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Antimicrobial and Antidiarrheal Activities of *Pterocarpus santalinoides* Stem Bark Extract in Rat Models

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ABSTRACT

Background and Objective: Diarrhea remains a major health challenge, often linked to microbial infections and managed using synthetic drugs that may cause side effects or resistance. This study aimed to evaluate the antimicrobial and antidiarrheal activities of *Pterocarpus santalinoides* stem bark ethanolic extract in albino rat models to validate its traditional use. **Materials and Methods:** The antimicrobial activity was assessed using the agar well diffusion method to measure zones of inhibition against *S. typhi, E. coli, S. dysenteriae, S. aureus*, and *C. jejuni*. The minimum inhibitory concentration (MIC) was determined via doubling dilution (1000-62.25 mg/mL). Ciprofloxacin served as a positive control. The antidiarrheal potential was tested in rats using castor oil-induced diarrhea and charcoal meal motility models. Four rat groups (n = 5 per group) were treated with distilled water, 0.5 mg/kg loperamide, and 500 or 1000 mg/kg extract. Acute toxicity (LD) was also determined. Data were analyzed using appropriate statistical tests at $p<0.05$ significance. **Results:** The extract showed significant ($p<0.05$) antimicrobial activity with inhibition zones at 1000 mg/mL measuring 25.00±1.00 mm (*S. typhi*), 15.67±0.78 mm (*E. coli*), 9.00±1.00 mm (*S. dysenteriae*), 16.67±0.58 mm (*S. aureus*), and 12.67±0.58 mm (*C. jejuni*). The MIC was 62.25 mg/mL for *S. typhi* and 250 mg/mL for the other pathogens. In the castor oil model, extract-treated groups had significantly reduced stool frequency and severity, and decreased intestinal motility in the charcoal test. Loperamide showed the highest reduction, but extract effects were comparable **Conclusion:** *Pterocarpus santalinoides* stem bark extract exhibits notable antimicrobial and antidiarrheal activities, supporting its traditional use as an alternative remedy for infectious diarrhea. Further studies are recommended to isolate active compounds and assess long-term safety.

KEYWORDS

Acute-toxicity, enteropathogenic, castor-oil, induced, diarrhea, loperamide

INTRODUCTION

Natural remedies from plant sources in our environment have been of tremendous help in combating different types of diseases affecting man and livestock since ancient times. Despite the recent availability of several synthetic drugs, extracts from various parts of plants are preferably utilized by local folks to manage various ailments based on the belief that plants are the sources of unadulterated phytochemicals on which modern medical science principally relies to produce synthetic drugs. Hence, a large number of

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antimicrobial compounds have been discovered from natural products for the treatment and control of infectious agents[1]. Nevertheless, a relatively few portion of these were accessible to the target world's market[2]. Consequently, the exploration for novel antimicrobial compounds from natural sources has become an essential sector of contemporary medicine to mitigate the socio-economic and health impact caused by multidrug-resistant microbes[3]. Plants have a remarkable ability to yield an extensive diversity of bioactive principles, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins. These secondary metabolites are the basis of plant-derived antimicrobial substances[4]. It is hoped that plant extracts exhibiting active sites apart from those used by antibiotics will be effective against drug-resistant pathogens. Medicinal plants imbued with active ingredients have been of tremendous value in the treatment of a wide range of intractable diseases dating back to the time of early men, many thousands of years ago.

