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Pharmacognostic and antidysentery screening of mixed ethanol leaf extract of *Parkia biglobosa* and *Acanthus montanus* (50:50)

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Abstract

Introduction: *Parkia biglobosa* belong to the family mimosaceae and *Acanthus montanus* belong to the family acanthaceae. The plants both have a multipurpose use as herbal medicine.

Aim: This work investigate the pharmacognostic standard and anti-dysentery activity of the mixed ethanol extract of both plants on *E. histolytical* induced dysentery.

Method: The phytochemical, chemo microscopy and proximate analysis were carried out using the standard procedures. The mixed extract of ratio 50:50 was administered to different groups of rats at different doses. Loperamide was administered as standard drug. The change in faecal consistency was observed and recorded.

Result: The phytochemical analysis shows the presence of alkaloids, tannins, proteins, glycosides and carbohydrates. Chemomicroscopy of both plants showed the presence of starch, calcium oxalate, lignin, cellulose. While the extractive value analysis gave 25.0% and 32.7% for water extractive value and ethanol extractive value respectively (for *Acanthus montanus*) and 23.0% and 30.0% for water extractive value and ethanol extractive value respectively (for *Parkiabiglobosa*).

The absence of death at 5000mg/kg of the extract shows that the lethal dose of the ethanol extract of the plant mixture is higher than 5000mg/kg which may be an indication of safety of the mixture.

The anti-dysentery activity of the mixture of both plants with different concentration of 250mg/kg, 500mg/kg, 1000mg/kg body weight of ethanol extract showed a significant change in the faecal consistency of the rats. The effect obtained on the administration of 500mg/kg body weight of the extract mixture is more comparable to the standard (loperamide).

Conclusion: The synergistic use of the extract contains the secondary metabolites glycosides, alkaloids, flavonoids, which may be responsible for their anti-dysentery synergistic activity. Mixed extracts from both leaves of *P. biglobosa* and *A. montanus* can be recommended as an anti-dysentery agent.

Keywords: *Parkiabiglobosa; Acanthus motanus*; Anti-dysentery; Phytochemicals

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1. Introduction

1.1. Herbal medicine

The World Health Organization (WHO) defines herbal medicine as a practice which includes herbs, herbal materials, herbal preparations and finished herbal products, that contain as active ingredients parts of plants, or other plant materials, or combinations [1] These herbs are derived from plant parts such as leaves, stems, flowers, roots, and seeds [2]

Herbal drugs contain active ingredients, plant parts or plant materials in the processed or crude state with certain excipients, i.e., dilutions, solvents or preservatives [2][3] These active ingredients protect plants from damage and diseases and contribute to the plants aroma, flavor and color. Scientifically, they are known as phytochemicals which include several classes such as saponins, flavonoids, glycosides, tannins, alkaloids and terpenoids [4]

Today herbal medicine is still the primary healthcare system for about 80% of the world's population, especially in the developing countries [5][6][7]. There has been also a sudden increase in the utilization of herbs as prescription drugs in developed countries such as France and Germany [8][9]. However, there is a concern that not all herbal medicines are safe as reported [10]. Over the years the use oftraditional medicine has provided us with valuable formulas on the selection, preparation and application of herbal remedies. The same vigorous method clinically and scientifically must be implemented to verify the effectiveness and safety of curative products, to be viable alternative to western medicine [11]

1.2. Dysentry

1.2.1. Explanation of Dysentery

Dysentery is an intestinal infection that causes severe diarrhea with blood. It can be caused by a parasite or bacteria. Dysentery is usually spread as a result of poor hygiene. For example, if people who have dysentery don't wash their hands after using the toilet, anything they touch is at risk. The infection is also spread through contact with food or water that has been contaminated with fecal matter. Dysentery is an intestinal inflammatory primarily of the colon; it can lead to mild or severe stomach cramp and severe diarrhoea with mucus or blood in the faeces.[12]

1.2.2. Types of dysentery

According to World Health Organization there are two main types of dysentery.

