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*Original Research Article*

# Evaluation of Antidiarrhoeal Activity of Methanol Extract of The Leaf of *Margaritaria discoidea*

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ABSTRACT

*Margaritaria discoidea* leaves are used traditionally in the treatment of diarrhoea. The study aimed to evaluate the antidiarrhoea activity of the methanol extract of the leaves of *M. discoidea.* The fresh leaves of *M. discoidea* were collected, dried, and authenticated. The acute toxicity effect was determined using the castor oil-induced protocol. The plant extract was given orally at 10, 100, and 1000 mg/kg body weight. Loperamide was used at 2 mg/kg body weight orally as a positive control standard. Male adult albino Wistar rats (180-256 g) consisting of thirty (30) rats divided into six (6) groups of five (5) rats each, were acclimatized for 7 days in a standard animal house and then exposed to 12 h light and dark cycle at 25 ºC and humidity of 55 %, with free access to water and feed, were used in this experiment. The effects of *M. discoidea* methanol leaf extract on the number and total faecal output (NTF), number and weight faecal output (NWF), weight of the wet faecal output (WWF), and weight of total faecal output (WTF) on castor oil-induced diarrhoea in rats indicated that the rats pretreated with the vehicle (Tween 80) and administered with castor oil showed significantly ($p < 0.05$) higher rate of WWF in 2, 3 and 4 h post treatment. The crude extract significantly reduced the NWF output as the dose of the extract increased, with an increase in post-treatment time compared to the vehicle pretreated group.

***Keywords:*** *Margaritaria discoidea*, Antidiarrhoeal, Castor oil, Loperamide, Methanol extract.

## Introduction

Diarrhoea is the frequent passage of more than three (3) liquid stools daily.[1] There are clear distinctions in the definition of diarrhoea. One such is that diarrhoea does not involve frequent passing of formed stools or the passage of pasty stools.[2] Whenever the intestinal tract is infected, symptoms usually develop, leading to diarrhoea.[1] Food, water intake, and person-to-person contact are the most common sources of infection.[3] According to the World Health Organization (WHO), diarrhoea is classified into three clinical types: acute watery diarrhoea (which lasts for several hours or days), acute bloody diarrhoea (dysentery), and persistent diarrhoea (≤ 14 days).[3] Some pathogens associated with acute diarrhoea include: *Vibrio cholerae, Escherichia coli*, and rotavirus.[2] Bacterial pathogens associated with diarrhoea include: *Escherichia coli, Salmonella spp, Shigella spp,* and *Campylobacter spp.*[3,4] Due to the chronic nature of persistent diarrhoea, it can be accompanied by blood. Chronic diarrhoea is usually high in children who are malnourished or have co-morbidity diseases such as obesity, viral gastroenteritis, irritable bowel movement, inflammatory bowel disease, malabsorption syndrome, and chronic infections.[5] The third leading cause of death, especially in children under five (5) years old, is diarrhoea, and it has been documented that it kills around 443,832 children every year. [6,7]

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A survey that was carried out recently reported that 34 sub-Saharan countries recorded a high clustering of diarrhoeal disease (15.3 %) among children aged 5 years and below.[8] Although the sudden onset of diarrhoea in some cases can be self-limiting, the primary concerns are usually severe cases that result in excessive fluid loss and electrolyte imbalance. This leads to the need for both the pharmacological and non-pharmacological approaches. [9, 10] Some signs associated with severe diarrhoea include sunken eyes, lethargy, unconsciousness, and irritability.[11] Some key measures deployed to prevent diarrhoea include access to safe drinking water, improved sanitation, vaccination, health education, and good personal hygiene.[12]

There are challenges associated with some of the current drugs (Loperamide, bismuth subsalicylate, rifaximin, dicyclomine, alosetron, and eluxadoline) used in treating diarrhoea. They include drug-drug interactions, drug resistance (especially for Loperamide) and adverse effects such as constipation, nausea, headaches and farting.[13] These led scientists to investigate and look for alternative natural product medications. The dependence on traditional medicines is very high in some developing countries like Nigeria.[14] Five medicinal plants are commonly used in diarrhoeal treatment in Nigeria. They include: *Acacia nilotica (*Fabaceae), *Acanthospermum hispidum (*Asteraceae), *Parkia biglobosa* (Fabaceae) and *Margaritaria discoidea* (Phyllanthaceae).[15] *Margaritaria discoidea* is a tree in the family Phyllanthaceae, commonly known as the pheasant-berry, egossa red pear, or bushveld peacock-berry. These trees are native to the warmer, higher rainfall areas of Africa. A medium to tall tree in forest and riverine areas, where it can grow up to 30 meters tall, or a shrub or small tree in drier and more open areas.[15] The stem is usually straight with rough, flaking bark, grayish-brown on top and reddish beneath.[14] The branches of young trees grow horizontally from the stem. The leaves are alternate and produced on one plane. Male and female flowers are produced on separate trees, with both types of flowers being small, greenish-yellow in colour, and fragrant. The fruit is a three-lobed capsule about 10 mm in diameter and golden-brown

when ripe.[15] The inner part of the fruit is dark metallic blue-green, giving rise to the name bushveld peacock-berry.[15] These trees are used in traditional medicine across Africa: A leaf-decoction is taken in Ivory Coast for blennorrhoea and poisoning, while in Ubangi, a decoction of roots and leafy twigs is also used for blennorrhoea.[15] A decoction wash is used as a stimulant in case of general fatigue. The bark is a purgative in West Africa and an anthelmintic in Central Africa.[14] The Fula people use the bark for toothache, in the Central African Republic, a decoction is used for post-partum pains, and in the Republic of the Congo for stomach and kidney complaints and to facilitate parturition. In Malawi, the powdered bark extract is applied to swellings and inflammation for quick relief.[15] This study evaluated the antidiarrhoea activity of methanol extract of the leaves of *M. discoidea* using the castor oil induced protocol, and a standard antidiarrhoeal drug, Loperamide, as a positive control.

