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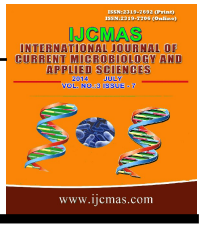

Original Research Article

Studies on the comparison of phytochemical constituents and antimicrobial activity of *Curcuma longa* varieties

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A B S T R A C T

Turmeric comes from the root of *curcuma longa* plant and has a tough brown skin and a deep orange flesh. Turmeric has long been used as a powerful anti-inflammatory in both the Chinese and Indian systems of medicine. Turmeric was traditionally Called "Indian saffron" because of its deep yellow-orange color and has been used throughout history and condiment, healing remedy and textile dye. Turmeric is rich in curcuminoids. Curcuminoids vary in chemical structures, physiochemical characteristics. The present work reports on extraction method using Soxhlet extractor, characterization and separation of phytochemicals using TLC and FT-IR methodology. The present study aimed at comparing the *in vitro* antimicrobial activity of two varieties (cosmetics and food) of turmeric and to screen in bacterial (*E.coli, Staphylococcus aureus*) and fungal (*Aspergillus niger, Aspergillus fumigatus*) species.

K e y w o r d s

Curcumin, Agar well diffusion, Antimicrobial activity, *Curcuma longa*, Minimum inhibitory concentration

Introduction

Medicinal Plant:

India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value (Gupta *et al.*, 2005; Sandhu *et al.*, 2005). Human beings have used plants for the treatment of diverse ailments for thousands years (Sofowara, 1982; Hill *et al.*, 1989).

According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rabe and Van Stoden, 2000), since they cannot afford the products of Western pharmaceutical industries (Salie *et al.*, 1996), together with their side effects and lack of healthcare facilities (Griggs *et al.*, 2001).

Rural areas of many developing countries still rely on traditional medicine for their

primary health care needs and have found a place in day-to-day life.

***Curcuma longa*:**

Turmeric is the rhizome or underground stem of ginger like plant. The plant is an herbaceous perrineal, 60–90 cm high with a short stem tufted leaf. Its flowers are yellow, between 10–15 cm in length and they group together in dense spikes, which appear from the end of spring until the middle session.

No fruits are known for this plant. The whole turmeric rhizome, with a rough, segmented skin. The rhizome is yellowish-brown with a dull orange interior that looks bright yellow when powdered.

Rhizome measures 2.5–7.0 cm (in length), and 2.5 cm (in diameter) with small tuber branching off. Turmeric held a place of honor in Indian traditional ayurvedic medicine (Fig.1).

Molecular constituents in turmeric:

Turmeric has hundreds of molecular constituents, each with a variety of biological activities. For instance, there are at least 20 molecules that are antibiotic, 14 are known cancer preventives, 12 that are anti-tumor, 12 are anti-inflammatory and there are at least 10 different anti-oxidants. Infect, 326 biological activities of turmeric are known. This is also testimony to the use of whole herbs and not just isolated molecules.

Speaking of molecules by far the most researcher in turmeric are the three gold-colored alkaloids curcuminoids viz. Curcumin, Demethoxycuccumin and Bisdemethoxycurcumin. Most of the research done is with 95% curcuminoids extract of turmeric, through in its raw state turmeric is only 3–5% curcuminoids. The yield of essential oil in various parts is 1.3% in leaf, 0.3% in flower, 4.3% in root and 3.8% in rhizome.

Minimum inhibitory concentration:

In microbiology, **Minimum Inhibitory Concentration (MIC)** is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism.

Antimicrobial activity:

An **antimicrobial** or **antibiotic** is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterial are used against bacteria and antifungal are used against fungi. They can also be classed according to their function. Antimicrobials that kill microbes are called *microbicidal*; those that merely inhibit their growth are called *micro biostatic*.

Nowadays, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial compounds (Hancock *et al.*, 2012).

The necessity to develop new drugs requires varied strategies, among them, the bio prospection of secondary metabolites produced by medicinal plants (Dionisi *et al.*, 2012; Benko-Iseppon *et al.*, 2010).

Materials and Methods

Collection & extraction:

The two varieties (cosmetic used turmeric and food used turmeric) plant material of *Curcuma longa* or turmeric rhizomes is used in this study was collected from provision market.

Fresh rhizomes were cleaned washed with deionized water. Sliced and dried in the sun/shade for one week and again dried at 50°C in a hot air oven for 6 hours. Dried rhizomes were cut in small pieces, and ground it into a powder.

