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

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: Healthy 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 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 test 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, UTI.

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]. According to WHO (as cited in [4]) and [5], 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.

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]. As opined by [7], curative potentials of plants materials are well documented in many literature, and is due particularly to the presence of pharmacologically important constituents (secondary metabolites) [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, the plant was originally found in Ghana and Brazil, but it is 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 [19-22], 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 and stem-bark and roots are used to treat jaundice, gastroenteritis, gonorrhea, tryphoenteritis, 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 use 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:

Healthy 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 [7] and [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 hrs with occasional swirling at interval while methanol and ethyl acetate extracts were occasionally shaken and allowed for 48 hrs. 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 70^{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 [53] and [54] was adopted with modification. One hundred and fifty (150) g of the coarse plant material was packed into a cellulose 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 70°C. Weights of the crude extracts were recorded, and the dried extracts stored in the refrigerator 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* sp (2), *K. pneumoniae* (15), *E. clocae* (7), *Citrobacter* sp (7), *Pseudononas* sp (3), *P. mirabilis* (5), Coagulase Negative Staphylococci (CoNS) (11) and *Enterococcus* sp (2). All the test organisms were obtained from urine samples of UTI 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 used.

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 (5) g of the crude extract was weighed and dissolved into 10 mL of 10% tween 80 in a sterile bottle and mix 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 and incubated at 37˚C for 18–24 hrs. 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 [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 distilled water (as control) was added to the fifth well. The plates were allowed to stand on the laboratory bench for 2 hrs to allow proper diffusion of the extract into the medium and thereafter incubated at 37°C for 18-24 hrs. 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 [58] with modification. The crude extracts were reconstituted to yield 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 [59] and [60] was used. 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 [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].

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

Figure 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 depicted 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 saponins, tannins, flavonoids, anthraquinone, terpenoids and steroids were detected. However, terpenoids was not found in the methanol and ethyl acetate extracts (Table 1).

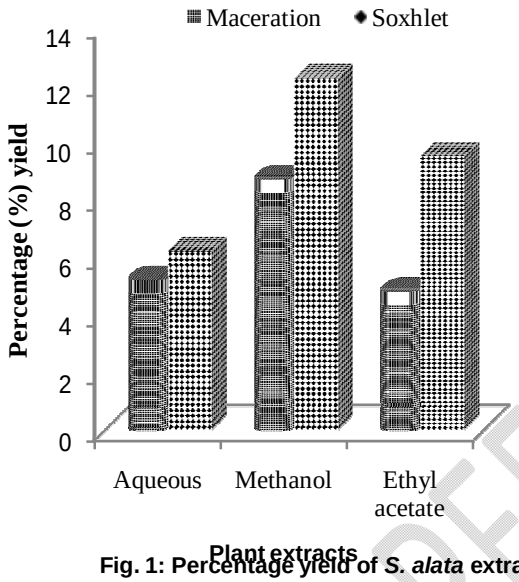

Fig. 1: Percentage yield of *S. alata* extracts

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 (Figure 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).

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	+	+	+	+	+	+

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

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, 5, 5, 5, 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).
Maceration methanol extract (SaMeMa) was more active on *Pseudomonas* sp compared to other extracts (Table 2a-b). At 500 mg/mL and 250 mg/mL concentrations, all isolates of *Pseudomonas* sp tested were inhibited with inhibition zones ranged from 12.0±0.0 to 19.0±1.41 mm and 8.5±0.71 to 14.5±0.71 mm respectively (Table 2a). SaMeSh, SaEaSh, SaEaMa, SaAqSh, and SaAqMa inhibited 2 isolates each of *Pseudomonas* sp at 500 mg/mL and 250 mg/mL concentrations. No inhibition zone was recorded against *Pseudomonas* sp at 62.5 - 125 mg/mL concentrations except SaMeMa and SaAqMa with activity at 125 mg/mL (Table 2a-b).
Soxhlet aqueous extract (SaAqSh) recorded the highest inhibition zone against *Klebseilla* sp 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 *Klebseilla* sp 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

TABLE 2a: Antibacterial activity of *Senna alata* extracts

Bacteria	Code	Mean zone of inhibition (mm)															
		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

Bacteria	Code	Mean zone of inhibition (mm)							
		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.

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).

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 (Table 3a-c).

