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# Physico-chemical properties of Perilla frutescens seeds

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### ABSTRACT

Seeds and their oil are used by people in different parts of the world by ancient time. Perilla frutescens belonging to the Lamiaceae family, is an edible plant who's seeds are used in some Asia countries as well as all over the world. seed oil of Perilla frutescens was extracted using soxhlet apparatus. The yield of seed oil is (40.1%). Physicochemical properties of extracted perilla seed oil were also determined. Protein content of seed is (18.12%), Carbohydrate content (18.91%), acid value of oil is 1.59. The iodine value of the oil is 136.3. Saponification value of Perilla frutescens oil is 185.3. The oil was analysed for its chemical composition by Gas chromatograph mass spectroscopy (GCMS), The fatty acids in seed oil were identified as Palmitic acid 12.76%, Stearic acid 2.62 %, Oleic acid 0.16%, Linoleic acid 15.40 %, a-linolenic acid 66.58 %, Arachidic acid 0.18 %, Hexadecanoic acid, 14-methyl- methyl ester 0.22%, 2,6-Heptadien-1-ol, 2,4-dimethyl 0.13 %, Lilial 0.15 %. Linoleic acid (omega 6) and a-linolenic acid (omega 3) are essential fatty acids. The high content of a-linolenic acid Linoleic acid suggests that Perilla frutescens seed oil can be excellent source of essential fatty acid.

Key words: Perilla frutescens, omega 3, omega 6, seed oil.

### INTRODUCTION

Plants are one of the most important sources of medicines. Medicines from plants are used by human being since prehistoric period. In India the references to the curative properties of some herbs in the Rig-veda seems to be the earliest records of use of plants in medicines. Various parts of plants such as leaves, roots, flowers and seed are used for different purpose in all around the world.

*Perilla frutescens* have been used as an important traditional herbal medicine for treating various disease including depression, anxiety, tumor, cough, antioxidant, allergy, intoxication, and some intestinal disorders. In northern India the stem of the plant is traditionally used as an analgesic and anti-abortive agent. The leaves are said to helpful for asthma, colds and flu's and regulate stomach function [1]. Perilla is listed in the Chinese Pharmacopoeia and has been used for centuries as a medicinal plant for asthma, influenza, cough, chronic bronchitis and vomiting [2-3]. Perilla seeds are a traditional source of oils produced in Korea [4]. A *perilla* line from Bangladesh is a potential commercial source of rosefuran, a compound of interest in flavoring and perfumery [5].Medicinal and Traditional use of *Perilla frutescens* in Garhwal Himalaya is that the plant is used for various purpose i.e. as a medicine, edible oil, garnish or flavouring agent, as vegetable and other traditional food items [6]. Due to medicinal and traditional

use of *Perilla frutescens* and its seeds, it motivate us to investigate Physico-chemical properties of Perilla *frutescens* seeds.

#### MATERIALS AND METHODS

#### 2.1.Collection of seeds

The seeds of *Perilla frutescens* were collected from Badowala, Deheradun, India at *Latitude*. 30.31° *and Longitude*. 78.03°. The voucher specimens were identified and authenticated by Dr. Dr. Sumer Chand, Ex-Scientist, Systematic Botany Division, Forest Research Institute, Dehradun, India. Dried fruits were collected in polythene bags and brought to the laboratory.

#### 2.2.Extraction of seed oil

A known weight of *Perilla frutescens* seeds were grinded into powder with high speed blender and dried in an air circulating oven at 50°C for 1 h. Oil was extracted from the seeds powder with petroleum ether (boiling point 60- $80^{\circ}$ C) using a Soxhlet extractor. The solvent was distilled off at  $80^{\circ}$ C and oil was dried over anhydrous sodium sulphate.

#### 2.3.Physical and Chemical analysis of seed oil

Physico-chemical analyses of the extracted oil was carried out by using AOAC, 1990 methods[7].Density of oil was determined picnometrically, refractive index was determined at 25°C using Abbey Refractometer, moisture was estimated by heating in oven at 105°C. Iodine value was determined using Wijj's method as reported in AOAC methods [7]. The procedures of [8]were adopted for the estimation of Saponification values, Unsaponifiable matter content and acid value of the oil sample.

