

Research Article



Screening Fresh, Dry and Processed Turmeric (*Curcuma longa* L.) Essential Oil 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. Volatile oil represents 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. Concentrated volatile oil of eight turmeric varieties in different forms (fresh, dry and processed) was evaluated for antimicrobial action on *Escherichia coli* and *Pseudomonas aeruginosa* by disc diffusion method. The inhibition zone (mm) was compared to standard antibiotics, Ampicillin and Streptomycin. The results uncovered that, turmeric essential oil extracted from fresh rhizome of Local-check displayed highest antibacterial activity against *Escherichia coli*, followed by kedaram in processed form and alleppey supreme in dried form as compared to standard antibiotics. Turmeric, may thus offer an effective alternative in prevention and treatment of *Escherichia coli* infections.

Keywords: Turmeric, Cultivars, Antimicrobial, Volatile Oil

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 Tibetan). Turmeric owes its aroma to the volatile oil (present upto 5%) present in the rhizome, which can be recovered by hydro-distillation process. The major volatile principles of the rhizome oil are α -turmerone (30-32%), β -turmerone (15-18%) and α -turmerone (17-26%) and minor components are cineole, α -phellandrene, β -caryophyllene and α -zingiberene.¹ Turmeric oil, has many applications in cosmetic sector, perfumes and soap industries. It is an antacid and in small doses acts as a carminative, stomachic, appetizer and tonic. It is also found to be effective against bronchial asthma in clinical trial.^{1,2} Furthermore, exhibits therapeutic properties which are responsible for anti-cancer, anti-oxidant, antimicrobial, antiparasitic, antimutagenic, immunomodulatory, anti-inflammatory, anti-protease and apoptosis inducing properties.³⁻⁷ The development of bacterial resistance to antibiotics has demanded the search for new antibacterial agents. 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.⁸ Most of the *E.coli* strains are resistant to different antibiotics and 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.^{9,10} On the other hand, *P.aeruginosa*, gram

negative bacteria carries multi-resistant plasmids and has exceptional ability to colonize in a wide variety of environments.^{11,12} In the present study we report the antibacterial effect of turmeric volatile oil obtained from eight varieties and three different forms against *E.coli* and *P.aeruginosa*. The zone of inhibition (mm) recorded by disc diffusion method was compared with standard antibiotics (Ampicillin and Streptomycin).

MATERIALS 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.^{13,14} 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, chopped 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 processed in excess of boiling water bath for 45 minutes. Later, the soft rhizomes were



chopped and dried in hot air oven at 40 °C for 48hrs and powdered in a laboratory blender (Oster). This sample is referred as 'processed rhizome'. All the samples (fresh, dry and processed) were stored in refrigerator till further analysis.

Extraction of Volatile oil from Turmeric Rhizomes

The powdered turmeric rhizomes of eight varieties in fresh, dry and processed forms were independently subjected to hydro-distillation in Clevenger's apparatus for 5hrs, 15min. The light yellow colored oil obtained was dried over minimum amount of anhydrous sodium sulphate to remove any traces of water.^{1,13,14} 30µl of the respective volatile oil obtained from 8 varieties and 3 forms (fresh, dry and processed) were used 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 (1.5×10^8 CFU/ml) were used as a reference to adjust the turbidity of microbial suspension.

Method of Screening

The sterilized Nutrient agar [Hi Media (M002)] (in autoclave at 121 °C for 15min) was poured into each petri-dish and allowed to solidify under aseptic conditions inside the Laminar Air Flow (LAF) chamber. Sterile paper disc of 6mm diameter was aseptically saturated with 30µl of the respective volatile oil obtained from 8 varieties and 3 forms (fresh, dry and processed). These discs, were allowed to dry for 1hour in LAF, for complete absorbance of the sample and later placed onto nutrient agar surface swabbed with 30µl of respective test organism (ca. 1.5×10^8 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/Sulbactam ($A/S^{10/10}$) were aseptically placed on the agar plate. 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/Sulbactam ($A/S^{10/10}$), and Streptomycin (S^{10} 10mcg/disc) procured from HiMedia.¹⁵

RESULTS AND DISCUSSION

The present investigation was carried out to screen essential oils for their antibacterial properties against two bacterial cultures (Fig. 1). Turmeric volatile oil from fresh, dry and processed forms was assessed against 30µl of

concentrated essential oil by adopting disc diffusion method of screening. The antibacterial activity observed was compared with standard antibiotics such as Ampicillin and Streptomycin.



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

Figure 1: Test organisms used for antimicrobial assay

Efficacy of Standard Antibiotics against *Escherichia coli* and *Pseudomonas aeruginosa*

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 *E.coli* displayed susceptibility towards Ampicillin as well as Streptomycin. However, *P.aeruginosa* was highly susceptible to Streptomycin and impervious to Ampicillin.^{8,11,12}

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

Antibiotic	Inhibition zone (mm): Mean ± SEM	
	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
Ampicillin	-----No Inhibition-----	31.6 ± 0.4
Streptomycin	25 ± 0.0	29.2 ± 0.4

[Values are represented as Mean±SEM]



A: Streptomycin B: Ampicillin

Figure 2: Antibacterial activity demonstrated by antibiotics (A&B) against *P. aeruginosa*.

