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Original Article

Comparative Antimicrobial Study of Ethanolic Extract of Leaf and Rhizome of *Curcuma longa* Linn

Nikhil Singh*, Sangeeta Gupta, Vaibhav Rathore

ABSTRACT

Introduction: *Curcuma longa L.*, botanically related to ginger belongs to the Zingiberaceae family. It is extensively used in Ayurveda, Unani and Siddha medicine as a home remedy for various diseases. **Aim:** The present study is to compare the antimicrobial activity of the ethanolic extracts from rhizome and leaf of *Curcuma longa Linn.* **Method:** *In vitro* disc diffusion method and serial dilution method has been used to determine the antimicrobial potency against various bacterial pathogens such as *Klebsiella pneumoniae Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* by comparing the zone of inhibition shown by both the test extracts at different concentration (25 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, 300 mg/ml) and there MIC value has been evaluated. Ethanol and Ampicillin were used as negative and positive control for the study. **Result:** The leaf and rhizome extract showed maximum zone of inhibition at 300 mg/ml concentration, which was about 7.6 mm and 8 mm on the bacterial strain *Pseudomonas aeruginosa* and least effectiveness for other bacterial strain such as *Staphylococcus aureus* and *Bacillus subtilis*. The recorded MIC value was at 6.25 mg/ml which showed 3.991% inhibition for rhizome extract and 3.129% inhibition for leaf extract. **Conclusion:** The rhizome extract was found to be more potent antimicrobial agent than the leaf extract and can be used in future studies.

Key words: *Curcuma longa L*, Minimum inhibitory concentration, antimicrobial agent, Bacterial stain, Antibacterial activity, Turmeric.

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INTRODUCTION

Now-a-days multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases.[1,2] In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immunesuppression and allergic reactions.[3] Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine.[4] However, the potential of higher plants as sources for new drugs is still largely unexplored. India is the largest producer of medicinal herbs and appropriately called the botanical garden of the world.[5] Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine[6] Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants.[7,8] Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the worlds.[9,10]

Turmeric (*Curcuma longa* L.) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases. *Curcuma longa L.*, botanically related to ginger belongs to the Zingiberaceae family.[11] It is a perennial plant having a short stem with large oblong leaves and bears ovate, fusiform 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 and is considered as auspicious and is a part of religious rituals.[12] The compounds showing yellow colour are three curcuminoid compounds; curcumin, demethoxycurcumin and bisdemethoxycurcumin. Curcumin a yellow bioactive pigment, is the major component of turmeric. It has been shown that curcumin have a wide spectrum of biological activities.[13] The extracts of turmeric rhizome has recently been used as an insect repellent ,anti-bacterial,[14] antidiabetic,[15] antioxidant,[16,17] anti-inflammatory,[18,19] anticancer,[20] antiallergic,[21] antiprotozoal[22] and wound healing activity.[23] The main objectives of the present study were to evaluate and compare the antimicrobial activity of the extracts from rhizome and leaf of *Curcuma longa* Linn against various bacterial pathogens.

MATERIALS AND METHODS

Preparation of plant material and extracts

Fresh leaves and rhizomes of *Curcuma longa* were collected from the local area and the sample was identified by Botanist. from G.B Pant University, Pantnagar, Moradabad. Fresh leaves and rhizome

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were cleaned and cut into small pieces and air dried for 2 days. The dried sample was again dried in a hot air oven at 50°C for 24 hrs, then pulverized by a mechanical grinder and passed through a 20 mesh sieve. The dried powdered was extracted with ethanol using a Soxhlet apparatus. The extraction was carried out for 24 hrs at room temperature. And the extracts were filtered and concentrated at 35°C under reduced pressure and stored at 4°C for further experiment.

Test microorganism

The test organisms were clinical isolates from the stock culture acquired from the Institute of Microbial Technology (IMTech), Chandigarh, India. Following four bacterial strains were used in the present study. Gram positive bacteria- *Klebsiella pneumoniae (MTCC 4032), Pseudomonas aeruginosa* (MTCC 6458) & Gram negative bacteria, *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 2757). Each of these microorganisms was subculture unto nutrient broth to test for viability and subsequently on nutrient agar slants and kept at 4° C prior to susceptibility testing

Antimicrobial activity

In-vitro antimicrobial screening was generally performed by paper disc diffusion method for the primary selection of the compound as therapeutic agent.[24] The antimicrobial activity was performed on the ethanol extract of the rhizome and leaf of *Curcuma longa*. Activity was performed against four pathogenic bacteria like *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Two Gram positive and two Gram negative bacteria). Sterile discs (6 mm diameter) made of Whatman filter paper were impregnated with 20µl of different concentration of both the test extract (25mg/ml, 50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml, 250mg/ml, 300mg/ml) and air dried to eliminate residual solvent and then was placed on the agar media In addition to these disc loaded with ethanol and disc loaded with Ampicillin were used as negative and positive control. Plates were then incubated in BOD incubator at 37°C for 24 h. After incubation both the plates were observed for zones of inhibition, and their diameter were measured including the diameter of the disc.

Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MIC) of the ethanol extract of rhizome and leaf of *Curcuma longa* were determined by Serial dilution method.[25] Nutrient broth media were prepared and 5ml of media is taken in each of the test tube. Ethanol extract of leaf and rhizome is prepared of different concentration (6.25 mg/ml, 12.50 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml). Freshly grown bacterial strains of Pseudomonas aeruginosa is inoculated in broth media and grown at 37°C for 18 hrs. Each tube was inoculated with different concentration of both the extracts prepared at different concentration. All the tubes were incubated at 37°C on a shaker with 140 rpm for 24 h. Presence of turbidity denoted presence of microorganism in the test tube after the period of incubation, whereas the complete absence of any turbidity indicates complete inhibition of microbial growth. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC. After incubation, the bacterial growth was measured at 660 nm. The % of inhibition was calculated by using the formula below:

% Inhibition = OD of culture with sample/ OD of culture without sample x 100

RESULT AND DISCUSSION

In the present investigation, comparison of the ethanol leaf extract and rhizome extract were evaluated for their antibacterial properties. It has been found that various ethanobotanical study of *Curcuma longa* revealed effective antibacterial properties.[26] Various researcher have also worked in exploring the antimicrobial properties present in the various parts of the plants such as leaf and rhizome extract of *Curcuma longa*. In the present investigation the ethanolic plant extract of *Curcuma longa* was tested against the four pathogenic bacterial strains viz. *Klebsiella pneumonia, Staphylococcus aureus*, Pseudomonas *aeruginosa* and *Bacillus subtilis*. The antibacterial efficacy of the extract of *Curcuma longa* (L.) leaves and rhizomes was quantitatively assessed on basis of inhibition zone and MIC (minimum inhibitory concentration). The zone of inhibition by disc diffusion method shown by both the ethanolic leaf and rhizome extract on various concentration are given below in Table 1 and 2.

The ethanol extract of leaf and rhizomes show maximum zone of inhibition at concentration (300mg/ml) on the bacterial strain *Bacillus subtilis*. The leaf ethanolic extract was more effective showing 7.6 mm zone of inhibition on *Bacillus subtilis* at concentration 300mg/ml while it was about 7.0 mm zone of inhibition for the pathogen *Pseudomonas aeruginosa* for the same concentration when compared with the standard and was less effective While the Ethanolic extract of rhizome show maximum zone of inhibition of about 8.0 mm at concentration 300 mg/ml on the same bacterial stain *Bacillus subtilis* while less effective for the pathogens *Staphylococcus aureus* showing 7.0 mm zone of inhibition when compared with the standard drug at the same concentration. This can be further seen that the present study result is satisfactory by investigating various similar works such as the alcoholic leaf extract of *Curcuma longa* had shown effective antibacterial effect on various strains with showing maximum zone of inhibition on the *Bacillus* bacterial strain.[27,28] And the rhizome extract of *Curcuma longa* was reported to show effective antibacterial activity in the study of various medicinal herbs showing antimicrobial properties in which *Curcuma longa* rhizome is taken as one of the herb.[29] It has been also reported that curcuminoids isolated from the rhizomes of *Curcuma longa* shows potent antimicrobial activity against *B. subtilis, E. coli, S. auresus and P. mirabilis,* whereas it showed moderate activity against *K. pneumonia, Enterobacter aerogenes and Pseudomonas aeruginosa.*[30]

The MIC value reported on ethanolic extract of leaf and rhizomes of *Curcuma longa* was performed on bacterial strain *Pseudomonas aeruginosa* on which antimicrobial activity was found to be more potent. The percentage inhibition at various concentration (200, 100, 50, 25, 12.5 and 6.5 mg/ml) was reported for both the extract is given in Table 3 below.

The MIC value is the lowest concentration that completely inhibited the growth of microorganisms grown aseptically. The percentage inhibition reported at various concentrations on the bacterial strain *Pseudomonas aeruginosa* with minimum concentration of about 6.25mg/ml obtain by various fold of dilution. The MIC value for leaf and rhizome ethanolic extract shows very less variation and was found to be 3.129 % and 3.991% whereas the percent inhibition at highest concentration 200mg/ml was found to be 93.125% for rhizome extract and 91.058% for leaf extract. The rhizome show more potent antimicrobial potency when compared with the leaf extract as observed in the Figure 1. The various phytochemical study on the leaf and rhizome of the plant *Curcuma longa* revealed the presence of various active constituents such as alkaloids, tannins, flavonoids, glycosides and saponins seen in both the leaf and the rhizome extract.[31,32] It has been also reported that the alkaloids and flavonoids are the responsible compounds for antibacterial activity in various plants.[33] It can also be said that the various components which are present both in the rhizomes and the leaf of the plant *Curcuma longa* is responsible for the antibacterial activity against pathogens. According to the various research it has been concluded that the leaf and the rhizome show the presence of active constituents Curcuminoids showing various bands of Curcumin, bisdemethoxycurcumin and demethoxycurcumin

