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The Fatty Acid Composition and Properties of Oil Extracted from Seeds of *Carum roxburghianum* Benth. (Radhuni)

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Abstract

Carum roxburghianum Benth. (Radhuni) seeds of three different places of Bangladesh were investigated to determine the fatty acid composition and physico-chemical properties of extracted oil. The seeds were found to contain oil ranging from 15.31 to 20.32%. The percentage compositions of fatty acids were identified and quantified by Gas Liquid Chromatography (GLC). The saturated and unsaturated fatty acids in extracts were 4.95 to 6.27% and 93.73 to 94.89%, respectively. Among six fatty acids identified in this study, oleic acid contributed the highest portion (76.44 to 79.16%), whereas, linoleic (1.38%), linolenic (15.28 - 15.40%), stearic (0.70%), palmitic (4.95 - 5.57%) and eicosanoic acid (0.51 - 15.73%) together contributed the rest. Physico-chemical properties of the oil extracted were also investigated. The specific gravity, refractive index, optical rotation were recorded as 0.903 to 0.918 at 30°C, 1.465 to 1.470 at 30°C and +8.54° to +9.56° at 26°C, respectively. The chemical properties like saponification value (183.52 - 193.45), iodine value (80.99 - 120.90), peroxide value (23.25 - 36.16), acid value (143.84 - 162.99), ester value (48.12 - 62.03), percentage of unsaponifiable matter (3.56 - 6.51%), Reicher-Meissel value (2.00 - 3.12), Polenske value (4.12 - 6.20) and Henher value (85.12 - 95.56) were determined. Overall Radhuni seeds oil can be considered as a good source of oleic acid.

Keywords: *Carum roxburghianum*, Radhuni seed oil, fatty acid composition, oleic acid, Gas Liquid Chromatography.

Introduction

Carum roxburghianum Benth. (locally known as Radhuni) belonging to the family Apiaceae (*Umbelliferae*) (Minosuke, 1958; Heinrich *et al.*, 2003) is native to tropical Asia and also cultivated in Bangladesh, India and Indo-China. The people of this region cultivates it as pot herb and use its seed to flavor curries. The herb Radhuni grows 90-95 cm high and is an annual winter crop. The seeds about 1/5 inches (1.5-3.0 mm) long (Anonymous, 1976). In Bangladesh Radhuni Yield: 1.0-1.5 t/hac (Personal communication with Regional Spice Research Center, BARI at Joydebpur).

Radhuni seed is used as condiment, stimulant and carmine (Uphof *et al.*, 1968). It is useful in hiccup, vomiting and pain in the bladder (Kirtikar and Basu, 1935) and forms an ingredient of carminative and stimulant preparations which are useful in dyspepsia and flatulence. It is often used in Indian cuisine, as a very strong spice with smell of parsley. A couple of pinches can flavor a curry (Joy *et al.*, 2001). It is a con-

stituent of panch phoran (Bengali five spice) mixture (Alluri *et al.*, 2005). Moreover, the Radhuni seed oil is used as scenting agents in detergents, soaps, air fresheners and insect repellents, whilst as an intermediate in the synthesis of perfume chemicals and as a pharmaceutical aid (Bakowski and Michalik, 1986).

Plant seeds are the most promising sources of lipid, a major constituent of human food. It provides essential fatty acids for body and serves as the structural material of cell membranes in all tissues (Saleh-E-In and Roy, 2007). The fatty acids or lipids class produced from any type of oil seed may vary with geographical location, cultivar, soil type, climate, moisture, temperature, maturity of the seed and agricultural practice (Galliard *et al.*, 1975; Kinsella *et al.*, 1975; Chu and Sheldon, 1979; Ching, 2000; Saleh-E-In and Roy, 2007).

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With the increasing demand of vegetable oils and spice in the country, the investigation on seeds of Radhuni has assumed to be of great importance.

The studies on fatty acid composition and other activities of the Radhuni oil are available (Gujral *et al.*, 1953; Shahid *et al.*, 2003). In the present study, Bangladeshi *C. roxburghianum* seeds have been considered to (a) estimate the chemical composition of extracted seed oil, (b) compare the quality and quantity of fatty acids and (c) evaluate whether the oil could be used in edible purpose and as pharmaceuticals aid.

Materials and Methods

Plant materials

Radhuni seeds were collected from Regional Spice Research Center at Joydebpur, Keranigonj in Dhaka and Regional Spice Research Center at Faridpur. The brownish green seeds were harvested in the month of March, 2009. The samples were cleaned to separate from dirt, sun-dried, steam-distilled to make it free from essential oil and again sun-dried.

