



<https://doi.org/10.47430/ujmr.2381.008>



Received: December 1, 2022

Accepted: December 31, 2022

Evaluation of the Antimicrobial Activity of *Ficus sycomorus* and *Hyphaene theibaica* leaf Extracts against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*

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Abstract

The effective use of *Ficus sycomorus* and *Hyphaene theibaica* traditionally in treatment of variety of illnesses has been widely reported. The aim of the study was to determine the antimicrobial activities of the leaf extracts of *Ficus sycomorus* and *Hyphaene theibaica* on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Fresh leaves of *Ficus sycomorus* and *Hyphaene theibaica* were collected, dried and subjected to ethanolic extraction, and screened for phytochemicals. Five different concentrations of each extract was prepared viz: 200, 160, 120, 80 and 40 mg/mL using distilled water as solvent and tested against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* using agar well diffusion method. Qualitative phytochemical screening revealed that *F. sycomorus* contained phenol saponins, tannins, flavonoids and steroids while *H. theibaica* contains Saponin, flavonoids, alkaloid, phenol and steroids. Antimicrobial activity of ethanolic leaf extracts of *Ficus sycomorus* was observed only against *Escherichia coli* at 200 mg/mL. While no zones of inhibition were observed against any of the test isolates for ethanolic leaf extracts of *Hyphaene theibaica*. The minimum inhibitory concentration (MIC) of *F. sycomorus* extracts on *E. coli* was 100 mg/mL and the minimum bactericidal concentration (MBC) was 200 mg/mL. The activity of *F. sycomorus* leaf extract on *E. coli* being an enteric bacteria, could justify the traditional claims of its use in effective treatment of diarrhea and other stomach complications.

Keywords: *Ficus sycomorus*, *Hyphaene theibaica*, Antimicrobial activity.

INTRODUCTION

Plants have for so long been utilized worldwide as characteristic drug to cure different human diseases. The medicinal use of extracts from *Ficus sycomorus* and *Hyphaene theibaica* plant materials in treatment of some infectious diseases has been reported in different parts of the world (Hossain, 2019; Ewasinha *et al.*, 2021).

Leaf extracts of plants have overtime been used traditionally for the treatment of different diseases. Plant as antimicrobials may inhibit bacteria by a mechanism different from that of antibiotics. They might help treat microbial diseases that are resistant as a result. A number of studies on the screening of plants as natural antimicrobials have been reported by writers in this regard (Anowi *et al.*, 2012;

Adebayo-Tayo and Odeniyi, 2012; Josephs *et al.*, 2012; Kashani *et al.*, 2012; Alves *et al.*, 2013, Ewasinha *et al.*, 2021). Treatment for gastroenteritis and other bacterially-caused infectious diseases is greatly aided by medicinal plants. Exploration of more recent antimicrobials in plants results in a new strategy for reducing antibiotic resistance and consequently offers potential advantages in contemporary chemotherapy.

Other studies revealed that the acetone, methanol and ethyl acetate stem bark extract of *Ficus* spp. exhibit antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, and *Staphylococcus aureus* at concentrations from 25, 50, 75 and 100 ug/mL (Manimozhi *et al.*, 2012).

With the exception of *Listeria monocytogenes*, which was only mildly inhibited, methanol and aqueous extracts of *H. theibaica* (dour fruit) demonstrated higher antibacterial activity against Gram-positive (*S. aureus* and *B. subtilis*) bacteria and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella Typhi*) (Mohamed *et al.*, 2010).