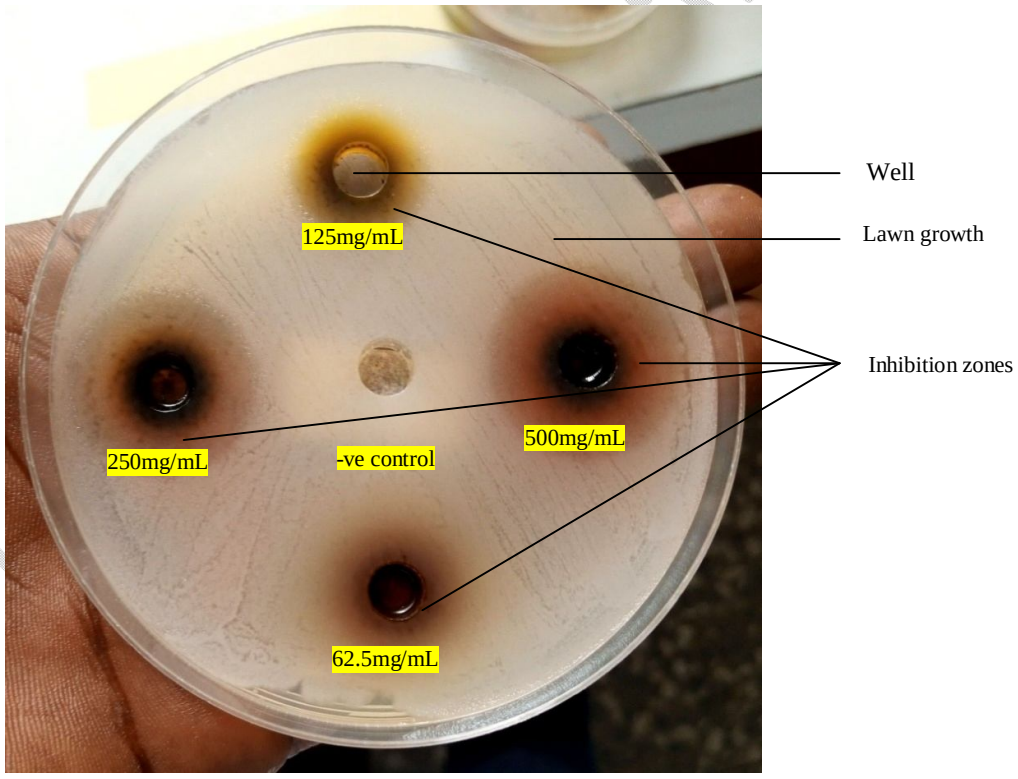

Fig. 2: Plate showing antibacterial activity of *S. alata* extracts

TABLE 2c: Antibacterial activity of *Senna alata* extracts against uropathogens

Bacteria	Code	Mean zone of inhibition (mm)															
		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. mirabilis	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. mirabilis	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.

DISCUSSION

In this study, maceration and soxhlet methods were employed in the extraction of *S. alata* leaves using different solvents. There existed, a variation in the percentage yield of the extracts obtained between various solvents with respect to extraction methods. 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%). The observed variation in the yield of extracts explains the solubility of plant metabolites in different solvent [49].

The phytochemicals (Table 1) were uniformly distributed in all the respective extracts obtained except in methanol and ethyl acetate extracts were terpenoids was not detected. The results for the phytochemicals screening of *S. alata* extracts is in conformity with previous finding though with some exceptions [8,62,63]. The absence of alkaloids in this study is supported by the work of [2] who reported absent of alkaloids in three species of Senna viz; *S. alata*, *S. hirsuta* and *S. obtusifolia*. Similarly, [7] and [9] reported the absent of alkaloids in extracts of *S. alata*. The absent of terpenoids in this study is in concordance with the results obtained by [63]. The secondary metabolites detected in the present study are known for various phamarcological properties.

Flavonoids are polyphenolic derivatives known to form complexes with soluble proteins and bacterial peptidoglycan and elicited a wide range of biological activities such as antioxidant, anticancer, anti-inflammatory, antimicrobial and anti-allergic [64,65]. Tannins are phenolic compound 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], anti-oxidant [67], antimicrobial [68,69,70] etc. According to Francis (as cited in [71]) saponins showed significant therapeutic properties and acts as membrane permeabilizing agents. Steroids were detected, and their therapeutic value according to Bell (as cited in [8]) may be linked to their relationship with such compounds as sex hormones. Terpenoids are among the most widely distributed secondary metabolites in plants. Terpenoids are useful in the treatment of several diseases caused by viruses, bacteria, parasites, fungi, including cancerous cells [72,73].

