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Preparation & evaluation of Polyherbal Nanoformulation for Memory Enhancer & Cytoprotective Activity.

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ABSTRACT

Curcumin, is the major constituent of *Curcuma longa* L. belonging to Zingiberaceae family or turmeric, commonly used for cooking in Asian cuisine, is known to possess a broad range of anti-inflamatory pharmacological properties at relatively nontoxic doses. Due to high curcumin content Indian turmeric is very popular as compared to other countries. Instead of turmeric rhizome , Turmeric leaf also contains many phytochemicals same as present in the Turmeric root. The presence of plant phytochemicals in the leaf possesses disease preventive properties. Curcumin is the main Phytochemical found in Turmeric. Curcumin is found to be effective against *Staphylococcus aureus* (*S. aureus*). The phytochemical screening revealed that the plant leaf extract contain saponin, tannins, steroids, phenols, etc.. *S. aureus* infections, particularly those caused by the multidrug-resistant strains, have emerged as a global health issue and urgent action is needed. We also attempt to highlight the potential of Turmeric leaf against S. aureus as therapeutic antibacterial agent.

Keywords- Curcuma longa, Curcumin, Staphylococcus aureus.

1.INTRODUCTION

1.1.ANTIMICROBIAL ACTIVITY

Curcumin or diferuloylmethane is the major phytochemical of *Curcuma longa* L. (Zingiberaceae family), which is commonly known as turmeric. Curcumin is the polyphenolic compound that gives the yellow colour of the herb. Turmeric is mainly cultivated in tropical and subtropical regions and is mainly produced by India. Traditionally, it has been used to flavour food, dye cloths, and treat various human ailments . Curcumin is extracted from turmeric by solvent extraction (preferably with ethanol) through various methods (e.g., Soxhlet, ultrasonic, microwave, and supercritical carbon dioxide) followed by purification via column chromatography . Ever since the identification of curcumin as the main constituent of turmeric, multiple pharmacological activities of curcumin that include antimicrobial, antidiabetic, anti-inflammatory, anticancer, and antioxidant have been reported . More excitingly, when combined with other drugs, curcumin has been found to enhance the effects of antibacterial , antifungal , anticancer , and antioxidant activities.

Nosocomial infections are acquired infections in hospitalized patients in the hospital. Staphylococcus aureus is a major cause of nosocomial infections such. Every person infected with Staphylococcus aureus infection, although certain groups of people are at greater risk, including those with chronic conditions such as diabetes, cancer, vascular disease, eczema, foodborne and lung disease. In health, the risk of Staphylococcus aureus infection is higher because patients often have a decreased immune system .

Curcumin inhibits the growth of both Gram-positive and Gram-negative bacteria . *S. aureus* is one of the Gram-positive strains that is susceptible to curcumin-mediated inhibition. *S. aureus* is a pathogen that causes various infections including infective endocarditis (IE), bacteremia, skin and soft tissue, osteoarticular, and pleuropulmonary infections . Over the years, *S. aureus* has evolved and developed multiple strategies to evade human immune system and to resist antibiotics treatment. This has given rise to the evolution of MRSA, and the emergence of healthcare-associated (HA) and community-associated (CA) MRSA has caused a major problem to the human society .

Meals ready-to-eat (MRE) is getting popularity in our daily life. These packed foods are designed to have a long shelf life, require very little preparation work and are perfect for emergency survival preparation. Meals are often vulnerable to contamination and subsequent growth by food borne pathogens (SALMONELLA ENTERITIDIS, STAPHYLOCOCCUS AUREUS, CAMPYLOBACTER JEJUNI, LISTERIA MONOCYTOGENES) during their preparation. There is a great concern of increasing antibiotic resistance of these pathogen. There is an immense need of

developing a drug which has same phytochemicals as that of turmeric rhizome and can be effective against the Staphllococcus aureus bacteria. Here we found that Turmeric leaf contains most of the phytochemicals similar to the Turmeric rhizome.

1.2 PHTOCHEMICALS PRESENT IN TURMERIC LEAF

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1.3 ANTIBACTERIAL ACTIVITY OF TURMERIC LEAF

This study aimed to determine the antimicrobial activity of turmeric leaf extract. Turmeric leaf extract gave an **inhibitory effect** to the growth of Escherichia coli, Staphylococcus aureus, and Shigella dysenteriae, and did not give inhibitory effect to the growth of Lactobacillus acidophilus.

