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ORIGINAL ARTICLE

Antimicrobial and toxicological evaluation of ethanol leaf extract of *Salacia lehmbachii*

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ABSTRACT

The leaves of *Salacia lehmbachii* are used ethnomedically across Africa for the treatment of different diseases its antimicrobial activity as well as toxicological profile were evaluated. Antimicrobial activity against clinical strains of *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococus aureus*, *Shigella species*, *Eschericha coli* and *Proteus mirabilis* were compared with Gentamycin. Toxicological investigation was determined by administering 100 mg/kg, 200 mg/kg and 400 mg/kg of the ethanol leaf extract to male Wistar rats for 21 days with distilled water as control. Hematological and biochemical parameters as well as the vital organs were examined. The ethanol extract inhibited the growth of *P. aeruginosa*, *S. typhi*, *S. aureus*, *Shigella species*, *E. coli* and *P. mirabilis* to varying extents. The LD_{50} in rats was greater than 5000 mg/kg. Toxicological evaluation of the extract did not produce any significant effect on hematological and biochemical parameters and vital organs in rats. *S. lehmbachii* ethanol leaf extract did not demonstrate antimicrobial activity against selected microorganisms. Neither did it show any non-toxic effect on the parameters investigated in rats. Thus the extract can be considered safe when administered orally.

KEY WORDS: *Salacia lehmbachii*; leaf extract; antimicrobial activity; safety assessment

Introduction

Medicinal plants are widely used in treating and preventing specific diseases and are known to play an important role in health care. The demand for herbal medicines for disease treatment is on the increase due to their efficacy, availability and affordability. Herbal medicines account for 70 to 80 percent of the health care needs of the world's population, especially for millions of people in major areas of developing countries (WHO, 2005; WHO, 2013). In Nigeria, a vast range of medicinal plants have been used for the treatment of different ailments without scientific investigation of their therapeutic potentials. In the last few decades, researchers have evaluated numerous medicinal plants for their bioactive and pharmacological properties. These efforts are being continually taken to investigate the advantages of herbal medicine in modern science with the aim to adopt effectively potential medical practice and prevent harmful effects (Abere *et al.*, 2010).

Salacia lehmbachii, locally known by the Efik people of South south Nigeria as 'Ebananganang', is a shrub-like small tree of about three meters high belonging to the family Celastraceae. It is found mostly in the tropical rain forest of Central, West and East Africa (Corstiaen & Sosef, 2007). The leaves are seasonally evergreen, firm and hard to slice. There are numerous therapeutic applications of *S. lehmbachii* justifying its folkloric background. The leaves are used for their antipyretic, anti-diarrheal, antimotility and anti-ulcer properties (Essien *et al.*, 2015; Essien *et al.*, 2016; Essien *et al.*, 2017).

The aim of the present study was to evaluate the possible *in vitro* antimicrobial activity and toxicological profiles of the ethanol leaf extract of *S. lehmbachii*, by assessing its physical, hematological and biochemical effects in rats.

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Materials and methods

Collection and preparation of plant materials

The leaves of *Salacia lehmbachii* Loes were collected in November 2013 from a farm land at Oruk Otong village in the Ukanafun Local Government Area of Akwa Ibom State, Nigeria. The leaves were identified and confirmed by a taxonomist at the Department of Botany, University of Calabar, where voucher specimen number (Unical/H/688) is maintained for future reference. The leaves were cleaned, air-dried at room temperature and powdered with the help of mortar and pestle.

Preparation of extract

Five hundred grams of the dried and powdered leaves were extracted in ethanol by soxhlet extraction. The extract was filtered and dried on a water bath at a temperature of 45 °C. The yield was 12.5% on dried weight. The extract was subsequently reconstituted in normal saline for routine use during the study.

Phytochemical studies

The phytochemical screening of ethanol leaf extract of *S. lehmbachii* was carried out for various secondary metabolites such as tannins (ferric chloride test), alkaloids (Mayer's and Draggendorff reagents), saponins (Froth test), steroids (Liebermann-Burchard test), terpenoids (Salkowski test), flavonoids (ammonia and sulphoric acid test) and anthraquinones (Borntrager's test) (Oloyede, 2005; Ajayi, 2008).

Test strains

The ethanol leaf extract was tested for possible antimicrobial activity using six pathogenic bacteria, *i.e. Pseudomonas aeruginosa, Salmonella typhi, Staphylococus aureus, Shigella species, Escherichia coli* and *Proteus mirabilis*. All clinical isolates were obtained from the Microbiology Department, University College Hospital, Calabar, Nigeria.

