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Research Article

ANTIMICROBIAL ACTIVITY OF THREE DIFFERENT RHIZOMES OF *CURCUMA LONGA* & *CURCUMA AROMATICA* ON UROPATHOGENS OF DIABETIC PATIENTS

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ABSTRACT

Urinary tract infection is a common infection prevalent among patients with diabetes. Diabetes raises a risk of UTI as a consequence of malfunctioned gentitourinary tract. The growing concern associated with the management of UTI in diabetic patients is the multi drug resistance in Uropathogens to conventional antibacterial therapy. Recent changing trends in antimicrobial resistance demands quick alternative regime for the control of frequent UTI in patients with Diabetes Mellitus. In this prospective study we investigated the rhizomes of *Curcuma longa* and *C.aromatica* commonly known as turmeric collected from three different regions of Tamil Nadu. Our results indicated good antimicrobial efficacy of the wild type rhizome of *C.aromatica* on various multi resistant UTI pathogens of *Pseudomonas aeruginosa*, Methicillin resistant *S.aureus*,(MRSA) Vancomycin resistant *Enterococcus Faecalis*,(VRE)and *E.coli.*

Keywords: Urinary tract infections (UTI), Multi drug resistance (MDR), *C. Longa, Diabetes* Mellitus (DM*) C.aromatica.omreagroma, golugupta*

INTRODUCTION

Urinary tract infections (UTIs) are frequently observed in clinical practice and result in significant morbidity with high medical costs.DM alters the genitourinary system and can be a cause of severe complications ranging from dysuria (pain or burning sensation during Urination) organ damage and sometimes even death due to complicated UTI (pyeleonephritis).UTI is more widespread in women with DM than in non diabetic women as a consequence of debilitated immune system. The risk factors for UTI involve colonization with a different uropathogen in cases of recurrent UTI, glucosuria and impaired granulocyte function[17].Diabetic patients are at a greater risk of developing acute pyelonephritis, renal abscess, abnormalities of bladder scarring and pyelitis. People with diabetes have dysfunctional bladders which contract poorly. Women are prone to UTIs for reasons that are not well understood.[18] UTI is uncommon in men but contributes to have larger complications after initial infection. Ninety five percent of UTIs are caused by uropathogens which tend to multiply at the opening of the urethra and migrate towards the bladder. UTI is a result of various risk factors which may trigger Infection. Recurrent UTI is a nasty infection in sexually active young women and patients with DM. Cystitis or bladder infection is commonly prevalent in women and young adolescent girls. The infection can be brief and acute (Cystitis) with classical symptoms of dysuria. In cases of continuous infection deeper layers of the bladder may be damaged (pyleonephritis). The risk of UTI increases with harmful changes in the immune system leading to the easier invasion and colonization of uropathogens in the lining of the bladder. DM is also a leading a cause of overactive bladder or neurogenic bladder. Hospitalization for pyleonephritis occurs 15 times more frequently in diabetic patients.

Symptomatic UTI may be present as a severe illness including higher frequency of bacteremia and bilateral renal involvement with pyleonephritis or unusual clinical presentations of emphysematous cystitis. Diabetic patients encounter urinary urgency and incontinence, during night. This condition is often manifested by the shape of painful urination and retention of urine in the bladder. Immunologic impairments such as defective migration, and phagocytic alterations of chemotaxis in polymorphonuclear leukocytes is well marked in diabetic patients.

The management of acute uncomplicated UTI has changed dramatically in the past few years. Attempts to decrease the costs of laboratory investigations have led to diminish the use of routine urine cultures for patients with acute cystitis. Patients are treated empirically with antimicrobials, and susceptibility tests are often carried out only when the therapy has failed. Outpatient treatment for acute uncomplicated pyelonephritis has been demonstrated to be considerably less expensive than inpatient treatment. Therefore, many patients with uncomplicated UTIs are managed in the outpatient setting with oral agents after being stabilized in an urgent care facility.

