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Phytochemical Screening and Antibacterial Activity of Bark Extracts of *Ficus Sycomorus*

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Ficus sycomorus has traditionally been used to treat dysentery and in dressing wounds. The research aimed to screen the phytochemicals and evaluate the antibacterial activities of the extracts using different solvents. The powder sample was extracted by soaking 200 ml of each ethanol and water in it. The ethanol and water extracts were screened for their phytochemical and antibacterial activity against Salmonella typhi, Escherichia coli and Pseudomonas aeruginosa. The phytochemical screening revealed the presence of flavonoids, tannins, cardiac glycosides, saponins and terpenoids in both extracts but alkaloids and reducing sugar are present only in the aqueous extract. Anthraquinone was present only in the ethanolic extract. The antibacterial result showed MIC value ranging from 250 – 500 µg/ml and the MBC ranging from 500 – 2000µg/ml. The extracts demonstrated good antibacterial activity against the tested bacteria but ethanolic extract showed less activity when compared with the aqueous extract. The results from the activity of the bark of *Ficus sycomorus* suggest that it can be used to treat diseases caused by these bacteria.

Keywords: phytochemical, antibacterial, Ficus sycomorus

1. Introduction

Antibiotic resistance occurs when bacteria alter their response to antibiotics, rendering them resistant to these medications. As a result, infections caused by these resistant bacteria become more challenging to treat compared to those caused by non-resistant strains [1]. Antimicrobial resistance (AMR) is a broader term, encompassing resistance to drugs to treat infections caused by other microbes as well, such as parasites (e.g. malaria), viruses (e.g. HIV), and fungi (e.g. Candida) [2–5]. By 2050, it is predicted that bacterial infections will account for 1.8 million more fatalities per year than cancer, and the current dearth of antibiotic treatment development is just making matters worse [1]. Despite disproportionately affecting people in low- and middle-income countries, antibacterial drug resistance is a serious worldwide health concern that impacts nations of all economic levels [6].

Plant parts, which have one or more of their organs containing substances that can be used for therapeutic purposes, are called medicinal plants [7]. Plants have been used as medical agents since the earliest days of human existence, necessitating detailed studies to identify those employed for various purposes [8]. Serving as the foundation of advanced traditional medicine systems for thousands of years, they continue to provide new treatments to humanity. Although some therapeutic claims have been debunked, medicinal plant-based therapies remain rooted in centuries of empirical knowledge [9]. The seeds of the Opium poppy (*Papayer Solnniferumi*) and castor oil seeds (*Ricinus cunnunisi*) were excavated from some ancient Egyptian tombs which indicated their use as far back as 1500 BC [10]. [11] reported the antimicrobial activity of the extracts of *Citrus aurantifolia* leaves. The fruit juice has also been shown to inactivate *Escherichia coli*. Garlic *(Allium Sativum*) is used to reduce cholesterol levels and boost the immune system; it lowers high blood pressure and its oil has been seen to have antibacterial properties [12]. Milk thistle *Silybum Marianum* has also been used to protect the liver by treating a wide range of liver conditions; it can be used for increasing breast milk production [12].

Ficus sycomorus is a medicinal plant with a long history of traditional use, originally native to Africa as shown in **figure 1**. Today, it is cultivated worldwide and found across most tropical regions. The *Moraceae* family includes

the short trees *Ficus sycomorus*, which are indigenous to Africa. Throughout the world, its fruits, known as figs, are widely utilised as food and medicine. Flavonoids, anthocyanins, and phenolic antioxidants are abundant in both fresh and dried figs. Fresh fig latex has historically been used to treat cough, snake bites, toothaches, haemorrhoids, warts, and epilepsy. Additionally, it might prevent mice's tumours from growing spontaneously or after transplantation [8,13]. In Nigeria, *Ficus Sycomorus* has been used by the *Igede* people as a treatment for dysentery and in wound dressing [14]. It is also used in circumcision, leprosy and epilepsy [15]. This research will utilize the bark of the *Ficus sycomorus* **(Fig. 2)** for the studies.

Phytochemicals are chemical compounds that occur naturally in plants. Some are responsible for colour and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic [16]. The term is generally used to refer to those chemicals that may have biological significance, (for example antioxidants) but are not established as essential nutrients [17]. Phytochemicals are non-nutritive plant chemicals that have protective or disease-preventive properties [16]. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life (Brown and Arthur, 2001). There are many phytochemicals and each works differently. Some of the possible actions are via antioxidants, hormonal action, stimulation of enzymes, interference with DNA replication, antibacterial effect and physical action [16,18].

