INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

VOLATILE CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF FRESH RHIZOME OF *CURCUMA LONGA* LINN.

Vijender Singh[1], Gunjan[1*], Mohammed Ali[2]

[1]*School of Pharmacy, Sharda University, 32, 34 Knowledge Park – III, Greater Noida, U.P., India.*

[2]*Department of Pharmacognosy, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi, India.*

ARTICLE INFO

Article history

Received 28/03/2017

Available online 30/04/2017

Keywords

Aspergillus niger,
β- eudesmol.

ABSTRACT

Curcuma longa Linn. syn. Indian saffron, curcuma in hindi known as haldi (*f* Zingiberaceae.) is a tall herb. It is a native of South Asia & is cultivated extensively throughout warmer parts of the world, including India. Volatile constituents of the fresh rhizomes of curcuma by GLC and GC-MS resulted in the identification of twenty seven components. The oil consists of seven monoterpenes, out of which three were hydrocarbons, three alcohols and one ester.Fifteen sesquiterpenes out of which nine hydrocarbons, three alcohols, one ketone, one oxide and one epoxide. Sesqueterpenes of which spanthulenol (46.6%) was the major components followed by *β*- eudesmol (15.1) and *ar* - turmerone (9.7%) and oil also consist of five non terpenic compounds. Volatile oil of *Curcuma longa* in higher concentration showed significant antibacterial activity against the strains of *Staphylococcus aureus* (16.4 mm) followed by *Escherichia coli* (14.2 mm), significant anti fungal activity against *Candida albicans* (13.1 mm) followed by *Aspergillus niger* (12.5 mm). The alcoholic extract of *Curcuma longa* showed more potent antioxidant activity in comparison to aqueous extract.

Corresponding author

Gunjan

School of Pharmacy,
Sharda University, 32, 34 Knowledge Park – III,
Greater Noida, U.P., India.
gunjan.singh2@sharda.ac.in
08800781217

*Please cite this article in press as **Vijender Singh** et al. Volatile Constituents and Biological Activities of Fresh Rhizome of Curcuma Longa Linn.. Indo American Journal of Pharmaceutical Research.2017:7(04).*

INTRODUCTION

Turmeric an important constituent of *Curcuma longa* Linn. *syn.* Indian saffron, curcuma (*f* Zingiberaceae.) is a tall herb. It is a gene of 70 species of rhizomatous herb which is cultivated from the warmer part of the world.[1] Plant is a native of South Asia & is cultivated extensively throughout warmer parts of the world, including India. Its secondary metabolites have various pharmacological activity *viz* anti-inflammatory, antimicrobial, wound healing, anticancer and antiviral disease.[2] Turmeric is a valuable antiseptic & its lotion is applied to small pox or chicken pox eruptions.[2] Turmeric and Curcumin have been especially useful in increasing the bile flow in infected bile ducts.[3] The ethanolic extract of rhizome exhibited blood sugar lowering activity in alloxan- induced diabetic rats.[4] Presence of *β*- sesquipheliandrene in volatile oil of curcuma has been reported for antitumor activity[5] .It has also been used for cardiovascular, metabolic, pulmonary and autoimmune disease. Volatile oils of Curcuma have been known to possess wound healing properties and inhibitory properties.[6]The essential oil of Curcuma has been reported for *ar*-turmerone, turmerol, atlantone. The main constituents were terpenoline, alpha phellandrene and 1,8 cineole.[7] It has various therapeutic effects including antimicrobial properties and has been validated experimentally by researcher. [8,9] It has been used from ancient time in medicine, cosmetic, food, and flavoring agent worldwide.[10]Turmeric powder and its extract oleoresin is used on commercial basis.[3]Curcumin, the active ingredient of the rhizome responsible for its biological activity, is the principle coloring matter & constitutes one-third of the oleoresin. The rhizomes contain curcumin oils demethoxy curcumin, dihydro curcumin.[11]

EXPERIMENTAL

Plant Material:

Fresh rhizomes of *Curcuma longa* Linn. were purchased from local vegetable market, Bhagalpur, Bihar, India. The plant material was identified & authenticated by Dr. John Department of Botany, Marwari College, Bhagalpur, Bihar, India. A voucher specimen was kept in the herbarium of the department of Botany, Marwari College, Bhagalpur, Bihar.

