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Phenolic Compounds and Seed Oil Composition of *Ziziphus mauritiana* L. Fruit

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Ber is a tropical fruit which grows from the tree species, *Ziziphus mauritiana* Lamk. The pericarp of this fruit is consumed either fresh or dried while its seeds are usually discarded as waste. The present study was undertaken to evaluate the antioxidant activity and phenolic content of the fruit, and to evaluate if any potential value-added phytochemicals can be extracted from seed waste. The edible portion of the fruit was extracted with 60% aqueous methanol by sonication and then assayed for total phenolic content, antioxidant activity, and individual phenolic compounds by HPLC-DAD. The seed oil extracted with n-hexane was assayed for fatty acid composition, sterols, and tocopherols content by GC-MS. The total phenolic content of the fresh fruit was 12.8 mg/g as gallic acid equivalent, with an antioxidant activity of 0.5 μmol/g as quercetin equivalent by Folin-Ciocalteu and DPPH assays respectively. Hydroxybenzoic acid, vanillin, *ortho*- and *para*-coumaric acid, epicatechin, quercetin, and naringenin were tentatively identified by matching retention time and UV spectra with those of commercial reference standards. GC-MS analysis of the TMS derivative of fruit extract showed the presence of following compounds: propanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, dodecanoic, n-pentadecanoic, hexadecanoic, benzoic, and trihydroxybenzoic acids. In addition, D-fructose, galactofuranoside, gluconic acid, and β-sitosterol were also detected. In seed oil of ber, the fatty acids such as, hexanoic, octanoic, 7-octadecenoic, 9,12-octadecendoic, eicosanoic, 11-eicosenoic, and docosanoic acid with 7-octadecenoic acid, were found to make up 55% of total fatty acids. Squalene, γ-tocopherol and stigmasterol were identified as minor constituents in the unsaponifiable fraction of seed oil. Current study shows that ber fruit is a good source of healthy phytochemicals.

INTRODUCTION

Ziziphus mauritiana L. belongs to the *Rhamnaceae* family, fruit of which is commonly known as ber in India and Pakistan. Another domesticated species of the genus *Ziziphus* is *Z. jujuba* Mill, which is native to China and known as Chinese jujube. In the 1990s, the International Centre for Underutilized Crops (ICUC), following a number of consultations with national programs in Africa and Asia, highlighted *Z. mauritiana* as a priority species for enhanced research attention. ICUC has published a monograph on ber and other jujubes highlighting the research needs for this genus [Azam-Ali *et al.*, 2006]. *Z. mauritiana* L. is thought to be native to tropical Asia from where it was carried out to Africa and Australia. Ber fruit is exported to the Middle East from India, Thailand, and Pakistan.

Ber fruit is consumed fresh or dried and is used to prepare jams, candied fruit, beverages, and other food products. The ber fruit has a high sugar content (sucrose, glucose fructose and starch); it is therefore high in carbohydrates, which provide energy. The fresh ber fruits contain 81% of water and also have protein with many essential amino acids (asparginine, arginine, glutamic acid, aspartic acid, glycine, serine and threonine) [Morton *et al*., 1987; Azam-Ali *et al.*, 2006]. Besides fruits; roots, bark, leaves, wood, and seeds are also reported to have medicinal uses [Azam-Ali *et al.*, 2006]. At the present time, the market for jujube/ber fruits is restricted to producing regions; one of the reasons for slow export growth besides other marketing reasons is the lack of awareness regarding the fruit and its nutritional values. Annual production of ber fruit in Pakistan is 23.225 million tons [Anonymous, 2006–07].

After ICUC initiatives, ber fruit has been considered for nutritional and phytochemical studies. The ber fruit is reported to be more rich in vitamin C and phosphorus than apples and oranges [Azam-Ali *et al.*, 2006; Obeed *et al.*, 2008]. Recently sugars, organic acids, phenolic compounds (in purified extracts) and triterpenoids are also reported in *Z. mauritiana* L. fruit [Guo *et al.*, 2010; Muchuweti *et al.*, 2005]. Mucilage content of the fruit has also been characterized to be used in the food industry [Sepúlveda *et al.*, 2007].