Curcuma longa commonly known as turmeric, is an herbaceous perennial plant which belongs to the family *zingiberaceae*. The plant is distributed throughout the tropical and subtropical regions of the world and widely cultivated in South East Asian countries. The genus of *Curcuma* contains thirty species. It grows in rich humid and clay soils. Turmeric is a tuberous rhizome with rough, segmented skin and is also a traditional ayurvedic medicine. The component of turmeric contains *Curcumoids, Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin.*

C. aromatica locally known as "Ban Haldi" in Hindi, is found as a wild species throughout India, though cultivated in West Bengal and south India. *C. aromatica* has vast botanical value, already known in India as tonic, carminative, antidote to snake bites and astringent. It is used for bruises, corns and sprains. Paste of rhizome with milk is used for dysentery and gastric ailments. Extract of *C. aromatica* is given for curing indigestion, rheumatism and dysentery. Plant parts are also used for healing wounds and fractured bones. It is also used to remove stillborn baby from womb. Khasi and Garo tribes of Meghalaya use the paste of Curcuma rhizomes and consume it with water to prevent helminthes infections. *C. aromatica* possesses wide range of activities as it's a potent antifungal, antimicrobial, mosquito repellent, and anti-inflammatory.[5] The oil of Curcumin exhibits inhibitory effect on sarcoma in mice. *Curcumin* has a potential activity for treating early cervical cancer. The major component of the turmeric is *Curcumin* 60% of which is present in the rhizomes. Comparative study of turmeric fractions with standard antibiotics shows that ionic, resin, and ethanloic fractions of turmeric are 100% effective against all tested gram positive and gram negative organisms resistant to broad spectrum antibiotics that are used for the empirical treatment of UTI.[9] Aim of this study was to investigate the antibacterial activity of three different rhizomes of *Curcumin* grown in different regions of Tamil Nadu tested against the multi drug resistant Uropathogens isolated from patients with type2 DM.

MATERIALS AND METHODS

Collection of Urine samples

A total of 120 midstream urine samples were collected using sterile uricols (Hi media pvt India Ltd) from the diabetology department of Bangalore Hospital. Urine samples were processed in lab within two hours of collection. Isolation of Uropathogens was followed by a pour plate method on Hi.Chrome UTI agar. Plates were incubated at

37c for a period of 24 hours. Patients were educated before the sample collection with the assistance of medical staff.

Colonies were counted using the digital colony counter to identify significant bacteruria(>105 cfu/ml) to compare the results with non diabetic patients.UTI pathogens were identified by gram staining following standard laboratory biochemical Tests.

Determination of Antimicrobial resistance

Antibiotic resistance in UTI isolates was determined by Disc diffusion method with commercially available antibiotic discs(Hi media Pvt India ltd).Test organisms were grown in 5ml of Brain heart Infusion broth , incubated at 37c overnight and swabbed on Mueller Hinton Agar plates .Using sterile forceps antibiotic discs were placed on the surface of the agar and incubated overnight. The plates were checked for the zone of inhibition and results were tabulated according to standard CLSI guidelines.

Sample preparation

Rhizomes of turmeric (*Curcuma longa* Linn.) were collected from the field at different places in Tamilnadu (Athanoor, dharmapuri – Commercial type and kolli hills - wild type). The rhizomes were thoroughly washed with water to remove the soil particles. Rhizomes were chopped into small slices, surface sterilized with 10% of Mercuric chloride and sun dried in a tray for 2 to 3 days. Dried samples were stored at room temperature in a plastic zip lock bag till used.

Determination of minimum inhibitory concentrations (MICs)

The MIC was determined using the agar well diffusion method. Concentrated ethanolic extracts (100ul) of turmeric were added at two-fold serial dilution (0.244 to 1000 ppt) in approximately 2mm wide agar wells on assay plates, and incubated at 37ºC for 24 h. MICs values were taken as the lowest concentration of extract which completely inhibited bacterial growth after 24 h of incubation at 37 °C.

Crude extracts preparation

Using a grinder fully dried rhizomes were powdered. 1.0kg of the powdered samples were weighed and transferred to a paper cylinder placed in to the soxhlet apparatus. The samples were macerated (soaked) in ethyl acetate for 3 to 4 hours, at temperature of 50^{o}c, matching the boiling point of the solvent. The extraction was followed by using re-flux method. *Reflux* is a technique involving the condensation of vapors and the return of the condensate to the system from which it originated. Water was supplied continuously to the condenser to cool the solvent, in order to prevent the evaporation and facilitate the process of extraction. The extract was distilled to remove the solvent in order to get a concentrated extract. Solvent thus extracted was evaporated by water bath and the extract was stored in the refrigerator.

