European Journal of Medicinal Plants

33(2): 43-51, 2022; Article no.EJMP.84479
ISSN: 2231-0894, NLM ID: 101583475

Evaluation of *In vitro* Activity of Antimicrobial Potential and Phytochemical Screening of Methanol Extract of *Curcuma longa* Linn against Some Clinical Isolates

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2022/v33i230452

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/84479

Original Research Article

Received 15 January 2022
Accepted 21 March 2022
Published 30 March 2022

ABSTRACT

Since ancient times, plants have been used as a source of medicinal compounds. This study was carried out to assess the antimicrobial potential of the methanol extract of *Curcuma longa* rhizome against some clinical isolates. A total of seven clinical isolates including *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans, Aspergillus flavus,* and *Fusarium* species were used. Different concentrations of the extracts were made. Agar well diffusion methods were used for bacterial and *Candida albicans* while agar dilution technique was used for the mold strains. Ciprofloxacin and Fluconazole were used as a standard positive control against the bacteria and fungi respectively. Dimethyl sulfoxide (DMSO) was used as a negative control. The result showed that methanol extract of *Curcuma longa* rhizome inhibited the growth of all tested organisms. The zone of inhibition of *K. pneumoniae* ranged from 9.33-13.33mm, *S. aureus,* 6.33-12.67mm, *E. coli*, 8- 11.67mm, *P. aeruginosa*, 7.67-10mm, and *Candida albicans* 8.33-13mm while the control drug Ciprofloxacin ranged from 25.33-41.33mm and fluconazole was 20mm. The percentage inhibition of diameter of growth of *Fusarium spp* ranged from 74.61-100% and that of *A. flavus* ranged from 32.44-100%. The positive control drug

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(Fluconazole) and 250mg/ml of the extract showed complete inhibition of the test organisms. Qualitative phytochemical studies of the extract of *Curcuma longa* in different solvents (N-hexane, water, methanol, and acetone) showed the presence of alkaloids, phenols, saponins, steroids, tannins, glycosides, and flavonoids. This result is indicative of its broad-spectrum antimicrobial potential and could be employed in the management and treatment of infections. This study corroborates the plant's historic use and lays the groundwork for potential therapeutic development.

Keywords: Curcuma longa; antimicrobial potential; phytochemicals; methanolic extract.

1. INTRODUCTION

Antimicrobials have a critical role in lowering the global burden of infectious diseases [1,2]. However, the development and spread of multidrug-resistant (MDR) strains in pathogenic bacteria has become a serious public health risk, as there are fewer, or possibly no effective antimicrobial agents available for infections caused by pathogenic bacteria [3,4]. As a corollary, based upon the evidence of the rapid global spread of resistant clinical isolates, finding novel antimicrobial agents is imperative. A large variety of medicinal plants have been identified as important sources of natural antimicrobial compounds that may be beneficial in the treatment of these challenging bacterial infections [5]. Approximately 50% of contemporary pharmaceuticals are derived directly or indirectly from plants, with approximately 80% of the population in developing nations dependent only on plants for their medical needs [6-9]. Many plants have been utilized for their antibacterial properties, which are attributed to phytochemicals produced in the plant's secondary metabolism and these include tannins, alkaloids, phenolic compounds, and flavonoids, which have been found in vitro to have antimicrobial properties [2].

Curcuma longa (Turmeric) is a member of the Zingiberaceae family. It's a rhizomatous perennial plant of Southern Asian origin. It is generally known in India as "Haldi" and is widely grown throughout the country [10]. It is a perennial plant with a luxuriant and upright stem. It flourishes in areas with little rainfall and a mild, warm, and humid climate [11]. It has thin-bladed leaves with an oval sheath-like long petiole with an entire border. It is a tuberous rhizome or a modified stem that is branching and fleshy, has segmented skin with finger-like projections, and is yellowish-brown in color with a dull orange within. Its rhizome powder has been used commercially as a spice, pigment, flavor, preservative, and additive, and it is also utilized extensively in curries and mustards [12]. It also aids digestion [10]. It is used to flavor and preserves food in Nigeria [11]. The rhizomes have been utilized as insect repellent, antibacterial, and anti-diabetic for ages. Rheumatism, bodily aches, genital discharges, dyspepsia, inflammations, constipation, leukoderma, amenorrhoea, and colic inflammatory conditions have all been treated with it [13]. Turmeric, which contains natural phytochemical components including curcuminoids, is said to have a wide range of therapeutic benefits and biological functions. Curcumin and other curcuminoids are the major compounds found in the rhizome of *Curcuma longa* [13].

