World Journal of Pharmaceutical and Life Sciences

WJPLS

SJIF Impact Factor: 4.223

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *CURCUMA LONGA* L. (ZINGIBERACEAE) FIXED OIL

Abdel Karim. M.[1*], Abdel Wahab[2],A. Osman

[1]Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.
[2]University of Zalingi, Sudan.

***Corresponding Author: Dr. Abdel Karim. M.**

Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.

Article Received on 16/12/2016 Article Revised on 06/01/2017 Article Accepted on 26/01/2017

ABSTRACT

This study was set to investigate the chemical constituents of the medicinally important species *Curcuma longa* fixed oil and to evaluate its potential antimicrobial activity.61 components were detected by GC-MS analysis being dominated by: 2-(2-phenyl-2-propyl)-1,3-dioxolane(15.5%), 5-(4-ethylphenyl)-imadazole-4-propenoic acid(7.51%), 2-methyl-3-trimethylsilyl-1,3,7-octatriene(7.31%), curlone(5.80%), 9,12-octadecadienoic acid methyl ester (5.47%), hexadecanoic acid methyl ester(4.73%) and methyl stearate(3.66%). In cup plate agar diffusion bioassay, the oil exhibited excellent activity against *Bacillus subtilis* at concentrations: 25-100 mg/ml. It also showed activity against all test organisms in this range of concentration, but it was inactive against *Staphylococcus aureus* at 25mg/ml. At a concentration of 12.5mg/ml it showed activity only against *Pseudomonas aeruginosa* and *Escherichia coli.*

KEYWORDS: *Curcuma longa,* Essential Oil, GC-MS, Antimicrobial Activity.

INTRODUCTION

Natural products from different plants, continue to be used in pharmaceutical preparations either as plant extracts or as pure isolates. The isolation of many physiologically active natural products like artemisinin, harmaline, morphin, atropine showed the real importance to investigate plants that can be sources of new compounds with potent clinical activities. Plants can produce a large number of compounds that can provide a wide spectrum of biological properties.

Curcuma longa L. (Zingiberaceae) is a perennial herb up to 1 m high with a short stem. The plant is distributed throughout tropical and subtropical regions, being widely cultivated in China and India and other countries. *Curcuma longa* rhizomes are oblong, ovate, pyriform, often short-branched and they are a household remedy in Nepal.[1] As a powder *Curcuma longa* has been used for its flavoring and digestive properties.[2] In Indian traditional medicine the plant is used against biliary disorders, anorexia, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis.[3] In old Hindu medicine, *Curcuma longa* was extensively used for the treatment of sprains and swellings caused by injury.[4] The traditional medicine in China uses *C. longa* L. for abdominal pains. The coloring matter in *Curcuma longa*, which is a major constituent, is known as curcumin and its chemical structure was established by Roughley and Whiting.[5] Curcumin and other semi-synthetic analogues were evaluated for anti-inflammatory activity in model animals.[6] Curcumin and its analogues showed similar responses in carrageenin-induced paw edema ; however the sodium curcuminate was the most potent analogue. In another study, the anti-inflammatory activity of different fractions of the rhizomes was investigated *in vivo.*[7] Different fractions reduced the granuloma growth and no toxic effects were observed. The potential anti-inflammatory potency was also investigated by Ghatak and Basu.[8] The inhibitory effects of curcumin on the proliferation of blood mononuclear cells and vascular smooth muscle cells was studied.[9] Curcumin was able to impair the response of cells to mitogen, PHA and the response to alloantigen, MLR. The authors suggested that curcumin could be used clinically in transplant atherosclerosis. In another study, Ammon et al.[3] claimed that curcumin can inhibit leucotriene formation in rat peritoneal polymorphonuclear neutrophils (PMNL). Unnikrishnan and Rao[10] assessed the antioxidant capacity of curcumin and some of its derivatives. It was shown that these substances can protect hemoglobin from oxidation even at low concentrations. Pulla and Lokesh[11] demonstrated that curcumin is a potent antioxidant and inhibits lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates. Lipid peroxidation

