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Detection of Antimicrobial Effect of *Curcuma longa*

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ABSTRACT

Curcuma longa (Zingiberaceae family) is a robust perennial herb with a short stem and tufted leaves. Curcumin, the major constituent of *Curcuma longa*. Turmeric commonly used for cooking in Asian cuisine is known to possess a broad range of pharmacological properties at relatively nontoxic doses. Curcumin is found to be effective against *Staphylococcus aureus* and *E.coli*. As demonstrated by *in vitro* experiments, curcumin exerts even more potent effects when used in combination with various other antimicrobial agent. Hence, curcumin which is a natural product derived from plant is believed to have profound medicinal benefits and could be potentially developed into a naturally derived antibiotic in the future. *S. aureus* infections, *E.coli* infections particularly those caused by multidrug resistant strains, have emerged as a global health issue and urgent action is needed this project focuses on the antimicrobial effect of Curcumin against both *S.aureus* and *E.coli* .This study provides significant insighs into the therapeutic effect of curcumin against microorganisms. The main aim of this study was to detect the inhibitory effect of *Curcuma longa* on *S.aureus* in comparison with antibiotic gentamicin using diffusion method. Minimum inhibitory concentration of showed that curcumin mediated growth inhibition of *S.aureus*. Results showed that *Curcuma longa* significantly inhibited the activity of *S.aureus* growth with inhibition zones.

INTRODUCTION:

Botanical Name: *Curcuma longa*

Common Name: Turmeric

Synonyms: Turmeric, *Curcuma longa*, Curcuma domestica, Curcumin.

PLANT PROFILE

Figure No. 1:

SCIENTIFIC CLASSIFICATION:

Kingdom: Plantae

Division: Magnoliophyte

Class: Liliopside

Sub Class: Zingiberidae

Order: Zingiberales

Family: Zingiberaceae

Genus: Curcuma

Species: *C. longa*

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is native to the Indian subcontinent and Southeast Asia. It is a powdered

rhizome which obtained by cleaning, boiling and drying in oven. It is an important herb in Indian Ayurvedic system and Chinese traditional medicine. It is a symbol prosperity; it has been used from centuries in Indian Ayurveda and Herbal systems of medicinal preparations. Besides, it is used as a spice and natural colourant in food preparations.

Habitat and Distribution

The origin of *Curcuma Longa* is Southern Asia, most probably from India. Actually, this plant does not found in a true wild region, even though it appears in some region to have become naturalized. This plant is a sterile triploid, and it grows up continued selection and vegetative propagation of hybrid between the diploid wild Turmeric.

It is a tropical plant, which grows in a humid warm weather with a lot of rainfall. Appropriate temperature for Turmeric is between 20°C and 30°C (68°F and 86°F). It needs light for growing, open fields are the best for this plant. **India** is the center for producing this plant. **Nizamabad** is the city in India, which is the largest center for producing *Curcuma longa* in Asia. Tamil Nadu, is the second largest city in producing Turmeric. Sangli is important center for trading Turmeric. Moreover, it is found in Pakistan, especially in Kasur, that is the largest city for *Curcuma Longa* trading in Pakistan. In addition, it is grown in China, Jamaica, Indonesia, and Malaysia.

It has been grown in India since long time. It reached to China at 7th century. After that, it reached to east Africa at 8th century. It reached to Jamaica at 18th century. Turmeric is grown on a large scale, but the trade production just in India and South East of Asia.

Geographical Location

The origin of *Curcuma Longa* is Southern Asia, most probably from India. It is grown in Cambodia, China, India, Indonesia, Lao People's Democratic Republic, Madagascar, Malaysia, the Philippines, and Vietnam. It is extensively cultivated in China, India, Indonesia, and Thailand and throughout the tropics, including tropical regions of Africa.

Reproductive biology:

Turmeric plant is planted in the month of September to October. It grows in light black, black clayey loams, and red soils with irrigated and rain fed conditions. It is harvested after 9 -10 months of planting. The lower leaves turn yellow and fall with age.

Plant Description

Turmeric is a perennial herbaceous plant that reaches up to 1 m (3 ft. 3 in) tall. Highly branched, yellow to orange, cylindrical, aromatic rhizomes are found. The leaves are alternate and arranged in two rows. They are divided into leaf sheath, petiole, and leaf blade. From the leaf sheaths, a false stem is formed. The petiole is 50 to 115 cm (20–45 in) long. The simple leaf blades are usually 76 to 115 cm (30–45 in) long and rarely up to 230 cm (91 in). They have a width of 38 to 45 cm (15–18 in) and are oblong to elliptic, narrowing at the tip.

