



Original Research Article

Fatty Acid Profile and Phytochemical Characterization of Bael Seed (*Aegle marmelos L.*) Oil

V.K.Bajaniya*, U.K.Kandoliya, N.H.Bodar, N.V.Bhadja and B.A.Golakiya

Separation Cell, Food Testing Laboratory, Department of Biotechnology
Junagadh Agricultural University, Junagadh, India

*Corresponding author

ABSTRACT

Keywords

Aegle marmelos L., Oil, Fatty acid profile, Unsaturated fatty acids

The seed oil of *Aegle marmelos* was analyzed to establish its physicochemical properties and fatty acids profile as part of an on-going screening process for plant constituents of nutritional and economic significance. Physicochemical characteristics showed that the light yellow oil had refractive index of 1.468, The iodine value was 114.81 ± 0.07 mg iodine/g, saponification value was 183.69 ± 2.41 mg KOH/g, acid value was 19.05 ± 0.09 mg KOH/g, and peroxide value is absent in oil analyzed. The seed oil was found to contain predominantly in the composition of unsaturated fatty acids linoleic acid (2452.06 ppm), oleic acid (961.52 ppm) and linolenic acid observed (37.55 ppm).

Introduction

Aegle marmelos (L.) or Bael is a deciduous holy tree, popular in temples of 'Lord Shiva' in India, which has enormous potential of medicinal plants and traditional uses against various diseases. The tree is native to India and is found growing wild in Sub-Himalayan tracts from Jhelum eastwards to West Bengal, in central and south India. Seeds are beneficial to in treating diabetes, high blood pressure and high cholesterol levels. Seed oil exhibits antibacterial activity against different strains of *Vibrios* (Kulkarni *et al.*, 2012). In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Upon

compiling the available literature on research work done mainly on physicochemical characterization of oil from the seed of *Aegle marmelos L.*, the present work is planned to fill the niche areas which has to be systematically filled in research works related to *Aegle marmelos L.*

Materials and Methods

The seeds *Aegle marmelos L.* was collected from local market of Junagadh. Oil was extracted from seeds by soxhlet extraction in hexane as solvent and analytical work for all the parameters was carried out at separation cell, and MS-MS cell of Food Testing Laboratory, Department of Biotechnology, Junagadh Agricultural University, Junagadh.

The extracted oils were dried under reduce pressure in rotary evaporator to make free from solvent. Oils were stored at -20°C until prior to use for further evaluation of acid value, iodine value, peroxide value saponification values, Refractive index and moisture content.

Moisture was determined by oven drying at 105°C for 3 hours. Ash and total fat contents were determined according to AOAC (2005). Physicochemical characteristics of *Aegle marmelos L.* The ordinary oil constants, e.g., acid value, iodine, saponification, and peroxide number, and refractive index, were estimated according to the AOAC (2005). The fatty acids profiles were determined by GC-MS. Fatty acid methyl esters were prepared using BF₃ methanolic solution and extracted with hexane (Viorica *et al.*, 2012).

GC-MS analyses were performed as per method described by Viorica *et al.* (2012) with some modification. GC-MS analyses were performed by Food Testing Laboratories using a Shimadzu model QP2010 quadruple mass spectrometer detector. The GC column was a DB-5, 30m, 0.25µ m capillary. The initial column temperature was 60°C. The temperature program was 12°C per minute with one minute hold time when rich at 150°C. A final temperature was 240°C per minutes with hold time at five minutes and the mass spectrometer detector analyses. The ion source temp was 230°C.

Interface temp was 240°C and the solvent cut time was 2 minutes. For the identification of the compounds the mass spectra of the samples were compared with those Mass Spectral Library as well as the fatty acids composition was quantified using appropriate standards.

Results and Discussion

Oils and fats and their products are rarely delivered completely dry to customers. The solubility of water in common fats and oils is as high as 0.05–0.30% without physical evidence of its presence (Sonntag, 1982). In view of the enormous volume of fat and oil and their products traded and exchanged internationally, rapid determination of various quality parameters such as moisture content assumes considerable importance. The result of present study showed that the moisture content was less (0.05 %) in the oil of *Aegle marmelos L.* (Table 1). Acid value is an important indicator of vegetable oil quality. Acid value is expressed as the amount of KOH (in milligrams) necessary to neutralize free fatty acids contained in 1 g of oil (ISO, 1983). The increase in acid value should be taken as an indicator of oxidation of oil which may lead to gum and sludge formation besides corrosion. The present study showed that oil of *Aegle marmelos L.* has less acid value (19.05 ± 0.09 mg KOH/g). The amount of unsaturation of the constituent fatty acids has been measured by the iodine value. The iodine value of oils can provide very useful information in other scientific fields. For example, iodine value is used for the determination of oil quality of different plant species for the study of the effects of insecticides on plants, and for the determination of the quality of diesel fuel derived from vegetable oils (Misra *et al.*, 1988; Bergman *et al.*, 1989). Although many methods have been developed, the Wijs method is the most widely used as a standard method.

