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Antimicrobial Activity, Phytochemistry and Acute Toxicity Profile of *Sarcocephalius latifolius* Root Bark

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ABSTRACT

Sarcocephalius latifolius root bark is used in ethnomedicine to treat urinary tract infections, malaria, diarrhea, and typhoid fever. The present study was aimed at investigating the antimicrobial activity of the plant. Clinical isolates of *Klebsiella spp, Proteus mirabilis, and* type isolates including *Candida albicans* ATCC 7596, *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 1200 and *Salmonella kintambo* SSRL113 were used in the study. Agar well diffusion technique and broth dilution method were used to determine the antimicrobial properties and minimum inhibitory concentrations (MIC). The results obtained revealed that the ethanol extract significantly ($P < 0.05$) exhibited more antimicrobial activity than the aqueous extract. Fractionation of the ethanol extract was carried out with chloroform, ethyl-acetate, acetone, and methanol to obtain corresponding fractions. Antimicrobial activity testing of the fractions showed that the ethyl-acetate fraction exhibited the highest antimicrobial effect. Phytochemical analysis of the crude extracts and the various fractions revealed the varied content of alkaloids, saponins, essential oils, tannins, glycosides and flavonoids, which are reported to be mainly responsible for the recorded antimicrobial effect. Acute toxicity studies performed on the ethanol extract using albino rats suggested that the extract is non-toxic ($LD_{50} > 5000$ mg/kg body weight). The results authenticate the folklore claim of medicinal properties of the plant.

***Keywords*:** *Sarcocephalius latifolius*, Antimcrobial activity, Phytochemical analysis, Acute toxicity.

Introduction

Medicinal plants have been a source of medicinal agents for many years, and many chemotherapeutic drugs have their origin from plants.[1] The world health Organization (WHO) stated that medicinal plants are the best precursors for synthesizing new drugs.[2] In recent times, there is an increase in plant-based therapeutic products in both developed and developing countries because they are mostly non-toxic, have fewer side effects, and are available at affordable prices.[3] Medicinal plants are used widely in many parts of the world, especially in rural areas with limited access to modern medical and health facilities.[4] Even though orthodox medicines are now available for many debilitating ailments and disease conditions, there is a need to develop new therapeutic antimicrobial agents from medicinal plants due to the increasing toxicity and failure of many chemotherapeutics and antibiotics resistance exhibited by pathogenic microorganisms.[5-7] Medicinal plants have also been reported to have minimal or no side effects and have been found useful for practically almost every known ailment.

Sarcocephalus latifolius (Smith), also called African peach, is a member of the plant family Rubiaceae. The plant is a small multi-stemmed tree with rounded-ovate leaves and bears red ball-like fruits.[8] The plant is widely distributed in several African countries, including, but not limited to, Uganda, Gambia, Nigeria, Cameroon, Democratic Republic of Congo, Ghana, Congo, Gabon, Zaire, Ivory Coast, Ghana, Liberia, Mali, Senegal, Sierra Leone. The use of *Sarcocephalus latifolius* in herbal medical practice is widespread in South-Eastern Nigeria, where the root of the plant is used to treat diabetes, gastrointestinal disorders, urinary tract infections, malaria, and typhoid fevers, either singly or in combination with other herbs. The wide range of medicinal uses and antimicrobial properties of this plant was reported by Oluremi *et al*.,[9] and Aderibigbe *et al*.,[10] Ethnomedical use of *Sarcocephalus latifolius* in the treatment of malaria has also been reported by Ahoyo *et al*.,[11] Therefore, the objective of the study was to investigate the antimicrobial properties of the crude extracts and various fractions of *Sarcocephalus latifolius* (root bark) and determine its acute toxicity in experimental animal models.

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Materials and Methods

Collection of plant material

The root bark of *Sarcocephalus latifolius* was harvested from a forest in Idah, Kogi State, Nigeria, in August 2020 and authenticated by Mr. Felix Nwafor, a plant taxonomist at the herbarium unit of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria. A voucher specimen with specimen number PCG/UNN/0348 was deposited at the herbarium for reference purposes.

