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Antimicrobial activity, cytotoxic test and phytochemical screening of extracts of the stem of *Fadogia agrestis*

O. M. Ameen[1], G. A. Olatunji[1], R. F. Atata[2] and L. A. Usman[1]

[1]Department of Chemistry, University of Ilorin, PMB 1515, Ilorin, Nigeria.
[2] Department of Biological Sciences (Microbiology Unit), Fountain University, Osogbo, Nigeria.

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ABSTRACT: The use of medicinal plants with therapeutic properties represents a secular tradition in different cultures, mainly in underdeveloped countries. *Fadogia agrestis* is commonly used in the management of erectile dysfunction. In this study, the cytotoxicity using brine shrimp lethality test (BST) and antibacterial activity against gram-positive and gram-negative bacteria strains of the chloroform, ethyl acetate and methanolic extracts of *Fadogia agrestis* stem are presented. The minimum inhibitory concentration (MIC) was also determined. Preliminary phytochemical screening of all the extract revealed the presence of reducing sugar, carbohydrates and alkaloids. In addition, the chloroform extract showed presence of saponins and flavanoids. In ethyl acetate extract; terpenoids was found, while methanolic extracts contain saponins, steroids, terpenoids and flavanoids. Tannins, anthraquinone and glycosides were not detected in the three extracts. The extracts exhibited low toxicity against the brine shrimp *Artemia salina* but demonstrated antibacterial activities against the tested bacteria, with chloroform extract showing high activity against *S. aureus*, *S. spp*, *B. subtilis* and *E. coli* (MIC 6.75 mg/ml). These results should prompt new researches in order to isolate the constituents responsible for the activity.

Key words: *Fadogia agrestis*, antimicrobial activity, Phytochemistry.

Introduction

The use of medicinal herb in the treatment and prevention of diseases is attracting scientist's attention worldwide. This is corroborated by World Health Organization in its quest to bring primary health care to the populace. The plant kingdom has long served as a prolific source of useful drugs, foods, additives, flavouring agents, colourants, binders and lubricants. As a matter of fact, it has been estimated that about 25% of all prescribed medicines today are substances derived from plants. The use of traditional medicine and medicinal plants in most developing countries, as a normotive basis for the maintenance of good health, has been widely observed. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized countries has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (Falodun *et al* 2006).

*To whom correspondence should be addressed.
E-mail: moameen@unilorin.edu.ng; aommohd@yahoo.com
Tel> +2348035019199

Traditional medicine is an important part of African cultures and local medicinal systems vary between different cultural groups and regions (Makhubu 2006). Herbs are now very popular in developing countries on account of improved knowledge about the safety, efficacy and quality assurance of ethno- medicine. In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of the children with high fevers, resulting from malaria, is the use of herbal medicines at home. Similarly, in industrialized countries, adaptations of traditional medicine often termed complementary or alternative medicine (CAM), also play an important role in the health care system of 20% of the population (WHO 2003).

In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with good antibacterial activity will be used for the treatment of bacterial infections. This is because, according to Arora and Keur *(*1999), the success story of chemotherapy lies in the continuous search of new drugs to counter the challenges posed by resistant strains of microorganisms.

Fadogia agrestis, Rubiaceae is a woody herb or shrub with yellowish stem and leaves, 1 – 3 feet high. It grows in the savannah part of Africa (Yakubu *et al* 2005). In the African traditional medicine, the decoction of this plant is extensively used as a febrifuge which could be associated with its use as an antimalarial drug (Anero *et al.*, 2008). In vitro antiplasmodial activity has been reported for extracts from leaves collected in Burkina Faso (Sanon *et al*., 2003), while its use as diuretic plant and in the treatment of kidney pain and convulsions has also been reported (Adjanohoun *et al*., 1986). The aphrodisiac potentials of *Fadogia agnestis* was recenlty proved scientifically by Yakubu *et al.*, (2005), similarly, two monoterpene glycosides were isolated from the leaves of *F. Agrestis* found in Guinea (Anero *et a.l* 2008).

