Composition and Antimicrobial Activity of the Essential Oil from Leaves of *Curcuma longa* L. Kasur Variety

Z. PARVEEN*, S. NAWAZ, S. SIDDIQUE AND K. SHAHZAD
PCSIR Laboratories Complex, Ferozpur Road, Lahore-54600, Pakistan

***Address for correspondence**
E-mail: Zahidaparveen18@gmail.com

The essential oil from the leaves of *Curcuma longa* L. Kasur variety grown in Pakistan was extracted by hydro-distillation. Chemical constituents of the essential oil were identified by gas chromatography/mass spectrometry. The chromatographic analysis of oil showed 25 constituents, out of which nine chemical constituents were identified. The eucalyptol (10.27%) was the major component of the essential oil. α-pinene (1.50%), β-phellandrene (2.49%), β-pinene (3.57%), limonene (2.73%), 1,3,8-p-menthatriene (1.76%), ascaridole epoxide (1.452%), 2-methylisoborneol (2.92%), 5-isopropyl-6-methyl-hepta-3, dien-2-ol (2.07%) were also present in considerable quantity. The antimicrobial properties of leaves of *Curcuma longa* were tested by disc diffusion method against various human pathogens, including eight fungal and five bacterial strains. Essential oil showed maximum resistance against *Fusarium miniformes* MAY 3629 followed by *Bacillus subtilis* ATCC 6633 whereas; it exhibited least resistance against *Fusarium oxysporium* ATCC 48122. The results of the antimicrobial assay revealed that essential oil showed significant inhibitory activity against the tested organisms.

Key words: Antimicrobial effects, *Curcuma longa* L, essential oil, gas chromatography/mass spectrometry, hydro-distillation, leaves

Due to negative consumer perceptions of artificial preservatives, attention is shifting towards natural preservatives[1]. The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum, both for the growing interest of consumers in ingredients from natural sources and also because of increasing concern about potentially harmful synthetic additives/preservatives[2].

Therefore, much attention has been focused on utilisation of plant derived antimicrobials to control pathogens in foods. Consequently, alternative preservatives are needed, which possess antimicrobial activity and cause no health problems[3,4].

Spices and condiments have been used for centuries to enhance the flavour and aroma of foods and their medicinal values. Spices are known for antimicrobial properties[5] and are most commonly used natural antimicrobial agents in foods[6,7]. Essential oils from different spices were found to possess antimicrobial activity[8-10] and majority of the essential oils are classified by FDA as 'Generally Recognised as Safe'[11].

Curcuma longa L. commonly known as 'turmeric' belongs to Zingiberaceae family, is a genus of 70 species of rhizomateous herbs. It is a perennial herb, cultivated extensively throughout the warmer parts of the world[12]. It is known worldwide for its multipurpose use in medicine, cosmetics, food flavouring and textile industries[13]. Turmeric powder, extracts and oleoresins are some of the widely used commercial products of *C. longa* plant[14]. The oils and the diarylheptanoid curcumin, which are the major secondary metabolites for *C. longa* have been shown to be largely responsible for the pharmacological activities of turmeric powder, extracts and oleoresins. The main activities have been found to be antiinflammatory, hepatoprotective, antimicrobial, wound healing, anticancer, antitumor and antiviral[15]. Furthermore, it has potential therapeutic effects against neurodegenerative, cardiovascular, pulmonary, metabolic and autoimmune diseases[16]. The oil of *C. longa* has been shown to possess the antiinflammatory activity, increase the bile flow and effective against bronchial asthma in a clinical trial[15]. Both the curcumin and the oil have been shown to possess wound healing properties and inhibitory activities against pathogenic fungi both *in vitro* and *in vivo*[15].

The essential oil of *C. longa* rhizome has been studied in detail by a number of workers[17,18] and the main constituents were ar-turmerone, turmerol and atlantone[18]. Leaves of *Curcuma* species are a waste product during postharvest operations. Traditionally, the leaves of *C. longa* extensively used in culinary preparation are aromatic and contain essential oil. *C. longa* leaves oil bestowed with medicinal values, has been used for treatment of various ailments and many of its therapeutic properties have been experimentally validated including its antimicrobial activity[19,20].