Extraction procedure

Extraction was carried out by a modification of the method described by Ezeonu *et al*.,[12] Within three hours after the collection, the root bark was washed with clean tap water, chopped into smaller bits, and air-dried under a shade for ten days before being pulverized into a coarse powder using Hammer pulverizer MS-Body milling machine. A 500 g portion of powder was respectively cold macerated in 2 liters

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of distilled water and 2 liters of ethanol at ambient temperature for 48 h. The extracts were filtered successively with a muslin cloth and Whatman No. 1 filter paper. The resulting ethanol filtrate was then concentrated using a vacuum rotary evaporator at 40^{0}C to get a dry brown crude extract, while the aqueous extract was concentrated to dryness using a Redline forced air convention drying oven at 45^{o}C and 40 rpm. The resulting aqueous and ethanol extracts were stored at 4^{o}C until required for use.

Percentage yield of the extraction

Percentage yield (%) = $\frac{Weight\ of\ Extract}{Weight\ of\ pulverized\ plant\ material}$ (g) X 100

Fractionation of the crude extract

Dried ethanol extract (50 g) was fractionated by column chromatography. Silica gel (200 g) was homogenized with the dried ethanol extract and then parked inside a chromatographic column. The column was eluted in succession with 1.5 liters of n-hexane, chloroform, ethyl-acetate, acetone, and methanol to obtain their corresponding fractions, thus, n-hexane soluble fraction, Chloroform soluble fraction, ethyl-acetate soluble fraction, and acetone soluble fraction. The resulting fractions were concentrated using a rotary evaporator at 45^{o}C and 40 rpm.

Test organisms

The test microorganisms were all collected from the Medical Microbiology Laboratory unit, University of Nigeria. The test organisms used were *Klebsiella spp, Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 10145), *Salmonella kintambo* (SSRL113), *Proteus mirabilis,* and *Candida albicans* (ATCC 7596). Pure cultures of the isolates were stored at 4^{o}C until when required for use.

Phytochemical screening

The phytochemical screening for the bioactive constituents was carried out using the methods as described by Soforowa.[13]

Antimicrobial screening of the crude extracts

The antimicrobial activity was performed using a modified agar well diffusion method described by Yusuf *et al*.,.[14] The crude extracts were dissolved with 5% dimethylsulphoxide (DMSO) to get various concentrations of 250, 125, 100 and 50 mg/ml. Before use, the test organisms' stock cultures were subcultured on Muller Hinton broth for the bacterial isolates and Sabouraud Dextrose broth for the yeast and incubated at 37^{0}C for 24 hours for the bacterial isolates, while the yeast was incubated for 48 hours at 30^{0}C. After this period, the cultures were diluted with sterile Muller Hinton broth and Sabouraud Dextrose broth to achieve turbidity of 0.5 Mcfarland standard equivalent to a cell density of 1.5 x 10^8 cfu/ml. Muller Hinton agar and Sabouraud Dextrose agar plates were inoculated with 0.1 ml of the respective test organisms' standardized culture and carefully spread out on the agar plates with a sterile bent glass rod in order to achieve confluent growth. Five wells measuring 6 mm in diameter were made in each of the plates with a sterile cork borer. Approximately100 ml (0.1 ml) of each concentration of the extract were respectively introduced into the different wells using sterile pipettes. The sixth hole contained 5% DMSO as a negative control. The plates were left on the bench to diffuse at ambient temperature for 1 hour, after which they were incubated in the upright position at 37^{0}C for 24 hours and 30^{0}C for 48 hours, respectively. Antibiotics discs of ciprofloxacin (10 mg/ml) and fluconazole discs (20 mg/ml) were used as a positive control for the bacterial and fungal test microorganisms, respectively. The experiment was performed in triplicate. After incubation, the diameters of inhibition zones were determined using a transparent rule in millimeters (mm).

Antimicrobial screening of the fractions

The different fractions recovered were reconstituted with 15% DMSO to obtain the following 50, 25, 12.5 and 6.25 mg/ml concentrations. The antimicrobial assay was conducted as previously described for the crude extracts.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC) of Crude Extracts

This was carried out by a modification of the broth dilution method as described by Tsado *et al*.,[15] The MIC of the extract was determined for the test organisms in triplicates at varying concentrations of 50.0, 25.0, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391 and 0.195 mg/ml. A 5 µl inoculum of the various test organisms, earlier diluted to 0.5 McFarland turbidity standard, was introduced into the various test tubes. Tubes containing only nutrient broth were inoculated with the different test organisms and designated as the positive control. All the bacterial culture tubes were incubated at 37^{0}C for 24 hours, while the fungal tubes were incubated at 30^{0}C for 48 hours. After incubation, the culture tubes were examined for the presence or absence of microbial growth. The test microorganisms grew in the control tubes and other tubes lacking sufficient extract to stop their proliferation. The lowest concentration of the extract that stops the proliferation of the organism was designated the MIC. The MMC was taken as the minimum concentration of the extracts that is microbicidal to the microorganism. A portion of liquid (5 µl) from each test tube showed no visible turbidity after 24 hours of incubation in the MIC determination were plated on sterile Muller Hinton agar plates for the bacterial test organisms and SDA plates for the yeast. The plates were incubated appropriately. After incubation, the lowest concentration at which no visible microbial growth was observed was taken as the MMC.

