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Phytochemical analysis and antimicrobial activity of *Curcuma longa* (rhizome extract) against human pathogens

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ABSTRACT

Curcuma longa (Turmeric) is the rhizomatous perennial spice plant which is used as antioxidant and traditional medicine. In the present study qualitative photochemical analysis of Curcuma longa rhizome extract was performed for detection of carbohydrates, amino acids, starch, glycosides, steroids, tannins, saponins and phenols. Also the antimicrobial activity was tested as per the zone of inhibition formed by rhizome extract against human pathogens like E. Coli, Basillus subtilis, Staphylococcus aureus, Salmonella typhimurium, Micrococcus lateus and Proteus valgaris at 70mg/ml concentration. The Phytochemical analysis revealed the presence of steroids, amino acids, glycosides, tannins and saponin in the given methanolic extract and the extract showed the antimicrobial activity against all the pathogens studied like E. Coli (25nm), Basillus subtilis (24nm), Staphylococcus aureus (23nm) , Salmonella typhimurium (23nm), Micrococcus lateus(26nm) and Proteus valgaris (22nm). Besides this the antioxidant activity of dried rhizome extract of spice Curcuma longa was studied by DPPH assay method at various concentration of extract. In antioxidant assay concentration of 1mg/ml shows the higher scavenging percentage i.e 42.39% while concentration of 0.4 mg/ml shows lower scavenging percentage i.e 3.91%.

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INTRODUCTION

The fad of ready mix food is getting increased day by day. The popularity of ready to meal food is also getting increased as it requires less time for preparation. No. of ingredients are being added in these products for the improvement of its self life and other beneficial properties. The most of the meal products will get contaminated with food borne pathogens during the preparation. The contaminants will be *Salmonella Enteritidis, Staphylococcus aureus, Campylobacter jejuni, Listeria monocytogenes.* The resistance of antibiotics for these pathogens is a serious concern now a days (Perreten *et al.* 1998; Stermitz *et al.* 2000). Use of natural antibacterial com- pound such as extracts of spices and herbs etc., for food preservation is getting immense interest among researchers (Smid and Gorris 1999; Estevez *et al.* 2007; Pezeshk *et al.* 2011). *Curcuma longa* rhizome has been traditionally used as antimicrobial agent as well as an insect repellant (Rudrappa and Bais, 2008). Member of *Zingiberaceae* family are found to be a rich source of substances of phytochemical interest. They are rich in curcuminoids, and recognized for their broad spectrum of biological activities, curcuminoids vary in chemicalstructures, physico-chemical characteristics as well as the functional properties (Revathy *et al.*, 2011). Several studies have reported the broad-spectrum antimicrobial activity for curcumin including antibacterial, antiviral, antifungal, and antimalarial activities. Because of the extended antimicrobial activity of curcumin and safety property even at high doses (12 g/day) assessed by clinical trials in human, it was used as a structural sample to design the new antimicrobial agents with modified and increased antimicrobial activities through the synthesis of various derivatives related to curcumin (LaColla *et al.*, 1998 ; Anand *et al.*, 2007). Rhizome extract containing curcumin has the highest antimicrobial activity. This work supports development of drug from *Curcuma longa* (turmeric) which is helpful for pharmaceutical industry.

MATERIALS AND METHODS

Preparation of rhizome extract

Turmeric rhizomes were obtained from local market of shahada region of nandurbar district. The collected rhizomes were shade dried. The dried plant materials were finely powdered by tissue homogenizer (Infinigen™ Tissue Mixer Mill). Approximately 30gm of powder was extracted with methanol by Soxhlation and solvent is recovered by distillation. The extract was concentrated under reduced pressure and air dried. All the solvents and chemicals are of AR/HPLC grade and obtained from HIMEDIA. The reference curcumin was obtained from MODERN SCIENCE.

Phytochemical analysis of *Curcuma longa* rhizome extract

The individual extract was subjected to the qualitative phytochemical screening for the presence of phytochemicals. The test of starch, amino acids, and phenols was carried out adopting standard procedure given by Saxena *et. al.*, 2012. Carbohydrates were determined by molisch's test. Steroids were tested by salkowski test. The glycosides were detected by keller- kiliani test. Tannin detection was by ferric chloride test and bromine water test, while saponins were determined with the froth tests and haemolytic test.