plays an important role in the event of inflammation, heart diseases and cancer. The operating mechanism is probably through activation of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase.[12] Another article about curcuminoids as potent inhibitors of lipid peroxidation was described by Sreejayan Rao.[13] The ethanolic extract of *C. longa* was evaluated against *Plasmodium falciparum* and *L. major*. The extract was able to inhibit the *in vitro* growth of these parasites.[14] *C. longa* oil was evaluated against cultures of *Staphylococcus albus*, *S. aureus* and *Bacillus typhosus* and growth inhibition was observed in different concentrations.[15]

MATERIALS AND METHODS

Plant material

The rhizomes of *C. longa* were collected from Faioom, Eggypt. The plant was authenticated by direct comparison with a herbarium sample.

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness) was used for GC-MS analysis .

Test organisms

C. longa oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in Table(1).

Table 1: Test organisms.

S. No	Microorganism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
6	*Candida albicans*	fungus

Methods

Extraction of oil from *Curcuma longa*

Dry-powdered rhizomes of *Curcuma longa* (300g) were macerated with n-hexane at room temperature for 48h. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4°C for further manipulation.

Esterification of oil

A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol. A stock solution of methanolic sulphuric acid was prepared by mixing (1ml) of concentrated sulphuric acid with (99ml) methanol. The oil (2ml) was placed in a test tube and (7ml) of alcoholic sodium hydroxide were added followed by (7ml) of alcoholic sulphuric acid. The tube was stoppered and shaked vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of n- hexane were added and the tube was vigorously shaked for five minutes .The hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate(1µl) was injected in the GC-MS vial.

GC-MS analysis

Curcuma longa fixed oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness) was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is given in Table 2, while other chromatographic conditions are depicted in Table 3.

Table 2: Oven temperature program.

Rate	Temperature(C)	Hold time (mim.$^{-1}$)
-	60.0	0.00
10.00	300.0	0.00

Table 3: Chromatographic conditions.

Column oven temperature	1300.0 °C
Injection temperature	280.0 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml/ min
Column flow	1.50ml/sec
Linear velocity.	44.7cm/sec
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial assay

Preparation of bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in (100 ml) of normal saline to produce a suspension containing about 108-109 colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Preparation of fungal suspensions

Fungal cultures were maintained on potato dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antibacterial activity

The cup-plate agar diffusion method was adopted, with some minor modifications, to assess the antibacterial activity. (2ml) of the standardized bacterial stock suspension were mixed with (200 ml) of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle. Each of these plates was divided into two halves. Two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the test samples. Separate Petri dishes were designed for standard antimicrobial chemotherapeutics.

The agar discs were removed, alternate cups were filled with(0.1 ml) samples of each test solution using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

The above procedure was repeated for different concentrations of the test solutions and the standard chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

RESULTS AND DISCUSSION

GC-MS analysis of *Curcuma longa* oil

GC-MS analysis of *Curcuma longa* oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 61 components (Table 4). The typical total ion chromatograms (TIC) is depicted in Fig.1.

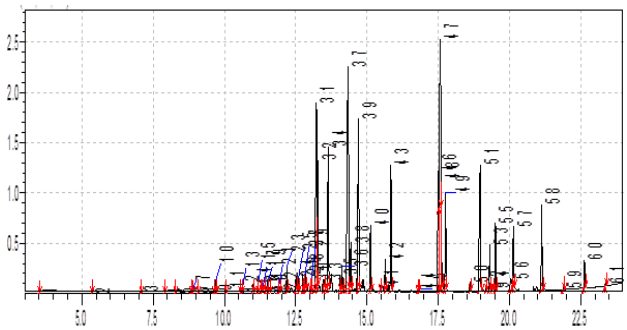

Fig. 1: Total ions chromatograms of *Curcuma longa* oil.