Approximately 10g of sample were taken into a thimble and placed in a Soxhlet Apparatus. 150ml of solvent was added and extracted according to their boiling point for 6 hours. The solvents used were aqueous (Boiling Point – 70 to 90°C) and ethanol (Boiling Point – 40 to 50°C) for the extraction of plant material. After completion of extraction the dark brown extract was stored in refrigerated condition until use.

Antimicrobial activity:

Method performed by a sterile cotton swab was dipped into the respective microbial suspension and surplus removed by rotation of the swab against the sides of the tube above the fluid level. The agar media plates were inoculated with the respective organisms by even streaking of the swab over the entire surface of the plate three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculums. Finally, it was swabbed all around the edge of the agar surface. Wells of 7 mm size were made with sterile borer into agar plates containing the bacterial and fungal inoculums. 0.1 ml/100µl volume of each of the plant extract prepared in four concentrations was dispensed into the wells of inoculated plates.

Sterilized distilled water and ethanol were used as a control which were introduced into the well instead of plant extract. The plates thus prepared were refrigerated for 60 minutes allowing the diffusion of the extract into the agar. After incubation for 24 hr at 37°C, the plates were observed.

If antimicrobial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the extract. The zone of inhibition was measured and expressed in millimeters (mm).

Separation by TLC:

Slurry of silica gel is prepared by dissolving 20 g of gel and 50 mg of calcium sulphate in about 50 ml of distilled water. This is coated uniformly over a glass plate and the plate is actively by heating at 100°C. Standards and unknown solution are spotted at the base of the plate. Then the plate is then developed in a chamber saturated with the solvent. The solvent is marked and the plate is dried in air. The lipids are located by keeping the plate in an iodine chamber and keeping the plate in a hot air oven at 100°C for 15 minutes. The sample is the unknown solutions are identified by comparing their Rf value with those of the standard.

Characterization by FT-IR:

Accurately weighed quantity of plant extract about 10 g was dissolved in ethanol by using volumetric flask. To this 1.5ml of each sample (Cosmetic used turmeric and Food used turmeric) are measured by transmittance of composition present in clove and it performed under Fourier Transform Infrared Spectroscopy. Instrument name is CPU – 32 with KBr spectrum.

Different wavelength shows presence of particular functional group of compounds. From above data one can confirm the presence of eugenol in clove by phytochemical analysis and chemical test which is further reconfirmed by maximum wavelength, Refractive Index (Rf) value and FT-IR.

The identification for the chemical components was confirmed by comparison of wavelength and authentic standards.

Results and Discussion

Maximum zone of inhibition in antimicrobial activity

Among all the zone of inhibition for extracts of plant part, Food used turmeric ethanol extract found more effective against bacterial and also fungal microbes. Other extracts were also found effective against microbes except *Staphylococcus aureus* due to its inhibition zone formation (Table. 1 and Fig. 2).

TLC method using two different mobile phases:

Different compositions of mobile phase were tested in TLC (Thin Layer Chromatography) for the separation and its Rf values were determined. Chloroform: Methanol in 95:5 ratios is a Mobile Phase for the First method to determination of TLC in Cosmetic used and Food used *Curcuma longa* varieties. As a result of Rf values for Chloroform: Methanol solvent is about 0.2 and 0.6 for cosmetic used extract such as aqueous and ethanol respectively.

And also 0.5 and 0.6 for food used extracts such as aqueous and ethanol respectively.

Petroleum ether: Ethyl ether: Glacial acetic acid in 90:10:1 ratio is a Mobile Phase for the Second method to determination of TLC in Cosmetic used and Food used *Curcuma longa* varieties.

As a result of Rf values for Petroleum ether: Ethyl ether: Glacial acetic acid solvent is about 0.3 and 0.7 for cosmetic used extract such as aqueous and ethanol respectively.

And also 0.2 and 0.5 for food used extracts such as aqueous and ethanol respectively. The results were shown in (Table.2 and Fig. 3 & 4).

Better resolution of Rf value showed that Petroleum ether, Ethyl ether and glacial acetic acid can be suitable solvent for the separation using TLC method in this study.

Using FT-IR to characterized two different varieties of turmeric:

In Fourier Transform-Infrared Spectroscopy Analysis the Cosmetic and Food used ethanolic extract of *Curcuma longa* analyzed trough FT-IR Spectrophotometer where it showed 10 wavelengths in cm-1with their respective transmittance at %T in Cosmetic used turmeric extracted (ethanol extract) sample and also 12 wavelengths in cm-1 their respective transmittance at %T in Food used turmeric extracted (ethanol extract) sample.

Graph showed many peaks for different functional groups present in plant extracts (Fig. 5 and 6).

