Original Research Article

Phytochemical properties and antimicrobial activities of aqueous Extract of *Curcuma longa* (Turmeric) rhizome extract

Abstract

Curcuma longa (Zingiberaceae) is a native plant of Southern Asia and is cultivated extensively throughout the warmer parts of the world. The aim of the study was to determine the phytochemical properties and antimicrobial activities of Turmeric plant extract. The antimicrobial activities of the plant extract of *Curcuma longa* were determined using agar well diffusion method. The antimicrobial activity of the extract was tested at various concentrations of 0g/ml, 0.1g/ml, 0.15g/ml and 0.2g/ml respectively against *Escherichia coli* and *staphylococcus aureus.* The zone of inhibition exhibited by ethanol extract against the test organisms ranged from 11.0 to 26.00mm. The zone of inhibition exhibited by the aqueous extract ranged from13.0mm to 21.33mm. The tetracycline standard antibiotic showed highest grand inhibitory effect of 29.67mm followed by the ethanol extract which showed 26.00mm. The phytochemical screening revealed that the plant extract contained saponins, tannins, flavonoids, phenols and steroids. Turmeric plant shows antimicrobial potential and may be of great use to pharmaceutical industries for the development of medicines to cure ailments.

Keywords: Turmeric, Phytochemical, antimicrobial, plant extract.

1. INTRODUCTION

A medicinal plant is any plant in which one or more of its organs contains substance that can be used for therapeutic purposes or which contains substances that can be used as precursors for the synthesis of useful drugs [1]. The use of medicinal plants predates the introduction of antibiotics and other modern drugs into the African continent. In addition, certain antibiotics present undesirable side effects such as nausea, depression of bone marrow, thrombocytopenic purpura and agranulocytosis leading to the emergence of previously uncommon diseases. The diarrheagenic strain of *Escherichia coli* and *Staphylococcus aureus* are known to cause gastrointestinal illness in humans and other animals. Recently, it has been reported that 79% of *E. coli* strains resistant to ampicillin and 30% of strains were resistant to ciprofloxacin while 37% of *S. aureus* strains resistant to ciprofloxacin[2]. This has given scientists the impetus to search for newer and alternative microbial compounds from medicinal plants [3]. Turmeric, a dried rhizome of an herbaceous plant, is closely related to ginger. The spice is also sometimes called "Indian saffron" thanks to its yellow colour. The underground rhizome imparts a distinctive flavor to food but it is also used to provide food with a deep, indelible orange color. In the form of this fine, dried, yellow powder, turmeric is mostly sold to customers in

developed countries. Turmeric is used in a wide variety of foods of the cuisines of Southern Asia but locally it also applies as an antiseptic for skin abrasions and cuts [4].

Information in literature on the antimicrobial properties, phytochemical composition of Turmeric plant and its use internationally as a natural preservative and as components of functional foods to promote health abound. It is therefore necessary to evaluate the phytochemical properties and antimicrobial activities of turmeric plant to diversify its use in promoting good health and reducing the risk of diseases. This study was aimed at determining the phytochemical properties and antimicrobial activities of Turmeric plant extract.

2. MATERIALS AND METHODS

3.0 Study Area

This research work was carried out in Makurdi metropolis, the capital of Benue State. The city is sited within Benue state and situated at the heart end of guinea savannah vegetation zone of Nigeria (middle Belt) The town is located between latitude 7^0 30, 7^0 45 N and longitude 8^0 30, 8^0 35 E. the town has a monthly temperature range of 27- 37^0C with mean annual rainfall ranging from 15-18mm. The rainy season occurs from the month of April- October and is more prolonged than the dry season which is usually from November –March.

3.1 Collection of Plant Material

The turmeric rhizomes used in this work were purchased from Wurukum market, Makurdi, packaged in polythene envelopes and taken to the laboratory of the Benue State University for further studies.