Diarrhea has become a pressing challenge in our society of today, as many people have been finding it difficult to get proper treatment for such health challenges. Ahsanul Haque *et al*.[5] reported that in developing nations, diarrheal illnesses are among the major causes of morbidity and mortality and are responsible for the loss of hundreds of thousands of people yearly. Similarly, it was demonstrated in 2015 that diarrhea was one of the main killers of children, and it accounted for 9% of all deaths among children below the age of 5 years worldwide. According to the data, sub-Saharan Africa and southern Asia regions recorded the highest child death toll as a result of diarrhea[6], which is mainly attributed to poor hygiene and sanitary measures. According to the WHO, diarrhea is expressed by increased frequency and volume of loose or liquid stools passed out three or more times daily. The most common cause of diarrhea is the disturbance or washing of the intestinal lining (epithelium) by various types of enteropathogenic bacteria, viruses, and parasites. However, in many clinical cases, the actual cause of the disease is unknown. The incidence of diarrhea remains high, despite the efforts of international organizations to control the disease[7]. In diarrhea, there is an extraordinary peristalsis of the bowel that leads to loss of a huge amount of electrolytes in frequent stooling[7]. For thousands of years, plants have provided a reservoir of compounds used as therapeutic agents. Active metabolites found in plants are responsible for their medicinal properties[8]. Medicinal plants with antidiarrheal properties have been widely used by traditional healers. However, the effectiveness of many of these plants has scarcely been scientifically evaluated and proven. One of such plant is *Pterocarpus santalinoides*.

Azamthulla *et al*.[9] gave a detailed description of *Pterocarpus santalinoides*, a tree of small to medium size, nine-twelve meters tall, with characteristically hard, dark purple heartwood of bitter flavor. The bark is blackish brown, its thickness ranges from 1-1.5 cm, and it flakes in small patches. It is a shadow tolerant tree usually found beside the riverine forests in Africa and tropical South America[10]. In Nigeria, many indigenous plants, including *P. santalinoides,* are used as food or medicine. In Southeast Nigeria, the fresh, tender leaves of *Pterocarpus santalinoides* are used as a vegetable in soup making, while the stem bark is used in making pepper soup. *Pterocarpus santalinoides* is a plant believed to possess potent antibacterial properties in ethnomedicine. The application of botanical extracts or plant-derived pure chemicals to treat disease in Nigeria is not new. It has, since ages, been a special alternative to synthetic drugs in therapeutic modality that has stood the test of time. Nevertheless, limited scientific authentication has been done on these species to justify their medicinal uses.

Castor oil tends to produce bowel movement that relaxes constipation, acting through ricinoleic acid, a hydroxylated fatty acid derived from castor oil through the action of the intestinal enzyme lipase[11]. The ricinoleic acid thus liberated causes irritation and swelling of the intestinal mucous membrane, leading to the release of prostaglandins and nitric oxide, which stimulate gastrointestinal secretion, motility, epithelial permeability, and oedema of the intestinal mucosa[12].

To address this gap, the present study aims to determine the potential *in vitro* antimicrobial activities of ethanol extract of the stem bark of *Pterocarpus santalinoides* on some enteropathogenic microbes often associated with diarrhea and its *in vivo* antidiarrheal effect in castor oil-induced diarrhea in rats.

MATERIALS AND METHODS

Collection of plant material: The stem bark 2 kg of *Pterocarpus santalinoides* was collected during the flowering period of the plant from the Afoeke community, Timber Umuahia, Abia State, Nigeria, in May, 2022. The plant stem bark was identified and authenticated by Dr. F.N. Ugwuja, Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria. The stem bark was air-dried at room temperature in the laboratory for two weeks, and they were used for antimicrobial activities and anti-diarrhea evaluations.

Other materials used: The weighing balance, beakers, glass jar, and filter paper were collected from the Department of Plant Science and Biotechnology Laboratory, while the buckets used were bought from Ndioru market. The loperamide and charcoal meal were bought from Grace and Mercy pharmacy, Umuahia. Pasture pipette, hand glove, cotton wool, plastic petri-dishes, methylated spirit, swab stick, ethanol, and castor oil were bought from Jochem Chemical Stores Limited.

Preparation of plant extract: The stem bark was initially air-dried under shade for two weeks. Thereafter, the materials were placed inside an envelope and dried in the oven for 1 hr at 40°C to achieve brittleness. The material was then pulverized using a milling machine (Thomas Wiley Mill, model: ED-5), and the resultant powdered bark (150 g) was soaked in 450 mL of 96 % ethanol for 48 hrs and filtered through Whatman No. 1 filter paper. The excess solvent in the extract obtained was removed by concentrating under reduced pressure using a rotary evaporator (Yarong RE-5). Concentrated extracts were dried at 40°C on a water bath, and 10.06 g crude extract representing 6.70% was collected and preserved under refrigeration (4±2°C) for further experiments.