## Materials and Methods

*Materials*

Loperamide hydrochloride, Imodium® (Teva Pharmaceuticals, USA), was used as the standard antidiarrhoeal drug, Glycerin (Humanwell Pharmaceuticals PLC, India), Castor oil (*Ricinus communis*), and Tween 80 (Alkest TW 20®, Nigeria). Chloroform (Lucas Oil Products, Inc. USA, 95.6% purity, 2% concentration), Sodium Chloride (ChemQuest International Inc. USA, 96% purity), Acetone (Naphtha Pharmaceuticals USA, 99.9% purity, 750 ppm), Ethanol (Lucas Oil Products, Inc. USA, 95.6% purity, 2% concentration). Glycerin, Naphthol solution (ChemQuest International Inc., USA), Phloroglucinol, Acetic acid, Ruthenium red, Potassium hydroxide solution (Humanwell Pharmaceuticals PLC, India). All other reagents used were of analytical grade.

*Experimental animals*

Adult albino male Wistar rats between 10 and 14 weeks old, weighing between 180 and 256 g, were used for the experiment. The animals were obtained from the central animal house of the Department of Veterinary Pathology, University of Nigeria, Nsukka. The animals were acclimatized for 7 days in a standard animal house and then exposed to a 12 h light and dark cycle at 24 –25 ºC and a humidity of 55%. The animals were allowed free access to water and feed. Before the start of the experiment, the animal ethics committee at the University of Nigeria, Nsukka, approved the research protocol involving the use of laboratory animals (approval number, FPSRE/UNN/20/0050). All the experiments were carried out according to the International Code of Practices for Care and the Use of Animals for Scientific Purposes (ICPCS).

*Plant material*

The fresh leaves of *Margaritaria discoidea* were collected from Nru village in Nsukka Local Government Area of Enugu State, 6.842942ºN latitude and 7.373266ºE longitude, Nigeria in January 2020. The leaves were authenticated by Mr. Felix Nwafor, a taxonomist at the Department of Pharmacognosy, University of Nigeria, Nsukka, with the voucher number PCG/UNN/0308, assigned to it. The leaves were washed with running tap water and air-dried under shade at 25 ºC for 14 days. The dried leaves were pulverized to a 1 mm mesh size with a mechanical grinder (Gx160 Delmer 5.5 HP).

*Macroscopical examination of the leaves*

Macroscopical studies of the specimens, which comprised organoleptic characters (colour, odour, appearance, taste, shape, texture, venation, presence or absence of petiole, the apex, margin, base, lamina) of the fresh leaves, were evaluated following standard procedures as described by Trease and Evans. [16]

*Fresh leaf microscopy*

The leaves' foliar epidermis of the adaxial (upper surface) and abaxial (lower surface) surfaces was prepared using the clearing method.[17] The leaf samples were cleared by soaking in commercial bleach containing 3.5 % sodium hypochlorite for 18 hours. Then, the epidermal strips of the leaf samples were scraped gently with a pair of forceps and placed on a clean slide, stained with Safranin solution, and covered with a cover slip. The slides were viewed under a light Olympus Tokyo (Japan No.271961) microscope at x40, x100, and x400 magnifications, and photomicrographs were taken with a Motican Camera 2.0. The following parameters were observed and assessed.[17,18]

Epidermal cells: the type and number of epidermal cells were counted and recorded, Stomata type: the stomatal complex types were recorded following the terminologies, Stomata size (length and width): the stomata length and width were measured using Motic microscope software, a total of ten (10) field of views for each sample. Stomatal density: the stomatal density was determined as the number of stomata per square millimetre.

Stomatal index (S.I.): the stomatal index was determined as follows:

$$S.I = (S/S + E)\ x\ 100 \quad (1)$$

Where: S = Number of stomata in a field of view, E = Number of epidermal cells in the same field of view.

Trichome parameters: the trichome types, size, density, and index were determined following the same procedures as the stomata above, vein islet number, and vein islet termination number. All parameters were observed on the leaves' adaxial and abaxial surfaces.[16]

*Microscopy of the Transverse Section of the Leaf*

A transverse section (TS) of the leaf was made using a Reichert sledge microtome following the following procedures.[19] The sections were microtomed at 10 – 15 unimicrons and picked with a camel hair brush from the tip of the microtome knife into separate petri dishes containing 70 % absolute alcohol and labeled appropriately. Safranine and fast green served as biological stains in differentiating lignified tissues.

