

# Antidiarrhoeal effects of the methanol extract of the aerial parts of *Mitracarpus villosus*

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**Abstract:** *Mitracarpus villosus* (S.W) DC is used as treatment for diarrhoea and dysentery in some communities of West Africa. This study aimed to investigate the antidiarrhoeal effects of the methanol extract of the aerial parts of *Mitracarpus villosus* in mice to substantiate its use as an antidiarrhoeal preparation in traditional medicine. The mean lethal dose (LD<sub>50</sub>) of the extract was assessed in mice. The effect of the extract (100, 200 and 400 mg/kg body weight) on diarrhoea was evaluated against castor oil- induced diarrhoea. Castor oil-induced enteropooling was used to determine the effect of the plant extract on intestinal intraluminal fluid accumulation and the activity of *Mitracarpus villosus* extract on intestinal motility was investigated by means of the charcoal meal test and distal colonic bead expulsion time. Effect on gastric emptying was also evaluated. The extract decreased the number of wet faeces produced in a significant (P<0.05), dose-related manner. The extract also caused significant reduction of both the quantity of fluid accumulated in the intestinal lumen and small intestinal propulsion. The decreases were dose-dependent. Distal colonic propulsion time was delayed; however, the values obtained were not significantly different from control. Likewise, gastric emptying was significantly delayed. The results from this study showed that the methanol extract of the aerial parts of *Mitracarpus villosus* exhibited significant antidiarrhoeal activities.

**Keywords:** Intestinal propulsion, fluid accumulation, gastric emptying.

## INTRODUCTION

Diarrhoea is change in bowel habits that result in more frequent and less formed stools than is usual for an individual, with quantity of about 200g of stool in 24 h or at least 3 defecations in a day which may be accompanied by abdominal cramps (Nathan, 2008). Diarrhoeal diseases have been placed as the second major cause of death in children less than five years of age and worldwide about 1.7 billion cases were reported yearly with over 760,000 deaths recorded annually in children; especially in developing countries where it has been estimated that children under three years of age experience about 3 episodes each year (WHO, 2013). A significant number of the adult population has been reported to be affected with the condition (Barr and Smith, 2014) thus placing diarrhea as a significant health issue in developing countries. Diarrhoea is common where there is unhealthy environment such as inadequate sanitation, inadequate access to clean water and general poor hygiene; conditions that prevail in rural and poor semi-urban settlements; consequently, bacteria, fungi, viruses, protozoa and other microbial organisms have been implicated in the aetiology of diarrhoea (Bulled *et al*, 2014; Karambu *et al*, 2015). The fatality of diarrhoea is due to the rapid onset of dehydration and metabolic acidosis because damage to intestinal mucosa causes inflammation and prevents absorption of water from intestine into blood stream thus water, sodium, potassium and bicarbonates are evacuated in watery stools (Wittenberg, 2012).

The Pneumonia and Diarrhoea progress report of the International Vaccine Access Centre revealed that between 2000 and 2013 the number of deaths caused by diarrhoea have been reduced to 54% globally; however, the annual reduction rates of this disease in the highest-burden countries have remained unimpressive (IVAC, 2014). Also diarrhoea continues to be a cause of ill-health and death among older children, adolescents and adults in socio-economically challenged countries (Lamberti *et al*, 2014), thus one of the goals of the integrated Global Action Plan for the Prevention and control of Pneumonia and Diarrhoea (GAPPD) is to reduce mortality resulting from diarrhea in children less than five years to less than 1/1000 live births by 2025 and coordinated multi-sectorial approach have been advocated to meet this target (World Health Organization/United Nations Children's Fund, 2013).

