

Saturated and Polyunsaturated Fatty Acids Rich Populations of *Prinsepia utilis* Royle in Western Himalaya

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Abstract: The study aims to identify the fatty acid composition in *Prinsepia utilis* seeds collected from diverse populations of Western Himalaya, India. The seeds contained higher saturated fatty acid (SFA-43.09%; Average of all populations) followed by polyunsaturated fatty acid (PUFA - 29.57%) and monounsaturated fatty acid (MUFA - 25.53%), however variations among growing populations was measured. The correlation analysis reveals a significant ($p < 0.05$) negative connection between 10-Octadecenoic acid methyl ester (FA1), and altitude. Edaphic factors like available nitrogen showed significantly ($p < 0.05$) but the negative correlation with *cis*-11-Eicosenoic acid, methyl ester (FA10; $p < 0.001$), Docosanoic acid, methyl ester (FA11; $p < 0.05$), Heptadecanoic acid, methyl ester (FA14; $p < 0.05$), and Tricosanoic acid, methyl ester (FA29; $p < 0.05$). Antioxidant activity did not show any variation in the seed oil of different populations but varied among the concentrations (5-50 μ L) used. IC₅₀ values of DPPH activity was significantly ($p < 0.05$) varied connect ranged from 11.31-24.21 μ L/mL among the different populations. These variations in fatty acid composition and IC₅₀ values of the DPPH activity can be one of the indicators for selecting a promising population that would be harnessed for its potential in nutraceuticals and cosmeceuticals industries.

Key words: altitude, fatty acids, antioxidant, edaphic factors

1 Introduction

Fatty acids play an important role in human nutrition as a source of health-promoting biochemical functions such as energetic, metabolites, and structural activities¹. Among others, omega fatty acids are known to maintain cell membrane, brain functions, and transmission of nerve impulses in humans for hemoglobin synthesis, cell division, and transfer of ambient oxygen to blood plasma². The quality of edible oil is determined by fatty acids composition; however, this varies among the source of collection³. This becomes more important in the present scenario when the global market of fatty acids is expected to reach \$ 17.5 Billion by 2023⁴. Therefore, the identification of novel sources for fatty acids is the need of the hour. Studies reported that wild edible plants are a good source of natural fatty acids compared to cultivated ones⁵⁻⁷. This is true for the Himalayan region, where the diversity of wild edible plants (WEP) is much more than any other region⁸. These

plants are reported to be good sources of health-beneficial compounds such as fatty acid, which can generate livelihood and address food and nutritional insecurity⁹.

Among others, *Prinsepia utilis* belongs to the family Rosaceae and is commonly known as Himalayan Cherry (English); Bhekhal, Jatalu, and Dhatila (Hindi) is one of the WEP found in the Himalayan region. It is a deciduous shrub found between 1000-3000 m asl and used in Chinese & Indian medicine to cure skin diseases, rheumatism, inflammation, and leprosy¹⁰. The seeds of the species are a source of edible oil and are reported as good as Mediterranean olive oil¹¹. The seeds are rich in palmitic, oleic, linoleic, and erucic acid⁹. Besides cooking, the oil is used in arthritis, body pain, and muscular pain¹¹. The seed oil is used in cosmeceuticals, skincare formulations and works as a skin barrier due to the presence of unsaturated fatty acids and vitamins¹². The whole plant is traditionally used in many ailments such as infantile eczema, myogenic toxic-

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ity, skin burns, and scalds; leaves used in puss-filled boils; roots and seeds are useful in rheumatism, diarrhea, and stomachache; seed oil in high blood pressure; roots of the plant used to cure menorrhagia and bark in diabetes^{13–17}. The fermented seeds are reported to contain high phenolic content, which is used in gastrointestinal problems¹¹. Several studies of the species reported its use in developing various cosmetics products. For instance, *P. utilis* extract has been used in developing anti-aging and skin disease products^{12, 18}.

It is well known that environmental and edaphic factors influence the fatty acids of seed¹⁹. In addition, the genetic composition of plant²⁰, variety, geographical locations, edaphic factors, climatic conditions, stage of fruit development, and their interaction are also known to influence the fatty acid composition^{19, 20}. Studies reported that fatty acids of *Ocimum basilicum* are affected by geographical and environmental variation²¹; type of variety, climatic conditions, soil type and maturity difference in plant affect fatty acids of Mango²²; different geographic locations affect the fatty acids of *Pinus pinea*²³; *Linum usitatissimum*²⁴, and *Prunus sibirica*²⁵. However, such types of studies are lacking in *P. utilis*. Therefore, the objective of the present study is to understand (i) the composition of fatty acid in the seeds of different populations, (ii) the influence of different growing locations and edaphic factors on the fatty acid composition, and (iii) the antioxidant activity of the seed oil of different populations using *in vitro* antioxidant assay. The data obtained from the study will help to identify the suitable habitat and populations for the further reintroduction of the species and will serve as baseline information for selecting elite germplasm for further domestication and breeding programme. This will lead towards harnessing the potential of species in the right perspectives at an industrial scale.

2 Materials and Methods

2.1 Sample collection

Ripened fruits were collected during the growing period from April to May 2019 from different locations of Kumaun Himalaya, representing diverse altitudes (1407 to 2750 m asl). The fruits were collected considering the geographical variation such as altitude, latitude, and longitude and coded as GBN-1 to GBN-9 (Table 1). The fruits were transported in a zipper bag in a cool ice pack and brought to the Center for Biodiversity Conservation and Management of G.B Pant National Institute of Himalayan Environment (GBP-NIHE) laboratory. The seeds were separated from the pulp and dried at room temperature (25°C) for 5–7 days.