*Powdered Leaf Microscopy*

The leaves were dried in the shade and pulverized with a local mortar and pestle. Chemical microscopy was conducted on the powdered leaf to determine the presence of starch, calcium oxalate crystals, and lignified vessels. A 0.5 g quantity of the sample was placed on a glass slide, and two (2) drops of chloral hydrate were dropped and passed over a Bunsen burner repeatedly until bubbles formed. This ensured that the colored pigments in the powdered leaf were cleared.[19]

*Test for Starch*

A 1 mL quantity of iodine was added to a 1.0 g quantity of the cleared leaf powder on a glass slide. One (1) drop of glycerin was added and observed under a light Olympus Tokyo (Japan No.271961) microscope at x400 magnification.[19]

*Test for Lignin*

A 1.0 g quantity of the cleared powder was added to a glass slide, 1:1 phloroglucinol and concentrated hydrochloric acid were dropped, and a drop of glycerin was added and observed under a light Olympus Tokyo (Japan No.271961) microscope at x 100 magnification.[19]

*Test for Calcium oxalate crystals*

A 1 mL quantity of concentrated acetic acid was added to a 1.0 g quantity of the cleared leaf powder on a glass slide. A drop of glycerin was added and observed under an Olympus (LM) light microscope at x 400 magnification.[19, 20]

*Test for Gums and Mucilage*

A drop of red Ruthenium was added to a 1.0 g of cleared powder sample. The appearance of pink coloration indicated the presence of gums and mucilage.

*Determination of physicochemical parameters (Proximate analysis)*

The physicochemical constants of the powdered leaf samples were determined to evaluate the quality and purity of the extract.[19]

Parameters, such as total ash, water-soluble ash, and acid-insoluble ash values, were calculated per World Health Organization (WHO) guidelines. Alcohol and water-soluble extractive values were determined to determine the amount of water and alcohol soluble components. The determination of the total ash value, acid-insoluble ash, and water-soluble ash, respectively, followed established protocols.[17,16,17] The results were expressed in percentages calculated with reference to the air-dried plant sample.

*Determination of moisture content*

The powdered leaf (2 g) was weighed into a China dish, and the contents were evenly distributed at a depth not exceeding 10 mm. The loaded plate was heated at 105 ºC in a hot air oven and weighed at different intervals until a constant weight was obtained. The difference in weight after drying and the initial weight was the moisture content.[16, 17]

Determination of extractive yields

*Alcohol soluble extractive value*

The powdered leaf sample (5 g) was macerated with 100 mL of ethanol for 24 h with intermittent shaking using a mechanical shaker during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered through a filter paper. A 25 mL filtrate was evaporated in a tarred dish at 105 ºC until a constant weight was obtained. The percentage of alcohol-soluble extractive value was calculated with reference to the air-dried material.[16]

*Water-soluble extractive value*

The same procedure was followed for the alcohol extractive, except that water replaced alcohol.[16]

*Hexane soluble extractive value*

The powdered leaf sample (5 g) was macerated with 100 mL n-hexane with frequent shaking during the first 6 hours with a mechanical shaker and allowed to stand for 18 hours. The extract was filtered, and 25 mL of filtrate was evaporated in a tarred dish at 105 ℃ and weighed. The percentage of hexane-soluble extractives was calculated with reference to the air-dried substance.[16]

*Extraction of plant material*

The dried leaf sample (1 kg) of *Margaritaria discoidea* was ground into powder and macerated with 2.5 L of methanol with intermittent shaking after every 2 hours for 48 hours. The macerated was filtered through a cotton wool plug, and the filtrate was then concentrated under reduced pressure using a rotary evaporator (Powai, Mumbai-400076) to obtain the crude extract.[17, 19]

*Pharmacological studies*

*Acute-toxicity test*

The acute toxicity test of the methanol extract of the leaves of *Margaritaria discoidea* was determined using Lorke's modified method.[21] Adult albino male Wistar rats weighing 180 –256 g were used for the acute toxicity test. The animals were obtained from the central animal house of the Department of Veterinary Pathology, University of Nigeria, Nsukka. The animals were acclimatized for 7 days in a standard animal house and then exposed to 12 12-hour light and dark cycle at 24 –25 ºC and a humidity of 55 %. The animals were allowed free access to water and feed. A batch of 30 randomly selected adult rats that weighed between 180 and 256 g was used for this investigation. They were assigned to six (6) groups of five rats per group (n = 5). The first three groups received oral doses of 10, 100, and 1,000 mg/kg body weight of the extract in the first phase. In the second phase, the rats in groups 4, 5, and 6 received single oral doses of 1600, 2900, and 5000 mg/kg body weight, respectively. Signs of toxicity and the number of deaths were observed for 24 hours.

*Antidiarrhoea activity (Castor oil-induced diarrhoea test)*

The effect of the methanol extract of *Margaritaria discoidea* on castor oil-induced diarrhoea was evaluated in rats using the Awouters method.[22] In this method, twenty (20) adult albino rats that had previously been fed a standard Pfizer diet and had free access to drinking water were used. After 18 hours of fasting, the rats were assigned to four groups of five rats per group and treated as follows with single oral doses:

Group 1 (control): Received 5 mL/kg body weight of Tween 80 (vehicle) per rat.

Group 2: Received 2.5 mg/kg body weight of Loperamide (standard antidiarrhoeal drug).

