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ANTIOXIDANT AND ANTIDIARRHOEAL ACTIVITY OF *Manniophyton africanum* LEAF EXTRACT IN MICE

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ABSTRACT
The antidiarrhoeic activity of the methanolic leaf extract of *Manniophyton africanum* (MEMA) has been evaluated out in mice using different models (Castor oil-induced diarrhoea, effects on gastrointestinal motility, and castor oil-induced gastric enteropooling). MEMA (200,400 and 600 mg/kg, p.o.) inhibited at 5% significance level, the frequency of defecation and reduced the wetness of faecal droppings in castor oil-induced diarrhoea, markedly inhibited the movement of charcoal meal plug through the gastrointestinal tract, in a dose dependent manner, comparable to diphenoxylate and atropine. It however had no effect in the intra luminal fluid content in the castor oil-induced gastric enteropooling. Diphenoxylate (5 mg/kg) and atropine (3 mg/kg) were used as the reference drugs. MEMA were also found to be possessing marginal (52-66%) free radical scavenging activities *in vitro* using the DPPH and FRAP models.
The remarkable antidiarrhoeal property of MEMA *in vivo* gives credence to its use in the management of a wide range of diarrhoeal state.

Keywords: Manniophyton africanum; antioxidants; antidiarrhoeal activity; castor oil-induced gastric enteropooling

INTRODUCTION

Diarrhoea is defined as the frequent passage of liquid faeces and involves both an increase in the motility of the gastrointestinal tract along with increased secretions and a decrease in the absorption of fluid and thus a loss of electrolytes (particularly Na^+) and water (Rang *et al*, 2003). Causes of diarrhea include infectious agents, plant toxins, gastrointestinal disorders such as inflammatory and dysmotility problems of gastrointestinal tract and substances that increase gastrointestinal tract secretions (Alquist *et al*, 2001).

Diarrhoea has been recognized as one of the most important health problems in developing countries (Snyder and Merson, 1982). In Nigeria, diarrhea remains the number one killer among children aged 1 –5 years and worldwide, the disease accounts for 4-5 million deaths among humans annually (Audu *et al*, 2000). Also in Veterinary medicine, diarrhoea occurs as symptoms or complications of most viral, bacteria, parasitic and nutritional diseases (Susan and Mays, 2005).

To overcome the menace of diarrhoeal diseases in developing countries, the World Health Organization (WHO) has included a programme for control of diarrhoea which involves the use of traditional herbal medicine (Snyder and Merson, 1982) and medicinal plants have been documented to have advantage in toxicity considerations based on their long term use and one might expect bioactive compounds obtained from such plants to have low animal and human toxicity (Fabricant and Farnsworth, 2001). Based on this, many people have embarked on the use of indigenous plants as remedy against diarrhoeal diseases (Etuk, *et al*, 2009) and several plants have been reported to be used in treating and managing diarrhoea (Agunu *et al*, 2005).

Manniophyton africanum var. fulvum (Mull. Arg.) belongs to the family Euphorbiaceae, made up of shrubs or climber of about 30m. The flowers are usually pale to yellow in colour, and the Genus is made up of approximately 20 species.

This study was carried out to establish the pharmacological basis for the use of *M. africanum* for diarrhoea management in Umuaga-Udi, Enugu state, South-east Nigerian folklore medicine using various models.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Manniophyton africanum* were collected from Umuaga community of Udi local government Area in Enugu State, Nigeria. The plant was identified taxonomically by the botanical section of the Michael

Okpara University of Agriculture, Umudike, Nigeria. A voucher specimen was deposited in our laboratory for further references.

Preparation of extract

The fresh leaves were dried under mild sunlight, and then pulverized into coarse powder. Seven hundred (700) grams of the pulverised sample were extracted in 80% methanol for 48 h with intermittent shaking. Thereafter, filtration was done using filter papers and funnel into an already weighed beaker. The solvent was allowed to evaporate in an electric oven at 40^{o}C. The percentage yield (w/w) of the extract was determined.

Animals

Out bred mature albino Wistar mice (25-43 g) of both sexes were used. They were housed in standard environmental conditions of temperature, lighting hour, relative humidity, and provided with standard commercial pelleted feed (Vital feed®, Nigeria) and drinking water *ad libitum*. Ethical conditions governing the conducts of experiments with life animals were strictly observed as stipulated by Zimmerman (1983) and Ward and Elsea (1997). The research protocol was approved by the University's ethical committee.

Acute toxicity testing

The plant was assessed for acute toxic effects. Twenty mice of both sexes weighing between 20 g and 35 g were randomly selected and grouped into five groups of 5 mice each. Group A served as control with each rat receiving 10 ml/kg of distilled water. Four groups (B-E) were treated orally with varying doses of the extract (100 mg/kg, 500 mg/kg, 1000mg/kg and 5000 mg/kg). The mice were allowed free access to feed and water *ad libitum* for 48 hours. They were observed for signs of acute toxicity and death.

