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Fatty Acid Profile, Tocopherol Content of Seed Oil, and Nutritional Analysis of Seed Cake of Wood Apple (*Limonia acidissima* L.), an Underutilized Fruit-Yielding Tree Species

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Abstract: The present study was aimed at analyzing the fatty acid composition, tocopherols, and physico-chemical characterization of wood apple (*Limonia acidissima* L.) seed oil and the nutritional profile of seed cake. The fatty acids in seed oil were analyzed by gas chromatography–mass spectrometry (GC-MS), and the total seed oil was 32.02 ± 0.08%, comprising oleic (21.56 ± 0.57%), alpha-linolenic (16.28 ± 0.29%), and linoleic acid (10.02 ± 0.43%), whereas saturated fatty acid content was 33.38 ± 0.60% including palmitic (17.68 ± 0.65%) and stearic acid (14.15 ± 0.27%). A greater amount of unsaturated fatty acids (52.37%) were noticed compared to saturated fatty acids (33.38%); hence the seed is highly suitable for nutritional and industrial applications. Gamma-tocopherol was present in a higher quantity (39.27 ± 0.07 mg/100 g) as compared to alpha (12.64 ± 0.01 mg/100 g) and delta (3.77 ± 0.00 mg/100 g) tocopherols, which are considered as natural antioxidants. The spectrophotometric technique was used for quantitative analysis of total phenolic content, and it revealed 135.42 ± 1.47 mg gallic acid equivalent /100 g DW in seed cake. All the results of the studied seed oil and cake showed a good source of natural functional ingredients for several health benefits.

Keywords: wood apple; fatty acid profile; tocopherol; nutritional; phenolics; GC-MS; HPLC

1. Introduction

Plant seeds are an important source of oils and fats to meet nutritional, industrial, and pharmaceutical needs [1]. Oils and fats are composed of neutral lipids, majorly triglycerides, which are the sources of nutraceutical compounds that are an essential part of the human diet and major constituents for the storage of energy, structural and functional composition of cells [2]. Seed oil is used for the preparation of soap and detergents, cosmetics, and also used as ingredients for paint and varnishes, lubricants, and organic pesticides [3]. Oils rich in polyunsaturated fats have been related to the prevention of coronary heart diseases, diabetes, cancer, and depression, whereas cholesterol and saturated fats cause chronic diseases [4]. Many seed oils are reported to contain tocopherols, and they are considered as effective fat-soluble antioxidants present in the oil, which helps to protect cell membranes, improvement in blood circulation, and treating various diseases [5]. Most seeds and vegetable oils, such as groundnut, sunflower, soybean, and peanut, are the most important oil sources for cooking, canning, and preparations of emulsions and margarine [4].

The increasing population of the world is creating a shortage of food sources which permits the interest toward underutilized fruits. Seed oil and cake is the major portion of the human diet due to abundant nutrients, protection from oxidative stress, and several diseases. Seeds have beengiven special attention throughout the world, particularly underutilized fruits. As a biodiversity country, India has been a habitat for thousands of wild underutilized fruit seeds, which could be exploited directly as foods or used to obtain valuable natural compounds and derivatives [6].

Wood apple (*Limonia acidissima* L.) is an underutilized fruit-yielding tree species native to India and Sri Lanka (Figure 1A). Wood apple (Figure 1B) is used by the tribal and rural population of the developing world, which contributes to the traditional health system [6]. Extracts of wood apple are used traditionally for curing various diseases, such as antimicrobial, antifungal, liver, and cardiac tonic, dysentery, hiccough, sore throat, and is a good antidote for snakebite and used as a face cream to remove small spots and lesions on the skin. The phytochemical analysis of *L. acidissima* plant parts showed the presence of alkaloids, flavonoids, phenols, terpenoids, tannins, fats, sterols, saponins, glycosides, gum, mucilage, and fixed oils [7,8]. The small, numerous, and white seeds scattered throughout fruits(Figure 1C) showed an abundance in protein, carbohydrate, amino acid content and high amount of iron (Fe), zinc (Zn), sodium (Na), potassium (K), phosphorus (P), copper (Cu), magnesium (Mg) and manganese (Mn) [9]. However, no detailed reports are available on wood apple seed oil (Figure 1D). Therefore, the present study was aimed atanalyzing the fatty acid composition by GC-MS, tocopherols by HPLC/FD, and physico-chemical properties of seed oil, and nutritional analysis of wood apple seed cake.

