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Phytochemical analysis, kill kinetics and in vivo toxicity evaluation of crude methanolic extract of *Senna alata* (Fabaceae) (L.) Roxb. (Bark)

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ABSTRACT

The rapid emergence of anti-microbial resistance by pathogenic agents, has been a continuous challenge, which has called for the development of an alternative therapeutic agent. *Senna alata*, as a medicinal plant, has been extensively exploited in meeting the healthcare needs of the indigenous people in many parts of the world. This study was designed to analyze the phytochemical composition, determine the kill kinetics, and evaluate the toxicity potentials of *Senna alata* (leaf, flower, and bark), a promising plant source for novel antibiotics. Dried powdered samples of *S. alata* leaf, flower, and bark were extracted to obtain crude methanol extracts of each part. The extracts were analyzed for the presence of secondary metabolites. Based on the minimum inhibitory concentration (MIC) of the extract, the kill kinetics of the susceptible pathogens were determined at MIC, 2 x MIC, 4 x MIC, and 8 x MIC concentrations. The *in vivo* toxicity evaluation was carried out using Wistar rats, to determine the highest concentration of the extract that would kill half of the treated animals (LD_{50}). The animals were given various concentrations via oral gavage for 28 days for subacute toxicity, after which liver function and haematological parameters were analyzed for signs of toxicity. The phytochemical profile revealed the presence of glycosides, alkaloids, tannins, saponins, flavonoids, anthraquinones, volatile oils and phenol in all the plant parts, while carbohydrates were found only in the bark and leaf. The kill-kinetics showed a time- and concentration-dependent trend, when the log cfu/mL was plotted against time. The result of the acute toxicity gave an LD_{50} > 6000 mg/kg body weight. The sub-acute toxicity evaluation on the Wistar rats revealed that the liver function parameters, such as alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP) did not vary significantly ($p<0.05$) as well as the creatinine of the treated rats. Haematological parameters (white blood cells, red blood cells, and platelets) were also not affected. The results showed the potential of *S. alata* as a source of broad-spectrum antimicrobial compounds that can be administered without fear of toxicity or side effects.

INTRODUCTION

It has been over 80 years since the emergence of antimicrobial chemotherapy. An era that saw the revolutionization of the healthcare system across the world (Bebrone et al., 2010). Antimicrobials' use drastically reduced mortality from infections caused by many microorganisms, such as bacteria, fungi, and protozoa.

These infections formerly carried a high mortality rates, like puerperal sepsis, meningitis, tuberculosis, candidiasis, cholera, and amoebiasis. These diseases have become amenable to treatment with a range of potent antimicrobial agents (Okonkwo et al., 2008; Oyim et al., 2021).

Antimicrobial agents are chemicals or drugs used to inhibit the growth of or outrightly kill pathogenic microorganisms already established in the tissues of the host. The basic principle upon which chemotherapy is premised is selective toxicity.

The place of plants in history as a good source of anti-infective agents, with compounds that are highly effective in the fight against microbial infections, is well documented. Phytomedicines of plant origins have shown great promise in the treatment of intractable infectious diseases. Plants containing protuberances and related alkaloids, picrolematype indole, alkaloids, and garcinia flavones used in African traditional medicine, have been found to be active against a wide range of microorganisms (Oyim et al., 2021). Though soil microorganisms produce most of the clinically used antibiotics, higher plants have also been a source of antibiotics (Omar et al., 2000). Over the years, the use of plant materials to prevent and successfully treat infectious diseases has attracted the attention of scientists worldwide. Many investigations are being conducted on medicinal plants based on their ethnobotanical uses as indicated by the local populace, with the object of finding bioactive phytochemical constituents for the treatment and prevention of infectious diseases and other diseases of non-microbial etiology.

The upsurge of interest in natural products in the developed economies led to the development of several drugs and chemotherapeutic agents from plants as well as traditionally used rural herbal remedies. Plant-based bioactive compounds have great therapeutic potential as they can serve the purpose without the side effects often associated with synthetic drugs. They also allow little chance for the development of resistance by pathogens. The common notion in society and the medical community is that plant-based products are healthier, safer, and more reliable than synthetic products. Plants are believed to act generally to stimulate and supplement the body's healing forces. (Van Wyk and Wink, 2018).

