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Research Article

Evaluation of Extractive Value and In-Vitro antimicrobial potential of *Curcuma longa* using disk diffusion method

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Abstract

The efficiency of turmeric fractions, such as petroleum ether, chloroform, benzene, methanol and aqueous were evaluated for their inhibitory effect on clinical and standard strains of pathogenic bacteria and fungus. The methanolic fraction of *Curcuma longa* rhizome had high potential to inhibit some pathogenic bacteria i.e. *S. aureus* to a greater degree than other fractions of *Curcuma longa*. In our study the results show that the different fractions (petroleum ether, methanol etc.) of *Curcuma longa* rhizome were more effective antimicrobial agents than the crude extract of *Curcuma longa*. Methanolic extract of *curcuma longa* was evaluated against gram positive and gram-negative bacteria. The bacteria isolates include *Bacillus subtilis* and *Escherichia coli*. Antimicrobial activities were estimated by Well diffusion method and antifungal activity was evaluated against *Candida Albicans* and *Aspergillus Niger*. The results were pointed as the methanol extract was more effective on both bacteria and fungus then water also similar effective on both bacteria and fungus but against *Aspergillus Niger* it has outstanding results.

Keywords: *Curcuma longa*, Phytochemical, Antimicrobial, Free radical, Scavenging Potential

INTRODUCTION

Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation, and acts by removing injurious stimuli and initiating the healing process¹. Inflammation is therefore a defence mechanism that is vital to health. Inflammation can be classified as either *acute* or *chronic*². Herbal medicine has been commonly used over the years for treatment and prevention of diseases and health promotion as well as for enhancement of the span and quality of life³. Ayurveda literally means the Science of life. It is presumed that the fundamental and applied principles of Ayurveda got organized the last 5000 year⁴. *Curcuma longa* Linn is popularly known as "turmeric" or Haldi (Hindi) Turmeric plant has been used in traditional medicine as a remedy for various diseases including cough, diabetes and hepatic disorders, Turmeric is one such herb⁵. Turmeric is used as an herbal medicine for rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, small pox, chicken pox, wound healing, urinary tract infections, and liver ailments⁶. Throughout the Orient, turmeric is traditionally used for both prevention and therapy of diseases⁷. Modern *in-vitro* studies reveal that turmeric is a potent antioxidant, anti-inflammatory, antimutagenic, antimicrobial, and anticancer agent⁸.

Development of antibiotic drugs have been one of the most discussing topic in the present scenario because resistance is main reason due to which the antibiotics are fail to treat infection and antifungal activity, there is a lot need

development of new antibiotics applications of phytoconstituents are frequently considered be nowadays⁹ because plant constituents, the aim and objective of this study highlights the *in-vitro* antimicrobial and anti-inflammatory effect of *curcuma longa*.

MATERIALS AND METHOD

Collection and Drying of plant material: The leaves, flowers and root of *curcuma longa* were collected from Gwalior, Madhya Pradesh India. The leaves, flowers and root of *curcuma longa* were dried under in college laboratory. Then, the leaves were hot-air dried at 50°C for 24 hr using a convection oven for storage and further extraction. They were pulverized to make fine powder. The fine powder was passed through sieve no. 17 to maintain uniformity and were ground as powder and stored at room temperature.

Physiochemical analysis (screening of powder): Physiochemical screening of powdered fruit was done by the standard methods.

Extraction of *C. longa* rhizome: Extraction of *C. longa* was done by Soxhlet extraction method.

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 alkaloids and tannins, Cardiac glycosides, saponins and flavonoids and terpenoids.

Pharmacological Screening:

DPPH Free Radical-Scavenging Activity: The antioxidant activities of all turmeric extracts were evaluated according to the DPPH radical-scavenging activity, 1 ml of the extract was mixed with 1.2 mL of 0.003% DPPH in methanol at varying concentrations (2.5–80.0 $\mu\text{g/ml}$). DPPH scavenging activity is expressed as the concentration of a sample required to decrease DPPH absorbance by 50% (IC_{50}). This value can be graphically determined by plotting the absorbance (the percentage of inhibition of DPPH radicals) against the log concentration of DPPH and determining the slope of the nonlinear regression.

