

## **Phytochemical screening and comparative study of anti microbial activity of leaves and rhizomes of turmeric varieties**

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### **ABSTRACT**

*Curcuma longa* is a major spice crop grown abundantly in India and other tropical countries. Its major constituent is curcumin which gives turmeric its unique aroma, flavor and medicinal properties. The present study aimed at comparing the *in vitro* antimicrobial activity of two varieties of turmeric, the PTS and Erode variety and to screen the phytochemicals present in turmeric leaves. Six Gram positive and Gram negative bacteria namely *Serratia marcescens*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Streptococcus pyrogens* and *Staphylococcus aureus* were subjected to test the antimicrobial activity along with two fungi namely, *Candida albicans* and *Aspergillus niger*. The ethanolic and methanolic extracts of rhizomes and leaves were subjected to microbial susceptibility assays using agar well diffusion method. Phytochemical screening of two leaf varieties was done to test the presence of phytochemicals responsible for the antimicrobial potential of leaves of *C. longa*. The results of the present study revealed that both ethanol and methanol extracts showed antimicrobial activity on rhizome and leaf extracts. The rhizome extracts showed high inhibition over *E. coli*, *S. pyrogens*, *B. subtilis* and *C. albicans*. The leaf extracts possessed antimicrobial potential against *S. pyrogens*, *B. subtilis* and *C. albicans*. The phytochemical screening of leaf extracts showed the presence of flavanoids, cardiac glycosides and phenols in both the leaf varieties. The present study indicated the antimicrobial property of turmeric leaves which can also be used for therapeutic purposes along with other medicinal plants. Among the two varieties tested, the Erode variety was found superior in its antimicrobial potential.

**Keywords:** Antibacterial activity, zone of inhibition, turmeric, methanol extract.

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### **INTRODUCTION**

According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants [1]. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Various medicinal plants have been used for years in daily life to treat disease all over the world [2]. They have been used as a source of potent and powerful drugs [3]. There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom [4]. This worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding

the value of natural products in health care. [5]. Herbal products are suitable for treating a wide range of infections and diseases [6].

Plants are rich in a wide variety of secondary metabolite such as tannins, terpenoids, alkaloids, and flavanoids which have been proved *invitro* to have anti microbial properties. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments [7]. In the last few years, a number of studies have been conducted in different countries to prove such efficiency.

India has a rich history of using plants for medicinal purposes. Turmeric (*Curcuma longa* L.) is a medicinal plant extensively used in Ayurvedha, Unani and Siddha medicine as home remedy for various diseases. *C. longa* L., botanically related to ginger belongs to the Zingiberaceae family[8] is a perennial plant having a short stem with large oblong leaves and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow in colour. Turmeric rhizome is used as a food additive (spice), preservative and colouring agent [9] in Asian countries, including China and South East Asia. It is also considered as auspicious and is a part of religious rituals. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury. In recent times, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Various sesquiterpenes and curcuminoids have been isolated from the rhizome of *C. longa*, attributing a wide array of biological activities such as antioxidant [10] anti-inflammatory [11] wound healing [12], anticancer [13] and antibacterial activity [14].

Being the world's largest manufacturer of turmeric, India produces tones of turmeric plants every year. The rhizomes are mostly used and leaves are not given care. The leaves of turmeric, known as Haldi leaves are used in Indian and Malaysian cookery as both fresh and dried-extracted form. It is also a good colouring agent and a basic ingredient in curry powders and are purported to improve digestion and reduce gas and bloating.

Many species of *Curcuma* are traditionally used for their medicinal properties. Antifungal, antibacterial and anti inflammatory activity has been reported for species such as *C.longa*, *C.zedoria*, *C.aromatica* and *C.amada* [11]. The pharmacology of *C.longa* was studied by Ammon *et al*, (1991) [16] in detail.

The present study aimed in comparing the antimicrobial activity of turmeric leaves and rhizomes of two varieties cultivated in Tamil Nadu, India namely the Erode variety and PTS Variety commonly called as Andra turmeric and to screen the phytochemical constituents present in both varieties which imparts the antimicrobial potential. The PTS variety is a hybrid variety from Paradeep Turmeric Station and is a famous cultivar of Andra Pradesh which is now preferred by most farmers for its high yield and its high tolerance to disease and pest attack even though its curcumin content is less when compared to Erode variety. The Erode variety is native to Tamil Nadu and is a popular variety because of its high curcumin content over other varieties.

