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COMPOSITION OF ESSENTIAL OILS OF CUMINUM CYMINUM UNRIPE FRUITS, PIPER BETLE VARIETY BANGLA LEAVES AND VITEX NEGUNDO LEAVES, FLOWERS AND FRUITS

Mohammed Ali¹* and Shahnaz Sultana^{1,2}

¹Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi-110 062, India.

²College of Pharmacy, Jazan University, Jazan, Saudi Arabia.

*Corresponding Author: Prof. Mohammed Ali

Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi-110 062, India.

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ABSTRACT

Cuminum cyminum L. (Apiaceae) seeds are used as a spice and to treat cough, diarrhea, digestive disorders, dyspepsia, epilepsy, fever, headache, liver and lung diseases, morning sickness and toothache. Piper betle L. (Piperaceae) leaves are beneficial to cure asthma, blood diseases, bronchitis, conjunctivitis, constipation, coughs, headache, itches, leucorrhoea, gum inflammation, rheumatism, malaria, night blindness, stomach disorders and throat irritation. Vitex negundo L. (Lamiaceae) is used to relieve arthritic joints, asthma, bronchitis, consumption, eye diseases, headaches, leucoderma, spleen enlargement, children teeth diseases, scorpion sting, snake bite, sinusitis and tubercular neck swellings, to control mosquitoes and to promote the growth of hair. The present study was carried out to analyze essential oils of these plants. The materials were hydro-distilled to get the essential oils which were analyzed by GC and GC-MS techniques. The essential oil from the unripe green seeds of C. cyminum was consisted of mainly cymene (37.20%), α-phellandrene (22.26%), sabinene (18.35%), (1,1-dimethylethyl)-3methyl benzene (3.65%), (2E,4E)-2,4-heptadienoic acid (3.61%) and y-himachalene (2.19%). The prominent constituents of the essential oil of the leaves of P. betle var. Bangla were acetyl eugenol (22.79%), valencene (18.01%), eugenol (16.15%), α -amorphene (8. 19%) and γ -cadinene (6.34%). The main constituents of the oil of the leaves of V. negundo were viridoflorol (15.8%), 4-terpineol (13.6%), β -caryophyllene (12.8%), β -phellandrene (10.9%), sabinene (7.3%) and palmitic acid (4.9%). The predominant components of the oil of the flowers of V. *negundo* were sabinene (22.7 %), viridoflorol (15.9%), globulol (11.6%), α -selinene (10.7%), caryophyllene oxide (9.8%) and β -caryophyllene (9.1%). The fruit essential oil of V. negudo contained mainly n-hexadecanoic acid (19.1%), β-selinene (15.2%), β-caryophyllene (14.5%), α-cedrene (10.4%) and viridiflorol (7.1%).

KEYWORDS: Cuminum Cyminum, Piper betle variety Bangla, Vitex negundo, essential oils, analysis.

INTRODUCTION

Cuminum cyminum L. (Family Apiaceae), known as cumin and jeera, is a small annual herb with slender, angular branched stem. The plant is indigenous to Egypt and Syria and one of the most cultivated popular spices throughout the world. Cumin seeds resemble caraway seeds, but are slightly smaller having oblong shape, thicker in the middle, compressed laterally with nine ridges and yellow-brown in color. Cumin is the second most popular spice in the world after black pepper. Cumin seeds are added in foods for its distinctive aroma and flavor. The seeds are considered as an analgesic, antispasmodic, aphrodisiac, appetizer. astringent, carminative, digestive, diuretic, emmenagogue, eupeptic and thermogenic; used to treat bloating, colic, cough, diarrhea, digestive disorders, dyspepsia, epilepsy, fever, flatulence, headache, indigestion, liver and lung diseases, morning sickness and toothache.^[1] A decoction of cumin and leaves of *Phyllanthus fraternus* with sugar is drunk to cure gonorrhea and syphilis. A decoction of cumin and leaves of *Ficus glomerata* is given to relieve stomach complaints. Cumin mixed with *Hemidumus indicus* is administered orally to treat gonorrhea. A seed paste is applied on the forehead to calm down headache and ingested to relieve arthritic rheumatism. Powdered fruits of cumin and *Trachyspermum ammi* are consumed to cure scorpion sting.^[2] Cumin oil is used for a variety of flavors, perfumes and in some cosmetics. The seeds are rich in iron, promote digestion and weight loss, improve blood cholesterol and start menstruation. The essential oil possessed anticonvulsant, antinociceptive, antiinflammatory, antidiabetic, antimicrobial, antifungal, sedative, stimulant and stomachic properties.^[1,2]