Experimental animals: Adult albino rats 42 of male and female genders acquired from the Veterinary Animal Facility of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were used for the study. The animals were housed under standard conditions (25±2°C and 12 hrs light/dark cycle). The rats were sustained on standard pellets (Livestock feed®: Finisher mash from Chikkun Feeds Nigeria, with crude protein of 19.90% and metabolizable energy of 3209.64 Kcal). All animals were allowed unlimited access to drinking water and were acclimatized for seven days.

Ethical statement: The use and care of laboratory animals in the study were in accordance with the ethical guidelines as contained in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (EEC Directive of 1986; 86/609/EEC) and amended in the European Treaty Series (ETS No. 170) of 2005. Ethical clearance with approval number CREC/001/25 was given by the COLNAS Research Ethics Committee (CREC), Michael Okpara University of Agriculture, Umudike, in accordance with the International Standard on the Care and Use of Experimental Animals.

Enteropathogenic microbial cultures: The five microbial isolates used in the study were *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae,* and *Campylobacter jejuni*. They were obtained from the Royal Medical Diagnostics and Research Laboratory, Olokoro, Umuahia, Abia State, Nigeria.

Antimicrobial screening of extract on experimental microorganisms: The agar well diffusion technique was used to screen the plant extracts for antimicrobial activities. An aliquot (10 g) of the extract was weighed and dissolved in 10 mL of water to obtain a concentration of 10,000 mg/mL. The extract was doubly diluted further to obtain 1000 and 500 mg/mL concentrations. Microorganisms were placed on solidified nutrient agar media by a process known as seeding, and a 6 mm diameter well was cut at the center of the plate using a cork borer. A sample (0.1 mL) of the given concentration of extract was introduced into the agar well. The agar plate was incubated (37°C, 24 hrs) and observed for a zone of inhibition[13].

Determination of minimum inhibitory concentration: The minimum inhibitory concentration of the extracts was determined by incorporating constant volumes (0.2) of each dilution of the extract into the punch-holes on pre-seeded nutrient agar. An aliquot of the extract 1 g was dissolved in 1 mL of sterile distilled water to obtain 1000 mg/mL. This 1000 mg/mL concentration was then doubly diluted in sterile distilled water to obtain a concentration of 1000, 500, 250, 125, and 62.25 mg/mL. Incubated at 37°C for 24 hrs in the incubator. Following the incubation, the diameter of the zone of inhibition was recorded. The minimum inhibitory concentration was determined by comparing the different concentrations of the extract having different zones of inhibition and then selecting the lowest concentration of the extract.

Determination of the lethal dosage (LD_{50}): This assay was divided into three stages, with the outcome of each stage determining whether to terminate testing or proceed to the next stage. A confirmatory (confidence) test was used to validate the final test result[14].

Stage 1: This stage requires nine animals. The nine animals are divided into three groups of three animals each. Each group of animals was administered different doses (10, 100, and 1000 mg/kg) of the test substance. The animals are placed under observation for 24 hrs to monitor their behavior as well as if mortality will occur.

Stage 2: This phase involves the use of another set of nine animals, which were distributed into three groups of three animals each. The animals are administered higher doses (1600, 2900, and 5000 mg/kg) of test stem bark extract and then observed for 24 hrs for behavior as well as mortality.

Final stage/confirmatory test: A 5000 mg/kg was repeated on another set of three rats still, no mortality was recorded within 24 hrs and a further seven days.

Accurate concentration for inducement was calculated as:

$$\frac{\text{Dose} \times \text{Body weight}}{\text{Concentration}} \times 100$$

Then the LD_{50} is calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where:

D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

Phytochemical composition of *pterocarpus santalinoides* bark ethanolic extract: The phytochemical content of *P. santalinoides* bark ethanolic extract was determined[15,16].