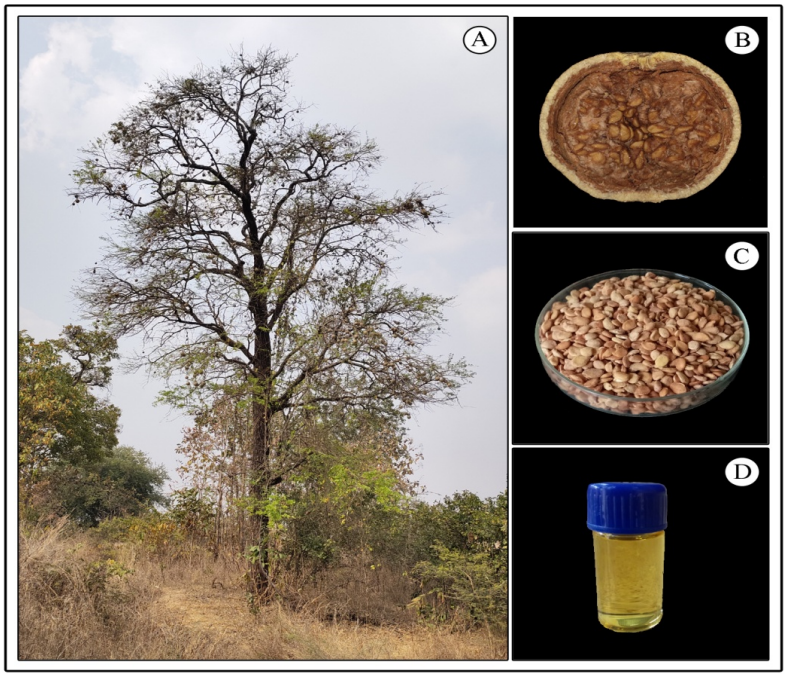

Figure 1. Wood apple (*Limonia acidissima* L.)-Tree (**A**), Fruit (**B**), Seeds (**C**), and Oil (**D**).

2. Materials and Methods

2.1. Samples

Ripened fruits of *Limonia acidissima* L. were collected from Itagi, Koppal District, Karnataka, India, during February 2019. The collected fruits were washed under running tap water to remove the pulp content. Seeds were dried in a hot air oven at 48 ± 2 °C to remove moisture and stored at −20 °C until future analysis.

2.2. Standards and Chemicals

n-Hexane, methanol, potassium hydroxide (KOH), hydrochloric acid, 14% methanolic boron trifluoride (w/v), helium, 5% ethanolic pyrogallol (w/v), diethyl ether, ethanol, milli-Q-water, acetone, phenolphthalein, chloroform, Hanus iodine solution, potassium iodide, sodium thiosulfate, sodium hydroxide, phosphate-buffered saline, bovine serum albumin, copper sulfate, Folin-Ciocalteu's reagent, anthrone reagent, and sodium carbonate were of analytical grade from Merck, Bangalore (India). Glucose, gallic acid, α, γ, and δ-tocopherols were purchased from Sigma–Aldrich, Bangalore, India.

2.3. Soxhlet Extraction of Wood Apple Seeds

Moisture-free wood apple seeds were pulverized into a fine powder in a mixer grinder and extracted with n-hexane using soxhlet apparatus for 8 h. The solvent was evaporated by using rotary evaporation, and the oil was collected in a glass vial. Concentrated oil was dried under a nitrogen air atmosphere. The extracted wood apple seed oil (WASO) was used for fatty acid composition, tocopherol, and physico-chemical characterizations, and defatted wood apple seed cake (DWASC) was used for nutritional analysis.

2.4. Investigation of Physico-Chemical Properties of WASO

Physico-chemical properties of WASO were achieved by different standard methods described by [10,11]. The viscosity of the oil was measured using a Brookfield viscometer (Model DV-III, Stoughton, MA, USA). Viscosity was reported in centipoise (cP) at a temperature of 28 ± 2 °C. The Refractive index was determined at 28 ± 2 °C using a hand refractometer (Atago Co. RX-500, Tokyo, Japan). The specific gravity was estimated using a 10 mL Pycnometer (Borosil, India) at 28 ± 2 °C temperature. The content of wax was determined gravimetrically using the standard method described [12]. Briefly, 25 mL of n-hexanewas added toseed oil (1 g) and vortexed. The acetone and n-hexane (3:1) were added to the mixture and refrigerated for 24 h at −20 °C. The liquid phase was separated and re-dissolved in n-hexane to removethe residue of the undissolved matter, and the wax content was expressed as mg/g oil.

The acid value (AV), iodine value (IV), free fatty acid value (FFA), saponification value (SV), and unsaponifiable matter (USM) of WASO were assayed by the method described by [11]. For AV determination, 0.1 g of oil was neutralized by ethyl alcohol. The mixture was incubated in a water bath until the oil dissolved completely. Using phenolphthalein, the mixture was titrated against 0.1 N potassium hydroxide. IV results were expressed as g I_2/100 g. Briefly, 0.1 g of oil was dissolved in 10 mL of chloroform, and 30 mL of Hanus iodine solution was added. Flasks were incubated in the dark for 30 min with occasional shaking. One milliliter of 15% potassium iodide was added to the incubated mixture and titrated against 0.1 N sodium thiosulfate. Once the yellow color existed, few drops of starch were added, and the titration was continued until the yellow color disappeared. SV was evaluated by adding 5% alcoholic potassium hydroxide to 0.1 g of oil and incubated in a water bath for 1 h to saponify. The solution was titrated against 0.1 N hydrochloric acid using a phenolphthalein indicator and expressed as mg KOH/g. The USM was assayed and expressed in percentage, where 15 mL of ethanol and 5 mL of 60% aqueous potassium hydroxide was added to 1 g of oil. The mixture was refluxed until the oil was saponified. The saponified matter was extracted with diethyl ether, andthe supernatant was collected. After evaporating to dryness, the residue was desiccated and weighed. The percentage of free fatty acid was measured by dissolving 1 g of oil in ethanol and diethyl ether (1:1). It was then mixed thoroughly and neutralized with 0.1 N potassium hydroxide, and the mixture was titrated against 0.1 N sodium hydroxide using a phenolphthalein indicator.