Isolation:

The fresh rhizome (1.0 kg each) were hydro distilled for three hours according to the method recommended in the British Pharmacopoeia 2003. The pale yellow to very light orange colored oil(1.60 %v/w) was obtained from leaves and peels respectively. The collected volatile oil was dried over anhydrous sodium sulphate and stored at 4 ^{0}C in the dark.

GC Analysis:

Analytical GC was carried out on a Varian 3300 GC fitted with a silicone DB-1 capillary column (30m × 0.25mm), film thickness 0.25µm, carrier gas Nitrogen, flow rate 1.5 ml/min., split mode, temperature programmed 80-250 ^{0}C at 4 ^{0}C/min. Injector temperature and detector temperature were 250 ^{0}C and 300 ^{0}C respectively. Detector used was FID. Injection volume for all samples was 0.1µ.

GC-MS Analysis:

GC-MS Analysis was carried out on a QP-2000 instrument at 70eV and 250^{0}C. GC column Ulbon HR-1 fused silica capillary 0.25mm × 50m with film thickness 0.25µm. The initial temperature was 100 ^{0}C for six minutes and then heated at a rate of 10 ^{0}C per min. to 250 ^{0}C. Carrier gas Helium, flow rate 2ml/min., detector used was FID.

Identification of volatile constituents:

The individual compounds were identified by comparing their retention indices (RI) of the peaks on ULBON HR-1 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 made by comparison of fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K.L, WILEY8 libraries and also with those reported in the literature.[12-18] Relative amounts of identical components were based on peak areas obtained without FID response factor correction. The components of the oil, the percentage of each constituent and their RI values are summarized in Table 1. The constituents were arranged in order of GLC and GC-MS elution on silicon DB-1 and ULBON HR-1 fused silica column, respectively.

ANTI-MICROBIAL ACTIVITY

Preparation of sample:

The volatile oil (0.1% v/v, 0.5 % v/v, 1% v/v) and dried alcoholic extract (5.0%w/w) were dissolved in dimethyl sulfoxide (DMSO) for anti-microbial activity.

Preparation of Standard Drugs Solution:

Chloramphenicol and Ketoconazole were used as standard solutions for comparison of anti-bacterial and anti-fungal studies. Both the standard drugs were taken in DMSO. The concentration of both standard drug solutions was 10 mg / ml.

Anti-microbial Activity:

The antimicrobial activities of volatile oil and dried alcoholic extract of fresh rhizome of Curcuma longa were collected and the experiments were performed in Microbiology laboratory, School of Medical Science and Research, Sharda University, Greater Noida. The identification of microbial strains was based on morphological, cultural and biochemical tests. The antibacterial activities of various oil concentrations and dried alcoholic extract of the fresh rhizomes of Curcuma longa were studied by the cup plate method[19-21] against various microorganisms mentioned in the Table 2. Chloramphenicol and Ketoconazole were used as standard and the activity of each concentration was compared with corresponding concentration of standard drugs. The plates were incubated at 37 $\pm$ 2 ºC for antibacterial activity and 25 $\pm$ 2 ºC for anti fungal activity, after 48 hrs of incubation. The Petri dishes were taken out from the incubator and the anti-microbial activity of different concentrations of oil and dried alcoholic extract of fresh rhizomes of Curcuma longa were compared by measuring the diameter of the zone of inhibition. (Table 2).

ANTI OXIDANT ACTIVITY

Preparation of DPPH Solution:

DPPH solution (0.1mM in methanol) was prepared by dissolving 1.9 mg of DPPH in methanol and volume was made up to 100 ml with methanol. The solution was kept in darkness for 30 minutes for the completion of reaction.

