International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol. 3, No.1, pp 175-179, Jan-Mar 2011

Chemical characterization by GLC & GC-MS and antimicrobial activity of essential oil from leaves of *Chrysanthemum indicum* Linn

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Abstract: A steam distilled volatile oil obtained from the leaves of *Chrysanthemum indicum* Linn. (Family- Compositae) was analyzed by capillary GC and GC-MS. The volatile oil mainly comprises of thirty nine (39) components out of which thirty one (31) constituents comprising 82.6% of the oil were identified. The volatile oil mainly contains monoterpenes (48.1%). Isoborneol, a monoterpene, was the predominant characterized constituent (14.40 %) among all isolated constituents of volatile oil. Out of the twelve (12) monoterpenes, there were five (5) hydrocarbons (9.6%) and seven (7) oxygenated monoterpenes (38.5%). In sesquiterpenic group, nineteen (19) components were isolated (34.5%) and out of nineteen (19) sesquiterpenes, there were sixteen hydrocarbon (33.5%) and three oxygenated sesquiterpenes (1%) and among these sesquiterpenes, δ-cadinene (5.5%) was the predominant characterized constituent. Eight (8) other components comprising 17.4% of all the isolates were unidentified.
The volatile oil has shown maximum antibacterial activity against *Pseudomonas aeruginosa* (16.5 mm) at a concentration of 1%v/v followed by against *Micrococcus luteus* (13 mm) on comparison with erythromycin as standard antibacterial agent. The volatile oil has also shown antifungal activity against *Candida albicans* (18 mm) at the same concentration of 1%v/v, when compared with ketoconazole as standard antifungal agent.
Keywords: *Chrysanthemum indicum* Linn. (CHI), essential oil, antimicrobial activity.

Introduction

Chrysanthemum indicum L. is an important medicinal plant spreading widely in Korea, native of China and Japan and occasionally grown in Indian gardens for its ornamental multi-colored flowers, belonging to Family- Compositae (1). Though almost all of its parts are used in Ayurvedic and modern systems of medicine, leaves and flowers are the most important part in the field of medicine and drug. The other species of *Chrysanthemum* are *Chrysanthemum cinerariifolium* (Trev.)Vis., *C. leucanthemum* L., *C. morifolium* Ramat., *C. coronarium* and *C. balsamita* L. (2). *Chrysanthemum indicum* Linn. is an shrub, medium size, 60-90 cm high, perennial, with leafy stem. Leaves are dark green in colour, slightly bitter in taste and of aromatic odour. Flowers are deep yellow in color (3). *Chrysanthemum indicum* L. is traditionally reported for treatment of pneumonia,

colitis, stomatitis, cancer, fever, to treat vertigo, pertussis, hypertensive symptoms, inflammation, parkinson's diseases, and also used as antibacterial (oral bacteria) agent (4). Plant contains yejuhua lactone, asteglasine A, sesquiterp. alk., α-,β-Amyrins, lupeol, γ-taraxasterol & sitosterol, a new guaianolide – angeloylajadin with angeloylcumambrin B and arteglasin A, acacetin, chrysanthemol isolated from flowers and characterised; acacetin, cumambrin A, daucosterol, glycerol-1-monobehenate and palmitic acid (5). Flavanoids, 5,7-dihydroxy chromone, diosmetin, apigenin, eriodictyol and luteolin present in flower. dl-Camphor, azulene and β-3-carene obtained by distillation of flowers (6) . New sesquiterpene lactone – yehuja lactone along with chamazulene also isolated from flowers. Yejuhua lactone; diguaianolide identical with handelin isolated from flowers. Essential oil of flowers yeilds camphor (16%), trans-carene-trns-2-ol (15%), borneol acetate (12%), sabinene (7%), chrysanthenone, chrysanthemin glucoside. Camphene, δ-carene, 1,8- cineole, p-cymene, limonene, linalool, myrcene, α, β-pinenes, β-selinene, α-terpinene, α-terpeneol and thymol, pinocarvone, α-humulene, β-caryophyllene, α-copaene, umbellulone and trans-chrysanthenyl acetate are also reported in the plant (7).

As a part of over investigation on aromatic and medicinal plant of India, we describe in this communication, the chemical composition of the oil isolated from leaves by modern sophisticated technique.

