

Antimicrobial and Preliminary Phytochemical studies of Methanol Extract of Root Bark of *Crossopteryx febrifuga* (Rubiaceae)

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ABSTRACT

Crossopteryx febrifuga is one of the useful plants used in Hausa traditional medicine in North Western Nigeria. It belongs to the family *Rubiaceae*. The phytochemical studies of the root bark of the plant was carried out using standard procedure. The was found to contain: steroids, flavonoids, terpenoids, anthraquinones, cardiac glycosides, tannins, alkaloids and saponins. The antimicrobial activity screening was carried out using both bacterial and fungal strains. The bacterial strains include: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Eschericia coli*. The fungal strains include: *Aspergillus fumigatus*, *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*. In general, the extract showed considerable activity on the bacterial species. It inhibited the growth of both gram positive and gram negative microorganism with zones of inhibition ranging from 7- 23 mm at concentrations of 50 µg/ml, 100 µg/ml and 200 µg/ml. The plant extract did not show significant activity on fungal strains. It inhibited the growth of *Aspergillus fumigatus* at 400 µg/ml and 500 µg/ml which produced zones of inhibition of 8 mm and 12 mm respectively at the stated concentration. It can be concluded that the activity showed by the methanolic extract of the plant is as a result of the phytochemicals present in the plant.

INTRODUCTION

Since ancient time, plants, animals and mineral sources have been used by the early man to cure and prevent diseases. Plant is the most researched and plant have been the major source of drugs that we use in modern medicine today and will continue to provide cure for man kind. *Crossopteryx febrifuga* is one of the numerous plants used in Hausa traditional medicine in North Western Nigeria. It belongs to the family *Rubiaceae*. The Hausa people of North Western Nigeria call it Kasfiya, Kashin Awaki or Giyayyata. *C. febrifuga* is widely used in the management of malaria, fever and painful inflammatory disorders (Adeola *et al.*, 2011). The decoction of the root bark is used in the treatment of coughs and gastrointestinal complaints (Adeola *et al.*, 2011). Also, the decoction of the leaves is applied as a lotion on itching

part of the body (Adeola *et al.*, 2011). In Mali, the powder of the fruits is used as sedative in the treatment of cough (Yahaya *et al.*, 2011). The decoction of the stem bark is used in central Africa as an antipruritic lotion. The infusion of the root bark is used in treatment of syphilitic ulcer, while the leaf infusion is used for treatment of the inflammation of the eye. In tropical Africa the stem bark is used in treatment of fever, malaria, diarrhoea, intestinal worms and ophtalmia and for application to wounds (Edeoga *et al.*, 2005). Previous studies have shown that the methanolic extract of the stem bark of *C. febrifuga* possesses significant analgesic and anti-inflammatory properties possibly mediated via non-selective inhibition of cyclo-oxygenase pathways (Yahaya *et al.*, 2011). The antimicrobial properties of the decoction of methanol extract of the fruit of *C. febrifuga* showed moderate activity against *Staphylococcus aureus* (Olusamni *et al.*, 2010). The polyphenolic extracts of the leaves of *C. febrifuga* was found to inhibit *Entamoeba histolytica* growth with MAC<10 µg/ml.

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The same extracts, at a concentration of 80µg/ml in an organ bath, also exhibited more than 70% inhibition of acetylcholine and/or KCl solution-induced contractions on isolated guinea-pig ileum (Sanon *et al.*, 2003). Alkaloids extract of *C. febrifuga* present a significant activity on *Plasmodium falciparum* chloroquine resistant W2 strain, with $IC_{50} < 4 \mu\text{g/ml}$, confirming the traditional use of plant against malaria (Sanon *et al.*, 2003). *Crossopteryx febrifuga* seeds extracts of different fractions were assayed for radical scavenging activity, using the stable free radical diphenylpicrylhydrazyl (DPPH), and for inhibition of enzymatic lipid peroxidation mediated by soybean 15-lipoxygenase. The plant investigated showed activity. *C. febrifuga* appear to be excellent sources of antioxidants (Maiga *et al.*, 2006). Although, several parts of the plant have been evaluated for pharmacological activity, this present study is aimed at evaluating the phytochemical constituents and antimicrobial activity of the methanolic extract of the root bark of the plant.

MATERIALS AND METHODS

Methods

Collection and Identification of the plant

The leaves, the flower, the fruit and the root bark of *C. febrifuga* were collected in May, 2011 from Zuru local government of Kebbi state-Nigeria. The plant was identified by A. M. Umar, (Taxonomist) in the Department of Biological Science, Usmanu Danfodiyo University, Sokoto. The herbarium specimen is deposited at the herbarium unit of the department for future reference.

Drying of Sample

The root bark of *Crossopteryx febrifuga* was air dried under shed. During the drying period, the sample was weighed continuously until a constant mass was obtained. The sample was then grounded into fine powder using pestle and mortar. The powdered plant sample was then stored in appropriate container until it was required for use.

Extraction of Plant Material

The powdered plant material (200 g) was extracted using 750 cm³ of methanol with the aid soxhlet extractor at a temperature of 64°C for 8 hours. The extract was then concentrated over water bath at the same temperature.

