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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *SARCOCEPHALUS POBEGUINII* L. LEAF EXTRACT

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ABSTRACT

People still rely on traditional plant-based medicine as their primary source of health care. The infusion of *Sarcocephalus pobeguinii* leaves is taken as a remedy for fever. This study investigated the phytochemical and antimicrobial properties of ethanol: water (4:1) extract of *Sarcocephalus pobeguinii* leaf. The phytochemical evaluation was carried out using standard methods while agar well diffusion method was used to study antimicrobial activity. Results obtained reveal that the extract contained some detectable phytochemicals such as alkaloids, flavonoids, glycosides, tannins and saponins, whereas steroids and terpenoids were absent. The extract was effective against bacterial species, namely *Staphylococcus aureus*, *Bacillus substilis*, *Salmonella typhi, and Pseudomonas aeruginosa* while fungi (*Candida albicans* and *Saccharomyces cerevisiae*) were resistant to the extract. The MIC and MBC values were in the range of 25 – 100 mg/ml of the extract. These results support the use of the extract as herbal medicine for the treatment of various diseases.

KEYWORDS: Antimicrobials, Antibiotics, Extract, Phytochemicals, *Sarcocephalus pobeguinii*

INTRODUCTION

Herbal medicine or phytomedicine refers to the use of plant parts such as seeds, berries, roots, leaves, bark or flowers for medicinal purposes [1]. Plants and plant products have been used as medicines since the start of history [2]. In many parts of the developing countries, especially in the rural areas, herbal medicines continue to be used as the primary source of health care for the treatment and alleviation of ailments and symptoms like cough, malaria, fever, diabetes, cold, diarrhea, dysentery, acne, scalds, allergic dermatitis, etc. According to the World Health Organization estimates, about 80% of the people living in the developing countries use traditional medicines as their primary health care [3]. History has shown that Traditional medicine practitioners have contributed immensely to the elucidation of many potent compounds [4].

Presently, substances derived from plants are attracting a lot of attention due to their numerous functionalities [5]. Phytomedicine is now preferred by many people due their milder and fewer side effects when compared to orthodox medicine, and due to affordability [6]. In West African countries such as Ghana and Nigeria, herbal medicine is greatly patronized due to beliefs, convenience and privacy.

The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds; these are phytochemical components, the most important of which are alkaloids, tannins, flavonoids and phenolic compounds [7] which on interaction

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with human systems produce important physiological actions. They are also a great source of new lead chemicals for pharmaceuticals. There have been numerous reports of the use of plants and natural products for the treatment of diseases. Drugs which have been derived from natural resources include taxol (anti-cancerous), camptothecin (anti-cancerous) and artemisinin (antimalarial).

Many plant-derived medicines used in traditional medicinal systems have been found to be effective against microbial pathogens. Many researchers have conducted research on plant products to check their antimicrobial effects [8].

Sarcocephalus pobeguinii pobeguin ex pelleger, named after the French botanist Charles Henri Oliver Pobeguin, belongs to the plant division Magnoliophyta which consists of order Gentianales, a large family of Rubiaceae and a genus of *Sarcocephalus.* The genus *Sarcocephalus* is a flowering plant (angiosperm) which consists of about 48 plant species [9]. *Sarcocephalus pobeguinii* is a savannah tree up to 30 m high, trunk straight, cylindrical, 3 m in girth, not buttressed, found near river-banks, lake-sides and marsh-forest. *S. pobeguinii* trees are widely distributed in Senegal, Guinea, Gabon, disjointedly to North and South Nigeria, Cameroun and Zambia. It flowers in April– July, and bears fruits in September–November in West Africa. The fruits are edible and are a favored food for chimpanzees in Guinea and gorillas in Central Africa. The seeds are recorded to be dispersed by water. Three closely related species are *Sarcocephalus latifolius, Sarcocephalus diderichi* and *Sarcocephalus vandergushtii* [10, 11].

Sarcocephalus pobeguinii plant has not been studied for its phytochemical screening and antimicrobial activity. Therefore, the present study highlights for the first time the antibacterial activities of alcohol/water solvent fractions of this plant (Figure 1).

Figure 1: *Sarcocephalus pobeguinii*

MATERIALS AND METHODS

Reagents and Chemicals

All chemicals and reagents used in this study were of analytical grade and purchased from certified suppliers.

Collection and Identification of Plant Samples

Sarcocephalus pobeguinii leaves were collected from Galadimawa, Giwa L.G.A of Kaduna State and identified (voucher number 547) by a Botanist at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria.

Preparation and Extraction of Plant Material

S. pobeguinii leaves were shade dried in the laboratory at room temperature for seven days. The dried leaves were then pulverized using wooden pestle and mortar. The pulverized sample (600 g) was moistened with little amount of water and then microwaved three times for three minutes, with intermittent cooling and then extracted with 500 mL of ethanol/water in the ratio of 4:1 and then filtered with Whatman No. 1 filter paper. The extraction was carried out four times. The extract obtained was evaporated using rotary evaporator at 40^{o}C to prevent the degradation of plant material and dried at room temperature to a constant weight. The extract was then stored in an air tight container at room temperature until required for analysis.