Acute Toxicity Studies (LD_{50})

The LD50 determination was conducted using the procedure described by Lorke[16] with little modification. The evaluation was done in two phases. Three groups of three rats, each weighing 150-200g were treated with 10, 100, and 1000g extract/kg body weight orally in the first phase. The rats were observed for clinical signs and toxicity symptoms such as an acute change in behavioral response, irritability, tremors, convulsion, salivation, weight loss, and diarrhea within 24 hours and monitored for any mortality within 72 hours. Another group of three rats was administered with normal saline to serve as the control. Based on the results of phase one (no sign of toxicity), a new set of three fresh rats per group were treated with 1600, 2900, and 5000mg extract/kg body weight orally in the second phase. Clinical signs and symptoms of toxic effects and mortality were observed for seven days. Throughout the experiment, the experimental rats were kept in well-ventilated cages in an animal house and allowed free access to animal feed (Vital Feed, Nig. Ltd) and clean water. The animals were handled with humane care according to National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011).[17] Ethical approval for this study was obtained from the Research Ethics Committee of the Faculty of Pharmaceutical Sciences, University of Nigeria (Approval Number: FPSRE/UNN/20/0024).

Data analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) statistical software. Significance was set at $P < 0.05$.

Results and Discussion

The ethanol extract was found to be generally more effective than the aqueous extract against the test organisms. This observation is consistent with the findings of Oko *et al*.,[18] and Ahoyo *et al*.,[19] who also reported that the ethanol extract of *Sarcocephalus latifolius* exhibited antimicrobial activity than the aqueous extract. However, standard antibiotics/antifungal drug demonstrated the highest activity against all organisms tested with inhibition zone in the range 19 to 30 mm. *Staphylococcus aureus* ATCC 2600 and *Proteus mirabilis* recorded the highest zone of inhibition for the ethanol extract (22 mm and 19 mm) respectively, while *Candida ablicans* ATCC 7596 and *Klebsiella spp* were not inhibited (Figure 1). The aqueous extract exhibited

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varying degrees of activity against the test microorganisms except for *Klebsiella spp, Salmonella kintambo* SSRL113, *Psendomonas aeruginosa* ATCC 10145, and *Candida albicans* ATCC 7596, which were not inhibited. *Staphylococcus aureus* ATCC 2600 and *Proteus mirabilis* recorded the highest inhibition zone diameter (20 mm and 15 mm, respectively), while *Escherichia coli* ATCC 11775 recorded the least inhibition zone diameter of 12 mm (Figure 2). The extracts' antimicrobial activity was dose-dependent, with the extracts exhibiting more significant activity at higher concentrations (Figures 1 and 2). The MIC of the ethanol extract for different organisms ranged between 1.63 mg/ml and >50 mg/ml, which is the highest tested concentration, while that of the aqueous extract ranged between 6.25 mg/ml and >50 mg/ml (Table 1). The low activity of the aqueous extract against the test microorganisms is consistent with some previous works' findings using other plants.[20-24] This supports the prevalent use of locally distilled gin in preparing many medicinal plant decoctions in Nigeria. Since the ethanol crude extract of *Sarcocephalus latifolius* exhibited more significant antimicrobial activity against the organisms tested than the aqueous, it was subjected to fractionation by partitioning successively with chloroform, ethyl-acetate, acetone, and methanol to obtain their corresponding fractions. The fractionation of the ethanol extract improved the antimicrobial activity against the tested organisms. This revealed that the fractionation process might have concentrated the active ingredients leading to more excellent antimicrobial activity. Similar results where fractionation enhanced antimicrobial effect were reported by Mbahi *et al.*,[25] The susceptibility of the different test microorganisms to the different fractions of the ethanol extract varied from one organism to another. These differences could be due to variations in the chemical constituents and amounts of phytochemicals extracted by the solvents. Ethyl-acetate fraction exhibited the most significant antimicrobial effect against the test organisms (Table 2).

