Original Research Article

ANTIBACTERIAL, ANTIFUNGAL AND PHYTOCHEMICAL SCREENING OF BITTER STEM BARK (*Sacoglottis gabonensis).*

ABSTRACT

The antimicrobial effects of bitter stem bark (Sacoglottis gabonensis) was evaluated using both ethanol and aqueous extracts against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Penicillium spp. Agar-well diffusion method was employed in determining the antimicrobial activity and broth dilution method for determining the Minimum Inhibitory Concentration. All the isolates investigated were susceptible to both the ethanol, aqueous and the combined aqueous and ethanol (synergy) extracts of the bitter stem bark.

The zone of inhibition ranged from 7.0 $\pm 0.00^{b}$ mm to 13.0 $\pm 0.01^{a}$ mm with *Escherichia coli* being the most susceptible at 13.0 $\pm 0.01^{a}$ mm to the ethanol and at 12.0 $\pm 0.02^{a}$ mm to the aqueous extracts at 200 mg/ml concentration while *P. aeruginosa* and *Penicillin* spp. were the least susceptible at 11.0 $\pm 0.02^{a}$ mm to the ethanol and at 10.0 $\pm 0.02^{a}$ mm to the aqueous extract at 200 mg/ml concentration. The control/ standard antimicrobial agent (Gentamicin and Ketoconazole) showed higher inhibitory activity than the plant extracts. The least inhibitory (MIC) value of 6.25 mg/ml was produced against *P. aeruginosa* by the ethanolic extract and against *C. albicans* by the combined (aqueous and ethanol) extract of the plant. The qualitative and quantitative phytochemical screening of the stem bark of *Sacoglottis gabonensis* reveals the presence of flavonoid, tannin, saponin and alkaloid. The most abundant percentage composition observed was flavonoid (7.61 %) while tannin had the least component (1.26 %). The findings from this study show that the stem bark extract possesses appreciable antimicrobial activity against commonly encountered microorganisms in the environment. This therefore implies that it can be used as a chemotherapeutic agent which will contribute to the development of antibiotic drugs against the test organisms.

KEYWORDS: Antimicrobial, Sacoglottis gabonensis, extracts, phytochemical.

1. INTRODUCTION

For many years, nature has been a source of medicinal agent with most modern drugs been isolated from natural sources. Plants are the basic sources of knowledge of modern medicine as several synthetic drugs are made from starting molecules extracted from plants and play important role in drug development programs in the pharmaceutical industry based on their use in traditional medicine and natural products [1]. Thus, plants remain the most abundant natural primary source of active drugs and are invaluable in the ethnomedical treatment of diverse ailments. Medicinal plants are generally sources of various phytochemicals, some of which are usually responsible for their biological activities [2].

Medicinal plants are abundant source of antimicrobial compounds and a wide range of these plant extracts are used to treat several infections caused by resistant microorganisms as they have potential antimicrobial activity [3]. They could have effects such as bacteriostatic, bacteriocidal, sterilizing disinfectant, antiseptic and preservative. Various plants have been shown to possess natural antimicrobial activities of great importance and therapeutic potentials in medicine and preservation [3].

Sacoglottis gabonensis is a large evergreen tree found in South America and Africa that occurs mostly in evergreen forest, savanna edges and on river banks. It belongs to the family – Humiriaceae and is commonly known as bitter bark tree. In Nigeria, it is known as nche /ntala /okpi-uta in Igbo, atala in Yoruba and úgu in Edo and most prevalent in certain rural communities of Nigeria especially Abia, Akwa-Ibom, Cross River, Delta, Bayelsa, Edo, Imo and Rivers State. It has been extensively used in Africa as most parts of the tree possess economic importance. The stem bark is used as additive in drinks (e.g., palmwine) to prolong its shelf life [4] and its addition to palm-wine or gin prevents fever and eradicates body pains. In Gabon, an extract of the stem bark is drunk as an emetic. In Sierra Leone and Nigeria (mostly among the Ngwa people of Abia state), the bark is used to treat stomach-ache and serve as a spice for its heating effect in pregnant and nursing mothers. In coastal Côte d'Ivoire, the stem extract is used in hipbaths in women after child delivery; but less attention have been paid to the health effect of these practices. The fruit seed is eaten as food and the sap used as pain-killers [5; 6].