In obese persons with high cholesterol levels, turmeric is used orally to lower total cholesterol, low-density lipoprotein, and triglycerides [11]. It can be used as an antibacterial agent for textiles, as a clothing dye, and to keep clothes from fading. Curcumin is combined with other antimicrobial compounds to produce antimicrobial skin gels and emulsions that provide better skin protection and wound dressing qualities. [14]. It is often used as a disinfectant or antiseptic, for the treatment of burns, blistering wounds, parasitic skin infections, and acne [11].

Curcuma longa's antibacterial potential has not been properly studied in Nigeria, despite various evidence of its folk medicinal use in the alleviation and healing of ailments. This research was done because of the need to provide scientific evidence on the inhibitory effects of *Curcuma longa* on pathogenic microbes and its phytochemical constituents to support the use for antimicrobial treatment.

2. MATERIALS AND METHODS

2.1 Plant Collection and Taxonomy

Fresh rhizomes of *Curcuma longa* were bought from New market Enugu, Enugu State, Nigeria between the months of February and March 2019. The rhizomes were identified and

authenticated at the Department of Botany, University of Nigeria, Nsukka (UNN).

2.2 Extraction of Plant Material

The fresh rhizomes of *Curcuma longa* were washed, cut into tiny pieces and left to air dry. The dried rhizomes were ground into fine powder using a gasoline powered grinding machine. 400g of the powdered rhizome was placed in a soxhlet extractor with 1 liter of methanol. The solvent was refluxed under gentle heat for 5 hours. The solvent-extract (liquid) was concentrated using mild heat. The extracts were placed on Petri dishes to evaporate to dryness. The extract was kept in a refrigerator at 4^{o}C until required.

2.3 Collection of Microbial Isolates

A total of seven clinical isolates were used for this study. The isolates consist of a Gram positive strain (*Staphylococcus aureus*), three Gram negative strains (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*), a yeast strain (*Candida albicans*), and two mould strains (*Aspergillus flavus* and *Fusarium spp.*). The bacterial isolates were collected from the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu state, while the fungal isolates were collected from Emmanuel Research Laboratory, Enugu state. The bacterial isolates were inoculated in nutrient agar and the fungal isolates were inoculated in Sabouraud Dextrose Agar (SDA).

2.4 Antimicrobial Susceptibility Test

2.4.1 Antibacterial activity

The susceptibility test of the bacteria isolates to methanol extract of *Curcuma longa* rhizome was carried out using agar well diffusion method. Different concentrations of the extract were made by two fold serial dilutions (500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml) using DMSO (Dimethyl Sulfoxide). The bacterial strains were seeded on a well-dried Nutrient agar plate. With a sterile 5mm cork borer, holes were made aseptically and labeled according to the varied concentrations. The wells were filled with 30microlitre of the different extract concentrations. As a negative control, a single well in each plate was filled with DMSO. Ciprofloxacin (5mg/ml) was used as a standard antibiotic for positive control. The plates were left at room temperature for 30minutes for proper diffusion of the extract into the agar. The plates were incubated at 37^{o}C for 24hours. With a meter rule, the clear zones of inhibition were checked and measured. The experiment was carried out three times.

