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RESEARCH ARTICLE

Antibacterial effect of the leaf extracts of *Ficus thonningii* on *Escherichia coli* and *Staphylococcus aureus* in Kebbi State, Northern Nigeria

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ABSTRACT

The use of ethnomedicinal plants in the treatment of many ailments is very popular in the developing world. *Ficus thonningii*, commonly called the wild fig, has been used in numerous communities in Nigeria as an ethnomedicinal regimen. The phytochemical and antibacterial activities of the plant were carried out in this study. The aqueous extract of the leaves of *F. thonningii* was screened for antibacterial activity carried out by disc diffusion method while the minimum inhibitory concentrations and the minimum bactericidal concentrations were determined using broth dilution method. Phytochemical constituents present in the extract were alkaloids, tannins, flavonoids, anthraquinones, saponins, phlobatannins, and resins. In this analysis, the leave extracts inhibited the growth of test organisms at all concentration (30, 60, and 90 mg/ml) although with significant differences. The larger zone of inhibition was observed on *Staphylococcus aureus* which signifies that it is more susceptible to the leaves extract compared to *Escherichia coli*. The high antibacterial activity of the extract recorded on *E. coli* and *S. aureus* was encouraging at 90 mg/ml with inhibition zone of 3.9 ± 0.33 and 4.4 ± 0.26. The antibacterial activity of the extract was also compared with that of ciprofloxacin. This could be due to the secondary metabolites present in *F. thonningii*, it has been well documented that they possess significant antimicrobial activities that could invariably be useful in the development of safe chemotherapeutic agents for the treatment of many bacterial infections.

Keywords: Antibacterial, Ethnomedicinal, *Ficus thonningii* North Nigeria, Leaf extracts

INTRODUCTION

Ethnomedicine has over the years played an important role in sustaining the health of people in developing countries due to arguably limited access to adequate health care (Yadav and Singh, 2011). Plants have been used across several cultures in the world to manage and treat a lot of diseases and this is as old as life itself. However, in recent years, there have been a considerable number of researches that studied the medicinal values of many plants (Sofowora, 2008). In many rural communities in Africa and several developing nations, conventional drugs have been tagged expensive and not readily available. This has necessitated the use of traditional medicines tin an attempt to cure illnesses (Dangarembizi *et al*., 2014). A high percentage of the world population still depend on plants and plant extracts for the production of regular medicines and local herbs (Benzie and Watchel-Galor, 2011).

Plant-derived phytomedicines used traditionally to treat diseases may contain certain active ingredients that may act individually or in synergy to produce an efficacious result (Van-Wyk and Wink, 2004).

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Beliefs, experiences, practices, and information that are transmitted from one generation to another in different cultures whether scientifically rationale or not has been used in the safeguarding of health and well-being of people in low-income communities (WHO, 2000). With these descriptions, various methods and forms of therapies have been employed by herbalists that are not just limited to herbal medicines but may also include minor invasive procedures, wound dressing, bone luxation reduction, and therapeutic massages among others (Adeshina, 2008).[1-10]

Most Nigerians, especially those living in rural communities, do not have access to orthodox medicine and it is estimated that about 80% of the populace still prefer to solve their health problems consulting traditional healers (Adekannbi *et al*., 2014).

In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug are of paramount importance. Hence, work in both mixture of traditional medicine and single active compounds is very important (Kumar *et al*., 1997).[11-21]

MATERIALS AND METHODS

Study area

The study was carried out in Aliero Local Government area of Kebbi State, Nigeria. The area is located in the extreme North-Western corner of Nigeria within latitude 10°–15° and longitude 33°–603° East of the equator, Kebbi North is geographically located in the northwest part of the state. The estimated population of the L.G.A is 232,846 people in 2006. It has an area of 51 km^2 (Anon; 2018).

Plant collection

The leaves of *Ficus thonningii* were collected in Aliero town, Kebbi state, Nigeria, in the month on July 2, 2019. The plant was identified and authenticated by the taxonomist at the Department of Plant Science and Bio-technology, Kebbi State University of Science and Technology, Aliero in person of Professor Dharmendra Singh. Its voucher number is 511B. It is in the family of Moraceae species *F. thonningii*. In Hausa language, it is called “Chediya.”

Preparation of plant extract

The procedure described by Yakubu *et al*. (2005) was adopted for the preparation of aqueous extract of *F. thonningii* leaves. The fresh leaves were dried at room temperature in the absence of sunlight for 7 days, after which they were grounded to powder using mortar and pestle to a constant weight, and then stored in a plastic container. A known weight (50.0 g) of powder was diluted in of distilled water and then left undisturbed for 48 h after an initials vigorous stirring. This was later filtered with muslin cloth and the filtrate concentrated using the steam from Soxhlet extractor set at 100°C and kept for further used.