Group 3: Received 200 mg/kg body weight of the methanol leaf extract of *Margaritaria discoidea*

Group 4: Received 400 mg/kg body weight of the methanol leaf extract of *Margaritaria discoidea*

One (1) hour after the above treatments, each rat in groups (1–4) received 1 mL of castor oil orally, and the rats were separated into their respective cages. The rats were observed for fecal discharge consistency and defecation frequency. The faeces were collected on a white paper sheet placed beneath the cages. The wet and dry droppings numbers for each rat were counted every 1 hour for 4 hours, and the white papers were changed periodically for each evaluation. The number of wet and dry faeces counted indicated the degree of wetness and the frequency of defecation.[22]

*Preparation of fluid samples for electrolyte test*

Twenty (20) adult albino rats who had previously been fed a standard Pfizer diet and allowed free access to water were used in this experiment. Rats were fasted for 18 hours and divided into four groups of five rats (n = 5).[22] Each rat in groups (1-4) received 1 mL of castor oil orally using a suitable stomach tube, and after 1 hour, rats in different groups were treated as follows:

Group 1: Received 5 mL/kg body weight of Tween (80) (vehicle) orally.

Group 2: Received 2.5 mg/kg body weight of Loperamide orally.

Group 3: Received 200 mg/kg body weight of the methanol leaf extract of *Margaritaria discoidea* orally.

Group 4: Received 400 mg/kg body weight of the methanol leaf extract of *Margaritaria discoidea* orally.

Two (2) hours after the above treatment, the rats were anaesthetized and humanely sacrificed by chloroform inhalation.[23] The small intestine of each rat was located and tied at the pylorus and ileocecal junction, then cut out, and the contents were milked out into a measuring cylinder. The effluents of the rats' intestinal loops (serosal solutions) were centrifuged at 3000 x g for 30 minutes. The supernatants were obtained and analyzed for concentrations of sodium (Na) and potassium (K) ions.

*Determination of sodium ion ($Na^+$) concentration (Teco Diagnostic Kit)*

The determination of sodium concentration was carried out using the method of Dosso *et al*.[23]

*Determination of potassium ion ($K^+$) concentration (Teco Diagnostic Kit)*

The determination of potassium concentration was carried out using the method of Dosso *et al.*[23]

*Statistical analysis*

The results obtained were analyzed using one-way analysis of variance (ANOVA) and expressed as mean ± standard deviation. The difference between means of treated and control groups was further evaluated using the least significant difference (LSD) Post hoc test and considered significant at $p < 0.05$.

## Results and Discussion

The macroscopic analysis of *M. discoidea* is presented in Table 1, which includes the leaves' habitat and the alternate arrangement, while Table 2 show the organoleptic characters of the leaf powder of *Margaritaria discoidea* which showed that the odour, taste and colour of the leaves were slightly aromatic, slightly bitter and light green, respectively.

The microscopic evaluation of the powdered leaf of *M. discoidea* in Table 3 showed the presence of epidermal cells containing stomata, bundle of phloem parenchyma cells, and an epidermal cell with anomocytic type of stomata. Subsidiary cells and trichomes were

absent. The photomicrographs of the leaf of *M. discoidea* are presented in Figure 1 (a-d). The adaxial surface of the leaf of *M. discoidea* showed polygonal-shaped epidermal cells and the absence of stomata, the abaxial surface of the leaf of *M. discoidea* showed epidermal cells and anomocytic type of stomata, the transverse section of the leaf of *M. discoidea*, and the detailed transverse section of the leaf of *M. discoidea* were present. The photomicrographs of the leaf powder of *M. discoidea* are presented in Figure 2 (e-i). The chemo microscopy of the leaf powder shows lignified tissues and cork cells, and the leaf fragment in chemo microscopy shows an anomocytic type of stomata. The chemomicrograph of the powder showed a large crystal of calcium oxalate, the chemomicrograph of the leaf powder showed a mass of palisade cells, and the chemomicrograph of the leaf powder showed cork cells and epidermal cells, respectively. The result of the microscopy of the leaf powder of *M. discoidea* is presented in Table 4. The microscopical examination shows the presence of lignin, starch, and calcium oxalate, while gum and mucilage were absent. The analytical standards of the leaf, which showed the percentage composition of total ash, water soluble ash, acid insoluble ash, moisture content, water soluble yield, alcohol soluble yield, hexane soluble yield, chloroform soluble yield, and ethyl acetate soluble yield, are presented in Table 5.

**Table 1:** Macroscopic study of *Margaritaria discoidea*

| | |
|---|---|
| Habit and Habitat | *M. discoidea* is a tropical tree that grows up to 26 feet tall. Samples of the leaf were collected from a tree growing within the Nru village in Nsukka Local Government Area of Enugu State, Nigeria |
| Leaf | The leaves alternate in arrangement. They are ovate-elliptic in shape, acute at the base, and acuminate at the apex with an entire margin. They are glabrous (lack hairs), green on the upper surface and grey on the lower surface. They measure up to 11.9 cm in length and 6.3 cm in width. |

**Table 2:** Organoleptic properties of the leaf powder of *Margaritaria discoidea*

| | |
|---|---|
| Odour | Slightly aromatic |
| Taste | Slightly bitter |
| Colour | Light green |

