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Antidiarrheal and Antibacterial Activities of *Calopogonium mucunoides* Desv Leaf Extracts

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Abstract: *Calopogonium mucunoides* Desv is an annual legume indigenous to tropical regions of the world. The plant leaves are widely used in South-Eastern Nigeria for the management of bacterial infections, diarrhea and ulcer. Among compounds of pharmacological interest occurring in relative abundance in the plant are alkaloids and flavonoids. The aim of the present study was to investigate the antidiarrheal and antibacterial activities of the ethanol extract of the leaves of the plant as well as the antibacterial activity of alkaloids and flavonoids extracts of the plant leaves. Plant extract was prepared by maceration in ethanol. Its alkaloids and flavonoids were isolated using standard laboratory procedures. Castor oil- induced diarrhea model in rats was used to assay the antidiarrheal activity of the plant extract. The antibacterial activity assay was performed, using the Agar-well diffusion method, against pathogenic strains of *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus cereus*. Quantitative phytochemical analysis of plant revealed the presence of flavonoids and alkaloids in relative abundance while tannins and terpenes were present in moderate concentrations. The results of antidiarrheal studies showed that over four hours, the extract significantly ($p<0.05$) protected the rats against castor oil-induced diarrhea, inhibited gastrointestinal transit, reduced the frequency of defecation in the treated groups compared with the untreated group. The crude ethanol extract as well as the flavonoid and alkaloid constituents had very good antibacterial activity against the test organisms. The crude extract as well as the isolated test phyto-constituents inhibited the growth of bacterial isolates in a concentration- dependent manner with concentrations ranging between 6.2-100 mg/ml. It can be concluded that the findings from this study showed that *Calopogonium mucunoides* possesses antidiarrheal activity as well as antibacterial potential against the test pathogens. The study suggests that the plant may serve as a source for the development of novel drugs for the treatment of diarrhea and other diseases associated with the test organisms used in this study. The study has also given credence to the traditional usage of this plant as anti- diarrheal and antibacterial agent.

Key words: Antidiarrheal Activity · Antibacterial Activity · Calopogonium Mucunoides · Phytochemistry · Bacterial Isolates

INTRODUCTION

The medicinal value of plants have assumed a more important dimension in the past few decades owing largely to the discovery that extracts from plants contain not only minerals and primary metabolites, but also a diverse array of secondary metabolites with vast pharmacological potentials [1]. The most important of these bioactive constituents of plants include alkaloids and flavonoids [2, 3]. Flavonoids are reputed to possess many useful pharmacological properties including antibacterial, antidiarrheal, antifungal activities as well as anti-inflammatory activity [4, 5] and are also known to block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, diabetes, Alzheimer's disease, Parkinson's disease and in the aging process [6]. Alkaloids, on the other hand are known to have diverse pharmacological activities, including antibacterial, antimalarial and anti-inflammatory activities [7, 8].

Several plants have been reported to possess antibacterial activity [9-15] while others have been reported to have antidiarrheal activity [4, 5]. Diarrheal disease is a leading cause of mortality and morbidity, especially among children in developing countries,

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resulting in a major health problem [4]. The major causative agents of infectious diarrhea and other enteric diseases include *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*) *Salmonella typhi* (*S. typhi*) and *Escherichia coli* (*E. coli*) [4].

Infections caused by multidrug-resistant Gram-positive bacteria such as *S. aureus* and *B. cereus* represent a major public health problem, not just in terms of morbidity and mortality, but also in terms of management and implementation of infection control measures [16]. Gram-negative bacteria such as *S. typhi* and *E. coli* are associated with cell envelope which contains an additional outer membrane composed of phospholipids and highly charged lipopolysaccharides which confer pathogenicity to the gram-negative bacteria [17]. This outer membrane prevents certain drugs and antibiotics from penetrating the cell, partially accounting for why gram-negative bacteria are resistant to multiple drugs and are generally more resistant to antibiotics than gram-positive bacteria. In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment infectious diarrhea and other infectious diseases, making it a global growing problem. In addition to this problem, antibiotics are sometimes associated with adverse effects on host [18]. Hence, there is need for a continuous search for new antimicrobial agents. The present study is an effort to explore new sources of natural and potent antibacterial agents. Plant derived drugs remain an important resource to combat serious diseases as plant herbal mixtures have made large contribution to human health and well being [19].