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], which is evident in the present study (Table 1). The crude extracts of *S. alata* demonstrated broad spectrum of activity against the tested uropathogens (Table 2a-f). This reaffirmed the traditional usage of *S. alata* in disease management in Nigeria [18,17].

The results showed that SaMeSh was active against all the tested uropathogens except *Cronobacter* sp where no inhibition zone was recorded at 500 mg/mL concentration (Table 2e). However, CoNS was the most sensitive uropathogen at 62.5 mg/mL concentration (Table 2e). On the other hands, SaMeMa inhibited all isolates of *K. pneumoniae*, *E. clocae*, *Citrobacter* sp, *Pseudomonas* sp, *Cronobacter* sp and *Enterococcus* sp at 500 mg/mL, whereas 8, 6 and 2 isolates of CoNS, *E. coli* and *P. mirabilis* were inhibited at 500 mg/mL respectively. This is in agreement with [50] who observed that bacterial species varied widely in their degree of susceptibility. The maceration methanol extracts (SaMeMa) showed better activity than the soxhlet methanol extracts (SaMeSh). This may be due to the liberation of active phytochemical constituents in the extracts. However, there was no significant difference (P =.254) between the results obtained by SaMeMa and SaMeSh. This finding is in conformity with previous studies [58,7], and disagreed with Alalor *et al* (as cited in [74]) who reported no activity against Gram negative bacteria. Contrary to previous reports by [75] the organic extract of *S. alata* demonstrated significant activity against *K. pneumoniae*. However, the difference in the activity of SaMeSh and SaMeMa and other studies may be attributed to variation in the concentrations of bioactive constituents in the extracts used.

Soxhlet and maceration ethyl acetate extracts of *S. alata* were effective against both Gram positive and Gram negative uropathogens tested. Both extracts completely inhibited the growth of *E. coli*, *Cronobacter* sp, and *Enterococcus* sp at 500 mg/mL (Table 2a-f). However, the antibacterial activity was significantly higher against *Enterococcus* sp and *Cronobacter* sp at 125 mg/mL compared to other isolates. The maceration ethyl acetate extract (SaEaMa) was slightly effective at lower concentrations against the tested uropathogens compared to the soxhlet ethyl acetate extract (SaEaSh). However, the difference was not statistically significant (P = .802). The present study supported the work of [4] who reported inhibitory activity of ethyl acetate extract of *S. alata* against stains of pathogenic bacteria.

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. alata*ethyl 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

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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. alata*ethyl acetate extract (soxhlet), SaEaMa = *S. alata* ethyl acetate extract (maceration), SaAqSh = *S. alata* aqueous extract (soxhlet), SaAqMa = *S. alata* aqueous extract (maceration).

The soxhlet and maceration aqueous extract of *S. alata* (SaAqSh and SaAqMa) showed antibacterial activity against tested uropathogens at various concentrations. However, *Cronobacter* sp and *Enterococcus* sp was not inhibited by SaAqSh at 500 mg/mL (Table 2f). The most susceptible isolate inhibited by SaAqSh was *Citrobacter* sp where all isolates were inhibited at 500 mg/mL. Meanwhile, all the tested uropathogens were inhibited by SaAqMa at 500 mg/mL (Table 2b, d, f). This study is in line with previous works [63,76,50]. Contrary to the present finding, [49] reported no activity with aqueous extract against *S. 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 MIC and MBC. The lower the MIC and MBC of plant extract or drug against bacterial strain, the better its potency [77]. In general, the MICs values ranged from 31.25 to 250 mg/mL and MBCs values from 62.5 to 500 mg/mL (Table 3a-c). However, MIC and MBC were undetected against *Cronobacter* sp with SaMeSh and SaAqSh (Table 3c). However, 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 varied MICs and MBCs [76,75]. 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 bacteria respectively. The MICs values reported in Ghana (3.13-12.5 mg/mL) is lower compared to the present study [77], however, the MBC values obtained in this study is comparable to the same authors and [76]. The difference in MIC and MBC values in the present study with previously documented works may be attributed to factors such as sources of the isolates, age and geographical location of the plant, extraction methods and intrinsic resistance mechanisms of the test organisms.

