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Composition and Antibacterial Activity of the Essential Oil from the Rhizome of Turmeric (*Curcuma longa* L.)

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The essential oil from the rhizome of turmeric (*Curcuma longa* L. Bannu var.) grown in Pakistan was isolated by the hydro-distillation. Chemical constituents of the essential oil were separated and identified by means of gas chromatography/mass spectrometry (GC-MS). The chromatographic analysis of oil showed 17 constituents of which, 5 chemical constituents contributing 80 % of the total oil constituents could be identified. The main components of the essential oil were ar-turmerone (38.59 %), turmerone (8.88 %), curlone (12.9 %). eucalyptol (1.59 %) and caryophyllene (0.99 %) are the minor class of essential oil. Therefore, in the volatile oil from rhizomes of turmeric, sesquiterpenoids are major compounds, accounting for 46 %. The antibacterial activity of Bannu oil was also evaluated against four pathogenic bacterial strains (*Bacillus subtilis*, *B. macerans*, *B. licheniformis* and *Azotobacter* sp.) using agar well diffusion method. Ethanol, hexane and ethyl acetate extracts exhibited antibacterial activity as indicated by minimum inhibitory concentration (MIC) values, but other extracts of butanol and water did not exhibit any antibacterial activity. Ethanolic extract is most active against tested microorganisms. The MIC values of different strains and extracts ranged from 0.01-1.0 mm in diameter.

Key Words: Rhizome, *Curcuma longa* L., Essential oil, GC-MS, Antibacterial activity.

INTRODUCTION

Turmeric (*Curcuma longa* L. syn. *C. domestica*) is cultivated for the underground rhizome, which is widely used as condiments, dye stuff, drug, cosmetic, flavour and food industry[1]. The major constituents are essential oil and curcuminoids which are present in leaf and rhizomes. Turmeric oleoresin contained 30-55 % of curcuminoids pigments and 15-25 % of volatile oil. The active principles are synthesized in leaves, translocated and stored in rhizomes. Growth and development of leaves and rhizomes are dependent on several factors such as nutrition[2], cultivation practices[3], genotype[4] and environmental factors.

Volatile oil from turmeric has activity against snake venom[5] and ar-turmerone is responsible for acting on hemorrhagic activity and lethal effect of Bothrops venom tested in mice. Ar-turmerone presents antiplatelet activity and it has more potent inhibitors than aspirin against platelet aggregation induced by collagen[6]. Volatile oil from turmeric shows antifungal activity against *Colletotrichum falcatum*, *Fusarium moniliforme*, *Fusarium exysporium*, *Curvularia pallescens* and *Aspergillus niger*; the tested extract has 52 % of ar-turmerone and 12 % of ar-turmerol in its composition[7].

Several techniques can be used to remove the volatile oil from turmeric. Extraction of the volatile oil from the ground turmeric can be performed with solvents like hexane or petroleum ether or by steam distillation[8-10]. Hydrodistillation is a traditional method for removal of essential oils[11]. It is a versatile process that can be employed for small or large industries[11,8]. Hydrodistillation using the Clevenger apparatus is the official AOAC method for the analysis of volatile oils from spices[12]. Several researchers have used this technique to obtain volatile oil from different plant sources[13]. In every study involving hydrodistillation, the volatile oil was the product analyzed and the remaining turmeric was not of interest.

Medicinal plants have a long history of use and their use is widespread in both developing and developed countries. According to the report of the world health organization, 80 % of the world population relies mainly on traditional therapies which involved the use of plant extracts or their active substances[14]. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs[15]. Medicinal and spice plants are renewable raw materials. Their production is an alternative to the overproduction of traditional crops in agriculture. They also have an increasing economic importance[16].

The rhizome of turmeric has a rich history as spice, food preservative and colouring agent. Turmeric is a well-known indigenous herbal medicine having many biological activities[17]. Long before the time of cheaper synthetic food preservatives and colouring agents, spices like turmeric played a key role as food additive[18]. Its use as a remedy for hypercholesterolemia, arthritis, indigestion and liver problem has been known since long[19]. The continuing research indicates that turmeric have unique antioxidant, antimutagenic, antitumorigenic and anticarcinogenic, antiinflammatory, antiarthritic, antimicrobial and hypocholesterolemic properties as reviewed elsewhere[18,20].

