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PHYTOCHEMICAL AND ANTIBACTERIAL SUSCEPTIBILITY STUDIES OF THE STEM BARK EXTRACT OF *Fadogia erythrophloea* (K. Schum. and K. Krause) HUTCH. and DALZIEL

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ABSTRACT
This study is designed to explore the phytochemistry and antimicrobial activity of the stem bark of Fadogia erythrophloea. The powdered stem bark material was subjected to Soxhlet extraction using methanol. The methanol extract was then partitioned using chloroform and ethyl acetate. The fractions were also concentrated using rotary evaporator. Phytochemical screening of the extract and fractions of Fadogia erythrophloea stem bark revealed the presence of cardiac glycosides, flavonoids, tannins, saponins and alkaloids. The antimicrobial analysis revealed that the crude methanol extract, chloroform and ethyl acetate fractions were active against most of the test microorganisms. The mean zones of inhibition against the test microorganisms ranged between 20 – 33 mm. The highest zone of inhibition was obtained with ethyl acetate for Bacillus cereus as 33 mm. The Minimum Inhibitory Concentration (MIC) ranges from 2.75 mg/ml to 50 mg/ml, the MIC for both methanol and chloroform extracts were both found to be 12.5 mg/ml whereas ethyl acetate has 6.25 mg/ml. Minimum Bactericidal Concentration (MBC) of the ethyl acetate fraction was determined to12.5 mg/ml for most of the microorganisms tested. The broad range of inhibition detected implied that the extract has measurable antibacterial properties. This may be due to the presence of active principles which were detected in the phytochemical screening.

KEY WORDS: Fadogia erythrophloea, Stem bark, phytochemistry, antimicrobial activity, Soxhlet extraction.

INTRODUCTION

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. Encompassing concepts and methods for protection and restoration of health, traditional medicine has served as a fount of alternative medicine, new pharmaceuticals and health care-products. Recently, public, scientific and medical interest in this key topic has soared as the importance of the indigenous collective wisdom in the use of herbal preparations, has been recognized (Sulaiman *et al*., 2016).

Until recently, plants were important sources for the discovery of novel pharmacological active compounds, with many drugs being derived directly or indirectly from plants (Cordell, 2000). There are hundreds of species recognized to possess therapeutic value. Although these are not purified into separate compounds, many are believed to exert therapeutic effects good enough to be proven effective by modern analysis (Cordell, 2000).

The search for components with novel biological properties has gained increasing importance due to growing worldwide concern about the alarming increase in the rate of deadly diseases. Considering the bio-prospectus of medicinal flora, the present study was conducted to establish the phytochemistry of the stem bark of *Fadogia erythrophloea* and to investigate its antimicrobial properties against a wide array of microorganisms. The choice of *Fadogia erythrophloea (K. Schum. & K. Krause)* Hutch. & Dalziel as the plant of interest in this work is based on its vast claimed ethno-medicinal importance in the tropics, including West Africa. These include, but not limited to the use of the leaves, stem bark, and fruits as antidotes (venomous stings, bites, arrow poison, etc.), emetics, anti malaria, vermifuge and for childhood fever. The Root is equally used as medicine for diarrhoea, dysentery, colitis and constipation (Michel, 2004).

MATERIALS AND METHODS

Sampling of Plant Material

The plant materials were collected from the bushes around Faka, Igabi local government area of Kaduna State-Nigeria, in the month of November 2015.

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It was authenticated with specimen voucher number 940 at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria-Nigeria. The stem bark were separated from the leaves, air-dried for 35 days and crushed to coarse powder.

Extraction of The Plant Material

The dried powdered stem bark (800 g) were extracted exhaustively with methanol using Soxhlet extractor at a temperature of about 60 ^{0}C. The extract was concentrated in vacuo at 40 ^{0}C using rotary evaporator, and the crude extract (residue) was air-dried.

The methanol extract was partitioned with chloroform and ethyl acetate (in an order of increasing solvent polarity).

Preliminary Phytochemical Screening

Portion each of the methanol, chloroform and ethyl acetate fractions were subjected to preliminary phytochemical screening using standard procedures (Ndukwe *et al.*, 2011).

Antibacterial Screening

The Test Organisms

The microorganisms tested were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria. All the isolates were checked for purity and maintained in a slant of nutrient agar.

