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Nutritional quality of *Moringa oleifera* for its bioactivity and antibacterial properties

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Abstract

Need to search for edible plants with antimicrobial and nutraceutical properties is increasing due to reduced efficacy, high cost and increased resistance of conventional medicine and for food quality and safety applications. This study analysed proximate, amino acids, minerals, secondary metabolites, and phytochemical composition of *Moringa oleifera* from two geographical locations in India and there was wide variation between two regions. Proximate composition revealed high protein, fibre and less fat content of leaves. Glutamate, arginine and isoleucine were the major amino acids. Calcium and potassium was found to be high in leaves and copper was high in seeds. Leaves and seeds had high vitamin E and C. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed 185 compounds from seeds and 50 compounds from leaves. 1,30-triacontanediol, octacosane, Z-14-nonacosane were found to be major compounds of leaves from Madurai and nonacosane, .gamma.-sitosterol were found to be major compounds of leaves from Chennai. 6-octadecenoic acid, n-hexadecanoic acid, oleic acid were found to be major compounds of seeds from Madurai and 13-docosenamide, (Z)-, propionamide, ethyl oleate were found to be major compounds of seeds from Chennai. Leaves had high total polyphenols and antioxidant activity. High antimicrobial activity against dysentery causing entero-pathogens was found in leaves and seeds from Madurai.

Introduction

Moringa is the sole genus in the flowering plant family Moringaceae and is native to Asia and Africa. Among the 14 species, *M. oleifera* Lam. (syn. *M. pterygosperma* Gaertn.) is most utilized and widely known (Sengupta, 1970; Morton, 1991; Steinitz *et al*., 2009). The leaves and immature pods (drumstick) are extensively used as vegetable accompaniments and as health foods in southern India. The leaves are cooked and eaten like spinach or used to prepare soups and salads. It is consumed by pregnant women to enhance breast milk production.

In rural parts, it is used in the preparation of cow and buffalo ghee from butter fat to enhance the flavour, taste and extend shelf life (Anwar and Bhanger, 2003). In folk medicine, leaves are rubbed against the temple to relieve headaches and poultice of fresh leaves are applied to stop bleeding from a shallow cut. The leaf juice is believed to control glycemia and is applied for swollen glands. They are traditionally used as purgatives and in the treatment of haemorrhoids, fevers and inflammation of nose and throat, bronchitis, eye and ear infections and to combat vitamin C deficiency. They are also reported to control hypertension and hypercholesterolemia (Asare *et al.*, 2012).

Moringa oil has a high content of oleic acid (72%) and is used in cosmetic products to increase the health and strength of hair and skin (Anwar and Rashid, 2007). Despite the extensive food and medicinal uses of Moringa, there is little data on the phytochemical composition of the local varieties. Therefore, the objective of the present study was to determine the chemical and nutrient composition of the extracts from the leaves and seeds of *M. oleifera* Lam from Madurai and Chennai districts of the southern part of India and the comparison of the phytochemical constituents present in the hexane and methanol extracts of the Indian species of *M. oleifera* Lam using GC-MS.

Materials and Methods

Plant materials

Leaves and seeds of *M. oleifera* were collected from Madurai and Chennai districts of Tamilnadu, India. The samples were washed, air-dried (30°C) and powdered separately.

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Proximate analysis

The protein, fat, crude fibre and ash content were determined by AOAC (2005) methods. The moisture content of the samples was determined using a moisture analyser (Sartorius MA35).

Determination of amino acid composition

Amino acids were estimated according to Bassler and Buchholz (1993).

Mineral analysis

Minerals were determined by AOAC (2005) method.

Vitamin analysis

The B-complex and other water soluble vitamins determined included B_1, B_2, B_3, B_5, B_9 and C. Fat soluble vitamins analysed were vitamin A and E (McMurray *et al.*, 1980; Thompson and Duval, 1989).

Detection of secondary metabolites

Tannins, phlobatannins and flavonoids were estimated according to Kanimozhi *et al.* (2011) and Evans (2009) respectively. Saponins were detected by AOAC method (2005).

Steroids

Acetic anhydride (2 mL) was added to 0.5 g ethanol extract followed by sulphuric acid (2 mL). The colour changed from violet to blue or green confirming the presence of steroids.

Terpenoids (Salkowski test)

The extract (5 mL) was mixed with 2 mL of chloroform. Concentrated sulphuric acid (3 mL) was added to form a layer. Reddish brown colour at the interface indicated terpenoids.

Glycosides (Keller-Kiliani test)

The extract (5 mL) was treated with glacial acetic acid (2 mL), ferric chloride (1 drop) and concentrated sulphuric acid (1 mL) was overlaid. Brown ring at the interface indicated glycosides.

Determination of alkaloids

Alkaloids were estimated according to Evans (2009).

Determination of total polyphenols

Phenolic acids were estimated according to Singleton and Rossi (1965).

Determination of antioxidant activity

DPPH scavenging method was followed according to Ratshilivha *et al.* (2014).

Preparation of crude extracts

Samples (2 g) were extracted with 20 mL of hexane followed by water at 30°C using a magnetic stirrer for 3 h and centrifuged at 3500 rpm for 30 min. The supernatant was separated, filtered and air-dried (Bodlund *et al*., 2014). The above procedure was repeated with methanol.

Bacterial strains

Eleven bacterial strains *Escherichia coli, Salmonella typhi, Salmonella typhimurium, Shigella dysentery, Enterobacter feacalis, Proteus vulgaris, Staphylococcus aureus, Shigella flexneri, Salmonella paratyphi-B, Klebsella pnemoniaea, Salmonella paratyphi-A* were isolated from clinical cases provided by Sri Ramachandra Medical Centre and Hospital, Chennai. Bacteria were grown and maintained in nutrient broth (Himedia, M002) (Holt *et al*., 1994).