Fig 1: *Ficus sycomorus tree*

Fig 2: Bark of *Ficus Sycomorus*

Ficus sycomorus belongs to the family Moraceae [8]. It is commonly called white fig, sycamore fig, and mulberry fig. Its local names are; Farin Baure (Hausa), Borihi (Fulfulde), Bandiboku (Nupe), Tarmu (kanuri), Opoto (Yoruba).
The leaves *Ficus sycomorus* have been reported to have antidiabetic and antioxidant (70% methanol extract) properties. It also exhibits anti-tumour activity and antibacterial activity, but no anti-fungal activity [19]. Aqueous extract of stem bark exhibits sedative, anticonvulsant and muscular activities [20].

The root bark of *Ficus sycomorus* is used in Northern Nigeria for the treatment of epilepsy, diarrhoea, dysentery, painful urination and vaginal infections without any scientific validation [10].

2. Materials and Methods

2.1 Sampling and Sample Treatment

The *Ficus Sycomorus* stem bark was obtained from the old market in Sokoto State Nigeria. The sample was identified at the herbarium unit department of biological sciences and was given a voucher number UDU/AND/0256 at, Usmanu Danfodiyo University, Sokoto. The bark of the plant was rinsed in water and cut into smaller pieces for easy drying. The samples were air-dried in the laboratory and crushed using a wooden pestle and mortar. The crushed sample was sieved through a 20-mesh sieve and the powdery sample was packed into a polythene bag before further analysis.

2.2 Preparation of Extract

The powdered sample (70g) in two places was soaked separately in 200 ml each of distilled water and ethanol for 72 hours. The barks were then filtered and concentrated using a rotary evaporator at 50^{o}C. The resultant extracts were stored in air-tight containers in a refrigerator until subsequent use.

2.3 Phytochemical Screening

a. Test for Saponins

The plant extracts (1 ml) were transferred into a test tube. Distilled water (1 ml) was added to the test tube and shaken vigorously. Persistent froth that lasts for about 15 minutes would indicate the presence of saponins [21].

b. Test for Tannins

The extracts (2ml) of each extract were diluted with distilled water in separate test tubes and 2-3 drops of 5% ferric chloride ($FeCl_3$) were added. A green–black or blue colouration would indicate the presence of tannin [21].

c. Test for Flavonoids

Each of the extracts (2 ml) was transferred into a different test tube. 10% sodium hydroxide (1 ml) was added to each of the test tubes with the extracts. Three drops of dilute hydrochloric acid (HCl) were added to each of the extracts. A change in colour from yellow to colourless indicates a positive result [22].

d. Test for Alkaloids
Each of the extracts (1 ml) was stirred with 1% aqueous HCl (3 ml) in a hot water bath and then filtered. The filtrates were treated with Meyer's reagent. A buff precipitate indicates the presence of alkaloids [21].
e. Test for Steroids
Salkowski test: the extracts (2 ml) each were transferred into separate test tubes containing chloroform (2 ml), and concentrated sulphuric acid (H_2SO_4) was subsequently added to form a lower layer. A reddish-brown ring at the interface of the two liquids and a violet colour in the supernatant layer indicated the presence of steroids [21].

2.4 Antibacterial Assays
a. Test organisms
The stock culture for the clinical isolates of *Salmonella Typhi, Pseudomonas aeruginosa, and Escherichia coli* was obtained from the Department of Microbiology, Usmanu Danfodiyo University Teaching Hospital, Sokoto (UDUTH Sokoto) which was isolated from patients that were tested and confirmed to have clinical cases of gastrointestinal tract infections, upper respiratory tract infections and urinary tract infections. They were subjected to further biochemical tests for confirmation.
b. Preparation of plant extracts
The plant extracts were dissolved in 10% dimethyl sulphoxide (DMSO) to make different concentrations i.e. 40 mg/ml, 30 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml and 2 mg/ml.
c. Preparation of inoculum
Bacterial strains were streaked over Luria broth (LB) agar plates and incubated at 37°C for 24 hours. Single colonies were selected and inoculated in Luria broth at 37°C overnight. The density of inoculum required for the test was adjusted to McFarland Standard 0.5.