The pyhtochemical screening of *Sacoglottis gabonensis* have shown the bark extracts of the tree to possess various active compounds with bergenin and gallic acid as the most active compounds [7]. The bark extract possesses antioxidant properties against peroxyl radical-induced lysis of mammalian erythrocytes as shown by *in-vivo* and *in-vitro* evidence [8]. Also, its antioxidant potential on 2, 4- dinitrophenylhydrazine-induced membrane peroxidation *in-vivo* has been reported [9].

The increasing resistance of major diseases to synthetic pharmaceutical products and the need to discover new molecular structures as lead compounds from the plant kingdom have led to a revival of interest in herbal medicines leading to a global growing interest in medicinal plants reflecting the authenticity of many traditional claims regarding the value of natural products in health care [1]. Therefore, this study was designed to investigate the antimicrobial properties and phytochemical constituents of the stem bark extract of *Sacoglottis gabonensis*.

2. MATERIALS AND METHODS

2.1. Collection and identification of Plant material

The stem bark of *S. gabonensis* was collected from Ikwano Local Government Area of Abia State of Nigeria and was identified and authenticated by Mr. O. F Udogu, a botanist in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Abia state, Nigeria.

2.1.1. Preparation of extract

The stem bark was washed with sterilized water, dried and ground to a fine powder using a mechanical blender. The ethanol and aqueous extracts were prepared by soaking fifty grams (50 g) of the powdered stem bark in 500 ml of 95 % ethanol and 500 ml distilled water respectively at room temperature for 24 h. The extracts were filtered separately through whatman filter paper (No. 1) and concentrated using rotary evaporator, warmed on water bath at 70 °C for the aqueous extract and temperature of 50 °C for ethanol extracts, to obtain crude extracts [10]. The extracts were stored at 4 ^{o}C in a refrigerator till further use. A combination of both extracts (ethanol and aqueous) was used in the synergistic assessment.

2.2. Collection and Maintenance of Test Organisms

Stock cultures of bacterial isolates of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and fungal isolates of *Candida albicans* and *Penicillium* spp., where obtained from the laboratory stock of the Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State. They were resuscitated and their identities confirmed using standard procedures according to the methods of [11] and [12]. Pure culture of the bacteria species were maintained on nutrient agar slant and the fungi species on potato dextrose agar slant at 4^{o}C before use.

2.3. Antimicrobial Assay

The agar well diffusion method was employed for determining the antimicrobial activities of the stem bark extract. Sterile Muller-Hinton agar plates and potato dextrose agar plates were inoculated with

standardized 50µl inoculum of each selected test organisms using spread plate technique. Wells of 6mm in diameter were made and 0.1 ml of the various concentrations (200 mg/ml, 100 mg/ml and 50 mg/ml) of the plant extracts dispensed into the wells. Gentamycin/ ketoconazole 200 mg/ml was used as a positive control. The plates were incubated at 37° C for 24h for bacteria and room temperature (25^{o}C) for 72 hours for fungi. The antimicrobial activity was evaluated by measuring the diameter of the resulting zones of inhibition in milimeters (mm) around the wells [13].

2.3.1. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of the extracts was carried out using the broth dilution method. Variable concentrations of the extract was prepared by serial dilution to obtain concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml 1.5625 mg/ml. Two milliliter (2 ml) of nutrient/ potato dextrose broth containing the test organisms was each dispensed into sterilized test tubes and a 1 ml extract of varying concentrations added to the test tubes containing the test organisms. The tubes were corked and incubated aerobically at 37°C for 24 hours for bacteria and 25^{o}C for 72 hours for fungi. The tubes were observed for turbidity after incubation to determine the MIC. The MICs for each organism were expressed as the lowest concentrations which inhibit the growth of the test microbial isolates [10; 14].