Determination of the effect of extract on small intestinal transit time of charcoal meal in rats: A 36 adult male albino rats were assigned to 4 treatment groups, with each group containing 3 rats, and replicated thrice in a completely randomized design. The rats were fasted for 18 hrs preceding the beginning of the test but were permitted unlimited access to water. Group 1 got no treatment and represented the control, group 2 was administered Loperamide (0.5 mg/kg body weight), while groups 3 and 4 received 500 and 1000 mg/kg body weight of the extract, respectively. All treatments were via the oral route. After thirty minutes of the first treatment, the animals received 1 mL of charcoal

meal (10% charcoal suspended in 10% gum acacia) orally. In another 30 min, the animals were ethically sacrificed, and the small intestine was carefully harvested and its full length measured from the pyloric sphincter to the ileocecal junction. For each animal, the distance travelled by the charcoal meal was also measured and expressed as a percentage of the full length using the relationship below:

$$\text{Gastrointestinal transit (\%)} = \frac{\text{Distance moved by charcoal meal}}{\text{The whole length of the small intestine}} \times 100$$

The inhibitory effect of the extract on gastrointestinal transit was calculated relative to the control as:

$$\text{Inhibition (\%)} = \frac{\text{Gastrointestinal transit of control} - \text{Gastrointestianl transit of test}}{\text{Gastrointestinal transit of control}} \times 100$$

Determination of the effect of extract on castor oil-induced diarrhea in albino rats

Principle: Castor oil has a laxative effect which is mediated by ricinoleic acid, a hydroxylated fatty acid released from castor oil by the intestinal lipase[11,17]. The liberated ricinoleic acid causes irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins and nitric oxide, which stimulate gastrointestinal secretion, motility, epithelial permeability, and oedema of the intestinal mucosa[12]. Thirty-six adult male albino rats assigned to 4 groups with three replicates of rats each were used. The rats were fasted for 18 hours before commencement of the experiment, but were allowed free access to water. Group 1 received distilled water and served as the control, group 2 was administered Loperamide (0.5 mg/kg body weight), while groups 3 and 4 received 500 and 1000 mg/kg body weight of the extract, respectively. All treatments were via the oral route. Thirty minutes after treatments, animals received 1mL of castor oil orally and were placed individually in a cage with the bottom lined with weighed absorbent paper, and diarrhea episodes were observed for a period of 3 hrs. The parameters recorded included the onset of diarrheal stool (latent period), the number of both wet and dry stools, and the weight of the wet stools. All these were measured every 1 hr, and the paper was changed after each evaluation. The percentage of rats that responded to diarrhea in each group was calculated. The mean number of stools passed by the treated groups was compared with that of the control, and the mean number of diarrhea faeces pooled by the control group was considered as 100%.

The percentage inhibition of wet faeces and frequency of stool caused by the extract was calculated relative to the control using the relation:

$$\text{Inhibition of defecation (\%)} = \frac{N_C - N_T}{N_C} \times 100$$

Where:

N_C = Mean number of faeces of the control group

N_T = Mean number of feaces of the treated group

The level of reduction (%) in defecation of watery faeces was calculated using the relation:

$$\text{Inhibition of diarrheafaeces (\%)} = \frac{N_C - N_T}{N_C} \times 10$$

Where:

N_C = Mean number of diarrhea faeces of control group

N_T = Mean number of diarrheic faeces of treated group

Statistical analysis: The data obtained were subjected to both one-way and two-way Analysis of Variance (ANOVA) using Statistical Product and Service Solutions (SPSS) version 22 at a 0.05 level of significance. The means were compared using Duncan multiple range comparisons test and statistical significance were established at 95% confidence level.

RESULTS

Results of the acute toxicity test (LD_{50}) reveal that *Pterocapus santalinoides* extract is non-toxic and caused no death or signs of acute intoxication in rats at a dose up to 5000 mg/kg administered orally (Table 1). The rats that received the highest dose of the extract were initially inactive, but they recovered their activeness within 24 hrs and survived the test.

Results of the antimicrobial activity and inhibitory zone effects of *P. santalinoides* stem bark on the test organisms (Table 2) showed that 1000 mg/ml extract exhibited significant ($p<0.05$) and varying inhibitory effects on the different micro-organisms in the range of 12.67±0.58-25.00±1.00 with the exception of the inhibition on *Escherichia coli* and *Staphylococcus aureus* which are statistically at par. However, the standard drug Ciprofloxacin had the highest mean inhibition values ranging from 36.00±1.00-59.00±1.00. The highest inhibition of the extract and standard drug was recorded against *Salmonella typhi*.