d. Determination of antibacterial activities
Preparation of discs and Disc diffusion method
The Whatman No.1 filter paper was used for the preparation of discs and then subjected to different concentrations of extracts and dried at room temperature overnight under aseptic conditions. A disc diffusion assay was done to determine the antibacterial activity of plant extracts. The method given by Kirby and Bauer was used. The test tube containing 0.1ml inoculum with 2 ml top agar was spread on a nutrient agar medium. The discs impregnated with different concentrations of plant extracts were placed on the nutrient agar plate with the help of sterile forceps. Plates were incubated at 37°C overnight. The zone of inhibition was measured using a scale. In this assay, Amoxicillin was used as a positive control, whereas DMSO was used as a negative control.

e. Luria broth dilution method
Minimum inhibitory concentrations (MIC) were determined using the Luria broth dilution method. In this, 10 test tubes containing Luria broth were taken and different concentrations of plant extracts were added to them. Test tubes were then inoculated with bacterial suspension and incubated at 35°C for 18 - 24 hours. The turbidity of the culture was observed as a turbidity value using a turbidity meter (Aqualytic Germany). The lowest concentration of extract that inhibited the growth of bacteria was considered as MIC value for each bacterial strain.
f. Determination of Minimal Bactericidal Concentration (MBC)
Samples were taken from the nutrient agar plates that showed no visible growth after 24 h incubation and subcultured into freshly prepared sterile nutrient agar. The least concentration that did not produce growth after 24 h was regarded as the MBC [23].

3. Results and Discussion

3.1 Results
3.1.1 Phytochemical studies
Table 1 presents the phytochemical screening results for *Ficus sycomorus*. The analysis identified the presence of alkaloids, flavonoids, terpenoids, Anthraquinone, tannins, saponins, cardiac glycosides and reducing sugar. All these compounds, except steroids, were detected in either ethanol or aqueous extract which was absent in all the solvents. However, alkaloids and reducing sugar were absent in the ethanol extract. These results support the idea that different solvents have different effects on the kinds of phytochemicals that are extracted from plants.

Table 1: Phytochemical screening of bark of *Ficus sycomorus*

Solvent Extract	Flavonoid	Tannin	Cardiac glycoside	Saponins	Steroids	Terpenoids	Anthraquinone	Alkaloid Wagner's	Reducing sugar
Aqueous extract	++	++	+	++	-	+	-	+	+
Ethanol extract	+	+++	+++	+	-	+++	+	-	-

Key: + Present, - Absent, ++ moderately present, +++ highly present

3.1.2 Antibacterial Studies

Table 2: Antibacterial activity of ethanolic bark extract of *Ficus sycomorus* (Zone Inhibition)

Name of organisms	Conc. of extract (mg/ml)	Zone of inhibition(mm)
Salmonella typhi	0.2	16
	0.1	14
	0.05	12
	0.025	10
Pseudomonas aeruginosa	0.2	16
	0.1	14
	0.05	12
	0.025	10
Escherichia coli	0.2	25
	0.1	20
	0.05	15
	0.025	13

Table 2 presents the antibacterial activity of *Ficus sycomorus* ethanol extracts, showing inhibition zones ranging from 10 to 25 mm. Specifically, *Escherichia coli* exhibited inhibition zones between 13 and 25 mm, while *Salmonella typhi* and *Pseudomonas aeruginosa* had zones between 10 and 16 mm. The ethanol extract displayed the most effective inhibitory performance. However, the extracts did not inhibit *Escherichia coli*, a multi-resistant bacterium, but were effective against *Salmonella typhi* and *Pseudomonas aeruginosa*. A zone of inhibition measuring 16 mm or more is generally associated with microbial susceptibility [24,25]. Therefore, this study confirms the antibacterial potential of the ethanol extract from *Ficus sycomorus*. Considering the findings by [24,25], the aqueous extract exhibited weak inhibitory activity as shown in table 3. The result in **Table 3** indicates the zone of inhibition in the range of 12 to 28 mm.

Table 3: Antibacterial activity of aqueous bark extract of *Ficus sycomorus* (Zone Inhibition)

Name of organisms	Conc. of extract (mg/ml)	Zone of inhibition(mm)
Salmonella typhi	0.2	28
	0.1	22
	0.05	17
	0.025	12
Pseudomonas aeruginosa	0.2	27
	0.1	24
	0.05	22
	0.025	19
Escherichia coli	0.2	24
	0.1	21
	0.05	17
	0.025	14

The efficacy of the antimicrobial agent of the extracts is represented in Table 4 in (µg/ml)

Table 4: Minimum inhibitory concentration (MIC) of bark of *Ficus sycomorus*

Organisms	Ethanol extract (µg/ml)	Aqueous extract (µg/ml)
Escherichia coli	500	500
Pseudomonas aeruginosa	500	250
Salmonella typhi	250	250

Tables 5 and 6 reveal the observation of bark extract of ethanol and aqueous of *Ficus sycomorus*. The duo has shown that in some concentrations (mg/ml) of the extract, colonies are observed while in some, no colony was observed.

