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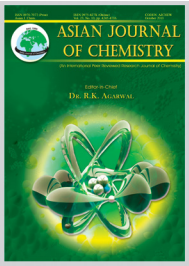

Antibacterial Activity of Different Varieties of *Curcuma longa* Rhizome Extracts Against Pathogenic Bacteria

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Curcuma longa rhizome extracts of different samples (A, B and C) were evaluated for antibacterial activity against four pathogenic bacterial strains, which are *Bacillus subtilis, Bacillus macerans, Bacillus licheniformis* and *Azotobacter sp*. using agar well diffusion method. The different solvents used to determine antibacterial activity were ethanol, hexane, ethyl acetate, butanol and water. 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 show any antibacterial activity. Ethanolic extract of all samples is most active against all pathogens. Hexane and ethyl acetate soluble extract of all samples exhibit most promising antibacterial activity. Hexane and ethyl acetate extracts of sample B were the most resistant against *B. licheniformis* and *Azotobacter* respectively. Among all the samples, the ethanolic extract of sample C (Kasur variety) was found to be the most effective against all tested strains. The minimum inhibitory concentration values of different strains, extracts and varieties ranged from 0.01-0.83 mg/mL.

Key Words: *Curcuma longa* rhizome, Antibacterial activity.

INTRODUCTION

Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants[1]. Since ancient times, plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many different human disorders. They are also used to boost flavour, herbs and spices are also known for their preservative[2] and medicinal value, which forms one of the oldest sciences[3].

Curcuma longa (Zingibraceae) is the well known traditional medicinal plant. The antibacterial activity of *Curcuma longa* was reported as early as 1956[4]. This is a tropical plant grown in southern and south eastern tropical Asia including China, India, Indonesia, Jamica, Malaysia and Penu[5]. Recently curcumin diferuloylmethane present in the extract was found to be responsible for anti HIV activity[6] and has great potential for inhibition of tumor promotion. The antibacterial effects of ionic, resin and ethanolic fraction of *Curcuma longa* was studied against urinary track infection isolates. Ethanolic fraction possessed antibacterial activity against four *Streptococcus* species.

Turmeric extract can inhibit the growth of a variety of bacteria, parasites and fungi[7]. Recently, it is used worldwide as a natural medicine[8,9]. Curcuma sp. is also used as an antiinflammatory and antimicrobial agent has been recognized for more than a century[10]. The most common chemical constituents of essential oil of turmeric from Kasur region of Pakistan are aromatic turmerone, α-turmerone, curlone, caryophyllene, eucalyptol and α-phellandrene[5]. The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents[11].

Bacillus subtilis causes food poisoning due to food spoilage while *B. macerans* causes brain abscess due to intracranial penetration[12]. *B. licheniformis* causes infection in leukemia patients having catheter[13]. The previously reported pharmacological studies on *Curcuma longa* from different regions of South Asia and other parts of world prompted to explore the antibacterial activity of this plant from three different ecological zones of Pakistan. So this study is focused on the determination of antibacterial activity of three samples of turmeric collected from Kasur, Faisalabad and Bannu of Pakistan.

EXPERIMENTAL

For the preparation of plant extract, three varieties of turmeric Bannu (sample A), Faisalabad (sample B) and Kasur (sample C) were obtained from Ayub Agriculture Research Institute, Faisalabad, Pakistan.

Extraction and isolation: The shade dried rhizomes (250 g) were chopped into small pieces and extracted with ethanol three times at room temperature for 5 days. The combined ethanolic extract was evaporated under reduced pressure to obtain dark brown viscous residue under vacuum. This extract was shaked with hexane and hexane soluble fraction was separated and dried.

Defatted extract was dissolved in 300 mL distilled water and shaked with ethyl acetate and butanol respectively. All fractions were dried under vacuum and stored at 4 ºC.

Microorganisms: The microorganisms employed in the current study were procured from the Government College University Lahore, Pakistan which includes *Bacillus subtilis*, *Bacillus macerans, Bacillus licheniformis* and *Azotobacter*. The strains were maintained on nutrient agar slants at 4 ºC.