Animals

Sixty male Wistar rats (170–200 g) were obtained from the Animal House of the Pharmacology Department, Faculty of Basic Medical Sciences, University of Calabar. The rats had free access to rat chow and water *ad libitum*. All the animals were kept under standard environmental conditions and were handled according to international guidelines on the Use and Handling of Experimental Animals (NIH, 1985). Approval for the study was obtained from the Research and Ethical Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria.

Antimicrobial activity

The ethanol leaf extract was tested for possible antimicrobial activity using six pathogenic bacteria, viz. *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Shigella species, Eschericha coli* and *Proteus mirabilis*. All clinical isolates were obtained from the Microbiology Department, University College Hospital, Calabar, Nigeria. Purity plates of microbial isolates were obtained by culturing on respective selective media. Biochemical tests were performed to confirm the identity of the organisms used in this study. Discrete colonies of fresh cultures from different bacterial isolates were carefully mixed in 5 ml nutrient broth and incubated for 24 h at 37 °C prior to antimicrobial susceptibility testing. The antimicrobial activity of the ethanol leaf extract of *S. lehmbachii* was assayed using agar well diffusion method of Kumar *et al.* (2012) and Akuodor *et al.* (2011). Broth cultures, 0.5 ml, of the isolate containing 10^5 cfu/ml of organism were properly introduced into sterile petri-dish and 15 ml of Muller Hinton agar was added. The content was thoroughly mixed and allowed to solidify. Holes were bored in the plates with a standard sterile cork borer of 8 mm in diameter and the ethanol leaf extract reconstituted in distilled water at different concentrations of 50, 100, 150, 200 and 250 µg/ml, solvent blank and standard antibiotic (gentamycin) were applied in each of the wells in the culture plates. The studies were performed in duplicate and plates allowed to stand for 2 h for pre-diffusion of the extract and incubated at 37 °C for 24 h. Thereafter, the diameters of the zones of inhibition were measured against the test organisms. Gentamycin disc (30 µg/ml) was used as a reference standard, while distilled water was used as control. The growth was compared with the reference as well as the control.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the ethanol leaf extract of *S. lehmbachii* was determined as described by Andrews (2001). Six sterile tubes were arranged in a test tube rack and 0.5 ml of sterile nutrient broth was transferred into each test tube. Thereafter, there was a serial dilution of the extract to obtain concentrations of 250, 200, 150, 100 and 50 µg/ml. The 0.5 ml test tube organism was taken and transferred into each test tube containing the mixture of the broth and the extract and then incubated at 37 °C for 24 h. The MIC was recorded as the least concentration of the leaf extract that showed no visible growth of the test organism.

Toxicological evaluation

Acute toxicity test

Male Wistar rats weighing 170–200 g (4 rats per group) were orally treated with the ethanol extract of *S. lehmbachii* at doses of 100 mg/kg, 600 mg/kg, 1000 mg/kg, 2000 mg/kg, 3000 mg/kg and 5000 mg/kg. Each group of rats was placed in a test cage for a 45-min adaptation period before oral administration of the extract using an orogastric cannula. The animals were observed for 72 h. Toxicological features and lethality were noted for each group at the end of the observation period (OECD, 2002). The rats were further monitored for seven days.

Subacute toxicity test

A total of twenty-four male Wistar rats were weighed and divided into 4 groups of 6 rats each. The rats were orally treated daily with *S. lehmbachii* leaf extract at doses of 100, 200 and 400 mg/kg and distilled water for 21 consecutive

days. At the end of the treatment period, the rats were sacrificed under inhaled chloroform anesthesia (OECD, 2008). Blood samples were collected through cardiac puncture into EDTA and nonheparinized containers for hematological and biochemical analysis. The organs were excised, weighed and macroscopically examined.

Statistical analysis

The data are represented as mean ± SEM. Statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Tukey post hoc test, where $p<0.05$ was statistically considered significant.

Table 1. Inhibition zones of ethanol leaf extract of *S. lehmbachii* and Gentamycin.

Organisms	Inhibition zones (mm)	
	Extract	Gentamycin
Pseudomonas aeruginosa	17	25
Salmonella typhi	15	22
Staphylococus aureus	18	26
Eschericha coli	14	20
Shigella species	16	5
Proteus mirabilis	19	31

Table 2. Minimum inhibitory concentration (MIC) of ethanol leaf extract of *S. lehmbachii*.

Organisms	Minimum inhibitory concentration (µg/ml)
Pseudomonas aeruginosa	30
Salmonella typhi	31
Staphylococus aureus	29
Eschericha coli	60
Shigella species	33
Proteus mirabilis	35

Results

Phytochemical screening

Phytochemical analysis of the leaves of *S. lehmbachii* revealed the presence of alkaloids, saponins, tannins, terpenoides, flavonoids, phenols, steroids and anthraquinones

Antimicrobial activity

The antimicrobial activity of the ethanol extract of *S. lehmbachii* leaf was assessed to determine its efficacy against pathogenic bacterial organisms. The extract exhibited strong activity against all bacterial strains tested. Of all the microorganisms tested, the leaf extract showed the highest activity against *P. aeruginosa, S. aureus* and *P. mirabilis*. Gentamycin at a concentration of 30 µg/ml fully inhibited the growth of all the bacterial strains except *Shigella spp* with minimal inhibition (Table 1). Minimum inhibitory concentration (MIC) is shown in Table 2.