Table1: Zone of inhibition shown by Ethanolic extract of leaves of *Curcuma longa* on different bacterial strain

Pathogens Extract dose	Zone of Inhibition (mm) at different concentration								
	25	50	100	150	200	250	300	Positive control	Negative control
Bacillus subtilis	6.2	6.2	6.3	6.3	7.0	7.0	8	8.0	Nil
Klebsiella Pneumonia	6.1	6.1	6.2	6.2	6.4	6.7	6.8	7.0	Nil
Staphylococcus aureus	Nil	Nil	Nil	Nil	Nil	Nil	Nil	9.0	Nil
Pseudomonas aeruginosa	6.1	Nil	Nil	Nil	Nil	7.0	7.0	7.0	Nil

Table 2: Zone of inhibition shown by Ethanolic extract of rhizomes of *Curcuma longa* on different bacterial strain

Pathogens Extract dose	Zone of Inhibition (mm) at different concentration								
	25	50	100	150	200	250	300	Positive control	Negative control
Bacillus subtilis	6.4	6.4	6.4	6.4	6.4	7.0	7.5	9.0	Nil
KlebsiellaPneumonia	Nil	Nil	Nil	Nil	Nil	6.5	6.5	7.5	Nil
Staphylococcus aureus	6.1	6.2	6.3	6.3	6.7	6.7	7.0	1cm	Nil
Pseudomonas aeruginosa	6.1	6.1	6.1	6.1	6.2	6.2	6.6	9	Nil

Table 3: MIC value for ethanolic extract of rhizomes and leaf of *Curcuma longa* at various concentrations on the bacterial strain Pseudomonas aeruginosa

S.No	Concentration of extract(mg/ml)	Bacterial Percentage Inhibition	
		Ethanolic rhizome extract	Ethanolic leaf extract
1	Control	0	0
2	200	93.125	91.058
3	100	69.812	69.746
4	50	67.239	66.998
5	25	43.123	42.399
6	12.5	10.012	10.432
7	6.25	3.991	3.129

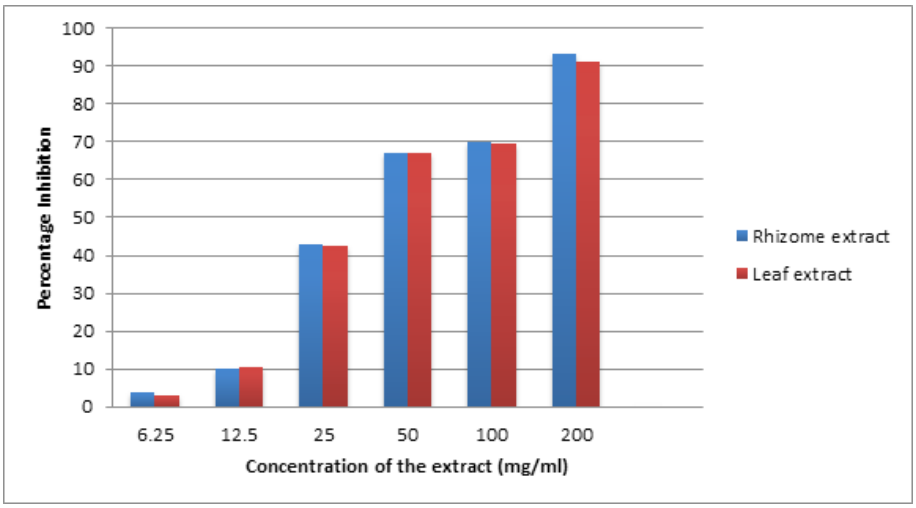

Figure 1: Graphical representation of percentage inhibition of both the extract of *Curcuma longa* at various concentration of the extract on Pathogen Pseudomonas aeruginosa

in the isolation and characterization of curcuminoids by various techniques.[30] The active constituents present in the *Curcuma longa* leaf and rhizome leads to plasmolysis, cell disruption and death of the cell wall of the specific pathogen as seen in the evaluation of antimicrobial properties in *Curcuma longa* rhizome extract against *Staphylococcus aureus* strain by electron microscopy.[32]

CONCLUSION

In this study, the comparative efficiency of rhizome and leaf extract of the crude drug *Curcuma longa Linn* were evaluated for its microbial inhibitory effect on various pathogenic bacterial strains such as *Klebsiella pneumonia, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* was observed. Both the extract show potent antimicrobial agent but not on all the bacterial strains but on specific bacterial stain such as *Pseudomonas aeruginosa* and *Bacillus subtilis* show effective inhibitory result. If we compare the antimicrobial activity of both the ethanolic extract from leaf as well as from the rhizome. The rhizome extract found to be more potent in its activity than the leaf extract.

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