2.4.2 Antifungal Activity

The antifungal activity of methanol extract *Curcuma longa* against mould strains was determined using the agar dilution method. SDA containing Chloramphenicol was used as culture media. Stock solution of 1000mg/ml of the extract was made by dissolving 10g of the extract in 10ml of DMSO. Dilutions of the SDA with the stock solution of the extract were made in a sterile universal container to get different concentrations (250 mg/ml, 125mg/ml and 62.5mg/ml) of 10ml agar. The different concentrations were poured on Petri dishes and left to solidify. The plates were well dried. The culture media of different concentrations were inoculated with 5mm agar blocks which were cut from a 3 day-culture of moulds (*A. flavus* and *Fusarium spp.*). Undiluted agar was used as negative control and agar diluted with Fluconazole at 5mg/ml was used as positive control. The plates were incubated at room temperature and monitored daily for growth. The diameter of growth extension was measured daily for 3 consecutive days.

Percentage inhibition of diameter growth was calculated by employing the following formula:

$$\% \text{ Inhibition} = C\text{-}T \div C \times 100$$

Where C = diameter growth of negative control; T = diameter growth of test

Antifungal activity test of the extract against the yeast strain (*Candida albicans*) was carried out in triplicate the same way as the bacterial strain (agar well diffusion method). SDA was used as culture media.

2.5 Phytochemical Analysis (Qualitative)

For 48 hours, 20g of powdered extract was soaked in 100mls of various solvents including n-hexane, distilled water, methanol, and ethanol. To concentrate it, the extract was decanted and boiled for 3 minutes. Preliminary phytochemical screening was carried out using standard methodologies that had been adopted by various researchers [9,11,15]. Plant secondary metabolites such as alkaloids, saponin,

flavonoids, steroids, phenols, glycosides, and tannins were determined [11]

2.6 Statistical Analysis

Graph Pad prism version 7.0 was used for all statistical analyses (Graph Pad, San Diego, CA, USA).Ordinary One-way Analysis of variance (ANOVA) and Student t-test were used for comparison of mean differences between and among groups respectively at 95% confidence interval. P-value ≤ 0.05 was considered statistically significant.

3. RESULTS

Fig. 1 shows the comparison of the Zone of inhibition diameters (ZID) for the antimicrobial activity of methanol extract of *Curcuma longa* rhizome and control drugs against the tested organisms. The positive controls are Ciprofloxacin for bacteria and Fluconazole for yeast. From the table, *Klebsiella pneumoniae* showed the highest ZID in all the tested extract concentrations, while *Pseudomonas aeruginosa* showed the least ZID in all tested extract concentrations (Plates 1 and 2). *Staphylococcus aureus* was resistant (i.e. has no ZID) at the extract concentration of 62.5mg/ml and *Pseudomonas aeruginosa* was resistant at the extract concentration of 125mg/ml and 62.5mg/ml. ZID of *K. pneumonia* ranged from 9.33-13.33mm, *S. aureus* ranged from 6.33-12.67mm, *E. coli* ranged from 8-11.67mm, *P. aeruginosa* ranged from 7.67-10mm and *Candida albicans* ranged from 8.33-13mm (Fig 2), while the control drug Ciprofloxacin ranged from 25.33-41.33mm and that of Fluconazole was 20mm. Ordinary one-way ANOVA showed a statistically significant difference ($p<0.0001$) in the mean ZIDs of individual test organisms with respect to different extract concentrations and control drugs. Similarly, there were significant differences ($p<0.05$) in the antimicrobial activity of different concentrations of the extract when compared across the different microbial isolates.

Fig. 3 shows the Percentage Inhibition of Diameter of Growth (PIDG) of moulds on agar plates containing different concentrations of the extract and Fluconazole (positive control). At 250mg/ml of the extract there was complete inhibition of both *Aspergillus flavus* and *Fusarium species. Fusarium spp.* was more sensitive to the extract with a PIDG ranging from 74.61-100%. *Aspergillus flavus* had a PIDG ranging from 32.44-100%. Fluconazole also showed complete inhibition of the test organisms. ANOVA showed a statistically significant difference in the PIDG of the two tested organisms when compared according to different extract concentration and with Fluconazole ($p<0.001$). Student t-test showed no statistically significant difference ($p>0.05$) in the PIDG of the two tested organisms when compared across 250mg/ml extract concentration and Fluconazole. But showed a statistically significant difference ($p<0.001$) when compared across 125mg/ml and 62.5mg/ml extract concentration.