2.2 Fat extraction

The dried seeds were grounded to a fine powder using a mixer grinder (Baltra, India). Extraction of fat was done with petroleum ether at 40°C for 8 h using a soxhlet extractor. Fatty acids composition was analyzed, and fatty acid methyl esters (FAMES) were prepared with minor modification²⁶. Briefly, 100 mg of *P. utilis* seed oil was added into a mixture (2 mL) of petroleum ether and methylbenzene (v/v 1:1) and shaken up to ~5 min until the oil completely dissolved. Thereafter, 2 mL KOH was added in 0.5 M in methanol and shaken for another 1–2 min to saponify and methyl esterify the oil. The solution was incubated with 2 mL HCl (2 M) for 3 min to neutralize the excessive potassium hydroxide. The final organic solution on the top was filtered using a 0.45 µm filter for GC-MS analysis.

2.3 Radical scavenging activity

Antioxidant activity of the seed oil was carried out by *in vitro* assay 2,2-diphenyl-1-picrylhydrazyl (DPPH) method²⁷. Inhibition percentage was calculated and expressed as IC₅₀ value about control. The different concentrations of oil (5 to 50 µL/mL) were used for calculating IC₅₀ value using the following formula.

$$\% \text{ inhibition} = (\text{control} - \text{sample}) / \text{control} \times 100$$

Table 1 Physico-chemical characteristics of study sites and soil of *Prinsepia utilis* in western Himalaya.

Populations	Code	Altitude (m asl)	Latitude (N)	Longitude (E)	Moisture (%)	pH	OC (%)	AN (kg/ha)	AK (kg/ha)	AP (mg/g)
Dawarahat	GBN 1	1407	29° 45' 35"	79° 26' 17"	3.46 ± 0.22 ^a	6.43 ± 0.15 ^{de}	1.10 ± 0.10 ^d	173.13 ± 2.46 ^b	135.63 ± 2.63 ⁱ	0.20 ± 0.01 ^c
Badecheena	GBN 2	1606	29° 64' 92"	79° 77' 48"	1.63 ± 0.17 ^c	5.53 ± 0.21 ^h	2.93 ± 0.28 ^c	49.33 ± 1.47 ^f	158.38 ± 6.75 ^h	0.15 ± 0.01 ^d
Ranikhet	GBN 3	1725	29° 67' 04"	79° 49' 23"	2.46 ± 0.30 ^b	6.13 ± 0.15 ^{ef}	3.36 ± 1.27 ^c	107.02 ± 6.31 ^d	256.54 ± 5.53 ^c	0.12 ± 0.01 ^c
Jageswar	GBN 4	1786	29° 63' 84"	79° 85' 28"	1.16 ± 0.08 ^d	6.57 ± 0.15 ^d	1.83 ± 0.09 ^d	66.35 ± 4.34 ^c	220.16 ± 5.67 ^f	0.13 ± 0.02 ^{de}
Dunagiri	GBN 5	1911	29° 79' 22"	79° 46' 35"	1.11 ± 0.09 ^d	7.00 ± 0.20 ^c	5.56 ± 0.23 ^a	280.17 ± 5.59 ^a	401.97 ± 6.89 ^c	0.23 ± 0.02 ^b
Nainital	GBN 6	2025	29° 39' 29"	79° 44' 72"	2.80 ± 0.48 ^b	7.83 ± 0.21 ^a	5.69 ± 0.07 ^a	158.04 ± 2.4 ^c	448.38 ± 2.51 ^b	0.18 ± 0.01 ^c
Pangu	GBN 7	2165	29° 58' 59"	80° 36' 55"	2.73 ± 0.25 ^b	6.07 ± 0.06 ^{fg}	4.55 ± 0.28 ^b	161.53 ± 3.08 ^{bc}	570.53 ± 3.40 ^a	0.24 ± 0.01 ^b
Mukteswar	GBN 8	2244	29° 43' 45"	79° 60' 45"	1.04 ± 0.07 ^d	7.37 ± 0.21 ^b	4.44 ± 0.19 ^b	114.74 ± 1.63 ^d	193.83 ± 2.08 ^g	0.29 ± 0.02 ^a
Narayan Ashram	GBN 9	2750	29° 59' 22"	80° 39' 31"	3.62 ± 0.25 ^a	5.77 ± 0.25 ^{gh}	6.07 ± 0.53 ^a	170.86 ± 2.61 ^{bc}	348.41 ± 5.31 ^d	0.27 ± 0.01 ^a

OC- organic carbon, AN- available nitrogen, AK- available potassium and AP- available phosphorus

2.4 GC-MS analysis

The fatty acid profile, i.e., seed oil's fatty acid methyl esters (FAMES), was determined by gas chromatography (model GCMS-QP2010 Plus) coupled with a mass spectrometer. Different compounds were separated through capillary column DB-5MS (30 m × 0.32 mm, 0.25 µm of film thickness). Helium (99.99%) was used as a carrier gas with a 1.21 mL/min flow rate. The column temperature was set at 140 to 280°C at a rate of 4°C/min. Injector and detector temperature was set at 260°C. A sample volume (0.1 µL) was injected in a split mode, with a split ratio of 1:10. The mass spectrometer was set to scan in the range of m/z 40–650 with electron impact (EI) ionization mode. The chemical compositions of the samples were identified by matching the mass spectra with the data in the NIST Mass Spectral Library.