The killing increased with an increase in the dosage of curcumin I as well as exposure time. For instance, exposure to 25 μM curcumin I for 30 min showed **12%–60% killing** of all the tested bacteria, while the same concentration of curcumin. I showed 20%–92% killing after 2h of incubation against all the four genera.

2.MATERIALS AND METHOD

2.1 Collection of plant material: The Plant material (Leaves) were collected from the agriculture land of Paonta Sahib, Dist. Sirmaur, Himachal Pradesh . The leaves were brought to the laboratory and washed thoroughly in running tapwater to clean the adhering particulate matter and then rinsed in distilled water, Shade dried, Coarsely powdered and stored in air tight container for further use.

2.2Preparation of Leaf Extract: Ten grams of Turmeric leaf powder were put into the soxhlet apparatus and the extract were taken after 18 hours using ethanol as solvent. It was further concentrated by rotary evaporator and stored at 4 degree C until the use. The leaf sample is now stored for phytochemical screening.

2.3Screening of phytochemicals: The Turmeric leaf extract were screened for the presence of phytochemical constituents which may be the reason for the microbial properties of C. longa leaves. The methods followed were slightly modified methods of swadhini et al (2011)[18] and Pathak et al (2011) [19] .

2.4 Test for Alkaloids: This was tested by using dragendroff's reagent and confirmed by using Wagner's reagent and confirmed by using Wagner's reagent. 3 ml of crude ethanolic extract was put in a test –tube and 1 ml conc. HCl acid was added gently. Mixture was warmed for about 20 min. , allowed to cool and then filtered using Whattman's filter paper. The filtrate was then subjected to the Dragendroff's test and then Wagner's test.

DRAGENDROFF'S TEST: Two drops of Dragendroff's reagent were added to 1ml of the filtrate. A creamy precipitate was observed indicating the presence of alkaloids. This was confirmed by using the Wagner's test.

WAGNER'S TEST :1ml of the filterate was mixed with Wagner's reagent. A brownish precipitate was formed revealing the presence of alkaloids.

SAPONIN:5ml of the ethanolic plant extract was mixed with 20ml of sterile distilled water and then vigorously agitated in a test –tube and left to stand. Frothing was observed which lasted for about 15 minutes. This shows the presence of saponin.

TANNIN:3ml of the plant extract was mixed with equal proportion of freshly prepared Fecl3.

A green colouration appeared indicating the presence of tannin.

COUMARIN: 1.5ml of 0.25M NaOH was mixed with 2ml portion of Turmeric extract. The formation of yellow colour showed indication of the presence of coumarin.

FLAVANOIDS: The turmeric ethanolic extract was treated with 0.25M NaOH solution. The formation of a bright yellow colour indicated the presence of flavanoids.

DITERPENES: This test was oerformed by using copper acetate test. 1ml of turmeric extract was mixed with an equal proportion of water and 10 drops of copper acetate solution were added using a dropper. A deep green colouration was observed showing that diterpenes is present.

PHLOBATANNINS: 1ml of the plant extract was mixed with 1% HCl acid and the mixture was slightly boiled.A reddish brown precipitate was formed,indicating the preence of phylobatannins.

CARDIAC GLYCOSIDES: The Legal's test was used to test for cardiac glycosides,this was performed by the addition of 1ml of pyridine to 3ml of the ethanolic turmeric extract.5 drops of freshly prepared 2% sodium nitroprusside solution and 5 drops of 20% NaOH solution were then added using a dropper.A pinkish –red colouration was observed which gradually faded after standing for a while into brownish-yellow,an evidence of the presence of cardiac gylcosides.

PHENOL: 1ml of the extract was placed in attest tube and then mi.xed with 4 drops of freshly prepared alcoholic FeCl3 solution.A bluishblack colouration was observed which shows that a phenol is present in the plant extract.

STEROIDS: 1ml of the extract was added to 10ml of chloroform in a test-tube.Concentrated H2SO4 acid was poured gently through the walls of the tube into the mixture without agitation.The presence of a red interface and yellow-greenish flouroscencein the H2SO4 acid layer showed indication of the presence of steroid in the plant extract.

ANTHRAQUINONES: This was performed by using the Borntrager's test. 0.5g of turmeric extract was agitaed with 10ml portion of benzene in a test-tube. The resulting mixture was filtered and 5ml of 10% ammonia solution was added to the filtrate. This mixture was then thoroughly agitated and then left to stand. A pinkish-red colouration appeared in the lower phase , which shows the presence of free hydroxy-anthraquinones.

