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Characterization and Fatty Acid Composition of *Amoora rohituka* Seed and Leaf Oils

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ABSTRACT

Fatty acid content of each of the petroleum ether (40-60°C) extracted oils of mature seeds and leaves of *Amoora rohituka*, has been analyzed for the first time by GC using standard fatty acids. Oil contents of the seeds and leaves were found to be 160.58 g/kg and 50.45 g/kg respectively (weight per kg dry materials). The oil of the seeds contained five identified fatty acids, accounting 88.85% of the total fatty acids and five unidentified compounds. The predominant fatty acids were oleic acid (46.7%), stearic acid (21.23%) and palmitic acid (19.46%).The leaf oil contained six identified fatty acids, accounting 84.21% of the total fatty acids and four unidentified compounds. The predominant fatty acids were palmitic acid (42.19%), oleic acid (19.27%), stearic acid (13.64%). It has been found that both seed and leaf oils of *Amoora rohituka* are rich in essential fatty acids and consequently the oils especially the seed oil may prove its worth as edible oil if considered from nutritional point of view.

Key words: Fatty acids, seed oil, leaf oil, *Amoora rohituka*, GLC analysis.

INTRODUCTION

Recent studies on seeds from a variety of plants indicate that they are potential sources of oil for nutritional, medicinal and industrial purposes[1-7]. New oilseeds with nutritional and pharmaceutical values have been rapidly explored since decades ago. The importance of unsaturated fatty acids came under limelight as they proved to be essential for human health. As human body can not synthesize these fatty acids, they may be obtained from vegetations and marine life. It is also well known that the suitability of oil for a particular purpose is determined by its fatty acid composition. However, no oil from any single source has been found to be suitable for all purposes as oils from different sources generally differ in their fatty acid compositions. The patterns of fatty acid variation in plant seed oils have also been used as an important tool in taxonomic and phytogenetic studies[8]. In search for a new source of oil, a large number of non-conventional plants have been surveyed[9]. One such plant *Aphanamixis polystachya* syn. *Amoora rohituka* (Pittaraj), which 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[10]. This plant has several folk medicinal uses in Indian villages[11]. Its bark is astringent and used also in spleen and liver diseases, tumors and abdominal complaints and seed oil is used as liniment in rheumatism[11]. A number of compounds have been isolated from the plant, some of which are active also. The leaves contain a diterpene named aphanamixol[10], and stem bark of the plant contains a triterpinoid, amooranin, with cytotoxic activity and this compound also showed anticancer effect against colon carcinoma cell line *in vitro*[12-13]. Aphanamixin lactone, aphanamixolide, rohitukin, some guaiane derive sesquiterpenoids were also isolated from this plant[10, 14]. A flavone glycoside isolated from the root of the

plant, and a keto fatty acid, namely 7-keto-octadec-cis-11-enoic acid was also isolated from the seed oil of this plant[15-16]. Some other compounds like triterpenoids, limonoids, alkaloids and saponin were also isolated from this plant[17-21]. Seed extracts of the plant also evaluated as a source of repellents, antifeedant, toxicants and protectants in storage against *Tribolium castaneum* (Herbst)[22]. But no details studies have been conducted on *Amoora rohituka* seed and leaf oils and its' edible use as well as for its medicinal purposes.

The present communication deals with some investigations for characterization and fatty acid composition of the oil of *A. rohituka* seeds and leaves. Such information could have potential for possible future domestication and use for human consumption[23].

MATERIAL AND METHODS

Plant materials and chemicals

All reagents used in this study were of analytical grade. Fresh and matured fruits and leaves of *A. rohituka* were collected from Burdwan Forest Department, Burdwan, West Bengal, India, and authenticated by Prof. A. Mukherjee, Department of Botany, Burdwan University, West Bengal, India. A voucher specimen (Burdwan, Moumita 245) has been deposited at the herbarium of the Botany Department, Burdwan University, Burdwan, bearing acronym BURD. Standard fatty acids used in the experiment were purchased from Sigma Chemical Co., USA.