The cumin seeds contained a volatile oil composed of mono- and sesquiterpenes mainly thymol,

cuminaldehyde, limonene, α - and β -pinenes, 1,8-cineole, *o*- and *p*-cymenes, α - and γ -terpinenes, safranal, linalool, *trans*-dihydrocarvone, α -phellandrene and cuminic alcohol,^[3-13] flavones, 2-C-methyl-D-erythritol, alkyl glycosides, amino acids, fatty acids and aromatic compounds,^[14-17] nutrients, e.g., vitamins, amino acids, proteins, minerals, starch, sugars, tannins, phytic acid and dietary fiber components.^[18]

Piper betle L. (family Piperaceae), known as tambula, paan, vettilai and betel, is an evergreen, dioecious, glabrous and perennial climbing vine found in Indonesia, Pakistan, Philippines, China, Sri Lanka, India, Madagascar, Indo-China and Papua New Guinea.^[19,20] Its leaves are glossy, ovate-oblong with white catkin, petiole 2-5 cm long, powdery pubescent; base cordate, sometimes rounded in leaf blades toward apex of stem, symmetric, apex acuminate; drupes fused to form terete, fleshy, reddish, compound fruits, apices tomentose and prominent. The leaves are antiseptic, aphrodisiac, appetizer, aromatic, astringent, carminative, stimulant, tonic and used to treat asthma, blood diseases, bronchitis, conjunctivitis, constipation, coughs, dyspnea, filarial, headache, itches, leucorrhoea, gum inflammation, rheumatism, malaria, night blindness, stomach disorders and throat irritation; externally applied to relieve arthritis, boils, bruises, cuts, flatulence, injuries, headache and ulcers. The leaf oil is used as an antiseptic, cardiotonic, counter irritant, hypotensive and to releieve bruises, sores and swellings.^[19,21-22] The leaf essential oil has antiseptic, hypotensive, respiratory depressant and cardiotonic properties. The leaf mixed with lime and areca nut is taken as betel quid with or without tobacco as a stimulant masticatory and in euphoria-inducing formulation with adverse health effects in the East Indies, India and Philippines. Betel is notable for staining the teeth of regular users. In southern India and Sri Lanka, a sheaf of betel leaves is traditionally offered as a mark of respect and auspicious beginnings during wedding ceremonies and for payment to Ayurvedic physicians, astrologers and given to the elders for their blessings.

The betel leaves contained essential oils composed of safrole, allyl pyrocatechol, eugenol, terpinen-4-ol, eugenyl acetate, farnesol, valencene, estragole, chavicol and chavibetol acetate as the major components,^[23-28] aliphatic constituents, tannins, flavonoids, aromatic and fatty acids, piperlonguminine, phenolic compounds, sesquiterpenes, piperine, piperbetol, phytosterols, bis-chavicol dodecanoyl esters, allyl pyrocatechol, diosgenin and ursolic acid.^[28-35]

Vitex negundo L., syn. Agnus-castus negundo (L.) Carrière, Vitex chinensis Mill; V. arborea Desf., V. arborea Desf., V. gracilis Salisb., V. incise Lam., V. laciniata Schauer, V. sinuate Medik., V. spicata Lour. (family Lamiaceae), known as nirgundi, nisinda, Chinese chaste tree, five-leaved chaste tree and horseshoe vitex, is distributed in moist area throughout India,

