



Antidiarrhoeal and antimicrobial effects of ethanol root bark extract from *Salacia lehmbachii*

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ABSTRACT

Introduction: The roots of *Salacia lehmbachii* are used in Nigerian folklore medicine without scientific basis. The present study was aimed to investigate the antidiarrheal and antimicrobial activities of the ethanol extract of *S. lehmbachii* root bark.

Methods: The antidiarrheal activity was examined using castor oil induced diarrhoea method. The ethanol root bark extract effects on intestinal transit time and enteropooling were also evaluated in rats, while antimicrobial activity was conducted on selected microorganisms. The acute toxicity test and phytochemical screening of the extract were also carried out.

Results: The extract produced significant ($P < 0.05$) dose dependent protection on rats against castor oil induced diarrhoea. The extract inhibited intestinal transit time and caused significant dose related inhibition of castor oil induced enteropooling in rats, comparable to the standard drug, atropine ($P < 0.05$). The root bark extract significantly and dose dependently delayed the onset of castor oil induced diarrhoea, reduced the frequency of defecation and decreased the severity of diarrhoea in rats. *S. lehmbachii* ethanol root bark extract significantly and dose dependently decreased the volume of intestinal fluid accumulation in the castor oil induced enteropooling. The extract also significantly inhibited the growth of test organisms. The acute toxicity test produced no lethality in rats, whereas the phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, cardiac glycosides, resins and balsam.

Conclusion: The results of this study confirm the ethnomedicinal use of *S. lehmbachii* root bark as a valuable natural agent for the treatment of diarrhoea and microbial infections.

Implication for health policy/practice/research/medical education:

Salacia lehmbachii ethanol root bark extract significantly demonstrated high antidiarrheal, antimotility, antienteropooling as well as broad spectrum antibacterial activities. Thus, this plant can be considered as a candidate for bioassay guided and isolation of compounds which could possibly be developed into new lead structures for drug development programs against gastrointestinal disorders and infectious diseases.

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Introduction

Medicinal plants are used commonly by most people in rural areas in treatment of diseases traditionally and these are considered beneficial in healthcare. Medicinal plants are generally, considered to possess innumerable bioactive constituents which steadily increase their demand as an

alternative to synthetic agents (1-3). More so, formulated products derived from these plants are safer with less harmful effect for human than the synthetic drugs (4). However, pharmacological evaluation of these agents is important for drug development. Thus, different plant parts have been on the focus for intense scientific

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researches, globally.

Salacia lehmbachii (Celastraceae) with many local names in Nigeria, Cameroon and Tanzania is a small tree with a height of about three meters, always green with firm and difficult to slice. The leaves are important in such conditions as pyrexia, diarrhea, motility and ulcers (5,6). The extracts from the root have antioxidants (7), analgesic, anti-inflammatory (8), anti-cholinergic (9), anti-infertility (10) and anti-hemorrhoid effects (11). The present study investigated the antidiarrheal and antimicrobial activities of ethanol extract of *S. lehmbachii* root bark.

Materials and Methods

Plant collection

The roots of *S. lehmbachii* were collected from Akwa Ibom State, Nigeria. The roots were taxonomically identified in the Herbarium Unit of Department of Botany, University of Calabar, Calabar and voucher specimen deposited with number 688.

Preparation of root bark

The root barks (*S. lehmbachii*) were washed, chopped into small pieces, air-dried at room temperature. The dried root barks were milled into fine powder with the aid of mortar and pestle. The powder was macerated in 2.5 L of ethanol at room temperature ($25 \pm 1^\circ\text{C}$) for 24 hours with occasional stirring and shaking. The filtrate concentrated on water bath at reduced temperature of 40°C to recover the extract, the gathered yield was 10.5% w/w light brown, powdery crude *S. lehmbachii* root bark ethanol extract. Aliquot portions residue of the extract weighed and dissolved in distilled water for use on each day of our experiments.

