Chemical Composition of Turmeric Oil (*Curcuma longa L.* cv. Roma) and its Antimicrobial Activity against Eye Infecting Pathogens

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Abstract

The essential oil from the rhizomes of Roma cultivar of turmeric (*Curcuma longa*) from Orissa was examined for its antimicrobial activity against the pathogens causing eye infections. The oil was obtained by hydrodistillation extraction method using Clevenger apparatus. Chemical analysis of the oil was done by using gas chromatography and mass spectrometry (GC/MS). The antimicrobial effects of oil towards *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger* were tested by inhibition zone diameter (IZD) test to screen the antimicrobial activity, minimum inhibitory concentration (MIC) test and minimum killing time (MKT) test to determine the minimum concentration of oil and minimum time required to kill the pathogens. Oil showed very good activity against all four microbial strains used at concentration of 10 µL except *Pseudomonas aerugenosa.* Very low concentration of 1.95 µL/mL oil was needed to inhibit the growth of most highly infecting pathogen *Staphylococcus aureus* within 15 min of its exposure in comparison to other microbial strains. High turmerone content (49.76%) of elite turmeric cultivar Roma released from Orissa (India) might be assigned to be responsible for such excellent anti microbial activity against the tested pathogens. The purpose of this study is to authenticate the use of turmeric rhizome oil against eye infections so as to giving an approach to formulate turmeric rhizome oil as potential eye drop in place of traditional antibiotics after undertaking its *in vivo* pharmacological studies.

Key Word Index

Curcuma longa L., essential oil, GC/MS analysis, ar-tumerone, microbicidal, eye ailments.

Introduction

Turmeric (*Curcuma longa* L) belongs to the Zingiberaceae family grown in warm rainy regions of India. It is widely used as an Indian folk medicine for the treatment of various illnesses such as cough, wounds, rheumatism, sinusitis, digestive disorders and various eye disesses (1-2). The aroma of turmeric is due to its volatile oil which is an aromatic stimulant and carminative (3).The essential oil from rhizomes of *Curcuma longa* shows a wide range of biological activities in terms of antibacterial, antifungal, anticancer, insect repellant and anti snake venom activity (4-5). Ailments related to eye due to microbial infections continue to be a major health problem worldwide (6). Severe eye infections like blepharo conjuctivities, corneal ulcers, abscesses, styes, dacryocystitis, periorbital-celluties, orbitalcellutitis and blebs are mainly caused by *Staphylococcus aureus*, a normal flora and *Pseudomonas aeroginosa*, an opportunistic pathogen (7-8). *Candida sps* and *Aspergillus sps* are other most common cause of endogenous endophthalmitis, leading to scarring of the chorioretina and blindness (9). At present, the treatment of choice for these pathogens is antibiotics which causes severe side effects like hypersensitivity reactions, gastric disturbances, ototoxicity and nephrotoxicity (10) and incites resistance against these pathogens (11) on the other hand there exists many advantages in using antimicrobial compounds from medicinal plants such as fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (12-13). Use of turmeric, for treating eye infections by different ethnological groups like common people in different remote villages, traditional healers and tribal community Kond, Kui and Dongria of Kadhamal and Bondas, Bhunia, Paroja and Dora of Koraput districts of Orissa (14) as well as qualified medicinal

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practitioners of Ayurveda, Naturopathy, Unani and Sidha and Aromatherapists nationwide, is well known to our ancient civilization and has been in practice even till today. Turmeric is powdered and boiled in water for a few minutes and drops of strained water extract has been used for treatments of eye problems in general by these tribal groups of Orissa which has been in common practice in many a village community. Orissa, the second largest producer of turmeric of India (15) largely witnesses such ethnological use of turmeric in treatment of many common ailments. Orissa is the home state for releasing of many promising turmeric cultivars including Roma, which is popularly cultivated in North and South Eastern Ghat for high biomass yield and high oil. The composition of volatile oil of turmeric rhizome from various geographical regions has been reported by several authors (16-17) which may differ in quantity and quality with respect to varietal and environmental difference. Compositions of turmeric rhizome volatile oil are known to influence the quality and utility of a variety with respect to its use as a source of drug against diseases like eye and skin infection (1). So far, no information is available either on the constituent analysis of rhizome essential oil of Roma variety of Orissa or on its potential antimicrobial properties against eye infecting pathogens. In the present study validation of ethnological use of turmeric (cv roma) essential oil *in vitro* is done through performing all the basic tests: determination of IZD (Inhibition Zone Diameter), MIC (minimum inhibitory concentration), bactericidal and fungicidal effect, MKT (Minimum killing time) so as to enable an opening to formulate turmeric oil as potential eye drop in place of traditional antibiotics after undertaking it's *in vivo* pharmacological studies.

Experimental

Plant material: The rhizomes of *Curcuma longa* (cv. roma) were collected from the High Altitude Research Station Pottangi and grown in medicinal plant garden of Centre of Biotechnology, Bhubaneswar, Orissa (India). Fresh leaves and rhizomes on harvest were collected, washed under running tap water and were used immediately to extract the essential oils.