Senna (Linn), a common name for a genus of the legume family with about 535 species is widely distributed in temperate and tropical areas. The genus contains shrubs and trees as well as annuals and perennial herbs. Many are ornamental. Some woody tropical species of the plant are among the most important flowering trees. Some species of *Senna* are ecologically important for being host to some butterfly species, for example, the cloudless sulfur butterflies (Sandford, 2000).

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The economic importance of the genus includes its uses in the chemical industries, such as the cosmetic industries, it also serves as a source of food, such as vegetables (Abdulwaliyu et al., 2018). *Senna alata* is a tropical shrub, having yellow flowers and large leaves whose juice is used as a cure for ringworm and poisonous bites (Figure 1).

S. alata has been identified as a medicinal plant used in the cure of many ailments and diseases in many parts of the world. The leaves are taken internally as an effective laxative (Abdulwaliyu et al., 2018). In Ghana and Cote D'Ivore, the decoction of the leaves, flowers, bark, and root of the plant is used to treat diarrhea, dysentery, and other gastrointestinal problems. The macerated juices of young fresh leaves are used to treat eye infections and parasitic diseases (Oladeji et al., 2020). The decoction of stem bark and roots is used to treat urinary tract infections (UTI), bronchitis, and asthma (Essiett and Bassey, 2013). In some places in Northern Nigeria, especially in Adamawa and Taraba States, the root, stem, and leaves are used by practitioners of herbal medicine to treat burns, wounds, and skin diseases such as purities, eczema, and itching (Oladeji et al., 2020). An infusion of flowers is used for asthma and bronchitis (Essiett and Bassey, 2013). A decoction of flowers is also used as an expectorant in bronchitis and dysphoria as an astringent and as a mouthwash in stomatitis.

This research was designed to provide a scientific framework to ascertain and put these numerous claims in the proper perspective, establishing a verifiable backup to the antimicrobial claims and toxicity status of *S. alata*.

MATERIALS AND METHODS

Collection and authentication of plant material

The fresh mature leaves, flowers, and bark of *S. alata* were collected from the Botanical Garden of the University of Ibadan, Ibadan, Nigeria. The leaves, flowers, and bark were identified in the University of Ibadan Herbarium, where a voucher specimen was also deposited with the number, UIH22376.

Preparation and extraction of plant material

Leaves, flowers, and bark of *S. alata* were air-dried separately for about three weeks after which they were crisp-dried in the oven at 40ºC for four days. The plant parts were pulverized with a grinding mill separately and stored in an airtight container for further use. One hundred grams of each of the powdered plant parts were extracted with 100ml of 70% methanol, ether, xylene, and distilled water in a bowl and covered for 3 days with frequent stirring or agitation with a mixer. The resulting solution in each case was filtered off and concentrated to dryness

Figure 1. Leaves and flowers of *Senna alata*. (photograph by H. Joseph) (Hennebelle et al., 2009).

with the rotary evaporator. The extracts were stored in sealed labeled containers and kept in the refrigerator at 4°C, until needed.

pH of the extracts

The pH of the extracts was determined using a pH meter according to the method of Altemimi et al. (2017).

Yield of extracts

The percentage yield was calculated as follows:

$$\%\,Yield = \frac{initial\ weight\ of\ plant\ part - final\ weight\ of\ plant\ part}{initail\ weight\ of\ plant\ part} X100$$

Concentration of extracts

The concentration of the various plant parts was calculated as follows:

$$Concentration = \frac{Weight\ of\ plant\ parts}{Volume\ of\ solvents}\ X\ 1000$$

Phytochemical screening

The phytochemical screening was carried out to determine the presence of active constituents present in the plant extracts. The phytochemical assay for alkaloids, flavonoids, glycosides, tannins, saponins, and anthraquinones was carried out according to established procedures (Essiett and Bassey, 2013).

