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In Vitro Study on the Antimicrobial Activity of *Curcuma longa* Rhizome on Some Microorganism

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Abstract: The present study investigates the antimicrobial activity, phytochemical and minimum inhibitory concentration (MIC) of *Curcuma longa* rhizome extract on *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus salivarius.* Methanol, and chloroform extracts of the plant rhizome were collected and obtained by standard methods. All the solvent extracts were evaporated to dryness, dry residues were dissolved in dimethyl sulfoxide (DMSO) and tested for antibacterial activity. The plant extract was distinctively applied as antibacterial agent through agar well diffusion method on aseptically prepared nutrient agar. It was determined in the result of this study, that the chloroform extract of *Curcuma longa* rhizome was found to show more activity than the methanol extract on all the isolates. The inhibition zone diameter (IZD) of the chloroform extracts ranged between 7-34mm while ethanol extract ranged between 3-13mm. The MIC varied between 1.56 - 3.125mg/ml and 1.56-6.25mg/ml for methanol and chloroform extract respectively while *Staphylococcus epidermid is* showed the least sensitivity of all the isolates The chloroform extracts exhibited higher inhibitory activity on the test organisms than the positive control ciprofloxacin. Phytochemical analyses of the extracts revealed the presence of alkaloids, tannins, phenolic compounds, terpenoids, saponins and flavonoids. According to this study, *Curcuma longa* rhizome can be used for the treatment of diseases caused by Staphylococcus sppas well as *Streptococcus salivarius.*

Keywords: Curcuma Longa, Staphylococcus Aureus, Staphylococcus Epidermidis, Streptococcus Salivarius, Extracts

1. Introduction

The clinical efficacy of many existing antibiotics is being threatened by the multidrug-resistant pathogens [1]. The increasing failure of chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial agents has led to the screening of several medicinal plants for their potential antimicrobial activity [2], [3]. The use of plant extracts in the treatment of diseases have become of important interest over the years [1]. Medicinal plants generally contain a number of compounds, which may be potential natural antibacterial for the treatment of common bacterial infections [11]. *Curcuma longa* (turmeric) is a rhizomatous herbaceous perennial plant of the ginger family. Tumeric is a spice that has received much interest from both the medical/scientific worlds as well as from the culinary world. Turmeric is a natural medicament with a wide spectrum of biologic actions which include anti-inflammatory, antioxidant, anti-carcinogenic, antimutagenic, anticoagulant, antidiabetic, infertility, antibacterial and antifungal activities [4]. Components of turmeric are namedcurcuminoids [5]. These components are polyphenols with a strong antioxidant function [30]. Curcumin, (diferuloylmethane) the main yellow bioactive component of turmeric has a wide spectrum of biological actions and this provides a basis for exploring its endodontic applications. Aromatic tumerone is another bioactive compound found in turmeric and recently is a promising player in regeneration of neurological diseases [31]. The aim of the study was to evaluate the bactericidal property of turmeric extract as alternative to synthetic antibiotics and to determine the minimum inhibitory concentration as well as the phytochemical contents of *Curcuma longa* extract.

2. Materials and Methods

2.1. Collection of Plant Materials

Turmeric rhizomes were purchasedfrom Ogbete main market, Enugu State and authenticated at Biology department, Enugu State University of Science and Technology. The collected rhizomes were washed thoroughly and shade dried. The dried plant material was finely powdered and grounded using sterile blender.

2.2. Preparation of Turmeric Extract

2.2.1. Methanol Extract

200g of air-driedpowdered*Curcuma longa* rhizomewas weighed and mixed in 1L of methanol. The mixture wasleftat room temperature for 7 days for maceration, with daily intermittent shaking. After 7 days, the solution was filtered using mushin clothand the filtratesevaporated to dryness using a rotary evaporator at 40°C. They were stored at 20°C until use.