2.5 Soil analysis

Soil samples were collected during May 2019 simultaneously at the time of fruit samples collection. Samples were collected at 0–30 cm depth from each site, and the three samples were bulked to form one sample per site. Roots and stones were removed from each sample. These were dried at room temperature for 5 days. Dried soils were then passed through a 0.5 mm sieve and stored to determine soil physicochemical analysis. Standard methods were used^(28, 29) for analysis of soil parameters such as soil moisture, pH (measured by digital pH meter), organic carbon, available nitrogen, available potassium, and available phosphorus. The moisture content and organic carbon value was represented in percentage (%), available nitrogen and potassium in kg/ha, and available phosphorus in mg/g.

2.6 Statistical analysis

The analysis was performed using R software to understand the relationship between fatty acids, altitude, and edaphic factors by Pearson correlation coefficient analysis. Neighbor-joining (NJ) cluster and principal component analysis (PCA) was done through PAST 3.0 (Paleontological Statistics Software Package for Education and Data Analysis) software for understanding the variation in fatty acids among populations and prioritizing the most promising location for fatty acid composition. Canonical correspondence analysis was conducted using fatty acids as response variables and edaphic factors as explanatory variables to check their effect on fatty acid composition using R software. The significant mean values among populations were tested using Duncan's multiple range test (DMRT; $p < 0.05$) by using IBM SPSS software Version 26 for different measured parameters.

3 Results

3.1 Fatty acid composition

Analysis of fatty acid using GC-MS showed a presence of 29 compounds in the seed oil of *P. utilis*, amounting to 88.93 – 98.66% (Table 2). The major fatty acids was Methyl 10-*trans*,12-*cis*-octadecadienoate (32.68%); Hexadecanoic acid, methyl ester (27.88%); 10-octadecenoic acid methyl ester (25.29%); 9,12-Octadecadienoic acid (*Z,Z*) (15.34%); Methyl stearate (11.92%); 9,12-Octadecadienoic acid (*Z,Z*)-methyl ester (10.87%); 9-Hexadecenoic acid, methyl ester, (*Z*)- (1.29%) and Eicosanoic acid, methyl ester (1.71%). GC-MS analysis showed the variation in fatty acid composition among different populations. For instance, the 10-octadecenoic acid methyl ester was present in all the populations except the GBN-9, while the 9-octadecenoic acid methyl ester was detected in the GBN-9 population only. The 9,12-octadecadienoic acid (*Z,Z*) present in all the populations except GBN7, GBN-8, and GBN-9, while methyl 10-*trans*,12-*cis*-octadecadienoate was only present in GBN-7, GBN-8, and GBN-9 populations. Further, when the total fatty acids were categorized in saturated, polyunsaturated, and monounsaturated fatty acids (SFA, PUFA, and MUFA), the highest value of SFA content was found in the GBN-2 population (64.58%), whereas the lowest content was detected in GBN-5 (31.53%). Similarly, MUFA was maximum in GBN-8 (32.19%), whereas the GBN-9 (1.31%) population contains the minimum. PUFA was higher in GBN-9 (51.00%) than other populations (Fig. 1).

3.2 Soil composition

Soil collected from the different populations revealed variation in physicochemical properties (Table 1). Maximum moisture content (3.62%) was recorded at the GBN-9 population, which was significantly higher than other populations except for GBN-1. The moisture content ranged from 1.04 (GBN-8) to 3.62% (GBN-9). Maximum pH (7.83) was found in the GBN-6 population and ranged from 5.53 ± 0.21 (GBN-2) to 7.83 ± 0.21 (GBN-6). Similarly, organic carbon ranged between 1.10 ± 0.10 (GBN-1) to $6.07 \pm 0.53\%$ (GBN-9), and a maximum was found at the GBN-9 population. Available nitrogen was significantly ($p < 0.05$) higher in GBN-5 (280.17 ± 5.59 kg/ha) as compared to other populations. Available potassium showed a significantly higher value in the GBN-7 population (570.53 ± 3.40 kg/ha). Available phosphorus was found maximum in GBN-8 (0.29 ± 0.02 mg/g) and minimum in GBN-3 (0.12 ± 0.01 mg/g) population.

3.3 Antioxidant activity of oil

The scavenging ability of edible oil measured by DPPH antioxidant assay reveals the significant variation in antioxidant activity among the populations (Fig. 2). Increasing the oil concentration from 5 to 50 µL/mL showed a reduction in free radicals scavenging activity. The maximum in-

Table 2 Fatty acid compositions(%) in seed oil of different populations of *Prinsepia utilis* in western Himalaya (– not detected).

