# *IN-VITRO* ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACTS OF *CURCUMA LONGA* L.

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**Summary**

The present study is to detect the antibacterial property in the extracts of *Curcuma longa*. The antibacterial activity of *Curcuma longa* was tested on pathogenic bacteria, i.e., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Micrococcus luteus*, *Bacillus subtilis*, *Enterococcus faecalis* and *Streprtococcus faecalis* causing infectious diseases diseases in human beings. Cold as well as hot methanol extracts were used for the study of antibacterial activity by agar well diffusion method. The bacterial plates were prepared by nutrient agar. The extracts of two different concentrations, 100mg/ml and 300mg/ml were loaded in the 6mm wells prepared in the nutrient agar. ZOI was measured around the wells to check the antibacterial activity of extracts. Results showed the hot methanol extracts to have higher antibacterial property in comparison to the cold methanol extract. Relatively higher zone of inhibitory values were obtained for both Gram-negative bacteria, *Micrococcus luteus, Proteus vulgaris* and Gram-positive bacteria, *Enterococcus faecalis* with hot methanol extract. Antibacterial activity of hot methanol extract of *Curcuma longa* was carried out to validate the use of traditional herbal medicine and the results of this study tend to give credence to the common use of *Curcuma longa* rhizome.

**Keywords:** *Curcuma longa*, turmeric, rhizome, cold methanol extract, hot methanol extract, antibacterial activity.

The science of pharmacognosy is enjoying a vigorous renaissance due to the wide spread use of herbal medicine and natural products as supplements but challenges are being faced to authenticate and standardize these products. On the other hand re-emerging diseases require new approaches and solutions. As history indicates, the best source for new chemical entities is the natural source which provides the basic molecular and active texts like the Vedas and Bible has been traced to the occurrence of natural products with medicinal properties. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms (1, 2, 3), animals (4, 5) and plants (6, 7, 8, 9, 10). Herbs from around world have been traditionally used for centuries to treat bacterial diseases. Herbs continue to play a major role in primary health care as therapeutic remedies in many developing countries. Due to several undesirable effects of synthetic drugs, the world population is turned towards medicines derived from herbs or herbal products.

*Curcuma longa* L. a perennial herb which is commonly known as turmeric, it is a member of the Zingiberaceae family. *Curcuma longa* an indigenous plant grows widely all over India. It is known by different names in different languages viz., Haridra (Sanskrit), Haldi (Bengali, Oriya and Hindi), Manjul (Tamil), Paspu (Telugu), Aurukesthur kurcum (Arabic) and Turmeric (English). The underground rhizomes of the plant are used for medicinal and food preparation. The rhizome is an underground stem that is thick and fleshy ringed with the bases of old leaves. Rhizomes are boiled and then dried make the distinctive bright yellow spice, turmeric (11, 12).

Turmeric is used as a food additive (spice), preservative and coloring agent in Asian countries, including China and South East Asia. It is also considered as auspicious and is a part of religious rituals and was widely used in Ayurvedic, Siddha and Unani systems. In old Hindu texts it is described as an aromatic, stimulant and carminative. Mixed with slaked (hydrated) lime, turmeric is a well known household remedy for spains and swellings caused by injury (13). In traditional Indian Ayurvedic medicine turmeric has been used as a tonic for the digestive system and the liver; to dispel worms, strengthen the body and dissolve gallstones; and for menstrual irregularity and arthritis. In China, *Curcuma longa* is used for diseases associated with abdominal pains (14). Its root has traditionally been used as an insect repellant, antimicrobial (15), antidiabetic (16) and colic inflammatory disorders (17). It possesses antiseptic, anti-inflammatory and detoxifying properties (12). According to the folklore used of this plant extract it was interesting to investigated antibacterial activity of the plant root extract. This study aim to investigate antibacterial activity of cold and hot methanol extracts of *Curcuma longa* rhizome against various pathogenic bacteria.

## Materials And Methods

**Plant material:**

Rhizomes of the *Curcuma longa* were collected from Vizianagaram district farms and the identification was done by Prof. M. Venkaiah, Department of Botany, Andhra University, Visakhapatnam. A voucher specimen has been deposited in the same Department.

**Extraction preparation:**

Rhizomes were washed thoroughly under running tap water, dried on paper towel, then kept in shade for 25-30 days and finally crushed to fine powder in mixer grinder. The dried powder of rhizome (300g) was dissolved in 1000ml of methanol. The extract was collected after three days, filtered and kept in sterilized dark bottles. This procedure was repeated three times for proper extraction. The extract was evaporated to dryness using rotary evaporator. The residue from cold extraction was packed in Soxhlet apparatus for hot extraction. The extract was filtered and concentrated in rotary evaporator at 35-40$^0$C under reduced pressure to obtain a semisolid material which was then lyophilized to get a powder. The dried materials were then diluted with an inert solvent, dimethyl sulfoxide (DMSO) to obtain two different (100mg/ml and 300mg/ml) concentrations (18). They were stored at 4$^0$C for further studies.

**Bacterial strains:**

Pure bacterial strains (Table 1) were obtained from microbial type culture collection (MTCC), Institute of microbial technology (IMTECH), Chandigarh, India and were maintained on nutrient agar.

