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Phytochemical and Bioassay Guided Antimicrobial Evaluation of Methanol Leaf Extract of *Ficus sycomorus* Linn (Moraceae)

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Abstract This research investigated *in-vitro* antimicrobial activity and phytochemical constituents of *Ficus sycomorus* Linn (morecae) leaves. 700 g of dry pulverized *Ficus sycomorus* leaves were extracted with methanol using soxhlet extractor and a gummy dark green mass of 124.8 g crude extract was obtained. The crude methanol extract (80 g) was fractionated through column chromatography and four pooled fractions (A to D) were obtained weighing 3.25, 3.50, 1.00 and 4.52 g respectively. The preliminary phytochemical evaluation was carried out using standard methods of analysis and this investigation revealed the presence of alkaloids, carbohydrates, tannins, cardiac glycoside, cardinolides, saponins, terpenoids and flavonoids. Anthraquinones and combine anthraquinones were absent. The antimicrobial activity of the plant extracts was assayed using the agar plate disc diffusion and nutrient broth dilution techniques. The extracts were subjected for antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae* and *Candida albicans*. The crude extract inhibited the growth of all the tested organisms at different concentrations especially *Salmonella typhi* and *Streptococcus pyogenes* which had mean inhibition zone of 22.00±0.00 mm and 21.23±0.58 mm respectively. Among the column fractions, fraction C the most active, exhibited broader activity on the tested organisms with highest activity on *Streptococcus pyogenes* and *Escherichia coli* having inhibition zones of 26.00±0.00 mm and 27.00±0.00 mm respectively. The crude extract was susceptible to *Salmonella typhi* with MIC and MBC of 50 mg/mL and 100 mg/mL respectively. And to *Streptococcus pyogenes*, the crude extract was bactericidal at 100 mg/mL. The outcome of this study offers support to the Ethno-medicinal uses of *Ficus sycomorus* in the treatment of various ailments.

Keywords Phytochemical, *Ficus sycomorus*, Antimicrobial, Ethno-medicinal, phytochemicals

Introduction

The dependence of humans and animals on vegetation for food, shelter, oxygen and medicine is as ancient as humanity itself; hence the common saying that "all flesh is grass" cannot be disputed. Nature has been a potential source of therapeutic agents for thousands of years, and the use of natural products, especially plants for healing is as ancient and universal as medicine itself [1]. Thus, medicinal plants which form the backbone of traditional medicine have in the last few decades been the subject of pharmacological studies. *Ficus sycomorus* belongs to moraceae, a family that is reputable for its medicinal value and consist of about 40 genera and over 1,400 species of

trees, shrubs, vine and herbs, often with milky latex juices [2]. It is a common savannah tree that grows in high water table areas, and found along watercourses such as streams, rivers, swamps and waterholes. The leaves of *Ficus sycomorus* are broadly ovate with base cordate and apex rounded. Their petioles are 1-5 cm in length with 5-7 pairs of yellow lateral veins. The figs are arranged in leaf axils or on leafless branches up to 10 cm in length. They are solitary or paired, globose in shape and ranges in color between yellow-red to reddish purple when ripped. Their seeds are numerous, round and very tiny in nature. *Ficus sycomorus* is reported to have many traditional medicine uses in the treatment of snake bites, jaundice, chest pain, dysentery, cool, coughs and throat infections [3]. *Ficus sycomorus* roots have laxative and anthelmintic properties. It finds relevance in the treatment of diabetes mellitus and other infectious diseases in the Northern part of Nigeria [4]. The plant has been reported to be a potent antimicrobial agent against ciprofloxacin resistant *Salmonella typhi* [5]. The *in Vitro* antimicrobial screening of the methanol root-bark extract of *Ficus sycomorus* revealed that the extract exhibited varying activity against *Enterococcus faecalis, Escherichia coli, Salmonella typhi, Shigella dysenteria* and *Candida albicans* [6]. (Also, aqueous extract of stem bark exhibits sedative and muscular activities [7]. It is therefore, imperative to screen the said part of the plant against some selected pathogenic organisms responsible for such diseases.

