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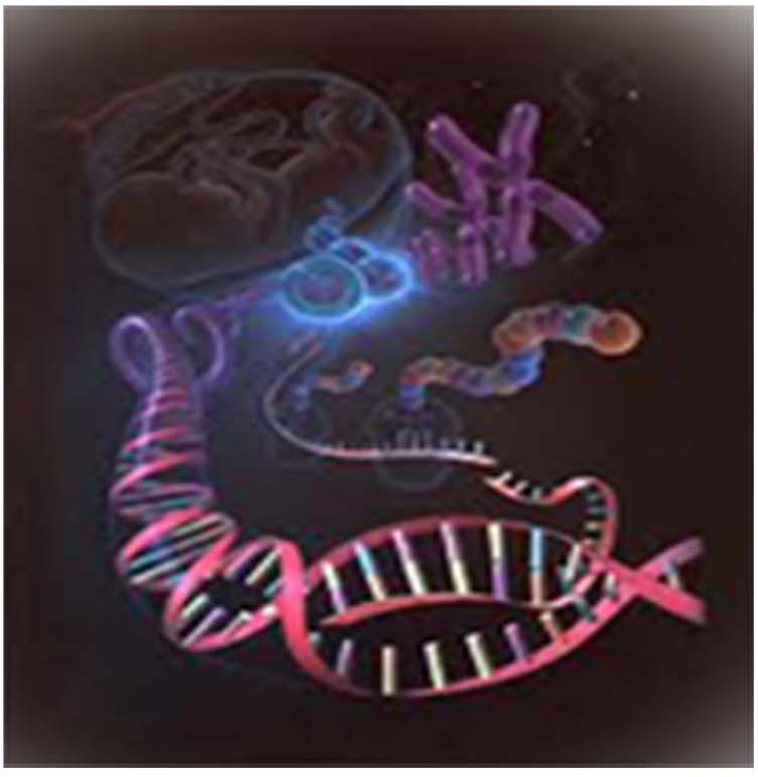

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Research Paper

ANTIMICROBIAL ACTIVITY OF RHIZOME OF SELECTED *CURCUMA* VARIETY

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The genus *Curcuma* belongs to the family Zingiberaceae consist of 40 species, in tropical Asia. Most of the genus of this family is well-known medicinal plant. The ethanolic and aqueous extracts of four *Curcuma* variety, i.e. *Curcuma longa* (turmeric), *Curcuma caesia* (Black turmeric), *Curcuma amada* (Mango ginger) and *Curcuma aromatica* (Van turmeric) were prepared from the rhizome. The antimicrobial properties of both of the extract were studied by testing the antibacterial as well as antifungal activity. The antibacterial activity test was done by agar well diffusion method against two gram positive, i.e., *Staphylococcus aureus and Bacillus subtilis* and one gram negative *Pseudomonas aeruginosa* bacterial strain. For antifungal activity *Candida albicans and Aspergillus flavus* were used as a test fungal strains. The zone of inhibition of the extracts were determined and compared with the standard drugs tetracycline and fluconazole to know the efficiency. The ethanolic extract of *Curcuma longa* and *Curcuma aromatica* was found to have both antibacterial and antifungal activity. The aqueous extract of all plant did not show any significant inhibitory activity against microbes.

Keywords: Antimicrobial activity, *Curcuma* species., Rhizome, Agar well diffusion method, Thin layer chromatography

INTRODUCTION

From ancient time many medicinal plants represent an excellent source of antimicrobial agents (Mahesh and Satish, 2008). In rural areas many plant materials used as a traditional medicine which are readily available and relatively cheaper than modern medicine (Rashedul *et al*., 2010). Plants generally produce many secondary metabolites (compound related to groups like phenol, alkaloid, terpenoids, glycosides, etc.) which constitute an important source of microbicides, pesticides, fungicides and many pharmaceutical drugs. Plant products still remain the principle source of pharmaceutical agents used in traditional medicine (Srinivasan *et al*., 2001).