Aromatic turmerone (20-30 %) was reported to be the major compound present in turmeric volatile oil[1], which is a mosquito repellent[21] and may be an effective drug for the treatment of respiratory disease[22] and dermatophytosis[23]. Synthetic turmerone appears to act as anticarcinogenic[24]. Antivenom activity of turmerone isolated from turmeric has also been reported[5]. Recently, turmeric oil isolated from CRTO was found to be both antifungal[25] and antibacterial[26].

The objective of the present study is to evaluate the chemical constituents and antibacterial activity of essential oil of Bannu variety of *Curcuma longa*. The findings also support the use of *C. longa* varieties in traditional medicines for the treatment of bacterial infections.

EXPERIMENTAL

The rhizomes of *C. longa* Bannu variety were collected from Ayub Agriculture research centre, Faisalabad.

Extraction of essential oils: The cut pieces of rhizome were subjected to hydro-distillation.

Steam distillation: Known weight of rhizomes were taken in reaction vessel and attached to steam generator. A water cool condenser was also attached with reaction vessel. Steam generator produced the steam which passed through the sample condensed and collected with essential oils. The oil was dried over anhydrous sodium sulphate and stored in a sealed vial at 4 ºC till GC-MS analysis was carried out. The yield of the oil is calculated on the basis of fresh weight of sample.

GC-MS Analysis: GC-MS of Varian, Saturn model 2000, equipped with ion trap detector (ITD) was used for the identification of different components of essential oil of *C. longa*. Sample was injected on a DB-5MS (30 m × 0.25 mm i.d., 0.25 μ film thickness) column. Helium was used as a carrier gas with a flow rate of 7.0-9.5 psi and split ratio 1:5. The column temperature was maintained at 75 ºC for 5 min with a 2.5 ºC rise per min to 250 ºC.

Various components were identified by their retention time and peak enhancement with standard samples in gas chromatographic mode and MS library search from the derived mass fragmentation pattern of various components of the essential oil.

Antibacterial activity

Microorganisms: Four different strains were used for testing antibacterial activity included *Bacillus subtilis*, *Bacillus macerans*, *Bacillus licheniformis* (gram positive bacteria) and *Azotobacter* (gram negative bacterium). The test organisms used in this study were obtained from G.C.U, Lahore, Pakistan. The bacteria were cultured on nutrient agar slants. The cultures were maintained by subculturing periodically and preserved at 4 ºC prior to use.

Screening for antibacterial activity: Antibacterial activity was tested by agar well diffusion method[27]. Different concentrations of the turmeric oil were prepared in different organic solvents *i.e*., ethanol, hexane, ethyl acetate, dichloromethane and water by using serial dilution method. The test organisms were seeded into respective medium by gently mixing 0.1 mL of the 24 h fresh cultures with 35 mL sterile melted agar in sterile Petri-plates. After harding four 7 mm diameter wells were made using sterile borer. The wells were filled with 0.1 mL of the sample extract. The antibacterial assay plates were incubated at 37 ºC for 24 h. The diameter of the zones of inhibition around each of the well was taken as measure of the antibacterial activity. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was recorded.

Minimum inhibitory concentration (MIC): The oil dilutions which showed antibacterial activity in agar well assay were subjected to MIC assay. In order to determine MIC serial dilutions of the extracts and oil were prepared with concentration ranged from 0.01-28.00 mg/mL. The MIC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear zone. All tests performed in triplicate.

RESULTS AND DISCUSSION

Hydro-distillation of rhizomes of *C. longa* Bannu variety gave a pale yellow coloured liquid. The results obtained from the analysis of essential oil of *C. longa* are presented in Table-1. Analysis revealed the presence of 17 chemical constituents, out of which 5 have been identified on the basis of their fragmentation pattern by mass spectroscopy. Sesquiterpenoids are the major constituents of *C. longa* which includes ar-turmerone (38.59 %), turmerone (8.88 %) curlone (12.93 %) where eucalyptol (1.59 %) and caryophyllene (0.99 %) are the minor class of essential oil.

We have already reported chemical profile of rhizome essential oil of Kasur variety of turmeric. The most abundant components were aromatic turmerone (25.3 %), α-tumerone (18.3 %) and curlone (12.5 %). Other constituents are caryophyllene (2.26 %) and eucalyptol (1.6 %). The component present in lowest amount is α-phellandrene (0.42 %)[28]. α-phellandrene is absent in essential oil of bannu variety of *C. longa*. There are significant qualitative and quantitative differences between the chemical profiles of different varieties of the essential oils of *C. longa*.

