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Studies on the Antibacterial Susceptibility of Uropathogens to *Senna alata* Extracts in Calabar, Cross River State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Senna alata* is an underutilized shrub found in many countries and is known for its traditional use in the treatment of dermatophytes and other related diseases. Therefore, this study aimed at evaluating the phytochemical and antibacterial effects of *S. alat*a leaves extracts against bacterial isolates obtained from urinary tract infection patients in Calabar.
Methodology: Matured fresh leaves of *Senna alata* were collected within Calabar, Cross River state, Nigeria, in May 2022 and identified by a botanist in the Department of Botany, University of Calabar. The leaves of *S. alata* were extracted with water, methanol and ethyl acetate using

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maceration and soxhlet methods. Phytochemical analysis was conducted to detect the presence of bioactive compounds using standard methods. The crude extracts of *S. alata* were investigated for antibacterial properties using agar well diffusion method and mechanisms of antibiosis determined using MBC/MIC ratio.

Results: In both methods of extraction, methanol yielded more extracts compared to other solvents. Soxhlet methanol extract (SaMeSh) had the highest (12.21%) percentage yield while maceration ethyl acetate extract (SaEaMa) had the least (4.77%) percentage yield. The phytochemicals assayed revealed the presence of saponins, tannins, flavonoids, anthraquinones, terpenoids and steroids. However, terpenoids was not detected in methanol and ethyl acetate extracts. *Senna alata* extracts demonstrated broad spectrum of activity against the tested isolates at various concentrations with organic solvents exhibiting the highest antibacterial activity. However, the observed activity varied with respect to concentration of extract and types of organisms. The MIC values ranged from 31.25 to 250 mg/mL and MBC values from 62.5 to 500 mg/mL. The MIC index of the crude extracts against the test uropathogens was ≤8.

Conclusion: This study indicates that *S. alata* could be a source of novel antimicrobial agent. Further research is required to isolate, characterize and identify bioactive constituents responsible for the observed activity.

Keywords: Uropathogens; Senna alata; antibacterial susceptibility; urinary tract infection.

1. INTRODUCTION

It is somewhat difficult to establish the exact time when man started using plants for medicinal purposes. However, evidence from archaeological studies showed that the use of plants for medicinal purposes dated back to the Paleolithic era, about 60,000 years ago [1]. Traditional medicine is gradually taking the core of health care system in many parts of the world, and in 2008 the World Health Organization (WHO) estimated that over 80% of population in many developing countries still depends on traditional medicine [2,3]. Medicinal plant is any plant which, one or more of its organs contains substances that can be used for therapeutic purposes, or which are precursors for the production of allopathic drugs [4,5].

Plants have the inherent potentials to synthesize diverse biologically active compounds (secondary metabolites), which may have both a defensive role against herbivores, pathogen attack, and interplay competition. Moreover, these metabolites act as attractant for pollinators or symbionts [6]. Curative potentials of plants materials are well documented in many literature, and are due particularly to the presence of pharmacologically important constituents (secondary metabolites) [7,8,9,10]. With the trend in antibiotic resistance, there is an increased interest in screening for alternative medicine in plant extracts with the view to discover biologically active compounds.

Senna alata (family, Fabaceae) is an erect tropical annual herb with compound leaves, originally found in Ghana and Brazil, but now widely distributed throughout the world, including Nigeria [11,12]. *S. alata* was previously named *Cassia alata*. It has so many local names. They are: ringworm weed in English, in the Southwest of Nigeria; *S. alata* is call 'Ewe Asunwon Oyinbo' [13]. The Óró ethnic group in Akwa Ibom state, Nigeria call the *S. alata* 'Udók-aya' (E. B. Ben, personal communication, 01- Sept. - 2019).

In traditional medicine, *S. alata* shows several therapeutic virtues; the decoction of leaves and stem-bark are used to treat dysentery, skin diseases, back ache, constipation and helminthic infection [14,15,16]. In Nigeria, Uwazie *et al*. [17] and Oluwole *et al*. [18] reported that the leaves, stem, and root are used to treat wound, skin diseases, respiratory tract infection, burns, diarrhoea and constipation. Its leaves are used against malaria pathogens [19,20,21,22], skin rashes and mycosis [9,23,24,25], diuretic and purgative [26], wound healing [27,28] and diarrhea [29]. The leaves, roots and stem-bark are also specific for the treatment of eczema [30].

In Cameroon, decoction of leaves, stem-bark and roots are used to treat jaundice, gastroenteritis, gonorrhea, ringworm and helminthiasis [31,32]. Jiofack *et al*. [33] reported that the leaves and roots decoction of the plant is used to aid quick delivery in Cameroon. In Thailand, the leaves of *S. alata* are used as laxative and in the treatment of topical disease [34]. The Ghanaian employed the plant for the treatment of malaria among other diseases [35]. The leaves are reported to be highly beneficial in the treatment of

convulsion, heart failure and oedema [36]. In Egypt, China, India and other west African regions, the plant parts were found useful in the treatment of constipation, syphilis, diabetes mellitus, haemorrhoids, asthma, malaria and parasitic infections [37,38,39,40].

The plant has also been reported for various pharmacological activities such as; antioxidant [41], anticancer [42,43,44], anti-osteoarthritic agents [45], anti-inflammatory agents [43,46], hypolipidemic agent [47], anti-microbial [48,49,50,51], anti-diabetic [52] etc. Despite various literatures establishing antimicrobial effect of *S. alata*, there are limited studies so far being conducted in Calabar to establish its in vitro activity against uropathogens. Therefore, this study is aimed at evaluating the antibacterial effects of *S. alat*a leaves extracts against uropathogens to promote its ethno-medicinal utilization in infectious disease management.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant

Matured fresh leaves of *Senna alata* were obtained within Calabar, Cross River state, Nigeria, in May 2022. The plant was identified and authenticated by a botanist in the Department of Botany, University of Calabar. Prior to extraction, the fresh leaves samples of *S. alata* collected were washed with tap water to remove surface contaminants and then air dried under shade for two weeks. The dried plant material was pulverized to get coarse powder. The crushed plant leaves were kept in air-tight plastic bags for further analysis.

2.2 Extraction of Plant Materials

2.2.1 Cold maceration extraction

The method described by Zige *et al*. [7] and Ogunjobi and Abiala [53] was adopted for extraction of *S. alata* with little modification. Approximately 200 g of the pulverized plant material was weighed into three conical flasks labeled W, M and EA with 2000 mL capacity, after which 1.5 L each of distilled water, methanol and ethyl acetate was added into the flasks W, M and EA respectively. Aqueous extract was allowed to stand at room temperature for 24 hours with occasional swirling at interval while methanol and ethyl acetate extracts were occasionally shaken and allowed for 48 hours. The macerated mixture was filtered using muslin cloth and finally filtered through Whatman No 1 filter paper. The filtrate was poured into a conical flask and concentrated by evaporation in a water bath set at 45^{o}C. The macerated crude extracts obtained were stored at -4^{o}C in a refrigerator for further use.

2.2.2 Soxhlet extraction

Soxhlet method as previously described by Ogunjobi and Abiala [53] and Kwapong [54] was adopted with modification. One hundred and fifty (150) g of the coarse plant material was packed into a glass thimble and placed into the extraction tube of the soxhlet apparatus. The extraction tube packed with the plant material was then secured onto a 5 L round bottomed flask and placed on a heating mantle. The setup was filled with solvent (1000 mL) and a reflux condenser was secured above the extraction tube of the soxhlet apparatus. The extraction was allowed to run at a heating temperature of 50°C to 80°C until extraction was completed. The resulting extracts were concentrated to dryness using water bath set at 45°C. Weights of the crude extracts were recorded, and the dried extracts stored in the refrigerator at -4^{o}C until required for further analysis.

2.3 Qualitative Phytochemical Screening

Preliminary phytochemical analysis was carried out on the *S. alata* extracts to detect the presence of bioactive compounds such as: saponnins, tannins, flavonoids, alkaloids, anthraquinones, terpenoids and steroids using standard methods [55,56], with little modification.