3.2 Preparation of Plant Extract

The rhizomes were carefully washed with clean water, steamed for 10minutes to remove the raw odour. The sample was dried in a hot air oven at 50^0C for 24hnours, and then grounded into powder and pass through a sieve with nominal mesh size of 2mm in diameter. 20g of *Curcuma longa* was dissolved in 100ml sterile distilled water and allowed to soak for 24hours. The mixture was filtered through whatman's filter paper no. 1, and kept for the analysis.

3.3 Phytochemical Analysis

Alkaloids and steroids were determined by the method described by Haborne [5]. Saponins were determined by the method described by AOAC [6]. Hydrogen cyanide was determined by the method described by Onwuka [7]. Flavonoid was determined by the method described by Haborne [5]. Phenols and Tannins were determined by the method described by Person [8]. Ethanol extract of the samples was used for the test. The dried sample were soaked in the solvent overnight and filtered before use [5].

3.3.1 Test for Alkaloids

The extract (1.0 ml) was shaken with 5.0 ml of 2 % HCl on a steam bath and filtered. To 1ml of the filtrate, Hagers reagent (iodine in potassium iodide solution) was added. The formation of orange red precipitate confirmed the presence of alkaloids.

3.3.2 Test for Saponins

Frothing test: 1ml of the filtrate was diluted in 1ml of water and shaken vigorously. Persistence foam indicated the presence of saponins.

3.3.3Test for Tannins

Ferric chloride test: 5ml of the extract was added to 2.0ml of 1% HCL. Deposition of a red precipitate showed the presence of tannins.

3.3.4 Test for Hydrogen cyanide

Spot paper test: 1ml of the extract was added with 2 - 3 drops of toluene solution. A change from the yellow colour of the paper to brick red colour indicated a positive result for hydrogen cyanide.

3.3.5 Test for Steroids

The extract (1 ml) was dissolved in 2.0 ml of chloroform in a test tube, and then 1 ml of concentrated sulfuric acid was added. Formation of reddish brown colour at the inter-phase indicated the presence of steroids.

3.3.6 Test for Phenols

Ferric chloride test: The extract (1.0 ml) was added with 1.0 ml of 10 % Ferric chloride. The formation of a greenish brown precipitate indicated the presence of phenols.

3.3.7 Test for Flavonoids

The extract (1.0 ml) was diluted in 1.0 ml of diluted NaOH. Formation of yellow precipitate indicated the presence of flavonoids.

3.4 Microbial Analysis

3.4.1 Preparation of microorganisms for the experiment

The clinical isolates were obtained from different medical laboratories within Makurdi metropolis, Benue State, Nigeria. The bacteria isolated include: Gram positive; *Staphylococcus aureus* and Gram negative bacteria; *Escherichia coli.* The isolates were sub cultured onto MaConkey agar and incubated at 37^0C for 24hrs. After identification, the remaining isolates that were not used were subcultured in nutrient agar slants and stored at 4^0C.

3.4.2 Isolation and identification of bacteria

Bacteria isolates were collected on nutrient agar slants and were sub-cultured on MaConkey agar and incubated at 37^0C for 24 hours.

The bacteria colonies were differentiated based on their morphological characteristics by gram staining, followed by other biochemical tests such as Coagulase test, Oxidase test, Catalase test, and Indole test. Identified organisms are gram positive; *staphylococcus aureus* and gram negative; *Escherichia coli*.

3.4.3 Preparation of McFarland

Standard

McFarland Standards are used to standardize the approximate number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland Standard. A McFarland Standard is chemical solution of barium chloride and sulfuric acid; the reaction between these two chemicals results in the production of a fine precipitate, barium sulfate. When

shaken well, the turbidity of McFarland Standard is visually comparable to a bacterial suspension of known concentration [9].

Procedure

1g of Barium chloride ($Bacl_2$) was weighed and dissolved in 99ml of distilled water to get 1% $Bacl_2$. 1ml of conc. Sulfuric acid (H_2SO_4) was dissolved in 99ml of distilled water to get 1% H_2SO_4. 9.95ml of 1% H_2SO_4 was dissolved in 0.05ml (50 ul) of 1% $Bacl_2$, the solution was dissolved with a stirring rod to become turbid. The isolate was dissolved in 10ml of normal saline to compare with the turbidity of the McFarland standard [10].