Determination of Anti-Oxidant Activity:

1ml of DPPH solution was added to each ml of different alc. & aq. extracts of curcuma and allowed to stand at room temperature for 30 min. Then absorbance was measured at 517 nm on a double beam U. V. spectrophotometer. Similarly 1ml Extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution and the absorbance was measured at 230 nm.
(Table 3). The percentage inhibition was measured by following formula.[22]

% inhibition = (Ac-At) ×100/Ac

Ac = Absorbance of control

At = Absorbance of test sample

Table 1: Chemical composition of volatile oil of the fresh rhizomes of *Curcuma longa* Linn.

S. No.	Component	RI	% age	S. No.	Component	RI	% age
1	tricyclene	912	0.4	15	Bisabolene	1473	0.8
2	*α*- thujene	922	0.7	16	*α* – zingiberene	1475	1.4
3	camphene	939	0.4	17	caryophyllene oxide	1554	1.1
4	linalool	1086	1.1	18	carotol	1566	1.3
5	4 - terpineol	1170	0.8	19	humulene epoxide	1574	1.1
6	*p*- cymen -7-ol	1270	2.9	20	spathulenol	1588	46.6
7	*α* – terpenyl acetate	1337	0.3	21	*β*- eudesmol	1660	15.1
8	*Trans* - *β*- caryophyllene	1403	1.6	22	benzyl benzoate	1781	0.3
9	*β*- elemene	1405	0.2	23	*ar*-turmerone	1803	9.7
10	*β*- curcumene	1416	1.7	24	2-hexadecenal	1866	1.7
11	*β*- copaene	1447	0.9	25	ethyl-6-hexadecanoate	1890	2.1
12	germacarene –D	1454	0.6	26	ethyl-6–octadecanoate	1903	2.6
13	*β*- selinene	1464	2.3	27	*n*-heptadec- 5, 9- diene	1905	0.9
14	α – selinene	1471	1.4				

RI – retention index;

Monoterpenes (7) = 6.60%; Hydrocarbons (3) =1.5%; Alcohol (3) = 4.8%, Acetate (1) = 0.3%;

Sesquiterpenes (15)= 85.8;Hydrocarbons(9) =10.9%, Alcohol(3) = 63%, Epoxide(1) = 1.1%,

Ketone(1) = 9.7%, Oxide(1) = 1.1%;

Non-terpenoid components (5) = 7.60 % ; Hydrocarbons(1) = 0.9% , Ester (3) = 5%, Aldehyde (1) = 1.7%.

Table 2: Anti-microbial activities of volatile constituents and dried alcoholic extract of fresh rhizomes of *Curcuma longa* Linn.

S. No.	Test Organism	Zone of Inhibition in mm[a] Conc. of Volatile Oil			Dried Alcoholic Extract 5.0 %w/v	Standard Chloramphenicol (0.1 mg/ml)	Standard Ketoconazole (0.1 mg/ml)
		0.1 %v/v	0.5 %v/v	1.0 %v/v			
1	*Staphylococcus aureus*	7.4	9.6	16.4	9.2	20.9	-
2	*Escherichia coli*	6.7	8.8	14.2	8.2	18.8	-
3	*Candida albicans*	6.4	7.9	13.1	7.2	16.8	17.9
4	*Aspergillus niger*	5.9	7.3	12.5	6.7	15.9	17.6

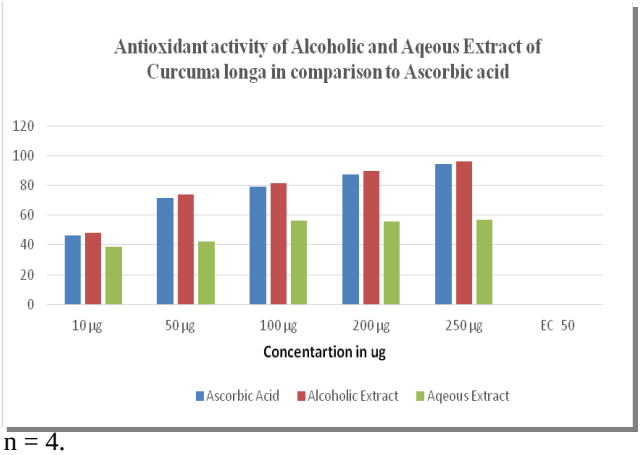
[a] an average of triplicate
Chloramphenicol - Against all micro-organism [gram $_+$ ve, gram $_-$ ve bacteria and fungal strains]
Ketoconazole - Against fungal strains only.