2.2.2. Chloroform Extract

100g of the air dried powdered*Curcuma longa* rhizome was weighed using weighing balance and dispensed in 500ml of chloroform. The mixture was leftat room temperature for 7 days for maceration, with daily intermittent shaking. After 7 days, the solution was filtered using mushin cloth and the filtrates evaporated to dryness using a rotary evaporator at 40°C. They were stored at 20°C until use.

2.3. Collection of the Selected Microorganisms

The test organisms were collected from the medical laboratory of microbiology and parasitological unit of the University of Nigeria teaching Hospital, (UNTH Enugu).

2.4. Antibacterial Activity Assay

Antibacterial activity of methanol and chloroform extracts of *Curcuma longa*was determined by agar-well diffusion method.

Agar-Well Diffusion Method

The antimicrobial screening was carried out using the agar well diffusion method as described by Lino and Deogracious, 2006. The test isolates were separately inoculated into the prepared nutrient agar plates. A sterile cork borer was then used to make four wells (6mm diameter each) for different concentrations of the extract on each of the plates containing cultures of different test isolate. The different concentrations of 25, 50, 100, and 200mg/ml of the extracts were then introduced into the four wells using sterile Pasteur pipettes. A known antibiotic was used to test bacteria isolates susceptibility to antibiotics. This was used to compare and contrast the anti-microbial activities of the plant extract. The culture plates were allowed to stand on the working bench for 30min for pre diffusion and were then incubated at 37^0C for 24h. After 24h, antibacterial activity was determined by measuring the diameter zones of inhibition (mm) (against the test isolate) around each of the extract and antibiotic.

2.5. Phytochemical Analysis of the Plant Extract

Phytochemical screening tests were carried out on the methanol extract of *Curcuma longa* according to methods used by Uthayarasa, 2010; the test sample was subjected to phytochemical analysis in order to determine the presence of phytochemical constituents. The phytochemical tests employed were for tannins, saponin, reducing sugar, flavonoids and terpenoids.

2.5.1. Test for Tannins

Lead test

20mg of turmeric was dissolved in 1ml of distilled water and heated on a water bath. The mixture was then filtered and3 drops of Ferric chloride were added to the solution. A green color indicated the presence of tannins on the extract.

2.5.2. Test for Saponins

Frothing test

40 mg of turmeric was dissolved with 5ml of distilled water and shaken vigorously till a stable persistent froth was obtained. The froth was mixed with 3 drops of olive oil and shaken vigorously.

The presence of emulsion indicated its presence on the extract.

2.5.3. Test for Terpeniods

Salkowaski's test

20mg of turmeric was dissolved in 1ml of chloroform and 1ml of concentrated sulphuric acid was added to it. A reddish brown discoloration at the interface showed the presence of terpenoids.

2.5.4. Test for Reducing Sugar

Fehling's test

Few drops of extract were heated with Fehling's A and B solution. The appearance of orange red precipitate showed the presence of carbohydrates.

2.5.5. Test for Flavoniods

Ferric chloride test

20mg of turmeric was dissolved in 1ml of distilled water. 0.5ml of dilute ammonia solution and Conc. Sulphuric acid was added later. A yellow color showed the presence of flavonoids. The yellow color became colorless on allowing the solution to stand.

2.6. Determination of Minimum Inhibitory Concentration (MIC) of the Plant Extract

Minimum inhibitory concentration was determined by agar well diffusion method as described by Ogata *et al.* (2000). The concentrations used in the antimicrobial susceptibility test were diluted further (2 fold-dilution) to get different concentrations of 12.5, 6.25, 3.125, and 1.5625mg/ml, which were incorporated into various test tubes. 0.1ml of the test organism previously diluted to 0.5 Mcfarland turbidity standard was introduced and spread into each plate containing Muller Hinton agar.6mm wells were cut using sterilized cork borer and each filled with 0.1ml of extracts. The plates were

incubated at 37°C for 24h and the diameter of resultant zone of inhibition was measured. The least concentration of the extacts that inhibits the growth of the test organism weredesignated as the minimum inhibitory concentration.