Calopo (*Calopogonium mucunoides* Desv.) is a vigorous, hairy annual trailing legume. It can reach several meters and form a dense, tangled mass of foliage, 30-50 cm deep. The root system is dense and shallow, at most 50 cm deep. The stems are succulent, covered with long, brown hairs. They are creeping in the lower parts, sometimes rooting at the nodes that come in contact with the soil. The upper part of the stem is twining. The leaves are up to 16 cm long and trifoliate. The hairy leaflets are 4-10 cm long and 2-5 cm broad, ovate to elliptical. The inflorescence is a slender hairy raceme that may be up to 20 cm long and that bears 2 to 12 blue or purple small flowers. The fruits are 3-8 seeded hairy pods, 2-4 cm long [20]. The plant leaf has been used in Nigerian folk medicine for the treatment of ulcer, diarrhea and bacterial infections [21]. The anti-ulcer activity of the plant leaf [22] has been reported. Also, qualitative phytochemical screening of the plant revealed the presence of alkaloids and flavonoids in relative abundance amongst other phytochemicals of pharmacological importance [22]. The aim of this study was to investigate the antidiarrheal activity of crude *Calopogonium mucunoides* ethanol extract, as well as the antibacterial activities of its alkaloid and flavonoid extracts, with the view to validating the therapeutic importance of the pant in the folklore management of diarrhea and microbial infections in Nigeria.

Fig. 1: Pictorial view of *Calopogonium mucunoides* leaves

MATERIALS AND METHODS

Plant Material: The fresh leaves of *Calopogonium mucunoides* were collected from Nsukka, Enugu State, Nigeria and identified by Mr. Alphred Ozioko of the Bioresource Development and Conservation Programme Research Centre, Nsukka, Nigeria; while the voucher specimens were deposited in the *herbarium* unit of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka, Nigeria.

Animals: Albino (Wistar) rats of both sexes between the ages of 8-10 weeks old with average weight of 150 -200g were used for the study. The animals were kept in well-aerated stainless steel wire cages in the Department of Biochemistry animal house and were allowed to acclimatize for two weeks in environmentally normal tropical housing (17+2°C, relative humidity 70 - 90% with natural light/dark cycles at approximately 12h periodicity). They were maintained on standard animal feeds and drinking water *ad libitum* during the stabilization period. The laboratory animals were used in accordance with laboratory practice regulation and the principle of humane laboratory animal care as documented by Zimmermann [23].

Chemicals: The chemicals used in this work were of analytical grade and were purchased from reputable manufacturers such as Riedel De Haen (RDH), Germany; British Drug Houses (BDH), Dorset, U.K; May and Baker, Dagenham, England; Sigma Chemical Company, St. Louis, U.S.A; Merck Darmstadt, England and Hopkin and Williams, Essex, England.

Preparation of the Crude Ethanol Extract: The leaves of *Calopogonium mucunoides* were washed with distilled water, shade-dried for three weeks and homogenized into fine powder using an electric blender. A quantity, 500g of the air-dried and powdered plant material was macerated in 2500 ml of 95% ethanol for 24 hours at room temperature. The mixture was filtered and the filtrate was concentrated in vacuum at 40°C. and yield was determined. It was stored in an air-tight container at 4°C for further use.

Quantitative Phytochemical Analysis: Quantitative phytochemical analysis was done using standard methods as described by Harbourne [24] and Onwuka [25].

Antidiarrheal Studies: Castor oil-induced diarrhea was evaluated using the method of Awouters *et al*. [26]. Wistar albino rats of either sex were divided into 5 groups. Group 1 served as the standard and received the standard antidiarrheal drug, diphenoxylate hydrochloride (Lomotil) at the dose of 2.5 mg/kg orally. Group 2 served as the control and received the vehicle (Normal saline) at the dose of 5ml/kg orally. Groups 3 and 4 served as the test groups and received the ethanol leaf extract of *Calapagonium mucunoides* at doses of 200 and 400 mg/kg orally, respectively. Diarrhea was induced by oral administration of 0.5 ml castor oil to each rat, one hour after the above treatments. During an observation time of 4h (Time of onset of diarrhea) frequency of defecation by the animals was recorded. Castor oil-induced intestinal motility test was evaluated using the method of Mascolo *et al*. [27].

