Pak. J. Bot., 42(1): 455-462, 2010.

ANTIBACTERIAL ACTIVITY OF *CURCUMA LONGA* VARIETIES AGAINST DIFFERENT STRAINS OF BACTERIA

SHAGUFTA NAZ[1*], SAFIA JABEEN[1], SAIQA ILYAS[1] FARKHANDA MANZOOR[2], FARAH ASLAM[1] AND AAMIR ALI[3]

[1]*Department of Botany, Lahore College for Women University, Lahore, Pakistan*
[2]*Department of Zoology, Lahore College for Women University, Lahore, Pakistan*
[3]*Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan*
[*]*E-mail: drsnaz31@hotmail.com*

Abstract

Crude extracts of curcuminoids and essential oil of *Curcuma longa* varieties Kasur, Faisalabad and Bannu were studied for their antibacterial activity against 4 bacterial strains viz., *Bacillus subtilis, Bacillus macerans, Bacillus licheniformis* and *Azotobacter* using agar well diffusion method. Solvents used to determine antibacterial activity were ethanol and methanol. Ethanol was used for the extraction of curcuminoids. Essential oil was extracted by hydrodistillation and diluted in methanol by serial dilution method. Both Curcuminoids and oil showed zone of inhibition against all tested strains of bacteria. Among all the three turmeric varieties, Kasur variety had the most inhibitory effect on the growth of all bacterial strains tested as compared to Faisalabad and Bannu varieties. Among all the bacterial strains *B. subtilis* was the most sensitive to turmeric extracts of curcuminoids and oil. The MIC value for different strains and varieties ranged from 3.0 to 20.6 mm in diameter.

Introduction

Medicinal plants are important source for the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents (Ushimaru *et al*., 2007). Different plant parts are used for medicinal purposes i.e., bulb, gel, leaves, roots, barks, peels etc. The use of plants to treat illness is found throughout human culture (Anne-Catherine, 2007). The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds. Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. It is known that more than 400,000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine (Odugbemi, 2006).

Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants. *Curcuma longa* is a medicinal plant that botanically is related to Zingiberaceae family (Chattopadhyay *et al*., 2004). *C. longa*, commonly known as 'turmeric', is widely used as a spice and colouring agent, and is well known for its medicinal properties (Luthra *et al*., 2001).

Components of turmeric are named curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin (Chainani-Wu, 2003). Curcumin is the most important fraction which is responsible for the biological activities of turmeric. The melting point of curcumin, $C_2H_2OO_6$, is 184 ºC. It is soluble in ethanol and acetone, but insoluble in water (Joe *et al*., 2004) Curcumin 95%, a potent antioxidant is believed to be the most bioactive and soothing portion of the herb turmeric and posses the properties like antioxidant, anti-inflammatory, anti-platelet, cholesterol-

lowering antibacterial and anti-fungal effects. It contains a mixture of powerful antioxidant phytonutrients known as curcuminoids and inhibits cancer at initiation, promotion and progression stages of tumor development. It is a strong anti-oxidant, which supports colon health, exerts neuroprotective activity and helps to maintain a healthy cardiovascular system (Luthra *et al*., 2001).

Curcumin was first isolated in 1815 (Vogel & Pelletier, 1815). Its chemical structure, was determined by Roughley & Whiting (1973). In the molecule of curcumin, the main chain is aliphatic, unsaturated and the aryl group can be substituted or not (Roughley & Whiting, 1973). *C. longa* oil was tested against cultures of *Staphylococcus albus*, *S. aureus* and *Bacillus typhosus*, inhibiting the growth of *S. albus* and *S. aureus* in concentrations up to 1 to 5,000 (Chopra *et al*., 1941).

Keeping in view the important role of turmeric in inhibition of different cultures of bacteria and its role as antioxidant and antibacterial, the present study was conducted to compare the antibacterial activity of the essential oils and extracts of *C. longa* varieties and potency of turmeric varieties on some bacteria. This study also supports the use of different varieties of turmeric in traditional medicines for the treatment of bacterial infections.