2.3.2. Determination of Minimum Bactericidal Concentrations / fungicidal Concentration (MBC /MFC)

The minimum bactericidal concentration (MBCs) / The minimum fungicidal concentrations (MFCs) was determined by subculturing of 2 µl from each of the tubes showing no growth (MIC culture tubes) onto nutrient and potato dextrose agar plates and incubating for 24 h at 37 ^{0}C and 72 h at 28 °C respectively. The lowest concentration with no visible growth was defined as MBC and MFC respectively, indicating 99.5% killing of the original inoculums [10].

2.4. Qualitative and Quantitative Phytochemical Analysis

Phytochemical screening of the plant extracts was carried out using standard methods as described by [15; 16] and [17] to ascertain the presence or absence of the different metabolites (flavonoids, tannins, saponins and alkaloids).

2.5. Statistical Analysis

Data was collected and analyzed using One-way analysis of variance (one-way ANOVA). Values were reported as means of triplicate determination ± standard deviation.

3. RESULTS AND DISCUSSION

The diameter of the zone of inhibition of the ethanol and aqueous extracts of *Sacoglottis gabonensis* against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Penicillin* species are presented in Table 1. It shows that both extracts had inhibitory activity against the isolates at higher concentrations of between 100 and 200 mg/ml, with the ethanolic extracts showing more activity.

Table 1: Antimicrobial Screening of Ethanol and Aqueous Extracts of *Sacoglottis gabonensis* against Selected Test Organisms

Test Organisms	Ethanol extract Concentration (mg/ml)			Aqueous Extract Concentration (mg/ml)			Control Gentamicin (200mg/ml)/ ketoconazole (200 mg/ml)
	200	100	50	200	100	50	

E.coli	13.0±0.01[a]	8.0±0.01[b]	0.0±0.01[c]	12.0±0.02[a]	0.0±0.00[b]	0.0±0.00[c]	22.0±0.04
S. aureus	12.0±0.02[a]	7.0±0.02[b]	0.0±0.00[c]	10.0±0.01[a]	0.0±0.00[b]	0.0±0.00[c]	19.0±0.02
P.aeruginosa	11.0±0.00[a]	7.0±0.00[b]	0.0±0.00[c]	10.0±0.02[a]	0.0±0.02[b]	0.0±0.00[c]	24.0±0.02
C. albicans	12.0±0.00[a]	8.0±0.00[b]	0.0±0.00[c]	10.0±0.02[a]	0.0±0.02[b]	0.0±0.00[c]	26.0 ±0.06
Penicillin spp	11.0±0.02[a]	7.0±0.02[b]	0.0±0.00[c]	10.0±0.02[a]	7.0±0.02[b]	0.0±0.00[c]	28.0 ±0.06

Values with different superscript are significantly different from each other (P<0.05)

The combined effects of the ethanol and aqueous extracts of *Sacoglottis gabonensis* against the test isolates (Table 2) shows that the highest *in-vitro* antimicrobial activity (14.0[a]±0.01 mm) was exhibited at the concentration of 200 mg/ml against *E.coli*, while the least antimicrobial activity (12.0[a]±0.00 mm) against *P. aeruginosa* and *Penicillin* spp. was also exhibited at the concentration of 200 mg/ml. The zone of inhibition produced by the standard antimicrobial agents (Gentamicin and Ketoconazole) against the test microorganisms showed higher inhibitory activities than the extracts of *Sacoglottis gabonensis.*

Table 2: Antimicrobial Screening of the Combined Effect of Aqueous and Ethanol Extracts of *Sacoglottis gabonensis* against Selected Test Organisms

Test Organisms	Ethanol/Aqueous extract Concentration (mg/ml)			Control Gentamicin (200mg/ml)/ ketoconazole (200 mg/ml)
	200	100	50	
E.coli	14.0±0.01[a]	11.0±0.01[b]	0.0±0.00[c]	22.0±0.04

