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Volatile Oils And Fatty Oils From Leaves And Seeds Of Moringa Olifera Lam. Cultivated In Southeast Vietnam

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ABSTRACT

Volatile oils and fatty oils from leaves and seeds of Moringa oleifera Lam. varieties cultivated in Ho Chi Minh City (HCM) Dong Nai province (DN), and Ninh Thuan province (NT) were extracted and evaluated. The volatile oil was extracted by hydrodistillation from 4-6 hours. The fatty oil of seeds was extracted by solvent extraction with hexane for 9 hours. The chemical composition of the oils was analysed by Gas Chromatography-Mass Spectrometry (GC/MS) and Gas Chromatography-Flame Ionization Detector (GC/FID) methods. The leaf/seed oil contents of HCM, DN, and NT varieties were obtained with yields of 0.10%/0.19%, 0.04%/0.13%, and 0.07%/0.15%, respectively. The leaf oil of the HCM variety contained 15 constituents of which the major is 7-isoprenyl oxycoumarin (26.11%) followed by n-tricosane (21.27%), n-tetracosane (15.65%), trans-nonadecene (8.14%), geranial (7.59%), and neral (6.89%). The leaf oil of DN variety contained 45 constituents of which the major is limonene (38.99%) followed by 7-isoprenyl oxycoumarin (26.11%), n-pentacosane (5.86%), and n-heptacosane (5.67%). The leaf oil of NT variety contained 26 constituents of which the major is 7-isoprenyl oxycoumarin (20.37%) followed by coumarin (18.12%), n-tricosane (16.54%), cabreuva D oxide (14.67%), and n-tetracosane (10.16%). M. oleifera plants of three varieties gave volatile seed oils containing particularly a homologous series of alkanes from C12 to C34 (58.05-81.28%). The seed fatty oils of three M. oleifera varieties obtained by solvent extraction with hexane gave 31-38% of the content. Their chemical compositions presented mainly C12-C26 fatty acids including saturated fatty acids (23-24%) and unsaturated fatty acids (64-77%) dominated by omega-7&9 fatty oils (60-76%).

Keywords: Moringa, Moringa oleifera, Moringaceae, volatile oils, limonene, coumarin, oleic acid, fatty oil, omega fatty acid, chemical composition.

1. INTRODUCTION

Moringa oleifera Lam. species (Moringacea) is cultivated widely and native to the sub-Himalayan tracts of India, Pakistan, Bangladesh, and Afghanistan. This small and rapidly-growing tree named as “drumstick” tree in India, “Moringa” or “Ben oil” tree in English, “horseradish” tree in Florida, “benzolive” tree in Haiti, “kelor” tree in Balinese, “arunggai” tree in Pagasinan, mulangay tree in Tagalog, nébéday (Senegal) tree in French, “saijan/saragvo/gujarati” tree in Hindi, “sajna” tree in

Bengali, “La mu” tree in Chinese, and “chùm ngây” tree in Vietnamese. It was utilised commonly by the ancient Romans, Greeks, and Egyptians. It is now widely cultivated and has become naturalised in many locations in the tropics. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses.[1-4] Because of its high nutrient values[1,3,5-7], high bioactivity[4,8-12], and its coagulability in water[11,13-20], the moringa tree has become one of the miracle trees for humans in the world. It has been used for nutrition, traditional medicine, nutraceutical purposes, water purifying, and industrial uses.[3,21]

Previous research showed that the chemical constituent of moringa trees is coupled with their ubiquitous distribution in the tree. Therefore, all parts of the plant are edible. The leaf contains vitamins (A, C, E, K, B2, B6, B12), macro-elements (Na, Ca, K, P, ..), micro-elements (Fe, Mg, Zn, ..), proteins, fibers, and carbohydrates at richer source levels than any other food plants contains those.[7,8,21,22] Previous researches reported that from 100 g of dry leaves of M. oleifera can be obtained 7 times more vitamin C than from oranges, 10 times more vitamin A than from carrots, 17 times more calcium than in milk, 9 times more protein than in yogurt, 15 times more potassium than from bananas, and 25 times more iron than the obtained from spinach.[23-25]

