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PHYSICOCHEMICAL EVALUATION AND ANTI-DIARRHOEA ACTIVITY OF *MONODORA MYRISTICA* SEED EXTRACTS ON ALBINO MICE

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ABSTRACT

Diarrhoea is one of the primary causes of illness and mortality in both children and adults. The aim of this study is to evaluate the quality of the crude drug and assess the anti-motility potentials of the crude and fractionated extracts of Monodora myristica (M. myristica) seeds. The pulverised seeds of M. myristica was extracted with 70% ethanol via marceration. Vacuum Liquid Chromatography (VLC) was carried out using hexane, dichloromethane, ethyl acetate and methanol. Phytochemical, physicochemical and anti-motility analysis of the crude extract and chromatographic fractions were carried out using standard methods. Phytochemical screening revealed the presence of saponins, alkaloids, cardiac glycosides, phenols, steroids, reducing sugar and flavonoids. Physicochemical analysis of the seed sample revealed that the moisture content of the seed was 6.60 ± 0.64%, while total ash, acid insoluble ash, alcohol soluble ash and water soluble ash of seed of M. myristica were 0.03 ± 0.00%, 0.01 ± 0.00%, 0.02 ± 0.00% and 0.00 ± 0.00%, respectively. The alcohol and aqueous extractive value were 0.37 ± 0.07 g and 0.13 ± 0.05 g, respectively. The groups that had the most significant reduction in the volume of stool passed were Groups 8, 4 and 5 treated with 400 mg/kg of fractions MM5, MM1 and MM2, respectively. The crude extract and fractions of M. myristica had good anti-motility activity, while the fractions exerted better activity. This study revealed the quality of the crude drug by exploring its physicochemical properties. Meanwhile, the seed extract of M. myristica exerts good antidiarrheal activity with MM1, MM2 and MM5 fractions as the most active fractions.

***Keywords*:** Diarrhoea, Physicochemical, Phytochemical, Anti-motility, Antidiarrheal

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INTRODUCTION

Diarrhoea is one of the major causes of morbidity and mortality amongst neonates, infants, children and adults in under-developed nations such as Nigeria. This is primarily due to a lack of cleanliness and proper sanitation. Studies have shown that more children are killed by diarrhoea than by malaria, measles and AIDS combined (Liu *et al*. 2012). Diarrhoea is a disease that primarily induces passage of loose or watery faeces three or more times in a 24 hour period (WHO 2024). It is best defined as an increase in the frequency of bowel evacuation. Diarrhoea is one of the most common and frequent gastrointestinal problems in the globe. Every year, almost 1.7 billion instances of diarrheal illness are reported. It is also the second biggest cause of death in children under the age of five, killing roughly 525,000 children under the age of five each year. Hypothetically, despite being avoidable, diarrhoea kills more individuals in this age range than measles, AIDS and malaria combined.

The most prevalent cause of diarrhoea is obstruction of the intestine's epithelium by numerous bacteria, viruses and parasites. Although the exact origin of the condition is uncertain in many clinical cases. Despite international organisations' efforts to control the disease, the prevalence of diarrhoea remains high. Diarrhoea is caused by an alteration in bowel movement, which results in an increase in water content and, as a result, a rise in the frequency of stools (Khalilur *et al*. 2015). The virus can spread through contaminated food, polluted drinking water and unsanitary environment. Apart from non-pharmacological measure such as good cleanliness, improved sanitation and rehydration, various orthodox drugs have been utilised to treat diarrhoea (Kola-Mustapha *et al*. 2019).

According to recent reports, approximately 2.2 million people are plagued by the disease worldwide, with children under the age of five being the most vulnerable groups. Due to the economic viability, accessibility and ancestral experiences reported on medicinal herbs usage, plant based natural products have become an overwhelming component of traditional medicines as they are practiced globally (Wendel *et al.* 2016). Thus, for gastrointestinal disorders (such as diarrhoea), investigating medicinal herbs has become critical in developing new therapies.

Monodora myristica (*M. myristica*) is a flowering plant in the Annonaceae (custard apple) family (Ojiako *et al.* 2010). The tree grows natively in the Sub-saharan African evergreen forests of West Africa, from Liberia to Nigeria, Cameroon and Ghana, as well as Angola, Uganda and West Kenya. *M. myristica*'s fruit is a 20 cm diameter berry that is smooth, green and spherical when mature, and turns woody. It is linked to a tall stalk that can grow to be 60 cm long. The numerous oblong, pale brown seeds, which are usually 1.5 cm long and immersed in a white sweet-smelling pulp, are found inside the fruit (Nwozo *et al.* 2015).