Experimental

Plant material

Fresh leaves of *Chrysanthemum indicum* L. were collected form botanical garden of Ram-Eesh Institute, Greater Noida. Plant material was authentified as *Chrysanthemum indicum* L. by the taxonomic department of National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi by Dr. Anjula Pandey (Taxonomist). A voucher specimen (Specimen No: 2009-8/893) is preserved in herbarium section of taxonomic department of NBPGR, New Delhi and also in the phytochemistry laboratory, Faculty of Pharmacy, Ram-Eesh Institute of Vocational and Technical Education, Greater Noida, U.P.

Isolation of volatile oil

The fresh leaves (500 gm) were hydro-distilled for 3 hours according to the method recommended in the British Pharmacopoeia, 2003 (8). The yield of volatile oil obtained was 0.6 % V/W. The yellow colored volatile oil was collected in the graduated tube. The collected volatile oil was dried over an anhydrous sodium sulphate and stored at 4ºC in the dark.

Volatile constituents were identified by comparing their Kovate indices and retention times with those of authentic standards available in the author's laboratory and with those of literature.

GC analysis

Analytical GC was carried out on a varion 3300 GC fitted with a silicone DB-1 capillary column (30 m X 0.25 mm), film thickness 0.25 µm, carrier gas nitrogen, flow rate 1.5 ml/min., split mode, temperature programmed 180º – 250ºC at 4ºC/min. Injector temperature and detector temperature were 250ºC and 300ºC, respectively. Detector used was FID. Injector volume for all samples was 0.1 µl.

GC-MS analysis

GC-MS analysis was carried out on a Schimadzu QP-2000 instrument at 70 eV and 250ºC. GC column Ublon HR-1 fused silica capillary 0.25 mm X 50 m with film thickness 0.25 µm. The initial temperature was 100ºC for 6 min and then heated at a rate of 10ºC/min. to 250ºC. Carrier gas, helium, flow rate 2 ml/min, detector used was FID.

Identification

The volatile components were identified by comparing their retention indices of GC chromatograph with those of literature. Further identification was done by GC-MS. The fragmentation patterns of mass spectra were compared with those of the spectrometer data base using NBS 54 AL and Wiley L – built libraries and also with those reported in the literature (9, 10).

Screening of antimicrobial activity

Preparation of test solutions

The volatile oil of leaves of *Chrysanthemum indicum* L. was dissolved in dimethyl sulfoxide (DMSO) and three different concentrations (0.1% v/v, 0.5% v/v and 1.0% v/v) of the volatile oil were taken for antimicrobial activities.

Preparation of standard

For the preparation of standard solution, pure erythromycin and ketoconazole were dissolved in DMSO.

Antimicrobial activity

The antibacterial and antifungal activities of volatile oil of *Chrysanthemum indicum* L. were performed by the Department of Microbiology, Ram-Eesh Institute of Vocational and Technical Education, Greater Noida. The identification of microbial strains was based on morphological, cultural and biochemical tests. The *invitro* antimicrobial activity of volatile oil was studied by cup plate method (10, 11, 12) against various microorganisms mentioned in the Table 2. Pure erythromycin (0.1 mg/ml) and ketoconazole (0.1 mg/ml) were used as standards. The plates were incubated at 37±2ºC, after 48 hrs of incubation. The Petri dishes were taken out from the incubator and the antimicrobial activities were compared by measuring the diameter of zone of inhibition.

Table No. 1: Chemical composition of volatile oil of leaves of *Chrysanthemum indicum* Linn.