Test for alkaloid

About 0.5 g of each of the plant parts (leaf, flower, and bark) extracts were stirred with 0.5 ml of dilute hydrochloric acid in a separate test tube in a steam bath (water bath) for two minutes and were filtered; the filtrates were treated with a few drops of Mayer's reagent and 1ml each of the filtrates was also treated with Draggendoff's reagent and 1 ml was treated with Wagner's reagent. Orange precipitates indicate the presence of alkaloids. (Shoaib et al., 2016).

Identification of flavonoids

Sodium hydroxide test: to 0.2 ml of each of the leaf, flower, and bark extracts in a separate test tube, 0.2 ml of Sodium hydroxide was added and gently shaken. A yellow solution that later turns colourless indicates the presence of flavonoids. (Górniak et al., 2019).

Test for tannins

Half of a gram (0.5 g), of each of the extracts; leaf, flower,

and bark were stirred with 10 ml of alcohol and distilled water respectively in a separate test tube and were filtered, ferric chloride reagent was added to the filtrates, and 1ml of the extracts was also treated with Bromine water. Green precipitates were taken as evidence of the presence of tannins (Raymond et al., 2011).

Test for glycosides

Each extract was hydrolyzed with diluted hydrochloric acid (HCl) solution and neutralized with sodium hydroxide (NaOH) solution. A drop of Fehling's solution A and B were added to each of the extracts. Red precipitate in the extract indicated the presence of glycosides (Tagousop et al., 2018).

Test for saponins

The ability of Saponins to produce frothing in an aqueous solution and to haemolyse red blood cells was used as screening tests for this compound (Jiang et al., 2018).

Frothing test: A little portion of the extracts was shaken with hot distilled water in a test tube for about five minutes.

Haemolysis test: A given quantity, 0.2 g, of the extracts each was dissolved in 10 ml of warm water and five drops of concentrated sodium chloride (NaCl) which was isotonic with blood serum was added to each test tube and were inverted gently to mix the content.

Test for reducing compounds: A mass of 0.5 g of each extract was diluted with 1 ml of water, and 1 ml of Fehling's solution A and B was added and then put in the hot water bath to heat.

Test for anthraquinones

Borntrager's test was used for the detection of anthraquinones: About 5 ml of each extract was shaken with 10 ml of benzene, filtered and 5 ml of 10% ammonia (NH_3) solution was added and shaken. The presence of pink precipitate indicates anthraquinones (Gul et al., 2017).

Test for phlorotannins

About 1 g of the powdered *S. alata* (leaf, flower, and bark), was boiled in water and filtered. The resistant extract was dried, and the aqueous extract was boiled with 1 litre of aqueous HCl. The formation of a red precipitate indicates the presence of phlorotannins (Karthikeyan and Vidya, 2019).

The kill-kinetics

The most active part was used to challenge the most susceptible organisms at MIC, 2X MIC, 4 X MIC, and 8 X MIC concentrations. Each concentration was mixed with a fresh overnight culture of the test organisms in a 1:1 ratio and vortexed. At each time interval of 0, 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, and 24 h, 0.2 ml was withdrawn and plated on a sterile agar plate. The plates were incubated at 37°C for 24 h (for bacteria), and 25°C for 48 h (for fungi). The log of the cell forming unit per milliliter was plotted against time, to determine the trend in the antimicrobial activity of *S. alata*.

Toxicity evaluation

The bioassay was conducted according to Organization for Economic Cooperation and Development (OECD) test guideline 407 with Good Laboratory Practice (GLP) application, and WHO research guidelines for evaluating the safety and efficacy of herbal medicines. Wistar albino rats were used for this study. Two to five months old, sexually matured Wistar albino rats, weighing between 120 to 150 g, were obtained from the animal laboratory of the Department of Biochemistry, University of Ibadan, Ibadan. All the animals were kept under standard environmental conditions. The rats had free access to water and food in situ. The principles of laboratory animal care were followed, and the Departmental ethical committee approved the use of the rats and the study design.

