PHYTOCHEMICAL AND ANTIBACTAERIAL ACTIVITIES OF *FICUS SYCOMORUS* (LINNEAUS) LEAVES AND UNRIPED FRUITS EXTRACTS

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Abstract

Microorganisms are increasingly developing resistance against commonly used antimicrobial agents and plants have provided a good source of antinfective agents. Therefore, there is need to isolate, characterize and standardize the active principles of these herbs for the production of newer and perhaps more effective drugs. Undertaken in this study, were the phytochemical screening of the methanolic extracts of *Ficus sycomorus* unripe fruits and stem-bark for bioactive principle(s) and the antibacterial effects of various solvent extracts against clinical isolates of *Escharichia coli*; *Staphylococcus aureus*, *Pasteurella aeroginosa* and *Bacillus cereus*. The punch hole method was used for the antibacterial screening of the various extracts and Ciprofloxacin as the reference antibacterial agent. The results were evaluated as inhibition zone around the holes impregnated with (100mg/ml) of the various extracts and ciprofloxacin (10 mg/ml) respectively. The phytochemical analysis of the methanolic extracts of the unriped fruits and stem-bark extracts of *F. sycomorus* gave positive results for flavonoids, tannins, alkaloids and glycosides. The zone of inhibition ranged from 3.5 mm – 14.7 mm for the unriped fruits (MEF, HEF and EEF) and 3.7 – 14.2 mm for the stem-bark (MES, HES and EES) extracts respectively. The extracts produced organ related antibacterial effects comparable to ciprofloxacin the reference antibacterial agent. The antibacterial effects observed could be attributed to the glycosides present in the extracts or the combined effects of the various phytochemicals. This study lend pharmacological support to the traditional use of *F. sycomorus* fruits and stem-bark extracts in the management or control of bacterial related diseases in man and his animals.

KEYWORDS: Phytochemical, Antibacterial, Activities, *Ficus sycomorus*, Fruits, Leaves.

Introduction

From the earliest times, man acquired knowledge of the beneficial and adverse effects of plants from observation on animals. The health, growth and productive performance of animals especially livestock are affected by the chemical composition of the plant they consume (Okpara, 2015). Plants are generally studied either for their beneficial or toxicological effects (Nwude, 1997., Kerry *et al*; 2008).

Plants have provided a good source of antiinfective agents with quinine, proberberine and berberine remaining highly effective instruments in the fight against microbial infection (Kwon *et al*. 2008). Phytochemicals such as auriculatin and prehyllentone, eryenegalensein A and O and erybrodin

A elucidated from *Erythrina senegalensis* are reported to have antimicrobial, antiulcerogenic and antidiarrhoeal properties (Okpara, 2015). Olaleye *et al*; (2004), attributed the the antidiarrhoeal, antioxidant and anti-inflammatory properties of the leaves of *Voacarga Africana* to its rich flavonoids contents. Similar reports came from Oyewole (2004) who attributed the antioxidant, anti-inflammatory and antidiarrhoeal effects of

Sclerocarya biruea stem-bark extracts to its rich flavonoids and tannins constituents. The floral biodiversity of Africa provide the African traditional medicine practioners with an impressive "natural pharmacy" from which plants are selected as remedies or as ingredients to prepare herbal medicine for an array of human and animal disorders (Okpara *et al*; 2007).

Ficus sycomorus (Moraceae) is a savannah tree belonging to the mulberry family and genus *Ficus*. It is called wild fig in English and *Baure* in Hausa (Sandabe, 2002). The fruits of the plant are eaten by livestock, wild animals, birds and humans as food (Wakil *et al*; 2016). The stem-bark extracts were reported to be used in folkloric medicine in the treatment of ailments such as mental disorder, dysentery, cough, tuberculosis, helminthosis and poisonous snake envenomation (Wakeel *et al*; 2014). There is paucity of information on the possible antibacterial and antidiarrhoeal properties of the unriped fruits and stem-bark extracts of this plant. This study evaluated the antibacterial activities of different fractions of the fruits and stem-bark extracts of *Ficus sycomorus* using standard protocols.