Extraction

The essential oil free dried samples were powdered by a waring blender. About 100 g of crushed seed-cake was then subjected to extraction with petroleum ether (b.p. 40-60°C) in a Soxhlet apparatus for 72 h to isolate oil from each variety with three replications. In each experiment, the mass filtered and filtrate was vacuum distilled to remove solvent completely. The yield of oils were calculated and stored in a refrigerator for further analyses using AOAC methods (Official Methods of Analysis, 1984).

Physico-chemical study of the oil

The physico-chemical properties of extracted oils of a particular place were determined with three replications using cited standard methods (Official Methods of Analysis, 1984; A Manual of Lab. Tech., 1976; British Pharmacopoeia, 2004; Kirk and Sawyer, 1991).

Identification and quantification of fatty acids

The fatty acid contents (qualitative and quantitative) were determined by GLC of methyl esters. The fatty acid methyl esters (FAMES) were prepared by complete esterification (checked by TLC) of oil using $\text{BF}_3\text{-MeOH}$ complex (Official

Methods of Analysis, 1984; Metcalfe *et al.*, 1966; Bannon *et al.*, 1982; Saleh-E-In and Roy, 2007). Standard FAMES (E. Merck) were used for the identification and quantification of the peaks.

For methylation, the lipid was at first, saponified with alcoholic sodium hydroxide, then cooled and diluted with water. After evaporation of alcohol, the acidified aqueous mixture was extracted with ether. Ether was then removed from ethereal solution to get fatty acid mixture. The fatty acid mixture was esterified with $\text{BF}_3\text{-MeOH}$ complex. After esterification, the reaction mixture was dissolved in diethyl ether in a separating funnel and was washed with dilute sodium carbonate solution until the effervescence was ceased. It was then washed with water, dried over anhydrous sodium sulphate and finally ether was removed to get methyl ester mixture (Official Methods of Analysis, 1984; Metcalfe *et al.*, 1966; Bannon *et al.*, 1982; Saleh-E-In and Roy, 2007).

Instrument and separation conditions

The FAMES were analyzed on a Trace GC ULTRA, Thermo Electron Corporation, Gas Chromatograph fitted with a flame ionization detector and an electronic integrator. An SE-54 quartz capillary column (30 m \times 0.25 mm *i.d.* and 0.25 μm film thicknesses) packed with 10% diethylene glycol succinate on 100-200 mesh solid support was used. Nitrogen was used as carrier gas at a flow rate of 1.5 mL/min. The separation was affected at 100°C-220°C. The following temperature program was chosen in GC analyses: Initial temperature 150°C, increasing at 7°C/min to 260°C for 30 min. The oven, injection and detection temperatures were fixed at 150°, 180° and 220°C respectively. The fatty acids were identified by comparison of relative retention times and peak positions of the chromatogram with that for the standard FAMES. The amounts of fatty acids were calculated from the peak areas computed by LKB 2220 electronic recording integrator.

Results and Discussion

The comparative results of the physico-chemical properties and fatty acid compositions of Radhuni seeds oil of different places of Bangladesh are presented in Tables I and II, respectively. Seeds contained 15.31 to 20.32% fatty oil. The lowest amount of oil was found in Joydebpur sample (15.31%) compared to 20.23% and 20.32% in Faridpur and Keranigonj samples, respectively.

Table I: Physico-chemical properties of *C. roxburghianum* (Radhuni) seeds of different regions of Bangladesh.

Characteristics	Values in the sample of		
	Joydebpur	Keranigonj, Dhaka	Faridpur
Oil yield (%)	15.31 ± 0.12	20.32 ± 0.13	20.23 ± 0.33
Appearance at Room temperature 30°C	A homogeneous, opaque, viscous oily liquid with dark greenish black color and lighter than water		
Odor and taste	Unpleasant spicy odor with bitter taste		
Miscibility and solubility	Insoluble in water but freely miscible in chloroform, carbon tetrachloride, petroleum ether, n-hexane, diethyl ether and alcohol		
Specific gravity at 30°C	0.903 ± 0.039	0.918 ± 0.027	0.904 ± 0.035
Refractive index [η] ^{30°C}	1.470 ± 0.123	1.465 ± 0.093	1.470 ± 0.095
Optical rotation [α] _D ^{26°C}	+9.03° ± 0.05	+8.54° ± 0.04	+9.56° ± 0.05
Acid value	162.99 ± 1.50	154.10 ± 1.36	143.84 ± 0.30
Ester value	48.12 ± 1.60	56.25 ± 1.35	62.03 ± 1.45
Saponification value (mg KOH/g)	193.45 ± 0.30	183.52 ± 0.75	189.30 ± 0.85
Unsaponifiable matter (%)	6.51 ± 0.39	4.23 ± 0.79	3.56 ± 0.96
Iodine value (Hanus method)	120.90 ± 0.79	110.39 ± 0.56	80.99 ± 0.98
Peroxide value	23.25 ± 0.30	36.16 ± 0.30	27.07 ± 0.35
Reichert Miessel (R.M.) value	2.59 ± 0.04	3.12 ± 0.05	2.00 ± 0.03
Poleneske value	5.07 ± 0.93	6.20 ± 0.56	4.12 ± 0.53
Henher value	93.68 ± 0.36	95.56 ± 0.49	85.12 ± 0.43