**Table 3:** Microscopy of the leaf of *M. discoidea*

| | |
|---|---|
| Epidermal cell | Epidermal cells are polygonal in shape on both the upper and lower surfaces of the leaves. |
| Stomata type | The leaf is hypostomatic (stomata only occur on the lower surface), and the anomocytic type of stomata – subsidiary cells are absent. Guard cells are directly surrounded by epidermal cells. |
| Trichome | Absent |
| Stomata density ($mm^{-2)}$ | 135.29 ± 3.40 |
| Stomata index (%) | 27.56 ± 1.04 |
| Stomata length (µm) | 19.13 ± 1.03 |
| Stomata width (µm) | 11.82 ± 0.63 |
| Stomata size ($\mu m^2$) | 227.28 ± 24.14 |
| Vein islet number ($mm^{-2}$) | 9.34 ± 0.05 |
| Veinlet termination number ($mm^{-2}$) | 11.26 ± 0.13 |

**Table 4:** Microscopy of the leaf powder of *M. discoidea*

| **Parameter** | ***M. discoidea*** |
|---|---|
| Lignin | Present |
| Starch | Present |
| Calcium oxalate | Present (prism shape) |
| Cystoliths | Present |
| Oil body | Absent |
| Gum and mucilage | Absent |

**Table 5:** Analytical Standards of *M. discoidea*

| **Test** | **Composition (%)** |
|---|---|
| Moisture content | 7.40 ± 0.21 |
| Total Ash | 4.85 ± 0.03 |
| Water-soluble ash | 2.45 ± 0.03 |
| Acid insoluble ash | 3.21 ± 0.04 |
| Water soluble yield | 12.70 ± 0.21 |
| Alcohol soluble yield | 17.40 ± 0.14 |
| Hexane soluble yield | 4.07 ± 0.33 |
| Chloroform soluble yield | 6.03 ± 0.05 |
| Ethyl acetate soluble yield | 6.33 ± 0.03 |

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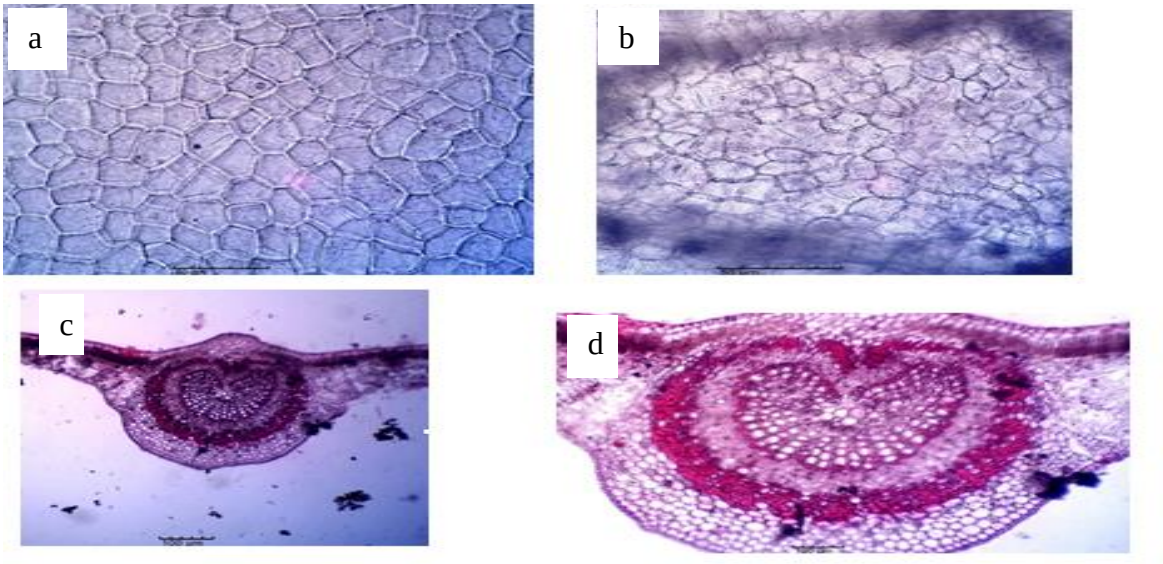


**Figure 1:** Photomicrographs of the leaf of *M. discoidea* (a) Adaxial surface of the leaf of *M. discoidea* showing polygonal-shaped epidermal cells (Ec) and absence of stomata (X 400), (b) Abaxial surface of the leaf of *M. discoidea* showing epidermal cells (Ec) and anomocytic type of stomata (St) (X 400). (c) Transverse section of the leaf of *M. discoidea (X 40) (d)* Transverse section of the leaf of *M. discoidea (X 40)*

