



# Comparison of nutritional quality of fourteen wild *Linum* species based on fatty acid composition, lipid health indices, and chemometric approaches unravelling their nutraceutical potential

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## ABSTRACT

Fatty acid profiles of 14 *Linum* species was determined by GC-MS analysis to study the nutritional quality of *Linum* species based on fatty acid composition, lipid health indices, and chemometric approaches. *L. lewisii* and *L. marginale* found to have the highest content of ALA i.e., 65.38 % and 62.79 %, respectively, *L. tenuifolium* recorded the highest linoleic acid content (69.69 %), while, *L. catharticum* recorded highest oleic acid (27.03 %). Health indices viz. polyunsaturated fatty acids/saturated fatty acids ratio, n-6/n-3 fatty acids ratio, atherogenicity, thrombogenicity, oxidability, oxidative stability, hypocholesterolemic/hypercholesterolemic fatty acids, and peroxidisability calculated based on the fatty acid composition revealed that all the linseed species except *L. aristatum*, *L. tenuifolium* and *L. hudsoniodes* have healthy fatty acid composition. *L. lewisii* clearly emerges as a promising species followed by *L. bienne* with great values across multiple indices, making them as a potential candidate for dietary or nutritional interests. The lipid profile of *Linum* species could be well distinguished by two principal components by Principal Component Analysis (PCA).

## 1. Introduction

Over the last few decades, focus on oilseed crops with high nutritional value to promote safe and healthy eating habits has gradually increased [1]. Consumers are becoming increasingly conscious of the nutritional value and health advantages of the products they consume [2]. Lipids are the essential dietary components and forms the only dietary proportions of essential fatty acids occurring naturally in the form of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA). Fatty acids are transported to cells, where they help in contraction of muscles and regulation of overall metabolic process [3]. However, studies investigating the significance of fatty acids in human and animal health put emphasis not only on polyunsaturated fatty acids, but also on monounsaturated and saturated fatty acids. Fatty acids could indeed play a positive or negative role in disease prevention and treatment. Saturated fatty acids, particularly palmitic, myristic, laurel, and, to a lesser extent, stearic acid, have been shown in studies to raise the level of cholesterol in low-density lipoproteins (LDL cholesterol). Saturated fatty acids are also thought to play a role in the

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development of certain malignant tumours in humans and other mammals. Therefore, changing or modifying the content and/or composition of fatty acids in diet may be one of the ways to enhance the nutritional wellbeing [4]. This may be possible by choosing the right source and recommended proportion of fatty acids.

Considering the numerous health benefits provided by fatty acids, the amount of scientific research on linseed, including their fatty acid composition, has grown during past few decades. Linseed is known as the richest plant source of  $\alpha$ -linolenic acid, which is an n-3 polyunsaturated fatty acid (n-3 PUFA) [5]. Due to high n-3 PUFAs, linseed has gained popularity as a superfood. It is already known that n-3 PUFAs are considered important in human nutrition and health [6]. Linseed oil is used as functional ingredient for the fortification of baked foods, salads, and dairy products [7]. There are substantial evidences that long-chain n-3 PUFAs plays a vital role in many regulatory processes at cell and tissue level including tissues of brain and retina. The greatest interest is the signalling role of n-3 PUFAs that helps in maintaining human health by regulating inflammatory processes, protection against several tumours and metabolic diseases [8]. Linseed is known for its wide range of applications in addition to its high nutritional value, and has gained attention as a potential strategic crop for nutrition and fibre [9,10]. Native to the Indian subcontinent, linseed is cultivated mainly in Russia federation, Kazakhstan, Canada, China and India [11]. Linseed represents a great genetic variability, with different species and cultivars adapted to growth in different environmental conditions around the world, which also contributes to its biochemical variability [12]. Thus, traceability studies based on fatty acids have the benefit of providing valuable information for consumers about the nutritional quality of the food, especially in terms of n-3 fatty acid content and the saturation, stability, and oxidability of the fatty acids in food products [13].

Realizing the nutritional value of flaxseed as an important plant based source of fatty acids, present work aims to evaluate and compare the nutritional value of 14 *Linum* species grown in different parts of world by determining the fatty acid profiles, analysing their nutritional value indices and health lipid indices such as, n-6/n-3 PUFA, PUFA/SFA, desirable fatty acids (DFA), atherogenic index (AI), thrombogenic index (TI), oxidability index (COX), oxidative stability (OS), hypocholesterolemic/hypercholesterolemic index (h/H index), and peroxidisability index (PI) using different chemometric approaches.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Analytical grade methanol, n-hexane and toluene were purchased from Merck (India). Acetyl chloride (purity >99 %) was purchased from Sigma-Aldrich (India). Helium gas (purity >99.999 %) was supplied by Sigma Gases, New Delhi, India.

### 2.2. Seed collection and field experiment design

The seeds of *Linum* wild germplasm (109 accessions) belonging to 14 different species were procured from USDA-ARS, Washington State University (USA) and USDA-ARS, Iowa State University (USA) by the Indian Council of Agricultural Research- National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India (Tables S1 and S2). Single plant selection was done to maintain uniformity among the accessions. The seed harvested from these accessions were multiplied through single plant progenies during the year 2019-20 at Indian Council for Agricultural Research-National Bureau of Plant Genetic Resources (NBPGR), experimental farm, IARI, New Delhi (28° 38' 53.7" N, 77° 09' 05.4" E, and 218 m above mean sea level). These seeds were used for fatty acid profiling in the present work. The field experiment was conducted in an Augmented Block Design [14]. The crop was fertilized with 60:40 kg/ha of N:P<sub>2</sub>O<sub>5</sub> and 20 kg/ha each of Sulphur and Zinc as per recommendations for linseed crop.

### 2.3. Extraction and derivatization of fatty acids

The extraction of fatty acid esters from the linseed germplasm was performed using the previously standardized method with a few modifications [15]. Briefly, 1g of dried linseedseed sample was weighed and powdered. 50 mg of the powdered seed sample was transferred to 15 ml Borosil vials fitted with a Teflon-lined screw cap. The sample was esterified with 0.5 mL of methanol-benzene-acetyl chloride (20: 4: 1, v/v) methylating reagent at 65 °C for 60 min in a hot water bath. Samples were allowed to cool down to room temperature, and 2 mL of n-hexane was added to the cooled down sample. The contents were mixed thoroughly and centrifuged at 5000 RPM for 10 min. The upper phase (n-hexane) containing derivatized fatty acids was separated and transferred to 1.5 mL autosampler vial for GC-MS analysis.

### 2.4. Fatty acid analysis using gas chromatography-mass spectroscopy (GC-MS)

Fatty acid methyl esters were analysed by gas chromatography-mass spectroscopy (GC-MS) (Model 8860/5977, Agilent Technologies, California, United States) using a DB-WAX capillary column (30 m × 0.25 mm; film thickness 0.25  $\mu$ m) and Helium (purity >99.999 %) as the carrier gas with the constant flow rate of 1.0 mL/min. An injection volume of 0.1  $\mu$ L was used for analysis. The temperature of injector port was maintained at 240 °C and oven temperature programming was used. The initial oven temperature was maintained at 200 °C for 2 min, and then ramped to 220 °C at a rate of 10 °C/min and held for 6 min. The mass spectroscopic system was operated in Electric Ionization (EI) mode at 70 eV. The source temperature and quad temperature was set at 230 °C and 150 °C, respectively with a solvent delay of 4 min. Mass of the compounds was analysed in the range of  $m/z$  35–600 amu and the fatty acids were identified by making use of spectral matching of each GC/MS spectra with the NIST library data for the GC-MS system. The

relative fatty acid composition (in percentage) of each linseed species was defined by the mean percentage and standard deviation of individual fatty acid.