Antibiotic sensitivity test

Antibiotics (Himedia) having different mode of actions such as tetracycline (SD037), norfloxacin (SD057), ciprofloxacin (SD060) and vancomycin (SD045) were used.

Determination of antibacterial activity using disc diffusion method

Sterile discs (Himedia-SD067) in nutrient agar (Himedia M001) were loaded with 40 µL of plant extracts and plates were incubated at 37°C for 24 hours (Holt *et al*., 1994).

Detection of phytochemicals by GC-MS analysis

The samples were injected into Agilent 6890N DB-5 ms capillary column (30 m x 250 mm x 0.25 µm). Compound identification was accomplished with NIST spectral library.

Detection of functional group by Fourier transform infrared spectrophotometer (FTIR)

FTIR (CARY 630, Agilent) was based on Universal Diamond ATR with spectral range 4000-650 cm^{-1}, spectral resolution 4 cm^{-1} and scan number 8 and DLaTGS detector.

Results and Discussion

Proximate analysis

Moringa is reported to have excellent nutritional properties, low seed toxicity, high quality of oil, ability to purify water and adapt to poor soils and dry

Table 1. Proximate, mineral, vitamin, secondary metabolites composition and antibacterial activity of *M.oleifera*

Nutrients (g/100g)	Leaf		Seed pod	
	Madurai	Chennai	Madurai	Chennai
Protein	28.40±0.2	27.10±0.3	3.10±0.1	3.80±0.3
Fat	1.90±0.3	1.70±0.1	0.10±0.4	0.10±0.1
Crude fibre	19.20±0.2	18±0.10	5.5±0.10	4.2±0.20
Total ash	3.00±0.10	2.90±0.2	2.50±0.2	2.30±0.1
Moisture	7.50±0.1	6.90±0.1	4.10±0.2	5.30±0.1
Calcium (Ca)	2225±0.1	2205±0.1	33±0.4	38±0.1
Magnesium (Mg)	3760.2±0.3	360±0.1	30±0.1	28.5±0.1
Phosphorous (P)	250±0.3	239±0.1	110±0.2	131±0.1
Potassium (K)	1391±0.1	1112±0.2	270±0.2	261±0.1
Copper (Cu)	0.6±0.1	0.3±0.1	3.9±0.1	4±0.1
Iron (Fe)	29.3±0.2	29.3±0.1	6.10±0.1	6.4±0.1
Sulfur (S)	870±0.1	900±0.4	141±0.5	155±0.5
Vitamin A / Retinol (mg)	6.8±0.1	6.3±0.1	0.3±0.1	0.8±0.1
Vitamin B_1 / Thiamine (mg)	2.64±0.4	2.59±0.2	0.05±0.2	0.06±0.3
Vitamin B_2 / Riboflavin (mg)	20.5±0.2	21±0.2	0.06±0.1	0.08±0.1
Vitamin B_3 / Nicotinic acid (mg)	8.2±0.1	9.6±0.1	1.2±0.3	1.9±0.5
Vitamin B_5 / Pantothenic acid (mg)	0.125±0.2	1.6±0.4	0.9±0.1	0.7±0.2
Vitamin B_9 / Folate (µg)	39.5±0.1	40±0.3	46±0.2	48±0.2
Vitamin C / Ascorbic acid (mg)	17.3±0.3	19.4±0.1	130±0.3	124±0.4
Vitamin E / Tocopherol (mg)	113±0.2	121±0.6	ND	ND
Tannins	ND	ND	+	+
Phlobatannins	ND	ND	ND	ND
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Terpenoids	+	+	ND	ND
Glycosides	+	+	+	+
Total Polyphenols (mg/g)	40.27 ± 0.9	36.57 ± 0.3	40.17 ± 0.01	38.21±0.7
Alkaloids	ND	ND	+	+
Antioxidant Activity (µg/ml)	29± 0.67	15 ± 0.92	36 ± 0.45	31 ± 0.33
	Zone of inhibition (mm)			
Escherichia coli	9.0	7.0	25.0	1.0
Salmonella typhi	7.0	7.0	23.0	1.0
Salmonella typhimurium	8.0	7.0	20.0	0.9
Shigella dysenteri	9.0	8.0	24.0	0.7
Enterobacter faecalis	ND	ND	28.0	0.0
Proteus vulgaris	ND	ND	26.0	ND
Staphylococcus aureus	17.0	12.0	25.0	12.0
Shigella flexneri	7.0	9.0	14.0	8.0
Salmonella para typhi-B	ND	ND	0.9	ND
Klebsiella pneumoniae	ND	ND	ND	ND
Salmonella para typhi-A	ND	ND	ND	ND

climates. Variation in the nutritive values depends on genetic background, environment and cultivation methods. The protein content of Moringa is a potential replacement for animal diets (Moyo *et al*., 2013). Leaves had high protein (28.4 g) and crude fibre (19.2 g) content but less fat (1.9 g) content whereas the fat content (0.1 g) was almost negligible in seeds. Among the two regions, Moringa from Madurai had slightly higher proximate composition (Table 1). Other studies have reported variable content 16, 22.42, 23.27, 27.4 and 40% (Nouala *et al.*, 2006).

Amino acid analysis

All ten essential amino acids were present in both leaves and seeds with similar leucine, phenylalanine, threonine and valine content. Leaves had high isoleucine and low tryptophan content. Seeds had high arginine and low valine content. Glutamate was high followed by aspartate, glycine, serine and alanine in leaves and seeds. Among the two regions, Moringa from Chennai had high amino acid content (Table 2). Roots, leaves and seeds of Moringa have appreciable amino acid content. The essential amino acid profile of both seeds and leaves are similar to the reports from World Health Organisation except for the higher leucine content and similar to studies by Anhwange *et al.* (2004) and slightly different from the findings of Foidl *et al*. (2001) and Sanchez *et al.* (2010).