Over the years, lack of standardisation and accurate dosing has been the major criticisms levied against traditional medical practictioners (Sofowora 1993). There is a strong link between lack of patronage of herbal drugs and lack of standardisation of herbal drugs. It has been observed that the lack of patronage of herbal medicines is basically due to the lack of appropriate quality control measures meted on them as well as insufficient knowledge of herbal formulations. In the early times, quantity or dosage of crude drugs to be administered was solely dependent on common sense principles not necessarily age factor (Sofowora 1993). Despite the avalanche of pharmacological properties associated with *M. myristica* seeds, it is pertinent to ascertain the quality by deducing the physicochemical properties, hence, in addition to ascertaining the anti-motility activity of the crude ethanol extract and chromatographic fractions of *M. myristica*, this study also aims to deduce the quality of *M. myristica* seeds.

METHODS

Materials

The materials and equipment used includes; cotton wool, beaker, test tubes, test tube racks, conical flask, measuring cylinder, petri dish, spatula, masking tape, glass rod, weighing balance, ruler, refrigerator, Whatman filter paper, sample bottle, funnel, oven, sinta glass (for VLC), vacuum pump, dessicator, hot air oven, water bath, Thin-Layer Chromatography (TLC) plates and round bottom flask while reagents used are of analytical grade and they include; ethyl acetate (JHD®, China), n-hexane (Sigma-Aldrich, Germany), dichloromethane (DCM) (JHD®, China), butanol (JHD®, China), ethanol (JHD®, China), methanol (JHD®, China) and distilled water.

Collection and Authentication of Plant Material

The fresh seeds of *M. myristica* were obtained from small forest at main market, Onitsha, Anambra State around February, 2022. The plant was authenticated at the Department of Botany Herbarium, Delta State University, Abraka by the taxonomist, Mr. Michael Ozioma where the voucher number DELSUH103 was obtained.

Preparation of Plant Materials for Extraction

The seeds were processed in the laboratory at the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, Delta State University, Abraka, they were separated from their shell and dried for 4 days. They were subsequently pulverised and stored in an air tight container prior to extraction.

Extraction of Plant Sample

A total of 1,000 g of the powdered seed sample of *M. myristica* was macerated in 70% ethanol with a total solvent of 3,000 ml. The whole mixture was allowed to stand for 3 days with constant daily agitation, thereafter the macerated sample was filtered and the resulting filtrate was concentrated on a water bath at a temperature of 70°C. The final extract was weighed and the yield calculated. It was then stored in a refrigerator at a temperature of 4°C until further use.

Phytochemical Screening

The phytochemical screening was conducted according to standard procedures to detect the presence of phytoconstuents in the crude and fractionated extracts (Ekaete *et al.* 2013).

Tests for saponins

One millilitre (1 ml) of each extract was mixed with 1 ml of water and was vigorously shaken for a stable persistent froth. The froth formed indicates the presence of saponin in the plant extraction. 1 ml of the extract was reacted with 1 ml of olive oil and shaken vigorously, the formation of an emulsion confirms the presence of saponin.

Test for tannins

One millilitre (1 ml) of each extract was transferred to a test tube and few drops of 0.1% ferric chloride were added and the solution was observed for brownish green or blue black colouration.

Test for flavonoids

One millilitre (1 ml) of each extract was transferred to a test tube and was treated with 1 ml of sodium hydroxide (NaOH), followed by addition of dilute hydrochloric acid (HCl). A yellow solution with NaOH which turned colourless upon addition of HCl indicates the presence of flavonoids.

Test for alkaloids

***Dragendorff's test*:** One millilitre (1 ml) of each extract was measured and transferred to a test tube. This sample was then treated with few drops of dilute HCl. One millilitre (1 ml) of dragendorff's reagent was added to the solution. The formation of an orange precipitate indicates the presence of alkaloids.

***Mayer's test*:** One millilitre (1 ml) of each extract was measured and transferred to a test tube. This sample was then treated with few drops of dilute HCl. One millilitre (1 ml) of Meyer's reagent was added to the solution and was observed for a colour change. The formation of a whitish or creamy precipitate indicates the presence of alkaloids.