Fourier Transform-Infrared Spectroscopy analysis showed different functional group mainly hydroxyl group at particular wavelength in graph. Modern instrumental technique play an indispensable role in the structural determination of organizes compounds. For example: FT-IR Spectroscopy is an invaluable tool for determining the presence or absence of

certain functional group or hydroxyl group such as carbon-carbon multiple bonds, aromatic rings, carbonyl group or hydroxyl group in a molecule (Berger and Sicker, 2009).

Previous estimation of *Curcuma longa* done by FT-IR spectrum of eugenol showed the presence of alcoholic group (Reddy *et al.*, 2009).

Table.1 Maximum Zone of inhibition of all Microbes

NAME OF MICROBES	ZONE OF INHIBITION (mm)			
	COSMETIC USED TURMERIC		FOOD USED TURMERIC	
	AQUEOUS	ETHANOL	AQUEOUS	ETHANOL
E.coli	7	7	3	7
Staphylococcus aureus	5	6	-	6
Aspergillus niger	6	12	8	16
Aspergillus fumigatus	12	19	12	15
Antibiotic	4	9	12	18
Control	-	-	-	-

Table.2 TLC Method in two different mobile phases

TLC Mobile Phase	RATIO	Rf VALUES			
		COSMITIC USED EXTRACT		FOOD USED EXTRACT	
		AQUEOUS	ETHANOL	AQUEOUS	ETHANOL
Chloroform: Methanol	95:5	0.2	0.6	0.5	0.6
Petroleum ether: Ethyl ether : Glacial acetic acid	90:10:1	0.3	0.7	0.2	0.5