Minerals

Leaves had high calcium, potassium, sulphur, magnesium, phosphorous and iron content whereas copper content was high in seeds. The mineral composition was slightly higher in Moringa from Madurai except for sulphur content which was high in Moringa from Chennai (Table 1). Calcium and iron content was high when compared with vegetables such as mushroom and leaves of cassava, amaranth, taro and pumpkin (Ibok and Deborah, 2008).

Vitamins

Vitamin E (tocopherol) content was high in leaves whereas seeds had high vitamin C (ascorbic acid) content. Vitamin B_2 (riboflavin) content of seeds was negligible and vitamin E was absent. Vitamin B_5 (pantothenic acid) and B_9 (folate) content were similar in leaves and seeds. Folate was found in µg in both leaves and seeds (Table 1). The low content of vitamin C in leaves may be due to oxidation loss during air drying at room temperature for two days. It is thus evident that Moringa leaves are nutrient dense providing essential micronutrients.

Secondary metabolites

Saponins, flavonoids, steroids, glycosides and

Table 2. Amino acid composition of *M. oleifera* leaves and seeds (g/16g N)

Amino acids	Leaf			Seed pod		
	Madurai	Chennai	WHO	Madurai	Chennai	WHO
Arginine	4.30±0.1	3.7±0.2	1.88	9.44±0.2	11.00±0.2	8.06
Histidine	2.50±0.2	3.12±0.3	1.90	6.3±0.3	5.7±0.2	2.01
Isoleucine	5.90±0.2	9.10±0.2	2.33	8.3±0.1	7.7±0.4	4.35
Leucine	4.80±0.1	4.40±0.1	5.22	4.8±0.1	4.7±0.3	5.27
Lysine	4.10±0.1	3.30±0.3	3.60	4.2±0.3	3.7±0.2	3.24
Methionine	1.90±0.2	3.40±0.1	0.95	3.5±0.4	3.1±0.2	0.97
Phenylalanine	4.20±0.3	4.60±0.4	4.26	3.50±0.2	3.9±03	4.53
Tryptophan	1.50±0.3	1.90±0.1	2.10	4.3±0.3	3.7±0.2	2.30
Threonine	4.10±0.1	4.50±0.2	4.38	3.2±0.1	3.8±0.3	3.22
Valine	4.55±0.1	4.75±0.1	3.36	3.36±0.2	2.37±0.5	3.09
Glycine	5.13±0.2	5.11±0.2	5.15	5.00±0.3	4.7±0.1	5.00
Glutamate	15.86±0.1	15.33±0.5	15.14	14.23±0.4	14.74±0.6	14.76
Serine	4.25±0.1	4.66±0.3	4.20	4.22±0.1	4.11±0.4	4.25
Alanine	3.23±0.1	3.90±0.3	3.43	4.29±0.1	3.55±0.3	3.23
Aspartate	6.44±0.2	6.86±0.3	6.86	6.02±0.1	6.37±0.2	6.14

polyphenols were present in leaves and seeds from Madurai and Chennai but Phlobatannins was absent. Tannins and Alkaloids were absent in leaves of both regions. Terpenoids was present in leaves but not in seeds. Polyphenol content was slightly high in Moringa from Madurai (Table 1). Secondary metabolites are activated by enzyme hydrolysis and are used as medications (Kashiwada *et al.*, 2012). This study established that M. oleifera leaves and seeds contained: tannins, steroids, terpenoids, flavonoids, glycosides, saponins, alkaloids and polyphenols which have been identified by other researchers in various plants and in different parts of plants (Bennett *et al*., 2003). The findings in this study agree with earlier studies that not all phytochemicals are present in all plant parts and that those present differ according to the type of the extracting solvent used. Flavonoids are also active in reducing high blood pressure (Ayinde *et al*., 2007). They are many in number as well as strong antioxidants and also found to be effective antimicrobial substances in vitro against a wide array of microorganisms by inhibiting the membrane bound enzymes (Sultana *et al*., 2007). Tannins are a group of polymeric phenolic substances capable of tanning leather, inactivating and killing microorganisms (Scalbert, 1991).

Antioxidant activity

Leaves from Madurai had 50% higher antioxidant activity than the leaves from Chennai whereas the antioxidant activity of seeds was similar for both the regions (Table 1). Moringa is known for its high antioxidant activity among all fruits and vegetables (Yang *et al.*, 2006). Shafaghat *et al*. (2011) have also documented high radical scavenging activity of *Hypericum scabrum* L., seed (hexane extract) containing omega-3 fatty acids, bis (2-ethylhexyl) phthalate, linoleic acid and nonacosane as major compounds.

Antibacterial activity

Among the eleven entero-pathogens tested it was found that *E. coli, S. typhi, S. typhimurium, S. dysentri, S. flexneri* and *S. aureus* were inhibited by both leaves and seeds from Madurai and Chennai. But seeds from Madurai showed highest inhibition against nine strains namely *E. coli, S. typhi, S. typhimurium, S. dysentri, S. flexneri, S. aureus, E. feacalis, P. vulgaris* and *S. para typhi – B* (Table 1). The pathogenesis results indicated that ciprofloxacin (targeted towards bacterial nucleic acid) and norfloxacin (targeted towards bacterial cell wall) inhibited all the 11 strains, but *S. typhi* was resistant towards tetracycline (targeted towards bacterial protein synthesis) and also *S. typhi, S. typhimurium, S. paratyphi-B, K. pnemoniaea* and *S. paratyphi-A* were resistant against vancomycin (targeted towards bacterial cell wall) (Table 1). Ethanolic extract of Cynodon dactylon L showed 2-Hexadecen-1-ol, 3,7,11,15 tetramethyl, hexadecanoic acid, Ethyl ester, gamma.-sitosterol, 9-Octadecenoic acid, methyl ester, tetracontane, N-nonacosane which had high anti-microbial activity (Kanimozhi *et al*., 2012). Yayli *et al*. (2006) reported the antibacterial activity of nonacosane (6.2%) from the essential oil of *M. meyeri* which was lower than our observation. Tesemma *et al*. (2013) compared the antibacterial activity against *E. coli* based on IR and NMR of n-octacosane isolated from *M. stenopetala* using column chromatography which showed similarity with the reference compound (ciprofloxacin). Nonacosane occurs naturally and has been reported to be a component of *Phyllanthus amarus* (Euphorbiaceae) with anti-pesticide property (Soetan *et al.*, 2010). Salmonella typhi was resistant to antibiotic targeting nucleic acid (tetracycline). But the Moringa seed from Madurai inhibited the pathogen. Similarly, *Salmonella* species were resistant to antibiotic targeting cell wall (vancomycin). But both the leaves and seeds (Madurai) inhibited the pathogen at very low concentrations 0.16, 0.15 mg/40