Bacillary dysentery

Amoebic dysentery

Bacillary Dysentery

Bacillary dysentery is the most common type of dysentery. It results from bacteria called *Shigella*. The disease is called shigellosis. The term bacillary dysentery etymologically might seem to refer to any dysentery caused by any bacilliform bacteria, but its meaning is restricted conventionaly to Shigella dysentery. The bacteria of the genus Shigella, secrete substances known as cytotoxins, which kill and damage intestinal tissue on contact. Shigella is thought to cause bleeding due to invasion rather than toxin, because even non-toxogenic strains can cause dysentery. It tends to be most prevalent when flies are at their most prolific [13].

1.2.3. Amoebic Dysentery

Amoebic dysentery is also called amoebiasis, it comes from a parasite called *Entamoeba histolytica.* [14]. When amoebae inside the bowel of an infected person are ready to leave the body, they group together into cyst and form a shell that surrounds and protects them, which is then passed out in the feces and can survive outside the body.

1.2.4. Mechanism of Action.

Under poor hygienic conditions of exposing feces, contamination of the surroundings take place such that nearby food and water are infected. People in the contaminated environment will then be infected with the amoebae. Amoebic dysentery is particularly common in parts of the world where human feces are used as fertilize. Once in the mouth, the cyst travels down into the stomach. The amoebae inside the cyst are protected from the stomach's digestive acid. From the stomach, the cyst travels to the intestines, where it breaks open and releases the amoebae, causing the infection.

The amoebae can burrow into the walls of the intestines and cause small abscesses and ulcers to form. The cycle then begins again. [15]

The common signs and symptoms of dysentery are as follows:

Fever and chills.
Abdominal pain.
Nausea and vomiting.
Fatigue.
Watery diarrhoea which can contain blood, mucus or pus.
Painful passing of stool.

Symptoms normally present themselves after 1 to 3 days, and are usually no longer present after a week.Temporary lactose intolerance can occur, as well. In some severe occasions, there are; severe abdominal cramps, fever, shock , and delirium can all be symptoms.[15]

1.2.5. Complications in Dysentery

The most common complication of dysentery, is dehydration. Other complications of dysentery may include:

Severely low potassium levels, which can cause life-threatening heartbeat changesSeizures; Hemolytic uraemic syndrome (a type of kidney damage).

1.3. Acanthus montanus

1.3.1. Description

The plant *Acanthus montanus* (Nees) T. Anders (also called mountain thistle or alligator plant) is a perennial herb that belongs to the family of Acanthaceae. It is a striking small shrub with sparse branches and soft stem.It is also reported to be one of the threatened and underutilized species of vegetables in Africa [16] due perhaps to its highly perishable nature. It is a vigorously thinly branched perennial with basal clusters of oblong to lance-shaped, glossy, dark green leaves reaching up to 12 inches long [17]. The leaves have silver marks with wavy margins. The plant grows up to 3 feet

Figure 1 Leaves of *Acanthus montanus* herbs

tall after which it starts growing horizontally bearing the weight of the leaves about 14 inches long, with spikes of pale pink flowers[17]

1.3.2 *Chemical Constituent*

Ethanol extract of *A. montanus* aerial parts has been reported to contain flavonoides (e.g. β-sitosterol glucoside), fatty acids (e.g. palmitic acid), sterol glucoside (e.g. linaroside, homoplantagenin), phenolic acids (e.g. shikimic acid and protochatecuic acid), phenyl ethanoid 6(e.g. acetoside) [18], phenylethanoid glycoside (acanmontanoside, decaffeoylverbascoside, verbascoside, isoverbascoside and leucosceptoside A), benzoxazinoid glucosides and aliphatic alcohol glycosides [19]

1.3.2. Medicinal Use

This plant is common in Nigeria and aside from its ornamental usage, In folk medicine, decoctions of this leaves are used for different therapeutic uses such as management of diabetes, treatment of body aches and pains, cough, inflammatory diseases, infectious diseases, cough, and others [20][21]. This plant is common in Nigeria. It has been used in folk medicine to relieve aches, pains and to treat furuncles [22]. In Benue State, Nigeria, the plant is utilized by the Etulo natives to treat worms in children and adults [23]

Pharmacological studies shows that the plant has spasmolytic [24], analgesic [25], anti-inflammatory and antipyretic [26] activities.