Phytochemical screening

The root bark extract was of *S. lehmbachii* tested for different secondary metabolites using standard methods (12,13).

Animals

Healthy Wistar rats of both male and female weighing 180–220 g, were used for this study. All animals housed and maintained under laboratory conditions of temperature and humidity, with 12 h natural light/12 h dark cycles. They had free access to rat chow and clean water, except when fasting was required in the course of the study. Their cages were cleaned daily of wastes. The experimental procedures and protocols used in this study were approved by the Ethics Committee of the University of Calabar, Nigeria and conform to the guide to the care and use of laboratory animals in research and teaching (14).

Test strains

Pseudomonas aeruginosa, *Salmonella typhi*, *Staphylococcus*

aureus, *Shigella species* and *Escherichia coli*, all clinical isolates obtained from the Microbiology Department, University College Hospital, Calabar, Nigeria were used in this study.

Acute toxicity test

Oral acute toxicity test was performed using the Organization of Economic Cooperation and Development (OECD) guideline for testing of chemicals 401 (15). Male and female rats weighing 180–220 g, were used for this study, and conducted in two phases. Three groups of 3 rats (all male separated from female) in each cage, were administered 100, 600 and 1000 mg/kg of the root bark extract orally. The rats were observed for signs of toxicity and mortality for 24 hours with special attention at first 4 hours. This was followed by administration of 2000, 3000 and 5000 mg/kg to the next three groups of 3 rats and observed for salivation, paw-licking, writhing (toxicity signs), change in body weight and mortality. The number of deaths was recorded in each group and the final LD₅₀ values were calculated.

Evaluation of antidiarrheal activity of the root bark

Castor oil induced diarrhea in rats

The procedure described previously (16,17) with some modifications was used for the study. The animals were kept at 25°C , fasted for 24 hours but had access to water prior to the experiment. Each rat was placed separately in a cage having blotting paper lined on the floor. The rats in the group 1 were orally treated with 20 mL/kg distilled water. The rats in groups 2–4 were treated orally with 50, 100, and 200 mg/kg of the extract of *S. lehmbachii* ethanol root bark accordingly. The rats in group 5 were individually treated with 4 mg/kg of loperamide, a standard antidiarrheal agent. Thirty minutes after pretreatment of the animals, each rat was given 1 mL of castor oil in each of the five groups. The severity of diarrhea was monitored for 5 hours for diarrheal droppings. The number of diarrhea (drops) was recorded and compared with the controls. The percentage (%) inhibition of diarrhea was calculated following the formula below (18):

$$\% \text{ inhibition of defecation} = \frac{\text{Control mean} - \text{treated (test) mean}}{\text{Control mean}} \times 100$$

Castor oil-induced enteropooling test

As described above, the rats used in this study were fasted for 24 hours (during which the animals had free access to clean water) and kept in five groups of 6 rats in a group. Each rat was separated and placed in a plastic cage lined with a transparent paper at the floor. The rats in group 1 were treated with 20 mL/kg of distilled water each. The rats in groups 2–4 received *S. lehmbachii* ethanol root bark extract doses of 50, 100 and 200 mg/kg, respectively. Group 5 rats received 4 mg/kg of loperamide, followed by 1 mL of

castor oil orally. Thirty minutes following administration of castor oil orally, each rat was sacrificed as previously described (19). The small intestine was excised, intestinal content expelled into a graduated measuring cylinder and its volume was determined using the formula. The intestinal content percentage inhibition was determined using the below formula (20):

$$\% \text{ inhibition of intestinal fluid} = \frac{\text{Control-test extract}}{\text{Control}} \times 100$$

