



UPDATE PUBLISHING

ISSN: 2521-3903

Evaluation of antidiarrhea and antimicrobial activities of methanol extract leaves of *Gmelina arborea*

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ABSTRACT

Since ancient times, plants have commonly been used in folk medicine for the treatment of various ailments. This work evaluated the efficacy of methanol extract of *Gmelina arborea* leaf as antidiarrheal and antimicrobial agent. The antimicrobial activity was conducted using standard microbiological techniques and antidiarrheal activity of the extract was carried out with 24 Wistar rats which were randomly grouped into 6 (n = 4). Group 1 served as control and received distilled water (10 ml/kg), group 2 received Loperamide, groups 3 was administered 10ml/kg of castor oil only (negative control), group 4 through 6 received *Gmelina arborea* at different doses of 200mg/kg-800mg/kg respectively. Diarrheal was induced using oral administration of 10 ml/kg of castor oil. Animals were kept in separate metabolic cages with transparent plastic container beneath the cage to collect faeces. Latency time, frequency of defecation, total surface of impregnation and fresh total stools weight were measured for 8 hrs. The results of the antimicrobial activity of *Gmelina arborea* leaf extract showed that *Staphylococcus aureus* and *E. coli* were the most susceptible strains to *Gmelina arborea* extract with zone of inhibition of 9.73 ± 0.64 mm at 1000mg/kg of the extract. The faecal drops at 2/3hrs was significantly different ($p < 0.05$) in all the extract groups when compared to the untreated group, however the extract treated groups showed non-significant ($p > 0.05$) difference when compared to the standard drug. The findings from this study suggested that methanol leaf extract of *Gmelina arborea* contain pharmacologically active substances with antidiarrhea and antimicrobial properties.

KEYWORDS: Antidiarrheal, Antimicrobial, Latency time, Frequency of defecation, Total surface of impregnation, Fresh total stools weight and extract

Received: March 7, 2020
Accepted: June 22, 2020
Published: July 12, 2020

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INTRODUCTION

Since ancient times, plants have commonly been used in folk medicine for the treatment of various ailments. Most of the scientists have investigated the plant products to find out their antimicrobial properties [1]. Using higher plants, various diseases including infectious diseases are treated [2]. Each and every plant on the earth contains an important compound which has antimicrobial properties [3]. On the earth, there are more than 300,000 plant species and only about 2% of plants have been checked so far, for their antimicrobial properties. Plant extracts from more than 157 plant families which have potential antimicrobial properties have been discovered. Therefore it is clearly indicated that the plants are main centre of interest in near future for the discovery of new antimicrobial drugs.

Diarrhea is characterized as rapid movement of faecal matter through intestine resulting in poor absorption of water,

nutritive elements and electrolytes producing abnormal frequent evacuation of watery stools. According to world health organization, it is the one of the most common cause of morbidity and mortality in many developing countries effecting mainly the infants and children's [4]. The major causative agents of diarrhoea in humans include: *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* [5].

Gmelina is an important genus in the family of Verbenaceae and is widespread across Southern China, Bangladesh, Myanmar, Thailand, Vietnam, Indonesia and Philippines [6]. *Gmelina arborea* is commonly called "beech wood" in English. It is an unarmed, moderately sized to large deciduous tree, about 30m or more in height and a diameter of up to 4.5m. The plant, *G. arborea* was reported to have several medicinal properties such as aphrodisiac, astringent, antidiabetic, diuretic, and tonic characteristics. In Indian folk medicine, the root decoction was used to treat abdominal tumors [7] (Chellappan and Pemiah, 2014), and as laxative. It is also a

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folk remedy for anthrax, bilious disorder, bites, blood disorder, cholera, convulsions, diarrhea, rheumatism, snake bite and sores [8]. The fruits were used to treat alopecia, anemia, and leprosy. The leaves were used to treat high blood pressure, malaria, scorpion and insect stings [9]. *Gmelina arborea* extracts have also been used by traditional practitioners in Nigeria to treat diarrhea [10].