This study revealed that the MIC index (MBC/MIC ratio) of SaAqSh and SaAqMa on the test organisms was ≤ 2, SaEaSh, SaEaMa and SaMeSh ≤ 4, while SaMeMa had MIC index of ≤ 8. Since the MBC/MIC ratio of the extracts on all the test uropathogens was ≤8, the extracts is considered to be bactericidal against the test uropathogens [61,80]. However, where MIC value equals MBC value, is an indication of broad spectrum bactericidal potential and hence, better therapeutic [80].

4. CONCLUSION

All the test uropathogens were found to be susceptible to *S. alata* extracts at various concentrations with organic solvents exhibiting the highest antibacterial activity. The lower MICs and MBCs values recorded against the test organisms indicated high therapeutic potency of the extracts. The MIC index values ≤8, recorded indicate broad spectrum bactericidal activity. Hence, *S. alata* is an important source of antimicrobial products. Further studies focus on isolation, characterization and identification of bioactive constituents found in the respective extracts is recommended.

REFERENCES

1. Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: Past history and future perspective. Journal of Herbmed Pharmacology. 2017;7(1):1-7. doi: 10.15171/jhp.2018.01.
2. Essiett UA, Bassey IE. Comparative phytochemical screening and nutritional potentials ofthe flowers (petals) of *Senna alata* (l) roxb, *Senna hirsuta* (l.) Irwin and barneby, and *Senna obtusifolia* (l.) Irwin and barneby (Fabaceae). Journal of Applied Pharmaceutical Science, 2013;3(08):097-101. DOI: 10.7324/JAPS.2013.3817.
3. Ita BN, Ndukwe GI. Antioxidant activity of *Senna alata* root extracts. Journal of Natural Products and Resources, 2017;3(1):94-96.
4. Afrin M. *Phytochemical and Biological Investigation of Senna alata leaves* (Thesis). East West University, Dhaka. Bangladesh. 2015;111p.
5. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety, Frontier of Pharmacology. 2013;4: 1–10.
6. Kalita D, Saikia J, Devasarmah HK. Ethonomedicinal, antibacterial and antifungal potentiality of *Centella asiatica, Nerium indicum* and *Cuscuta reflexa* widely used in Tiwa Tribe of Morigaon district of Assam, India. International Journal of Phytomedicine. 2012;4:380-385.

7. Zige DV, Ohimain EI, Nengimonyo B. Antimicrobial activity of ethanol extract of *Sennaalata* leaves against some selected microorganism in Bayelsa State, Nigeria. Greener Journal of Microbiology and Antimicrobials. 2014;2(2):026-031.

8. Abubakar I, Mann A, Mathew JT. Phytochemical composition, antioxidant and anti-nutritional properties of root-bark and leaf methanol extracts of *Senna alata* L. grown in Nigeria, African Journal of Pure and Applied Chemistry. 2015;9(5):91-97.

9. Fazwa FMA, Norhayati S, Nabilah SB, Keeren SR, Zunoliza A, Ling SK, et al. Selection of high yielding genotypes of *Senna alata* for future breeding programme. International Journal of Agriculture, Forestry and Plantation. 2020;10:379-385.

10. Naowaboot J, Wannasiri S. Anti-lipogenic effect of *Senna alata* leaf extract in high-fat diet-induced obese mice. Asian Pacific Journal of Tropical Biomedicine. 2016;6(3):232–238. doi:10.1016/j.apjtb.2015.12.006.

11. Kumar MP, Tanaya G, Sumanta G, Basudeb B, Prasenjit M. Isolation and characterization of a compound from the leaves of *Cassia alata* Linn. EC Chemistry. 2016;2(2):138-144.

12. Oladeji O. Thin-layer chromatographic analysis of flavonoids and total phenolics in methanolic and ethanolic extracts of *Senna alata*(L.) Roxb. (Fabales: Fabaceae). Brazilian Journal of Biological Sciences. 2016;3(5):221-225.

13. Adelowo F, Oladeji O. An overview of the phytochemical analysis of bioactive compounds in *Senna alata.* Advances in Biochemistry. 2017;5(5):102-109. doi: 10.11648/j.ab.20170505.14.

14. Abiolu OA. Ethnobotanical study of medicinal plants in southwestern Nigeria and traditional healers' perception of indigenous knowledge digitization. Inkanyiso Journal of Humanities and Social Sciences. 2018;10(1):90-102.