Considering the fact that several microorganisms become resistant to conventional antibiotics, the purpose of this study was to evaluate the cytotoxicity and *in vitro* antibacterial activity of the extracts from *F. agrestis* stem using the microdilution method to assay the susceptibilities of six bacteria strains.

Materials and Methods

Plant Material

Fadoga agrestis stem was purchased from a vendor at Sango in Ilorin, Nigeria; the stem was authenticated at the herbarium of the Plant Biology Department, University of Ilorin, Ilorin, Nigeria.

Extraction

The stem was air dried at ambient temperature for 3 weeks, ground into powder, 250 g each of the plant was extracted with ethyl acetate (EA), chloroform ($CHCl_3$) and methanol (MeOH). The extracts were concentrated, and kept in sample bottle for further analysis.

Phytochemical screening

The crude extracts were subjected to phytochemical, screening, testing for the presence of Alkaloids, Tannins, Flavonoids, Anthraquinones, Glycosides, Carbohydrates, Steroids and Saponins using standard experimental procedure (Harborne, 1973; Trease and Evans, 1989).

Brine Shrimp Lethality Bioassay

The modified method of McLaughlin *et al.*, (1998) was employed in this study. Natural sea water from Bar Beach, Lagos was poured into an improvised hatching chamber made of plastic dish, brine shrimp eggs were added at the closed section of the chamber. The open air section of the chamber was then exposed to fluorescent light for 48 hrs.

Sample bottles of the same size used were washed and sterilized before use. Different concentrations (10000, 1000, 100, 10 μg/ml) of the extracts of *F. agrestis* were prepared using the extracting solvents. The tests were carried out in triplicates.

After 48 hrs a drop of DMSO and 4 ml of sea water were added to each of the sample bottles containing the extract. Ten brine shrimp larvae were carefully counted into each of the sample bottles with the aid of dropping pipette and the volume of the sea water was made up to 5 ml.

A control experiment containing 5 ml of sea water, a drop of DMSO and ten brine shrimp larvae was set up. The experiment was maintained at room temperature for 24 hr under light after which number of surviving larvae were counted and recorded; the data obtained were subjected to Finney's Probit analysis to determine the LC_{50} of each extract. The toxicity is expressed by this LC_{50} which is defined as concentration of the oil that kills 50% of the larvae within 24 hr. percentage mortality was also calculated as number of dead larvae divided by initial number of larvae (10) multiply by 100.

$$\%\, mortality = \frac{No.\, of\ dead\ larvae}{initial\ no\ live\ larvae} \times 100$$

Antimicrobial Analysis

Antibacterial activity of various extracts of *F. agrestis* bark was evaluated by ditch-diffusion method employing 24 h old cultures of six test organisms including two Gram-positive bacteria; *Streptococcus spp* and *Staphylococcus aureus*, four Gram-negative bacteria; *Pseudomonas spp, Proteus vulgaris, Bacillus subtilis* and *Escherichia coli*. The standardized test organisms were inoculated into sterile nutrient agar medium by swabbing such that approximately 106cfu/ml of the test organism was delivered on each agar plate. Eight holes of uniform diameter [6 mm] were made by using a sterile borer. Three volumes of each of the test solutions as well as standard solution [Chloramphenicol] and the blank [respective solvents] were placed in each hole separately under specific condition and the plates were then maintained at room temperature for 2 h to allow the diffusion of the solution into the medium. All the plates were then incubated at 37°C for 24 h and the zone of inhibition was measured.

Determination of minimum inhibitory concentration (MIC)

These tests were performed on the bacteria that exhibited inhibition zones using the effective extracts. The extracts were diluted double fold (2:2) with Muller Hinton Broth (Merck) in a series of ten test tubes. An aliquot of 0.5 ml of the bacterial suspension (Mc Farland 0.5) was used. The same process was repeated using Gentamicin (Genta 120 mg – _.E. ULUGAY) as positive control. All tubes were incubated at 37^{o}C for 24 h. The lowest concentration that inhibits growth was considered as the minimum inhibitory concentration, MIC.