Antioxidants in fruits are evaluated worldwide to assess the health beneficial role and are reported for many fruits grown around the world. The antioxidant potential of plant extracts is usually assessed by a wide range of procedures

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with DPPH free radical scavenging and total polyphenols assay by Folin-Ciocalteu procedures being the two most commonly used [Memon *et al.,* 2010; Kredy *et al.*, 2010]. Phenolic phytochemicals are major contributors to antioxidant activity of fruits and over 8,000 different phenolic phytochemicals have been documented in the literature [Luthria, 2006a]. Phenolic compounds are classified as: phenolic acids (derivatives of benzoic and cinnamic acid), flavonoids (flavones, flavonols, flavanones, flavanols, anthocyanidins), tannins (gallic acid, catechin, or epicatechin polymers), and a miscellaneous group which comprises lignans, lignins, coumarins, stilbenes, and other phenolic compounds not included in these subgroups [Luthria, 2006b]. Phenolic acids and flavonoids have gained more attention in comparison to other phytochemicals due to numerous reports in recent literature on their health beneficial properties [Balasundram *et al.*, 2006].

Seeds of *Z. mauritiana* L. have not been investigated for their composition in detail but in monograph by ICUC they are reported for their medicinal uses. The dicot seed of *Z. mauritiana* L. is covered in hard core, the seeds looking like small almonds.

Objectives of the current study are; (1) to update earlier reported procedure for assaying phenolic acids and flavonoids in the fruit samples of *Z. mauritaina* L. simultaneously; (2) to evaluate the composition of fruit in terms of total phenolics, fatty acids, sugars, and antioxidant activity; and (3) to characterise seed oil extracts for fatty acids and unsaponifiables.

MATERIAL AND METHODS

Chemicals and reagents

All standards of phenolic acids and flavonoids were purchased from Tokyo Chemical Industry Ltd. (Japan). DPPH (MP Biomedicals Inc., Illkrich, France), Folin-Ciocalteu (Fluka, Steinheim, Switzerland), and sodium carbonate (Merck, Germany) were also purchased. BSTFA (*bis*-(trimethylsilyl) trifluoroacetamide) was bought from Arcos Organics, New Jersey, USA. Methanol (HPLC grade), ethanol, acetonitrile, ethyl acetate, and formic acid were purchased from Fischer Scientific (Leicestershire, UK). All chemicals were used as received.

Plant material

The fruit of *Z. mauritiana* L. was collected from Matiari, Sindh, Pakistan, in the month of February 2010. The species was confirmed by the Department of Plant Protection, Sindh Agriculture University, Tandojam, Sindh, Pakistan, and the plant was identified as a Gola Lemai variety of *Z. mauritiana* L. Fruit samples were stored at 4°C immediately after collection for not more than two days. Pericarp was then separated from the seed and frozen below -4°C. Separated seeds were taken out of the shell, ground in a mortar and pestle, and portions were used for oil extraction and analysis.

HPLC analysis of phenolic compounds

Sample preparation

Samples were prepared as reported by Lin & Harnly [2007]. Fruit material (5 g fresh plant materials) was extracted with 20 mL of methanol:water (60:40, v/v) using sonication (Model No. SC 121 TH, Sonicor Instrument Corp., Copiague, NY, USA) for 60 min at room temperature (<35°C at the end). The extract was filtered through a 0.45 μm Nylon membrane 13 mm filter (Micropore, San Diego, CA). In order to avoid error from unexpected degradation of the phenolics, the LC determinations were completed in less than 24 h after the extracts were prepared.

HPLC conditions

Phenolic compound analysis was carried out in a Spectra system SCM 1000 (ThermoFinnigan, California, USA) liquid chromatograph equipped with a vacuum degasser and a DAD system. A Hypersil Gold C-18 (250 mm × 4.6 mm, 5 μm) column (Thermo Corporation, USA) was used for separation. The mobile phase composed of A (0.1% aqueous formic acid) and B (methanol) run with a gradient elution of 5%-25% B for 10 min, 28–32% B from 10–23 min, 32–25% B from 23–35 min, 25–48% B from 35–50 min, and 48–70% B from 50–90 min at a flow rate of 0.7 mL/min with the injection volume of 20 μL. DAD detection range was set from 200–700 nm and detection windows were set at 270, 320 and 254 nm. The software used for data acquisition and evaluation was Chromquest, Version 4.2. Identification of phenolic compounds was based on retention time and UV spectrum comparison with those of the standards. Quantification was carried out at 270 nm for all phenolic compounds.

