# ANTIDIARRHOEAL PROPERTIES OF THE METHANOLIC FRUIT EXTRACT OF *MOMORDICA BALSAMINA* (LINNEAUS)

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## Abstract

Momordica balsamina (Linnaeus) family Cucurbitaceae is a Nigerian medicinal plant used traditionally to treat an array of diseases such as diarrhea and dysentery. The antidiarrhoeal effects of the fruit extract of M. balsamina were investigated in rodents. The fruit extract was obtained by Soxhlet extraction using methanol as solvent and subjected to phytochemical screening. The result of the phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponnins, resins and reducing sugars. The elemental analysis of the powdered fruit revealed high concentration of sodium, followed by magnesium, iron, zinc, potassium, manganese and lead. The orally determined LD50 of the fruit extract in Swiss albino mice was found to be 5,500 mg/kg indicating its relative safety. The methanolic extract at moderate doses (400 mg/kg and 800 mg/kg) significantly ($P < 0.05$) reduced the gastrointestinal transit of activated charcoal in mice comparable to loperamide, a potent antidarrhoeal agent. The oral administration of the extract (200 mg/kg, 400 mg/kg, and 800 mg/kg) also dose dependently protected Wistar rats against castor oil (2 ml/rat)-induced diarrhoea. It also significantly reduced castor oil-induced enteropooling in Wistar rats. This evidence clearly indicates that the methanolic fruit extract of M. balsamina possess strong antidiarrhoeal potential which may explain the use of the plant as popular antidiarrhoeal recipe.
**Keywords:** Antidiarrhoea activity, M. balsamina methanolic fruit extract.

## Introduction

Diarrhoea involves increased gastro-intestinal tract motility, increase in secretion and a decrease in absorption of fluid, and thus, loss of electrolytes (particularly $Na^+$) and fluid in faces (Jung *et al*., 2011; Okpara, 2015). Generally, the result of increased motility in the colon may be important symptoms of such underlying disorders as amoebic dysentery, lactose intolerance, gastrointestinal tumor, collibacillosis, and inflammatory bowel disease (Weber, 1996). Diarrhoea could also result from helminthes infestation, viral infections, malabsorption, fears and anxiety as well as oxidative stress (Furness and Speakman, 2008; Ambali *et al*., 2011).

Diarrhoea diseases caused several million of human deaths in the world annually (Salud *et al*., 2007). At the beginning of the 1980s, deaths caused by diarrhoea were estimated at 5.6 million every year for children under the age of 5 years (Das *et al*., 2007). Also, diarrhoea causes morbidity and mortality in livestock inflicting significant economic losses (Okpara *et al*., 2016).

To overcome the menace of diarrhoeal diseases in the developing countries, the World Health Organization (WHO) has evolved a programme for its control which includes the use of traditional herbal medicine (WHO, 2002, Magaji *et al*., 2007; N'gaman *et al*., 2011). Several plants have been reported to be effective in the treatment and control diarrhoea in man and his animals (Okpara, 2015). Of the many local herbs used in folkloric medicine for treatment of diseases in Nigeria, only very few have been properly identified and documented (Bosha *et al*., 2010). Out of

these only a very small portion has been subjected to verification, hence efficacy and toxicity of many remains in doubt (Bosha *et al*., 2010; Okpara, 2015). Screening for plant bioactivity based on folkloric use is the best approach to new drug discovery (Sofowora, 2006).

African Balsam (*Momordica balsamina*) family *Cucurbitaceae* is a creeping or climbing plant that grows wild in many countries in Africa. In Nigeria, the plant flowers in September and the fruit ripens between November and December. The fruit pulp is used in folkloric medicine for the treatment of gastrointestinal disorders in man and Newcastle disease in chicken (Mgbojikwe *et al*., 2000; 2002). This study evaluated the antidiarrhoeal activities of the aqueous fruit extract of *Momordica balsamina* (L) using standard protocols.

## Materials and Methods

### Plant Collection and Identification

Fresh matured fruits and leaves of *Momordica balsamina* were collected in January 2013 within the premises of NVRI, Vom, Plateau State, Nigeria and identified by a plant Taxonomist at the NVRI, Vom. Voucher Specimen OJOBC was deposited at the Institute Herbarium for reference purposes.

### Preparation of Plant Material

Each Balsam fruit was washed with deionized water, cut into sixteen sizes and the seeds separated from the pulp. Thereafter, the fruits were air dried under the shade for 10 hours per day for 14 days in the laboratory to avoid solar bleaching. Using pestle and motar, the fruit was ground into fine powder and sieved to remove excess coarse plant material yielding 205 g.

