TURMERIC: A PROMISING SPICE FOR PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITIES

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ABSTRACT:The present study stress on the medicinal properties of *Curcuma longa*, to find its effectiveness and efficiency against diverse pathogenic microorganisms and further contribute in enhancing phytology. The result of the present study revealed that bothaqueous and methanolic extract showed antibacterial activity against the test organism by Agar well diffusion method. Both the bacterial strains were sensitive to the turmeric extracts, although, it was observed that *S.aureus*showed more inhibition against methanolic extract as compared to the aqueous extract whereas, *E.coli* showed similar zone of inhibition against both the extracts. Phytochemical screening of the crude turmeric extract revealed the presence of carbohydrates, proteins, steroids, alkaloids and flavonoids. These bioactive principles are believed to be responsible for the antibacterial property of turmeric and therefore it could be used for therapeutic purposes along with other medicinal plants.

IndexTerms - Curcuma longa, Antimicrobial activity, Phytochemical properties

I. INTRODUCTION

Turmeric is one of most essential spices all over the world with a long and distinguished human use particularly in the Eastern civilization.[1] In India, *Curcuma longa* has been in use as a culinary ingredient since 3000 BC. It is used as a food coloring for curry and as a preservative for food. As a medicine it is used to treat a wide variety of ailments including stomach ache, skin problems, muscular problems and arthritis.

Turmeric (*Curcuma longa* L.) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases. Turmeric rhizome 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[2]. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury. In recent times, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Various sesquiterpenes and curcuminoids have been isolated from the rhizome of *C. longa*, attributing a wide array of biological activities such as antioxidant, anti-inflammatory, wound healing, anticancer and antibacterial activity [3]. Antifungal, antibacterial and anti-inflammatory activity has been reported for species such as *C.longa, C.zedoria, C.aromatica and C.amada.* Curcuma species, particularly turmeric (*C.longa*) exhibit a broad antibiotic activity against both Gram-positive and Gram-negative bacteria.

A bioactive compound in turmeric that has antibacterial activity is curcumin, Turmeric is a prompt source of bioactive compounds like antioxidants, polyphenols and flavonoids, which may be the substitute of antibiotics used in food and food products. Turmerone, zingiberene, ar-turmerone and curlone are included in volatile constituents of turmeric while the nonvolatile constituents are curcuminoids.[1]

II. REVIEW OF LITERATURE

Turmeric (*Curcuma longa*) is a perennial herb which belongs to family Zingiberaceae. It is cultivated in tropical and subtropical area of the earth mostly in India, Pakistan and China. It is normally famous as Haldi in Pakistan and India. From prehistoric time it is mostly used as a house hold therapy to manage several physiological ailments. The particular aroma of turmeric rhizome is due to the aromatic volatile oil like turmerone (25%), curdione (11.58%) and ar-turmerone (8.5%) whereas phenolic compound like curcumin impart specific yellow color to the rhizomes of *Curcuma longa.* Numerous plant based antibiotics and bioactive compounds have been known in herbaceous tropical plants by the scientists that play an important role in physiological and biochemical metabolic reactions of animal and human body[4].

A study published in July 2013 in the journal "Gut" found that turmeric protects liver cells from hepatitis C, a virus that causes liver disease. Using human liver cells, researchers incubated curcumin-the active component of turmeric with hepatitis C to examine its antiviral activity. They found that turmeric effectively prevented the hepatitis C virus from entering the human liver cells. Turmeric curcumin exerts antimicrobial activity against Vibrio vulnificus infection, according to the results of a study published in December 2011 in the journal "FEMS Immunology and Medical Microbiology." Vibrio vulnificus belongs to the same family of bacteria that cause cholera. Being exposed to contaminated seafood or seawater can cause vomiting, diarrhea and abdominal pain. [5]

Turmeric contains fat (5.1%), protein (6.3%), carbohydrates (69.4%), minerals (3.5%) and moisture (13.1%). Essential oil obtained through steam distillation of turmeric rhizomes possesses sabinene (0.6%), borneol (0.5%), *a*-phellandrene (1%), cineol (1%), sesquiterpines (53%), zingiberene (25%) and curcumin (diferuloylmethane) (3–4%). Turmeric comprises volatile as well as nonvolatile compounds. Volatile compounds are turmerone, zingiberene, curlone and arturmerone. The nonvolatile components include the curcuminoids.[6]