The DPPH radical-scavenging ability of fruit extracts

The assay of DPPH radical-scavenging potential was carried out according to the method reported by Rubens & Wagner [2004] with some modifications. Briefly, 2 mL of 0.1 mmol DPPH in methanol solution was added to 2 mL of plant extract (extracted by stirring) and then the mixture solution was placed in the dark for 30 min. The quercetin standards (1–10 μmol) were treated in a similar way and used as standard references to measure the scavenging potential of the sample. The absorbance was measured (Perkin Elmer lambda 35, UV/VIS Spectrophotometer) at 517 nm from 0–30 min with different time intervals. The absorbance after 30 min became constant and was used to construct a calibration graph. The amount equivalent to quercetin was calculated from the calibration graph using the equation of the straight line:

$$y = -0.0395\ \mu mol + 0.4778\ (R^2 = 0.9935)$$

and the expression for concentration was converted to μmol/g (quercetin equivalent) of the sample.

Determination of total phenolics by Folin-Ciocalteu method

The total phenolic content of *Ziziphus mauritiana* L. fruit was determined using the Folin-Ciocalteu (FC) reagent method [Iqbal *et al.*, 2005]. The reaction mixture contained 200 μL of diluted sample extracts, 800 μL of freshly prepared diluted FC reagent and 2 mL of 7.5% sodium carbonate. The final mixture was made-up to 7 mL with deionised water. Mixtures were kept in the dark at ambient temperature for

2 h to complete the reaction. The absorbance at 765 nm was measured on a Perkin Elmer lambda 35, UV/VIS Spectrophotometer, with 1 cm cells. Gallic acid was used as a standard, the amount of phenolic compounds was calculated from the calibration graph:

$$y = 0.0031 \text{ ppm} + 0.0802 \ (R^2 = 0.998)$$

and results were calculated as gallic acid equivalents (mg/g) of ber fruit sample. The reaction was conducted in triplicate and results were averaged.

Silyl derivatives of fruit extract

Dried fruit sample (200 mg) was extracted with 20 mL of 60% methanol using sonication, for 30 min, then filtered and evaporated to nearly 1 mL under a gentle stream of nitrogen. The liquid was transferred to a gas chromatography vial (nearly 3 mL volume) and the extract was brought to complete dryness under a nitrogen environment. To this end, 500 µL of BSTFA were added, and the vial was capped and the mixture was allowed to react at 70°C for 15 min. The resulting solution was used for gas chromatographic analysis.

GC-MS of silyl derivatives

The GC-MS analysis of silyl derivatives was performed on an Agilent 6890 N gas chromatography instrument coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent autosampler 7683-B injector (Agilent Technologies, Little Fall, NY, USA). A capillary column HP-5MS (5% phenyl methylsiloxane) with dimensions of 30 m x 0.25 mm i.d x 0.25 micron film thickness (Agilent Technologies, Palo Alto, CA, USA) was used for the separation. The sample was injected at an injector temperature of 300°C. The column oven was set at an initial temperature of 100°C for 5 min, ramped to 310°C at 5°C / min, and then maintained for 8 min. Split injection mode was used at 1:20, and helium was used as a carrier gas with a flow rate of 0.7 mL/min. The mass spectrometer was operated in the electron impact (EI) mode of 70 eV, ion source temperature of 230°C, quadrupole temperature of 150°C, translating line temperature of 270°C, and Em voltage 1076 V while the mass scan ranged from 50–800 m/z. The silyl derivatives were identified and authenticated using their MS spectra compared to those from the NIST05 mass spectral library. The quantification was done by Chemstation data handling software from Agilent-Technologies.

Oil extraction

Seeds of *Ziziphus mauritiana* L. (50 g) were ground and extracted with a Soxhlet apparatus. The extraction was carried out in a boiling hot water bath for 5–6 h with 0.5 L of *n*-hexane. The solvent was distilled off under a vacuum in a rotary evaporator at 80°C. The oil was then transferred to a desiccator and allowed to cool before being weighed. The drying, cooling, and weighing was repeated until a constant dry weight within ±0.01 g was obtained. The result was expressed as the percentage of oil extracted from dried seed powder. The extracted oil sample was stored below 5°C under a nitrogen atmosphere for further analysis.