Materials and Methods

1. Plant material: Three varieties of *Curcuma longa* Kasur, Faisalabad and Bannu were obtained from Ayub Agriculture Research Institute Faisalabad, Pakistan.

2. Extraction procedure: Soxhlet apparatus was used for the extraction of curcuminoid.

2.1 Soxhlet extraction: Curcuminoid was extracted by Soxhlet apparatus. The plant material was cut into small pieces and placed in the extraction thimble. Its weighed amount was placed in an extraction chamber which was suspended above the flask containing the solvent ethanol and below a condenser. The flask was heated and the ethanol evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the ethanol extract was removed and ethanol was evaporated by using rotary evaporator. The weight of the extract was measured and percentage yield of the plant material was calculated.

2.2 Extraction of oil: Oil was extracted by hydro-distillation by Reverse Dean-Stark method.

Reverse dean-stark method: A closed system of producing steam was also evolved which helped in the collection of oil without losing it into condensed water. The plant material was taken in a 5 dm^3 round bottom flask which was to be heated in an isomantle. The flask was filled to about half of its capacity with the plants material and then enough water was added into it to completely immerse the material. A reversed Dean-stark attachment 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 a separation of essential oil from plant material. The oil was separated from water by separatory funnel. The oil was dried with anhydrous Na_2SO_4 and weighed.

3. Microorganisms: Four different strains were used for testing antibacterial activity included *Bacillus subtilis, Bacillus macerans, Bacillus licheniformis and Azotobacter.* 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 4ºC prior to use.

4. Screening for antibacterial activity: Antibacterial activity was tested by agar well diffusion method (Mukherjee *et al.,* 1995). Different concentrations of the turmeric curcuminoids and oil were prepared in ethanol and methanol respectively 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 7mm diameter wells were made using sterile borer. The wells were filled with 0.1ml of the sample extract. The antibacterial assay plates were incubated at 37ºC 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.

5. Minimum inhibitory concentration (MIC): The extracts and oil which showed antibacterial activity in agar well assay were subjected to MIC assay (Jones *et al*., 1985). In order to determine MIC serial dilutions of the extracts and oil were prepared with concentration ranged from 4 to 28 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.

6. Statistical analysis: Analysis of variance of antibacterial activities of plant extracts from *C. longa* varieties were analyzed using the SAS program. Mean separation was performed by the protected LSD method at $p \leq 0.05$ (Anon., 1991).

Results and Discussion

Yield of extracts: Percentage yield of curcuminoid extracts of *C. longa* varieties was; Kasur (2.68%), Faisalabad (3.42%) and Bannu (0.18%) and for oil: Kasur (0.477%), Faisalabad (0.431%) and Bannu (0.695%) respectively (Table 1).

Table 1. Yield of different varieties of turmeric.

S. No.	Turmeric variety	Yield of curcuminoids (%)	Yield of oil (%)
01	Kasur	2.68	0.477
02	Faisalabad	3.42	0.431
03	Bannu	0.18	0.695

Antibacterial activity of different varieties of *Curcuma longa*: Antibacterial activity was studied with oil and curcuminoid extracts. Agar well diffusion method was used to determine the zone of inhibition of bacterial growth at particular concentration of both oil and curcuminoid. Both curcumin and the oil dilutions suppress growth of several bacteria (Bhavani & Sreenivasa, 1979). Turmeric is well known indigenous herbal medicine having many biological activities (Ammon & Wahl, 1991). All the varieties showed significant inhibitory activities. Inhibition was observed against all tested bacterial strains.