***Wagner's test*:** One millilitre (1 ml) of each extract was measured and transferred to a test tube. This sample was then treated with few drops of dilute HCl. One millilitre (1 ml) of Wagner's reagent was added to the solution and was observed for a colour change. The formation of reddish brown precipitate indicates the presence of alkaloids.

Test for terpenoids

One millilitre (1 ml) of each extract was measured and transferred to a test tube. One millilitre (1 ml) of chloroform was also measured and properly shaken together with the extract, a layer was formed. 1 ml of concentrated sulphuric acid was carefully added to the extract. A reddish brown colouration at the interface was formed upon the addition of the sulphuric acid indicating the presence of terpenoids.

Test for reducing sugars

One millilitre (1 ml) of Fehling A and B solutions were warmed together in a test tube. A freshly warmed One millilitre (1 ml) of each test sample was transferred to different test tubes containing the Fehling's solution. Observations were made for a dark green or red colour indicating the presence of reducing sugars.

Physicochemical Test

The method used was similar to that of Khandelwal (2008) but was subjected to slight modifications. Moisture content, ash values and extractive values were determined.

Determination of moisture (loss on drying)

Three empty porcelain dishes were weighed, labelled and weights where recorded, then 2 g of the powdered sample was weighed and transferred into each of labelled porcelain - dishes thereafter it was placed in a hot air oven at a temperature of 110°C for 15 minutes. After drying it was placed in a desiccator to cool and then all porcelain dishes were reweighed to obtain the final weight. The difference in weight of the final product was recorded as moisture.

Extractive Value

Determination of alcohol-oluble extractive

A total of 5 g of the powdered seed of *M. myristica* was weighed using a weighing bottle and transferred into a 250 ml flask, the flask was then filled with l00 ml of 90% ethanol and the flask was well covered with a cork. The flask was shaken and set aside to macerate for 24 hours as it was frequently shaken. After 24 hours, it was then filtered into a 50 ml cylinder and when sufficient filtrate was collected, 25 ml of the filtrate was then transferred into a weighed porcelain dish. Then it was evaporated to dryness over a water bath and the drying process was completed with hot air oven at 100°C. When it was completely dried, was then transferred into a desiccator to cool and was weighed. This procedure was repeated to obtain three values and average was calculated for. The percentage w/w of the extractive with reference to air dried drug was calculated.

Determination of water soluble extractive

A total of 5 g of the powdered seed of *M. myristica* was weighed using a weighing bottle and transferred into a 250 ml flask, the flask was then filled with l00 ml of chloroform water (1% chloroform in 99% water) and the flask was well covered with a cork. The flask was shaken and set aside to macerate for 24 hours as it was frequently shaken. After 24 hours it was then filtered into a 50 ml cylinder and when sufficient filtrate was collected, 25 ml of the filtrate was then transferred into a weighed porcelain dish. Then it was evaporated to dryness over a water bath and the drying process was completed with hot air oven at 100°C. When it was completely dried it was then transferred into a desiccator to cool and was weighed. This process was repeated to obtain three values and the average values was obtained. The percentage w/w of the extractive with reference to air dried drug was calculated.

Determination of Ash Value

Total ash value

A total of 4 g of *M. myristica* was placed on a crucible in a balance and the weight was noted. The crucible was covered with a lid and was placed in a muffle furnace, then the lid was removed to check the ash closely and few drops of distilled water was added to the ash content development of black colour indicates that the ash have not become carbon free, so there is need to burn again at 700°C until it becomes carbon free. The crucible was placed on a hot plate and the moisture ash was dried completely. The crucible was placed inside

the furnace for 40 minutes, removed and checked until no black is observed. Few drops of distilled water were added again to confirm. The absence of black colour means the ash is completely carbon free now.

Acid insoluble ash value

About 25 ml of 40% HC1 was added to the crucible containing total ash, covered with a lid and boiled for 5 minutes. The insoluble ash was collected on an ashless filter paper, washed with hot water until filtrate was neutral. The residues and the ashless was then transferred to the original crucible and ignited to constant weight. The crucible was cooled in a desiccator and weighed. The content of acid insoluble ash was calculated in mg/g of air dried material and expressed as percentage. The experiment was carried out in triplicate.

Water soluble ash value

About 25 ml of water was added to the crucible containing total ash and boiled for 15 minutes. The insoluble matter was collected in an ashless filter paper, washed with hot water and ignited on a heating mantle for 15 minutes at a temperature not exceeding 450°C. It is subjected to filtration; the crucible was then weighed to get constant weight. Water soluble ash was calculated by subtracting the weight of the residue in mg from the weight of total ash and expressed in percentage. The experiment was carried out in triplicate.