There are a few reports on constituents of *C. longa* leaf essential oil from different origins. Sixty-one compounds were identified from the leaf oil of *C. longa* constituting 99.8% of the oil and main constituents were α-phellandrene (53.4%), terpinolene (11.5%) and 1,8-cineole (10.5%)[17]. Leaves essential of elite genotype of *C. longa* L. from South Eastern Ghats of Orissa showed α-phellandrene (57.8%) as a major constituent[21]. The leaf oil of *C. longa* from Vietnam contained mainly α-phellandrene (24.5%), 1,8-cineole (15.9%), p-cymene (13.2%) and β-pinene (8.9%) (12), while

that of a Nigerian chemotype contained mainly α-phellandrene (47.7%) and terpinolene (28.9%)[22]. However, no information is known about the constituents and antimicrobial activity of *C. longa* Kasur var. leaves oil, which were collected from Ayub Agriculture Research Institute, Faisalabad, Pakistan.

The objectives of this study were to determine the chemical composition and antimicrobial activity of the essential oil of *C. longa* leaves against common food borne pathogens, in an attempt to contribute it as alternative product for microbial control and food preservation.

The leaves of *C. longa* Kasur var. were collected from Ayub Agriculture Research Institute, Faisalabad, Pakistan. The plant was identified at Department of Botany, Lahore College for Women University, Lahore, Pakistan. A voucher specimen is deposited in Prem Madan Herbarium (PMH) of Lahore College for Women University, Lahore Pakistan (Voucher No. PMH-12013).

Oil was extracted by hydro-distillation by reverse dean-stark method. A closed steam producing system was evolved which helped in the collection of oil without losing it into condensed water. The plant material was taken in a 5 l round bottom flask and heated in an isomantle. The flask was filled with the plants material (about half of its capacity) and then enough water was added to completely immerse the material. A reversed Dean-Stark assembly was fixed on the flask mouth and a coil condenser was attached on its top. The flask was heated and steam was produced in the flask, released oil which was carried away by the steam rising out of the flask. The steam carrying oil was led to the condenser on the top and condensed liquid dropped into the reverse dean stark apparatus. The oil floated on the top of water layer which on the addition of liquid coming from the condenser pushed the water in the bottom through the side arm back into the flask for recirculation. The same water was used again and again, which carried the essential oil from flask into straight arm and thus affected the extraction and separation of essential oil from plant material. The oil was separated from water by separatory funnel. The oil was dried over anhydrous Na_2SO_4 and weighed.

The analysis of the essential oil was carried out on gas chromatography/mass spectrometry (GC-MS) of Agilent Technologies Inc., USA, Model 6890 N, operating in electron ionization mode at 70 eV equipped with a split-less injector. Helium is used as a carrier gas at the flow rate of 1 ml/min, while HP-5 MS (30 m×0.25 mm id, 0.25 μ film thickness) capillary column was used. The initial temperature was programmed at 50-140° at the rate of 5°/min and then 100-250° at the rate of 3°/min followed by a constant temperature at 260° for period of 20 min. Sample (2 μl) was injected to column programmed at 200° and resolutions of components were attained. The mass spectrometer is capable of scanning from 35 to 500 AMU every second or less. The data acquisition system continuously acquires and stores all data analyses. The components were identified by their retention time and peak enhancement with standard samples in GC mode and NIST library search from the derived fragmentation pattern of the various components of the oil. The test organisms used in the study were Gram-negative (*Salmonella typhymurium* ATCC 14028, *Enterobacter areogene* ATCC 13048 and *Escherichia coli* ATCC 25922) and Gram-positive organisms (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) along with different fungal strains i.e. *Aspergillus niger* ATCC 16404, *Aspergillus flavus* ATCC 204304, *Aspergillus fumigatus* KM 8001, *Aspergillus ficuum* ATCC 66876, *Aspergillus oryzae* ATCC 10124, *Fusarium oxysporum* ATCC 48122, *Penicillum digitatum* ATCC 201167, *Fusarium miniformes* MAY 3629, *Fusarium saloni* MAY 3636. They were obtained from Food and Biotechnology Research Centre, PCSIR Labs, Lahore Pakistan and were stored initially at 4° in respective agar i.e., nutrient agar for bacteria and potato dextrose for fungal strains.