Detection of *Curcumin* and related compounds

The TLC plates were developed using glass slides pre-saturated with the mobile phase using different solvents and allowed to develop to a height of about 8 cm. The TLC runs were made under laboratory conditions of 25 ± *50^{o}C* and 50 % relative humidity. After development, the plate was removed before 5mm of the solvent elute reaches the end of the glass slide. Dried glass slides with pure *Curcumin* extract spots were visualized under a UV transilluminator.

RESULTS

Isolation of the clinical isolates from urine samples showed the prevalence of *E.coli* was 4.2%, *E. faecalis*(21%) *P. aeruginosa* (2.4%) and *S. aureus* (16.2%) In non-diabetic patients the prevalence of UTI was *E.coli* (3.6%), *E.Faeclis*(9.6%) *P.aeruginosa* (2.4%) and *S.aureus* (6.6%). Results from antimicrobial sensitivity tests indicated multidrug resistance (MDR) in all UTI isolates.

Table 1: Prevalence of UTI pathogens in diabetic patients

Uropathogens	Number of diabetic patients
E.faecalis	35
Staphylococcus aurues	27
E.coli	7
Pseudomonas aeruginosa	4

Table 2: Prevalence of UTI pathogens in non diabetic patients

Uropathogens	Number of non diabetic patients
E.faecalis	16
Staphylococcus aurues	11
E.coli	6
Pseudomonas aeruginosa	4

The results were tabulated on the basis of Zone of Inhibition observed on agar well diffusion method. 0.1% -0.4% concentration of the ethanolic extracts of rhizomes 1) Commercial type (*C.longa* Athanoor),2) Commercial type(*C.longa* - Dharmapuri) 3) Wild type(*C.aromatica* – Kolli Hills were tested . All the extracts were found effective against the MRSA isolates. Maximum activity of *C.aromatica* was observed (Zone of Inhibition 20mm) against VRE isolates. However limited antimicrobial activity of *Curcuma* rhizomes was observed in *E.coli* and *P.aeruginosa* Isolates(11mm and 10mm) respectively.

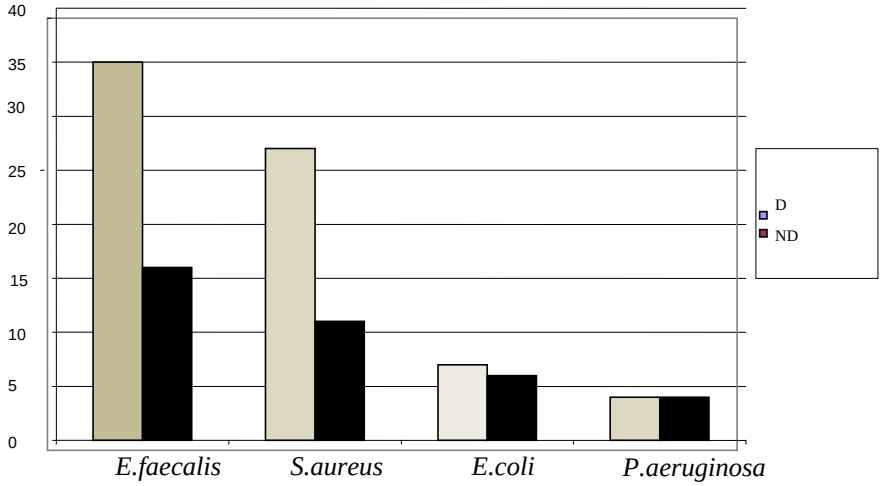

Fig. 1: Comparison of prevalence of Uropathogens (Diabetic vs. Non diabetic group)

A-Ampicillin,Ak-Amikacin,Cd-clindamycin,Ce-Cephotaxime,Cf-Ciprofloxacin,Cw-Clarithromycin,Cz-cefozolin,Do-Doxycycline,E-erythromycin,Fo-fosfomycin,G-gentamicin,K-kennamycin,N-Neomycin,Na-nalidixicacid,Nf-nitrofurantoin,Nt-netillin,Nv-novobiocin,P-penicillin,PB-Polymixin,R-Rifampin,S-streptomycin,T-tetracycline,Tb-tobramycin,Va-vancomycin.