* Each value represents the average of three replicate analyses ± SD

The physico-chemical properties of the oil showed that the oils were in general opaque and viscous liquid of dark greenish black in color with an unpleasant spicy odor and bitter taste. It was freely miscible in chloroform, carbon tetrachloride, petroleum ether, n-hexane, diethyl ether and alcohol but immiscible with water.

The optical rotations were within +8.54° to +9.56° at 26°C. The refractive indices (RI) varied from 1.465 to 1.470 at

30°C which were comparable to there of soybean oil (1.466 - 1.470) and rice bran oil (1.470 - 1.473) (Meyer, 1987; Lange, 1944; Mowla *et al.*, 1990; Kirk *et al.*, 1991). The refractive indices of the oils indicated that the oil contained fairly large amount of long chain unsaturated fatty acid (Mabaleha *et al.*, 2004; Ali *et al.*, 1998; Saleh-E-In and Roy, 2007).

Table II: Fatty acid composition (in wt %) of *C. roxburghianum* (Radhuni) seeds of different regions of Bangladesh determined by GLC

Name of fatty acid	Content (wt %) in the sample of		
	Joydebpur	Keranigonj, Dhaka	Faridpur
Palmitic acid (C _{16:0})	5.57 ± 0.14	5.11 ± 0.12	4.95 ± 0.51
Stearic acid (C _{18:0})	0.70 ± 0.32	Not found	Not found
Oleic acid (C _{18:1})	76.44 ± 0.56	79.16 ± 0.23	79.15 ± 0.43
Linoleic acid (C _{18:2})	1.38 ± 0.52	Not found	Not found
Linolenic acid (C _{18:3})	15.40 ± 0.42	Not found	15.28 ± 0.18
Ecosenoic acid (C _{20:1})	0.51 ± 0.02	15.73 ± 0.09	Not found

* Each value represents the average of three replicate analyses ± SD

The specific gravities of the oils were found within 0.903 to 0.918 at 30°C, which indicated that the oil contained higher molecular weight fatty acids as well they were closer to those of olive oil (0.914 - 0.918), cotton seed oil (0.917 - 0.918) and mustard oil (0.914 - 0.923) (Meyer, 1987; Lange, 1944; Mowla *et al.*, 1990; Ching, 2000).

Properties of the oil such as acid value, iodine value and saponification value usually gives the structural, stability and quality information about the oils (Saleh-E-In and Roy, 2007). Saponification values were found within 183.52 to 193.45 mg KOH/g indicating the presence of higher molecular weight fatty acids in the oils. High molecular weight fatty acids are not good for human health. In that sense, fats or oils having low saponification number should be preferred (Meyer, 1987). The saponification values were quite similar to the range of olive oil (185 - 196), corn oil (187 - 193), linseed oil (188 - 195) and cotton seed oil (189 - 199) (Meyer, 1987; Lange, 1944; Mowla *et al.*, 1990; Ching, 2000; Jacobs, 2006; Nollet, 2004) which were typical C₁₆ and C₁₈ oils.

The extracted fatty oils had the iodine value within 80.99 to 120.90 which were almost similar to those for olive oil (79 - 88), peanut oil (88 - 98), rapeseed oil (97 - 110), rice bran oil (92 - 109), cotton seed oil (99 - 121) and mustard oil (96 - 124) (Meyer, 1987; Lange, 1944; Mowla *et al.*, 1990; Ching, 2000; Jacobs, 2006; Nollet, 2004). Iodine value is a measure the degree of unsaturation of fatty acids content of any fat or oil (as the value increases unsaturation increases). So, the result indicates that the oil possesses high unsaturation (Saleh-E-In and Roy, 2007). Therefore, the extracted oil possesses high proportion of higher unsaturated fatty acids.