Microorganism and media: The test organisms used in this study were *Staphylococcus aureus* (MTCC-3160), *Pseudomonas aeruginosa* (MTCC-424), *Candida albicans* (MTCC-183) *and Aspergillus niger* (MTCC-281) obtained from the Microbial Type Culture Collection, Chandigarh, India. All the strains maintained in recommended media were purchased from Hi-Media India private Ltd., Mumbai.

Extraction of essential oils: The fresh rhizomes of turmeric were washed to remove soil, peeled and sliced. Sliced rhizomes of fresh turmeric (100 g) were mixed with distilled water. The essential oil was extracted by hydro-distillation using a Clevenger's apparatus following the method of (18). A flask containing the sliced rhizomes was heated for 6-10 h and the condensed vapor was separated throughout an auto-oil/water separator. The oil present at the upper most layers was collected in the ependroff tube. Each essential oil extraction was run in triplicate.

Oil yield: The total amount of oil in the rhizomes was calculated by the following method. Yield percentage was recorded as dry weight basis:

Rhizome oil % yield (v/w) (dry weight) =
volume of essential oils (mL) x 100%
Weight of raw materials

Analysis of the essential oils: GC analysis of the oils were carried out on a 6890 series instrument (Agilent Technologies, Palo Alto, CA, USA), equipped with FID and a HP-5 fused silica capillary column (30 m x 0.25 mm (internal diameter:,film thickness 0.25 mm). The temperature was programmed from 50–240°C at 4°C/min; from 240°C to 270°C at 15°C/min; held isothermal at 50°C for 1 min and at 270°C for 15 min. The temperatures of both auto-injector and detector were kept at 280°C; sample injection volume, 1µL; split ration was 100:1. The carrier gas was nitrogen at a flow rate of 1.2 mL/min. GC/MS (70eV) data were measured on the same gas chromatograph coupled with MSD 5973. MS source temperature at 230°C; MS quadrapole temperature at 150°C; interface temperature at 290°C; mass scan, 20-600 amu; carrier gas, He at a flow rate of 1.0mL/min. The retention index was calculated using a homologous series of n-alkanes C8-C18. Compounds were identified by comparison of their retention indices and mass spectra with the data given in the literature, National Institute of Standards and Technology (NIST), Wiley and our own created library (19-21).

Initial screening: For initial screening the disc diffusion method as described previously by (22) was followed with slight modifications. Briefly, Nutrient Agar NA) and Potato Dextrose Aagar (PDA) plates were swabbed with freshly grown cultures of the test pathogens by the help of a pre-sterilized cotton swab. Sterile filter paper discs (5 mm diameter) were kept on the above plates at equidistance. Varying volumes (2, 5 and 10 µl) of rhizome oil was loaded over the sterile filter paper discs. The plates were incubated at 37°C 18-24 hours for bacteria, 48 hours for fungi and observed for a zone of clearance around the discs which indicated positive microbicidal activity of the oil. All the experiments were carried out in triplicate.

Determination of MIC (minimum inhibitory concentration): Minimum inhibitory concentration of oil was determined by the tube dilution method (23) .The oil was diluted with NBT & PDBT (Nutrient and potato dextrose broth supplemented with 0.75% of Tween-20) to give oil concentration of 5 µg/ml to 100 µg/mL. 50 µL of (fresh culture) of the test organisms was inoculated into 1mL of NBT and PDT containing various concentrations of the oil. The tubes were incubated at 37°C, for 18–24 h, (48 h for fungi) and the lowest concentration inhibiting bacterial and fungal growth (no turbidity) was noted as MIC.

Test for bactericidal and fungicidal effect: In order to evaluate the effect (microbicidal /microbiostatic) of the oils, one loop from the MIC tube was sub cultured on to the NA and PDA plates which were then incubated at 37°C over night to check whether the oil merely had bactericidal or fungicidal activity i.e. no growth on subculturing.

Determination of MKT (minimum killing time): This experiment was designed to determine the time required to kill