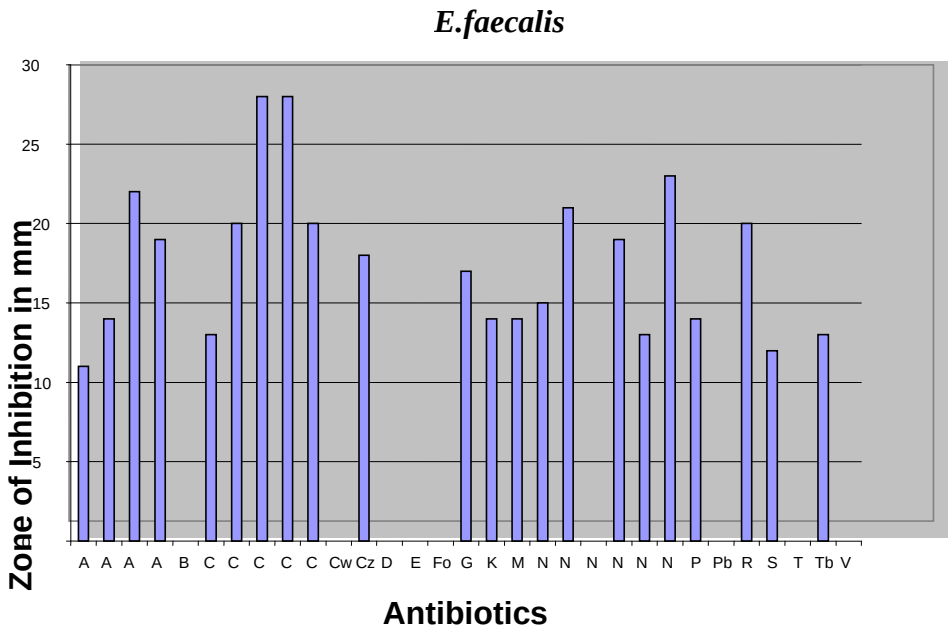

Fig. 2: Antibiotic resistance pattern of Vancomycin resistant *E.faecalis* (VRE)