#### 2.4Preparation of Fatty acid methyl esters

Fatty acids are polar compounds and are not volatile. For gas chromatographic analysis it is necessary that the sample to be analyzed must be volatile. In order to make fatty acids present in the oil volatile, derivatization is performed prior to GC-MS analysis. Methylation is the most general method of converting non-volatile fatty acids into volatile fatty acids methyl esters. Methylation of fatty acids was performed with  $BF_3$  – methanol as derivatizing reagent, which is the most accepted procedure for converting fatty acids into FAMEs. The Fatty acids were derivatized by using the boron trifluoride method as described by Hisil, 1988[9].

#### 2.5.Gas Chromatography/mass spectrometry Analysis (GC/MS)

Derivatized fatty acids methyl esters were analysed by using a Shimadzu GC-2010 equipped with a Shimadzu GCMS-QP2010 Plus mass selective detector having HP- MS capillary column (30m x 0.25mm, film thickness 0.25  $\mu$ m). The column oven initial temperature was 140°C, programmed at 4°C/min to final oven temperature 240°C and held for 10 min at this temperature, injector temperature was 270°C. Helium was used as carrier gas with column flow rate 1.21 ml/min and the split ratio 1:20. For GC/MS detection, an electron ionization system with ionization energy of 70eV was used, Ion source temperature was 230°C and Interface temperature was 280°C. The components were identified by comparing their relative retention times and mass spectra with those of standards , wiley 8 library data of the main system.

#### **RESULTS AND DISCUSSION**

The nutritional status of seeds is presented in Table-1, the yield of seed oil is (40.1%) that is comparable with Coconut Oil (*Cocos mucifera*) seed oil (39 %), Ground Nut Seed Oil (*Arachis hypogoes*) seed oil (40.1%). Protein content of seed is (18.12%) which is near to other conventionally used oils e.g. Mustard seed oil (20.0%) ,Cotton seed oil (19.4%), Linseed seed oil(20.3%), Sunflower seed oil (19.8%), Coconut seed oil (23.9%), Almond seed oil (20.8%),reported in Nutritive value of Indian foods [10]. Carbohydrate content (18.91%) also lies in the range of other oils which are used for food purposes and other applications [10].

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Figure 1. GC-MS chromatogram of Perilla frutescens seed oil

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Peak Report TIC							
Peak#	R.Time	Area	Area%	Name			
1	5.029	423320	0.13	2,6-Heptadien-1-ol, 2,4-dimethyl-			
2	6.149	227833	0.07	BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8-1			
3	7.385	479738	0.15	Lilial			
4	7.633	283593	0.09	ROSE PHENONE			
5	8.089	3021688	0.92	1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER			
6	9.009	354078	0.11	2-Naphthalenemethanol, decahydroalpha,.alpha.,4a-trimethy			
7	10,783	645964	0.20	1-TERT-BUTYL-2-METHOXY-4-METHYL-3,5-DINITROI			
8	11.552	650073	0.20	9-HEXADECENOIC ACID, METHYL ESTER, (Z)-			
9	11.761	41758743	12,76	HEXADECANOIC ACID, METHYL ESTER			
10	12,492	705028	0.22	Hexadecanoic acid, 14-methyl-, methyl ester			
11	12.774	395291	0.12	CYCLOPENTANETRIDECANOIC ACID, METHYL ESTE			
12	13.433	50417777	15,40	9,12-Octadecadienoic acid (Z,Z)-, methyl ester			
13	13.521	217963445	66,58	9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER			
14	13.725	8574411	2,62	Octadecanoic acid, methyl ester			
15	14.144	381979	0.12	Methyl 12-oxo-9-dodecenoate			
16	15.411	514035	0,16	9-OCTADECENOIC ACID (Z)-, METHYL ESTER			
17	15.623	586390	0,18	EICOSANOIC ACID, METHYL ESTER			
		327383386	100.00				