Inflorescence, Flower, Fruit

At the top of the inflorescence, stem bracts are present on which no flowers occur; these are white to green and sometimes, tinged reddish-purple, and the upper ends are tapered. The hermaphrodite flowers are zygomorphic and threefold. The three 0.8 to 1.2 cm (0.3–0.5 in) long sepals are fused, white, have fluffy hairs and the three calyx teeth are unequal. The three bright-yellow petals are fused into a corolla tube up to 3 cm (1.2 in) long. The three corolla lobes have a length of 1.0 to 1.5 cm (0.39–0.59 in) and are triangular with soft-spiny upper ends. While the average corolla lobe is larger than the two lateral, only the median stamen of the inner circle is fertile. The dust bag is spurred at its base. All other stamens are converted to staminodes. The outer staminodes are shorter than the labellum. The labellum is yellowish, with a yellow ribbon in its center and it is obovate, with a length from 1.2 to 2.0 cm (0.47–0.79 in). Three carpels are under a constant, trilobed ovary adherent, which is sparsely hairy. The fruit capsule opens with three compartments.

THERAPEUTIC USES

Anti-microbial Activity

The *Curcuma longa* rhizome extracts shows activity against pathogenic *Bacillus subtilis*, *Staphylococcus aureus* (gram +ve) *Salmonella typhimufium*, *Escherichia coli*, *Agrobacterium tumefaciens* (gram -ve) and one acid fast *Mycobacterium tuberculosis* strains and inhibits growth on these organisms.

Anti-oxidant activity

Biological activity of turmeric is attributed to the presence of curcuminoids. Turmeric was the second most active spice among 23 spices studied for antioxidant activity. Cell possesses

an elaborate defense system to destroy reactive oxygen species (free radicals) and lipid peroxidation products that cause considerable damage to the cell. Antioxidants neutralize free radicals and stop the chain reaction.

Anti inflammatory activity

In Indian system of medicine turmeric is a household remedy for reducing pain, swelling, wound injury and various types of inflammation. In the carrageenin induced oedema, the turmeric oil at 1.6 ml/kg body weight has shown as much anti-inflammatory activity as phenylbutazone.

Anti-cancer activity

Turmeric possesses anti-cancer activity because curcuminoids present in turmeric show tremendous free radical scavenging. The anticancer activity of turmeric has been reported in oral, skin,.colonic and mammary cancer. The development of oral neoplasma identified histopathologic ally as squamous cell carcinomas in animals painted with 4- nitroquinoline-l-oxide (NQO) on their cheek mucosa showed a decrease in lipid peroxidation and tumor burden when administered with turmeric extracts simultaneously.

Respiratory activity

The fresh juice of *curcuma longa* rhizomes is given in bronchitis. In rhinitis and cough boil haridra in milk and mixed with jiggery given internally. In catarrhal cough, sore throat and throat infection the decoction of rhizome is used for gargle and also the piece of rhizome is slightly burnt and given for chewing.

Diabetes mellitus

Turmeric rhizomes power is very useful with amla juice and honey in madhumeha (Diabetes mellitus).

Neuroprotective

Curcuma oil significantly reduces the ill effects of ischemia by attenuating nitrosative and oxidative stress. Curcuma oil as a neuroprotective with an excellent therapeutic window for the prevention of ischemic brain injury.

MATERIAL AND METHODS:

Chemicals and reagents: Methanol, Water, acetic anhydride, sulphuric acid, Wagner's reagent, Mayer's reagent, Hager's reagent, Dragendroff's reagent

Plant material collection

The rhizomes of *curcuma longa* were collected from Prof. Jaya Shankar Agriculture University, Rajendranagar, Hyderabad, Telangana.

Test Microorganism

The standard strains of *Staphylococcus aureus* used as test organism were obtained from microbiology lab Mallareddy Pharmacy College, Maisammaguda, Secunderabad. Cultures of bacteria were grown on nutrient broth maintained and persevered on nutrient agar slants prior to use.

Methods

Preparation of the rhizomes power sample of *curcuma longa*

- Collected rhizomes were cleaned and boiled for 6 hours using distilled water in mantle.
- Boiled rhizomes were dried in tray dryer for 3 days to remove moisture present in it.
- Rhizomes was made into small pieces.
- Rhizomes tray dried are kept in Hot air oven at 60° for 30 min to remove any moisture left over.
- The obtained rhizomes are blended using the blender and stored for further experiment.

