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Surface hydrocarbons from the leaves of *Amoora rohituka* W&A.

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ABSTRACT

Surface hydrocarbons from the fresh leaves of *Amoora rohituka* W & A. have been characterized and their relative distributions determined through GLC studies. The predominant occurrence of higher odd-number hydrocarbons and other experimental findings indicate this plant as a higher plant based on taxonomy through chemical characteristics.

Key words: *Amoora rohituka*, surface wax, leaves, hydrocarbons.

INTRODUCTION

Amoora rohituka syn. *Aphanamixis polystachya*, commonly known as Pittaraj in Sanskrit, belongs to the family Meliaceae is chosen for the present study. This plant is widely distributed in many parts of India, especially in Uttarpradesh, West Bengal, Assam, Sikim, Chota Nagpur region, and Western ghats. This plant has several folk medicinal uses in Indian villages. Its bark is astringent and used also in spleen and liver diseases, tumors and abdominal complaints. Seed oil is used as liniment in rheumatism[1]. A number of compounds have been isolated from the plant, some of which are active also. The leaves contain a diterpene named aphanamixol[2], and stem bark of the plant contains a triterpinoid, amooranin, with cytotoxic activity[3]. Aphanamixin lactone, aphanamixolide, rohitukin[2], some guaiane derive sesquiterpenoids[4] were also isolated from this plant. A flavone glycoside isolated from the root of the plant [5], and a keto fatty acid was also isolated from the seed oil of this plant[6]. Some more compounds like triterpenoids, limonoids, alkaloids and saponin were also isolated from this plant[7-11]. Amooranin, a triterpenoid isolated from stem bark of *Amoora rohituka* showed anticancer effect against colon carcinoma cell line in vitro[12]. Seed extracts of the plant also evaluated as a source of repellents, antifeedant, toxicants and protectants in storage against *Tribolium castaneum* (Herbst)[13].

It is well known that surface wax of the leaves of a plant plays an important role in affecting transportation and leaf surface properties although its composition may vary with environmental situations and also with the age of the plant[14-16]. The presence of normal alkanes as major constituents of leaf cuticular wax is well established and their distributions are considered as taxonomic markers[17-22]. But, this approach is not applicable to all plants[14, 15, 23-25]. This paper deals with the characterization and analysis of the surface hydrocarbons, mainly *n*-alkanes present in the leaves of the above mentioned plant.

MATERIAL AND METHODS

Plant materials and chemicals

Chloroform, used for solvent extraction and elution purpose, and carbon tetrachloride used for thin-layer chromatographic solvent were all of

analytical grades, obtained from E. Merck, India. The standard hydrocarbon samples were obtained from Sigma Chemical Co., USA. Fresh leaves of *Amoora rohituka* were collected from Burdwan Divisional Forest Department, Burdwan, West Bengal, India in the middle of may 2008 and authenticated by Prof. A. Mukherjee, Botany department, University of Burdwan, Burdwan, West Bengal, India.

Isolation and purification of surface wax

The surface wax was extracted in cold chloroform (1 min) from the fresh leaves (50 g) of *Amoora rohituka* at room temperature. The solvent was removed under reduced pressure. The crude extract thus obtained was purified by preparative thin layer chromatography using carbon tetrachloride as mobile phase. The thin layer chromatographic plates (thickness 0.5 mm) were prepared with silica gel-G (E. Merck, India) using Unoplan coating apparatus (Shandon, London). The hydrocarbon band was identified through co-TLC studies with standard hydrocarbon samples (Sigma, USA). The band obtained was eluted with chloroform. The eluted single band showed no absorption for any detectable functional group in infrared region and the absence of alkenes was further confirmed by argentometric TLC[26] indicating clearly the presence of only alkanes in it.

GC analysis

The purified hydrocarbon fraction was then analyzed by a programmed GLC run (oven temperature 170-300°C at 5° min^{-1} rise, initial period 1 min and final period 15 min) in a Hewlett-Packard (HP; Palo Alto, CA, USA) gas chromatography (Model 5890 Series) on a HP-1 capillary column (25 m long; Int. diameter 0.01 mm) with flame ionization detector (FID) and the carrier gas was nitrogen at a flow rate of 20ml/min. Peaks were identified by comparison of their retention times with those of standard samples of *n*-alkanes (Sigma, USA).

RESULTS AND DISCUSSION

The distribution of hydrocarbon constituents of the surface wax of the leaves of *A. rohituka* has been placed in Table 1.

This analysis revealed the presence of all the members of *n*-alkanes in the series C_{15}-C_{34}. The most predominant occurrence of alkane is C_{31} (26.22%) and the lowest occurrence are C_{19} (0.55%) and C_{21} (0.22%). The exhibited alkane distribution pattern of A. rohituka is very much in conformity with that expected for higher plants, namely the preponderance of odd-numbered alkanes in the range C_{25}-C_{33}. It is to be noted that *n*-alkanes are the major components in the surface hydrocarbons. Relative percentage of *n*-alkanes is 82.38, whereas branched chain alkanes is 17.62 and the ratio of odd and even numbered n- alkanes is 1.84:1 (Table 1). Moreover, the ratio of normal to branched hydrocarbons is 4.68:1 (Table 1). Considering all these above facts it may be concluded that this is the characteristic feature of a higher palnt[27, 28].

Table 1: Distribution of the hydrocarbon constituents of the leaf surface wax of *Amoora rohituka*

***n*-alkanes (Carbon number)**	**Relative %**
C_{15}	0.62
C_{16}	0.62
C_{17}	4.44
C_{18}	1.26
C_{19}	0.55
C_{20}	1.21
C_{21}	0.22
C_{22}	1.05
C_{23}	1.20
C_{24}	1.19
C_{25}	1.28
C_{26}	1.53
C_{27}	2.11
C_{28}	2.73
C_{29}	12.05
C_{30}	11.09
C_{31}	26.22
C_{32}	5.23
C_{33}	4.65
C_{34}	3.13
Total *n*-alkanes	82.38
Branched chain alkanes	17.62
Ratio of normal to branched hydrocarbons	4.68:1
Ratio of odd and even numbered hydrocarbons	1.84:1

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