Major constituents are:

2-(2-phenyl-2-propyl)-1,3-dioxolane(15.5%)

The EI mass spectrum of 2-(-phenyl—propenyl)-1,3-dioxolone is shown in Fig.2 .The peak at m/z192, which appeared at R.T. 14.33 in total ion chromatogram, corresponds to $M^+[C_{12}H_{16}O_2]^+$.

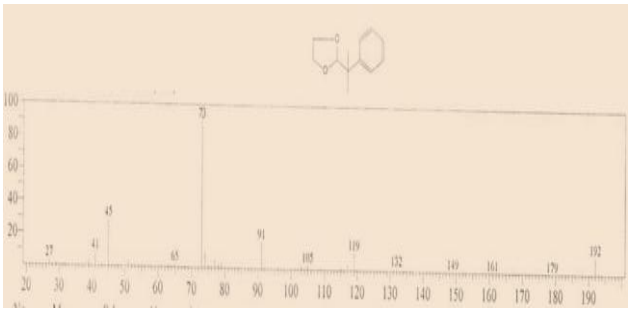

Fig. 2: Mass spectrum of 2-(2-phenyl-2-propyl)-1,3-dioxolane.

5-(4-ethylphenyl)-Imadazole-4-propenoic acid(7.51%)

The EI mass spectrum of 5-(4-ethylphenyl)-Imadazole-4-propenoic acid is shown in Fig.3 .The peak at m/z284, which appeared at R.T. 18.97 in total ion chromatogram, corresponds to $M^+[C_{15}H_{16}N_4O_2]^+$. The peak at m/z253 corresponds to loss of a methoxyl function.

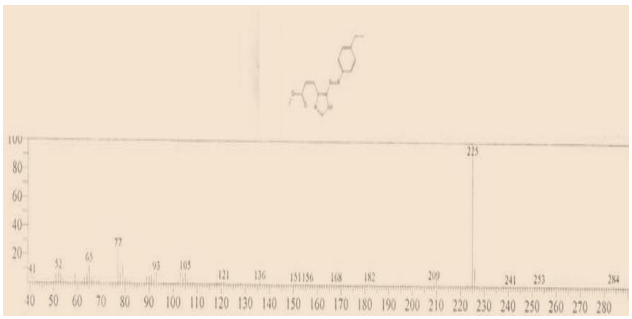

Fig.3: Mass spectrum of 5-(4-ethylphenyl)-Imadazole-4-propenoic acid.

Table 4 : Constituents of *Curcuma longa* oil.