Isolation of seed and leaf oil and their characterization

Fruits were initially de-coated and the seeds were dried in air. The finely powdered air dried seeds (550 g) were extracted with 4 L petroleum ether (40-60°C) in a soxhlet for 72 hours and after complete removal of the solvent under vacuum, seed oil was obtained. The total oil was weighed and stored under nitrogen at 4°C for further analysis. Oil from seeds was light yellow in color.

In the similar way leaves were air dried and finely powdered leaves (800 g) extracted with 5 L petroleum ether (40-60°C) in a soxhlet for 72 hours and after complete removal of the solvent under vacuum, leaf oil was obtained. The total oil was weighed and stored under nitrogen at 4°C for further analysis. The visible color of the leaf oil was dark green. The weight of oil extracted from per kg of seeds and leaves (powder form) were determined to calculate the oil content. Both seed and leaf oils were liquid in state at room temperature. The chemical analyses of the seed and leaf oils (including acid values, iodine values and saponification values) were performed according to the methods of Association of Official Analytical Chemists[24] and the results have been placed in Table 1. Densities and specific gravities of both the oils were also determined. Relative viscosities (relative to water) of seed and leaf oils were measured at room temperature (27°C) employing an Ostwald type viscometer.

All these measurements were performed in triplicate and means are reported in Table 1.

Preparation of FAME

Fatty acid methyl esters (FAME) of seed and leaf oils were prepared after alkaline hydrolysis, followed by methylation with 12.5% boron trifluoride (BF_3) in methanol catalyst[25]. Methyl esters of fatty acid mixtures of seed oil and leaf oil were purified separately by preparative TLC using Hexane : Ethyl acetate (1 :1) as chromatographic solvent and each of the fatty acid methyl ester bands was eluted with chloroform (Merck, India) and stored in a refrigerator for further analysis.

Gas-Liquid Chromatographic analysis

Analysis of the FAME by capillary gas-liquid chromatography (GLC) were carried out on a Hewlett Packard (HP, Palo Alto, CA, USA), Agilent 6890 chromatograph equipped with a flame-ionization detector (FID) on a split injector. A HP-5 capillary column (30 m × 0.25 mm i.d.) was used for FAME analysis. The temperatures of the injection and detector ports were set at 250°C. The oven temperature program was: initially 160°C held for 2 minutes, then raised at 3°C/min to 220°C and finally held for 18 minutes at 220°C. The carrier gas was nitrogen at a flow rate of 20 ml/min; volume injected 1µl; split ratio, 1:20. Peaks were identified by comparison of their retention times with those of standard fatty acid methyl esters (methyl esters of lauric acid, myristic acid, palmitic acid, linoleic acid,

oleic acid, stearic acid, arachidonic acid, eicosenoic acid and DHA). The percentage composition of the samples was computed from the GC peak areas.

RESULTS AND DISCUSSION

The physico-chemical properties of the seed and leaf oils of *A. rohituka* are shown in Table 1. The oil content of seed and leaf oil were 160.58 g/kg and 50.45 g/kg respectively. Density of seed and leaf oil were measured to be 0.92 g/ml and 0.87 g/ml respectively. Specific gravity for both the oils was also measured. The values were 0.88 for seed oil and 0.85 for leaf oil. Viscosity of seed oil was determined to be 14.54 cP and that of leaf oil was 8.68 cP. The fatty acid compositions of both the oils are placed in Table 2 and 3 respectively. It can be seen that that iodine values for seed and leaf oils (Table 1) are consistent with the corresponding total unsaturation of the fatty acids, which is 53.20% and 29.63% respectively. The acid values (Table 1) indicate the amount of free fatty acids present in these oils.