Ferric Reducing Antioxidant Power (FRAP) Assay: The FRAP assay was performed as described by Benjie and Strain. The reduction of a ferric tripyridyl triazine complex into its ferrous form produces an intense blue colour at low pH that can be monitored by measuring the absorbance at 593 nm. Briefly, 200 μL of the extract solution at different concentrations (62.5–1000.0 $\mu\text{g/mL}$) was mixed with 1.5 mL of the FRAP reagent, and the reaction mixture was incubated at 37°C for 4 min. The FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer (pH 3.6) with 1 volume of 10 mM TPTZ solution in 40 mM hydrochloric acid and 1 volume of 20 mM ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$). The FRAP reagent was prewarmed to 37°C and was always freshly prepared. A standard curve was plotted using an aqueous solution of ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) (100–1000 μmol).

Antimicrobial screening of extracts: A drug is considered as bacteriostatic or fungistatic when it inhibits the growth or multiplication of bacteria or fungi respectively and considered as bactericidal or fungicidal when it actually results in the

death of bacteria or fungi. Drugs that are bactericidal under certain circumstances may have an apparent bacteriostatic effect at the other times. Important factors for the antimicrobial activity are size of the inoculum, metabolic state of organisms, pH, temperature and duration of interaction, concentration of the inhibitor and presence of interfering substance. In vitro tests are used as screening procedure for new agents and for treating susceptibility of individual isolates from infection to determine which of the available drugs might be useful therapeutically. In general, minimum inhibitory concentration (MIC) and sensitivity tests are used to express the effectiveness of a compound as an antimicrobial agent. MIC is the smallest conditions. MIC can be determined by the tube dilution method. Sensitivity testing is done to determine the range of microorganism that are susceptible to the compound under specified conditions. This method is suitable for the organism that grows well overnight such as most of the common aerobes and facultative anaerobes and rapidly growing fungi such as *Candida albicans*. Several forms of diffusion methods have been advocated. Among these, the Kirby-Bauer method is the official method of the USA Food & Drug Administration.

Antimicrobial General Procedure: The cultures were prepared in sterile nutrient broth for 16–18 h at 37 °C. The cultures were aseptically swabbed on the surface of sterile Nutrient Agar plates. Different antibiotics (Ciprofloxacin at 50mg concentration for Gram-positive and Gram-negative bacteria; 50mg Clotrimazole for fungus in DMSO) were aseptically placed over the seeded agar plates. The plates were incubated at 37 °C for 24 h and the diameter of the inhibition zones.

RESULTS AND DISCUSSION

Preliminary Studies

Table 1: Morphological characteristics of *curcuma longa*

S. No.	Character	Fruit	Stem	Rhizomes
1	Color	Pale to buff yellow	Outer surface-brownish black and inner surface-reddish brown	Dark-brown and black(externally) ceramic-yellow (internally)
2	Odor	Like raw mango	No characteristics	Pleasant, Aromatic
3	Shape	Flattened, longitudinal wrinkled	Flat, slight curved	Bluntly conical
4	Size	Various	9cm	1-3cm length
5	Texture	Smooth	Rough	Rough

Table 2: Physiochemical analysis of powder of *curcuma longa*

S. No.	Parameters	Results (%)
1	Total Ash	7.68
2	Acid insoluble Ash	4.84
3	Water insoluble Ash	2.47
4	Water soluble extractive value	13.42
5	Alcohol soluble extractive value	5.28
6	Loss on Drying	11.08

Table 3: Physiochemical analysis of powder of *curcuma longa*

Extracts	Color	Consistency	% Yield
Petroleum ether (40-60 °C)	Yellow	Viscous	2.2
Benzene	Brown	solid	2.8
Chloroform	Reddish	solid	3.8
Ethanol	Reddish	solid	9.8
Water	Pale yellow	solid	15.8

Phytochemical screening of *Curcuma longa*Table 4: Phytochemical screening of *Curcuma longa* extract

