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A study on antibacterial property of *curcuma longa* – herbal and traditional medicine

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ABSTRACT

Objectives: The aim of the study was to study the antibacterial property of *Curcuma longa*. Human beings are dependent on the use of plants and herbs as a source of drugs for curing ailments since ancient times. Herbs are medicinal plants which are created by nature for curing human diseases. Ayurvedic Materia Medica is a rich repository of herbs and about 2000 plant species mentioned in Ayurveda are used to cure various diseases in human beings. In Veda and Atharvaveda, written around 2000 BC, there are a lot of medical references and prescriptions showing beneficial results in a cure for a number of human chronic diseases, including rheumatoid arthritis, neuralgia, jaundice, skin diseases, gout, tumors, encephalitis, and bronchitis. Most of the medicinal plants and herbs have been found to have antibacterial, antiviral, anti-inflammatory, analgesic, and protective properties. The use of the traditional system of medicines in India and China such as Ayurveda, Unani, and Siddha is around 5000 years old, which recommends management of lifestyle, including diet management, exercise, and meditation along with treatment, including specific herbs to cure several ailments. *Curcuma longa* a traditional herbal medicine is widely used in India since ancient times to cure several ailments.In the present study, antibacterial property of ethanolic extract, extracted from dried rhizomes of *Curcuma longa* was studied in microbiology laboratory against different bacterial strains.

Materials and Methods: The dried rhizomes of *Curcuma longa* were purchased from local market in Punjab. The collected roots of *Curcuma longa* were washed, shade dried, grounded to fine powder and was subjected to soxhlet extraction using ethanol as solvent. Two bacterial strains were used in current study; one was Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli. The method to access antibacterial activity was Cylinder Plate method.

Results: 50 gm. of dried powder was subjected to Soxhlet extraction using ethanol solvent to get the extract of curcumin for investigation of antibacterial activity of turmeric against different Gram-positive and Gram negative bacterial strains using cylindrical plate method. A distinguished zones of inhibition 6.5 mm, 7.5 mm, and 11 mm in diameter were seen under plates containing different concentrations of *Curcuma longa* extract.

Conclusion: The potentiality of extract of *Curcuma longa* to inhibit the growth of microbial strains indicates its broad-spectrum antibacterial property which can be used for the betterment of health of society to treat several infections. Turmeric and its constituents may be included in modern system of medicine for the development of new dosage forms to treat several diseases with natural herbs with lesser adverse effects in comparison to allopathic system of medicine and improve the health and wellness of our society.

Keywords: *Curcuma longa*, *Staphylococcus aureus*, *Escherichia coli*, Antibacterial, Disease, Extracts

INTRODUCTION

From ancient times, in India, turmeric is used as a spice, derived from the dried rhizomes of *Curcuma longa*, a member of the ginger family (Zingiberaceae). The herb is known as "Golden

Spice of India." Turmeric is used in our country for a number of medicinal purposes for centuries. In the traditional system of medicine, turmeric is used as an home remedy to cure a number of ailments, including anti-inflammatory, antineoplastic, anti-oxidant, anti-coagulant, antidiabetic, cardioprotective, antiulcer, hypotensive, neuroprotective, antivenin, hypocholesterolemic, and antiviral activities. *Curcuma longa* powder is known as most powerful healing herb in nature.[1]

From a number of years, the benefits of this natural drug are known among society. Kapha is reduced by turmeric extract, its powder is helpful to remove phlegm from the throat, leucorrhea – water discharge or any other discharge such as pus cells present in any open wound. Liver obstruction and jaundice are cured using turmeric in Unani System of medicines, its external application is beneficial for the removal of ulcers and reducing inflammation. The most common part of the plant used widely is rhizome. Hot water extracts of the rhizome are taken orally in to reduce inflammation in the oral cavity. In Rasayan herb, turmeric is used as an anti-aging agent to counteract aging process.[2,3]

Turmeric a native of South-East Asia, Asian countries along with Bangladesh, South East Asia, and China are using turmeric as natural coloring agent, in Spices and preservative.[4,5]

In Asia, our country stands at the first position in the production of turmeric. Turmeric is considered as fortunate, so it is included in several religious ceremonies in India. From ancient times, Turmeric powder was used to treat sprain and strain caused by injury.[5] Due to its brilliant yellow color, turmeric is also known as "Indian saffron."[6,7]