The agar disc diffusion method was employed for the determination of antibacterial and antifungal activity of *C. longa* leaves oil following the procedure of Baydar *et al*.[23], against different food borne pathogens including bacteria (*S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *S. typhymurium* ATCC14028 and *E. aerogenes* ATCC 13048) and fungi (*A. niger* ATCC 16404, *A. flavus* ATCC 204304, *A. fumigatus* KM 8001, *A. ficuum* ATCC 66876, *A. oryzae* ATCC 10124, *F. oxysporum* ATCC 48122, *P. digitatum* ATCC 201167, *F. miniformes* MAY 3629, *F. saloni* MAY3636). Standard culture media (CM 139, CM271, CM145, CM69, CM7 and CM201) from Oxoid were employed throughout the present investigation for the purpose of culture maintenance

at their respective temperatures that is 25° for fungi and 37° for bacteria. For dispensation of the medium, glass Perti plates of medium size (9 cm) were used. Approximately, 15 ml of the sterile standard culture medium, cooled to 45-50°, were poured into presterilized Petri plates. 0.1 ml of respective microbes was uniformly inoculated on the sterile plates. Agar formed a firm gel which on cooling gave a layer of 2-3 mm thickness.

Sterile and dried 4 mm paper discs (Difco) were impregnated with filtered (0.45 mm Millipore filter) and freshly extracted oil of *C. longa* L. leaves. These oil-impregnated discs were dried under laminar flow cabinet. The concentration of oil for each disc was 10 µl. The discs were placed on freshly seeded microbial lawns (4 discs in each plate) with a control. All experiments were conducted in triplicate. The petri plates were incubated at their respective temperatures and zones of inhibition thus developed against tested microorganisms were measured in millimetres after a period of 24, 48 and 96 h. The results of antimicrobial activity of oil against different microorganisms were expressed as resistant, intermediate and sensitive.

The essential oil was extracted by hydro-distillation from of *C. longa* leaves; yield was 1.45% (v/w). The GC coupled with MS revealed the presence of 25 compounds. Nine components, 28.76% of total were identified (Table 1). These components were further classified into two fractions i.e. hydrocarbon fraction and oxygenated fraction. Hydrocarbon fraction constituted α-pinene, β-pinene, β-phellandrene, limonene and 1,3,8-p-menthatriene while eucalyptol, ascaridole epoxide, 2-methylisoborneol, 5-isopropyl-6-methyl-hepta-3,5-dien-2-ol comprised the oxygenated fraction of the oil.

In present study, eucalyptol (10.27%) was the major component of the essential oil which was contrary to previous results in which α-phellandrene and terpinolene reported as major constituents[12,17,21,22,24]. It is interesting to note that α-phellandrene and terpinolene, which had been reported as major constituent of leaves oil[12,17,21,22,24] were absent in this study. α-inene (1.50%) and β-inene (3.57%) amounts were comparable to reports published earlier on leaf oil of *C. longa*[24]. β-phellandrene (2.49%), limonene (2.73%), 1,3,8-p-menthatriene (1.76%) ascaridole epoxide (1.452%), 2-methylisoborneol (2.92%), 5-isopropyl-6-methyl-hepta-3,5-dien-2-ol (2.07%) were present in considerable quantity.

Salient finding of the present investigation is first time report of six newly identified components of *C. longa* leaves essential oil including β-phellandrene, limonene, 1,3,8-p-menthatriene, ascaridole epoxide, 2-methylisoborneol, 5-isopropyl-6-methyl-hepta-3,5-dien-2-ol. The antibacterial and antifungal activities of *C. longa* leaves essential oil, investigated against different food borne pathogens by disc diffusion method, are presented in Table 2. The results showed different degrees of growth inhibition, depending on the microbial strains. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains[25].