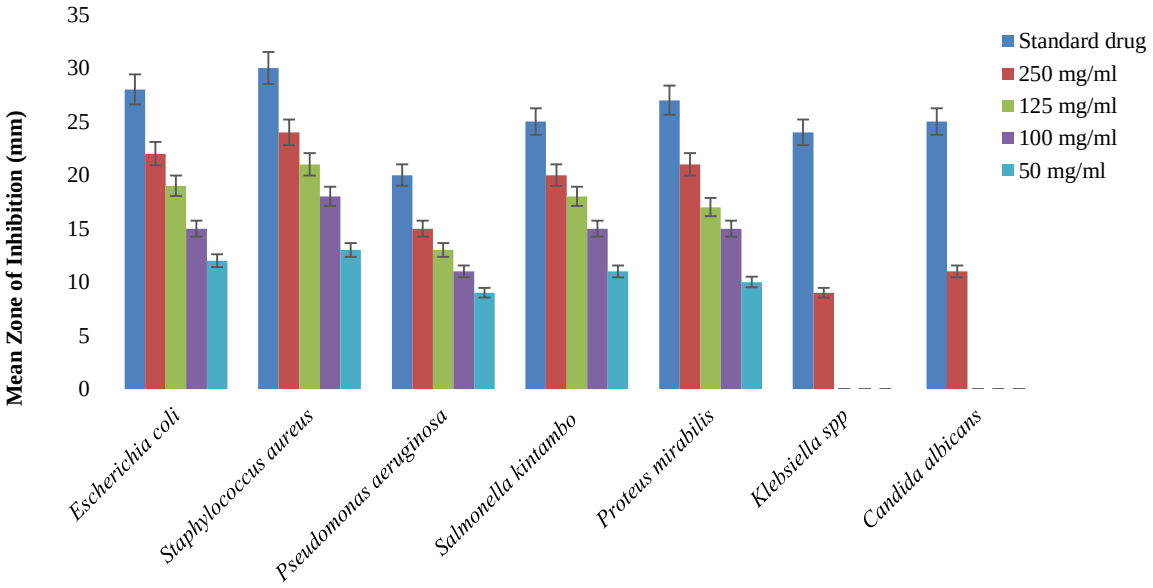

Figure 1: Antimicrobial Activity of Sarcocephalus latifolius Ethanol Extract

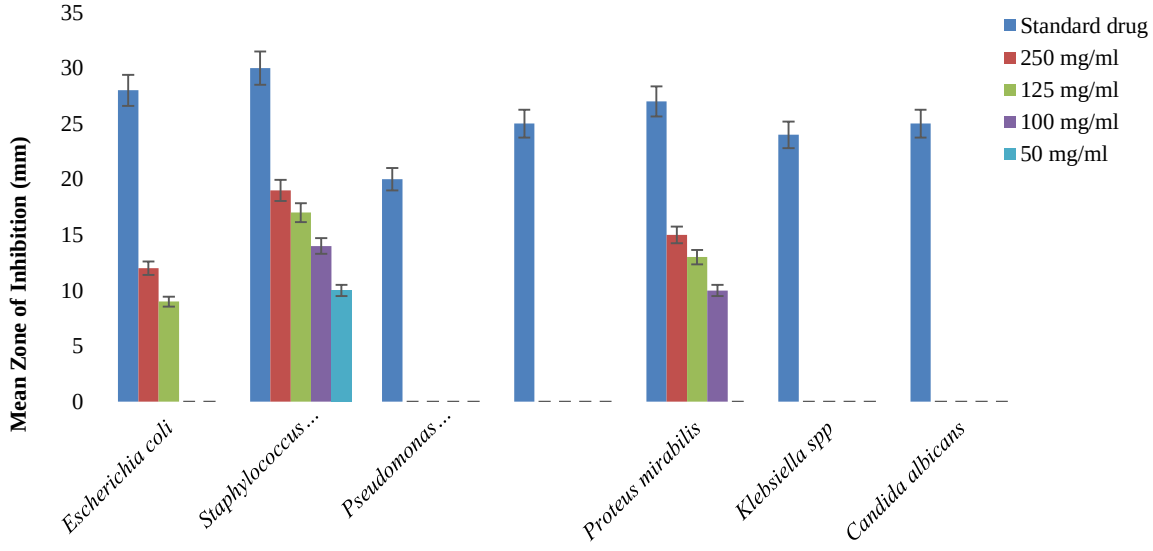

Figure 2: Antimicrobial Activity of *Sarcocephalus latifolius* Aqueous Extract

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Table 1: Mean MIC and MMC Values for *Sacocephalus latifolius* Aqueous and Ethanol Extracts