Flavonoids Extraction: Following the method of Subramanian and Nagarjan [28] one hundred grams of the finely powdered sample was Soxhlet -extracted with 80% hot methanol (300 ml) on a water bath for 24 hrs and filtered. The filtrate was re-extracted successively with petroleum ether (Fraction I), ethyl ether (Fraction II) and ethyl acetate (Fraction III) using separating funnel. Petroleum ether fraction was discarded as being rich in fatty substances, whereas ethyl ether and ethyl acetate fractions were analyzed for free and bound flavonoids respectively. The ethyl acetate fraction of each of the samples was hydrolyzed by refluxing with 7% H_2SO_4 for 2hrs (For removal of bounded sugars) and the filtrate was extracted with ethyl acetate in separating funnel. Ethyl acetate extract obtained was washed with distilled water to neutrality. Ethyl ether (Free flavonoids) and ethyl acetate fractions (Bound flavonoids) were dried in vacuum and weighed. The extracts thus obtained were stored at 4°C in air tight glass containers and were suspended in their respective solvents to get 10 mg/ml for antimicrobial assay.

Alkaloids Extraction: Alkaloids were extracted from the plant by well-established method [29] after preliminary detection of alkaloids. Finely powered sample (100 g) of plant leaves were extracted with 10% acetic acid in ethanol for 4hrs. Extracts were concentrated and were made alkaline by NH_4OH. The precipitate thus obtained was collected by centrifugation, washed with 1% NH_4OH, filtered, dried in vaccum and weighed. Extracts thus obtained were stored at 4°C in air tight glass vials for further use for antimicrobial assay.

Test Microorganisms: Clinical isolates of bacterial pathogens used for this work were collected from the Department of Microbiology, University of Nigeria, Nsukka; which are two gram- negative bacteria, *Escherichia coli* and *Salmonella typhimurium* and two gram- positive bacteria *Staphylococcus aureus* and *Bacillus cereus*. The bacteria isolates were further purified by sub culturing each isolate into fresh plates of nutrient agar. The pure isolates were identified using standard biochemical methods [30].

Antimicrobial Activity Assay: Antimicrobial activity of the plant extract was evaluated using the agar-well diffusion technique of Perez *et al*. [31]. A volume of 20ml of sterilized agar was poured into sterile Petri plates. After solidification were swabbed on the respective plates. The wells were punched over their agar plates using sterile gel puncher. The punched agars were filled with 100ml of respective plant extracts at concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml in each case. The plates were incubated at 38°C for 24 hours. Chloramphenicol (0.05%) was used as positive control and analysis was done in triplicates. Normal saline was used as negative control. The antibacterial activity was assayed by measuring the diameter of inhibition zone

(IZ) formed around the well. Minimum inhibitory concentration (MIC) was obtained by noting the lowest concentration of extract which had activity against the test microorganisms [11].

Statistical Analysis: The data obtained in this study, (Where appropriate) were evaluated using the one-way analysis of variance (ANOVA) test between two mean groups, control and test groups, followed by student's t-test. Significant levels were at $p<0.05$.

RESULTS

Percentage Yield and Phytochemical Composition of the Ethanol Extract of the Leaves of *Calopogonium mucunoides*: The percentage yield of ethanol extract was 30%. Table 1 shows the result of the quantitative phytochemical constituents of the sample. The ethanol extract constitutes were respectively; tannins (0.2987±0.306 mg/g), flavonoids (0.6600 ± 0.418 mg/g), alkaloids (0.3403 ± 0.279mg/g) and terpenoids (0.2300 ± 0.051mg/g).

Table 2 shows that rats treated with graded doses of the ethanol extract (200mg/kg, 400mg/kg), exhibited significant ($p <0.05$) inhibition of the frequency of defecation when compared with the untreated group of rats, in a dose-dependent manner. The Table further reveals that the inhibitory effects were similar to that of Lomotil (standard antidiarrhoeal drug) at 2.5mg/kg b.w.