### Preparation of Plant Extract

The extraction was as described by Harborne (1998); Okpara (2015). Briefly 200 g of the powdered fruit material of *Momordica balsamina* was packed into a thimble and transferred into Soxhlet Extractor. The extraction was carried out in 70% methanol at 60$^{o}$C until there was no more colour change in the methanol indicating that the extraction was complete. The methanolic extract harvested was then concentrated *in vacuo* using a rotary evaporator coupled to a thermocirculator. The extract was subsequently placed in the hot air oven at 80$^{o}$C to remove the residual methanol until a constant weight was obtained. After evaporation, the weight of the extract was taken and percentage yield calculated as weight of extract ÷ weight of powdered fruit x 100. The extract was stored in air and water tight glass container in the refrigerator at 4$^{o}$C until required. The yield was 6.8% (w/w), percentage yield was calculated using the formular:

$$\% \text{ Yield} = \frac{\text{weight of extract}}{\text{weight of powdered fruit}} \times 100$$

### Animal Acquisition and Management

Wistar rats (*Rattus norvagicus*) both sexes weighing 195 – 210 g and Swiss albino mice (*Mus musculus*) adult males weighing 25 – 30 g were obtained from the Experimental Animal Unit, NVRI, Vom. They were kept in steel rat and mice cages and allowed a two week period of acclimatization before the commencement of the experiment. The animals were fed with Chicken Growers Mash (Vital Feed PLc, Bukuru, Jos) and drinking water produced *ad libitum.* The animals were fed with Chicken Growers Mash (Vital Feed PLc, Bukuru, Jos) and drinking water provided *ad libitum.*

### Determination of Median Lethal Dose ($LD_{50}$)

The median lethal dose ($LD_{50}$) was determined through a two phase approach as described by Lorke (1983). The first phase

involved 9 albino mice divided into 3 groups of 3 mice each. Each group was administered *Momordica balsamina* fruit methanolic extract at doses of 4000 mg/kg, 5000 mg/kg and 6000 mg/kg body weight *per os* respectively. Signs of toxicity and death were observed over a period of 48 hours. The doses of the second phase which depended on the result obtained from phase I, consisted of three mice divided into three groups of one mouse each, administered with methanolic fruit extract of *M. balsamina* at 5000 mg/kg, 5500 mg/kg and 6000 mg/kg respectively. The $LD_{50}$ was caculated by obtaining the geometric mean between the highest lethal dose that produced 100% death (6000 mg/kg) and the lowest non-lethal dose 5000 mg/kg).

**Determination of the Elemental Content of *M. balsamina* fruit powder**

The powdered fruit of *M. balsamina* was screened for elemental constituents using the technique previously described by Bhatia (2005). Briefly, 5 g of dried fruit powder in an evaporating dish was placed in an oven at 80$^{o}$C and dried to constant weight. The sample was placed in a weighing crucible and ashed at 500$^{o}$C in hotspot furnace for 3 hours and screened for elemental constituents. The elements screened were potassium, zinc, lead, cupper, cadmium, sodium, calcium, manganese, magnesium and iron.

**Effects of Methanolic Fruit Extract of *M. balsamina* on Castor-oil-induced Enteropooling in Rats**

Twenty-five rats weighing (195 – 200) were used. The intra-luminal fluid accumulated was determined by the method of Umaru *et al.,* (2011) with slight modification. The rats were fasted overnight and separated into five groups of five rats each. Group I rats received 3 ml/kg of distilled water intraperitonally (*i.p*), Group II rats received 3 mg/kg of atropine sulphate (*i.p*) while Groups III, IV and V rats were given 200 mg/kg, 400 mg/kg and 800 mg/kg doses of the methanolic fruit extract of *M. balsamina* orally respectively.

After 1 hour, each rat was treated with 2 ml of castor-oil *per os*. One hour after - the castor-oil treatment, the rats were sacrificed and the small intestine removed, tied on both ends with cotton thread and weighed. The intestinal content was collected by milking and the volume measured. Thereafter, the intestine was re-weighed and the difference calculated and recorded (Umaru *et al.,* (2011).

**Effects of the Methanolic Fruit Extracts on Castor-oil-Induced Diarrhoea in Rats**

This was carried out as previously described by Okpara (2015), Okpara *et al*, (2016). The rats were fasted for 12hrs prior to the commencement of the experiment and were randomly divided into five groups (I, II, III, IV and V) of five rats each. The rats in group I received 3 ml/kg of distilled water p.o. while, those in groups II, III and IV received graded doses of the extract (200, 400 and 800mg/kg. The last group received 5 mg/kg of loperamide. After 30 mins of drug pre-treatment, castor oil (2 ml/rat) was administered intragastrically. The rats were placed in individual cages over clean filter papers. 3h after castor oil-challenge, the rat cages were inspected by independent observers for the presence of characteristic diarrhoeal droppings. Their absence was recorded as effective protection from diarrhoea (Okpara, 2015; Okpara *et al.,* 2016).

