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Studies on the Antibacterial Effect of Different Fractions of *Curcuma longa* Against Urinary Tract Infection Isolates

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Abstract: *Curcuma longa* belongs to the family Zingiberaceae, commonly known as turmeric. Antibacterial activity of ionic, oil, resins and ethanolic fractions of turmeric was checked against both gram positive and gram negative Urinary Tract Infection isolates. Sixty-five bacterial strains were isolated from urine of patients suffering from urinary tract infection and identified by conventional methods. Eighty percent of total isolated organisms were found to be gram negative while remaining 20% were gram positive. Ionic, resin and ethanolic fractions of turmeric were found to possessed antibacterial activity against *S. saprophyticus, S. aureus, S. epidermidis, S. pyogenes, E. faecalis* and *B. subtilis* while oil fraction exhibited no activity against the same organisms. No activity of any of the four fractions was found against gram negative isolates. The antibacterial activity of turmeric was also compared with fifteen standard antibiotics. Comparative study of turmeric fractions with standard antibiotics showed that ionic, resin and ethanolic fractions of turmeric are 100% effective against all tested gram positive organisms, which are resistant to most of the broad-spectrum antibiotics used.

Key words: Urinary tract infection, *Curcuma longa,* turmeric, antibiotic, antibacterial

INTRODUCTION

Curcuma longa belongs to the family Zingiberaceae, commonly known as turmeric. It is cultivated primarily in Bengal, China, Taiwan, Sri Lanka, Java, Peru, Australia and the West Indies[1]. Turmeric is the rhizome or underground stem of a ginger-like plant. The whole turmeric is a tuberous rhizome, with a rough, segmented skin. The rhizome is yellowish-brown with a dull orange interior that looks bright yellow when powdered. Rhizome measures 2.5-7 cm (length), 2.5 cm (diameter) with small tuber branching off[2]. Turmeric held a place of honor in India's traditional Ayurvedic medicine. In Ayurvedic it was prescribed for treatment of many medical problems ranging from constipation to skin disease. It was used as a digestive aid and treatment for fever, wounds, infections, dysentery, arthritis, jaundice and other liver problems. In Unani Tib turmeric is considered to be the safest herb of choice for all blood disorders since it purifies, stimulates and builds blood[3,4].

Turmeric is a well-known indigenous herbal medicine having many biological activities. It is an excellent anti-inflammatory herb[5] and therefore is very good in treatment of arthritis, rheumatoid arthritis, injuries and trauma[6]. Oxidation by free radicals induces aging and cause chronic diseases turmeric exhibits antioxidant activity and protect from free radical damage[7]. Curcumas also exhibit anti-tumor activities[8] and prevent cancer[9,10]. It inhibits the topoisomerase enzyme, which is required for the replication of cancer[11]. It directly helps a cell to retain its integrity if threatened by carcinogens[12,13]. *Curcumin longa* inhibit integrase, protease and TNF_KB production[14], inhibit Tat mediated trans-activation of type1 human immuno-deficiency virus long terminal repeat[15] and protease resistant prion protein[16]. It also prevents adriamycin induced organ injuries[17].

The aim of this study was to check the antibacterial activity of four fractions i.e. ionic, oil, resin and ethanolic of *Curcuma longa* against different UTI isolates and their comparative study with other standard antibiotics.

MATERIALS AND METHODS

Extraction of turmeric fractions: The dried and powdered plant material was extracted by taking 200 g of ground turmeric rhizome and dissolved in 1000 mL ethanol (95%) and soaked for 7 days. Then filtered, evaporated and concentrated. Total yield was 80 g of 20% solution. Separation of different fractions was done. Four fractions i.e. ionic, non-ionic, resin and ethanolic were recovered. Extracted fraction 1 mL was dissolved in 9 mL of ethanol to get 10% stock concentration. Dilutions of stock were prepared in water[1].

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Isolation and identification of UTI isolates: Sixty-five gram positive and gram negative bacterial strains were isolated from urine of patients suffering from urinary tract infection. They were identified on the bases of morphological, cultural and biochemical characteristics[18].