Phytochemical analysis of the extract[22-25]

Test for alkaloids

Two milliliters of *F. thonningii* extract were stirred with of Hcl and mixture was warmed and filtered. Two milliliters of filtrate were treated separately with a few drops of potassium mercuric iodide (Mayer’s reagent). Turbidity and precipitation with either of these reagents were observed and recorded (Tiwari *et al*., 2013; Harbourne *et al*., 2013).

Test for saponin

Two milliliters of the filtrate were mixed with 5 ml of distilled water and shaken vigorously and observed for froth formation (Tiwari *et al*., 2013; Harbourne *et al*., 2013).

Test for anthraquinones

Two milliliters of plant extract were boiled with 6 ml of Hcl and filtered. The filtrate was shaken with 5 ml of benzene, filtered, and 2 ml of ammonia solution was added to the filtrate.

The mixture was shaken and the presence of a pink, violet, or red color in the ammoniacal phase indicated the presence of free hydroxyl anthraquinones (Tiwari *et al*., 2013; Harbourne *et al*., 2013).

Test for glycoside

0.2 ml of Fehling's solution A and B were added to 5 ml of the filtrate until it turned alkaline and was heated on a water bath for 2 min. A brick red precipitated observed the presence of glycosides (Tiwari *et al*., 2013; Harbourne *et al*., 2013).

Test for phlobatannins

Two milliliters of extract were boiled in aqueous hydrochloric acid, the deposition of a red precipitate indicated the presence of the tested phytochemical (Tiwari *et al*., 2013; Harbourne *et al*., 2013).

Test for flavonoids

Five milliliters of dilute ammonia solution were added to 5 ml of filtrate followed by few drops of concentrated H_2SO_4. The presence of flavonoids was confirmed by fellow coloration (Tiwari *et al*., 2013; Harbourne *et al*., 2013).

Test for tannins

A few drops of Fecl3 were added in 2 ml of filtrate and observed for color change brownish-green or a blue-black coloration was taken as evidence for the presence of tannins (Tiwari *et al*., 2013; Harbourne *et al*., 2013).

Test for resins

Precipitation test

About 2 ml of the extract was extracted with 15 ml of ethanol. The alcoholic extract was poured into 20 ml of distilled water in a beaker. A formation of precipitation indicates the presence of resins (Tiwari *et al*., 2013; Harbourne *et al*., 2013).

Color test

Two milliliters of the extract were extracted using chloroform and the chloroform extract concentrated of dryness. The residue was redissolved in 3 ml acetone and 3 ml of concentrated HCl was added. The mixture was heated in water bath for 30 min. A pink coloration that changes to red indicates the presence of resins (Tiwari *et al*., 2013; Harbourne *et al*., 2013).[26-33]

Sterilization of glass wires

All glass wires were soaked overnight in ethanol, washed with detergent, rinsed with distilled water, and air dried. Petri dishes, glass slides, and Mcartney bottles were sterilized by dried heat oven at 160°C for 1 h.

Preparation of culture media

The medium that was used in the study is Mueller-Hinton agar and it was prepared according to the manufacturers' instruction. It has become the standard medium for the Bauer–Kirby method and its performance is specified by the NNCLS.

Preparation of Mueller-Hinton agar

37.0 g Mueller-Hinton agar powder was weighed and dissolved in 100 ml of distilled water contained in a sterile conical flask, the solution in the conical flask was then heated with hot plate so that powder Mueller-Hinton will dissolve in the distilled water and homogenous mixture is obtained. The mixture was then sterilized using the autoclave at 121°C for 15 min. It was allowed to cool to 45°C before dispensing it into Petri dishes. The molten agar was allowed to solidify in the plate and then incubated at 35°C for 24 h to check for the sterility of the medium (Isenberg, 2005; Cheesbrough, 2006).

Test organism collection

Isolates of *Staphylococcus aureus* and *Escherichia coli* were obtained from the Department of Microbiology, Faculty of Life Science, Kebbi State University of Science and Technology Aliero, Nigeria. It was inoculated into an already prepared agar plates using streaking technique, and the plates were labeled, respectively. The plate was then incubated at for 24 h to obtain a 24 h viable culture. The susceptibilities of the test organism (*S. aureus* and *E. coli*) to *F. thonningii* were assayed using disc diffusion assay (Isenberg, 2005; Cheesbrough, 2006).