Figure 1. GC/MS chromatogram of Rhizome oil of *Curcuma longa*

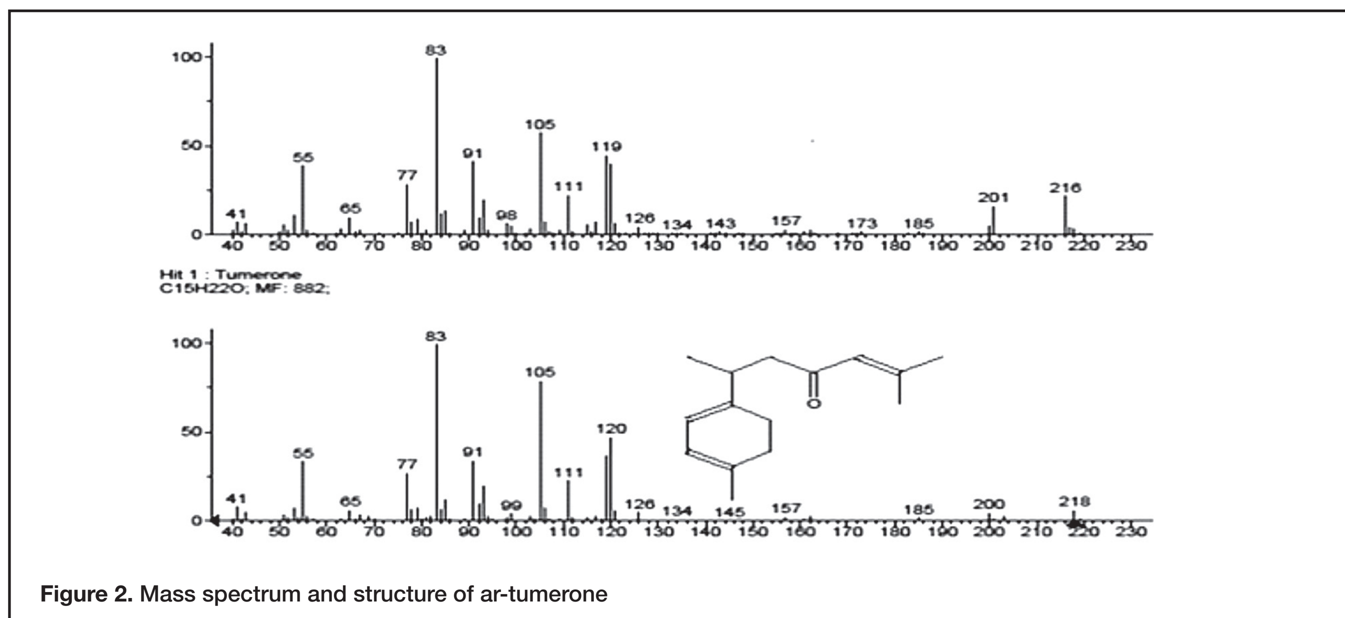

Figure 2. Mass spectrum and structure of ar-tumerone

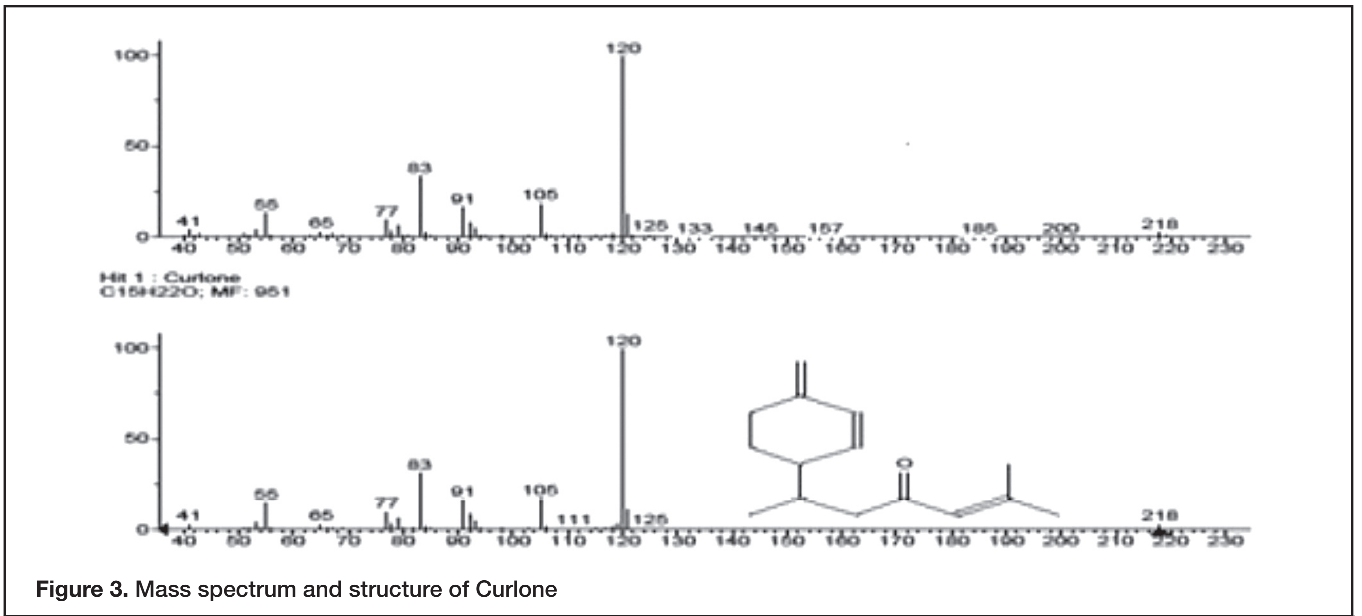

Figure 3. Mass spectrum and structure of Curlone

