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FATTY ACID AND NUTRIENT COMPOSITION OF PERILLA (*PERILLA FRUTESCENS* L.) ACCESSIONS COLLECTED FROM UTTARAKHAND

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ABSTRACT

With Perilla seeds as raw materials, this paper aims at the compositions of major active fatty oil ingredients, nutrients, mineral elements in order to characterize the nutritional properties of Perilla (*Perilla frutescens* L.) seeds collected from Uttarakhand, which could aid its finely processing and future application in the development of functional food. Edible vegetable oils are the chief source of nutritionally required fatty acids in human diet. Mustard oil, soybean oil, sunflower oil, sesame oil, coconut oil and groundnut oil are among the edible vegetable oils that are mostly consumed in India. Perilla seed oil is considered to be oil that has high amount of polyunsaturated fats compared to other cooking oils. Perilla, an oil seed crop is rich in omega-3 and omega-6 polyunsaturated fatty acids and minerals. The fatty acid and nutrient composition of twelve Perilla accessions, collected from different locations of Uttarakhand and subsequently cultivated under similar climatic conditions of Doon valley, Dehradun were analyzed. The methyl esters were analyzed by gas chromatography. The elements detection was done with the help of ICP/MS. It was determined that Perilla seed oil collected from Uttarakhand contains a higher amount of polyunsaturated fatty acids as compared to other oilseed crops. Polyunsaturated fatty acids (PUFA's) are termed "essential" because they cannot be synthesized by the body and must be supplied in the diet. Besides this, Perilla seed was also analyzed for proximate composition and mineral elements. It was found that Perilla seed is rich in fat as well as iron and magnesium as compared to other oilseed crops. It also provides a higher amount of energy as compared to others.

Key words: *Perilla frutescens*, Linoleic acid, α-Linolenic acid, Polyunsaturated fatty acids.

INTRODUCTION

Perilla (*Perilla frutescens*), a member of the family Lamiaceae, commonly known as "Bhanjira" is an underutilized crop of Indian Himalayas. The plant is a native to India and China and major producing countries of Perilla are China, India, Japan, Korea, Thailand and other East Asian countries (Asif, 2011). It is an annual species and is widely distributed in China. Recently, it has also been introduced into other countries like Europe, Russia and USA (Youfang *et al*., 2012). In Uttarakhand Himalaya region, the seeds of cultivated Perilla are eaten raw, seed oil is used for cooking purposes and oil cake consumed as raw or fed to cattle. The roasted seeds are grounded to prepare spicy 'sauce' (Pandey and Bhatt, 2008). Perilla seeds contain unsaturated fatty acids, different polypherols, minerals, protein and carbohydrates (Longvah, 2000). Some pharmaceutical properties like antimicrobial, antioxidant, cytotoxicity, anti-inflammatory and anti-allergic activities have also been reported (Verma *et al*., 2013). It has also been demonstrated that it is a potential source of food that is rich in protein and omega 3 and 6 poly unsaturated fatty acid, which can be considered nutritional food for human and animal (Longvah and Deosthale, 1991). Polyunsaturated fatty acids help to regulate the immune system. They are found

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in fish, flexseed, sunflower, soya, walnut and leafy vegetables (Kanchanamayoon and Kanenil, 2007). Previous report showed that fatty acid composition and oil content of seeds mainly depend on the plant species and also depend on the climatic conditions of a particular place and also on the maturation time of the seeds (Youfang *et al*., 2012).

Relatively high concentration of certain essential elements, such as Fe, Mn, Zn and Ca has been demonstrated to influence the retention of toxic elements in animals and human beings. The imbalance in human health has been linked with the excess or deficiency of trace elements in soils, water, plants and animals. The continuous intake of diets that are excessively high in a particular trace element can influence changes in the functioning, forms, activities of some organs or concentrations of such element in the body tissue and fluids can rise above the permissible limit (Haq and Ullah, 2011). Studies originally showed that optimal intakes of elements such as sodium, potassium, magnesium, calcium, manganese, copper, zinc and iodine could reduce individual risk factors, including those related to cardiovascular disease for both human beings and animals (Anke *et al*., 1984; Mertz, 1982; Sanchez-Castillo *et al*., 1998). However, there are little research which focuses on investigating the component characteristics and mineral nutrients of *Perilla frutescens* seeds. Since, perilla seed oil is known to have health benefits, the present study was designed to find out the fatty oil composition and nutrient content in different accessions collected from Uttarakhand.