Intestinal transit test

Adult rats of both sexes weighing 180-220 were allocated into five groups of 6 per group. Prior to the experiments, the rats were fasted for 24 hours (during this time they were given free access to clean water *ad libitum*). Group 1 rats were treated with 20 mL/kg of distilled water. Group 2-4 rats received doses of 50, 100 and 200 mg/kg root bark extract. Group 5 rats received 5 mg/kg atropine sulphate each. Thirty minutes later, the animals were given 1 mL of charcoal meal (10% charcoal suspension in 5% tragacanth) each, orally. All rats were sacrificed after half an hour of charcoal meal. The small intestine of each rat was dissected out from the pylorus to the caecum, and the total distance traversed by the charcoal plug along the small intestine, was determined in both the control and treated groups (expressed as a percentage) (21).

$$\% \text{ inhibition} = \frac{\text{Mean length of intestine} - \text{mean distance traveled by meal}}{\text{Mean length of intestine}} \times 100$$

Antimicrobial activity of the extract

The clinical isolates of 5 bacteria species, consisted of *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella* species and *Escherichia coli*, were sourced from Microbiology Department of University College Hospital, Calabar, Nigeria. Each organism maintained on nutrient agar slants was recovered by sub-culturing on nutrient broth after 24 hours. Before use, the bacterial culture was diluted each to 1:100 in fresh sterile nutrient broth (22). The organisms to be tested were streaked on sterile nutrient agar plates, incubated at 37°C, and then examined after 24 hours. Total growth suppression by specific concentration of the test extract was needed to be made active. The ethanol root bark *S. lehmbachii* extract was tested at final doses of 6.25, 12.5, 25, 50, 100 and 125 µg/mL. Plates in blank containing nutrient agar only and other sets having nutrient agar with ethanol were served as controls, while gentamicin (10 µg/mL) was used as standard drug. The treatment (each) was performed in triplicate, while inhibition of complete bacterial growth was required to declare the extract bioactive.

Determination of minimum inhibitory concentration
The minimum inhibitory concentration (MIC) of the

extract was assayed through micro dilution method (23). Ethanol root bark extract of *S. lehmbachii* which inhibits growth of one or more microorganisms was examined for MIC. Serial dilutions of the extract were prepared to the concentrations of 6.25, 12.5, 25, 50, 100 and 125 µg/mL. The wells for the test were inoculated with 0.1 mL aliquot containing the test organisms (1×10^6 cfu/mL) and serial dilution of the root bark extract containing 50 µL each. Thereafter, micro plate was incubated at 37°C for 24 hours and dilutions of the extract corresponding to each test organism showing no visible growth was considered as the MIC.

Statistical analysis

Data were expressed as the mean \pm SEM of six determinations. The significance of difference between means was determined using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test (24). Statistical significance was established at $P < 0.05$.

Results

Phytochemical screening

It is important to know the chemical nature of plant products when their pharmacological responses are screened (12). Phytochemical evaluation of *S. lehmbachii* root bark extract showed the presence of the following secondary metabolites; alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, cardiac glycosides, resins and balsam.

Acute toxicity test

There was no observed behavioral changes, mortality or signs of toxicity in 48-day period following observation in rats receiving 5000 mg/kg of the extract root bark. Hence, the median lethal dose (LD50) was found to be greater than 5000 mg/kg.

Effect of castor oil induced diarrhea test in rats

Pretreatment of the rats with *S. lehmbachii* ethanol root bark extract (50 mg/kg, 100 mg/kg and 200 mg/kg, orally) dose dependently and significantly ($P < 0.05 - 0.01$) delayed the onset of diarrhea, lowered rate of dropping and reduced the severity of diarrhea in rats. The standard drug, loperamide (4 mg/kg, orally), showed a marked and significant ($P < 0.01$) antidiarrheal effect than the highest dose of the extract (400 mg/kg) used (Table 1).