Mediterranean countries, central Asia, Sri Lanka, Afghanistan, tropical Africa, Madagascar, China and Philippines.^[36, 37] It is a large, erect, aromatic shrub, up to 8 m high, with quadrangular, densely whitish tomentose branchlets; bark is reddish brown; leaves are digitate, with 3-5 lanceolate leaflets: flowers numerous, borne in panicles, white to blue in color; fruit a succulent, oval, black or purple drupe.^[36,37] The plant has anthelmintic, antiseptic, astringent, carminative, cephalic. emmenagogue, expectorant, ophthalmic, stomachic, thermogenic, vermifuge, vulnerary properties and is used to cure arthritic joints, asthma, bronchitis, consumption, eve diseases, headaches, leucoderma, neck gland sores, spleen enlargement, children teeth diseases, scorpion sting, snake bite, sinusitis and tubercular neck swellings, to control mosquitoes and to promote the growth of hair. The leaves are aromatic, carminative, tonic and vermifuge, prescribed to treat dysmenorrhea, catarrhal fever, eye diseases, gonorrhea, headache, puerperal state of women, rheumatism, toothache and ulcers. Essential oil of the leaves is effective to relieve sexually transmitted diseases, sinuses and scrofulous sores. The root has expectorant, febrifuge, tonic, vermifuge effects and is antidote for snake bites. The fruits are taken as a nutrient, refrigerant, vermifuge and to prevent catarrh, headache, indigestion and watery eyes. Pulverized seeds as an aphrodisiac are taken orallv and in spermatorrhoea.^[36,37]

The leaves of *V. negundo* contained a volatile oil composed mainly of sabinene, linalool, viridiflorol, βcaryophyllene, terpinen-4-ol, γ-elemene, epi-laurenene, globulol, epiglobulol, α-guaiene, α-copaene, humulene epoxide II, δ-guaiene, caryophyllene epoxide and ethyl hexadecenoate.^[38-46] The predominant compounds of the essential oil of flowering tops were identified as viridiflorol, β-caryophyllene, 4-terpineol, linalool, αselinene, germacren-4-ol, carryophyllene epoxide, globulol and (E)-nerolidol.^[44,47,48] The fruit oil possessed β-selinene, α-cedrene, germacrene D and hexadecanoic acid as the prominent constituents.^[44] The leaves yielded casticin, iso-orientin, chrysophenol D, luteolin, phydroxybenzoic acid, D-fructose, negundoside, agnuside, vitegnoside, flavones and flavanones.^[50-52]

Keeping in view the various therapeutic values of the plants and the development of safer, ecofriendly and biodegradable herbal preparations, the essential oil compositions of *Cuminum cyminum* unripe fruits, *Piper betle* variety *Bangla* leaves and *Vitex negundo* leaves, flowers and fruits were analyzed.

MATERIALS AND METHODS Plant materials

The fresh unripe fruits of *Cuminum cyminum* were collected from the cultivated plants grown in the Herbal garden of Jamia Hamdard University. The leaves of *Piper betle* variety *Bangla* were purchased from a local vegetable market of Delhi. The leaves, flowers and fruits of *Vitex negundo* were procured from a wild region of

Roorkee, Uttarakhand. The plant materials were authenticated by Prof. M. P. Sharma, Department of Botany, Jamia Hamdard, New Delhi. The voucher specimens of the plant materials were deposited in the herbarium of the Phytochemistry Research Laboratory, Jamia Hamdard for future reference.

Isolation of the essential oils

The plant materials (500 g each) were hydrodistilled individually in a Clevenger type glass apparatus for 4 h. The essential oils were collected, measured, dried over anhydrous sodium sulphate and stored at 4^{0} C in the dark for GC and GC-MS analysis. The yields of essential oils obtained from the plant materials were between 2.35 - 1.14%.

GC analysis

The gas chromatographic analysis of each essential oil was carried out on a GC-2010 (Shimadzu) equipped with a flame ionization detector (FID) and ULBON HR-1 fused silica capillary column ($60 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ µm}$). The injector and detector (FID) temperatures were maintained at 250 and 270 °C, respectively. The carrier gas used was nitrogen at a flow rate of 1.21 ml/min with column pressure of 155.1 kPa. The samples (0.2 µl) were injected into the column with a split ratio of 80:1. Each component separation was achieved following a linear temperature programmed from 60 to 230 °C at a rate of 30 °C/min and then held at 230 °C for 9 min with a total run time of 55.14 min. Percentage of the constituents were calculated by the electronic integration of FID peak areas.