METHODS

Preparation of Extract: Soxhlet extraction

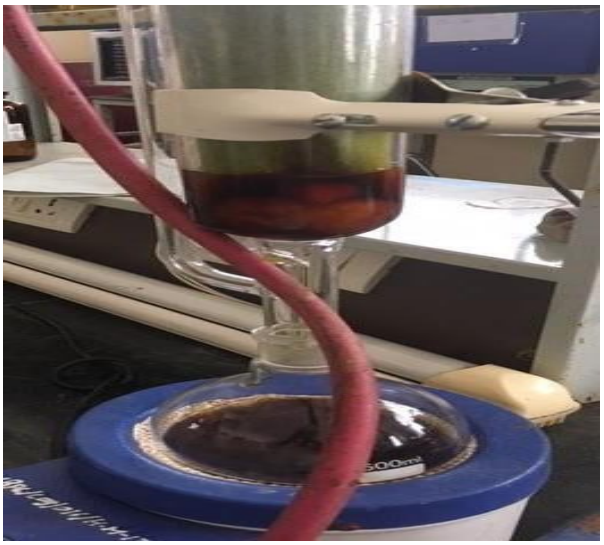

Figure No. 2: AQUEOUS EXTRACTION

Soxhlet extraction method is convenient and widely used for extraction because of its continuous process, less time and less solvent-consumption than maceration and percolation.

Procedure: The powdered plant material is placed in a Soxhlet apparatus, which is fitted on top of a collecting flask beneath a reflux condenser. Solvent is added to flask and the setup is heated under reflux below the boiling point of the solvent. The steam of the solvent, which contacts with the material, will dissolve metabolites and bring it back to round bottomed flask. Initially dried powdered rhizome of *Curcuma longa* was soaked in Methanol and water for one day. Plant material of *Curcuma longa* is about 2 Kg was taken and extracted with Methanol and water by using Soxhlet apparatus. 100g of powder sample was taken and extraction was carried out for 12hours. It was then filtered and concentrated.

Antimicrobial activity

To check the presence of antimicrobial substance, the antimicrobial susceptibility test were performed by standard disc diffusion method.in this method, antibiogram patterns were studied for Methanol extract and Aqueous extract for comparing 3 concentrations, 50mg/ml, 100mg/ml, 200mg/ml based on available literature. Empty sterile discs having a diameter of 6mm were impregnated with 25ul of each serial dilution of extract solution. These

impregnated discs, now were contain different concentration (1250, 2500, 5000µg/disc) respectively of extract and placed on to nutrient agar surface spread with 0.1 ml of bacterial culture. The plates were incubated at 37°C for 12-14h. The experiments were carried out in a triplicate. The results were recorded by measuring the zone of growth inhibition around the disc.

Principle: Minimum Inhibitory Concentration which inhibits the growth of microorganisms is denoted as MIC. The MIC amount is compared with known toxic quantities to ultimately determine a correct dosage for use. For practical reason it is not satisfactory to use agar diffusion method in order to determine this quantity. A more accurate approach is to serially dilute the antibiotic and test each separate concentration on standard inoculums of test organism, selecting the lowest concentration of antimicrobial agent that inhibit the growth of microbes.

Phytochemical tests:

Test for steroids:

I. Salkowski Test:

Chloroform solution of the extract when shaken with concentrated sulphuric acid and on standing yields red colour.

II. Lieberman Burchardt test:

Chloroform solution of the extract with few drops of acetic anhydride and one ml of concentrate sulphuric acid from the sides gives reddish ring at the junction of 2 layers.

Tests of Alkaloids:

The extracts were mixed with ammonia and then extracted with Methanol solution. To this dilute Hydrochloric acid was added. The acid layer was used for chemical tests for Alkaloids.

I. Mayer's test (Potassium Mercuric Iodide): The acid layer with few drops of Mayer's reagent gives a creamy white precipitate.

II. Wagner's Tests (Solution of Iodine in Potassium Iodide): The acid layer with few drops of Wagner's reagent gives reddish brown coloured precipitate.

III. Hager's Test (Saturated solution of picric acid): To 2ml test solution, and Hager's reagent (Qualigens, India) was added to it. A yellow coloured precipitate indicates the presence of alkaloids.

IV. Dragendroff's test (Solution of Potassium Bismuth Iodide): Acid layer with few drops of Dragendroff's reagent gives reddish brown precipitate.

RESULTS

The product obtained Soxhlet extract of Methanol was 16.5% and aqueous extract was 15.5%.