Effect of intestinal transit test in rats

The effect of *S. lehmbachii* root bark extract on the intestinal transit is shown on Table 2. The root bark (50 mg/kg, 100 mg/kg and 200 mg/kg, orally) dose dependently and significantly ($P < 0.05 - 0.01$) decreased the propulsive movement and transit charcoal meal. However, the standard drug, atropine sulphate (5 mg/kg) demonstrated marked and significant ($P < 0.01$) inhibition

Table 1. Effects of *Salacia lehmbachii* ethanol root bark extract of castor oil induced diarrhea in rats

Drug	Dose (mg/kg)	Mean diarrhea frequency (4 h)	% Protected
Control	0.2 mL	12.17 ± 0.87	0.0
<i>S. lehmbachii</i>	50	4.33 ± 0.67	64 ^a
<i>S. lehmbachii</i>	100	2.80 ± 0.65	77 ^b
<i>S. lehmbachii</i>	200	1.33 ± 0.56	89 ^b
Loperamide	4	1.00 ± 0.52	92 ^b

One-way ANOVA + Dunnett's post hoc test (n=6). ^a P<0.05, ^b P<0.01 compared to control.

in intestinal motility than the highest dose (400 mg/kg) of the root bark extract used in the experiment.

Effect of castor oil induced enteropooling test in rats

Pretreatment of the test groups of rats (50 mg/kg, 100 mg/kg and 200 mg/kg, orally) of the extract dose dependently and significantly ($P < 0.05 - 0.01$) inhibited castor oil induced fluid accumulation. The standard antidiarrhea drug, (loperamide 4 mg/kg) showed more marked ($P < 0.01$) inhibitory effect on castor oil induced fluid accumulation than the highest dose of the extract (400 mg/kg) employed (Table 3). The intestinal fluids of the rats pretreated with the ethanol root bark extract and loperamide were more viscous than control rats treated with distilled water.

Antibacterial activity

The antimicrobial activity of *S. lehmbachii* root bark extract is presented on Table 4. The extract showed activity against all the organisms tested. Gentamycin tested at 10 µg/mL, inhibited four of the organisms, while *Shigella* spp was moderately inhibited.

Minimum inhibitory concentration

The MIC of ethanol root bark extract of *S. lehmbachii* against the organisms were obtained as follows: *S. aureus* (26), *P. aeruginosa* (45), *S. typhi* (30), *Shigella* spp (30) and *E. coli* (55).

Discussion

Diarrhea, which due to altered motility and accumulation of fluid around the intestinal tract. Most agents with antidiarrheal property show activity by lowering

Table 3. Effect of *Salacia lehmbachii* ethanol root bark extract on castor oil induced enteropooling in rats

Drug	Dose (mg/kg)	Intestinal fluid (mL)	Inhibition of intestinal fluid volume (%)
Control	0.2 mL	2.68 ± 0.08	0.0
<i>S. lehmbachii</i>	50	1.05 ± 0.03	61 ^a
<i>S. lehmbachii</i>	100	0.87 ± 0.18	68 ^a
<i>S. lehmbachii</i>	200	0.42 ± 0.18	84 ^b
Loperamide	4	0.37 ± 0.12	86 ^b

One-way ANOVA + Dunnett's post hoc test (n=6). ^a P<0.05, ^b P<0.01 compared to control.

Table 4. Zone of inhibition (mm) of organisms by ethanol root bark extract of *Salacia lehmbachii* and gentamicin

Organisms	Zone of inhibition (mm)	
	<i>S. lehmbachii</i> extract	Gentamicin
<i>S. aureus</i>	20	24
<i>P. aeruginosa</i>	18	20
<i>S. typhi</i>	19	22
<i>Shigella</i> spp	15	19
<i>E. coli</i>	12	17