S.No.	Components	Percentage (%)	RI
1.	Unknown	0.1	-
2.	α- Pinene	1.3	928
3.	Sabinine	2.0	965
4.	Camphor	6.8	1126
5.	Borneol	4.0	1154
6.	Isoborneol	14.4	1149
7.	Limonene	0.7	1022
8.	α- Phellandrene	1.4	1000
9.	Chrysanthenol	3.4	1100
10.	Cis- β- Ocemene	4.2	1027
11.	Sabinaketone	5.3	1130
12.	4- Terpineol	3.4	1170
13.	Unknown	0.6	-
14.	α- Terpineol	1.2	1149
15.	t- Caryophyllene	1.2	1404
16.	Unknown	7.4	-
17.	Unknown	0.4	-
18.	Unknown	0.9	-
19.	α- Humulene	0.5	1440
20.	β- Farnesene	5.1	1454
21.	Unknown	3.6	-
22.	α- Copane	4.1	1370
23.	α- Gurjunene	5.3	1389
24.	β- Elemene	1.8	1382
25.	γ- Cadinene	0.7	1497
26.	β- Selinene	0.6	1471
27.	α- Cedrol	0.2	1575
28.	δ- Cadinene	5.5	1505
29.	β-Sesquiphellandrene	3.6	1515
30.	ar- Curcumene	0.9	1472
31.	α- Cedenol	0.4	1652
32.	Germacrene D	0.3	1465
33.	α- Zingiberene	0.6	1490
34.	Germacren -4-ol	0.5	1511
35.	Veridiflorol	1.8	1590
36.	t-β- Farnesene	1.0	1454
37.	Unknown	3.8	-
38.	Unknown	0.6	-
39.	Sapthulenol	0.4	1551

RI = Retention Index

Total monoterpenes = 48.1 %

Total sesquiterpenes = 34.5 %

Others components = 17.4 %

Table No. 2 : Antimicrobial activity of volatile oil of leaves of *Chrysanthemum indicum* Linn.

S. No.	Test Organism	Zone of Inhibition (mm)[a]				
		Dilution of volatile oil in DMSO (v/v) Acetone			Standards	
					Erythromycin	Ketoconazole
		0.1%v/v	0.5%v/v	1.0%v/v	0.1 mg/ml	0.1 mg/ml
1.	*Micrococcus luteus* (ATCC 9341)	10.5	11	13	28	_
2.	*P. aeruginosa* (ATCC 9027)	11	16	16.5	19	_
3.	*Candida albicans* (MTCC 227)	10	15	18	_	30

[a] an average of triplicate,

Erythromycin - against bacterial strains only, **Ketoconazole**- against fungal strains only.

Result and Discussion

The constituents are arranged in order of GC elution on Ublon HR-1 column. Analysis of the isolate by GC and GC-MS resulted in the identification of thirty one (31) components comprising 82.6 %, out of thirty nine (39) components isolated as the total volatile constituents comprising 100 % of volatile oil. Quantitatively as assessed from total peak areas, the volatile oil was characterized by a high amount of monoterpenes (48.1%). Isoborneol was the predominant characterized constituent (14.40 %) among all the isolated monoterpenes and also among all the isolated volatile constituents of oils. Out of the twelve (12) monoterpenes, there were five hydrocarbons (9.6%) and seven oxygenated monoterpenes (38.5%). In sesquiterpene group nineteen (19) components were isolated (34.5%). δ-cadinene was the predominant characterized constituent (5.5%) among sesquiterpenes. Out of the nineteen sesquiterpenes, there were sixteen hydrocarbons (33.5%) and three oxygenated sesquiterpenes (1%). Eight (8) other components comprising 17.4% of all the isolates were unidentified.

Volatile oil of leaves of *Chrysanthemum indicum* L. (CHI) have been tested against two bacterial strains and one fungal strain in comparison with Erythromycin and Ketoconazole as standard antibacterial and antifungal agent, respectively. Maximum activity was shown by 1.0% v/v concentration of CHI volatile oil against *Pseudomonas aeruginosa* (16.5 mm) followed by *Micrococcus luteus* (13 mm) in case of bacteria while zone of inhibition against *Candida albicans* was maximum as 18 mm at the same 1.0 %v/v concentration. It is concluded that volatile oil posses good antimicrobial activity at proportionate to its concentration.

Conclusion

From the above result, it can be deduced that the volatile oil comprises of thirty nine (39) components out of which thirty one (31) constituents comprising 82.6 % were positively identified and Isoborneol (14.4 %) was found to be predominant constituent. The maximum antibacterial activity was shown against *Pseudomonas aeruginosa* (16.5 mm) at 1% v/v concentration of volatile oil, whereas maximum antifungal activity was shown against *Candida albicans* (18.0 mm) at the same concentration.

Acknowledgement

The authors are thankful to Dr. Anjula Pandey, Taxonomist, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic and Resources, New Delhi for identification and authentication of the plant and to Dr. R. C. Sharma, Chairman, Ram-Eesh Institute of Vocational & Technical Education, Greater Noida, UP, for providing research facilities to carry out the work.

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