S. aureus	13.0±0.03 [a]	9.0±0.02 [b]	0.0±0.00 [c]	19.0±0.02
P. aeruginosa	12.0±0.00 [a]	7.0±0.00 [b]	0.0±0.00 [c]	24.0±0.02
C. albicans	13.0±0.00 [a]	8.0±0.00 [b]	0.0±0.00 [c]	26.0 ±0.06
Penicillin spp	12.0±0.02 [a]	7.0±0.01 [b]	0.0±0.00 [c]	28.0 ±0.06

Values with different superscript are significantly different from each other (P<0.05)

The minimum inhibitory concentration (MIC) of the extracts of *Sacoglottis gabonensis* against the test isolates are represented in Table 3 and Table 4. Both extracts (aqueous and ethanol) showed active inhibitory effects but with the ethanolic extracts showing more activity. The least inhibitory value of 6.25 mg/ml was produced against *P. aeruginosa* and *C. albicans* by the ethanolic extract and by the combined extract of the aqueous and ethanolic extracts of *S. gabonensis* respectively.

Table 3: MIC Values of Aqueous and Ethanol Extracts of *S. gabonensis* Against Test Isolates

	Test Organisms	Concs. (mg/ml) 50	25	12.5	6.25	3.12	1.56	MIC
Aqueous	*E. coli*	+	+	+	+	+	+	50
	S. aureus	+	+	+	+	+	+	50
	P.aeruginosa	+	+	+	+	+	+	50
	Penicillin spp	+	+	+	+	+	+	50
	C. albicans	+	+	+	+	+	+	50
Ethanol	*E. coli*	-	+	+	+	+	+	25
	S. aureus	-	+	+	+	+	+	25
	P.aeruginosa	-	-	-	+	+	+	6.25
	Penicillin spp	-	-	+	+	+	+	12.5
	C. albicans	-	-	+	+	+	+	12.5

Key: + =positive,

- =Negative

Table 4: MIC Values of Combined Extract (Aqueous and Ethanol) of *S. gabonensis* Against Test Isolates

	Test Organisms	Concs. (mglm) 50	25	12.5	6.25	3.12	1.56	MIC
A & E	*E. coli*	-	+	+	+	+	+	25
	S.aureus	-	+	+	+	+	+	25
	P.aeruginosa	-	-	+	+	+	+	12.5
	Penicillin spp	-	+	+	+	+	+	25
	C. albicans	-	-	-	+	+	+	6.25

Key: + = positive,

- = Negative

In Table 5, the minimum bactericidal concentration/ minimum fungicidal concentration revealed greater activity by the ethanolic extracts of *S. gabonensis* against *P. aeruginosa* at 12.5 mg/ml and the combined (ethanol and aqueous) extracts against *C. albicans* at 12.5 mg/ml (Table 6), while the aqueous plant extract had cidal activities at 50 mg/ml for all the isolates tested.

Table 5: MBC/MFC Values of Aqueous and Ethanol Extracts of *S. gabonensis* Against Test Isolates

	Test Organisms	Concs. (mg/ml) 50	25	12.5	6.25	3.12	1.56	MBC/MFC
Aqueous	*E.coli*	+	+	+	+	+	+	50
	S.aureus	+	+	+	+	+	+	50
	P.aeruginosa	+	+	+	+	+	+	50
	Penicillin spp	-	+	+	+	+	+	50
	C. albicans	+	+	+	+	+	+	50
Ethanol	*E.coli*	-	+	+	+	+	+	50
	S.aureus	-	+	+	+	+	+	50
	P.aeruginosa	-	-	-	-	+	+	12.5
	Penicillin spp	-	-	+	+	+	+	25
	C. albicans	-	-	+	+	+	+	25

Key: += positive,

- = Negative

Table 6: MBC/MFC Values of Combined Extract (Aqueous and Ethanol) of *S. gabonensis* Against Test Isolates

	Test Organisms	Concs. (mg/ml) 50	25	12.5	6.25	3.12	1.56	MBC/MFC

A & E	*E. coli*	-	+	+	+	+	+	50
	S.aureus	-	+	+	+	+	+	50
	P.aeruginosa	-	-	+	+	+	+	50
	Penicillin spp	-	+	+	+	+	+	50
	C. albicans	-	-	-	-	+	+	12.5

