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PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF STEM BARK AND LEAVES EXTRACTS FROM FICUS SYCOMORUS

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ABSTRACT

Introduction: Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. **Materials and methods:** Ethanolic extracts prepared from stem bark and leaves of *ficus sycomorus* as well as the bioactive compounds screened from these crude extracts, were tested for their antimicrobial activity against some gram –ve bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) , and gram +ve bacterial (*Bacillus subtitis*, *Staphyloccus aureus*) and two fungal species (*Aspergillus nigor*, *Candida albicans*) using agar diffusion method. **Results:** The study indicated that the crude extract was active against all pathogens tested and having broad spectrum of activity against bacteria and fungi, whereas screened compounds showed selective activities. These results provide promising baseline information for the potential use of these crude extracts as well as some of the phytochemicals screened secondary metabolites in the treatment of some bacterial and fungal infections.

KEYWORDS: Ficus sycomorus, antimicrobial activity, phytochemical screening, bioactive compounds, agar diffusion method.

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.[1] Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different part of the world. Medicinal plants were used as excellent antimicrobial agents because it possess a variety of chemical constituent is nature recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species. In recent years, secondary plant metabolites (Phytochemicals) previously with unknown pharmacological activities have been extensively investigated as source of pharmaceutical agents. Now a day's multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases.[2,3] In addition to this problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, immune-suppression and allergic reactions.[4] This situation forced scientists to search for new antimicrobial substances. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants .[5,6] There are several reports on the antimicrobial activity of different herbal and plants extracts in different regions of the world's.[7,8] A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance.[9] The medicinal value of plants lies in some chemical substances (secondary metabolites) that produce a definite physiological and/or pharmacological actions on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, cumarines and phenolic compounds.[10]

Ficus is a genus of about 800 species and 2000 varieties of *Ficus* of woody trees, shrubs and vines in the family

Moraceae occurring in most tropical and subtropical forests worldwide. It is collectively known as *fig* trees and the most well-known species in the genus is the common *Fig*, which produce commercial fruit called *fig*. It grows to 50 ft tall, but more typically to a height of 10 – 30ft. *Ficus sycomorus*, which has been known as the biblical *sycamore fig*,[11] is distributed from the African continent in the south to the Arabian Peninsula[12] in riverine forests of arid and semi-arid areas. Various secondary metabolites which constitute an important source of the pharmaceutical drugs have been isolated from different parts of plants. Some of these compounds have been reported to be present in the *Ficus species* such as tannins, saponins, flavonoids, steroids, anthraquinone, glycosides and reducing sugars.[13,14] Phytochemical investigations of some *Ficus species* revealed that phenolic compounds as their major components.[15] Considering the enormous potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents. *F.sycomorus* is used traditionally to treat fungal diseases, jaundice and dysentery, and also used in the treatment of snake bites, chest pains, cool, coughs and throat infections.[16] The stem bark extract partially inhibits microbial growth.[17] *Ficus sycomorus* have been suspected to possess anti-diarrheal activities.[18] The sedative and anticonvulsant properties of this plant have also been reported.[19] The plant has also been reported to be a potent antimicrobial agent against ciprofloxacin resistant *Salmonella typhi*.[20] Many studies reported antifungal activity of stem bark extract of *F.sycomorus* on some fungal isolates causing deep and superficial infections. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. In the continuation of this strategy of new drug discovery we have studied antimicrobial activity of alcoholic extract of *ficus sycomorus*.

MATERIALS AND METHODS

Plant material collection and identification

The stem bark and the leaves of *F. sycomorus* were collected. The plant was identified by a taxonomist at medicinal and aromatic plants institute, National Center for Research - Khartoum, Sudan. The stem bark was then ground into powder and was used for the subsequent experimentation.

Reagents

Chloroform (SD Fine India), Ferric Chloride (BDH England), Acetic anhydride (SD Fine England), Sulphuric acid (SD Fine India), Hydrochloric acid (Romile EU), Alumminum Chloride (BDH England),Potassium Hydroxide (Sharlau Spain),Hydrogen Peroxide (Sharlau Spain), Ammonium Hydroxide (SD Fine India), Benzene (Sharlau Spain), Sodium Chloride (Sharlau Spain), Gelatin salt (Sharlau Spain), Potassium chloride (BDH England), Mercuric iodide (BHD England), Ethanol (National Distillation Company).

Preparation of extract

200 g of the plant sample was shade dried grounded using mortar and pestle and extracted by soaking in 80 % ethanol for about seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extracts were combined together. Extracts allowed to air till complete dryness and the yield percentages were calculated as followed:

Weight of extract / weight of sample * 100

Table 1: Yield % of extracts.

Sample	Weight of sample	Weight of extract	Yield %
Leaves	200 g	18..42 g	9.21 %
Bark	200 g	24.56 g	12.18 %

Figure 1: Image showing the leaves and Bark extracts.

Figure 2: Rotary Evaporator apparatus.