Code	Compound name	Category of fatty acid	Retention time	GBN1	GBN2	GBN3	GBN4	GBN5	GBN6	GBN7	GBN8	GBN9
FA1	10-Octadecenoic acid, methyl ester	MUFA	21.6	27.37	20.5	25.52	28.64	26.41	22.15	20.92	30.78	–
FA2	15-Tetracosenoic acid, methyl ester, (Z)-	MUFA	33.42	–	0.12	–	–	–	0.05	0.03	0.06	–
FA3	2,5-Furandione, 3-(dodecenyl) dihydro-	MUFA	26.73	–	–	–	–	–	0.14	–	–	–
FA4	2-Methylhexacosane	Hydrocarbon	29.62	–	0.16	–	–	–	–	–	–	–
FA5	9,12-Octadecadienoic acid (Z,Z)-	PUFA	21.38	25.96	3.92	26.97	3.38	28.42	3.37	–	–	–
FA6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	PUFA	21.14	10.48	5.92	10.71	16.32	11.26	4.98	16.45	–	–
FA7	9-Hexadecenoic acid, methyl ester, (Z)-	MUFA	16.52	1.11	2.26	1.03	1.32	0.86	2.15	0.93	0.92	1.02
FA8	9-Octadecenoic acid, methyl ester, (E)-	Trans-MUFA	21.68	–	–	–	–	–	–	–	–	9.7
FA9	cis-10-Heptadecenoic acid, methyl ester	MUFA	18.91	0.07	0.14	0.06	0.06	0.04	0.01	0.03	0.04	0.08
FA10	cis-11-Eicosenoic acid, methyl ester	MUFA	25.58	0.33	0.57	0.31	0.48	0.03	0.28	0.25	0.39	0.21
FA11	Docosanoic acid, methyl ester	SFA	30.18	0.42	0.71	0.36	0.61	0.31	0.68	0.44	0.51	0.39
FA12	Eicosanoic acid, methyl ester	SFA	26.19	1.39	2.35	1.2	2.15	1.18	2.3	1.56	1.87	1.4
FA13	Heneicosane	Alkane	29.61	0.09	0.07	–	0.06	0.06	0.13	0.09	0.11	0.1
FA14	Heptadecanoic acid, methyl ester	SFA	19.52	0.16	0.45	0.18	0.29	0.15	0.32	0.19	0.24	0.21
FA15	Hexacosane	Alkane	35.11	0.18	0.22	0.14	0.22	0.15	0.15	0.14	–	0.17
FA16	Hexadecadienoic acid, methyl ester	SFA	24.95	–	–	0.15	–	–	–	–	–	–
FA17	Hexadecanoic acid, methyl ester	SFA	17.27	20.17	43.53	20.38	31.32	20.42	40.09	21.7	29.67	23.6
FA18	Hexatriacontane	Alkane	43.89	0.09	–	–	–	0.07	0.17	0.07	0.09	–
FA19	Methyl 10-trans,12-cis-octadecadienoate	Trans-PUFA	21.4	–	–	–	–	–	–	26.44	20.61	51.00
FA20	Methyl stearate	SFA	22.05	10.25	16.34	8.92	12.49	9.01	17.87	9.19	12.78	10.47
FA21	Methyl tetradecanoate	SFA	11.96	0.15	0.48	–	–	0.09	0.2	0.15	0.18	–
FA22	n-Hexadecanoic acid	SFA	18.26	–	–	0.7	–	–	0.65	–	–	–
FA23	Octadec-9-enoic acid	MUFA	22.4	–	–	1.05	–	–	1.42	–	–	–
FA24	Pentacosane	Alkane	33.34	0.12	–	–	0.15	–	0.16	0.14	0.11	0.13
FA25	Pentalene, octahydro-1-(2-octyldecyl)	–	26.73	–	0.15	–	–	–	–	–	–	–
FA26	Tetracontane	Alkane	36.82	0.25	0.31	0.21	0.3	0.2	0.29	0.16	0.18	0.21
FA27	Tetracosanoic acid, methyl ester	SFA	33.9	0.37	0.63	0.34	0.48	0.32	0.59	0.33	0.42	0.32
FA28	Tetradecanoic acid, methyl ester	SFA	11.96	–	–	0.11	0.2	–	–	–	–	0.17
FA29	Tricosanoic acid, methyl ester	SFA	32.08	0.05	0.09	0.06	0.09	0.05	0.09	0.05	0.07	0.06
Total fatty acid			–	98.28	98.01	98.05	97.83	98.55	97.43	98.66	98.54	88.93

SFA- saturated fatty acid, MUFA- monounsaturated fatty acid, PUFA- polyunsaturated fatty acid

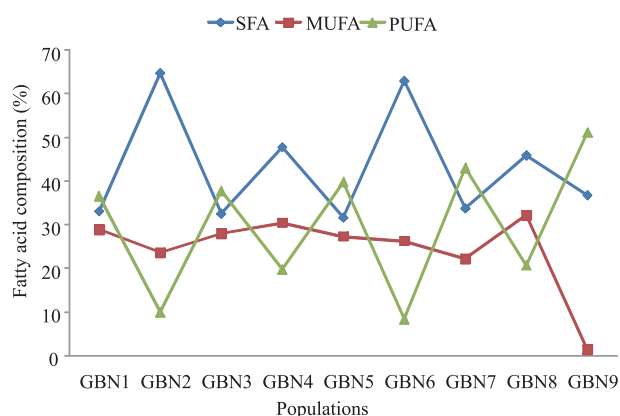
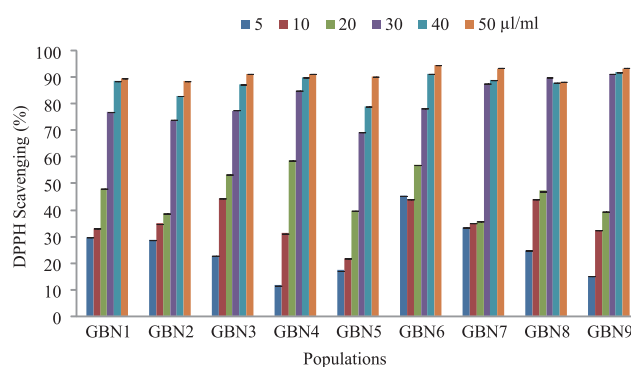
**Fig. 1** Ratio of fatty acids in the seed oils of *Prinsepia utilis*.**Fig. 2** Antioxidant activity of oil in different populations.