The oil produced from 5-10 month old rhizome of *C. longa* from northern plains of India was reported to contain 59.7 % of ar-turmerone[29] while the rhizome oil of another Indian chemotype[30] was characterized by ar-tumerone (41.4 %), tumerone (29.5 %) and turmerol (20 %). Rhizomes that were grown in Bhuttan was analyzed using GC and GC-MS[31]. The major compounds were found to be ar-turmerone (16.7-25.7 %), α-turmerone (30.1-32.0 %) and β-turmerone (14.7-18.4 %). The major constituents of the rhizome oil[32] were α-turmerone (44.1 %), β-tumerone (18.5 %) and ar-turmerone (5.4 %). The essential oil from turmeric rhizomes from Calicut-

TABLE-1
GC-MS ANALYSIS OF ESSENTIAL OIL OF BANNU TURMERIC VARIETY

Name of compounds	R:T	m.f.	m.w.	Yield (%)	m/e Value
Eucalyptol	7.75	$C_{10}H_{18O}$	154	1.590	M^+ 51(6 %), 55 (47 %), 58 (18 %), 67 (35 %), 71 (69 %), 81 (99 %), 84 (67 %), 93 (68 %), 96 (41 %), 108 (100 %), 111 (90 %), 121 (12 %), 125 (17 %), 136 (14 %), 139 (82 %), 154 (96 %)
Caryophyllene	13.92	$C_{15}H_{24}$	204	0.995	M^+ 51 (6 %), 55 (25 %), 65 (15 %), 69 (57 %), 79 (66 %), 93 (88 %), 105 (59 %), 109 (17 %), 120 (44 %), 133 (100 %), 147 (33 %), 161 (43 %), 175 (12 %), 189 (28 %), 204 (11 %)
Ar-tumerone	20.35	$C_{15}H_{20}O$	216	38.590	M^+ 51 (5 %), 55 (25 %), 65 (8 %), 77 (18 %), 83 (100 %), 91 (31 %), 105 (63 %), 111 (17 %), 119 (70 %), 132 (16 %), 201 (25 %), 216 (35 %)
Tumerone	20.25	$C_{15}H_{22}O$	218	8.880	M^+ 55 (24 %), 65 (7 %), 77 (27 %), 83 (86 %), 91 (37 %), 105 (100 %), 111 (33 %), 120 (59 %), 126 (8 %), 200 (9 %), 218 (8 %)
Curlone	20.83	$C_{15}H_{22}O$	218	12.930	M^+ 55 (11 %), 65 (3 %), 77 (7 %), 83 (27 %), 91 (16 %), 105 (18 %), 120 (100 %), 218 (4 %)

India[33] having components of ar-tumerone (31.1 %), tumerone (10.0 %), curlone (10.6 %) and ar-curcumerene (6.3 %).

The antibacterial activity of essential oil of bannu variety of *C. longa* was assayed by agar well diffusion method against four bacterial species and the results are expressed as MIC is given in Table-2. Dilutions of essential oil in ethanol solvent showed significant inhibitory activity. Inhibition was observed against all bacterial strains. Inhibition zone of bannu oil ranged from 0.3-8.0 mm in diameter. As observed in the case of solvent ethanol, dilutions of oil in hexane also showed antibacterial activity. Hexane extract of bannu oil dilutions showed higher activity and produced inhibition zone ranging from 0.6-8.0 mm in diameter. However the MIC was much lower than that of solvent ethanol (Table-3). Bannu oil dilutions in hexane showed higher MIC against *B. licheniformis* (0.4 mg/mL).The dilutions of essential oil in ethyl acetate solvent also showed antibacterial activity. Bannu variety showed higher activity against *Bacillus lichniformis* and its inhibition zone ranging from 1.3-8.8 mm. Bannu oil showed higher MIC against *B. subtilis* followed by *B. licheniformis*, *B. macerans* and *Azotobacter*. On the other hand, water and butanol extracts of oil did not show any inhibitory activity.