Acid values of extracted oils were found to vary within 143.84 - 162.99 indicating that the proportion of free fatty acid content was very high than those in edible oils like soybean oil (0.38 - 0.54), mustard oil (3.65 - 4.56) and palm oil (0.17 - 1.06) (Meyer, 1987; Lange, 1944; Mowla *et al.*, 1990; Ching, 2000; Jacobs, 2006; Nollet, 2004). If the concentration of free fatty acid in a fat or oil is very high then the oil is hazardous for human health (Saleh-E-In and Roy, 2007). So, this oil cannot be used for edible purpose directly. However, it might be used for edible purposes after refining or for industrial purposes.

Ester numbers were found from 48.12 to 62.03 and peroxide values were found from 23.25 to 36.16. Peroxide values showed that the oil had much free active oxygen enabling

autoxidation of the oil (Mowla *et al.*, 1990; Jacobs, 2006).

Unsaponifiable matter contents ranging from 3.56 to 6.51% were very much higher than those in any edible or non-edible oils like soybean oil (0.015%), rapeseed oil (0.02%), sesame seed oil (0.02%) and palm oil (0.012%) (Nollet, 2004; Mowla *et al.*, 1990; Jacobs, 2006). The oil is supposed to be contaminated with mineral oil, higher aliphatic alcohols, sterols, pigments, hydrocarbons and sufficient amount of tocopherols (Patterson, 1983; Ali *et al.*, 1998; Mowla *et al.*, 1990; Jacobs, 2006). Tocopherols play important role as antioxidant (Patterson, 1983; Ali *et al.*, 1998).

Reichert-Miessel (R.M.) values ranging from 2.00 to 3.12 were very much close to those in palm oil (0.9 - 1.9) and corn oil (1.5 - 2.8) (Mowla *et al.*, 1990; Jacobs, 2006; Meyer, 1987; Lange, 1944) and Poleneske values were found from 4.12 to 6.20, palm kernel oil (4.20) and coconut oil (4.40) indicating that the oil had not much volatile components and low chain fatty acids (Mowla *et al.*, 1990; Jacobs, 2006). Henher values from 85.12 to 95.56 showed that the oil's unsaponifiable matter and non-volatile components were very high and similar to palm oil (94 - 97) and corn oil (93 - 95) (Mowla *et al.*, 1990; Jacobs, 2006; Nollet, 2004). The drying property of the extracted oils were examined to be non-drying in nature.

GLC analyses showed that oleic acid (76.44 to 79.16%) was the major fatty acid found in the extract and the saturated fatty acids present in the oil sample were included palmitic acid (4.95 to 5.57%) and stearic acid (0.70%) but stearic acid was found only in Joydebpur sample. Other unsaturated fatty acid linolenic acid (15.28 to 15.40%) was not identified in Keranigonj sample and linoleic acid (1.38%) was found only in Joydebpur sample. Ecosenoic acid was identified as 0.51% in Joydebpur sample and 15.73% in Keranigonj sample but was not identified in Faridpur sample. The oleic acid percentage was comparable to those in olive oil (65 - 85%) and safflower oil (79.7%). On the other hand, the palmitic acid content was almost comparable to those in the grape seed oil (4 - 11%), linseed oil (4.8%) and sunflower oil (4.29%) (Nollet, 2004; Meyer, 1987; Lange, 1944; Mowla *et al.*, 1990; Ching, 2000).

Healthful fats are lower saturated and higher mono-unsaturated fatty acid containing. Poly-unsaturated such as linolenic acid containing fats are pron to oxidation; whilst a high oleic acid helps to reduce the raised level of total plas-

ma cholesterol without reducing the high density lipoprotein (HDL) cholesterol level (Francie *et al.*, 1995).

As common vegetable oils usually contain 6 - 15% saturated fatty acids (Norton, 1989; Golfman and Bohme, 2001; Kamal Eldin and Yanishlieva, 2002) and Radhuni seeds oil contain 4.95 to 6.45% saturated fatty acids of the total oil, so, it is slightly higher in respect of the lower limit of the said percentage range but it is comparatively better for human consumption owing to its high percentages of the unsaturated fatty acids (93.73 to 94.89%) especially the oleic acid (76.44% to 79.16%) content.

Conclusion

Demand of quality oils and fats is increasing all over the world. To cope with the increasing demand of oils and fats the non-conventional sources are getting importance. The present study shows that Radhuni seeds contain 15.31 to 20.32% oil, and this oil is better than other vegetable oils with respect to health benefit. The fatty acid composition of the extracted oil is found in good agreement with the content of unsaturated acids (76.44 to 79.16% oleic acid and other 15.27 to 15.40% linolenic acid). But the deviation in the content of saturated acids is noticeable.

It is concluded that Radhuni seeds of Bangladeshi origin has got suitable nutritional properties and therefore, may be used as edible oil after proper refining. Otherwise, the extracted oil may be tapped as a source of oleic acid with an aim to be used in the food and pharmaceutical industries.

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