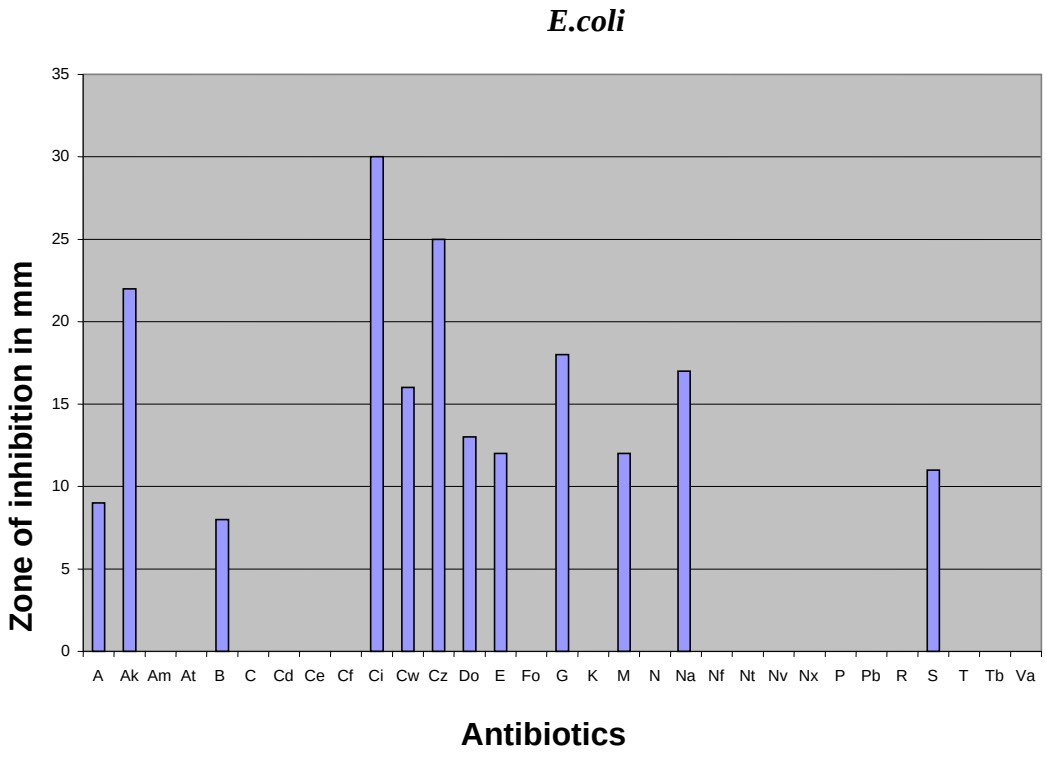

Fig. 3: Antibiotic resistance pattern of *E.coli.*

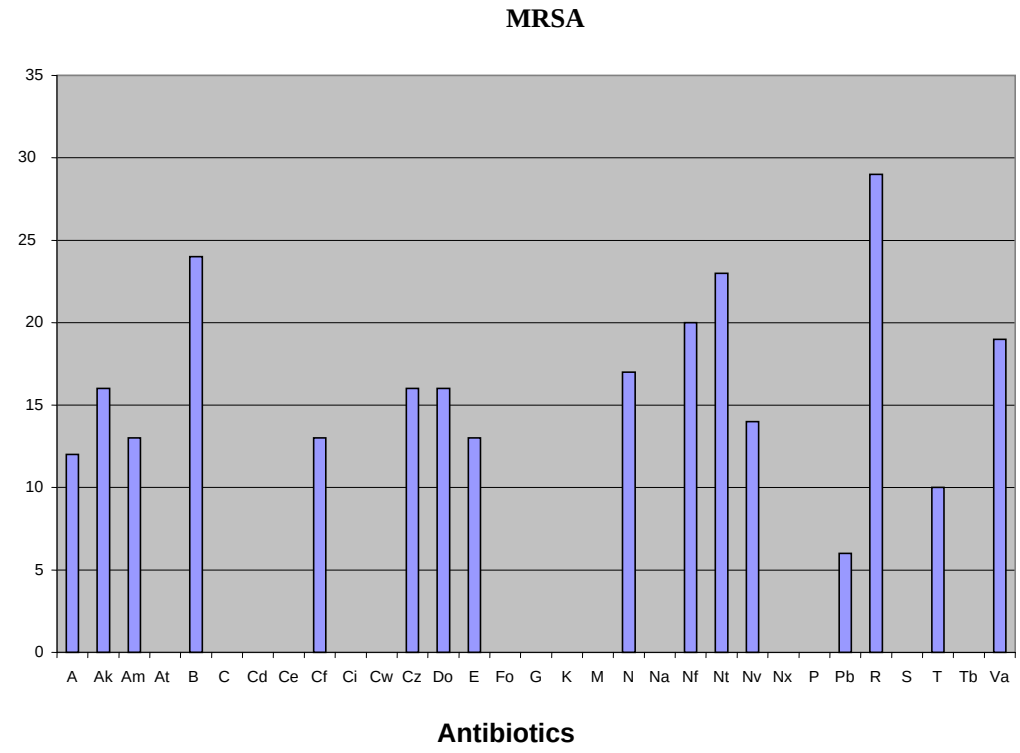

Fig. 4: Antibiotic Resistance pattern of Methicilin resistant *S.aureus*(MRSA)