2.5. Fatty Acids Profiling

2.5.1. Preparation of Fatty Acid Methyl Esters (FAMEs)

FAME was prepared according to standard methods [13]. WASO (10 mg) wassaponified with 1 mL methanolic potassium hydroxide (0.5 M, w/v) at 60 °C for 1 h in a boiling

water bath. The reaction was stopped by adding 1 mL of methanolic hydrochloric acid (0.5 N, v/v) and 2 mL of hexane. The hexane pool was collected (3 times) and evaporated to dryness under a nitrogen air atmosphere. Fatty acids were methylated with 0.7 mL of methanolic boron trifluoride (14%, w/v) and 0.3 mL of benzene. The extracted FAMEs were washed with hexane and water. The hexane pool was evaporated and re-dissolved in MS grade n-hexane.

2.5.2. GC-MS Characterization of Fatty Acids

Fatty acids analysis was achieved with Agilent 7890 B GC 5977 A MSD GC-MS (Agilent Technologies, Santa Clara, CA, USA). An Agilent DB-23 column (50%- Cyanopropyl-methylpolysiloxane; 60 m length, 0.25 µm, i.d. 250 µm) was used. Helium was used as a carrier gas with a flow rate of 1 mL/min. The injection volume was 1 µL, and the spilt ratio was 20:1. The GC oven program was initially set at 60 °C andheld for 1 min, and then increased at 20 °C/min to 130 °C, and 7.5 °C/min to 170 °C. Finally, the temperature reached 200 °C and was heldfor 3 min. MS data were collected in a 70 eV scanning electron ionization mode from m/z 40 to 500. Fatty acid methyl esters were identified with their mass spectrum data, and results were confirmed by a mass spectral library search (NIST 2.0 g).

2.6. Estimation of Tocopherols

2.6.1. Sample Preparation

The separation and quantification of tocopherols were carried out using High-performance liquid chromatography [14]. WASO (1 g) was saponified with 1 mL of potassium hydroxide (100%, w/v), 4 mL of ethanolic Pyrogallol (5%, w/v), and incubated in a water bath for 3 min at 80 °C. The reaction was neutralized by sudden cooling, and 30 mL of distilled water was added. Diethyl ether fractions were separated and collected in another tube and repeated the same thrice. The pooled extract was washed and evaporated to dryness under a vacuum at 40 °C. The residue was dissolved in 1 mL of ethanol and 4 mL of benzene and evaporated to dryness under nitrogen air. The dried extract was dissolved with 1 mL of n-hexane and used for the characterization of tocopherols.

2.6.2. Tocopherol Estimation

The quantification of tocopherols was carried out by using the Shimadzu LC 8A-HPLC system, SCL-10Avp system controller coupled with a RF-20A fluorescence detector. Separation was achieved with a reverse-phase Kinetex C18 column (250 × 4.6 mm, 5 µm), and the injection volume was 5 µL. The excitation wavelength used was 290 nm, whereas the emission wavelength was 330 nm. The mobile phase consisted of methanol and water (95:5, v/v) at a flow rate of 1mL per minute in an isocratic mode of elution [15]. Tocopherols were identified and quantified on retention time bases of known standards.

2.7. Nutritional Characterization of Defatted Seed Cake

2.7.1. Nutritional Analysis

The proximate analysis, including total soluble solids, titratable acidity, and pH was analyzed using the [16] methods. Briefly, the Defatted seed cake was homogenized with 10 mL of distilled water at room temperature, and the mixture was used to quantify. Titratable acidity was expressed as a percentage of citric acid. The pH was measured by directly immersing the electrode into the homogenized cake using a pH meter (CD Instrumental Pvt. Ltd., Bangalore, India). Total soluble solids were measured directly using a refractometer (Erma handheld refractometer, Japan) and expressed in °Brix.

Total moisture and ash content were assayed according to the methods described in the [17]. The moisture content was evaluated by drying 100 g of whole seeds at 102 °C in an air circulating oven (Serwell Instrument Incorporation, Bangalore, India) until it reached constant weight. The defatted seed cake (1 g) was taken into a preheated and weighed

crucible, and it was kept in a muffle furnace at 555 °C for 6 h to calculate the total ash content. The crucible was cooled in a desiccator, and the final weight was noted.