Table 1 shows the phytochemical constituents of the *Curcuma longa* in different solvents (N-hexane, water, methanol and acetone). The result reveals the presence of alkaloids, phenols, saponins, tannins, glycosides and flavonoids. N-hexane showed the presence of all the analytes. There was absence of phenols in water, absence of saponins in methanol and absence of saponins and tannins in acetone.

4. DISCUSSION

Plants have long been seen as a promising source of new medicinal compounds. They exhibit a diverse spectrum of biological functions and can be effectively used to treat diseases [16]. Traditional medicine may provide interesting options for combating drug resistance. The result of this study showed that methanol extract of *Curcuma longa* rhizome inhibited the growth of bacteria (Gram positive and Gram negative) and fungi (yeast and moulds). The results agree with the work of many researchers [13,17,18]

Table 1. Phytochemical analysis (qualitative) of various extracts of *Curcuma longa* rhizome

Parameters/Solvents	N-Hexane	Water	Methanol	Acetone
Alkaloids	+	+	+	+
Phenols	+	-	+	+
Saponins	+	+	-	-
Steroids	+	+	+	+
Tannins	+	+	+	-
Glycosides	+	+	+	+
Flavonoids	+	+	+	+

KEY: + = Presence; -=Absence

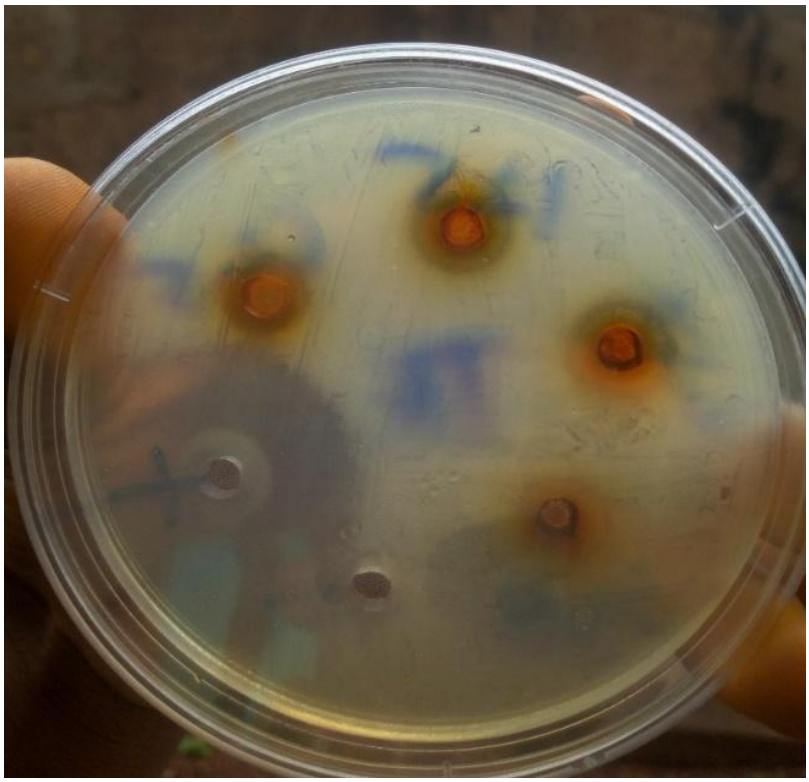

Plate 1. *Klebsiella pneumonia*

Plate 2. *Pseudomonas aeruginosa*

The antibacterial activity of methanolic extract of *Curcuma longa* as represented in Table 1 shows that the extract is very effective against the clinical isolates tested. The plant extract inhibited

all the organisms with the highest average zone of inhibition (13.33mm) against *Klebsiella pneumoniae* at 500mg/ml and the least average zone of inhibition (10.00 mm) against *Pseudomonas aeruginosa* at that same concentration. This inhibition zone seen at high conentation in *P. aeruginosa* may have been because these organisms are known to use high levels of inherent and acquired resistant mechanisms to counter the effect of most antimicrobials. At low concentration there was no effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa* and this does not agree with the work of Oghenejobo et al. that reported the average zone of inhibitions of 3.3mm and 4.3 mm respectively [11]. However, they conducted their research using ethanol as solvent which may have more activity than methanol extract. The authors chose methanol because it has been identified as the most effective extraction solvent, giving the maximum extraction yield and phytochemical content. Nevertheless, the work aligns with the work of Niamsa and Sittiwet in Thailand that recorded resistance for *Staphylococcus aureus* and *Pseudomonas aeruginosa* at low concentrations of the extract [12]. These differences could be due to the strains of microorganisms used, the solvent and inconsistency in the constituents of *Curcuma longa* [18].