Materials and Methods

Fresh mature unriped fruits and stem-bark of *Ficus sycomorus* (Linn) were obtained in Vom, Vwang district, Plateau State, Nigeria and duly authenticated by Mr. Sam Shwarpshakka of Agronomy unit, Department of Agricultural Technology, FCAH&PT, Vom. The unriped mature fruits and the stem-bark were separated, each separately air dried for two weeks, subsequently pulverized, sieved and stored in air and water tight containers in a refrigerator at (5^oC) until required.

Extraction

Five hundred gram (500 g) each of powdered unripe fruits and stem-bark of *Ficus sycomorus* were separately extracted with n-hexane, diethyl ether and methanol using Soxhlet apparatus for 6 hours each. The extracts were concentrated *in vacuo* using rotary evaporator at a temperature of 50^oC and then evaporated to dryness in a hot air oven at the same temperature. The methanolic extract of the unripe fruits of *Ficus sycomorus* was labelled MEF, while that of the stem-bark was labelled MES. Similarly, n – hexane and ether extracts of the unripe fruits and stem-bark were labelled HEF, HES, EEF and EES respectively. The dried extracts were stored in air and water tight plastic containers and kept in a refrigerator at 5^oC for subsequent use.

Phytochemical Analysis

Methanolic extracts of both the fruits (MEF) and the stem-bark (MES) were used in the phytochemical screening to test for the presence of alkaloids, flavonoids, tannins, saponnins, resin, anthraquinones and glycosides using the methods described by Sofowora (1985).

Chromatographic Studies

Chromatographic (TLC) studies of n – hexane extracts was carried out using hexane ethyl acetate (3:1) and n – butanol acetic acid-water (4:1:5) solvent mixtures. The separated components were viewed under UV light at wave length 254 nm and 366 nm and the respective R_F values were recorded.

Antibactrial Studies

Bacterial species used in the study

The organisms tested include: *Esherichia coli*, *Staphylococcus aureus* and *Pasteurella aeruginosa* which were clinical specimen and *Bacillus cereus* which was a non-clinical specimen. They were obtained as pure cultures from Diagnostic Division Laboratory, NVRI, Vom using standard methods of Cowan (1974) and Cheesbrough (1991).

Media Preparation

Nutrient agar plates were prepared asceptically according to the manufacturer's instruction

and the streaking done as described by Olawuyi *et al*. (2010).

Antibacterial Sensitivity Testing

The disc diffusion methods as described by Bauer *et al.*, (1996) was used. A uniform concentration of 100 mg/ml was used through-out the procedure so that the relative efficacy of the extracts could be measured. A positive test was indicated by a zone of inhibition of the bacterial growth around the disc. Ciprofloacin® (10 mg/ml) was used as standard antibiotic.

RESULTS

Table I: Results of the phytochemical screening of the fruits and stem-bark extracts of *Ficus sycomorus*.

Components	Alk.	Sap.	Tan.	Resins	Flavonoid	Antq.	Glyco
MEF	+	-	+	-	+	-	+
MES	+	+	+	-	+	-	+

Key: + = Present - = Absent, Alk = Alkaloids, Sap = Saponnins, Anta = Antraquinons, Glyco = glycosides.

Table 2: Antibacterial Activity of the Various Solvent Extracts of unripe Fruits of *Ficus sycomorus*. (n = 3; mean + SEM)

	Organisms			
	E. coli	***S. aureus***	***P. aeroginosa***	***B. cerus***
MEF	14.2 ± 0.01	5.0 ± 0.2	9.5 ± 0.2	8.5 ± 0.1
HEF	4.0 ± 0.04	0.0 ± 0.0	4.2 ± 0.0	3.7 ± 0.0
EEF	0.0 ± 0.0	0.0 ± 0.00	0.0 ± 0.0	4.2 ± 0.1
Ciprofloxacin	8.6 ± 0.3	16.5 ± 0.5	1.12 ± 0.1	9.5 ± 0.2

Table 3: Antibacterial Activity of the Stem-bark Extracts of *Ficus sycomorus*

	Organisms			
	E. coli	***S. aureus***	***P. aeroginosa***	***B. cerus***
MES	14.5 ± 0.2	8.0 ± 0.1	12.6 ± 0.0	9.7 ± 0.1
HES	6.7 ± 0.0	5.2 ± 0.1	0.0 ± 0.0	4.5 ± 0.1
EES	5.0 ± 0.1	3.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Ciprofloxacin	8.6 ± 0.3	14.6 ± 0.5	11.2 ± 0.1	9.5 ± 0.2

Table 4: R_F Values of the Chromatographic (TLC) analysis of the Hexane Extracts of *Ficus sycomorus* Fruits and Stem-bark.