Crude fat, protein, and carbohydrate contents were quantified bythe method reported by [18]. Defatted seed cake (1 g) was ground with 10 mL of phosphate-buffered saline and used for protein estimation. Aliquots of each sample were placedinto test tubes, and 4.5 mL of alkaline copper sulfate reagent was added. Tubes were mixed well and incubated at room temperature for 10 min. Then 0.5 mL of 2N Folin-Ciocalteau's reagent was added and incubated again for 30 min, and the optical density was measured at 660 nm. Bovine serum albumin was used as a standard, and the amount of the total protein content was calculated by regression analysis. Total carbohydrate content was achieved using the anthrone reagent method. Defatted seed cake (1 g) was hydrolyzed in a boiling water bath for 3 h with 5 mL of 2.5 N HCl and cooled at room temperature. It was then neutralized with sodium carbonate until effervesces ceased. The supernatant was centrifuged and collected for analysis. Aliquots of each were taken, and 4 mL of Anthrone reagent was added to each tube. Tubes were mixed well and incubated in aboiling water bath for 10 min, cooled rapidly, and the optical density was read at 630 nm. Glucose was used as a standard, and the amount of total carbohydrate content was calculated by regression analysis.

2.7.2. Extraction and Quantification of Total Phenolic Content

Total phenolic content was carried out as per apreviously described method [19]. Defatted WASC powder (2 g) was extracted with 20 mL of aqueous methanol (80%, v/v) and filtered the extract. Three milliliters of milli-Q-water and 0.5 mL of 2N Folin-Ciocalteu reagent were added toan aliquot of filtrate (0.5 mL) and mixed thoroughly. Then, 2mL of 20% (w/v) sodium carbonate was added, and the tubes were incubated for 30 min in the dark at room temperature. Absorbance was recorded at 760 nm against the blank using a UV-VIS Spectrophotometer. Results were expressed as mg gallic acid equivalent/100 g dry weight.

2.8. Statistical Analysis

All the determined values were estimated in triplicate using three lots; each contained 100 g of seeds (n = 3). Results are presented as mean ± standard deviation (SD). Statistical analysis was carried out by using SPSS software.

3. Results and Discussion

3.1. Soxhlet Extraction of Wood Apple Seed Oil

Oilseed crops that yield more than 15% (*w/w*) on a dry weight basis will be considered as a predominant source for edible oil production and/or industrial applications [20]. The total content of oil in wood apple seeds was 32.02 ± 0.08 g/100 g dry weight (Table 1). The oil content was relatively equal to commercially available oil sources, for example, palm (*Elaeis guineensis*) 37.19%, sunflower (*Helianthus annuus*) 32%, and kokum (*Garcinia indica*) 16.80% [12,21].

Table 1. Physico-chemical properties of wood apple (*Limonia acidissima* L.) seed oil *.

Sl. No.	Parameters (at 30 °C)	Composition
1	Color	Golden yellow
2	Physical state at 4 °C	Liquid
3	Viscosity (cP)	42.33 ± 0.57
4	Wax content (mg/g)	0.13 ± 0.00
5	Refractive index	1.44 ± 0.01
6	Specific gravity	0.92 ± 0.00
7	Free fatty acid (%)	1.38 ± 0.02
8	Acid value (mg KOH/g)	2.12 ± 0.01
9	Iodine value (g I_2/100 g)	116.16 ± 0.28
10	Saponification value (mg KOH/g)	186.50 ± 0.86
12	Unsaponifiable matter (%)	1.15 ± 0.01

* Mean ± standard deviation of triplicate determinations.

