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VOLATILE CONSTITUENTS OF ESSENTIAL OILS ISOLATED FROM LEAF AND INFLORESCENCES OF *PIPER LONGUM* LINN.

MD. NAZRUL ISLAM BHUIYAN, JARIPA BEGUM AND M. N. ANWAR *
BCSIR Laboratories, P.O. Chittagong Cantonment, Chittagong-4220, Bangladesh.
*Microbiology, University of Chittagong, Chittagong-4331, Bangladesh.

ABSTRACT

Essential oil compositions of the inflorescences and leaf of *Piper longum* Linn. were investigated by gas chromatography–mass spectrometry (GC-MS). *P. longum* oils were found to contain few monoterpene hydrocarbons, a moderate content of sesquiterpenes and high content of aliphatic hydrocarbons. The inflorescences oil rich in eugenol (33.11%), caryophyllene (9.29%), cinnamyl acetate (5.91%) and β-pinene (4.74%), whereas leaf oil rich in trans-nerolidol (19.08%), caryophyllene (12.25%), 3-heptene, 7-phenyl- (3.71%), benzyl benzoate (3.68%), caryophyllene oxide (3.62%) and β-elemene (3.28%). The compositions of both oils varied qualitatively and quantitatively.

Key Words : Piper longum, essential oils, GC-MS analysis, eugenol, trans-nerolidol.

INTRODUCTION

Piper longum Linn. Syn. *Chavica roxburghii,* Miq. members of the botanical family Piperaceae were among the first cultivated plants. Long pepper (*P. longum*) is the best known species in this family and is probably among the most recognized spices in the world. *P. longum* (long pepper), which is originated from South Asia, has been used since long as one of the most famous traditional Chinese medicines for curing coronary heart disease, vascular headache, trigeminal neuralgia and stopping pain (Ling *et al.* 2007). Long pepper acts as a general tonic and hematinic and widely used in Ayurveda as good rejuvenator (Rasayana). *P. longum* is known to enhance the bio-availability of food and drugs. In fact *P. longum* is taken along with Quinine for making it more effective. *P. longum* is used as a spice and also in pickles and preservers. *P. longum* is widely used in Ayurvedic and Unani systems of medicine particularly for diseases of respiratory tract most of them include cough, carminative, bronchitis, asthma etc.

* Corresponding author.

Long pepper is locally applied to counter-irritant and act as analgesic for muscular pains and inflammation. The Materia Medica of Ayurveda, which dates back to 6,000 B.C., has many references advocating the use of pepper in a variety of ailments, particularly those pertaining to the gastro-intestinal tract (Chopra and Chopra 1959, Akamasu 1970, Perry 1980, Vagbhat 1962, Kaviraj 1963, Raj and Nagarsheth 1978). Piperine is the active principle of long pepper (*P. longum* L.). *Piper* species have been used in traditional medicine for intermittent fevers and to promote the secretion of bile. They are also recommended for neurological, broncho-pulmonary and gastrointestinal disorders including dyspepsia, flatulence, constipation and hemorrhoids (Chopra and Chopra 1959, Akamasu 1970, Perry 1980, Vagbhat 1962, Kaviraj 1963, Raj and Nagarsheth 1978). It was reported that long pepper was used for patients with chronic malaria with splenomegaly (enlarged spleen). Long pepper fruits were given in an increasing dose from 3 to 30, starting with 3 and increasing daily by 3 fruits. Subsequently the dose was decreased from 30 to 3 fruits, by reducing 3 fruits daily. Long pepper was boiled in milk and water and drank once a day in the early morning. Drinking this decoction reportedly caused cessation of malarial parasite multiplication and regression of splenomegaly. Long pepper, and to a lesser extent trikatu, have been used in the treatment of asthma and chronic bronchitis in Ayurveda and Unani medicine (Dymock *et al.* 1972). It has broad antimicrobial, anti-parasitic and insecticidal properties. Peppers have been traditionally used as local anesthetics, but the mechanism of this analgesic (pain-relieving) action has only been recently described. The dried catkins and the root of *P. longum* are used in medicine. They are considered heating, stimulant, carminative, alterative, laxative and useful in cough, hoarseness, asthma, dyspepsia, paralysis, etc (Dutt, 1877). The fruits oil contains caryophyllene oxide and β-caryophyllene (Tewtrakul *et al,* 2000). The main components in fruits oil were β-caryophyllene (33.44%), 3-carene (7.58%), eugenol (7.39%), D-limonene (6.70%), zingiberene (6.68%) and cubenol (3.64%) (Ling *et al.* 2007). Regarding *P. longum,* there is no work available in our country. So the present study deals with the investigation of the chemical components in leaf and inflorescences oils of *P. longum* grown in Bangladesh.