Materials and Methods

Plant collection and Identification

Fresh leaves of *Ficus sycomorus* were collected from Alau-Dam, Jere Local Government Area of Borno State, Nigeria. The herbarium specimen was identified by a plant taxonomist from the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria. Specimen voucher number 8012B was allocated to the plant material and deposited for reference.

Extraction and preparation of plant extract

The plant leaves were air-dried under shade and care was taken to render it free of foreign materials through manual picking. The dried leaves were pulverized using wooden mortar and pestle. Seven hundred grams (700 g) of the pulverized sample material were extracted with absolute methanol using soxhlet extractor and 124.8 g crude extract was obtained.. The crude extract was concentrated over a water-bath and then exposed to air at 25^0C to dryness. The dried extract was weighed, labeled and stored in a desiccator. The crude methanol extract (80 g) was fractionated through column chromatography and four pooled fractions (A to D) were obtained weighing 3.25, 3.50, 1.00 and 4.52 g respectively.

Phytochemical Evaluation

The crude leaf extract and its column fractions were evaluated qualitatively for phytochemical constituents utilizing standard methods of analysis [3, 8-9]. This investigation revealed the presence of alkaloids, carbohydrates, tannins, cardiac glycoside, cardinolides, saponins, terpenoids and flavonoids. Anthraquinones and combine anthraquinones were absent.

Microorganisms

Test microorganisms were *Escherichia coli, Shigella dysenteriae, Candida albicans, Staphylococcus aureus, Streptococcus pyogenes* and *Salmonella typhi.* All the organisms were clinical isolates obtained from Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri. The organisms were stored at 2-8 ºC until required. Standard antibiotics (Ciprofloxacin 5 µg/disc and Erythromycin 15 µg/disc) were used as positive controls.

Preparation of Sample Extract for Pathogenic Assay

Stock solution of the extract was prepared by dissolving 10 g of extract in 10 mL of DMSO, and 1000 mg/mL solution was obtained. Working solutions of the extracts (both the crude and the various pooled fractions obtained

during column chromatography) were prepared at varying concentrations ranging from 400, 200, 100, 50, and 25 mg/mL using DMSO as the solvent of dissolution.

Susceptibility Activity

The sensitivity of the test organisms to the crude extract and the various column fractions were carried out using Agar disc diffusion method. The agar plates inoculated with test organisms were used in these assays. Antimicrobial disks were prepared by punching Whattman filter papers with a paper punching machine and the disks were autoclaved at 160 °C for 15 mins to sterilize them. Thereafter, the disks were impregnated with the various prepared concentrations of the extracts by the use of micropipettes. A sterile swap was used to inoculate evenly the test specie on the surface of the medium. The plates were allowed to dry for about 5 mins. Flame sterilized forceps were used to place the impregnated disk on the surface of the agar. The disks were placed at equal distances apart on the surface of the agar. The disks were also gently pressed onto the surface of the agar using a sterile forcep. The plates were inverted to dry and incubate for 24 hours at 37 °C. A measure of 0.5mL of pure solvent used in the extraction was used as negative control, and 0.5mL of 5µg/mL solution of Ciprofloxacin and 0.5 mL of 15µg/mL solution of Erythromycin were used as positive controls. Using caliper, the diameter of the zone of inhibition(s) was measured for each extract concentration used. Antibacterial activity was evaluated by the diameters of zones of growth inhibition in triplicates and results presented as Mean±SEM [10].

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined by the broth dilution method. The stock solution of the extracts was diluted to 400, 200, 100, 50, and 25 mg/mL respectively in nutrient broth. Duplicate tubes of each dilution were inoculated with 1mL of test organism and incubated at 37^0C for 24hrs. Solutions of Ciprofloxacin (5µg/mL) and Erythromycin (15µg/mL) were included in each experiment as positive controls, while pure solvent used for extraction was used as negative control. The minimum inhibitory concentration was taken as the lowest concentration of extracts that did not permit any turbidity.