Antibacterial screening: Three methods were used for the detection of antibacterial activity of different fractions of turmeric i.e. Agar-well diffusion method, Agar-disc diffusion method and Agar-dilution method[18]. Minimal Inhibitory Concentration (MIC) of different fractions of turmeric against UTI isolates was also determined.

Determination of antibiotic resistance or sensitivity profile: The antibiotic resistance or susceptibility of identified organisms were carried out by disc diffusion method with commercially available disc of penicillin G, amoxicillin, polymyxin B, gentamycin, kanamycin, neomycin, tetracycline, tobramycin, chloroamphenicol, lincomycin, ciprofloxacin, ofloxacin, norfloxacin, optochin and sulfamethoxazole-trimethoprim[18].

Effect of turmeric on macromolecule synthesis: Nutrient broth (10 mL) was added with 100 uL of overnight culture of *S. aureus*, *S. epidermidis*, *S. saprophyticus*, *S. pyogenes*, *E. faecalis* and *B. subtilis*, respectively and incubated for 1 h. After 1 h different fractions of turmeric was added and mixture was re-incubated for 24 h at 37°C. Following two methods were followed to check the effect of turmeric on macromolecule synthesis of above mentioned organisms.

Protein estimation: Next day mixture was centrifuged and supernatant was collected. Supernatant (1 mL) was added with ethanol (2 mL) to precipitate protein. Precipitates were harvested by centrifugation and re-suspended in 0.1 mL PBS. Protein concentration was estimated[19]. A standard curve was plotted using BSA (mg mL^{-1}) against optical density (550 nm) and by the help of this curve the protein of unknown sample was determined. Protein estimation of control culture (without mixing turmeric) was also estimated.

DNA estimation: Next day culture (3 mL) of *S. aureus*, *S. epidermidis*, *S. saprophyticus*, *S. pyogenes*, *E. faecalis* and *B. subtilis* was centrifuged at 5000 rpm. Supernatant was discarded and 0.1 mL of SDS and 0.2 mL of EDTA was added to a pellet and mixture was re-centrifuged. Supernatant was collected and mixed with phenol and chloroform (1:1) and again centrifuged at 5000 rpm for 15 min. Upper layer was then pipetted out and added with 1 mL of NaOH. Centrifuged again and pellet was re-suspended in ethanol. Incubation was done in refrigerator for 30 min. After incubation centrifuged again and pellet was collected which contained DNA. Pellet was re-suspended in PBS and optical density (760 nm) was noted and graph was plotted[20]. DNA estimation of control (without mixing turmeric) was also estimated.

RESULTS AND DISCUSSION

Herbs and spices have been used for generations by humans as food and to treat ailments. Many herbs and spices have the medicinal properties that prevent disease. Some commonly used herbs and spices such as garlic, black cumin, cloves, cinnamon, thyme, all spices, bay, leaves, mustard and rosemary possess antimicrobial properties and can be used as therapeutics[2]. In this study antibacterial activity of four fractions of turmeric i.e. ionic, resin, ethanolic and oil was monitored using agar-well diffusion, agar-disc diffusion and agar-dilution method. In agar-well and disc-diffusion methods activity was determined by noting the zones of inhibition around the wells or disc while in agar-dilution the activity was determined by inhibition of bacterial growth at particular concentration of fractions of turmeric. Turmeric is a well-known indigenous herbal medicine having many biological activities[5]. It is a well-known spice, which is used as a dye, medicine and flavoring agent and exhibits a wide range of biological activities[21]. Sixty-five bacterial strains were isolated from urine of patients suffering from urinary tract infection and identified by conventional methods. Antibacterial activity of four fractions of turmeric was checked against these isolates. Table 1 showed the percentage of gram negative isolates was as follows *E. coli* (47.6%) followed by *P. aeruginosa* (9.2%,) *K. pneumoniae* (7.6%), *E. aerogenes* (6.1%), *P. mirabilis* and *S. marcescens* (4.6% each). The percentage of gram positive isolates includes, *S. aureus* and *S. pyogenes* (4.6% each), *E. faecalis, S. epidermidis* and *B. subtilis* (3% each) and *S. saprophyticus* (1.5%). Present results are