**Effects of Methanolic Fruit Extract of *M. balsamina* on Gastro-intestinal transit of Activated Charcoal-Tragacanth Meal in Mice**

This was determined using the method previously described by Okpara *et al*.,

(2007); and Okpara *et al*., (2016) with slight modification. Briefly, the animals were randomly divided into five groups (I, II, III, IV and V) of five mice each. These animals were starved for 24 hours prior to the experiment but were allowed free access to water. Mice in groups I were given 3 ml/kg of distilled water i.p, group II, III and IV received *M. balsmanina* fruit extract (200, 400 and 800 mg/kg i.p) respectively. Group V mice were given 3 mg/kg of atropine (i.p). 5 minutes, after drug administration, 0.5 ml of a 5% charcoal suspension in a 10% suspension of tragacanth powder was administered p.o to each mice. All the mice were euthanized 40 minutes. later in a gas chamber with chloroform, the abdomen opened and the distance travelled by charcoal plug from the pylorus to the caecum determined and express as percentage of the total length of the small intestine (Abdullahi *et al*, 2001; Okpara *et al.,* 2016).

## RESULTS

### Extraction

The methanolic fruits extract of *M. balsamina* was dark brown and have slight bitter taste. The yield was 6.82% (w/w).

### Phytochemical Screening

The result of the phytochemical screening of the methanolic fruit extract of *M. balsamina* is shown in (Table I). The result revealed that the fruit extract contained flavonoids and alkaloids in very high concentration, while tannins, saponnins and reducing sugars occurred in moderate concentration and resins and glycosides were found in low concentration. Sterols, glycosides and anthraqinone derivations were absent.

### $LD_{50}$ Determination

The $LD_{50}$ was calculated to be 5500 mg/kg body weight by evaluating the geometric mean of the highest dose that killed the rats and the lowest dose that did not kill the rats (Table 2, 3).

### Effect of Methanolic Fruit Extract of *M. balsamina* on Castor oil-induced Enteropooling in Rats

The result of the effect of *M. balsamina* methanolic fruit extract on castor oil-induced enteropooling in rats is presented in Table 4. There was no significant difference ($P > 0.05$) in the fluid accumulated in the intestine of the rats in the control (DW) group and the group treated with the fruit extract at 200 mg/kg body weight. However, treatments with 400 mg/kg and 800 mg/kg and atropine 3 mg/kg significantly ($P < 0.05$) decreased the accumulated fluid when respectively compared with the control (DW). Although not significant ($P > 0.05$), atropine was observed to be more potent than the extracts (400 and 800 mg/kg) in decreasing the intestinal fluid contents.

**Table I: Phytochemical Composition and Concentration of Trace Elements in *Momordica balsamina* (Balsam) Fruit**

| Chemical Constituents | Fruit of Balsam | Elements | Concentration (PPM) |
|---|---|---|---|
| Glycosides | - | Calcium (Ca) | 0.00 |
| Saponnins | ++ | Lead (Pb) | 0.01 |
| Anthraquinone Derivatives | - | Cupper (Cu) | 0.00 |
| Flavonoids | +++ | Zinc (Zn) | 0.08 |
| Tannins | ++ | Iron (Fe) | 0.45 |
| Alkaliods | +++ | Sodium (Na) | 30.50 |
| Sterols and Triterpenes | - | Cadmium (Cd) | 0.00 |
| Carbohydrates | + | Manganese (Mn) | 0.03 |
| Reducing Sugars | ++ | Magnesium (Mg) | 0.61 |
| Resins | + | Potassium (K) | 0.06 |

**Table 2: Phase I of Test for Median Lethal Dose ($LD_{50}$) of *M. balsamina* Fruit Methanolic Extract (n=3)**

| Group | Dose (mg/kg) *p.o*) | No. Dead/Alive |
|---|---|---|
| I | 4000 | 0/3 |
| II | 5000 | 0/3 |
| III | 6000 | 1/3 |

**Table 3: Phase II of Test for Median Lethal Dose ($LD_{50}$) of Methanolic Fruit Extract of *Momordica balsamina* (n=1)**

| Group | Doses (mg/kg), *p.o*) | No. Dead/Alive |
|---|---|---|
| I | 5000 | 0/1 |
| II | 5500 | 0/1 |
| III | 6000 | 1/1 |