Member of *Zingiberaceae* family are found to be a rich source of substances of phytochemical interest. They are rich in curcuminoids, and recognized for their broad spectrum of biological

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activities, curcuminoids vary in chemical structures, physico-chemical characteristics as well as the functional properties (Revathy *et al.,* 2011). Numbers of plants from this family are used in traditional system of medicine because of its wide spectrum of pharmacological activities (Anand, 2008). Many species of *Curcuma longa* are traditionally used for their medicinal properties. In many literatures antifungal, antibacterial and antiflamatory activity has been reported for species such as *Curcuma longa, Curcuma zedoaria, Curcuma aromatic and Curcuma amada* (Apisariyakul *et al.*, 1995; Yoshioka *et al.*, 1998). From ancient time most people use the rhizome of these plants use as a traditional medicine due to their medicinal effect (Wibowo *et al.*, 2012).

Fungi and bacteria are significant destroyers of food stuffs and produced various types of diseases in human. The Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* are mainly responsible for post-operative wound infection, toxic shock syndrome and food poisoning. The Gram-negative bacterium such as *Pseudomonas aeruginosa* is present in human lung, urinary track and kidney; causes lower urinary tract infection, inflammation and sepsis (Nascimento, 2000). Also the toxic effects produced by *Aspergillus* includes carcinogenicity, genotoxicity, terratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immune suppression (Satish, 2007). *Candida albicans* is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in human (Enfert, 2007).

The present work was aimed to analyze the antimicrobial effect of different *Curcuma* variety and to identify the compound class in the aqueous and ethanolic extract of rhizomes. The findings could support further development of *Curcuma* as antimicrobial element to enrich the traditional medicine.

MATERIALS AND METHODS

Collection of Plant Material

Fresh rhizome of *Curcuma longa* (turmeric), *Curcuma caesia* (Black turmeric), *Curcuma amada* (Mango ginger) and *Curcuma aromatica* (Van turmeric) were collected from Government Agriculture College, Bilaspur, Chhattisgarh, India and were store in air tight plastic bag at 4^0C until use.

Preparation of Rhizome Extract of *Curcuma* Variety

The fresh rhizome of all four *Curcuma* spp. was washed with distilled water to remove soil and other impurities, cut into small pieces of ¼ inches size and air dried for 2 days. The dried sample was again dried in a hot air oven at 50 °C for 24 h, then ground into powder and pass through a sieve with nominal mesh size of 2 mm diameter. Powdered sample was extracted sequentially with ethanol and water using soxhlet apparatus. These extracts were evaporated to dryness using vacuum evaporator. Vacuum dried extract was stored in air tight vials until use. Stock solutions of each extract were prepared by dissolving 20 mg of dry powder in 1 ml of respective solvent (ethanol and water).

Screening of Plant Extract for Antimicrobial Activity

Agar well diffusion method was used for detection of antimicrobial activity of different extracts. Fluconazole and tetracycline were taken as a positive control for antifungal and antibacterial test.

Test Microorganisms

Bacterial cultures of *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC96) and fungal cultures of *Candida albicans* (MTCC 3017) and *Aspergillus flavus (*MTCC 277) were used as a test organism for antimicrobial activities. All microbial cultures were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India.

Antibacterial Activity

Nutrient Agar Media (all components were g/L NaCl 5; Peptone 5; Beef extract 3; Agar 15; pH 6.8) containing plates were prepared. Bacterial broth culture was prepared to a density of 10^8 cells ml^{-1} of 0.5 McFarland standards. Three different bacterial cultures were spread in separate NAM plates. In the plates, wells (6 mm diameter) were made using sterile borer, 2 mm from the edge of the plate. Ethanolic and aqueous extract of different *Curcuma* spp. (50 µl) were introduced in wells. Antibiotic disc having a concentration of tetracycline 30 µg/disc were introduced in well, which served as positive control. The extraction solvent ethanol and distilled water served as negative controls. The plates were allow to stand for 1 h to allow the diffusion of extract from wells and incubated for 12-48 h at 37 ºC. Results were recorded after every 24 h (Biruhalem, 2011).