Hit#:1 Entry:201917 Library:WILEY8.LIB

SI:97 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:0



CompName: HEXADECANOIC ACID, METHYL ESTER \$\$ METHYL HEXADECANOATE \$\$ PALMITIC ACID METHYL ESTER \$\$ AI3-03509 \$\$ AIDS-



Hit#:1 Entry:23480 Library:NIST05s.LIB

SI:98 Formula:C19H34O2 CAS:112-63-0 MolWeight:294 RetIndex:2093

CompName:9,12-Octadecadienoic acid (Z,Z)-, methyl ester \$\$ Linoleic acid, methyl ester \$\$ Methyl cis,cis-9,12-octadecadienoate \$\$ Methyl linoleate \$\$ Methyl acid, methyl ester \$\$ Linoleic acid, methyl ester \$\$ Linoleic acid, methyl ester \$\$ Methyl ester \$\$ Methyl acid, methyl ester \$\$ Methyl ester 100



Hit#:3 Entry:23400 Library:NIST05s.LIB

SI:91 Formula:C19H32O2 CAS:301-00-8 MolWeight:292 RetIndex:2101







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Hit#:9 Entry:100069 Library:NIST05.LIB SI:93 Formula:C19H38O2 CAS:112-61-8 MolWeight:298 RetIndex:2077 CompName:Octadecanoic acid, methyl ester \$\$ Stearic acid, methyl ester \$\$ n-Octadecanoic acid, methyl ester \$\$ Kemester 9718 \$\$ Methyl n-octadecanoate \$\$ 90 70 110 350 130210 230250310 330 370 390 150 290





Oil extracted from *Perilla frutescens* seeds had light yellow colour. The moisture content is 6.90%, which lies in the range of 3.0-9.9% of other used oils, this suggests that oil can be stored for a long period. Acid value of oil is 1.59, which falls within the recommended codex of 0.6 and 10 for virgin and non –virgin edible fats and oil, respectively[11-12]. This suggests that the *Perilla frutescens* seed oil is suitable for edible purposes and also in the manufacture of paints and varnishes [13]. The iodine value of the oil is 136.3, which lies in the category of drying oils. Iodine value of the oil is 136.3, near to those for drying oils, Iodine value shows the degree of unsaturation of fatty acids in an oil or fat and is thus a relative measure of the unsaturation. Drying oil dry rapidly on contact with atmospheric oxygen and makes the oil useful on commercial scale for paint and varnishes, oil paints. Saponification value of *Perilla frutescens* oil is 185.3, Saponification value is used in checking adulteration. The low saponification value of any oil suggests that the oil may not be industrially useful. The high saponification value suggests that the oils contain high molecular weight fatty acids and low level of impurities. This suggests that the oil could be used in soap making industry [14-15].

Table No.-1 Proximate analysis of Perilla frutescens seed

S.No.	Parameter	Result
1	Oil yield %	40.1
2	Carbohydrate content ,%	18.91
3	Protein content, %	18.12
4	Moisture content, %	6.90
5	Ash content, %	2.03
9	Crude Fiber, %	22.32

Table No.2 chemical properties of Perilla frutescens seed oil

S.No.	Chemical properties	Result
1	State at room temperature	liquid
2	Colour	Light yellow
3	Specific gravity (at 30°C)	0.92
4	Acid value (mgKOH/gm)	1.59
5	Iodine Value	136.3
6	Unsaponifiable matter %w/w	1.6
7	Saponification value	185.3

Seed oils are composed primarily of triglycerides which are glycerol esters of fatty acids. GC-MS examination of *Perilla frutescens* seed oil (Fig.1, and Table 3) shows that it contains Palmitic acid 12.76% Stearic acid 2.62 %, Oleic acid 0.16%, Linoleic acid 15.40 %,  $\alpha$ -linolenic acid 66.58 %, Arachidic acid 0.18 %, Hexadecanoic acid, 14-methyl-, methyl ester 0.22%, 2,6-Heptadien-1-ol, 2,4-dimethyl 0.13 %, Lilial 0.15 % as major components. Linoleic acid (omega 6) and  $\alpha$ -linolenic acid (omega 3) are essential fatty acids. Omega 3 fatty acids are necessary for human health but the body can't make them so we have to get them through food, they play a crucial role in brain function (brain memory and performance), as well as normal growth and development and behavioral function. Research shows that omega-3 fatty acids reduce inflammation and may help lower risk of chronic diseases such as heart disease, cancer, and arthritis. In fact, infants who do not get enough omega-3 fatty acids from their mothers during pregnancy are at risk for developing vision and nerve problems. Symptoms of omega-3 fatty acid