Fatty acid composition of oil

Fatty acid composition was determined by gas liquid chromatography after derivatization to methyl esters according to the IUPAC standard method [Paquot, 1992]. The analysis of fatty acid methyl esters (FAMEs) was carried out using an Agilent GC-MS as described in the previous section and a methyl lignocerate coated polar capillary column SP-2340 (60m × 0.25 mm) with 0.2 µm film thickness from Supelco (Bellefonte, PA, USA). Helium was used as a carrier gas at a flow rate of 1.2 mL/min. Column conditions were as follows: initial oven temperature, 130°C; ramp rate, 4°C/ min; final temperature, 220°C; injector temperature, 260°C; detector temperature, 270°C. A sample volume of 1.0 µL was injected with the split ratio of 1:40. FAMEs were identified using the Agilent 1036A (NIST molecular structure) library. The FA composition was reported as a relative percentage of the total peak area.

Analysis of sterol from unsaponified fraction of oil

Extraction and separation of total sterols (ST) was performed after saponification of the oil sample without derivatisation according to the method of Ramadan & Morsel [2003]. Oil (250 mg) was refluxed with 5 mL of ethanolic potassium hydroxide solution (6% w/v) and few anti-bumping granules for 60 min. The unsaponifiable fraction was extracted three times with 10 mL of petroleum ether; the extracts were combined and washed three times with 10 mL of neutral ethanol/water (1:1 v/v), and then dried overnight with anhydrous sodium sulphate. The extract was evaporated in a rotary evaporator at 25°C under reduced pressure, and then the solvent was completely evaporated under a nitrogen atmosphere and reconstituted with hexane for injection into GC-MS.

GC-MS conditions for sterol analysis

The GC-MS analysis of sterol was performed with an Agilent GC-MS system as described in the above section. A capillary column HP-5MS (5% phenyl methylsiloxane) with dimensions of 30 m x 0.25 mm i.d x 0.25 micron film thickness (Agilent Technologies, Palo Alto, CA, USA) was used. The sample was injected at the injector temperature of 280°C. The initial temperature of 150°C was ramped to 250°C at 15°C / min and maintained for 2 min, raised to 310°C at the rate of 15°C /min, and kept at 310°C for 10 min. The split ratio was 1:50, and helium was used as a carrier gas with a flow rate of 1.2 mL/min. Peaks were identified and authenticated using their MS spectra compared to those from the Agilent 1036A (NIST molecular structure) library.

RESULTS AND DISCUSSION

Ber fruit analysis

Total phenolic and antioxidant activity of ber extracts

Total phenolic compounds and antioxidant activity were assayed by FC-reagent and a DPPH free radical scavenging assay procedure. The ber fruit contains 12.8 mg/g phenolic compounds as gallic acid equivalents and 0.5 µmol/g antioxidant activity as quercetin equivalents.

TABLE 1. Retention time and UV spectral characteristics of selected phenolic standards separated by HPLC analysis using diode-array detection.

Compound	t_R (min)	λ (nm)
Gallic acid	8.2	271, 227
Catechin hydrate	14.3	234, 279
Protocatechuic acid	15.8	259, 294, 222
p-Hydroxybenzoic acid	15.8	255
Chlorogenic acid	16.2	324, 241
Epicatechin	16.4	234, 278
Caffeic acid	17.4	306, 222
Epicatechingallate	18.4	276, 233
Vanillin	19.6	281, 308, 230
p-Coumaric acid	22.2	309, 234
Ferrullic acid	24.1	323, 240
m-Coumaric acid	26.8	278, 233
Rutin	29.3	256, 354
o-Coumaric acid	32.9	277, 324, 233
Myricetin	34.9	371, 253
Morin	48.3	252, 353
Diosmin	53.6	298
Quercetin	53.7	257, 370
Naringenin	55.5	288, 235
Kaempferol	60.2	366, 265
Chrysin	74.6	267, 314
5-Hydroxyflavone	85.5	275, 239