Determination of Minimum Bactericidal Concentration (MBC)

A measure of 2 mL of inoculated culture was taken from each of the broth tubes that showed no turbidity in the minimum inhibitory concentration assay and inoculated onto fresh agar plates. These were incubated at 37 ºC for 24hrs. The plates were observed for growth, and the lowest concentration that shows no growth was recorded as the minimum bactericidal concentration.

Results

The results of the phytochemical evaluation of crude extract of *Ficus sycomorus* leaves is as presented in Table 1. The extract and the pooled column fractions were further subjected to antimicrobial studies. The susceptibility pattern against the test organisms is shown in Table 2-7. Meanwhile, the minimal inhibitory concentration and minimal bactericidal concentration are presented in Table 8 and Table 9 respectively.

Table 1: Phytochemical analysis of leaf extract and pooled column fractions of *Ficus sycomorus*

S/N	Secondary Metabolites	Crude	F_A	F_B	F_C	F_D
1	Alkaloids					
	a. Dragendorff's	+	-	+	+	-
	b. Mayer's	+	-	+	+	-
2	Carbohydrates					
	a. Molisch's	+	+	+	-	+
	b. Barfoed's	+	+	+	-	+
3	Flavonoids					
	a. Ferric chloride	+	-	+	+	-
	b. Sodium Hydroxide	+	-	+	+	-
4	Tannis					

	a. Lead Acetate	+	+	-	+	-
	b. Ferric Chloride	+	+	-	+	-
5	Reducing Sugar	+	+	-	-	+
6	Cardiac Glycosides	+	-	+	+	-
7	Saponins	+	+	+	-	+
8	Steriods	+	-	+	-	+
9	Terpenoids	+	-	+	+	-
10	Anthraquinones	-	-	-	-	-

Key: F_A= fraction A, F_B= fraction B, F_C= fraction C, F_D= fraction D, present = +, absent = -

Table 2: Sensitivity pattern of the crude extract and pooled column fractions against *Staphylococcus aureus*

Concentration (mg/disc)	**Inhibition zone (mean±sem)**						
	Crude	**F_A**	**F_B**	**F_C**	**F_D**	**CIP (5µg/disc)**	**ERY (15µg/disc)**
200	10.00±0.00	13.00±0.00	14.00±0.00	16.00±0.00	0.00±0.00	30.00±0.00	24.67±0.58
100	0.00±0.00	10.00±0.00	12.67±0.58	13.00±0.00	0.00±0.00		
50	0.00±0.00	0.00±0.00	7.00±0.00	9.00±0.00	0.00±0.00		
25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00		

Key: F_A= column fraction A, F_B= column fraction B, F_C= column fraction C, F_D= column fraction D, CIP= Ciprofloxacin, ERY= Erythromycin

Table 3: Sensitivity pattern of the crude extract and pooled column fractions against *Streptococcus pyogenes*

Concentration (mg/disc)	**Inhibition zone (mean±sem)**						
	Crude	**F_A**	**F_B**	**F_C**	**F_D**	**CIP (5µg/disc)**	**ERY (15µg/disc)**
200	21.23±0.58	12.00±0.00	7.00±0.00	26.00±0.00	0.00±0.00	25.33±0.58	20.67±0.58
100	18.00±0.00	00.00±0.00	0.00±0.00	20.00±0.00	0.00±0.00		
50	13.67±0.58	0.00±0.00	0.00±0.00	13.00±0.00	0.00±0.00		
25	9.33±0.58	0.00±0.00	0.00±0.00	8.67±0.58	0.00±0.00		

Key: F_A= column fraction A, F_B= column fraction B, F_C= column fraction C, F_D= column fraction D, CIP= Ciprofloxacin, ERY= Erythromycin

Table 4: Sensitivity pattern of the crude extract and pooled column fractions against *Escherichia coli*