Table 3 Antioxidant activity of oil in different populations.

Populations	IC ₅₀ (μL/mL)
GBN 1	18.65 ± 0.18 ^{cd}
GBN 2	20.65 ± 0.84 ^b
GBN 3	17.33 ± 0.60 ^d
GBN 4	19.62 ± 0.06 ^{bc}
GBN 5	24.21 ± 0.15 ^a
GBN 6	11.39 ± 0.07 ^f
GBN 7	18.05 ± 0.04 ^d
GBN 8	16.63 ± 0.15 ^c
GBN 9	20.23 ± 0.12 ^b

hibition was found at 50 μL/mL in all the populations except GBN-8, whereas 30 μL/mL showed maximum inhibition (Fig. 2). GBN-6 population showed the highest IC₅₀ (11.39 μL/mL) as compared to other populations, and the lowest IC₅₀ was recorded in the GBN-5 (24.21 μL/mL) population (Table 3).

3.4 Relationship of edaphic factors with fatty acids composition

Correlation analysis between environmental factors and fatty acids composition revealed that altitude is negatively but significantly ($p < 0.05$) correlated with 10-octadecenoic acid, methyl ester (FA1). While developing a correlation among soil nutrients and fatty acid composition, available nitrogen and *cis*-11-eicosenoic acid, methyl ester (FA10), docosanoic acid, methyl ester (FA11), heptadecanoic acid, methyl ester (FA14), and tricosanoic acid, methyl ester (FA29) were negatively but significantly ($p < 0.001$) correlated. Tetracontane (FA26) showed a significant negative ($p < 0.05$) correlation with available phosphorus (Fig. 3). Correlations among different fatty acid compositions reveal the positive and negative relationship. For instance, a significant ($p < 0.05$) negative relation of 9,12-octadecadienoic acid (*Z,Z*)-(FA5) with *cis*-11-eicosenoic acid, methyl ester (FA10); positive relationship of 9-hexadecenoic acid, methyl ester, (*Z*)-(FA7) with *cis*-10-heptadecenoic acid, methyl ester (FA9), docosanoic acid, methyl ester (FA11), eicosanoic acid, methyl ester (FA12), heptadecanoic acid, methyl ester (FA14), hexadecanoic acid, methyl ester (FA17), methyl stearate (FA20), tetracontane (FA26), tetracosanoic acid, methyl ester (FA27) and tricosanoic acid, methyl ester (FA29); *cis*-10-heptadecenoic acid, methyl

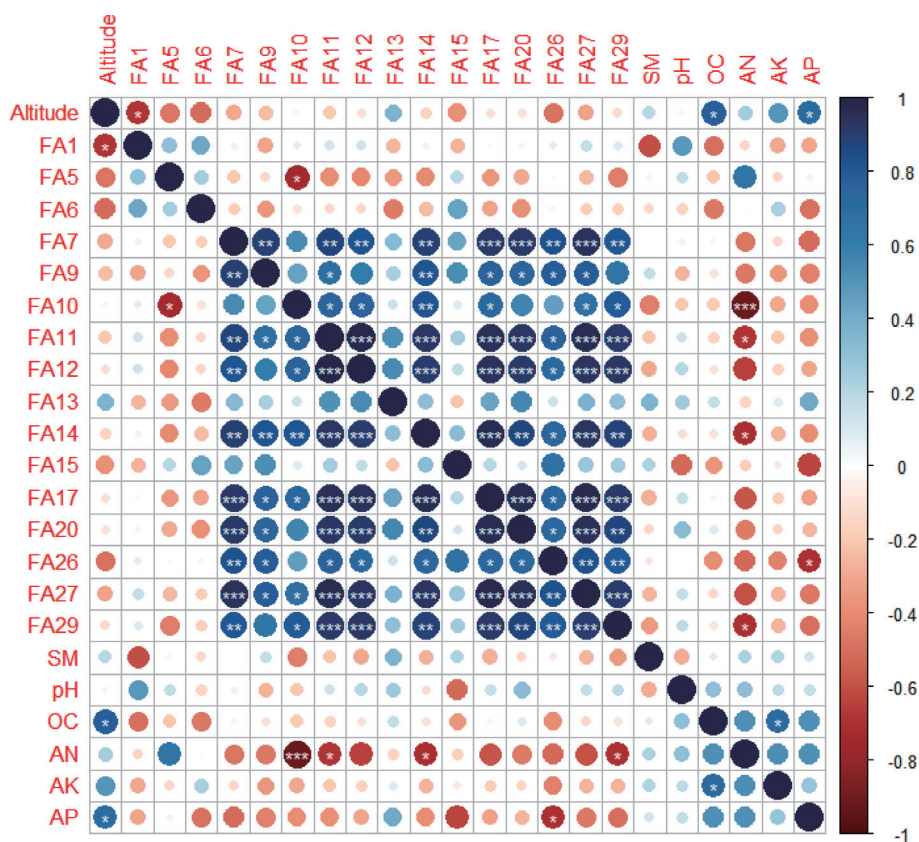


Fig. 3 Relationship between altitude, edaphic factors and fatty acid composition in *Prinsepia utilis* ($p < 0.05^*$, 0.01^{**} and 0.001^{***}).