The use of oral rehydration salts and zinc supplementation among other intervention strategies is promoted in the management of acute diarrhea, yet only 35% of diarrhoeal cases in children were managed with oral rehydration therapy as these practices have not been fully imbibed as routine community therapy to diminish the severity of the disease (WHO, 2004; WHO, 2013). Many African communities use herbal therapies alone as first line treatments or in combination with orthodox medicines for the treatment of diarrhoea considering that many conventional treatments may not produce the desired effects and may present with adverse effects and the inaccessibility of hospital in many parts of rural African countries has resulted in increasing dependence on

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indigenous medicines as remedies for diarrhoea in communities (Agbon *et al.*, 2010; Njume and Goduka, 2012). Furthermore, many plants have been reported to possess antidiarrhoeal properties (Pokale and Kuswaha, 2011).

*Mitracarpus villosus* (Sw.) DC. (Rubiaceae) is an annual herb that grows as weeds in old and uninhabited farmlands and is reported to be used in West African traditional medicine practices for the treatment of ailments that include diarrhoea and dysentery (Jegade *et al.*, 2005). Several studies have been carried out on the plant that demonstrates hepatoprotective, larvicidal, antifungal, antimicrobial, anti-nociceptive, anti-inflammatory and sedative activities (Ekipendu *et al.*, 1994; Germano *et al.*, 1999; Bisignano *et al.*, 2000; Kporou *et al.*, 2010; Abdullahi *et al.*, 2011; Makabila-Koubemba *et al.*, 2011; John-Africa, *et al.*, 2015). The objective of this study was to establish the effects of the plant extract on laboratory models of diarrhoea in rodents as part of our efforts to document the pharmacological activities of the plant *Mitracarpus villosus*.

## MATERIALS AND METHODS

### *Collection and preparation of plant material*

The plant *Mitracarpus villosus* was collected by Tanko Garba from Idu Abuja, Nigeria in September 2014. The plant was collected by cutting about 1cm above ground level. These were shaken to remove dead parts and foreign matter. The plant was identified and validated (Voucher No NIPRD/H/6606) by Dr. Grace Ugbabe of the Department of Medicinal Plant Research and Traditional Medicine (MPR&TM), National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja.

### *Plant extraction*

The aerial parts were cleaned of debris, air-dried and crushed with a mortar and pestle to obtain coarse powder. 200g of the material was subjected to extraction by maceration using 250L methanol for 24 h with occasional shaking. The filtrate was concentrated under reduced pressure using a rotary vacuum evaporator and the concentrate was evaporated to constant weight on a water bath to give a dark green semi-solid (the extract - MV) with yield of 10.8% w/w.

### *Animals*

Swiss albino mice bred and maintained at the Animal Facility Centre of the National Institute for Pharmaceutical Research and Development, Idu Abuja were used in these studies. They were housed in standard polypropylene cages with wood shavings as beddings, and maintained at ambient conditions. The animals were fed on mouse feed and water was given freely. The animals were handled according to the Institutional animal ethical committee guideline as approved by the NIPRD

committee for regulation of animal studies (SOP No 05:03:02). Food was withdrawn from the mice 3 h preceding the experiment to minimize excessive stress in the animals that may be caused by long periods of fasting (Salgado *et al.*, 2005).

### *Acute toxicity test*

This test was performed in two stages. In stage one; mice were randomized into 3 groups of 3 animals in each group and were given 10, 100 and 1000mg/kg of extract accordingly. These animals were subsequently monitored for signs of intoxication and mortality for duration of 24h. In stage two, extract administration was slated based on the results documented in stage one, thus the mice (n=1) received 1600, 2900 and 5000 mg/kg of extract (Lorke 1983; Okoli *et al.*, 2010). All animals were monitored for 14 days.

### *Gastrointestinal tract activity studies*

Swiss albino mice (25-30g) were weighed, randomized and grouped into 6 groups (n=5) and treated as follows:

- Group 1-Distilled water
- Group 2-MV 100 mg/kg (MV 100)
- Group 3-MV 200 mg/kg (MV 200)
- Group 4-MV 400 mg/kg (MV 400)
- Group 5-Loperamide 2 mg/kg
- Group 6-Atropine sulphate 1 mg/kg

### *Castor oil-induced diarrhoea test*

Thirty minutes after treatment, all mice were given 0.5ml of castor oil. The animals were placed separately in plastic cages covered with white paper which was changed periodically. The mice were monitored for a period of 4 hours. The cumulative numbers of wet defecations per hour and total number of droppings were recorded.