Acute toxicity: The acute toxicity study was carried out using Lorke`s method with some modifications: Three groups (A, B, and C) of three Wistar rats each, were administered three different concentrations of the crude extract of *S. alata* 10, 100, 1000 mg/kg body weight, respectively. The rats were monitored and observed for any sign of toxicity, change in behaviours, body temperature, etc. Based on the observations, another set of six rats, two rats in three groups, were administered with 2000, 2500, and 3000 mg/kg body weight, respectively. They were also observed for fourteen days, for signs of toxicity. Based on further observations, another two groups of two rats each were finally administered with 4000 and 6000 mg/kg body weight. In each case, the rats were observed for 14 days for signs of toxicity. In the end, the rats were sacrificed and the organs, such as the liver, kidney, pancreas, and gut, were harvested and macroscopically examined for signs of toxicity.

The LD_{50} was calculated as the dose that was able to cause the death of half (50%) of the number of rats in the

Table 1. Phytochemical analysis of leaf, flower, and bark of *Senna alata*.

Compounds	Leaf	Flower	Bark
Volatile oil	+	+	+
Glycoside	+	+	+
Alkaloids	+	+	+
Saponins	+	+	+
Anthraquinones	+	+	+
Flavonoid	+	+	+
Tannins	+	+	+
Carbohydrate	+	-	+
Protein	+	-	-

Note: + = present; - = absent.

study (WHO, 1992).

Sub-acute toxicity: The Wistar albino rats received intra-gastric gavages of the plant extract doses of 500 mg/kg and 1000 mg/kg every 48 h and the control group was given distilled water for 28 days. The behaviour and body weight were observed daily while the food and water intake were monitored. After 28 days the surviving rats were given no food overnight. The rats were sacrificed by decapitation and blood sample were collected into ethylene EDTA bottles for haematological analysis and lithium heparin bottles for biochemical analysis (WHO, 1993).

Statistical analysis

Values are expressed as mean ± standard deviation. The statistical analysis of variance was done by ANOVA; followed by the student-Newman kerls, test, and Duncan posthoc test. $p < 0.05$ was considered as the level of statistical significance.

RESULTS AND DISCUSSION

As part of the different approaches to the therapeutic assay of plants, herbal drugs are in great demand due to their varied pharmacological properties and as a source of novel bioactive compounds. As a part of natural plant products-based antibiotic drug discovery, the *S. alata* plant (Leaf, Flower, and Bark) was selected for evaluation in this study.

As expected, *S. alata* with its many claimed medicinal uses, has a significant amount of phenolics, together with other secondary metabolites (Table 1) (Kasiama et al 2022). However, it is believed that its phytochemicals, that is, the alkaloids are responsible in synergy with the phenolics, for the overall medicinal and therapeutic values of *S. alata* (Khurm et al 2021) The kill-kinetics which were carried out at MIC, 2 x MIC, 4 x MIC, and 8 x MIC concentrations, showed a time and concentration-dependent trend in the log CFU/ml reduction on the test organisms (Figures 2 and 3).This trend agrees with Tamo et al. (2016), who observed similar trend on extracts of the same plant against *E. coli*. In each case, the crude methanolic extract of *S. alata*, compared very well with the positive controls used. This shows that if the bioactive compounds are isolated, via bioassay-guided fractionation, the resultant drug would be as efficacious or even more effective than the drugs in use (Mulat et al 2020).

With an LD_{50} > 6000 mg/kg body weight, as revealed by the acute toxicity evaluation, *S. alata* is safe and has no negative side-effect (Table 2). This is in total agreement with Roy et al. (2016), who carried out a similar toxicity study on Swiss albino mice, using ethanolic leaf extract of *S. alata*, and recorded no mortality or any side effects. Similarly, Ugbogu et al. (2016), declared the aqueous stem extract of *S. alata* safe after administering up to 10 g/kg body weight on Wistar rats without any observable side effects or mortality. In the sub-acute toxicity evaluation, after 28 days of oral administration of methanolic extract of *S. alata*, Wistar rats treated with doses of 500 mg/kg and 1000 mg/kg body weight showed some behavioral changes such as sluggishness in their response to external stimuli and reduction in mobility, 60 min. after oral administration which later subsided after 24 h. This may be due to mild suppression of metabolism, and or hormonal functionalities (Omoniwa et al., 2022). There was a progressive increase in weight, which is significantly different in the treatment groups when compared to the control group (Table 3). In a review, Lim, (2014), found a contrary report. He presented the study of Sodipo et al. (1998), in which administration of aqueous leaf extract of *S. alata*, induced some adverse effects in Wistar rats. Such effects include loss of appetite, emaciation, and loss of weight. But Pieme et al. (2006), observed no side effects