1. Phytochemical screening[22,23,24,25,26]

1.1 Identification of tannins

0.5 g of each extract dissolved in 10 ml hot saline solution and divided in two tests tubes. To one tube 2-3 drops of ferric chloride added and to the other one 2 – 3 drops of gelatin salts reagent added. The occurrence of a blackish blue colour in the first test tube and turbidity in the second one denotes the presence of tannins.

1.2 Test of sterols and triterpenes

0.5 g each extract was dissolved in 10 of chloroform. To 5 ml of the solution, 0.5 ml acetic anhydride was added and then 3 drops of conc. Sulphuric acid at the bottom of the test tube. At the contact zone of the two liquids a The

gradual appearance of green, blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and or triterpenses (pink to purple) in the sample.

1.3 Test for Alkaloids

0.5 g of each extract was heated with 5 ml of 2N HCL in water bath and stirred for about 10 minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent was added while to the other tube few drops of Valser's reagent was added. A slight turbidity or heavy precipitate in either of the tow test tubes was tanked as presumptive evidence for the presence of alkaloids.

1.4 Tests for Flavonoids

0.5 g of each extract was dissolved in 30 ml of 80% ethanol. The filtrate was used for following tests: 3 ml of the filtrate in a test tube 1ml of 1% aluminum chloride solution was in methanol was added. Formation of a yellow color indicated the presence of Flavonoids. Flavones or and chalcone.

1.5 Test for Saponins

0.3 g of each extract was placed in a clean test tube. 10 ml of distilled water was added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for least an hour, was taken as evidence for presence of saponins.

1.6 Test for Cumarins

0.5 g of each extract dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH put on it. Then the filter paper was inspected under UV light, the presence of coumrins was indicated if the spot have found to be adsorbed the UV light.

1.7 Test for Anthraquinone glycosides

0.5 g of each extract was boiled with 10 ml of 0.5N KOH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5ml of the benzene solution was shacked with 3ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline layer was found to have assumed pink or red color.

2. Assessment of antimicrobial activity and bioassays (Preparation of the test organisms and Preparation of the standard bacterial suspensions)

One ml aliquots of a 24-hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37○C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108 -109 colony forming units (CFU) per ml. The suspension was stored in the refrigerator at 4○C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique.[27,28] Serial dilutions of the stock suspensions were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette to the surface of dried nutrient agar plates. The plates were allowed to stand for 2-hours at room temperature for the drops to dry, sand then incubated at 37○C for 24 hours .After incubation, the number of developed colonies in each drop was counted. The average number of the colonies per drop (0.02ml) was multiplied by 50 and by the dilution factor to give the viable count of stock suspensions, expressed as the number of colony forming unit per ml of suspension. Each time of fresh stock suspension will be prepared, all the above experimental condition will be maintained constant so that suspensions with very close viable count would be obtained.

3. Preparation of standard fungal organisms

The fungal standard cultures were obtained from the department of Microbiology and Parasitology, Medicinal and Aromatic Plant Research Institute, Khartoum and were maintained on Sabouraud dextrose agar, incubated at 25○C for 4 days. The fungal growth were harvested and washed with sterile normal saline and finally suspended in 100 ml of sterile normal saline and the suspension was stored in refrigerator till used.

4. In vitro testing of sycomorus extract for antimicrobial activity

4.1 Testing for antibacterial activity

The cup-plate agar diffusion method[29] was adopted, with some minor modifications; to assess the antibacterial activity of the prepared extracts (NCCLS 2000). In accordance with this method one ml of the isolated standardized and bacterial stock suspension (108-109 C.F.U per ml) were thoroughly mixed with 100 ml of sterile molten Mueller- Hinton agar which was maintained at 45○C. Twenty ml aliquots of the inoculated Mueller-Hinton agar were distributed onto sterile Petri-dishes. The agar was left to set, and in each of these plates, four cups (10 mm in diameter) were cut using a sterile cork borer (NO.4) and the agar discs were removed. Alternate cups were filled with 100µL of samples of each of the extract, using standard fine adjustable automatic pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position, at 37○C for 18 hours. Two replicates were carried out for each extract against each of the tested organisms. Simultaneously, positive control involving the addition of methanol instead of the extract was included. Upon the completion of incubation the diameter of the resultant inhibition zones were measured, averaged and then the mean values were tabulated.

4.2 Testing for antifungal activity

The same method as for antibacterial activity was used. Sabouraud dextrose agar was used instead of nutrient agar. The inoculated medium was incubated at 25○C for

three days for the *Aspergillus niger* and two days for *Candida albicans*.

4.3 Determination of Minimum Inhibitory Concentrations (MIC) by agar well dilution method

F. sycomorus extract was prepared in the series of decreasing concentrations in the following order 50, 25, 12.5 and 6.25 mg / ml. MIC is the least concentration of antimicrobial agent that completely inhibits the growth. Results were reported as MICs.

