ISSN : 0974 - 7508 Volume 6 Issue 4

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 6(4), 2010 [172-175]

Altitudinal and cultivation variation in fatty acid profile of *Perilla frutescens* L.

Devendra Singh Negi[1]*, Ashok Kumar[1], Mahendra Lal Tamta[1], Nivedita Shukla[1], Ranjan Banerji[2]

[1]Department of Chemistry, P.O. Box 75, HNB Garhwal University, Srinagar (Garhwal)-246 174, Uttarakhand, (INDIA)

[2]Phytochemistry Division, National Botanical Research Institute, Ranapratap Marg, Lucknow-226 001, (INDIA)

E-mail : devendra_negi@yahoo.com

Received: 30th September, 2010 ; Accepted: 9th October, 2010

ABSTRACT

The seeds of *P. frutescens* L., collected from different altitudes showed a maximum oil yield (43%) from Ghat Chamoli, while in cultivated samples from Pinder Valley an oil yield of 46% was observed. The fatty acid profile revealed more than 87% polyunsaturated fatty acids (PUFAs) having 74.3% omega-3 fatty acid (ALA) from cultivated seed oil of Dehradun, while in wild samples a maximum of 84.5% PUFAs with 68.8 % omega-3 fatty acid. The fatty acid profile revealed octadecatrienoic (omega-3) acid as the major component of these oils ranging from 65.96-74.29%, maximum being in cultivated seed oil from Dehradun region, therefore, we may conclude that *Perilla* should be cultivated for the oil vis-à-vis omega-3 fatty acid.

KEYWORDS

Perilla frutescens;
Cultivated;
Omega-3 fatty acid;
Linolenic acid.

INTRODUCTION

Humans do not synthesize two of the fatty acids essential for the health i.e., linoleic (18:2) and linolenic (18:3) acids. Therefore, these essential fatty acids must be obtained with diet. Alpha linoleic acid (ALA, omega-3) is one of them which has been reported from *Actinidia chinensis*, *Salvia hispanica*, *Mathiola incana* in more than 60.0 % of total fatty acids, however, *Perilla frutescens* contained only 56.8 %. Dietary lipids and fatty acid composition of our diet plays an important role in health and disease prevention[1-3]. The n-3 PUFAs are beneficial for improving ovulation, embryo quality and cardiovascular protective effects[4-6]. The ratio of n-6/n-3 PUFAs is directly involve when considering the prevention of cancers, heart disease, hypertension and auto-immune disorder[7,8]. WHO/FAO suggested a ratio of 5:1-10:1[9], in the Western diet 10 and 25 to 1 has been estimated[10] and NIH suggests a ratio of 2:3-3:1[11]. Literature revealed that perilla oil is rich source of protein, fats, PUFAs and its lipid contents ranged from 38.6 to 47.8%. It also prevents excessive growth of visceral adipose tissue[12-14].

The present investigation has been undertaken in view of the importance of omega-3 fatty acids which are beneficial in preventing many health problems including heart diseases, rheumatoid arthritis and can-

TABLE 1 : Oil yield of the sample

Sample	DH	GC	PV	SG	TH
Wild	41.1±1.53	43.0±2.26	37.2±1.79	41.8±2.29	38.9±1.71
Cultivated	42.9±1.54	41.3±1.05	46.2±1.08	45.2±1.32	43.0±2.03

DH, Dehradun; GC, Ghat Chamoli; PV, Pinder Valley; SG, Srinagar Garhwal; TH, Tehri; ±SD value; Analysis were carried out in triplicate

Full Paper

TABLE 2 : Fatty acid composition of wild and cultivated seed oils of *p. frutescens* (abbreviation letter between brackets)