Curcumin is one of the most researched bioflavonoids today,[8,9] and a number of studies have confirmed its antioxidant,[10,11] anti-inflammatory,[11] anti-cancer,[12] chemoprotective,[13] gastroprotective, and many other health properties[14,15] [Figure 1]. Most studies have proven that curcumin binds cyclooxygenase and lipoxygenase protein to produce its therapeutic response.[16-18] Due to anti-inflammatory properties, turmeric has been deemed a natural wonder by recent research,[19,20] giving evidences for its role in the cure of several ailments such as cancer and presenile dementia.[20-22] Turmeric powder has a warm, bitter, black pepper-like flavor and earthy, and mustard-like aroma.[23]

The most of the therapeutic activities of the turmeric are due to curcumin.[24,25] It also is used as hepatoprotective,[26] nephroprotective, anticoagulant, and anti-HIV to combat AIDS.[27,28] The curcumin reduces oxidative damage and amyloid pathology in an alzheimer mice[15] and is used as spices in India and Abroad.[21,22] The curcumin gold complex is obtained by combining curcumin with Auric chloride in presence of ethanol[29,30]. As per elemental analysis of curcumin gold complex it contains Au(CUR)2Cl[23,24] [Figure 2].

The main active constituent of turmeric is curcumin, which is yellowish in color and biologically active showing antimicrobial, antiviral, anticancer, and antifungal properties [Figure 3].[31,32]

MATERIALS AND METHODS

Plant material collection

The plant material was purchased from the local market at a well-known General Store in Bathinda region, Punjab, and India during the month of December 2018. The plant material identification was done by department of pharmacognosy. The collected roots of *Curcuma longa* were washed with running water. The roots were allowed to dry under shade for 15 days and cut into small pieces and dried in oven at 40°C for 48 h. After the drying process rhizomes of *Curcuma longa* was grounded to fine powder using tissue homogenizer. Fifty grams of dried plant powder were subjected to Soxhlet extraction using ethanol solvent. The extraction and antimicrobial activity tests were carried out between December 2018 and May 2019 at Department of Pharmaceutics and Pharmacognosy, Adesh Institute of Pharmacy and Biomedical Sciences, Adesh University, Bathinda.

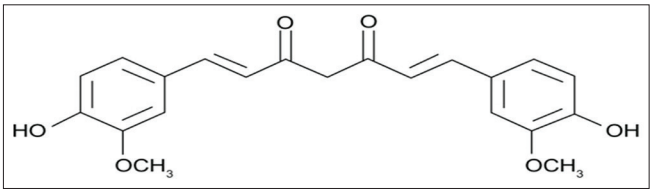

Figure 1: Structure of curcumin.

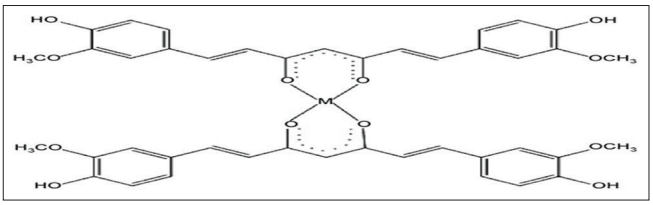

Figure 2: Structure of the curcumin gold complex.

Figure 3: Dried rhizome and powdered form of *Curcuma longa*.

Preparation of turmeric extract

Requirements

Fifty grams turmeric and 50 ml ethanol (95%), 150 ml distilled water, and a Soxhlet apparatus.

Soxhlet extraction

All the required materials were weighed accurately. Now, a clean round bottom flask was placed securely into the heating mantle. Then required quantity of ethanol and water was added in the round bottom flask in addition to some boiling stones to ensure gentle boiling of the solvent. The Soxhlet extractor is then placed on top of the round bottom flask. Then, a small cotton ball is placed at the bottom of the thimble. Now, 50 gm turmeric powder were packed into the thimble of Soxhlet extractor. Finally, another cotton ball is placed on top of the turmeric. Now, the reflux condenser is attached to the upper part of the Soxhlet extractor [Figure 4].

The water flow and the heating mantle are then turned on here; we see the Soxhlet apparatus is filled as the reflux proceeds. The temperature increases in RBF which leads to evaporation of the solvent into the condenser. The condenser converted solvent into liquid and drop down that into thimble containing powdered turmeric drug. At the end of the extraction process, the flask containing the ethanol extract was removed and ethanol was evaporated by using rotator evaporator. The turmeric extract was accurately weighed to calculate percentage yield.