The methanol extract of *Curcuma longa* rhizome significantly inhibited *Candida albicans* at all concentrations of the extract. Also, using agar dilution method, the growth of *Aspergillus flavus* and *Fusarium spp.* were significantly inhibited by the extract when compared with the positive and negative control. The extract inhibited the growth of *Fusarium spp.* more than that of *Aspergillus flavus*. The highest percentage radial growth inhibition was 100% and the least was 32.44%.The findings correlate with the work of Bader et al., done in Egypt which also recorded sensitivity of *Candida albicans* to methanol extract of *Curcuma longa* rhizome [12]. They also recorded a greater inhibition of *Fusarium spp*. than *Aspergillus flavus*, when concentrations of the extract were used in their study. Many reports have demonstrated that the antifungal activities of turmeric extracts are due to their poly cationic property, with the length of the polymer chain increasing the activity. Furthermore, turmeric extract may have an effect on the synthesis of certain fungal enzymes [12,19].

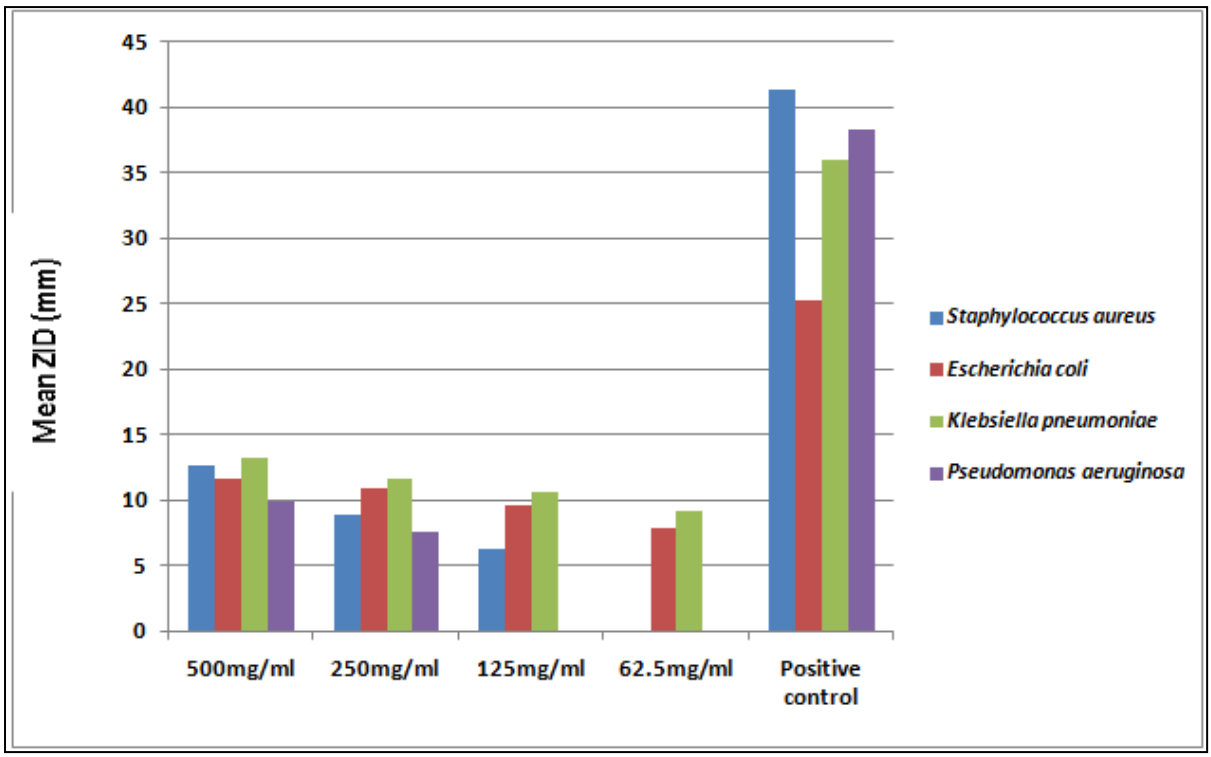

Fig. 1. Comparison of the zone of inhibition diameter for the bacteria and the positive control Ciprofloxacin