Test Microorganisms	Aqueous Extract		Ethanol Extract	
	MIC (mg/mL)	MMC (mg/mL)	MIC (mg/mL)	MMC (mg/mL)
Escherichia coli ATCC 11775	25	> 50	12.5	50
Staphylococous aureus ATCC 12600	6.25	25	1.563	12.5
Pseudomonas aeruginosa ATCC 10145	> 50	> 50	50	> 50
Salmonella kintambo SSRL113	50	> 50	25	50
Proteus mirabilis	6.25	12.5	3.125	12.5
Klebsiella spp	> 50	> 50	> 50	> 50
Candida albicans ATCC 7596	> 50	> 50	> 50	> 50

Table 2: Antimicrobial Activity of the Various Fractions of *Sacorcephalus latfolius*

Test Organisms	Conc	Mean zone of inhibition (mm)			
	mg/mL	Chloroform	Ethyl-acetate	Acetone	Methanol
Escherichia coli ATCC 11775	50	0 ± 0^{A}	16.00 ± 0^{B}	0 ± 0^{A}	0 ± 0^{A}
	25	0 ± 0^{A}	14.00 ± 0^{B}	0 ± 0^{A}	0 ± 0^{A}
	12.5	0 ± 0^{A}	10.00 ± 0^{B}	0 ± 0^{A}	0 ± 0^{A}
Staphylococcus aureus ATCC 12600	50	19.50 ± 0.707AB	21.50 ± 0.707B	19.50 ± 0.707AB	18.00 ± 0A
	25	16.50 ± 0.707AB	18.50 ± 0.707B	16.50 ± 0.707AB	15.00 ± 0A
	12.5	14.50 ± 0.707BC	16.50 ± 0.707C	13.50 ± 0.707B	10.00 ± 0A
Pseudomonas aeruginosa ATCC 10145	50	12.50 ± 0.707^{A}	15.00 ± 0^{B}	12.00 ± 0^{A}	15.00 ± 0^{B}
	25	9.50 ± 0.707^{AB}	13.00 ± 0^{BC}	0.0 ± 0^{A}	9.50 ± 0.707^{AB}
	12.5	0.0 ± 0^{A}	9.50 ± 0.707^{B}	0.0 ± 0^{A}	0.00 ± 0^{A}
Salmonella kintambo SSRL113	50	0 ± 0^{A}	13.00 ± 0^{B}	0.0 ± 0^{A}	13.50 ± 0.70711^{B}
	25	0.0 ± 0^{A}	9.00 ± 0^{B}	0.0 ± 0^{A}	0 ± 0^{A}
	12.5	0.0 ± 0^{A}	0.0 ± 0^{A}	0.0 ± 0^{A}	0.0 ± 0^{A}
Proteus mirabilis	50	21.50 ± 0.707^{C}	18.50 ± 0.707^{B}	18.50 ± 0.707^{B}	15.50 ± 0.707^{A}
	25	18.50 ± 0.707^{B}	16.50 ± 0.707^{B}	16.00 ± 0^{B}	12.50 ± 0.707^{A}
	12.5	14.00 ± 0^{C}	12.50 ± 0.707^{BC}	12.00 ± 0^{B}	9.50 ± 0.707^{A}
Klebsiella spp	50	0.0 ± 0^{A}	10.50 ± 0.707^{B}	0.0 ± 0^{A}	0.0 ± 0^{A}
	25	0.0 ± 0^{A}	8.50 ± 0.707^{B}	0.0 ± 0^{A}	0.0 ± 0^{A}
	12.5	0.0 ± 0^{A}	0.0 ± 0^{A}	0.0 ± 0^{A}	0.0 ± 0^{A}
Candida albicans ATCC 7596	50	0.0 ± 0^{A}	13.00 ± 0^{B}	0.0 ± 0^{A}	0.0 ± 0^{A}
	25	0.0 ± 0^{A}	10.00 ± 0^{B}	0.0 ± 0^{A}	0.0 ± 0^{A}
	12.5	0.0 ± 0^{A}	0.0 ± 0^{A}	0.0 ± 0^{A}	0.0 ± 0^{A}