It is becoming more and more popular to use herbal plant extracts with therapeutic properties in the creation of antibacterial agents. This is as a result of rising antimicrobial drug costs and an increase in microorganism resistance to currently accessible, commercially produced antimicrobial antibiotics. In this regard, extracts from medicinal plant materials can serve as alternatives to synthetic antibiotics as they are from natural sources and are easily obtainable, also they have minimal or lesser side effects if used appropriately as they contain natural compounds and they are also relatively cheaper to produce. Interestingly, preliminary observation indicated that the root bark and leaves of *F. sycomorus* are used in Northern Nigeria for the treatment of epilepsy, diarrhea, dysentery, painful urination and vaginal infections without any scientific validation of their antimicrobial effect and safety. Therefore, this study was conducted to determine the antimicrobial activities of the leaf extracts of *Ficus sycomorus* and *Hyphaene theibaica* on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

MATERIALS AND METHODS

Collection of Plant Material

Samples of *F. sycomorus* (fig tree) and *H. theibaica* (dour palm) leaves were obtained from live and healthy trees at Sabon Gari farm village, Girei Local Government Area, Adamawa State, Nigeria from May to July 2021. The plants were confirmed in the Department of Plant Science, Modibbo Adama University, Yola.

Sample Processing and Extraction

The leaves were washed thoroughly using distilled water, and shed-dried at room temperature for 7 days. The samples were pounded separately into powder form using clean mortar and pestle. Each powdered sample was poured into a sterile polythene bag and stored for analysis.

Extraction of the plants bioactive compounds was done using absolute ethanol as solvent. For each of the plant samples, 100 g was soaked in 500 mL conical flask containing 400 mL of the solvent for 72 hours. Each mixture was filtered using whatman's No. 1 filter paper. The filtrates were concentrated using rotary

evaporator to recover the crude ethanolic plant extracts as demonstrated by Isah *et al.* (2020).

Qualitative Phytochemical Screening

The extracts were screened for the presence of the following phytochemicals;

Test for Alkaloids

A mass of 0.5 g of the extract was dissolved in 5 mL of 1% hydrochloric acid; after which a few drops of Meyer's reagent were added to 1 mL of the filtrate. Alkaloids were detected when a white or creamy white precipitate formed (Enwa *et al.*, 2014).

Test for Saponins

One millilitre (1 mL) of filtrate was diluted with water and shaken vigorously. The formation of persistent foams indicated the presence of Saponins. (Mensah *et al.*, 2009#).

Test for Flavonoids:

A few drop of concentrated hydrochloric acid was added to a small amount of an alcoholic extracts of the plant materials, the immediate development of a red colour indicates the presence of flavonoid (Mensah *et al.*, 2009).

Test for Tannins:

Ten per cent (10%) alcoholic ferric chloride was added to (2 mL) of each extract fraction: formation of brownish blue or black colour indicated the presence of tannins (Enwa *et al.*, 2014).

Test for Steroids:

One millilitre (1 mL) of sulphuric acid was added to 1 mL solution of the extract. The appearance of red colour indicated the presence of steroids (Kalpana *et al.*, 2016).

Test for Phenols:

Two millilitres (2 mL) of each extract fraction, 2 mL of 5% aqueous ferric chloride was added; formation of blue colour indicated the presence of phenols in the sample (Enwa *et al.*, 2014).

Test Organisms

The test organisms (*Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* isolated from surface wounds) were obtained from stock cultures collections of the Department of Microbiology laboratory, Modibbo Adama University, Yola. *E. coli* and *S. aureus* were sub-cultured onto nutrient agar while *C. albicans* was subcultured onto Sabouraud dextrose agar. All the plates were incubated at 37°C for 24 hours.

Preparation of Concentrations of plant Extracts.

For each of the plant extracts, a stock concentration of 200 mg/mL was prepared using distilled water as solvent by weighing 10 g of the extract in 50 mL of solution. Subsequent concentrations viz; 160 mg/mL, 120 mg/mL, 80 mg/mL and 40 mg/mL were prepared from the

stock concentration in 4ml each using the relation;

$$V_a = \frac{\text{Concentration} \times 4}{200}$$

Where V_a = volume of the stock needed to prepare the desired concentration.

Concentration = The concentration needed to be prepared.