Kasur variety: The MIC of Kasur curcuminoid showed that *B. subtilis, B. macerans, B. licheniformis, Azotobacter* were inhibited at all concentrations and Kasur oil was also effective against all tested strains while *B. licheniformis* was resistant only at lower concentration ranged from 4 to 10 mg/ml. Kasur curcuminoids showed higher MIC against only *B. subtilis* as compared to all other tested organisms and its inhibition zone ranged from 4.5 to 20.6mm (Fig. 1a). Kasur oil showed MIC ranged from 3.0 to 18.0 mm in diameter (Fig. 1b).

Faisalabad variety: Faisalabad variety showed antibacterial activity against all tested microorganisms and its curcuminoid had large MIC than oil. Faisalabad curcuminoids gave higher zone of inhibition against *B. subtilis* (12.2 mm) and followed by *B. licheniformis* (8.1 mm), *B. macerans* (7.6 mm) and *Azotobacter* (7.1 mm) as shown in Fig. 2a. Its oil also gave higher MIC against *B. subtilis* (10.0 mm) and followed by *B. licheniformis* (7.0 mm), *Azotobacter* (6.0 mm) and *B. macerans* (5.0 mm) as shown in Fig. 2b.

Bannu variety: Bannu curcuminoid and oil was also effective against tested microorganisms at higher concentrations. It gave higher MIC against *B. subtilis* (7.0mm) and lower MIC against *Azotobacter* (5.3mm) (Fig. 3a). Bannu oil also showed higher MIC against *B. subtilis* (8.0 mm) and lower MIC against *Azotobacter* (5.5 mm) as shown in Fig. 3b.

Many *C. longa* species are traditionally used for their medicinal properties. Antifungal, antibacterial and antiflamatory activity has been reported for species such as *C. longa, C. zedoaria, C. aromatic* and *C. amada* (Apisariyakul *et al*., 1995; Yoshioka *et al*., 1998; Negi *et al*., 1999; Majumdar *et al*., 2000). It is evident from the results that *B. subtilis* was the most sensitive organism to *C. longa* extract of curcuminoid and oil. Wilson *et al*., (2005) reported that antibacterial activity of ethanol extract of *C. zedoaria* (0.15mg/ml) and *C. malabarica* (0.94mg/ml) showed higher inhibition against *B. subtilis* and their ethanol extracts were effective only at higher concentration of 3.75 mg/well. Both the species of turmeric gave MIC against *B. subtilis* was 8.0 mm in diameter. In has been reported that Gram positive bacteria are more sensitive to plant oil and extract (Cosentino *et al*., 1999; Karaman *et al*., 2003). Alzoreky & Nakahara, (2003) studied that among gram positive bacteria, *B. cereus* was the most sensitive organism to *C. longa* extract and its ethanol extract gave MIC 12.0 mm in diameter.

Conclusion

Based on these results, we may conclude that both curcuminoid and oil showed antibacterial activity against all tested organisms and had large inhibition against *B. subtilis*. The varying degrees of sensitivity of the bacterial test organisms may be due to the intrinsic tolerance of microorganisms. Kasur variety is the most resistant against the growth of tested microorganisms at the concentration ranged from 4 to 28 mg/ml and Faisalabad variety is most resistant from organisms than Bannu variety.