2.4 Source of Test Organisms

The test organisms were *S. marcescens* (5), *E. coli* (8), *Cronobacter* species (2), *K. pneumoniae* (15), *E. clocae* (7), *Citrobacter* species (7), *Pseudononas* species (3), *P. mirabilis* (5), Coagulase Negative Staphylococci (CoNS) (11) and *Enterococcus* species (2). All the test organisms were obtained from urine samples of infected patients from tertiary hospitals in Calabar. The test isolates were maintained on nutrient agar slants filled with liquid paraffin oil and kept under room temperature. The isolates were subsequently sub-cultured before use.

2.5 Reconstitution and Sterility Check of the Plant Extracts

Preparation of the various concentrations of the extracts was done using double-fold serial

dilution. Five grams (5 g) of the crude extract was weighed and dissolved into 10 mL of 10% tween 80 in a sterile bottle and mixed to give a stock concentration of 500 mg/mL. The stock extract of *S. alata* was stored inside sterile McCartney bottle and placed in a refrigerator at 5°C until required for the antibacterial test. Each extract (methanol, ethyl acetate and aqueous) was tested for the growth of microbes. This was carried out by inoculating 0.5 mL of each of the extract on sterile Mueller Hinton Agar (MHA) and incubated at 37˚C for 18–24 hours. The plates were observed for growth. The absence of growth in the extracts after incubation indicates sterility and thus was evaluated for antibacterial activity.

2.6 Antibacterial Susceptibility of *S. alata* Extracts

The agar well diffusion method as described by Mordi *et al*. [50] was used to determine the antibacterial activity of crude extracts as recommended by the CLSI [57]. One hundred (100) µL of the standardized bacterial suspension was spread on the surface of MHA plates and allowed to seed. With the aid of a sterile 6 mm cork borer, four equidistant wells were bored into the agar medium while the fifth well was bored at the center of the plate. The bottoms of the wells were sealed with one drop of the sterile molten MHA; to prevent diffusion of the extracts under the agar. With the aid of automated pipette, 50 µL of the extract concentrations (500, 250, 125 and 62.5 mg/mL) was dispensed into the wells. Equal amount of sterile distilled water (as control) was added to the fifth well. The plates were allowed to stand on the laboratory bench for 2 hours to allow proper diffusion of the extract into the medium and thereafter incubated at 37°C for 18-24 hours. Sensitivity of the test organisms to the plant extract was determined by measuring the diameter of the zones of inhibition after incubation. All experimental set up was carried out in duplicates.

2.7 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the plant extracts was determined for each susceptible isolates. The MIC was evaluated by broth dilution technique as described by Ehiowemwenguan *et al*. [58] with modification. The crude extracts were reconstituted to 500, 250, 125, 62.5, 31.25 and 15.625 mg/mL concentration in nutrient broth in tubes. Using a sterile wire loop, 10µL of the test isolate previously diluted to 0.5 McFarland was introduced to each extract concentration. The procedure was repeated using standard antibiotic (Ciprofloxacin 5µg/mL) as positive control. A tube containing nutrient broth only was seeded with the test organisms to serve as a negative control. All the tubes were incubated at 35 °C for 18-24 hours.

2.8 Determination of Minimum Bactericidal Concentration (MBC)

To determine the MBC, methods described by Adedayo *et al*. [59] and Evbuomwan *et al*. [60] was used. Briefly, a loopful of broth from the MIC tubes was inoculated on fresh nutrient agar plates and incubated at 37^{o}C for 24 hours. The minimum bacterial concentration was taken as the lowest concentration of the extract that did not allow any bacterial growth on the surface of the agar plate.

2.9 Determination of Mechanisms of Antibiosis (Bactericidal or Bacteriostatic)

The mechanism of antibiosis of *S. alata* extracts was calculated using the ratio of MBC/MIC or MIC index as described by Olajuyigbe and Afolayan [61] to elucidate whether the observed antibacterial activities were bactericidal or bacteriostatic. When the ratio of MBC/MIC was ≤2, the extract was considered bactericidal or otherwise bacteriostatic. If the ratio is ≥16, the extract was considered ineffective [61].

2.10 Statistical Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistics, such as frequencies, percentage, mean and standard deviation, were used. Associations between categorical variables were conducted using Pearson Chi-square test set at the 95% significant level. *P*-value of <.05 was considered to indicate statistically significant differences.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield of *S. alata* Extracts

Fig. 1 showed the percentage yield of *S. alata* extracts. For soxhlet method, *S. alata* leaves yielded 12.21%, 9.45% and 6.15% for methanol, ethyl acetate and aqueous respectively.

Similarly, methanol extract showed the highest yield of about 8.73% in maceration method, followed by aqueous (5.2%) and ethyl acetate showed the least yield (4.77%).

3.2 Preliminary Phytochemical Screening

The preliminary phytochemical analysis of the crude extracts is shown in (Table 1). The methanol extracts revealed the presence of saponins, tannins, anthraquinone, steroids and flavonoids. Also, saponins, tannins, flavonoids, anthraquinone, and steroids were found in ethyl acetate extracts, whereas in aqueous extraction saponins, tannins, flavonoids, anthraquinone, terpenoids and steroids were detected. However, terpenoids was not found in the methanol and ethyl acetate extracts (Table 1).

3.3 *In vitro* Antibacterial Activity of *S. alata* Extracts

Crude extracts of *S. alata* was tested against isolated uropathogens at different concentrations using agar well diffusion method (Fig. 2). The results revealed that *S. alata* extracts were effective against all the test uropathogens. However, the inhibition zones observed were concentration dependent and varied with respect to solvent of extraction and types of organisms (Table 2a-f).

At 500 mg/mL concentration, inhibition zones recorded against *S. marcescens* were 24.0±0.0, 20.5±0.71, 18.5±0.71, 15.0±1.41, 14.5±0.71 and 13.0±0.0 mm for SaAqMa, SaMeMa, SaAqSh, SaMeSh, SaEaSh, and SaEaMa respectively. Of all the tested extracts, only SaMeMa and SaAqMa were active against *S. marcescens* at 62.5 mg/mL concentration. However, out of 5 isolates of *S. marcescens* tested, SaMeSh and SaAqSh inhibited 4 isolates each, SaAqMa inhibited 3 while SaMeMa, SaEaSh and SaEaMa inhibited 2 isolates each at different concentration (Table 2a-b).

Maceration ethyl Maceration ethyl acetate extract (SaEaMa) was more effective against *E. clocae* compared to other extracts. The zones of inhibition at 500 mg/mL concentration ranged from 21.5±0.71 to 18.0±0.0 mm. All isolates of *E. clocae* were inhibited at 500 mg/mL concentration by SaMeMa whereas 6 isolates, 5 isolates, 5 isolates, 5 isolates, and 4 isolates each were inhibited by SaAqMa, SaAqSh, SaMeSh, SaEaMa and SaEaSh respectively (Table 2a-b).

SaMeMa, SaAqMa and SaAqSh were active against all isolates of *Citrobacter* sp at 500 mg/mL concentration. This was followed by SaMeSh, SaEaSh and SaEaMa with 6, 4, and 4 isolates inhibited respectively (Table 2a-b).

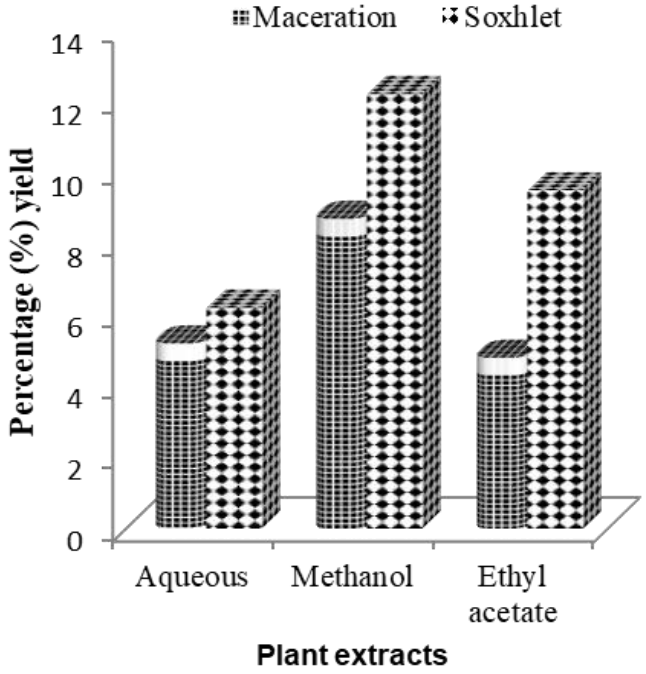

Fig. 1. Percentage yield of *S. alata* extracts

Maceration methanol extract (SaMeMa) was more active on *Pseudomonas* sp compared to other extracts (Table 2a-b).