It was found in the present study that leaves oil exhibited maximum zone of inhibition against *F. miniformes* MAY 3629 (22 mm) followed by *B. subtilis* ATCC 6633 (21 mm) and *A. flavus* ATCC 204304 (20 mm) after 48 h of incubation, whereas the minimum zone of inhibition was shown by *F. oxysporum* ATCC 48122 (10 mm) and *P. digitatum*

TABLE 1: GAS CHROMATOGRAPHY/MASS SPECTROMETRY ANALYSIS OF ESSENTIAL OIL OF *CURCUMA LONGA* LEAVES

Components	Retention time (min)	% age	M/Z values
α-pinene	5.40	1.50	M+(136,8) (121,17) (105,13) (93,100) (80,6) (77,31) (67,6) (65,6) (55,6) (53,8)
β-pinene	7.90	3.57	M+(136,5) (121,8) (107,4) (93,100) (79,19) (65,7) (53,10) (50,3)
β-phellandrene	8.95	2.49	M+(136,19) (121,7) (93,100) (80,11) (77,35) (69,8) (65,7) (53,6)
Limonene	10.41	2.73	M+(136,31) (121,34) (107,29) (103,3) (93,85) (79,44) (68,100) (63,4) (53,25)
Eucalyptol	12.16	10.27	M+(154,39) (139,28) (134,29) (119,100) (108,34) (93,24) (81,32) (71,20) (55,15)
1,3,8-p menthatriene	13.96	1.76	M+(134,85) (119,100) (105,32) (91,03) (77,24) (51,10)
Ascaridole epoxide	17.51	1.45	M+(150,40) (135,100) (125,33) (121,31) (107,76) (95,48) (91,47) (83,30) (79,37) (69,36) (6516) (51,10)
2-methylisoborneol	19.45	2.92	M+(168,8) (150,7) (135,8) (122,8) (95,100) (91,12) (79,11) (67,12) (55,7)
5-isopropyl-6-methyl-hepta-3, 5-dien-2-ol	25.35	2.07	M+(168,10) (150,11) (135,15) (122,12) (107,46) (95,100) (70,12) (59,40) (55,10)

TABLE 2: ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *CURCUMA LONGA* LEAVES AGAINST TESTED MICROORGANISMS

Tested microorganism	Colony morphology	Incubation temperature (°)	Culture media (oxoid)	Inhibition zone at 24 h (mm)	Inhibition zone at 48 h (mm)	Inhibition zone at 96 h (mm)	Efficiency
Aspergillus niger	White, later green/black	25	CM 139	18	18	18	S
Aspergillus flavus	White, later green/black	25	CM 139	20	20	20	S
Aspergillus fumigatus	White, later green/black	25	CM 139	16	16	16	I
Aspergillus ficuum	White, later green/black	25	CM 139	11	11	11	S
Aspergillus oryzae	White, later green/black	25	CM 139	15	15	15	I
Fusarium oxysporium	White, later pink, wrinkled	25	CM 139	10	10	10	R
Fusarium miniformes	White, later brown, irregular	25	CM 139	22	22	22	S
Fusarium saloni	White cottony	25	CM 139	15	15	15	I
Penicillium digitatum	White, later blue-green	25	CM 139	10	10	10	R
Staphylococcus aureus	Gram+ve, cocci	37	CM 145	17	17	17	S
Bacillus subtilis	Gram+ve, rods	37	CM 271	21	21	21	S
Escherichia coli	Gram−ve, rods	37	CM 69	16	16	16	S
Salmonella typhimurim	Gram−ve rods	37	CM 201	17	17	17	S
Enterobacter aerogenes	Gram−ve rods	37	CM 7	15	15	15	I