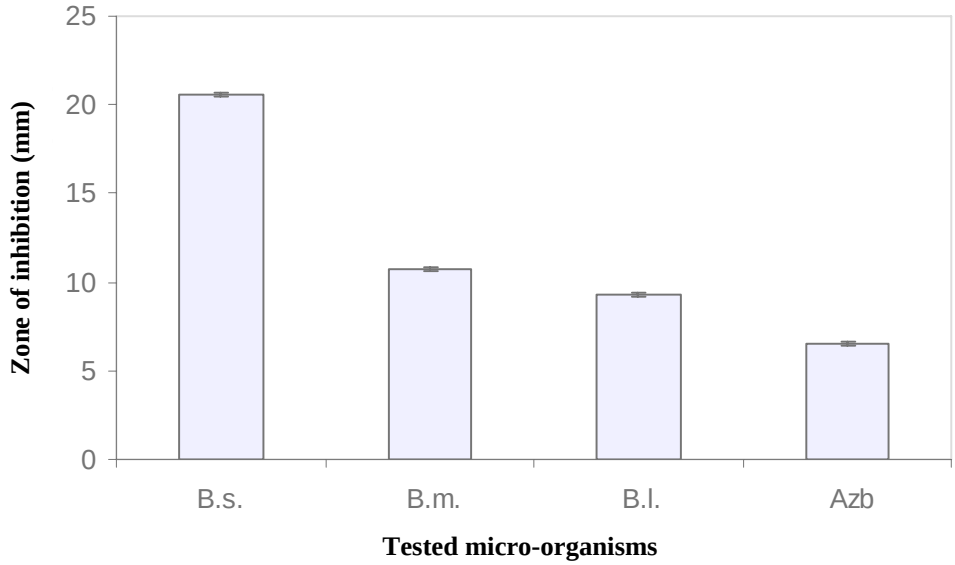

Fig. 1a. Minimum inhibitory concentration (MIC) of Kasur curcuminoids against different bacterial strains.

B.s. = Bacillus *subtilis* B.m. = Bacillus *macerans*
B.l. = Bacillus *licheniformis* Azb = Azotobacter

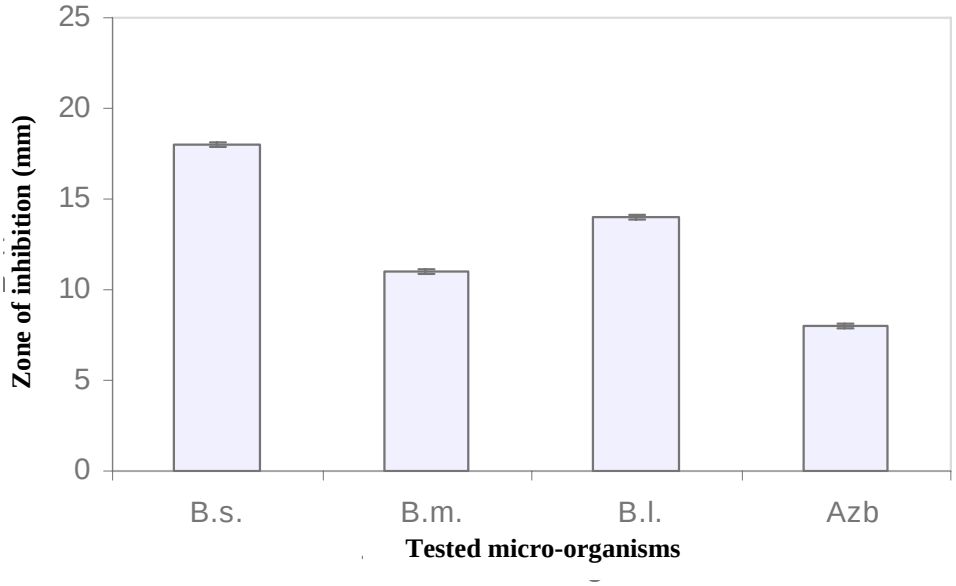

Fig. 1b. Minimum inhibitory concentration (MIC) of Kasur oil against different bacterial strains.