Acute toxicity study

In acute toxicity test, the ethanol leaf extract of *S. lehmbachii* did not produce any lethality in rats up to the oral dose level of 5000 mg/kg, as observed for 4 h after treatment. When monitored further for seven days, there were no visible signs of toxicity and mortality seen, hence the LD_{50} value is greater than 5000 mg/kg.

Hematological parameters

The data in Table 3 show the effects of ethanol leaf extract of *S. lehmbachii* on hematological parameters. At all doses examined, the extract showed non-significant changes in hematological parameters in rats when administered daily for 21 days.

Biochemical parameters

Table 4 shows the effects of the ethanol leaf extract of *S. lehmbachii* on liver enzymes, urea and creatinine in rats.

Table 3. Effects of ethanol leaf extract of *S. lehmbachii* on hematological parameters in rats.

Parameter	Control	100 mg/kg	200 mg/kg	400 mg/kg
White blood cell (x 10^9/L)	6.10 ± 0.52	6.16 ± 0.20	5.88 ± 0.69	5.91 ± 0.87
Pack cell volume (%)	41.62 ± 1.20	43.70 ± 1.19	42.62 ± 1.44	40.95 ± 1.45
Red blood cell (x10^{12}/L)	6.55 ± 0.31	7.66 ± 0.21	7.33 ± 0.61	7.50 ± 0.55
Hemoglobin (g/dL)	13.32 ± 0.54	13.46 ± 0.19	13.62 ± 0.18	13.73 ± 16
Mean corpuscular volume (fl)	20.35 ± 0.63	22.65 ± 1.20	21.48 ± 1.59	23.10 ± 1.08
Mean cell hemoglobin (pg)	34.44 ± 0.43	33.70 ± 0.21	35.19 ± 0.22	34.22 ± 0.46
Mean cell hemoglobin concentration (g/dl)	31.29 ± 0.51	30.54 ± 0.22	30.60 ± 0.59	31.23 ± 0.22
Platelet (x 10^9/L)	628.17 ± 11.22	633.18 ± 21.12	630.21 ± 31.48	632.45 ± 33.50
Neutrophils (%)	33.30 ± 1.90	34.85 ± 1.64	32.11 ± 2.49	36.02 ± 2.68*
Lymphocytes (%)	60.56 ± 2.44	62.39 ± 2.77	61.33 ± 2.67	60.30 ± 1.68
Monocytes (%)	1.10 ± 0.32	1.23 ± 0.28	1.18 ± 0.40	1.20 ± 0.36
Eosinophils (%)	0.11 ± 0.20	0.18 ± 0.21	0.30 ± 0.32	0.33 ± 0.24

Results are presented as means ± SEM (n=6).

The extract did not elicit any significant effect on various biochemical parameters, urea and creatinine.

Effect on vital organs

S. lehmbachii ethanol leaf extract did not produce any significant effect on the weight of different vital organs from rats after daily administration for 21 days (Table 5). All organs were macroscopically comparable to the control.

Discussion

Medicinal plants contain highly active pharmacological constituents which have been the basis for the treatment of different ailments (Wang *et al.*, 2014). Phytochemical analysis of the leaf extract revealed the presence of alkaloids, saponins, tannins, terpenoides, flavonoids, phenols, steroids and anthraquinones. Initial screening of medicinal plants secondary metabolites assists the detection of bioactive compound, which initiates drug discovery and development (Aziz, 2015). The extract displayed strong antimicrobial potential against the pathogenic microorganisms tested.

The purpose of an acute toxicity study is to ascertain the nature and level of the adverse reactions to either a single dose or overdose of the agent (Klein, 1996). *S. lehmbachii* did not produce any toxic effect in rats when administered orally up to 5000 mg/kg in divided doses. It is an indication of the low toxicity of the extract (Dietrich, 1983), hence the ethanol leaf extract can be considered to be non-toxic acutely. The high safety profile obtained may be responsible for its wide spread use in different ethnotherapeutic interventions.

The purpose of the subacute toxicity studies is to know the organs which are likely to be susceptible to toxicity by herbal agents. The tests provide information on target organ toxicity and are carried out to identify the extent of non-observable adverse effect (National Research Council, 2006). Subacute investigation can also help to ascertain appropriate dose regimens for long-term studies.