Soxhlet aqueous extract (SaAqSh) recorded the highest inhibition zone against *K. pneumoniae* at 500 mg/mL concentration with inhibition zone of 23.0±0.0 mm (Table 2d). The results revealed that SaAqMa showed the highest activity against *K. pneumoniae* at 125 mg/mL concentration with 80% (12/15) isolates inhibited. This was followed by SaMeSh, SaMeMa, SaEaSh, SaEaMa and SaAqSh were 11, 10, 10, 9, and 6 isolates each were inhibited at 125 mg/mL concentration respectively (Table 2c-d).

E. coli isolates were sensitive to *S. alata* extracts at different concentrations. SaEaMa had the highest inhibition of 23.5±0.71 mm at 500 mg/mL concentration. All the extracts tested were active against *E. coli* strains up to 125 mg/mL concentration. However, no inhibition was observed at 62.5 mg/mL concentration (Table 2c-d).

The results revealed that *P. mirabilis* were susceptible to extracts of *S. alata* with SaAqMa exhibiting the highest activity at 500 mg/mL concentration (18.5±0.71 mm). This was followed by SaEaMa with inhibition zone of 18.0±0.0 mm. Similarly, at 500 mg/mL concentration, 15.5±0.71 and 14.0±0.0 mm inhibition zones was recorded for SaAqSh and SaEaSh respectively. However, 2 out 5 isolates of *P. mirabilis* were susceptible to SaMeSh and SaMeMa at 500 mg/mL concentrations (Table 2e).

Cronobacter sp were sensitive to all the extracts at 500 mg/mL concentration except SaMeSh and SaAqSh. The most active extract was SaEaSh while the least activity was recorded for SaAqMa at 500 mg/mL concentration (Table 2e-f).

The results indicate that *S. alata* extracts were more effective against Gram positive isolates at lower concentrations compared to Gram negative. All the extracts were active against some strains of CoNS at 62.5 mg/mL concentration except SaEaMa. However, SaAqMa had the highest inhibition zone (24.0±0.0 mm) at 500 mg/mL concentration (Table 2f).

S. alata extracts were active against *Enterococcus* sp at various concentrations. However, SaEaMa showed better activity compared to other extracts (Table 2e-f). The results showed that SaAqSh was ineffective at 500 mg/mL concentrations (Table 2f).

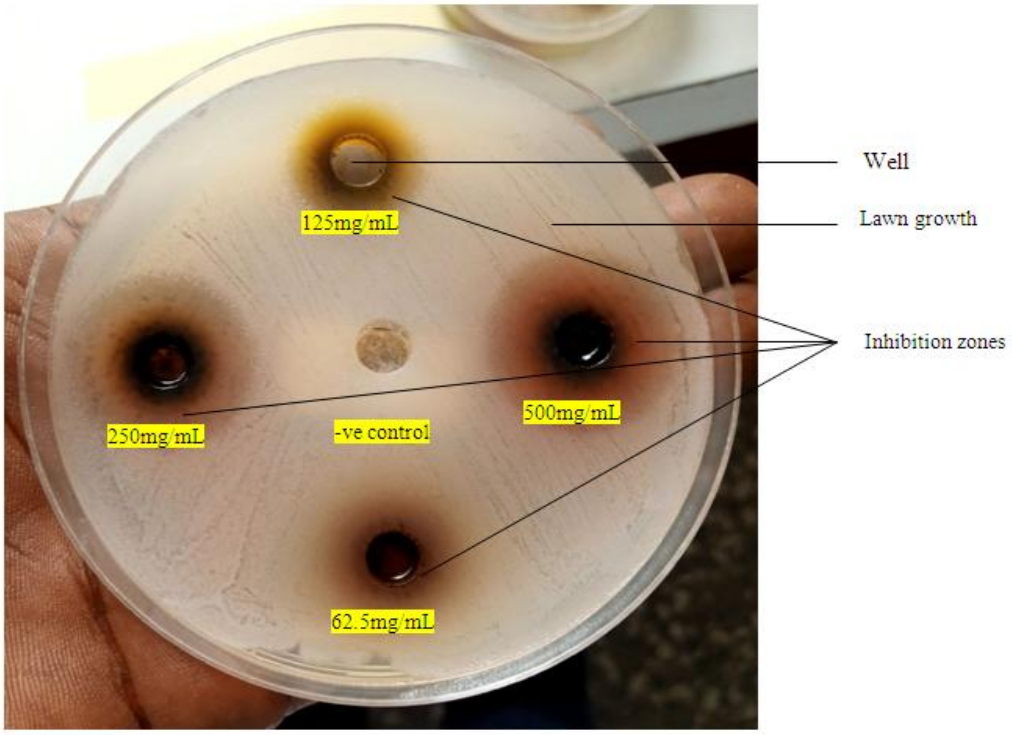

Fig. 2. Plate showing antibacterial activity of *S. alata* extracts

Table 1. Preliminary phytochemical analysis of *S. alata* leaves extracts

Phytochemical constituents	Chemical test	SaMeSh	SaMeMa	SaEaSh	SaEaMa	SaAqSh	SaAqMa
Saponins	Frothing test	+	+	+	+	+	+
Tannins	Ferric chloride test	+	+	+	+	+	+
Flavonoids	Magnesium metal test	-	-	-	-	-	-
	Sodium hydroxide test	+	+	+	+	+	+
Alkaloids	Dragendoff's test	-	-	-	-	-	-
Anthraquinone	Combined anthraquinone	+	+	+	+	+	+
	Free anthraquinone	+	+	+	+	+	+
Terpenoids	Salkwoski's test	-	-	-	-	+	+
Steroids	Liebermann-Burchard test	+	+	+	+	+	+

KEY: *SaMeSh = S. alata methanol extracts (soxhlet); SaMeMa = S. alata methanol extracts (maceration); SaEaSh = S. alata ethyl acetate extracts (soxhlet); SaEaMa = S. alata ethyl acetate extracts (maceration); SaAqSh = S. alata aqueous extracts (soxhlet); SaAqMa = S. alata aqueous extracts (maceration); - = Not present; + = Present*