Besides the nutritional value, the essential oils from moringa leaves have been interested by scientists.[8,9,10,21] By the mean of GC/MS and even Headspace-Solid Phase Microextraction (HS-SPME), the volatile compound of M. oleifera leaves from Mozambique, Taiwan, Brazil, China, Rwanda, were identified.[9,10,21] In these, the bioactivity of the essential oil was also determined. Chuang et al.[9] reported steam distillation of dried moringa leaves collected in Taiwan giving a clear brown essential oil yielding 0.24%. The analysis of the oil by GC/MS presented a total of 44 compounds dominated by pentacosane (17.41%), hexacosane (11.20%), (E)-phytol (7.66%), and 1-(2,3,6-trimethylphenyl)-2-butanone (3.44%) in the chemical composition. Barreto et al.[10] reported the chemical composition of essential oils from leaves collected in Brazil was rich in phytol (21.6%) and thymol (9.6%). Mukunzi et al.[21] presented the volatile profile analysis of moringa leaf samples from China and Rwanda by using HS-SPME and GC/MS methods. This work reported that a total of 93 volatiles consisting of aldehydes, alcohols, ketones, hydrocarbons, esters, terpenoids, and acids were identified. A total of 61 compounds were contained in the sample from China in which acetic acid (12.54% of total volatiles) played as the most abundant volatile compound. Rwandan samples contained 59 compounds having hexanoic acid (19.81% of total volatiles) as the most abundant one. Marrufo et al.[8] investigated the leaf essential oil of moringa grown in Mozambique. The chemical composition studied using GC and GC/MS analysis showed that hexacosane (13.9%), pentacosane (13.3%), and heptacosane (11.4%) were the main components.

Another important part of the moringa tree that can be mentioned is the seed, especially its fatty oils. Most previous studies showed that moringa seed oil has a very high nutritional value. The seed of the fatty oil amount from 2% to 4%.[3,5,15,16,26-28]. Lala et al. [29] reported the oil from moringa seeds variety Periyakulam 1 cultivated in India extracted using three different procedures including cold press, extraction with hexane, and extraction with a mixture of chloroform: methanol (1:1). The oil was found to contain high levels of unsaturated fatty acids, especially oleic (up to 71.60%). In addition, the dominant saturated acids as palmitic and behenic (both up to 6.4%) were presented. Rashid et al. [28] evaluated moringa seed oil for the first time as a potential feedstock for biodiesel. In this work, moringa oil was proven a high-oleic acid oil (>70%). Bhatnagar and Gopala Krishna [30] conducted that moringa seeds are endowed with a high oil content (~39%) which resembles olive oil because of its high oleic acid content (~65-75%). Leone et al. [31] stated that moringa seeds are a promising resource for food and non-food applications, due to their content of monounsaturated fatty acids with a high monounsaturated/saturated fatty acids ratio, sterols, and tocopherols, as well as proteins rich in

sulfated amino acids. This report was proved by investigations of the moringa seed oil studied by many scientists. Aly et al. [26] presented the profile of Egyptian moringa seed oils. Results showed that the oil was found to contain high levels of unsaturated fatty acids, especially omega-9-fatty acid (oleic) (up to 76.29 %). The dominant saturated acids were reported including palmitic acid, stearic acid, and arachidic acid (the three up to 12.66 %). Iranloye et al. [32] established the potential of moringa oil to improve the stability and nutritional quality of soybean oil. The soybean oil and moringa seed oil were extracted by using Soxhlet extraction techniques. The results showed that blending soybean/moringa oil offers a better option than the use of partial hydrogenation in commercial soybean oil.

Although the value brought by moringa species has been proven by several scientific reports, the moringa tree cultivated commonly for using leaves for food and tea without studying scientifically in Vietnam. Also, there has been little known in the literature about the composition of the leaf essential oil and seed oil as well as their bioactivities. In this paper, a study of the chemical composition of the essential oil hydrodistillated from leaves/seeds and fatty oil extracted from seeds of M. oleifera Lam. varieties (Figure 1) grown in the Southeast Vietnam is reported for the first time.

Figure 1. Moringa plant parts: (A) fruits and seeds; (B) branches, twigs, and leaves

2. RESULTS AND DISCUSSION

2.1. Extraction of essential oils

The essential oils of ground fresh leaves and seeds of M. oleifera were distilled by hydrodistillation method for 4-5 hours by Clevenger apparatus. The contents of these oils are given in Table 1.

Table 1. Contents of leaf and seed essential oils of M. oleifera collected from various locations in Southeast Vietnam.

Location	Contents of essential oils (%)	
	Leaves	Seeds
HCM	0.10 ± 0.010	0.19 ± 0.010
DN	0.04 ± 0.001	0.13 ± 0.002
NT	0.07 ± 0.002	0.15 ± 0.020

As shown in Table 1, the extraction result presented that the essential oils existed in leaves of M. oleifera at lower concentrations than those in seeds. In addition, the content of essential oils extracted from seeds and leaves of HCM samples was higher than other samples.

The chemical compositions of volatile essential oils of M. oleifera were analysed by GC/MS and GC/FID methods. A total of 49 and 32 compounds of essential oils from leaves (Table 2) and seeds (Table 3) were identified, respectively.

Table 2. Chemical compositions of leaf essential oils of M. oleifera collected from various locations in Southeast Vietnam.