**Table 4: Effects of Methanolic Fruit Extract of *M. balsamina* on Castor Oil-Induced Enteroprooling in Wistar Rats (n=5; mean ± SEM)**

| Extract Treatment (mg/kg) | Weight of Intestine + Content (gram) | Weight of Intestine | Weight of Accumulated Fluid (gram) | Percentage (%) of Fluid Accumulated |
|---|---|---|---|---|
| Control (DW) (3 mg/kg) | 9.75 ± 0.40 | 4.91 ± 0.11 | 4.84 ± 0.22 | $49.6^{a}$ |
| MFE 200 | 8.16 ± 0.05 | 4.60 ± 0.32 | 3.55 ± 0.22 | $43.5^{a}$ |
| MFE 400 | 5.60 ± 0.12 | 4.08 ± 0.17 | 1.52 ± 0.10 | $27.1^{b}$ |
| MFE 800 | 3.90 ± 0.07 | 3.10 ± 0.01 | 0.79 ± 0.08 | $20.2^{b}$ |
| Loperamide (5mg/kg) | 3.71 ± 0.5 | 3.14 ± 0.51 | 0.58 ± 0.85 | $15.6^{b}$ |

MFE = Methanolic fruit extract of *M. balsamina.*
DW = Distilled water.
Means denoted by different superscripts are significantly different ($P < 0.05$).

**Table 5: Effects of Methanolic Fruit Extract of *M. balsamina* on Intestinal Transit in Mice (n=5; Mean ± SEM).**

| Drug | Dose, mg/kg (*i.p.*) | Distance Travelled by Charcoal Mean (mm). |
|---|---|---|
| Distilled water (DW) | 3 ml/kg | $62.45 \pm 0.20^{a}$ |
| MFE 200 | 200 | $48.60 \pm 0.10^{a}$ |
| MFE 400 | 400 | $14.50 \pm 0.12b$ |
| MFE 800 | 800 | $11.63 \pm 0.31^{b}$ |
| Atropine | 3 | $7.55 \pm 0.10^{b}$ |

Values are mean ± standard error of the mean (SEM) n=5 Means denoted by different superscripts are significant different ($P < 0.05$).

The results of the effect of *M. balsamina* extract on intestinal transit in mice are shown in Table 6. The fruit extracts at 400 mg/kg, 800 mg/kg and atropine 3 mg/kg respectively significantly ($P < 0.05$) reduced the length travelled by the charcoal meal when compared with the length obtained for the control and the fruit extract at 200 mg/kg body weight.

**Table 6: Effects of Methanolic Extract of *M. balsamina* Fruits on Castor oil-induced Diarrhoea in Rats (n=5; Mean±SEM)**

| Treatment | Dose Mg/kg (*p.o*) | Number Showing Diarrhoea out of total number of rats | Latent time (min) | Percentage Protection (%) |
|---|---|---|---|---|
| DW (Control) | 3 ml/kg | 5/5 | $85.62 \pm 0.30^{a}$ | $0.00^{a}$ |
| MFE 200 | 200 | 4/5 | $9.1 \pm 42.03^{a}$ | $20^{a}$ |
| MFE 400 | 400 | 1/5 | $152.60 \pm 0.11^{b}$ | $80^{b}$ |
| MFE 800 | 800 | 0/5 | $155.40 \pm 0.25^{b}$ | $100^{b}$ |
| Loperamide | 5 | 0/5 | $167.20 \pm 0.15^{b}$ | $100^{b}$ |

Means denoted by same superscripts are significantly different ($P < 0.05$). MFE = methanolic extract of *M. balsamina*.

The result of the effect of the extract of *M. balsamina* fruit on Castor oil-induced diarrhoea is shown (Table 6). The methanolic extract 400 mg and 800 mg/kg as well as loperamide 5 mg/kg body weight significantly ($P < 0.05$) protected rats against castor oil-induced diarrhoea compared to the control (DW) and 200 mg/kg body weight. The extracts 400 mg/kg and 800 mg/kg body weight provided 100 to 80% protections to animals against diarrhoea induced by castor oil. The maximal effects of the extract (800 mg/kg) were similar to the effect of loperamide a potent antidiarrhoeal drug.