Peak#	R.Time	Area	Area%	Name
1	3.480	37094	0.01	Hexanoic acid, methyl ester
2	5.339	16634	0.00	1-Octanol
3	7.056	414464	0.08	Ethanone, 1-(3-methylphenyl)-
4	7.888	29762	0.01	(-)-Carvone
5	8.253	44138	0.01	7-Methoxymethyl-2,7-dimethylcyclohepta-
6	8.822	129582	0.02	Benzene, 1,1'-(1,1,2,2-tetramethyl-1,2-etha
7	8.849	89640	0.02	Decanoic acid, methyl ester
8	9.056	54245	0.01	Methyl 4-(prop-1-en-2-yl)cyclohex-1-eneca
9	9.607	461471	0.09	Cyclohexane, 1,2,4-tris(methylene)-
10	9.700	1923151	0.37	2-Pentanone, 4-methyl-4-phenyl-
11	10.039	781353	0.15	p-Toluidine, N-methyl-N-nitroso-
12	10.551	110293	0.02	1-Methyl-6-(3-methylbuta-1,3-dienyl)-7-ox
13	10.597	191322	0.04	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethy
14	11.001	1263245	0.24	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-met
15	11.152	728646	0.14	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexe
16	11.281	207423	0.04	Benzene, 1,1'-(1,1,2,2-tetramethyl-1,2-etha
17	11.321	158688	0.03	.beta.-Bisabolene
18	11.412	266140	0.05	Dodecanoic acid, methyl ester
19	11.522	1719645	0.33	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-
20	11.582	385151	0.07	Bergamotol, Z-.alpha.-trans-
21	11.878	645300	0.12	7-epi-trans-sesquisabinene hydrate
22	11.943	592130	0.11	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5
23	12.193	4492357	0.86	p-Menthane, 2,3-dibromo-8-phenyl-
24	12.511	3106940	0.60	Benzene, 1-(3-cyclopentylpropyl)-2,4-dimet
25	12.600	2184215	0.42	5,9-Undecadien-1-yne, 6,10-dimethyl-
26	12.749	3303846	0.64	Benzene, (1,1,4,6,6-pentamethylheptyl)-
27	12.800	1116029	0.21	7-epi-cis-sesquisabinene hydrate
28	12.852	2969375	0.57	Santalol, cis,.alpha.-
29	13.021	812580	0.16	Benzene, (1,1,4,6,6-pentamethylheptyl)-
30	13.055	1739242	0.33	1,4-Methanoazulene-9-methanol, decahydr
31	13.225	47216724	9.09	Ar-tumerone
32	13.268	21702046	4.18	Tumerone
33	13.493	1860154	0.36	Pregna-3,5-dien-9-ol-20-one
34	13.645	30157856	5.80	Curlone
35	14.053	2791009	0.54	Cyclopentanecarboxylic acid, 3-isopropylic
36	14.128	4887200	0.94	7-Oxabicyclo[4.1.0]heptane, 1-(1,3-dimethy
37	14.335	79227274	15.25	1,3-Dioxolane, 2-(2-phenyl-2-propyl)-
38	14.435	10052675	1.93	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4
39	14.704	37986396	7.31	1,3,7-Octatriene, 2-methyl-3-trimethylsilyl-
40	15.127	11364311	2.19	3-(1,5-Dimethyl-hexa-1,4-dienyl)-2,2-dimet
41	15.465	894185	0.17	Dicumyl peroxide
42	15.643	4996695	0.96	9-Hexadecenoic acid, methyl ester, (Z)-
43	15.841	24587297	4.73	Hexadecanoic acid, methyl ester
44	16.773	607125	0.12	Methoprene
45	16.812	405806	0.08	Heptadecanoic acid, methyl ester
46	17.500	28412767	5.47	9,12-Octadecadienoic acid (Z,Z)-, methyl e
47	17.577	46320198	8.92	9-Octadecenoic acid (Z)-, methyl ester
48	17.604	11529191	2.22	9-Octadecenoic acid, methyl ester, (E)-
49	17.758	19013087	3.66	Methyl stearate
50	18.614	1536089	0.30	Benzenamine, N-methyl-4-(phenylazo)-
51	18.974	39031820	7.51	Imidazole-4-propenoic acid, 5-(4-ethylphen
52	19.175	533540	0.10	Behenic alcohol
53	19.311	8921397	1.72	11-Eicosenoic acid, methyl ester
54	19.362	666406	0.13	cis-11-Eicosenoic acid, methyl ester
Peak#	R.Time	Area	Area%	Name
55	19.510	12998913	2.50	Methyl 18-methylnonadecanoate
56	20.051	4700420	0.90	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-
57	20.140	12099660	2.33	Benzaldehyde, 4-((4-(dimethylamino)pheny
58	21.132	18460893	3.55	Methyl 20-methyl-heneicosanoate
59	21.892	567810	0.11	Tricosanoic acid, methyl ester
60	22.629	5544456	1.07	Tetracosanoic acid, methyl ester
61	23.375	475208	0.09	Squalene
		519522709	100.00	

2-Methyl-3-trimethylsilyl-1,3,7-octatriene(7.31%)

The EI mass spectrum of 2-methyl-3-trimethylsilyl-1,3,7-octatriene is shown in Fig.4 .The peak at m/z194, which appeared at R.T. 14.70 in total ion chromatogram, corresponds to $M^+[C_{12}H_{22}Si]^+$ .The peak at m/z179 corresponds to loss of a methyl function.