GC-MS analysis

The GC-MS analysis of each oil was carried out on a GC-MS-QP 2010 Plus (Shimadzu) fitted with a Column AB-Innowax (60 m × 0.25 mm i.d., film thickness 0.25 μ m). The carrier gas was nitrogen at a flow rate 1.21 ml/min. The oven column temperature was initially kept at 60 °C for 10 min and increased up to 230 °C at a rate of 4 °C/min, held at 230 °C for 10 min and increased up to 260 °C at a rate of 1 °C/min and then held at 260 °C for 10 min. The split flow was 101 ml/min. The split ratio was 1:80. The injector temperature was 240 °C and detector temperature was 280 °C. Injection volume was 0.3 μ l. The ionization energy (voltage) was 70 eV and mass scan range (*m*/*z*) was 40-850 amu. The percentage composition of the oil was calculated automatically from the FID peak area without any correction.

Identification of compounds

The individual compounds were identified by comparing their Kovat's indices (KI) of the peaks on Innowax fused silica capillary column with literature values, matching against the standard library spectra, built up using pure substances and components of known essential oils. Further identification was carried out by comparison of fragmentation pattern of the mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K L, WILEY 8 libraries and published literature.^[53,54] Relative amounts of identical components were based on peak areas obtained without FID response factor correction.

RESULTS AND DISCUSSION

The essential oil of the unripe green seeds of C. cyminum was a light yellow liquid in a yield of 2.35% (v/w). Table 1 shows the components identified by both GC and GC-MS in the essential oil; 26 components were characterized representing 97.98% of the essential oil. The essential oil was composed of a high number of monoterpenic hydrocarbons (7) comprising 81.04%, monoterpenic alcohols (7) constituting 2.94% and sesquiterpene hydrocarbons (4) having 3.08%. The predominant identified component in essential oil was cymene (37.20%) followed by α -phellandrene (22.26%), (18.35%), (1,1-dimethylethyl)-3sabinene methvl benzene (3.65%), (2E,4E)-2,4-heptadienoic acid (3.61%) and y-himachalene (2.19%). Four compounds, viz., sorbic acid (0.11%), *n*-hept-3,5-diene-oic acid (0.71%), (2E,4E)-2,4-heptadienoic acid (3.61%) and (Z,Z)- 9,12octadecadienoic acid (linoleic acid) (0.17%) were the organic carboxylic acids detected in the essential oil. *n*-Tricosan-4,5-diol (0.13%) and *n*-dodec- 4- ene (0.82%) were the aliphatic constituents and the aromatic components included p-cymene (37.20%), (1,1dimethylethyl)-3- methyl benzene (3.65%) and p-cymen-8-ol (0.73%). Eight monoterpenes, one each of aliphatic alcohol and alkane, and three each of carboxylic acids and sesquiterpenes were present in trace amounts. Cuminaldehyde, limonene, thymol, β -pinene, 1,8cineole, o-cymene, α - and γ -terpinenes, safranal, linalool, trans-dihydrocarvone and cuminic alcohol were the major components in the essential oils of mature cumin seeds and all these constituents could not be detected in the essential oil of unripe green seeds.

The essential oil from the leaves of P. betle var. Bangla was a light green liquid in a yield of 1.98% (v/w). The chemical composition of the piper essential oil is tabulated in Table 1 with their Kovat's indices and respective percentage. Total 23 components (97.66%) were characterized including three each of monoterpenes (2.98%) and eugenol derivatives (39.59%), one isovanillin (4.09%) and sixteen sesquiterpenes (51.0%). The prominent constituents of the oil were acetyl (22.79%), valencene (18.01%), eugenol eugenol (16.15%), α -amorphene (8. 19%) and γ -cadinene (6.34%). Except humulene oxide II and patchulone, all sesquiterpenes were hydrocarbons. Eleven the components occurred in trace amounts. The essential oil was devoid of aliphatic constituents and fatty acids.