Effect of the Ethanol Extract of *Calopogonium mucunoides* on Gastrointestinal Motility in Rats: The data presented in Fig. 2 show that both the rats treated with graded doses of the ethanol extract (200mg/kg, 400mg/kg) and the rats treated with Lomotil (standard anti diarrheal drug) at 2.5mg/kg b.w., significantly ($p <0.05$) decreased the propulsive movement of the charcoal meal through the GIT compared to the rats administered normal saline (negative control group).

Antibacterial Activity Studies:

The Inhibitory Profile of Chloramphenicol (A Standard Antibiotic Drug) on *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*: Fig. 3 reveals that chloramphenicol had the highest inhibitory effect against E. coli with minimum inhibitory concentration (MIC) of 6.2 mg/ml and IZ=10.00 ±1.14mm., followed by *S. typhi* (MIC = 6.2mg/ml), *B. cereus* (MIC = 6.2 mg/ml) and *S. aureus* (MIC = 12.5 mg/ml) in that order. The figure further shows that the inhibitory effect was concentration- dependent.

Table 1: Percentage yield and phytochemical composition of the ethanol extract of the leaves of *Calopogonium mucunoides*

S/N	Phytochemical composition	Mean in mg/g ± SD
1	Tannins	0.2987 ± 0.306
2	Alkaloids	0.3403 ± 0.279
3	Flavonoids	0.6600 ± 0.418
4	Terpenoids	0.2300 ± 0.051

Each value is the mean of 3 determinations ± Standard deviation.

Table 2: Effect of the ethanol leaf extract of *Calopogonium mucunoides* on the frequency of defecation

S/N	Group	Pretreatment	Frequency of defecation Mean + SD (%)
1.	Lomotil	2.5 mg/kg	*0.50 = 0.31
2.	Normal Saline	5 ml/kg	3.14 = 0.42
3.	Methanol extract	200mg/kg	*1.63 = 0.19
4.	Ethanol extract	400mg/kg	*1.25 = 0.08

Each value represents the mean ±S. D. (n=4). *Significantly different at $p<0.05$ relative to control (Group 2)

The Inhibitory Profile of Free Flavonoids from *C. mucunoides* on *Bacillus cerus*, *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*: Fig. 4 shows that free flavonoids had highest inhibitory effect on *S. typhi* with minimum inhibitory concentration of 12.5 mg/ml and IZ=18.20 ±1.94mm, followed *by S. aureus, E. coli. and B. cereus* with minimum inhibitory concentrations of 12.5mg/ml, 25mg/ml and 25mg/ml respectively. Free flavonoids showed better antibacterial activity at 25-100 mg/ml against *B. cereus* and *S. typhi* than chloramphenicol, the standard antibiotic drug (Fig. 3), but had less inhibitory activity against *E. coli* and *S. aureus* when compared with the standard drug. The figure further shows that the inhibitory effect was concentration- dependent.

The Inhibitory Profile of Bound Flavonoids from *C. mucunoides* on *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*: Table 3 shows that the bound flavonoids of *C. mucunoides* had inhibitory effect only against *B. cereus* at 25-100 mg/ml (IZ=0 9 ± 0.50mm, 10 ± 0.5mm and 13±0 .51mm) with MIC of 25mg/ml but showed no inhibition at 6.25-12.5 mg/ml. It showed no activity against the rest of the spectrum of bacteria used. The inhibitory activity of bound flavonoids against *B. cereus* was less than that of chloramphenicol (Fig. 3).

The Inhibitory Profile of Alkaloids from *C. mucunoides* on *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*: Fig. 5 shows that alkaloids from *C. mucunoides* had highest efficacy on *S. typhi*

Table 3: The inhibitory profile of bound flavonoid (BF) of *C. Mucunoides*

Zones of inhibition (mm)				
Conc mg/ml	*B. cereus*	*S. typhi*	*E. coli*	*S. aureus*
100	13± 0.51	nil	nil	nil
50	10 ± 5.5	nil	Nil	nil
25	0 9 ± 0.50	nil	nil	nil
12.5	nil	nil	Nil	nil
6.25	Nil	nil	nil	nil