Antimicrobial screening test

The bacterial strains used were *E. Coli*, *Basillus subtilis*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Micrococcus lateus* and *Proteus valgaris.* The strains were obtained from Mahatma Phule Krishi Vidhyapeeth, Rahuri. The agar well diffusion method was adopted to assess the antimicrobial activity of the selected plant extract. Mueller Hinton media was distributed in Petri plates. The Petri plates were labeled and the media was allowed to set. After setting 30µl of each of the bacterial suspension was spread on the surface of the agar in the labeled Petri dishes. Using a sterile tip three wells made at media surface. Then 70µl of extract was added to first well and antibiotic (+ve control) and methanol (-ve control) in second and third well. The plates were then incubated at 37 ^{0}C for 18 to 24 hours. After the incubation the diameter of the growth inhibition zone were measured (Rambir *et al.*, 2002).

Antioxidant assay

Antioxidant compounds may scavenge reactive oxygen species (ROS) and peroxide radicals, thereby preventing or treating certain pathogenic conditions. DPPH is a well known radical to monitor chemical reactions involving radicals and recently it is most widely used for antioxidant assay (Bondet *et al*., 1997). The extracts of rhizomes were tested for the scavenging effect on DPPH radical according to the method of Rachana *et al*. In this method, 0.2 mL of extract solution in methanol (95%) at different concentrations (0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 mg mL) was added to 8 mL of 0.004% (w/v) stock solution of DPPH in ethanol (95%). The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a Biospectrometer. All determinations were performed in triplicate. The DPPH radical scavenging activity (S%) was calculated using the following equation

Formula for calculation of Scavenging percent (S%)

$S\% = ((A_{control} - A_{sample})/A_{control}) \times 100$

RESULT AND DISCUSSION

Curcuma is gaining importance worldwide as a potential source of new drugs to combat a variety of ailments as the species contains molecules credited with anti-inflammatory, hypocholestremic, choleratic, antimicrobial, insect repellent, antirheumatic, antifibrotic, antivenomous, antiviral, antidiabetic, antihepatotoxic as well as anticancerous properties (Sukari *et al.*, 2010). For the extraction of curcumin rhizomes were collected, dried and fine powder was prepared. Extraction was done by using methanol as solvent with the help of soxhlet apparatus. After the extraction the dry extract weight and recovery percentage was measured which was about 4.10gm and the yield was 13.66 % . Lawand and Gandhi (2013) reported that the plant extract could be obtained by soxhlet extraction method by using methanol as solvent thus in our study we have also used soxhlet extraction method. Different solvents like chloroform, ethyl acetate, methanol, acetone can be used for extraction of curcuminoids from turmeric rhizomes but methanol has been proven to give high yield of curcuminoids (Kulkarni *et al.* 2012) so methanol was a solvent used for extraction. Phytochemical screening of the rhizome extract confirmed the presence of steroids, saponins, tannins, glycosides and amino acids. Among these compounds, tannins and flavonoids are the substances which can give the colour. Tannins are the most important ingredients which are necessary for dyeing. Antibacterial activity of rhizome extract of *Curcuma longa* and the separated components of the plant studied revealed the presence of bioactive properties. Results of the agar well diffusion method of *Curcuma longa* are summarized in graph no 1. The antimicrobial results showed that the methanolic extract of plants have more potential to inhibit test pathogenic microbes. The most pronounced activity with inhibition zones of more than 25.0 mm was shown by rhizome extract (inhibition zone 26 mm against *Micrococcus luteus*) of *C. longa*. In addition, the rhizome extract of *C. longa* had showed significant activity against *E Coli, B. subtilis , S. aureus, Salmonella typhi and proteus vulgaricus*

with inhibition zones 24.0, 23.0, 23.0 and 22.0 mm respectively (Graph no.1). Yasodamma *et al*., (2013) reported antibacterial activity of rhizome and leaf extracts of the *C*. *neilgherrensis*, *C. longa*, *C*. *zedoaria*, *C. malabarica*, *C. xanthorrhiza* on mouth and dental pathogens *S. aureus* and other human pathogens like *Klebsiella, Vibrio, Bacillus, Streptococcus, Azatobacter, Enterobacter, Pseudomonas and E. coli.* In antioxidant assay concentration of 1mg/ml shows the higher scavenging percentage i.e 42.39% while concentration of 0.4 mg/ml shows lower scavenging percentage i.e 3.91% (Graph no. 2).

CONCLUSION

Phytochemical screening provides knowledge of the chemical constituents of plants not only for the discovery of new therapeutic agents, but also for information in discovering new sources of other economic materials. Phytochemical analysis reveals the presence of major phytochemicals like glycosides, tannin, saponin etc. The rhizome extract exhibited *in vitro* antibacterial activity against all the microbs used for study. Some of these bacteria's are associated with food borne infection. Phytochemical analysis and antibacterial studies of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies in the manufacturing of the new drugs for treatment of various diseases.

