European Journal of Medicinal Plants
4(4): 473-482, 2014

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Phytochemical and Anti-microbial Screening of the Leaves and Twigs of *Sclerocarpus africanus* (Jacq)

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Authors' contributions

This work was carried out in collaboration between all authors. Author RGA designed and supervised the study, author JAN wrote the first draft and managed the analyses, author TAT managed the literature searches, proof reading, advised and guided the final draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 24th September 2013
Accepted 13th December 2013
Published 13th January 2014

ABSTRACT

Aim: To determine phytochemical constituents and antimicrobial activity of leaves and twigs of *Sclerocarpus africanus* (Jacq); prove or otherwise ethno-medicinal claims on *S. africanus*.
Place and Duration of Study: Departments of Chemistry, Biological sciences and Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, between June to October, 2010.
Methodology: Petroleum ether, ethyl acetate, ethanol and methanol extracts of leaves and twigs of *S. africanus* were phytochemically screened for the presence of carbohydrates, saponins, tannins, alkaloids, anthraquinone glycosides and flavonoids. Minimum bactericidal/fungicidal concentrations (MBC)/(MFC) and Minimum inhibition concentration (MIC) were carried out on the extracts using Broth dilution method.
Results: Phytochemical screening showed presence of carbohydrates, tannins and saponins. Flavonoids and anthraquinone glycosides were found only in the ethanol and methanol extracts. Anti-microbial screening of methanol and ethanol extracts showed activity against the following human pathogens: *Staphylococcus aureus, Salmonella typhi, Streptococcus pyogenes, Shigella dysenteriae, Candida albicans* and *Candida thrusei*, with MIC value of 2.5 mg/ml; while *Neisseria gonorrhea* was inhibited at MIC 1.25 mg/ml.

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MBC/MFC value of 10 mg/ ml was observed for all the pathogens, except *N. gonorrhea* which had an observered MBC of 5 mg/ ml for ethanol extract. Similar MBC/MFC values were obtained for methanol extract except *Shigella dysentereae* which had MBC of 5 mg/ ml. Petroleum ether extract was active against *S. aureus, S. typhi, S. dysenteriae and N. gonorrhoea* with MIC value of 5 mg/ml and MBC/MFC value 10 mg/ml; no activity was observed for *S. pyogenes, C. albicans and C. thrusei*; *N. gonorrhea* was most inhibited.
Conclusion: Results obtained justify the ethno-medicinal use of this plant in treatment of gonorrhea and other venereal diseases caused by the test micro organisms.

Keywords: Sclerocarpus africanus (Jacq); Neisseria gonorrhea; Minimum inhibitory concentration; minimum bactericidal concentration.

1. INTRODUCTION

Man is engaged in an eternal battle against disease [1]. In African trado-medicine, various means have been employed in times past to fight pathogens; plants and other natural products are still being utilized for this purpose [2,3,4]. These plants contain compounds which are believed to be curative. For example, *Hymenocardia acida* is used extensively for the treatment of a wide variety of ailments [5].

The plant *Sclerocarpus africanus* (Jacq), locally known in Igbo dialect (Nigeria) as "*nli-atulu"* and Manding language (Senegal) as *Bambara-goni* is used for treating gonorhoea and other venereal diseases in Nigerian ethno-medicine [6,7]. Its medicinal value is yet to be fully studied. This study investigates the leaves and twigs for the presence of secondary metabolites, medicinal property and antimicrobial activity to verify the tradomedicinal claims of this plant as a remedy for gonorrhea.

S. africanus commonly known as African bone bract is of the family Asteraceae (Compositae). It is an annual herb up to 130 cm high and is widely dispersed in tropical Africa and India. Asteraceae, (also known as aster, dewey or sunflower family) is the largest family of vascular plants. It has more than 22,750 currently accepted species spread across 1,620 genera and 12 subfamilies. *Compositae* means composite and refers to the characteristic inflorescence: a special type of pseudanthium found in only a few other angiosperm families. Most members are herbaceous but a significant number are shrubs, vines and trees [6].