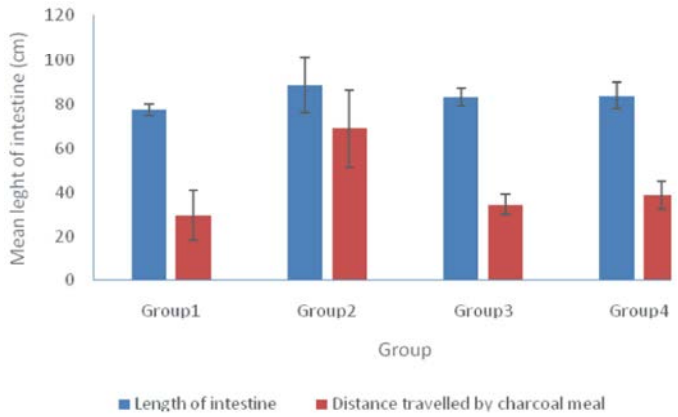

Fig. 2: Effect of the ethanol extract of *Calopogonium mucunoides* on the distance traveled by charcoal meal in rats
Group 1= 2.5mg/kg b.w of lomotil (standard antidiarrheal drug)
Group 2= 5ml/kg b.w. of normal saline (negative control)
Group 3= 200mg/kg b.w of extract
Group 4= 400mg/kg b.w of extract