Results and Discussion

Phytochemical screening helps reveal the chemical nature of the constituents of plant extracts; it may also be used to search for bioactive agents that could be used in the synthesis of very useful drugs. Phytochemical analysis of the stem of *Fadogia agrestis* revealed the presence of terpenoids, in the ethyl acetate extract, while saponins, was detected in the chloroform extract and steroids in the methanolic extract. Saponins and flavonoids were detected in the chloroform and methanol extracts, terpenoids in the ethyl acetate and methanol extract while carbohydrates, reducing sugar and alkaloids were common to the three extracts (Table 1). Alkaloids, saponins, anthraquinones and flavonoids had earlier been reported to be present in the aqueous extracts of the stem of *F. agrestis* by Yakubu *et al* (2005).

Table1: Phytochemical analysis of the crude extracts of *F. agrestis.*

Metabolites	**$CHCl_3$**	**EA**	**MeOH**
Tannins	-ve	-ve	-ve
Anthraquinones	-ve	-ve	-ve
Saponins	+ve	-ve	+ve
Steroids	-ve	-ve	+ve
Reducing sugar	+ve	+ve	+ve
Terpenoids	-ve	+ve	+ve
Glycosides	-ve	-ve	-ve
Flavonoids	+ve	-ve	+ve
Carbohydrates	+ve	+ve	+ve
Alkaloids	+ve	+ve	+ve

$CHCl_3$ = chloroform extract; EA = ethyl acetate extract; MeOH = methanol extract

Brine shrimp larvae have been used as a bioassay for a variety of toxic substances. The method has also been applied to plant extracts in order to facilitate the isolation of biologically active compounds (Pisutthanan *et al* 2004). The bioactivity of different extracts of *F. agrestis* against *A. salina* is presented in Table 2. At 10000 ppm for the $CHCl_3$ extracts, the ten brine shrimps used in the triplicate tests died after 24 h, which gave 100% mortality and by implication 10000 µg/ml of the $CHCl_3$ extracts is highly toxic to the brine shrimps. On the other hand, ethyl acetate and methanol extracts had 77 and 76.7 % mortality respectively. At 1000 µg/ml, 83.33% mortality was recorded for $CHCl_3$ extract. Ethyl acetate and methanol showed a very low mortality which is less than 10%. At 100 µg/ml, the extract showed very low mortality, while at 10 µg/ml there was no mortality. Though the $CHCl_3$ extract has the highest toxicity of all the extracts, but with a LC_{50} value of 400.59 µg/ml the extract may not contain cytotoxic compound of significant interest. Brine shrimp lethality assay is a rapid inexpensive and simple bioassay for testing plant extracts bioactivity, the result in most cases correlate with cytotoxic and antitumor properties of the plant and it has been reported that extracts resulting in LC_{50} values of less than 250 µg/ml were considered significantly active (Rieser *et al*., 1996).

Table 2: Bioactivity of *Fadogia agrestis* extracts using brine shrimp (*Artemia salina*) lethality assay.

Extract	**Conc 10000 µg/ml**	**% mortality**	**Conc 1000 µg/ml**	**% mortality**	**Conc 100 µg/ml**	**% mortality**	**Conc 10 µg/ml**	**% mortality**	**Lc_{50} µg/ml, 24h**
$CHCl_3$	0, 0, 0	100	1, 2, 2	83.33	10, 10, 8	6.67	10, 10, 10	0	400.59
EA	3, 3, 1	77	8, 10, 10	7	10, 10, 10	0		0	4496.67
MeOH	2, 4, 1	76.7	10, 10, 9	3.3	10, 10, 10	0	10, 10, 10	0	4803
							10, 10, 10		