4mls = the volume of each subsequent concentration

200 mg/mL is the concentration of stock solution (Helmenstine, 2019).

Screening of Extracts for Antimicrobial Activity

Extracts of both *Ficus sycomorus* and *Hyphaene theibaica* were tested separately against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* using agar well diffusion method as described by Cheesbrough (2004).

The turbidity of 24 hours broth culture of each test isolate was adjusted to 0.5 McFarland standard. Using a sterile pipette, 0.1 mL of each standardized bacteria was aseptically introduced unto freshly prepared Mueller-Hinton agar plates and was gently spread using sterile glass rod. Same method was employed for *C. albicans* on Saboraud dextrose agar. All the plates were allowed to rest for 15-20 minutes at room temperature. Wells of 6.0 mm diameter with sterile cork borer were aseptically made on each agar plate using a sterile cork borer. A sterile micropipette was used to dispense 50 μ L of the prepared concentrations into the appropriate wells labelled against each concentration (i.e. 200, 160, 120, 80 and 40 mg/mL). Sterile distilled water was used as negative control. Ciprofloxacin (3 mg/mL) and Fluconazole (2.5 mg/mL) were used as positive control for bacterial and fungal isolates respectively. Following a 30-minute pre-diffusion of extract in laminar flow, all plates underwent a 24-hour incubation period at 37°C. Antimicrobial activity was gauged by measuring the diameter of the zone of inhibition surrounding each well in millimetres.

Determination of Minimum Inhibitory Concentration (MIC)

Broth culture of each isolate was adjusted to 0.5 MacFarland standard (1.5×10^8 CFU/mL) and 0.1 mL of each cellular suspension was diluted into 10 mL of nutrient broth (for *E. coli* and *S. aureus*) and 10 ml Saboraud dextrose broth (for *C. albicans*) to yield approximate cell suspension of 10^6 CFU/mL as described by Donkor et al. (2016).

A stock concentration of 400mg/mL of the extract were prepared in 20 mL using sterile broth (sterile Saboraud dextrose broth, for *C.*

albicans) as diluent. Subsequent concentrations of 400, 200, 100, 50 and 25 mg/mL were prepared each in 1 mL using two fold serial dilution. To each tube, 0.1 mL of 10^6 CFU/mL was added to yield approximate cell suspension of 10^5 CFU/mL. All the tubes were incubated at 37°C for 24 hours and were observed for growth.

For each extract per organism, the lowest concentration that showed no growth (turbidity) was considered as the minimum inhibitory concentration (MIC) (Usman et al., 2007).

Determination of MBC and MFC

Minimum Bactericidal Concentration and Minimum Fungicidal Concentration were determined by using the method described by Usman et al. (2007). A loop of the content of each test tube starting from the MIC and higher concentrations was inoculated by streaking on nutrient agar plate (for *E. coli* and *S. aureus*) and Saboraud dextrose agar (for *C. albicans*). All the plates were incubated at 37 °C for 24 hours. The lowest concentration under which no growth was observed was considered as the minimum bactericidal or fungicidal concentration.

RESULTS

The results show that both extracts of *Ficus sycomorus* and *Hyphaene theibaica* maintained their original (greenish) colour after extraction with ethanol. And both of the plant materials have same percentage yield of 25% (Table 1).

Phytochemical screening indicated that both the two plant extracts contain tannins, saponins, flavonoids and steroids, while only *Ficus sycomorus* contain phenols and *Hyphaene theibaica* contain flavonoids (Table 2).

The result of the antimicrobial activity testing of *Ficus sycomorus* leaf extract on the test organisms indicated that *E. coli* was found to be the only organism susceptible to *F. sycomorus* with the plant producing 20mm diameter zone of inhibition around the well at 200 mg/mL concentration. Whereas, *S. aureus* and *C. albicans* showed no susceptibility to the extract at all the concentrations prepared (Table 3). The result also revealed that none of the test organisms were susceptible to *H. theibaica* extracts at all concentrations prepared (Table 4).