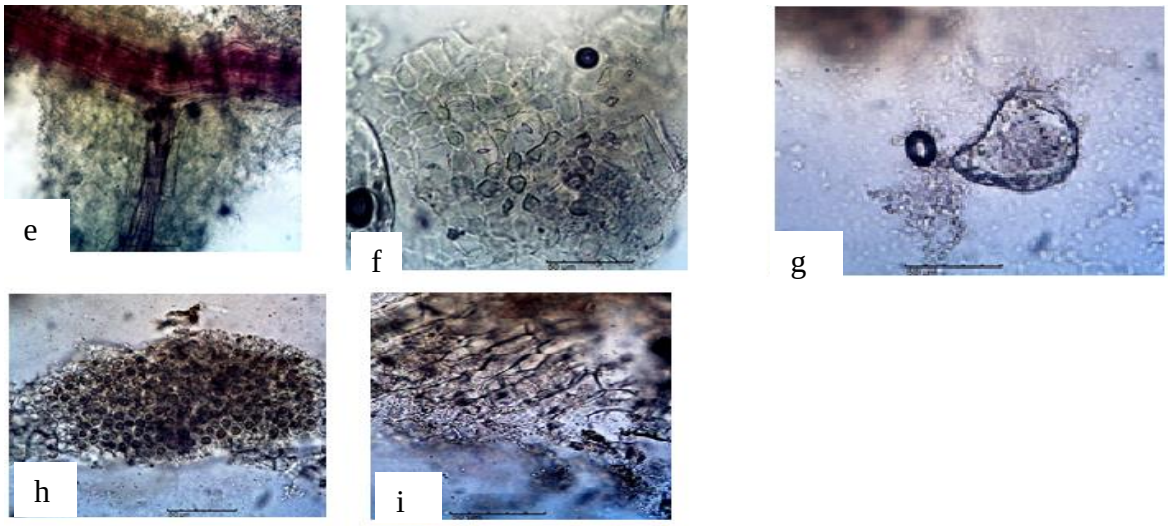


**Figure 2:** Photomicrographs of the leaf powder of *M. discoidea* (e) Chemomicroscopy of the powder showing lignified tissues (Lt - vessels and fibres) and cork cells (Cc)
(f) Fragment of the leaf in chemomicroscopy showing anomocytic type of stomata (St), epidermal cell (Ec), and crystal of calcium oxalate (CaOx)
(g) Chemomicrograph of the powder showing a large crystal of calcium oxalate (CaOx)
(h) Chemomicrograph of the powder showing a mass of palisade cells (Pc)
(i) Chemomicrograph of the powder showing cork cells and epidermal cell

The acute toxicity and lethality of the extract doses of 10, 100, and 1000 mg/kg are represented in Table 6. Zero mortality rate was recorded at extract doses of 10-1000 mg/kg, and no mortality was recorded at extract doses of 1600, 2900, and 5000 mg/kg.[24] Furthermore, the antidiarrhoeal activity of the crude extract of *Margaritaria discoidea*, one hour post-treatment, is presented in Table 7. The number and total faecal output (NTF) of the normal and standard samples were 10.50 ± 1.50 and 9.50 ± 0.50, respectively, while the untreated batch recorded 11.50 ± 1.50.

In the acute toxicity test, none of the animals that received 10, 100, and 1000 mg/kg of the crude extract died. Also, in the second phase of the experiment, there was no mortality after 24 hours of oral doses of 1600, 2900, and 5000 mg/kg. The acute toxicity test in mice using *M. discoidea* extract did not show any observable toxic effect, and there was no recorded mortality at the highest dose of 5000 mg/kg.[25] This finding was subsequently correlated with the report of Adedapo,[26] which is in tandem with the present study, thus indicating that the extract could be considered safe at the different doses tested. Studies have reported that no dose-related toxicity should be reported above 5000 mg/kg body weight by the oral route.[21] Acute toxicity test of the extract showed that even at 5000 mg/kg body weight, the rats showed no sign of toxicity, suggesting that the *M. discoidea* should generally be considered safe. The results on the effects of *M. discoidea* methanol leaf extract on the number and total faecal output (NTF), number and weight of faecal output (NWF), weight of the wet faecal output (WWF), and weight of total faecal output(WTF) on castor oil-induced diarrhoea in

rats indicated that the rats pretreated with the vehicle (Tween 80) and administered with castor oil showed significantly ($p< 0.05$) higher rate of WWF in one (1) hour post treatment when compared to those in 2, 3 and 4 hours post treatment (Tables 8, 9 and 10). The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, releasing prostaglandins, which stimulate motility and secretion.[21] Diarrhoea results from an imbalance between the absorptive and the secretory mechanisms in the intestinal tract, resulting in excess fluid loss in faeces. In some diarrhoea, the secretory mechanism dominates; others are characterized by hypermotility. Pretreatment with methanol leaf extract of *M. discoidea* in the 3rd and 4th hours significantly ($p < 0.05$) reduced the incidences of both NWF and WWF in castor oil-induced diarrheic rats compared to the vehicle pretreated group (Tables 9 and 10, respectively). The results obtained by pretreatment with 400 mg/kg body weight of the methanol leaf extract of *M. discoidea* were statistically significant ($p < 0.05$) with that of Loperamide (2.5 mg/kg), a standard drug used in diarrhea cases. The evidence presented here revealed that the methanol leaf extract of *M. discoidea* inhibits both the incidence and severity of diarrhoea.[26] The decrease in wetness of faeces can be attributed to the anti-enteropooling activity of *M. discoidea*. In contrast, the reduction in NTF, NWF, and WTF can be attributed to the decreased propulsive contraction of the intestine.[27] These observations indicate that the extract in graded doses reduced diarrhoea, probably by inhibiting peristalsis and gastrointestinal (GI) motility in castor oil-induced enteropooling.[28]