	Solvent System	
	n-butanol-acetic acid-water (5:1:5)	Benzene-ethyl acetate (5:1)
Fraction		
HEF	0.95, 0.08, 0.74	0.60, 0.85 0.80
HES	0.95, 0.05, 0.07	0.75, 0.82 0.80

RESULTS AND DISCUSSION

Flavonoids were detected in both the methaonlic extracts of the unripe fruits and the stem-bark. Flavonoids are the pigments that colour most fruits, flowers and seeds. They are formed in plants and participate in the light dependant phase of photosynthesis during which they catalyze electron transport (Okpara, 2015). Some plant derived flavonoids, such as quercetin, rutin and silymarin are reputed for their antioxidant, anti-inflammatory and antidiarrhoeal effects (Chen *et al*., Okpara, *et al*., 2016). These pharmacological activities of flavonoids are linked to their ability to inhibit

cyclooxygenase and lipooxygenase, which act on arachidonic acid metabolism in cell membrane to form potent inflammatory prostaglandin (Okpara, 2015).

Alkaloids were also present in both extracts. Alkanoids are used as analgesics, stimulants, hallucinogen, and antibacterial agents (Okpara *et al*., 2007). Furthermore, tannins were equally present in both fruit and stem-bark methanolic extracts. Helminthosis is a common disease of animals caused by gastrointestinal nematodes which has been recognized as constituting a major constraint to profitable production of stock animals and birds (Chasisi *et al*., 2003; Lasisi *et al*., 2003). Control of gastrointestinal nematode has traditionally being by the use of chemical anthelmintics. There is no doubt that this has associated problem. The search for suitable and economical means of control has suggested that certain animal fodder and herbs may contain anthelmintic agents. Pronthocyanidin (condense tannins) isolated from some plants have been shown to be capable of limiting helminths protective enzyme activity (Lasisi *et al*., 2003).

Glycosides were found to be present in both MEF and MES. The presence of glycosides lend pharmacological credence to the use of this herb for bacterial related illness such as scouring in calves, diarrhoea, and stomach disorders. A range of antibacterial agents such as streptomycin, neomycin, kenomycin, and gentamycin are glycosidic agents (Aliu, 2007).

The inhibition zone in mm against each bacterium was recorded the highest antibacterial activity was noticed against *Escheridica coli* (14.5 mm) which was sensitive to both the fruit and stem-bark extracts. The least was 3.5 mm which was noticed against *Staphylococcus aureus* in EES. The *B. cerus* and *P. aeroginosa* were also sensitive to the methanolic extracts. The antibacterial activity of the stem-bark extracts in various solvents is presented in Table 3. This indicated that *E. coli* was highly sensitive to the stem-bark extract in methanol whereas all others are insensitive. Comparing the results, that is the fruit and stem-bark in various solvents, the stem-bark extracts were found to be more potent than the unriped fruits extracts. Also, more antibacterial activity was recorded with MEF and MES than the HEF, EEF. Therefore, it could be concluded that the active principle resides in the polar solvents. This agrees with the findings of Sofowora (1993). Dangoggo *et al*. (2002). Explaining the use of the plant in the treatment of gastrointestinal disorders.

The organs of the plant in which particular constituents reside differ widely. Some are accumulated exclusively in some particular organs. However, in some cases a particular phytochemical may be found in different organs such as the leaves, stem-bark, roots, fruits and flowers (Dangoggo *et al*., 2002, Okpara, 2015). Furthermore, there are other phytochemicals that are found all over the organs of plants. Thus, the appearance of identical R_F values in the chromatographic studies in both HEF and HES in benzene and other (BAW) solvent chromatogram is indicative that the plant contain the phytochemical represented by the R_F value 0.95 likewise in the benzene-ether chromatogram these appears identical R_F value of 0.80 for both HEF, HES. This value may be representing the same compound as in BAW and the difference in RF values of 0.95 in BAW and 0.80 in benzene-ether solvent may be accounted for by the difference in the developing solvent.

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