Table 1: Percentage of gram negative and gram positive UTI isolates

Isolates	Total number of organism	Total percentage
Gram negative	**52**	
Escherichia coli	31	47.6
Pseudomonas aeruginosa	6	9.2
Klebsiella pneumoniae	5	7.6
Enterobacter aerogenes	4	6.1
Proteus mirabilis	3	4.6
Serratia marcescens	3	4.6
Gram positive	**13**	
Staphylococcus aureus	3	4.6
Streptococcus pyogenes	3	4.6
Staphylococcu epidermidis	2	3.0
Enterococcus faecalis	2	3.0
Staphylococcu saprophyticus	1	1.5
Bacillus subtilis	2	3.0

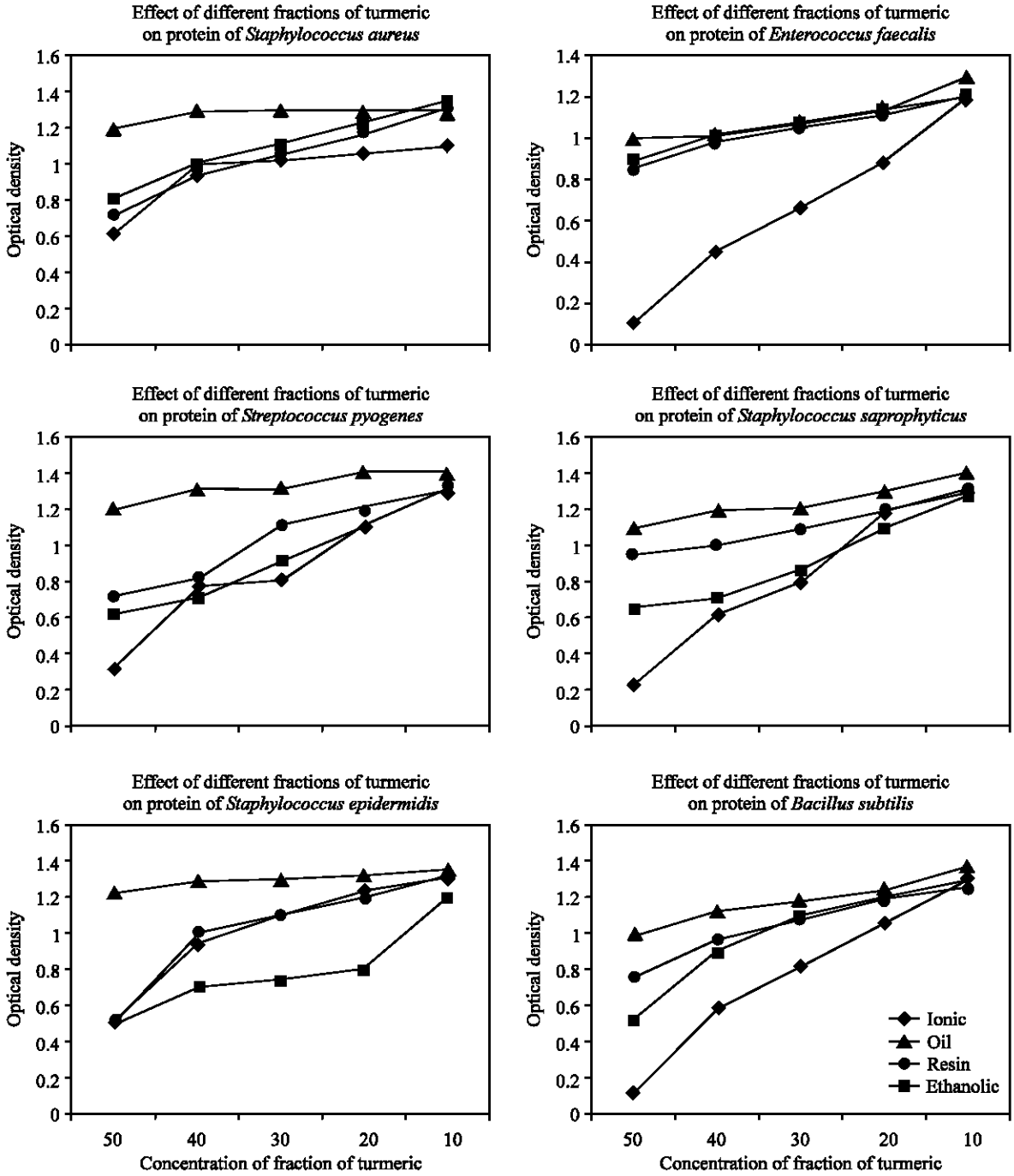

Fig. 1: Effect of turmeric fractions on protein concentration of gram positive UTI isolates

