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Screening Fresh, Dry and Processed Turmeric (*Curcuma longa* L.) Extract Against Pathogenic Bacteria.

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ABSTRACT

Turmeric (*Curcuma longa* L.) is a rhizomatous perennial plant related to Zingiberaceae family, commonly known as Curcuma, Curcum, Haridra and Indian saffron. Curcuminoids represent the key bioactive principles of turmeric, which are of great significance as health beneficial molecules. In the current study, eight cultivars namely, *Local (check), Alleppey supreme, Kedaram, Prabha, Prathibha*, *Suvarna, Suguna and Sudharshana*, maintained at Sanjeevani Vatika, Department of Horticulture, UAS (B), GKVK, Bangalore were utilized for the study. Rhizome extract of turmeric in different forms (fresh, dry and Processed) was evaluated for antimicrobial action on *Pseudomonas aeruginosa* and *Escherichia coli* by disc diffusion method. The inhibition zone (mm) was compared to standard antibiotics, Ampicillin and Streptomycin.The results uncovered that the dry and processed form of rhizome extract effectively inhibited *P.aeruginosa* which is resistant to most broad spectrum antibiotics. Turmeric, may thus offer an effective alternative in prevention and treatment of bacterial infections.

Keywords: turmeric, cultivars, antimicrobial, extract

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INTRODUCTION

Turmeric is accepted as a Wonder compound", as it has a plethora of beneficial effects. In South Asian countries, turmeric is being used since ancient times as a spice, food preservative, coloring agent, cosmetic and in traditional systems of medicine (Ayurveda, Siddha, Unani and Tibetian). The major phyto-constituent of rhizome extract are Curcuminoids namely, Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin [1-3]. These Curcuminoids have been valued as a functional foods because of its health promoting properties due to pharmacological activities such as antimicrobial (against bacteria, fungal and viruses)[4-10] antioxidant, antiparasitic, antimutagenic, anti-cancer, antimalarial, anti-inflammatory, and for treating Alzheimers disease [11-15]

The development of bacterial resistance to antibiotics has demanded the search for new antibacterial agents [16]. The gram negative bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli*pose a great challenge to control through antibiotics as they have the genetic ability to transmit and acquire resistance to therapeutic agents [17]. *P.aeruginosa,* gram negative bacteria carries multi-resistant plasmids and has exceptional ability to colonize in a wide variety of environments and have the genetic capability to express a wide repertoire of resistance mechanisms [18,19]. This pathogen also causes severe eye infections, corneal ulcers, abscesses, styes and dacryocystitis [5]. Pseudomonas infection is more prevalent among patients with burn wounds, cystic fibrosis, acute leukemia and organ transplants [20]. On the other hand, most of the *E.coli* strains, which is also resistant to different antibiotics, can cause Bloody diarrhea, Stomach cramps, urinary tract infections, anemia and kidney failure. Additionally, susceptibility to colonization of small intestine by an *E.coli* strain causes oedema disease, food borne disease and many more[21,22]. In the present study we report the antibacterial effect of turmeric extracts obtained from eight varieties and three different forms against *P.aeruginosa* and *E.coli.* The zone of inhibition (*mm*) recorded by disc diffusion method was compared with standard antibiotics (Ampicillin and Streptomycin).

MATERIAL AND METHODS

Plant material

The *C.longa* rhizomes procured from IISR were maintained at Sanjeevani Vatika, Dept. of Horticulture, UAS (B), GKVK, Bangalore. The crop was raised in the month of May, 2013 and harvested in January, 2014, as per the maturation period of the cultivar.Suguna, Sudharshana and Prabha (Short duration), Alleppey supreme, Prathibha, Suvarna and Kedaram (Medium duration) and Local-check (Long duration) were considered for the present study.