Table 5: Ethanolic observation of bark extract of *Ficus sycomorus*

Name of organisms	Conc of extract (mg/ml)	Observation
Escherichia coli	0.2	No Colony observed
	0.1	No Colony observed
	0.05	Colony observed
	0.025	Colony observed
Pseudomonas aeruginosa	0.2	No Colony observed
	0.1	Colony observed
	0.05	Colony observed
	0.025	Colony observed
Salmonella typhi	0.2	No Colony observed
	0.1	No Colony observed
	0.05	No colony observed
	0.025	Colony observed

Table 6: Aqueous Observation of bark extract of *Ficus sycomorus*

Name of organisms	Conc of extract (mg/ml)	Observation
Escherichia coli	0.2	No Colony observed
	0.1	No Colony observed
	0.05	Colony observed
	0.025	Colony observed
Pseudomonas aeruginosa	0.2	No Colony observed
	0.1	No Colony observed
	0.05	Colony observed
	0.025	Colony observed
Salmonella typhi	0.2	No Colony observed
	0.1	No Colony observed
	0.05	No colony observed
	0.025	Colony observed

Table 7: MBC Result

Organisms	Ethanolic extract (µg/ml)	Aqueous extract (µg/ml)
Pseudomonas aeruginosa	2000	1000
Salmonella typhi	500	500
Escherichia coli	1000	1000

The results for the minimum bacterial concentration (MBC) are shown in Table 7. The lowest concentration necessary for an antimicrobial agent to eradicate a bacterium is known as the minimum bacterial concentration.

3.2 Discussion

Plants are a valuable source of potential compounds for developing new antimicrobial agents. Numerous studies highlight the antimicrobial properties of various plant species. These findings can aid in identifying the bioactive compounds responsible for the activity, paving the way for the development of new therapeutic drugs [7,9,26]. The preliminary phytochemical screening of the plant extract showed that steroids were not detected in all the extracts. However, only flavonoids were present in the aqueous extract but not as much as in the methanolic extract. Tannins were present in small quantities in the aqueous extract and higher in the ethanol extract. Both alkaloids and reducing sugar are found present in the aqueous extract while absent in the methanolic extract, glycosides, terpenoids and saponins were present. The above findings correspond to that of sandabe et al [20] and Ibrahim et al [27]. The presence of flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radicals. compounds such as alkaloids, saponins and steroids which are used as anti-inflammatory and anti-oxidant agents [28].

The ethanolic and aqueous extract of bark of *Ficus sycomorus* showed activity against all the tested bacteria, that is *Salmonella typhi*, *Escherichia coli* and *Pseudomona aeruginosa*. The aqueous extract possessed a moderate inhibitory effect against *Salmonella typhi* (28 – 12 mm) followed by *Pseudomona aeruginosa* and *Escherichia coli*. The ethanolic extract shows comparatively less activity against the tested bacteria. The result indicated that the bark extract showed anti-bacterial activities at variable degrees against the tested bacteria, with MIC values ranging from 250 – 500µg/ml. the present study indicated that *Salmonella typhi* was more sensitive to the bark extract of *Ficus sycomorus* with a MIC value of 250µg/ml. Therefore, the antimicrobial properties of these extracts lend credence to the plant's historic medicinal use in the treatment of infectious disorders. These findings tally with the work of Magbool et al [29] that revealed the extract of *Ficus sycomorus* demonstrated strong antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Candida albicans*. Khan and co-workers [30,31], however, reports the opposite activity, indicating a weak inhibition against gram-positive bacteria [31].

4. Conclusion

In summary, the plant extracts showed efficacy against every tested bacterium. The findings are promising enough to warrant additional investigation into these extracts in order to identify and isolate the bioactive substances that are causing the action. Thus, it has been confirmed that traditional healers can use this plant to cure a variety of illnesses. However, more research on the plant's toxicity and dosage is soon to be required.

Conflict of interest

The authors declare no conflict of interest.

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