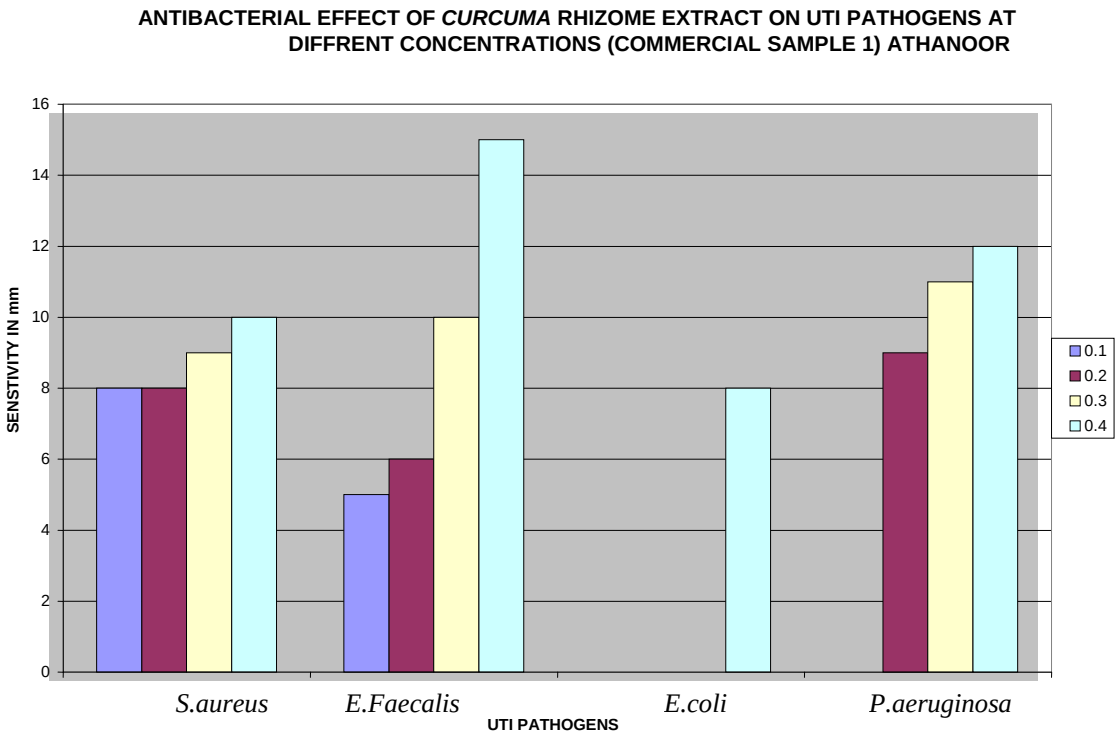

Fig. 5: Shows the antimicrobial efficacy of *Curcuma* Rhizomes on various multi drug resistant UTI isolates when tested with Commercial type (Athanoor) 0.1%-0.4% concentration of Curcumin extracts.

Maximum zone of inhibition was found at 0.4% concentration.