Effect of the extract on castor oil-induced diarrhoea.

The methods of Biswas *et al* (2002) as modified by Ezekwesili *et al* (2004) were used. Briefly, mice fasted for 18 h were randomly allotted to five groups of five animals each. Three groups were administered orally with MEMA (200, 400 or 600 mg/kg, respectively). The fourth group received diphenoxylate (5 mg/kg, p.o.) as standard drug, while the fifth group received distilled water (10 ml/kg, p.o.) and served as control. After 60 min, each animal was administered with 0.5 ml of castor oil by gavage and placed in separate cage. Animals were observed for defecation up to 4 h. Transparent blotting papers were placed beneath each locally fabricated metabolic cage and characteristic droppings were noted.

Effect of *M. africanum* extract on gastrointestinal motility

The effect of *M. africanum* on charcoal meal transit time was evaluated using the method of Mascola *et al* (1994), modified by Chidume *et al* (2001). Twenty five female mice weighing between 27-35 g were fasted for 12 h but allowed free access to drinking water. They were randomly divided into five groups (1-5) of five mice each. They were treated as follows: The mice in group 1 received distilled water (10 ml/ kg b.w, p.o.). Group 2 received diphenoxylate (5 mg /kg b.w, p.o.) and those in groups 3, 4, and 5 received MEMA (200, 400 and 600 mg/ kg b.w., p.o.) respectively, all by stomach intubations. Five minutes after drug administration, 0.5 ml of 10% charcoal suspension in 5% acacia gum was administered to each mouse by stomach intubations. Thirty minutes later, all the mice were sacrificed by cervical dislocation, the abdomen opened and the total length of the small intestine measured with a calibrated ruler. The distance travelled by the charcoal plug from the pylorus to caecum was determined and expressed as a percentage of the total length of the small intestine. Also the percent inhibition of movement was calculated by subtracting the percentage travelled from 100%.

Effect of *M. africanum* extract on Castor oil - induced enteropooling.

Intraluminal fluid accumulation was determined by the method of Robert *et al* (1976). Twenty five mature male mice were fasted for 12 h with free access to clean water. They were randomly divided into five groups (1 – 5) with 5 mice each.

Group 1 received distilled water (10 ml/ kg b.w) and served as untreated control while, group 2 received atropine sulphate (3 mg/kg b.w) (reference standard) and groups 3, 4 and 5 received 200, 400 and 600 mg/kg b.w of the extract respectively. They were treated by stomach intubations. One hour post treatment, each mouse was given 0.5 ml castor oil by oral gavage. Two hours after administration of castor oil, the mice were sacrificed by cervical dislocation, laparatomized, the small intestine located and tied at the pyloric and ceacal junction and dissected out. The small intestine was weighed with analytical weighing balance (Mettler H_{30}). The content of each intestine was milked into a graduated test tube and its content was measured.

Estimation of the antioxidant activity of *M. africanum* extract
The total antioxidant activity of the methanolic leaf extract of *Manniophyton africanum* was estimated by Ferric Reducing Antioxidant Power (FRAP) assay of Benzie and Strain (1999) and 1,1 –diphenyl-2-picryl-hydrazyl (DPPH) photometric assay of Iwalewa *et al*., (2008).

Data analysis
Data obtained were presented as mean ± SEM and analyzed using one-way analysis of variance (ANOVA) and post-hoc comparisons were carried out using Dunnet's *t*-test. Values of $P<0.05$ were considered significant in the study

RESULTS
Extraction
The percentage yield was 6.19% w/w dry matter. The extract was ox-blood in colour and had a slight pungent odour

Effect of the extract on castor oil-induced diarrhoea.
The percentage antidiarrhoeal activity of *M. africanum* extract in mice is presented in Table1. The extract (200, 400 or 600 mg/kg) showed a dose dependent activity respectively.

Effect of the extract on charcoal transit time.
The result of *M. africanum* extract on charcoal meal transit time in mice is presented in Table 2. The result showed that the all the doses of the extract (200, 400 and 600 mg/kg) reduced the distance travelled by the charcoal plug in a dose dependent manner. The reference drug, diphenoxylate (5 mg/kg) and the extract at 500 and 1000 mg/kg significantly ($P < 0.05$) inhibited the charcoal movement in a dose dependent manner when compared with the negative control group with the extract at the dose of 600 mg/kg having a little higher percent inhibition than the reference drug.