Concentration (mg/disc)	**Inhibition zone (mean±sem)**						
	Crude	**F_A**	**F_B**	**F_C**	**F_D**	**CIP (5µg/disc)**	**ERY (15µg/disc)**
200	15.00±0.00	0.00±0.00	0.00±0.00	27.00±0.00	0.00±0.00	23.67±0.58	11.33±0.58
100	11.67±0.58	00.00±0.00	0.00±0.00	21.00±0.00	0.00±0.00		
50	7.00±0.00	0.00±0.00	0.00±0.00	15.00±0.00	0.00±0.00		
25	0.00±0.00	0.00±0.00	0.00±0.00	10.33±0.58	0.00±0.00		

Key: F_A= column fraction A, F_B= column fraction B, F_C= column fraction C, F_D= column fraction D, CIP= Ciprofloxacin, ERY= Erythromycin

Table 5: Sensitivity pattern of the crude extract and pooled column fraction against *Salmonella typhi*

Concentration (mg/disc)	**Inhibition zone (mean±sem)**						
	Crude	**F_A**	**F_B**	**F_C**	**F_D**	**CIP (5µg/disc)**	**ERY (15µg/disc)**
200	22.00±0.00	15.00±0.00	0.00±0.00	20.00±0.00	0.00±0.00	28.00±0.00	7.00±0.00
100	20.00±0.00	12.00±0.00	0.00±0.00	17.00±0.00	0.00±0.00		
50	15.00±0.00	8.67±0.58	0.00±0.00	13.00±0.00	0.00±0.00		
25	9.67±0.58	0.00±0.00	0.00±0.00	8.67±0.58	0.00±0.00		

Key: F_A= column fraction A, F_B= column fraction B, F_C= column fraction C, F_D= column fraction D, CIP= Ciprofloxacin, ERY= Erythromycin

Table 6: Sensitivity pattern of the crude extract and pooled column fractions against *Shigella dysenteriae*

Concentration (mg/disc)	Inhibition zone (mean±sem)						
	Crude	F_A	F_B	F_C	F_D	CIP (5µg/disc)	ERY (15µg/disc)
200	18.00±0.00	0.00±0.00	0.00±0.00	21.00±0.00	0.00±0.00	30.67±0.58	28.33±0.58
100	15.00±0.00	0.00±0.00	0.00±0.00	17.67±0.58	0.00±0.00		
50	10.00±0.00	0.00±0.00	0.00±0.00	13.33±0.58	0.00±0.00		
25	0.00±0.00	0.00±0.00	0.00±0.00	9.00±0.00	0.00±0.00		

Key: F_A= column fraction A, F_B= column fraction B, F_C= column fraction C, F_D= column fraction D, CIP= Ciprofloxacin, ERY= Erythromycin

Table 7: Sensitivity pattern of the crude extract and pooled column fractions against *Candida albicans*

Concentration (mg/disc)	Inhibition zone (mean±sem)						
	Crude	F_A	F_B	F_C	F_D	CIP (5µg/disc)	ERY (15µg/disc)
200	9.67±0.58	13.00±0.00	0.00±0.00	13.00±0.00	0.00±0.00	32.00±0.00	32.00±0.00
100	0.00±0.00	10.33±0.58	0.00±0.00	10.00±0.00	0.00±0.00		
50	0.00±0.00	8.00±0.00	0.00±0.00	7.00±0.00	0.00±0.00		
25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00		

Key: F_A= column fraction A, F_B= column fraction B, F_C= column fraction C, F_D= column fraction D, CIP= Ciprofloxacin, ERY= Erythromycin

Table 8: Minimum inhibitory concentrations of the crude extract of *Ficus sycomorus*

Organism	400mg/mL	200mg/mL	100mg/mL	50mg/mL	25mg/mL
Staphylococcus aureus	-	β	+	+	+
Streptococcus pyogenes	-	-	β	+	+
Escherichia coli	-	-	β	+	+
Salmonella typhi	-	-	-	β	+
Shigelladysenteriae	-	-	β	+	+
Candida albicans	-	β	+	+	+

Key: positive (+) means turbid or cloudy, Negative (-) means not turbid, β means minimum inhibition concentration