MATERIALS AND METHODS

Plant Materials

The leaves of *Gmelina arborea* was collected in the month of September 2019 from National Root Crop Research Institute, Umudike (NRCRI) Abia State, Nigeria and taxonomically identified by Dr. G. Omosun of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. The leave was washed with distilled water and dried for about seven days at room temperature.

The dried leaves were pulverized into fine powder using Pulverizer (5126 TP) and preserved in cellophane bags until when used.

Extract Preparation

Five hundred gram (500g) of powdered leave was macerated in 2.5L of 98% methanol at room temperature for 72h. It was continuously mixed and then filtered using a filter paper (Whatman size No.1). The filtrate was dried in a water bath at 45°C and concentrate was kept in air tight bottle at 4°C until used [11].

Experimental Animals

Healthy adult wister albino rats weighing 100–150g were procured from animal house farm of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. They were housed in standard transparent cages with wheat husk bedding, renewed every 24h. They were kept under controlled room temperature and humidity (18 to 29 °C; 30 to 70%) in a 12h light-dark cycle. Animals were acclimatized for two weeks to laboratory conditions before starting the experiment. The animals were given standard laboratory diet and water *ad libitum*. Care of experimental animals was taken as per the guidelines given by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Animal Welfare Division), Umuahia Abia State, Nigeria.

Experimental Design

The animals were divided into six different groups with 4 animals in each groups. The groups are thus;

- Group 1 – Normal (Not induced) – Feed + water
- Group 2 – (Control Positive) – Castor oil + Loperamide + feed + water
- Group 3 – (Control Negative) – Castor oil + feed + water

- Group 4 – Castor oil + 200mg/kg extract + feed + water [200mg/kg]
- Group 5 – Castor oil + 400mg/kg extract + feed + water [400mg/kg]
- Group 6 – Castor oil + 800mg/kg extract + feed + water [800mg/kg]

Induction of Diarrhea in Rats

The animals were starved for 18 hours but had free access to water. 10 ml/kg of castor oil were orally administered to all groups. Animals were kept in separate metabolic cages with transparent plastic container beneath the cage and lined with Whatmann paper to collect faces. Following castor oil administration, parameters such as latency time, frequency of defecation, total surface of impregnation and fresh total stools weight were measured for an 8 h period and compared with those of the control.

Test Organisms (Energetic Bacteria)

The microbial cultures *Escherichia coli*, *staphylococcus aureus*, *salmonella spp*, *candida Albican*, *strep. pneumoniae* were obtained from the Department of Microbiology, Michael Okpara University of Agriculture, Umudike. They were properly identified and preserved on agar slants at 37°C.

Determinations of Antimicrobial Susceptibility of the Plant Extracts

The agar diffusion method by Lino and Deogracious (2006) and 0.5 McFarland for the test organisms according to Baker and Thornsberg (1983) was used for this purpose. The concentration of the extract used were 1000, 500, 250, and 125 mg/ml) and Pefloxacin (12.5 mg/ml) was used as standard drug control. Antimicrobial activity was determined by measurement of the diameter of zone of inhibition (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined by the macro broth dilution methods [12,13] at various concentrations (1000 - 125mg/ml).

RESULT AND DISCUSSION

The results of the antimicrobial activity of *Gmelina arborea* leaf extract suggested that *Candida albican* was the most resistant strain to the plant extract followed by *Strep pneumonia* and *Samonella SPP* while *Staph. aureus* and *E. coli* were the most susceptible strains to *Gmelina arborea* extract

In the figure above the *Gmelina arborea* leaf extract showed a significant decrease ($p < 0.05$) when compared to the standard drug. Though the *Gmelina arborea* leaf extract was able to inhibit the growth of microorganisms but not as potent as the standard drug.

Table 1: Diameter of zone of inhibition for various concentration of *Gmelina arborea* extract.