R=Resistant, S=Sensitive, I=Intermediate, CM139=Potato dextrose agar, CM 145=Staphlococcus medium 110, CM 271=Blood agar base, CM 69=Eosin methylene blue, CM 201=Bismithsulphite agar, CM 7=Macconkey's agar pH of the assay medium ranges from 6.4 to 7.3 depending upon the tested organisms, CM=Culture media

ATCC 201167 (10 mm) after 48 h of incubation. However, *A. niger* ATCC16404, *S. aureus* ATCC 25923, *S. typhymurium* 14028, *E. coli* ATCC 25922, *A. fumigatus* KM 8001, *E. aerogenes* ATCC 13048, *A. oryzae* ATCC 10124, *F. saloni* MAY 3636, *A. ficuum* ATCC 66876 and gave 18, 17, 17, 16, 16, 15, 15, 15 and 11 mm of zone of inhibition, respectively after 48 h of incubation at respective temperature.

Previous studies showed that *C. longa* inhibited the growth and activity of some bacteria and fungi. However, the antimicrobial efficacy results strictly dependent on concentration, microbial species, and essential oil fraction and especially on modality of extraction used. The antimicrobial activity of *C. longa* extract has been attributed to compounds belonging to flavonoids and terpens particularly to borneol, cymene, cuparene, and careen[26].

Gram-positive bacteria are more sensitive to plant oils and extracts than Gram-negative bacteria[27]. 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 phytocompounds present in the essential oil. The bioassay guided fractionation procedure showed that the plant essential oil was rich in terpenes (monoterpenes, oxygenated monoterpenes and sesquiterpenes). At present, however, the mode of action of terpenic constituents on microorganisms is not fully understood[28].

In the present study, *C. longa* leaves oil showed significant antimicrobial activity against all the tested organisms. This is because of the fact that some essential oils contain active components that influence certain metabolic functions of microbial cells. Wilkins and Board,[29] suggested that antimicrobial activity of oils is may be due to impairment of variety of enzymes systems that are involved in the production of energy or synthesis of structural components in the microbial cells. The differences in the sensitivity of food borne pathogens may be due to differences in methods used in study[30].

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Zia ur Rehman, Senior Scientific Officer, Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Ferozpur Road, Lahore-54600, Pakistan for GC-MS analysis and to Dr. Shagufta Naz, Department of Botany, Lahore College for Women University, Lahore, Pakistan for plant identification and authentication.