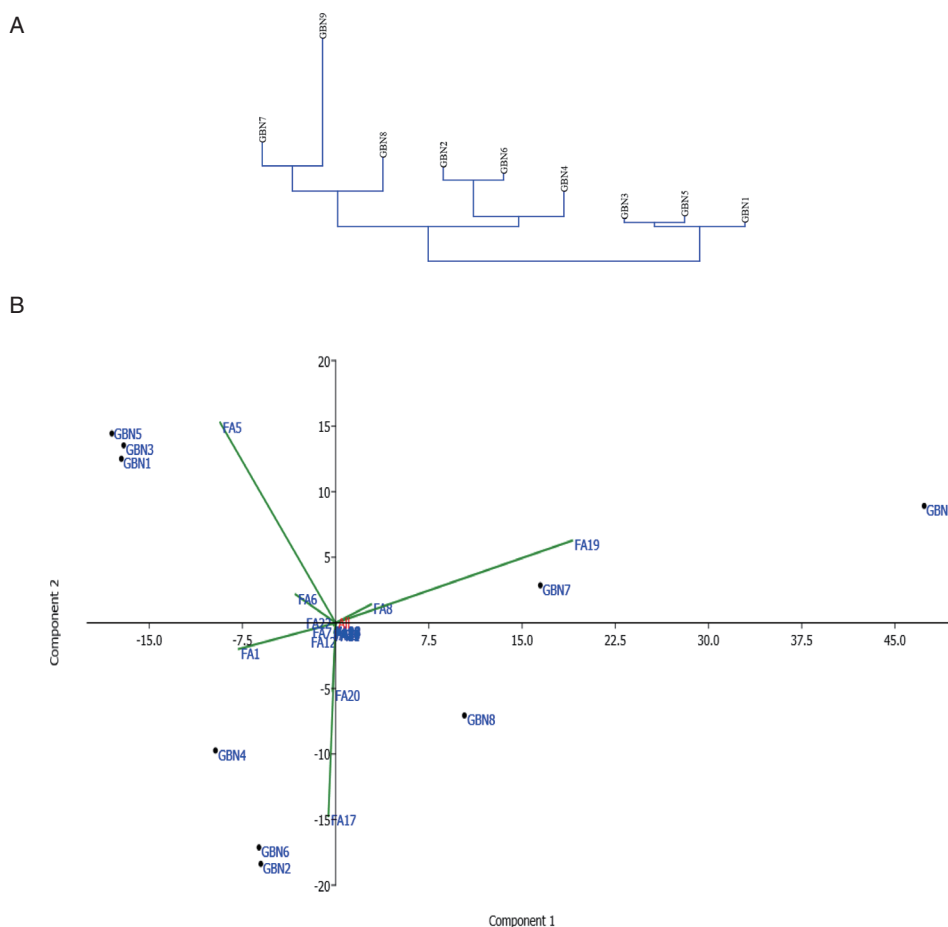


Fig. 4 A: Neighbor joining Cluster analysis of fatty acid composition; B: Principal component analysis of fatty acid composition.

ester (FA9) with heptadecanoic acid, methyl ester (FA14), docosanoic acid, methyl ester (FA11), hexadecanoic acid, methyl ester (FA17), methyl stearate (FA20), tetracontane (FA26) and tetracosanoic acid, methyl ester (FA27); *cis*-11-eicosenoic acid, methyl ester (FA10) with docosanoic acid, methyl ester (FA11), eicosanoic acid, methyl ester (FA12), heptadecanoic acid, methyl ester (FA14), hexadecanoic acid, methyl ester (FA17), tetracosanoic acid, methyl ester (FA27) and tricosanoic acid, methyl ester (FA29); docosanoic acid, methyl ester (FA11) eicosanoic acid, methyl ester (FA12), heptadecanoic acid, methyl ester (FA14), hexadecanoic acid, methyl ester (FA17), methyl stearate (FA20), tetracontane (FA26), tetracosanoic acid, methyl ester (FA27) and tricosanoic acid, methyl ester (FA29); eicosanoic acid, methyl ester (FA12) with heptadecanoic acid, methyl ester (FA14), hexadecanoic acid, methyl ester (FA17), methyl stearate (FA20), tetracontane (FA26), tetracosanoic acid, methyl ester (FA27) and tricosanoic acid, methyl ester (FA29); heptadecanoic acid, methyl ester (FA14) with hexadecanoic acid, methyl ester (FA17), tetracosanoic acid, methyl ester (FA27), methyl stearate (FA20), tetracontane (FA26) and tricosanoic acid,

methyl ester (FA29); hexadecanoic acid, methyl ester (FA17) with methyl stearate (FA20), tetracontane (FA26), tetracosanoic acid, methyl ester (FA27), tricosanoic acid, methyl ester (FA29); methyl stearate (FA20) with tetracontane (FA26) and tetracosanoic acid, methyl ester (FA27), tricosanoic acid, methyl ester (FA29). Similarly, tetracontane (FA26), significantly ($p < 0.01$) correlated with tetracosanoic acid, methyl ester (FA27), and tricosanoic acid, methyl ester (FA29), and tetracosanoic acid, methyl ester (FA27) with tricosanoic acid, methyl ester (FA29). All these indicated that the fatty acid compounds are strongly inter-linked.