Compounds	LRI		% GC			Method of identification
	Lit.[34]	Obs.	HCM	DN	NT	
Hydrocarbon compounds			**54.12**	**62.78**	**40.29**	
Monoterpene hydrocarbons			**4.41**	**40.56**	**3.78**	
β-Pinene	974	975	0.58		2.74	LRI, MS
β-Myrcene	988	984	0.87	0.73		LRI, MS
Limonene	1024	1026	2.97	38.99	0.29	LRI, MS
(Z)-β-Ocimene	1032	1033			0.45	LRI, MS
(E)-β-Ocimene	1044	1042			0.30	LRI, MS
γ-Terpinene	1054	1052		0.84		LRI, MS
Sesquiterpene hydrocarbons			**1.82**	**0.91**	**2.10**	
β-Elemene	1389	1385	1.82	0.14	2.10	LRI, MS
trans-Caryophyllene	1417	1414		0.42		LRI, MS
γ-Cadinene	1513	1509		0.34		LRI, MS
Straight chain alkanes			**39.74**	**20.68**	**28.83**	
n-Tetradecane	1400	1400		0.10	0.24	LRI, MS
n-Nonadecane	1900	1900		0.55		LRI, MS
n-Eicosane	2000	2000		0.20		LRI, MS
n-Heneicosane	2100	2100		2.04		LRI, MS
n-Docosane	2200	2200		0.21		LRI, MS
n-Tricosane	2300	2300	21.27	0.95	16.54	LRI, MS
n-Tetracosane	2400	2400	15.65	0.38	10.16	LRI, MS
n-Pentacosane	2500	2500	1.10	5.86	0.91	LRI, MS
n-Hexacosane	2600	2600		0.31	0.38	LRI, MS
n-Heptacosane	2700	2700	1.72	5.67	0.32	LRI, MS
n-Octacosane	2800	2800		0.25	0.17	LRI, MS
n-Nonacosane	2900	2900		4.16	0.11	LRI, MS
Alkenes			**8.14**	**0.64**	**5.57**	
1,21-Docosadiene		2428		0.54	2.79	MS
trans-Nonadecene		2471	8.14	0.10	2.79	MS
Oxygenated compounds			**45.37**	**37.22**	**59.71**	
Oxides				**1.18**	**15.81**	
cis-Linalool oxide	1063	1065			0.51	LRI, MS
trans-Linalool oxide	1084	1081		0.38	0.63	LRI, MS
Cabreuva B oxide	1462	1461		0.45		LRI, MS
Cabreuva C oxide	1466	1467		0.17		LRI, MS

Cabreuva D oxide	1479	1472		0.18	14.67	LRI, MS
Alcohols			**0.83**	**0.76**	**3.42**	
Linalool	1095	1096		0.16	3.35	LRI, MS
α-Terpineol	1186	1188	0.83	0.43	0.07	LRI, MS
n-Hexadecanol	1874	1875		0.17		LRI, MS
Carbonyls			**17.93**	**3.00**	**1.50**	
n-Nonanal	1100	1101	3.44	0.38	0.12	LRI, MS
Neral (citral b)	1235	1231	6.89	0.78	0.13	LRI, MS
Geranial (citral a)	1264	1262	7.59	1.54	1.25	LRI, MS
Octadecanal		2225		0.31		MS
Phenylpropanoids				**1.13**		
β-Ionol	1412	1412		0.41		LRI, MS
(Z)-Asarone	1616	1612		0.71		LRI, MS
Coumarins			**26.61**	**27.77**	**38.49**	
Coumarin	1432	1432		0.21	18.12	LRI, MS
7-Isoprenyl oxycoumarin	2115	2112	26.11	27.05	20.37	LRI, MS
Osthole	2140	2137	0.49	0.51		LRI, MS
Esters				**3.09**	**0.49**	
Isoamyl 3-(2-furan)propionate	1430	1426		0.27		LRI, MS
(2E)-Hexenyl phenyl acetate	1635	1636		0.25		LRI, MS
Eudesm-7(11)-en-4-yl acetate	1839	1837		0.29		LRI, MS
(Z)-Lanceol acetate	1854	1853		0.29		LRI, MS
Methyl hexadecanoate	1921	1925		0.17		LRI, MS
Geranyl benzoate	1951	1951		1.39		LRI, MS
Methyl linoleate	2095	2095		0.23		LRI, MS
Bis(2-ethylhexyl) phthalate		2524		0.21	0.49	MS
Lactones				**0.30**		
Cyclopentadecanolide	1832	1832		0.30		LRI, MS

Notes: Percentage of FID peak area normalization without the use of the response factor. LRI (linear retention index) for ZB-5 [Lit.: literature[34], Obs.: observed].