MATERIAL AND METHODS

Plant material

The plant materials of *Piper longum* were collected from the plants grown in the campus of BCSIR Laboratory, Chittagong during June 2007. The specimen was identified by Dr. Mohammad Yusuf, Ex-Director-in-charge,BCSIR Labs.Ctg.

One-voucher specimen (Y-685) was deposited in the herbarium of BCSIR Laboratory, Chittagong.

Extraction of essential oil

Samples of leaf were harvested from healthy, well-grown, two-year-old plants. Samples of fresh leaves (700g) and the fresh inflorencences (500g) were ground in a blender. The materials were subjected to hydrodistillation using a Clevenger glass apparatus upto 4 h for isolation of oils separately from the two parts. The oil samples were stored at 0°C in air-tight containers after drying them over anhydrous sodium sulfate, filtered and concentrated under reduced pressure at room temperature to obtain the essential oil for GC-MS analyses.

GC-MS analysis

The essential oil from leaf and inflorencences of *Piper longum* were analyzed by GC-MS electron impact ionization (EI) method on GC-17A gas chromatograph (Shimadzu) coupled to a GC-MS QP 5050A Mass Spectrometer (Shimadzu); fused silica capillary column (30m x 0.25mm; 0.25 μm film thickness), coated with DB-5 (J&W); column temperature 100^{o}C (2 min) to 250^{o}C at the rate of 3^{o}C/min; carrier gas, helium at constant pressure of 90Kpa. Acquisition parameters full scan; scan range 40-350 amu.

Identification of the compounds

Compound identification was done by comparing the NIST library data of the peaks with those reported in literature , mass spectra of the peaks with literature data . Percentage composition was computed from GC peak areas on DB-5 column without applying correction factors.

RESULTS AND DISCUSSION

Seventy volatile compounds in leaf oil and thirty-two volatile compounds in inflorescences oil were identified by mass spectra library and listed in Table 1 and 2 respectively. Their relative contents were calculated on the basis of peak area ratio, and also shown in Table 1. The leaf oil rich in trans-nerolidol (19.08%), caryophyllene (12.25%), 3-heptene, 7-phenyl- (3.71%), benzyl benzoate (3.68%), caryophyllene oxide (3.62%), β-elemene (3.28%), δ-guaiene (2.96%), octacosane (2.86%), nonane, 5-propyl- (2.77%), retinal (4.15%), 2-heptanol, acetate (1.73%), α-caryophyllene (1.50%), cyclohexanone, 2-cyclohexylidene- (1.42%) and lineoleoyl chloride (1.11%). On the other hand, the inflorescences oil rich in eugenol (33.11%), caryophyllene (9.29%), cinnamyl

acetate (5.91%), β-pinene (4.74%), nerolidol, acetate (3.82%), 2-heptanol, acetate (2.63%), humulen-(v1) (2.62%), phytol (1.98%), γ-elemene (1.77%), limonene (1.61%) and pinene (1.12%). The study reveals that composition of two oils differs from the earlier reports and may, therefore be treated as different chemotypes. On the basis of above fact, it may be concluded that *P. longum* growing widely in Bangladesh, may be utilized as a source for the isolation of natural eugenol and trans-nerolidol respectively. As a result of this study, the essential oil of *P. longum* has been extracted and its components identified. The high concentration of eugenol and trans-nerolidol in leaf and inflorescences oil makes it respectively potentially useful in the medicines because they exhibit antibacterial activities (Reddy *et al*., 2001). However, further study has to be conducted. Eugenol is used in perfumeries, flavorings, essential oils and in medicine as a local antiseptic and anesthetic. It is also used in combination with astringents as a mouth wash after tooth extraction. An antiseptic ointment of eugenol with hydrous wool fat has been used for eczema. Eugenol is administered, in the same manner as oil of cloves in phthisis, as a carminative and antiseptic (Jadhav *et al.* 2004). It is a key ingredient in Indonesian kretek (clove) cigarettes. It was used in the production of isoeugenol for the manufacture of vanillin, though most vanillin when eugenol mixed with zinc oxide, zinc oxide eugenol forms which has restorative and prosthodontic applications in dentistry. Although attempts have been made to develop eugenol derivatives for intravenous injection, the toxicity of propanidid (Right and Payne 1962) in human patients was not acceptable. Clove oil is growing in popularity as an anaesthetic for use on aquarium fish. It is one of many compounds that are attractive to males of various species of orchid bees, who apparently gather the chemical to synthesize pheromones; it is commonly used as bait to attract and collect these bees for study (Schiestl and Roubik 2003). It is worth noting that the oil of *P. longum* has been reported to be used in folk medicine in the treatment of eczema and analgesics.