Table 3. Chemical composition *M. oleifera* leaves

S.No.	Madurai Moringa Leaves	Area %	RT
1	Bicyclo[3.1.1]	0.59	15.46
2	Phytol	0.94	20.40
3	9,12,15-Octadecatrienoic acid	0.44	21.35
4	Ethylidenecyclooctane	0.83	26.31
5	Cyclopropaneoctanal, 2-octyl-1,5,9,13-Tetradecatetraene	2.08	26.38
6	9-Octadecenoic acid	0.93	26.51
7	Hexadecane	1.07	26.71
8	1H-Indene	0.58	27.47
9	Pyridine-3-carboxamide	0.37	27.59
10	Cyclopropane carboxamide	1.02	27.73
11	cis-11,12-Epoxytetradecen-1-olDihydroartemisinin	0.59	27.78
12	Z,Z-8,10-Hexadecadien-1-ol acetate	1.16	27.87
13	Cyclopropane carboxamide	0.78	27.95
14	Cyclopropane carboxamide	0.52	28.00
15	2-Myristynoyl-glycinamide	1.33	28.17
16	Cyclopropane carboxamide	1.79	28.26
17	Cyclopropane carboxamide	1.87	28.37
18	1,30-Triacontanediol	14.98	28.63
19	Pyridine-3-carboxamide, oxime	1.18	28.73
20	1,3,2-Dioxaphospholane	3.49	28.81
21	Pyridine-3-carboxamide	1.66	28.88
22	Eicosane	3.66	28.95
23	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15	1.40	29.01
24	13-Docosenamide, (Z)-	7.15	29.77
25	Z-14-Nonacosane	8.30	30.18
26	5-Acetamido-4,7-dioxo-4,7-dihydrobenzofurazan	6.51	30.33
27	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15	3.79	30.63
28	2,2-Dimethyl-1-oxa-2-silacyclotrid ecanone-13	8.28	30.90
29	Octacosane	8.57	30.98
30	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	5.79	31.15
31	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15	2.41	31.69
32	13-Hexacosyne	1.86	32.85
33	3,4-Seco-5.alpha.-cholestan-3-oicacid	1.05	33.17
34	1-Monolinoleoylglycerol trimethylsilyl ether	0.40	33.28
35	No matches found	0.86	33.50
36	9H-Fluorene-4-carboxyl ic acid, 9-oxo-,	0.52	33.69
37	Cobalt, [1,1',1'',1'''-[(1,2,3,4-.eta.)	1.26	33.91
	Chennai Moringa Leaves		
1	Eicosane	0.12	3.86
2	Sulfurous acid, butyl nonyl ester	0.11	6.51
3	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-,	0.32	15.46
4	1,2,3,4,4a,5,6,8a-Octahydro-naphthalene	0.38	21.34
5	Heptadecane, 4-propyl-Hexadecanoic acid	0.24	23.51
6	Ergosta-5,22-dien-3-ol, (3.beta.,22E,24S)-	0.66	23.87
7	Ergosta-4,22-dien-3.beta.-ol	0.34	23.97
8	5,14,23-Octadecatrien-14,15-diol	0.27	25.64
9	4-Tetradecyne	0.72	26.31
10	11,13-Dimethyl-12-tetradecen-1-olacetate	1.94	26.38
11	1H-Indene, 5-butyl-6-hexyloctahydro-o-	0.24	26.53
12	Eicosane	0.99	26.70
13	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	0.54	27.21
14	Benzene, 1-isothiocyanato-2-methyl4-Thiazolidinone	1.08	27.46
15	Z,E-7,11-Hexadecadien-1-yl acetatedl-Thioctic acid	0.30	27.55
16	Campesterol	5.00	27.86
17	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	0.48	27.97
18	AcetamideN-(6-acetylaminobenzothiazol-2-yl)-2-(adamantan-1-yl)-	0.62	28.01
19	N-Methyl-1-adamantaneacetamide	0.82	28.09
20	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	0.91	28.18
21	Pyridine-3-carboxamide	0.87	28.22
22	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15	1.08	28.32
23	13-Methyl-Z-14-nonacosene Hexasiloxane	1.35	28.38
24	5-(p-Aminophenyl)-4-(p-tolyl)-2-thiazolamine	1.26	28.46
25	Perhydro-htx-2-one, 2-dependyl-, acetate ester	2.42	28.56
26	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15	4.06	28.69
27	Methadone N-oxide	3.08	28.81
28	Heptacosane	5.12	28.94
29	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,15-hexadecamethyl-	2.98	29.09
30	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,15-hexadecamethyl-	1.78	29.18
31	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,15-hexadecamethyl-	2.52	29.27
32	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,15-hexadecamethyl-	2.66	29.43
33	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,15-hexadecamethyl-	1.30	29.49
34	trans-13-Docosenamide	3.43	29.77
35	Eicosane	2.78	29.90
36	.gamma.-Sitosterol	9.56	30.16
37	Pyridine-3-carboxamide	5.07	30.33
38	Cyclohexanecarboxylic acid	6.03	30.89
39	Nonacosane	15.55	30.99
40	Pyridine-3-carboxamide	3.77	31.14
41	2-(4-Chlorophenyl)-5,7-dimethylimidazo	1.14	31.65
42	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,15-hexadecamethyl-	1.24	32.52
43	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]-	0.95	32.86
44	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,15-hexadecamethyl-	0.31	33.15
45	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,15-hexadecamethyl-	0.14	33.22
46	Purine-2,6-dione, 8-(3-ethoxypropylamino)-1,3-dimethyl-3	0.40	33.36
47	3-Phenylpropionic acid, 5-methoxy-2-[3,4-dimethoxyphenyl]-	0.82	33.50
48	No matches found	0.61	33.53
49	2-Myristynoyl-glycinamide	0.49	33.71
50	Cobalt, [1,1',1'',1'''-[(1,2,3,4-eta.)-1.	1.15	33.89

µL respectively.