Antifungal Activity-

Potato Dextrose Agar (PDA) media (Potato extract 200 ml, Dextrose 20 gL^{-1}, Agar 15 gL^{-1}, pH-5.6) was prepared for antifungal test. The concentration of fungal suspensions were adjusted to 10^7 cells/ml. Fungal cultures were spread on PDA plates. In the plates, wells (6 mm diameter) were made using borer. Ethanolic and aqueous extract (50 µl from stock [20 mg/ml]) of each *Curcuma* spp. were introduced in wells. Antifungal agent fluconazole (50 µl), having a concentration of 1 mg/ml, were introduced in well, which served as positive control. Ethanol and sterilized distilled water served as negative controls which were poured in wells of petriplates. All the plates were incubated for 48-72 h at 27ºC. Results were recorded after 72 h (Murray, 1995).

Thin Layer Chromatography

Thin Layer Chromatography (TLC) was performed for qualitative detection of rhizome alkaloids. Methanol:Chloroform (5:95 v/v) was used as solvent system. 10 µl of sample was loaded on the TLC plate 15 mm from the bottom and kept in solvent development chamber saturated with solvent. TLC was performed under conditions of 25 ± 5 ^{0}C and 50% relative humidity. Spot were visualized by iodine vapor. The Rf value of all samples were calculated (Revathy *et al.*, 2011). After TLC compounds were reisolated from the silicagel using the same solvent in which compound were extracted from the plant sample. Then the wavelength scan was done in the range of 400-700 nm to find out the absorption maxima of compound.

RESULTS AND DISCUSSION

Curcuma is gaining importance worldwide as a potential source of new drugs to combat a variety of ailments as the species contains molecules credited with anti-inflammatory, hypocholestremic, choleratic, antimicrobial, insect repellent, antirheumatic, antifibrotic, antivenomous, antiviral, antidiabetic, antihepatotoxic as well as anticancerous properties (Sukari *et al.*, 2010). In this study ethanolic and aqueous extract from four

different plants from same species were investigated on two gram-positive, one gram-negative bacteria and two fungus including (*B. subtilis*, *P. aeruginosa*, *S. aureus*, *C. albicans* and *A. flavus*). The inhibition effect was determined by agar well diffusion method. Results (Table 1) showed that the ethanolic extract of plants have more potential to inhibit test pathogenic bacteria and fungi than aqueous plant rhizome extract. The water fractions of all the four selected plants showed no inhibition against all the bacteria and fungi tested in this study, which means the aqueous extraction procedure was not effective for the isolation of active antimicrobial component from *curcuma*.

The most pronounced activity with inhibition zones of more than 11.0 mm was shown by ethanol extract (inhibition zone 13 mm against *B. subtilis* at concentration 20 mg ml^{-1}) of *C. longa*. In addition, the ethanol extract of *C. longa* had showed significant activity against *S. aureus, A. flavus* and *C. albicans* with inhibition zones 12.0, 11.0 and 10.0 mm at concentration 20 mg ml^{-1} respectively. The ethanol extracts of *C. aromatica* at the concentration of 20 mg ml^{-1}, exhibited modest inhibition against *B. subtilis, S. aureus* and *C. albicans* at 11 mm each. Ethanolic extract of the *C. aromatic* was the only extract which shows inhibition activity against *P. aeruginosa* at 10 mm. while the ethanolic and aqueous extract other three plant did not show any activity against *P. aeruginosa.* The ethanolic extract of *C. amada* show antifungal activity against *C. albicans* and *A. flavus.* Its ethanolic as well as aqueous extract did not show any antimicrobial activity against any type of test gram bacteria, whereas *C. caesia* showed least activity against microbes. Only ethanolic extract of *C. caesia* was effective against *A. flavus.*

A recent phytochemical study of *C. longa* revealed the presence of various types terpenoids and glycosides like compounds and these compounds are similar to those that have been reported to possess strong antimicrobial activity against gram positive, gram negative bacteria and pathogenic fungi. It is likely that the presence of this type of compounds may have contributed to the antimicrobial activity of *C. longa* (Chhetri, 2008; Okigbo, 2009). Ara *et al*. (2011) reported that the extract of *Curcuma aromatica* showed antibacterial activity against *Basillus lichaniformis*, *Micrococcus leutus* and *Salmonella thyphorium*. They also reported that, this antimicrobial activity is due to presence of large phenolic contents. Wilson *et al*. (2005) has been reported that *B. subtilis* was the most sensitive organism to *C. longa* extract of curcuminoid and oil. Antibacterial activity of ethanol extract of *C. longa* showed higher inhibition against *B. subtilis* and their ethanol extracts were effective only at higher concentration of 3.75 mg/well.