5. Antifungal activity of reference drugs against standard microorganisms

5.1 Antifungal activity of reference drugs

The antifungal drugs were also tested at different concentrations obtained by taking 0.1 g of each powdered drug and dissolved in 100 ml sterile distilled water to give a concentration of 1000 µg/ml followed by serial dilutions to give concentrations of 12.5, 25 and 50 µg/ml Nystatin against reference fungi *Candida albicans* and *Aspergillus niger* using 5, 10 and 20 µg/ml Clotrimazole against the same organisms.

RESULTS AND DISCUSSION

Table 2: Phytochemical screening of *ficus sycomorus* extracts.

Test	Leaves	Bark	Observation
Saponins	+	++	Foam
Cumarins	++	++	UV absorption
Alkaloids	-	-	No observation
Anthraquinones	-	+++	Pink colour
Tannins	++	+++	Green colour
Flavonoids	++	++	Yellow color
Sterols	+++	+++	Green / blue colour
Triterpenes	+	++	Purple colour

Table 3: Antimicrobial activity of the two extract against Standard Organisms using (100 mg/ml).

Part Used	Solvent	Standard tested organisms* /M.D.I.Z (mm)**					
		B. s	E. c	Ps. A	S. a	C. a	A. n
Leaves	Ethanol	14	13	14	13	12	12
Stem	Ethanol	21	20	20	31	14	13

*Standard organisms tested: *B.S. = Bacillus subtilis, S.a. =Staphylococcus aureus, E.c. = Escherichia coli, Ps.a. = Pseudomonas aeruginosa, A.n= Aspergillus niger, C.a= Candida albicans.*

Table 4: Antifungal activity of reference antibiotics against standard microorganisms.

S. No	Drugs	Concentrations (µg/ml)	Standard microorganisms used MDIZ* (mm)	
			Tested fungi used (M. D. I. Z mm)	
			A. n	C. a
	Clotrimazole	40	30	42
		20	22	40
		10	19	33
		5	16	30
4	Nystatin	50	28	17
		25	26	14
		12.5	23	-

An investigation on the phytochemical screening of *ficus sycomorus* stem bark and leaves extracts revealed the presence of medicinally active constituents. The phytochemical active compounds of *ficus sycomorus* were screened and the results are presented in table 2. In analysis of tannin compounds the occurrence of a blackish blue colour in the first test tube and turbidity in the second one denotes the presence of tannins. Similarly based on the presence or absence of colour change indicate positive and negative results are indicate. In these screening process tannins, saponins, sterols, anthraquinones, cumarine, triterpenes and flavonoids gave positive results and alkaloids gave negative results. The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. These secondary metabolites exert antimicrobial activity through different mechanisms. Plant phenolic compounds especially flavonoids are currently of growing interest owning to their supposed properties in promoting health (anti-oxidants),[30] Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets

and macrophages.[31] Tannins are reported to possess physiological astringent and haemostatic properties, which hasten wound healing and ameliorate inflamed mucus membrane and also inhibit the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them; they form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. They have important roles such as stable and potent anti-oxidants,[32,33,34] They act as binders and for treatment of diarrhea and dysentery.[35] Tannins also reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectivity and is also used as diuretic.[36] Plant tannin has been recognized for their pharmacological properties and is known to make trees and shrubs a difficult meal for many caterpillars.[37] A large number of studies have been done in recent years on the antifungal and antibacterial activity of terpenoids of natural origin. The mechanism of action of triterpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic nature. Coumarins have been reported to stimulate macrophages which could have an indirect negative effect on infections. Plant steroids are known to be important for their cardiotonic, insecticidal and anti-microbial properties. They are also used in nutrition, herbal medicines, cosmetics and they are routinely used in medicine because of their profound biological activities.[38] Saponins have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiotonic in nature and are reported to have anti-diabetic and anti-fungal properties.[39,40] They are stored in plant cells as inactive precursors but are readily converted into biological active antibiotics by enzymes in response to pathogen attack. Extracts of the plant tested showed varying degree of antibacterial and antimycotic activities against the test organisms (Table.3). The antibacterial activities of the ethanol extracts compared favourably with that of standard antibiotic and have appeared to be broad spectrum as its activities were independent on gram reaction. The inhibition zone for *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans* of the two extracts (stem and leaves extracts) against Standard Organisms using concentration of 100 mg/ml are illustrated bellow (Table 3). The stem extract showed high antibacterial and antifungal activities with inhibition zones ranging between 14 and 31 mm for different microorganisms tested, and the leaves extract showed low antimicrobial activity with inhibition zones ranging between 12 and 14 mm for different bacteria and fungi tested. The inhibition zone for *aspergillus nigor* and *candida albicans* was much less as compared to bacteria, and inhibition zone for stem extracts showing greatest effectiveness than leaves extracts for both bacteria and fungi. Along all tested bacteria the stem extract was found to be more effective against *Staphylococcus aureus* than the other tested organisms.

CONCLUSION

This study has revealed the presence of many secondary metabolites in stem and leaves of *ficus sycomorus* it has further confirmed that the plant extracts could be used for various infections including bacterial and fungal infections the results lend credence to the folkloric use if this plant in treating microbial infections and shows that *ficus sycomorus* could be exploited for new potent antimicrobial agents.

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