3.2. Investigation of Physico-Chemical Properties of WASO

Physico-chemical characterization of WASO is depicted in Table 1. Physical and chemical properties of oil indicated the overall acceptance with respect to oil purity, quality, stability, flavor, degree of unsaturation, and natural antioxidants. The quality of the oil was decided based on the physico-chemical properties, including viscosity, iodine value, saponification value, specific gravity, and peroxide value of edible oil [22]. WASO showed interesting results as compared to the other accepted edible oils on the market. The color of the oil was clear golden yellow. This indicated that oil didnot consist of chlorophyll and carotenoid pigments, and the physical state at 4 °C was semi-liquid. Viscosity was 42.33 ± 0.57 cP, which is in line with sunflower (48.20 cP), soybean (48.70 cP), and cotton (53.50 cP). This indicated that the degree of saturated fatty acids was less compared to unsaturated fatty acids, and the quality of the oil is suitable for cooking and industrial purpose [23]. WASO showed a lower content of wax (0.13 ± 0.00%) which agreed well with reported sunflower oil (0.12%). A higher percentage of waxes in oil tended to crystallize, and the oil becameturbid when cooled at room temperature [24]. WASO exhibited a refractive index of 1.44 ± 0.01, and values were comparably similar to soybean and sunflower, which are1.46 and 1.46,respectively [25] and more than palm oil, 1.40 [26]. The specific gravity was 0.92 ± 0.00, comparatively similar to palm (0.99) and soybean (0.95) [25,26]. Free fatty acid content was 1.38 ± 0.02% as oleic acid, whereas the other edible oils contain 0.81% in soybean, 1.36% in peanut [27]. Any oil consisting of less than 5% of free fatty acid content will be considered as edible oil, whereas high FFA content in oils, directly permitting oxidation, will become rancid in a short period of time [28]. The acid value was 2.12 ± 0.01 mg KOH/g, and the result obtained was less than the maximum acceptance level (4 mg KOH/g) [29]. Low AV represents the stability of oil at room temperature, whereas high acid value in oil leads to unpleasant flavor and odor generation. IV represents the total unsaturated fatty acids. The presence of double/triple bonds in fatty acids was the cause for high IV in the oil samples. Seed oil tested for IV showed 116.16 ± 0.28 g I_2/100 g, whereas palm oil has low IV (50.0 g I_2/100 g) and soybean have ahigh IV (123.42 g I_2/100 g) [26]. This represents that the percentage of unsaturated fatty acid content was more in WASO. SV in WASO showed 186.50 ± 0.86 mg KOH/g, which is approximately similar to soybean, groundnut, and palm oil, which are 188, 198, and 214.17 mg KOH/g, respectively [25,26]. More SV is useful in industrial applications, such as the preparation of soap, shampoo, and paints [26]. When the SV of oil is less than 190 mg KOH/g, it is an indication of the presence of high molecular weight of triglycerides, such as linoleic and linolenic acids [30]. The unsaponifiable matter was 1.15 ± 0.01%, and the other reported oils showed 1.23% in olive oil and 0.81% in sunflower [27]. The presence of lignans, crude fiber, proteins, and mineral elements are causes for the high unsaponifiable matter [31].

3.3. Fatty Acid Profiling

The fatty acid profile of WASO ispresented in Table 2 andshown in Figure 2. This showed the five major fatty acids, namely palmitic acid, stearic acid, oleic acid, linoleic, and linolenic acids. The monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA) content was almost equal, 25.51 ± 0.67% and 26.86 ± 0.13%, respectively. At the same time, saturated fatty acid (SFA) wasrelatively higher (33.38 ± 0.60%) among the total fatty acid content. The saturated fatty acid content was approximately equal to the oil-yielding tree species, for example, African mangosteen (*Garcinia livingstonei*) 38.23%, palm oil 49.9%, and less than kokum 61.20% [12,32,33]. Palm oil shares 38% of marketing in India because it constitutes a higherpercentage of saturated fatty acids (50.70%) [34]. Palmitic acid (17.68 ± 0.65%) was the highest among the saturated fatty acid content; subsequently, stearic acid (14.15 ± 0.27%), behenic acid (1.24 ± 0.10%), and margaric acid (0.30 ± 0.04%). Oils rich in saturated fatty acids had high oxidative stability and are suitable for frying and baking [35]. These fatty acids areconsidered a major component of cell membranes, secretary and transport lipids, with crucial roles in protein palmitoylation

and palmitoylated signal molecules [36]. Hence, WASO can be used as a substitute for palm oil because it has comparably more or less saturated fatty acids.

Table 2. Fatty acid profiling of wood apple (*Limonia acidissima* L.) seed oil (Mean ± standard deviation, *n* = 3).

Peak	t_R (min)	Identified Compounds		Common Name	Rel. Percentage (%)
1	14.66	Hexadecanoic acid, methyl ester	C16:0	Palmitic acid	17.68 ± 0.65
2	15.61	9-Hexadecenoic acid, methyl ester, (Z)-	C16:1	Palmitoleic acid	0.38 ± 0.14
3	16.76	Heptadecanoic acid, methyl ester	C17:0	Margaric acid	0.30 ± 0.04
4	17.56	Methyl stearate	C18:0	Stearic acid	14.15 ± 0.27
5	18.00	9-Octadecenoic acid, methyl ester, (E)-	C18:1n9t	Elaidic acid	1.36 ± 0.04
6	18.23	9-Octadecenoic acid (Z), methyl ester	C18:1n9c	Oleic acid	21.56 ± 0.57
7	18.33	11-Octadecenoic acid, methyl ester	C18:1n7	Vaccenic acid	1.70 ± 0.04
8	19.33	9,12-Octadecadienoic acid, methyl ester (Z,Z)-	C18:2n6	Linoleic acid (Omega-6)	10.02 ± 0.43
9	20.05	6,9,12- Octadecatrienoic acid, methyl ester	C18:3n6	γ- Linolenic acid	0.23 ± 0.04
10	20.72	9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z)-	C18:3n3	α- Linolenic acid (Omega-3)	16.28 ± 0.29
11	21.34	Cholesta-3,5-diene		Cholesterilene	0.50 ± 0.06
12	21.53	cis-13-Eicosenoic acid, methyl ester	C20:1n7	Paullinic acid	0.50 ± 0.02
13	22.61	Octadecanoic acid, 10-hydroxy-, methyl ester		Rosilic acid	1.63 ± 0.30
14	22.68	Phosphine oxide, methyldiphenyl-			2.10 ± 0.09
15	23.00	Octadecanoic acid,9,10,12-trimethoxy-, methyl ester			1.37 ± 0.05
16	23.38	Ethyl iso-allocholate			0.37 ± 0.05
17	23.50	7-Methyl-Z-tetradecen-1-ol aceatate			0.85 ± 0.07
18	23.69	Cholesta-5,7,9(11)-trien-3-ol acetate			0.63 ± 0.05
19	23.71	1-Heptatriacotanol			0.50 ± 0.05
20	24.82	Docosanoic acid, methyl ester	C22:0	Behenic acid	1.24 ± 0.10
21	26.05	7,10,13- Eicosatrienoic acid, methyl ester	C22:3	Eicosatrienoic acid	0.32 ± 0.06
22	27.81	Cholesteryl benzoate			1.41 ± 0.19
23	29.04	Z-5-Methyl-6-heneicosen-11-one			1.17 ± 0.14
24	29.19	Cholesteryl formate			0.57 ± 0.05
25	29.21	Phorbol			0.64 ± 0.06
26	29.30	Cholest-5-en-3-ol (3.beta.)-, 9-octadecenoate, (Z)-			2.30 ± 0.18
∑ Saturated fatty acids					33.38 ± 0.60
∑ Monounsaturated fatty acids					25.51 ± 0.67
∑ Polyunsaturated fatty acids					26.86 ± 0.13