### *Castor oil-induced intestinal fluid accumulation test*

Thirty minutes after extract/drug administration, each of the animals in groups 2 - 6 was given 0.5ml of castor oil orally. All mice were euthanized after further 30min; the small intestine was ligated at the pyloric sphincter and the ileo-cecal junction. The whole small intestine from each mouse was dissected out and weighed. The difference in the weight of intestine in the control and castor oil treated groups was considered as the intestinal intraluminal fluid accumulation induced by castor oil.

### *Charcoal meal test*

In this experiment, thirty min after treatment each mouse was administered orally 0.5ml of charcoal meal consisting of 3% deactivated charcoal suspended in 10% aqueous tragacanth (Antwi *et al.*, 2009). After 30min, each animal was euthanized by diethyl ether inhalation. The abdominal region was dissected and the intestine was isolated. The length covered by the leading head of the charcoal meal from the pylorus and the whole length of the small intestine were measured. Intestinal propulsion

was calculated as percentage of the distance moved by the charcoal from the pylorus relative to the ileo-caecal junction.

#### ***Colonic bead expulsion test***

In this test, 1h after animals had received treatment, a bright coloured bead of about 3mm in diameter, pre-warmed in a water bath maintained at 37°C was inserted into the rectum to a depth of 2cm from the anus with the aid of a catheter. Mice were individually paced in cages lined with white paper to facilitate visualization of the bead. The time taken for the inserted glass bead to be excreted was recorded: this was taken as the distal colonic transit time.

#### ***Gastric emptying activity test***

This test was carried out in mice fasted for 18h of food and 4h of water prior to the experiment. After a pre-treatment period of 1hr, each animal received 0.5ml of charcoal meal consisting of 3% activated charcoal in 10 % aqueous tragacanth. Fifteen minutes later mice were euthanized by diethyl ether inhalation. The abdominal region was dissected and the stomach was carefully ligated at the cardiac and pyloric sphincters. The stomach was isolated, weighed, opened along the greater curvature and the contents emptied. The stomach was carefully rinsed under a slow running tap. Excess water was blotted off using filter paper and the empty stomach re-weighed. The difference in weight between the stomach before and after expulsion of the contents was taken as weight of the stomach contents (Endo and Kumagai, 1998; Charoenthongtrakul 2009).

### **STATISTICAL ANALYSIS**

Results were expressed as mean  $\pm$  SEM. Significance was determined using one-way analysis of variance (ANOVA) followed by Dunnet's post hoc test. Results were regarded as significant at  $P < 0.05$ . The software package Graph pad PRISM 5 was used to analyse the data.

### **RESULTS**

#### ***Effect on Castor oil-induced diarrhoea***

The castor oil administered to the animals induced production of copious stools in the mice with the most number of wet stools produced during the second hour (fig. 1). Pretreatment of the mice with the extract caused a significant dose-dependent reduction in the number of total and wet stools produced showing 26.83, 48.78, 53.66 % inhibition. There was also significant difference in the time of onset of diarrhoea in the extract treated groups when compared to control (table 1).

#### ***Effect on Castor oil-induced intestinal fluid accumulation***

The extract caused a reduction in the quantity of fluid accumulated in the lumen of the small intestine. The

effect was dose-related and significant at 400 mg/kg on comparison to control. Loperamide produced a significant reduction of intraluminal fluid accumulated (table 2)

#### ***Effect on Charcoal meal transit time***

Dose dependent decrease in propulsion of the charcoal meal through the small intestine was observed in animals that received the methanolic extract of *Mitracarpus villosus* and the reference drugs loperamide and atropine as shown in table 3. The effect was significant for MV at 400 mg/kg, loperamide and atropine.