The collected extract was further subjected to antibacterial studies against micro-organisms using cylinder plate method.

Preparation of media to check the Antibacterial properties of turmeric

Culture media provide artificially all essential nutrients for growth of micro-organisms. Basically, there are two types of culture media for cultivation of bacteria. A synthetic or chemically defined medium is compared by entirely chemical nutrients, whereas a complex media are composed of several ingredients of unknown chemical composition. The nutrient agar medium is relatively simple media which support the growth of most of micro-organisms.

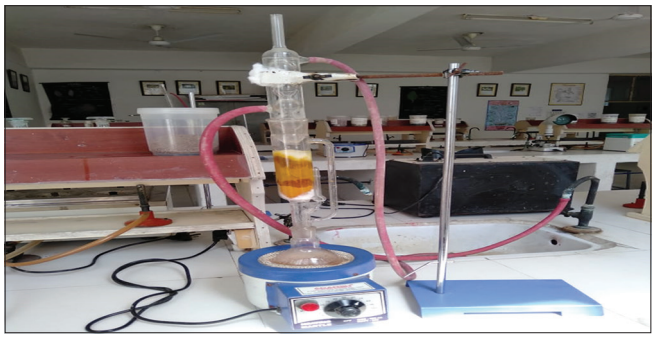

Figure 4: Soxhlet assembly in pharmacognosy department during extraction.

The constituents of Nutrient Agar Media are such as:

- Water: In general, tap water is used, but it should have low mineral content.
- Beef extract: It is an aqueous extract of beef tissue cone to a part and is a rich source of carbohydrates organize nitrogen compound, water-soluble vitamins, and salts.
- Peptone: It is a water-soluble compound resulting from the digestion of protein material such as casein, meat, and gelatin peptone. It acts as a rich source of organic nitrogen and vitamins.
- Sodium chloride (NaCl): It acts as electrolytes.
- Agar: It is a complex carbohydrates obtained from marine algae. It is used as solidifying agent in concentration of 1–2% due to its unique property of getting dissolved by heating at 100°C for 4 h and gives clear solution and it becomes a solid-like gel on the reduction of temperature below 40°C.
- Official formula: Composition of Nutrient Agar Medium.

Ingredients	Quantity for 1000 ml	Quantity for 50 ml
Beef extract	10 g	0.50 g
Peptone	10 g	0.50 g
Sodium chloride	5 g	0.25 g
Agar	1.5–2 g	1 g
Distilled water	q.s. to 1000 ml	q.s. to 50 ml

Method for preparation of media

1. Weigh accurately required quantity of each ingredient on a separate paper using physical balance.
2. Wash all the glassware's properly, dry and sterilize at 165°C for 1 h using hot air oven.
3. Add small quantity of water 10–15 ml in a clean conical flask.
4. Add accurately measured quantity of all the ingredient into a clean conical flask of 100 ml and dissolve them with the help of glass rod. Heat may be added with stirring, if required until agar gets completely dissolved.
5. Make the volume up to required quantity using distilled water, if necessary filter it.
6. Adjust to pH 7.2–7.4 using buffers and then sterilize the whole media by autoclave using 15 lb pressure at 121°C for 20 min.
7. Sterilized media were transferred aseptically to the sterile Petri plates under the laminar air flow cabinet [Figure 5].

Micro-organisms used for antibacterial activities

Different micro-organisms tested for antimicrobial activities were *Staphylococcus aureus* ATCC 6538 (Gram-positive) *Escherichia coli* (Gram-negative) ATCC 25922.

Antibacterial activity of turmeric extract

The antibacterial activity of turmeric extract was carried out by cylindrical plated method of microbial assay. The basic principle of microbial assay lies in comparison of inhibition of growth of micro-organisms by measured concentration of antibiotic to be investigated with that produced by known concentration of the standard antibiotic whose activity is already known.

Cylindrical plate method or cup-plate method

This method depends on the diffusion of an antibiotic from a vertical cylinder or a cavity, through the solidified agar layer of a Petri dish or plate, to an extent such that growth of the added micro-organism is prevented entirely in a circular area or zone around the cylinder or cavity containing a solution of the antibiotic. A previously liquefied medium, appropriate to the assay, is inoculated with the requisite quantity of suspension of the micro-organisms, the suspension is added to the medium at a temperature between 40 and 50°C and the inoculated medium is poured immediately into Petri dishes or large rectangular plates to occupy a depth of 3–4 mm.