Phytochemical Screening

The presence or absence of the phytochemical constituents in the plant material was investigated. Tests for alkaloids, flavonoids, glycosides, and steroids/triterpenes were carried out in accordance with methods described by Trease and Evans [12], while tests for tannins and saponins were carried out using the procedures of Harborne [13].

Antimicrobial Assay of *S. pobeguinii* Leaf Extract

Test Microorganisms

The test microorganisms used for this analysis, including six species of bacteria, viz. *Staphylococcus aureus, Salmonella typhi*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*, and two fungal entities, viz. *Candida albicans* and *Saccharomyces cerevisiae* were pure clinical isolates obtained from the Department of Microbiology, Ahmadu Bello University Zaria.

Culture Media

The culture media used for the analysis includes Mueller Hinton agar (MHA), Mueller Hinton broth (MHB), potato dextrose agar, (PDA) and nutrient agar (NA). The mentioned media were used for zone of inhibition, minimal inhibitory concentration and minimal bactericidal concentration. All media were prepared according to manufacturer's instructions and sterilized by autoclaving at 121^{o}C.

Determination of Inhibitory Activity of the *S. pobeguinii* Leaf Extract Using Agar Well Diffusion Method

The standard inoculate of both the bacterial and the fungal isolates were streaked on sterilized Mueller Hinton and potato dextrose agar plates respectively with the help of sterile swab sticks. Exactly 0.5 g of the extract was weighed and dissolved in 10 ml of dimethyl sulfoxide (DMSO) to obtain a concentration of 50 mg/ml. Wells were punctured on each inoculated agar plate with a sterile cork borer. Each well was filled up with approximately 0.2 ml of the 50 mg/ml concentration of the extract. The inoculated plates of Mueller Hinton agar were then incubated at 37^{o}C for 24 hours while the plates of potato dextrose agar were incubated at room temperature for 3 days. At the end of the incubation periods, the plates were observed for any evidence of inhibition which appeared as clear zones that were completely devoid of colony growth around the wells. The diameter of the zones were measured and recorded in millimeters.

Determination of Minimal Inhibitory Concentration of the *S. pobeguinii* Leaf Extract

The minimal inhibitory concentration of the *S. pobeguinii* extract was determined using the tube dilution method, with the Mueller Hinton broth used as diluent in accordance with National Committee for Clinical Laboratory recommendation [14]. Dilutions of the extract in the sterile broth were made to obtain the concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. The organisms were inoculated into each tube containing the broth and the extract. The inoculated tubes were then incubated at 37^{o}C for 24 hours. At the end of the incubation period, the tubes were examined for the presence or absence of colony growth using turbidity as a criterion. The lowest concentration in the series without visible sign of growth was considered to be the minimal inhibitory concentration (MIC).

Determination of Minimal Bactericidal Concentration (MBC)

Experiments were carried out to determine whether the test microbes were killed or only their growth was inhibited during the MIC experiments. This MIC result was used to determine the minimal bactericidal concentration (MBC) of the extract. A sterilized wire loop was dipped into the test tube that did not show turbidity on the MIC test and a loopful was taken and streaked on sterile nutrient agar plates. The plates were incubated at 37^{o}C for 18 hours. At the end of the incubation period,

the plates were examined for the presence or absence of colony growth.

Determination of Activity Index

The activity index (ratio of the average zone of inhibition of the extract over the zone of inhibition of standard antibiotic) was used to compare the inhibitory activity of the crude extract to that of the standard antibiotic.

RESULTS AND DISCUSSION

The leaf extract of *S. pobeguinii* using ethanol:water (4:1) was obtained as brick red solid mass with extraction yield of 14.5%.
The qualitative phytochemical investigation on the extract of this plant showed the presence of alkaloid, flavoniod, saponin, tannin, and glycosides, while triterpenoids and steroids were absent (Table 1).

Table 1: Phytochemicals of *S. pobeguinii* leaf extract

S/No	*Phytochemicals*	*Result*
1	Flavonoids	+
2	Steroid	_
3	Triterpenoid	_
4	Saponins	+
5	Tannins	+
6	Glycosides	+
7	Alkaloid (Wagner's) Alkaloid (Mayer's)	+ +

+ = Positive (present), - = negative (absent)