## 2.5. Classification of fatty acid

Different classes of fatty acids viz. saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were calculated using following formula:

$$SFA = [PA] + [SA]$$

$$MUFA = [OA]$$

$$PUFA = [LA] + [ALA]$$

where, PA = palmitic acid, SA = stearic acid, OA, oleic acid, LA = linoleic acid, ALA =  $\alpha$ -linolenic acid, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids and PUFA = polyunsaturated fatty acids.

## 2.6. Determination of lipid health/nutritional indices

Health indices such as the polyunsaturated fatty acids/saturated fatty acids (PUFA/SFA) ratio, n-6/n-3 PUFA, atherogenicity index (AI), thrombogenicity index (TI), oxidability (COX), oxidative stability (OS), hypocholesterolemic/hypercholesterolemic index (h/H index), and peroxidisability index (PI) were calculated based on fatty acid composition using the following equations [4,16]:

$$\frac{n-6 \text{ PUFA}}{n-3 \text{ PUFA}} = \frac{[LA]}{[ALA]}$$

$$\frac{PUFA}{SFA} = \frac{[LA] + [ALA]}{[PA] + [SA]}$$

$$DFA = [SA] + [OA] + [LA] + [ALA]$$

$$\text{Atherogenicity Index (AI)} = \frac{[PA] + [SA]}{[MUFA] + [PUFA]_{n-6} + [PUFA]_{n-3}}$$

$$\text{Thrombogenicity Index (TI)} = \frac{[PA] + [SA]}{0.5 \times [MUFA] + 0.5 \times [PUFA]_{n-6} + 3 \times [PUFA]_{n-3} + \frac{[PUFA]_{n-3}}{[PUFA]_{n-6}}}$$

$$\text{Oxidability (COX)} = \frac{[OA] + 10.3 \times [LA] + 21.6 \times [ALA]}{100}$$

$$\text{Oxidative stability (OS)} = [MUFA] + 45 \times [LA] + 100 \times [ALA]$$

$$\frac{\text{Hypocholesterolemic}}{\text{Hypercholesterolemic}} \text{ index } \left( \frac{h}{H} \text{ index} \right) = \frac{[OA] + [LA] + [ALA]}{[PA]}$$

$$\text{Peroxidisability index (PI)} = 0.025 \times [OA] + [LA] + 2 \times [ALA]$$

where, PA = palmitic acid, SA = stearic acid, OA, oleic acid, LA = linoleic acid, ALA =  $\alpha$ -linolenic acid, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids and PUFA = polyunsaturated fatty acids.

## 2.7. Statistical analysis

Descriptive analysis was performed to obtain mean  $\pm$  SD values of all the fatty acids, nutritional value indices and lipid health indices. One-way analysis of variance was used for intra-class comparison of individual as well as class of fatty acids, nutritional value indices and lipid health indices of linseed species, and were performed using Graph pad by Prism version 9.5.1.

Correlation study of all the individual as well as classes of fatty acids was as performed using the Graph pad by Prism version 9.5.1 and the results were represented in the form of r value (pearson's coefficient). Similarly, hierarchical clustering of the linseed species on the basis of their fatty acid profile was performed on the basis of Euclidian distance and the clusters were studied graphically using dendrogram prepared online using DATAtab statistical calculator (<https://datatab.net/statistics-calculator/cluster>).

The classification and discrimination of linseed species using fatty acid profiles were achieved by PCA using Graphpad Prism version 9.5.1. PCA plots mapped variables (eight fatty acids, including five individual fatty acids and three classes of fatty acids, SFA, MUFA, and PUFA) and samples (109 samples belonging to 14 species of linseed) using loadings and scores in dimensional spaces determined by PCs with eigenvalues  $>1.0$  in accordance with the Kaiser's rule [17]. The score plot reveals whether samples are

comparable or distinct, typical or an outlier, while the loading plot shows the identification of significant variables.

### 3. Results and discussion

#### 3.1. Fatty acid profiles

Fatty acid composition of 109 linseed germplasm belonging to 14 species of genus *Linum*, revealed large variability in the fatty acid profiles (Fig. 1 and Table S3). The value of ALA which is also the major fatty acid present in linseed species, ranged from 1.70 % to 65.38 % with a mean value of 43.38 % (Table 1). The LA content ranged from 7.30 % to 81.91 % with average value of 23.49 %. Oleic acid ranged from 6.34 % to 40.36 % with an average value of 21.42 %. Palmitic and stearic acids found to be the minor fatty acids with average value of 7.12 % and 4.06 % respectively, ranging from 1.49 % to 14.41 %, and 1.51 % to 10.29 %, respectively.

The ALA content of different *Linum* species seeds showed a large variability. While most of the *Linum* species (eight out of fourteen) recorded high ALA (>45 %); *L. tenuifolium*, and *L. hudsonioides* recorded very low ALA (<5 %); and *L. aristatum* recorded low ALA (<10 %). These very low and low ALA species recorded very high LA (>60 %). *L. lewisii* and *L. marginale* recorded very high (>55 %) ALA while widely cultivated *L. usitatissimum* recorded average 49.28 % ALA. *L. strictum*, *L. catharticum*, and *L. flavum* recorded moderate ALA (20 to 30 %). Wild accessions W6-56977 and EC1073090 of *L. lewisii* found to have highest ALA of 65.38 % and 63.68 %, respectively while EC1073114 of *L. marginale* recorded 62.79 % ALA. The accessions EC1073077 of *L. tenuifolium* and EC1073124 of *L. hudsonioides* showed the lowest ALA content of 1.70 % and 2.16 %, respectively. The high ALA content is the characteristic feature of seeds of *Linum* species [18]. High ALA has been a key molecule for the nutritional value of linseed. High levels of ALA also improve fatty acid profile of the seeds, making linseed a valuable source of n-3 PUFA. Intake of n-3 PUFA dietary supplementation has been shown to have cardioprotective role by lessening endothelial cell apoptosis and mitochondrial dysfunction caused by oxidative stress [19]. The species with higher concentrations of ALA thus may have potentially higher antioxidant activity, since ALA is ascribed to have an antioxidant role [19].

With regard to LA content, very low ALA species *L. tenuifolium*, *L. hudsonioides* and *L. aristatum* recorded very high LA (>60 %). Thus, showing an inverse relationship between the two fatty acids. Accessions EC1073077 of *L. tenuifolium* and EC1073121 of