Phytochemical Screening

The phytochemical screening was carried out for the following: steroids, flavonoids, terpenoids, anthraquinones, cardiac glycosides, tannins, alkaloids, saponins and carbohydrate using standard procedures:

Qualitative Phytochemical Screening

Detection of alkaloids

Extract was dissolved in dilute Hydrochloric acid and filtered. The filtrate was divided into 3 portions and the following reagents were used to test for the presence of alkaloids.

a) Mayer's Test

The filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). The formation of a yellow coloured precipitate indicated the presence of alkaloids.

b) Wagner's Test

The filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of brown/reddish precipitate indicated the presence of alkaloids.

c) Dragendroff's Test

The filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). The formation of reddish precipitate indicated the presence of alkaloids.

Detection of carbohydrates

Extract was dissolved in 5 mLs of distilled water and filtered. The filtrate was divided into 2 portions and was used to test for the presence of carbohydrates using the following reagents.

a) Molisch's Test

The filtrate was treated with 2 drops of alcoholic α -naphthol solution in a test tube. The formation of the violet ring at the junction indicates the presence of carbohydrates.

c) Fehling's Test

Filtrate was hydrolysed with dil. HCl, and then neutralized with alkali and warmed with Fehling's A & B solutions. The formation of red precipitate indicates the presence of reducing sugars.

Detection of phytosterols / Triterpenoids

The extract was dissolved in water and then treated with chloroform. The liquids were separated using separating funnel. The chloroform portion was collected and then divided into 2 portions and was used for the test.

a) Salkowski's Test

A few drops of Conc. Sulphuric acid was added to the filtrate by the wall of the test tube. The appearance of golden yellow colour indicated the presence of triterpenes.

b) Libermann Burchard's test

A few drops of acetic anhydride was added to the filtrate in a test tube, then followed by the addition of Conc. Sulphuric acid by the wall of the test tube. The formation of brown ring at the junction indicates the presence of phytosterols.

Detection of Saponins

Frothing Test

The extract (2 mLs) was diluted with twice its volume of water and shaken in a test tube for 5 mins. The occurrence of a honeycombs froth, which lasts for about 45 minutes, indicates the presence of saponins (Sofowora, 1984).

Detection of Cardiac Glycosides

Keller- Killiani's Test

The extract (1 mL) was diluted with 20 mLs of water, 1 mL of strong lead sub-acetate solution was added to precipitate pigments, which were filtered off. The filtrate obtained was shaken with equal volume of chloroform and allowed to separate into two layers in a separating funnel. The chloroform layer was removed and evaporated to dryness over a water bath. The residue was dissolved in 3ml of ferric chloride in glacial acetic acid, and then transferred to a dry test tube. Few drops of concentrated sulphuric acid were added by the wall of the test tube. On standing, a brown colour at the interphase (due to deoxysugars) and a pale green colour in the upper layer (due to steroidal nucleus) is a preliminary test for digitoxose .

Detection of Flavonoids Test

a) Shinoda's Test.

The methanolic extract was dissolved in 2 mLs of distilled water and warmed. A 4 drops of concentrated hydrochloric acid were then added followed by addition of some magnesium chips. Immediate appearance of orange colour denotes flavones, red crimson colour denotes flavonols, and pink magenta colour denotes flavones .

b) Sodium hydroxide test

The extract were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Detection Tannins

a) Lead acetate test

To 1 mL of the extract, 2 drops of lead sub acetate solution was added. A coloured precipitate indicates the presence of tannins .

b) Bromine water test

The plant extract was treated with 3 drops of bromine water. Non-formation of coloured precipitate indicates the presence of hydrolysable tannins

Detection of Phenolic compounds

Ferric chloride test.

The extract (1 mL) was diluted with water. Few drops of a solution of ferric chloride were added. The appearance of blue-black colour indicates the presence of tannins.

Detection of Anthraquinones

Borntrager's Test

The extract was extracted with petroleum ether. The petroleum ether fraction was concentrated and shaken with 5 mLs

of 25% ammonia solution. A cherry-red colour of the alkaline solution indicates emodins in an oxidized form.

Antimicrobial Activity

Test Organisms

Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus fumigatus*, *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger* were obtained from the microbiology laboratory of Usmanu Danfodiyo University Teaching Hospital, Sokoto. The isolates were identified using standard microbiological procedure as described by Ganapathi *et al.* (2011).

Screening for Antibacterial and Antifungal Activities

The antibacterial and antifungal activities of the extracts were assayed as described by Adekunle (2009). The standardized suspensions were used to inoculate the surfaces of Muller Hinton agar plates (90 mm in diameter) using sterile cotton swab. 6 mm diameter wells were bored using sterile cork borer in agar and filled with the desired concentrations of the plant extract (50µg/ml, 100 µg/ml, and 200 µg/ml).

Commercial antibiotics (ciprofloxacin 30µg/ml) was used as reference standard to determine the sensitivity of the isolates. The plates were allowed to stand for 5 hours at room temperature for extracts to diffuse into the agar and then incubated at 37°C over night. The zone of inhibition was measured using metric rule.