The result for Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *F. sycomorus* extract against the isolates indicated that the MIC for *E. coli* was 100 mg/mL and the MBC was 200 mg/mL, whereas no activity of the extract was observed on *S. aureus* and *C. albicans* (Table 5).

Table 1: Physical Characteristics and Yield Extracts of *Ficus sycomorus* and *Hyphaene theibaica*

Extracts	Weight (g)	Yield (g)	Percentage Yield (%)	Color
ELEF	400	100	25.00	Green
ELEH	400	100	25.00	Green

KEY: ELEF = Ethanolic Leave Extract of *Ficus sycomorus*; ELEH = Ethanolic Leave Extract of *Hyphaene theibaica*

Table 2: Qualitative Phytochemical Compositions of Crude Leaves Extracts *Ficus sycomorus* and *Hyphaene theibaica*

Phytochemicals	<i>Ficus sycomorus</i>	<i>Hyphaene theibaica</i>
Phenol	+	-
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Alkaloids	-	+
Steroids	+	+

Note: (+) Present; (-) absent

Table 3: Antimicrobial Activity of *Ficus sycomorus* Leaf Extracts on Test Organisms using Agar Well Diffusion

S/N	Concentrations (mg/mL)	Zone of Inhibition (mm)		
		<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
1	40	0.00	0.00	0.00
2	80	0.00	0.00	0.00
3	120	0.00	0.00	0.00
4	160	0.00	0.00	0.00
5	200	0.00	20.00	0.00
6	Cip. (3 mg/mL)	30.00	28.00	NT
7	Flu. (2.5 mg/mL)	NT	NT	32.00
8	Dil. H ₂ O	0.00	0.00	0.00

Key: Cip.: Ciprofloxacin, Flu.: Fluconazole, Dil. H₂O: Distilled Water, NT: Not Tested.

Table 4: Antimicrobial Activity of *Hyphene theibaica* Leaf Extracts on Test organisms Using Agar Well Diffusion.

S/N	Concentrations (mg/mL)	Zone of Inhibition (mm)		
		<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
1	40	0.00	0.00	0.00
2	80	0.00	0.00	0.00
3	120	0.00	0.00	0.00
4	160	0.00	0.00	0.00
5	200	0.00	0.00	0.00
6	Cip. (3 mg/mL)	29.00	26.00	NT
7	Flu. (2.5 mg/mL)	NT	NT	28.00
8	Dil. H ₂ O	0.00	0.00	0.00

Key: Cip.: Ciprofloxacin, Flu.: Fluconazole, Dil. H₂O: Distilled Water, NT: Not Tested.

Table 5: Minimum Inhibitory Concentration and Minimum Bactericidal/Fungicidal concentration of *Ficus sycomorus* Leaf Extracts on Test Organisms

S/N	Concentrations (mg/mL)	Isolates					
		<i>S. aureus</i>		<i>E. coli</i>		<i>C. albicans</i>	
		MIC	MBC	MIC	MBC	MIC	MFC
1	400	+	+	-	-	+	+
2	200	+	+	-	-	+	+
3	100	+	+	-	+	+	+
4	50	+	+	+	+	+	+
5	25	+	+	+	+	+	+

Key: +: Presence of growth, - : absence of growth, MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal concentration, MFC: Minimum Fungicidal Concentration.

DISCUSSION

Bioactive plants have played indispensable roles in the treatment of a variety of infections traditionally in different parts of the country and the world at large. In this study, ethanolic leaf extracts of *Ficus sycomorus* and *Hyphaene theibaica* were tested for antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans* isolates from surface wound.