Key: + = positive,

- = Negative

Table 7 and Table 8 shows the qualitative and quantitative phytochemical screening of the stem bark of *Sacoglottis gabonensis* revealing the presence of Flavonoid, Tannin, Saponin and Alkaloid. The most abundant percentage composition observed was flavonoid (7.61 %), followed by Alkaloid (6.81 %), and saponin (6.48 %), and Tannin having the least component (1.26 %).

Table 7: Qualitative Phytochemical Analysis of the Stem Bark of *Sacoglottis gabonensis*

Phytochemical	**% Composition**
Flavonoid	+++
Tannin	+
Saponin	+++
Alkaloid	+++

Table 8: Quantitative Phytochemical Composition of the Stem Bark of *Sacoglottis gabonensis*

Phytochemical	**% Composition**
Flavonoid	7.61 $\pm$0.14
Tannin	1.26 $\pm$0.18
Saponin	6.48 $\pm$0.12
Alkaloid	6.81 $\pm$0.02

Values shows means of triplicate analysis

Antibiotics have remained the mainstay of drug therapy of infectious diseases worldwide. Identification of natural products from plants that may serve as valuable sources of antimicrobial agents for medicinal

uses seems to be a viable alternative to the conventional antibiotics in the face of increasing antibiotics resistance. In the present study, antimicrobial potency and synergistic effect of crude aqueous and ethanolic extracts of *Sacoglottis gabonensis* against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Penicillium* species were investigated (Table 1 and Table 2).

The results obtained from the antimicrobial activities of the extract of the stem bark of *Sacoglottis gabonensis,* individually and in combination (synergy) posses appreciable antimicrobial activity against commonly encountered microorganisms investigated.

The antimicrobial activities of the extracts were observed to be concentration-dependent and the activity varied with concentration against the tested pathogens. All the organisms tested were sensitive to the extracts of *Sacoglottis gabonensis* between the concentrations of 100 mg/ml and 200 mg/ml of the extracts. The inhibition zone ranged from 7.0 ± 0.00^{b} mm to 14.0 ± 0.01^{a} mm with *Escherichia coli* being the most susceptible (at 14.0 ± 0.01^{a} mm, 13.0 ± 0.01^{a} mm and 12.0 ± 0.02^{a} mm) to the synergy, ethanol and aqueous extracts at 200mg/ml concentration respectively while *P. aeruginosa* and *Penicillin* spp. were the least susceptible (at 12.0 ± 0.02^{a} mm, 11.0 ± 0.02^{a} mm and 10.0 ± 0.02^{a} mm) to the synergy, ethanol and aqueous extract at 200mg/ml concentration respectively. This correlate with a similar study by [18], who reported that, the aqueous extract of *S. gabonensis* showed promising inhibitory activity against microorganisms such as *M. ulcerans in vitro.* [19], also reported *S. gabonensis* to possess antimicrobial activities, against the growth of *Pseudomonas aeruginosa*, *Shigella flexnerii*, *Staphylococcus aureus* and *Streptococcus* spp.

The fact that the various microbial isolates subjected to the extract of *Sacoglottis gabonensis* were susceptible shows that the stem bark of this plant has antimicrobial potency. This finding agree with the work of [20], who reported that preparation from stem bark of *Sacoglottis gabonensis* could be useful chemotherapeutic agent against infectious diseases. The results obtained shows that the ethanol extracts showed higher sensitivity of the extract on all microorganisms tested than the aqueous extracts. This probably indicates that ethanol is a better solvent than water in the extraction of the active principles of the plant. This corroborates with the report of [21]. Furthermore, ethanol extract produced the highest zones of inhibition agree with the previous report of [22], who stated that many factors influence the

active components present in plants which included: the age of the plants, extracting solvent, method of extraction and time of harvesting plant material. The result obtained in this study is comparable to those recorded in similar studies by [23; 24] and [25]. *Moringa oleifera* extracts have been reported to demonstrate antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Candida albican*, and *Mucor* species [23].