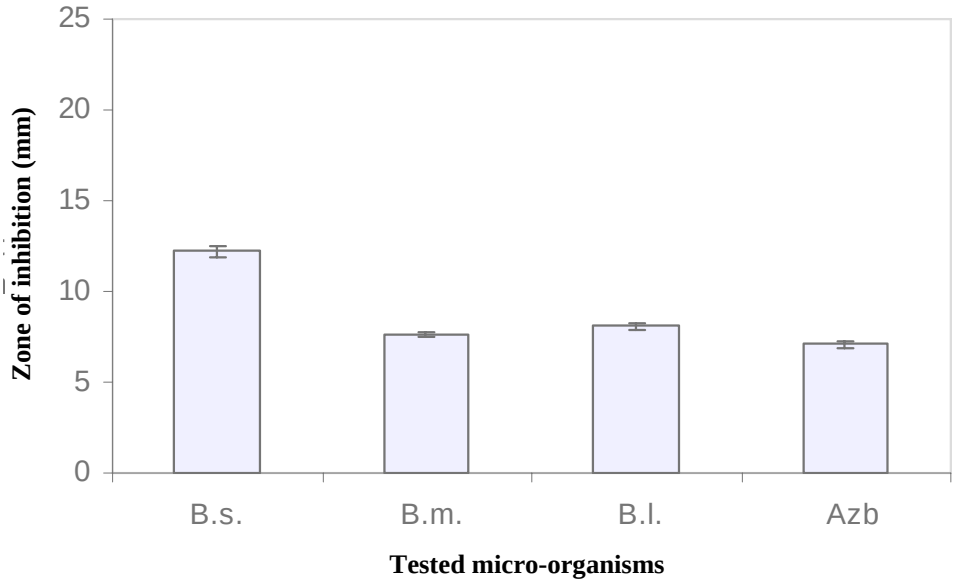

Fig. 2a. Minimum inhibitory concentration (MIC) of Faisalabad curcuminoids against different bacterial strains.

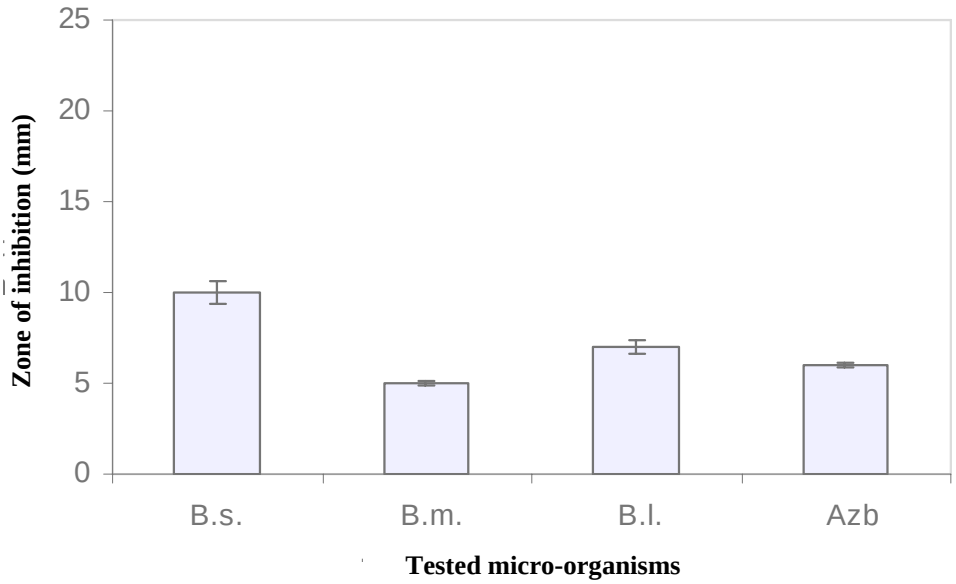

Fig. 2b. Minimum inhibitory concentration (MIC) of Faisalabad oil against different bacterial strains.