The Minimum Inhibitory Concentration (MIC) of ethanol stem bark extract of *P. santalinoides* on the test microorganisms was expressed at 250 mg/mL on *E. coli*, *S. aureus, S. dysenteriae,* and *C. jejuni,* respectively, while *S. typhi* had its minimum inhibition at 62.25 mg/mL (Table 3).

Table 1: Acute toxicity evaluation of stem bark extract of (*Pterocarpus santalinoides*)

Group	No. of rats per test	Doses of extract (mg/kg)	No. of death	Survival	Mortality ratio
1	3	10	0	3	0/3
2	3	100	0	3	0/3
3	3	1000	0	3	0/3
4	3	1600	0	3	0/3
5	3	2900	0	3	0/3
6	3	5000	0	3	0/3

Table 2: Antimicrobial activities (zone of inhibition (mm) of *P. santalinoides* stem bark extract on enteropathogenic microorganisms

Groups	Microorganisms	1000 mg/mL ciprofloxacin	1000 mg/mL *P. santalinoides* stem bark extract
1	*Salmonella typhi*	59.00 ± 1.00^{c}	25.00 ± 1.00^{d}
2	*Escherichia coli*	50.00 ± 2.00^{b}	15.67 ± 0.78^{c}
3	*Shigella dysenteriae*	49.00 ± 1.00^{b}	9.00 ± 1.00^{a}
4	*Staphylococcus aureus*	33.67 ± 2.08^{a}	16.67 ± 0.58^{c}
5	*Campylobacter jejuni*	36.00 ± 1.00^{a}	12.67 ± 0.58^{b}

Values are presented as Mean±Standard Deviation (n = 3) and Means on the same column with different letter superscripts are significantly different ($p<0.05$)

Table 3: Minimum inhibitory concentration (MIC) of *Pterocarpus santalinoides* ethanol stem bark extract on enteropathogenic microorganism

Microorganisms	1000 mg/mL extract	500 mg/mL extract	250 mg/mL extract	125 mg/mL extract	62.25 mg/mL extract	MIC
Salmonella typhi	25	12.3	7.3	2.3	0.6	62.25 mg/mL
Escherichia coli	15.6	8.3	5	0	0	250 mg/mL
Shigella dysenteriae	9	4.6	1.6	0	0	250 mg/mL
Staphylococcus aureus	16.6	7.6	3	0	0	250 mg/mL
Campilobacter jejuni	12.6	6	2.3	0	0	250 mg/mL

The least MIC 62.25 was expressed against *S. typhi,* showing it to be more sensitive than other test organisms to the extract

Table 4: Effect of *P. santalinoides* ethanol stem bark extract on charcoal meal transit in rats

Treatments	Length of intestine (cm)	Distance travelled by charcoal meal (cm)	Movement of charcoal (%)	Inhibition of charcoal movement (%)
Control	94.20±2.78^{b}	78.20±2.95^{b}	82.99±3.13^{b}	0.00±0.00^{a}
0.5 mg/kg loperamide	88.40±4.28^{a}	59.60±7.30^{a}	67.44±8.36^{a}	19.36±0.96^{c}
500 mg/kg extract	90.20±5.31^{ab}	56.80±4.66^{a}	63.58±8.15^{a}	23.16±1.06^{d}
1000 mg/k extract	90.60±2.07^{ab}	43.00±2.92^{b}	40.66±3.03^{b}	33.29±0.55^{b}

Values are presented as Mean±Standard Deviation (n = 3) and Means on the same column with different letter superscripts are significantly different (p<0.05)

Table 5: Effect of *P. santalinoides* stem bark extract on the weight of wet stool from castor oil-induced diarrheal rats