Monounsaturated fatty acids, especially oleic acid, is well known for human health benefits and also important for lowering the effectiveness of low-density lipoprotein (LDL) cholesterol level which reduces the risk for coronary heart diseases. Further, it is documented as an insulin resistance contrary promoter to the polyunsaturated fatty acids with the protection against insulin resistance [37]. The oleic acid (21.56 ± 0.57%) is the dominant fatty acid, and this could be comparably more or less than in sunflower, soybean, and orange at 28.0%, 21.3%, and 26.1%, respectively [38,39]. Next to this, vaccenic acid (1.70 ± 0.04%), elaidic acid (1.36 ± 0.4%), palmitoleic, and paullinic acids were present in traces. Oil-rich in monounsaturated fatty acids makes the oil advisable in terms of nutrition and provides enough stability to be used forbaking purposes [40]. Hence, the higher oleic acid content in oil, which has high oxidative stability, can be used as a healthy alternative to partially hydrogenated vegetable oils.

Polyunsaturated fatty acid compositions of the obtained WASO werecomparatively equal to saturated and monounsaturated fatty acids. Linoleic and alpha-linolenic acids are long-chain polyunsaturated fatty acids, which are considered to be essential fatty acids because they cannot be synthesized endogenously [41]. α-Linolenic acid (16.28 ± 0.29%) is a major omega-3 fatty acid, whereas soybean (9.4%), rapeseed (8.6%), and flaxseed (52.46%)

are close to the total content of alpha-linolenic acid [38,42]. Next to this, a loweramount of gamma-linolenic acid was detected. These fatty acids are well known for preventing heart and blood vessels related disease treatments and for selective antitumor properties with negligible systemic toxicity [43]. Linoleic acid (10.02 ± 0.43%) was the next highest among the total polyunsaturated fatty acids, whereas flaxseed, olive, and palm oil are 16.16%, 8.50%, and 9.3%, respectively [27,43]. WASO wasa good source for essential fatty acids, whereas high content of omega-3 and omega-6 fatty acids in the diet increases HDL-cholesterol and decreases LDL-cholesterol. In contrast, a higher rate of oleic acid decreases LDL-cholesterol in the diet and does not affect HDL-cholesterol [44].