Table. 1: Results of Phytochemical Screening of Methanol Extract of *C. febrifuga* .

Phytoconstituent Groups Test		Result
Tannins		
a)	Lead sub – acetate Test	+
b)	Bromine water Test	+ (Hydrolysable Tannins)
Alkaloids		
a)	Mayer's Reagent	+
b)	Dragendoff's Reagent	+
c)	Wagner's Reagent	+
Saponins		
a)	Frothing Test	+
Flavonoids		
a)	Ferric Chloride Test	+
b)	Shinoda's Test	+
c)	Sodium Hydroxide Test	+
Steroids		
a)	Liebermann – Bauchard's	+
b)	Salkawaski's Test	+
Carbohydrates		
a)	Molisch Test	+
b)	Fehling's Test	+
Phenolics		
a)	Ferric Chloride Test	+
Cardiac Glycosides		
a)	Keller- Killiani's Test	+
Anthraquinones		
a)	Borntrager's Test	ND

Key: + = Present ; N.D = Not Detected

Table 2: Results of Antibacterial Activity of Methanol Extract of *C. febrifuga*

S/No.	Test Organisms	Concentration/ Zone of inhibition (mm)			+ve Control (ciprofloxacin) 30µg/ml
		50 µg	100 µg	200 µg	
	<i>Pseudomonas aeruginosa</i>	-	14	19	25
	<i>Staphylococcus aureus</i>	9	17	23	27
	<i>Eschericia coli</i>	7	15	19	25

Table 3: Antifungal activity of Methanol Extract of *C. febrifuga*

S/No.	Test Organisms	Concentration/ Zone of inhibition (mm)			+ve Control (ciprofloxacin) 30µg/ml
		200 µg	400 µg	500 µg	
1.	<i>Aspergillus fumigatus</i>	-	8	12	24
2.	<i>Candida albicans</i>	-	-	-	23
3.	<i>Aspergillus flavus</i>	-	-	-	22
4.	<i>Aspergillus niger</i>	-	-	-	24

Table 4: MIC and MBC of Antibacterial Activity of Methanol Extract of *C. febrifuga*

S/No.	Test Organisms	MIC µg/ml	MBC µg/ml
1.	<i>Pseudomonas aeruginosa</i>	5×10^4	10×10^4
2.	<i>Staphylococcus aureus</i>	5×10^4	1×10^4
3.	<i>Eschericia coli</i>	1×10^4	1×10^4

Table 5: MIC and MFC of Antifungal Activity of Methanol Extract of *C. febrifuga*

S/No.	Test Organisms	MIC µg/ml	MFC µg/ml
1.	<i>Aspergillus fumigatus</i>	5×10^4	10×10^4

DISCUSSION

Secondary metabolites are mostly produced by plant during adverse condition for protection against herbivores (Chitra *et al.*, 2009). Result of phytochemical screening (table 1), revealed the presence of tannins, alkaloids, saponins, flavonoids, steroids and cardiac glycosides. These secondary metabolites, although they are of no primary function to the plant, they show some pharmacological activity on human beings and other animals. The presence of flavonoids, tannins and saponins in the plant may be responsible for the antimicrobial activity demonstrated by the plant extract. Methanolic extract of *Crossopteryx febrifuga* root bark inhibited the growth of micro-organisms. From this result, the methanolic extract of *Crossopteryx febrifuga* root bark had shown antibacterial activity at 50 µg/ml, 100 µg/ml and 200 µg/ml on *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* with zone of inhibition which ranges between 7 mm to 23 mm. From the table 2, it can be deduced that the gram positive microorganism (*Staphylococcus aureus*) was more susceptible to the extract As the highest zone of inhibition can be observed on it. From table 2, the zone of inhibition produced by the extract, can be compared fairly with that of the standard antibiotic (ciprofloxacin) .. The extract did not show significant activity on the fungal strains this can be seen from table 3. The extract only inhibited the growth of *Aspergillus fumigatus* with zone inhibition between 8 mm to 12 mm. It can be deduced from table 3 that the extract does not have a good antifungal activity. Also, it can not be used as abroad spectrum antibiotics. The Minimum inhibitory Concentration (MIC), is the lowest concentration of the extract that inhibits the growth of the microorganisms. The extract had a good MIC on both the bacterial and the fungal (*Aspergillus fumigatus*) strains (tables 4 and 5. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration

(MFC) is the lowest concentration that kills about 99.9 % of the microorganisms. This can be seen also in tables 4 and 5. The extract also had a significance MBCs and MFCs.

CONCLUSION

The qualitative phytochemical analysis revealed the presence of tannins, alkaloids, saponins, flavonoids, steroids and cardiac glycosides. The methanol extract the root bark of *Crossopteryx febrifuga* showed some antimicrobial activity. The activity was significant on bacterial strains. Very little activity was observed on fungal strains. It can be concluded that the extract may not be used as a broad spectrum antibiotics as it did not show significant activity fungal strains.

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