Phytochemical screening of the plants revealed the presence of saponins, flavonoids, alkaloids, steroids and tannins are all present in ethanolic leaf extracts of both plants, with alkaloids and phenols absent in *F. sycomorus* and *H. theibaica* respectively. The presence of flavonoids and tannins in *Ficus sycomorus* and *Hyphaene theibaica* as found in this study agrees with several other findings, in which similar phytochemical groups were present in *Ficus sycomorus* and *Hyphaene theibaica* (Osama and Abdelkarim, 2015; Bello *et al.*, 2013; Salem *et al.*, 2013; Sandabe *et al.*, 2006). Other studies also reported the presence of alkaloids, terpenoids, and phenols in the fractions of *Ficus sycomorus* and *Hyphaene theibaica* (Wurochekke *et al.*, 2013) as observed in this study. Ahmed *et al.* (2017) stated that the presence of these phytochemicals in plants is what accounts for their potential in the treatment of wide array of both infectious and non-infectious diseases. Similarly, Enwa *et al.* (2014) also reported that these phytochemicals found in the plants extracts are responsible for their antimicrobial activity. Monte *et al.* (2014) also stated that phytochemicals are able to inhibit peptidoglycan synthesis, damage microbial membrane structures modify bacterial membrane surface hydrophobicity and also modulate quorum-sensing (QS).

On accessing the antimicrobial activity of *F. sycomorus* extract on the tests organisms, the findings of the study shows that *F. sycomorus* only exhibited antimicrobial activity on *E. coli* at the concentration of 200 mg/mL. The antimicrobial effect of *F. sycomorus* leaf extracts against some enteric bacteria including *E. coli* and *K. pneumoniae* and its use in the treatment of infected wounds and diarrhoea has been reported by Hossain (2019). *F. sycomorus* was observed to have no activity against *S. aureus* and *C. albicans*. However, Saleh *et al.* (2015) reported that methanolic and acetonitrile extracts from *F. sycomorus* at a concentration of 100 mg/mL has considerable antimicrobial effect on *S. aureus*. The inactivity of the extracts against *C. albicans* was consistent with the report of Mousa *et al.*

(1994) who reported that that *F. sycomorus* extracts have no antifungal activity.

The leaf extracts of *Hyphaene theibaica* completely showed no antimicrobial effect against the test isolates. Most studies on the antimicrobial activity of this plant were carried out on the seeds extracts rather than the leaves. Reports of other researches such as Mohamed *et al.* (2010) and Ewasinha *et al.* (2021) indicated that fruit extract of *Hyphaene theibaica* possess antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans* as well as other bacterial and fungal isolates. Although the leaf extracts have similar phytochemicals composition with the fruit extracts as indicated in the literature, the concentrations of these phytochemicals may be less in the leaves than in the fruits. This may account for the lack of antimicrobial activity observed in this study. Therefore, higher concentrations of the leaf extracts may be required to achieve observable antimicrobial activity.

The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of *F. sycomorus* extract against *E. coli* were observed to be 100 mg/mL and 200 mg/mL respectively. This finding is consistent to that of Isah *et al.* (2020) who also reported extracts of *F. sycomorus* to be both inhibitory and bactericidal not only on *E. coli*, but also other bacterial isolates including *Salmonella Typhi*.

CONCLUSION

Based on the results obtained from this study, it can be concluded that, both *Ficus sycomorus* and *Hyphaene theibaica* leaf extracts contain bioactive compounds which are known to be naturally antimicrobial. *Ficus sycomorus* leaf extract was found to exhibit antimicrobial activity against *Escherichia coli* and has no activity on *Staphylococcus aureus* and *Candida albicans* at a concentration of 200mg/ml. *Hyphaene theibaica* leaf extracts have no antimicrobial effect on any of the test organisms. The activity of *F. sycomorus* leaf extract against *E. coli* being an enteric bacteria, justifies the traditional claims of the use of the plant material in effective treatment of diarrhea and other stomach complications.

Acknowledgement

Authors wish to acknowledge the Department of Microbiology, Modibbo Adama University, Yola, for providing laboratory facilities and the test Organisms used in this Research.

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