Vacuum Liquid Chromatography

Ethanol seed extract of *M. myristica* was subjected to Vacuum Liquid Chromatographic (VLC) procedure according to the methods used by Ikpefan *et al.* (2014) with slight modification. The VLC fraction of the seeds extract of *M. myristica* was carried out using 300 ml of hexane (100%), hexane – dichloromethane (75:25, 50:50, 25:75), dichloromethane (100%), dichloromethane – ethyl acetate (75:25, 50:50, 25:75), ethyl acetate (100%), ethyl acetate – methanol (75:25, 50:50, 25:75) and methanol (100%). A total of 13 fractions was obtained, these fractions were then analysed using TLC techniques and similar fractions were bulked. A total of four fractions were obtained from the TLC analysis, these were further concentrated and subjected to biological assay.

Acute toxicity study

According to the OECD-423 guidelines (2002), Nine albino mice were purchased from the animal house in the Faculty of Basic Medical Sciences, Delta State University, Abraka. The animals were kept fasting overnight and provided with water only. 2,000 mg/kg body weight of the extract was administered orally by gastric intubations, and the mice were observed for 14 days. Three animals were used for the toxicity test, mortality was observed in all three animals used; therefore, the dose administered was termed as a toxic dose. The concentration of the extract administered was stepped down to 400 mg/kg body weight, and the mice were observed for 14 days. No mortality was observed. Therefore, the same dose was repeated to confirm the safe dose of the extract.

Test for Antidiarrheal Activity

Test animals

Male albino mice (22 g–25 g) were collected and acclimatised to normal laboratory conditions for 1 week prior to study and given pellet diet and water ad libitum. Animals were handled according to the guideline of National Institute of Health Guide for Care and Use of Laboratory Animal. Ethical approval was granted by the Research, Ethics and Grants Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. The approval number is RBC/FBMC/DELSU/24/326.

Castor oil induced diarrhoea in mice

Male mice were divided into nine groups of five mice each. The animals were fasted for 24 hours prior to the test. Group 1 mice were treated with distilled water, which served as negative control, while Group 2 which served as the positive control group, received loperamide (3 mg/kg). Group 3 received 200 mg/kg crude extracts of *M. myristica*, Group 4 received 400 mg/kg MM1 fraction, Group 5 received 400 mg/kg of MM2 fraction, Group 6 received 400 mg/kg of MM3 fraction, Group 7 received 400 mg/kg of MM4 fraction and Group 8 received 400 mg/kg MM5 fraction. The animals in Group 9 was neither treated nor induced and they served as the normal control group. All doses were administered orally. After one hour, all groups except Group 9, received 1 ml of castor oil orally. The animals were placed in cages lined with adsorbent papers and observed for 4 hours for the presence of watery (wet), unformed stool. The average weight of stool in each group was noted. To ascertain the percentage inhibition, the control group result was considered as 100%. The activity of each group was expressed as percent inhibition (%) of diarrhoea. The percentage inhibition of defecation was calculated as follows:

$$\%\ inhibition = \frac{A-B}{A} \times 100$$

where, A indicates the mean number of defecations caused by castor oil and B indicates the mean number of defecations caused by drug extract.

Gastrointestinal Motility Test

In this test, selected mice were divided into nine groups of five mice in each. At first, 1 ml of castor oil was given orally to every rat of each group to produce diarrhoea. After one hour, Group 1 (negative control group) received distilled water orally. Group 2 received standard drug (loperamide 3 mg/kg) and Group 3 received 200 mg/kg extract of *M. myristica* while groups 4 to 8 received 400 mg/kg of fractions MM1, MM2, MM3, MM4 and MM5, respectively. The mice in Group 9 made up the normal control group. After one hour, all animals across all groups except Group 9, received 1 ml of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour later, following the charcoal meal administration, all animals were euthanised, and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum, was measured and expressed as percentage of the total length of the small intestine from pylorus to the caecum (Rahman *et al.* 2012).

$$\% \text{ intestinal transit} = \frac{\text{Length traveled by charcoal}}{\text{Total length of small intestine}} \times 100$$

$$\text{Transit inhibition} = T_0 - T_1$$

where,

T_0 = intestinal transit of negative control
T_1 = intestinal transit of test drug

Statistical Analysis

The data obtained were evaluated using Microsoft Excel 2016. Data were represented as mean and standard error of mean (SEM).