15. Aslam MS. Application of genus Cassia in the treatment of constipation: A systematic review [version 1; referees: Awaiting peer review]. F1000Research. 2019;8:256.https://doi.org/10.12688/f1000research.17893.1.

16. Chukwuma EC, Soladoye MO, Feyisola RT. Traditional medicine and the future of medicinal plants in Nigeria. Journal of Medicinal Plants Studies. 2015;3(4):23-29.

17. Uwazie JN, Yakubu MT, Ashafa AOT, Ajiboye TO. Identification of characterization of anti-diabetic principle in Senna alata (Linn) flower using alloxan-induced diabetic male Wister rats. Journal of Ethnopharmacology. 2020;261(28). Https://doi.org/10.1016/j.jep.2020.112997.

18. Oluwole SO, Funmilayo EA, Abimbola PO, Deborah TB. Ethnobotanical description and biological activities of *Senna alata.* Evidence-Based Complementary and Alternative Medicine. 2020.https://doi.org/10.1155/2020/2580259.

19. Angaye TCN, Konmeze O, Seibokuro B, Adulphus P. Biolarvicidal control of malaria using solvent extracts of *Senna alata.* MOJ Toxicology. 2019;5(1):40-42.

20. Edwin UPM, Nyiutaha IG, Essien AE, Nnamdi OK, Sunday EM. Larvicidal effect of aqueous and ethanolic extracts of *Senna alata* on *Anopheles gambiae*, *Culex quinquefasciatus* and *Aedes aegypti*. Pakistan Journal of Pharmaceutical Sciences. 2013;26(3):561-566.

21. Josué WP, Nadia NAC, Claire KM, Jeannette Y, Mbida M. *In vitro* sensitivity of *Plasmodium falciparum* field isolates to methanolic and aqueous extracts of *Cassia alata* (Fabaceae). Alternative and Integretive Medicine. 2014;3(2):159. doi:10.4172/2327-5162.1000159.

22. Komlaga G, Agyare C, Dickson RA, Mensah MLK, Annan K, Loiseau PM, Champy P. Medicinal plants and finished marketed herbal products used in the treatment of malaria in the Ashanti region, Ghana. Journal of Ethnopharmacology. 2015;172(2015):333–346. http://dx.doi.org/10.1016/j.jep.2015.06.041.

23. Guerra F, Ansari AA, Kurup R, Subramanian G. Antifungal activity of *Senna alata*, *Senna bicapsularis* and *Pityrogramma calomelanos*. Journal of Complementary and Alternative Medical Research. 2020;10(3):11-21. DOI: 10.9734/JOCAMR/2020/v10i330164.

24. Legaspi CLB, Maramba-Lazarte CC. The phytochemical content and the in vitro antifungal properties of *Senna alata* (Linn.) Roxb: A Review. ACTA Medica Philippina. 2020;54(1):86-93.

25. Saidin SH, Ali NAM, Hirmizi NM, Yusoff N, Abdullah Z, Markandan S, et al. Skin care active ingredients from *Senna alata* (L.) Roxb extracts. Asian Journal of Pharmacognosy. 2019;3(1):23-31.

26. Matthew OA, Gambo NN. Phytochemistry and antimicrobial activity of the leaf of *Cassia alata* Linn. Chemistry and Materials Research. 2013;3(3):96-101.

27. Kanedi M, Rokiban A, Widodo S, Nopiyansah, Isbiyantoro, Fauziah L. Healing effect of leaf extract of Candlebush (*Cassia alata* L.) on cutaneous wound infected with *Trichophyton rubrum*. World Journal of Pharmaceutical and Life Sciences. 2016;2(5):42-50.

28. Nasution SLR, Putri M, Hulu W, Girsang E, Nasution AN. Healing potential of *Senna alata* leaves extract in Rats. Journal of Education, Health and Sport. 2019;9(5):127-136. DOI: http://dx.doi.org/10.5281/zenodo.2707605.

29. Yakubu MT, Musa IF. Liver and Kidney functional indices of pregnant Rats following the administration of the crude alkaloids from *Senna alata* (Linn. Roxb) leaves. Iranian Journal of Toxicology. 2012;6(16):615-625.

30. Shiddamallayya N, Yasmeen A, Gopakumar K. Medicobotanical survey of Kumar pavatha Kukke Subramanya, Manglore, Karnataka, Indian J. Tradit. Knowl. 2010;9:96–99.