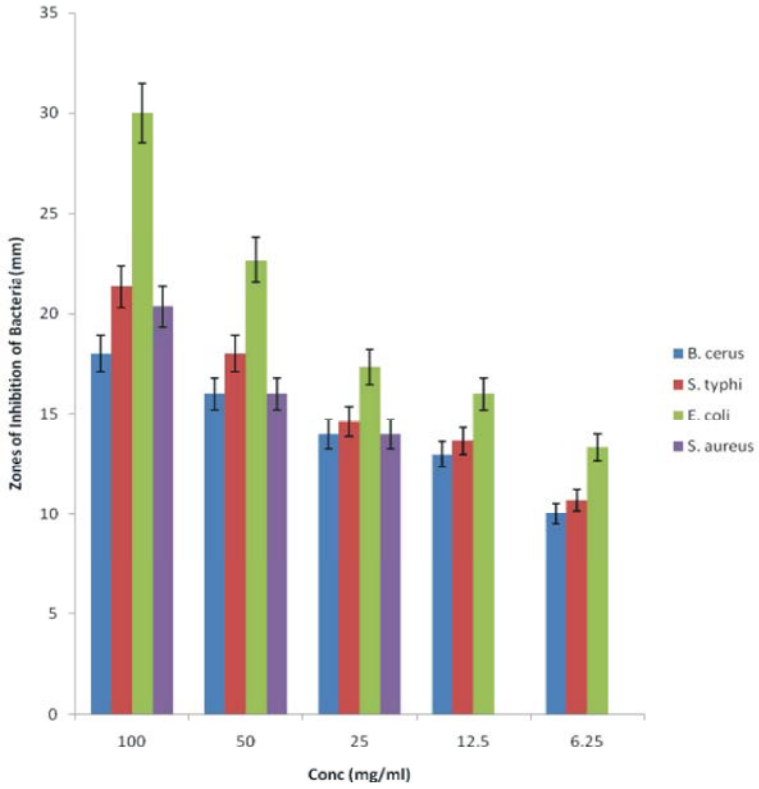

Fig. 3: The inhibitory profile of chloramphenicol

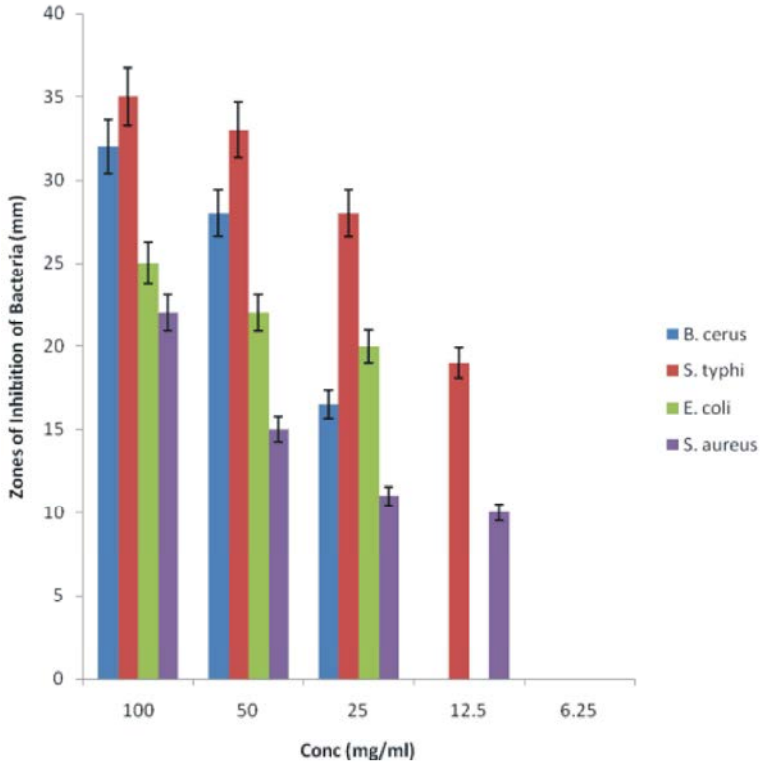

Fig. 4: The inhibitory profile of free flavonoids of *Calapagonium mucunoides*

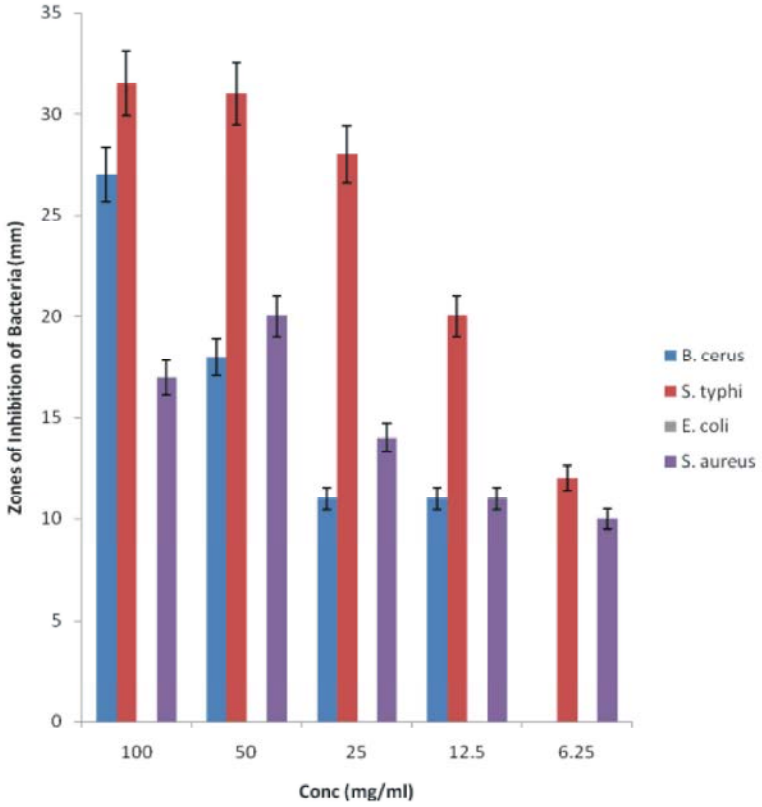

Fig. 5: The inhibitory profile of alkaloids of *Calapagonium mucunoides*

Table 4: The inhibitory profile of ethanol extract of *Calapagonium mucunoides*

Zones of inhibition (mm)				
Conc mg/ml	*B. cereus*	*S. typhi*	*E. coli*	*S. aureus*
100	nil	16 ±1.22	17±0.03	12±2.57
50	nil	10.5 ±0.58	12.5±1.53	nil
25	nil	nil	nil	nil
12.5	nil	Nil	Nil	nil
6.25	Nil	nil	nil	nil

Table 5: The Minimium inhibitory concentrations (MIC) of chloramphenicol and *Calopogonium mucunoides* extracts