Secondary metabolites	Name of test	Various extracts of <i>Curcuma longa</i>				
		Pet. ether	Benzene	Chloro	Methanol	Water
Alkaloid	Mayer's test	-	-	-	+	+
	Hager's test	-	-	-	-	-
Tannins and Phenolics	Ferric chloride	+	+	+	+	+
	Vanillin HCl	+	+	+	+	+
Protein	Ninhydrin test	-	-	-	-	-
	Biuret test	-	-	-	-	-
Flavonoids	Shinoda test	+	+	+	+	+
Steroids and triterpenoids	Salkowski test	-	-	-	-	-
	Lieberman-Bouchard test	-	-	-	-	-
Glycosides	Legal Test	+	+	+	+	+
	Nitroprusside	+	+	+	+	+
Carbohydrates	Benedict's test	+	+	+	+	+
	Fehling's test	+	+	+	+	+

Antioxidant activity by DPPH assay

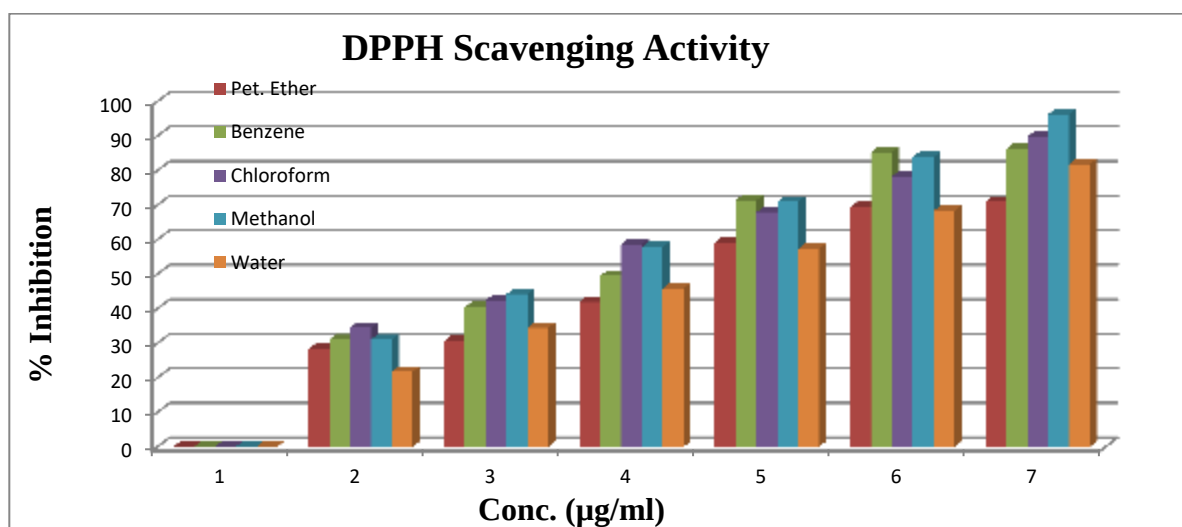


Figure 1: DPPH Scavenging Assay

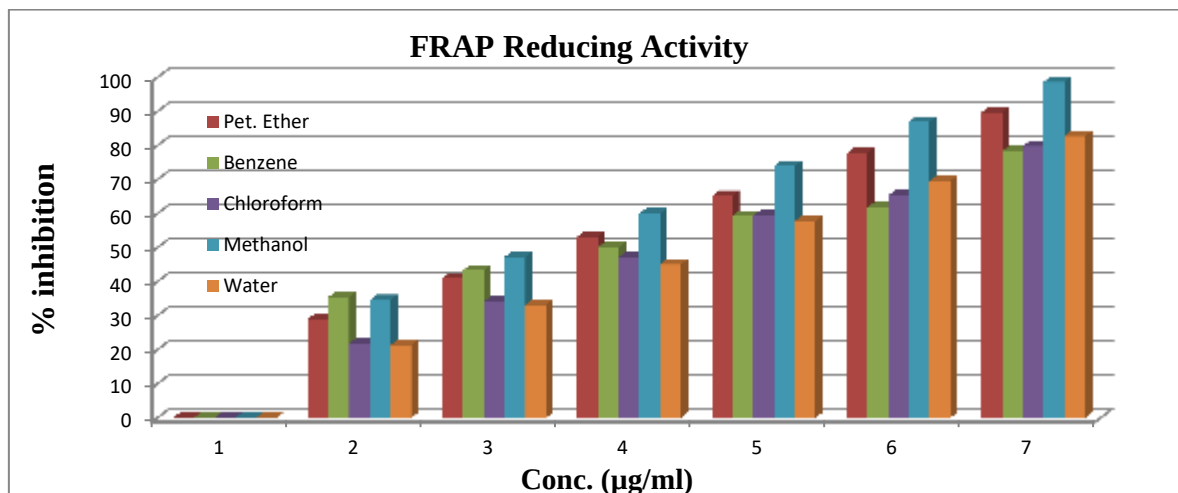


Figure 2: FRAP Scavenging Assay

Evaluation of antimicrobial activity of *curcuma longa*Table 5: Antibacterial activity of *Curcuma longa* rhizomes

Sample applied	Diameter of zone of inhibition (mm)	
	<i>B. subtilis</i> (A)	<i>E. coli</i> (A)
Chloroform extract	16	13
Methanol extract	24	27
Water extract	23	25
Ciproflaxacin	27	26