## MATERIALS AND METHODS

**Collection of Plant Material:** The two plant materials were collected from the agricultural gardens of Erode district, Tamil Nadu. The plants were brought to the laboratory and leaves and rhizomes were separated and washed thoroughly in running tap water to clean the adhering sand particles and then rinsed in distilled water, shade dried, coarsely powdered and stored in air tight containers for further use.

**Preparation of Leaf Extracts:** Ten grams of each turmeric leaf variety were put into the Soxhlet apparatus and the extracts were taken after 18 hours using ethanol and methanol as solvents. It was further concentrated by rotary evaporator and stored at 4°C until use. The leaf samples were also dried, powdered and stored for phytochemical screening.

**Preparation of Rhizome Extracts:** The rhizomes were descaled and cut into small pieces and crushed in a mortar and pestle. Ten grams of each rhizome sample were taken in separate containers and 10 ml of 95% solvents were added and kept in a rotary shaker for 24 hours. The extracts were filtered using a Whatmann No 1 filter paper and the extracts were stored in 4 °C until use.

**Microbial samples:** The bacterial and fungal cultures were obtained from the microbial type culture collection (MTCC) of Institute of Microbial Technology (IMTECH), Chandigarh, India for the present study. Microorganisms

used were *Serratia marcesens* (MTCC97), *Escherichia coli* (MTCC433) *Bacillus subtilis* (MTCC121), *Klebsiella pneumoniae* (MTCC3384), *Streptococcus pyrogens* (MTCC442) and *Staphylococcus aureus* (MTCC 96), *Aspergillus niger* (MTCC 281) and *Candida albicans* (MTCC 183). They were subcultured in recommended media purchased from Hi-Media, India private Ltd, Mumbai and stored in 4°C for further use.

**Culture media and antibiotics:** Mueller-Hinton agar media (Hi Media laboratories, Mumbai) was used for the growth of bacterial cultures and for fungi, Sabouraud dextrose agar was used. Gentamycin was used as the standard antibiotic and Fluconazole was used for fungi.

**Screening for Antibacterial Activity:** Antibacterial activity of all leaf and rhizome extracts was tested by agar well diffusion method [17]. The culture plates were prepared by pouring 20 ml of Mueller-Hinton agar medium into sterile petri plates. The test bacteria were then swabbed over the agar media using sterile cotton swabs to get uniform distribution of the bacterial cultures. 5mm diameter wells were made using sterile cork borer. The wells were filled with the sample extracts. A sterile antibiotic disc was placed on another end. For each bacterial strain, Gentamycin served as positive control and negative controls were maintained where ethanol and methanol alone was used instead of the extract. The anti bacterial assay plates were then incubated in 37°C for 24 hours. The diameter of the zone of inhibition around each well was taken as a measure of antibacterial activity.

**Screening for Antifungal Activity:** To evaluate the antifungal activity, sterile agar plates were used according to disc diffusion assay. Activated fungal cultures in Sabouraud broth were adjusted to  $1 \times 10^8$  cfu/ml as per McFarland standard. Sterile filter paper discs (5 mm diameter) were impregnated with leaf and rhizome extracts. The discs were placed in fungal seeded plates and incubated at 25°C for 48-72 hours. Fluconazole (10 mg/disc) was used as a control.

**Minimum Inhibition Concentration (MIC) of the Extract:** The MIC was defined as the lowest concentration that completely inhibited the growth of microorganisms for 24 hours. The MIC of the extracts was done using the agar well diffusion technique. Two fold dilution series of each extract was prepared to achieve a decreasing concentration range of 200 to 12.5% (V/V). A 0.5ml volume of each solution was added aseptically into the wells of Mueller Hinton agar plates that were already inoculated with standardized bacterial isolates ( $10^6$  cfu/ml). The plates were incubated at 37°C for 24 hours. MIC was calculated as the lowest concentration at which a clear zone of inhibition was observed.

**Screening of Phytochemicals:** The turmeric leaf extracts were screened for the presence of phytochemical constituents which may be the reason for the antimicrobial properties of *C.longa* leaves. The methods followed were slightly modified methods of Swadhini et al (2011) [18] and Pathak et al (2011) [19].