Effect of the extract on castor oil-induced enteroprooling in mice.
The result of the effect of *M. africanum* extract on castor oil enteropooling is presented in Table 3. Studies on the castor oil induced enteropooling showed that both the extract at all doses did not decrease the intra luminal volume of fluid accumulation in test mice when compared to the control group given reference drug atropine (3 mg/kg).

Antioxidant Assay
Figure (1) shows the antioxidant effects of *M. africanum* extract *in vitro* in the DPPH photometric assay after 30 minutes of incubation in the dark. Results showed a concentration-dependent antioxidant activity, though very marginal. The FRAP (Figure 2) which compares the time-dependent ferric reducing potential of various concentrations of test extract as showed similar findings, especially when compared with the ascorbic acid standard. Response represents Mean ± SEM. The experiments were done in triplicates.

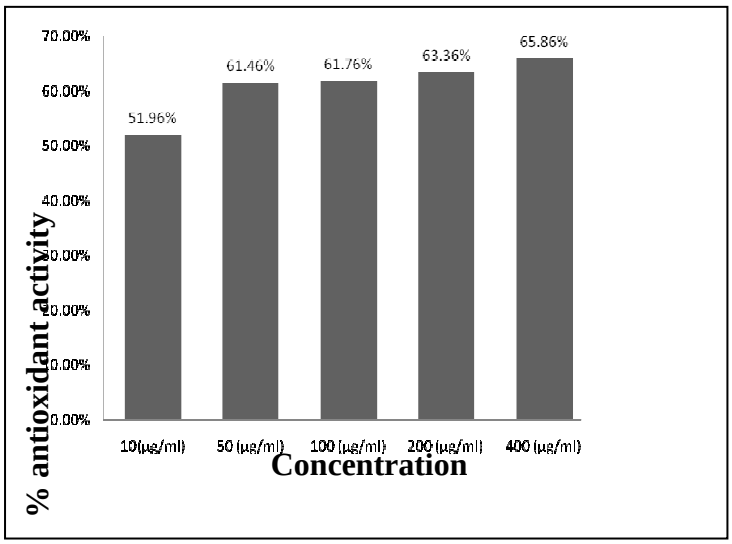

Fig. I : In vitro antioxidant activity of concentrations of M. africanum using the DPPH photometric assay.

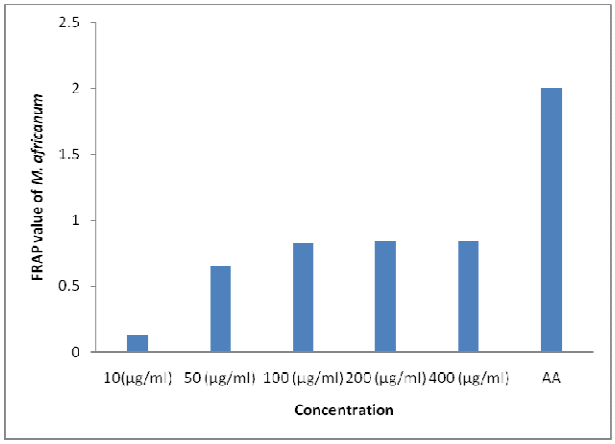

Fig 2. *In vitro* reducing potentials of concentrations of *M. africanum*

Table 1. Effect of the extract on castor oil-induced diarrhoea.

Group	Treatment	Faeces (n/mice)	Wet Faeces (n/mice)	% antidiarrhoeal activity
1	Distilled water (10ml/kg)	4.02± 0.01	15.03± 0.12	78.95
2	Diphenoxylate(5mg/kg)	21.60± 0.65	5.12± 0.11	19.23
3	MEMA (200 mg/kg)	13.22± 0.20*	5.03± 0.01	27.77
4	MEMA (400 mg/kg)	12.02± 0.10*	5.07± 0.21	29.41
5	MEMA (600mg/kg)	18.01± 0.03	6.12± 0.08*	25.00

Values are expressed as mean ±SEM; * Significance level (p <0.005)

Table 2. Effect of *M. africanum* extract on charcoal transit time in mice

Group	Treatment	Length of stomach (cm) Mean ± S.E.M	Distance covered (cm) mean ±S.E.M	% Inhibition
1	Distilled water (10ml/kg)	36.6±1.10	36.6±1.28	0
2	Diphenoxylate(5mg/kg)	34.73±2.46	19.17±3.98	44.80
3	MEMA (200 mg/kg)	44.17 ± 1.07	27.25 ± 2.00*	38.31
4	MEMA (400 mg/kg)	36.97 ± 1.12	21.00 ±3.02	43.20
5	MEMA (600mg/kg)	38.83±1.12*	18.67±0.94	51.92

Values are expressed as mean ±SEM; Level of significance (p <0.05)

Table 3. Effect of *M. africanum* extract of on castor oil-induced enteroprooling in mice.