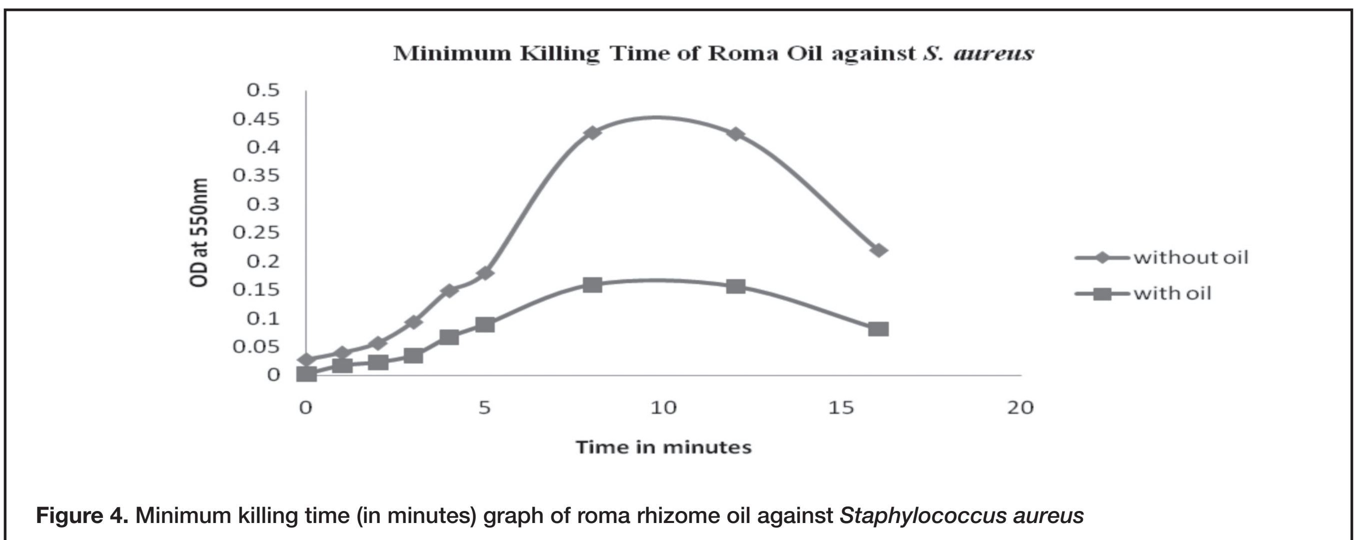

Figure 4. Minimum killing time (in minutes) graph of roma rhizome oil against *Staphylococcus aureus*

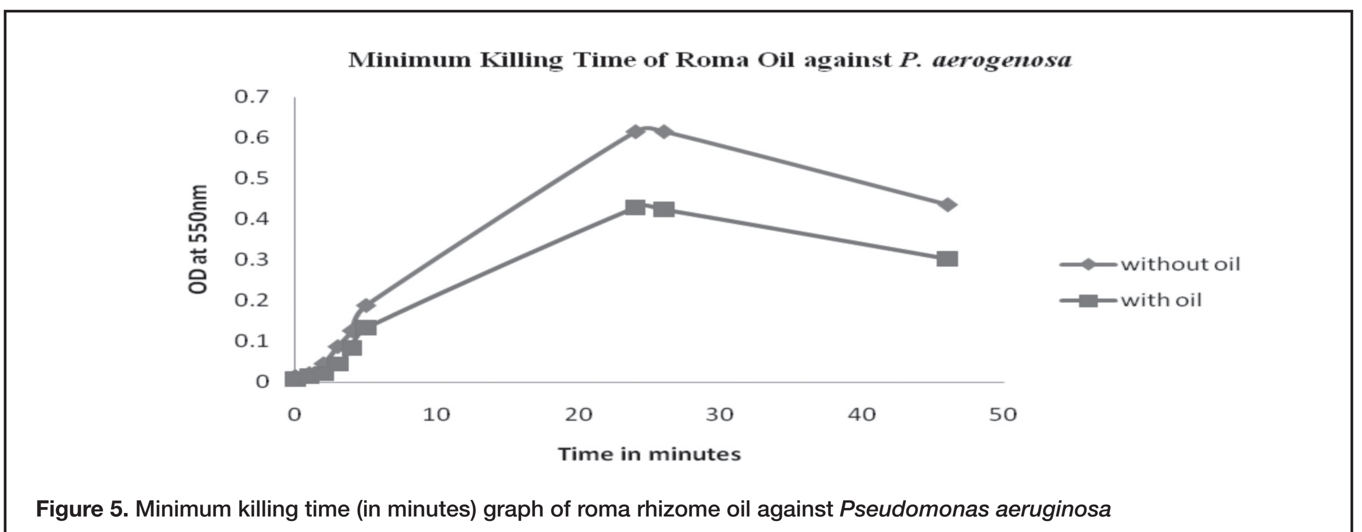

Figure 5. Minimum killing time (in minutes) graph of roma rhizome oil against *Pseudomonas aeruginosa*

