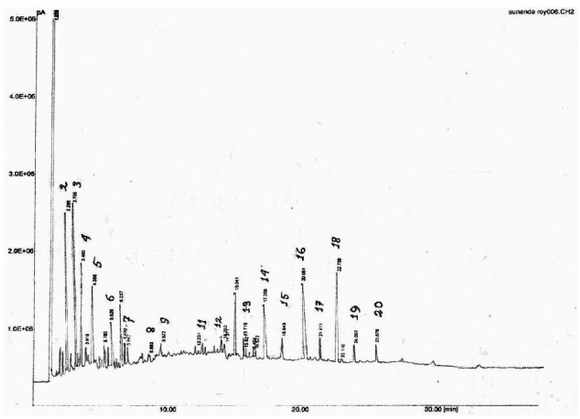

Fig.1. *GC Chromatogram of the purified hydrocarbon fraction.*

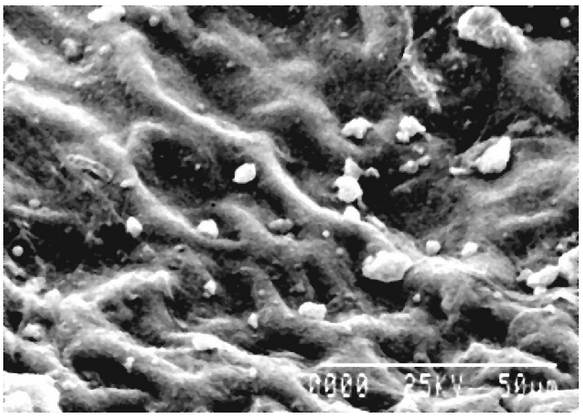

Fig.2. *SEM picture of epicuticular wax of upper surface of F. glomerata leaf.*

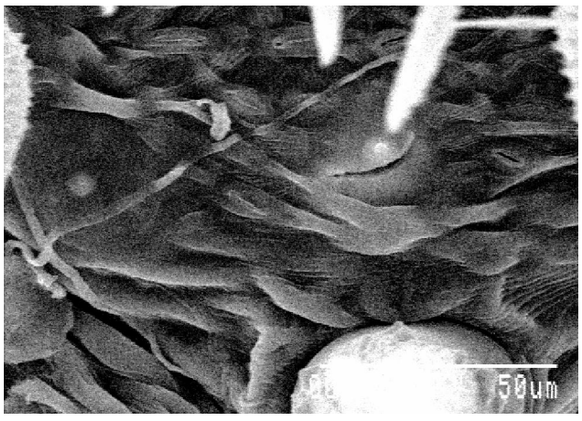

Fig.3. *SEM picture of epicuticular wax of lower surface of F. glomerata leaf.*

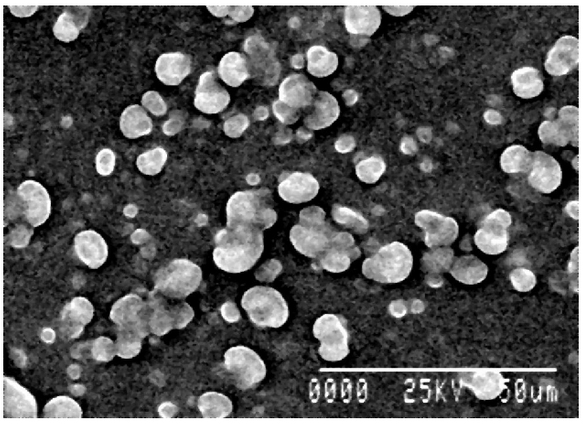

Fig.4. *SEM picture of hydrocarbon isolated from the crude extract of F. glomerata leaf.*

(Fig. 2) and lower surface (Fig. 3) of *F. glomerata* leaves showed deposition of wax on the leaf surface. In Fig. 2, it is evident from the electron micrographs with a few stomata and rudimentary cells of trichomes. A few dust particles were also found in the micrograph picture. It was distinct in upper surface features having mesophyll cells and guard cells and a limited clogging of stomata (Lerman and Darley, 1975). In Fig. 3, the present observation is well supported by Prasad and Inamdar (1990) and Prasad *et al*. (1991) that the long trichomes may act as filters or serve as a platform to gather the particulate matter in the lower surface features. There was no stomatal appearance found in the micrographs. SEM picture of pure hydrocarbon (Fig. 4) isolated from the crude extract of leaves showed crystalline waxy droplets, which were more or less scatterly arranged with varied shapes and sizes.

Conclusions

From the analyses of n-alkanes it was concluded that odd numbered n-alkanes were greater in relative amount than even number n-alkanes. The predominant character of C_{29} and C_{31} indicates that the plant belongs to higher plant group. SEM pictures differentiate upper and lower surfaces of the leaf and nature of alkanes in the surface wax. The total work again reveals that the motto of this experimental works will be useful as a taxonomic marker of the plant.

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