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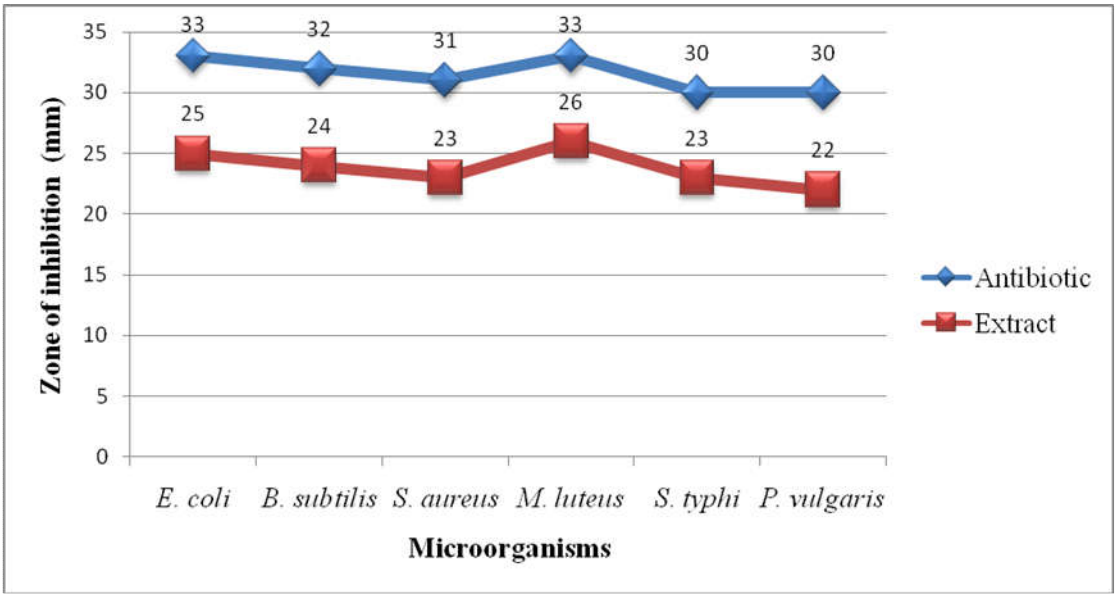
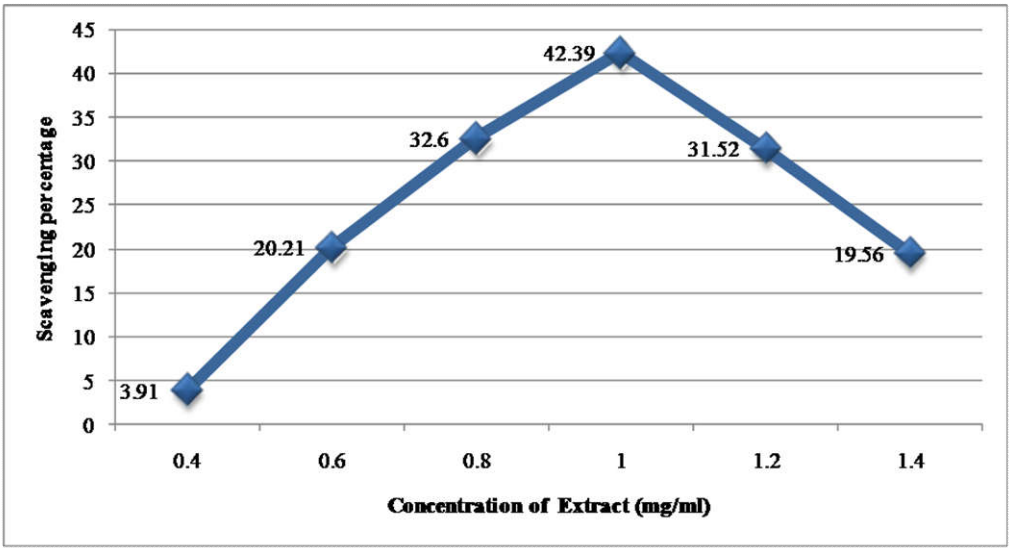