Table 2a. Antibacterial activity of *Senna alata* extracts

		Mean zone of inhibition (mm)															
Bacteria	**Code**	**SaMeSh**				**SaMeMa**				**SaEaSh**				**SaEaMa**			
		500 mg/mL	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**
S. marcescens	NH 23	11.0±1.41	-	-	-	20.5±0.71	18.5±0.71	15.5±0.71	10.0±0.0	10.0±0.0	-	-	-	-	-	-	-
	NH 17	11.0±1.41	-	-	-	9.5±0.71	-	-	-	14.5±0.71	13.0±0.0	-	-	11.5±2.12	-	-	-
	GH 1	15.0±1.41	9±1.41	-	-	13.5±0.71	10.5±0.71	-	-	14.0±0.0	9.0±1.41	-	-	13.0±0.0	9.5±0.71	-	-
	GH 14	14.0±1.41	10±0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	UC 40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. clocae	NH 2	16.5±2.12	11±1.41	7.5±0.71	-	17.0±1.41	12.5±0.71	9.5±0.71	-	14.5±0.71	10.0±0.0	-		12.5±0.71	-		
	NH 25	19.0±1.41	10.5±0.71	-	-	16.0±0.0	14.0±0.0	12.0±0.0	-	16.0±0.0	13.0±1.41	11.0±1.41	-	16.5±0.71	13.5±0.71	10.5±0.71	-
	NH 34	15.0±1.41	9.5±0.71	-	-	9.0±1.41	-	-	-	-	-	-	-	-	-	-	-
	NH 29	15.0±1.41	9.5±0.71	-	-	10.0±0.0	-	-	-	-	-	-	-	-	-	-	-
	GH 25	-	-	-	-	18.0±0.0	13.5±0.71	8.0±1.41	-	-	-	-	-	19.0±0.0	14.5±0.71	10.5±0.71	-
	GH 9	20.0±2.83	12.5±3.34	10±1.41	-	15.5±0.71	12.5±0.71	9.5±0.71	-	20.0±0.0	13.5±2.12	9.0±1.41	-	21.5±0.71	16.5±0.71	12.5±0.71	-
	UC 32	-	-	-	-	17.0±0.0	13.0±0.0	12.0±0.0	-	12.0±1.41	-	-	-	14.0±1.41	9.5±0.71		-
Citrobacter sp	NH 10	15.0±1.41	14±0.0	13.5±0.71	-	12.5±0.71	9.0±0.0	-	-	19.5±0.71	13.5±0.71	10.0±0.0	-	19.5±0.71	14.0±1.41	10.5±0.71	-
	NH 22	19.5±0.71	16.5±0.71	10.5±0.71	-	20.0±0.0	16.5±0.71	10.5±0.71	-	-	-	-	-	18.5±0.71	15.0±1.41	11.0±1.41	-
	GH 23	14.5±0.71	10±0.0	7.5±0.71	-	10.0±0.0	8.5±0.71	-	-	12.5±2.12	7.5±2.12	-	-	-	-	-	-
	GH 11	-	-	-	-	9.0±1.41	-	-	-	-	-	-	-	-	-	-	-
	GH10	19.0±1.41	16±1.41	10.5±0.71	-	18.0±1.41	14.0±1.41	10.0±0.0	-	13.0±0.0	10.0±0.0	-	-	15.0±0.0	10.0±0.0	-	-
	GH 7	12.0±0.0	-	-	-	14.5±0.71	10.5±0.71	-	-	-	-	-	-	-	-	-	-
	UC 20	17.0±1.41	11.5±0.71	-	-	17.5±2.12	12.0±1.41	-	-	18.0±0.0	12.5±0.71	-	-	20.0±0.0	17.5±0.71	12.5±0.71	-
Pseud. sp	NH 20	17.0±1.41	11.5±2.12	-	-	17.5±2.12	12.0±1.41	-	-	15.5±0.71	12.0±0.0	-	-	15.5±0.71	11.5±0.71	-	-
	NH 15	12.0±2.83	7.5±2.12	-	-	19.0±1.41	14.5±0.71	10.5±0.71	-	11.0±1.41	8.0±0.0	-	-	11.0±1.41	-	-	-
	UC 23	-	-	-	-	12.0±0.0	8.5±0.71	-	-	-	-	-	-	-	-	-	-

Data are means of two replicates (n=2) mean ± SD; ***KEY****: NH = Nigeria Navy Reference Hospital, Calabar, GH = General Hospital, Calabar, UC = University of Calabar Teaching Hospital, SaMeSh = S. alata methanol extract (soxhlet), SaMeMa = S. alata methanol extract (maceration), SaEaSh = S. alata ethyl acetate extract (soxhlet), SaEaMa = S. alata ethyl acetate extract (maceration); - = No inhibition zone; Pseud. sp = Pseudomonas sp.*

Table 2b. Antibacterial activity of *Senna alata* extracts against uropathogens

		Mean zone of inhibition (mm)							
Bacteria	**Code**	**SaAqSh**				**SaAqMa**			
		500 mg/mL	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**
S. marcescens	NH 23	18.5±0.71	13.0±0.0	9.5±0.71	-	24.0±0.0	20.5±0.71	18.5±0.71	11.5±0.71
	NH 17	14.0±0.0	9.5±0.71	-	-	15.0±1.41	10.5±0.71	-	-
	GH 1	17.5±0.71	13.5±0.71	-	-	15.0±0.0	13.5±0.71	-	-
	GH 14	9.5±0.71	-	-	-	-	-	-	-
	UC 40	-	-	-	-	-	-	-	-
E. clocae	NH 2	16.0±0.0	10.5±0.71	-	-	15.0±0.0	15.0±0.0	11.0±1.41	-
	NH 25	10.0±1.41		-	-	14.5±0.71	9.0±1.41		-
	NH 34	-	-	-	-	-	-	-	-
	NH 29	-	-	-	-	12.5±0.71	-	-	-
	GH 25	17.0±1.41	12.0±0.0	7.5±0.71	-	9.5±0.71	-	-	-
	GH 9	20.5±0.71	15.5±0.71	11.0±1.41	-	19.5±0.71	15.0±1.41	10.5±0.71	-
	UC 32	19.0±0.0	13.0±0.0	9.5±0.71	-	18.0±1.41	14.0±1.41	12.5±0.71	-
Citrobacter sp	NH 10	11.5±0.71	-	-	-	13.5±0.71	9.5±0.71	-	-
	NH 22	18.0±0.0	13.5±0.71	8.5±0.71	4.5±2.27	21.0±1.41	18.0±0.0	13.5±0.71	8.0±0.0
	GH 23	14.0±1.41	10.0±0.0	-	-	13.0±0.0	8.0±0.0	-	-
	GH 11	15.0±0.0	11.0±0.0	-	-	9.0±1.41	-	-	-
	GH10	18.5±0.71	14.5±0.71	11.0±1.41	-	18.0±0.0	14.5±0.71	10.0±1.41	-
	GH 7	13.5±0.71	10.5±0.71	-	-	13.0±1.41	9.5±0.71		-
	UC 20	19.0±1.41	14.0±1.41	9.5±0.71	-	19.0±0.0	14.5±0.71	10.0±0.0	-
Pseud. sp	NH 20	13.0±0.0	10.0±0.0	-	-	15.0±1.41	10.0±0.0	-	-
	NH 15	-	-	-	-	-	-	-	-
	UC 23	12.0±0.0	7.5±0.71	-	-	14.0±0.0	13.0±1.41	11.0±1.41	-

Data are means of two replicates (n=2) mean ± SD; KEY: NH = Nigeria Navy Reference Hospital, Calabar, GH = General Hospital, Calabar, UC = University of Calabar Teaching Hospital, SaAqSh = S. alata aqueous extract (soxhlet), SaAqMa = S. alata aqueous extract (maceration); - = No inhibition zone; Pseud. sp = Pseudomonas sp.