**Test for Alkaloids:** 0.5 g of leaf extract was dissolved in 5 ml of 1 % HCl in steam bath. To 1 ml of this, 6 drops of Dragendorff's reagent was added; Precipitate or turbidity indicated the presence of alkaloids.

**Test for Flavanoid:** To 1 ml of leaf extract, 5 ml of diluted ammonia was added followed by conc. Sulphuric acid. Appearance of yellow colour indicates the presence of flavanoids.

**Test for Tannins:** To 2 ml of leaf filtrate obtained by boiling 0.5 g leaf and 20 ml water, few drops of Ferric Chloride was added and the presence of tannins was confirmed on observation of a blue or black precipitate.

**Test for Saponins:** 50 mg of the dried leaf sample was diluted with distilled water and made up to 20 ml. Frothing test was done by pouring the suspension in a graduated cylinder and shaken for few minutes. The formation of 2 cm foam and its persistence indicates the presence of saponins.

**Test for Cardiac glycosides:** The presence of cardiac glycosides was confirmed by Keller –Kilani test which is performed by the addition of 1 ml glacial acetic acid, 1 drop of 1%  $\text{FeCl}_3$  and conc.  $\text{H}_2\text{SO}_4$  to 2 ml of leaf filtrate resulting in the appearance green blue colour.

**Test for Phenols:** 50 mg of leaf powder was dissolved in 5 ml of distilled water. A few drops of neutral ferric chloride solution was added and the appearance of dark green colour indicated the presence of Phenols.

**Statistical Analysis:** Each experiment was carried out in triplicates and the mean diameter of the zone inhibition zone was recorded. The data were statistically analyzed by using software SPSS 15.0. A least significance (LSD 0.05) was used to test the efficiency of the extracts through a general linear model. The test was statistically significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The aim of the present study was to compare the antimicrobial activity of turmeric leaves with rhizomes. Also a comparison was made between the two preferred varieties in Erode region, Tamil Nadu, India which is famously called as the “Yellow city” because of its abundant production and export of turmeric in India. The antimicrobial activity of leaves and rhizome extracts of *C.longa* were assayed *in vitro*. The result obtained in the present study provides a scientific support to the ethnomedical uses of the leaf extracts of turmeric plants in the treatment of microbial diseases.

Various researchers have worked in exploring the antimicrobial potency of leaf extracts against infectious bacteria. Suree and Pana (2005) [20] found ethanolic extracts and essential oil of *Zingiber officinale* and *Myristica fragrans* to be effective against the Enterobacteriaceae and concluded that the varying degree of sensitivity of microbes may be due the nature and combination of the phytochemicals present in the extracts. Graph 1 and 3 summarizes the bacterial growth inhibition of both ethanolic and methanolic extracts of rhizomes and leaves of PTS and Erode varieties of turmeric. The results showed that methanol extract of rhizomes had more inhibitory effect than ethanolic extracts except PTS extract on *S.pyrogens* and *S. aureus*. In Erode variety, a mild variation alone was observed in case of *E.coli* which showed a zone of inhibition of 19.8 mm. All the other methanol extracts showed high zones than ethanol extracts in both PTS and Erode varieties. The highest zone of inhibition was recorded in PTS methanol extract with a zone of 21.33 mm and the least zone was observed in Erode methanol extract on *S.marcesens* (6.5 mm). Both ethanolic extracts did not produce inhibition zones on *S.marcesens*.

The ethanol extract of PTS leaves had more inhibitory effect on *S.pyrogens*, *E.coli* and *S.aureus* with a zone of 10.17, 11.3 and 8.7 mm respectively when compared to methanol extracts which showed zones of 9.67, no zone and 10.7 mm respectively. In Erode variety, the ethanol extract produced more growth inhibition on *S. aureus* alone; no zone was observed in case of *Klebshiella* and *Serratia*. *K. pneumonia* had the least microbial activity against methanol alone and had no inhibitory effect on other leaf extracts. The highest inhibition was observed in PTS methanol over *Bacillus subtilis* (13.7 mm) and the least by PTS ethanol on *S.marcesens* (4.7 mm). Even though *Bacillus subtilis* produces resting spores and are more resistant to environmental stresses, the turmeric leaf extract was able to control its growth. The results were highly significant for all pathogens. An overall agreement was seen between the zone levels and MIC values. The MIC values for rhizomes and leaf extracts are given in Table 1 and 2 respectively.