Graph No 1. Graph showing antimicrobial activity of Extract and Antibiotic

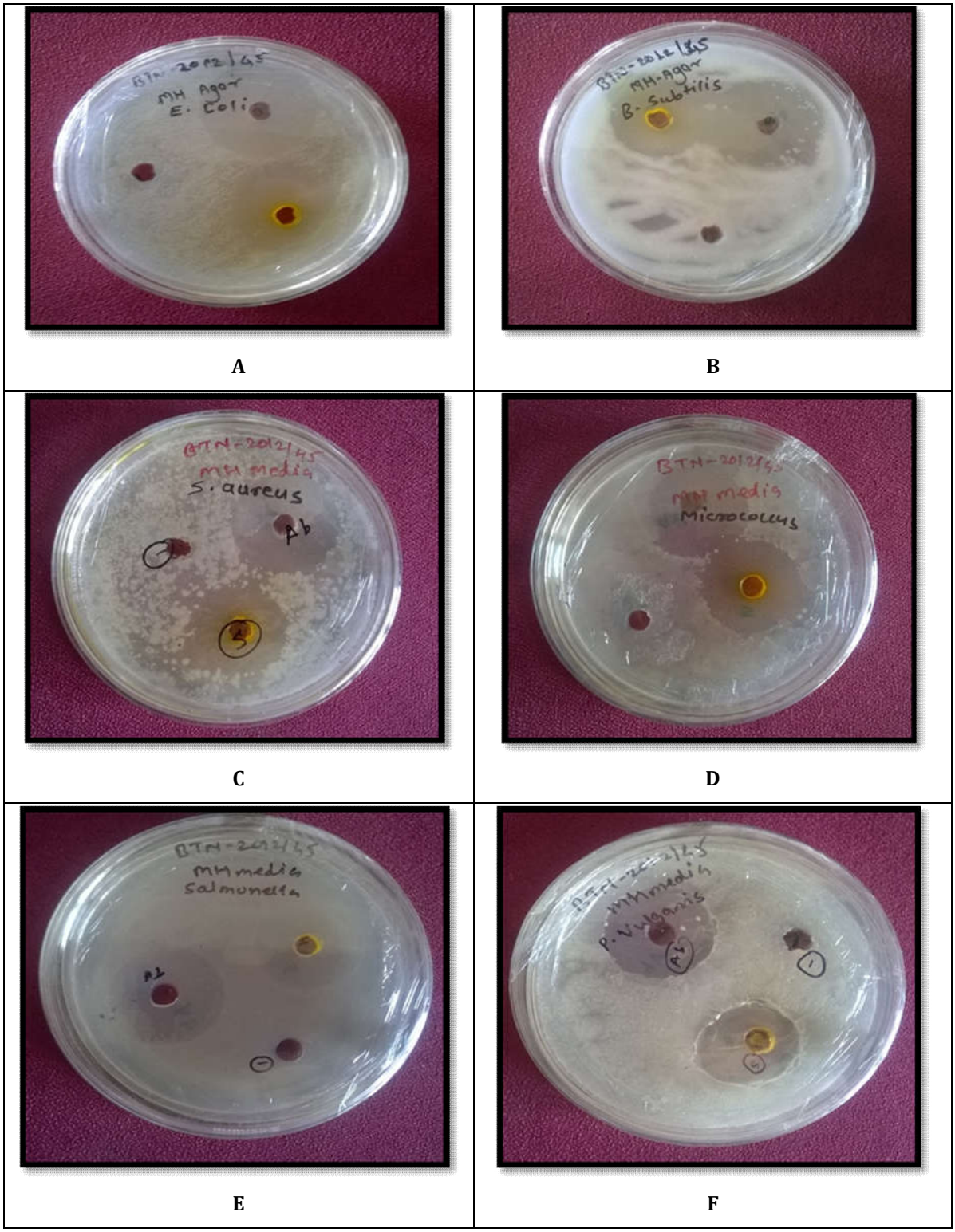


Graph No 2. DPPH scavenging activity (Scavenging percentage against concentration)

Table No 1. Phytochemical analysis of *Curcuma longa rhizome extract*

Sr. No.	Phytochemicals	Result
1	Carbohydrates	-
2	Starch	-
3	Amino acid	+
4	Steroids	+
5	Glycosides	+
6	Tannin	+
7	Saponin	+
8	Phenols	-

Note: + sign indicates the positive test for the compound and – sign indicates the negative test for the compound



A **B**

C **D**

E **F**

Fig. 1. Antibacterial activity of *C. longa* extract against A) *E. Coli* B) *B. substilis* C) *S. aureus* D) *M. luteus* E) *S. typhimurium* F) *P. vulgaris*

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