Antibacterial activity assay: Agar well diffusion method[14] was used to study antibacterial activity. Each of the tested organisms was grown in nutrient broth for a period of 24 h at 37 ºC. Sterile agar plates were prepared and each of the test organisms was streaked on the surface of the respected agar plates. These were allowed for 45 min to pre-diffuse and 7 mm sterile cork borer was used to bore holes on the agar plate. 0.1 mL of the extract of the solvents were introduced into the wells and allowed for 45 min to diffuse into the agar. The inoculated agar plates were incubated at 37 ºC for 24 h and the inhibition zone diameter was measured. The experiment was done three times and the mean values are used for further analysis.

Determination of minimum inhibitory concentration: The minimal inhibition concentration values of extracts of *Curcuma longa* varieties were determined by using the agar well diffusion method. Serial dilutions of the extracts were prepared with concentration ranged from 4 to 28 mg/mL. The wells were filled with 0.1 mL of extracts dilutions. All test plates were incubated at 37 ºC for 24 h. At the end of this period, inhibition zones were measured as mm. The least concentration of each compounds showing a clear zone of inhibition were taken as the minimum inhibitory concentration. The assays were performed three times with three triplicates.

RESULTS AND DISCUSSION

The antibacterial activity of *Curcuma longa* collected from three different areas as mentioned above was studied shown in Figs. 1-3. The ethanolic extract of all samples were prepared and fractionated into hexane, ethyl acetate, butanol and water soluble fractions. All these extracts were tested against bacterial strains *Bacillus subtilis, B. macerans, B. licheniformis* and *Azotobacter*.

Ethanolic extract of all rhizomes of *Curcuma longa* showed maximum antibacterial activity against all microbes (Fig. 1). Ethanol extract of sample B showed higher activity than sample A and C and produced inhibition zone ranging from 5.6 to 20.6 mm in diameter. Ethanol extract of sample C, showed higher activity than sample A and its inhibition zone ranging from 6.6 to 10 mm. Inhibition zone of sample A ranged from 5.6 to 7 mm in diameter. Ethanol extracts of all samples showed minimum inhibitory concentration against *B. subtilis* (0.01 mg/mL) than followed by *Bacillus macerans* (0.03 mg/mL),

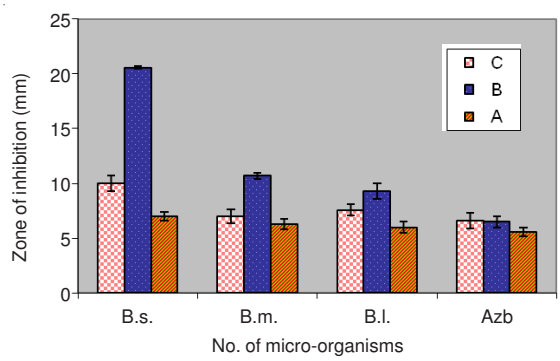

Fig. 1. Inhibition zones of ethanolic extract of curcuminoids against different bacterial strains

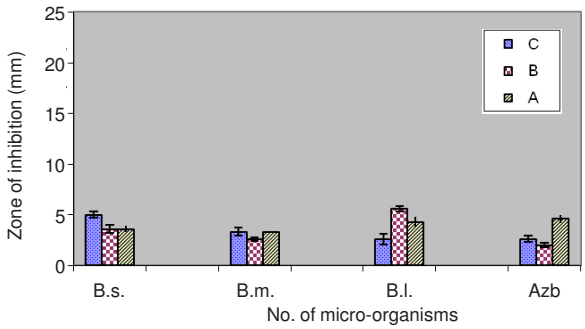

Fig. 2. Inhibition zones of hexane extract of curcuminoids against different bacterial strains

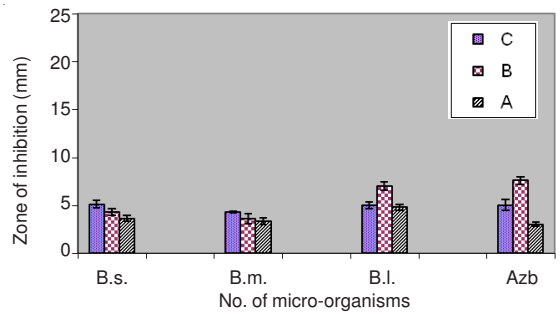

Fig. 3. Inhibition zones of ethyl acetate extract of curcuminoids against different bacterial strains; A= Bannu var; B= Faisalabad var. C= Kasur var; B.s. = *Bacillus subtilis*; B.m. = *Bacillus macerans*; B.l. = *Bacillus licheniformis*; Azb = *Azotobacter*

Bacillus licheniformis (0.08 mg/mL) and *Azotobacter* (0.15 mg/mL) respectively.