MATERIALS AND METHODS

Collection of Seeds

Seeds of *Perilla frutescens* var. *frutescens* were collected from 12 distant locations of Uttarakhand Himalaya (India) and grown in the experimental field of Centre for Aromatic Plants, Selaqui, Dehradun Uttarakhand (India) located at 30°21′46″N latitude and 77°50′59″ longitude at 680m (asl). The seeds were sown in the month of May, 2010 in the sandy-loam soil. Five to six plants from each accession were selected randomly for seed harvesting in the month of October. Botanical identity of the species was authenticated from the Botanical Survey of India (BSI), Dehradun and the specimens were deposited at BSI and CAP, Selaqui, Dehradun herbarium.

Fatty oil extraction

The seeds (50 g) were crushed using mortar and pestle. The seed powder was refluxed with 500 ml of hexane (RHCL India Pvt. Limited)) for 6 hrs using Soxhlet apparatus. The refluxed mixture was then filtered in order to remove impurities. The solvent in the filtrate was removed by Rotatary Evaporator (BUCHI make) and the remaining oil was weighed and stored. The oil content of the seeds was calculated. The methyl esters of fats were prepared (Lowenstein, 1975).

Chromatographic technique used (Gas-Chromatography)

The fatty acid methyl esters (FAMEs) were separated by capillary gas chromatography (NUCON GC-5765) using a 60 m × 0.25 mm (FID) fused silica bonded phase column (BPX70, SGE) with Flame Ionization Detector (FID). The column oven was programmed from 125^{o}C for 3 min to 220^{o}C at 8^{o}C/min. using Nitrogen as carrier gas with flow rate of 43 cm/s.0.1µL of sample injected for quantification. Fatty acids were tentatively identified by comparing them with standard mixtures of fatty acid methyl esters and the results calculated using response factors derived from chromatograph.

Nutrient analysis of the seeds

The raw seeds were pulverized with mortar and pestle. Aliquots of the pulverized sample of *Perilla frutescens* were taken for the determination of nutrient composition. Protein content (N*6.25) was estimated by the Kjeldahl method: fat and ash were determined by AOAC methods (Anonymous, 1975).Total carbohydrates, including fiber, were calculated by difference. Minerals were determined in Perkin Elmer model Inductively Coupled Plasma-Mass Spectrometer (ICP-MS).

RESULTS AND DISCUSSION

The fatty acid composition of Perilla oil was analyzed and presented in Table-1. The fatty acid composition in the oils characterized by GC, stated three dominant fatty acids: α-linolenic acid (C18:3 n-3), linoleic acid (C18:2 n-6) and oleic acid (C18:0), which ranged from 51.2-63.3%, 12.2-18.6% and 11.9-23.8% of the total FA's, respectively. Earlier reports from Italy reported α-linolenic acid, linoleic acid and palmitic acid which ranged from 52.0-55.0%, 11.5-12.1% and 8.5-9.7%, respectively (Peirett, 2011). It was found that saturated, monounsaturated and polyunsaturated fatty acids accounted for 12.3%, 17.9% and 72.7% of the total fatty acid profile. The Perilla seed oil was compared with other seed oil samples.

The results are presented in table 1 and 2. It was found that Perilla oil contains a higher amount of linoleic and linolenic acid as compared to linseed, sesame, mustard, soya and sunflower (Chowdhury *et al*., 2007; Kiralan *et al*., 2010; Longvah and Deosthale, 1991; Nzikou *et al*., 2009). It is well established that Omega-3 and omega-6 fatty acids are major constituents used to reduce cholesterol level, glyceride contents in the blood and also in cancer treatment .Perilla oil is a rich source of PUFAs and is useful for the treatment of various diseases as well as maintenance of health(Asif and Kumar, 2010). The omega 3 fatty acids are mainly consumed by vegans for heart health (Calder, 2004). Omega- fatty acids have largely been derived from flaxseed (*Linum usitatissimum*)

in West Asia and Europe (Sayanova and Napier, 2004). The proximate composition and nutrient content of *Perilla frutescens* was also determined. The results are presented in table-3. The protein and total ash content was comparable to the reported values (Longvah and Deosthale, 1991). The fat content was found to be more when compared with the reported results. Compared to common oilseeds, the proximate composition of Perilla was similar to that of sunflower. The results showed a significant content of magnesium (261.7mg/100g) and iron (9.54mg/100g). Manganese (4.93mg/100g) was also found in more quantity as compared to other oilseed crops which can help to assist the body in metabolizing protein and carbohydrates. Magnesium (Mg) improves insulin sensitivity, protects against diabetes and its complications, reduces blood pressure, prevents heart rhythm abnormalities and is found in chlorophyll (Khan *et al*., 2011; Obiajunwa *et al*., 2002). The elements like copper and chromium were also found in low content as compared to other oilseeds crops (0.20 mg/100g and 17.6µg/100g, respectively). Copper (Cu) is an essential redox-active transition element that plays vital role in various metabolic processes. Being toxic, its quantity in plants should be very low. It is essential to the human body since it forms a component in many enzyme systems, such as cytochrome oxidase, lysyl oxidase and an iron-oxidizing enzyme in blood. Chromium (Cr) is known to regulate carbohydrate, nucleic acid and lipoprotein metabolism and also potentiates insulin action.