31. Pieme CA, Penlap VN, Nkegoum B, Taziebou PCL, Tekwu EM, Etoa FX, et al. Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L.) Roxb (Ceasalpiniaceae). African Journal of Biotechnology. 2006;5(3):283-289. DOI: 10.5897/AJB05.197.

32. Tatsimo SJN, Tamokou JD, Tsague VT, Lamshoft M, Sarkar P, Bag PK, et al. Antibacterial-guided isolation of constituents from *Senna alata* leaves with a particular reference against Multi-Drug-Resistant *Vibrio cholerae* and *Shigella flexneri*. International Journal of Biological and Chemical Sciences. 2017;11(1):46-53.

33. Jiofack T, Fokunang C, Guedje N, Kemeuze V, Fongnzossie E, Nkongmeneck B.A, et al. Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. International Journal of Medicine and Medical Sciences. 2010;2(3):60-79.

34. Chewchindaa S, Sithisarnb P. Simultaneous HPTLC determination of Rhein and Aloe-emodin in *Senna alata* leaves from Thailand and their commercial products. Natural Product Communications. 2017;12(3):399-401.

35. Appiah KS, Oppong CP, Mardani HK, Omari RA, Kpabitey S, Amoatey CA et al. Medicinal Plants Used in the Ejisu-Juaben Municipality, Southern Ghana: An ethnobotanical study. Medicines. 2018;6:1.doi:10.3390/medicines6010001.

36. Parveen S, Shahzad A. A review on in vitro culture of *Cassia alata* Linn. (*Senna alata*): Analysis of metabolites and biological activities. Journal of Functional and Environmental Botany. 2015;5(2):78-86. DOI: 10.5958/2231-1750.2015.00016.5.

37. Abo KA, Fred-Jaiyesimi AA, Jaiyesimi AEA. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. Journal of Ethnopharmacology. 2008;115(1):67–71.

38. Leung L, Riutta T, Kotecha J, Rosser W. Chronic constipation: An evidence-based review. Be Journal of the American Board of Family Medicine. 2011;24(4):436–451.

39. Oladeji, O. S., Adelowo, F. E., Oluyori, P. A., Bankole, D. T. (2020). Ethnobotanical description and biological activities of *Senna alata*. *Evidence-Based Complementary and Alternative Medicine*, 1-12. https://doi.org/10.1155/2020/2580259.

40. Selvi V, Isaivani V, Karpagam S. Studies on antimicrobial activities from flower extract of *Cassia alata* Linn. International Journal of Current Science. 2012;2:299–303.

41. Vedekoi J, Selestin SD. In vitro antioxidant property and phytochemical constituents of *Senna alata* leaves aqueous extract collected in Ngaoundéré (Cameroon). Pharmaceutical and Biosciences Journal. 2020;8(3):15-21.

42. Kudatarkar NM, Nayak YK. Pharmacological screening of *Cassia alata* leaves on colorectal cancer. Colorectal Cancer. 2018;4(1):02. DOI: 10.21767/2471-9943.100049.

43. Lewis A, Levy A. Anti-inflammatory activities of *Cassia alata* leaf extract in complete freund's adjuvant arthritis in Rats. West Indian Medical Journal. 2011;60(6):615–621.

44. Yuenyongsawad S, Bunluepuech K, Wattanapiromsakul C, Tewtrakul S. Anti-cancer activity of compounds from *Cassia garrettiana* heartwood. *Songklanakarin.* Journal of Science and Technology. 2014;36(2):189–194.

45. Ongchai S, Chokchaitaweesuk C, Kongdang P, Chomdej S, Buddhachat K. In vitro chondroprotective potential of *Senna alata* and *Senna tora* in porcine cartilage explants and their species differentiation by DNA barcoding-high resolution melting (Bar-HRM) analysis. PLoS ONE. 2019;14(4):e0215664. https://doi.org/10.1371/journal.pone.0215664.

46. Villasenor IM, Sanchez AC. Cassia indoline, a new analgesic and anti-inflammatory alkaloid from *Cassia alata*. zeitschrift für naturforschung C. 2009;*64*(5,6):335-338.

47. Patil UK, Saraf S, Dixit VK. Hypolipidemic activity of seeds of *Cassia tora* Linn. Journal of Ethnopharmacology. 2004;90(2-3):249–252.