As shown in Table 2, the typical compound groups of leaf oil from three M. oleifera including HCM, DN, and NT varieties were identical, accounting for 15 components (7 functional groups), 45 components (10 functional groups), and 26 components (8 functional groups), respectively. The difference in the number of components/compound groups came from the difference in the geographical site. M. oleifera DN variety studied grew in the area belonging to Long Khanh, a town of Dong Nai province and a trading hub between provinces of the Central Highlands and the Southeast regions where moringa trees were planted on basaltc soil with from medium to heavy soil texture, having a high total content of clay, rich organic substance, medium to rich macronutrient content and very high micronutrient content.[35] The geography, climate, and red soil of Long Khanh have created the specific quality for plant growth. Despite belonging to Southeast Vietnam, Ninh Thuan province is located in the hottest area of Vietnam. The geological structure of Ho Chi Minh City is weak soil, high groundwater, and surrounded by a system of canals and rivers. Thus, plant growth of M. oleifera species in Ho Chi Minh City is more suitable compared to that in Ninh Thuan province. This feature was proved by the higher content of HCM seed oils than the other oils studied (Table 1).

Significant substances among the major compounds in the chemical composition of leaf oils (Table 2) were coumarin and its derivatives (7-isoprenyl oxycoumarin and osthole, Figure 2), which both forms account for 26.61% from HCM variety, for 28.50% from DN variety, and for 38.49% from NT variety.

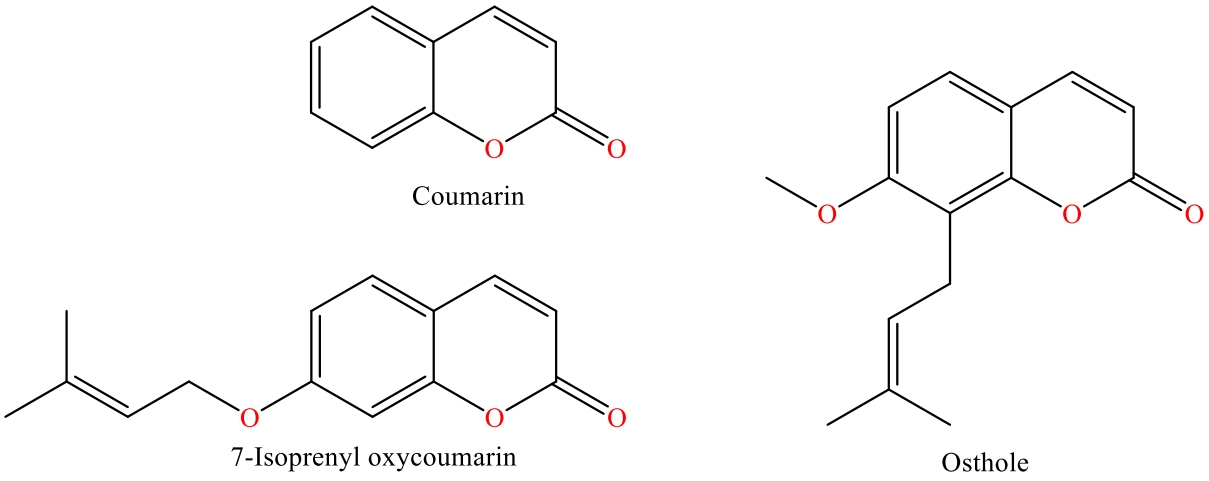


Figure 2. Coumarin and its derivatives

Besides coumarins, in DN variety, mono-sesquiterpenes were found in high concentration (> 42%), which limonene (Figure 3) dominated as a major constituent (40.02%), while in HCM and NT varieties, these terpenes were found in small amounts. n-Nonanal (3.34%) and monoterpenoids (Figure 3) such as neral (6.89%) and geranial (7.59%) were the dominant components in HCM leaf oils, while β-pinene (2.74%) and linalool (3.35%) as dominant ones belonging monoterpenes in NT leaf oils.

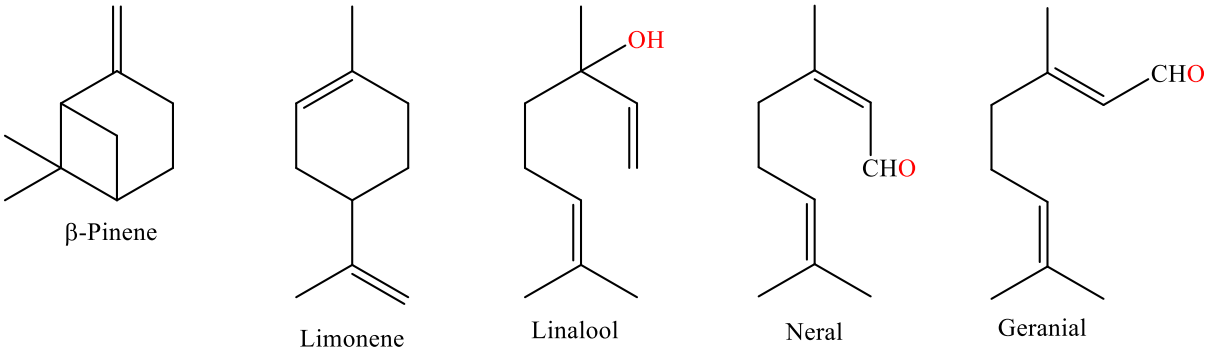


Figure 3. Some monoterpene hydrocarbons and oxygenated monoterpenes were found in leaf oils as main constituents.