Test organisms	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml
<i>Staph Aureus</i>	9.73±0.64 ^a	5.37±0.60 ^c	2.47±0.80 ^b	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a
<i>E. Coli</i>	9.13±0.23 ^e	5.80±0.20 ^d	4.07±0.12 ^c	2.30±0.44 ^b	0.0±0.0 ^a	0.0±0.0 ^a
<i>Samonella SPP</i>	8.93±0.12 ^c	5.20±0.34 ^b	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a
<i>Strep pneumonia</i>	6.20±0.20 ^c	2.03±0.58 ^b	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a
<i>Candida albican</i>	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a

Means with the same alphabets as superscripts are non-significantly (p>0.05) different

The faecal drops at 1hr were not significantly (p>0.05) different in all the groups. At 2/3hrs the faecal drop was significantly different (p<0.05) in all the extract groups when compared to the untreated group, however the extract groups was not significantly (p>0.05) different when compared to the standard drug.

The weight of the stool at 1 hr in all the groups was not significantly (p>0.05) different. At 2hrs group administered standard drug, 200mg/kg and 400mg/kg of the extract were not significantly (p>0.05) different when compared to the untreated group, however the group administered 800mg/kg of the extract was significantly (p<0.05) different when compared to the untreated group. At 3hrs group administered 200mg/kg were not significantly (p>0.05) different when compared to the untreated group and the standard drug while group 400mg/kg and 800mg/kg of the extract were significantly (p<0.05) different when compared to the untreated group.

Castor oil induced diarrhea was inhibited after 3hrs in group administered 400mg/kg and 800mg/kg of the extract when compared to the untreated group.

DISCUSSION

The use of plants as natural therapies in management of diseases is getting much attention [14]. Many studies have established the usefulness of medicinal plants as a great source for the isolation of active principles for drug formulation [15,16,17]. *G. arborea* plant extracts demonstrated antimicrobial activity against *E. coli*, *Staph. aureus*, *Samonella SPP* (Table 1). *Candida albican* was the most resistant strain to the plant extract followed by *Strep Pneumonia*. At concentrations of 500mg/ml, 250mg/ml and 125mg/ml, *E. coli* had the highest zones of inhibitions (5.80±0.20mm) followed by *Staph aureus*. This indicated that *E. coli* was the most susceptible to the leaf extract of *G. arborea* at the various concentrations used. At concentration of 1000 mg/ml, all the test microorganisms had significant decrease of inhibition when compared to the commonly used antibiotic (Figure 1).

The minimum inhibition concentration of the test organisms were 125mg/ml for *E. coli*, 500mg/ml for *Staph aureus* and *Samonella SPP*, but no inhibitory effect on *Candida albican* (Table 2). This is similar to the finding of Oboh *et al.* [18] who reported inhibitory effects of ethanolic extract of *T. occidentalis* on *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus spp.* but no inhibitory effect on *Salmonella typhi*. Diarrhea results from an imbalance between the absorptive and secretory mechanisms

Table 2: Microbial Inhibition Concentration of *Gmelina arborea* leaf extract against microrganisms (mg/ml)

Test Organisms	MIC Value (mg/ml)
<i>Staph aureus</i>	250 mg/ml
<i>E. coli</i>	125 mg/ml
<i>Samonella SPP</i>	500 mg/ml
<i>Strep pneumonia</i>	500 mg/ml
<i>Candida albican</i>	<1000 mg/ml

The inhibitory effect of *Gmelina arborea* leaf extract started at 125mg/ml with inhibition zones of 2.30±0.44mm against *E. coli* and 250mg/ml with inhibition zones of 2.47±0.80mm against *Staph aureus*

Table 3: Effect of methanol leaf extract of *Gmelina arborea* on Faecal drops of castor oil induced diarrhea in wistar rat

Groups	Faecal drops		
	1hr	2hrs	3hrs
Untreated	5.40±2.07 ^a	3.80±0.84 ^b	3.20±0.45 ^b
Std Drug	4.00±0.71 ^a	1.60±1.34 ^a	0.60±0.89 ^a
200 mg/kg	4.20±0.84 ^a	2.60±0.89 ^{a,b}	1.80±1.30 ^{a,b}
400 mg/kg	3.80±0.84 ^a	2.00±1.00 ^{a,b}	0.80±0.84 ^a
800 mg/kg	4.40±0.55 ^a	1.00±1.00 ^a	0.20±0.45 ^a

Means with the same alphabets as superscripts are non-significantly (p>0.05) different.