3.4.4 Sensitivity test for turmeric extract on the microorganisms

The sensitivity test of the plant extract was determined using agar well diffusion method as describe by ICMSF [11] with some slight modification in which the bacteria was dissolved in normal saline to compare with McFarland standard. The bacteria were first grown on MaConkey agar at 37^0C for 24hrs before use. The isolates were later sub cultured onto nutrient agar after comparing with McFarland standard (0.5). Wells were then bored into the agar medium using a sterile 6mm cork borer. The wells were then filled up with the solution of the extract and care was taken not to allow the solution to spill to surface of the medium. The plates were allowed to stand on the laboratory for 1 hour to allow proper inflow of the solution into the medium before incubating the plates in the incubator at 37^0C for 24hrs. The plates were later observed for zones of inhibition. The effect of the extracts on bacteria isolates were compared with those of standard antibiotics (Tetracycline) at a concentration of 5g/ml, 3g/ml and 1g/ml. Test organisms were treated with 90% ethanol and distilled water as control.

3.4.5 Gram's staining

Each organism in the stock culture bottle was picked with an inoculating loop and used to make a smear on a clean grease free glass slide with a drop of distilled water. The slide was placed on a staining well and floated with crystal violet for about 30 seconds after heating the smear through a Bunsen burner flame for a few seconds. The wire loop was heated to ensure sterilization. The colony of each test organism was picked with the loop and emulsified on the microscopic slide that contained the drop of distilled water. After emulsification, the slide was swirled in the air three times and then passed through the flame 5-7 times to ensure that the smear was heat fixed. The crystal violet was washed with distilled water and iodine and allowed to stand for 30 seconds. 70% alcohol was used to rinse off the iodine. The slide was finally flooded with safranine for 30 seconds. Rinsed with distilled water and allowed to dry off before viewing under the microscope at 40x objective.

3.4.6 Citrate Test

The test was done by preparing Simon's citrate agar as recommended by the manufacturers. The isolates were streaked on it using a wire loop and incubating at 35^0c for 48 hours. A bright blue colour in the medium indicates a positive test while

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no colour change indicates a negative result

3.4.7 Catalase Test

Three percent of hydrogen peroxide was drooped on a glass slide. A bit of growth was removed from a solid medium with a wire loop. A positive test was indicated by budding and frothing.

3.4.8 Indole Test

The organisms were grown in 5ml of peptone water for 24hours. After 24hours of incubation, 2-3drops of kovacs Indole reagent was added. It was shake gently. A positive reaction was indicated by the development of a red colour in the reagent layer above the broth within I minute. In a negative reaction, the Indole reagent retained its yellow colour.

3.4.9 Coagulase Test

Two drops of saline was placed 2 cm apart on a clean glass slide that has been divided into two with a grease pedicel. Each colony was carefully emulsified in a drop of saline. A loop of citrated human plasma was added to the bacteria suspension on one slide and mixed with the loop. Clumping of cells indicated coagulase positive.

4 RESULTS

4.1 Phytochemical analysis of the Turmeric Rhizome

The phytochemical analysis of the turmeric extract showed the presence of alkaloids, tannins, steroids, phenols and flavonoids. Hydrogen cyanide was absent. The presence of this phytochemicals confirmed the medicinal properties of the turmeric plant as shown in table 1.

Table 1: The qualitative test on photochemical properties of the Turmeric rhizome.

Phytochemical Components	Test	Observations	Inferences
Alkaloid	Dragendroffs test	Orange red precipitate	+
Saponin	Froth	Stable froth emulsion foam	+
Tannin	Acid test	Reddish brown	+
Hydrogen cyanide	Sodium picrate	Yellow to reddish brown colour was not observed	_
Steroids	Salkowskis test	Red colour interface	+
Phenol	Ferric chloride	Bluish black colour	+
Flavonoid	Sodium hydroxide	Yellowish precipitate.	+

4.2 Antimicrobial Activity (zone of inhibition in mm) of Turmeric extract against pathogens

The antimicrobial activity of the plant was studied using the extract of the turmeric as shown in the table below. Agar well diffusion method was used to determine the zone of inhibition of the bacteria growth. The mean effect of both aqueous and ethanol extract was used on *Staphylococcus aureus* and *Escherichia* coli.