TABLE-1. COMPOSITION OF ESTIMATED VOLATILE OILOF THE LEAF OF *PIPER LONGUM*

SL. NO	Name of Constituents	%
1.	β -Pinene	0.22
2.	β.-Myrcene	0.04
3.	Limonene	0.74

4.	2-Heptanol, acetate	1.73
5.	Z-Ocimene	0.11
6.	8-Pentadecanone	0.10
7.	Linalool	0.32
8.	Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene-	0.16
9.	2-Acetoxytetradecane	0.92
10.	3-tert-Butylphenol	0.11
11.	Tridecane	0.17
12.	Thujone	0.17
13.	2-Methyl-oct-2-enedial	0.11
14.	Nonane, 5-propyl-	2.77
15.	1-Tridecene	0.28
16.	Tetradecane	0.19
17.	β-Elemene	3.28
18.	Cyclooctene, 1,2-dimethyl-	0.27
19.	Caryophyllene	12.25
20.	α-Cubebene	0.41
21.	Isobornyl acetate	0.68
22.	α-Caryophyllene	1.50
23.	Naphthalene, decahydro-	0.43
24.	β-Selinene	0.54
25.	Curcumene	0.35
26.	Ylangene	0.18
27.	Neoisomenthol	0.32
28.	Butylated Hydroxytoluene	0.59

29.	α-Himachalene	0.32
30.	Guaiene	0.26
31.	Isocaryophillene	0.79
32.	trans-Nerolidol	19.08
33.	Cyclohexanone, 2-cyclohexylidene-	1.42
34.	Caryophyllene oxide	3.62
35.	Cubenol	0.26
36.	Triquinacene, 1,4-bis(methoxy)-	0.26
37.	Humulen	0.66
38.	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	0.18
39.	α-Cadinol	0.18
40.	delta.-Cadinol	0.47
41.	Lineoleoyl chloride	1.11
42.	δ-Guaiene	2.96
43.	Thujopsene	0.91
44.	3-Tridecen-1-yne, (Z)	0.51
45.	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	0.28
46.	Benzaldehyde, 2,4,5-trimethoxy-	0.16
47.	Ledene alcohol	0.33
48.	Carane, 4,5-epoxy-, trans	0.21
49.	Methyl 3,4-dimethoxycinnamate	0.20
50.	Benzyl Benzoate	3.68
51.	Methylconiferylaldehyde	0.25
52.	Patchoulane	0.27
53.	Methyl (Z)-5,11,14,17-eicosatetraenoate	0.26

54.	3-Heptene, 7-phenyl-	3.71
55.	Octacosane	2.86
56.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-	0.08
57.	l-Ascorbic acid 2,6-dihexadecanoate	0.48
58.	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	0.20
59.	Verrucarol	0.15
60.	2-Methoxybenzyl acetate	0.33
61.	Nerolidyl acetate	1.55
62.	Tigogenin lactone acetate	0.25
63.	Benzene, 5-heptenyl-	0.36
64.	8-Tetradecyn-1-ol	0.09
65.	Benzene, (3,3-dimethyl-4-pentenyl)-	2.95
66.	Ledol	0.08
67.	Glaucyl alcohol	0.24
68.	Caryophyllene-(I1)	2.27
69.	26,27-Dinorergosta-5,23-dien-3-ol, (3.beta.)-	0.39
70.	Retinal	4.15

TABLE-2. COMPOSITION OF ESTIMATED VOLATILE OILOF THE INFLORESCENCES OF *PIPER LONGUM*

SL. No	Name of constituents	%
1.	Pinene	1.12
2.	β-pinene	4.74
3.	Limonene	1.61
4.	2-Heptanol, acetate	2.63
5.	Linalool	0.69
6.	Cyclopropanecarboxylic acid, nonyl ester	0.37
7.	Cinnamaldehyde, (E)-	0.34

8.	Phytol	1.98
9.	γ-Nonanolactone	0.29
10.	Eugenol	33.11
11.	Benzenepropyl acetate	0.31
12.	Caryophyllene	9.29
13.	α-Cubebene	0.60
14.	Cinnamyl acetate	5.91
15.	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	0.73
16.	α-Caryophyllene	0.95
17.	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,4.alpha.,7.alpha.)]-	0.46
18.	Cedrene	0.56
19.	Zingiberene	0.83
20.	γ-Elemene	1.77
21.	Butylated Hydroxytoluene	0.35
22.	β-Bisabolene	1.61
23.	2,4-Dodecadienal, (E,E)-	0.40
24.	α-Farnesene	0.70
25.	Nerolidol, acetate	3.82
26.	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, cis-	2.87
27.	Humulen-(v1)	2.62
28.	Linoleoyl chloride	0.71
29.	β-Selinene	1.87
30.	Benzyl Benzoate	1.70
31.	n-Hexadecanoic acid	0.32
32.	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E)-	1.95

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