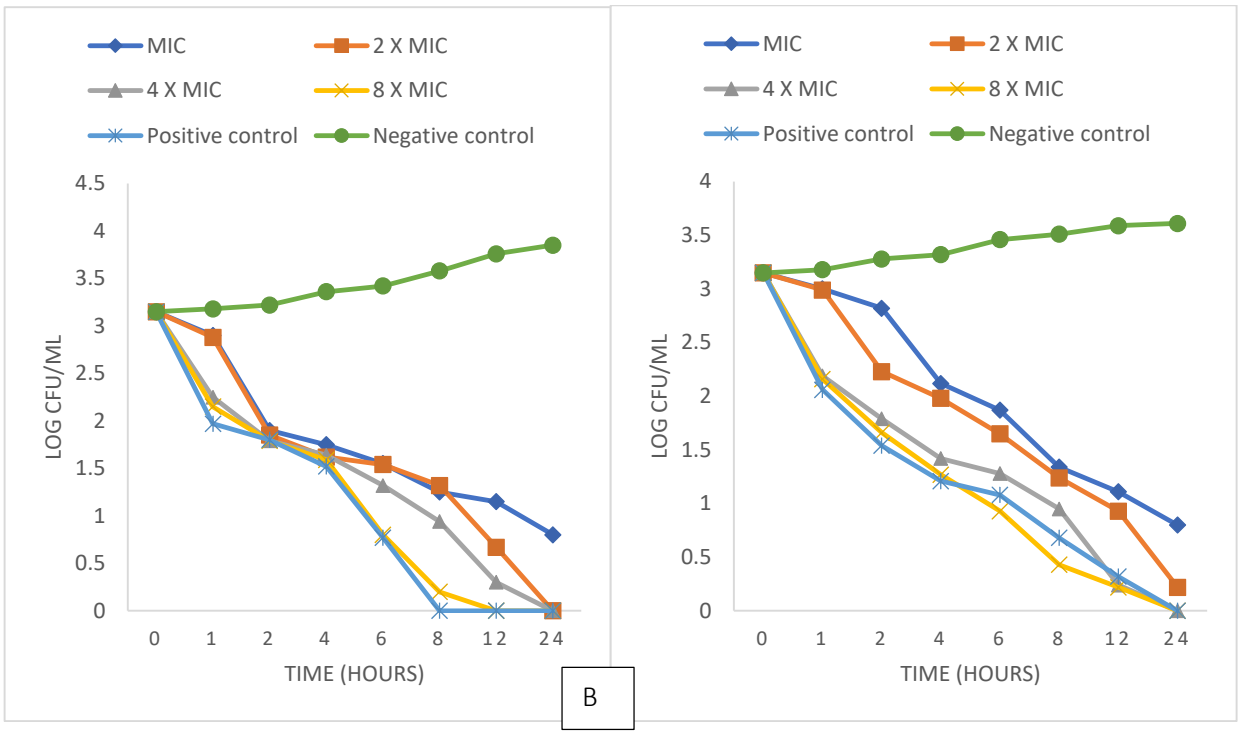

Figure 2. The kill-kinetics of the crude methanolic extract of *Senna alata* (Bark) on (A) *Salmonella typhi* and (B*) E. coli*.