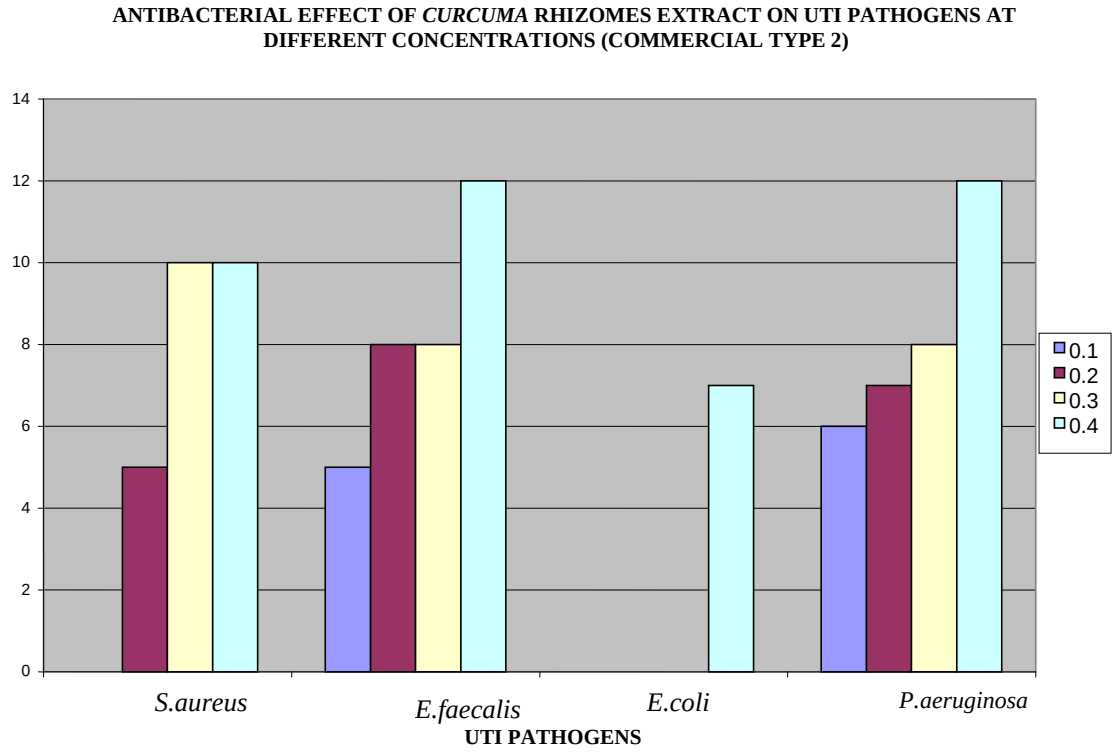

Fig6: Shows the antimicrobial efficacy of Curcuma Rhizomes on various multi drug resistant UTI isolates when tested with Commercial Sample 2 (Dharmapuri) 0.1%-0.4% concentration of Curcumin extracts.

Maximum zone of Inhibition was observed at 0.4% concentration. No antimicrobial activity was observed at 0.1-0.3% concentration when tested with *E.coli*.0.4% concentration of curcumin extract indicated a good antimicrobial activity

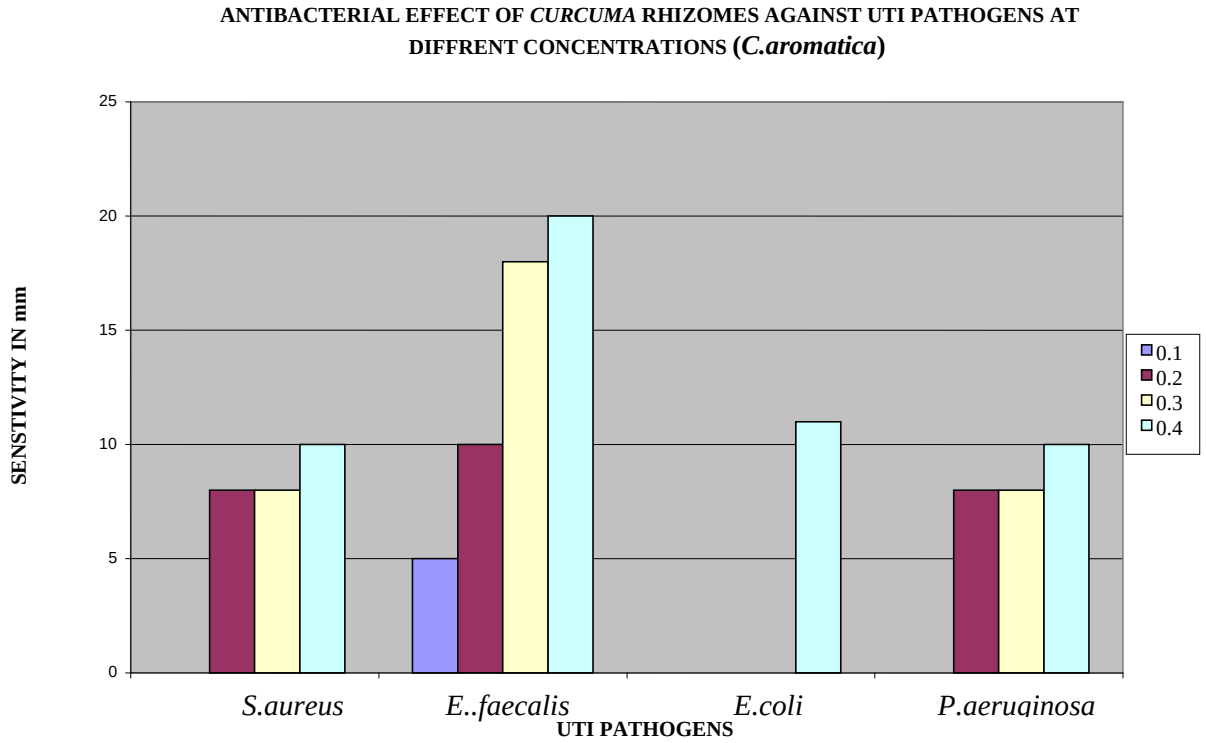

Fig. 7: Antibacterial activity of Wild Type curcuma aromitica (Kolli hills) when tested on MDR UTI isolates