Disc diffusion assay

Preparation and impregnation with plant extract
Disc of diameter 6 mm was bored in the laboratory, the disc was then wrapped in a foil paper and sterilized in a hot air oven at 100°C for 1 h. The disc sterilized was now soaked in different concentration (30 mg, 60 mg, and 90 mg) of the plant extract for 1 h so as to ensure that they were fully saturated; the control disc was, however, soaked in distilled water as negative control. The disc was then aseptically removed from the plant solution and allowed to dry in a drying oven at 25°C then they were packed into a sterile bottle, corked, and stored in the refrigerator and ready for use.

Determination of inhibition (Isenberg, 2005; Cheesbrough, 2006)

Inoculation
Already prepared Mueller-Hinton agar media plates with viable cultures of *S. aureus* and *E. coli*, each test organism was inoculated aseptically into four different agar plates by streaking technique and labeled, respectively. The already prepared disc was applied to the surface of the inoculated agar using sterile forceps, that is, each organism was tested at various concentration of the plant. And also, the disc impregnated with distilled water was also inoculated on all the plates as the negative control. The plates were then incubated at 37°C for 24 h after which the plates were observed for zone of inhibition around the disc and also the zone of inhibition was measured and recorded using a transparent meter rule (Isenberg, 2005; Cheesbrough, 2006).

Determination of minimum inhibition concentration (MIC)

The MIC of the extracts was carried out on the test organisms. This was done by broth dilution method. Nutrient broth was prepared according to manufacturer's instruction. Ten milliliters of the broth were dispensed into each test tube and sterilized at 121°C for 15 min, this was allowed to cool. Concentrations of extracts showing inhibition for the organisms when tested during sensitivity test were 2-fold serially diluted in the test tube containing nutrient broth. Two-fold serial dilutions of the extracts in the sterile broth gave the following concentrations; 50, 25, 12.5, 6.25, and 3.125 mg/ml. The initial concentration was obtained by dissolving 0.5 g of the extract in 10 ml of sterile broth. From the standardized microorganisms in normal saline, 0.1 mL was inoculated into the different concentrations of the extract in the nutrient broth. Tube containing nutrient broth only was seeded with a loop full of standardized test organisms to serve as positive control. Another tube containing nutrient broth and the extract was used as a negative control. The inoculated tubes were incubated at 37°C for 24 h (bacteria) and 2–3 days (fungi). At the end of the incubation period, the test tubes were observed for turbidity (growth). The lowest concentration in the series which showed no turbidity when compared with the control was considered and recorded as MIC (Isenberg, 2005; Cheesbrough, 2006).

Determination of minimum bactericidal (MBC)

The result from the MIC was used to determine the MBC of the extract. A loop fall from the test tubes that did not show turbidity (clear) was aseptically subcultured on to nutrient agar plates. The plates were incubated at 37°C for 24 h. At the end of the incubation period, the plates were observed for the presence or absence of growth. The lowest concentration from MIC 39 tubes that showed no growth on the MHA and PDA plates was regarded as the MBC (Isenberg, 2005; Cheesbrough, 2006).

Statistical analysis

Plant extract was grouped according to their concentrations extracted from the main plant powder and expressed as milligram per millimeter of the total to show the inhibition rate for each parasite species. Inhibition rates were expressed in a descriptive statistics (Mean ± MD) (Versace, 1966).

RESULTS

Phytochemical properties of aqueous extract of *F. thonningii*

The result of the phytochemical quantitative analysis of aqueous plant extract of *F. thonningii* showed

the presence of alkaloid, tannins, flavonoids, saponins, anthraquinones, phlobatannin, and resins only glycoside is absent, as shown in Table 1.

Antibacterial activity

The aqueous leaves extract of *F. thonningii* exhibited antibacterial activity against *E. coli* and *S. aureus* portrayed in Table 2; the antibacterial activity of *F. thonningii* had the highest antibacterial activity on *S. aureus* and had the least antibacterial activity on *E. coli.*

The MIC and minimum bactericidal concentration (MBC)

The result of MIC and MBC of the extract that will be able to inhibit the growth of the bacteria is 12.5 mg/ml and 25 mg/ml on *E. coli* followed by 6.25 mg/ml and 12.5 mg/ml on *S. aureus*. The result shown in Table 3 indicates that *S. aureus* has the lowest MIC and MBC compared to *E. coli.*

DISCUSSION

Ficus thonningii leaf extract has shown an antibacterial property in this study. This has supported previous and ongoing investigations on plants with antibacterial properties in an attempt to bring about fine substances that could deliver effective and efficacious results in management of diseases (Kadam *et al*., 2011). Furthermore, phytochemical properties such as saponins, alkaloids, tannins, flavonoids, steroid, glycoside, and anthraquinone were present in the leave extracts. The previous studies on the phytochemistry of *F. thonningii* have also shown the presence of this phytochemicals (Argal *et al*., 2006). This, therefore, supports the reason why herbalists achieve positive results whenever these plants are used to cure diseases of bacterial etiology (Tom *et al*., 2008). There are studies that described the hypocholesterolemic and antidiabetic properties of saponins. Numerous plant families, genera, and species produce a characteristic mix of these chemicals, and they can sometimes be used as taxonomic basis in classifying plants (Rupasinghe *et al*., 2003).