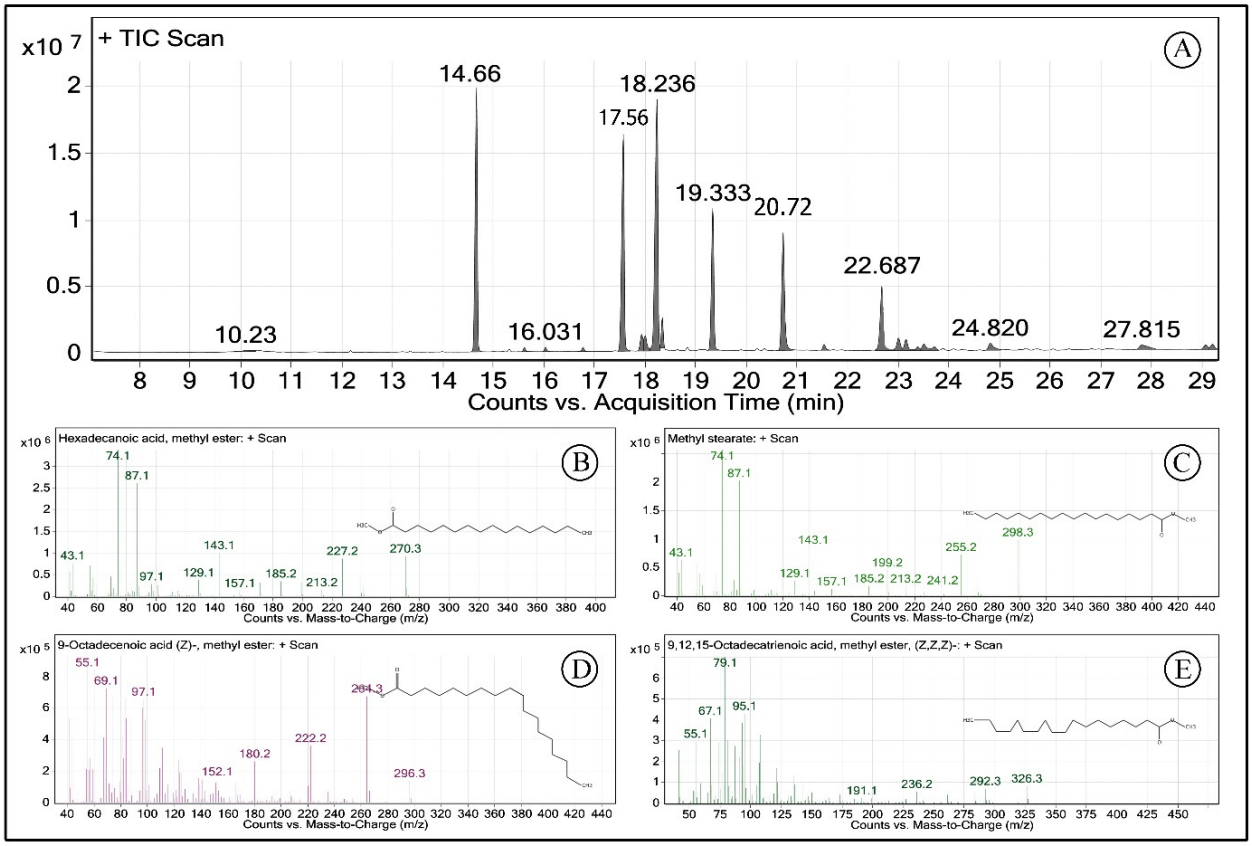

Figure 2. GC-MS chromatograms of wood apple (*Limonia acidissima* L.) seed oil fatty acid methyl esters (FAMEs) (**A**) and MS spectra of major FAMEs of analyzed fatty acids. Methyl palmitate (**B**), methyl stearate (**C**), methyl oleate (**D**), methyl linolenate (**E**).

3.4. Estimation of Tocopherols

Tocopherols are nothing but vitamin E, a fat-soluble vitamin and essential for human health. Tocopherols have beengiven much interest because of their overall health impact and arrest of oxidation at cellular levels [45]. Tocopherols are important natural antioxidants [46]. The total tocopherol content in WASO was (55.68 ± 0.08 mg/100 g) which showed as a comparatively good source to obtain vitamin E. Gamma-tocopherol content was present in greaterquantity, whereas the content of alpha-tocopherol and delta-tocopherol was 12.64 ± 0.01 mg/100 g and 3.77 ± 0.00 mg/100 g, respectively (Table 3, Figure 3). High gamma-tocopherol content in the oil indicates the abundance of α-linolenic acid [47]. The result obtained in WASO was approximately more or less in other plant sources, such assunflower and flaxseed oil, which are 57.97, 42.24 mg/100 g, respectively [48].

Table 3. Tocopherol composition (mg/100 g) in wood apple seed oil *.

Sl. No.	t_R	Tocopherols	Composition
1	9.54	δ-Tocopherol	12.64 ± 0.01
2	10.92	γ-Tocopherol	39.27 ± 0.07
3	12.25	α-Tocopherol	3.77 ± 0.00
	Total tocopherol content		55.68 ± 0.08

* Mean ± standard deviation of triplicates.