Fig. 1 Medicinal properties of Curcumin

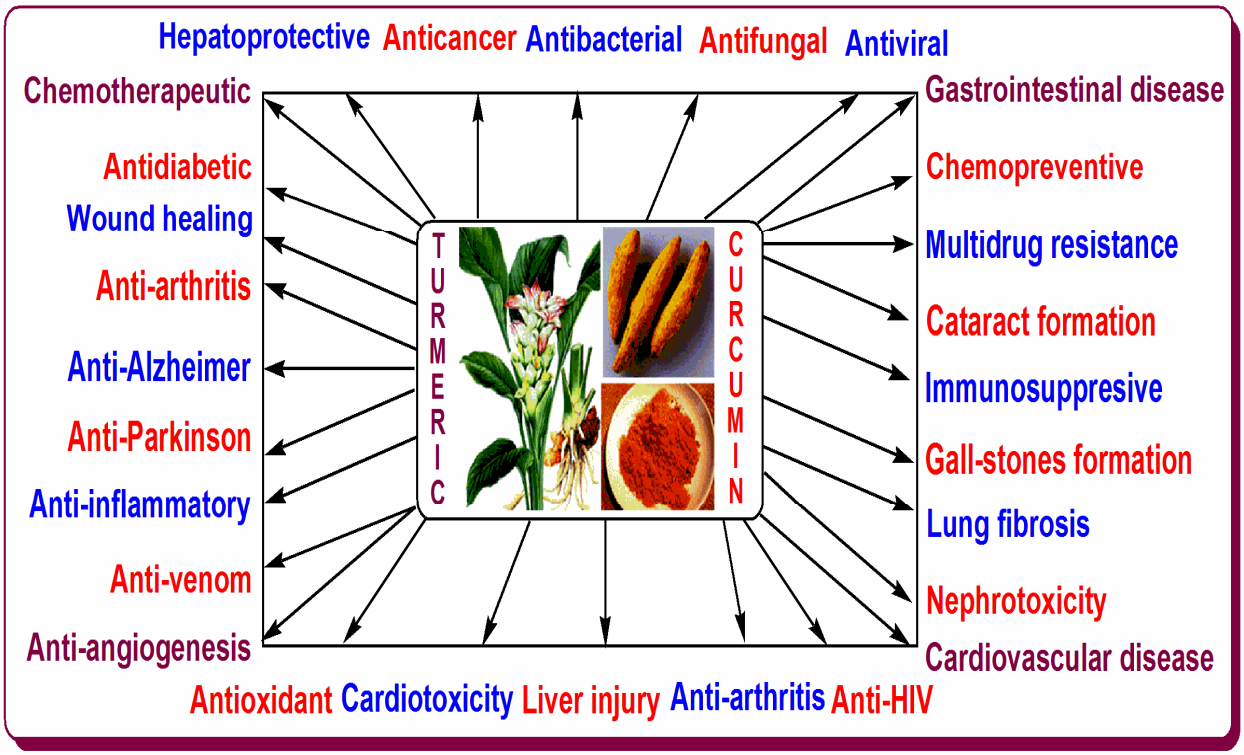


Fig. 2 Maximum Zone of Inhibition

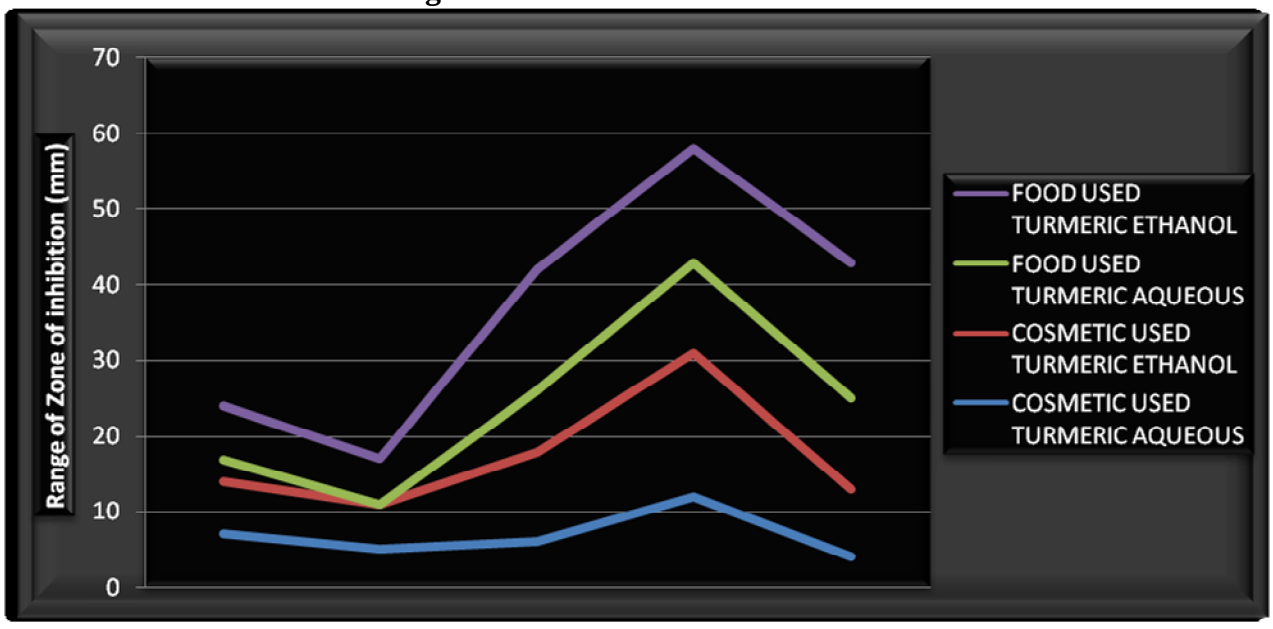


Fig. 3 Mobile Phase – Chloroform: Methanol in 95:5 Ratios

Fig. 4 Mobile Phase – Petroleum ether: Ethyl ether: Glacial acetic acid in 90:10:1 Ratio