This herb belongs to the tribe Heliantheae. It is the third largest tribe in the sunflower family. Most genera and species are found in North America and South America, particularly in Mexico. A few genera are pantropical. Most Heliantheae are herbs or shrubs but there are some that grow to the size of small trees [8,9]. The genus Sclerocarpus includes 8 species of annuals and procumbent perennials which occur in the Americas, Africa and Asia. Sclerocarpus is placed by molecular data as a relatively basal member of the sunflower subtribe, Heliantheae [10]. Previous phytochemical investigations of Heliantheae have led to the isolation and identification of important medicinal compounds [11-15].

1.1 The Origin

Sclerocarpus africanus originated from Senegal and then spread to other parts of Africa like Sudan, Mozambique and south of Namibia [6].

Habitat: It occurs in sandy soils, woodlands and bushes or along river banks and lake shores [6].

Description: *Sclerocarpus* is an annual herb, erect, 15-120 cm high stems, hispid-pubescent leaves opposite on the lower part of the stem, alternate, higher up, ovate, 3-12 cm long, 1-6.5 cm wide, base crenate margins serrate or crenate, apex acute, scabrid or hispid on both surfaces, petioles to 4 cm long [6]. Capitula terminal, involuare with 5 spreading leafy green bract, 0.7– 3cm long, appressed pubescent, paleae tubular with large bulge caused by the ovary 9-13mm long. Ray florets few or absent, yellow to deep yellow with a narrow tube 2.5-6mm long. Ray 2.5-4.5 mm long, disc florets greenish yellow, 6.5-7.5 cm long puberulous near apex [7].

1.2 Medicinal Uses

The leaf is used to cure venereal diseases. An extract of the leaves in water in which mutton has been cooked is taken by the Igbo as a remedy for gonorrhea [6].

2. MATERIALS AND METHODS

The leaves and twigs of *S. africanus* were collected from a village in Zaria city, Kaduna state, Nigeria, in the month of June, 2010. It was confirmed and identified by Mal. Musa Gala of Department of Biological science, Ahmadu Bello University Zaria. The plant was given a specimen voucher number 667 and a sample deposited in the Herbarium.

2.1 Preparation of Plant Sample and Extraction

The leaves and twigs were air dried and pulverised with a mortar and pestle and weighed. Leaves (81.50 g) was packed in a soxhlet extractor and extracted with four different solvents of increasing polarity: 800 ml petroleum ether (60 – 80ºC), ethyl acetate (400 ml), ethanol (400 ml) and methanol (400 ml) in a sequential manner. After this, 65.60 g of twigs was extracted using methanol (500 ml). Each extract was concentrated using a rotary evaporator. The extracts were then kept in a fume cupboard to dry for further analysis.

2.2 Preliminary Phytochemical Screening

Preliminary phytochemical screening for secondary metabolites was carried out on the extracts in accordance with described methods [16-18]. Molisch's, Barfoed's, Fehling's tests, Resorcinol test (for Ketoses), tests for starch, soluble starch and standard test for combined reducing sugar were used in the test for Carbohydrates. Salkowski's test was used in the test for steroidal nucleus, anthraquinone glycosides test utilized Borntrager's test. Sulphuric acid test and Shinoda's test was used to test for flavonoids. For alkaloids, Mayer, Wagner and Picric acid reagents were used. Ferric chloride and lead subacetate tests were used to assay for tannins. Froth test was used to detect the presence of saponins.

2.3 Antimicrobial Studies

Antimicrobial screening of *S.africanus* methanol (SCME), ethanol (SCET) and petroleum ether extracts (SCPE) was determined using some pathogens. Pathogenic micro-organisms used were S. aureus, S. pyogenes, C. ulcerans for Gram positive; S. typhi, P. fluorescens,

P. mirabilis, S. dysenteriae, N. gonorrhea for Gram negative and C. albicans, C. thrusei for fungi. Microorganisms were obtained from Department of Microbiology A.B.U. Teaching Hospital, Zaria. All isolates were checked and maintained in slants of nutrient agar. Agar Well diffusion method was used to determine the antimicrobial activities of extracts. Each extract (0.1 g) was weighed and dissolved in 10 ml of solvent of extraction to obtain a concentration of 10 mg/ml, this was the initial concentration of extract used to determine the antimicrobial activities.