Chronic exposure to Cr may result in liver, kidney and lung damage (Saraf and Samant, 2013). Minor elements have very important functions and it is believed a key component of proteins such as haemoprotein and haemoglobin which play role in biochemical functions and essential enzyme system even in low doses. Trace elements play both restorative and protective role in skirmishing diseases. There is an immense scope to develop the preventive medicinal aspects of various trace elements (Haq and Ullah, 2011).

Table 1. Fatty acid composition of *P. frutescens* seed oil compared to other seed oils

Components	Perilla*	Linseed [a,b]	Sesame [a,c]	Mustard[a,d]	Soya[a,d]	Sunflower[a,d]
C 16:0 Palmitic	6.0-11.0	5.5-7.0	8.6-9.7	2.9 -4.51	9.8-14.04	6.52-7.8
C 18:0 Stearic	2.1-5.5	3.5-4.0	2.4-5.5	0.9- 2.78	2.4-4.07	1.98-2.1
C 18:1 Oleic	11.9-23.8	15.0-22.1	28.9-38.9	8.9- 38.21	23.27-28.9	17.7-45.39
C 18:2 Linoleic	12.2-18.6	18.0-20.5	46.2-50.7	18.1- 25.31	50.7-52.18	46.02-78.5
C 18:3 Linolenic	51.2-63.3	47.5 -56.0	1.0-6.5	11.30- 14.5	5.63-6.5	-

*Range of twelve *P. frutescens* accessions collected from Uttarakhand; [a]Longvah and Deosthale, 1991; [b]Kiralan *et al*.,2010; [c]Nzikou *et al*., 2009; [d]Chowdhury *et al*., 2007.

Table 2. Content (%) of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and total unsaturated (MUFA+ PUFA) fatty acids of *P. frutescens* oil compared to other seed oils

Fatty acids	Perilla oil	Linseed oil	Sesame oil	Mustard oil	Soya oil	Sunflower oil
SFA	12.3	10.1	13.2	5.6	18.5	11.30
MUFA	17.9	18.6	33.9	23.6	26.08	31.55
PUFA	72.7	71.1	53.3	34.61	63.6	62.3
MUFA+ PUFA	90.6	89.7	87.2	58.21	89.68	93.85

Mean values given (%)

Table 3. Proximate composition and mineral content of *P. frutescens* oil compared to other seed oils (per 100g seeds)

Content	Perilla	Groundnut[a]	Linseed[a]	Mustard seed[a]	Sunflower[a]
Moisture (g)	6.8	5.5	6.5	8.5	5.5
Protein (g)	18.5	18.3	20.3	20.0	19.8
Fat (g)	52.0	43.3	37.1	39.7	52.1
Ash (g)	3.4	5.2	2.4	4.2	3.7
Carbohydrate (g)	22.8	25	28.9	23.8	17.9
Energy (Kcal)	630	563	530	541	620
Calcium (mg)	249.9	1450	170	490	280
Magnesium (mg)	261.7	-	-	-	-
Phosphorous (mg)	677.2	570	370	700	670
Iron(mg)	9.54	9.3	2.7	7.9	5.6
Manganese(mg)	4.93	1.32	-	2.56	-
Zinc (mg)	4.22	12.2	-	4.8	-
Copper (mg)	0.20	2.29	-	0.83	-
Chromium (µg)	17.6	87	-	63	-

[a]Longvah and Deosthale, 1991

CONCLUSION

On the basis of our present investigation it is reflected that *Perilla frutescens* cultivated in Uttarakhand can be a good source of essential polyunsaturated fatty acids (ω-3 and ω-6) which are needed by a human body. Perilla oil can be promoted as a good supplement nutritional food for both vegetarian and non-vegetarian persons. It also showed that Perilla is a good source of protein, comparable in quality to some other oilseed proteins. This oil may be used to nutritional advantage. The present study gives a new perspective about the presence of some major and trace elements in *Perilla frutescens*. This work attempts to enrich knowledge of the nutritional properties of this plant as well as fatty oil.

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