GC-MS analysis

The extraction efficiency was higher for hexane than methanol. Hence, the hexane extract was subjected to GC-MS analysis. Thirty seven compounds in leaves from Madurai and fifty compounds from Chennai were identified (Table 3). 1,30-triacontanediol (14.98%), octacosane (8.57%), Z-14-nonacosane (8.3%) and 2,2-dimethyl-1-oxa-2-silacyclotrid ecanone-13 (8.28%) were the major compounds in leaves from Madurai. Nonacosane (15.55%) and gamma.-Sitosterol (9.56%) were the major compounds in leaves from Chennai. Pyridine-3-carboxamide, 2-myristynoyl-glycinamide, eicosane and octasiloxane-1,1,3,3,5,5,7,7,9,9,11,11,13,13,15 were common in leaves of both regions.

Seeds from Madurai had 161 compounds and 185 compounds were found in seeds from Chennai (Table 4). 6-octadecenoic acid (52.24%), n-hexadecanoic acid (6.17%), oleic acid (5.12%) were found to be the major compounds of seeds from Madurai and 13-docosenamide, (Z)- (13.62%), propionamide (4.48%), ethyl oleate (4.33%) were found to be the major compounds of seeds from Chennai. Eicosane, 13-docosenamide, (Z)-,octacosane, hexadecane and 9-octadecenoic acid were found to be the common fractions in seeds. Seed extracts contained a larger number of phytochemicals than leaves and seeds from Chennai had more phytochemicals. Tocopherol and .gamma.-sitosterol in this study were consistent with the study of Sánchez *et al*. (2010). Pyridine-3-carboxamide and 2-myristynoyl-glycinamide have been recognized with medicinal properties and uses against approximately 80 diseases such as cancer, cystic fibrosis, cardiovascular diseases, cell membrane and DNA damage by free radicals, oxidation of low density lipoproteins and disorders of the skin, eye, lungs, and other lipid body constituents (Cohen and Grifo, 2007).