in close agreement with Ali[22], who reported organisms responsible for UTI include *E. coli*, *P. mirabilis*, *K. pneumoniae*, Staphylococcus spp. and *P. aeruginosa*. Ionic, resin and ethanolic fractions showed 100% activity against all tested gram positives and 0% activity against gram negative bacteria. Table 2 showed the MIC of ionic, resin and ethanolic fractions against gram positive isolates. Turmeric extract and turmeric oil have shown chemo-protective effect against chemically induced malignancies in experimental animal[21]. It was previously reported[23] that plant antimicrobials usually possessed low level of antibacterial activity against gram negative organisms, because major plant pathogens are gram negatives and they have an effective

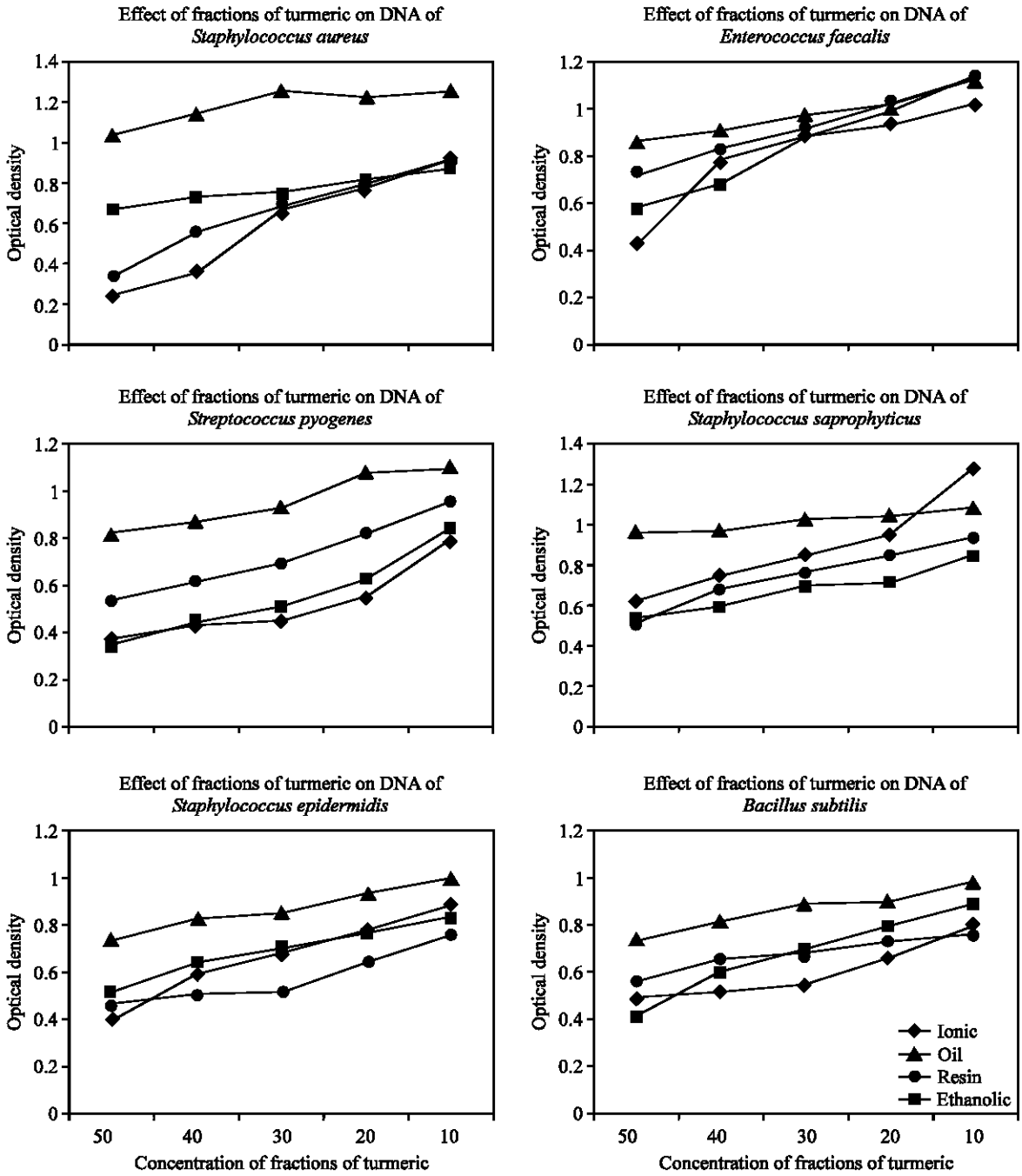

Fig. 2: Effect of turmeric fractions on DNA concentration of gram positive UTI isolates

permeability barrier i.e. outer membrane, which restricts the penetration of compounds and MDR pumps. Table 3 showed the comparative study of four fractions of turmeric with standard antibiotics. Results shows that ionic, resin and ethanolic fractions of turmeric are (100%) effective against all tested gram positive bacteria but 0% effective against tested gram negative bacteria. Antibiotic chloroamphenicol showed highest antibacterial activity i.e. 84.6% against gram positive bacteria and gentamycin showed highest i.e. 69.2% against gram negative bacteria. The effect of different fractions of turmeric on macromolecular synthesis was also carried out. In this aspect protein (Fig. 1) and DNA estimation (Fig. 2) were carried out. The concentration of proteins and DNA when