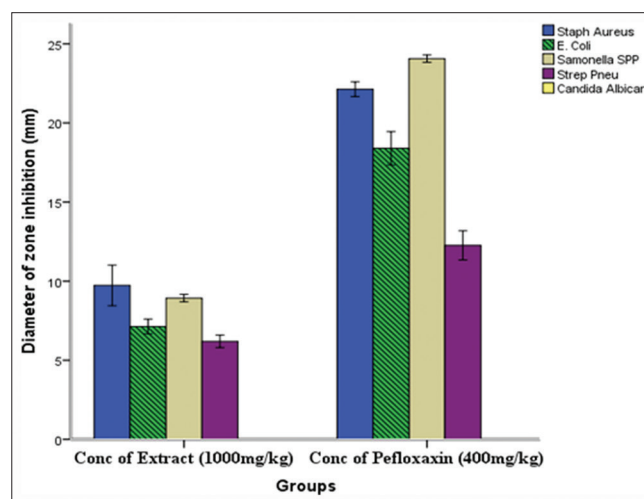


Figure 1: Antimicrobial activity of ethanol leaf extract of *Gmelina arborea*

in the intestinal tract accompanied by hurry, leading to an excess loss of fluid in the feces [19].

The use of castor oil for the study of diarrhea in animal model has been reported. Diarrhea induced by castor oil

Table 4: Effect of methanol leaf extract of *Gmelina arborea* on Weight of stool (g) of castor oil induced diarrhea in wistar rats

Groups	Weight of stool (g)		
	1hr	2hrs	3hrs
Untreated	3.88±0.84 ^a	2.74±0.62 ^b	2.48±0.72 ^b
Std Drug	4.08±1.55 ^a	1.59±1.03 ^{a, b}	1.14±1.07 ^{a, b}
200 mg/kg	3.88±0.19 ^a	2.52±1.06 ^{a, b}	1.32±0.93 ^{a, b}
400 mg/kg	3.76±0.53 ^a	2.27±1.34 ^{a, b}	0.62±0.65 ^a
800 mg/kg	4.10±0.16 ^a	0.79±0.35 ^a	0.16±0.36 ^a

Means with the same alphabets as superscripts are non-significantly ($p > 0.05$) different

Table 5: Effect of methanol leaf extract of *Gmelina arborea* on % inhibition of diarrhea of castor oil induced diarrhea in wistar rats

Groups	% inhibition of diarrhea		
	1hr	2hrs	3hrs
Untreated	- ^a	- ^a	- ^a
Std Drug	28.00 ^a	51.00 ^{a, b}	81.66 ^{b, c}
200 mg/kg	17.88 ^a	28.00 ^{a, b}	49.99 ^{a, b}
400 mg/kg	21.33 ^a	49.66 ^{a, b}	76.66 ^{b, c}
800 mg/kg	8.88 ^a	68.33 ^b	93.33 ^c

Means with the same alphabets as superscripts are non-significantly ($p > 0.05$) different

results from the action of ricinoleic acid which causes the irritation and inflammation of the intestinal mucosa leading to prostaglandins (PGE₂) release. The released PGE₂ stimulates gastrointestinal motility and secretion of water and electrolytes [20], thus inducing an increase in the peristalsis and an intestinal hyper secretion of fluid. The fecal drops at 1hr were not significantly ($p > 0.05$) different in all the groups. At 2/3hrs the fecal drop and stool weight was significantly different ($p < 0.05$) in all the extract treated groups when compared to the untreated group (Tables 3 and 4), the extract at 400mg/kg and 800mg/kg showed significant ($p < 0.05$) increased in the % inhibition of diarrhea when compared to the untreated group (Table 5). This could be attributed to the reported bioactive compound in *G. arborea*.

CONCLUSION

The methanol leaf extract of *Gmelina arborea* contain pharmacologically active substances with antidiarrhea and antimicrobial properties. This finding from this study suggested that the extract may be useful in management of diarrheal and infection.

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