Similarly, the extract's effect on electrolytes' intestinal fluid concentration showed that the extract caused significant ($p < 0.05$) reductions in electrolytes (concentration of intestinal sodium ions) compared to the control. The extract may also have exerted its antidiarrhoeal effect by promoting water and electrolyte absorption.[29,30] The antidiarrhoeal activity of the crude drug significantly ($p < 0.05$) reduced the number and weight of wet faecal output as the extract dose increased with an increase in post-treatment time, as shown in Tables 7- 10. Also, the study's results showed that the NTF and WTF were the same for all the treatment groups. However, the NWF was different only in the normal control group, while weight of the WWF showed the most variation within the treatment groups (Table 7). The effects of the methanol leaf extract of *Margaritaria discoidea* were observed two (2) hours post-treatment. The result showed that NTF and WTF were the same for all the treatment groups, while NWF and WWF showed a significant difference ($p < 0.05$) for the treatment groups (Table 8). Also, the extract's effects three hours post-treatment showed that NTF and WTF were the same for all the treatment groups, while NWF and WWF showed a significant difference ($p < 0.05$) for the treatment groups (Table 9). The study also showed that NTF and WTF were the same for all the treatment groups, while NWF and WWF showed significant differences ($p < 0.05$) for the treatment groups, as shown in Table 10.

**Table 6:** Acute toxicity and lethality

| Dose of Extract (mg/kg) | Mortality | Dose of Extract (mg/kg) | Mortality |
|---|---|---|---|
| 10 | 0/3 | 1600 | 0/1 |
| 100 | 0/3 | 2900 | 0/1 |
| 1000 | 0/3 | 5000 | 0/1 |

**Table 7:** Antidiarrhoeal activity of the crude extract of *Margaritaria discoidea*, one hour post-treatment (mg/kg)

| Treatment groups | NTF | NWF | WWF | WTF |
|---|---|---|---|---|
| Normal | $10.50\pm1.50^{a}$ | $0.50\pm0.50^{b}$ | $0.00\pm0.00^{b}$ | $2.00\pm0.00^{a}$ |
| Standard | $9.50\pm0.50^{a}$ | $4.50\pm0.50^{a}$ | $1.00\pm0.00^{ab}$ | $2.00\pm0.00^{a}$ |
| Untreated | $11.50\pm1.50^{a}$ | $8.00\pm2.00^{a}$ | $1.50\pm0.00^{a}$ | $2.50\pm0.50^{a}$ |
| 200 mg/kg | $10.00\pm1.00^{a}$ | $7.00\pm1.00^{a}$ | $1.50\pm0.50^{a}$ | $2.00\pm0.00^{a}$ |
| 400 mg/kg | $10.50\pm0.50^{a}$ | $5.50\pm0.50^{a}$ | $1.00\pm0.00^{ab}$ | $2.00\pm0.00^{a}$ |
| 600 mg/kg | $9.50\pm0.50^{a}$ | $5.00\pm0.00^{a}$ | $1.00\pm0.00^{ab}$ | $2.00\pm0.00^{a}$ |

Values expressed as means ± standard error of 4 replicate data
Means with different letters as superscripts are significantly different at $p < 0.05$
NTF = Number and Total Faecal output, NWF = Number and Weight Faecal output
WWF = Weight of the Wet Faecal output, WTF = Weight of Total Faecal output
Normal = vehicle, Standard = Loperamide 2 mg/kg

**Table 8:** Antidiarrhoeal activity of the crude extract of *Margaritaria discoidea* two hours post-treatment (mg/kg)

| Treatment groups | NTF | NWF | WWF | WTF |
|---|---|---|---|---|
| Normal | $11.50\pm0.50^{a}$ | $0.00\pm0.00^{c}$ | $0.00\pm0.70^{c}$ | $2.49\pm0.07^{a}$ |
| Standard | $12.50\pm0.50^{a}$ | $4.00\pm0.00^{bc}$ | $0.57\pm0.01^{bc}$ | $2.64\pm0.06^{a}$ |
| Untreated | $11.00\pm2.00^{a}$ | $9.50\pm1.50^{a}$ | $2.09\pm0.35^{a}$ | $2.33\pm0.35^{a}$ |
| 200mg/kg | $10.50\pm1.50^{a}$ | $6.00\pm1.00^{ab}$ | $1.11\pm0.46^{b}$ | $2.33\pm0.95^{a}$ |
| 400mg/kg | $10.00\pm0.00^{a}$ | $5.50\pm0.50^{ab}$ | $0.78\pm0.09^{bc}$ | $2.41\pm0.12^{a}$ |
| 600mg/kg | $10.50\pm0.50^{a}$ | $2.70\pm2.30^{bc}$ | $0.66\pm0.06^{bc}$ | $2.39\pm0.60^{a}$ |

Values expressed as means ± standard error of 4 replicate data.
Means with different letters as superscripts are significantly different at $p < 0.05$
NTF = Number and Total Faecal output, NWF = Number and Weight of Faecal output
WWF = Weight of the Wet Faecal output, WTF = Weight of Total Faecal output
Normal = vehicle, Standard = Loperamide 2 mg/kg