gastrointestinal motility or its secretions. Ricinoleic acid, a castor oil metabolite could bring about diarrhea (25, 26) which might increase motility tone in small intestine and alter electrolyte permeability in the membrane of intestinal mucosa (27,28). However, Ricinoleate stimulates endogenous secretion of the prostaglandin (29). Prostaglandins E series are reported as good agents for diarrhea in both humans and animals (30). Moreover, disruption of prostaglandin biosynthesis may shorten castor oil induced diarrhea (31). The root bark extract of *S. lehmbachii* exhibited significant antidiarrheal activity. Tannins, flavonoids, saponins and steroids as plants secondary metabolites have shown antidiarrheal properties (32- 34). The root bark extract exhibited significant dose dependent reductions in castor oil induced diarrhea, which is a clear evidence of its antidiarrheal potential when compared with the standard antidiarrheal drug, loperamide at 10 mg/kg. Apart from regulating gastrointestinal tract, loperamide also reduce intestinal transit time, the rate of colon out-flow and colonic motility (35).

Table 2. Effect of *Salacia lehmbachii* ethanol root bark extract on intestinal transit in rats

Drug	Dose (mg/kg)	Intestinal length (cm)	Distance travelled by charcoal meal (cm)	Inhibition of transit (%)
Control	0.2 mL	88.83±1.81	86.17±2.07	0.0
<i>S. lehmbachii</i>	50	86.50 ± 2.46	27.17 ± 2.07	69 ^a
<i>S. lehmbachii</i>	100	86.00 ± 1.88	21.17 ± 2.75	75 ^b
<i>S. lehmbachii</i>	200	82.50 ± 1.84	13.50 ± 1.35	84 ^b
Atropine	5	84.50±1.61	11.83±1.05	86 ^b

One-way ANOVA + Dunnett's post hoc test (n=6). ^a P<0.05, ^b P<0.01 compared to control.

The graded doses of the root bark extract significantly decreased intestinal transit time by a reduction in distance travelled charcoal meal. Atropine at 5 mg/kg, decreased propulsive movement in charcoal meal test following its anticholinergic effect (35). In castor oil induced enteropooling study, the root bark extract demonstrated significant reductions of water contents at different doses, as well as frequency of defecation and intestinal fluid accumulation. The remarkable inhibition of castor oil induced enteropooling in rats shows that the extract may produce relief in diarrhea through its spasmolytic and antienteropooling effects.

The extract of *S. lehmbachii* (root bark) possessed broad spectrum of antimicrobial activity against *S. aureus*, *shigella spp*, *P. aeruginosa*, *S. typhi* and *E. coli*. The results suggest that the plant extract could be used in the treatment of dysentery and diarrhea (36). Increased inhibition was found against *S. aureus* and *S. typhi*. The findings have shown that the extract could possess antagonizing qualities capable of preventing microbial survival.

Plants synthesize phytoconstituents which have health stimulating properties and the constituents of these plants possess health stimulating effect, whose importance increases their intake in our diet (37). Moreso, investigation of their initial secondary metabolites might help in detecting bioactive components which could assist in drug discovery and development (12). The oral acute toxicity test of the root bark extract could not be determined, as neither toxicity signs nor mortality was observed up to 5000 mg/kg, suggesting the extract to be safe with a wide therapeutic range.

Conclusion

The discussed results in this experimental study indicates that *S. lehmbachii* root bark extract possesses antidiarrheal and antimicrobial activities. The findings therefore validate the use of *Salacia lehmbachii* root bark as antidiarrheal and antimicrobial agents in traditional medicine in Nigeria. However, further studies are now under way to isolate, characterize and determine the structure of the plant active constituents.

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Authors' contributions

GCA conceived the research idea and designed the work, GAE and JAE wrote the first draft of the manuscript, EDO and FON carried out the literature search, GAI carried out the statistical analysis, while FVU supervised the study. All authors read and approved the final manuscript.

Conflict of interests

No conflict of interest is associated with this study.

Ethical considerations

The protocol for this study was confirmed by Animal Research Ethical Committee, University of Calabar (ERN/025PA30617) and the authors of this manuscript observed ethical issues. Animals were handled according to the International Guidelines for Care and Handling of Experimental Animals.

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