The mechanism behind the antimicrobial action of different spices include the hydrogen bonding and hydrophobic interaction of various phenolic compounds to the membrane proteins, which cause cell membrane disturbance, disruption of cell wall and damage of electron transport chain. The antibacterial potential of aqueous extracts is possibly due to the anionic constituents like nitrate, chlorides, sulphates and thiocyanate in addition to several other compounds that are present naturally in plants. The methanolic extracts exhibited better effects as compared to the aqueous extracts as organic solvent dissolves organic compounds quickly resulting in release of larger amount of vigorous antimicrobial constituents. The thick structural components of gram-positive bacteria in this case can be accountable for the more interaction between curcumin, active components and the structural lipoproteins. The increased collaboration may outcomes in the inhibition of the gram-positive bacteria[7].

A change in the cell membrane permeability is the main factor in antimicrobial action of a particular compound. Phenolic compounds may completely disrupt the cellular membranes, affect the cellular integrity and cause ultimate cell death·

III.RESEARCH METHODOLOGY

Collection of Plant part

Turmeric (*Curcuma longa*) tubers were collected from the local market. These tubers were skinned, cut into small pieces and washed thoroughly. 50 g of the finely chopped turmeric were weighed and kept for drying in an incubator. The dried turmeric was then smashed in a surface sterilized mortar and pestle and the powder was used to make the aqueous and methanolic extract.

Preparation of aqueous and methanolic extracts

Organic Solvent Extraction

Air-dried powder of Turmeric sample (50 g) was thoroughly mixed with 50 ml organic solvent (viz. methanol). The mixture was placed at room temperature for 72 h on shaker with 150 rpm. Solution was filtered through muslin cloth and then re-filtered by passing through Whatman Filter No. 1. The filtrate thus obtained was concentrated by complete evaporation of solvent in oven at 50'C to yield the pure extract. Stock solutions of crude extracts were prepared by mixing well the appropriate amount of dried extracts (400mg/4ml) with respective solvent to obtain a final concentration of 100 mg/ml. Different concentration were prepared from the stock solutions and each solution was stored at 4°C after collecting in sterilized bottles until further use. (Table 2)

Aqueous extract preparation

The method to prepare the aqueous is the same like methanolic except the diluent used which is distilled water rather than methanol in this case. The concentrations prepared were 5%, 10%, 20%, 30%, and 40% from the stock solution (100mg/ml). (Table 2)

Table 2- Dilution table for preparation of aqueous and methanolic extract of Turmeric.

Concentration (%)	Volume of distilled water for aqueous extract/ volume of methanol for methanolic extract (ml)	Volume of sample (ml)	Total volume (ml)
5	4.75	0.25	5
10	4.5	0.5	5
20	4.0	1.0	5
30	3.5	1.5	5
40	3.0	2.0	5

Test microorganism

The bacterial strains used as test organisms are *Escherichia coli* (Gram negative, coccobacilli, facultative anaerobe) and *Streptococcus aureus* (Gram positive, cocci).

Antimicrobial analysis

Antibacterial activities of methanolic and aqueous extract of *Curcuma longa* were identified using the standard Agar Well Diffusion assay. Petri dishes (100mm) containing 20ml of Mueller-Hinton Agar (MHA) seeded with 0.2ml test organism were prepared (inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 CFU/ml). Media was allowed to solidify and the petri plates were labelled according to the bacterial strain used as inoculum. After solidification of the media,

wells of about 10mm diameter were made using a sterile cork borer. 50 µl of different concentration of methanolic and aqueous extracts were poured into labelled wells respectively and the plates were refrigerated for pre-diffusion at $4°C$ for about half an hour followed by incubation at $37°C$ for 18-24 hours. Distilled water and organic solvent were poured into the well as negative control while Ampoxin antibiotic (10µg/ml) was used as positive control. The experiment was performed under aseptic conditions and the diameter zone of inhibition was measured in terms of millimeters.

Preparation of crude extract for phytochemical tests

The turmeric tuber was skinned, cut into small pieces and washed properly. The chopped turmeric was then smashed in a surface sterilized mortar and pestle. The smashed paste was then squeezed into a beaker using a wet muslin cloth. The freshly prepared crude was then used for phytochemical tests.

Phytochemical analysis.