Sample preparation

The harvested fresh rhizomes were processed as per the standard protocol. These rhizomes were sorted into 3 different sets: Fresh, Dry and Processed rhizomes. The first set of fresh rhizomes was manually cleaned, grated and subjected to blend in a laboratory blender (Oster) to obtain a fine paste and this sample is referred as 'fresh rhizome'. The second set of fresh rhizomes was manually cleaned, grated, chopped into thin slices and dried in hot air oven at 40°C for 48 hrs and powdered in a laboratory blender (Oster) and this sample is referred to as 'dried rhizome'. The third set of fresh rhizomes was manually cleaned and was processed in excess of boiling water bath for 45 minutes. Later, excess water was drained out and the soft rhizomes were chopped into thin slices, dried in hot air oven at 40°C for 48 hrs and powdered in a laboratory blender (Oster). This sample is referred as 'processed rhizome' [23,24]. All the samples (fresh, dry and processed) were stored in refrigerator till further analysis.

Extraction of curcuminoids from turmeric rhizomes

The powdered turmeric rhizomes of eight varieties in fresh, dry and processed forms were independently subjected to soxhlet apparatus for the extraction of curcuminoids. A known amount (10g) of respective sample was loaded into Soxhlet extractor using methanol as a solvent as per the available literature [25,26] The sample was further concentrated in a rotary vacuum evaporator. Extracts were stored at 4°C in refrigerator. 10,000ppm concentration of the respective extract obtained from 8 varieties and 3 forms (Fresh, Dry and Processed) were prepared in methanol for antimicrobial assay.

Test microorganism used in the study

Two bacterial strains *Escherichia coli* (MCC 2079) and *Pseudomonas aeruginosa* (MCC 2080) were obtained from National Centre for Cell Science (NCCS), Pune.

Inoculum preparation

A 24 hour old pure culture of *E.coli* and *P.aeruginosa* were used for the preparation of bacterial suspension as per Mac-Farland Nephelometer Standard. Suspensions of organisms were made in sterile isotonic solution of sodium chloride (0.9%w/v). 0.5 McFarland standards (1.5 x 10^{8} CFU/ml)were used as a reference to adjust the turbidity of microbial suspension[5].

Method of screening

Sterilization of media, peptone water, distilled water, petri-plates, L-shaped glass rod, micro-tips were carried out in autoclave at 121°C for 15min.The sterilized Nutrient agar was poured into each petri-dishes and allowed to solidify under aseptic conditions inside the Laminar Air Flow (LAF) chamber.Sterile paper disc of 6*mm* diameter was aseptically saturated with 30µl of the respective extract obtained from 8 varieties and 3forms (Fresh, Dry and Processed).These discs, were allowed to dry for 1hour in Laminar Air Flow (LAF) chamber for complete absorbance of the sample and later placed onto nutrient agar[[Hi Media (M002)] surfaceswabbed with 30µl of respective test organism (ca. 1.5 x 10^{8} CFU/ml using 0.5 McFarland's standard)with the help of a sterilized forceps. The plates were incubated for 24h at 37°C (Fig. 6). Similarly, standard antibiotic disc of Streptomycin (S^{10} 10mcg/disc) and Ampicillin/Sulbactum ($A/S^{10/10}$) were aseptically placed on the agar plate, swabbed with the respective test organism. The results were recorded as five independent observations by measuring the zone of growth inhibition (*mm*) around the disc. The recorded inhibition zone (*mm*) of the sample were compared with the inhibition zone of the standard antibiotics (Ampicillin/Sulbactum ($A/S^{10/10}$), and Streptomycin (S^{10} 10mcg/disc),) procured from HiMedia[5].

RESULT AND DISCUSSION

The present investigation was carried out to screen curcuminoids for their antibacterial properties against two bacterial cultures (Fig. 1). Turmeric extract from eight varieties, and in fresh, dry and processed forms were assessed against 10,000ppm concentration of extract by adopting disc diffusion method of screening. The antibacterial activities observed were compared with standard antibiotics such as Ampicillin and Streptomycin.

Efficacy of standard antibiotics against *Pseudomonas aeruginosa* and *Escherichia coli*

The antibacterial activity recorded as inhibition zone (*mm*) exhibited by antibiotics; Ampicillin and Streptomycin against the test organism after 24hrs of incubation are shown in (Table 1; Fig. 2 and 3). The results revealed that *P.aeruginosa* is highly susceptible to Streptomycin and impervious to Ampicillin[27, 18, 19] However, *E.coli* displayed susceptibility towards Ampicillin as well as Streptomycin.