Table 9: Minimum bactericidal concentrations of the crude extract of *Ficus sycomorus*

Organism	400mg/mL	200mg/mL	100mg/mL	50mg/mL	25mg/mL
Staphylococcus aureus	α	-	+	+	+
Streptococcus pyogenes	-	-	α	+	+
Escherichia coli	-	α	-	+	+
Salmonella typhi	-	-	α	-	+
Shigelladysenteriae	-	α	-	+	+
Candida albicans	α	-	+	+	+

Key: positive (+) means turbid or cloudy, Negative (-) means not turbid, α means minimum bactericidal concentration

Discussions

One hundred and twenty four point eight grams (124.8 g) of crude was recovered from 700 g of pulverized *Ficus sycomorus* leaves. It was dark army green in colour and gummy in texture. Eighty grams (80 g) of the extract was subjected to column chromatography and four pooled fractions were obtained. The phytochemical test of the crude methanol leaf extract of *Ficus sycomorus* revealed the presence of Alkaloids, Flavonoids, Tannins, Cardiac glycosides, Saponins, Steriods, Terpenoids and Carbohydrates. Anthraquinone and combine Anthraquinone were found to be absent. The *in-vitro* antimicrobial test presented in Tables 2-7 showed the susceptibility activity against Gram positive, Gram negative and Fungi organisms. Evaluation of the antimicrobial activity of methanol leaf

extract and the column fractions of *Ficus sycomorus* revealed that the crude extract and fraction F_C were susceptible to all the selected microorganisms' tested, while fractions F_A, F_B and F_D were susceptible to some organisms and had no activity on others. The diameter zones of inhibitions for methanol crude extract were found to be within the range of 9.33±0.58 to 21.23±0.58, 7.00±0.00 to 22.00±0.00 and 9.67±0.58 mm for Gram-positive, Gram-negative and fungi specie respectively. The result of the pooled column fractions (F_A to F_D) reveals that the highest susceptibility values expressed by fraction F_A for Gram-positive, Gram-negative and fungi specie were 13.00±0.00, 15.00±0.00 and 13.00±0.00 mm respectively. The highest value observed from fraction F_B for Gram-positive was 14.00±0.00 mm. there was no activity for Gram-negative and fungi specie. Fraction F_C had the highest value of 26.00±0.00, 27.00±0.00 and 13.00±0.00 mm for Gram-positive, Gram-negative and fungi specie. For fraction F_D, there was no activity for all the tested organisms. These differences in zones of inhibition may be directly related to the susceptibility of each organism to the crude extract and the various fractions obtained from column chromatography. The crude methanol extract and pooled column fraction F_C showed moderate to high antimicrobial activity towards all the tested organisms at high concentrations and the activity decreases as the concentration decreases, thus they are concentration dependent. At high concentrations (200 mg/mL above), crude extract and fraction F_C portions insignificantly ($P > 0.05$) differ with Ciprofloxacin and Erythromycin; this is suggestive of the presence of some compounds or groups in the extracts with similar mechanism of action to that of Ciprofloxacin and Erythromycin. The data obtained from crude methanol extract against the tested organisms as MIC and MBC range from 50-200 and 100-400 mg/mL respectively. The crude extract was susceptible to *Salmonella typhi* since it has the least MIC and MBC values of 50 and 100 mg/mL. The crude extract was considered bactericidal on *Streptococcus pyogenes* since both the MIC and MBC values were the same at 100mg/mL. For *Staphylococcus aureus* and *Candida albicans* their MIC and MBC were 200 and 400 mg/mL respectively. The remaining tested organisms (*Escherichia coli* and *Shigella dysenteriae*) have their MIC and MBC at 100 and 200 mg/mL respectively.

Conclusion

The crude extract and fraction Fc showed varying inhibitory activities against all the test organisms. The results are encouraging enough to pursue isolation and characterization of the active compounds from the extract that can serve as possible anti-bacterial agents. Hence, the uses of this part of the plant by the traditional healers for the treatment of the aforementioned diseases have been validated.

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