The figure displays susceptibility of *P. aeruginosa* to Streptomycin; resistance towards Ampicillin.



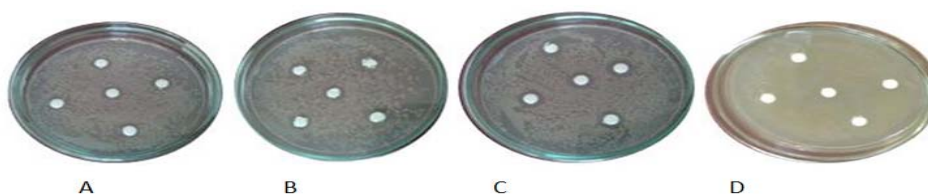
A: Streptomycin B: Ampicillin

Figure 3: Antibacterial activity demonstrated by antibiotics (A&B) against *E. coli*.

The figure displays susceptibility of *E. coli* to both Streptomycin and Ampicillin.

Table 2: Antibacterial activity of volatile oil against *Escherichia coli* and *Pseudomonas aeruginosa*

Forms →	Fresh		Dry		Processed	
Sample ↓						
Micro-organism →	<i>E.Coli</i>	<i>P.aeruginosa</i>	<i>E.Coli</i>	<i>P.aeruginosa</i>	<i>E.Coli</i>	<i>P.aeruginosa</i>
Local-Check	16.8 ± 2.26	RESISTANT	11.0 ± 1	RESISTANT	11.6 ± 0.92	RESISTANT
Alleppey supreme	14.6 ± 3.02		12.6 ± 2.11		0.0 ± 0	
Kedaram	11 ± 0.54		12.0 ± 0.70		13.2 ± 1.74	
Prabha	10 ± 0		9.4 ± 0.4		8.6 ± 0.24	
Prathibha	9 ± 0		8.8 ± 0.2		8.6 ± 0.24	
Suvarna	8.2 ± 0.37		10.6 ± 0.4		8.4 ± 0.24	
Suguna	0 ± 0		9.2 ± 0.2		10.0 ± 0	
Sudharshana	9.4 ± 0.24		11.6 ± 1.6		11.6 ± 1.6	



A: FRESH form B: DRY form C: Processed form D: Resistance exhibited by *P.aeruginosa* towards Turmeric volatile oil

Figure 4: Anti-bacterial activity of Turmeric extract (mm) against *E.coli* and *P.aeruginosa*

Efficacy of volatile oil 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 volatile oil against *Escherichia coli* and *Pseudomonas aeruginosa* after 24hrs of incubation.

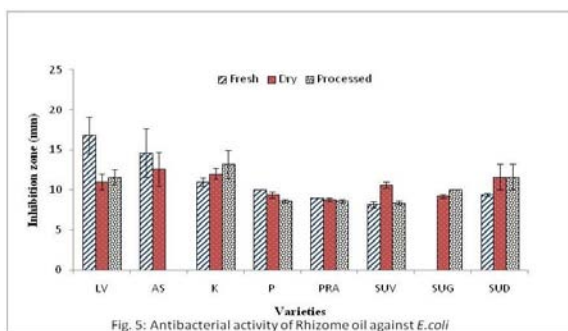


Fig. 5: Antibacterial activity of Rhizome oil against *E.coli*

Note: Data are given as mean ± SEM

Figure 5: Antibacterial activity of Rhizome oil against *E.coli*

It was observed from Table 2 that the results obtained are encouraging as 30µl of concentrated turmeric oil, extracted from fresh, dry and processed forms from all the eight varieties displayed antibacterial activity against *E.coli*. Undiluted oil obtained from fresh rhizome of Local-check displayed highest antibacterial activity (16.8 ± 2.26), followed by Kedaram (13.2 ± 1.74) in processed form and subsequently by Alleppey supreme (12.6 ± 2.11) in dried form as compared to standard antibiotics. On the other hand essential oil extracted from turmeric varieties

in 3 forms was ineffective against *P.aeruginosa*. The resistance may be due to the restricted permeability of cell wall by efflux systems.^{11,12,15} Varying degree of sensitivity of bacterial test organism with forms and varieties may also be due to intrinsic tolerance of microorganism, nature of phytochemicals present in essential oils.^{16,17,18}

Also, factors such as volume and composition of essential oil, method of oil extraction, thickness of agar, inoculum size, bacterial species, and even strains also influence the susceptibility of essential oil against test bacterium.^{19,20}

The mechanism of antibacterial action involves impairment of variety of enzyme systems involved in synthesis of components in microbial cells.^{21,22}

This antibacterial property of volatile oil from *C.longa* has been attributed to the presence of number of secondary metabolites such as sesquiterpenes (Ar-turmerone, α-turmerone and β-turmerone) and monoterpenes.^{23,24} Further, this was supported by^{25,15} who reported that turmerone and curlone of turmeric oil possess excellent antibacterial action against *E.coli* and *B.coagulans*.

CONCLUSION

The findings confirmed the efficacy of turmeric oil against *Escherichia coli*, which is resistant to different antibiotics. Thus, essential oil extracted from turmeric varieties in fresh, dry and processed forms, might indeed be a potential antibacterial agent against *E.coli* infections and can also be employed as natural preservative to improve the quality of food and prevent food borne diseases as they are considered as GRAS (Generally Recognized As Safe) by FDA.

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