Table 3: Shows the antimicrobial activity of Ethyl acetate Extract of Curcuma rhizomes against *S.aureus*, *E.faecalis, E.coli* and *P.aeruginosa.*

UTI isolates	Zone of Inhibition in Mm Concentrations											
	***C.longa* Commercial Extract**				***C.longa* Commercial Extract 2**				***C.aromatica* Commercial Extract 3**			
	0.1%	0.2%	0.3%	0.4%	0.1%	0.2%	0.3%	0.4%	0.1%	0.2%	0.3%	0.4%
S.aureus	8	8	9	10	-	5	10	10	-	8	8	10
E.faecalis	5	6	10	15	5	8	8	12	5	10	18	20
E.coli	-	-	-	8	-	-	-	7	-	-	-	11
P. aeruginosa	-	9	11	12	6	7	8	12	-	8	8	10

Table 4: MIC of *Curcuma longa* at different concentrations of extract 0.4%, 0.3%,0.2%and 0.1% commercial Ethanolic Extract 1

UTI Isolates	Extract	Antimicrobial activity	MIC(µg/ml)
S. aureus	Commercial type1	+	100
		+	66.66
E. faecalis		+	33.33
		-	13.66
E.coli		+	100
		+	60.66
P. aeruginosa		-	30.33
		-	14.66
+ = have inhibition zone		+	100
_ = no inhibition zone		+	60.66
		-	55.33
		-	16.66
		+	100
		+	66.66
		+	50.33
		-	18.66

Table 5: MIC of *Curcuma longa* at different concentrations of extract 0.4%,0.3%, 0.2%and 0.1% commercial Ethanolic Extract 2

UTI isolates	Extract	Antimicrobial activity	MIC(µg/ml)
S.aureus	Commercial type2	+	100
		+	66.66
E.faecalis		-	45.33
		-	14.66
E.coli		+	100
		+	40.66
P.aeroginosa		-	30.13
		-	12.66
		+	100
		+	70.86
		-	23.33
		-	19.66
		+	100
		+	60.66
		-	33.33
		-	17.66

Table 6: MIC of *C.aromatica* at different concentrations of extract 0.4%, 0.3%,0.2%and 0.1% Ethanolic Extract 3

UTI isolates	Extract	Antimicrobial activity	MIC(µg/ml)
S. aureus	Wild type	+	100
		+	80.66
E. faecalis		+	68.33
		+	60.60
E.coli		+	100
		+	47.66
P.aeruginosa		+	59.33
		+	14.00
		+	100
		+	86.00
		+	33.33
		+	10.00
		+	100
		+	56.40
		+	42.04
		+	13.00.

DISCUSSION

UTI is a common bacterial infection encountered throughout the lifetime of an individual. It is a significant burden on the healthcare system and accounts for 8 million doctor visits every year in US. UTI is often perplexing and painful. Treatment is usually initiated before urine cultures and sensitivity test results are available. Therefore, it is important to monitor the status of antimicrobial resistance among uropathogens in order to improve treatment recommendations. Increased antimicrobial resistance in UTI isolates is a major concern, commonly encountered in diabetic patients as a result of failure of conventional antibiotic therapy and suppressed immune system. This can be attributed to the fact that Uropathogens possess effective permeability barriers in the outer membrane restricting the penetration of antibacterial compounds and MDR pumps. Phytochemicals in plants have been used for generations to treat various infections and can be extensively studied. Turmeric extracts have shown a chemo protective effect against Uropathogens. With the investigations undertaken in this study we propose the potential bactericidal activity and medicinal use of *C. longa* and *C. aromatica.* These extracts can be used to develop an efficient therapeutic antimicrobial alternative therapy against UTI.

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