Table 3: DPPH free radical scavenging activities of dried alcoholic and aqueous extracts of fresh rhizome of *Curcuma longa* Linn.

Concentration	Absorbance		
	Ascorbic Acid	Dried alcoholic extract	Dried aqueous extract
10 µg	46.35±3.31	48.05±5.22	38.61±3.31
50 µg	71.66±6.11	73.82±2.46	42.08±2.27
100 µg	79.10±5.80	81.76±5.60	56.40±3.23
200 µg	87.44±4.74	89.66±6.11	55.64±5.78
250 µg	94.44±4.13	96.17±5.45	56.89±4.17
EC 50	14.9 µg	278.5 µg	189.8 µg

Graph showing the Antioxidant activity for fresh rhizome of *Curcuma longa* Linn.

ALCOHOLIC & AQUEOUS EXTRACTS USING ASCORBIC ACID AS STANDARD



Values are expressed as mean ± S.D., n = 4.

RESULT & DISCUSSION

Hydrodistilled volatile oil obtained from the rhizome of Curcuma, was analyzed by GC and GC-MS. The oil composition is summarized in Table 1. Twenty seven constituents were detected in the volatile oil out of which seven were monoterpenes (6.6%), fifteen sesqueterpenes (85.8%) and five non-terpenic compounds. Out of seven monoterpenes, three hydrocarbon (1.5%), three alcohol (4.8%) and one acetate (0.3%) were obtained. The major component identified in monoterpenes was p-cymen -7-ol (2.9%) followed by linalool (1.1%). Out of fifteen sesqueterpenes, nine hydrocarbons (10.9%), three alcohols (63%), one epoxide (1.1%), one ketone (9.7%) and one oxide (1.1%) were found. The oil contained spanthulenol (46.6%) being the major components identified followed by β – eudesmol(15.1%), ar–turmerone (9.7%), β - selinene (2.3%), five non- terpenic compounds (7.6%) and one hydrocarbon (0.9%). Out of seven monoterpenes three were hydrocarbons (1.5%) , three esters (5%), one aldehyde (1.7%) and one non-terpenic ester that is ethyl-6–octadecanoate (2.6%) which was the major component followed by ethyl-6-hexadecanoate (2.1 %) and 2-hexadecenal (1.7 %).

The control DMSO showed no inhibition of growth, while all the concentrations of oil were effective against bacteria viz *Escherichia coli*, *Staphylococcus aureus* and against gungus viz *Candida albicans* followed by *Aspergillus niger* when compared to Chloramphenicol and Ketoconazole. Volatile oil of *Curcuma longa* in higher concentration showed significant antibacterial activity against the strains of *Staphylococcus aureus* (16.4 mm) followed by *Escherichia coli* (14.2 mm), significant anti fungal activity against *Candida albicans* (13.1 mm) followed by *Aspergillus niger* (12.5 mm).

Alcoholic extracts of *Curcuma longa* Linn possess more potent free radical scavenging activity with increasing concentrations. Thus the results were comparable to the standards.

The results can prove that fresh rhizome of *Curcuma longa* Linn can be used in Phytotherapy.

ACKNOWLEDGEMENTS

The authors thank Sharda University especially to Sh. P. K. Gupta, Chancellor and Sh. Y. K. Gupta, Pro Chancellor, Sharda University, Greater Noida, U. P. for their positive attitude towards research along with Dr. Robin Singh, Senior Scientist, Indian Pharmacopoeia Commission, Ghaziabad, U. P. for the spectral analysis.