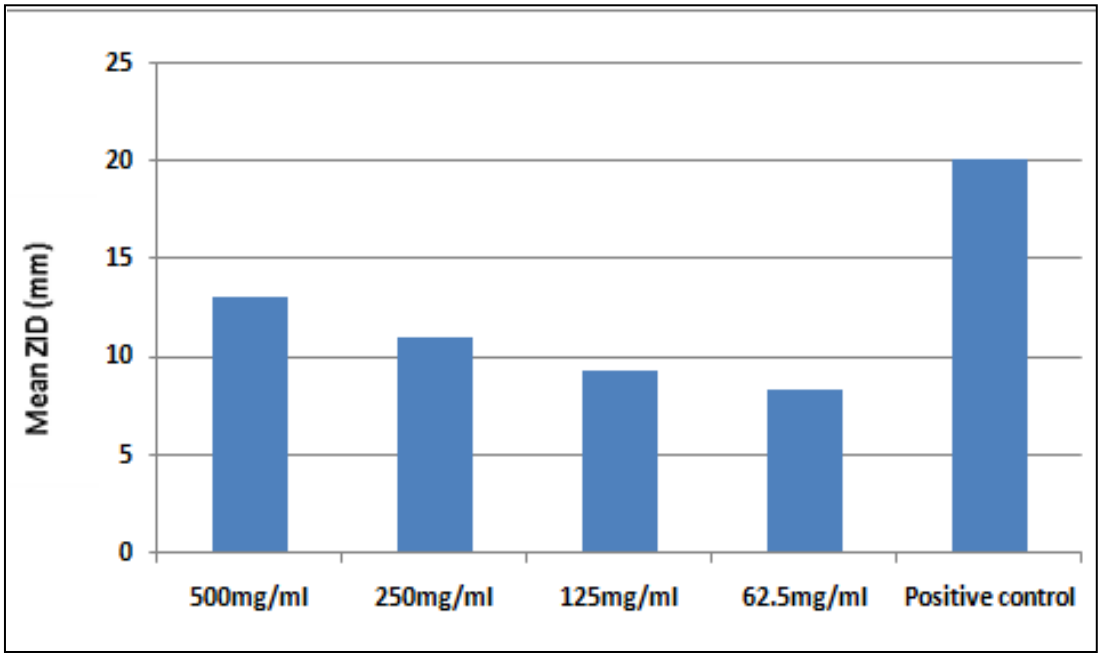

Fig. 2. Percentage Inhibition Diameter of Growth of Candida albicans and positive control Fluconazole