Susceptibility Test of the Extract Using Agar Well Diffusion Method

The disc diffusion method was used (Nostro *et al.,* 2000). The antimicrobial activities of the methanol and n-butanol fractions of the leaves of *Fadogia erythrophloea* was determined using stock concentration of 100 mg/ml. The standardised inoculate of the isolates were uniformly streaked unto freshly prepared Mueller-Hinton agar plates with the aid of a sterile swab stick. Five appropriately labelled wells were punched into each agar plate using a sterile cork borer (8 mm in diameter). The extract (0.2 ml) of appropriate concentration was placed in each well and then allowed to diffuse into the agar. An extra plate was streaked with the isolate inocula and ciprofloxacin standard (10 µ/disc) was placed on it. The plates were incubated at 37 °C for 24 h. While for the fungi, Sabouraud's Dextrose agar was used and the incubation period was 48 h. The antimicrobial activities were expressed as diameter of inhibition zones produced by the plant extracts. The interpretation of the measurements as sensitivity, intermediate and resistant were made according to Clinical Laboratory Standards Institute (CLSI, 2012) manual. The intermediate readings were considered as sensitive for the assessment of the data and recorded as sensitive or resistant depending on their respective MIC (minimum inhibitory concentration) breakpoint. Zones of inhibition of ≥ 18 mm were considered sensitive, 13 to 17 mm intermediate or marginally sensitive and < 13 mm resistant (Cheesebrough, 2006)

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations of the extracts were determined using the tube dilution method as outlined by the National Committee for Clinical Laboratory Standards (NCCLS, 2000). Varying concentrations of the extract (1.57-25 mg/ml) that exhibited sensitivity against the test organisms were prepared in test tubes containing Mueller-Hinton broth (MHB). The organisms were inoculated into each tube containing the diluted extracts. The tubes were incubated at 37 °C for 24 h for bacteria and 48 h for fungi. The lowest concentration in the series showing no visible growth of the test organisms was considered to be the Minimum Inhibitory Concentration.

Determination of Minimum Bactericidal and Fungicidal Concentrations (MBC & MFC)

The minimum bactericidal concentration of the extract was determined as outlined by the NCCLS (2000), on the nutrient agar plates. Minimum bactericidal concentrations were determined by assaying the test tube content of the MIC determinations. A loopful of the content of each tube was inoculated by streaking on a solidified nutrient agar plate and then incubated at 37 ^{0}C for 24 and 48 h for bacteria and fungi respectively after which it was observed for microbial growth. The lowest concentration of the subculture with no growth was considered as minimum bactericidal concentration.

RESULTS

About 95.20 g of methanolic extract was obtained, which represent 11.90 % of the plant material. The phytochemical screening of the stem bark extract of the plant *Fadogia erythrophloea* revealed the presence of glycosides, alkaloids, saponins, flavonoids, and tannins. The results are shown below in Table 1.

Table 1: Phytochemical Constituents of the Stem Bark Extracts of *Fadogia erythrophloea*

Phytochemical	Test	Extract and Fractions		
		CF	EA	ME
Glycosides	Modified Bontrager's	+	+	+
Tannins	Lead acetate Ferric Chloride	–	–	+
Saponins	Frothing	–	–	–
Flavonoids	NaOH	+	+	+
Alkaloids	Dragendoff's	+	+	+
	Wagner's	+	+	+
	Picric acid	+	+	+

Key: + = Present – = Absent Chloroform = CF Ethyl acetate = EA Methanol = ME

ANTIBACTERIAL SUSCEPTIBILITY TEST

The results of the antibacterial activity tests, expressed in terms of diameter of zones of inhibition, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal (MBC) of the test organisms are summarized in Tables 2, 3 and 4 respectively for crude methanol extract, chloroform fraction and ethyl acetate fraction.

Table 2: Inhibition Zone (mm) of the Crude Extract and Fractions

Test organism	ME	CF	EA	DMSO	Cipro
Staphylococcus aureus	8	22	29	8	35
Streptococcus feacalis	26	28	28	8	40
Bacillus cereus	29	25	33	8	40
Escherichia coli	25	26	30	8	29
Shigella dysenteriae	27	25	29	8	32
Klebsiella pneumonia	23	20	25	8	35

Key: 8 = Resistant, ME= Methanol, CF= Chloroform, EA= Ethyl acetate, DMSO= Dimethyl sulphoxide, Cipro= Ciprofloxacine.

Minimum Inhibitory Concentration MIC of the Extract and Fractions

The Minimum Inhibitory Concentrations of the stem-bark extracts of *Fadogia erythrophloea* are reported in Table 3. The results showed MIC ranging from 2.75 mg/ml to 50 mg/ml.

Table 3: MIC of *Fadogia erythrophloea* Extract and Fractions

Extract/Fractions	Conc. (mg/ml)	*S. aureus*	*S. faecalis*	*B. cereus*	*E. coli*	*S. dysenteriae*	*K. pneumonia*
Methanol	25	ND	–	–	–	–	–
	12.5	ND	MIC	MIC	MIC	MIC	MIC
	6.25	ND	+	+	+	+	+
	2.75	ND	++	++	++	++	++
Chloroform	25	–	–	–	–	–	–
	12.5	MIC	–	MIC	MIC	MIC	MIC
	6.25	+	MIC	+	+	+	+
	2.75	++	+	++	++	++	++
Ethyl acetate	25	–	–	–	–	–	–
	12.5	–	–	–	–	–	MIC
	6.25	MIC	MIC	MIC	MIC	MIC	+
	2.75	+	+	+	+	+	++

Key: – = No turbidity (no growth), MIC= Minimum inhibitory concentration, + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity, ND= Not Determined

Minimum Bactericidal Concentration MBC of the Crude Extract

The Minimum Bactericidal Concentrations of the fractions of the crude extracts of *Fadogia erythrophloea* are reported in Table 4. The results showed MBC ranging from 2.75 mg/ml to 50 mg/ml.