Fig. 5 Characterization of compounds in cosmetic used turmeric extract

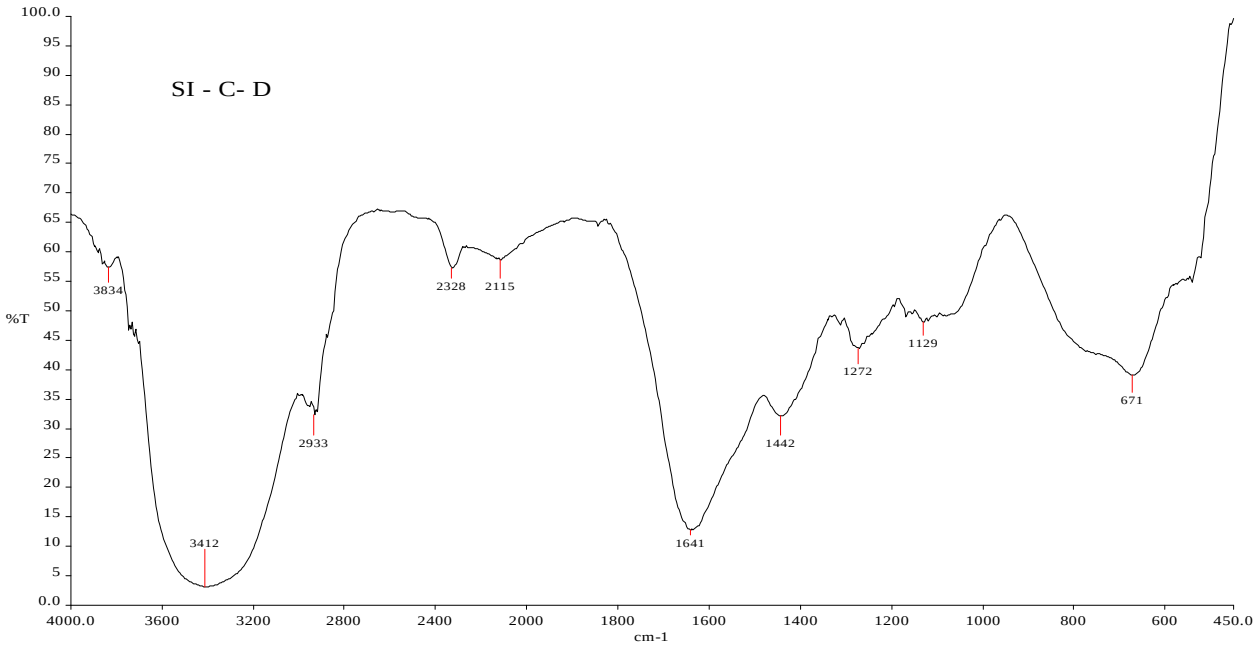
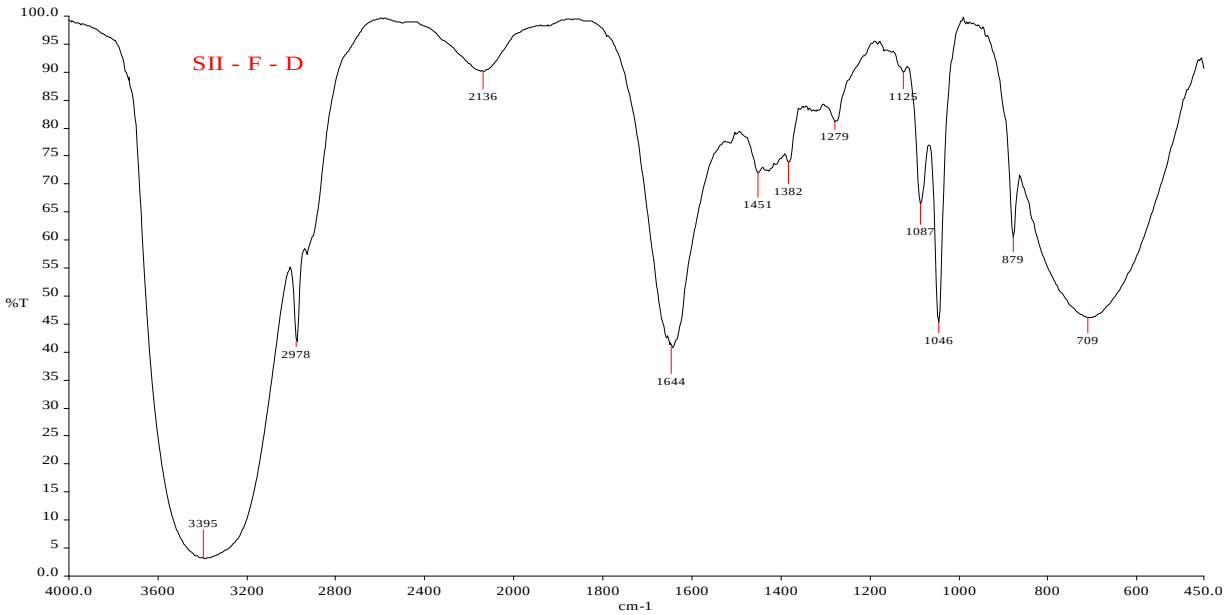


Fig. 6 Characterization of compounds in food used turmeric extract



Concluded from the present study is ethanolic extract of food used turmeric have the most potential antimicrobial activity when compare to other extracts. However, ethanolic and aqueous extract was found to be inhibiting fungi (*Aspergillus niger*), but slightly less inhibition for bacteria (*Staphylococcus aureus*). Comparison with Gentamycin and Fluconazole showed 18 mm which is nearer inhibition zone of food used turmeric extract against *Aspergillus niger* and *Staphylococcus aureus.* And also in component separation and characterization using TLC and FT-IR the food used turmeric is rich in phytochemical components when compare to cosmetic used turmeric. The finding of the present study clearly indicates that the extract of food used rhizome which has proved evidence for its antimicrobial potential.

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