It is difficult to provide a specific guideline relating peroxide value to rancidity. High peroxide values are a definite indication of a rancid fat, but values is absent indicates no rancidity in this oil of *Aegle marmelos L.* Saponification of oils is the applied term to

the operation in which ethanolic KOH reacts with oil to form glycerol and fatty acids. Glycerol and fatty acids are widely used as raw materials in food, cosmetics, pharmaceutical industries, soap production, synthetic detergents, greases, cosmetics, and several other products. The soap production starting from triglycerides and alkalis is accomplished for more than 2000 years by (Serri *et al.*, 2008; Hermansyah *et al.*, 2006). The present study showed that oil of *Aegle marmelos L.* have potential to be used in the cosmetic industries. Most of the physicochemical properties of the studied oils were favorably compared with other conventional seed oils like palm kernel oil, peanut oil, and soybean oil.

The Fig.1 and Table 2 representing GC-MS chromatogram and mass spectra, are shown the results obtained for samples of fatty acids from *Aegle marmelos* oil. The mass spectrum of Lauric acid methyl ester (1), myristic acid methyl ester (2), Palmitic acid methyl ester (3), Palmitoleic acid methyl ester (4), stearic acid methyl ester (5), oleic acid methyl ester (6), linoleic acid methyl ester (7), linolenic acid methyl ester (8), archidic acid methyl ester (9), and behenic acid methyl ester (10). It noted that samples of *Aegle marmelos* oil are major unsaturated fatty acids, than saturated fatty acids. Predominantly in the composition of unsaturated fatty acids is linoleic acid (2452.06 ppm), also the category of unsaturated fatty acids were oleic acid (961.52 ppm) and linolenic acid observed (37.55 ppm). The high linoleic acid value is similar to those found in other oils, such as hempseed oils (Ryu and MacCoss, 1979).

A total of 23 components were identified from iron chromatogram in *Aegle marmelos* (L.) by their retention indices RI, as well as by GC-MS (Table 3). The more than The oil comprised of fatty acid derivatives, octanoic acid ester (SI 92%), dodecanoic acid, methyl ester (SI 93%), Methyl tetradecanoate, methyl ester (SI 96%), Pentadecanoic acid, methyl ester (SI 95%), 9-Hexadecanoic acid, methyl ester, (SI 95%), Hexadecanoic acid, 14-methyl-, methyl ester (SI 90), 9,12-Hexadecadienoic acid, methyl ester (SI 92%), Heptadecanoic acid, methyl ester (SI 95%), 9-Hexadecenoic acid, methyl ester (SI 90%), 9-Octadecenoic acid (Z)-, methyl ester (SI 94%), 9,12- Octadecadienoic acid, methyl ester (SI 96%), 9,12,15-Octadecatrienoic acid, ethyl ester (SI 93%), Eicosanoic acid, methyl ester (SI 94%), 11-Eicosenoic acid, methyl ester (SI 93%), Docosanoic acid, methyl ester (SI 93%).

Among all the compounds; majority compounds were saturated fatty acids but they all are very important for their pharmacological activities viz. decanoic acid, methyl ester. Isopropyl myristate is used in cosmetic and topical medicinal preparations where good absorption through the skin is desired. 9,12,15-Octadecatrienoic acid, methyl ester (alpha linolenic acid) is an essential omega-3 fatty acid and organic compound found in seeds, nuts (notably walnuts), and many common vegetable oils (Chapman *et al.*, 1983).

Over all, seed oil of *Aegle marmelos L.* is a comparable good quality having number of valued fatty acids which is medicinally important.