	Fatty acid composition (%)							
Sample	**16:0$^{(P)}$**	**16:1$^{(H)}$**	**18:0$^{(S)}$**	**18:1$^{(O)}$**	**18:2$^{(L)}$**	**18:3$^{(Ln)}$**	**20:0$^{(Ar)}$**	**n-6/ n-3**
DH^{W}	9.67±0.143	0.12±0.015	2.49±0.308	0.23±0.029	15.92±0.283	67.88±1.473	0.18±0.050	4.26
DH^{C}	8.33±0.583	0.13±0.05	2.43±0.242	0.09±0.009	12.80±0.138	74.29±1.916	0.11±0.021	5.80
GC^{W}	9.94±0.380	0.11±0.02	2.33±0.146	0.11±0.021	14.51±0.129	68.65±1.463	0.11±0.006	4.73
GC^{C}	9.07±0.106	0.14±0.01	3.09±0.009	0.11±0.85	13.80±0.425	70.56±0.382	0.11±0.046	5.11
PV^{W}	11.9±0.500	0.11±0.01	2.62±0.063	0.13±0.040	15.41±0.361	66.85±0.793	0.12±0.019	4.34
PV^{C}	8.95±0.149	0.10±0.004	2.72±0.068	0.10±0.014	13.58±0.293	70.67±0.977	0.11±0.009	5.20
SG^{W}	9.69±0.049	0.10±0.002	2.29±0.099	0.20±0.135	15.67±0.076	68.82±0.925	0.16±0.059	4.39
SG^{C}	8.99±0.116	0.10±0.007	2.40±0.043	0.09±0.004	14.26±0.502	71.78±0.746	0.09±0.004	5.03
TH^{W}	10.66±0.232	0.10±0.008	2.81±0.020	0.10±0.027	16.69±0.364	65.96±0.621	0.12±0.009	3.95
TH^{C}	9.34±0.400	0.10±0.001	3.03±0.227	0.08±0.029	13.71±0.074	70.28±0.055	0.09±0.004	5.12

PUFAs in Cultivated 83.99-87.09 %
PUFAs in Wild 82.26-84.49 %

DH = Dehradun; GC = Ghat Chamoli; PV = Pinder Valley; SG = Srinagar Garhwal; TH = Tehri; ±SD value; ^{W}Wild; ^{C}Cultivated; ^{P}Palmitic acid; ^{H}Hexadecenoic; ^{S}Stearic acid; ^{O}Oleic acid; ^{L}Linoleic acid; ^{Ln}Linolenic acid; ^{Ar}Arachidic acid; Analysis were carried out in triplicate

cer[1,15-17] taking into account the altitudinal, cultivation parameters and ratio of n-6/n-3 PUFAs.

MATERIALS AND METHODS

The seeds of *P. frutescens* L. were collected from Dehradun (DH), Ghat Chamoli (GC), Pinder Valley (PV), Srinagar Garhwal (SG), and Tehri Gahrwal (TH) varying in their altitudes 600 m, 1443 m, 990 m, 590 m and 1378 m respectively and these samples were also cultivated. The plant was identified by Botanical Survey of India, Dehradun and a voucher specimen has been deposited there (BSD Accession No. 112748).

C4-C24 even carbon standards were obtained from Supelco Analytical USA and purchased from Sigma Aldrich, while solvents (Methanol HPLC grade) and chemicals analytical grade were purchased from Merk India Ltd.

Cultivation

The seeds of *P. frutescens* L. were collected as above and cultivated using five treatment consisting plant spacing (30×45, 30×60, 45×45, 45×60 and 60×60 cm) were studies in randomized block design to evaluate the effect of different plant spacing on oil yield and quality of chemical constituents of the oil.

Extraction

The oil was extracted from wild as well as cultivated seeds (100 g) in soxhlet extractors with petroleum ether (b.p. 40-60°) and the oil yield are presented in percent (w/w) TABLE 1.

Transesterification

The oils from wild as well as cultivated seeds were transesterified by Bureau of Indian Standard (BIS) method[18,19] with some modification. Hexane fraction was reflex with 0.5 N methanolic NaOH and extracted with diethyl ether, 5 ml water containing 1 ml concentrated HCL was added in remaining part and extracted with petroleum ether. Solvent was evaporated under reduced pressure and reflex with methanol containing two drops of H_2SO_4, diluted with water and extracted with petroleum ether.

GC and GC-MS analysis of methyl esters

Methyl esters were analyzed by using Agilent 6890N gas chromatograph equipped with FID and data handling system. Analytical conditions were Perkin Elmer® (Precisely) Cat # N 9316013 Phase Elite-I (Crossbond 100 % dimethyl Polysiloxane) Capillary Column (60 m×0.25 mm, film thickness 0.25µm), injector and detector temperatures were 210°C and 280°C respectively while nitrogen was used as carrier gas. Oven temperature was held for 5 minutes at 50°C, with 10 min solvent delay then Programmed at 3°C / min up to 230°C and then held isothermal at 230°C for 20 min. Identification of constituents was based on the com-

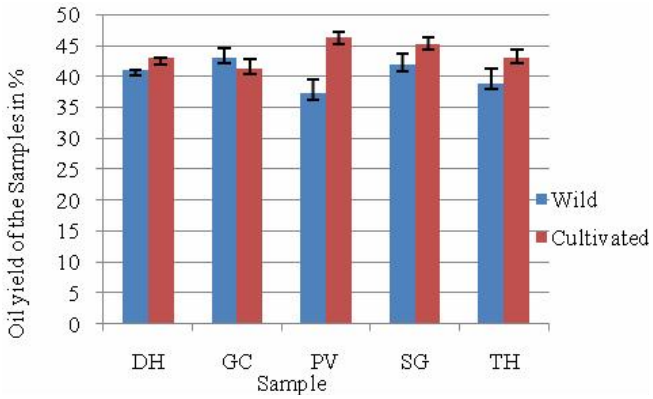
Full Paper

parison of their retention times with those of standard samples.