As observed in the case of ethanol extracts, hexane extracts of all varieties, showed antibacterial activity (Fig. 2). Hexane extract of sample B showed higher activity than sample A and C and produced inhibition zone ranging from 2 to 5.6 mm in diameter. Inhibition zone of sample A ranged from 3.3 to 4.6 mm in diameter. Hexane extract of sample B showed minimum inhibitory concentration against *B. licheniformis* (0.50 mg/mL). Hexane extracts of sample A and C showed minimum inhibitory concentration against *Azotobacter* (0.20 mg/mL and *B. subtilis* (0.30 mg/mL)) respectively.

Ethyl acetate soluble fractions of sample B exhibited remarkable inhibition (Fig. 3). Sample B showed higher activity than sample C and A and produced inhibition zone ranging from 3.6 to 7.6 mm in diameter. Sample C showed higher activity than sample A and its inhibition zone ranging

TABLE-1
MICS OF DIFFERENT SOLVENT EXTRACTS OF *Curcuma longa*

Solvents extract	MICs of solvent extracts (mg/mL)				
	Samples of *C. longa* var.	*Bacillus subtilis*	*Bacillus macerans*	*Bacillus licheniformis*	*Azotobacter*
Ethanol	A	0.16	0.16	0.20	0.20
	B	0.15	0.15	0.10	0.10
	C	0.01	0.03	0.08	0.15
Hexane	A	0.4	0.4	0.3	0.2
	B	0.50	0.50	0.83	0.50
	C	0.30	0.50	0.34	0.34
Ethyl acetate	A	0.67	0.67	0.41	0.67
	B	0.7	0.7	0.29	0.7
	C	0.15	0.1	0.51	0.51
Butanol	A	-	-	-	-
	B	-	-	-	-
	C	-	-	-	-
Water	A	-	-	-	-
	B	-	-	-	-
	C	-	-	-	-

from 4.3 to 5.1 mm. Inhibition zone of sample A ranged from 3.0 to 4.8 mm in diameter. Ethyl acetate extract of sample B showed minimum inhibitory concentration against *Azotobacter* (0.70 mg/mL). Ethyl acetate extracts of A and C samples showed minimum inhibitory concentration against *B. subtilis* (0.41 mg/mL) and *B. macerans* (0.1 mg/mL) respectively.

While butanol and water soluble extracts of *C. longa* were found to be inactive against any microbe (Table-1).

Among all the samples, the hexane and ethyl acetate soluble extracts of sample C were found to be most active against *Bacillus subtilis* and *B. macerans* where sample B showed maximum inhibition against *B. licheniformis*. For sample A hexane soluble extract was more efficient against *Azotobacter* while ethyl acetate soluble extract inhibit the growth of *B. licheniformis*.

In the present study, ethanol, hexane and ethyl acetate extract of all turmeric varieties showed antibacterial activity against all tested strains. On the contrary, observed that water and butanol extracts remain inactive against bacterial strains. It is evident from all figures that *B. subtilis* was the most sensitive organism to ethanol extract of sample C of *C. longa* rhizome extracts. As antibacterial activity of ethanol extract of sample C showed high minimum inhibitory concentration (20.6 mm) against *B. subtilis*[15] and antibacterial activity of ethanol extract of *C. zedoaria* (0.15 mg/mL) and *C. malabarica* (0.94 mg/mL) showed higher inhibition against *B. subtilis* and their ethanolic extracts were effective only at higher concentration of 3.75 mg/well. These both species of turmeric gave minimum inhibitory concentration against *B. subtilis* was 8.0 mm in diameter and water extract of turmeric did not show any activity[16]. Ethanol extract of turmeric is the most effective against *B. subtilis* and gave large inhibition zones[17,18]. However on the other hand, ethanol extracts of turmeric was inactive against all bacterial strains tested. It did not give minimum inhibitory concentration against microorganisms[19,20]. This variation may be because of the dose used in this study, the method of extraction of plants, the method of antibacterial study, the genetic variation of plant, age of the plant or the environment.

Conclusion

The difference in activities of sample A, B, C may lead to the conclusion that they are different varieties of *Curcuma longa* as occur in different regions of Pakistan. However, chemotaxanomic studies will be required to support this conclusion. Furthermore, these extracts of different *C. longa* samples would be effective against tested microorganisms.

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