Five fatty acids were identified from seed oil and quantified (Table 2), and that represent

Table 1: Some characteristics of the *Amoora rohituka* seed and leaf oil

Parameter	*A. rohituka* seed oil*	*A. rohituka* leaf oil*
Physical state at room temperature	Liquid	Liquid
Color	Light yellow	Dark green
Total oil content (g/kg)	160.58 ± 0.63	50.45 ± 0.48
Water content (%)	8.33 ± 0.017	11.54 ± 0.023
Density (g/ml)	0.92 ± 0.006	0.87 ± 0.004
Specific gravity	0.88 ± 0.002	0.85 ± 0.001
Viscosity (cP)	14.54 ± 0.35	8.68 ± 0.19
Acid value (mg KOH/g)	29.98 ± 0.11	28.56 ± 0.14
Iodine value (g/100g)	112.17 ± 0.53	74.57 ± 0.40
Saponification value (mg KOH)	205.30 ± 0.33	195.04 ± 0.27
Unsaponificable matter	2.19 ± 0.44	3.05 ± 0.63

* Values are means ± S.D., n = 3

Table 2: Fatty acid composition of the seed oil of *Amoora rohituka*

Name of the fatty acid	RT (Retention time) in minutes	Relative percentage*
Unidentified-1	3.260	1.86 ± 0.029
Lauric acid (C12:0)	3.977	0.89 ± 0.012
Palmitic acid (C16:0)	11.557	19.46 ± 0.022
Unidentified-2	12.587	0.48 ± 0.037
Unidentified-3	13.281	1.87 ± 0.010
Oleic acid (C18:1 n-9)	16.281	46.70 ± 0.036
Stearic acid (C18:0)	16.904	21.23 ± 0.019
Unidentified-4	17.966	4.47 ± 0.033
Unidentified-5	18.711	2.49 ± 0.025
Eicosenoic acid (C20:1)	22.362	0.57± 0.008

* Values are means ± S.D., n = 3

88.85% of the total fatty acids. The balance was made up of five unidentified compounds. Similarly, for leaf oil six fatty acids were identified, represent 84.21% of total fatty acids (Table 3). The balance was made up of four unidentified compounds.

Five fatty acids were identified in *A. rohituka* seed oil of which the most abundant unsaturated and saturated fatty acids are oleic (46.7%) and stearic (21.23%) respectively. The latter is comparable to the value of *Haematostaphis barteri* seed oil (15.40%)[2], but significantly higher than the ones for Samh seed oil (2.90%)[26], *Chrysophyllum albidum* (7.31%) and *Piper guineese* (5.34%)[27]. Palmitic acid (19.46%) was also present in considerable amount. Other identified fatty acids

Table 3: Fatty acid composition of the leaf oil of *Amoora rohituka*

Name of the fatty acid	RT (Retention time) in minutes	Relative percentage*
Myristic acid (C14:0)	7.058	3.43±0.017
Palmitic acid (C16:0)	11.541	42.19±0.019
Unidentified-1	13.385	5.82±0.026
Unidentified-2	13.939	2.48±0.030
Unidentified-3	14.140	3.19±0.012
Unidentified-4	15.475	4.31±0.010
Linoleic acid (C18:2 n-6)	15.960	4.83±0.022
Oleic acid (C18:1 n-9)	16.136	19.27±0.015
Stearic acid (C18:0)	16.864	13.64±0.023
Eicosenoic acid (C20:1)	22.383	0.85±0.011

* Values are means ± S.D., n = 3

present in minor amounts were lauric acid (0.89%) and eicosenoic acid (0.57%).The considerable high level of monounsaturated fatty acids in seed oil is nutritionally desirable since they do not accentuate serum cholesterol levels.

Six fatty acids were identified from *A. rohituka* leaf oil of which the most abundant unsaturated and saturated fatty acids are oleic (19.27%) and palmitic (42.19%) respectively. The presence of one very essential fatty acid (linoleic acid, 4.83%) confers the oil having considerable nutritional value. It is well known that dietary fats rich in linoleic acid prevent cardiovascular disorders such as coronary heart diseases and high blood pressure. Linoleic acid is also important for its metabolic role in the synthesis of prostaglandins. Its derivatives also serve as structural components of the plasma membrane and as precursors of some metabolic regulatory compounds. The level of linoleic acid is significantly high here, almost four times higher than that of *Ximenia americana* seed oil[28]. Myristic acid (3.43%) and stearic acid (13.64%) were present in considerable amount. Eicosenoic acid was also present in very minor amount (0.85%).

From the above results, it may be concluded that both seed and leaf oil of *Amoora rohituka* may be nutritionally valuable.

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