4.2.1 Mean inhibitory effect of ethanol extract of turmeric (*Curcuma longa*) on test organism.

The mean inhibitory concentration of ethanol extract has 26.00 as highest zone of inhibition on *S. aureus* at (0.2 g/ml) concentration.

Table2. Mean inhibitory effect of ethanol extract on bacteria isolates.

	Zone of inhibition (mm)	
Conc. plant extract (g/ml)	***Escherichia coli***	***Staphylococcus aureus***
0	11.00	12.00
0.1	19.33[b]	18.07
0.15	22.00[ab]	23.67[a]
0.2	25.33[a]	26.00[a]
LSD(p≤05)	**5.54**	**N.S**

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Foot note: Means tagged with same letter alphabets are not significant.
NS = no significant difference.

4.2.2. Mean inhibitory effect of Aqueous extract of turmeric (*Curcuma longa*) on test organisms.

The highest significant inhibitory effect was observed on *Escherichia coli* at 0.2g/ml (21.33) while the least inhibition was observed at 0.15g/ml (13.00) as shown in the table below.

Table3: Mean inhibitory effect of Aqueous extract of turmeric (*Curcuma longa*) on bacteria isolates.

	Zones of inhibition(mm)	
Conc. of plant extract(g/ml)	***Escherichia coli***	***Staphylococcus aureus***
0	0.67	4.0
0.1	17.33[b]	13.0[b]
0.15	20.67[b]	15.7[ab]
0.2	21.33[b]	21.3[a]
LSD(p≤ 0.05)	**NS**	**7.80**

Foot note: means tagged with same alphabets are not significant, **NS-** No significance.

4.3 Mean inhibitory effect of Tetracycline on test organisms.

The highest significant inhibitory effect was observed at 0.05g/ml (29.67) on *E. coli* and the least was at 0.1g/ml (22.33) on *S. aureus* as shown in the table below.

Table 4: Mean inhibitory effect of Tetracycline on bacteria isolates.

	Zones of inhibition(mm)	
Conc. of tetracycline(g/ml)	***Escherichia coli***	***Staphylococcus aureus***
0.1	22.67^{b}	22.33^{c}
0.3	25.67^{ab}	27.00^{b}
0.5	29.67^{a}	28.65^{b}
LSD(p< 0.05)	**5. 435**	**4.104**

Foot note: means not tagged with the same alphabets are significant.

4.1.4 Comparative means inhibitory effect of ethanol, water (aqueous) and tetracycline (positive control) on the two tests organisms.

The grand inhibitory effect of ethanol, water and tetracycline, the tetracycline has the highest inhibitory effect over the ethanol and water extract on both organisms at 29.67 and 28.67 respectively and ethanol has 26.00 on *S. aureus* and the aqueous extract has the least inhibitory effect on both organisms (21.33).

Table 5: Comparative mean inhibitory effect of ethanol, water (aqueous) and tetracycline (positive control) on the two tests organisms.

Extract	***E. coli***	***S. aureus***
Water	21.33^{b}	21.33^{b}
Ethanol	25.33^{ab}	26.00^{ab}
Tetracycline	29.67^{a}	28.67^{a}
LSD (p≤0.05)	**4.756**	**5.115**

4.2 Biochemical characteristics of bacteria isolates

Table 6 shows the biochemical characteristics of the isolates use to study the antimicrobial effect of the turmeric extract.

Table 6: Biochemical characteristics of isolates.