Table 4. Chemical composition of *M. oleifera* seeds

S.No	Madurai Moringa Seeds	Area %	RT
1	Hydroxylamine, o-decyl-	0.01	4.87
2	Docosanoic acid 1-methyl-butyl ester	0.03	5.00
3	Tetradecane, 2,6,10-trimethyl-	0.03	5.36
4	1-Iodo-2-methylundecane	0.04	5.47
5	Dodecane	0.03	5.56
6	Dodecane	0.01	5.84
7	Hexadecane, 2,6,11,15-tetramethyl-	0.05	5.97
8	Oxalic acid	0.06	6.08
9	Heptadecane	0.12	6.28
10	Tridecane	0.03	6.38
11	Nonane	0.03	6.46
12	Heptadecane	0.02	6.55
13	Dodecane	0.03	6.63
14	Cyclohexasiloxane, dodecamethyl-	0.01	6.78
15	Octane, 5-ethyl-2-methyl-	0.04	6.85
16	1-Iodo-2-methylundecane	0.01	6.97
17	Decane	0.01	7.09
18	Heptadecane	0.01	7.31
19	Eicosane	0.01	7.43
20	3-Allyl-6-methoxyphenol	0.01	7.53
21	Eugenol	0.02	7.60
22	Tetradecane	0.09	7.79
23	Heptadecane	0.04	7.96
24	Hexacosane	0.04	8.09
25	2-(4'-Methoxyphenyl)-2-(2'-methoxyphenyl)propane	0.05	8.19
26	Eicosane	0.05	8.31
27	Heptadecane, 9-octyl-	0.06	8.50
28	Nonane	0.19	8.64
29	1,2,3-Benzenetriol nonane	0.05	8.82
30	1,2,3-Benzenetriol	0.03	8.94
31	Decane	0.10	9.05
32	Hentriacontane	0.16	9.15
33	Nonane	0.03	9.29
34	Heptadecane, 9-octyl-	0.03	9.40
35	Phenol, 2,4-bis(1,1-dimethylethyl)	0.06	9.63
36	Hexadecane	0.02	9.73
37	Heptacosane	0.06	9.82
38	Nonadecane	0.02	9.98
39	Docosane	0.05	10.16
40	Hexadecane	0.07	10.28
41	Dodecanoic acid	0.08	10.49
42	1-Hexadecene	0.02	10.64
43	Hexadecane	0.12	10.75
44	Heptadecane	0.04	10.88
45	Diethyl Phthalate	0.07	11.02
46	Docosane	0.06	11.33
47	Hexadecanoic acid	0.07	11.61
48	Eicosane	0.04	11.72
49	Octadecane	0.04	11.81
50	Octacosane	0.05	11.99
51	Eicosane	0.04	11.99
52	Octacosane	0.06	12.19
53	Docosane	0.03	12.29
54	Heptadecane	0.10	12.44
55	Octadecane	0.17	12.61
56	Hentriacontane	0.04	12.75
57	Docosane	0.04	12.85
58	Heptacosane	0.03	13.03
59	Octadecane	0.05	13.18
60	Docosane	0.08	13.36
61	Tricosane, 2-methyl-	0.05	13.51
62	Heptadecane, 3-methyl-	0.03	13.66
63	Tetradecanoic acid	0.06	13.78
64	Tetradecanoic acid	0.15	13.91
65	1-Octadecene	0.03	14.06
66	Octadecane	0.10	14.17
67	Dodecane, 1,1'-oxybis-	0.02	14.26
68	Dodecane	0.02	14.33
69	Cyclododecane	0.01	14.42
70	Cyclononasiloxane	0.04	14.56
71	Cyclohexadecane, 1,2-diethyl-	0.01	14.70
72	Cyclohexadecane	0.01	14.81
73	Eicosane	0.04	14.93
74	Heneicosane	0.06	15.03
75	Methoxyacetic acid	0.02	15.21
76	Heneicosane	0.04	15.30
77	Phthalic acid, butyl isohexyl este	0.25	15.70
78	1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-	0.83	15.92
79	1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-	0.05	16.10
80	1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-	0.04	16.18
81	Triacontane	0.05	16.26
82	Heptacosane	0.15	16.39
83	Hexadecanoic acid	0.11	16.52
84	7,9-Di-tert-butyl-1-oxaspiro(4,5)eca-6,9-diene-2,8-dione	0.04	16.67
85	Benzenepropanoic acid	0.06	16.99
86	Docosane	0.10	17.14
87	Octadec-9-enoic acid	0.10	17.23
88	Dibutyl phthalate	0.37	17.39
89	Hexadecanoic acid	1.48	17.76
90	n-Hexadecanoic acid	6.17	18.24
91	Tricosane	0.48	18.82
92	n-Hexadecanoic acidl-(+)-Ascorbic acid	0.42	19.09
93	n-Hexadecanoic acidl-(+)-Ascorbic acid	0.22	19.21
94	n-Hexadecanoic acid Ethanol	0.49	19.41
95	Hexatriacontane	0.40	19.50
96	9-Octadecenoic acid, methyl ester,(E)-	0.47	19.69
97	l-(+)-Ascorbic acid 2,6-dihexadecanoate	0.38	19.75
98	1-Chloroeicosane	0.15	19.91
99	Dotriacontane	0.25	19.98
100	Heptadecane, 9-octyl-	0.81	20.14
101	Ethyl Oleate	4.93	20.92
102	Oleic Acid	5.12	21.34
103	6-Octadecenoic acid	52.24	22.29
104	Oleic Acid	0.24	23.05
105	Oleic Acid	0.39	23.18
106	Oleic Acid	0.53	23.25
107	Oleic Acid	0.27	23.38
108	Oleic Acid	0.41	23.51
109	Oleic Acid	0.92	23.68
110	Oleic Acid	0.92	24.03
111	Oleic Acid	0.36	24.28
149	.beta.-Tocopherol	0.42	30.21
150	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)	0.07	30.35
151	Ergosta-4,7,22-trien-3.beta.-olAnthraergostatrienol	0.08	30.45
152	17,21-Dimethylheptatriacontane	0.06	30.58
153	6-Chloro-4-phenyl-2-propylquinolin	0.16	30.74
154	Ergosta-4,6,22-trien-3.beta.-ol	0.28	30.95
155	Eicosane, 10-heptyl-10-octyl-Octacosanol	0.10	31.23
156	Vitamin E	0.28	31.36
157	Tetratriacontane	0.06	31.71
158	Cycloprop[7,8]ergost-22-en-3-one,3',7-dihydro-, (5.alpha.,7.beta.,8.alpha.,22E)-	0.05	32.08
159	Cyclodecasiloxane	0.04	32.40
160	Campesterol	0.17	33.15
161	Stigmasterol	0.31	33.76
	Chennai Moringa Seeds		
1	Silane, cyclohexyldimethoxymethyl-	0.11	4.96
2	Tetratetracontane	0.12	5.36
3	Decane, 3,6-dimethyl-	0.16	5.46
4	Dodecane, 2-methyl-	0.12	5.56
5	Decane, 2,4,6-trimethyl-	0.05	5.83
6	Tridecane	0.20	5.96
7	Undecane, 2,4-dimethyl-	0.23	6.08
8	Nonane, 1-iodo-	0.48	6.28
9	Nonadecane	0.10	6.38
10	Dodecane, 4,6-dimethyl-	0.11	6.45
11	Dodecane, 4,6-dimethyl-	0.11	6.55
12	Oxalic acid, 6-ethyloct-3-yl heptyl ester	0.11	6.62
13	Cyclohexasiloxane, dodecamethyl-	0.09	6.78
14	Heptadecane, 8-methyl-	0.22	6.85
15	Nonane, 5-butyl-	0.07	6.97
16	Pentadecane	0.07	7.08
17	Eicosane, 10-methyl-	0.03	7.31
18	Decane, 3,8-dimethyl-	0.07	7.42
19	Phenol, 2-methoxy-3-(2-propenyl)-	0.30	7.54
20	Tetradecane	0.26	7.78
21	Pentadecane	0.21	7.95
22	Dotriacontane	0.20	8.08
23	Caryophyllene	0.17	8.31