Three cabreuva oxides B-D (Figure 4), oxygenated nerolidol derivatives, were found in DN leaf oils in small quantities, while only cabreuva oxide D presented a percentage of 14.46% in NT leaf oils. Until now, there have been three reports concerning the presence of these oxides in the essential oil.

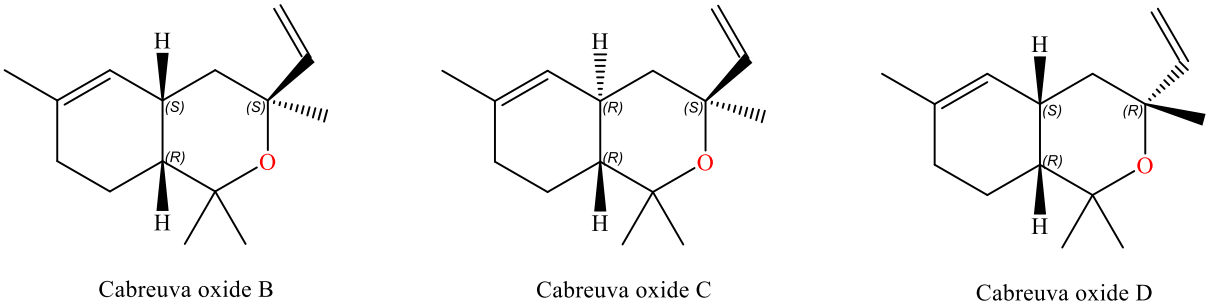


Figure 4. Cabreuva oxides

As shown in Table 2, alkanes were also mentioned as the dominating compounds occurring in leaf oils extracted from moringa varieties studied.

The chemical compositions of moringa leaf essential oils from southeast Vietnam and other countries such as Mozambique[8], Brazil[10], Taiwan[9], Rwanda, and China[21] conducted from similar comparative studies are identical in the abundant chemical ingredients. Marrufo et al[8] identified 29 compounds accounting for 92.3% of the total leaf oil of Mozambique moringa. The chemical composition of this oil was dominated by 91.1% of hydrocarbons. Mukunzi et al.[10] reported that phytol (21.6%) and thymol (9.6%) as the most abundant compounds in the leaf oils of M. oleifera from Brazil, while Chuang et al.[9] reported that C_{13}-C_{26} alkanes (30.91%, pentacosane (17.4%), hexacosane (11.2%), and (E)-phytol (7.7%) were presented as major components in the leaf oils of M. oleifera from Taiwan. Mukunzi et al[21] reported that the most abundant compounds of the oil from M. oleifera leaves collected in Rwanda and China were hexanoic acid (19.8%), and acetic acid (12.5%), respectively. Besides the climate conditions and geographical conditions, the large difference in the main components is also due to the differences between the fresh and dried materials. The characteristic feature of moringa leaf oils from Southeast Vietnam can be distinguished from other countries by a high concentration of limonene as well as by the presence of coumarin and its derivative. Coumarins have not been recorded and reported from Moringaceae family before.[33].

The chemical composition of seed essential oils of M. oleifera analysed by GC/MS (Table 3) reveals that the products extracted by hydrodistillation of moringa were not real essential oils because of lacking terpenes. The ingredient of the oils was almost dominated by alkanes, carboxylic acids, and the following by esters.

Table 3. Chemical compositions of seed essential oils of M. oleifera from various locations in Southeast Vietnam.

Name of compounds	LRI		% GC			Method of identification
	Lit.[34]	Obs.	HCM	DN	NT	
Alkanes			**58.12**	**76.32**	**81.29**	
Branched alkanes			**0.08**	**5.96**		
2,2,4,6,6-Pentamethylheptane		983	0.08	3.23		MS
2,2,4,4-Tetramethyloctane		1020		0.45		MS
1,2,4,5-Tetramethylbezene		1114		0.94		MS
2,6,10-Trimethyldodecane		1375		1.34		MS
Straight-chain alkanes (C12-C34)			**58.04**	**70.36**	**81.29**	
n-Dodecane	1200	1200	0.07	2.26		LRI, MS
n-Tridecane	1300	1300	0.03	1.03		LRI, MS
n-Tetradecane	1400	1400	0.77	3.93	1.04	LRI, MS
n-Pentadecane	1500	1500	1.48	3.45	3.21	LRI, MS
n-Hexadecane	1600	1600	16.19	3.91	35.48	LRI, MS
n-Heptadecane	1700	1700	0.37	2.82	0.69	LRI, MS
n-Octadecane	1800	1800	Trace	1.81		LRI, MS
n-Eicosane	2000	2000	0.08	2.43	Trace	LRI, MS
n-Heneicosane	2100	2100	17.25	2.34	Trace	LRI, MS
n-Docosane	2200	2200	0.14	3.54	Trace	LRI, MS
n-Tricosane	2300	2300	1.42	4.16	Trace	LRI, MS
n-Tetracosane	2400	2400	0.09	5.19	2.59	LRI, MS