Phytochemical analysis of various extracts of *Curcuma longa* (rhizome).

S.no	Secondary metabolites	Name of test	Extracts of Curcuma longa	
			Methanol	Water
1	Alkaloid	Mayer's test	+	+
		Hager's test	-	-
2	Tannins and	Ferric chloride test	+	+
	Phenolic compounds	Vanillin hydrochloride test	+	+
3	Proteins	Ninhydrin test	-	-
		Biuret test	-	-
4	Flavonoids	Shinoda test	+	+
		Alkaline reagent test	+	+
5	Steroids and	Salkowski test	-	-
	Triterpenoids	Libermann–Buchard test	-	-
6	Glycosides	Legal test	+	+
		Sodium nitroprusside test	+	+
7	Carbohydrates	Benedict's test	+	+
		Fehling's test	+	+

Figure No. 3

TESTS FOR ALKALOIDS:

Figure: 4 SAPONINS TEST

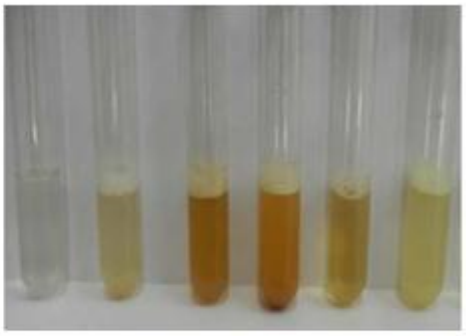

Figure:5 SALKOWSKI TEST

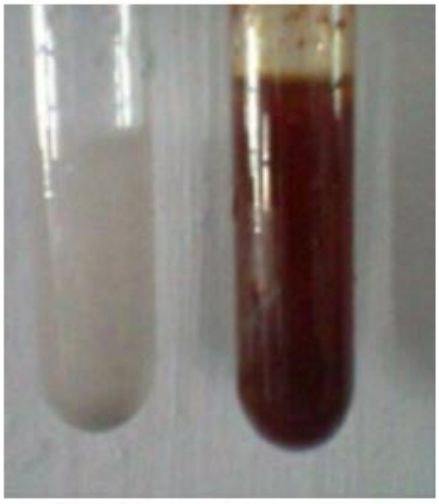

Figure: 6

Figure: 7 M.I.C of Methanol extracts

Figure: 8 M.I.C of Aqueous extracts

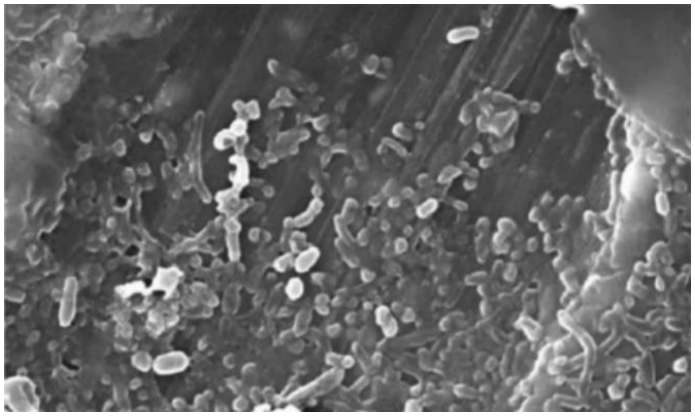

Figure No. 9: Untreated *Staphylococcus aureus*

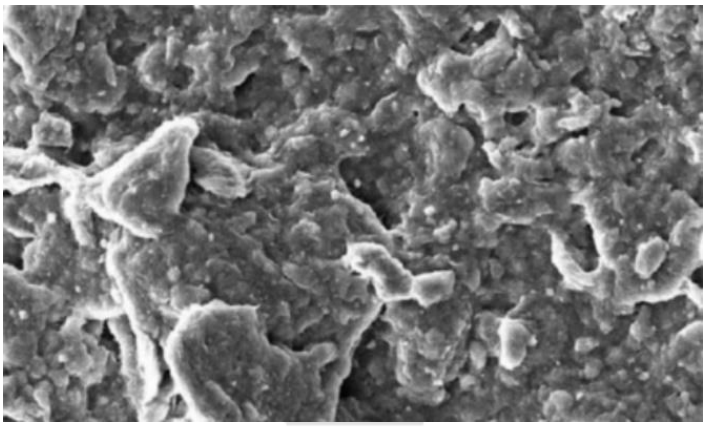

Figure No. 10: *Staphylococcus aureus* supplemented with *Curcuma longa* active compound indicates partial membrane damage