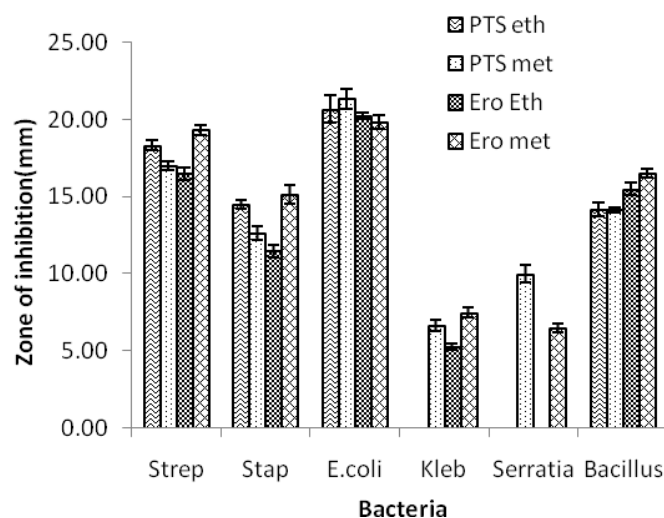
**Table 1: MIC values for rhizome extracts for bacteria expressed in %**

Plant extracts/ Bacteria	PTS Ethanol	PTS Methanol	Erode Ethanol	Erode Methanol
<i>Streptococcus pyrogens</i>	0.05	0.05	0.02	0.05
<i>Staphylococcus aureus</i>	0.02	0.02	0.02	0.02
<i>Escherichia coli</i>	0.05	0.05	0.05	0.05
<i>Klebshiella pneumoniae</i>	-	0.5	0.5	0.1
<i>Serratia marcesens</i>	-	0.02	-	0.5
<i>Bacillus subtilis</i>	0.02	0.02	0.02	0.02

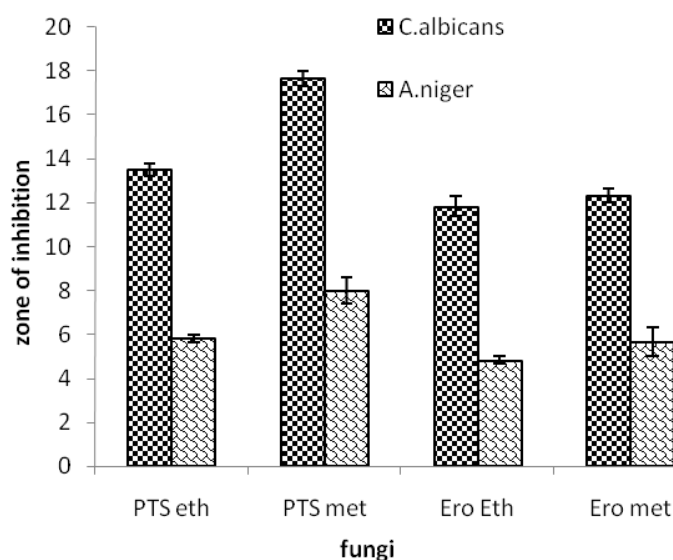
**Table 2: MIC values for leaf extracts for bacteria expressed in %**

Plant extracts/ Bacteria	PTS Ethanol	PTS Methanol	Erode Ethanol	Erode Methanol
<i>Streptococcus pyrogens</i>	0.02	0.1	0.02	0.02
<i>Staphylococcus aureus</i>	0.1	-	0.5	-
<i>Escherichia coli</i>	0.1	0.1	0.1	0.1
<i>Klebshiella pneumoniae</i>	-	-	-	0.5
<i>Serratia marcesens</i>	0.5	0.5	-	0.5
<i>Bacillus subtilis</i>	0.02	0.05	0.1	0.02

Graph-1 Anti microbial activity of rhizome extracts of PTS and Erode variety turmeric plants