**Table 1. List of tested bacterial strains and their catalogue numbers.**

| Bacteria | Catalogue number |
|---|---|
| *Escherichia coli* | MTCC B9637 |
| *Klebsiella pneumoniae* | MTCC B2405 |
| *Proteus vulgaris* | MTCC B0426 |
| *Micrococcus luteus* | MTCC B1538 |
| *Bacillus subtilis* | MTCC B2274 |
| *Enterococcus faecalis* and | MTCC B0439 |
| *Streprtococcus faecalis* | MTCC B0459 |

**Assessment of inhibition of bacterial growth:**

The antibacterial assessment tests were carried out using agar well diffusion method (19). The freshly prepared inoculums of bacterial strains were swabbed all over the surface of the nutrient agar plates. Two wells of 6mm diameter were made on the inoculated media with the help of a sterile metal borer and were numbered properly. The two different concentrations of cold as well as hot methanol extract solutions of rhizome of *Curcuma longa* were delivered into well by using micropipette. Then the plates were incubated at $37^0$C for 24 hours. The presence of zone of inhibition (ZOI) was regarded as the indicator of antimicrobial action and antimicrobial activity was expressed in terms of mean diameter of the ZOI measured in millimeter. Each test was carried out in triplicate. Penicillin (5µg) was used as a positive control and DMSO as a negative control.

## Results

The results of the ZOI values of cold and hot methanol extracts of *Curcuma longa* are presented in Table 2. Cold as well as hot methanol extracts of two concentrations were showed antibacterial activity against all tested bacteria except *Bacillus subtilis*, which was resistant to both extracts. The ZOI values were obtained more with hot methanol extract than cold methanol extract of *Curcuma longa*. Gram-negative bacteria, *Escherichia coli, Klebsiella pneumoniae* and Gram-positive bacteria *Streprtococcus faecalis* were found resistant to 100mg/ml concentration of extract. In the ZOI values were increased when increased in the concentration of extract. Cold methanol extract exhibited the high ZOI against Gram-negative bacteria, *Micrococcus luteus* and Gram-positive bacteria *Enterococcus faecalis*. Where as hot methanol extract exhibited maximum ZOI against Gram-negative bacteria, *Micrococcus luteus* and *Proteus vulgaris*. *Micrococcus luteus* and *Proteus vulgaris* were the most susceptible bacteria to both cold and hot methanol extracts. From the above results it shows that Gram-negative bacteria were more susceptible than Gram-positive bacteria for cold as well as hot methanol extracts of *Curcuma longa*. 300mg/ml of hot methanol extract exhibited the high ZOI values against *Enterococcus faecalis* was more than standard antibiotic (5µg) penicillin and similar value with *Escherichia coli*. Hence, it indicated that hot methanol extract had a broad spectrum of antibacterial activity. DMSO, a negative control did not prevented the bacterial growth, hence it indicated that it does not interfere in the formation of ZOI.

**Table 2. Inhibition zones values of cold and hot methanol extracts of *Curcuma longa*.**

| Bacterial strain | 'Inhibition Zones(mm)' | | | | | |
|---|---|---|---|---|---|---|
| | Cold methanol extract | | Hot methanol extract | | Penicillin | DMSO |
| | 100mg | 300mg | 100mg | 300mg | 5 µg | 100% |
| *Escherichia coli* | -- | 10 | 12 | 15 | 15 | -- |
| *Klebsiella pneumoniae* | -- | 10 | 12 | 14 | 20 | -- |
| *Proteus vulgaris* | 12 | 13 | 17 | 19 | 20 | -- |
| *Micrococcus luteus* | 14 | 16 | 18 | 21 | 22 | -- |
| *Bacillus subtilis* | -- | -- | -- | -- | 17 | -- |
| *Enterococcus faecalis* | 12 | 13 | 14 | 16 | 11 | -- |
| *Streprtococcus faecalis* | -- | 10 | 10 | 13 | 16 | -- |

--: no zone of inhibition.

Rhizomes of *Curcuma longa* were showed a potent antibacterial activity. This may be due to the presence of chemical components are named curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin and bisdemethoxycurcumin (20). Curcumin is the most important fraction which is responsible for the biological activities of turmeric. Curcumin 95%, a potent antioxidant is believed to be the most bioactive and soothing portion of the herb turmeric and possess the properties like antioxidant, anti-inflammatory, anti platelet, cholesterol lowering, antimicrobial effects. The antimicrobial activity of *Curcuma longa* has been reported in previous study. It was found that the *Curcuma longa* have inhibitory effect against *Pseudomonas aeruginosa* (15, 21), *Aeromaonas hydrophila* (22), *Helicobacer pylori* (23), *Escherichia coli* (24), *Listeria aureus, Salmonella typhimurium* and methicillin – resistant *Staphylococcus aureus* (25). In this study, the results showed antimicrobial activity of cold and hot methanol extracts of *Curcuma longa* against *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Micrococcus luteus, Bacillus subtilis, Enterococcus faecalis* and *Streprtococcus faecalis*. The result from this study may supported that the antimicrobial activity of *Curcuma longa*. Moreover, it may support the use of *Curcuma longa* for antimicrobial treatment disease of prevention of bacterial growth.

## Conclusion

The results of present assay have proved *Curcuma longa* to hold excellent potential as an antibacterial agent and it can be used in the treatment of infectious diseases caused by *Proteus vulgaris*, *Micrococcus luteus* and *Enterococcus faecalis*. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

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