24	Pentadecane	0.17	8.50
25	Hexadecane	0.47	8.63
26	2,3-Dimethyldodecane	0.09	8.82
27	Silane, trichlorooctadecyl-	0.41	9.04
28	Heneicosane	1.00	9.14
29	Heneicosane	0.14	9.28
30	Hexadecane	0.13	9.39
31	Phenol, 2,4-bis(1,1-dimethylethyl)	0.42	9.63
32	Docosane	0.40	9.82
33	Pentacosane	0.21	9.97
34	Dodecane	0.15	10.12
35	Heneicosane	0.14	10.27
36	Tetracosane	0.03	10.39
37	Pentacosane	0.06	10.48
38	Tetratetracontane	0.04	10.56
39	1-Hexadecene	0.04	10.63
40	Hexadecane	0.23	10.74
41	Dodecane, 2,6,11-trimethyl-	0.16	10.88
42	Tridecane, 2-methyl-	0.07	10.97
43	Diethyl Phthalate	0.09	11.08
44	Tridecane	0.14	11.25
45	Heptadecane, 9-octyl-	0.21	11.32
46	Dodecane	0.20	11.55
47	Heptadecane	0.10	11.61
48	10-Methylnonadecane	0.12	11.72
49	Benzeneacetic acid, .alpha.,3,4-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester	0.28	11.81
50	Heptacosane	0.19	11.98
51	Tetratriacontane	0.23	12.06
52	Heptadecane	0.32	12.19
53	Heptadecane	0.13	12.28
54	Heptadecane, 9-hexyl-	0.23	12.38
55	Nonacosane	0.31	12.44
56	2-Bromo dodecane	0.96	12.60
57	Heptadecane	0.21	12.74
58	Octadecane	0.17	12.85
59	Eicosane	0.22	13.01
60	2-Bromo dodecane	0.32	13.16
61	Tetracosane	0.52	13.35
62	Heneicosane	0.21	13.51
63	Heptadecane, 3-methyl-	0.14	13.66
64	Octacosane	0.12	13.77
65	3-Octadecene, (E)-	0.14	13.88
66	6-Tetradecanesulfonic acid, butyl ester	0.08	14.07
67	Pentadecane	0.20	14.16
68	Eicosane	0.12	14.25
69	Eicosane, 10-methyl-	0.08	14.33
70	Octacosane	0.04	14.42
71	Cyclononasiloxane, octadecamethyl-	0.25	14.60
72	Decane, 1-iodo-	0.06	14.70
73	Dodecane, 2,6,10-trimethyl-	0.03	14.80
74	Heptadecane	0.19	14.93
75	Octacosane	0.33	15.02
76	Octacosane	0.28	15.28
77	Tetratriacontane	0.04	15.44
78	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.85	15.68
79	Triacontane	0.30	15.80
80	Octadecane	0.33	15.94
81	Docosane, 7-hexyl-	0.14	16.11
82	Heptadecane, 9-octyl-	0.15	16.17
83	Octadecane, 1-iodo-	0.19	16.25
84	Nonacosane	0.87	16.39
85	Hexadecanoic acid, methyl ester	0.39	16.52
86	2H-Isoindole-2-acetic acid, 1,3-dihydro-.alpha.-methyl-1,3-dioxo-, methyl ester	0.38	16.64
87	Nonane, 1-iodo-	0.21	16.93
88	Docosane	0.15	17.02
89	Triacontane	0.44	17.13
90	Decane, 3,8-dimethyl-	0.17	17.21
91	Dibutyl phthalate	1.03	17.37
92	Hexadecanoic acid, ethyl ester	0.61	17.72
93	Octadecane	0.07	17.99
94	Eicosane	0.03	18.08
95	Heptadecane, 2-methyl-	0.14	18.38
96	Heptadecane, 3-methyl-	0.11	18.52
97	Octacosane	0.04	18.65
98	Docosane	0.30	18.78
99	Docosane, 7-hexyl-	0.14	19.04
100	Docosane	0.18	19.25
101	1-Heptadecene	0.25	19.33
102	Heptadecane, 9-octyl-	0.39	19.45
103	9-Octadecenoic acid (Z)-, methyl ester	0.35	19.61
104	2-Nonadecanone	0.28	19.67
105	Docosane, 11-decyl-	0.19	19.75
106	Eicosane	0.17	19.86
107	Triacontane	0.27	19.94
108	Hentriacontane	0.88	20.11
109	1-Ethylsulfanylmethyl-2,8,9-trioxa-5-aza-1-sila-bicyclo[3.3.3]undecane	0.72	20.23
110	Ethyl Oleate	4.33	20.77
111	Dodecane, 1,1'-oxybis-	0.78	20.85
112	Oleic Acid	0.52	20.95
113	Oleic Acid	0.35	21.09
114	Octadecanoic acid, ethyl ester	0.59	21.19
115	Oleic Acid	0.35	21.31
116	Oleic Acid	0.22	21.43
117	Octadec-9-enoic acid	0.25	21.53
118	6-Octadecenoic acid, (Z)-	0.35	21.82
119	6-Octadecenoic acid, (Z)-	0.26	21.89
120	Oleic Acid	0.14	22.05
121	Eicosane	0.20	22.14
122	Oleic Acid	0.28	22.27
123	Docosane, 11-decyl-	0.18	22.37
124	Cyclononasiloxane, octadecamethyl-	0.29	22.45
125	9-Octadecenoic acid, (E)-	0.16	22.57
126	Octadec-9-enoic acid	0.16	22.65
127	Oleic Acid	0.16	22.74
128	Docosane	0.13	22.81
129	Hentriacontane	0.63	22.96
130	Heptadecane, 2,6,10,15-tetramethyl	0.83	23.09
131	2-t-Butyl-6-ethylidene-5-(1-hydroxyethyl)[1,3]dioxan-4-one	1.42	23.32
132	Docosane	0.73	23.51
133	Heneicosane	0.82	23.58
134	.beta.-Sitosterol	0.55	23.68
135	Stigmasterol, 22,23-dihydro-	2.30	23.88
136	9-Octadecenamide, (Z)-	0.93	24.03
137	Heneicosane	0.47	24.16
138	9-Octadecenoic acid, (E)-	0.43	24.28
139	Hexasiloxane, tetradecamethyl-	0.55	24.35
140	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	0.47	24.49
141	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	0.72	24.56
142	Stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z)-	1.07	24.67
143	S-[2-[N,N-Dimethylamino]ethylpyrollidine-N-carbonylthiocarbohydroximate	1.19	24.82
144	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2.76	24.98
145	Triacontane, 1-bromo-	1.55	25.21
146	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	2.08	25.32
147	Triacontane, 1-bromo-	0.92	25.41
148	Eicosane, 9-octyl-	1.25	25.49
149	1,2-Benzenedicarboxylic acid, diisooctyl ester	1.70	25.55
150	9-Octadecenamide, (Z)-	3.79	25.79
151	Nonadecane	1.25	25.94
152	Tricosane	0.37	26.10
153	9-Octadecenoic acid, (E)-	0.39	26.16
154	9-Octadecenoic acid, (E)-	1.26	26.24
155	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.98	26.40
156	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	1.02	26.46
157	Dotriacontane, 1,32-dibromo-	0.51	26.57
158	Nonadecane	1.33	26.68
159	Heptadecane, 2-methyl-	1.20	26.79
160	Propionamide, 2,2-dimethyl-N-(5,6,7,9-tetrahydro-9-oxo-1,2,3,10-tetramethoxybenzo(a)heptalen-7-yl)-, (S)-	4.48	27.15
161	13-Docosenamide, (Z)-	13.62	27.45
162	9-Octadecenamide, (Z)-	0.80	27.53
163	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-, (all-E)-	0.97	27.63
164	17,21-Dimethylheptatriacontane	0.35	27.84
165	17,21-Dimethylheptatriacontane	0.74	27.90
166	Eicosane	0.42	28.15
167	1-Nonadecene	0.50	28.38