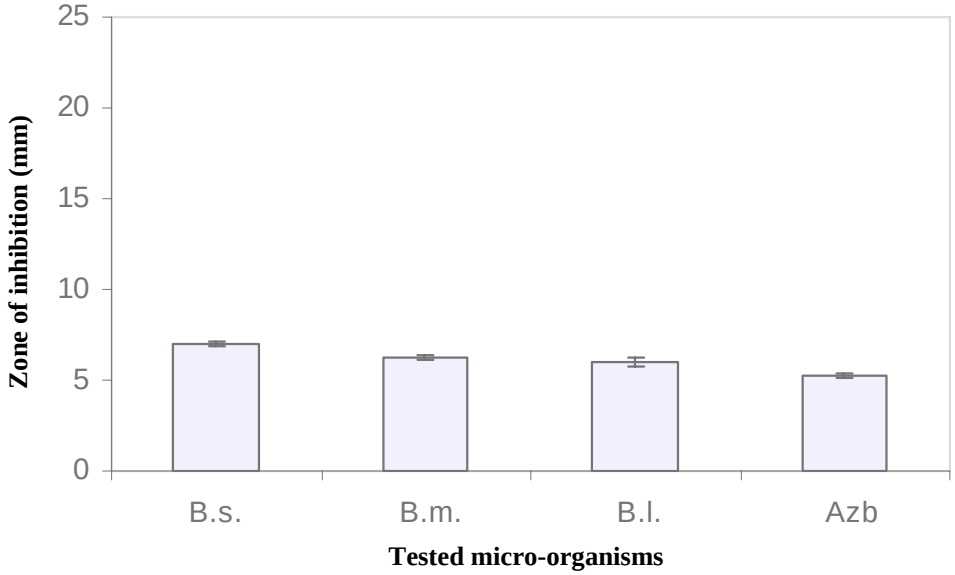

Fig. 3a. Minimum inhibitory concentration (MIC) of Bannu curcuminoids against different bacterial strains.

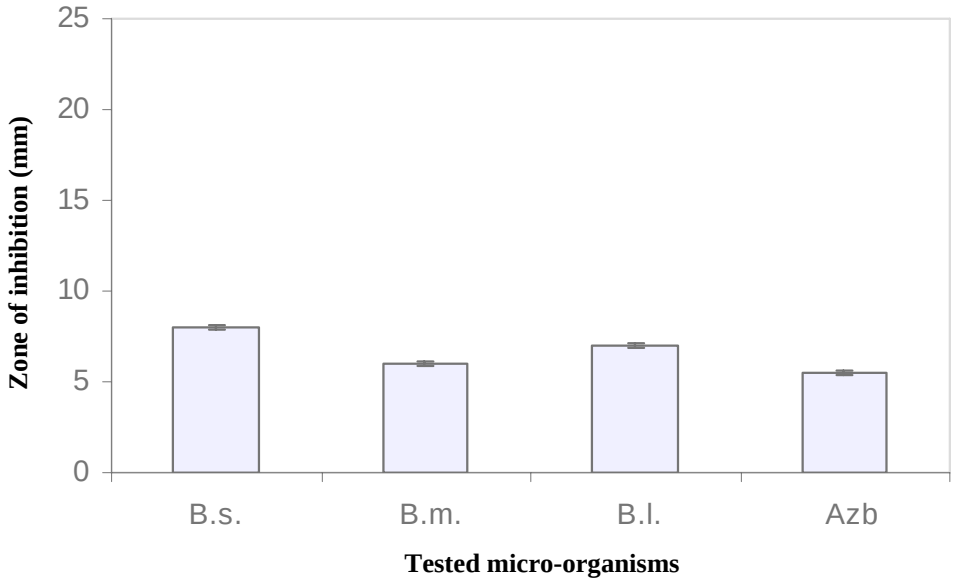

Fig. 3b. Minimum inhibitory concentration (MIC) of Bannu oil against different bacterial strains.

References

Alzoreky, N.S. and K. Nakahara. 2003. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *Int. J. Food Microbiol*., 80: 223-230.

Ammon, H.P.T. and M.A. Wahl. 1991.Pharmacology of *Curcuma longa*. *Planta Med*., 57: 1-7.

Anne-Catherine, F. 2007. Medicinal Plants: A Botanic Garden for the Nation. *Bot. Garden*., 121.

Anonymous. 1996. SAS User's guides statistics; SAS Institute, Inc., Cary, NC. 558.