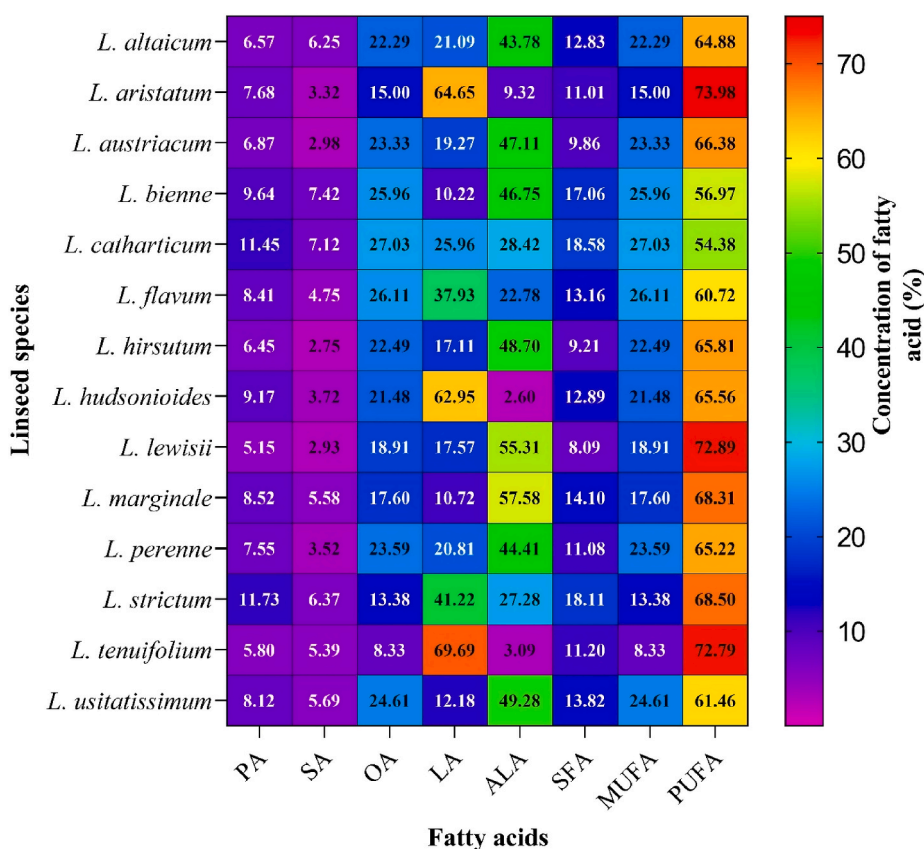


Fig. 1. Heat map representing fatty acid profile of wild *Linum* species, where fatty acids are showed in percentage concentration. The, X-axis represents name of fatty acids viz, palmitic acid (PA), stearic acid (SA), oleic acid (OA), linoleic acid (LA),  $\alpha$ -linolenic acid (ALA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) and Y-axis represents the name of *Linum* species.

**Table 1**  
Average and range of fatty acids of wild *Linum* species.

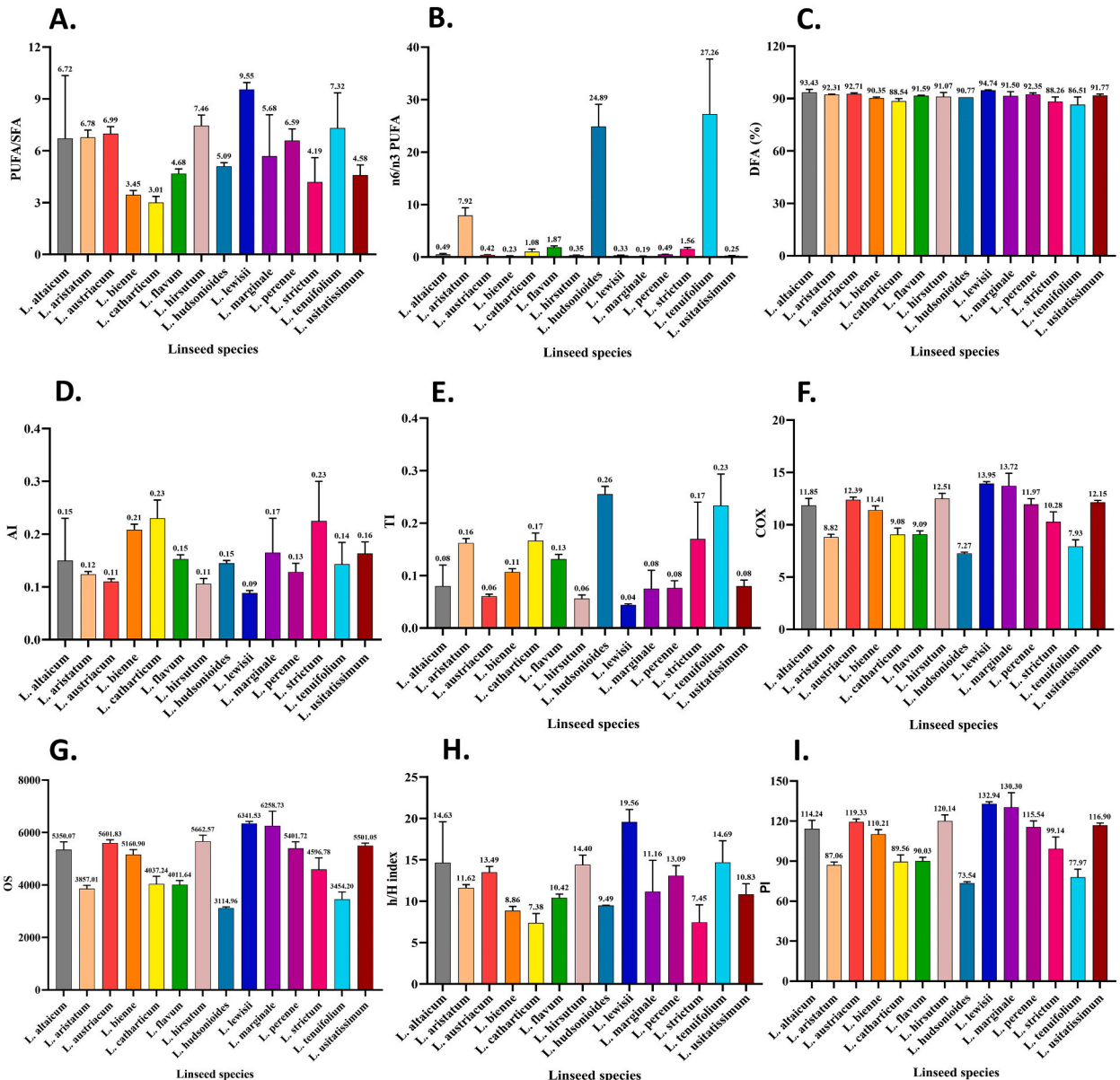
Species	Palmitic acid (%)			Stearic acid (%)			Oleic acid (%)			Linoleic acid (%)			Linolenic acid (%)			SFA (%)			MUFA (%)			PUFA (%)		
	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD
<i>L. altaicum</i>	4.75	8.40	6.57 ± 2.58	2.22	10.22	6.25 ± 5.71	20.89	23.69	22.29 ± 1.98	13.30	28.89	21.09 ± 11.02	43.24	44.33	43.78 ± 0.77	6.97	18.69	12.83 ± 8.29	20.89	23.69	22.29 ± 1.98	57.63	72.13	64.88 ± 10.25
<i>L. aristatum</i>	6.91	8.18	7.68 ± 0.47	2.91	4.69	3.32 ± 0.76	9.18	18.86	15.00 ± 4.01	56.55	70.80	64.65 ± 5.88	5.26	14.70	9.32 ± 3.59	9.85	12.40	11.01 ± 0.92	9.18	18.86	15.00 ± 4.01	70.30	80.97	73.98 ± 4.64
<i>L. austriacum</i>	4.87	9.18	6.87 ± 1.21	1.62	4.75	2.98 ± 1.02	16.33	30.95	23.33 ± 3.63	13.28	29.00	19.27 ± 3.74	38.63	60.52	47.11 ± 5.83	6.49	13.16	9.86 ± 1.77	16.33	30.95	23.33 ± 3.63	59.57	76.35	66.38 ± 4.12
<i>L. bienne</i>	7.15	11.78	9.64 ± 1.60	3.76	9.69	7.42 ± 2.18	19.01	40.36	25.96 ± 6.07	7.30	13.03	10.22 ± 1.92	32.70	55.36	46.75 ± 6.93	11.36	19.77	17.065 ± 2.57	19.01	40.36	25.96 ± 6.07	43.19	63.05	56.97 ± 6.23
<i>L. catharticum</i>	9.01	13.95	11.45 ± 2.47	5.83	8.23	7.12 ± 1.21	23.25	30.83	27.03 ± 3.79	17.12	35.78	25.96 ± 9.37	18.55	37.12	28.42 ± 9.34	14.84	22.18	18.58 ± 3.67	23.25	30.83	27.03 ± 3.79	54.24	54.57	54.38 ± 0.76
<i>L. flavum</i>	7.35	9.53	8.41 ± 0.80	3.32	6.43	4.75 ± 1.01	23.92	27.40	26.11 ± 1.27	16.52	43.26	37.93 ± 9.58	17.61	41.03	22.78 ± 8.20	10.84	15.05	13.16 ± 1.47	23.92	27.40	26.11 ± 1.27	57.55	63.83	60.72 ± 2.44
<i>L. hirsutum</i>	5.16	10.62	6.45 ± 1.76	1.51	3.80	2.75 ± 0.76	15.92	31.42	22.49 ± 5.50	13.66	20.85	17.11 ± 2.73	40.55	55.08	48.70 ± 5.67	7.97	14.18	9.21 ± 2.10	15.92	31.42	22.49 ± 5.50	54.40	75.93	65.81 ± 7.88
<i>L. hudsonioides</i>	9.12	9.23	9.17 ± 0.08	3.22	4.22	3.72 ± 0.71	21.45	21.52	21.48 ± 0.05	62.87	63.04	62.95 ± 0.12	2.16	3.05	2.60 ± 0.63	12.45	13.34	12.89 ± 0.63	21.45	21.52	21.48 ± 0.05	65.03	66.09	65.56 ± 0.75
<i>L. lewisii</i>	1.49	9.31	5.15 ± 1.39	1.76	5.68	2.93 ± 0.96	8.36	30.12	18.91 ± 3.69	9.24	34.69	17.57 ± 4.76	37.08	65.38	55.31 ± 6.03	4.65	14.99	8.09 ± 2.14	8.36	30.12	18.91 ± 3.69	58.95	83.08	72.89 ± 4.26
<i>L. marginale</i>	6.08	10.96	8.52 ± 3.45	3.12	8.05	5.58 ± 3.49	16.45	18.75	17.60 ± 1.63	9.89	11.56	10.72 ± 1.18	52.38	62.79	57.58 ± 7.36	9.20	19.01	14.10 ± 6.94	16.45	18.75	17.60 ± 1.63	62.27	74.35	68.31 ± 8.54
<i>L. perenne</i>	5.15	14.41	7.55 ± 2.86	2.38	6.82	3.52 ± 1.26	18.35	36.17	23.59 ± 5.09	16.68	27.96	20.81 ± 3.70	25.88	56.90	44.41 ± 8.18	7.88	21.23	11.08 ± 4.03	18.35	36.17	23.59 ± 5.09	42.60	73.58	65.22 ± 8.70
<i>L. strictum</i>	9.10	14.37	11.73 ± 3.73	3.86	8.89	6.37 ± 3.56	12.32	14.44	13.38 ± 1.50	40.68	41.76	41.22 ± 0.76	22.66	31.91	27.28 ± 6.54	12.96	23.26	18.11 ± 7.28	12.32	14.44	13.38 ± 1.50	64.42	72.59	68.50 ± 5.78
<i>L. tenuifolium</i>	4.81	7.46	5.80 ± 1.44	2.51	8.14	5.39 ± 2.82	6.34	10.28	8.33 ± 1.97	57.56	81.91	69.69 ± 12.18	1.70	4.24	3.09 ± 1.29	7.66	15.60	11.20 ± 4.04	6.34	10.28	8.33 ± 1.97	60.91	83.61	72.79 ± 11.39
<i>L. usitatissimum</i>	6.68	9.18	8.12 ± 1.30	4.08	6.52	5.69 ± 1.40	23.11	27.20	24.61 ± 2.25	9.14	14.20	12.18 ± 2.68	47.57	52.44	49.28 ± 2.74	10.76	15.70	13.82 ± 2.67	23.11	27.20	24.61 ± 2.25	60.77	62.04	61.46 ± 0.64
<i>P-value</i>			****			****			****			****			****			****			****			****