3.5 Neighbor-joining (NJ) cluster, principal component analysis (PCA), and canonical correspondence analysis (CCA)

NJ cluster and PCA analysis are multivariate analyses and work based on the distance-based matrix. In the present study, a cluster analysis of the fatty acid composition of nine populations of *P. utilis* grouped in two groups (Fig. 4A). 1st cluster contains GBN-1, GBN-3, and GBN-5 fall in one group and contains a similar composition of

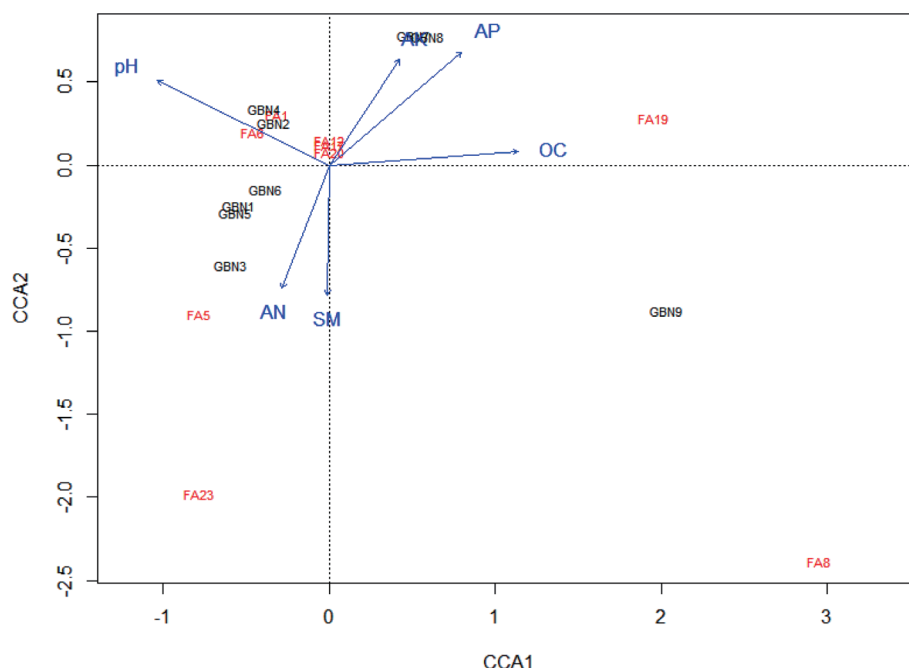


Fig. 5 Canonical correlation analysis of fatty acids and edaphic factors.

9,12-octadecadienoic acid (*Z,Z*)-(FA5) and 9,12-Octadecadienoic acid (*Z,Z*)-, methyl ester (FA6), fatty acid. The 2nd cluster conation two sub-clusters (A & B), cluster 'A' contain GBN2, GBN4 & GBN6 and cluster 'B' contain GBN7, GBN-9, and GBN-8 populations. Cluster 'A' is similar due to 10-octadecenoic acid, methyl ester (FA1), 9-hexadecenoic acid, methyl ester, (*Z*)-(FA7) and eicosanoic acid, methyl ester (FA12), but in cluster 'A' GBN2 & GBN6 have a more similar composition. The cluster 'B' contains GBN7, GBN9 & GBN8 population, which contain methyl 10-*trans*,12-*cis*-octadecadienoate (FA19) in different composition. PCA biplot analysis was conducted to further group 9 populations based on fatty acid composition. The analysis explained PC1 variance with 64.38%, PC2 with 24.56%, PC3 with 7.11%, and PC4 showed 3.64% variance (Fig. 4B).

The CCA analysis analyzed the relationship between major fatty acid and edaphic factors, displaying the triplot and reflecting major patterns. As shown in Fig. 5, populations GBN1, 3, 5, 6 dominated by 9,12-octadecadienoic acid (*Z,Z*)-(FA5) were influenced by available nitrogen, and it showed little influence on octadec-9-enoic acid (FA23) while fatty acid like 10-Octadecenoic acid, methyl ester (FA1), 9,12-Octadecadienoic acid (*Z,Z*)-, methyl ester (FA6) in GBN2 and GBN4 populations were influenced by pH. Population GBN7 and GBN8 represent Methyl 10-*trans*,12-*cis*-octadecadienoate (FA 19) compositions influenced by soil available potassium, available phosphorus, and organic carbon. Other fatty acids such as eicosanoic acid, methyl ester (FA12), hexadecanoic acid, methyl ester (FA17), and methyl stearate (FA20) followed a similar

pattern in GBN2 & 4 and GBN7 & 8 also influenced by pH, organic carbon, available nitrogen and available phosphorus.

4 Discussion

The present study, for the first time conducted to understand the association between altitude, edaphic factors, and fatty acid profile of *P. utilis* collected from different locations of western Himalaya. The species is known for its edible oil; therefore, it is essential to identify the population with high-yielding individuals/populations that adapt well to diverse environmental conditions and accumulate the desired quantity of the target fatty acids. It is well known that the quantity and quality of fatty acids largely depend on soil condition, genetic and environmental factors²⁰. Studies reported that the fatty acid composition varied among the plant seed and genotype of various plant species in different environmental and climatic conditions³⁰. For instance, the *Pistacia lentiscus* edible oil from a diverse population of Tunisia showed variability in fatty acids, carotenoids, and tocopherols³¹.