Apisariyakul A., N. Vanittanakom and D. Buddhasukh. 1995. Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae). *J. Ethnopharmacol*., 49: 163-169.

Bhavani, S.T.N. and M.V. Sreenivasa. 1979. Effect of turmeric (*Curcuma longa*) fractions on the growth of some intestinal and pathogenic bacteria *in vitro*. *Indian J. Exp. Biol*., 17: 1363-1366.

Chainani-Wu, N. 2003. Safety and anti-inflammatory activity of curcumin: a component of turmeric (*Curcuma longa*). *J. Altern. Complement Med*., 9: 161-8.

Chattopadhyay, I., K. Biswas, U. Bandyopadhyay and R.K. Banerjee. 2004.Turmeric and curcumin: Biological actions and medicinal applications. *Curr. Sci*., 87: 44-53.

Chopra, R.N., J.C. Gupta and G.S. Chopra. 1941. Pharmacological action of the essential oil of *Curcuma longa*. *Indian J Med Res.*, 29: 769-772.

Cosentino, S., C.I.G. Tuberoso, B. Pisano, M. Satta, V. Mascia, E. Arzedi and F. Palmas. 1999. *In-vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Lett. Appl. Microbiol*., 29: 130-135.

Joe, B., M. Vijaykumar and B.R. Lokesh. 2004. Biological properties of curcumin-cellular and molecular mechanisms of action. *Critical Reviews in Food Science and Nutrition*, 44: 97-111.

Jones, R.N., A.L. Barry, T.L. Gavan and J.A.ll. Washington. 1985. Microdilution and macrodilution broth procedures. *Manual of Clinical Microbiology*, 972-977.

Karaman, I., F. Sahin, M. Gulluce, H. Qgutcu, M. Sengul and A. Adiguzel. 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. *J. Ethnopharmacol*, 85: 231-235.

Luthra, P.M., R. Singh and R. Chandra. 2001. Therapeutic uses of *Curcuma longa* (Turmeric). *Indian J. Clin. Biochem*., 16: 153-160.

Majumdar, A.M., D.G. Naik, C.N. Dandge and H.M. Puntambekar. 2000. Antiflammatory activity of *Curcuma amada* in albino rats. *Indian journal of Pharmacology*, 32: 375-377.

Mukherjee, P.K., P. Balasubramanian, K. Saha, B.P. Saha and M. Pal. 1995. Antibacterial efficiency of *Nelumbo nucifera* (Nymphaeaceae) rhizomes extract. *Indian Drugs*, 32: 274-276.

Negi, P. S., G.K. Jayaprakasha, L. Jaganmohan and K.K. RaoSakariah. 1999. Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. *J. Agric. Food Chem*., 47: 4297-4300

Odugbemi, T. 2006. Medicinal plants as antimicrobials In: *Outline and pictures of Medicinal plants from Nigeria*. University of Lagos press, 53-64.

Roughley, P.J. and D.A. Whiting. 1973. Experiments in the biosynthesis of curcumin. *J. Chem. Soc*., 20: 2379-2388.

Ushimaru, P.I., T.N. Mariama, C. Luiz, B. Di Luciano and F.J. Ary. 2007. Antibacterial activity of medicinal plant extract. *Braz. J. Microbiol*., 38: 717-719.

Wilson, B., G. Abraham, S. Manjuv, M. Mathew, B. Vimala, S. Sundaresan and B. Nambisa. 2005. Antimicrobial activity of *Curcuma zedoaria* and *Curcuma malabarica* tubers. *J. Ethnophamacol*., 99: 147-151.

Yoshioka, T., E. Fujii, M. Endo, K. Wada, Y. Tokunage, N. Shiba, H. Hohsho, H. Shibuya and T. Muraki. 1998. Antiflammatory potency of dehydrocurdione, a zedoary-derived sesquiterpene. *Inflammation Research*, 47: 476-481.

(Received for publication 15 August 2009)