Table 2c. Antibacterial activity of *Senna alata* extracts against uropathogens

		Mean zone of inhibition (mm)															
Bacteria	**Code**	**SaMeSh**				**SaMeMa**				**SaEaSh**				**SaEaMa**			
		500 mg/mL	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**
K. pneumoniae	NH 3	20.0±1.41	16.0±2.83	10.5±2.12	-	21.5±0.71	18.5±0.71	11.0±1.41	-	19.0±0.0	15.5±0.71	11.0±1.41	-	19.5±0.71	15.5±0.71	11.5±2.12	-
	NH 33	14.5±0.71	10.5±0.71	7.5±0.71	-	12.5±0.71	8.5±0.71	-	-	12.5±2.12	7.5±2.12	-	-	17.5±0.71	11.5±0.71	-	-
	NH 74	11.5±2.12	-	-	-	8.5±0.71	-	-	-	10.5±0.71	-	-	-	-	-	-	-
	NH 78	17.0±1.41	11.5±0.71	-	-	17.0±1.41	12.0±1.41	-	-	17.5±0.71	12±0.0	-	-	20.0±0.0	17.5±0.71	12.5±0.71	-
	NH 32	20.0±1.41	16±2.83	10.5±2.12	-	21.5±0.71	18.5±0.71	12.0±1.41	-	18.0±1.41	14.5±1.41	10.0±1.41	-	19.5±0.71	16.0±0.0	12.5±0.71	-
	NH 63	12.5±0.71	9.5±2.12	-	-	20.5±0.71	17.5±0.71	14.0±0.0		21.0±1.41	17.0±1.41	12.5±0.71	-	18.5±0.71	14.0±1.41	10.5±0.71	-
	NH 69	20.0±1.41	16.0±2.83	10.5±2.12	-	21.5±0.71	17.5±0.71	11.5±0.71	8.0±1.41	18.5±0.71	15.5±0.71	11.0±1.41	-	19.5±0.71	15.5±0.71	11.5±2.12	-
	NH 66	20.0±1.41	16.0±2.83	10.5±2.12	-	21.5±0.71	18.0±1.41	15.0±0.0	11.5±0.71	18.5±0.71	15.5±0.71	11.0±1.41	-	19.0±0.0	14.5±0.71	11.0±1.41	-
	GH37	19.0±1.41	16.0±1.41	10.5±0.71	-	18.0±1.41	14.01.41	9.5±0.71	-	13.0±0.0	9.5±0.71	-	-	14.5±0.71	10.5±0.71	-	-
	GH 63	13.0±0.0	10.5±0.71	-	-	18.5±0.71	14.0±1.41	8.0±1.41	-	20.5±0.71	17.5±2.12	12.0±0.0	9.5±0.71	16.0±0.0	11.5±0.71	-	-
	UC 25	18.5±0.71	15.5±0.71	13.5±0.71	-	16.0±0.0	13.0±1.41	-	-	19.0±1.41	16.0±1.41	11.0±1.41	-	11.5±2.12	11.0±1.41	-	-
	UC 13	20.5±0.71	17.0±1.41	12.5±3.53	-	20.5±0.71	16.5±0.71	11.5±0.71	-	17.0±1.41	15.0±1.41	11.0±1.41	-	20.5±0.71	18.0±0.0	11.0±1.41	-
	UC 48	20.5±0.71	16.5±0.71	12.5±0.71	-	18.0±1.41	13.0±1.41	9.0±1.41	-	-	-	-	-	11.5±2.12	9.0±0.0	-	-
	UC 33	19.0±1.41	14.0±1.41	10.5±0.71	-	20.5±0.71	18.5±0.71	13.0±1.41	-	19.5±0.71	16.0±1.41	11.5±2.12	-	18.5±0.71	14.5±0.71	11.0±1.41	-
	UC 41	15.0±1.41	14.0±0.0	13.5±0.71	-	12.5±0.71	9.0±0.0	-	-	19.5±0.71	13.5±0.71	9.5±0.71	-	19.5±0.71	14.0±1.41	11.0±1.41	-
E. coli	NH 5	13.5±0.71	10.5±2.12	6.0±8.49	-	-	-	-	-	16.0±0.0	10.5±0.71	-	-	23.5±0.71	18.5±0.71	14.0±1.41	-
	NH 30	12.5±0.71	8.0±1.41	-	-	17.0±0.0	13.5±0.71	9.5±0.71	-	12.0±0.0	8.5±0.71	-	-	15.5±0.71	11.5±0.71	9.5±0.71	-
	NH 85	12.5±0.71	8.0±1.41	-	-	15.5±0.71	13.0±0.0	-	-	13.5±0.71	10.0±0.0	-	-	15.0±0.0	12.0±0.0	-	-
	GH 8	12.5±0.71	4.5±6.36	-	-	17.0±0.0	14.0±0.0	10.0±0.0	-	13.0±1.41	10.0±0.0	-	-	15.0±0.0	11.5±0.71	9.0±0.0	-
	GH 3	15.0±0.0	13.0±0.0	-	-	15.0±0.0	12.5±0.71	-	-	14.5±0.71	11.5±2.12	4.0±5.66	-	12.5±0.71	9.0±1.41	-	-
	GH 61	13.0±1.41	9.0±0.0	-	-	16.5±0.71	13.5±0.71	-	-	14.0±0.0	-	-	-	15.5±0.71	12.5±0.71	9.5±0.71	-
	UC 31	15.5±0.71	12.5±0.71	-	-	-	-	-	-	14.5±0.71	11.5±2.12	8.5±0.71	-	12.5±0.71	9.5±0.71	-	-
	UC 19	-	-	-	-	19.5±0.71	16.0±0.0	10.5±0.71	-	18.0±1.41	14.0±1.41	5.0±7.07	-	15.0±0.0	12.0±0.0	-	-

Data are means of two replicates (n=2) mean ± SD; KEY: NH = Nigeria Navy Reference Hospital, Calabar, GH = General Hospital, Calabar, UC = University of Calabar Teaching Hospital, SaMeSh = S. alata methanol extract (soxhlet), SaMeMa = S. alata methanol extract (maceration), SaEaSh = S. alata ethyl acetate extract (soxhlet), SaEaMa = S. alata ethyl acetate extract (maceration); -= No inhibition zone.

Table 2d. Antibacterial activity of *Senna alata* extracts against uropathogens

		Mean zone of inhibition (mm)							
Bacteria	**Code**	**SaAqSh**				**SaAqMa**			
		500 mg/Ml	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**
K. pneumoniae	NH 3	12.5±0.71	9.5±0.71	-	-	16.0±0.0	13.5±0.71	8.0±1.41	-
	NH 33	-	-	-	-	14.5±0.71	10.0±0.0	7.5±0.71	-
	NH 74	14.0±0.0	10.5±0.71	-	-	9.5±0.71	-	-	-
	NH 78	19.0±1.41	14.0±1.41	9.0±1.41	-	19.0±0.0	15.5±0.71	10.0±0.0	-
	NH 32	11.5±0.71	8.5±0.71	-	-	15.5±0.71	13.0±0.0	9.0±0.0	-
	NH 63	17.0±1.41	12.5±2.12	10.0±1.41	-	20.5±0.71	18.0±1.41	13.5±0.71	8.0±1.41
	NH 69	13.0±1.41	8.5±2.12	-	-	14.5±0.71	9.0±1.41	6.0±0.0	-
	NH 66	12.0±0.0	8.0±0.0	-	-	16.5±0.71	13.5±0.71	8.5±0.71	-
	GH 37	18.5±0.71	14.0±1.41	9.5±0.71	-	17.5±0.71	13.0±0.0	10.0±0.0	-
	GH 63	18.0±0.0	13.5±0.71	8.5±0.71	-	17.5±0.71	14.5±0.71	9.0±1.41	-
	UC 25	23.0±0.0	17.5±0.71	14.0±0.0	11.5±2.75	18.0±0.0	14.5±0.71	13.0±0.0	10.0±0.0
	UC 13	15.0±0.0	12.5±0.71	-	-	19.0±0.0	14.5±0.71	9.5±0.71	-
	UC 48	18.5±0.71	12.5±0.71	8.5±2.12	-	20.0±0.0	16.5±0.71	10.5±0.71	-
	UC 33	9.0±1.41	-	-	-	-	-	-	-
	UC 41	10.0±0.0	3.5±4.95	-	-	13.5±0.71	9.5±0.71	-	-
E. coli	NH 5	-	-	-	-	-	-	-	-
	NH 30	15.5±0.71	11.0±1.41	8.0±1.41	-	13.5±0.71	10.0±0.0	-	-
	NH 85	15.0±0.0	11.0±0.0	7.0±0.0	-	12.5±0.71	8.0±1.41	-	-
	GH 8	15.5±0.71	11.5±0.71	8.0±0.0	-	12.5±0.71	8.5±0.71	-	-
	GH 3	14.5±0.71	11.0±1.41	8.5±0.71	-	14.0±0.0	10.5±0.71	-	-
	GH 61	15.0±0.0	10.5±0.71	7.5±0.71	-	13.0±1.41	9.5±0.71	-	-
	UC 31	14.0±0.0	10.5±0.71	7.0±1.41	-	13.5±0.71	10.5±0.71	-	-
	UC 19	16.0±0.0	11.5±0.71	-	-	16.5±0.71	15.0±0.0	9.0±1.41	-

*Data are means of two replicates (n=2) mean ± SD; **KEY**: NH = Nigeria Navy Reference Hospital, Calabar, GH = General Hospital, Calabar, UC = University of Calabar Teaching Hospital, SaAqSh = S. alata aqueous extract (soxhlet), SaAqMa = S. alata aqueous extract (maceration); - = No inhibition zone.*