	Weight of wet stool (g)				
Treatment	After 1 hr	After 2 hrs	After 3 hrs	Total weight of wet stool (g)	Inhibition of weight of wet stool (%)
Control	2.40±0.16^{c,3}	0.88±0.23^{b,1}	1.58±0.16^{c,2}	4.86±0.53^{c}	0.00±0.00^{a}
0.5 mg/kg loperamide	0.32±0.13^{a,2}	0.46±0.11^{a,3}	0.00±0.00^{a,1}	0.78±0.18^{a}	82.52±1.18^{d}
500 mg/kg extract	1.08±0.16^{b,2}	1.70±0.45^{c,3}	0.56±0.13^{b,1}	3.34±0.62^{b}	32.61±1.53^{c}
1000 mg/kg extract	1.38±0.28^{c,3}	1.44±0.18^{c,2}	0.10±0.02^{a,1}	2.92±0.33^{b}	39.91±1.00^{b}

Values are presented as Mean±Standard Deviation (n = 3) and Means on the same row with different number superscripts are significantly different (p<0.05) while means on the same column with different letter superscripts are significantly different (p<0.05)

Table 6: Inhibitory effect of *P. santalinoides* stem bark on the number of wet stool

	Number of wet stools				
Treatment	After 1 hr	After 2 hrs	After 3 hrs	Total numberof wet stool (g)	Inhibition of numbers of wet stool (%)
Control	3.20±0.88^{c,1}	3.60±0.14^{b,1}	5.40±0.19^{b,2}	12.20±1.30^{c}	0.00±0.00^{a}
0.5 mg/kg loperamide	0.00±0.00^{a,1}	2.40±0.55^{a,1}	0.20±0.04^{a,2}	2.60±0.55^{a}	77.05±1.24^{c}
500 mg/kg extract	2.60±0.00^{c,3}	1.80±0.45^{a,2}	0.20±0.03^{a,1}	4.60±0.89^{b}	62.75±1.14^{b}
1000 mg/kg extract	1.00±0.00^{b,1}	2.60±0.60^{a,2}	1.00±0.00^{a,1}	4.60±0.48^{b}	62.87±1.22^{b}

Values are presented as Mean±Standard Deviation (n = 3) and Means on the same row with different number superscripts are significantly different (p<0.05) while means on the same column with different letter superscripts are significantly different (p<0.05)

Results of the charcoal meal transit test showed that the distance traveled by the charcoal meal, % movement of the meal and % inhibition of charcoal movement were significantly different in the rats that received a 1000 mg/kg dose of the extract compared to others (Table 4). The distance travelled by the charcoal meal in this group (43.00±2.29 cm) was shorter than 56.80±4.66 cm and 59.60±7.30 cm observed in the 500 mg/kg and Loperamide groups, respectively, giving rise to a significant % inhibition of charcoal meal movement. In the control group, which was administered only distilled water, the percentage inhibition of charcoal movement was zero and differed significantly (p 0.05) compared to all other groups.

The result of the inhibitory effect of *P. santalinoides* stem bark extract on castor oil induced diarrheal rats showed a progressive decline in the weight of wet stool in the extract (1000 and 500 mg/kg) and Loperamide (0.5 mg/kg) treated rats from the 1st to the 3rd hrs of diarrhea commencement (Table 5). These effects significantly differed (p 0.05) from those of the control (distilled water) group. Similarly, the total weight and percentage inhibition of wet stool also differed significantly in the extract and Loperamide-treated groups compared to the control. However, the group administered with Loperamide had significantly (p 0.05) the smallest amount of wet stool and the highest % inhibition of stool compared to all the other groups.

Effect of extract on the number of wet stools: Results of the effect of treatments on the number of wet stools are shown in Table 6. There was a decline and significant (p 0.05) contrast in the number and % inhibition of wet stool in the Loperamide group compared to the 1000 and 500 mg/kg groups after 1 hr, 2, and 3 hrs commencement of diarrhea in the rats. The mean number of wet stool (2.60±0.55) in this group was the smallest, with a corresponding highest %inhibition 77.05±1.24% compared to 4.60±0.48; 62.87±1.22% and 4.60±0.89; 62.75±1.14% recorded in the 1000 and 500 mg/kg group, respectively. In a stark contrast, there was no inhibition of wet stool in the control group as the number of wet stools increased from 3.20±0.88 in the first hour after diarrhea commencement to 5.40±0.19 in the 3 hrs.