Where, SFA= Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA= Polyunsaturated fatty acids; \*\*\*\* =  $p \leq 0.000$  (ANNOVA).

*L. aristatum* recorded the highest content with value as high as 81.91 % and 70.80 %, respectively. The LA recorded in *L. tenuifolium* is higher than the reported content in safflower and sunflower oil [20,21]. The LA content assessed in *Linum* spp. in the current study, found to be significantly higher than reported so far in *L. usitatissimum* and even higher than the LA reported in previous studies on wild *Linum* species [5,22–26]. However, *L. bienne*, *L. marginale* and *L. usitatissimum* recorded LA content less than 15 %, with all other species presenting LA content ranging from 15 % to 60 %.

The OA content in *Linum* species recorded from moderate to low. *L. catharticum*, *L. flavum* and *L. bienne* recorded average OA content more than 25 %. Accession EC1073071 of *L. bienne* recorded the highest OA content i.e., 40.36 %. This accession also has an ideal fatty acid composition for culinary purposes with moderate ALA (32.70 %), low LA (10.49 %), desirable n-6/n-3 ratio (0.32), low SFAs (16.44 %) and moderate PUFAs (43.19 %). OA rich diets have been reported to decrease central adiposity and improved the risk factors related to metabolic syndromes simultaneously [27].

The PA and SA content in the different wild *Linum* species showed very less variability. The low content of PA in *Linum* seeds is a characteristic trait and is found consistently among the species belonging to *Linum* which is suitable for the human nutrition and health [20]. Accession EC1073111 of species *L. lewisii* showed lowest PA content of 1.49 %. While around ten accessions from *L. hirsutum*, *L.*



**Fig. 2.** Graphical representation of lipid health/nutritional indices of wild *Linum* species: (A) Polyunsaturated fatty acids/saturated fatty acids (PUFA/SFA), (B) nn-6/n-3 PUFA, (C) Desirable fatty acids (DFA), (D) Atherogenicity index (AI), (E) Thrombogenicity index (TI), (F) Oxidability (COX), (G) Oxidative stability (OS), (H) Hypocholesterolemic/hypercholesterolemic index (h/H index), (I) Peroxidisability index (PI).

*austriacum* and *L. lewisii* recorded SA content around 1 %.

Moreover, a large variability in the fatty acid profile of accessions of different *Linum* species can be exploited for wide range of nutritional and therapeutic applications. Grouping the analysed fatty acids into different classes based on unsaturation gave some interesting outcomes. Linoleic acid and  $\alpha$ -linolenic acid contribute to the polyunsaturated fatty acids (PUFA) content of *Linum* seeds. The accessions from species *L. tenuifolium* (EC1073077), *L. lewisii* (W6-56060) and *L. aristatum* (EC1073121) recorded highest PUFA content i.e., 83.61 %, 83.08 % and 80.97 %, respectively, which is the highest ever reported for any *Linum* species. However, accessions from species *L. bienne* (EC1073071) and *L. perenne* (EC1073049) recorded with the lowest PUFAs i.e., less than 45 %. The PUFAs of plant source are ingested and acts as the precursors for animal PUFAs i.e., Eicosapentaenoic acid, Docosahexaenoic acid and Arachidonic acid [28].

The accession EC1073071 of *L. bienne* showed maximum monounsaturated fatty acids (MUFA) content of about 40 % among all the analysed species, while the seeds of *L. tenuifolium* (EC1073078) presented the lowest concentration (6 %) of MUFAs. A huge variability was observed in the MUFA content among all the species of *Linum*.

The saturated fatty acids (SFAs) were the least abundant class of fatty acids in *Linum* species. The highest SFAs were observed to be about 23 % in *L. strictum* accession EC1073080 and the least SFAs about 4 % in *L. lewisii* (EC1073111). SFAs are more likely known to be responsible to induce the obesity-associated inflammation and anxiety-like behaviour [29]. Thus, *Linum* species with low SFAs can be useful for appropriate nutritional purposes.

### 3.2. Lipid health/nutritional indices

Assessment of the role of fatty acids in human and animal health is important to consider the type of fat that should be consumed. Fatty acids can be obtained from a variety of dietary sources, each of which has distinctive properties; thus, fatty acid composition of dietary source should be investigated. Nutritional value indices like n-6/n-3 PUFA, MUFA/PUFA, PUFA/SFA, desirable fatty acids (DFA) and lipid health indices such as atherogenic index (AI), thrombogenic index (TI), oxidability index (COX), oxidative stability (OS), hypocholesterolemic/hypercholesterolemic index (h/H index), and peroxidisability index (PI) are also used to determine the nutritional and medicinal value of various food products and raw materials. They represent the connections between individual fatty acids and/or groups of fatty acids.