	Minimium inhibitory concentrations (mg/ml)				
Bacteria isolates	Chloramphenicol	Free flavonoids	Bound flavonoids	Alkaloids	Ethanol extract
B. cereus	6.25	25	25	12.5	-
S. typhi	6.25	12.25	-	6.25	50
E. coli	6.25	25	-	-	50
S. aureus	25	12.25	12.25	-	100

(MIC = 6.25mg/ml, IZ=12 ± 1.08mm). This is followed by *S. aureus* (MIC = 6.25mg/ml, IZ=10±1.16) and B. *cereus* (MIC =12.5mg/ml, IZ=12 ±1.15). The figure further reveals that the alkaloids had no inhibitory effect against *E. coli* but showed higher activity at 50-100 mg/ml against *B. cereus* and *S. typhi* and generally less activity in others when compared to the standard drug (Fig. 3). The figure further shows that the inhibitory effect was concentration- dependent.

The Inhibitory Profile of Ethanolic Extract on *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*: Table 4 shows that the ethanol extract of *C. mucunoides* inhibited the growth of *S. typhi* (MIC=50mg/ml, IZ=10.5 ±0.58), *E. coli* (MIC =50mg/ml, IZ =12.5±1.53) and *S. aureus* (MIC = 100mg/ml, IZ =12±2.57) at higher concentrations, but had no effect on *B. cerus*. At conccentraion of 100mg/ml, its activity was against all but *B. cereus* but was less when compared to Chloramphenicol, the standard drug (Fig. 3).

The data presented in Table 5 reveal that chloramphenicol, (the standard antibacterial drug) exhibited higher potency against *B. cereus, S. typhi and E. coli*, than the plant extracts as shown by their respective minimum inhibitory concentrations. However, alkaloids showed similar activity against S.typhi to the standard antibacterial drug. The data further show that free flavonoids and bound flavonoids had higher activity against *S. aureus* than the standard antibacterial drug.

DISCUSSION

In this study, the phytochemical constituents, antidiarrheal effect and antibacterial activity of the leaf extracts of *Calopogonium mucunoides* were investigated with a view to validating their potential in folkloric management of diarrhoea and bacterial infections. Previous phytochemical screening of the plant extract revealed the presence of glycosides, alkaloids, cardiac glycosides, carbohydrates, flavonoids, tannins, saponins, resins, steroidal rings and steroidal terpenes. In the present study, quantitative phytochemical analysis of the plant leaves revealed the presence of alkaloids and flavonoids in high concentrations; tannins and terpenoids in moderate concentrations, while saponins were present in low concentrations. Several studies have validated the use of antidiarrhoeal medicinal plants by investigating the biological activity of extracts of such plants [32, 33]. Antidiarrheal activity of medicinal plants has been ascribed to the presence of bioactive constituents such as flavonoids and tannins [4]. Diarrhea may be characterized as abnormally frequent defecation of faeces of low consistency which may be due to a disturbance in the transport of water and electrolytes in the intestines. Despite the multiplicity of etiologies, the 4 major mechanisms responsible for the pathophysiology in water and electrolytes transport are (i), increased luminal osmolarity (ii), increased electrolytes secretion (iii), decreased electrolytes adsorption and (iv), deranged intestinal motility [34]. The ethanol extract of the plant leaves exhibited antidiarrheal activity in castor oil- induced model of diarrhoea in rats. It showed significant ($p < 0.05$) inhibition of gastrointestinal transit as well as a significant ($p < 0.05$) reduction in the frequency of defecation when compared to the negative control. This result is consistent with the findings of previous studies [32, 33]. It is widely known that castor oil is metabolized by lipase into ricinoleic acid in the intestinal mucosa, resulting in the release of inflammatory mediators such as prostaglandins, histamine

and leucotrienes. The prostaglandins thus released promote vasodilation, smooth muscle contraction and mucus secretion in the small intestines [34] that are characteristic of diarrhoeic condition. Flavonoids present in anti-diarrhoeal plants are known to inhibit the activities of cycloxygenase 1 and 2 (COX -1, COX – 2) and lipo –oxygenase (LOX) [35] with the consequent inhibition of synthesis of prostaglandins and other inflammatory mediators. Anti-diarrheal activity of flavonoids has also been ascribed to their ability to inhibit intestinal motility and hydroelecrolytic secretions which are known to be altered in diarrheic condition [36]. Though several constituents are present in the ethanol leaf extract of *Calapagonium mucunoides*, it is possible that flavonoids, acting singly or in combination with other constituents present in the plant extract produced the observed anti –diarrheal effect. The antidiarrheal effect exhibited by the extract was similar to that of lomotil (a standard antidiarrhea drug), suggesting similar mechanism of action.