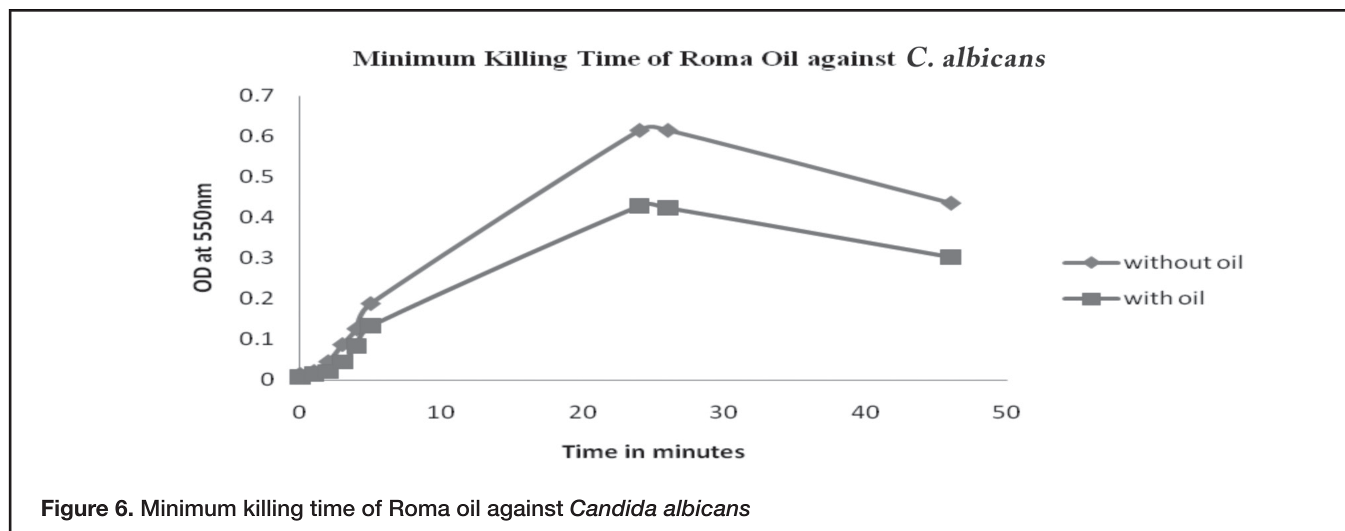

Figure 6. Minimum killing time of Roma oil against *Candida albicans*

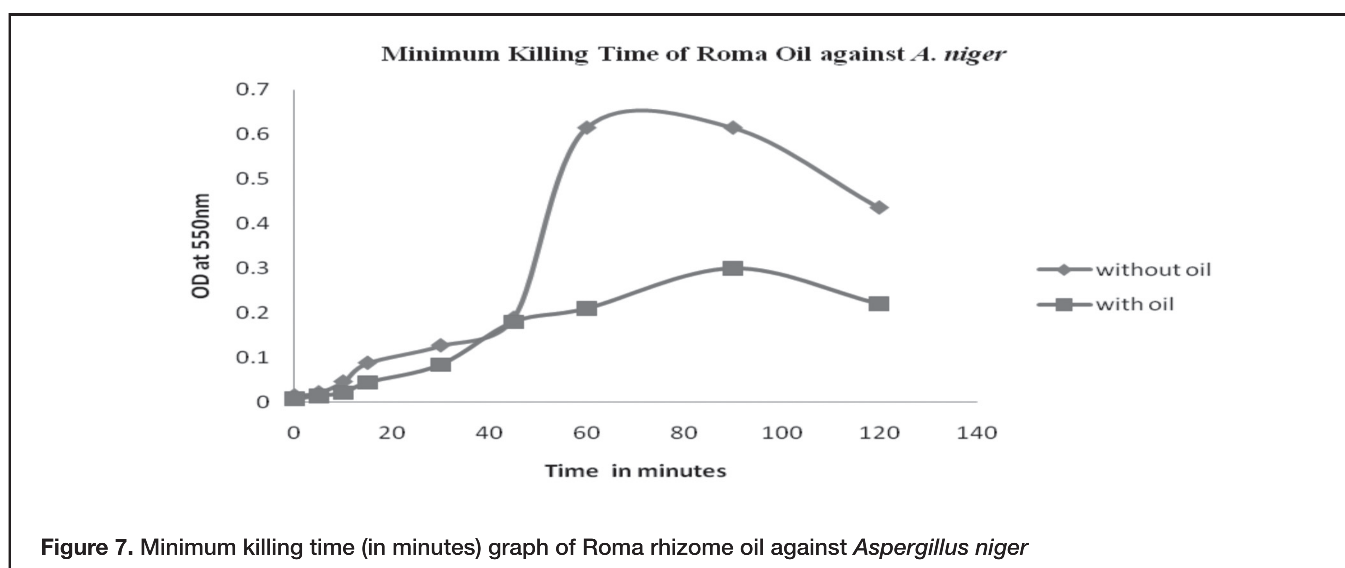

Figure 7. Minimum killing time (in minutes) graph of Roma rhizome oil against *Aspergillus niger*

the bacteria in vitro by the oil. One mL of NB, supplemented with 0.75% of DMSO and 1 mLof PDB supplemented with 0.75% of DMSO at MIC level of the oil was prepared and inoculated with 0.1 mL of freshly grown test organisms and incubated at 37°C. One loop of the sample from the above test tubes were sub cultured onto NA plates at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180 min. intervals and incubated overnight. Two sets of tubes were incubated for each test organisms from which subculturing were carried out alternatively (to avoid time lapse during subculture).

Determination of possible mode of action of the oil: Possible mode of action of the oils was determined through antibiotic sensitivity studies (studying antibiogram with group specific antibiotics following the method described previously (24). Specific antibiotic (Gentanycin: 10 µg), with specified concentrations was procured from Hi-Media, Ind. Ltd., and used to study the antibiogram of the isolates.