#### ***Effect on Colonic bead expulsion***

In the colonic bead expulsion test, the expulsion time for the bead was prolonged in all extract and drug treated mice. The extract produced a dose-dependent delay in expulsion of the beads. The inhibitory activity of the extract on distal colonic transit was significant at 400mg/kg as shown in table 4.

#### ***Effect on Gastric emptying activity***

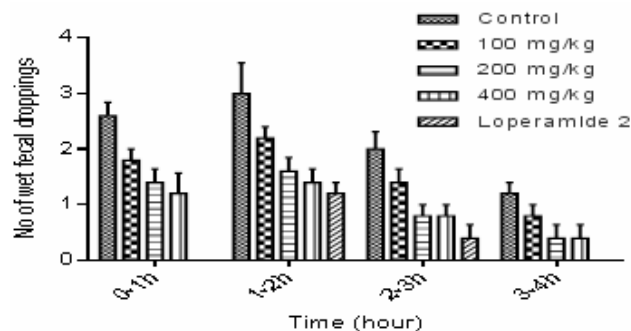
The weight of the stomach contents was increased on administration of the extract. This effect was not dose-dependent but the effect was significant in mice treated with 400mg/kg of extract, likewise loperamide and atropine exhibited significantly increase in weight of the stomach contents when compared to the group that received distilled water (table 5).

### **DISCUSSION**

The antidiarrhoeal activity of the methanol extract of *Mitracarpus villosus* was studied using castor oil induced diarrhoea; castor oil induced enteropooling was used to determine its effects on intestinal fluid accumulation and secretion and the activity of the extract on intestinal motility was investigated using the charcoal meal test and colonic bead expulsion time. The effect of the extract on gastric emptying was also evaluated. These laboratory animal models are commonly used for the evaluation of anti-diarrhoeal agents.

Pretreatment of the mice with the extract decreased both the quantity and frequency of stooling and similarly inhibited enteropooling. Previous reports state that the mechanism of diarrhoea caused by castor oil is as a result of the metabolism of castor oil to ricinoleic; an irritant that causes inflammation of the intestinal mucosa thereby leading to the release of inflammatory mediators like prostaglandins which cause vasodilatation, increase smooth muscle contraction and stimulate secretion by causing permeability alterations in the intestinal mucosa to electrolytes ( $\text{Na}^+$ ,  $\text{Cl}^-$ ) and water (Magaji *et al*, 2007). The results obtained showed that treatment with the methanolic extract of *Mitracarpus villosus* diminished the severity of diarrhea by causing a decrease in the number of wet fecal droppings; the onset of diarrhea was delayed

and the weight of intestinal contents was also decreased. It has been proposed that agents that inhibit prostaglandins cause delay of castor oil induced diarrhoea and interfere with intraluminal fluid accumulation therefore it is possible that the plant extract maybe mediating the observed effect by interference with prostaglandin synthesis and release (Chandrashekar *et al*, 2004; Umer *et al*, 2013).



