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Analysis of Oil and Fatty Acids from the Seeds of *Erythrina variegata* Linn

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Extraction and analysis of oil (petroleum ether 60-80ºC extract) from *Erythrina variegata* Linn. seeds and fatty acid analysis of the oil have been done. The seeds contain a fixed oil, which is orange yellow in color with 11.67 % yield. The seeds are rich in protein, which is fairly comparable with high protein animal sources like beef, pork, oyster and marine fishes. The iodine value of the oil place it in the non-drying group of oils, and the composition of the oils compares with common edible oils, which supports it to have use as edible oil. Gas-liquid chromatographic (GLC) analysis of the fatty acids shows eight naturally occurring fatty acids present in the oil, including Linoleic acid in good amount, which is one of the three essential fatty acids. Oleic acid is present in major amount, others being Palmitic acid and Stearic acid. Two polyunsaturated fatty acids, Linoleic acid and Linolenic acid are also present. The GC-MS analysis of the methyl esters of the mixture of fatty acids supports the presence of the fatty acids in the seeds.

Key words: Fatty acids, seed oil, *Erythrina variegata*, GLC analysis.

Non-traditional plant products may be made viable economically, if both oil and meal from the fruit seeds are utilized[1]. Wildly occurring trees and plants can be chosen for such purpose, which will solve crisis of nutritional supplement of low-income group people. Many such trees and plants have seeds with good protein and oil content, but are not known to use. Such an effort has been undertaken in this case with a species, which is widely and wildly available in many parts of India. *Erythrina variegata* Linn. is a legume, belongs to *Leguminosae* family. A medium sized, quick growing tree, with trifoliate leaves, large coral red flowers in dense racemes, and oblong seeds red to dark brown in color. The tree is found wild in deciduous forests through out India and Andaman and Nicobar Islands[2]. This is also cultivated in gardens as ornaments. *Erythrina variegata* has a reputation for medicinal properties in India, China and Southeast Asia. Roots, barks, and leaves are used as folk medicine, barks are used in diabetes and dysentery, leaves are used in olorrhoea, leucorrhoea, rheumatic joints, and roots are used in spleen disease[3].

No significant medicinal properties of the seeds have been reported yet, but the seeds can be ingested after boiling and roasting by human beings[4,5]. This observation lead to the study of the oil obtained from the seeds of *Erythrina variegata* Linn. The seeds contain a fixed oil. It is a matter of study to find the properties of the oil and the fatty acids contained in it. Such type of study could have potential for possible future domestication and safe use for human consumption[6]. Analysis of the fatty acids shows the presence of some unsaturated fatty acids (n-3 & n-6 PUFA and two MUFA) supports to the edibility of the oil.

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MATERIALS AND METHODS

Plant materials and chemicals

All reagents and chemicals used in this investigation were of analytical grades. Seeds of *E. variegata* Linn. were collected from Budwan Divisional Forest Department, Burdwan, West Bengal, India in the middle of 2011 and authenticated by Prof. A. Mukherjee, Botany Department, University of Burdwan, Burdwan, West Bengal, India. Voucher specimen Burdwan, Titil 4 has been deposited at the herbarium of the Department of Botany, University of Burdwan, Burdwan, bearing acronym BURD. Standard fatty acids used in the experiment were purchased from Sigma Chemical Co., USA.

Isolation and Characteristics of the Seed Oil

The seeds were crushed to powder by hand crusher, and the seed oil was extracted using continuous soxhlet extraction with petroleum ether 60-80°C for 72 hours and after complete removal of the solvent under vacuum, seed oil was obtained. Color and state of the oil was noted by visual inspection. The weight of the oil extracted from per kilogram of the seeds was determined to calculate the oil content. Oil was stored under nitrogen at 4°C for further analysis. The oil was orange yellow in color.

Specific gravity of the oil was measured at room temperature using specific gravity bottle. The saponification value, acid value and iodine value were determined by the method described by the Association of Official Analytical Chemists (1995)[7].

All the measurements were made in triplicate and placed in Table 1.