Statistical analyses: Each experiment was carried out in triplicate. The data were statistically analyzed using SPSS 10.0. A least significant difference (LSD0.05) was used to test the effects of EOs through a general linear model. The test was statistically significant at $p<0.05$.

Results and Discussion

The essential oil from the rhizome of roma cultivar have spicy aromatic odor, is insoluble in water and soluble in acetone and ethanol. The chemical constituent of the oil is reported in (**Table I**). GC/MS analysis of the oil showed the presence of 9 major components, accounting for the 87% of the total peak area in *C.longa* (cv.) roma (**Figure 1**). Compounds from rhizome oil were ar-tumerone (49.1%, mass spectra and structure shown in **Figure 2**) comprising maximum peak area followed by curlone (16.8%, mass spectra and structure showed in **Figure 3**), α-phellandrene (5.3%), ar-curcumene (3.5%), eucalyptol (2.6%), β-sesquiphellandrene (1.8%), β-caryophyllene (0.8%), β-bisabolene (0.6%) and δ-3-carene

Table I. Chemical composition of rhizome oil of *Curcuma longa* L. cv. Roma

Sl. No.	Oil constituents	MF	Area (%)± (SD)	RI[a]	RI[b]
1.	α-phellandrene	$C_{10}H_{16}$	5.3 ± 0.15	1005	1004
2.	δ-3-carene	$C_{10}H_{16}$	0.3± 0.10	1011	1011
3.	Eucalyptol	$C_{10}H_{16}$	2.6 ± 0.30	1032	1035
4.	β Caryophyllene	$C_{15}H_{24}$	0.8 ± 0.15	1418	1416
5.	β-farnesene	$C_{15}H_{24}$	0.6 ± 0.10	1458	1456
6.	ar-curcumene	$C_{10}H_{16}$	3.5 ± 0.25	1483	1483
7.	β-bisabolene	$C_{15}H_{24}$	0.6 ± 0.10	1509	1503
8.	β-sesquiphellandrene	$C_{15}H_{24}$	1.7± 0.15	1524	1524
9.	ar-turmerone	$C_{15}H_{24}$	49.1± 3.5	1668	1665
10.	Curlone (β-tumerone)	$C_{15}H_{22}O$	16.8 ± 0.41	1672	1670

MF: Molecular Formula, RI[a]: Retention Indices from literature.19 RI[b]: Experimental value
Average value (area %) of four replicate samples. Standard deviation (±) is given besides area percent in bracket.

Table II. Antimicrobial activity of roma rhizome oil by Disc Diffusion Method (DDM)

Microorganism	Mean zone sizes in mm by DDM* using different volumes			Inhibition zone diameter of gentamycin in mm	
	2.5 µL	5 µL	10 µL		F-Value
S. aureus	28 ± 1	35.33 ± 0.57	49.83 ± 0.76	17±1	789
P. aeruginosa	8 ± 1	12.33 ± 0.57	14 ± 1	25.67±0.57	256.3
C. albicans	14.67 ± 0.57	24.67 ± 0.57	30 ± 1	27.67±0.57	273.5
A. niger	10.33 ± 0.57	16.33 ± 0.57	25.33 ± 0.57	25±1	316.2

* Mean ± standard deviation (SD) where n=3 and data is significant at $P<0.05$.

(0.3%). Ar-tumerone has been reported as the major constituent of rhizome oil of turmeric of different origin (16). This result of GC/MS evaluation of essential oil of rhizome was compared with previous studies. Ref. 25 reported ar-tumerone (8.4%) in rhizome oil of *C. longa*, Zhu et al. (26) reported ar -tumerone (18%), Li et al. (27) reported ar-tumerone (6.4%), Sharma et al. (28) reported ar-tumerone (16.7–25.7%) in rhizome oil of *C. longa.* Presence of significantly high percentage (49.76%) of ar-tumerone in the cultivar roma will increase its demand and use in the national and international market as the high ar-tumerone content would increase the medicinal value of the oil. From the preliminary screening studies by disc diffusion method, it was observed that the test pathogens were susceptible to the oil. However a difference in the zone sizes were observed with different pathogens (**Table II**). Oil showed better activity against *S. aureus*, followed by *P. aeruginosa*, *C. albicans*, and least activity against *A. niger*. The MIC of the oil were ranged between 1-6 µL/mL (**Table III**).Though a variance was observed in the zones of inhibition and the MIC values, the three test pathogens except *S. aureus* were killed at same time i.e. took more than 24 h while *S. aureus* was killed after 15 min.(**Figures 4 through 7**). All the test pathogens were sensitive to all the antibiotics tested.