In thin layer chromatography performed for determining the presence of secondary metabolites, the Rf value for each sample was calculated and presented in the Table 2. The desired resolution of separation was achieved using methanol:chloroform (5:95 v/v) as the mobile phase. Three major bands were observed in all curcuminoids extract having Rf of 0.9, 0.55 and 0.37 respectively. The Rf value of 0.55 corresponds to demethyoxycurcumin (Revathy, 2011) and 0.37 respected to bis-demethoxycurcumin (Pothitirat, 2005). The spectrum analysis of reisolated compound, confirmed the presence of di-O-methylated and di-O- benzylated substituted curcumin and curcuminoids in the extract (Bong, 2000). By the spectrum study it was assumed that the possible

Table 1: Result of Antimicrobial Test of the Investigated Plants in Agar Diffusion Assay

Plant Species	Extracts	Inhibition zone (mm)* against				
		Bacillus subtilis (MTCC 441)	*Pseudomonas aeruginosa* (MTCC 424)	*Staphylococcus aureus* (MTCC 96)	*Candida albicans* (MTCC 3017)	*Aspergillus flavus* (MTCC 277)
Curcuma longa	Aqueous (20 mg/ml)	-	-	-	-	-
	Ethanolic (20 mg/ml)	13	-	12	10	11
Curcuma caesia	Aqueous (20 mg/ml)	-	-	-	-	-
	Ethanolic (20 mg/ml)	-	-	-	-	11
Curcuma amada	Aqueous (20 mg/ml)	-	-	-	-	-
	Ethanolic (20 mg/ml)		-	-	10	10
Curcuma aromatica	Aqueous (20 mg/ml)	-	-	-	-	-
	Ethanolic (20 mg/ml)	11	10	11	11	-

Note: * Inhibition zones including the diameter of the paper disc (6 mm).

Table 2: Rf Value of Different Rhizome Extract (Solvent Methanol: Chloroform (5:95 v/v), Total Solvent Run 8 cm)

S. No.	Sample	Rf value
1.	C. *longa* aqueous extract band 1	0.5
2.	C. *longa* aqueous extract band 2	0.318
3.	C. *aromatic* ethanol extract band 1	0.909
4.	C. *aromatic* ethanol extract band 2	0.545
5.	C. *aromatic* ethanol extract band 3	0.363
6.	C. *longa* ethanol extract band 1	0.909
7.	C. *longa* ethanol extract band 2	0.5
8.	C. *longa* ethanol extract band 3	0.363
9.	C. *amada* ethanol extract band 1	0.909
10.	C. *amada* ethanol extract band 2	0.49
11.	C. *amada* ethanol extract band 3	0.363
12.	C. *caesia* ethanol extract band 1	0.909
13.	C. *caesia* ethanol extract band 2	0.52
14.	C. *caesia* ethanol extract band 3	0.37

antimicrobial activity is due to this type of substituted curcumin compound, present in extract.

CONCLUSION

Natural plant compounds have also been shown to antimicrobial properties and may be an alternative to synthetic chemical agents. The ethanolic extract of *Curcuma* rhizome exhibited significant antibacterial activity, whereas the aqueous extract has weakest activity against these microorganisms. In the present conducted studies it is concluded that ethanolic extract is effective then aqueous extract against bacteria and fungi. The antimicrobial activity of ethanolic extract was due to the presence of various type of curcumoid like substrate, which is confirmed by the TLC.

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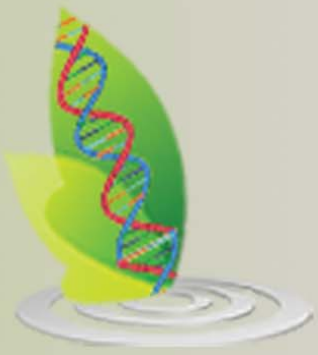

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