The prepared plates or dishes must be stored in such a way that no significant growth or death of the test micro-organism occurs before use. Solutions of known concentrations of the standard preparation, that is, azithromycin 30 µg/ml and the test antibiotic, that is, turmeric extract at a concentration of 1000 µg/ml, 2500 µg/ml, and 5000 µg/ml were prepared using appropriate buffer solutions. These solutions were applied to the surface of the solid medium in sterile cylinders prepared in the agar. Same and sufficient quantity (15–25 µL) of solution of test and standard drug was added to each well. When paper discs are used, these should be sterilized by exposure of both sides under a sterilizing lamp and then impregnated with the solutions and placed on the surface of the medium [Figure 6].

The Petri plates were left standing for 1–4 h, at room temperature or at 4°C in a refrigerator, as a period of pre-incubation for proper diffusion of antibiotic solution through agar. The plates were incubated in upright position at 37°C in bacteriological incubator for 18–24 h for growth of bacteria. After Incubation period, well-defined zone of inhibition was seen around the well containing *Curcuma longa* extract. Diameter of inhibition zones was measured in mm.

RESULTS

In the present study, the roots of *Curcuma longa* were purchased from the local market, they were washed to remove debris, dust particles. The roots were allowed to dry under shade for 15 days. The plant material was cut into small pieces and dried in oven at 40°C for 48 h. After the drying process, *Curcuma longa* was grounded into fine powder by the grinder.

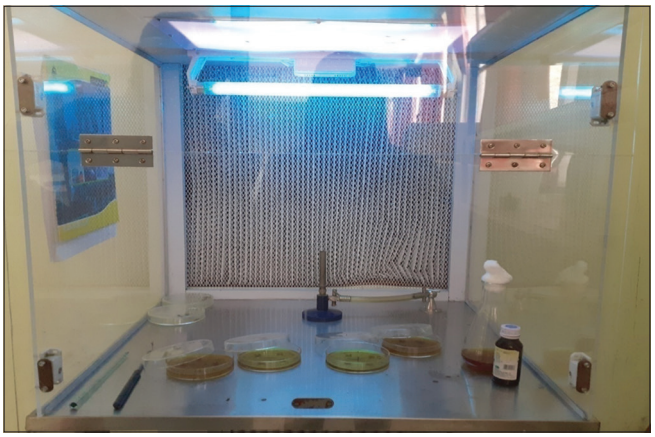

Figure 5: Aseptic transfer of media in Petri plates.

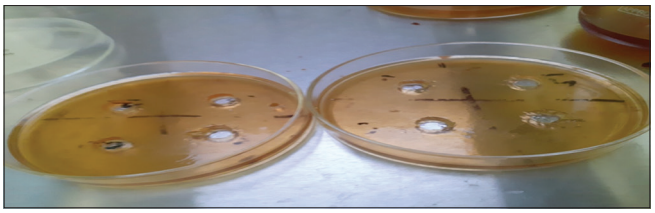

Figure 6: Preparation of wells in agar plates.

Fifty grams of dried powder were subjected to Soxhlet extraction using ethanol solvent to get the extract of curcumin for further investigation of antibacterial activity of turmeric against different Gram-positive and Gram-negative bacterial strains using cylindrical plate method or cup and plate method. A distinguished zone of inhibition 6.5 mm, 7.5 mm, and 11 mm in diameter was seen under plates containing different concentrations of *Curcuma longa* extract, namely, 1000 µg/ml, 2500 µg/ml, and 5000 µg/ml, respectively..

DISCUSSION

The results indicate that the *Curcuma longa* aqueous extract showed good antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* at low concentration.

The results of our study show that the *Curcuma longa* may have good antibacterial activity against various strains of Gram-positive and Gram-negative bacteria. Further, the investigation is required to study antibacterial activity of *Curcuma longa* in-depth for the eradication of bacteria, improvement of health and wellness of the society and nation.[26,31,32]

CONCLUSION

In this study the ethanolic extract of Curcuma longa rhizomes showed a promising inhibitory action against different

strains of microorganisms such as Staphylococcus aureus and Escherichia coli. Turmeric and its constituents may be included in modern system of medicine for the development of new dosage forms to treat several diseases with natural herbs with lesser adverse effects in comparison to allopathic system of medicine and improve the health and wellness of our society.

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Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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