The results of this study suggest that the antimicrobial activity of the essential oil of rhizome of roma cultivar is bactericidal against *S. aureus*. The oil was significantly effective ($p<0.05$) against the test pathogens. Bacterial and fungal susceptibility towards the oil was observed at 2.5 µL per disc but higher concentrations showed larger zones of inhibition, when tested by agar plate technique. The results were highly significant for all the treatments, determining MICs, MKTs and even when the activities compared with standard antibiotic. In general, there seemed to be overall agreement between the size of inhibition zones obtained by the disc diffusion method (DDM) and the minimum inhibitory concentration (MIC) values, i.e. larger zones of inhibition correlated with lower MIC values. This relationship between inhibition zones and MIC values has been reported in literature while studying the antibacterial activity of essential oils (29-30). Better activity of roma rhizome oil could be due to presence of high perentage of ar-tumerone (49.05%). Some reports support our inference that turmerone is responsible for showing antimicrobial activity against specific pathogens (31). Since, all the test pathogens were sensitive to the specific antibiotic studied; the microbicidal activity of the oil could be attributed as multidimensional. Whereas, the killing of *S. aureus* may be due to cell wall synthesis/protein synthesis inhibition as it showed a resistance to flucloxacillin and oxacillin (32). Antibacterial activity of essential oils and fixed seed oils of *Azadirachta indica* (Neem) and *Pongamia pinnata* (Karanj) through inhibition of cell wall synthesis has been reported against both Gram-positive and Gram-negative bacteria (22,33). Reference 23 has also stated that the antibac-

Table III. MIC values for oil against tested organisms

Microorganism	MIC of oil (µL/mL)
Staphylococcus aureus	1.95
Pseudomonas aeruginosa	7.81
Candida albicans	5.5
Aspergillus niger	6.7

terial activity of *Eucalyptus* and Lemongrass oil against *E. coli* was due to inhibition of cell wall synthesis. Previous studies have already shown the growth inhibition activity of *Curcuma longa* rhizome essential oil on different microorganisms. However, this is the first time that the bactericidal activities of *Curcuma longa* rhizome essential oil have been demonstrated against eye infection causing pathogens. The results appear promising, for possible use of this rhizome oil of roma cultivar as bactericidal agents, more particularly in eye, skin and ear infections which are very sensitive organs. The use of antibiotics causes severe side effects like hypersensitivity reactions, gastric disturbances, ototoxicity and nephrotoxicity (34). Different antibiotics such as chlorotetracyclin, oxytetracycline and chloramphenicol at low concentrations have been used to prevent these infections. These antibiotics have been proved to have severe side effects; nowadays consumers are concerned about the use of synthetic antibiotics. When safety of synthetic products is questioned natural compounds of plant origin may appeal to the public. The antimicrobial activity of some essential oils and their components against food-borne pathogens, including mycotoxin producing fungi, has also been tested (35-36). Probably in our investigation, for the first time we have documented the antibacterial activity of rhizome oil of roma cultivar against eye infection causing pathogens. Furthermore, essential oil of rhizome of roma cultivar proved to have bactericidal properties at low concentrations, and most of the essential oil components possess antioxidant properties (37) holds a promise as an alternate to expensive, harmful antibiotics against these pathogens. Of course, other studies are highly necessary to study the toxicity of these oils in order to set an appropriate formulation like eye drop for this purpose.