Evaluation of hematological parameters can be used to explain blood relating functions of a plant extract or its products (Yakubu *et al.*, 2007). More so, the analysis is essential to risk investigation as changes in the hematological system have a marked predictive value for human toxicity when data are translated from animal tests. A hemogram was carried out for all the *S. lehmbachii* leaf extracts treated and control rats. The results did not indicate significant effects. The non-significant effect of the extract on the hematological parameters analyzed showed that the extract did not affect the erythropoiesis, morphology or osmotic fragility of the red blood cells (Guyton & Hall, 2000). White blood cells are the first line of cellular defence responding to infectious agents, tissue injury and inflammatory processes. In addition, the non-significant changes observed in neutrophils, lymphocytes, monocytes and eosinophils in the leaf extract further confirmed the findings.

The clinical chemistry analysis was carried out to evaluate the possible alterations in hepatic and renal functions of the extract treated rats compared to controls. *S. lehmbachii* ethanol leaf extract showed no significant changes in any of the biochemical parameters in the 21-day treatment period. Liver and kidney function analysis is highly essential in the toxicity evaluation of drugs and plant extracts as they are both necessary for the survival

Table 4. Effects of ethanol leaf extract of *S. lehmbachii* on biochemical parameters in rats.

Parameter	Control	100 mg/kg	200 mg/kg	400 mg/kg
Aspatate transaminase (μ/L)	33.18 ± 3.63	33.25 ± 3.50	34.07 ± 3.41	34.11 ± 3.29
Alanine transaminase (μ/L)	23.30 ± 2.32	23.44 ± 2.30	23.50 ± 2.60	23.68 ± 2.72
Alkaline phosphatase (μ/L)	55.28 ± 2.85	56.30 ± 2.61	57.06 ± 2.29	57.08 ± 1.46
Urea (mmol/L)	15.60 ± 3.96	15.66 ± 2.80	16.33 ± 3.40	16.40 ± 3.23
Creatinine (μmol/L)	1.23 ± 0.31	1.48 ± 0.22	1.43 ± 0.43	1.45 ± 0.28

Results are presented as the means ± SEM (n=6).

Table 5. Effect of ethanol leaf extract of *S. lehmbachii* on vital organ weights in rats.

Organs	Control	100 mg/kg	200 mg/kg	400 mg/kg
Heart	0.33 ± 0.13	0.31 ± 0.23	0.32 ± 0.04	0.33 ± 0.04
Lungs	0.60 ± 0.15	0.61 ± 0.10	0.59 ± 0.06	0.61 ± 0.14
Kidneys	0.42 ± 0.04	0.42 ± 0.02	0.41 ± 0.05	0.42 ± 0.04
Liver	3.54 ± 0.11	3.49 ± 0.18	3.55 ± 0.22	3.48 ± 0.25
Spleen	0.40 ± 0.03	0.43 ± 0.03	0.42 ± 0.02	0.41 ± 0.03
Testes	1.32 ± 0.25	1.33 ± 0.09	1.32 ± 0.06	1.33 ± 0.03

Results are presented as means ± SEM (n=6).

of an organism (Olorunnisola *et al.*, 2012). High levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are seen in liver diseases or hepatotoxicity (Brautbar & William, 2002). The non-significant changes in AST, ALT and ALP at all doses studied suggest that subacute administration of *S. lehmbachii* leaf extract does not affect the hepatocyte function in rats. Equally, no significant increase in urea and creatinine in subacute administration of *S. lehmbachii* leaf extract was observed when compared to the control group. Any rise in urea and creatinine levels is only identified if there is damage to functional nephrons (Lameire *et al.*, 2005).

There were no significant changes observed in the weights of vital organs, suggesting that oral administration of *S. lehmbachii* ethanol extract at sub-acute doses had no effect on normal growth. In toxicity tests, the weights of vital organs have been observed to be a sensitive indicator for particular organs, defining toxicity as significant changes shown in those organs (Kluwe, 1981). The findings of this study revealed that the vital organs examined were neither adversely affected nor showed any clinical signs of toxicity. There was no reduction in vital organ weights of the treated animals at any of the doses administered, suggesting that the extract is non-toxic to the organs. Macroscopic examinations of the organs treated with different doses of *S. lehmbachii* leaf extract did not show any changes compared with organs of the control group.

Conclusion

The findings of the present study indicate that the ethanol leaf extract of *S. lehmbachii* possesses a broad spectrum of antimicrobial activity and suggest that the plant leaf extract could be exploited in management of diseases caused by the investigated microorganisms in humans. The results obtained in respect to the subacute toxicity study suggest that the leaf extract is relatively safe when administered orally. The displayed high antimicrobial activity and lack of toxic effect render *S. lehmbachii* a candidate for bioassay-guided isolation of compounds which could develop into new lead structures and candidates for drug development programs against diseases.

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