RESULTS

Percentage Yield of Extracts of *M. myristica*

The percentage yield of the crude extract of the seed of *M. myristica* is presented in Table 1. The result shows the yield of the crude extract and four bulked chromatographic fractions of the crude extract of *M. myristica*. The percentage yield of crude extract was 11.04% while those of the five chromatographic fractions were 44.89, 7.77, 10.11, 7.09 and 10.57%, respectively.

Table 1: Percentage yield of crude extract and chromatographic fractions of *M. myristica*.

Sample	Weight (g)	Yield (%)
Crude extract	91.00	11.04
MM 1	15.71	44.89
MM 2	2.72	7.77
MM 3	3.54	10.11
MM 4	2.48	7.09
MM 5	3.70	10.57

Phytochemical Analysis

The preliminary phytochemical screening of the crude extract and chromatographic fractions of the seed of *M. myristica* was carried out and the result showed the presence of seven phytoconstituents which includes; saponins, flavonoids, steroids, reducing sugar, phenol, cardiac glycoside and alkaloids in the crude extract while tannins and terpenoids were absent (Table 2).

Table 2: Result of preliminary phytochemical screening of *M. myristica.*

Phytoconstituents	Crude extracts	Fraction MM1	Fraction MM2	Fraction MM3	Fraction MM4	Fraction MM5
Saponin	+	+	+	–	+	+
Tannins	–	–	+	+	–	+
Flavonoids	+	+	+	+	+	+
Cardiac glycosides	+	–	+	+	–	+
Terpenoids	–	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Steroid	+	+	+	–	+	+
Reducing sugar	+	–	+	–	–	+
Phenol	+	+	+	+	+	+

Note: + = present, – = absent.

Physicochemical Evaluation of the Seed of *M. myristica*

Physicochemical evaluation of the seed of *M. myristica* shows that the dried sample has percentage moisture content of 6.60 ± 0.64% while the total ash content was 0.03 ± 0.00%, acid soluble and alcohol soluble and water soluble ash content were 0.01 ± 0.00 and 0.02 ± 0.00 and 0.00 ± 0.00%, respectively. Extractive values using alcohol and water was 0.37 ± 0.07 and 0.13 ± 0.05 g, respectively (see Table 3).

Table 3: Result of physicochemical evaluation of the seed sample of *M. myristica.*

Parameter	Pulverised *Monodora myristica*
Moisture content (%)	6.60 ± 0.64
Ash content (%)	
Total ash content	0.03 ± 0.00
Acid soluble ash	0.01 ± 0.00
Alcohol soluble ash	0.02 ± 0.00
Water soluble ash	0.00 ± 0.00
Extractive value (g)	
Alcohol extractive value	0.37 ± 0.07
Aqueous extractive value	0.13 ± 0.05

Antidiarrheal Evaluation

The result of the antidiarrheal evaluation of the crude extract and chromatographic fractions are presented in Table 4 and Figure 1. The result shows that the volume of stool passed by the grouped given with normal saline increase significantly when compared to the treatment group. Group 3 treated with 200 mg/kg of the crude extract reduce the volume of stool passed by the mice when compared to positive control group administered with 3 mg/kg

of loperamide. The group that had significant reduction in the volume of stool passed are group 8, 4 and 5 treated with 400 mg/kg of MM5 fraction, 400 mg/kg of MM1 fraction and 400 mg/kg of MM2 fraction, respectively.

Table 4: Effect of *M. myristica* seed extracts on castor oil induced diarrhea.

Group	Initial weight of mice (g)	Final weight of mice (g)	Weight of stool (g)	Percentage inhibition of diarrhoea (%)
Group 1	19.16 ± 0.08	18.15 ± 0.31	0.64 ± 0.08	Nil
Group 2	18.81 ± 0.37	17.05 ± 0.44	0.30 ± 0.18	87.50
Group 3	21.34 ± 0.89	20.42 ± 0.88	0.10 ± 0.07	50.00
Group 4	24.33 ± 0.64	23.74 ± 0.78	0.04 ± 0.02	66.67
Group 5	24.62 ± 0.06	24.33 ± 0.06	0.02 ± 0.00	83.33
Group 6	23.28 ± 0.73	22.69 ± 0.54	0.22 ± 0.12	33.33
Group 7	22.52 ± 0.98	21.88 ± 1.20	0.54 ± 0.10	50.00
Group 8	21.75 ± 2.17	19.67 ± 0.87	0.01 ± 0.01	83.33
Group 9	21.92 ± 0.15	21.99 ± 0.62	0.06 ± 0.01	0.00