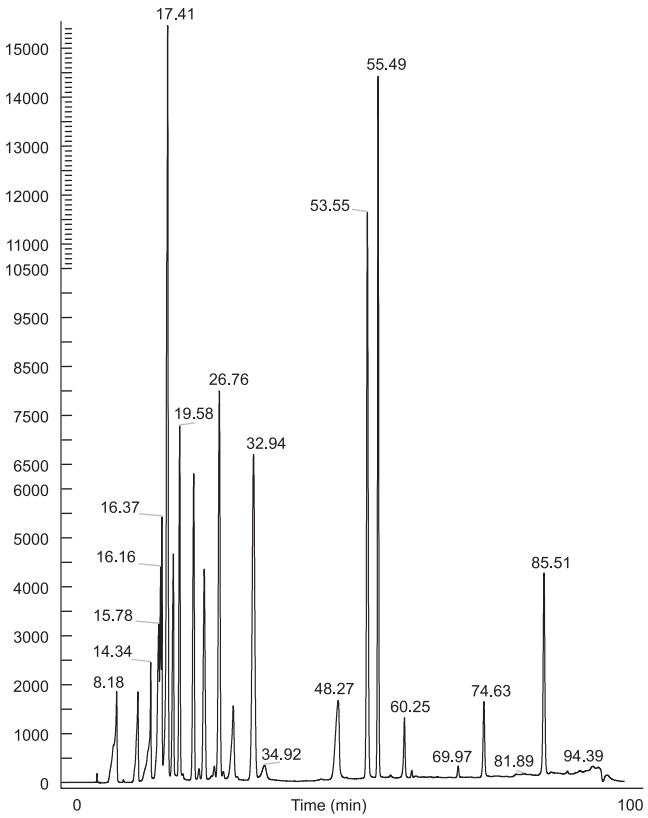

FIGURE 1. An HPLC chromatogram showing separation of twenty two standard phenolic compounds.

Phenolic compounds extracted and identified in ber samples

A standard mixture of ten phenolic acids (gallic acid, vanillic acid, *m*-coumaric acid, ferrulic acid, protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, and *o*-coumaric acid), nine flavonoids (myricitin, rutin, morin, diosmin, quercetin, naringenin, kaempferol, chrysin, and 5-hydroxyflavone), and three catechins (catechin hydrate, epigallocatechingallate, and epicatechin) were used to optimise separation conditions on a C-18 Thermo gold column with a flow rate of 0.7 mL/min.

Various mobile phase compositions using methanol (B) and 0.1% formic acid (A) were tried to separate all compounds mentioned above in a single run. The gradient programming of 0–22.5 min 10–28% B, 22.5–28.8 min 28–30% B, 28.8–60 min 30% B, 60–90 min 30–70% B, 90–100 min 70–10% B was found to separate most of the compounds but *o*-coumaric acid and myricetin could not be separated with severe overlapping. To increase the retention of myricetin which is relatively hydrophobic, reverse-gradient was employed after 32 min followed by forward gradient to reduce the overall run time. Using forward-reverse-forward gradient system, the best resolution was achieved with a mobile phase composed of A (0.1% aqueous formic acid) and B (methanol) and a gradient elution of 5%-25% B for 10 min, 28–32% B from 10–23 min, 32–25% B from 23–35 min, 25–48% B from 35–50 min, and 48–70% B from 50–90 min. Figure 1 shows the resolved peaks of all standards included in the study with coelution of epigallocatechingallate and caffeic acid. These two compounds could be discriminated by their UV spectral characteristics. The names of the compounds with their retention times are given in Table 1.

The identification of phenolic compounds from the ber sample was based on the comparison of retention times and UV spectra (Table 2). The quantification of *p*-hydroxybenzoic acid, *p* and *o*-coumaric acid, vanillin, ferulic acid

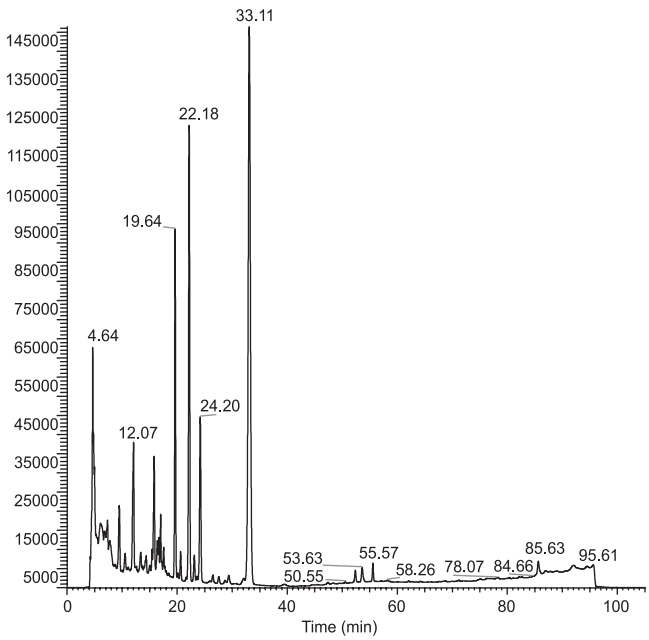

FIGURE 2. A typical HPLC chromatogram of phenolic compounds extracted by ultrasonic irradiation from ber fruit.