n-Pentacosane	2500	2500	Trace	3.96	0.22	LRI, MS
n-Hexacosane	2600	2600	0.17	2.29	0.35	LRI, MS
n-Heptacosane	2700	2700	0.02	3.75	Trace	LRI, MS
n-Octacosane	2800	2800	0.08	4.21	0.2	LRI, MS
n-Nonacosane	2900	2900	0.02	3.82	Trace	LRI, MS
n-Triacontane	3000	3000	16.2	3.89	27.9	LRI, MS
n-Untriacontane	3100	3100	0.23	2.81	0.65	LRI, MS
n-Dotriacontane	3200	3200	3.21	4.04	8.35	LRI, MS
n-Tritriacontane	3300	3300	0.22	2.62	0.61	LRI, MS
n-Tetratriacontane	3400	3400	Trace	2.1	Trace	LRI, MS
Carboxylic acids			**10.24**	**14.5**	**18.47**	
2-Hexadecenoic acid		1761	Trace	2.47	Trace	MS
1,2-Benzenedicarboxylic acid		2526	10.24	12.03	18.47	MS
Esters			**31.37**	**4.04**		
Butyl-2-methylpropyl 1,2-benzenedicarboxylate		1856		0.65		MS
Dibutyl phthalate		1952	31.37	3.39		MS

Notes: Trace: < 0.01%.

Hydrodistillation of moringa seed powder gave the oil, which was rich in hydrocarbons, especially in a homologous series of alkanes (C12-C34) at very high concentration (58-81%).

2.2. Extraction of fatty oils

Each fatty oil from seed moringa collected in Ho Chi Minh, Dong Nai, and Ninh Thuan was obtained by macerating 100 g dried powder with 1000 mL of hexane for 9 hours in the Soxhlet apparatus. The contents of the fatty oils were studied and shown in Table 4.

Table 4. Contents of fatty oils of M. oleifera seeds collected from various locations in Southeast Vietnam

Location	HCM	DN	NT
Contents of fatty oils (%)	31.28 ± 0.457	33.27 ± 0.369	38.08 ± 0.100

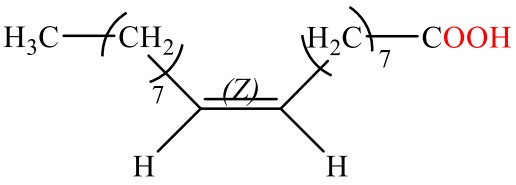
The oil obtained was stored and was subjected further for the preparation of fatty acid methyl esters (FAMEs) for GC. The marc of M. oleifera powder was used for the next studies. With the content being from 310-380 g kg^{-1}, the seed oils of M. oleifera varieties cultivated in Southeast Vietnam were found to agree well with previous literature reports on Indian M. oleifera varieties in Sri Lanka and India, 394 g kg^{-1} and 383 g kg^{-1}, reported by Bhatnagar [30] and Tsaknis [29], respectively.

The seed oils extracted from M. oleifera varieties methylated before using GC/MS for analysis of the chemical composition. The fatty acid profiles of HCM, DN, and NT oils are presented in Table 5. A total of 20 fatty acids were identified. The fat ingredient of oils was dominated by unsaturated fatty acids (64-77%).

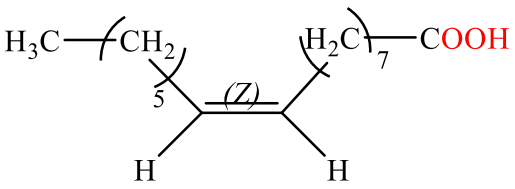
Table 5. The fatty acid profiles of seed oil extracted from M. oleifera from various locations in Southeast Vietnam.