The nutritional value and healthiness of linseed lipids for human consumption were assessed for the first time and results are

**Table 2**

Lipid health/Nutritional indices of fourteen wild *Linum* species.

Species	PUFA/SFA	n-6/n-3 PUFA	DFA	AI	TI	COX	OS	h/H index	PI
<b>Desirable values</b>	>0.45	<4.00	–	<1.00	<0.50	–	–	>1.00	>80.00
<i>L. altaicum</i>	6.72 ± 5.14	0.48 ± 0.26	93.43 ± 2.57	0.15 ± 0.11	0.08 ± 0.05	11.85 ± 0.95	5350.07 ± 417.02	14.63 ± 7.00	114.24 ± 8.99
<i>L. aristatum</i>	6.72 ± 0.94	6.93 ± 3.30	92.29 ± 0.47	0.12 ± 0.01	0.16 ± 0.02	8.82 ± 0.59	3856.25 ± 283.48	11.58 ± 0.83	87.04 ± 5.01
<i>L. austriacum</i>	6.73 ± 1.64	0.40 ± 0.13	92.69 ± 1.90	0.11 ± 0.02	0.06 ± 0.01	12.39 ± 1.00	5601.48 ± 475.78	13.06 ± 2.65	119.32 ± 8.73
<i>L. bienne</i>	3.34 ± 0.89	0.22 ± 0.07	90.35 ± 1.60	0.21 ± 0.04	0.10 ± 0.02	11.41 ± 1.36	5160.86 ± 650.04	8.60 ± 1.75	110.21 ± 11.70
<i>L. catharticum</i>	2.93 ± 0.61	0.91 ± 0.76	88.53 ± 2.47	0.23 ± 0.06	0.16 ± 0.03	9.08 ± 1.03	4037.23 ± 510.27	7.11 ± 1.95	89.56 ± 8.77
<i>L. flavum</i>	4.61 ± 0.71	1.67 ± 0.68	91.57 ± 0.80	0.15 ± 0.02	0.13 ± 0.02	9.09 ± 0.84	4010.96 ± 411.29	10.32 ± 1.15	90.02 ± 7.41
<i>L. hirsutum</i>	7.15 ± 1.74	0.35 ± 0.04	91.05 ± 6.76	0.10 ± 0.03	0.05 ± 0.02	12.51 ± 1.41	5662.44 ± 658.46	13.69 ± 3.27	120.13 ± 12.74
<i>L. hudsoniodes</i>	5.09 ± 0.31	24.21 ± 5.97	90.75 ± 0.01	0.15 ± 0.01	0.26 ± 0.02	7.26 ± 0.15	3114.23 ± 68.29	9.49 ± 0.01	73.52 ± 1.37
<i>L. lewisii</i>	9.01 ± 2.31	0.32 ± 0.14	94.72 ± 1.39	0.09 ± 0.03	0.04 ± 0.01	13.95 ± 0.98	6340.56 ± 471.64	17.82 ± 8.69	132.92 ± 8.54
<i>L. marginale</i>	4.84 ± 3.40	0.19 ± 0.01	91.48 ± 3.43	0.16 ± 0.09	0.07 ± 0.04	13.72 ± 1.70	6258.00 ± 787.61	10.08 ± 5.33	130.28 ± 15.50
<i>L. perenne</i>	5.89 ± 2.23	0.47 ± 0.13	92.33 ± 2.83	0.12 ± 0.60	0.07 ± 0.05	11.97 ± 1.73	5401.04 ± 816.53	11.76 ± 4.01	115.53 ± 15.30
<i>L. strictum</i>	3.78 ± 2.00	1.51 ± 0.40	88.25 ± 3.72	0.22 ± 0.11	0.16 ± 0.10	10.27 ± 1.35	4596.28 ± 621.21	6.98 ± 2.99	99.13 ± 12.69
<i>L. tenuifolium</i>	6.50 ± 3.50	22.55 ± 18.12	86.50 ± 7.61	0.14 ± 0.07	0.23 ± 0.10	7.93 ± 1.08	3453.38 ± 473.84	13.98 ± 4.50	77.95 ± 10.40
<i>L. usitatissimum</i>	4.45 ± 1.03	0.25 ± 0.07	91.76 ± 1.35	0.16 ± 0.04	0.08 ± 0.02	12.15 ± 0.32	5500.71 ± 158.19	10.60 ± 2.23	116.90 ± 2.79

Where, PUFA/SFA= Polyunsaturated fatty acids/saturated fatty acids; n6/n3 PUFA= Omega 6/omega 3; DFA are desirable fatty acids; AI= Atherogenic index; TI= Thrombogenic index; COX= Oxidability index; OS= Oxidative stability; h/H index = Hypocholesterolemic/hypercholesterolemic index; PI= Peroxidisability index.

presented in Fig. 2 and Table 2.

A balanced composition of PUFA to SFA in food is crucial for controlling serum cholesterol levels [30]. Food products having a PUFA/SFA ratio of less than 0.45 are generally not recommended for human consumption, due to their potential to induce an increase in serum cholesterol. Thus, PUFA/SFA higher than 0.45 is recommended in human diets to prevent the cardiovascular diseases and cancerous tumours [31]. In this study, the PUFA/SFA index of the *Linum* species ranged from 2.93 (*L. catharticum*) to 9.01 (*L. lewisii*). Though all the species observed to have PUFA/SFA more than 0.45, which indicates the appropriate balance of PUFA and SFA in these species (Fig. 2A), however, nutritionists have recently concentrated on the PUFA type and the balance between n-3 PUFA and n-6 PUFA in the diet. With diet having high PUFAs, a balanced and well proportionate n-6/n-3 PUFAs is necessary for its nutritional value. In plants n-6 and n-3 PUFAs comprises of LA and ALA, respectively. These n-6 and n-3 PUFAs are the main fatty acids regulating the hypocholesterolemic index. Dietary amounts of n-6/n-3 PUFAs below 4.0 are preferable for reducing the risk of cardiovascular diseases [31]. The results from present study indicates the n-6/n-3 PUFAs ranging from 0.19 to 24.21 (Fig. 2B). The majority of *Linum* species (11 species) shows a n-6/n-3 PUFA in the permissible limits except three species, *L. hudsonioides*, *L. tenuifolium* and *L. aristatum* having n-6/n-3 PUFAs ratio of 24.21, 22.55 and 6.95, respectively (Fig. 2B and Table 2). These values suggests that *L. hudsonioides*, *L. tenuifolium* and *L. aristatum* may not be suitable for human consumption and may increase the risk of cardiovascular disease upon intake, while all other species may be recommended for human consumption [32]. However, these species can be used as a potential donor germplasm for lowering ALA content. Moreover, these species are not feasibly crossable with the cultivated spp. Thus, they have potential roles as a donor germplasm in *Linum* improvement programme.

Similarly, this study also assessed total desirable fatty acids in *Linum* species. All the species shows DFA above 85 %, which makes these species suitable for the dietary purposes except the *L. hudsonioides*, *L. tenuifolium* and *L. aristatum* due to high n-6/n-3 PUFAs (Fig. 2C). The DFA in *Linum* species were significantly higher than the DFA reported in other food sources [4].

Polyunsaturated fatty acids (PUFAs) have a significant role in cardiovascular disease, and atherogenicity (AI) and thrombogenicity (TI) indices are frequently employed to estimate the prospective health advantages of consuming a particular food. The AI demonstrates the correlation between the sum of the major SFA and the major UFA classes, with the SFAs being proatherogenic that favours the formation of atherosclerotic plaque in arteries and the UFA being antiatherogenic that inhibits the formation of atherosclerotic plaques in arteries thereby preventing the appearance of coronary diseases. Thus, a low AI value is recommended for healthy diet. The TI shows tendency to form clots in the blood vessels. It compares pro-thrombogenic (SFA) and anti-thrombogenic (MUFA as well as n6 and n3 PUFAs). Therefore, a low TI value is also desirable. The TI and AI suggest the possibility of promoting platelet aggregation. It is advised to consume diets that have an AI and TI of less than 1.0 and 0.5, respectively, in terms of human health [4,16]. The AI and TI values for all the *Linum* species are observed under the advisable limits (Fig. 2D and E). These findings indicate that these species are favourable for human diet and health. The AI and TI indices of *Linum* species were close to the indices reported for polish goose varieties, walnut processed cheese, and edible oils [4,33].