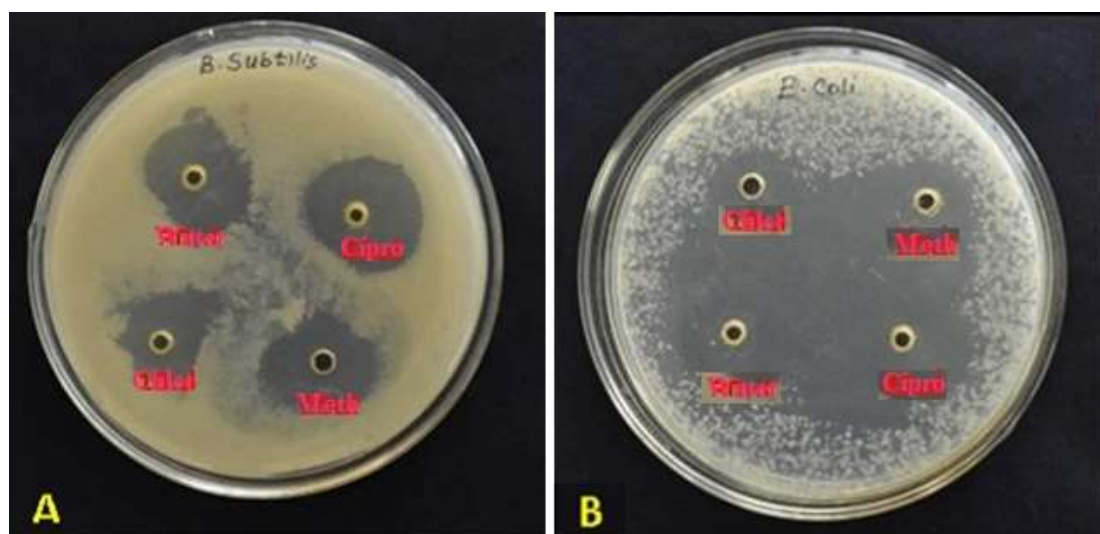
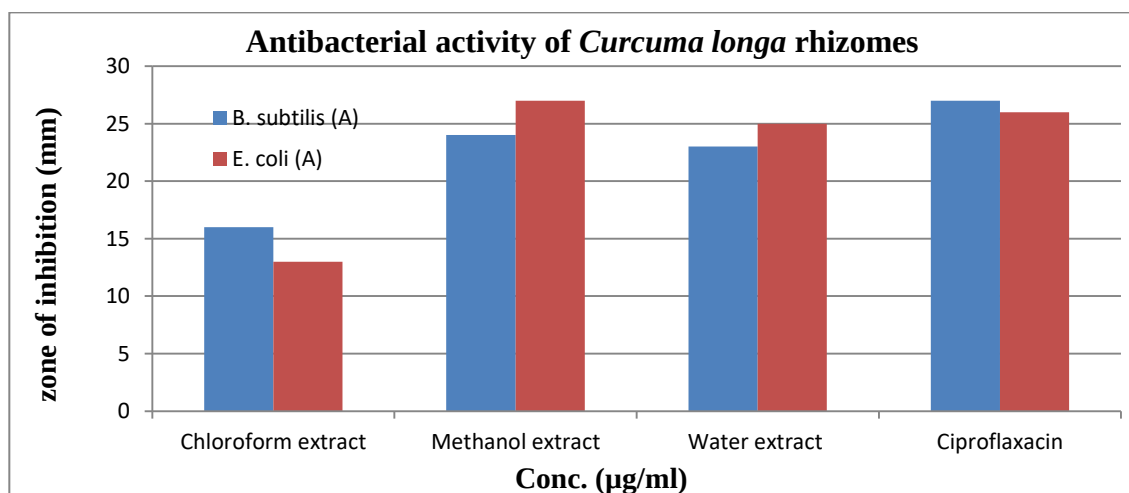
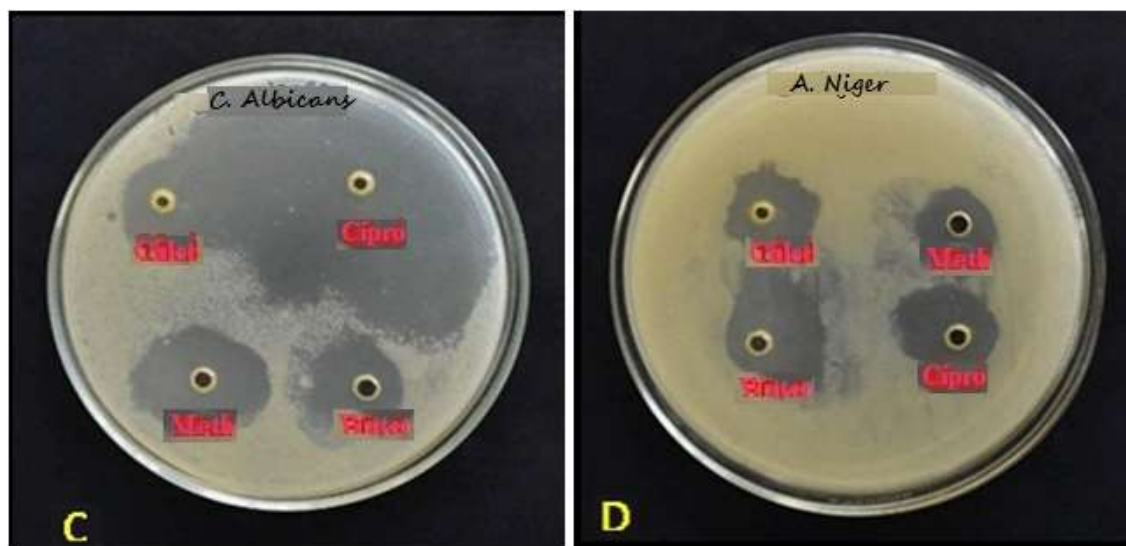
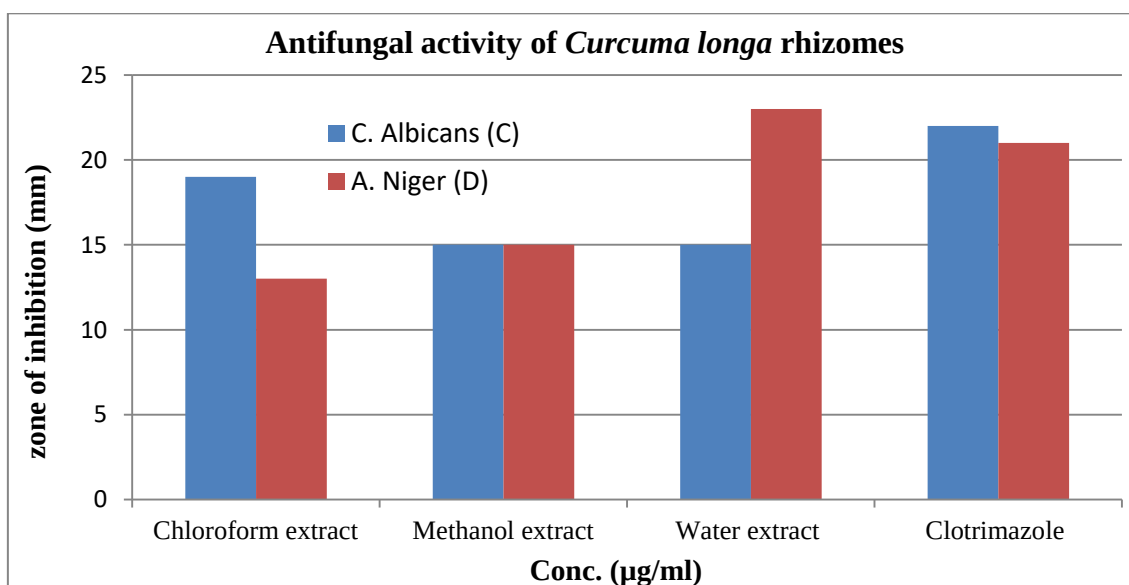
Figure 3: Antibacterial activity of *Curcuma longa* rhizomesFigure 4: Comparison of antibacterial activity of *Curcuma longa* rhizomes