Efficacy of extract obtained from eight varieties, in fresh, dry and Processed form against *Pseudomonas aeruginosa* and *Escherichia coli*

The data recorded as inhibition zone (*mm*) (Table 2; Fig 4 and 5) represents the antibacterial activity of extract against *Pseudomonas aeruginosa* and *Escherichia coli* after 24hrs of incubation.

It was observed from Table 2 that fresh extract of Prabha and Prathibha effectively inhibited pathogenic bacteria (*P.aeruginosa*) with 13.6 ± 1.46 and 20.8 ± 0.20 respectively. All other turmeric extracts in fresh form were ineffective against this pathogen. The resistance developed against these fresh extract might be due to spontaneous mutations or due to restricted permeability of cell wall through efflux pump system [19]. On the other hand turmeric extract of dry and processed turmeric exhibited toxicity against *P.aeruginosa,* but the degree of sensitivity varied with varieties. Highest activity was shown by dried form of Suvarna cultivar and processed form of Local-check with 31±1.1832 and 90±0.0 zone of inhibition respectively as compared to the standard antibiotics.Themechanism of antibacterial action involves, hydrophobic and hydrogen bonding of

phenolic compounds to membrane proteins which results in membrane disruption, destruction of electron transport systems and cell wall disruption [28].

However, on the other hand *E.coli* was observed to be completely resistant to all the extracts. [22] have reported that plant antimicrobials usually possessed low level of antibacterial activity against gram negative bacteria, such as *E.coli* due to restricted permeability by the outer membrane. Susceptibility of microorganism against plant extract is also strain dependent [29].

The present observations might also possibly be imputed to the variability among the varieties and the content of curcuminoids [29]. Other reasons such as volume of inoculum, growth phase of microorganisms, culture medium, type of solvent also influences the antimicrobial efficacy [30]. Further, Research works over the last few decades have validated that antimicrobial property of turmeric is been attributed mainly to curcumin [5, 31, 32].

Table 1: Inhibition zone exhibited by *Pseudomonas aeruginosa* and *Escherichia coli* against Ampicillin and Streptomycin

Antibiotic	Inhibition zone (mm)	
	Pseudomonas aeruginosa	*Escherichia coli*
Ampicillin	---------No Inhibition------	31.6±0.4
Streptomycin	25±0.0	29.2±0.4

[Values are represented as Mean±SEM]

Table 2: Inhibition zone (mm) of fresh, dry and processed extracts from *C.longa* against *Pseudomonas aeruginosa* and *Escherichia coli*

Forms →	Fresh form		Dry form		Processed form	
Micro-organism → / Sample ↓	*P.aeruginosa*	*E.coli*	*P.aeruginosa*	*E.coli*	*P.aeruginosa*	*E.coli*
Local-Check	0 ± 0	RESISTANT	13.6 ± 2.03	RESISTANT	90.0 ± 0	RESISTANT
Alleppey supreme	0 ± 0		11.6 ± 2.71		0.0 ± 0	
Kedaram	0 ± 0		26.0 ± 4.30		0.0 ± 0	
Prabha	13.6 ± 1.46		20.4 ± 3.55		23.0 ± 2.50	
Prathibha	20.8 ± 0.20		29.2 ± 1.06		19.8 ± 1.2	
Suvarna	0 ± 0		31.0 ± 1.18		13.6 ± 1.32	
Suguna	0 ± 0		25.2 ± 0.80		25.4 ± 1.02	
Sudharshana	0 ± 0		29.8 ± 1.46		18.0 ± 1.00	

[Values are represented as Mean±SEM]

Figure 1: Test organisms used for antimicrobial assay

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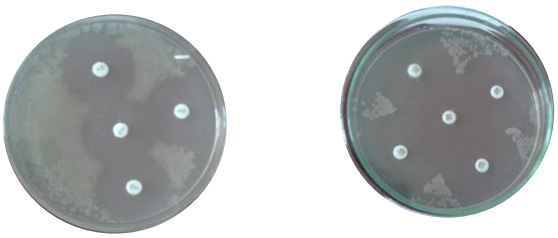
A: *Escherichia coli;* B: *Pseudomonas aeruginosa*