The essential oil from the leaves of *V. negundo* was a pale yellow liquid (2.14%, v/w). The chemical constituents of the essential oil were identified by analysis of GC and GC-MS. The chemical composition of the leaf oil, analyzed by GC and GC-MS, is tabulated in Table 1 with their Kovat's indices and respective percentage. The essential oil of the leaves contained total

26 components (96.3%) including seven monoterpenes (36.8%), fifteen sesquiterpenes (52.0%) and one each of fatty acid (4.9%) and fatty ester (1.5%). The major constituents of the oil were viridoflorol (15.8%), 4terpineol (13.6%), β -caryophyllene (12.8%), βphellandrene (10.9%), sabinene (7.3%) and palmitic acid (4.9%). Except citronellal and 4-terpineol, all the monoterpenes were hydrocarbons. The monoterpenes pcymene and citronellal occurred in trace amounts. β-Caryophyllene, guaia-3,7-diene, α -guaiene, valencene and β -curcumene were the five sesquiterpenic hydrocarbons. There were eight sesquiterpenic alcohols including elemol, epiglobulol, (E)-nerolidol, caryophyllene alcohol, globulol, viridoflorol, epi-acadinol and α -murolol and four of them were present in less them 1% amount. Two sesquiterpenic oxides, viz., caryophyllene oxide and humulene 1,2-epoxide and one ester, α -bisabolol acetate, were also present in the oil. One fatty acid, palmitic acid, and fatty ester, ethyl-9hexadecenoate, were the aliphatic constituent detected in the oil. Except p-cymene, no other aromatic compound was present in the oil.

The essential oil from the flowers of *V. negundo* was a colourless liquid (1.87 %, v/w). There were fifteen chemical constituents (95.1%) characterized in the essential oil of flowers of *V. negundo*. There were four monoterpenes (25.7%), nine sesquiterpenes (65.5%) and one each of an aliphatic hydrocarbon, heptane, (3.1%) and an aromatic compound, p -(1,1-dimethylethyl) toluene, (0.80%) present in the oil. Two each

monoterpenic hydrocarbons (23.4%) and alcohols (2.3%) and four each sesquiterpene hydrocarbons (22.0%) and alcohols (33.7%) and one oxide (9.8%) were detected in the oil. The predominant components of the oil were sabinene (22.7%), viridoflorol (15.9%), globulol (11.6%), α -selinene (10.7%), caryophyllene oxide (9.8%) and β -caryophyllene (9.1%). Only four components, viz., *p*-cymene, *p* -(1,1-dimethylethyl) toluene, linalool and *trans*- α -bergamotene were present in less than 1% amounts. *p*-Cymene and *p*-(1,1-dimethylethyl) toluene were the two aromatic compounds present in the essential oil of *V. negudo* flowers (Table 1).

The fruit essential oil (1.14%) of V. negudo, obtained as a colourless liquid, contained fifteen compounds (93.4%) and except sabinene (2.2%) and two fatty acids (22.0%), all the constituents were sesquiterpenes (69.2%). There were eight sesquiterpene hydrocarbons (58.5%), three sesquiterpene alcohols (9.4%) and one caryophyllene oxide (2.3%) component in the oil. The prominent constituent was n-hexadecanoic acid (19.1%) followed by β -selinene (15.2%), β -caryophyllene (14.5%), α cedrene (10.4%) and viridiflorol (7.1%). Elemol, viridiflorol and eudesmol were the sesquiterpenic alcohols. Except the fatty acids palmitoleic acid (2.9%) and n-hexadecanoic acid (19.1%), no other aliphatic constituent was present in the essential oil. The sesquiterpenes α -guaiene, elemol and eudesmol were present in trace amounts. The oil was devoid of aromatic compounds and fatty esters (Table -1).

 Table 1: Percentage Composition of Essential Oils of Cuminum Cyminum Unripe Fruits, Piper Betle Variety

 Bangla Leaves and Vitex Negundo Leaves, Flowers and Fruits.