Group	Treatment	Volume of intestinal content (ml) Mean ± SEM
1	Distilled water (10ml/kg)	0.25 ± 0.02
2	Atropine (3mg/kg)	0.07 ± 0.03
3	MEMA (200 mg/kg)	0.31 ± 0.04
4	MEMA (400 mg/kg)	0.52 ± 0.17
5	MEMA(600mg/kg)	0.45 ± 0.11

Values are expressed as mean ±SEM

DISCUSSION

The antidiarrhoeal properties of *M. Africanum* was studied using models justified by works of Havagiray, *et al*, (2004) such as castor oil induced diarrhoea, charcoal meal transit time and castor oil induced enteropooling in mice. In some diarrhoea, the secretory component predominates while other diarrhoeas are characterized by hypermotility of the gastrointestinal tract.

Studies have shown that activated charcoal readily adsorbs drugs and chemicals on the surface of the intestines, thereby preventing absorption (Levy, 1982), hence charcoal meal study was employed to study the effect of

MEMA on peristaltic movement. The extract and the reference drug diphenoxylate significantly ($P < 0.05$) produced antidiarrhoeal effects, decreased the distance travelled by the charcoal plug in a dose dependent manner with the percent inhibition of movement being a bit higher in the highest dose of the extract (600 mg/kg) than the reference drug. According to Bruton (1996), the property of reducing intestinal contractions (and consequently, intestinal transit) is demonstrated by most antidiarrhoeal drugs, as with MEMA. Reduction of intestinal transit time may possibly be due to anti-cholinergic effects (Brown and Taylor, 2000), if any. Diphenoxylate used in the symptomatic control of diarrhoea, works through its antimotiliy effects (Aliu, 2007). *M. africanum* may also be acting through the same mechanism.

The effect of *M. africanum* on castor oil induced enteropooling showed that the reference drug atropine (3 mg/kg) and the extract in all the doses used significantly ($p < 0.05$) reduced the intraluminal fluid accumulation in a dose dependent manner. Castor oil increases volume of intestinal content by prevention of the re- absorption of water and the liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa leading to release of prostaglandins which results in stimulation of motility and secretion and the prevention of re- absorption of NaCl and water (Pierce *et al*, 1971). The prevention of intraluminal fluid secretion by *M. africanum* in this study may be due to inhibition of prostaglandin biosynthesis with resultant decrease in secretion of fluid into the lumen or may be due to promotion of absorption of water and electrolytes in the gut. Suppression of intestinal fluid accumulation by the extract might also suggest inhibition of gastrointestinal function (Nwafor *et al*, 2000).

Previous phytochemical screening of the extract revealed the presence of carbohydrates, tannins, glycosides, terpenes, saponins and flavonoids (Uduak and Kola, 2010). Some of the chemical constituents present in the leaf extract have been shown to have antidiarrhoeal activity. Antidiarrhoeal and antidysentric properties of medicinal plants have been reported to be due to tannins, flavonoids, reducing sugars/glycosides among others (Longanga *et al*, 2000). Flavonoids and sugars obtained from selected medicinal plants were shown to exhibit antidirrhoeal properties (Palombo, 2005) and the inhibitory activity of flavonoids on intestinal motility in a dose related manner was earlier reported (Dicarlo *et al*, 1994).

This study further assessed the antioxidant activities using the DPPH photometric assay and the reducing potential of MEMA. Results showed that the extracts possessed very marginal antioxidant activity in both the DPPH and FRAP assays when compared to the ascorbic acid standard, although they were concentration-dependent, *in vitro*. Flavonoids, which were reported in present in the extract among others (Uduak and Kola, 2010), may be responsible for the observed antioxidant ability. They are phenolic substances isolated from vascular plants, possessing the ability to reduce free radical formation and scavenge free radicals *in vitro* (Pietta, 2000; Robak and Gryglewski, 1988). Although little or no documented information is available for their *in vivo* activity, most ingested flavonoids are extensively degraded to various phenolic acids, some of which still possess free radical-scavenging ability (Rice-Evans, 2001; Pietta, 2000). Phenolic compounds such as flavonoids, could easily donate hydroxyl hydrogen due to resonance stabilization (Sofidiya *et al*., 2006; Meir *et al*., 1995).

We therefore suggest that flavonoids and or other chemical constituents present in the extract might be responsible for both the antioxidant activity and the antidiarrhoeal activity of the *M. africanum* extract.

CONCLUSION

In conclusion, the results of this study indicate that *M. africanum* possesses antioxidant and antidiarrhoeal properties. However, more work is required to determine the exact mechanism(s) of action of the extract and to isolate and characterize the active principle.

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