Table.1 Physicochemical properties of the *Aegle marmelos L.* oil

Sr. No.	Different Oil Parameters	Value
1	Moisture content (%)	0.05
2	Acid Value	19.05± 0.09
3	Iodine value (I2/g)	114.81 ± 0.07
4	Peroxide value (m eq Peroxide/Kg)	Absent
5	Saponification number (mg KOH/g)	183.69 ± 2.41
6	Refractive index value	1.468

Table.2 Quantitative analysis of free fatty acid content in *Aegle marmelos L.*

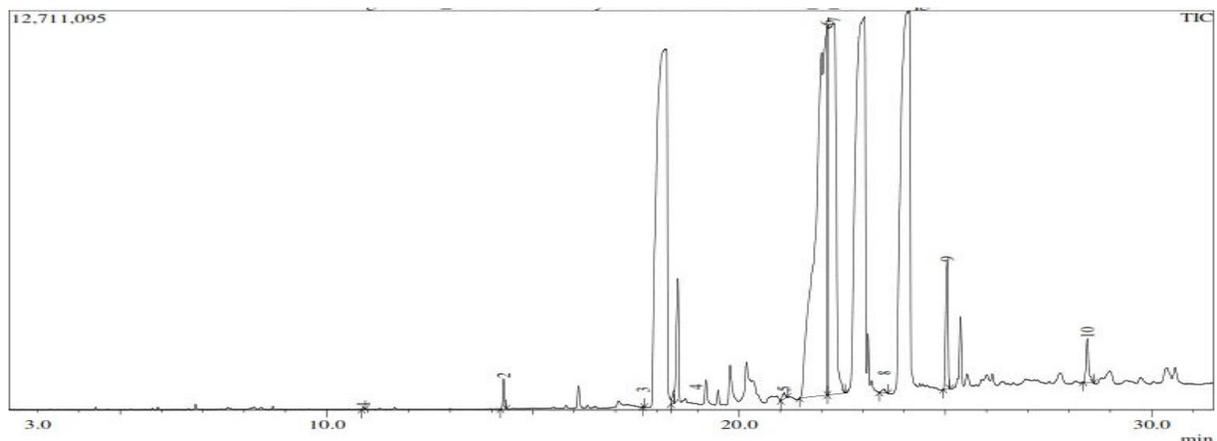
Peak	R.Time	Area	Height	Conc. (ppm)	Name
1	10.89	203642	80770	8.70	Lauric acid, methyl Ester
2	14.29	2778366	952984	146.08	Myristic acid, methyl Ester
3	17.68	20570	16261	12.08	Palmitic acid, methyl Ester
4	18.42	1788523	446281	68.26	Palmitoleic acid, methyl ester
5	21.09	1551611	264253	71.54	Stearic acid, methyl Ester
6	22.00	23016298	3431447	961.52	Oleic acid, methyl Ester
7	22.29	65092899	7051224	2452.06	Linoleic acid, methyl Ester
8	23.51	568888	112051	37.55	Linolenic acid, methyl Ester
9	25.04	13553712	4001769	544.81	Arachidic acid methyl Ester
10	28.31	123399	58104	29.98	Behenic acid, methyl Ester

Table.3 Qualitative analysis of free fatty acid content in *Aegle marmelos L.* seed oil