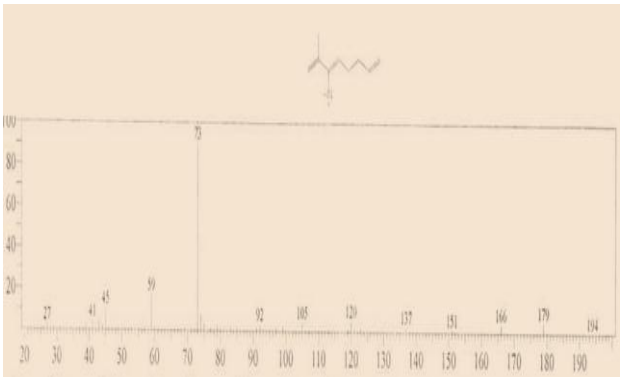

Fig. 4: Mass spectrum of 2-methyl-3-trimethylsilyl-1,3,7-octatriene.

Curlone(5.80%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.5 .The peak at m/z218, which appeared at R.T. 13.64 in total ion chromatogram, corresponds to $M^+[C_{15}H_{22}O]^+$

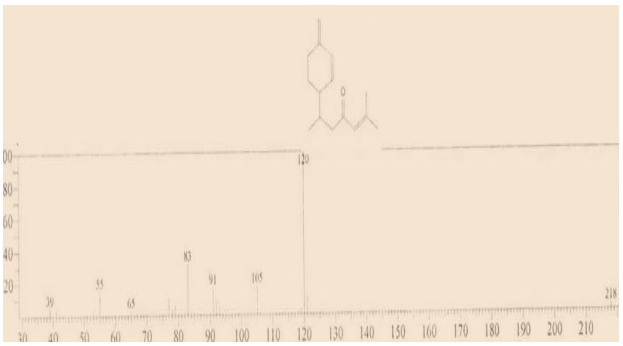

Fig.5: Mass spectrum of Curlone

9,12-Octadecadienoic acid methyl ester (5.47%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.6 .The peak at m/z294, which appeared at R.T. 17.500 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl function.

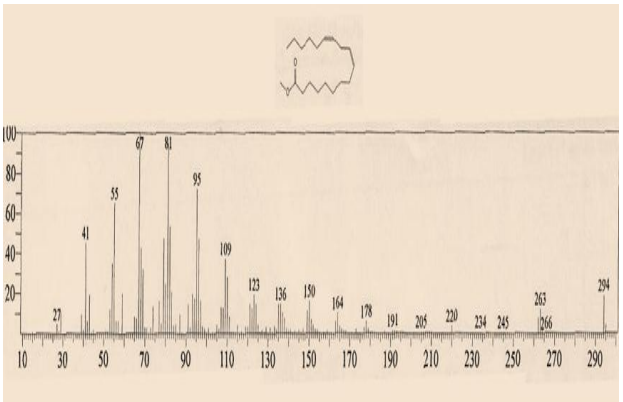

Fig. 6: Mass spectrum of 9,12-octadecadienoic acid methyl ester

Hexadecanoic acid methyl ester(4.73%)

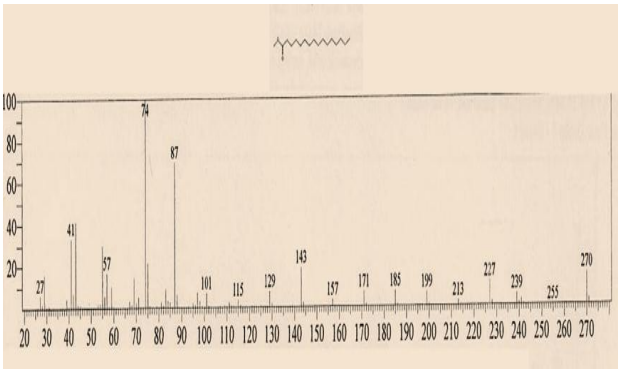

Fig. 7: Mass spectrum of hexadecanoic acid methyl ester.

The EI mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 7.The peak at m/z 270, which appeared at R.T. 15.841 in total ion chromatogram, corresponds to $M^+[C_{17}H_{34}O_2]^+$ .The peak at m/z239 is due to loss of a methoxyl function.