TABLE 2. Analysis of polyphenols from ber samples extracted by ultrasonic irradiation.

t_R(min)	λ (nm)	Identification (Amount in μg/g dry matter)
4.7	234	ni*
9.5	288, 231	narigenin glycoside
12.1	259, 293	protocatechuic acid isomer
15.8	255	*p*-hydroxybenzoic acid (83.0)
19.6	280, 309	vanillin (773)
22.2	308, 234	*p*-comaric acid (699.2)
24.1	323, 241	ferrulic acid (621.6)
33.0	276, 324	*o*-coumaric acid (131.2)
55.5	289, 231	naringenin (20.4)

* ni – not identified

and naringenin was achieved by calibration with purified standards (Table 2). Vanillin (773 μg/g dry matter basis (DMB)) and *p*-coumaric acid (699 μg/g DMB) were the two abundant phenolic compounds extracted from the ber sample with ultrasonication. The yield of the other flavonoids varied between 20 and 621 μg/g DMB. The compounds eluting at retention time 9.47 and 12.07 were tentatively identified as naringenin glycoside and a protocatechuic acid isomer based on their UV spectral resemblances (Figure 2). In current study *o*-coumaric acid, naringenin glycoside and naringenin were additionally identified as compared to a previous study [Muchuweti *et al.*, 2005].

Silyl derivatives of fruit extract

Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was used to prepare trimethylsilyl (TMS) derivatives of organic compounds. GC-MS analysis of the TMS derivatives showed the presence of carboxylic acids (propanoic to octadecanoic acid), benzoic acid, hexose sugars, and β-sitosterol in the crude extract of *Ziziphus mauritiana* L. fruit (Table 3). Hexadecanoic acid, D-ribofuranose, and β-sitosterol were identified as a prominent fatty acid, sugar, and sterol respectively, in ber fruit extract (Figure 3).

TABLE 3. GC-MS analysis of trimethylsilyl derivative of organic compounds extracted from ber fruits.

t_R	Name of compound	*Relative percentage
5.241	Hexanoic acid	6.2
7.963	Heptanoic acid	4.6
10.071	Octanoic acid	6.3
13.206	Nonanoic acid	3.3
15.767	Decanoic acid	1.8
20.482	Dodecanoic acid	0.7
28.548	Hexadecanoic acid	16.3
9.801	Benzoic acid	1.1
18.190	Trihydroxybutyric acid	0.2
10.312	Glycerol	1.3
24.245	D-ribofuranose	6.3
24.482	D-fructose	2.2
24.433	Glucofuranose	1.4
25.666	Galactofuranoside	1.4
26.213	Gluconic acid	0.8
47.892	β-Sitosterol	3.6

* The relative percentages are expressed as percentage of total identified and unidentified components (unidentified components are not mentioned here).