3. Results

3.1. Antimicrobial Activity of Methanol Extract of Curcuma longa on Isolates

The methanol extract of curcuma longa exhibited various antibacterial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus salivarius*. At the highest concentration of 200mg/ml *Streptococcus salivarius*showed the highest average zone of inhibition (9.0mm) (Table 1).

3.2. Antimicrobial Activity of Chloroform Extract of Curcuma longa on Isolates

The test organisms were inhibited by chloroform extract at concentrations of 200-50mg/ml. At the highest concentration of 200mg/ml, *Streptococcus salivarius* showed the highest average zone of inhibition at 34mm, *Staphylococcus aureus* (16mm), and *Staphylococcus epidermidis* (7mm) while*Staphylococcus epidermidis*showed no inhibition at 25mg/ml (Table 2).

3.3. Minimum Inhibitory Concentration (MIC) of Curcuma longa Extracts

Table 3 and 4 shows the minimum inhibitory concentration of methanol and choloform*Curcuma longa* extract on the test organisms. The methanol and chloroform extracts showed similar results, the least concentration that inhibited the growth of *Staphylococcus aureus*and*Streptococcussalivarius* was 1.56 mg/ml while the least concentration that inhibited the growth of *Staphylococcus epidermidis* was 3.125 mg/ml and 6.25mg/ml for methanol and chloroform extracts respectively.

Table 1. *Zone of Inhibition (mm) of Methanol Extract of Curcuma longaon Isolates. Extract inhibitory concentration (mg/ml).*

Test organisms	200	100	50	25	ControlCIPX
Staphylococcus aureus	8.0	4.0	3.0	3.0	20.0
Streptococcus salivarius	9.0	5.0	4.0	3.0	19.0
Staphylococcus epidermidis	7.0	12.0	4.0	-	17.0

KEY: - =No inhibition, CIPX=Ciprofloxacin

Table 2. *Zone of inhibition (mm) of chloroform extract of Curcuma longa on isolatesExtract inhibitory concentration (mg/ml).*

Test organisms	200	100	50	25	ControlCIPX
Staphylococcus aureus	16.0	14.0	12.0	10.0	20.0
Streptococcus salivarius	34.0	23.0	13.0	13.0	19.0
Staphylococcus epidermidis	13.0	3.0	1.0	-	17.0

KEY: - =No inhibition, CIPX=Ciprofloxacin

Table 3. *Minimum Inhibitory Concentration (MIC) of Curcuma longa Methanol extract. Extract inhibitory Concentration.*

Test Organisms	25	12.5	6.25	3.125	1.56	MIC (mg/ml)
Staphylococcus aureus	-	-	-	-	+	1.56
Streptococcus Salivarius	-	-	-	-	+	1.56
Staphylococcus epidermidis	-	-	-	+	+	3.125

Key: + Visible turbidity signifying growth, - No visible turbidity.

Table 4. *Average Minimium Inhibitory Concentration (MIC) of Curcuma longa Chloroform extract. Extract inhibitory Concentration.*

Test Organism	25	12.5	6.25	3.125	1.56	MIC (mg/ ml)
Staphylococcus aureus	-	-	-	-	+	1.56
Streptococcus Salivarius	-	-	-	-	+	1.56
Staphylococcus epidermidis	-	-	+	+	+	6.25

Key: + Visible turbidity signifying growth, - No visible turbidity.

3.4. Phytochemical Analysis of Curcuma longa

The preliminary phytochemical analysis of methanol extract of *Curcuma longa* showed presence of some important Phytochemicals like alkaloids, tannins, phenolic compounds, terpenoids, saponins and flavonoids. These phytoconstituents have important pharmacological activities like anti mutagenic, anti-inflammatory, antibacterial, antiprotozoal, and antioxidant properties (Table 5).

