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Phytochemical Screening and Antibacterial Activity of the Aqueous Extract of *Curcuma longa* (Turmeric) Rhizome

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Abstract: The development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants. One traditional medicine in that category is turmeric (*Curcuma longa*) which belongs to the ginger (Zingiberaceae) family. Its major constituents are fat soluble, polyphenolic pigments known as curcuminoids which give turmeric its unique aroma, flavour and medicinal properties. In this study, the *in-vitro* antimicrobial activity of the aqueous extract of *Curcuma longa* rhizome was investigated against standard strains of *Staphylococcus aureus* (ATCC 6571), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 6539), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus subtilis* (6633) using the agar well diffusion method. Different concentrations of the extract were prepared ranging from 12.5 mg/ml to 400 mg/ml. *Staphylococcus aureus* was more sensitive to the extract with zones of inhibition ranging from 13 mm to 27 mm. *Escherichia coli* was the least sensitive with zones of inhibition ranging from 7 mm to 22 mm. Mean Minimum Inhibitory Concentration (MIC) values were as follows: *S. aureus* (12.5 mg/ml), *E. coli* (25 mg/ml), *S. typhi* (50 mg/ml), *P. aeruginosa* (50 mg/ml) and *B. subtilis* (12.5 mg/ml). Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, glycosides and tannins.

Key words: Bacterial resistance, *Curcuma longa*, curcuminoids, antibacterial activity, minimum inhibitory concentration, phytochemical screening.

INTRODUCTION

Traditional medicine is known to be a fertile ground for the source of modern medicines [1]. Different plant parts such as the bulb, gel, leaves, seeds, roots, barks and peels are used for medicinal purposes. The use of plants to treat illness is found throughout human culture [2]. The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds. Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. It is known that more than 400,000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine [3]. One traditional medicine in that category is turmeric (*Curcuma longa*) which belongs to the ginger (Zingiberaceae) family. It is a perennial rhizomatous shrub native to Southern Asia and is extensively used as a spice, food preservative and colouring material in India, China and South East Asia. Turmeric is cultivated most extensively in India, followed by Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia, and Philippines. On a small scale, it is also grown in most tropical regions in Africa, America, and Pacific Ocean Islands. India is the largest producer, consumer and exporter of turmeric [4].

Components of turmeric are named curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin [5]. Curcumin is the most important fraction which is responsible for the biological activities of turmeric [2].

Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [6]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically

active compounds isolated from plant species used in herbal medicine [4].

Since the time of Ayurveda (1900 BC), turmeric has been used for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders. Extensive research on curcumin have demonstrated a wide spectrum of therapeutic effects such as anti-inflammatory, antibacterial, antiviral, antifungal, anti-diabetic, anti-coagulant, hepato-protective, anti-ulcer, hypotensive and hypocholesteremic [7, 8]. How a single agent could exhibit all these effects is an enigma under intense scrutiny [9]. Curcumin possesses antibacterial property against a number of Gram positive and Gram negative bacteria [10]. Also, its anti-inflammatory properties are well documented [8, 11].

MATERIALS AND METHODS

Collection of plant material

The rhizome of *C. longa* (turmeric) was purchased from market and authenticated at the Biological Science department of Ahmadu Bello University, Zaria. The rhizomes were washed thoroughly in running tap water to clean off the adhering sand particles and then rinsed with distilled water.

Preparation of Plant Extract

The scales on the rhizome were removed and the rhizomes were cut into small pieces and crushed using mortar and pestle. Exactly ten grams (10 g) of the rhizome was weighed and transferred into a beaker containing 10 ml distilled water. The solution was kept in a rotary shaker for 24 hours and filtered using a Whatmann No. 1 filter paper. The aqueous extract was stored at 4^{o}C.

Test Microorganisms

The standard strains of *Staphylococcus aureus* (ATCC 6571), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 6539), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus subtilis* (6633) were obtained from National Research Institute for Chemical Technology, Zaria, Nigeria. The bacteria were cultured on nutrient broth (Oxoid, United Kingdom) at 37^{o}C for 12 hours and were maintained and preserved on nutrient agar slants (Oxoid, United Kingdom) at 4^{o}C prior to use.

Phytochemical Screening of the Plant Extract

The aqueous extract of the plant was subjected to phytochemical tests for determination of plant secondary metabolites such as flavonoids, tannins, saponins, alkaloids and glycosides in accordance with standard procedures [12].

Screening for Antibacterial activity

The antibacterial effectiveness of the plant extract was measured by comparing the zone of inhibition values (in mm) of the extract with standard antibiotic Ciprofloxacin on the agar media by agar well diffusion method. Different concentrations of the aqueous extract were prepared ranging from 25mg/ml to 400mg/ml using the double serial dilution method. The organisms were sub cultured on nutrient broth for 24 hours after which 0.1ml of the broth containing each organism was introduced into sterile Petri dishes. Twenty five millilitres (25ml) of nutrient agar was introduced into the plates and mixed by gently swirling the Petri dishes. The plates were allowed to solidify and 5 mm wells were bored on the plates using a sterile cork borer, after which 0.1ml of the extract at different concentrations was introduced into the wells and the plates were incubated at 37^{o}C for 24 hours. The diameter of the zones of inhibition around each of the well was taken as measure of the antibacterial activity using metre rule [2].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC of the aqueous extract of the plant was determined by carrying out serial dilution of the extract to give concentrations ranging from to 3.125 mg/ml to 25 mg/ml, equal volume of the extract and nutrient broth were mixed in the test tube and 0.1 ml of the each organism in nutrient broth was introduced into each test tube. Two test tubes were used as control; one tube containing the aqueous extract and nutrient broth without the organism and another tube containing the nutrient broth and the organism. The tubes were incubated at 37^{0}C for 24 hr. The MIC was determined as the lowest concentration of the extract that did not show visible growth (no turbidity) when compared with the control tubes. The MBC was determined by sub-culturing the prepared dilutions on nutrient agar and further incubated at 37^{0}C for 24 hr. The lowest concentration of MIC tubes with no visible bacterial growth on nutrient agar was regarded as the MBC.