GC-MS was done by Perkin Elmer make Clarus 500 GC-MS equipped with data handling system. Analytical condition and temperature programming was the same as described above, helium used as carrier gas and GC-MS operating in EI mode at 70 eV. Identification of the constituents was based the comparison of their retention times with those of standard samples, and by comparison of their mass spectral fragmentation patterns matching against Commercial Library mass spectra (Nist, Pfleger, Wiley etc.). The fatty acid chemical constituents are listed in TABLE 2.

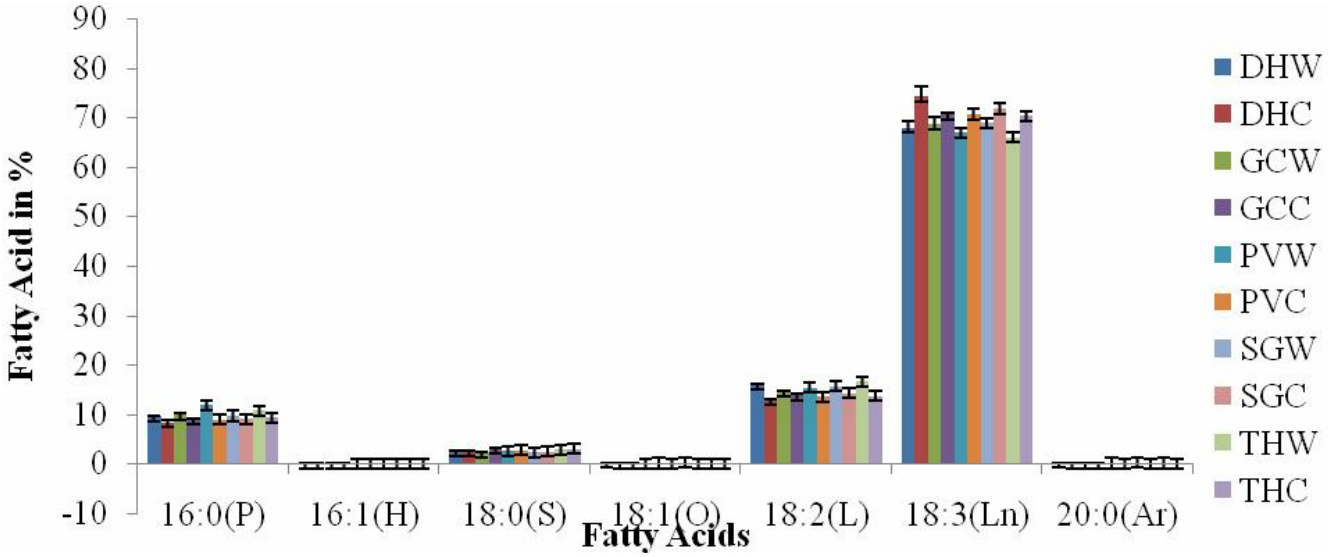
RESULTS AND DISCUSSION

The oil content from wild and cultivated seeds (TABLE 1) ranged from 38.9-46.2%, maximum being in cultivated seeds from Pinder Valley (PV). It is interesting to note that the wildly grown seeds of Pinder valley had minimum oil content as well. However, in the present study, it was observed that in general cultivated seeds had more oil than their wild habitats (Figure 1), the only exception being the seeds from Ghat Chamoli (GC).The fatty acid composition of the oils from Perilla is presented in TABLE 2. The fatty acid profile revealed octadecatrienoic (ALA, omega-3) acid as the major component of these oils ranging from 65.96-74.29 %, maximum being in cultivated seed oil from Dehradun region, whereas minimum was found in the seed oil from Tehri Garhwal in wild origin (Figure 2). However, 56.8 % omega-3 fatty acid was reported in Perilla. It is noteworthy to mention here that all the cultivated samples showed ratio of n-6/n-3 as recommended by WHO/FAO[9]. The total polyunsaturated fatty acids (pufa) ranged from 82.26-84.49% in seed oils from wild environment while 83.99-87.09% in cultivated form. Overall, 87.09% of pufa was observed in cultivated seed oil from Dehradun. Further, hexadecenoic (16:1) and dodecanoic (20:0) acids were found almost in equal