**Fig. 1:** Effect of methanolic extract of *Mitracarpus villosus* on castor oil induced diarrhea

The methanolic extract of *Mitracarpus villosus* inhibited intestinal transit as indicated by the delay of both charcoal meal transit and bead expulsion from the gastrointestinal tract. One of the factors implicated in the aetiology of diarrhoea is over stimulation of bowel movements by the parasympathetic nervous system which promotes digestive activity, thus increased peristalsis with subsequent increased propulsion through the gastrointestinal tract is associated with loose and frequent stooling (Blows, 2012). Anti-motility agents produce antidiarrhoeal effects by reducing gastrointestinal motility and/or secretions (Al-Maamori, 2011). The delay in transit of contents along the intestinal lumen therefore promotes the reabsorption of water and electrolytes thus enhancing drug absorption and slowing down the flow of fecal matter through the gastrointestinal tract and thereby affecting the consistency of fecal contents (Story, 2014). The viscosity of intraluminal contents generally increases with propulsion from the caecum to the rectum conversely water content decreases thus harder stools are formed when transit time increases (Spiller 2006). In this investigation, the methanolic extract of *Mitracarpus villosus* demonstrated a significant decrease of gastrointestinal transit time in both the small intestine and colon, manifested as inhibition of the charcoal movement and delay in bead expulsion from the rectum in the extract treated animals; thereby causing retention of stool in the intestine over a longer period. The extract by slowing down intestinal motility and increasing transit time of contents through the gastrointestinal tract probably afforded more time for water and electrolyte to be reabsorbed thus decreasing the frequency of passage of loose stools. This action could therefore be a probable mode by which the plant extract exerted antidiarrhoeal action. Although the application of antimotility drugs in

the management and cure of diarrhoea is generally discouraged especially in children because in infective diarrhoea, substantial decreases in motility can predispose to bacteremia (Bope and Kellerman, 2015), in adults however, use of antimotility and antisecretory drugs decrease the frequency of passage of diarrhoeal stools. The combination of treatment with both antimotility and antisecretory properties may provide faster and more complete relief of acute non-specific diarrhoea than either medication alone (Barr and Smith, 2014). The results presented here show that the plant extract tested possibly possesses both properties.

The results indicated that the extract caused delay in gastric emptying as denoted by increased gastric contents in treated mice. The standard drugs loperamide and atropine are agents reported to reduce contractility and consequently decrease normal peristaltic movements in the gastrointestinal tract (Parkman *et al*, 1999; Khansari *et al*, 2013). In this study, these agents have decreased gastric emptying which is a function of the co-ordination between the frequency and force of contraction of gastric peristalsis and the resistance to flow offered by the pyloric sphincter (Ezeja and Anaga 2011), thus the delayed gastric emptying exhibited by the extract maybe similarly as a result of inhibition of gastric peristaltic activity. Drugs that delay gastric emptying facilitate accumulation of gastric acid which could inactivate or destroy bacteria ingested with food as suggested by Endo and Kumagai (1998).

Antibiotic use in infectious diarrhoea can decrease the duration of illness and prevent complications (Dupont 2014). The extracts of *Mitracarpus villosus* in other studies have demonstrated both antibacterial and antifungal activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Okoye and Okafor 2013); these organisms are associated with diarrhoea (Nkuo-Akenji *et al*, 2002, Ifeanyi *et al*, 2010). These properties shown by the plant extract may be attributed to why the plant has been use in traditional medicine as a treatment for diarrhoeal conditions.

The preliminary phytochemical screening was carried out to identify the phytochemicals that are present in the plant extract as pharmacological activities exhibited by plants have been ascribed to the presence of these phytoconstituents (Saxena *et al*, 2013). Primary phytochemical examination revealed the presence of secondary plant metabolites that include terpenoids, phenols, sterols, anthraquinone, carbohydrates, volatile oils resins, flavonoids, tannins.

In other plants, these constituents have been associated with biological activities that include antispasmodic, anti-secretory and antidiarrhoeal effects (Al-Maamori, 2011). The antidiarrhoeal effects observed may be attributed to the synergistic actions of anti-motility, anti-secretory and anti-microbial effects of these plant constituents.