Table 2e. Antibacterial activity of *Senna alata* extracts against uropathogens

		Mean zone of inhibition (mm)															
Bacteria	**Code**	**SaMeSh**				**SaMeMa**				**SaEaSh**				**SaEaMa**			
		500 mg/mL	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**
P. mirabililis	NH 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NH 26	14.0±1.41	9.5±0.71	-	-	13.0±1.41	8.5±0.71	-	-	14.0±0.0	8.5±0.71	-	-	18.0±0.0	13.5±0.71	9.5±0.71	-
	NH 28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	GH 15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	GH 67	13.5±2.12	-	-	-	9.5±0.71	-	-	-	12.0±1.41	8.0±0.0	-	-	14.5±0.71	10.5±0.71	-	-
Cro. sp	NH 59	-	-	-	-	11.0±1.41	8.5±0.71	-	-	15.5±0.71	13.5±0.71	11.0±1.41	-	13.5±0.71	10.5±0.71	-	-
	GH 50	-	-	-	-	11.0±1.41	8.5±2.12	-	-	15.5±0.71	13.5±0.71	10.0±0.0	-	13.5±0.71	9.5±0.71	-	-
Staph. sp	GH 36	11.0±1.41	-	-	-	20.0±0.0	19.0±0.0	15.0±0.0	10.0±0.0	10.5±0.71	-	-	-	-	-	-	-
	GH 55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	UC 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	UC 15	20.0±0.0	17.0±1.41	15.0±4.24	5.0±7.07	20.5±0.71	18.0±0.0	17.0±1.41	11.0±1.41	17.5±3.53	13.0±4.24	11.5±2.12	-	19.0±1.41	16.5±0.71	11.5±0.71	-
	UC 18	11.0±1.41	-	-	-	12.5±0.71	-	-	-	17.0±1.41	11.5±0.71	-	-	17.0±1.41	14.0±2.83	-	-
	UC 11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NH 1	21.0±0.0	17.0±1.41	13.5±2.12	8.0±2.83	14.5±0.71	10.0±0.0	-	-	18.0±0.0	17.0±1.41	13.5±2.12	-	16.0±0.0	12.0±0.0	-	-
	NH 40	20.5±0.71	17.0±1.41	13.5±2.12	8.0±2.83	14.0±0.0	9.5±0.71	-	-	20.5±0.71	17.0±1.41	13.5±2.12	8.0±2.83	15.5±0.71	12.5±0.71	-	-
	NH 37	11.0±1.41	-	-	-	20.5±0.71	18.5±0.71	13.5±2.12	8.5±0.71	10.5±0.71	-	-	-	-	-	-	-
	NH 38	19.5±0.71	17.0±1.41	15.0±4.24	5.0±7.07	20.5±0.71	18.0±0.0	14.0±1.41	10.5±0.71	18.0±2.83	15.5±0.71	10.0±0.0	-	19.0±1.41	16.5±0.71	11.5±0.71	-
	NH 62	20.5±0.71	17.0±1.41	12.5±3.54	-	20.5±0.71	16.5±0.71	11.5±0.71	-	20.5±0.71	17.0±1.41	12.5±3.54	-	20.0±0.0	17.0±0.0	11.0±1.41	-
Entero. sp	NH 64	13.0±1.41	9.0±1.41	-	-	16.5±0.71	12.0±0.0	-	-	18.5±0.71	16.0±0.0	12.5±0.71	-	19.0±1.41	15.5±0.71	12.0±0.0	-
	GH 12	13.0±1.41	9.0±1.41	-	-	15.5±0.71	12.0±1.41	-	-	18.0±0.0	17.0±1.41	11.5±2.12	-	19.0±1.41	15.5±0.71	11.5±0.71	-

Data are means of two replicates (n=2) mean ± SD; KEY: NH = Nigeria Navy Reference Hospital, Calabar, GH = General Hospital, Calabar, UC = University of Calabar Teaching Hospital, SaMeSh = S. alata methanol extract (soxhlet), SaMeMa = S. alata methanol extract (maceration), SaEaSh = S. alata ethyl acetate extract (soxhlet) , SaEaMa = S. alata ethyl acetate extract (maceration); - = No inhibition zone.

Table 2f. Antibacterial activity of *Senna alata* extracts against uropathogens

		Mean zone of inhibition (mm)							
Bacteria	**Code**	**SaAqSh**				**SaAqMa**			
		500 mg/mL	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**
P. mirabililis	NH 4	-	-	-	-	-	-	-	-
	NH 26	15.5±0.71	11.5±0.71	-	-	18.5±0.71	11.5±0.71	-	-
	NH 28	-	-	-	-	9.0±1.41	-	-	-
	GH 15	-	-	-	-	-	-	-	-
	GH 67	12.0±0.0	4.0±5.66	-	-	9.0±1.41	-	-	-
Cro. sp	NH 59	-	-	-	-	9.5±0.71	-	-	-
	GH 50	-	-	-	-	9.5±0.71	-	-	-
Staph. sp	GH 36	18.5±0.71	12.5±0.71	10.0±0.0	-	23.5±0.71	20.5±0.71	18.0±1.41	10.0±0.0
	GH 55	-	-	-	-	-	-	-	-
	UC 2	-	-	-	-	-	-	-	-
	UC 15	9.5±0.71	-	-	-	-	-	-	-
	UC 18	20.0±0.0	16.5±0.71	14.0±1.41	10.0±2.75	20.0±0.0	19.0±1.41	14.0±1.41	10.0±0.0
	UC 11	-	-	-	-	-	-	-	-
	NH 1	20.5±0.71	17.0±0.0	12.0±1.41	8.5±1.37	24.0±0.0	16.5±0.71	12.0±0.0	8.5±0.71
	NH 40	21.0±1.41	18.5±0.71	15.0±1.41	10.5±1.35	21.0±0.0	17.5±0.71	13.5±2.12	9.5±0.71
	NH 37	17.5±2.12	13.0±1.41	10.5±0.71	-	24.0±0.0	20.5±0.71	18.5±0.71	11.5±0.71
	NH 38	9.0±1.41	-	-	-	-	-	-	-
	NH 62	14.5±0.71	10.5±0.71	-	-	19.0±0.0	14.5±0.71	10.0±0.0	-
Entero. sp	NH 64	-	-	-	-	13.0±1.41	9.5±0.71	-	-
	GH 12	-	-	-	-	10.5±0.71	-	-	-

Data are means of two replicates (n=2) mean ± SD; KEY: NH = Nigeria Navy Reference Hospital, Calabar, GH = General Hospital, Calabar, UC = University of Calabar Teaching Hospital, SaAqSh = S. alata aqueous extract (soxhlet), SaAqMa = S. alata aqueous extract (maceration); - = No inhibition zone.

3.4 MIC, MBC and MIC-Index

Table 3a-c showed results of MIC, MBC and MIC-index of *S. alata* extracts against uropathogens. The observed MIC and MBC of *S. alata* varied with respect to solvent of extraction used and the test organisms. The overall MICs of the extracts against uropathogens in this study ranged between 31.25 and 500 mg/mL and the MBCs values between 62.5 and 500 mg/mL. However, Gram positive uropathogens were more susceptible to the extracts with lower MICs compared to Gram negative uropathogens (Table 3a-c). As depicted in Table 16a-c, the results showed that the MBC/MIC ratio of SaAqSh and SaAqMa on uropathogens was ≤ 2, SaEaSh, SaEaMa and SaMeSh ≤ 4, while SaMeMa had MBC/MIC ratio of ≤ 8 (Tabla 3a-c).