Blood agar was used as growth medium, it was boiled to dissolve and sterilized at 21ºC for 15 min, medium was cooled to 45ºC and 20 ml of sterilized medium was poured into sterile Petri-dishes. The cooled and solidified plates were seeded with 0.1 ml inoculums of test micro-organism which were evenly spread over the surface of the agar medium using sterile swab and allowed to dry at 137ºC for 30 min.

A standard cork borer of 6 mm diameter was used to cut a well at the centre of each inoculated plate and 0.1 ml of extract was then introduced into each well on the medium. The inoculated plate was incubated at 37ºC for 24 hrs after which plates was observed for zone of growth inhibition. For antimicrobial activity against micro organism Neisseria gonorrhoeae, same procedure was used but the medium was a chocolate blood agar; a broad spectrum antibiotic drug, Sparfloxacin$^{(R)}$ with concentration of 2 mg/ml was used as standard (Positive control).

2.3.1 Minimum inhibitory concentration (MIC)

Minimum inhibition concentration (MIC) was carried out on the extract that had shown growth inhibitory effects on test organisms. It was done using broth dilution method as modified by Usman, et al. [19], where 10 ml of the broth was dispensed into test tubes and sterilized at 121ºC for 15 min. Mac - Farland's turbidity standard scale number 0.5 was prepared to give a turbid solution, normal saline was prepared and used to make a turbid suspension of the microorganism. Incubation was made at 37ºC for 6 hrs, dilution of the microorganism in normal saline was done continuously until the turbidity marched that of the Mac - Farland's scale by visual comparison.

The initial concentration was obtained by dissolving 0.1 g of the extract in 10 ml of broth. Having obtained different concentrations of extract in the broth, 0.1 ml of standard inoculums of the microorganism in normal saline was then inoculated in to the different concentrations of extract in the broth. The broth was then incubated at 37ºC for 24 hrs. The lowest concentration of extract in the broth which showed no turbidity represents the MIC.

2.3.2 Minimum bactericidal/fungicidal concentration (MBC)/(MFC)

Minimum bactericidal concentration (MBC) of extracts was carried out according to methods described by Usman et al. [19] and Vollekova [20]; to determine whether the microorganisms were killed or only their growth was inhibited. Blood agar was prepared, sterilized at 121ºC for 15 min cooled to 45ºC and 20 ml of sterilized agar was poured into sterile Petri dishes, plates were allowed to cool and solidify. Contents of MIC in the serial dilution were sub-cultured onto the agar and agar plates were incubated at 37ºC for 24 hrs, after which plates were observed for colony growth. The MBC was plate with lowest concentration of extract without growth.

3. RESULTS AND DISCUSSION

Phytochemical screening of plants usually reveals common secondary metabolites present in such plants. For *S. africanus*, it showed the presence of carbohydrate, tannins, saponins, alkaloids, flavonoids and anthraquinone glycosides (Table 1). That flavonoids were present in methanol extract of the twigs but not leaves is really puzzling; we considered our observations on the basis of test results derived using simple chromogenic methods. This disparity, however, could be an area for further research**.** Tor-Anyiin et al. [5] and Prashant et al. [21] have stated that flavonoids posses antimicrobial and antidiarrhoeal behavior by complexing with cell walls, binding to adhesins, inhibiting release of autocoids and prostaglandins, inhibiting contractions caused by spasmogens, stimulating normalization of deranged water transport across the mucosal cells and inhibiting GI release of acetylcholine. Tests for tannins was positive for methanol extract but negative for ethanol extract and similarly free reducing sugar test negative for ethanol extract. Permeability of methanol because of less steric hindrance compared to ethanol, methanol's low boiling point translating into more circulation circles for a given time in the soxhlet extractor, higher vapour pressure of methanol as against ethanol, the slightly higher polarity of methanol; [22], may account for the selectivity for tannins and free reducing sugars noticed here.