Note: Group 1 = distilled water (negative control); Group 2 = loperamide 3 mg/kg (positive control); Group 3 = 200 mg/kg crude extract; Group 4 = 400 mg/kg MM1 fraction; Group 5 = 400 mg/kg MM2 fraction; Group 6 = 400 mg/kg MM3 fraction; Group 7 = 400 mg/kg MM4 fraction; Group 8 = 400 mg/kg MM5 fraction; Group 9 = Normal control.

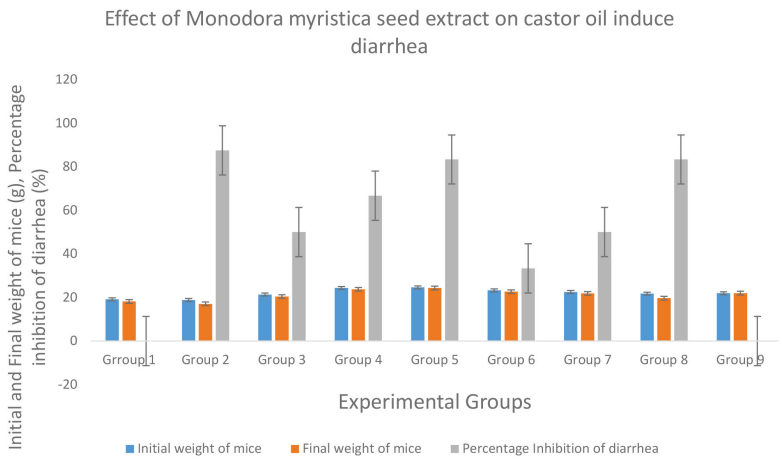

Figure 1: Effect of *Monodora myristica* seed extract on castor oil induced diarrhea

Note: Group 1 = distilled water (negative control); Group 2 = loperamide 3 mg/kg (positive control); Group 3 = 200 mg/kg crude extract; Group 4 = 400 mg/kg MM1 fraction; Group 5 = 400 mg/kg MM2 fraction; Group 6 = 400 mg/kg MM3 fraction; Group 7 = 400 mg/kg MM4 fraction; Group 8 = 400 mg/kg MM5 fraction; Group 9 = normal control.

Anti-motility Evaluation

The intestinal transit of the activate charcoal was measured and the result showed that group treated with 3 mg/kg of loperamide reduce the motility of the charcoal with mean weight travelled 0.47 ± 0.01 m and percentage inhibition of 29.29% when compared to the

crude extract and fractions with percentage inhibition of 27.07%, 15.17%, 22.67%, 22.83%, 27.17% and 18.67%, respectively. Groups treated with MM3 fraction, 200 mg/kg of crude extract, MM4 and MM5 fraction also reduced the motility of the charcoal with mean weight travelled of 0.57 ± 0.04, 0.65 ± 0.03, 0.72 ± 0.09 and 0.72 ± 0.05 m (see Table 5 and Figure 2).

Table 5: Result of castor oil induced intestinal transit in mice.

Groups	Length of intestine (mm)	Length travelled by activated charcoal (mm)	Whole weight (g)	Weight travelled (g)	Transit inhibition (mm)	% Intestinal transit
Group 1	53.00 ± 1.25	50.50 ± 0.21	1.22 ± 0.02	0.73 ± 0.01	–	95.28
Group 2	53.00 ± 0.47	21.21 ± 0.14	1.27 ± 0.01	0.47 ± 0.01	29.29	40.02
Group 3	56.00 ± 1.70	23.43 ± 3.73	1.61 ± 0.08	0.65 ± 0.03	27.07	41.84
Group 4	57.33 ± 2.88	35.33 ± 2.33	1.90 ± 0.21	1.04 ± 0.11	15.17	61.63
Group 5	57.00 ± 0.94	27.83 ± 3.95	1.73 ± 0.03	0.65 ± 0.07	22.67	48.82
Group 6	55.40 ± 2.32	27.67 ± 1.19	1.59 ± 0.04	0.57 ± 0.04	22.83	49.94
Group 7	60.67 ± 1.79	23.33 ± 1.92	2.11 ± 0.03	0.72 ± 0.09	27.17	38.45
Group 8	57.17 ± 1.57	31.83 ± 1.30	1.56 ± 0.08	0.72 ± 0.05	18.67	55.67
Group 9	56.14 ± 0.54	Nil	2.13 ± 0.60	Nil	Nil	Nil