Table 6: Antifungal activity of *Curcuma longa* rhizomes

Sample applied	Diameter of zone of inhibition (mm)	
	<i>C. Albicans</i> (C)	<i>A. Niger</i> (D)
Chloroform extract	19	13
Methanol extract	15	15
Water extract	15	23
Clotrimazole	22	21

Figure 5: Antifungal activity of *Curcuma longa* rhizomesFigure 6: Comparison of Antifungal activity of *Curcuma longa* rhizomes

DISCUSSION

Morphological characteristics of *curcuma longa* revealed that fruits were Pale to buff yellow color, odor like raw mango, shape flattened, longitudinal wrinkled with smooth texture in various size, Stem were color outer surface-brownish black and inner surface-reddish brown, odor characteristics, shape flat, slight curved, 9cm in size and rough in texture, Rhizomes were Dark-brown and black (externally) ceramic-yellow (internally), Pleasant, Aromatic odor, Bluntly conical shape, rough and 1-3cm in length. Physiochemical analysis of

powder of *curcuma longa* rhizomes showed Total Ash value 7.68, Acid insoluble Ash 4.84, Water insoluble Ash 2.47, Water soluble extractive value 13.42, Alcohol soluble extractive value 5.28 and Loss on Drying 11.08. Physical properties and % Yield of different extracts were found as Petroleum ether extract (2.2) was Yellow, viscous, Benzene extract (2.8) was solid brown, Chloroform extract (3.8) was reddish solid, Ethanol extract (9.8) was reddish solid and Water extract (15.8) was Pale yellow.

Phytochemical analysis of *C. longa* extract showing antimicrobial activity revealed the presence of different active

constituents in different extracts *Curcuma longa* extract contained alkaloids, tannin, flavonoid, glycoside and carbohydrate. There are reports showing that alkaloids and flavonoids are the responsible compounds for the antibacterial activities in higher plants. Antioxidant activity of extracts. The result of the free radical scavenging potential of standard and extract tested by DPPH method are depicted in table. DPPH assay for antioxidant activity has been widely used as *in-vitro* to study antioxidant property in experimental research. 50 % inhibition of (IC₅₀ Value) were obtained as Pet. Ether (<400 µg/ml), Benzene (>300 µg/ml), Chloroform (<300 µg/ml), Methanol (<300 µg/ml) and Water (>300 µg/ml). Antioxidant activity of extracts using FRAP Reducing method 50 % inhibition of (IC₅₀ Value) were obtained as Pet. Ether (>300 µg/ml), Benzene (<300 µg/ml), Chloroform (<400 µg/ml), Methanol (>200 µg/ml) and Water (<300 µg/ml). Antimicrobial Activity study, effects of extracts of *curcuma longa* was evaluated against gram positive and gram-negative bacteria. The bacteria isolates include Gram positive: *Bacillus subtilis*, and Gram-negative bacteria; *Escherichia coli*. Antimicrobial activities were estimated by Well diffusion method, and antifungal activity was evaluated against *Candida Albicans* and *Aspergillus Niger*. The results were pointed as the methanol extract was more effective on both bacteria and fungus then water also similar effective on both bacteria and fungus but against A Niger it has outstand results.