Methyl stearate(3.66%)

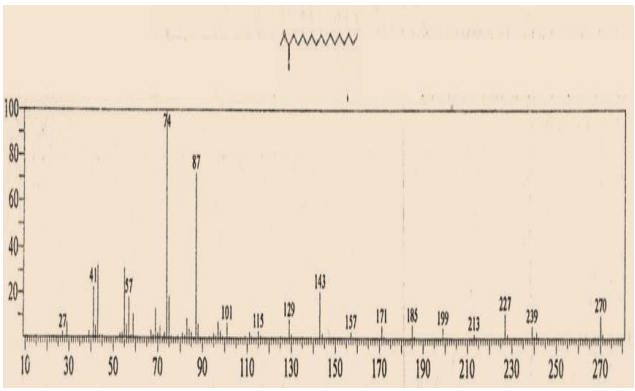

Fig. 8: Mass spectrum of methyl stearate.

The EI mass spectrum of methyl stearate is shown in Fig. 8.The peak at m/z 270, which appeared at R.T. 17.758 in total ion chromatogram, corresponds to $M^+[C_{19}H_{38}O_2]^+$ .The peak at m/z239 corresponds to loss of a methoxyl function.

Antibacterial activity

In cup plate agar diffusion assay, the oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones is depicted in Table (5). The results were interpreted in terms of the commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active). Tables (6) and (7) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 5: Antimicrobial activity of *Curcuma longa* oil.

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	16	31	16	15	15
	50	15	20	15	14	14
	25	-	19	14	14	13
	12.5	-	07	13	13	-

The oil exhibited excellent activity against *Bacillus subtilis* at concentrations: 25-100 mg/ml. In this range of concentration it also showed activity against all test organisms, but it was inactive against *Staphylococcus aureus* at 25mg/ml. At a concentration of 12.5mg/ml it showed activity only against *Pseudomonas aeruginosa* and *Escherichia coli*.

Table 6: Antibacterial activity of standard chemotherapeutic agents.

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 7: Antifungal activity of standard chemotherapeutic agent.

Drug	Conc.(mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

REFERENCES

1. Eigner, D., Scholz ,D., *J. Ethnopharmacol*, 1999; 67: 1.
2. Govindarajan, V.S., *CRC Cr Rev In Fd Sci Nut*,1980; 5: 199.
3. Ammon, H.P.T., Anazodo, M.I., Safayhi, H., Dhawan, B.N., Srimal, R.C., *Planta Med*, 1992; 58: 26.
4. Ammon, H.P.T., Wahl, M.A. *Planta Med*, 1991; 57: 1.
5. Roughley, P.J., Whiting, D.A., *J Chem Soc*, 1973; 20: 2379.
6. Mukophadhyay A., Basu, N., Ghatak, N., Gujral, P.K. *Agents and Actions*, 1982; 12: 508.
7. Arora, R.B., Basu, N., Kapoor, V., Jain, A.P., *Indian J Med Res*, 1971; 59: 1289.
8. Ghatak, N., Basu, N., *Indian J Exp Biol*, 1972; 10: 235.
9. Huang, H.C., Jan, T.R., Yeh, S.F., *Eur J Pharmacol*, 1992; 221: 381.
10. Unnikrishnan, M.K., Rao, M.N., *Pharmazie*, 1995; 50: 490.
11. Pulla, R. A., Lokesh, B.R., *Mol Cell Biochem*, 1992; 111: 117.
12. Pulla, R. A., Lokesh, B.R.. *Fd Chem Toxic*, 1994; 32: 279.
13. Sreejayan - Rao, M.N., *J Pharm Pharmacol*, 1994; 46: 1013.
14. Rasmussen ,H.B., Christensen, S.B., Kvist ,L.P., Karazmi, A., *Planta Med*, 2000; 66: 396.
15. Chopra, R.N., Gupta, J.C., Chopra, G.S., *Indian J Med Res*, 1941; 29: 769.