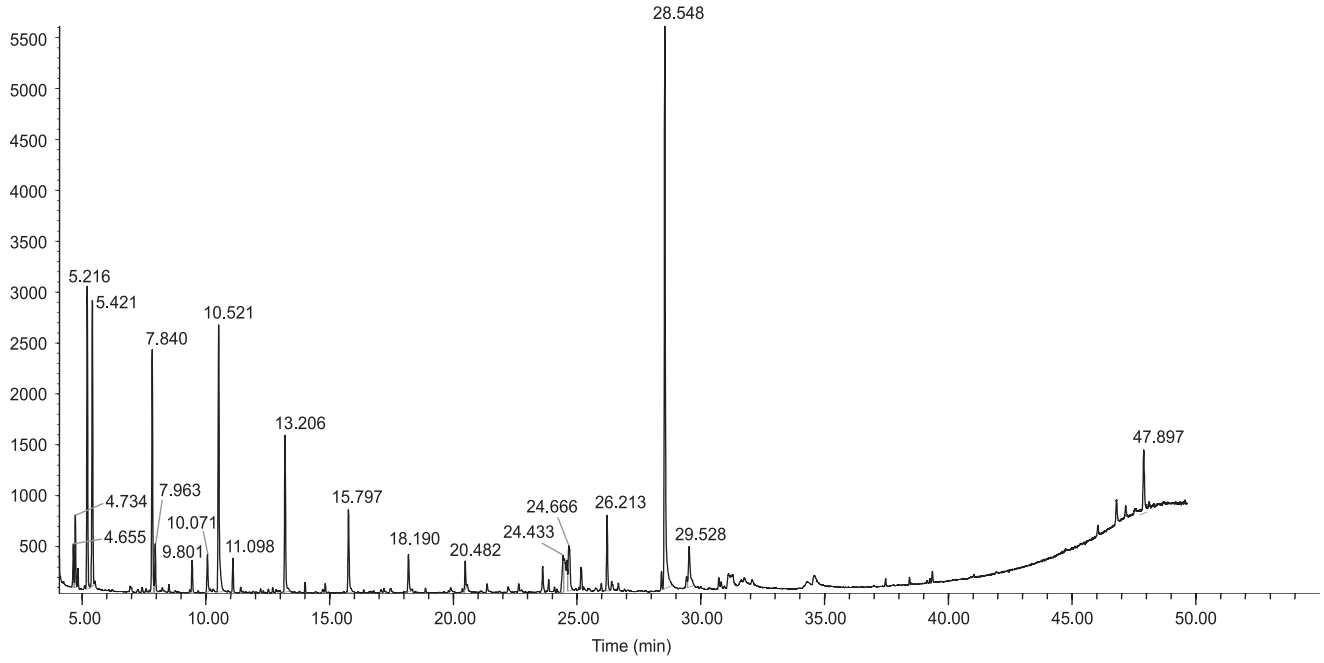

FIGURE 3. GC-MS analysis of trimethylsilyl derivative of organic compounds extracted from ber fruits.

TABLE 4. The identification of the individual fatty acid methyl esters from saponified fraction of seed oil by GC-MS analysis.

t_R(min)	FAMEs*	% of TAMEs ^
11.4	Hexadecanoic acid	7.2
13.3	Octadecanoic acid	6.9
14.1	7- Octadecenoic acid	55.2
15.2	9, 12- Octadecenoic acid	25.3
15.6	Eicosanoic acid	2.1
16.4	11-Eicosanoic acid	1.9
18.2	Docosanoic acid	1.5

* FAMEs=fatty acid methyl esters; ^ TAMEs= total assayed fatty acid methyl esters.

Ber seed oil analysis

Crude fat extraction and analysis from seed oil of ber samples

The crude fat content of the ground seeds of *Ziziphus mauritiana L.* was determined by Soxhlet extraction with hexane as 38.6%. The fatty acid composition of the crude fat was assayed by saponification of crude fat followed by preparation of their methyl esters. The fatty acid methyl esters (FAMEs) were assayed by GC-MS. The fatty acid profile of the ber oil is shown in Figure 4. The identification of the individual fatty acid methyl esters was carried out by spectral library comparison and by the mass spectral fragmentation pattern as hexadecanoic acid (7.2%), octadecanoic acid (6.9%), 7-octadecenoic (55.2%), 9,12-octadecenoic acid (25.3%), eicosanoic acid (2.1%), 11-eicosenoic acid (1.9%), and docosanoic acid (1.5%) (Table 4). The ratio of saturated to unsaturated fatty acids was calculated by dividing total of saturated with unsaturated fatty acids percentage and was found to be 5.3. Olive oil has a similar unsaturated to saturated fatty acids ratio (4.7)