48. Angelina M, Hanafi M, Suyatna FD, Mirawati TS, Ratnasari S, Dewi BE. Antiviral effect of sub fraction *Cassia alata* leaves extract to dengue virus serotype-2 strain New Guinea C in human cell line Huh-7 it-1. *IOP Conference Series:* Earth and Environmental Science. 2017;101:012004. doi:10.1088/1755-1315/101/1/012004.

49. Faruq ZU, Rahman UA, Bello M, Obianke M, Atiku FA. Antibacterial activity of the active Component of *Cassia alata* (Linn) Leaves. Nigerian Journal of Basic and Applied Science. 2010;18(1):97-100.

50. Mordi RM, Osula AN, Igeleke CL, Odjadjare EE, Oboh FOJ, Uwadiae EO. The evaluation of the antimicrobial property of *Cassia alata* leaves in Benin city, Nigeria. Biomedicine and Nursing. 2016;2(3):14-23.

51. Roy S, Lyndem LM. An in vitro confirmation of the ethonopharmacological use of Senna plants as anthelmintic against rumen fluke *Paramphistomum gracile*. BMC Veterinary Research. 2019; https://doi.org/10.1186/s12917-019-2094-3, 1-17.

52. Kazeem MI, Azeez GA, Ashafa AOT. Effect of *Senna alata* (L) Roxb (Fabaceae) leaf extracts onalpha-Amylase, Alpha-Glucosidase and Postprandial Hyperglycemia in Rats. Tropical Journal of Pharmaceutical Research. 2015;14(10):1843-1848.

53. Ogunjobi AA, Abiala MA. Antimicrobial activity of *Senna alata* and *Phyllanthus amarus*. Global Journal of Pharmacology. 2013;7(2):198-202. DOI: 10.5829/idosi.gjp.2013.7.2.74179.

54. Kwapong AA. *Natural product inhibitors of bacterial Type-IV secretion systems and efflux pumps* (Ph.D thesis). UCL School of Pharmacy, London. 2016;272p.

55. Anibijuwon II, Gbala ID, Nnadozie BI, Ifayefunmi OO. Susceptibility of selected multi-drug resistant clinical isolates to different leaf extracts of *Senna alata*. Notulae Scientia Biologicae. 2018;10(1):26-32. DOI: 15835/nsb10110176.

56. Ekpiken SE, Nfongeh JF, Bassey EE. Antibacterial properties of *Senna alata* and *Citrus sinensis* leaves extracts against some selected bacteria isolated from fish ponds in Calabar metropolis. World Journal of Pharmacy and Pharmaceutical Sciences. 2021;10(3):1777-1793. DOI: 10.20959/wjpps20213-17867.

57. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Approved Guideline- 26 *Edition*. CLSI supplement M100S; Wayne, Pennsylvania USA. 2017;37:1-249.

58. Ehiowemwenguan G, Inetianbor JE, Yakubu JM. Antimicrobial qualities of *Senna alata*. IOSR Journal of Pharmacy and Biological Sciences. 2014;9(2):47-52. DOI: 10.9790/3008-09244752.

59. Adedayo O, Anderson WA, Moo-Young M, Snieckus V, Patil PA, Kolawole DO. Phytochemistry and antibacterial activity of Senna alata flower. Pharmaceutical Biology. 2001;39:6, 408-412. DOI: 10.1076/phbi.39.6.408.5880.

60. Evbuomwan L, Chukwuka EP, Obazenu EI, Ilevbare L. Antibacterial activity of *Vernonia amygdalina* leaf extracts against multidrug resistant bacterial isolates. *Journal of* Applied Science and Environmental Management. 2018;22(1):17-21. DOI: https://dx.doi.org/10.4314/jasem.v22i1.4.

61. Olajuyigbe OO, Afolayan AJ. In vitro antibacterial and time-kill evaluation of the *Erythrina caffra* Thunb. extract against bacteria associated with diarrhoea. The Scientific World Journal. 2012;1-8. doi:10.1100/2012/738314.

62. Jayachitra J, Pavithra K, Pandian RS, Bharathi V, Shanthi S. Phytochemical and antimicrobial analysis of *Senna alata* leaves extract. World Journal of Pharmaceutical Research. 2018;7(14):1030-1044. DOI: 10.20959/wjpr201814-12879.

63. Karthika C, Rafi KM, Manivannan S. Phytochemical analysis and evaluation of antimicrobial potential of *Senna alata* Linn. leaves extract. Asian Journal of Pharmaceutical and Clinical Research. 2016;9(2):253-257.