DISCUSSION

The results of the present study have revealed the unique and invaluable attributes of *Pterocarpus santalinoides* stem bark ethanol extract, as demonstrated by the acute toxicity, MIC, antimicrobial activity, and its antidiarrheal activity in albino rats. Results of the acute toxicity test (LD_{50}) clearly revealed that the extract is safe, as no sign of toxicity was observed in the rats administered orally with a limited dose of 5000 mg/kg of the plant extract. Besides, during the seven-day post-treatment period, no deaths and no delayed noxiousness were observed across all the tested doses. These results are in tandem with the findings[18] reported that at a single dose of 5000 mg/kg none of the extracts (leaf, trunk bark, and root) of *P. stantalinoides* caused death in experimental rats. Similarly, the previous report[10] corroborates the present results. This entails that the oral acute toxicity value of the extract is beyond 5000 mg/ kg and that the extract can be administered in the management of human or animal infections without harmful effects or injuriousness at the controlled dose of 5000 mg/kg. Generally, if the acute toxicity value of a test substance is greater than 3 times the minimum effective dose, the substance is considered safe and is a good candidate for further studies[19,20]. This confirms that the extract is harmless and acceptable for oral administration, certifying its use in traditional medicine.

The results also indicated significant growth inhibition zones on the test enteropathogenic microorganisms via the minimum inhibitory concentration (MIC), which ranged from 62.25-250 mg/mL, and the antimicrobial Zone of Inhibition (ZI), which ranged from 12.67-25.00 mm. The inhibitory zone of the extract on *Salmonella typhi, Escherichia coli, Shigella dysenteriae, Staphylococcus aureus,* and *Campylobacter jejuni,* thus reported, varied from a study[21] reported that MICs of the stem bark extracts were 6.5 to 25 µg/mL for five strains (*E. coli* 0157H, S. aureus ATCC 25922, *Salmonella* sp., *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853) and 3.125 mg/mL for *S. aureus*. The discrepancies may be attributed to the methods of extraction and the types of solvents used during extraction. However, the antimicrobial activities exhibited by the extract is likely due to the presence of essential phytochemicals, such as alkaloids, flavonoids, saponins, tannins, terpenoids, phenols, cyanogenic glycosides, and steroids in the extract as reported by earlier researchers[15,16,22]. Similarly, Fred *et al.*[10] in their studies, preliminary phytochemical screening, evaluation of acute toxicity and antipyretic activity of methanolic extract of Pterocarpus santalinoides (Fabaceae) revealed the presence of tannins, flavonoids, terpenoids, steroids, alkaloids, glycosides, saponins, and resins.

The Minimum Inhibitory Concentration (MIC) of ethanol stem bark extract of *P. santalinoides* on the test microorganisms was expressed at 62.25 mg/mL on *Salmonella typhi,* while *E. coli*, *S. aureus, S. dysenteriae,* and *C. jejuni* had their MIC at 250 mg/mL. This suggests that *S. typhii* was the most sensitive of all the test microbes to the extract because it was inhibited at the lowest concentration of the extract than others. Similarly, there were significant and varying zones of inhibition of the organisms at 1000 mg/mL, with *S. typhi* also being the most inhibited. The significant and varying inhibitory effects on the different micro-organisms at a given concentration suggests that the sensitivity or susceptibility of the microbes to the extract is subject to their different innate/genetic characters. Conversely, the study revealed significantly ($p<0.05$) higher antimicrobial activities of the standard antibiotics (Ciprofloxacin) against all the test microbes compared to the extract. The lower antimicrobial activities of the *P. santilinoides* stem bark extract extracts compared to Ciprofloxacin may be as a result of the unpurified nature of the extract. The presence of impurities might have hindered full effectiveness of the active ingredients.