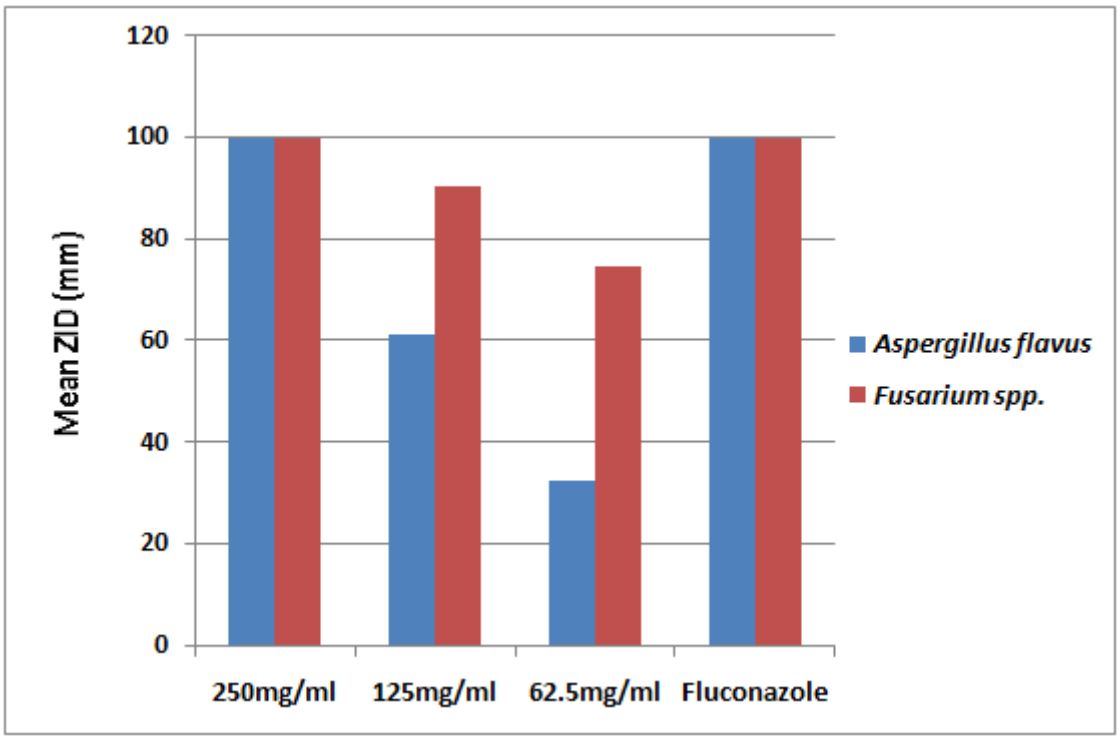

Fig. 3. Comparison of Percentage Inhibition Diameter of Growth of moulds and positive control Fluconazole

Qualitative phytochemical analysis carried out on extracts of *Curcuma longa* in different solvents (N-hexane, water, methanol and acetone) revealed the presence of alkaloids, phenols, saponins, steroids, tannins, glycosides and flavonoids. There was absence of phenol in water, absence of saponins in methanol, and absence of saponins and tannins in acetone. Rajesh et al., reported that solvents used could determine the phytochemical constituents of an

extract [20]. This work is in contrast to the work of Sawant and Godghate in India which reported their presence [9]**.** This could be as a result of different climatic conditions and soil nutrients in the area where the plant thrives. Rajesh et al. reported that the phytochemical constituents could be responsible for the biological and pharmacological actions of plants [20]. The antibacterial activity of plant extracts is due to phenolic chemicals [21]. The H-bonding and perhaps hydrophobic interactions of phenolic compounds present in the plant with membrane proteins, membrane damage, disruption of the electron transport chain, and cell wall breakdown could all be contributory factors. The extract was effective against Gram positive and Gram negative organisms showing its broad spectrum activities. Turmeric's antibacterial activities are not only linked to phenolic compounds but also essential oils such as curcumin, tumerol, and veleric acid [22]. Thus, these antimicrobial effects could be attributed to these secondary metabolites. Scientific phytochemical screening can provide a solid scientific foundation for its usage in the treatment of a variety of diseases.

5. CONCLUSION

Curcuma longa showed in-vitro antimicrobial activity against all tested organisms indicating its broad spectrum antimicrobial potential. The activity increased with increase in concentration. The extract contains various secondary metabolites like alkaloids, phenols, saponins, steroids, tannins, glycosides and flavonoids which could be responsible for the antimicrobial inhibitory effect. The findings suggest that *Curcuma longa* could be useful in the management and treatment of infection. It also serves as a stronger base for the traditional usage of this medicinal plant and the creation of novel pharmaceuticals. The results provide promising information for the use of *Curcuma longa* in management and treatment of infection. Also, it provides a good support to the traditional use of this medicinal plant and as a base for development of new drugs. Quantitative estimation of all the secondary metabolites is suggested. More investigation is needed to establish specific observation regarding the inhibitory activity of individual bioactive compound against the test organisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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