**Discussion**

The phytochemical analysis of the methanolic crude extract of *Momordica balsamina* fruits gave positive results for flavonoids, tannins, saponnins, alkaloids and reducing sugars. Flavonoids and alkaloids were relatively higher than the other phytochemicals. This is in agreement with earlier reports of Mgbojikwe *et al*., (2002). They reported high flavonoid content in *M. balsamina* fruit pulp. Flavonoids and their conjugates form a large group of naturally occurring compounds, found in fruits, vegetables, flowers and roots. The

antispasmodic, antibacterial and anti-diarrhoeal effects of an array of medicinal plants have been attributed to their rich flavonoids, tannins or alkaloids rich extracts (Abdullahi *et al*., 2001; Viana *et al*., 2003; Okpara, 2015; Okpara *et al*., 2016. The concentration of essential and non-essential elements – lead, zinc, iron, sodium, magnesium, potassium, manganese in the fruit powder of *M. balsamina* appears to be within the safety limit (WHO, 1996; Umaru *et al*., 2011). The relatively high oral median lethal dose ($LD_{50}$) of 5500 mg/kg body weight suggests that the methanolic fruit extract of *M. balsamina* was relatively safe in mice in terms of acute lethality, according to Lorke (1983).

Castor oil-induced diarrhoea has been widely used to analyze and evaluate the antidiarrhoeal potential of drugs in experimental model (Okpara *et al*., 2007; Okpara, 2015). Castor oil is triglyceride of fatty acids. It causes watery stool due to its active metabolite-ricinolic acid within one hour of oral administration (Abdullahi *et al*.,

2001; Okpara *et al*., 2016). When released, ricinolic acid induces inflammation, cause increased fluid secretion and enhance motility of the gastrointestinal tract resulting in diarrhoea (Ammon *et al*., 1974; Okpara, 2015). The antidiarrhoeal effect of loperamide is believed to be due to its antimotility and antisecretory properties (Janseen, 2001). Loperamide also increases the tone of anal sphincter muscle thereby reducing incontinence and urgency (Karim and Adaikan, 1997; Abdullahi *et al*., 2001).

Since the methanolic fruit extract of *M. balsamina* has the ability to inhibit the castor oil-induced diarrhoea in the mechanism of action of the extract may include decreased gastrointestinal secretion and or inhibition of gastrointestinal motility. Flavonoids highly present in this extract has been demonstrated to inhibit contraction induced by spasmogens (Okpara, 2015), inhibit intestinal secretion and transit time in mice comparable to loperamide hydrochloride a potent antidiarrhoeal drug. From this result, it is possible that the extract of *M. balsamina* fruit mediate its effects through similar mechanism.

The percentage motility f charcoal meal in comparison to the entire length of the intestinal tract from the pyloric sphincter to the ileo-caecum was used as index of GIT motility in mice (Abdullahi *et al*., 2001; Okpara *et al*., 2007). The result of this study also revealed that the extract dose dependently prolonged the gastrointestinal transit period of activated charcoal-tragacanth meal. The extract at 400 mg/kg and 800 mg/kg reduced the distance travelled by charcoal meal to 80% and 100% respectively. This compared favourably with the antimotility effect of loperamide (5 mg/kg) an anticholinergic drug. Due to their constituent charcoal meals increases gastric acid secretions through the release of gastrin and acetylcholine, which signals the release of histamine (Okpara, 2015), thereby increasing propulsiua peristalsis and reducing intestinal transit time. The decrease of intestinal motility exhibit by the methanolic fruit extract of *M. balsamina* may have been mediated by inhibition of gastric acid secretions through the inhibition of ACh and gastrin releases, and prevention of histamine release and subsequent spasmodic effects (Okpara, 2015; Okpara *et al*., 2016).

Furthermore, the result revealed that the methanolic extract of *M. balsamina* fruit also, dose dependently reduced the castor oil-induced enteropooling. The extract at the dose of 400 mg/kg and 800 mg/kg body weigh reduced the weight of the intestinal content comparable to atropine and antimuscarinic drug. The ability of the extract to reduce the weight of the intestinal content could be by preventing fluid and electrolyte secretion into the intestinal lumen and or allow the content enough time to be exposed to the intestinal tract (Schullthesis, 1998; Umaru *et al*., 2011. Atropine and its related compounds are competitive antagonists of the action of ACh and other muscarinic agents. Muscarinic receptor antagonists prevent the effect of ACh by blocking its binding to muscarinic receptors at the neuroeffector sites on the smooth muscles, gland cells and peripheral ganglia (Aliu, 2007). Some standard antidiarrhoeal agents such as loperamide are known to exert their effects by binding to the opiate receptors in the gut wall (Janssen, 2001). It is therefore considerable that the methanolic fruit extract of balsamina mediated its antidiarrhoeal effects through similar mechanism as loperamide. The results suggest that fruits are valuable not only for nutritional purposes but also as good source of medicinal ingredients. This study tends credence to the use of this plant

in folkloric medicine for the treatment and control of diarrhoeal diseases.

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