The minimum inhibitory concentration (MIC) of the various extracts were observed to be between the concentrations of 6.25 mg/ml and 12.5 mg/ml for the ethanolic and combined (synergy) extracts on both bacterial and fungal isolates and 50 mg/ml for the aqueous extracts on both bacterial and fungal isolates (Table 3 and table 4). These observations are in agreement with similar report by [26], who recorded remarkable activity against the growth of *E. coli, S. aureus* and *K. pneumonia* with zones ranging from 13mm to 26mm and MIC values of 25mg/ml, 50mg/ml and 100mg/ml for the test organisms respectively.

The minimum bactericidal/fungicidal concentration (MBC/MFC) values reflects greater activity of the ethanol extracts against *P. aeruginosa* at 12.5 mg/ml, *Penicillin spp* and *C. albicans* at 25 mg/ml when compared to the aqueous extracts at concentrations of 50 mg/ml against all tested isolates (Table 5**).** The combined (synergy) extracts had values of 12.5 mg/ml against *C. albicans* and 50 mg/ml against all bacterial isolates tested (*E. coli, S. aureus, P. aeruginosa)* and *Penicillin* spp. (Table 6). This present findings show that the ethanolic extracts have more inhibitory effects as an antimicrobial agent than their aqueous extracts and when compared with the control (standard antibiotics), the control produced higher inhibition than the various extracts against all the test organisms.

Phytochemical screening of the stem bark of *Sacoglottis gabonensis* as shown in (table 7), shows that the study plant materials contained alkaloids, terpenoid, flavonoids, tannins and saponin but to varying degrees. These phytoconstituents has been proven to possess various medicinal or herbal importance which further explains its antimicrobial activities [27]. Quantitatively, the percentage yields of phytochemical content of the stem bark of *Sacoglottis gabonensis* were as follows: alkaloids (6.81%), flavonoids (7.61%), saponins (6.48%), and tannins (1.26%) which shows that the stem bark of this plant have appreciable amount of these phytochemicals, hence their medicinal value. This is in contrast to the findings of [18], who reported the stem bark of *S. gabonensis* to have tannins, sterols, polyterpenes, polyphenols, flavonoids, and alkaloids in appreciable amounts with a trace of saponins. Other related

studies have reported the presence of tannins, flavonoids, saponins and phenols from extracts of *Sacoglottis gabonensis* plants [28]. These compounds have been shown to be active against potentially significant pathogens including those that are responsible for enteric infections [29]. Flavonoids have anti-fungal, antibacterial and anti-inflammatory properties [30]. Both alkaloids and flavonoids have antimicrobial activities [31], and phytoconstituents such as saponins and phenolics compounds are reported to inhibit bacterial growth. The pharmaceutical and therapeutic potentials of plants and their products are as a result of the presence of these phytochemicals (flavonoids, tannins, saponins, and alkaloids) in them [32].

CONCLUSION

The *in-vitro* antimicrobial activity demonstrated by *Sacoglottis gabonensis* stem bark extracts against the test microbial isolates indicates that the plant possess potential antimicrobial strength. Thereby, validating the local use of *Sacoglottis gabonensis* for medicinal purposes in treating infectious diseases such as gastro intestinal disorders, gonorrhea, diarrhea, typhoid and other infections in which the tested pathogens may be implicated. The presence of phytochemicals and the ability of the stem bark extracts to inhibit the growth of several microbial species is an indication of the broad spectrum antimicrobial potential of *Sacoglottis gabonensis* which makes it a potential candidate for a prospective antimicrobial drug. Hence, these plants are efficacious and contain natural compounds that could be used in the treatment of infections.

RECOMMENDATION

Further studies should be carried out on *Sacoglottis gabonensis* due to its efficacy against diseases as these plant extracts could be a potent raw material for pharmaceutical applications and help promote alternative medicine as better substitutes to synthetic antimicrobials.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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