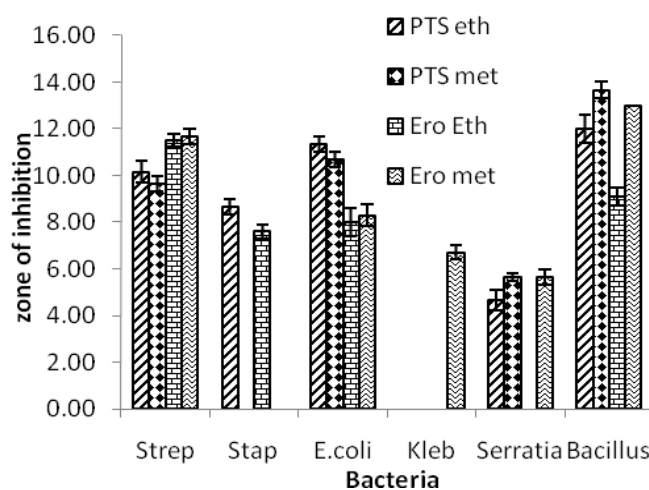


Graph 2. Anti fungal activity of rhizome extracts of PTS and Erode variety turmeric plants

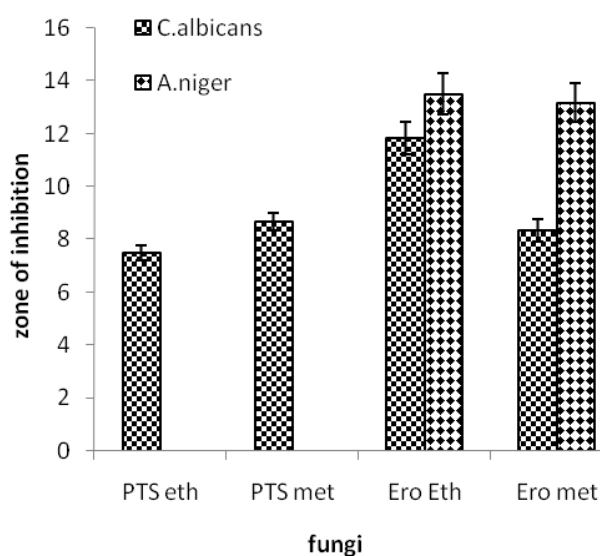


The fungal pathogens showed inhibitory effect to some extent. The results obtained is shown in Graph 2 and 4. Among the two fungi tested in the present study, *C.albicans* was found to be promising when compared to *A.niger*. *C.albicans* produced the highest zone in PTS methanol rhizome extract (17.67mm) and the least count was 7.5 mm with PTS ethanol extract. *A.niger* did not show any inhibition zone on PTS ethanol leaf extract. *A.niger* showed no zone of inhibition on PTS leaf extracts and recorded a highest zone of 13.5 mm on Erode ethanol leaf extract. The varying degrees of the zones clearly indicates that the statement given by Walsh et al (2003) [21] that the mechanism of antimicrobial properties of plant extracts involves various cellular processes, like synthesis of structural components in microbial cells.

Graph 3. Antibacterial activity of leaf extracts of PTS and Erode variety turmeric plants



Graph 4. Anti fungal activity of leaf extracts of PTS and Erode variety turmeric plants



A similar study of screening natural plant extracts against different fungal pathogens was recorded by Rani and murthy (2006) [22]. The phytochemical analysis given in Table-3 shows the presence of Flavanoids, Cardiac glycosides and Phenols in both the varieties of turmeric leaves. The results of the present study combats with the results of Swadhini et al (2011) [18] who had proved that the ethanol and methanol *C.longa* extracts were found to be active against fungi. The leaf extract was significantly effective ( $p < 0.05$ ) against the test pathogens. Of all the extracts, the maximum zone was exhibited by PTS methanol extract against *E.coli* and the least one by PTS ethanol leaf extract against *S.marcesens* (6.5 mm). The leaves of *C.longa* showed zone of inhibition against most of the test microbes in a comparable quantity with the rhizomes.



**Table 3: Screening of phytochemical constituents of PTS and Erode variety leaves of *C.longa***

Phytochemicals	PTS	Erode
Alkaloids	×	×
Flavanoids	√	√
Tannins	×	×
Saponins	×	×
Cardiac glycosides	√	√
Phenols	√	√

√ - positive, × - negative.

### CONCLUSION

The objective of this research was to evaluate the potential of turmeric leaf extracts on standard microorganism strains. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The findings of the present study clearly indicate that the leaves of turmeric plants also possess the anti microbial activity like that of the rhizome which has proved evidence for its antimicrobial potential.

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