The test sample was subjected to phytochemical analysis in order to find out the presence of phytochemical constituents. The phytochemical tests employed for carbohydrates, proteins, cardiac glycosides, steroids, alkaloids, flavonoids, saponins, anthraquinone, tannins and terpenoids

A. Test for carbohydrates- Benedict's test

Equal volumes of Benedict's reagent and test solution were mixed in attest tube. The mixture was heated in boiling water bath for 5 minutes. Solution appeared green showing the presence of reducing sugar.

B. Tests for Proteins: Xanthoproteic test:

To 1ml of extract, 1ml of conc.H_2SO_4 was added. This resulted in the formation of white precipitate which on boiling turned yellow. On addition of NH_4OH, yellow ppt. turned orange.

C. Test for glycosides: Borntrager's Test:

To the 3ml of aqueous extract, dil.H_2SO_4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. This solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

D. Test for Steroids: Salkowski Test:

To 2ml of aqueous extract, 2ml of chloroform and 2ml of conc.H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

E. Tests for alkaloids- The aqueous extract was evaporated in a test tube. To the residue dilute HCl was added, shaken well and filtered.**Hager's Test**- To the 2-3ml of filtrate Hager's reagent was added. Yellow precipitation was formed showing the presence of alkaloids.

F. Tests for flavonoids- **With Sodium Hydroxide**- On addition of an increasing amount of sodium hydroxide, the aqueous extract showed yellow coloration, this decolorized after addition of acid

G. Test for Anthraquinones- 0.5 gm of the extract was boiled with 10ml of sulphuric acid and filtered while hot.The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for color changes.

H. Test for Tannins- For 2ml of extract add few drops of 1% lead acetate. A yellowish precipitate showed the presence of tannins.

I. Test for Terpenoids- 2m l of aqueous extract was added to 2ml of acetic anhydride and concentration of H_2SO_4.Formation of blue, green rings indicated the presence of terpenoids

IV.RESULT AND DISCUSSION

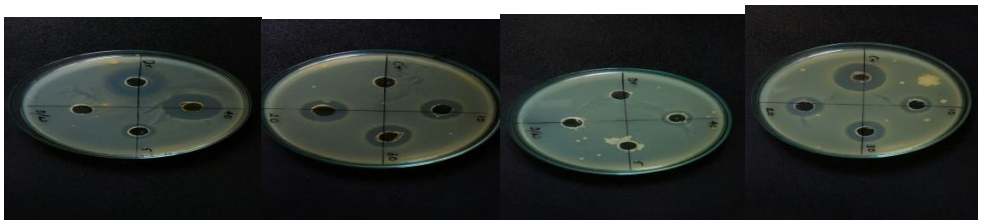
For antibacterial assay- The findings of the present study revealed that *Curcuma longa* contain potent antimicrobial property against tested organisms. The antimicrobial activity of the turmeric extracts (methnolic and aqueous) was initially evaluated by Agar Well Diffusion method using two strains of pathogenic bacteria *Escherichia Coli*, *Staphylococcus aureus*(Table 3). The presence of zone of inhibition revealed the antibacterial activity of turmeric.

Table 3- Measurement of zone of inhibition of the test organisms for aqueous and methanolic extract of Turmeric.

Concentration (mg/ml)	**Aqueous extract Zone of inhibition (mm)**		**Methanolic extract Zone of inhibition (mm)**	
	S.aureus	*E.coli*	*S.aureus*	*E.coli*
5	-	-	12	-
10	12	11	14	12
20	14	12	15	12
30	14	13	16	13
40	15	15	20	15
Crude extract (100mg/ml)	19	-	-	20
PC(Ampoxin)	-	-	20	-
NC(Distilled water)/(Methanol)	-	-	-	-

It was observed that the zone of inhibition of *S.aureus*was found to be more in methanolic extract as compared to the aqueous extract (Figure 1) while E.coli (Fig 2) showed almost similar zone of inhibition in both the extracts .