No	Fatty acids		C/C=C	% GC		
	IUPAC name	Common name		HCM	DN	NT
1	n-Dodecanoic acid	Lauric acid	C12:0	0.06	0.45	0.06
2	n-Tetradecanoic acid	Myristic acid	C14:0	0.20	0.67	0.22
3	n-Hexadecanoic acid	Palmitic acid	C16:0	7.43	8.08	6.61
4	(9Z)-Hexadecenoic acid	Palmitoleic acid Omega-7-fatty acid	C16:1	2.07	1.78	2.19
5	(Z)-7-Hexadecenoic acid		C17:1	0.14		0.10
6	(E)-7-Hexadecenoic acid		C17:1			0.08
7	n-Octadecanoic acid	Stearic acid	C18:0		6.49	
8	(Z)-9-Octadecenoic acid	Oleic acid Omega-9-fatty acid	C18:1	59.31	72.73	57.83
9	(E)-10-Octadecenoic acid		C18:1			0.08
10	(9Z,12Z)-9,12-Octadecadienoic acid	Linoleic acid	C18:2		0.77	
11	n-Eicosanoic acid	Arachidic acid	C20:0	5.19	3.16	2.33
12	(Z)-11-Eicosenoic acid		C20:1	2.84		3.11
13	(Z)-13-Eicosenoic acid	Paullinic acid Omega-7-fatty acid	C20:1	0.14	1.50	0.21
14	14-Methyleicosanoic acid		C21:0	0.09		0.09
15	n-Docosanoic acid	Behenic acid	C22:0	8.05	4.37	7.74
16	21-Methyldocosanoic acid	Isotricosanoic acid	C23:0			0.12
17	Tricosanoic acid	Tricosylic acid	C23:0	0.15		
18	Tetracosanoic acid	Lignoceric acid	C24:0	2.39		6.05
19	Pentacosanoic acid	Hyenic acid	C25:0			0.08
20	Hexacosanoic acid	Cerotic acid	C26:0			0.26
	Saturated fatty acids			23.56	23.22	23.54
	Unsaturated fatty acids			64.51	76.78	63.60
	Omega fatty acids			61.53	76.01	60.23
	- Omega-7 fatty acids			2.21	3.28	2.40
	- Omega-9 fatty acids			59.31	72.73	57.83

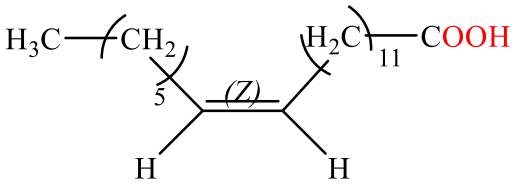
Oleic acid, palmitic acid, behenic acid, arachidic acid were the major unsaturated fatty acids. Stearic acid was only presented in the DN variety oil sample. The characteristic feature of high-oleic oils is the clear dominance of moringa seed fatty oils. As well as the previous studies[3,5,15,16,26-28], the predominant fatty acid in the seed oils extracted from moringa of HCM, DN, and NT varieties was oleic acid, classified as a monounsaturated omega-9 fatty acid (Figure 4), making up 59.31%, 72.73%, and 57.83%, respectively. Palmitoleic acid and paullinic acid, omega-7 fatty acids (Figure 5), were found presence in three oil samples in small amounts. These results show that, with the same climatic conditions, the formation of fatty oils in moringa plants is similar. Omega-9 fatty acids are believed to have many beneficial effects and are a healthy choice for cooking, while omega-7 fatty acids have beneficial health effects, such as increasing levels of HDL cholesterol and lowering levels of LDL cholesterol. The extraction product presenting the nutritional value of M. oleifera seed oils which is similar to olive oils.[36-38] This human nutrition has attracted the attention of several researchers in the production of both food purposes and non-food purposes such as biodiesel.[27,28]



Oleic acid
Omega-9 fatty oil



Palmitoleic acid
Omega-7 fatty oil



Palmitoleic acid
Omega-7 fatty oil

Figure 5. Omega fatty oils

3. EXPERIMENTAL

3.1. Plant materials

The moringa materials were collected in 2015 March (from Long Khanh-Dong Nai province) and in 2021 April (from Cu Chi-Ho Chi Minh city and Phan Rang-Ninh Thuan). The fresh leaves and fresh powdered coat-removed seeds were used for hydrodistillation of essential oils. The dried powdered seed was used for the extraction of fatty oils with hexane solvent.

3.2. Isolation of essential oils and fatty oils

Hydrodistillation of essential oils: 100 g of each sample of fresh ground material was charged into a 2000 mL round flask. The Clevenger apparatus was set up for hydrodistillation. The leaf oil was distilled with 1200 mL of water for 4 hours. A volume of 1000 mL of water was added to the seed sample for hydrodistillation for 5 hours. Each of the distillate oil samples was extracted with three 10 mL portions of diethyl ether. Anhydrous sodium sulfate was used for drying ethereal solutions and removed. The ether was recovered from the filtrate by rotary evaporation. The resulting essential oils were weighed for calculating the yields and analyzing the chemical compositions.