168	Hentriacontane	0.30	28.53
169	Octadec-9-enoic acid	0.27	28.63
170	Cyclodecasiloxane, eicosamethyl-	0.74	28.78
171	Tetratriacontane, 17-hexadecyl-	0.28	28.94
172	Tricosane	0.20	29.04
173	1-Nonadecene	0.16	29.16
174	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	0.30	29.38
175	.gamma.-Tocopherol	0.28	30.20
176	Cyclononasiloxane, octadecamethyl-	0.21	30.34
177	1-Nonadecene	0.04	30.50
178	Ascorbyl Palmitate	0.07	30.88
179	Tetrapentacontane, 1,54-dibromo-	0.08	31.09
180	Vitamin E	0.59	31.32
181	17,21-Dimethylheptatriacontane	0.06	31.67
182	Cyclononasiloxane, octadecamethyl-	0.21	32.37
183	Campesterol	1.21	33.10
184	Pyridine-3-carbonitrile, 1,2-dihydro-6-(1,3-benzodioxol-5-yl)-2-oxo-4-phenyl-	0.62	33.30
185	Stigmasterol	1.44	33.69

FTIR analysis

Leaves and seeds from both regions had similar fingerprints but different functional groups. The seeds had more functional groups than the leaves. The results confirmed the presence of primary alcohol, secondary alcohol and phenol in the range 3800-2000 (wavenumber). Alkanes, aldehyde, aromatic compound, aromatic amines and halogen compounds were present in the range 2000-1000 in leaves and 2000-800 in seeds. The alkynyl and carbonyl peaks were common in case of seed and leaf extracts. Amine peak is observed in case of seeds from Madurai whereas alcohol/phenol peak in seeds from Chennai. Apart from these numerous new peaks were observed in the finger print region of both leaf and seed from Madurai and Chennai. In leaves from both Madurai and Chennai similar peaks were found at 1891.190, -0.000; 1607.456, 658.740; 1400.314, 14.189; 1313.259, 31.578; 1238.598, 126.757; 1020.719, 2673.696. In seeds from both the regions similar peaks were found at 2107.539, 0.0739; 1743.103, 143.232; 1642.424, 1999.837; 1534.885, 643.529; 1447.072, 434.725; 1230.938, 573.831; 1047.216, 3128.011; 787.752, 234.920 (Fig. 1). Total phenols present in Moringa leaves extracts were reported using FTIR spectrum which showed intense bands characteristic of phenol groups. In Moringa major bands between 625-581 cm^{-1} and minor bands between 560 and 400 cm^{-1} in FTIR spectra of starch was attributed to skeletal modes of glucose pyranose ring since starch exhibit very similar spectra characteristics with glucose in this region (Adebisi *et al.*, 2014).

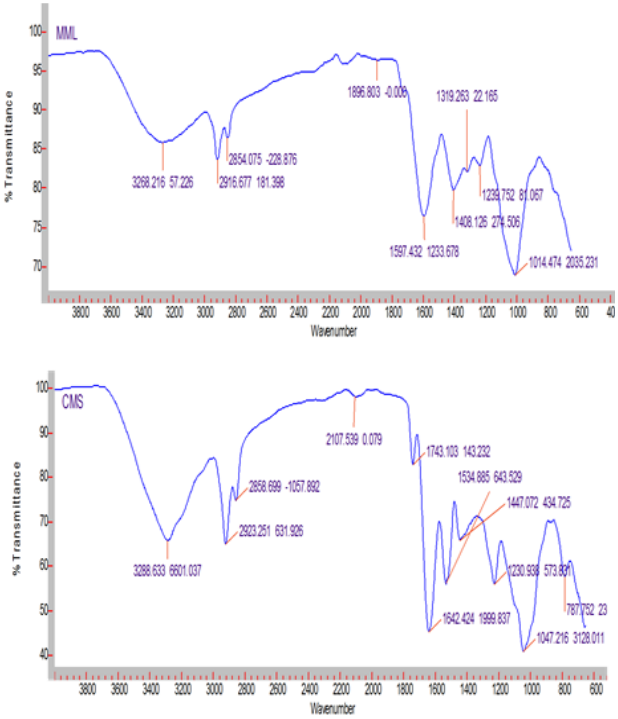

Figure 1. FTIR analysis of *M. oleifera* leaves and seeds

Conclusions

In conclusion, the presence of numerous secondary metabolites and high content of phytochemicals with other essential components could be useful to design food products for consumers with specific needs, to ensure the practice of traditional medicine and suggest new areas of research. The difference in the activity of Moringa based on geographical locations will broaden the boundaries to explore.

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