Preparation of FAME

Fatty acid methyl ester (FAME) of *E. variegata* seed oil was prepared after alkaline hydrolysis with 0.5N KOH alcoholic solution, followed by methylation with 12.5 % boron trifluoride (BF_3) in methanol[8]. Methyl esters of fatty acid mixture of the seed oil were purified by preparative thin-layer chromatography using Hexane : Ethyl acetate (1:1) as chromatographic solvent and the FAME band was eluted with chloroform A.R (Merck, India) and stored in a refrigerator.

GC Analysis

Fatty acid methyl ester (FAME) was analysed using Gas Liquid Chromatography of Agilent 6890 series plus system fitted with a HP-5 (Hewlett Packard, Palo Alto, USA) capillary column (30m × 0.25mm i.d; coating thickness 0.25µm and equipped with a flame ionization detector (FID). The temperatures of the injection and detector ports were set at 250ºC. The oven temperature program was: initially 160ºC held for 2 minutes, then raised at 3ºC/min to 220ºC and finally held for 18 minutes at 220°C. The carrier gas was nitrogen at a flow rate of 20ml/min; volume injected 1µL; and split ratio 1:20. Peaks were identified by comparison of their retention times with those of standard fatty acid methyl esters. The percentage compositions of the samples were computed from their GC peak areas.

GC-MS analysis

The FAME of the seed oil was further analysed by Gas Chromatography-Mass Spectrometry on a Shimadzu GCMS-QP 5050A fitted with a DB-5 (J and W Scientific, USA) capillary column (length 30 m, thickness 0.25 mm i.d., film thickness 0.25 mm), GC operating conditions were similar to those in our above study. MS condition: ionization voltage 70 ev; ion source temperature, 270°C and mass range was 30-700 mass units. The individual peaks were identified by comparison of their retention indices by comparing their mass with the NIST/WILEY library mass spectral database.

RESULTS AND DISSCUSSION

The oil extracted from the seeds of *E. variegata* is orange yellow in color with 11.67 % yield. Specific gravity of the oil was 0.88. Acid value, Saponification value, Iodine value of the oil were measured and placed in Table 1. The crude mixture of fatty acids was characterized by IR spectrum (2923.56, 2849.31cm^{-1} for carboxylic hydroxyl, 1747.19cm^{-1} for C=O stretching).

The acid value measures the total acidity present in the lipid, involving contributions from all the fatty acids that constitute the glyceride molecule. Whereas free fatty acid content is the measure of free fatty acids present in the oil or fat. The iodine value is below 100 mg/100 g, which indicates that the oil is of non-drying group[9] of oils. In tropical countries, vegetable oils are mostly used as dietary lipids. Free fatty acid content of

cooking oils should be within 0.0-3.0 %[10,11]. Low level of free fatty acids in the oil indicates that it may probably be used as edible oil and also may be stored for long time without spoilage via oxidative rancidity.

Eight fatty acids were identified and quantified from the GC analysis followed by GC-MS analysis (Table 2). Two peaks remained unidentified (Figure 1). The fatty acids present in the mixture commonly found in plant seed oil, Oleic acid is present (relative amount, 38.51 %) as the major unsaturated fatty (mono unsaturated fatty acid: MUFA) acid (Table 2). Another mono-unsaturated fatty acid, Eicosenoic acid has been found to be present (relative amount, 1.17 %) in the oil. Good amount of mono unsaturated fatty acid in the oil is desirable nutritionally since they do not accentuate serum cholesterol levels[12]. One poly unsaturated fatty acid (PUFA), Linoleic acid (13.13 %) is present as third major fatty acid (Table 2). It is one of the most important fatty acids required in human food for its role in prevention of distinct heart vascular diseases[13,14]. Linolenic acid is the second poly unsaturated fatty acid is also present in minute amount (relative amount, 0.57 %). About 80 to 90% of PUFA in the human diet is provided by Linoleic acid (18:2n-6) found in the vegetable oils such as sunflower oil, corn oil and soybean oil. Linoleic acid is considered one of the two essential fatty acids for human health[15]. Saturated fatty acids like Behenic acid (relative amount, 14.26 %), Palmitic acid (relative amount, 10.62 %) are found to be present in moderate amount, Stearic acid (relative amount, 1.52 %) and Arachidic acid (relative amount, 0.80 %) are seen to be present in lower amount. *E. variegata* seed