64. Mutha RE, Tatiya AU, Surana SJ. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. Future Journal of Pharmaceutical Sciences. 2021;7:25. https://doi.org/10.1186/s43094-020-00161-8.

65. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. Journal of Nutritional Science. 2016;5(e47):1-15. doi:10.1017/jns.2016.41.

66. Liu JB, Ding YS, Zhang Y, Chen JB, Cui BS, Bai JY. Anti-inflammatory hydrolyzable tannins from *Myricaria bracteata*. Journal of Natural Products. 2015;78(5):1015-25.

67. Lopez-Andres P, Luciano G, Vasta V, Gibson TM, Biondi L, Priolo A. Dietary quebracho tannins are not absorbed, but increase the antioxidant capacity of liver and plasma in sheep. British Journal of Nutrition. 2013;14:1-8.

68. Ajala OS, Jukov A, Ma CM. Hepatitis C virus inhibitory hydrolysable tannins from the fruits of *Terminalia chebula*. Fitoterapia. 2014;99:117–123.

69. Hoste HC, Martinez-Ortiz-De-Montellano F, Manolaraki S, Brunet N, OjedaRobertos I, Fourquaux JF. Direct and indirect effects of bioactive tannin rich tropical and temperate legumes against nematode infections. Veterinary Parasitology. 2012;186:18-27.

70. Liu XL, Hao YQ, Jin L, Xu ZJ, McAllister TA, Wang Y. Anti-*Escherichia coli* O157: H7 properties of purple prairie clover and sainfoin condensed tannins. Molecules. 2013;18:2183-99.

71. Moses T, Papadopoulou KK, Osbourn A. Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. Critical Reviews in Biochemistry and Molecular Biology. 2014;49(6):439-462. DOI: 10.3109/10409238.2014.953628.

72. Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X. Advances in pharmacological activities of terpenoids. Natural Product Communications. 2019;15(3):1–13. DOI: 10.1177/1934578X20903555.

73. Kayembe JS, Taba KM, Ntumba K, Kazadi TK. In vitro antimalarial activity of 11 terpenes isolated from *Ocimum gratissimum* and *Cassia alata* leaves. Screening of their binding affinity with Haemin. Journal of Plant Studies. 2012;1(2):168-172. doi:10.5539/jps. v1n2p168.

74. Goh MPY, Basri AM, Yasin H, Taha H, Ahmad N. Ethnobotanical review and pharmacological properties of selected medicinal plants in Brunei Darussalam: *Litsea elliptica, Dillenia*

suffruticosa, Dillenia excelsa, Aidia racemosa, Vitex pinnata and *Senna alata*. Asian Pacific Journal of Tropical Biomedicine. 2016;7(2):173–180. http://dx.doi.org/10.1016/j.apjtb.2016.11.026.

75. Okoko FJ. Antimicrobial activity of aqueous and ethanol leaf extracts of *Cassia alata* on some clinical bacteria isolates. International Research Journal of Microbiology. 2011;2(11):455-459.

76. Mosobil RB. *Comparative studies and synergistic effect of Senna alata, Ricinus communis leaves and Lannea barteri stembark extracts and their formulation on pathogenic bacteria and fungus (Thesis). University for Development Studies*. Tamale. 2018;1-165. *www.udsspace.uds.edu.gh*.

77. Donkor AM, Mosobil R, Suurbaar J. In vitro bacteriostatic and bactericidal activities of *Senna alata*, *Ricinus communis* and *Lannea barteri* extracts against wound and skin disease causing bacteria. Journal of Analytical & Pharmaceutical Research. 2016;3(1):11–12. DOI: 10.15406/japlr.2016.02.00046.

78. Doughari JH, Okafor B. Antimicrobial activity of *Senna alata* Linn. East and Central African Journal of Pharmaceutical Sciences. 2007;10:17-21.

79. Promgool T, Pancharoen O, Deachathai S. Antibacterial and antioxidative compounds from *Cassia alata* Linn. Songklanakarin Journal of Science and Technology. 2014;36(4):459-463.

80. Saeloh D, Visutthi M. Efficacy of Thai plant extracts for antibacterial and anti-biofilm activities against pathogenic bacteria. Antibiotics. 2021;10:1470. https://doi.org/10.3390/antibiotics10121470.