deficiency include fatigue, poor memory, dry skin, heart problems, mood swings or depression, and poor circulation [16-18]. Another polyunsaturated fatty acid in the sample was Linoleic acid 15.40 % (omega 6). Research studies shows that there should proper intake balance of omega 6 and omega 3 fatty acid. Omega 6 fatty acids are good for normal immune function and clotting but too much omega 6 fatty acid may promote abnormal clotting and overactive immune system [19].

		% Composition						
No.	Compounds	Present study (Dehradun)	India <sup>1</sup>	China <sup>2</sup>	Japan <sup>3</sup>	Korea <sup>4</sup>	Thailand⁵	
1.	Myristic acid	-	-	0.37	-	-	-	
2.	Palmitic acid	12.76	9.9	7.23	7.7	7.4	4.62	
3.	Stearic acid	2.62	2.33	2.89	3.8	3.6	0.37	
4.	Oleic acid	0.16	0.10	20.77	10.2	9.5	7.0	
5.	Linoleic acid	15.40	14.5	10.54	17.9	16.5	13.9	
6.	α-linolenic acid	66.58	68.6	52.58	60.4	63.0	40.8	
7.	Arachidic acid	0.18	0.10	-	-	-	-	
8.	Cis-11-eicosenoic acid	-	-	0.16	-	-	-	
9.	Hexadecenoic (16:1)	-	0.11	-	-	-	-	
10.	Hexadecanoic acid, 14- methyl-, methyl ester	0.22	-	-	-	-	-	
11.	2,6-Heptadien-1-ol, 2,4- dimethyl	0.13	-	-	-	-	-	
12.	Lilial	0.15	-	-	-	-	-	
Ref.			Saklani	Ding	Bhandari	Bhandari	Kanchanamayoon and	
			et al	et al	et al	et al	Chiang	
			2011	2012	2011	2011	2007	
			[1]	[20]	[21]	[21]	[22]	

Table 3 Comparison of chemical composition of seed oil from Perilla frutescensL.(Dehradun) with various countries of the world

On comparison of the chemical composition of of *Perilla frutescens* seed oil with other study Table 3 myristic acid 0.37% only found in seed oil from China. Palmitic acid 12.76% found in present study followed by 9.9% in another study Saklani 2011(India), then 7.7% (Japan), 7.4% b (Korea), 7.23% (China), and 4.62% (Thailand). In the case of Stearic acid maximum 3.8% is obtained fron seed oil from Japan and minimum 0.37% seed oil from Thailand. The order of percentage of Oleic acid 20.7% (China), 10.2% (Japan), 9.5% (Korea), 7.0% (Thailand), 0.16% (present study) and 0.10 another study (India). Linoleic acid is 17.9% (Japan), 16.5% (Korea), 15.40% (present study), 14.5% another study (India), 13.9% (Thailand) and minimum 10.54% (China). The percentage of  $\alpha$ -linolenic acid follows the order 68.6% (India), 66.58% (present study), 63.0% (Korea), 60.4% (Japan), followed China 52.58% and Thailand 40.8%. The variation in fatty acid composition as discussed above from different countries may be possible differences in climate, latitude, soil and growth conditions, which could affect the chemical components and growth form of Perilla [20].

#### CONCLUSION

Many of the physico-chemical properties studied of *Perilla frutescens* seed oil have very close similarity with other conventionally used oils and it is an important source of essential omega 3 fatty acid linolenic acid. Omega 3 fatty acid are necessary for human health but the body can't make it. So *Perilla frutescens* seed oil is an important source of essential fatty acid such as Omega 3 and Omega 6. Comparison of chemical composition of Perilla *frutescens* seed oil with other study shows that there is variation in the fatty acid composition.

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