1.4. Parkia biglobosa

1.4.1. Description

Parkia biglobosa (Jacq.) G. Don belongs to the family Memosaceae, it is a tree that propagates by roots and stems. It is a rain forest deciduous perennial plant grown as shade plant in compounds and in village squares and it grows even on walls of dilapidated houses as the roots spread. [27]. The leaves of the plant is an alternate, greenish in colour with oval-shaped, entire-margin, and a rounded base. The leaves produce whitish latex when cut from the stem, this latex has a pharmacological activity [28]. It is a that grows to between 7 and 20 metres high, in some cases up to 30 metres [29]

In Burkina Faso, *Parkia biglobosa* is a tree of utmost importance as a source of edible products and income for the vast majority of rural households [29] [30]. The species, known as néré in Francophone Africa, is indigenous to sub-Saharan Africa [31] and has a very wide distribution range.It has been ranked by local people among the top priority tree species in Burkina Faso [32][33]

1.4.2. Chemical Constituent

Qualitative determination of chemical and nutritional composition of *Parkia biglobosa* was carried out. Seeds and leaves of *P. biglobosa* were found to be rich in protein, carbohydrates, soluble sugars, glycosides, flavonoids and ascorbic acid. Phenolic compounds found in the leaves, roots, seeds are of minute quantity and are grouped into simple phenols, phenolic acids, flavones, flavonone and methoxyflavonol [34]

Figure 2 Leaf of *Parkia biglobosa* (Jacq.) G. Don

1.4.3. Medicinal Uses

Parkia is found in many countries of the world especially along the West Africa coast where the seeds are known to be rich in protein and vitamin B2. A decoction of the stem bark is used as a hot mouthwash to relieve toothache as well as a bath for fever by the Hausa people of Northern Nigeria and other part of West Africa [35]. *P. biglobosa* is also used among the Hausa people against bronchitis, pneumonia, diarrhoea, vomiting, sores and ulcers. The leaves are also used for toothache, burns and for sore eyes in Gambia [36]. The roots of *P. biglobosa* has been reported to be used in lotions for sore eyes when combined with leaves, they are active against bronchitis, pile, amoebiasis [37]

1.5. Plant Taxonomy of *Acanthus montanus* (Nees) T.Anderson

1.5.1. Scientific classification

Kingdom Plantae
Phylum Tracheophyta
Class Magnoliopsida
Oder Lamiales
Family Acanthaceae
Genus *Acanthus*
Species *Acanthus montanus* (Nees) T.Anderson
Binomial Name: *Acanthus montanus*
Common names: Bear's breech, white's ginger, leopard's tongue
Local Names: Ahon-ekun, irunmu-arugbo, Inyinyiogwu. Ogwunwaokuko.

1.5.2. Morphology

Acanthus montanus (Nees) T. Anders is a small stinging shrub belonging to the family of Acanthaceae and growing wild in grassland woods and rocky hills. Geographical distribution in Africa, Benin, Ghana, Nigeria, Togo, Central African Republic, Cameroon Equatorial, Guinea Gabon, the Balkans, Romania, Greece and Eastern Mediterranean [38] *Acanthus montanus* is a native of West Africa and has been introduced to the rest of the world as an ornamental plant.It can grow up to 2m tall. Leaves are opposite, glossy and papery in texture, deeply pinnately-lobed, up to 20cm long and 10cm wide. The lobes have spines and the upper surface is glossy dark green in colour. Flowers are showy, pinkish-white with large bracts having spiny teeth. The upper bract is larger than the lower. Calyx is bilabiate the upper being larger. The corolla is unilabiate, the upper one being rudimentary. The fruit is a capsule 2.5cm long.

1.5.3. Ethnopharmacology

Traditional uses

Cardiovascular Diseases: In various countries of west Africa where this plant is endemic, the leaves of *A. montanus* had been used in the treatment of hypertension and cardiac dysfunctions. Nigerians use the leaves, while the Geviya people of Gabon makes use of the young shoots eaten with salt to treat their heart diseases. Hypertension is treated by giving the patient a decoction of the leaves [39][40][41].