Oxidability (COX) and oxidative stability (OS) indices indicate the oxidative stability of the fatty acid. While OS should be as high and COX readings should be as low as possible to show that fatty acids are less likely to oxidise. The COX value, which is based on the percentages of unsaturated fatty acids (UFAs) present in the oils, is a beneficial component typically considered as an assessment of the oil's propensity to undergo autoxidation. However, OS value, depicts the stability of oil against the oxidation. All the species have COX value less than 15, while *L. hudsonioides* and *L. tenuifolium* having the least COX value around 7, which is the best desirable COX values observed in *Linum* species, however their very high n-6/n-3 makes it nutritionally undesirable, however, their characteristics to avoid the autoxidation may be useful for *Linum* improvement program (Fig. 2F). Similarly, all the species have OS values more than 3000, ranging from 3114 in *L. hudsonioides* to 6340 in *L. lewisii* (Fig. 2G). Although, bearing a low COX value, *L. hudsonioides* and *L. tenuifolium* display a comparatively low oxidative stability inferring these species to be susceptible to oxidation through external factors. On the other hand, *L. lewisii* is found to be suitable for human nutrition and susceptible to oxidation and auto-oxidation due to low COX and highest OS among *Linum* species.

The hypocholesterolemic and hypercholesterolemic fatty acids index (h/H) evaluates the effect of hypocholesterolemic and hypercholesterolemic fatty acids on cholesterol. The proportion of hypocholesterolemic fatty acids (h) represent more than 80 % of total fatty acids in all *Linum* species. *L. hirsutum*, *L. lewisii* and *L. austriacum* contains about 90 % hypocholesterolemic fatty acids. The lower percentage of hypercholesterolemic fatty acids (H) is desirable to give higher h/H ratio. The desirable h/H ratio considered as beneficial for the human consumption is above 1 [34]. The h/H index found to be above 7 in all the *Linum* species, ranging from 7.11 in *L. catharticum* to 17.82 in *L. lewisii* (Fig. 2H). All the *Linum* species can be considered nutritionally rich for human consumption due to its significantly high h/H index in comparison to animal lipid sources [4,16]. However, all the species except *L. strictum* presents an h/H index higher than sesame, olive, primrose and soyabean oil [33]. While, *L. lewisii* showed h/H index of 17.82, better than the h/H for fatty acid profile of sunflower oil reported in previous studies [35]. Such high h/H index of all the wild *Linum* species, may be useful to avoid the increase in cholesterol level and not harm the cardiovascular health of consumer due to low hypercholesterolemic and high hypocholesterolemic fatty acids levels.

The peroxidisability index demonstrates the association between oil's fatty acid content and its oxidation stability. It is of paramount value when considering shelf-life and potential health risks associated with rancid oils [36]. According to the research, lipid peroxidation susceptibility increases with increasing PI values. However, approximately 80–90 is the minimum desirable value for PI [37]. Greater the PI value, greater is the protection against atherosclerosis and coronary artery conditions [4]. The PI values for *Linum* species ranged from 73.52 in *L. hudsonioides* to 132.92 in *L. lewisii* (Fig. 2I). The high PI of wild *Linum* species ensures the lower peroxidation, which in turn lowers or avoids the formation and deposition of lipoxidation by-products in coronary arteries and thus protects from atherosclerosis. The *Linum* species shows significantly high PI in comparison to soyabean oil, corn oil, palm oil and sesame oil, which infer that oil from the studies species may be safer and more suitable for human consumption. Fish oil reported to



have highest PI of 262 in lipids [37].

### 3.3. Correlation among fatty acids profiles in different *Linum* species

Correlation among the fatty acids of different *Linum* species is provided in Table 3. ALA and LA were statistically ( $p < 0.0001$ ) found to be highly negatively correlated ( $r = -0.88$ ). This inverse relation of ALA and LA was found to be consistent with the earlier reports in flax [38]. This inverse relationship suggests that LA and ALA synthesis is dependent on each other due to the involvement of endoplasmic fatty acid desaturase 3 (FAD3) genes coding for FAD3 enzyme, which catalyses the conversion of LA to ALA in plant cells by desaturation at C-15 position. The inverse relation of ALA and LA is also significantly convenient with the previous study reporting the same observations in the lines carrying FAD3A isoforms D and E and FAD3B isoforms B, C and F individually as well as in combinations (EF, DC and EB) [39]. Nevertheless, both ALA and LA present a positive correlation with total PUFA content with  $r = 0.27$  and  $0.23$ , respectively. This may be due to increase in a negative correlation between both the PUFAs present in *Linum* seeds, that cause significant increase in one or the other PUFA resulting in a positive correlation with total PUFA content. Also, a significantly inverse relationship of ALA was observed with both PA and SA with  $r = -0.39$  and  $-0.24$ , respectively, which collectively shows a negative correlation of ALA and SFAs with  $r = -0.35$ . PA is elongated into SA through elongation catalysed by Elongase 1 and 2 enzymes [40]. Further, SA is converted into LA and ALA through a series of desaturation reactions. This may be an inference for negative correlation of ALA and total PUFA with both SFAs. Similarly, LA and the major MUFA of linseed, OA are negatively correlated ( $r = -0.37$ ) and shares an inverse relationship. In fatty acid metabolism, OA act as precursor for synthesis of LA. This conversion is catalysed by the enzyme encoded by Fatty acid desaturase 2 enzyme that desaturated OA at C-12 position to synthesise LA [41]. Similar observations have also been reported earlier in flax through FAD2 gene silencing, which resulted in accumulation of OA in linseed rather than getting converted into LA [38]. Similarly, OA shows a significantly inverse correlation to total PUFA. Which may also be due to the ultimate conversion of OA into PUFAs via desaturation catalysed by FAD2 and FAD3 enzymes [41].

### 3.4. Hierarchical clustering of *Linum* species

A dendrogram constructed for hierarchical clustering using complete linkage on the basis of Euclidian distance is given in Fig. 3. The clustering was mainly observed on the basis of content of important fatty acids of *Linum*. The current study reports the hierarchical clustering of such large number of *Linum* species on the basis of fatty acid profile for the very first time. The 14 *Linum* species were clustered into two main clusters in the dendrogram. There was total 11 species in the clusters I and only 3 species in cluster-II. While, all the species have mean PA and SA content less than/around 10 %, the clustering was observed mainly on the basis of ALA, LA and OA content.

Cluster-II contains the species with low ALA ( $<10\%$ ) and high LA content ( $>60\%$ ). Cluster-II was further divided into two groups, group-A and group-B. Group-A of cluster-II contains species *L. aristatum* and *L. tenuifolium*, both these species have very similar fatty acid profile with ALA content of 9.32 and 3.09 %, LA content of 64.65 and 69.69 %, and OA of 15.00 and 8.33 %, respectively. However, in previous studies *L. aristatum* was reported to have 4.70 % ALA, 63.40 % LA, 21.60 % OA [42], while 2.60 % ALA, 79.90 % LA and 10.00 % OA was reported in *L. tenuifolium* in a previous study [25]. Group-B of cluster-II contains *L. hudsonioides* with ALA of 2.60 %, LA of 62.95 %, and OA of 21.48 %. However previous study also reports the similar fatty acid profile with 2.08 % ALA, 68.92 % LA, 16.79 % OA [26]. It is also observed to have fatty acid profile similar to sunflower oil (0.17–0.43 % ALA, 55.53–67.58 % LA, 20.91–25.54 % OA, 6.29–6.35 % PA and 3.44–3.92 % SA) reported in literature [43,44]. Thus, *L. hudsonioides* may be a useful donor species for improvement of cooking oil. Both, group-A and group-B of cluster-II contains species with low ALA, high LA, with group-B species having comparatively high OA from group-A species.