In the antibacterial activity studies, the ethanol extract, alkaloid extract, flavonoid extract of *Calapagonium mucunoides* as well as chloramphenicol (a reference antibiotic drug) showed reasonable but varied antibacterial activities against the pathogenic microorganisms used in this study, as demonstrated by measuring the diameters of inhibition zone and determining the minimum inhibitory concentrations. This is consistent with several reports showing the antimicrobial properties of plant phytochemicals under laboratory conditions [37-39]. The free flavonoids from *Calapagonium mucunoides* was most efficacious among the plant extracts, (exhibiting the widest inhibition zone against both gram- positive and gram- negative bacteria) and showed a better antibacterial activity at 25-100 mg/ml against *B. cereus* and *S. typhi* than chloramphenicol (a standard antibiotic drug), but had a less activity against *E. coli* and *S. aureus* when compared to the standard drug. This was followed by its alkaloids which showed higher activity at 50-100 mg/ml against *B. cereus* and *S. typhi* and generally less activity in others when compared with the standard drug. The ethanol extract had activity against all test organisms but *B. cereus,* but the activity was less than that of chloramphenicol (the standard drug). It is likely that flavonoids and alkaloids, acting dually or in combination with other phytochemicals caused the observed effect of the extract, Bound flavonoids showed the least activity against the spectrum of bacteria used, showing activity only against *B. cereus* which was less when compared to the reference standard drug. In the present study, free flavonoids showed a higher antimicrobial activity compared to bound flavonoids. This may be attributed to the functional groups in flavonoids [9, 10].The selective antibacterial activity observed points to the fact that hydroxyl (OH) and aldehyde (-CHO), among other groups present in flavonoids are very important for their reactions to other molecules as stated before [40]. It seemed that binding of other moieties to these functional groups in flavonoids greatly reduced their antibacterial activity [2]. Alkaloids and flavonoids were shown to possess more antibacterial activity than ethanol extract, but the inability of the ethanol extract to inhibit *B. cereus* at all and *S. aureus* reasonably could be as a result of interference of other bioactive components in the extract which might not be efficacious in inhibiting bacterial growth, giving rise to antagonistic effect instead of a synergistic effect. The variations observed in the susceptibility pattern of the test organisms to the bioactive molecules in this study may be attributed to their chemical nature and to their different mechanisms of antibacterial action. Some flavonoids are known to affect bacterial cell membrane permeability by disrupting the membrane integrity while some others inhibit DNA gyrase, thereby inhibiting DNA synthesis [10]. Tanaka *et al* [41] reported that bisindole monoterpenoid alkaloids exert their antibacterial action by acting as DNA intercalating agents or like topoisomerase inhibitors. The outer membrane lipopolisaccharides of gram- negative bacteria prevents certain drugs and antibiotics from penetrating the cell, partially accounting for why gram-negative bacteria are resistant to multiple drugs and are generally more resistant to antibiotics than are gram-positive bacteria [17].These differences observed seemed to suggest different mechanisms of action for the antibacterial agents.

CONCLUSION

Results of the study showed that *Calopogonium mucunoides* possesses antidiarrheal activity as well as antibacterial potential against the test pathogens (gram-positive and gram-negative bacteria), which are major causative organisms of various human diseases, including diarrhea. The antibacterial activity exhibited by the plant extracts against the pathogenic test organisms used in this study suggests the potentials of the plant extracts in the therapy of infectious diarrhoea and other enteric diseases. The findings further reveal that the plant

contains phytochemical substances that can be used as components of new antimicrobial agents with potential activity against multi-drug resistant bacteria pathogens. The study has also given credence to the traditional usage of this plant as anti- diarrhoeal and antibacterial agent.

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