In the present study, dilutions of essential oil of bannu variety in ethanol, hexane and ethyl acetate showed antibacterial activity against all tested strains. On the contrary, observed that dilutions of essential oil in water and butanol remain inactive against bacterial strains. It is evident from table that gram positive bacterium *B. subtilis* was the most sensitive organism

TABLE-3
MICS OF DIFFERENT SOLVENT EXTRACTS OF *Curcuma longa*

Solvents extract	MICs of solvent extracts (mg/mL)			
	Bacillus subtilis	*B. macerans*	*B. licheniformis*	*Azotobacter* sp.
Ethonal	0.01	0.03	0.03	0.05
Hexane	0.80	0.80	0.40	1.00
Ethyl acetate	0.20	0.50	0.30	0.80
Butanol	–	–	–	–
Water	–	–	–	–

to oil dilutions in solvent ethanol and gram negative bacterium *Azotobacter* was less sensitive organism as antibacterial activity of *C. longa* essential oil serial dilutions in methanol showed high MIC (18.0 mm) against gram positive bacterium *B. subtilis*[34] and antibacterial activity of ethanol extract of turmeric showed high MIC (12.0 mm) against gram positive bacterium *B. cereus* and it did not show any MIC against gram negative bacterium *E. coli*[35]. So, present study showed that gram-positive bacteria were the most sensitive organism to the plant essential oils and gram negative bacteria were less sensitive to the plant essential oils. In general gram-positive bacteria are more sensitive to plant oil and extract than gram-negative bacteria[36-38]. The varying degrees of sensitivity of the bacterial test organisms may be due to both the intrinsic tolerance of microorganisms and the nature and combinations of phyto-compounds present in the essential oil[39].

TABLE-2
INHIBITION ZONES OF DIFFERENT SOLVENT EXTRACTS OF *Curcuma longa*

Strains	Solvent type	Inhibition zones induced by turmeric oil dilutions (mm)				
		4 (mg/mL)	10 (mg/mL)	16 (mg/mL)	22 (mg/mL)	28 (mg/mL)
Bacillus subtilus	Ethanol	2.6	5.3	6.0	6.3	8.0
B. macerus		2.0	3.3	4.3	5.3	6.0
B. lichniformis		2.3	3.0	5.0	5.3	7.0
Azotobactor sp.		0.3	2.0	3.6	4.0	5.5
Bacillus subtilus	Hexane	1.0	2.0	3.0	3.3	4.3
B. macerus		1.0	1.0	2.6	3.6	5.0
B. lichniformis		1.0	2.3	4.0	5.6	5.6
Azotobactor sp.		0.60	1.6	4.6	6.0	8.0
Bacillus subtilus	Ethyl acetate	2.3	4.6	5.6	7.0	8.0
B. macerus		1.3	5.0	4.0	4.0	3.3
B. lichniformis		3.3	5.3	7.3	7.6	9.0
Azotobactor sp.		2.0	4.0	5.0	6.0	6.3