REFERENCES

1. Awasthi PK, Dixit SC (2009). Chemical composition of *Curcuma Longa* leaves and rhizome oil from the plains of Northern India. J Young Pharm.;1:312–6.
2. Srimal RC (1997). Turmeric-a brief review of medicinal properties. Fitoterapia. ;68:483–93.
3. Zachariah TJ, Baby KN (1992). Effect of storage of fresh turmeric rhizomes on oleoresion and curcumin contents. J Spices Arom Crops. ;1:55–8.
4. DK Patel, SK Prasad, R Kumar, and S Hemalatha (2012).An overview on antidiabetic medicinal plants having insulin mimetic property Asian Pac J Trop Biomed. Apr; 2(4): 320–330.
5. Lee CC, Houghton P (2005). Cytotoxicity of plants from Malaysia and Thailand used traditionally to treat cancer. *Journal of Ethnopharmacology*.;100 (3):237–243.
6. Aggarwal BB, Harikumar KB (2009). Potential therapeutic effects of curcumin, the antiinflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol.;41:40–59.
7. Zwaving JH, Bos R (1992). Analysis of the essential oils of five Curcuma species. Flavour Fragr J. ;7:19–22.
8. Tripathi AK, Prajapati V, Verma N, Bahl JR, Bansal RP, Khanuja SP, et al (2002). Bioactivities of the leaf essential oil of *Curcuma longa* (var. ch-66) on three species of stored-product beetles (Coleoptera) J Econ Entomol. ;95:183–9
9. Roses IA (1999). Medicinal plants of the World: chemical Constituents, Traditional and Modern Medicinal Uses. New Jersey: Human Press; pp. 139–53.
10. Mc-Carron M, Mills AJ, Whittaker D, Sunny TP, Verghese .J (1995). Comparison of the monoterpenes derived from green leaves and fresh rhizomes of *Curcuma longa* L. from India. Flavour Fragr J.;10:355–7.
11. Zwaving JH, Bos R (1992). Analysis of the essential oils of five Curcuma species. Flavour Fragr J;7:19–22.
12. Adams R.P (1995). Identification of Essential Oil components by Gas Chromatography/Mass spectrometry, Allured publications Corp. Curol Steane, IL,.
13. Libey L.M. (1991). A paradox database for GC/MS data components of essential oil and other volatiles, J. Essent. Oils. Res.,;3:193-194.
14. Jennings W. and Shibamoo T (1980). Qualitative Analysis of Flavour and Fragrance Volatiles by Capillary Gas Chromatography, Academic Press, New York,.
15. Swigar A.A. and Silverstein R.M (1981). Monoterpenes, Aldrich Chem. Co., Milwakee WI,.
16. Anderson N.H. and Falcone M.S (1969). The Identification of sesquiterpenes hydrocarbons from GLC retention data, J.Chromatography,;44:52- 59.
17. Ali M (2001). Techniques in Terpenoid Identification, Birla Publishers, New Delhi,
18. Pharmacopoeia of India, Controller of Publication, Ministry of Health and Family Welfare, Govt of India, New Delhi (1996), Vol II, p. A-105
19. Mackie W. and McCartney L (1980). Practical Medical Microbiology, 13Th Edn. Churchill Livingstone, Edinburg, London, p.162,

20. Singh,V (2008). et.al, Volatile Constituents and Antimicrobial and Antifungal activities of Immature Green Seeds of *Trachyspermum ammi* Linn.' Journal of Essential Oil Bearing Plants,;11(1): 120- 123.
21. Apisariyakul A, Vanittanakom N, Buddhasukh D (1995). Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae) J Ethnopharmacol.; 49:163–9.
22. Gunjan et al (2015). Anti-Oxidant activity of some Indigenous medicinal plant – An Approach to Arthritic Therapy. Indo American Journal of Pharm Research.;5(06):2359-62

5487847845**1170338**