Cluster-I is divided into two groups, group-A and group-B. Group-B of Cluster I includes only three species, characterised with medium ALA (20–40 %) and medium LA (20–45 %). The subgroup-I includes species *L. catharticum* and *L. flavum*, having ALA of 28.42 and 22.78 % and LA of 25.96 and 37.93 %, and OA of 23.25 and 23.92 %, respectively. However, in a previous study 18.10 % ALA, 55.10 % LA and 18.30 % OA was reported in *L. flavum* [25], while 9.64 % ALA, 68.29 % LA and 9.70 % OA was reported in *L. catharticum* [26]. The species *L. strictum* is classified in subgroup-II of Group-B. It contains 27.28 % ALA, 41.22 % LA and low OA content of 13.38 %. In previous study, it is reported to have 39.70 % ALA, 41.20 % LA, 8.70 % OA [42].

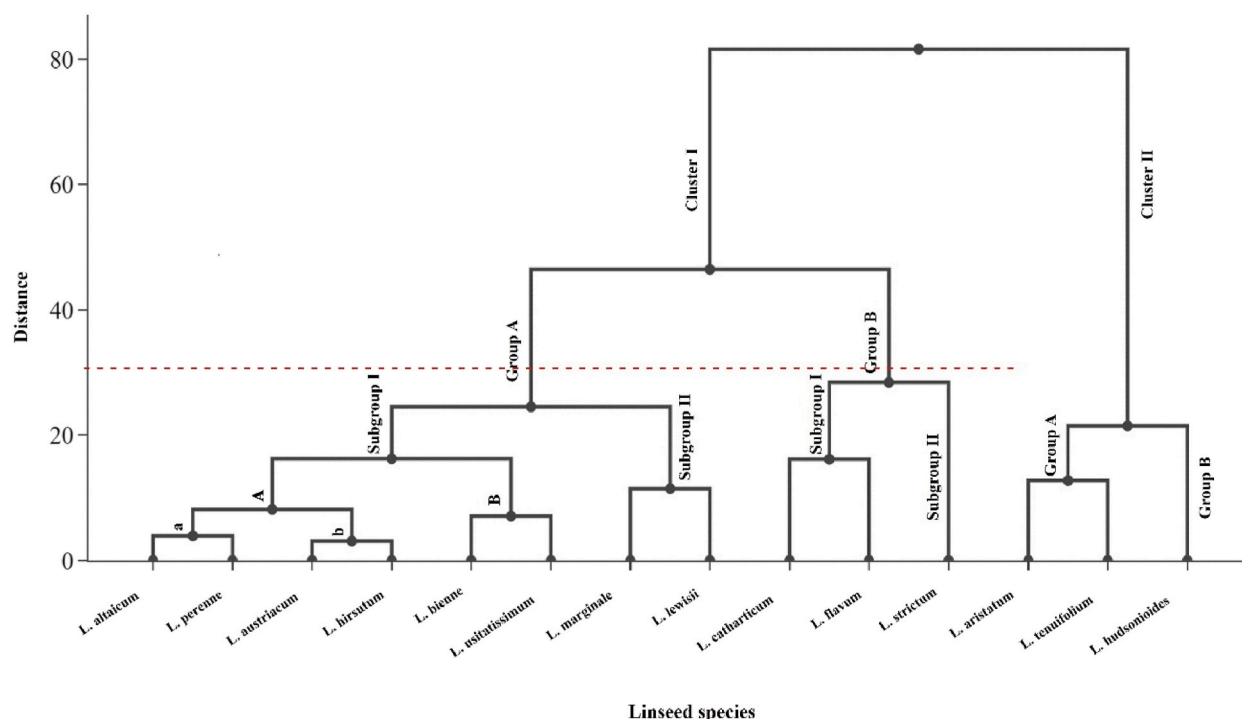
The group-A of cluster I includes 8 species and is further classified into subgroup-I and subgroup-II. subgroup-II of Group-A includes

**Table 3**

Pearson coefficients for the correlation between fatty acids in *Linum* species.

	PA	SA	OA	LA	ALA	SFA	MUFA	PUFA
PA	1.00	0.67****	0.34***	0.04 <sup>ns</sup>	-0.39****	0.92****	0.34***	-0.71****
SA	0.67****	1.00	0.26**	-0.09 <sup>ns</sup>	-0.24**	0.90****	0.26**	-0.66****
OA	0.34***	0.26**	1.00	-0.37****	-0.02 <sup>ns</sup>	0.33***	1.00****	-0.81****
LA	0.04 <sup>ns</sup>	-0.09 <sup>ns</sup>	-0.37****	1.00	-0.88****	-0.02 <sup>ns</sup>	-0.37****	0.23**
ALA	-0.39****	-0.24**	-0.02 <sup>ns</sup>	-0.88****	1.00	-0.35***	-0.02 <sup>ns</sup>	0.27**
SFA	0.92****	0.90****	0.33***	-0.02 <sup>ns</sup>	-0.35***	1.00	0.33***	-0.75****
MUFA	0.34***	0.26**	1.00****	-0.37****	-0.02 <sup>ns</sup>	0.33***	1.00	-0.81****
PUFA	-0.71****	-0.66****	-0.81****	0.23**	0.27**	-0.75****	-0.81****	1.00

Where, ns =  $p > 0.05$ , \* =  $p \leq 0.05$ , \*\* =  $p \leq 0.01$ , \*\*\* =  $p \leq 0.001$ , and \*\*\*\* =  $p \leq 0.000$ .



**Fig. 3.** Dendrogram of hierarchical clustering of 14 wild *Linum* species on the basis of their fatty acid composition. The central horizontal line shows Euclidean distance among the clusters and value of Euclidian distance was about 30 %.

two species viz. *L. lewisii* and *L. marginale* having very high mean ALA content of 57.58 and 55.31 %, low LA of 10.72 % and 17.57 %, and low OA content of 17.60 % and 18.91 %, respectively. However, in previous studies, *L. lewisii* was observed to have 51.20%–62.80 % ALA, 13.80%–21.20 % LA, 13.50%–22.10 % OA, 3.70%–5.30 % PA and 1.40%–4.40 % SA, while *L. marginale* is reported to have 53.98 % ALA, 11.45 % LA, 22.77 % OA, 8.13 % PA and 3.40 % SA. The results were in accordance with previous studies [22,25,26]. Subgroup-I includes six species, viz. *L. altaicum*, *L. perenne*, *L. austriacum*, *L. hirsutum*, *L. bienne* and *L. usitatissimum*, having high ALA content ranging from 43.00 % to 49.00 %, low LA content ranging from 10.00 % to 21.00 % and medium OA content ranging from 22.00 % to 25.00 %. Moreover, in previous study *L. altaicum* reported to have 52.90 % ALA with 23.40 % LA and 16.80 % OA. *L. perenne* reported to have 52.00 % ALA, 22.10%–24.30 % LA and 16.00%–20.70 % OA [23,25]. *L. austriacum* reported to have 51.40%–55.5 ALA, 18.87%–22.90 % LA and 16.30%–20.30 % OA [23–25]. *L. hirsutum* reported to contain 62.80–63.40 % ALA, 20.80–38.76 % LA and 6.36–12.30 % OA [23,25,45]. *L. bienne* reported to have 54.90 % ALA, 14.60 % LA and 18.20 % OA was reported [23]. *L. usitatissimum* reported to have 31–59 % ALA, 10.30–36.00 % LA and 15.00–27.00 % OA [5].