**Table 9:** Antidiarrhoea activity of the crude extract of *Margaritaria discoidea* three hours post-treatment (mg/kg)

| Treatment groups | NTF | NWF | WWF | WTF |
|---|---|---|---|---|
| Normal | 14.00±1.00$^{a}$ | 0.50±0.50$^{d}$ | 0.00±0.00$^{c}$ | 3.00±0.00$^{a}$ |
| Standard | 13.50±0.50$^{a}$ | 3.50±0.50$^{c}$ | 0.50±0.50$^{bc}$ | 3.00±0.00$^{a}$ |
| Untreated | 11.00±1.00$^{a}$ | 8.50±0.50$^{a}$ | 2.00±0.00$^{a}$ | 2.50±0.50$^{a}$ |
| 200mg/kg | 12.50±1.50$^{a}$ | 7.00±1.00$^{ab}$ | 1.50±0.50$^{ab}$ | 2.50±0.50$^{a}$ |
| 400mg/kg | 13.00±1.00$^{a}$ | 6.00±1.00$^{bc}$ | 1.50±0.50$^{ab}$ | 3.00±0.00$^{a}$ |
| 600mg/kg | 13.50±1.50$^{a}$ | 5.00±1.00$^{ab}$ | 1.00±0.00$^{ab}$ | 3.00±0.00$^{a}$ |

Values expressed as means ± standard error of 4 replicate data
Means with different letters as superscripts are significantly different at $p < 0.05$
NTF = Number and Total Faecal output, NWF = Number and Weight Faecal output
WWF = Weight of the Wet faecal output, WTF = Weight of Total Faecal output
Normal = vehicle, Standard = Loperamide 2 mg/kg

**Table 10:** Antidiarrhoeal activity of the crude extract of *Margaritaria discoidea* four hours post-treatment (mg/kg)

| Treatment groups | NTF | NWF | WWF | WTF |
|---|---|---|---|---|
| Normal | 14.50±0.50$^{a}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{b}$ | 2.50±0.50$^{a}$ |
| Standard | 14.00±0.00$^{a}$ | 2.50±0.50$^{cd}$ | 0.00±0.00$^{b}$ | 3.00±0.00$^{a}$ |
| Untreated | 14.00±1.00$^{a}$ | 2.50±1.50$^{a}$ | 2.50±0.50$^{a}$ | 3.00±0.00$^{a}$ |
| 200 mg/kg | 14.50±0.50$^{a}$ | 5.50±0.50$^{b}$ | 1.00±0.00$^{b}$ | 3.00±0.00$^{a}$ |
| 400 mg/kg | 14.50±1.50$^{a}$ | 4.00±1.00$^{bc}$ | 0.50±0.50$^{b}$ | 3.00±0.00$^{a}$ |
| 600 mg/kg | 15.00±0.00$^{a}$ | 3.50±0.50$^{bc}$ | 0.50±0.50$^{b}$ | 3.00±0.00$^{a}$ |

Values expressed as means ± standard error of 4 replicate data
Means with different letters as superscripts are significantly different at $p < 0.05$
NTF = Number and Total Faecal output
NWF = Number and Weight of Faecal output
WWF = Weight of the Wet Faecal output
WTF = Weight of Total Faecal output
Normal = vehicle
Standard = Loperamide 2 mg/kg

The effect of methanol leaf extract of *Margaritaria discoidea* on intestinal fluid potassium ion ($K^+$) and sodium ion ($Na^+$) concentration is presented in Table 11. The results for the crude extract of *Margaritaria discoidea* on potassium and sodium ion concentrations revealed that in the normal group, the K and Na ion concentrations were 4.00 ± 0.00 and 138.00 ± 2.00, respectively. However, the highest sodium ion concentration (148.00 ± 3.06) was observed in the treatment group compared to the control.

**Table 11:** Effect of the crude extract of *Margaritaria discoidea* on potassium ion and sodium ion concentration (mg/kg)

| Treatment groups | $K^+$ (MEq/L) | ($Na^+$) (MEq/L) |
|---|---|---|
| Normal | 4.00±0.00$^{a}$ | 138.00±2.00$^{bc}$ |
| Standard | 4.00±0.00$^{a}$ | 136.50±3.50$^{c}$ |
| Untreated | 5.50±1.50$^{a}$ | 163.00±3.00$^{a}$ |
| 200 mg/kg | 4.00±0.00$^{a}$ | 134.50±1.50$^{c}$ |
| 400 mg/kg | 4.00±0.00$^{a}$ | 141.50±5.00$^{bc}$ |
| 600 mg/kg | 4.50±0.50$^{a}$ | 148.00±3.06$^{b}$ |

Values expressed as means ± standard error of 4 replicate data
Means with different letters as superscripts are significantly different at $p < 0.05$
Normal = vehicle, Standard = Loperamide 2 mg/kg

## Conclusion

The methanol crude extract of the leaf of *Margaritaria discoidea* has been employed in folk medicine for its antidiarrhoeal activities. Pretreatment with the methanol leaf extract of *Margaritaria discoidea*, especially in the 3$^{rd}$ and 4$^{th}$ hours, significantly ($p < 0.05$) reduced the incidences of both the number and weight of wet fecal output in castor-induced diarrhoea in Wistar rats compared to the vehicle pretreated group. This justifies the ethno-medicinal claim of using the plant (leaf) in managing gastrointestinal disorders such as diarrhoea.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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