RESULTS AND DISCUSSION

Phytochemical analysis of *Curcuma longa* extract

Phytochemical analysis of aqueous *C. longa* extract revealed the presence of different active constituents (Table 1). *Curcuma longa* aqueous extract contained alkaloids, tannin, flavonoid, saponin and cardiac glycoside. There are reports showing that alkaloids and flavonoids are the compounds responsible for the antibacterial activities in higher plants [13]. It had long been documented that saponins, tannins and alkaloids are plant metabolites known for antimicrobial activity [14]. Some of the secondary metabolites detected in this plant extract may be responsible for the antibacterial activity observed and thus justifying their traditional use as medicinal plants for the treatment of various bacterial and fungal diseases.

Table-1: Phytochemical analysis of various extracts of *Curcuma longa* (rhizome)

Secondary metabolite	aqueous extract
Alkaloid	+
Tannins	+++
Flavonoids	++
Saponin	++
Cardiac glycosides	++

Key: + = mild, ++ = moderate, +++ = abundant

In vitro antibacterial activity of *Curcuma longa* extracts

The result of antibacterial susceptibility testing showed that all the strains of the five pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Bacillus subtilis*) (Table 2), were highly susceptible to ciprofloxacin and the highest concentration of the extract (400 mg/ml) with zones of inhibition ranging from 7 mm to 30 mm. Ciprofloxacin had a higher zone of inhibition (30 mm) than the highest concentration of the aqueous tumeric extract (27 mm) against *Staphylococcus aureus*. Also, ciprofloxacin had a higher zone of inhibition compared to the different concentrations of the extracts in the treatment of *Escherichia coli.* The zone of inhibition of the aqueous extract of turmeric on *Salmonella typhi* ranged from 7 mm to 25 mm. Four hundred milligrams (400mg/ml) of the aqueous extract gave the same zone of inhibition as ciprofloxacin. *Pseudomonas aeruginosa* and *Bacillus subtilis* gave zones of inhibition ranging from 13mm to 23mm and 10mm to 24mm respectively. *Pseudomonas aeruginosa* was more sensitive to the aqueous extract of turmeric rhizome. The result is similar to the study of [10] who reported the inhibitory effects of aqueous, ethanol and hexane extract of turmeric against *S. aureus*. Similar observations have been reported for species such as *Curcuma zedoaria*, *Curcuma aromatic* and *Curcuma amada* [15, 16]. It has been reported that the presence of curcumin in turmeric could be responsible for the antibacterial activity of turmeric [17]. Curcumin has been shown to kill several pathogenic Gram-positive bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis* and *Enterococcus* by inhibiting bacterial cell division [10].

Table-2: Antibacterial susceptibility test of aqueous extract of *Curcuma longa* rhizome against Gram positive and Gram negative organisms

Organisms	Zone of inhibition (mm)/ Concentration of extracts (mg/ml)					
	Positive control (Ciprofloxacin)	400	200	100	50	25
Staphylococcus aureus	30	27	20	14	13	13
Escherichia coli	27	22	19	12	10	8
Salmonella typhi	25	25	13	12	9	7
Pseudomonas aeruginosa	22	23	19	16	15	13
Bacillus subtilis	25	24	18	15	12	10

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the aqueous extract of *Curcuma longa* rhizome

The results showed that the aqueous extract of the plant exerted good antibacterial activity on all the bacteria at different concentrations. The MIC and MBC of the extract against *S. Aureus* and *B. Subtilis* were 12.5 mg/ml and 25 mg/ml respectively. For *E. coli* the MIC and MBC were 25 mg/ml and 100 mg/ml respectively. *Salmonella typhi* and *P. Aeruginosa* recorded MIC value of 50 mg/ml while the MBC was 100 mg/ml. The MBC values obtained for the aqueous extract against the pathogens are higher than MIC, indicating that the aqueous extract is bacteriostatic at lower concentrations and bactericidal at higher concentrations. This suggests that when the extract is used traditionally as an antimicrobial agent, it inhibits the growth of bacteria without necessarily killing the bacteria. Since most of the traditional preparations lack specific concentrations, this may account for the use of large quantity of the extracts by traditional medical practitioners for the treatment of their patients [18].

Table-3: Minimum Inhibitory and Bactericidal Concentrations of the aqueous extract of *Curcuma longa* rhizome

Organism	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus	12.5	25.0
Escherichia coli	25.0	100.0
Salmonellatyphi	50.0	100.0
Pseudomonas aeruginosa	50.0	100.0
Bacillus subtilis	12.5	25.0

CONCLUSION

In this study, the efficacy of aqueous extract of turmeric was evaluated for their inhibitory effect on standard strains of *S. aureus, E. coli, S. typhi, P. Aeruginosa* and *B. subtilis*. The aqueous extract of *C. longa* rhizome had a high potential to inhibit *S. aureus* to a greater degree than *S. typhi* which supports the local use of this plant in traditional therapy. This study paves the path for the application of turmeric as an alternative to conventional antibiotics.

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