The ethanol stem bark extracts (500 and 1000 mg/kg) produced a significant ($p<0.05$) delay in onset of diarrhea and frequency of stooling with the resultant reduction in number of wet stools and total number of stools which was comparable with that of Loperamide the standard drug. These effects from the extracts were produced in a dose dependent manner but Loperamide was rather different and unique in producing the highest percentage inhibition of wet stool output at a very low quantity of 0.5 mg/kg. The actual amounts of active ingredient in the crude extracts, which may also contain large amounts of impurities, are not known. Given the presence of impurities, the bioactive principle may likely be less than the amount of Loperamide administered to the rats.

All the rats in the control (distilled water-treated) group produced significantly the highest output of wet stool after 3 hrs of commencement of diarrhea compared to the groups administered with the extract and the standard drug. This confirms the antidiarrheal potentials of *P. santalinoides* stem bark extract.

In the length of intestine, the ethanol extracts of *P. santalinoides* stem bark and 0.5 mg/kg Loperamide induced a substantial reduction in the rate at which the charcoal meal moved through the small intestine compared to the effect produced by the control. The number of wet stools and total number of stools were also decreased in a non-dose-dependent manner, with the highest effect observed at 0.5 mg/kg Loperamide. The effect of the extract on the aforementioned parameters was significantly greater than that produced by the control; hence, the percentage inhibition of the number of wet stools of the stem bark ethanol extract (500 and 1000 mg/kg) was 62.87% and 62.75% and was comparable to 77.05% induced by Loperamide.

Results of the effect of *P. santalinoides* ethanol stem bark extracts on castor oil-induced diarrhea in experimental rats showed that the extract doses markedly reduced the frequency of defecation, number of diarrhea stools, and wetness of the fecal droppings. It also significantly reduced the intestinal propulsive movement in the charcoal meal-treated model[23]. Inhibition of intestinal motility and reduction in the severity and frequency of wet defecation could be useful actions in the treatment of diarrhea. In these two experimental models, the extract showed activity similar to that of Loperamide.

Earlier findings have established the antidiarrheal activity of tannins, flavonoids, alkaloids, saponins, reducing sugars, steroids, and/or terpenes-containing plant extracts[24]. An initial phytochemical analysis of the extract revealed the presence of alkaloids, saponins, tannins, reducing sugars, and flavonoids. These constituents may be responsible for the antidiarrheal activity of the ethanolic extract of *P. santalinoides*.

The results have established that the stem bark ethanolic extract of *Pterocarpus santalinoides* possesses significant antidiarrheal and antimicrobial activity due to its inhibitory effect on gastrointestinal propulsion. These results justify the use of the plant as an antidiarrheal and antimicrobial agent in folk medicine.

CONCLUSION

The study has established the antimicrobial activity of *Pterocarpus santalinoides* stem bark ethanol extract on five enteropathogenic organisms: *S. typhi, E. coli, S. dysenteriae, S. aureus, and C. jejuni* used in the study. The extract also induced a statistically significant reduction in the severity and frequency of diarrhea in the rats, as well as the intestinal propulsive movement in the charcoal meal-treated model. Inhibition of intestinal motility and reduction in the severity and frequency of wet defecation could be useful actions in the treatment of diarrhea. In these two experimental models, the extract showed activity similar to that of Loperamide. There is a need to isolate and characterize by GC-MS analysis the active chemical compounds of *Pterocarpus santalinoides* stem bark, which are responsible for the antidiarrheal and antimicrobial activities.

SIGNIFICANCE STATEMENT

This study discovered the potent antimicrobial and antidiarrheal activities of *Pterocarpus santalinoides* stem bark ethanolic extract that can be beneficial for developing alternative therapies against infectious diarrhea. the extract demonstrated remarkable inhibition *against S. typhi, E. coli, S. dysenteriae, S. aureus, and C. jejuni*, along with significant protection in castor oil-induced diarrhea and charcoal meal motility models in albino rats. these findings highlight the therapeutic potential of plant-based remedies, offering safer and more accessible solutions compared to synthetic drugs that often cause resistance or adverse effects. This study will help researchers to uncover the critical areas of medicinal plant pharmacology that many researchers were not able to explore. Thus, a new theory on natural antidiarrheal drug discovery may be arrived at.

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