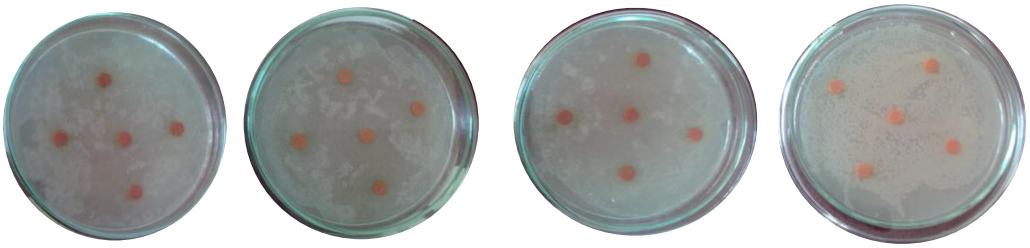
A: Streptomycin B: Ampicillin

Fig 2: Antibacterial activity demonstrated by antibiotics(A&B) against *P. aeruginosa*.
The figure displays susceptibility of *P. aeruginosa* to Streptomycin; resistance towards Ampicillin



A: Streptomycin B: Ampicillin

Fig 3: Antibacterial activity demonstrated by antibiotics(A&B) against *E. coli*.
The figure displays susceptilbility of E.coli to both Streptomycin and Ampicillin.



A B C D

Fig 4: Anti-bacterial activity of Turmeric extract (mm) against *P.aeruginosa* and *E.coli*
A: FRESH form;B: DRY form; C: Processed form
D: Resistance exhibited by *E.coli* towards Turmeric extract

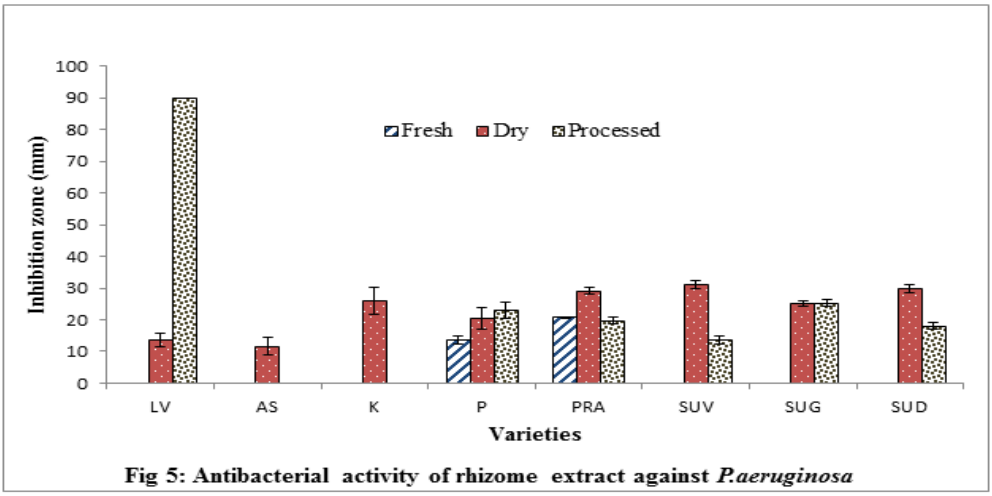


Fig 5: Antibacterial activity of rhizome extract against *P.aeruginosa*

Note: Data are given as mean ± SEM
LV: Local-Check; AS: Alleppey Supreme; K:Kedaram; P: Prabha; PRA: Prathibha; SUV: Suvarna; SUG: Suguna; SUD: Sudharshana

CONCLUSION

The results appear promising for possible use of turmeric extract as bactericidal agent against *P.aeruginosa,* which, pose a great challenge to control with antibiotics or disinfectants due to multi-resistant plasmids. Antimicrobialsobtained from turmeric thus offers many advantages such as fewer side effects, less expensive, better acceptance as turmeric is classified as GRAS (Generally Recognized As Safe) by National Cancer Institute and FAO/WHO.

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