**Table 1:** Effect of methanolic extract of *Mitracarpus villosus* (MV) on castor oil induced diarrhea

Treatment (mg/kg)	Onset of diarrhoea (min)	Total No. of defecations	Total No. of wet defecations	% Inhibition
Control	41.40 ± 1.12	12.40 ± 0.51	8.20 ± 0.37	-
MV 100	47.60 ± 2.50	8.40 ± 0.68 <sup>c</sup>	6.00 ± 0.32 <sup>b</sup>	26.83
MV 200	53.60 ± 1.91 <sup>a</sup>	7.00 ± 0.32 <sup>d</sup>	4.20 ± 0.49 <sup>d</sup>	48.78
MV 400	56.40 ± 2.93 <sup>b</sup>	6.60 ± 0.68 <sup>d</sup>	3.80 ± 0.37 <sup>d</sup>	53.66
Loperamide 2	105.0 ± 4.92 <sup>d</sup>	5.00 ± 0.32 <sup>d</sup>	1.40 ± 0.24 <sup>d</sup>	82.93

**Table 2:** Effect of methanolic extract of *Mitracarpus villosus* (MV) on castor oil (CO) induced intraluminal fluid accumulation.

Treatment (Dose mg/kg)	Weight of Intestine and contents (g)	Weight of Intestinal contents (g)	% Inhibition of intraluminal fluid accumulation
Distilled water 10 ml/kg + CO	2.59 ± 0.11	-	-
MV 100 + CO	2.34 ± 0.27	0.25	9.65
MV 200 + CO	2.15 ± 0.13	0.44	16.99
MV 400 + CO	2.08 ± 0.12	0.51	19.69
Loperamide 2 + CO	2.00 ± 0.09 <sup>a</sup>	0.59	22.78
Atropine 1 + CO	1.87 ± 0.12 <sup>b</sup>	0.72	27.80

**Table 3:** Effect of the methanolic extract of *Mitracarpus villosus* (MV) on gastrointestinal tract motility

Treatment	Dose (mg/kg)	Distance travelled by charcoal head	% Inhibition
Distilled water		75.59 ± 3.98	-
MV	100	61.06 ± 6.03	19.22
MV	200	55.42 ± 6.74	26.68
MV	400	40.13 ± 10.15 <sup>b</sup>	46.91
Loperamide	2	20.73 ± 3.42 <sup>c</sup>	72.58
Atropine	1.0	21.63 ± 4.80 <sup>c</sup>	71.39

Each value represents mean ± S.E.M, n = 5, P < 0.05 significant difference from control.

**Table 4:** Effect of the methanolic extract of *Mitracarpus villosus* (MV) on distal colonic transit

Treatment	Dose (mg/kg)	Time of bead expulsion (s)	% Time Delay
Distilled water		162.00 ± 25.50	-
MV	100	211.80 ± 14.50	30.74
MV	200	241.00 ± 38.86	48.77
MV	400	285.72 ± 27.25 <sup>b</sup>	76.37
Loperamide	2	302.00 ± 19.76 <sup>b</sup>	86.42
Atropine	1	247.20 ± 22.85 <sup>a</sup>	52.59

**Table 5:** Effect of the methanolic extract of *Mitracarpus villosus* (MV) on gastric emptying

Treatment	Dose (mg/kg)	Gastric Content (mg)
Distilled water	10 ml/kg	0.14 ± 0.05
MV	100	0.27 ± 0.07
MV	200	0.25 ± 0.02
MV	400	0.30 ± 0.03 <sup>a</sup>
Loperamide	2	0.38 ± 0.03 <sup>b</sup>
Atropine	1	0.36 ± 0.02 <sup>b</sup>

Each value represents mean ± S.E.M, n = 5, P < 0.05 significant difference from control.

In the oral acute toxicity test, no mortality was observed in all animals administered with doses up to 5g/kg of the methanol extract of *Mitracarpus villosus* for the duration of observation, therefore the oral LD<sub>50</sub> of the extract was approximated to be greater than 5g/kg suggesting that MV is not acutely toxic on oral administration.

## CONCLUSION

The results from this study indicate that the reduction of the severity of diarrhoea by the methanolic extract of *Mitracarpus villosus* may involve anti-motility and antisecretory mechanisms.

## ACKNOWLEDGEMENT

The Author of the manuscript wishes to acknowledge the technical assistance of Tamara Enimire.

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