Table 5. *Phytochemical Analysis of Methanol Extract of curcuma longa Rhizomes.*

Phytochemical	Test	Observation	Inference
Alkaloids	Wagner's test	Red precipitate	+
Tannins and Phenolic compounds	Lead test	Green colour	+
Terpenoids and Phytosterols	Salkowaski's test	Reddish-brown colour	+
Saponins	Foam test	Presence of emulsion	+
Flavonoids	Ferric chloride test	White precipitate	+
Glycosides	Brown ring	-	+
Carbohydrate	Fehling's test	-	-

4. Discussion

Over the years, plants and plant materials have been used in the treatment of many diseases and infections. In the present study, the chloroform and methanol extracts of *Curcuma longa* exhibited different ranges of activity against *S. aureus, Streptococcus salivarius*and *Staphylococcoccus epidermidis* (Tables1 and 2) indicating that the plant extracts had broad antibacterial spectrums [10]. Zones of inhibitions produced by chloroform and methanol extracts in the present study ranged between 7mm-34mm. The data obtained showed that the inhibitory effects on the various tested organisms were dose-dependent. This is in agreement with the work of Gupta *et al*. (2015) who reported that fractions of *C. longa* rhizome had an inhibitory effect on*S. aureus* with zone diameter between 8mm and 16mm. Chandrana *et al*. (2010) and Kim *et al*. (2012) also reported that turmeric extract was effective against *E.coli, Bacillus subtilis*and *S. aureus* which may be due to the presence of curcuminiod, a phenolic compound. Also Negi et al. (1999) reported that tumerone and curlone components of turmeric possessed better antibacterial activity against a wide range of microbes including *Bacillus subtilis, Bacillus coagulans, E.coli, Staphylococcus aureus*and *Pseudomonas aeruginosa*. Cikricki *et al*. (2008); Rai*et al*. (2008) and Basniwal*et al*.(2011) also reported that the antimicrobial activity of turmeric could be due to the presence of essential oil, curcumins, turmeric oil, tumerone and veleric acid. From the study, the antimicrobial activity of chloroform compared well with the standard drug ciprofloxacin. This is in agreement with the studies of Negi*et al*.(1999);Gupta *et al*. (2015), Parveen and Jehan (2015) who revealed very significant antimicrobial activity with the extracts of *C. longa* rhizome demonstrating broad spectrum of activity against the test organism. The chloroform extracts showed good antibacterial activity with zone of inhibition greater than or equal to 10mm indicating good antibacterial activity. Based on the limited spectrum of activity of methanol extracts when compared with the chloroform extracts, it suggests that the active component is more soluble in chloroform than in methanol. Studies have shown that some extracts have the ability to extract more of the essential oils and secondary plant metabolites which are believed to exert antibacterial activity on the test organism [1, 34].

The MIC values of the *C. longa* rhizome extracts were similar at 1.56mg/ml for *S.aureus* and *Streptococcus salivarius*while the MIC values for chloroform and methanol extract varied between 3.125 and 6.25mg/ml respectively (Tables 3 and 4);thus, indicating that evaluation of MIC is sufficient for measuring bactericidal activity [30].

From the study, the preliminary phytochemical analysis of methanol extract of *C. longa* showed the presence of alkaloids, tannins, phenolic compounds, terpenoids, saponins and flavonoid (Table 5). The phytochemical constituents are responsible for the biological and pharmacological actions of plants. Curcumin, demethoxycurcumin and bis-demetho-hydroxycurcumin are three pharmacologically important curcuminoids that have been isolated from *Curcuma longa* [6]. The phytochemical results obtained in the study are similar to those obtained by Ankur*et al*. (2015). The antimicrobial activity demonstrated by *C. longa* extract could be attributed to the presence of tannins and saponins which have been reported to possess antimicrobial activity [23, 24]. The metabolites such as phenolics, glycosides and flavonoids have been reported to be potent free radical scavengers and antioxidants [24]. The presence of flavonoids and phenolic compounds in *C. longa* rhizome in the study could suggest its antioxidant activity.

5. Conclusions

From this study, it was observed that the chloroform extract exhibited higher inhibitory activity on the test organism than the methanol extract and compared well with the standard drug ciprofloxacin. This is significant because of the possibility of incorporating its use against multidrug resistant organisms. The present work has shown phytochemical compositions andantimicrobial activity of *C. longa* rhizome extracts confirming the great potential of bioactive compounds.

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