Table 4: MBC/MFC of the Crude Methanol Extract and Fractions of *F. erythrophloea*

Extract/Fractions	Conc. (mg/ml)	*S. aureus*	*S. faecalis*	*B. cereus*	*E. coli*	*S. dysenteriae*	*K. pneumonia*
Methanol	25	ND	+	MBC	+	MBC	+
	12.5	ND	++	+	++	+	++
	6.25	ND	+++	++	+++	++	+++
	2.75	ND	++++	+++	++++	+++	++++
Chloroform	25	MBC	-	MBC	MBC	MBC	+
	12.5	+	MBC	+	+	+	++
	6.25	++	+	++	++	++	+++
	2.75	+++	++	+++	+++	+++	++++
Ethyl acetate	25	-	-	-	-	MBC	MBC
	12.5	MBC	MBC	MBC	MBC	+	+
	6.25	+	+	+	+	++	++
	2.75	++	++	++	++	+++	+++

Key: - = No colony growth, MBC = Minimum Bacterial Concentration, + = Scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth, ++++ = Very heavy

DISCUSSION

The phytochemical screening of the stem-bark extracts of *Fadogia erythrophloea* (Table 1) revealed the presence of glycosides, saponins, flavonoids, tannins and alkaloids in the plant materials called for immense research, since the metabolites are of various pharmacological importance (Ndukwe, *et al*., 2011).

Flavonoids have been reported to have immense antimicrobial, antifungal and antiviral activities. Thus, numerous research groups have south to elucidate the mechanism of action of some selected flavonoids. The presence of saponins in the plant confirms the claim by ethno-medicine that it is used in the treatment of fever (antipyretic) and also as an analgesic. The traditional use of antipyretic properties is a common worldwide feature of many ethno-botanical cultural systems. In ethno-botany, plants with naturally occurring antipyretic properties are commonly referred to as febrifuges. The fact that the phytochemical screening reveal the presence of cardiac glycosides confirm the ethno-medicinal claim that the plant is used in the treatment of arrow head poison (Fillipos *et al*., 2007).

The mean zones of inhibition against the test microorganisms ranged between 20 – 30 mm (Table 2). The highest zone of inhibition was obtained with ethyl acetate for *Bacillus cereus* as 33 mm. *Streptococcus feacalis, Bacillus cereus, Escherichia coli, Shigella dysenteria,* and *Klebsiella pneumoniae* were the most susceptible micro-organisms. Only *Staphylococcus aureus* was found to be resistant to methanol extract. The extracts were compared to standard antibiotics.

All the fractions of the extract inhibited the growth of *Streptococcus feacalis, Bacillus cereus, Escherichia coli, Shigella dysenteriae and Klebsiella pneumonia* (Table 3). The minimum inhibition concentration MIC for both methanol and chloroform extracts was found to be 12.5 mg/ml for all the test organisms except *Streptococcus feacalis* with MIC 6.25 mg/ml. Also, all the fractions except methanol inhibit the growth of *Staphylococcus aureus*. The ethyl acatate fraction was confirmed to have a broader spectrum than any of the fractions because it inhibits the growth of all the micro organisms at a relatively lower concentration of 6.25 mg/ml except for *Klebsiella pneumonia* (Table 3).

All the extracts exhibited very significant antibacterial activity against the test organisms. This can be deduced from Table 4. The MBC for the methanol extract was only recorded for *Bacillus cereus* and *Shigella dysenteriae* at 25 mg/ml, comparable with the MBC for the Chloroform extract against test microorganisms. Whereas for the ethyl acetate extract, most of the test organisms recorded MBC at 12.5 mg/ml except *Shigella dysenteriae* and *Klebsiella pneumonia* (Table 4).

CONCLUSION

The findings from this research work justify the ethno-medicinal use of the plant materials for the treatment of bacteria induced ailments, as stated by Michel, (2004). The results showed that the stem bark of *Fadogia erythrophloea* possessed measurable *in-vitro* antibacterial activity against many of the microorganisms implicated in the pathogenesis of human infections. The broad range of inhibitions observed implied that the extract has a comparable antibacterial activity, which may be due to the presence of active principles which were detected in an appreciable amount in the phytochemical screening. This research

work compares with the results from previous researches on the leaves extract of *Fadogia erythrophloea* by Sulaiman *et al*. (2016).

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