Extraction of fatty oils: The mature fresh pods of moringa trees were obtained from the farm in Long Khanh-Dong Nai province, Vietnam. Moringa seeds were collected manually from the pod. The seed wings and coat were removed. The kernels were dried at room temperature for many days until constant weight. The dried moringa seed was grounded in a domestic blender and sieved through a 180 μm stainless steel sieve. 100 g of dried moringa seed powder and 1000 mL of hexane were charged into an extraction thimble and round bottom flask, respectively. A 2000 ml Soxhlet apparatus was set up for the extraction of fatty oils. The heating for extraction took 9 hours. The hexane solution was filtered for removing the solid and separated from the moringa extract by the rotary evaporator.
All experiments were performed in triplicate.

3.3. GC analyses

Preparation of FAMEs for GC: Fatty acids in moringa seed oils were methylated in the presence of BF3 in methanol according to the procedure published by the Vietnamese standard (QCVN 4-4:2010/BYT).[39,40]

Gas Chromatography-Mass Spectrometry (GC/MS)

The GC/MS analysis of essential oil was performed on an Agilent gas chromatography 7890A, equipped with a Phenomenex 7HG-G010-11 Zebron ZB-5 GC capillary column (30 m x 0.32 mm x 0.25 µm), coupled to an Agilent Mass Selective Detector 5975C VL MSD Triple-Axis. Helium was used as the carrier gas at a flow rate of 1.20 mL/min. The constant pressure mode at 13.209 psi was chosen for the GC program. The injection temperature was 250 ^{o}C; the injection volume was 1.0 µL; the split ratio was 1:25, and the ionization voltage was 70 eV. The oven temperature was programmed from 60 ^{o}C to 240 ^{o}C at the rate of 3 ^{o}C/min. The detector was set at 250 ^{o}C.

The GC/MS analysis of FAMEs was performed on an Agilent gas chromatography 7890A, equipped with a DB-23 capillary column (60 m x 0.25mm x 0.15 µm), coupled to an Agilent Mass Selective Detector 5975C VL MSD Triple-Axis. Helium was used as the carrier gas at a flow rate of 0.9 mL/min. The constant pressure mode at 13.209 psi was chosen on the GC program. The injection temperature was 250 ^{o}C; the injection volume was 1.0 µL, splitless mode; and the ionization voltage was 70 eV. The column temperature program was set at 50 ^{o}C for 1 min, 50-175 ^{o}C at 25 ^{o}C/min, 175-235 ^{o}C at 4 ^{o}C/min, and 235 ^{o}C for 5 min.

Gas Chromatography

GC analyses were performed on an Agilent gas chromatography 6890N GC, equipped with a Phenomenex 7HG-G010-11 Zebron ZB-5 GC capillary column (30 m x 0.32 mm x 0.25 µm). Nitrogen was used as the carrier gas at the flow rate of 1.74 mL/ min. The constant pressure mode at 9.32 psi was used and the oven temperature was programmed from 60 ^{o}C to 240 ^{o}C at the rate of 3 ^{o}C/min. The injector and detector temperature were set at 250 ^{o}C. Oil samples of 0.1 µL were injected in splitless mode.

3.4. Identification of the essential oil and fatty oil components

The component of the oils was identified by gas chromatography by comparison of their linear retention indices (programmed retention indices) calculated relative to the homologous series of C_8-C_{22} n-alkanes by using the equation propounded by van den Dool and Kratz in 1963.[34,41,42] The identification of these constituents was based on comparing their mass spectra with those of standard compounds registered in the NIST 2020 library [43]. Moreover, the identification was also confirmed by collating the calculated retention indices of each constituent with those reported in the compilation of retention indices published by Adams [34]. The relative percentage of each constituent in the oil was determined by peak area normalization. No response factors were calculated.

4. CONCLUSIONS

The study showed the information on the extraction, analysis of the chemical composition of essential oils and fatty oils extracted from Moringa oleifera species cultivated in three areas of Southeast Vietnam. The geographical factor affecting the chemical composition of the leaf essential oil from moringa growing in Southeast Vietnam was proven. The essential oil of the leaves was dominated by coumarin and its two derivatives, 7-isoprenyl oxycoumarin and osthole, and alkanes. The presence of

coumarins with a high percentage in the moringa leaf essential oil was recorded and reported for the first time. These coumarins can be considered as compounds well distributed to plant species belonging to the Moringaceae family. Analyzing the chemical composition of products extracted from moringa seeds by hydrodistillation method showed that the compounds identified were not responsible for the essential oil because of lacking terpenes. Extracting moringa seeds with hexane by solvent extraction method gave fatty oil products rich in unsaturated acids, especially in omega fatty acids like omega-9 and omega-7 fatty acids. Regardless of geographical diversity, the dominant acids in moringa fatty oils were oleic acid and other C16-C20 unsaturated acids. Again, Moringa oleifera species was proven not only a nutritional plant but also an aromatic plant.

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Disclosure statement

There is no potential conflict of interest among authors in this work.

Data availability

The authors confirm that the data supporting the findings of this study are available in the Mendeley repository, Mendeley Data at DOI: 10.17632/fs58ng4wz6.1.

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