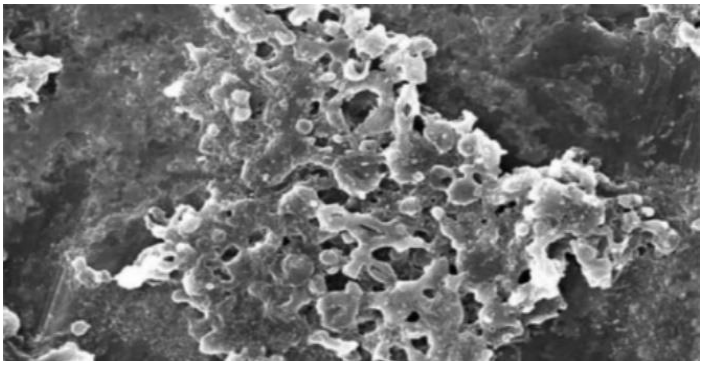

Figure No. 11: *Staphylococcus aureus* supplemented with *Curcuma longa* active compound indicates the cytoplasm tends to spill out of the bacterial cells.

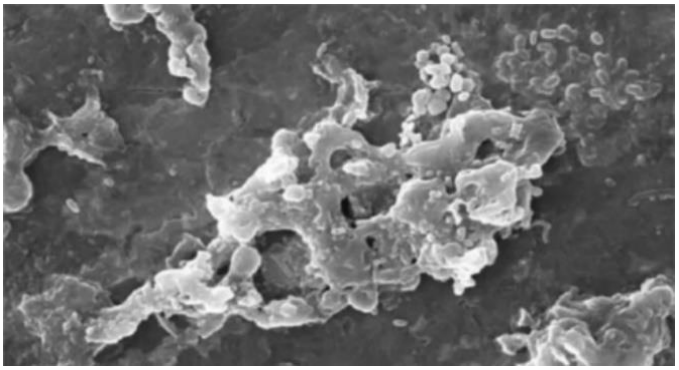

Figure No. 12: *Staphylococcus aureus* supplemented with *Curcuma longa* active compound indicates partial membrane damage.

DISCUSSION

Phytochemical analysis of *Curcuma longa L* extract

Phytochemical analysis of C.longa *L extract* shows the anti-microbial activity reveled by presence of five active constituents in two extracts. *C.longa L* extract contained alkaloids, carbohydrates flavonoids, tannins and glycosides. There are reports showing that alkaloids and flavonoids responsible for anti-bacterial activity.

Invitro analysis of *Curcuma longa L* extracts

Antimicrobial susceptibility tests on different fractions of *C.longa* extract against *S.aureus* show that C.longa rhizome extracts are highly active against *S.aureus* and gram positive showing 17- 25 Showing similar to Nigi et al.(1999) who invented the inhibitory effect of ethanol and hexane extract of turmeric against *S.aureus*. Further, it was observed that methanol is more reactive than all extracts of turmeric. Methanolic extract of turmeric show the highest result of inhibition.

The MIC (Minimum Inhibitory Concentration) was studied for different concentrations of *C.longa* extracts of methanol and Aqueous extract. The value ranges from 17-25 mm. Methanolic extract shows the most and aqueous shows the least value for inhibition.

Electron microscopy determined the morphology of bacteria after treatment with C.longa extract. The cells placed in solution of 0.1ml placed in the C.longa extract solution showed the normal morphology of *S.aureus* with multi layered membrane consisting outer membrane. In contrast the cells exposed to 0.g/ml various phases of cell death. In fig.9 and fig.

10 showed intermediate from stage cell disruption, plasmolysis and partial disapperarment of cytoplasmic membrane observed. The structure of outer membrane is unaffected in this stage and cell takes on deformed morphology, with partial lack of cytoplasmic membrane. In fig. 11 which previously corresponds to final stage of cell disruption, the outer membrane is significantly lost and cytoplasam tends to spill out of cell and finally leads to cell death.

CONCLUSION

In this study efficiency of turmeric fractions of Methanol and aqueous extract of C.longa is evaluated against gram negative bacteria of *S.aureus*. Methanolic fraction *curcuma longa* rhizome extract has high potential to inhibit pathogenic bacteria of *S.aureus* to a greater degree than other fractions of *Curcuma longa*. In our study, the results show aqueous fractions of *Curcuma longa* extract has antimicrobial activity is more effective than crude extract of *Curcuma longa*.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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