Organisms	**Catalase**	**Coagulase**	**Indole**	**Oxidase**
Escherichia coli	+	-	+	-
Staphylococcus aureus	+	+	-	-

+ = positive, - = negative

3. DISCUSSION

The result in (Table 1) shows the presence alkaloids, saponins, tannins, steroids, phenols and flavonoids. The presence of this phytochemicals confirmed the medicinal properties of the turmeric plant. It has also been shown that saponins are active antifungal agents. Tannins are also known as antimicrobial agents. Tannins are water soluble plant polyphenols that precipitate proteins [12]. Tannins have been reported to prevent the development of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them. The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins. [13]. The potent anti-oxidant activity of flavonoids is their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals and superoxide anions may be the most important functions of flavonoids [14]. It was reported that turmeric also contains some minerals and vitamins such as riboflavin, naicine, calcium, phosphorus, potassium, iron and thiamine. Constant feeding on turmeric plant could be important in sustaining strong bones, muscle contraction and relaxation, blood clothing and absorption of vitamins, B12, potassium and magnesium are known to reduce blood pressure. Potassium plays a role in controlling skeletal muscle contraction and nerve impulse transmission. Patients with soft bone problems are usually placed on high calcium and potassium meals [15]. The iron content present in the extract can help in hemoglobin formation [16] and hence recommended for iron deficiency called aneamia. *Curcuma* is gaining importance worldwide as a potential source of new drugs to combat a variety of ailment as the species contains molecules that are important for health such antihepathoxi, antivenomous, anti-viral such as anti-cancerous properties [17]. In this study, ethanol and aqueous extracts of *Curcuma longa* were investigated and compared with tetracycline on one gram positive and one gram negative bacteria (*E. coli* and *S. aureus)*. The inhibitory effect was determined by agar well diffusion method. The result revealed that turmeric posses antimicrobial activities against tested bacterial isolates at different concentrations. The turmeric extract was active against two bacterial isolates comprising both gram negative and gram positive organisms which include *Escherichia Coli* and *Staphylococcus aureus* .Tetracycline was used as standard antibiotic to compare the ethanolic and aqueous extract. Tetracycline (29.67mm) had significantly ($p < 0.05$) higher zone of inhibition than turmeric extract for both ethanol and aqueous. Ethanol extract of *Curcuma longa* had (26.00mm) higher zone of inhibition on S. *aureus* as compared to aqueous extract (25.33mm) but there was no significant difference between them ($p > 0.05$). The aqueous extract had the least zone of inhibition of 21.33mm ($p > 0.05$) as compared with both ethanol and tetracycline. In all indications, the result in table 2 to 5 showed that if turmeric extract becomes purified, it could have higher zones of inhibition than most standard antibiotics. According to Prasad *et al*., [12], a zone of diameter less than 22mm is not susceptible. The result in table 6 showed the biochemical characteristics of isolates used to study the anti-microbial effect of the turmeric extract. Both *Escherichia coli* and *Staphylococcus aureus* were catalase positive. Catalase test is useful to differentiate between the staphylococci

and the Streptococci [13]. *Staphylococcus aureus* was positive to coagulase test while *Escherichia coli* were negative. In the laboratory, coagulase test is used to distinguish between different types of *staphylococcus* isolates. Both *Escherichia coli* and *staphylococcus aureus* were negative to oxidase test. The oxidase test is a useful test for distinguishing the gram negative rods *Pseudomonas* and the enterobacteriaceae [7]. Only *Escherichia coli* was indole positive [18]. Testing for indole production is important in the identification of entero bacteria. *Escherichia coli* break down the amino acid tryptophan with the release of indole [12]. A recent phytochemical study of *Curcuma longa* revealed the presence of various types of compounds that may have contributed to the antimicrobial activity of *Curcuma longa*. This antimicrobial activity is due to the presence of phenolic contents. Onwuka [7] has reported that *B. subtilis* was the most sensitive organism to *Curcuma longa* extract of curcumoid and oil.

5. CONCLUSION

The results obtained from this study shows that the turmeric contains potential antimicrobial components that may be of great use for the development of medicines by pharmaceutical industries as a therapy against various diseases. The turmeric extract possess significant inhibitory effect against test pathogens.

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