REDUCING SUGAR'S: 1gm of the extract was put in a test-tube and this was dissolved with 10 ml of distilled water. The resulting mixture was filtered and the filtrate was subjected to Fehling's test and Benedict's test for the presence of reducing sugars. For the Fehling's test, 2ml of the filtrate was hydrolysed with dilute HCl acid and then neutralized with an alkali and then heated with Fehling's solutions A and B. A reddish precipitate was formed showing the presence of reducing sugars. This was confirmed by using the Benedict's test by mixing 2ml of the

filtrate from the extract with 2ml of Benedict's reagent and then gently heating. An orange-red precipitate appeared indicating the presence of reducing sugars.

Anthocyanin: Anthocyanin was tested for by treating 1ml of the turmeric extract with an equal volume of 2M HCl acid and ammonia solution. A pinkish colour was observed which immediately changed to blue/violet colour, an indication of the presence of anthocyanin in the plant extract.

3.RESULTS AND DISCUSSION

The Aim of the present study was to compare the antimicrobial activity of turmeric leaves with rhizomes . The leaves were collected from the plain region of Himachal Pradesh , District Sirmaur (Paonta Sahib).The Antimicrobial activity of leaf of *Curcuma longa* will assay in vitro.The result obtained in the present study provides a scientific support to the uses of the leaf extracts of turmeric plants in the treatment of microbial diseases. Various researchers have worked in exploring the antimicrobial activity of turmeric leaf extracts against infectious bacteria. In recent studies it was foumd that ethanolic extract of Curcuma longa to be effective against some strains of bacteria and concluded that the varying degree of sensitivity of microbes may be due to nature and combination of the phytocompounds present in the extracts. India has about 15% of the 20,000 known traditional medicinal plants in the world . Rural population depend on the medicinal plant for treatment of the ailments. The result of the preliminary phytochemical screening provide an empirical basis for the use of medicinal plants in traditional therapy. The phytochemical constituents are responsible for the biological and pharmacological actions of these plants. Alkaloids have antibacterial activity. Curcumin , demethpxy curcumin and bis-demetho-hydroxycurcumin, are three pharmacologically important curcuminoids that have been isolated from curcuma longa. They have been shown to posses antioxidant, anti-inflammatory ,anti-carcinogenic, anti-mutagenic, anti-fungal ,anti-viral anti anti-cancer properties. Saponins have been reported to have antimicrobial properties and they may act as important precursor for steroidal substances. The terpenoids and sesquiterpenes found is also exhibit anti nflammatory and antimicrobial effects. The result of the preliminary phytochemical screening of ethanolic extract of turmeric showed the presence of Alkaloids, Tnnins, Coumarin, Diterpenes, Phlobatannin, Cardiac glycosides, Phenols, Steroids etc. as shown in the table below. Solvent used also determine the phyto-chemical constituents. More polar solvent have lesser components compared to the least polar. Turmeric is widely used in traditional healing. The scientific phytochemical screening can provide a sound scientific rationale for its use in curing numetous ailments.

TABLE 1 : Result of preliminary phytochemical analysis of ethanolic extract of *Curcuma longa* Leaf.

Phytochemicals	**Test**	**Observation**	**Inference**
Alkaloids	Draggendroff's test	Creamy precipitate	+
	Wagner's test	Brownish precipitate	+
Tannin and Phenolic compounds	Lead test	Green colouration Bluishblack colouration	+ +
Terpenoids	Salkowaski's test	Reddish-brown colour	+
Saponin	Foam test	Presence of emulsion	+
Flavanoids	Ferric chloride test	White precipitate	+
Cardiac Glycosides		Brown ring	_
Reducing sugars	Fehling's test	Orange red precipitate	_
Anthocyanin		Pinkish colour	_
Phlobatannin		Reddish brown precipitate	+
Coumarin		Presence of yellow colour	+

3.2 CONCLUSION

The objective of this research was to evaluate the potential of the Turmeric leaf extract. The selection of crude plant extract for screening program has the potential being more succesful . Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, specially those related to the control of antibiotic resistance microbes. The findings of the present study clearly indicate that the leaves of Turmeric plants also posses the phytochemicals same like that of the rhizome which will prove evidence for its antimicrobial activity in future.

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