Sr. No	Compound name	SI	Structure	M. W.	R. T.	Area
1	Octanoic acid, methyl Ester	92	C ₉ H ₁₈ O ₂	158	5.776	84446
2	Dodecanoic acid, methyl ester	93	C ₁₃ H ₂₆ O ₂	214	10.892	164065
3	Methyl tetradecanoate	96	C ₁₅ H ₃₀ O ₂	242	14.294	2722466
4	Pentadecanoic acid, methyl ester	95	C ₁₆ H ₃₂ O ₂	256	16.107	2277883
5	30-Norlupan-28-oic acid,	58	C ₃₁ H ₅₀ O ₅	502	17.676	20570
6	Hexadecanoic acid, methyl ester	96	C ₁₇ H ₃₄ O ₂	270	18.194	196478763
7	Benzoic acid, 4-aminocarbonyl	52	C ₉ H ₉ NO ₃	179	18.442	1805298
8	9-Hexadecenoic acid, methyl ester,	95	C ₁₇ H ₃₂ O ₂	268	18.513	14927097
9	Hexadecanoic acid, 14-methyl-, methyl ester	90	C ₁₈ H ₃₆ O ₂	284	19.197	2811274
10	9,12-Hexadecadienoic acid, methyl ester	92	C ₁₇ H ₃₀ O ₂	266	19.49	1330014
11	Heptadecanoic acid, methyl ester	95	C ₁₈ H ₃₆ O ₂	284	19.782	6302324
12	9-Hexadecenoic acid, methyl ester,	90	C ₁₇ H ₃₂ O ₂	268	20.18	8478398
13	Tetracosanoic acid,methyl ester	84	C ₂₅ H ₅₀ O ₂	382	20.317	6374306
14	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	90	C ₁₉ H ₃₄ O ₂	294	21.091	963458
15	2-(4-Chlorophenyl)-3- cyclopropyl	50	C ₁₅ H ₁₈ ClN ₃ O	291	22.142	226425011
16	9-Octadecenoic acid (Z)-, methyl ester	94	C ₁₉ H ₃₆ O ₂	296	22.292	157431043
17	9,12-Octadecadienoic acid, methyl ester	96	C ₁₉ H ₃₄ O ₂	294	23.043	188924690
18	11,14-Eicosadienoic acid, methyl ester	90	C ₂₁ H ₃₈ O ₂	322	23.125	4051543
19	Nonadecanoic acid, methyl ester	87	C ₂₀ H ₄₀ O ₂	312	23.511	606552
20	9,12,15- Octadecatrienoic acid, ethyl ester	93	C ₂₀ H ₃₄ O ₂	306	24.125	183445190
21	Eicosanoic acid, methyl ester	94	C ₂₁ H ₄₂ O ₂	326	25.042	15844727
22	11-Eicosenoic acid, methyl ester	93	C ₂₁ H ₄₀ O ₂	324	25.369	7857618
23	Docosanoic acid, methyl ester	93	C ₂₃ H ₄₆ O ₂	354	28.441	6542623

SI-Similarity Index, M. W.-Molecular Weight, R.T.-Retention Time

Fig.1 Chromatogram of GCMS of *Aegle marmelos* L. seed oil



References

- Association of Official Analytical Chemists (AOAC) (2005). Official methods of analysis of AOAC international, 18th edn. AOAC International, Maryland, USA.
- Bergman, J.W., Carlson, G., Kushnak, G., Riveland, N.R., Stallknecht, G., Welty, L.E., Wichman, D. 1989. Registration of finch safflower. *Crop Sci.*, 29: 829–832.
- Chapman, D.J., De-Felice, J., Barber, J. 1983. Growth temperature effects on thylakoid membrane lipid and protein content of pea chloroplasts. *Plant Physiol.*, 72(1): 225–228.
- Hermansyah, H., Kubo, M., Shibasaki-Kitakawa, N., Yonemoto, T. 2006. Mathematical model for stepwise hydrolysis of triolein using *Candida rugosa* lipase in biphasic oil water system. *Biochem. Eng. J.*, 31: 125–132.
- ISO 660, 1983. Animal and vegetable fats and oils. Determination of acid value and acidity, ISO, Geneva.
- Kulkarni, A.S., Khotpal, R.R., Karadbhajane, V.Y., More, V.I., 2012. Physico-chemical Composition and lipid classes of *Aegle marmelos* (Bael) and *Citrullus colocynthis* (Tumba) seed oils. *J. Chem. Pharm. Res.*, 4(3): 1486–1488.
- Misra, N., Sangita-Batra, Rathore, A., Batra, S. 1988. Effects of storage moulds on the nutritional components of foeniculum vulgare mill. *Int. J. Trop. Plant Dis.*, 6: 67–72.
- Ryu, E.K., MacCoss, M. 1979. Modification of the Dittmer-Lester reagent for the detection of phospholipid derivatives on thin-layer chromatograms. *J. Lipid Res.*, 20: 561–563.
- Serri, N.A., Kamarudin A.H., Abdul Rahaman, S.N. 2008. Preliminary studies for production of fatty acids from hydrolysis of cooking palm oil using *C. rugosa* lipase. *J. Phys. Sci.*, 19: 79–88.
- Sonntag, N. 1982. Analytical methods. In: Bailey's industrial oil and fat products, 4th edn. John Wiley & Sons, New York, Vol. 2. Pp. 484–487.
- Viorica, M.P., Alexandra, G., Diana, N.R., Delia, D., Camelia, M., Despina, B., Constantin, M. 2012. *J. Agroaliment. Process. Technol.*, 2012, 18 (2), 136–140.