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References

1. B. B. Aggarwal, C. Sundaram, N. Malani, H. Ichikawa, *Curcumin: the Indian solid gold.* Adv Exp Med Biol., **595**, 1-75 (2007).
2. *www.shroong.com.*
3. K. Nadkarni, *Indian Materia Medica.* Popular Prakashan, Bombay, 414-418, (1976).
4. 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, *Antivenom and bio- logical effects of ar-turmerone isolated from Curcuma longa (Zingiberaceae),* Toxicon, **30**, 1211-1218 (1992).
5. R. Singh, R. Chandra, M. Bose, P.M. Luthra, *Antibacterial activity of Curcuma longa rhizome extract on pathogenic bacteria.* Current Science, **83(6)**, 737-740, (2002).
6. F. D. Lowy, *Staphylococcus aureus infections.* N Engl J Med., **339**, 520-532, (1998).
7. L. R. Groden, B. Murphy, J. Rodnite, *Lid Flora in Blepharitis. Cornea,* **10**, 50-53, (1991).
8. F. Stapleton, J. K. Dart, D. V. Seal, M. Matheson, *Epidemiology of Pseudomonas aeruginosa keratitis in contact lens wearers.* Epidemiol Infect., **114(3)**, 395–402, (1995).
9. Anonymous, *Data on blindness throughout the world.* World Health Organisation Chronicle, **33**, 275–83, (1979).
10. S. E. Cosgrove, G. A. Vigliani, M. Campion, *Initial low dose gentamicin for Staphylococcus aureus bacteremia and endocarditis is nephrotoxic.* Clin Infect Dis., **48(6)**, 713–721, (2009).
11. K. Poole, *Efflux mediated multiresistance in gram negative bacteria. Clinical microbiology & infection.* The official publication of the European society of clinical microbiology & infectious disease, **10(1)**, 12-26, (2004).
12. H. S. Kim, *Do not put too much value on conventional medicines.* J Ethnopharmacol, **100**, 37–39, (2005).
13. G. R. Prabu, A. Gnanamani, S. Sadulla Guaijaverin, *A plant flavonoid as potential antiplaque agent against Streptococcus mutans.* J Appl Microbiol, **101**, 487–495, (2006).
14. *www.infinityfoundation.com,* 66 Witherspoon Street, Suite 400, Princeton, NJ 08542, (1994).
15. *www.dacnet.nic.in/spices,* Directorate of Arecanut and Spices Development Ministry of Agriculture (Dept. of Agriculture and Cooperation) Government of India, Calicut, Kerala, (1966).
16. N. K. Leela, A. Tava, P.M. Shafi, S.P. John and B. Chempakam, *Chemical composition of essential oils of turmeric (Curcuma longa L.).* Acta Pharmaceutica, **52**, 137-141, (2002).
17. S.N. Garg, R.P. Bansal M. M.Gupta, S. Kumar, *Variation in the rhizome essential oil and curcumin contents and oil quality in the land races of turmeric Curcuma longa of North Indian Plains.* Flavour Fragrance Journal, **14**, 315-318, (1999).
18. E. Guenther, *The Essential Oils.* D. Van Nostrand Co. Priceton, **1**, 316-318, (1948).
19. R. P. Adams, *Identification of Essential Oils by Ion Trap Mass Spectroscopy.* Academic Press: New York. (1989).
20. F. W. McLafferty, *Registry of Mass Spectral Data;* 5th edn. Wiley: New York, (1989).
21. S. E. Stein, National Institute of Standards and Technology (NIST) Mass Spectral Database and Software, Version 3.02, USA, (1990).
22. S. Pattnaik, V. R. Subramanyam, C. C. Rath, *Effect of essential oils on the viability and morphology of E. coli (SP-11).* Microbios, **84**, 195-199, (1995).
23. S. Pattnaik, V.R. Subramanyam, M. Bapuji, C. R. Kole, *Antibacterial activity of aromatic constituents of essential oils.* Microbios, **89**, 39-47, (1997).
24. C. C. Rath, S.K.Dash, R.K. Mishra, O.S. Ramachandraiah, G. Azeemoddin, J.K. Charyulu, *A note on the characterization of susceptibility of turmeric (Curcuma longa L.) leaf oil against Shigella sp.* Indian Drugs, **36 (2)**,133-136, (1999).
25. J. Chen, Y. Chen, J. Yu, *Studies on chinese Curcuma-IV. Quantitative determination of curcuminoids in the root and tuber Zhongcaoyao.* Chinese Traditional Herbal Drugs, **14 (2)**, 59-63, (1983).
26. L.F. Zhu, Y.H. Li, B. L.Li, B.Y. Ju, W.L. Zhang, *Aromatic Plants and Essential Constituents (Supplements1). South China Institute of Botany.* Chinese Academy of Sciences, Hai, Feng.Publ.Co. Distributed by Peace Book Co.Ltd., Hong Kong. (1995).
27. Li, L. Z-Q. Luo, G-Y. Ma, Y-O Zhang, G-J. Zhao, *GC/MS study on chemical constituents from zingiberaceae plants.* Guangpu Shiyanshi, **14**, 1-5, (1997).
28. R.K. Sharma, B. R. Misra, T.C. Sharma, A.K Bordoloi, M.G. Pathak, R. A. Leclercq, *Essential oils of Curcuma longa L from Bhutan.* J. Essent. oil. Res., **9**, 589- 592, (1997).
29. R. Gupta, C. C. Rath, S. K. Dash, R. K. Mishra, *In vitro antibacterial potential assessment of carrot (Daucus carota) and celery (Apium graveolens) seed essential oils against twenty one bacteria.* Journal of Essential Oil-Bearing Plants, **7**, 79-86, (2004).
30. C. C. Rath, S. Mishra, S. K. Dash, R.K. Mishra, *Antistaphylococcal activity of lime and juniper essential oils against MRSA.* Indian Drugs **42**, 797-801, (2005).
31. A. Amphawan, N. Vanittanakom, D. Buddhasukh, *Antifungal activity of turmeric oil extracted from Curcuma longa (Zingiberaceae).* J. Ethnopharmacol., **49**, 163 –169, (1995).

32. O. Korzeniowski, M. A. Sande, *Combination antimicrobial therapy for Staphylococcus aureus endocarditis in patients addicted to parenteral drugs and in nonaddicts: a prospective study.* Ann Intern Med, *97 (4)*, 496–503, (1982).
33. M. Baswa, C. C. Rath, S. K. Dash, R. K. Mishra, *Antibacterial activity of karanj (Pongamia pinnata) and neem (Azadirachta indica) seed oil: A preliminary report.* Microbios, **105**, 183-189, (2001).
34. S. E. Cosgrove, G. A. Vigliani, M. Campion, *Initial low dose gentamicin for Staphylococcus aureus bacteremia and endocarditis is nephrotoxic.* Clin Infect Dis., **48(6)**, 713–721, (2009).
35. A. Ultee, R. A. Slump, G. Steging, E. J. Smid, *Antimicrobial activity of carvacrol towards Bacillus cerus on rice.* Journal of Food Protection, **63**, 620-624, (2000).
36. O. Senhaji, F. Mohmad, K. Ichraq, *Inactivation of Escherichia coli 0157:H7 by essential oil from Cinnamomum zeylanicum.* Brazilian Journal of Infectious Diseases, **11**, 234-236, (2007).
37. M. T. Barrat, H. J. D. Dorman, S. G. Deans, C. Figueiredo, J. G. Barroso, G. Roberto, *Antimicrobial and antioxidant properties of some commercial essential oils.* Flavour and Fragrance Journal, **13**, 235-244, (1998).

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