$CHCl_3$ = chloroform extract; EA = ethyl acetate extract; MeOH = methanol extract

Several new antibacterial agents are currently being developed in response to the emergence of bacterial resistance to existing drug. New vegetal sources presenting antimicrobial activity and low toxicity could be a viable alternative, with low cost and easily accessible to poor communities where the species are found. In many developing countries about 80% of available drugs come from medicinal plants and in industrialized countries plants make up the raw material for processes, which synthesize pure chemical derivatives (Penso 1980). The various extracts from *F. agrestis* bark showed moderate inhibiting activity on disease causing Gram-negative and Gram-positive bacteria, the most inhibited being *Proteus vulgaris*, *Bacillus subtilis*, and *Escherichia coli* (Table 3). Table 3 revealed that *P. vulgaris*, and *E. coli* were highly sensitive, while *B. subtilis* and *P. spp* were moderately sensitive to the chloroform extract, but *S. spp* and *S. aureus* exhibit low sensitivity. *P. vulgaris*, *S. spp* and *B. subtilis* were highly sensitive to the ethyl acetate extract while *S. aureus*, *P. spp* and *E. coli* were moderately sensitive. *B. subtilis* was moderately sensitive to the methanolic extract, *P. spp*, *S. spp* and *E. coli* showed low sensitivity while *P. vulgaris* and *S. aureus* were not sensitive. This is particularly interesting from a medical point of view because these microbial agents are responsible for severe opportunistic infections (Basile *et al* 2005).

Table 3: The antimicrobial activity of *Fadogia agrestis* extracts on some Gram – positive and Gram – negative bacteria.

Extracts	Gram – positive bacteria		Gram – negative bacteria			
	Proteus vulgaris	*Staphylococcus aureus*	*Pseudomonas spp*	*Streptococcus spp*	*Bacillus subtilis*	*Escherichia coli*
$CHCl_3$	+++	+	++	+	++	+++
EA	+++	++	++	+++	+++	++
MeOH	--	--	+	+	++	+

KEY: +++ = very sensitive; ++ = moderately sensitive; + = low sensitive; -- = no sensitive;
$CHCl_3$ = chloroform extract; EA = ethyl acetate extract; MeOH = methanol extract

Table 4 presents the results of the minimum inhibitory concentration (MIC) tests of the various extract of *F. agrestis* on the test organisms at the end of incubation periods of 24 h. From the table it is clearly shown that the chloroform extract of *F. agrestis* has the lowest MIC value of 6.75 mg/ml on *S. aureus*, *S. spp*, *B. subtilis* and *E. coli* while the methanolic extract recorded the highest MIC value of 137 mg/ml on *E. coli*.

Table 4: Minimum inhibitory concentration of *Fadogia agrestis* extracts on some Gram – positive and Gram – negative bacteria.

Extracts (mg/ml)	Gram – positive bacteria		Gram – negative bacteria			
	Proteus vulgaris	*Staphylococcus aureus*	*Pseudomonas spp*	*Streptococcus spp*	*Bacillus subtilis*	*Escherichia coli*
$CHCl_3$	27	6.75	13.5	6.75	6.75	6.75
EA	10.07	20.15	20.15	10.07	10.07	20.15
MeOH	--	--	34.25	68.50	34.25	137.00

$CHCl_3$ = chloroform extract; EA = ethyl acetate extract; MeOH = methanol extract

Conclusion

This study indicates that *F. agrestis* have potential antimicrobial activity but there are differences in the activities of the extracts based on the solvents that are used for extraction, it is natural that there are differences in their antimicrobial activities since the solvents are of different polarity and different compounds are extracted. The

important thing is the determination of the antimicrobial activities that plants have and their usability in the preparation of new drugs. The determination of the active compounds in this plant can serve as lead compounds for new chemotherapeutics. Even at trace level, presence of antibacterial active agents in the plant will facilitate preparation of new drugs with unique biological activities.

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