DH = Dehradun; GC = Ghat Chamoli; PV = Pinder Valley; SG = Srinagar Garhwal; TH = Tehri; I=SD value

Figure 1 : Oil yield of the sample



DHW = Wild Dehradun, DHC = Cultivated Dehradun, GCW = Wild Ghat Chamoli, GCC = Cultivated Ghat Chamoli, PVW = Wild Pinder Valley, PVC = Cultivated Pinder Valley, SGW = Wild Srinagar Garhwal, SGC = Cultivated Srinagar Garhwal, THW = Wild Tehri, THC = Tehri, I = SD value , H = Hexadecenoic, S = Stearic acid, O = Oleic acid, L = Linoleic acid, Ln = Linolenic acid, Ar = Arachidic acid

Figure 2 : Fatty acid composition of wild and cultivated seed oils of *p. frutescens*

amounts (TABLE 2) in wild and cultivated seed oils, whereas hexadecanoic (16:0) and octadecadienoic (18:2) acids was found according to the reported values[12]. In the present study we observed octadecenoic acid (18:1) in the range of 0.8-2.3 % as against the reported values. From the present study we may conclude that *Perilla* should be cultivated for the oil vis-à-vis omega-3 fatty acid.

ACKNOWLEDGEMENTS

The authors are thankful to Uttarakhand Council for Science and Technology (UCOST), Uttarakhand India for providing financial assistance.

REFERENCES

[1] A.P.Simopoulos; 'n-3 Fatty Acids and Human Health: Defining Strategies For Public Policy', Lipid, **36**, S83 **(2001)**.
[2] M.B.Schultz, K.Hoffmann; Br.J.Nutr., **95**, 860-589 **(2006)**.
[3] M.B.Katan, P.L.Zock, R.P.Mensink; Am.J.Clin. Nut., **61**, 1368s-1373s **(1995)**.
[4] T.Kojima, Y.Zeniya, T.Aoyama, A.Kondo, J.Yoshino; J.Rep.and Dev., **43(2)**, 121-127 **(1997)**.
[5] M.C.Morris; J.Cardiovasc Risk, **1**, 21-30 **(1994)**.
[6] F.M.Sacks, P.Hebert, L.J.Appel, N.O.Borhani, W.B.Applegate, J.D.Cohen, J.A.Cutter, K.A.Kirchner, L.H.Kuller K.J.Roth; J.Hyperten., **12**, S23-S31 **(1994)**.
[7] W.E.Conner; Am.J.Clin.Nutr., **71(Suppl.1)**, 171s-175s **(2000)**.
[8] P.Hung, K.Y.Gu, Kaku, S.Yunoki, K.Ottkura, I.Ikeda, H.Tachibana, M.Sugano, K.Yazawa, K.Yamada; Biosci.Biotechnol.Biochem., **64**, 2588-2593 **(2000)**.
[9] FAO and WHO Joint Consultation; Fats and Oils In Human Nutrition, Nut.Rev., **53**, 202-205 **(1995)**.
[10] J.Parry, L.Su, M.Luther, K.Zhou, M.M.Yurawecz, P.Whittaker, L.Yo; J.Agric.Food Chem., **53**, 566-573 **(2005)**.
[11] A.P.Simopolos, A.Leaf, N.Salem; J.Am.Coll.Nutr., **18(5)**, 487-489 **(1999)**.
[12] T.Longvah. Y.G.Deosthale; J.Am.Oil Chem.Soc., **68**, 781-784 **(1991)**.
[13] H.S.Shin, S.W.Kim; J.Am.Oil Chem.Soc., **71**, 619-622 **(1994)**.
[14] M.Okuno, K.Kajiwara, S.Imai, T.Kobayashi, N.Honma, T.Maki, K.Suruga, T.Goda, S.Takase, Y.Muto, H.Moriwaki; The Journal of Nutrition, **127(9)**, 1752-1757 **(1997)**.
[15] J.M.Kremer; Am.J.Clin.Nutr., **71**, 349S-351s **(2000)**.
[16] Anonymous; 'FDA Announces Qualified Health Claims for Omega-3 Fatty Acids', September 8, **(2004)**.
[17] R.Banerji; J.Lipid Sci.and Technol., **40**, 75-82 **(2008)**.
[18] U.Schuchardt, R.Sercheli, R.M.Vargas; J.Braz. Chem.Soc., **9**, 199-210 **(1998)**.
[19] Bureau of Indian Standards, New Delhi, Methods of Sampling and Test for Oils and Fats Part 3, Analysis by Gas Liquid Chromatography, BIS, 548, Part 3, **(1964)**.