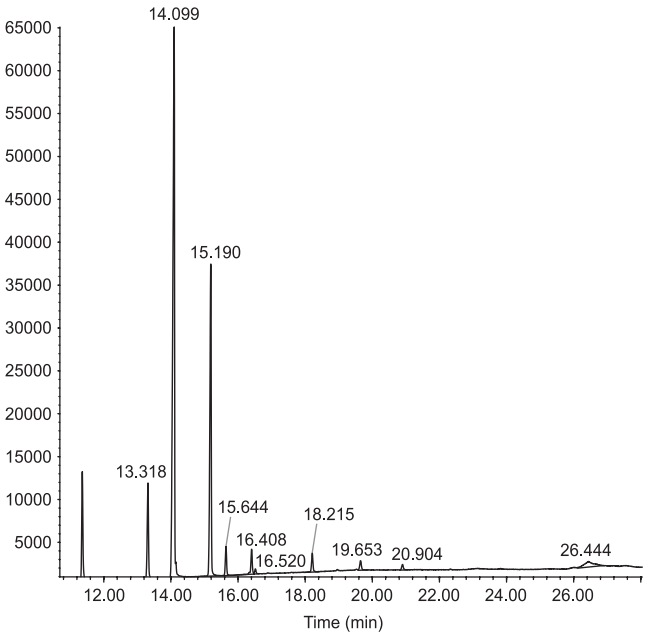

FIGURE 4. GC-MS analysis of fatty acid methyl methyl esters extracted from ber seed oil by Soxhlet procedure.

TABLE 5. Five major organic compounds from unsaponified fraction of seed oil by GC-MS analysis.

t_R(min)	Compounds	% of TUC*
4.8	Squalene	14.0
5.9	γ-tocopherol	4.3
6.8	Campesterol	5.8
7.3	Stigmasterol	23.6
8.3	Δ 4- sitosterol-3-one	6.8

* TUC=total unsaponified compounds.

and a higher percentage of 9-octadecenoic acid (77%). Thus ber seed oil may have significant promise for nutraceutical and pharmaceutical industries.

Analysis of unsaponified fraction of seed oil by GC-MS

The unsaponifiable fraction of seed oil showed five major compounds that were identified by GC-MS analysis (Figure 5) as: three sterols, γ-tocopherol (vitamin-E), and a hydrocarbon (squalene). Stigmasterol was a major component constituting 23.58% of total unsaponifiable compounds (Table 5). The results for sterols analysis are quite different from other oils as *Z. mauritina* L. seed oil showed the presence of stigmasterol in significant amounts while other vegetable oils normally contain sitosterol as a major sterol [Phillips *et al.*, 2002]. The composition of unsaponifiable fraction resembles olive oil in hydrocarbon content which is also rich in squalene [Cert *et al.*, 2000]. The oil seed is also unique due to the presence of γ-tocopherol only, while other oils commonly contain α-tocopherol as a major tocopherol. The exception is pumpkin seed oil in which γ-tocopherol is a major tocopherol [Stevenson *et al.*, 2007].

Tocopherols sterols and monounsaturated fat are of nutritional and medicinal importance, which often tends to increase the commercial value of natural oils [Reichert, 2002; Arain *et al.,* 2010].

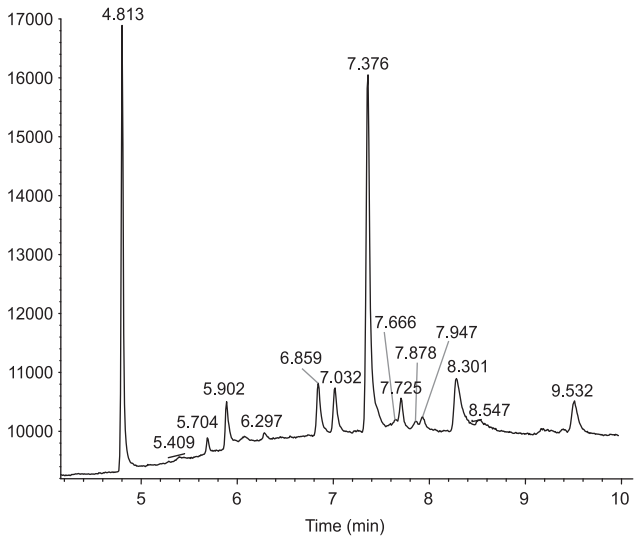

FIGURE 5. GC-MS chromatogram of unsaponified fraction of seed-oil extracted from ber fruit.

CONCLUSIONS

Ber fruit is rich in phenolic phytochemicals, with naringenin as a major flavonoid and *p*-coumaric acids as predominant phenolic acids. Besides sugars and fatty acids, β-sitosterol was also identified in the edible fruit portion. Seeds of Z. *mauritiana* L. contain significant quantity of oil, which is rich in monounsaturated fat, with unique minor components like γ-tocopherol and stigmasterol. Thus ber fruit and seed oil shows significant promise for alternative source of phytochemicals of nutritional and medicinal importance.

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