Trease and Evans [17] as well as Prashant et al. [21], have stated that polyphenols and tannins have antimicrobial, antidiarrhoeal and antihelmintic tendencies via the agency of complexation, they bind to adhesins, inhibit enzymes by substrate deprivation, complex with cell walls, disrupt membranes, complex with metal ion, make intestinal mucosa more resistant and reduce secretion, stimulate normalization of deranged water transport across the mucosal cells and reduce intestinal transit, block the binding of B subunit of heat-labile enterotoxin to GM1, resulting in the suppression of heat-labile enterotoxin-induced diarrhea, astringent action, increase supply of digestible proteins by animals by forming protein complexes in rumen, interfere with energy generation by uncoupling oxidative phosphorylation and cause a decrease in G.I. metabolism.

Alkaloids have antimicrobial, antidiarrhoeal, antihelmintic properties. They exhibit this behaviour by Intercalating into cell wall and DNA of parasites and inhibiting release of autocoids and prostaglandins. Alkaloids possess anti-oxidizing effects, thus reducing nitrate generation which is useful for protein synthesis and suppress transfer of sucrose from stomach to small intestine, diminishing the supply of glucose to the helminthes, act on CNS causing paralysis, according to literature sources: Ndukwe et al. [2], Prashant et al. [21], Sofowora [23] and Edeoga et al. [24].

3.1 Microbial Sensitivity Test

Microbial sensitivity tests showed methanol and ethanol extracts were sensitive to seven microbes whereas petroleum ether extract was sensitive to four microbes (Table 2).

Table 1. Result of preliminary phytochemical screening

CONSTITUENTS	TEST	OBSERVATION	INFERENCE				
			Pet-Ether	EtOA	CH_3OH	EtOH	CH_3OH (Twigs)
Carbohydrate	a. Molisch test	Purple colour at interphase	-	-	+	+	+
	b. Barfoed test	Red precipitate	-	-	-	-	-
	c. Fehling test(Free Reducing sugar)	Brick red precipitate	+	+	+	+	+
	d. Fehling test(Combined Reducing sugar)	red precipitate	+	+	+	+	+
	e. Starch	yellow colour	+	+	+	+	+
	f. Soluble Starch	yellow colour	+	+	+	+	+
	g. Resorcinol test	Red colour	+	+	+	+	+
Saponin	a. Frothing test	Frothing which persist > 30 min	+	+	+	+	+
Tannins	a. Lead subacetate	Yellow coloration	+	+	+	-	-
	b. $FeCl_3$ test	Greenish black coloration	+	+	+	-	-
Alkaloids	a. Mayer's test	cream precipitate	+	+	+	+	+
	b. Wagner's test	No precipitate	-	-	-	-	-
	c. Picric acid test	yellowish – brown ppt	+	+	+	+	+
Anthraquinone gycosides	a. Borntrager's Test	violet colour in ammoniacal lower phase	-	-	+	+	+
Steroidal nucleus	a. Salkowski test	Cherry red ring	-	-	+	+	+
Flavonoid	a. Sulphuric acid test	deep yellow solution	-	-	-	+	+
	b. Shinoda's test	red colour	-	+	+	+	+

Key: EtOH = Ethanol extract, EtOAc = ethyl acetate extract, - = absent, + = present, ppt = precipitate

Table 2. Microbial sensitivity test (for methanol, ethanol and petroleum ether) leaf extract of *S. africanus*

Test organism	CH_3OH	EtOH	Pet-ether
Staphylococcus aureus	S	S	S
Streptococcus pyogenes	S	S	R
Corynebacterium ulcerans	R	R	R
Salmonella typhi	S	S	S
Pseudomonas fluorescens	R	R	R
Proteus mirabilis	R	R	R
Shigella dysenteria	S	S	S
Candida albicans	S	S	R
Candida thrusei	S	S	R
Neisseria gonorrhea	S	S	S

KEY S = sensitive R= resistant

The methanol extract, using inhibition zone diameter of the extracts as criteria for inhibitory strength showed highest inhibition against pathogens that were sensitive, with the pathogen, *Neisseria gonorrhea,* being most inhibited (Table 3).

Table 3. Result for diameter zone of inhibition of extracts against test microorganisms (in mm).