Table 2: MIC of ionic, resins and ethanolic fractions of turmeric against gram positive UTI isolates

	MIC (mg mL^{-1})		
Isolate No.	Ionic fraction	Resin fraction	Ethanolic fraction
S. aureus-1	2.0	2.5	2.5
S. aureus-2	2.2	2.5	2.8
S. aureus-3	2.2	2.5	2.8
S. saprophyticus-1	2.5	2.8	2.8
S. saprophyticus-2	2.2	2.5	4.0
S. epidermidis-1	2.8	2.8	3.3
S. pyogenes-1	4.0	2.5	4.0
S. pyogenes-2	4.0	3.3	4.0
S. pyogenes-3	4.0	3.3	6.0
E. faecalis-1	3.3	4.0	5.0
E. faecalis-2	3.3	4.0	5.0
B. subtilis-1	2.2	3.3	3.3
B. subtilis-2	2.5	3.3	3.3

Table 3: Comparative study of ionic, oil, resins and ethanolic fractions of turmeric with standard antibiotics against gram positive and gram negative UTI isolates

	Percentage effectiveness (%)	
Antibiotic	Gram positive	Gram negative
Penicillin G	15.0	0.0
Amoxicillin	53.8	3.8
Polymyxin B	7.6	0.0
Gentamycin	76.9	69.2
Kanamycin	61.5	50.0
Neomycin	7.6	13.4
Tetracycline	7.6	1.9
Tobramycin	46.1	32.6
Chloroamphenicol	84.6	50.0
Lincomycin	15.3	0.0
Ciprofloxacin	46.1	48.0
Norfloxacin	69.2	48.0
Ofloxacin	76.9	40.3
Optochin	0.0	0.0
Sulfamethoxazole-trimethoprim	30.7	55.7
Turmeric fractions		
Ionic fraction	100.0	0.0
Oil fraction	0.0	0.0
Resin fraction	100.0	0.0
Ethanolic fraction	100.0	0.0

compared with control significantly decreased with the increase in concentration of turmeric fractions. Indicates, turmeric fractions affect the protein and DNA synthesis of gram positive bacteria in any way.

Present results reveal the potential medicinal use of turmeric as antimicrobial agents. *Curcuma longa* (turmeric) may provide a valuable tool for the development of a therapeutic agent against gram positive UTI isolates.

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