4. DISCUSSION

Of the two methods of extraction used, it was observed that soxhlet method produced the highest percentage of the phytochemicals in which methanol gave the highest yield in both soxhlet and maceration methods. In soxhlet it was 12.21% while in maceration it yielded 8.73%. Aqueous extraction using both methods is poor comparatively giving 6.15% in soxhlet and 5.2% in maceration. The two methods of extraction revealed the presence of phytochemicals uniformly distributed in all the crude extracts. However, terpenoids were not detected in methanol and ethyl acetate extracts. This observation was in conformity with previous findings of other workers [8,62,63]. The absence of alkaloids in this study is supported by the work of Essiet and Bassey [2] who looked at the flower of Senna and reported the absence of alkaloids in three species viz; *S. alata*, *S. hirsuta* and *S. obtusifolia*. Zige *et a*l. and Fazwa *et al*. [7,9] also reported absence of alkaloids in *S. alata*. Karthika *et al* reported the absence of terpenoids in the leaves of *S. alata* [63].

Flavonoids are polyphenolic derivatives known to form complexes with soluble proteins and bacterial peptidoglycan that elicited a wide range of biological activities such as antioxidant, anticancer, anti-inflammatory, antimicrobial and anti-allergic as reported by Mutha *et al*. and Panche *et al*. [64,65]. Interestingly, it was detected in our study using sodium hydroxide method (Table 1).

Tannins are phenolic compounds with astringent properties. Tannins act as defensive agents against plants pathogens and by extension possess significant therapeutic importance some of which include; anti-inflammatory [66], antioxidant [67], antimicrobial [68,69,70]. Tannins were harvested in good quantity in this work. Saponins is another bioactive substance harvested. It is said to have therapeutic property and acts as membrane permeabilizing agent [71]. It is one of phytochemicals extracted. Steroids were detected and their therapeutic value may be linked to their relationship with such compounds as sex hormones [8]. It must be emphasized that terpenoids are useful in the treatment of several diseases caused by viruses, bacteria, parasites, fungi including cancerous cells [72,73]. The terpenoids could only be extracted using aqueous menstruum both in soxhlet and maceration methods. The absence of terpenoids in methanol and ethyl acetate extract could be attributed to low polarity of the solvents [49].

In this study, the crude extracts of *S. alata* showed activity against the tested uropathogens (Table 2a-f). The therapeutic potential of medicinal plants is attributed to its inherent phytochemicals [64], as evidenced in the present study (Table 1). The crude extracts of *S. alata* demonstrated broad spectrum of activity against uropathogens. This probably affirmed the traditional usage of *S. alata* in disease management in Nigeria [18,17].

SaMeSh extract was active against the tested uropathogens except in *Cronobacter* species where no zone of inhibition was recorded at 500 mg/mL concentration (Table 2e). On the other hands, SaMeMa inhibited all isolates of *K. pneumoniae*, *E. clocae*, *Citrobacter* species, *Pseudomonas* species, *Cronobacter* species and *Enterococcus* species at 500 mg/mL. The implication is that *S. alata* extract can also manifest resistance in certain organisms even at high concentrations. This observation is in concordance with the work of Mordi *et al*. [50] who reported that bacteria species varied widely in their degree of susceptibility to antibacterial agents.

The maceration methanol extracts (SaMeMa) showed better activity than the soxhlet methanol extracts (SaMeSh) however there was no significant difference (*P* =.254) between the results. This is in conformity with previous studies of Ehiowemwenguan *et al*. [58] and Zige et al. [7]. and disagreed with Alalor et al. [74] who reported no activity against Gram negative

Table 3a. MIC, MBC and MIC index of *S. alata* extracts (Conc. (mg/mL)

Bacteria	Code	SaMeSh			SaMeMa			SaEaSh			SaEaMa			SaAqSh			SaAqMa		
		MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}
S. marcescens	NH 23	250	250	1	31.25	125	4	250	500	2	-	-	-	125	125	1	31.25	62.5	2
	NH 17	250	500	2	250	500	2	250	250	1	250	Nil	Nil	125	250	2	125	250	2
	GH 1	125	250	2	62.5	250	4	250	250	1	125	250	2	250	250	1	125	250	2
	GH 14	250	500	2	-	-	-	-	-	-	-	-	-	250	Nil	Nil	-	-	-
E. clocae	NH 2	125	250	2	62.5	250	4	250	250	1	250	500	2	125	250	2	125	125	1
	NH 25	125	250	2	62.5	250	4	31.25	125	4	125	125	1	250	500	2	125	250	2
	NH 34	125	250	2	250	Nil	Nil	-	-	-	-	-	-	-	-	-	-	-	-
	NH 29	125	250	2	125	250	2	-	-	-	-	-	-	-	-	-	250	500	2
	GH 25	-	-	-	62.5	125	2	62.5	62.5	1	125	125	1	125	250	2	250	500	2
	GH 9	125	125	1	62.5	250	4	62.5	125	2	125	125	1	125	125	1	125	125	1
Citrobacter sp	NH 10	125	125	1	125	250	2	62.5	125	2	62.5	125	2	250	500	2	250	250	1
	NH 22	62.5	125	2	62.5	250	4	62.5	62.5	1	125	125	1	62.5	125	2	62.5	62.5	1
	GH 23	125	250	2	125	500	4	125	250	2	125	250	2	250	250	1	125	250	2
	GH 11	-	-	-	250	250	1	-	-	-	-	-	-	125	250	2	250	250	1
	GH10	62.5	125	2	125	250	2	125	250	2	125	250	2	125	125	1	125	125	1
	GH 7	500	Nil	Nil	62.5	250	4	-	-	-	-	-	-	125	250	2	125	250	2
	UC 20	125	125	1	125	125	1	125	250	2	62.5	125	2	125	125	1	125	125	1
Pseud. sp	NH 20	250	500	2	125	500	4	125	250	2	125	500	4	250	500	2	250	500	2
	NH 15	125	500	2	125	250	2	125	250	2	250	500	2	-	-	-	-	-	-
	UC 23	-	-	-	62.5	250	4	-	-	-	-	-	-	250	500	2	125	125	1

KEY: NH = Nigeria Navy Reference Hospital, Calabar, GH = General Hospital, Calabar, UC = University of Calabar Teaching Hospital, ND = Not detected up to 500 mg/Ml, SaMeSh = S. alata methanol extract (soxhlet), SaMeMa = S. alata methanol extract (maceration), SaEaSh = S. alataethyl acetate extract (soxhlet), SaEaMa = S. alata ethyl acetate extract (maceration), SaAqSh = S. alata aqueous extract (soxhlet), SaAqMa = S. alata aqueous extract (maceration).

Table 3b. MIC, MBC and MIC index of *S. alata* extracts (Conc. (mg/mL)

Bacteria	Code	SaMeSh			SaMeMa			SaEaSh			SaEaMa			SaAqSh			SaAqMa		
		MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}
K. pneumoniae	NH 3	125	250	2	62.5	250	4	62.5	125	2	125	250	2	250	250	1	125	125	1
	NH 33	250	500	2	125	250	2	125	250	2	125	250	2	250	500	2	125	125	1
	NH 74	250	500	2	250	Nil	Nil	125	500	4	-	-	-	125	250	2	250	500	2
	NH 78	125	250	2	250	Nil	Nil	125	250	2	125	250	2	125	125	1	125	125	1
	NH 32	62.5	125	2	125	500	4	62.5	125	2	62.5	125	2	250	250	1	125	250	2
	NH 63	250	500	2	125	500	4	31.25	125	4	62.5	125	2	125	125	1	62.5	62.5	1
	NH 69	62.5	125	2	31.25	125	4	62.5	125	2	125	250	2	250	250	1	125	250	2
	NH 66	62.5	125	2	31.25	125	4	62.5	125	2	125	250	2	250	500	2	125	250	2
	GH37	62.5	125	2	62.5	250	4	125	250	2	125	500	4	125	125	1	125	125	1
	GH 63	250	500	2	62.5	250	4	62.5	62.5	1	125	250	2	125	500	2	62.5	125	2
	UC 25	62.5	125	2	125	500	4	62.5	125	2	125	125	1	125	250	2	62.5	125	2
	UC 13	125	250	2	31.25	250	8	62.5	125	2	125	250	2	250	250	1	125	125	1
	UC 48	62.5	125	2	62.5	250	4	250	500	2	125	250	2	125	125	1	125	125	1
	UC 33	125	250	2	62.5	250	4	62.5	125	2	125	125	1	250	Nil	Nil	-	-	-
	UC 41	125	250	2	125	250	2	125	250	2	125	125	1	250	500	2	125	250	2
E. coli	NH 5	125	250	2	-	-	-	125	250	2	125	125	1	-	-	-	-	-	-
	NH 30	250	Nil	Nil	125	500	2	125	250	2	125	250	2	125	125	1	125	250	2
	NH 85	250	500	2	250	500	2	125	250	2	125	250	2	125	125	1	250	250	1
	GH 8	250	Nil	Nil	62.5	250	4	125	250	2	125	125	1	125	250	2	125	250	2
	GH 3	250	500	2	125	500	4	62.5	125	2	125	250	2	125	250	2	125	250	2
	GH 61	250	500	2	125	500	4	250	Nil	Nil	125	125	1	125	250	2	125	250	2
	UC 31	125	250	2	62.5	250	4	62.5	125	2	125	250	2	125	125	1	125	250	2
	UC 19	62.5	250	4	62.5	250	4	62.5	125	2	125	250	2	250	250	1	125	125	1