Many studies have demonstrated that the antimicrobial properties of plants are conferred by the presence of secondary metabolites (Edeoga *et al*., 2005; Enaba *et al*., 2007; Bishnu *et al*., 2009). Thomas and Veda (2007) reported that the antimicrobial activity can be considered when the diameter zone of inhibition is 9 mm or more around the paper disk. And as a result, it can be deduced that the extracts tested strongly against the test organism (*S. aureus* and *E. coli*) but *C. albicans,* MRSA, and *P. mirabilis* were not susceptible. The antimicrobial activity of some members of the genus *Ficus* has been reported; leaves of *F. abutilifolia* (Ukwubile, 2010), *F. exasperate* (Odunbaku *et al*., 2008), *F. ingens* (Aliyu *et al*., 2008), and *F. sycomorus* (Njume *et al*., 2009) have been demonstrated to show the growth inhibitory effects on microorganisms.

This study depicts a high antibacterial effect of the extract on *E. coli* and *S. aureus* and has invariably been encouraging at 90 mg/ml with inhibition zone

Table 1: Phytochemical screening of *Ficus thonningii*

Phytochemical	Result
Test for alkaloid	+
Test for saponin	+
Test for anthraquinones	+
Test for glycoside	–
Test for phlobatannins	+
Test for flavonoids	+
Test for tannins	+
Test for resins	
Precipitation	+
Color test	+

+: Present, –: Absent

Table 2: Antibacterial activity of *Ficus thonningii* leaves extract on each of the test organisms

Test organism	Zone of inhibition in mg/ml			
	Concentration of the leaves extract			
	30 mg/ml	60 mg/ml	90 mg/ml	Positive control
Escherichia coli	2.5±0.47	3.0±0.23	3.9±0.33	8.3±0.00
Staphylococcus aureus	3.1±0.13	4.0±0.30	4.4±0.26	8.5±0.00

Table 3: MIC and MBC of *Ficus thonningii* leaves extract

Test bacteria	Concentrations (mg/ml) (MIC)	MBC (mg/ml)
Escherichia coli	12.5	25
Staphylococcus aureus	6.25	12.5

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

of 3.9 ± 0.33 and 4.4 ± 0.26. This could be due to the secondary metabolite present in *F. thonningii,* it is well documented that they possess antimicrobial activities. In this investigation, the leaves extract was found to inhibit the test organisms at all concentration (30, 60, and 90 mg/ml) although with significant differences. The highest zone of inhibition was observed on *S. aureus* which signifies that it is more susceptible to the leaves extract compared to *E. coli* [Table 2]. The activity of the extract was also compared with antibiotic ciprofloxacin as a positive control. The zone of inhibition observed on ciprofloxacin is almost twice as effective as the extract on *E. coli.* MIC is defined as the highest dilution or least concentration of 70 samples that inhibit the growth of microorganisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of microorganism to an antimicrobial agent and it monitors the activity of new antimicrobial agents. The MBC was determined by sub-culturing the test dilution (used in MIC) on to a fresh solid medium and incubated further for 24 h. The concentrations of the plant extracts that completely killed the bacteria were taken as MBC (Sen and Batra, 2012). The result of MIC and MBC of the extract that will be able to inhibit the growth of the bacteria is 12.5 mg/ml and 25 mg/ml on *E. coli* followed by 6.25 mg/ml and 12.5 mg/ml on *S. aureus*. The result shown in Table 3 indicates that *S. aureus* has the lowest MIC and MBC compared to *E. coli.*

The results obtained from this study have contributed in justifying the ethnopharmacological and cultural uses of *F. thonningii* Blume in the treatment of bacterial diseases. The plant extracts could serve as a lead for or be developed into standard chemotherapeutic agents that can be used as a therapeutically effective, safe, relatively inexpensive, highly tolerated, and convenient for many patients.

CONCLUSION

The results obtained from the study have justified the ethno-pharmacological and cultural uses of *Ficus thonningii* in the treatment of infectious and inflammation based diseases. The plant extracts could serve as a lead for or be developed into standard chemotherapeutic agents that can be used as anti-infective which are therapeutically effective, safe, relatively inexpensive, highly tolerated and convenient for many patients.

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