Values are represented as mean plus or minus (±) standard deviation. Mean values with different alphabets as superscripts in a row differ significantly ($p \leq 0.05$). Mean values with different alphabets as superscripts for a parameter in a column differ significantly ($p \leq 0.05$)

This result is not surprising since flavonoids, alkaloids, saponins, and tannins are the major antimicrobial metabolites found in higher concentrations in the ethyl-acetate fraction. This could be due to ethyl-acetate's greater extraction capacity, enabling it to extract more bioactive compounds responsible for the observed antimicrobial activity. Dikko *et al*.,[26] and Okokon *et al*.,[27] had also reported that ethyl-acetate fractions exhibit more excellent antimicrobial activity compared to other fractions. However, despite the higher zone of inhibition demonstrated by the ethyl-acetate fraction of *Sarcocephalus latifolius*, the zones of inhibitions were lower on all test organisms compared to the zone of inhibitions demonstrated by standard antimicrobial drug (ciprofloxacin/fluconazole) used as a positive control in this study. The phytochemical analysis of the crude extracts and the various fractions revealed the presence of saponins, tannins, glycosides, alkaloids, flavonoids, and oils in varying proportions. Ajiboye *et al*.,[28] and Ene *et al*.,[29] had also reported the presence of these phytochemicals in the root of this plant, consistent with the present study findings. Furthermore, Ajiboye *et al*., were able to further elucidate the structure of some of these phytochemicals, which will undoubtedly aid in developing therapeutic drugs from the plant. These compounds are secondary metabolites of plants and have variously been reported to possess antimicrobial activity and, therefore, contribute to the plant's observed antimicrobial effects. Their mode of action could partly be due to their ability to inactivate several microbial enzymes and transmembrane transport proteins. The acute toxicity studies conducted using the plant's ethanol extract

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indicated that it is practically non-toxic (LD50 > 5000 mg/kg body weight). Oral administration of *Sarcocephalus latifolius* ethanol extract (5000 mg/kg body weight) did not result in mortality (Table 4) or produce any observable signs or symptoms of acute toxicity such as tremors, convulsion, and mortality after 24 h and seven days of observation. The high oral LD50 (> 5000 mg/kg body weight) suggests that the plant extract is non-toxic when administered through the oral route.

Table 3: Phytochemical Screening of *Sarcocephalus latifolius* Crude Extracts and Fractions

phytochemicals	Aqueous extract	ethanol extract	chloroform fraction	ethyl-acetate fraction	acetone fraction	methanol fraction
Alkaloids	+	+++	+	+++	++	++
Flavoniods	_	++	++	+++	+++	++
Glycosides	+	++	+	+	++	+
Saponins	++	+++	+	+++	++	+++
Tannins	+++	+++	+	+++	++	++
Essential oil	_	+	++	+	+	+

- = Absent, + = Slightly present, ++ = Fairly present, +++ = Abundantly present

Table 4: Mortality rates due to oral administration of *Sarcocephalus latifolius* ethanol extract at different doses

		Phase One		
Groups of rats	**Dosage**	**No. of deaths**	**Mortality %**	**Total No. of rats**
1	10 mg/kg	0 of 3	0	3
2	100 mg/kg	0 of 3	0	3
3	1000 mg/kg	0 of 3	0	3
Control group (distilled water)	1ml/kg	0 of 3	0	3
		Phase Two		
Groups of rats	**Dosage**	**No. of deaths**	**Mortality %**	**Total No. of rats**
1	1500 mg/kg	0 of 3	0	3
2	3000 mg/kg	0 of 3	0	3
3	5000 mg/kg	0 of 3	0	3
Control group (distilled water)	1ml/kg	0 of 3	0	3

Conclusion

The results from this study demonstrated the antimicrobial potential of *Sarcocephalus latifolius* (root bark). This plant possesses significant invitro antimicrobial activity, and thus it may provide an excellent opportunity for drug development. The observed antimicrobial action is probably caused by the phytochemicals present in the extracts. The ethanol extract exerted a significant antimicrobial effect against *Escherichia coli*, *Staphylococcus aureus*, and *Proteus mirabilis*, which are among the major pathogens associated with urinary tract infections (UTI). This study, therefore, presents a scientific basis for the use of the plant in ethnomedicine.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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