KEY: NH = Nigeria Navy Reference Hospital, Calabar, GH = General Hospital, Calabar, UC = University of Calabar Teaching Hospital, ND = Not detected up to 500 mg/mL, SaMeSh = S. alata methanol extract (soxhlet), SaMeMa = S. alata methanol extract (maceration), SaEaSh = S. alataethyl acetate extract (soxhlet), SaEaMa = S. alata ethyl acetate extract (maceration), SaAqSh = S. alata aqueous extract (soxhlet), SaAqMa = S. alata aqueous extract (maceration).

Table 3c. MIC, MBC and MIC index of *S. alata* extracts (Conc. (mg/mL)

Bacteria	Code	SaMeSh			SaMeMa			SaEaSh			SaEaMa			SaAqSh			SaAqMa		
		MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}	MIC	MBC	MIC_{index}
P. mirabililis	NH 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NH 26	250	250	1	125	250	2	62.5	250	4	125	125	1	125	250	2	125	250	2
	NH 28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	250	500	2
	GH 15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	GH 67	250	500	2	250	500	2	125	250	2	62.5	125	2	250	500	2	250	500	2
Cro. sp	NH 59	-	-	-	125	250	2	62.5	125	2	250	250	1	-	-	-	250	250	1
	GH 50	-	-	-	125	250	2	62.5	125	2	125	250	2	-	-	-	250	500	2
Staph. sp	GH 36	500	Nil	Nil	31.25	125	4	250	500	2	-	-	-	125	125	1	31.25	62.5	2
	GH 55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	UC 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	UC 15	31.25	125	4	31.25	125	4	62.5	125	2	62.5	125	2	62.5	125	2	-	-	-
	UC 18	250	250	1	250	Nil	Nil	125	250	2	125	125	1	125	250	2	31.25	62.5	2
	UC 11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NH 1	31.25	125	4	125	250	2	62.5	125	2	250	250	1	62.5	125	2	62.5	125	2
	NH 40	31.25	125	4	250	Nil	Nil	31.25	62.5	2	125	250	2	31.25	62.5	2	62.5	62.5	1
	NH 37	500	Nil	Nil	62.5	125	2	125	250	2	-	-	-	125	250	2	62.5	62.5	1
	NH 38	62.5	125	2	31.25	250	8	62.5	125	2	62.5	125	2	62.5	125	2	-	-	-
	NH 62	62.5	125	2	125	250	2	62.5	125	2	125	125	1	62.5	125	2	125	125	1
Entero. sp	NH 64	250	Nil	Nil	125	250	2	125	125	1	62.5	125	2	125	125	1	250	250	1
	GH 12	250	250	1	125	250	2	62.5	125	2	62.5	125	2	62.5	125	2	250	500	2

KEY:NH = Nigeria Navy Reference Hospital, Calabar, GH = General Hospital, Calabar, UC = University of Calabar Teaching Hospital, ND = Not detected up to 500 mg/Ml, SaMeSh = S. alata methanol extract (soxhlet), SaMeMa = S. alata methanol extract (maceration), SaEaSh = S. alataethyl acetate extract (soxhlet), SaEaMa = S. alata ethyl acetate extract (maceration), SaAqSh = S. alata aqueous extract (soxhlet), SaAqMa = S. alata aqueous extract (maceration).

bacteria. In contrast to previous reports by Okoko [75] the organic extract of *S. alata* demonstrated significant activity against *K. pneumonia*e.

In this study, soxhlet and maceration ethyl acetate extracts were effective against both Gram positive and Gram negative uropathogens tested. The difference in activity may be attributed to variation in the concentrations of bioactive constituents in the extracts used. Both extracts completely inhibited the growth of *E. coli*, *Cronobacter* species, and *Enterococcus* species at 500 mg/mL (Table 2a-f). However, the antibacterial activity was significantly higher against *Enterococcus* species and *Cronobacter* species at 125 mg/mL when compared with other isolates. This study supported the work of Afrin [4] who reported inhibitory activity of ethyl acetate extract of *S. alata* against stains of pathogenic bacteria.

The soxhlet and maceration aqueous extract of *S. alata* (SaAqSh and SaAqMa) showed antibacterial activity against tested uropathogens at various concentrations. *Cronobacter* species and *Enterococcus* species were not inhibited by SaAqSh at 500 mg/mL (Table 2f). The most susceptible isolate inhibited by SaAqSh was *Citrobacter* species where all isolates were inhibited at 500 mg/mL. All the tested uropathogens were inhibited by SaAqMa at 500 mg/mL (Table 2b, d, f). This study is in consonant with previous studies [63,76,50]. In contrast to the present finding is the work of Faruq *et al*. [49] that reported no activity with aqueous extract against *Staphylococcus aureus*. This discrepancy may be attributed to concentration of the extracts used, age of the plant and geographical location of the plant.

The effectiveness of an antimicrobial agent is an inverse measurement of its Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The lower the MIC and MBC of plant extract or drug against bacterial strain, the better is its potency [77]. In this study, the MIC values range from 31.25 to 250 mg/mL and MBC values from 62.5 to 500 mg/mL (Table 3a-c). The observed MICs and MBCs varied with respect to solvent, extraction method and the type of bacteria.

Crude extracts of *S. alata* was previously showed to be active against both Gram positive and Gram negative bacteria with various values of MIC and MBC [75, 76]. Doughari and Okafor [78] reported MIC and MBC values between 12-20 mg/mL which is lower compared to the present study. Promgool *et al*. [79] in Thailand reported MIC values of 160-320 µg/mL and 640-1280 µg/mL for Gram positive and Gram negative respectively. Donkor *et al*. [77] in Ghana reported MIC values of 3.13-12.5 mg/mL which is lower than the result obtained in this study. The difference values obtained by different authors may be attributed to factors such as sources of the bacterial isolates, age and geographical location of the plant, extraction methods and intrinsic resistance mechanisms of the test organisms.

This study revealed the MIC index (MBC/MIC ratio) of ≤2, ≤4 and ≤8 for SaAqSh and SaAqMa, SaEaSh, SaEaMa and SaMeSh respectively while SaMeMa had MIC index of ≤8 on the test organism. This is an indication of bactericidal action against the test uropathogens as observed by Olajuyigbe and Afolayan [61] and Saeloh and Visutthi who worked on Thai plants [80]. These workers have reported that where MIC value equals MBC value, broad spectrum bactericidal potential is possible and hence better therapeutic [61,80].

4. CONCLUSION

Most of the tested uropathogens were found to be susceptible to *S. alata* extracts at various concentrations with organic solvents extracts exhibiting high antibacterial activity. The lower MIC and MBC values recorded against the test organisms indicated high therapeutic potency of the extracts. The MIC index values of ≤8 recorded indicates broad spectrum bactericidal activity. Hence, *S. alata* is an important source of antimicrobial products when processed professionally under a more purified entity and would make *S. alata* a good source of antibiotics in the changing era where old antibiotics are no longer effective against most clinical pathogens. More so as a broad spectrum antibiotic the pharmaceutical industry will be hosting a new antibiotic to challenge more diseases.

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COMPETING INTERESTS

Authors have declared that no competing interest exists.

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