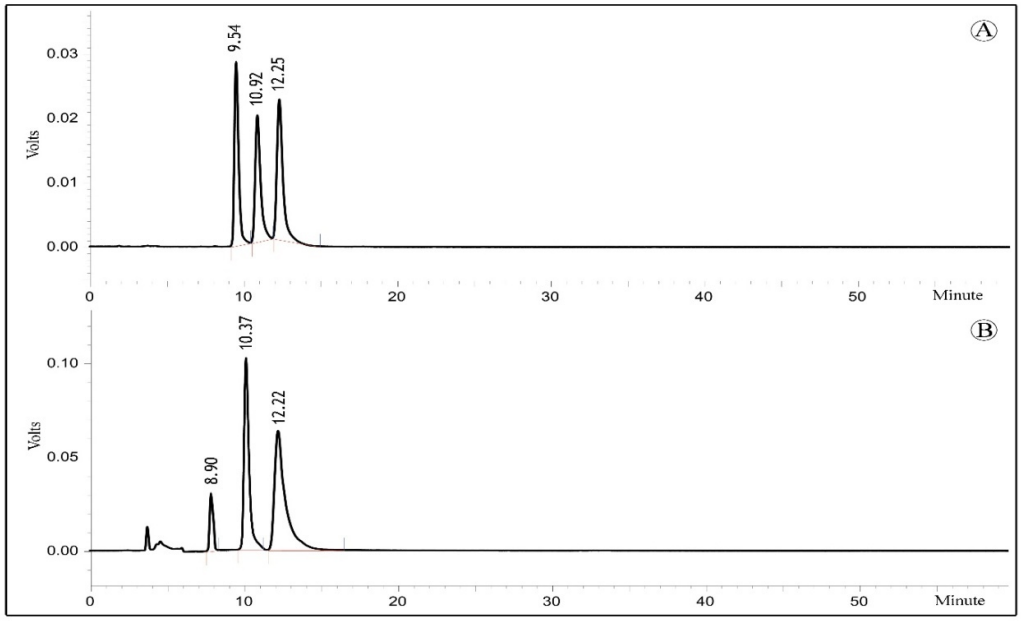

Figure 3. HPLC/FD chromatograms of tocopherol standards (**A**) and wood apple (*Limonia acidissima* L.) seed oil (**B**). Standard peak at RT-9.54 is δ-Tocopherol, peak RT-10.92 is γ-Tocopherol, and peak RT-12.25 is α-Tocopherol.

3.5. Nutritional Characterization of Defatted Seed Cake

The nutritional composition of defatted seed cake is presented in Table 4. Total moisture, fat, ash, protein, and carbohydrate contents agreed with the earlier report [49], whereas the total soluble solids, pH, acidity, and total carbohydrate content were reported for the first time in this present study. Total soluble solids, pH, and acidity reported in WASC were 1.52 ± 0.29°Brix, 1.76 ± 0.19, and 1.83 ± 0.02%, respectively. The composition of total carbohydrate content was (31.66 ± 0.04%), quite high when compared to groundnut (25.41%) and palm (23.1%) seeds [50]. Moisture content in seeds was slightly lower (4.83%) as compared to the other edible oils, such as sunflower (5.50%), groundnut (4.45%), and palm kernel (14.26%) [50,51]. Low moisture content suggests the ability to store for a longer period of time, whereas high moisture value leads to spoilage [52]. Ash content was a major indication of the presence of mineral elements. The ash content of seed cake (3.76 ± 0.18%) was comparable to reported values of groundnut (2.77%), palm (1.50%), and kokum (2.62%) [12,50]. The total 25.24 ± 0.07% crude protein present in seed cake was comparable to groundnut and African mangosteen, which are26.5% and 31.76%, respectively [32,50]. The proximate results of defatted cake showed an excellent source for nutritional properties. The high content of crude fat, protein, and carbohydrate in defatted WASC is an excellent source and ingredient for food products to meet the requirements of a growing population. The °Brix content of WASC was very low compared to other seed cakes. However, the seed cakes with low °Brix values could be used for biogas production [53]. Phenolic acids are the major bioactive constituents that are available from different plant sources. WASC showed 135.42 mg gallic acid equivalent/100 g of total phenolic content, and the results of African mangosteen, linseed, and rapeseed were 206.39,

102.0, and 830 mg gallic acid equivalent/100 g DW, respectively [32,54]. Food that consists of phenolic acids, which are principal constituents to protect our body from free radicals and chronic diseases [55], are beneficial to human health.

Table 4. Nutritional and nutraceutical composition of wood apple (*Limonia acidissima* L.) seeds *.

Sl. No.	Parameters	Composition
1	Moisture (%)	4.83 ± 0.01
2	Oil (%)	32.02 ± 0.08
3	TSS (°Brix)	1.52 ± 0.29
4	pH	1.76 ± 0.19
5	Acidity (%)	1.83 ± 0.02
6	Ash (%)	3.76 ± 0.18
7	Total Protein (%)	25.24 ± 0.07
8	Total Carbohydrate (%)	31.66 ± 0.04
9	Total phenolics (mg GAE/100 g)	135.42 ± 1.47

* Mean ± standard deviation of triplicate determinations.

4. Conclusions

The quality of edible oil was decided based on physico-chemical properties. Wood apple seed oil values were represented an overall acceptance with respect to oil purity, stability, flavor, degree of unsaturation, and natural antioxidants. The viscosity of oil indicated that the degree of saturated fatty acids was approximately equal to the degree of unsaturated fatty acids and is assessed as of suitable quality for cooking and industrial purposes. GCMS characterization resulted in a greater amount of unsaturated fatty acids compared to saturated fatty acids. Hence, the seed is a remarkably good source to obtain essential fats and for cooking and frying. The presence of tocopherol in the seed oil indicated its potential as a good source for scavenging the free radicals. Nutritional and nutraceutical analysis of seed cake revealed a rich content of protein, carbohydrate, and total phenols, which are helpful in maintaining good health. The utilization of such underutilized fruit seeds facilitates an extra economic benefit to the local people.

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