These secondary metabolites are secreted by plants in response to environmental pressure or as a defense mechanism to animal attacks or plant diseases [15]. The presence of these compounds gives the plants potential applications in the treatment of microbial diseases. Flavonoids act on prostaglandins which are involved in the late phase of acute inflammation [16]. Many alkaloids are used as antibacterial for the treatment of various bacterial ailments [17]. Tannins are one the components in herbs that provide for their astringent characteristic and are used for treating intestinal disorders such as diarrhea and dysentery, and have promising antibacterial activity [18]. The antipyretic potentials of alkaloid, tannins, flavonoid and glycosides have been reported [19].
The sensitivity test of *S. pobeguinii* extract against microorganisms such as *Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli*, *Candida albicans, and Saccharomyces cerevisiae* was carried out and potency of the extract against susceptible organisms was quantitatively assessed by Zone of inhibition, Minimal Inhibitory Concentration (MIC), and Minimal Bactericidal Concentration (MBC) values at concentrations of 12.5-100 mg/ml.
Table 2 shows the organisms that were sensitive or resistant to the *S. pobeguinii* leaf extract. All the fungi were resistant to the extract. The result showed antimicrobial activities on 4 out of 6 bacterial species tested, namely: *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* with the zone of inhibition at maximum concentration of 100 mg/ml ranging from 19 – 22 mm (Table 3). The highest zone of growth inhibition was shown against *S. aureus and B. substilis* (22 mm), followed by *Salmonella typhi* (20 mm) *and Pseudomonas aeruginosa* (19 mm). The relatively large zone sizes produced by the plant extract against the test bacteria was an indication of the potency of the bioactive components of the plant against these species. Ciprofloxacin was used as a positive control (Table 3).

Table 2: Sensitivity test of *S. pobeguinii* leaf extract compared with Ciprofloxacin and Econazole

Test organisms	*S. pobeguinii* Extract	Ciprofloxacin	Econazole
S. aureus	S	S	ND
S. typhi	S	S	ND
K. pneumonia	R	S	ND
B. subtilis	S	S	ND
P. aeruginosa	S	S	ND
E. coli	R	S	ND
S. cerevisiae	R	ND	S
C. albicans	R	ND	S

R = Resistant, S = sensitive, ND = Not determined

Table 3: Measured Zones of inhibition (mm) at varying concentrations (mg/ml) of the extract as compared with standard antibiotics

	S. pobeguinii L. extract Conc. (mg/ml)				Ciprofloxacin	Econazole
Organism	**100**	**50**	**25**	**12.5**	**Conc. 10 µg/ml**	
S. aureus	22	19	17	15	35	ND
S. typhi	20	17	15	13	38	ND
K. pneumoniae	-	-	-	-	40	ND
B. subtilis	22	18	16	14	36	ND
P. aeruginosa	19	16	13	-	36	ND
E. coli	-	-	-	-	36	ND
S. cerevisiae	-	-	-	-	ND	35
C. albicans	-	-	-	-	ND	35

- = no activity, ND = not determined

Table 4: Activity index of the *S. pobeguinii* leaf extract

Test organism	Activity index
Staphylococcus aureus	0.52
Salmonella typhi	0.43
Bacillus subtilis	0.49
Pseudomonas aeruginosa	0.44

Table 5: Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (mg/ml) of the *S. pobeguinii* leaf extract against test microorganisms

Test organism	Concentration (mg/ml)			
	100	**50**	**25**	12.5
S. aureus	-	ǂ	*	+
S. typhi	-	ǂ	*	+
B. subtilis	-	ǂ	*	+
P. aeruginosa	ǂ	*	+	+

- = no colony growth, + = colony growth, * = MIC, ǂ = MBC

Our findings on the effect of the crude extract correlate with reports that microorganisms varied widely in their degree of susceptibility [20]. The extract inhibited *S. aureus*, *S. typhi*, *B. subtilis*, and *P. aeruginosa* which when compared with activity of ciprofloxacin had corresponding activity index of 0.52, 0.43, 0.49, and 0.49 respectively (Table 4). Tegos *et al.* [21] explained that the observed difference is attributable to the fact that while synthetic antibiotics are in a pure form, crude plant extracts contains some impure substances that may be inert and do not have any antibacterial activities. Determination of MIC and MBC (Table 5) was performed in order to evaluate the efficacies of antimicrobial agents, and in this study the MIC values obtained were between 25 and 50 mg/ml (Table 5) while the MBC values were in the range of 50–100 mg/ml. The MIC for *S. aureus*, *S. typhi* and *B. subtilis,* was 25 mg/ml and for *P. aeruginosa* it was 50 mg/ml.

The concentration of the extract that was required to kill *S. aureus*, *S. typhi*, and *B. subtilis* was 50 mg/ml while for *P. aeruginosa* it was 100 mg/ml as shown by the MBC results in Table 5. The antibacterial activities of alkaloid, flavonoids, saponins and tannins have been reported [22]. Therefore, the antibacterial activity of *Sarcocephalus pobeguinii* leaf extract may be attributed to the presence of bioactive constituents.

CONCLUSION

This present study revealed the presence of a number of phytochemicals such saponins, tannins, flavonoids glycosides, and alkaloids which may be responsible for inhibiting and destroying the growth of microorganisms such as *S. aureus*, *S. typhi*, *B. subtilis*, and *P. aeruginosa.* Our findings are vital for the validation of the use of *S. pobeguinii* leaf infusion in the treatment of typhoid fever and related ailments.

CONFLICT OF INTEREST

authors declared no conflict of interests in te conduct and reporting of this study

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