Table 1. Physical and chemical characteristics of oil extracted from the seeds of *Eythrina variegata*

Parameters	*Erythrina variegata* seed oil*
State at room temperature	Liquid
Colour	Orange yellow
Total oil content (g/100 g)	11.67 ± 0.012
Specific gravity	0.88 ± 0.022
Acid value (mg KOH/g)	162.40 ± 0.033
Saponification value (mg KOH)	175.70 ± 0.014
Iodine value (g/100 g)	63.23 ± 0.015
FFA (%)	0.84 ± 0.011

*Values are means ± S.D., n=3

Table 2. Fatty acid composition of *Erythrina variegata* seed oil

Peak No.	Name of the fatty acid	Retention Time(RT) (in minutes)	Relative percentage*
1	Palmitic acid (C16: 0)	9.064	10.62 ± 0.036
2	Stearic acid (C18: 0)	15.575	1.52 ± 0.022
3	Oleic acid (C18:1 n-9)	15.956	38.51 ± 0.010
4	Linoleic acid (C18:2 n-6)	16.880	13.13 ± 0.030
5	Unidentified	17.316	12.92 ± 0.012
6	Linolenic acid (C18:3 n-3)	18.203	0.57 ± 0.017
7	Arachidic acid (C20 : 0)	20.467	0.80 ± 0.021
8	Eicosenoic acid (C20:1)	20.777	1.17 ± 0.032
9	Behenic acid (C22: 0)	24.559	14.26 ± 0.014
10	Unidentified	25.530	6.49 ± 0.021

*Values are means ± S.D., n=3

Legends to figures:

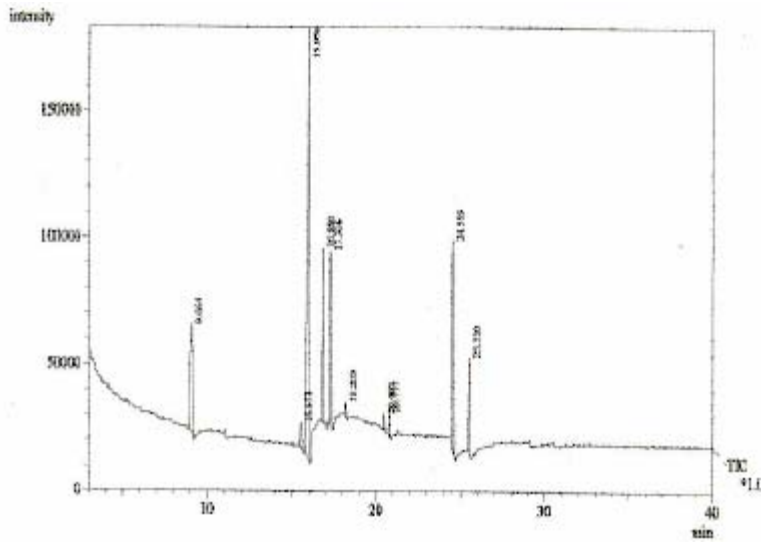

Fig. 1. Gas-chromatography of FAME of *Erythrina variegata* seed oil

oil was similar to soybean, cottonseed or rapeseed oil with respect to the high amount of 18:2, n-6 and low amount of 18:3, n-3 fatty acids[16]. The amounts of saturated and unsaturated fatty acids are 27.20 % and 53.38 % respectively without the unidentified ones. Presence of essential fatty acid regulates many body functions and play a role in immune system and also it is necessary for proper skin functioning[17,18].

CONCLUSION

Erythrina variegata seed oil contains a good amount of unsaturated fatty acids (Oleic acid, Linoleic acid, Linolenic acid and Eicosenoic acid) as well as saturated acids like Palmitic acid, Stearic acid, Arachidic acid and Behenic acid. Eight fatty acids were identified by GC and GC-MS analyses. It has been found that the fatty acids identified represent 80.58 % of the total fatty acids. The presence of one of the essential fatty acids (PUFA), Linoleic acid along with two mono-unsaturated fatty acids can make the *E. variegata* seed oil nutritionally viable.

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