S.No	Constituents	KI	Essential oil % area				
5.110			Α	В	С	D	Ε
1	<i>n</i> -Heptane	700	-	-	-	3.1	-
2	α-Thujene	922	0.43	-	-	-	-
3	α-Pinene	934	0.61	-	-	-	-
4	β-Phellandrene	964	-	-	10.9	-	-
5	Sabinene	971	18.3	0.49	7.3	22.7	2.2
6	β -Pinene	972	1.1	-	-	-	-
7	β -Myrcene	983	1.02	-	-	-	-
8	α-Phellendrene	1003	22.6	-	-	-	
9	<i>p</i> -Cymene	1015	37.2	-	0.6	0.7	-
10	1,8-Cineole	1018	-	2.21	-	-	-
11	<i>cis</i> -β-Ocimene	1037	-	-	1.7	-	-
12	Sorbic acid	1045	0.11	-	-	-	-
13	γ-Terpinene	1047	-	1.9	-	-	-
14	<i>n</i> -Tricosan-4,5-diol	1050	0.13	-	-	-	-
15	(1,1-Dimethyl ethyl)-3- methyl benzene	1067	3.65	-	-	-	-
16	<i>p</i> -(1,1-Dimethyl ethyl)- toluene	1072	-	-	-	0.8	-
17	<i>n</i> -Hept-3,5-diene-oic acid	-	0.71	-	-	-	-
18	Linalool	1085	-	-	-	0.5	-
19	Camphor	1125	1.1	-	-	-	-
20	trans-Verbenol	1133	0.4	-	-	-	-
21	Citronellal	1153	-	-	0.8	-	-
22	<i>p</i> -Cymen- 8-ol	1164	0.73	-	-	-	-
23	(E)-3-Pinen-2-ol	1170	0.17	-	-	-	-

$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	3.6 1.8	-
26 α-Terpineol 1175 1.08 - 27 cis-Myrtenol 1182 0.24 -	5.0 1.0	
27 <i>cis</i> -Myrtenol 1182 0.24 -		-
		-
		-
		-
		-
		-
		-
		5.9
		-
	2.8 9.1	14.5
35 α-Cedrene 1409 -		10.4
36 Aristolene 1416 -		6.2
).7 -	1.9
38 trans-α-Bergamotene 1433	- 0.3	-
39 α-Guaiene 1439 1	- 1.1	0.7
40 Aromadendrene 1441 - 4.32		-
41 (E)- Isoeugenol 1447 - 0.65		-
		3.7
		-
		-
		-
		-
		15.2
		-
		-
	3.6 1.9	-
).6 -	-
	- 10.7	-
		-
		-
		-
		-
		-
		-
58 Eudesm-4(14),11- diene 1517 - 0.53		-
59 Epiglobulol 1530 4	4.9 -	-
60 α- Cadinene 1534 - 1.21	-	-
61 Elemol 1548 1	.1 -	0.6
).8 5.1	-
	- 1.1	-
).9 -	-
	3.7 9.8	2.3
	5.8 15.9	
	1.1 11.6	-
		-
, I	1.3 -	-
		-
		0.7
).6 -	-
	l.7 -	-
	2.1 -	-
).3 -	-
76Palmitoleic acid1953-		2.9
77 Ethyl 9- hexadecenoate 1955 1	1.5 -	-
78 Palmitic acid 1965 4	4.9 -	9.1
1/00		-

KI: Kovat's index; A: *Cuminum cyminum* unripe fruits; B: *Piper betle* variety *Bangla* leaves; C: *Vitex negundo* leaves; D: *Vitex negundo* flowers; E: *Vitex negundo* fruits

CONCLUSION

The essential oil of the unripe green seeds of C. cyminum was consisted of mainly cymene. α -phellandrene. sabinene. (1,1-dimethylethyl)-3- methyl benzene. (2E,4E)-2,4-heptadienoic acid and y-himachalene. The prominent constituents of the essential oil of the leaves of P. betle var. Bangla were acetyl eugenol, valencene, eugenol, α -amorphene and γ -cadinene. The major constituents of the oil of the leaves of V. negundo were viridoflorol, 4-terpineol, β-caryophyllene, ßpalmitic sabinene and phellandrene. acid. The predominant components of the oil of the flowers of V. negundo were sabinene, viridoflorol, globulol, aselinene, caryophyllene oxide and β -caryophyllene. The fruit essential oil of V. negudo contained mainly nhexadecanoic acid, β -selinene, β -caryophyllene, α cedrene and viridiflorol.

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