Test organisms	Pet-ether	EtOH	CH_3OH
Staphylococcus aureus	14	18	20
Streptococcus pyogenes	0	22	24
Corynebacterium ulcerans	0	0	0
Salmonella typhi	16	24	21
Pseudomonas fluorescens	0	0	0
Proteus mirabilis	0	0	0
Shigella dysenteria	16	24	27
Candida albicans	0	22	20
Candida thrusei	0	21	21
Neisseria gonorrhea	17	25	31

For methanol extract, MIC was 2.5 mg/ml for all the pathogens that were sensitive (Table 4) except *N. gonorrhea* which was 1.25 mg/ml. Similar results were obtained for ethanol extract except the pathogen, *staphylococcus aureus* that had MIC value of 5 mg/ml. The petroleum ether extract showed minimum inhibition against four pathogens with MIC value of 5 mg/ml (Table 4).

The value of minimum bactericidal/fungicidal concentrations of methanol extract for the pathogens was 10 mg/ml, 5 mg/ml and 2.5 mg/ml, for S.aureus, S. pyogenes, S. tyhpi, C.albicans and C. thrusei, but 5 mg/ml for S. dysentereae and N. gonorrhea. The MBC/MFC for ethanol extract had values of 10 mg/ml for all the pathogens except N. gonorrhea, which was 5 mg/ml. Petroleum ether extract gave MBC/MFC value of 10 mg/ml (Table 5) for all the microbes that it showed sensitivity.

Table 4. Minimum inhibition concentration (MIC) of the extract against microbes (in mg/ml)

	Plant Extract														
	Pet-ether					EtOH					CH_3OH				
Test Organisms	10	5	2.5	1.25	0.625	10	5	2.5	1.25	0.625	10	5	2.5	1.25	0.625
Staphylococcus aureus	-	o*	+	++	+++	-	o*	+	++	+++	-	-	o*	+	++
Streptococcus pyogenes						-	-	o*	+	++	-	-	o*	+	++
Salmonella typhi	-	o*	+	++	+++	-	-	o*	+	++	-	-	o*	+	++
Shigella dysentereae	-	o*	+	++	+++	-	-	o*	+	++	-	-	o*	+	++
Candida albicans						-	-	o*	+	++	-	-	o*	+	++
Candida thrusei						-	-	o*	+	++	-	-	o*	+	++
Neisseria gonorrhea	-	o*	+	++	+++	-	-	o*	+	++	-	-	-	o*	+

KEY: - = No turbidity (no growth), + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity, o = MIC*

Table 5. Minimum bactericidal/fungicidal concentration (MBC/MFC) of extracts against test microbes (in mg/ml)

	Plant Extracts														
	Pet-ether					EtOH					CH_3OH				
Test Organisms	10	5	2.5	1.25	0.625	10	5	2.5	1.25	0.625	10	5	2.5	1.25	0.625
Staphylococcus aureus	o*	+	++	+++	++++	o*	+	++	+++	++++	o*	+	++	+++	+++
Streptococcus pyogenes						o*	+	++	+++	++++	o*	+	++	+++	++++
Salmonella typhi	o*	+	++	+++	++++	o*	+	++	+++	++++	o*	+	++	+++	++++
Shigella dysentereae	o*	+	++	+++	++++	o*	+	++	+++	++++	-	o*	+	++	+++
Candida albicans						o*	+	++	+++	++++	o*	+	++	+++	++++
Candida thrusei						o*	+	++	+++	++++	o*	+	++	+++	++++
Neisseria gonorrhea	o*	+	++	+++	++++	-	o*	+	++	+++	-	o*	+	+	++

KEY:- = No colony growth, + = Scanty colonies growth,++ = moderate colonies, +++ = Heavy colonies, o = MBC/MFC*

4. CONCLUSION

The anti-microbial screening of methanol, ethanol and petroleum ether extracts of *S. africanus* showed activities against the following microorganisms *S. aureus, S. pyogenes, S. typhi, S. dysenteriae, C.albicans, C. thrusei* and *N. gonorrhea.* Also from diameter zone of inhibition, the microbe, *N. gonorrhea* had the highest value (was most inhibited) and hence, this justifies traditional usage of this plant as a remedy for gonorrhea as well as other venereal diseases.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

We would like to acknowledge kindly and selfless advice given by Professor G.I. Ndukwe and Dr. O.T. Audu both of Ahmadu Bello University Zaria, Nigeria; during the experimental stages of this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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