### 3.5. Principal component analysis

Principal component analysis (PCA) was performed on all *Linum* species and variables (analysed fatty acids) to investigate the structure and regularity in the relationships between *Linum* species and fatty acids [46] (Fig. 4 and Table S4).

The variance eigen value was greater than 1 for both the principal components. A variance of 80.27 % in the data was explained by first two principal components (PCs). The principal components 1 had an eigenvalue of 4.18 and contributed 52.27 % variance (Fig. 4C–D). PC 1 was composed of PA, SA, OA, SFA, MUFA and PUFA. The second principal component had an eigen value of 2.24 and accounted for 28 % variance. PC 2 was composed of LA and ALA (Fig. 4C–D). PC scores represents the similarities or variance between *Linum* species on the basis of their fatty acid profiles (Fig. 4A). Each dot depicts the fatty acid profile of a *Linum* germplasm. The same-coloured dots represent the same species, clustering together or situated far from each other on the basis of their similar or variable fatty acid profile. Correlation between the original variables and the direction and length of the vectors indicates to what extent the given variables affect the principal components (Fig. 4B). A highly negative correlation was observed between LA and ALA. Similarly, A highly negative correlation was also observed between total PUFA and MUFA, while SFA, PA and SA were positively correlated with each other. The observed correlation is similar to the one observed in section 3.3. The correlation may be due to the interplay of enzymes responsible for fatty acid metabolism. From the current study, we observed that two variables, ALA and total PUFAs are located near the centre of axis indicating that information contained in them is transferred to a higher extent by the principal components. However, in other variables, a small extent of information was transferred by principal components. Additionally, it can be observed that the germplasm of species *L. flavum* and *L. perenne* were the most diverse in terms of fatty acids. This can be well explained from Fig. 4A, the loading of germplasm from species *L. flavum* and *L. perenne* were lying far away from the loading of other germplasm

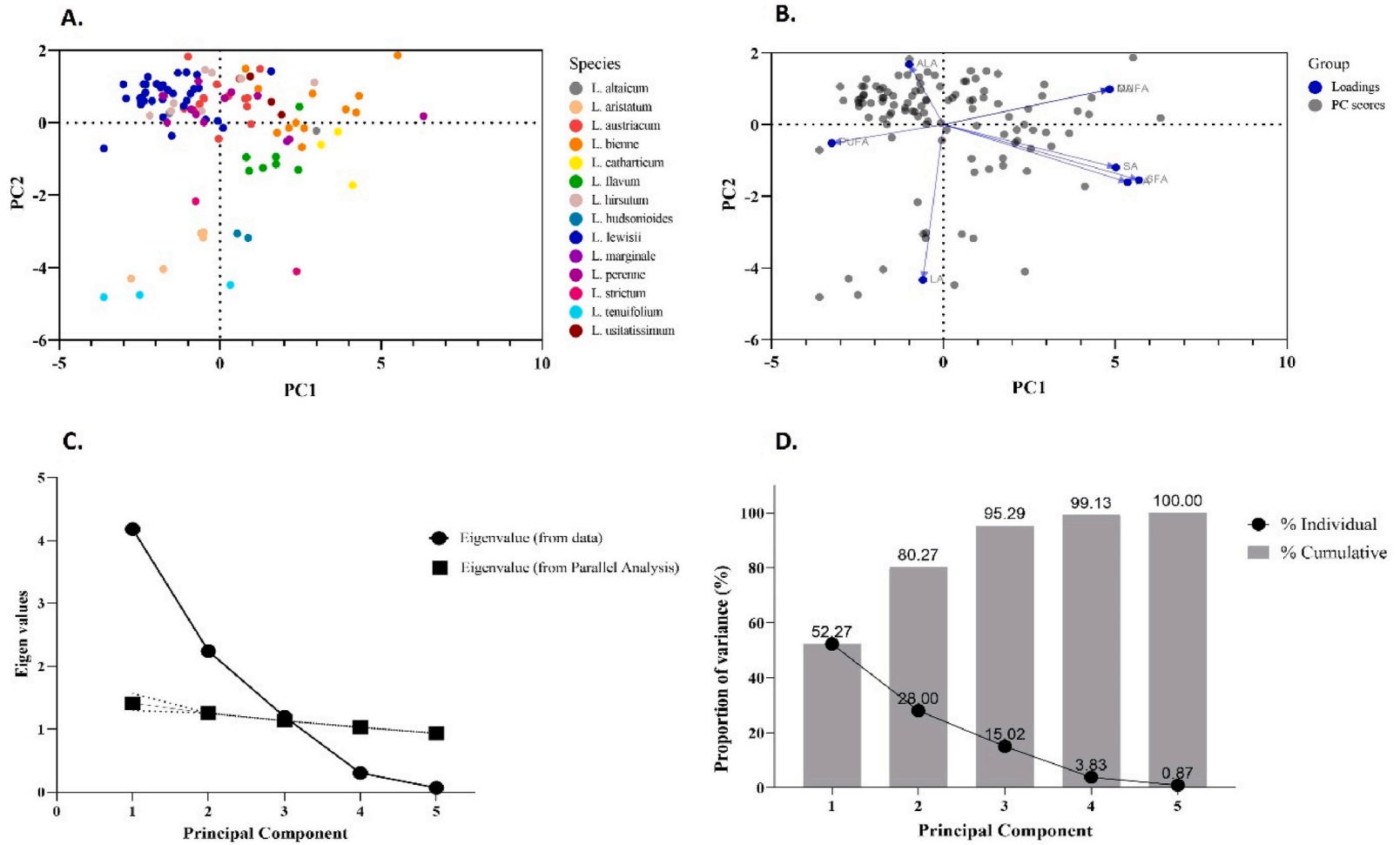


Fig. 4. Principal component analysis of fatty acid profiles of 14 wild *Linum* species, (A) PC scores, (B) Biplot of PC scores, (C) Eigen values and (D) Proportion of variance.

from these species.

#### 4. Conclusion

The results obtained from the current study revealed that germplasms of all the *Linum* species displayed a huge variability in their fatty acid profiles. *L. marginale* and *L. lewisii* recorded to have highest mean ALA content of 57.58 and 55.31 %, respectively. However, *L. tenuifolium* recorded the highest LA content (69.69 %), while, *L. catharticum* recorded highest oleic acid (27.03 %). *L. hudsonioides* is identified as a species with comparatively low mean ALA content (2.6 %) and a lipid profile similar to sunflower oil, which may be useful for culinary purpose. Thus, based on health indices all linseed species have polyunsaturated fatty acids to saturated fatty acids ratio value more than the desirable value of >0.45 and could be excellent sources of polyunsaturated fatty acids. However, *L. tenuifolium* and *L. hudsonioides* having very high value of n-6/n-3 ratio are ideal for human health as it is linked with inflammatory processes. Thus, these despite having good PUFA and SFA ratio pose risk of inflammation. *L. lewisii* clearly emerges as a promising species followed by *L. bienne* with great values across multiple indices, making them as a potential candidate for dietary or nutritional interests.

Based on health indices all linseed species except *L. tenuifolium* and *L. hudsonioides* have healthy fatty acid composition. *L. lewisii* clearly emerges as a promising species followed by *L. bienne* with great values across multiple indices, making them as a potential candidate for dietary or nutritional interests.

In summary, this study underscores the potential health benefits associated with diverse *Linum* species, particularly *L. lewisii*. Future research should delve into the feasibility of harnessing these species for broader human consumption, factoring in the ecological and agricultural implications of such endeavours.

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#### CRediT authorship contribution statement

**Navdeep Singh Plaha:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Nutan Kaushik:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Sumegha Awasthi:** Data curation, Investigation, Methodology. **Mamta Singh:** Methodology, Resources. **Vikender Kaur:** Resources, Writing – review & editing. **Sapna Langyan:** Writing – review & editing. **Ashok Kumar:** Writing – review & editing. **Sanjay Kalia:** Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2023.e21192>.

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