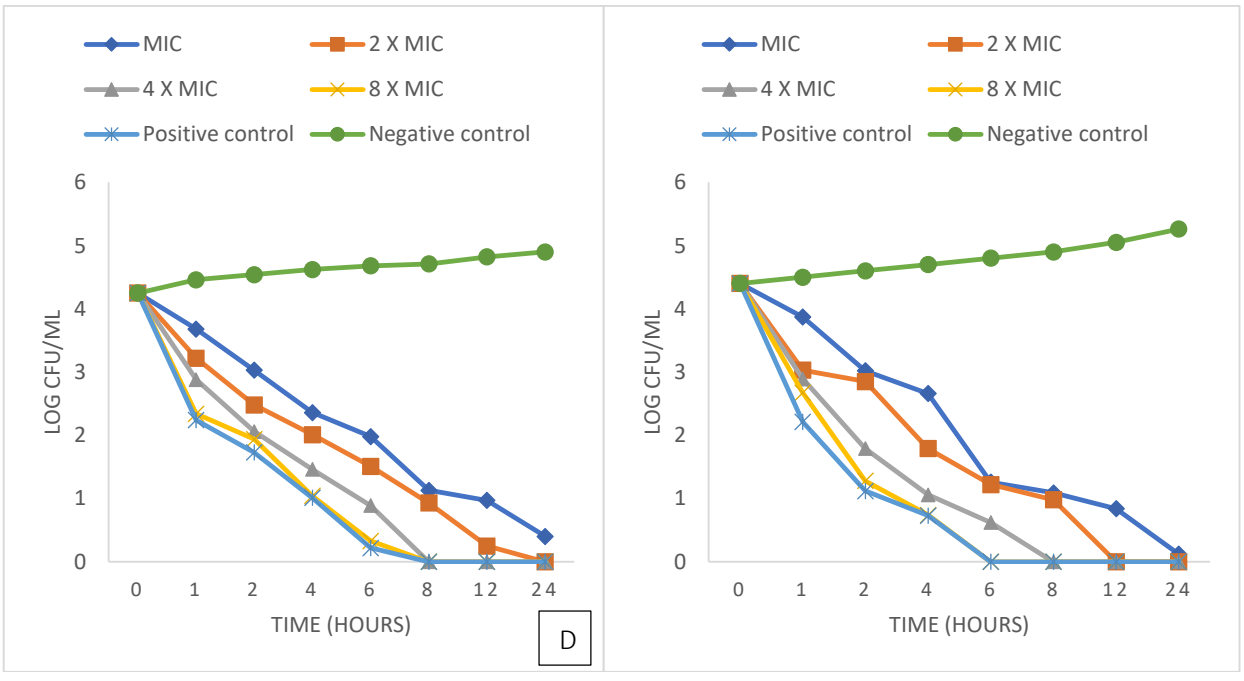

Figure 3. The kill-kinetics of the crude methanolic extract of *Senna alata* on (C) *Candida albicans* and (D) *Aspergillus niger*.

Table 2. The result of the minimum inhibitory and bactericidal/fungicidal concentration (MIC, MBC/MFC), of the crude methanol extract of *Senna alata*.

Organisms	MIC (µg/mL)	MBC/MFC (µg/mL)
Bacillus subtilis	25.0	50.0
Escherichia coli	25.0	50.0
Staphylococcus aureus	25.0	50.0
Pseudomonas aeruginosa	25.0	50.0
Salmonella typhi	12.5	25.0
Candida albicans	12.5	25.0
Aspergillus niger	12.5	25.0

Table 3. Result of acute toxicity of crude extract *Senna alata* on Wistar rats.

Phase 1/Doses (mg/kg bwt)	Number of mice	Average weight of the mice	Number of deaths (x3)	Percentage mortality	Log of dose
10	3	21.83	0	0	1
100	3	22.53	0	0	2
1000	3	21.67	0	0	3
Control	3	23.02	0	0	-
Phase 2					
2000	2	23.63	0	0	3.18
2500	2	23.12	0	0	3.30
3000	2	22.87	0	0	3.40
Control	2	23.62	0	0	-
Phase 3					
4000	2	23.33	0	0	3.48
6000	2	24.01	0	0	3.78
Control	2	23.81	0	0	-

Table 4. Body weight of Wistar rats in sub-acute toxicity after 28 days of oral administration of methanolic extracts of *Senna alata* (Bark).