REFERENCES

1. Smith-Palmer A, Stewart J, Fyfe L. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett Appl Microbiol 1998;26:118-22.
2. Reische DW, Lillard DA, Eitenmiller RR. Antioxidants in food lipids. In: Ahoh CC, Min DB, editors. Chemistry, Nutrition and Biotechnology. New York: Marcel Dekker; 1998. p. 423-48.
3. Aureli P, Costantini A, Zolea S. Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*. J Food Prot 1992;55:344-8.
4. Dorman HJ, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J Appl Microbiol 2000;88:308-16.
5. Brul S, Coote P. Preservative agents in foods. Mode of action and microbial resistance mechanisms. Int J Food Microbiol 1999;50:1-17.
6. Nanasombat S, Lohasupthawee P. Antibacterial activity of crude ethanolic extracts and essential oils of spices against Salmonellae and other Enterobacteria. KMITL Sci Technol J 2005;5:527-38.
7. Del Campo J, Amiot MJ, Nguyen-The C. Antimicrobial effect of rosemary extracts. J Food Prot 2000;63:1359-68.
8. Ozcan MM, Sağdiç O, Ozkan G. Inhibitory effects of spice essential oils on the growth of *Bacillus* species. J Med Food 2006;9:418-21.
9. Cafarchia C, De Laurentis N, Milillo MA, Losacco V, Puccini V. Antifungal activity of essential oils from leaves and flowers of *Inula viscosa* (Asteraceae) by Apulian region. Parassitologia 2002;44:153-6.
10. Salehi P, Sonboli A, Eftekhar F, Nejad-Ebrahimi S, Yousefzadi M. Essential oil composition, antibacterial and antioxidant activity of the oil and various extracts of *Ziziphora clinopodioides* subsp. rigida (BOISS.) RECH. f. from Iran. Biol Pharm Bull 2005;28:1892-6.
11. Kabara JJ. Phenols and chelators. In: Russell NJ, Gould GW, editors. Food Preservatives. London: Blackie; 1991. p. 200-14.
12. Awasthi PK, Dixit SC. Chemical composition of Curcuma Longa leaves and rhizome oil from the plains of Northern India. J Young Pharm 2009;1:312-6.
13. Roses IA. Medicinal plants of the World: chemical Constituents, Traditional and Modern Medicinal Uses. New Jersey: Human Press; 1999. p. 139-53.
14. Zachariah TJ, Baby KN. Effect of storage of fresh turmeric rhizomes on oleoresion and curcumin contents. J Spices Arom Crops 1992;1:55-8.
15. Srimal RC. Turmeric-a brief review of medicinal properties. Fitoterapia 1997;68:483-93.
16. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the antiinflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol 2009;41:40-59.
17. Mc-Carron M, Mills AJ, Whittaker D, Sunny TP, Verghese J. Comparison of the monoterpenes derived from green leaves and fresh rhizomes of *Curcuma longa* L. from India. Flavour Fragr J 1995;10:355-7.
18. Zwaving JH, Bos R. Analysis of the essential oils of five Curcuma species. Flavour Fragr J 1992;7:19-22.
19. Apisariyakul A, Vanittanakom N, Buddhasukh D. Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae). J Ethnopharmacol 1995;49:163-9.
20. Tripathi AK, Prajapati V, Verma N, Bahl JR, Bansal RP, Khanuja SP, *et al*. Bioactivities of the leaf essential oil of *Curcuma longa* (var. ch-66) on three species of stored-product beetles (Coleoptera). J Econ Entomol 2002;95:183-9.
21. Shikha S, Manoj KP, Enketeswar S, Sanghamitra N. Chemical composition of leaf and rhizome oil of an elite genotype *Curcuma longa* L. from South Eastern Ghats of Orissa. J Pharm Res 2010;3:1630-3.
22. Oguntimein BO, Weyerstahl P, Marschall-Weyerstahl H. Essential oil of *Curcuma longa* L. leaves. Flavour Fragr J 1990;5:89-90.
23. Baydar H, Sagdic O, Ozkan, G, Karadogan T. Antibacterial activity and composition of essential oil from *Origanam*, *Thymbra* and *Satureja* species with commercial importance in Turkey. Food Control 2004;15:169-72.
24. Behura S, Sahoo S, Srivastava VK. Major constituents in leaf essential oils of *Curcuma longa* L. and *Curcuma aromatica* Salisb. Curr Sci 2002;83:1312-3.
25. Koné WM, Atindehou KK, Terreaux C, Hostettmann K, Traoré D, Dosso M. Traditional medicine in north Côte-d'Ivoire: Screening of 50 medicinal plants for antibacterial activity. J Ethnopharmacol 2004;93:43-9.
26. Thongson C, Davidson PM, Mahakarnchanakul W, Vibulsresth P. Antimicrobial effect of Thai spices against *Listeria monocytogenes* and *Salmonella typhimurium* DT104. J Food Prot 2005;68:2054-8.
27. Karaman I, Sahin F, Güllüce M, Ogütçü H, Sengül M, Adigüzel A. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J Ethnopharmacol 2003;85:231-5.
28. Norajit K, Laohakunjit N, Kerdchoechuen O. Antibacterial effect of five Zingiberaceae essential oils. Molecules 2007;12:2047-60.
29. Wilkins KM, Board RG. Natural antimicrobial systems. In: Gould GW, editor. Mechanisms of Action of Food Preservation Procedures. London: Elsevier; 1989. p. 285.
30. Kumar S, Bahchi GD, Darokar MP. Antimicrobial activity observed in seeds of some coprophilus plants. Int J Pharm 1997;35:179-84.

Accepted 13 February 2013
Revised 11 February 2013
Received 09 August 2012
Indian J Pharm Sci 2013;75(1):117-122