Environmental factors such as altitude, temperature, humidity, and light intensity are reported to significantly affect the accumulation of saturated, monounsaturated, and polyunsaturated fatty acids in plants³². It is well known that change of fatty acid content of the plant, maintain appropriate membrane fluidity, which mainly depends on many environmental variables such as nutrient availability, salinity, moisture, rainfall, humidity and daylight as

well as geographical locations latitude, longitude and altitude³³). For instance, Fatty acids such as palmitoleic, linoleic, stearic, and palmitic acids in avocado and hazelnut oils are affected with changing altitude³⁴). In the present study, altitude was found to have a negative correlation in fatty acid profile as altitude increases fatty acid decrease, however, varies across the altitudinal gradient might be due to the temperature because as altitude increase temperature decrease and low-temperature increase unsaturation of fatty acids. The reports are available on the affect of altitude on fatty acids composition in coffee beans from Ethiopia³⁵), and seabuckthorn from Iran³⁶). The highest FA 19 (Methyl 10-*trans*,12-*cis*-octadecadienoate) content was found at high altitude populations, might be because in high altitude night temperature is generally low, which affects fatty acids composition and this is also reported that at low temperature the ratio of fatty acids composition can be changed as in the membrane of mulberry plants³⁷). In our study, the unsaturated fatty acid such as MUFA (GBN 8) and PUFA (GBN9) found higher while lower SFA content at high altitude populations. This might be because of increasing altitude decrease temperature and higher exposure of light at high altitude³⁸⁻⁴⁰). A significant ($p < 0.05$) negative correlation between FA10 and FA 5 ($r = -0.72$) might be due to the increase of one fatty acid leads to a corresponding decrease in the other⁴¹). The positive correlation ($p < 0.001$) between the fatty acids composition such as FA17 (Palmitic acid methyl ester) with FA20 (Stearic acid methyl ester) can help breeders to select such populations for domestication and further breeding programme²⁴). The differences in fatty acid compositions might be due to different phases of biosynthesis of those compounds and the accumulation of fatty acids. The higher altitude population (GBN7, GBN8 & GBN9) showed higher unsaturated fatty acids because cold stress such as low temperature has a strong effect on unsaturated fatty acids accumulation⁴²). It is well known that fatty acids have different medicinal properties. For instance, linoleic acid (FA5) is the essential fatty acid that is known for anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocidal, insectifuge, antihistaminic, antiacne, anti-coronary activities, 5-Alpha reductase inhibitor, antiarthritic, and many other properties⁴³). Similarly, palmitic and stearic fatty acids (FA20) are the most abundant dietary fatty acids under the saturated fatty acids. Palmitic acid, oleic acid, and stearic acid are free fatty acids and important energy sources for the body⁴⁴). In our study, FA17 (palmitic acid methyl ester) ranged between 20.17 - 43.53%, which is the most abundantly available saturated fatty acid in nature and used as an antioxidant, a novel vasodilator against cardiac arrest induced brain injury, having hypocholesterolemic, hemolytic, anticancer, antimicrobial, antiandrogenic, a 5-alpha reductase inhibitor properties and many more^{45, 46}). The arachidic acid methyl ester (FA12) is known

for preventing cholesterol gallstones by acting as a cholesterol solubilizer⁴⁷); Methyl linoleate (FA6) used in dermatological cosmetics, soaps, and emulsifier and play a direct role in epidermal permeability barrier; and Lignoceric acid (FA27) is reported to promote the growth of cotton fiber cell elongation⁴⁸). Stearic acid is the most abundant saturated fatty acid in nature, which plays an important role in regulating human body functions⁴⁹). Palmitoleic acid (FA7) is known for its insulin sensitivity and prevents beta-cell apoptosis induced by glucose or saturated fatty acids⁵⁰). A linoleic acid most common form of PUFA, is an unsaturated fatty acid involved in lipid metabolism and maintains the integrity of the integuments. It is also effective in reducing blood cholesterol concentrations, HDL-cholesterol, VLDL cholesterol, and repair cell membrane due to the attack of free radicals³⁶). Soil macronutrients are important for plant growth and biological processes and the development of seed quality. Nitrogen is important in fat synthesis, and both carbon and nitrogen are required in seed development⁵¹). In the present study, a negative correlation between available nitrogen and saturated fat (FA11, 14, 29) might be due to the different habitat conditions that do not adequately supply the nutrients. 1st cluster contains GBN-1, GBN-3, and GBN-5; similarly, the 2nd cluster A contains GBN2, GBN4 & GBN6, and cluster B contain GBN7, GBN-8, and GBN-9 might be due to a similar genetic structure. The presence of these fatty acids in the seeds of the species may be potentially used in the preparation of different health-promoting products. As the species is growing abundantly in the Himalayan region, proper utilization of its fruits and seeds may have an additional source of income for the Himalayan people.

5 Conclusion

A great difference was observed among the populations to fatty acids such as lower altitude population rich in SFA and high altitude population rich in PUFA. Among the different fatty acids, Methyl 10-*trans*,12-*cis*-octadecadienoate was only observed in GBN7, GBN8, and GBN9 populations. The altitude and soil nutrients like available nitrogen and phosphorus showed significant negative relation with fatty acids. A significant variation in the antioxidant activity among different populations showed its potential to use those populations with higher activity. The Cluster and PCA analysis revealed GBN7, GBN8, and GBN9 populations rich in unsaturated fatty acids and these populations can be utilized for mass propagation and cultivation. The variation observed in saturated and monounsaturated and polyunsaturated fatty acids offer new possibilities of developing novel cultivars with high-quality edible oil. Fats are used in the food industry for the production of a wide variety of products, ranging from margarine to chocolate, or used di-

rectly as a salad and cooking oil. Mass plantation of this species at degraded lands may help in providing livelihood to rural people and restoration of the degraded lands in the Himalayan region being as good soil binder.

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Conflicts of Interest

The authors declare no conflict of interest.

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