Note: Group 1 = distilled water (negative control); Group 2 = loperamide 3 mg/kg (positive control); Group 3 = 200 mg/kg crude extract; Group 4 = 400 mg/kg MM1 fraction; Group 5 = 400 mg/kg MM2 fraction; Group 6 = 400 mg/kg MM3 fraction; Group 7 = 400 mg/kg MM4 fraction; Group 8 = 400 mg/kg MM5 fraction; Group 9 = normal control.

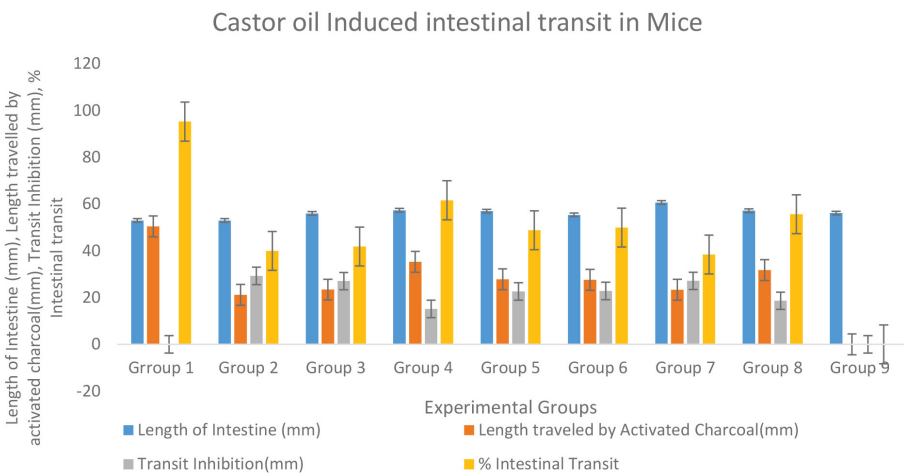

Figure 2: Castor oil induced intestinal transit in mice

Note: Group 1 = distilled water (negative control); Group 2 = loperamide 3 mg/kg (positive control); Group 3 = 200 mg/kg crude extract; Group 4 = 400 mg/kg MM1 fraction; Group 5 = 400 mg/kg MM2 fraction; Group 6 = 400 mg/kg MM3 fraction; Group 7 = 400 mg/kg MM4 fraction; Group 8 = 400 mg/kg MM5 fraction; Group 9 = normal control.

DISCUSSION

The use of plant derived medicines for the treatment of diarrhoea is a common practice in many folk medicines. Many people in the developing countries still rely on this treatment option. This study has evaluated the antidiarrheal property of the crude extract and chromatographic fraction of the *M. myristica* on male albino mice. Antidiarrheal agents mostly act by decreasing secretion and/or reducing the propulsive movement of gastrointestinal smooth muscles. Castor oil induces diarrhoea through a pathophysiological mechanism induced by its active metabolite, ricinoleic acid. Once castor oil is administered orally, it is broken down into ricinoleic acid; a hydroxylated fatty acid by the action of intestinal lipases in the intestinal lumen and considerable amounts of ricinoleic acid is absorbed in the intestine. Ricinoleic acid then mediates its action by binding with EP3 prostanoid receptors on smooth muscle of the intestine (Tunaru *et al.* 2012).

The presence of castor oil in the gastrointestinal tract triggers prostaglandin secretion, which in turn induces gastrointestinal motility (Bahekar and Kale 2015). The results obtained showed that the extract and chromatographic fractions of *M. myristica* has a great potential to reduce the frequency and quantity of stool at a dose comparable to that of the conventional drug used as control. This might be ascribed to the presence of biochemical constituent present in the samples.

Researchers have discovered that phytoconstituents such as tannins, flavonoids, alkaloids, sterols, terpenes, saponins and reducing sugars have been found to be responsible for antidiarrheal effects of medicinal plants (Abu *et al.* 2013). Flavonoids present antioxidant properties which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism (Chen *et al.* 2018) and consequently prostanglandin synthesis. The active metabolite of castor oil (ricinoleic acid) might also activate the nitric oxide pathway and induce nitric oxide dependent gut secretion (Machado *et al.* 2014).