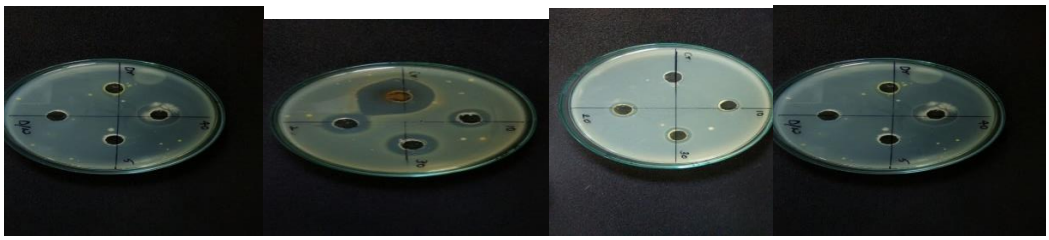
Figure 1- MH agar plate shows zone of inhibition of *S.aureus*in methanolic (A) and aqueous (B) extract of Turmeric.



A B

Figure 3- MH agar plate shows zone of inhibition of *S.aureus*in aqueous extract of Turmeric.

Figure 2- MH agar plate shows zone of inhibition of *E.coli*in methanolic and aqueous extract of Turmeric.



For phytochemical analysis-

A B

Phytochemical screening of the crude extract of *Curcuma longa* was performed and the presence of secondary metabolites was detected. According to the results, the tests for carbohydrates, proteins, steroids, alkaloids and flavonoids were positive while the tests for glycosides, anthraquinones, tannins and terpenoids were negative (Table 4). It is the believed that the presence of these secondary metabolites is responsible for the antibacterial property of turmeric.

Table 4- Phytochemical tests shows the following secondary metabolites in crude extract of Turmeric.

Phytochemical	**Result**
Carbohydrates	+
Proteins	+
Cardiac Glycosides	-
Steroids	+
Alkaloids	+
Flavonoids	+
Anthraquinones	-
Tannins	-
Terpenoids	-

Control experiments using methanol and distilled water (negative control) showed no inhibition by any bacteria indicating that the turmeric is solely responsible for inhibiting the growth of the bacteria and not organic solvent and distilled water. Ampoxin, the drug, used as a positive control showed zone of inhibition ranging from 18-20mm diameter. (Table 3).

Our results were found in agreement with some earlier studies in which the methanol extract of *C. longa* showed antimicrobial activity against different bacterial strains, namely,*S.intermedius*, *S.aureus* and *S.epidermidi*[0]. In comparison with this, considered bacterial strain (wiz.*S.aureus*) showed inhibitory effect in the present study as well. Turmeric oil a byproduct of curcumin was also found active against *S.aureus*, and *E.coli*.

In a study, dried rhizome of turmeric and Oleo resins of *Boswelliaserrata*were evaluated for their antimicrobial activities against Gram negative (*Escherichia coli* and *Salmonella typhi*) and Gram positive (*Staphylococcusaureus*, *Bacillus subtilis*) microorganisms. The zones of inhibition for *Curcuma longa* were 13±0.16mm for *S.typhi*, 12±0.22 mm for *E.coli*and 11±0.24

mm for *S. aureus*[22]. This is an alternative method to identify the antibacterial activity of turmeric. Although, some studies were carried out to evaluate the antimicrobial activity and chemical analysis of essential oil and olioresins of this plant against various oral and food-borne bacterial and fungal pathogens; however, the present study was focused mainly to investigate the antibacterial potential of crude extracts of rhizome of *Curcuma longa* in terms of zone of inhibition against pathogenic bacteria. [8]

Methanol extract was interpreted as most significant inhibitory against bacterial species evaluated, thus screened phytochemical analysis revealed the presence of terpenoids, flavanoids, saponins, gylcosides, anthraquinones, tannins, steroids, phenols and cardiac glycoside were quantitatively determined by adopting the procedure described by Harborne[23]. Whilst, the phytochemical analysis of the present study showed the presence of steroids, alkaloids and flavonoids.[9]

CONCLUSION:

It was concluded that *Curcuma longa* is highly considered as a universal panacea in herbal medicine with varied pharmacological and antimicrobial activities. The overall assessment concludes that turmeric possesses strong antibacterial, anticancer, antiseptic, anti-oxidative, anti-inflammatory, anti-tumor, antiviral properties. It is expected that *Curcuma longa* may find use as a novel herbal drug in the upcoming future to combat several diseases, including carcinogenesis, inflammatory disorders and oxidative stress-induced pathogenesis. Additional evaluations need to be done on *Curcuma longa* in order to explore to its other countless medicinal uses.The*Curcuma longa* (Turmeric) have been screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health.

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