	Bodyweight (kg)		
Female	**Day 1**	**Day 28**	**Average weight gained**
Control	143.59 ±7.37[a]	169.24±8.01 [a]	26.63± 5.30[a]
500mg/kg	124.12 ± 18.65 [b]	156.36 ±14.85[b]	32.27± 16.15[b]
1000mg/kg	132.81± 11.36 [c]	157.36 ±15.75[c]	24.55± 6.51 [c]
Male			
Control	126.34± 20.53[a]	178.26± 29.26[a]	49.72± 16.55 [a]
500mg/kg	147.45± 6.82 [b]	176.26± 8.46[b]	29.45± 12.72 [b]
1000mg/kg	161.42± 15.93[c]	162.38± 6.35[c]	33.93± 16.65 [c]

Values are expressed as means ± SD. Parameters are expressed as range values n = 5. Means with the same letter superscript within a column in each group (sexes) are not significantly different (P < 0.05).

in a similar study. Table 4 on the other hand, shows the haematological status of rats treated with oral administration of methanolic extracts of *S. alata*. No significant variation was observed for red blood cells

Table 5. Haematological values of rats` toxicity after 28 days of oral administration of methanolic extracts of *Senna alata* (Bark).

Female	WBC (10 /mm)	RBC (10 /mm)	Platelets (10 /mm)
Control	1.80± 0.56 [a]	3.29± 1.64 [a]	1.83± 0.34 [a]
500mg/kg	2.18± 0.54 [b]	3.35± 1.39 [a]	2.87± 0.74 [b]
1000mg/kg	2.33± 0.56 [b]	4.50± 1.09 [a]	3.05± 0.13 [c]
Male			
Control	2.60± 0.70 [a]	3.19± 0.43 [a]	1.18± 0.31 [a]
500mg/kg	2.73± 1.34 [a]	3.36± 0.34 [a]	1.31± 0.34 [a]
1000mg/kg	2.84± 0.36 [a]	3.45± 0.67 [a]	3.45± 0.67 [b]

Values are expressed as mean ± SD. Mean with the same letter superscript within a column in each group (sexes) are not significantly different (P < 0.05) according to Duncan's multiple test range.

Table 6. Biochemical values of rats in toxicity evaluation after 28 days of oral administration of methanolic extracts of *Senna alata*.

Parameters	Control	500 mg/kg	1000 mg/kg
Female			
ALT (U/L)	40.01±0.10[a]	40.06±0.25 [a]	38.55±0.16 [a]
AST(U/L)	81.40±0.04 [a]	80.50±0.12 [a]	83.60±0.05 [a]
ALP (U/L)	44.04±1.50 [a]	42.48±1.40 [a]	43.49±1.00 [a]
Creatinine(mg/dl)	14.60±1.00 [a]	13.55±0.40 [a]	16.34±0.50 [ab]
Male			
ALT (U/L)	42.01±0.10 [a]	42.06±0.25 [a]	38.75±0.16 [a]
AST (U/L)	80.60±0.14 [a]	78.88±0.26[a]	80.40±0.05 [a]
ALP (U/L)	44.56±0.70 [a]	44.56±0.45 [a]	44.70±0.50 [a]
Creatinine (mg/dl)	14.20±0.40 [a]	13.60±0.70 [a]	17.40±1.00 [b]

Values are expressed as means ± SD. Parameters are expressed as range values, n = 5. Mean with the same letter superscript within a row across the groups (sexes) are not significantly different (P < 0.05) according to Duncan multiple test range.

(RBC), and white blood cells (WBC). This was in tandem with the report of Pieme et al. (2006). However, the variation was significantly different for platelets. A slight increase in values was observed in RBC and WBC in the treatment groups compared to the control group.

Results of liver function analysis are shown in Table 5. There were no significant changes in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and creatinine. Again, this finding aligned with that of Pieme et al. (2006).

Conclusion

The discovery of a potent herbal therapy that is safe and active against bacterial and fungal infections would mark a milestone in the advancement of the world's healthcare system. The result of this study shows that in addition to its appreciable antimicrobial activity, methanolic extracts of *S. alata* when taken for a specific period of time may not cause any negative side-effects relating to liver functions or haematology.

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