According to scientific studies, phytoconstituents such flavonoids and alkaloids are responsible for reducing nitric oxide generation (Perri *et al.* 2019). The presence of these phytoconstituents may have increased the reabsorption of electrolytes and water by impeding castor oil mediated nitric oxide synthesis as nitric oxide has been reported to play a significant role in castor oil induced diarrhoea, explaining the pronounced inhibition of castor oil mediated intestinal fluid secretion observed with crude extract and fractions *M. myristica* (Hu *et al.* 2009). The study revealed a better anti-diarrhoea activity of fractions than that of the crude extract with MM1, MM2 and MM5 possessing better activity than other fractions.

Anti-motility study revealed a significant inhibition in the transit of the activate charcoal along the gastrointestinal tract which shows a decrease motility of the charcoal at a concentration 400 mg/kg of fraction MM3 than other fractions and the crude extract. A reduction in the weight of the activated charcoal travelled shows that the extract and fractions were able to reduce the motility of the gastrointestinal tract and thus reduce diarrhoea. Distilled water, which was the negative control, showed a lower inhibition of the charcoal, as it will normally not have effects on diarrhoea. This finding is comparable to the study of Ofokansi *et al.* (2020) who reported an elicited significant reduction in the number of wet stool in a dose–dependent manner with dichloromethane fraction (DCMF) (500mg/kg) offering the highest on the antidiarrheal activity.

The anti-motility activity can also be ascribed to the presence of phytochemical in the crude extract and chromatographic fractions of the seed sample. Previous study has shown that tannins inhibits gastrointestinal movement by reducing the intracellular calcium ions inward current or by the activation of calcium pumping system as well as

forming tannates which make the intestinal mucosa more resistant to movement and hence reduction in peristaltic movement (Belemtougri *et al.* 2006). More so, alkaloids have also been demonstrated to have inhibitory effect on gastrointestinal motility (Aleem & Janbaz, 2018).

The physicochemical parameters of the seed sample revealed the percentage moisture content to be 6.6%. Lowering the moisture content of crude pharmaceuticals can increase their shelf life and make them easier to carry. Drug deterioration thrives well in products with high moisture content, which shortens their shelf life (Emebu & Anyika 2011). The percentage mean value of the moisture content for this study was lower than that reported by Nkwocha *et al.* (2012) (14.50±0.52%). According to Faleyimu and Oluwalana (2008), who reported the moisture content of *M. myristica* as 10%. The results of this investigation are consistent with those of Enwereuzoh *et al.* (2015), who found that *M. myristica* had a moisture content of 6%.

Ash content describes the persistent inorganic residues after the full oxidation or burning of the organic materials in the sample and provides a summary of the mineral content of the substance, the ash content also help determines the purity and authenticity of crude drugs (Nkwocha *et al.* 2012) A high amount of ash denotes a sample with a high mineral content since the ash value of plant material measures the amount of minerals and earthy material adhering to the plant material. According to the permissible range for ash in seeds, nuts, and tubers (1.5%–5%), the amount of ash found in the plant sample was relatively low. As a result, *M. myristica* is unlikely to be a good source of minerals in the diet of humans. The results support those of Nkwocha *et al.* (2012), who also reported that *M. myristica* seeds had a low ash concentration.

The extractive value that is water soluble shows the presence of inorganic, organic and organic components. The extractive values that were soluble in alcohol suggested the existence of polar components. As a result, the study's findings show that the plant sample has a high alcohol extractive value because it has more polar constituents than there is sugar, acids or inorganic chemicals. This is in line with the findings of Ahlam *et al.* (2014), who noted that methanol had a higher extractive value than water.

CONCLUSION

From this study, it can be suggested that the seed extract of *M. myristica* possess antidiarrheal activity. Fractions MM1, MM2 and MM5 had better antidiarrheal activity when compared to the crude extract and fraction MM3 and MM4 as they reduce the level of stooling and inhibited intestinal motility in the mice. The antidiarrheal activities could be attributed to the presence of flavonoids and alkaloids which has been reported to possess antidiarrheal property. The physicochemical parameters revealed that the crude drugs were within standard values and also that it is not prone to microbial contamination as a result of the low moisture content. These results, therefore validates the folkloric use of this plant extract in the treatment of diarrhoea. Further study is recommended to explore lower dosage of the extract and fractions that produce best activity.

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