REFERENCES

1. V.S. Govindarajan, *CRC Crit. Rev. Food Sci. Nutr*., **12**, 199 (1980).
2. P. Rethinum, K. Sivaraman and P.K. Sushama, *Adv. Horticult. Plant. Spice Crops.*, **9**, 477 (1994).
3. J.S. Randhawa and R.K. Mahey, *Adv. Bot. Horticult. Pharmacol.*, **3**, 71 (1988).
4. M.R. Rao and D.V.R. Rao, *Adv. Horticult. Plant. Spice Crops.*, **9**, 131 (1994).
5. L.A.F. Ferreira, O.B. Henriques, A.A.S. Andreoni, G.R.F. Vital, M.M.C. Campos, G.G. Habermehl and V.L.G. Demoraes, *Toxicon*, **30**, 1211 (1992).
6. H.S. Lee, *Bioresour. Tech*., **97**, 1372 (2006).
7. G. Singh, O.P. Singh and S. Maurya, *Prog. Cryst. Growth Charac. Mater*., **45**, 75 (2002).
8. A.C. Manzan, F.S. Toniolo, E. Bredow and N.P. Povh, *J. Agric. Food Chem*., **51**, 6802 (2003).
9. D.L. Mata, Nelson, R.J.C.F. Afonso, M.B.A. Gloria and R.G. Junqueira, *Cienc. Technol. Alimen.*, **24**, 151 (2004).
10. C.R.A. Souza and M.B.A. Glória, *Braz. Arch. Biol. Technol.*, **41**, 218 (1998).
11. E. Guenther, The Essential Oil, D. van Nostrand Company Inc. New York, edn. 3, pp. 12-15 (1948).
12. G. Ertl, *J. Assoc. Off. Anal. Chem. Int.*, **80**, 901 (1997).
13. D.Q. Tuan and S.G. Ilangantileke, *J. Food Eng*., **31**, 47 (1997).
14. World Health Organization, Summary WHO Guidelines for the Assessment of Herbal Medicines, Herbal gram, Vol. 28, pp. 13-14 (1993).
15. J. Srivastava, J. Lambert and N. Vietmeyer, Medicinal Plants: An Expanding Role in Development, World Bank Technical Paper No. 320 (1996).
16. D. Srinivasan, S. Nathan, T. Suresh and L. Perumalsamy, *J. Ethnopharmacol*., **74**, 217 (2001).
17. H.P.T. Ammon and M.A. Wahl, *Planta Med*., **57**, 1 (1991).
18. M. Majeed, V. Badmaev, U. Shivakumar and R. Rajendran, Curcuminoids-Antioxidant Phytonutrients, Nutriscience Publishers, Inc., Piscataway, New Jersey (1995).
19. R.C. Srimal, *Fitoterapia*, **68**, 483 (1997).
20. J. Miquel, A. Bernd, J.M. Sempere and R.A. Diaz-Alperi, *Rev. Arch. Gerontol. Geriatr.*, **34**, 37 (2002).
21. A. Tawastin, S.D. Wratten, R.R. Scott, Y. Techadamrongsin and U. Thavara, *J. Vector Ecol.*, **26**, 76 (2001).
22. C. Li, L. Li, J. Luo and N. Huang, *Zhongguo Zhong Yao Za Zhi*, **23**, 624 (1998).
23. A. Apisariyakul, N. Vanittanakom and D. Buddhasukh, *J. Ethnopharmacol*., **49**, 163 (1995).
24. K.U. Baik, S.H. Jung and B.Z. Ahn, *Arch. Pharmacol. Res.*, **16**, 254 (1993).
25. G.K. Jayaprakasha, P.S. Negi, C. Anandharamakrishnan and K.K. Sakariah, *Z. Naturforsch*., **56c**, 40 (2001).
26. P.S. Negi, G.K. Jayaprakasha, R.L. Jaganmohan and K.K. Sakariah, *J. Agric. Food Chem*., **47**, 4297 (1999).
27. P.K. Mukherjee, P. Balasubramanian, K. Saha, B.P. Saha and M. Pal, *Indian Drugs*, **32**, 274 (1995).
28. S. Naz, S. Ilyas, Z. Parveen and S. Javed, *Asian J. Chem*., **22**, 3153 (2010).
29. M.C. Nigam and A. Ahmad, *Indian Perfumer*, **35**, 255 (1990).
30. J.H. Zwaving and R. Bos, *J. Flav. Fragr*., **7**, 19 (1992).
31. O.P. Sharma, *Biochem. Pharmacol*., **25**, 1811 (1976).
32. V.K. Raina, S.K. Srivastava and K.V. Syamsundar, *J. Essent. Oil Res*., (2005).
33. N.K. Leela, A. Tava, P.M. Shafi, S.P. John and B. Chempakam, *Acta Pharm*., **52**, 137 (2002).
34. S. Naz, S. Jabeen, S. Ilyas, F. Manzoor, F. Aslam and A. Ali, *Pak. J. Bot*., **42**, 455 (2010).
35. K. Norajit, N. Laohakunjit and O. Kerdchoechuen, *Molecules*, **12**, 2047 (2007).
36. I. Karaman, F. Sahin, M. Gulluce, H. Qgutcu, M. Sengul and A. Adiguzel, *J. Ethnopharmacol*., **85**, 231 (2003).
37. S. Cosentino, C.I.G. Tuberoso, B. Pisano, M. Satta, V. Mascia, E. Arzedi and F. Palmas, *Lett. Appl. Microbiol*., **29**, 130 (1999).
38. R.S. Farag, Z.Y. Daw, F.M. Hewed and G.S.A. El-Baroty, *J. Food Prot.*, **52**, 665 (1989).
39. S. Burt, *Int. J. Food Microbiol*., **94**, 223 (2004).