CONCLUSION

Thus, in this study, the efficiency of turmeric fractions, such as petroleum ether, chloroform, benzene, methanol and aqueous were evaluated for their inhibitory effect on clinical and standard strains of pathogenic bacteria *S. aureus*. The methanolic fraction of *C. longa* rhizome had high potential to inhibit some pathogenic bacteria *S. aureus* to a greater degree than other fractions of *C. longa*. In our study the results show that the different fractions (petroleum ether, methanol etc.) of *C. longa* rhizome were more effective antimicrobial agents than the crude extract of *C. longa*.

Methanolic extract of *curcuma longa* was evaluated against gram positive and gram-negative bacteria. The bacteria isolates include *Bacillus subtilis* and *Escherichia coli*.

Antimicrobial activities were estimated by Well diffusion method and antifungal activity was evaluated against *Candida Albicans* and *Aspergillus Niger*. The results were pointed as the methanol extract was more effective on both bacteria and fungus then water also similar effective on both bacteria and fungus but against A Niger it has outstand results.

CONFLICTS OF INTERESTS

There are no conflicts of interests.

REFERENCES

1. Ferrero-Miliani L, Nielsen O, Andersen P, Girardin S. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1 β generation, Clin Exp Immunol. 2007; 147:227-235. <https://doi.org/10.1111/j.1365-2249.2006.03261.x>
2. Takeuchi O, Akira S. Pattern Recognition Receptors and Inflammation. Cell, 2010; 140:805-20. <https://doi.org/10.1016/j.cell.2010.01.022>
3. Abdullahi A.A. Trends and challenges of traditional medicine in Africa. Afr. J. Tradit. Complement. Altern. Med. 2011; 8:115-123. <https://doi.org/10.4314/ajtcam.v8i5S.5>
4. Parasuraman S., Thing G.S., Dhanaraj S.A. Polyherbal formation: Concept of ayurveda. Pharmacogn. Rev. 2014; 8:73-80. <https://doi.org/10.4103/0973-7847.134229>
5. Duke J. Turmeric-The Genus *Curcuma*, Medicinal and Aromatic Plants-Industrial Profiles. Eco Bot. 2007; 61(4):397-398. [https://doi.org/10.1663/0013-0001\(2007\)61\[397b:TGCMMA\]2.0.CO;2](https://doi.org/10.1663/0013-0001(2007)61[397b:TGCMMA]2.0.CO;2)
6. Araujo C. C, Leon L. L. Biological activities of *Curcuma longa* L. Mem Inst Oswaldo Cruz. 2001; 96:723-8. <https://doi.org/10.1590/S0074-02762001000500026>
7. Tilak J. C, Banerjee M, Mohan H, Devasagayam T. P. Antioxidant availability of turmeric in relation to its medicinal and culinary uses. Phytother Res. 2004; 18:798-804. <https://doi.org/10.1002/ptr.1553>
8. Parasuraman S., Thing G.S., Dhanaraj S.A. Polyherbal formation: Concept of ayurveda. Pharmacogn. Rev. 2014; 8:73-80. <https://doi.org/10.4103/0973-7847.134229>
9. Wanda C Reygaert. An overview of the antimicrobial resistance mechanisms of bacteria, AIMS microbial .2018; 4(3):482-501. <https://doi.org/10.3934/microbiol.2018.3.482>