Respiratory Disease: The leaves of *A. montanus* in the form of tea is used by the people of Gabon and those of southeastern Nigeria to treat cough.[40]. Another society in Africa advocates the use of the leaves and the bark of the stem [41]

Gastrointestinal Diseases: The macerated leaves of *A. montanus* is used to induce vomiting in children among the Geviya tribe of Gabon. Women with stomach-ache and nausea is given young shoots cooked with peanut butter which is called mo-dika to provide them with relieve of the complaint [40] Abdominal pains are relieved by drinking the decoction of the leaves. The leaves and stems had been used to ease the pains of acute gastritis and is believed to be an antacid [42]. Leaves are also used in the treatement of Hepatitis and Hepatosplenomegaly in certain areas of Africa.

Inflammatory & Infectious Diseases: *A. montanus* is used in the treatment of inflammatory conditions by scarification using the thorns. This is done in similar manners by the Geviya people of Gabon [40] and the people of Aguambu-Bamumbu of the Cameroon. [43]. The same group of people make use of parts of the plant to treat gonorrhoea and syphilis. In this case the macerated stems are given to the patient. The roots is highly acclaimed by various society in Africa as an effective remedy for abscesses. To the people of southeastern Nigeria the roots are macerated and applied over the boils.

Pharmacological activities

It has been reported to be effective in the treatment of urogenital infections, urethral pain, endometritis, urinary disease,cystitis, leucorrhoea, aches and pains (Okoli *et al*., 2008)[38]. A. montanus leaves have been shown to display analgesic, anti-inflammatory and antipyretic activities [24] [25] [26][38].

Antimicrobial, anti-inflammatory and immunological activity: Some African societies had used the leaves in the treatment of various inflammatory conditions. Asongalem EA., reported, in the Journal of Ethnopharmacology in November 2004, that the aqueous extract of the leaves of A. montanus indeed has significant anti-inflammatory activity.

This is evidenced by the significant reduction in oedema induced by carrageenan within 30 minutes of application of the extract in the effective dose of 200mg/kg. [26] The methanol extract of leaves of *A. montanus* showed significant inhibitory effects on the growth of Helicobacter pylori. [25] This could possibly substantiate the use of the leaves as an antiulcer treatment as practiced by some traditional healers in Africa.

Analgesic activity: The analgesic properties of *A. montanus* was first reported [25] They found that the methanolic extract of the leaves has analgesic effects which could possibly be due to both centrally and peripherally mediated. [44]. Asongalem., *et al*., reported similar findings with their aqueous extracts of the leaves, however the aqueous extracts did not show any centrally mediated analgesic properties.[26]

Antipyretic activity: The leaves of *A. montanus* had been advocated in the treatment of fever in traditional medical practices by various communities globally. It was found in a study published in 2004 that the aqueous extracts of the leaves was able to reduce fever at doses greater than 100mg/kg within 6 hours. [26]

1.6 Plant Taxonomy of *Parkia biglobosa*

1.6.1 Scientific classification

Kingdom Plantae
Phylum Magnoliophyta
Class Magonoliopsida
Oder fabales
Family Mimosaceae
Genus *Parkia*
Species *Parkia biglobosa* (Jacq.) G. Don
Binomial Name: *Parkia biglobosa*
Local Names: Hausa- Dorowa, Igbo- Orgbu (Ogbu)

1.5.4. Morphology

Parkia biglobosa is a multipurpose fodder tree that belongs to the family mimosaceae [45],it is known by several vernacular names such as nareje in Fulfulde, Orgbu in Igbo,Wupga in Igala and Nere in Francophone Africa [31][46] illustrating its socio-economic potential in local communities. The stem is woody and sparsely branching. Leaves are alternate, smooth in texture with a cuspidate apex and a rounded base [32][47].

Parkia biglobosa is a medium-sized tree that reaches 7-10 m high. It has a smallfruits, which is green and orange in colour when unripe and yellow to brownish when ripe. The bark is longitudinally fissured, scaly between the fissures, thick, ash-grey to greyish-brown in colour. The leaves exudes a whitish latex when cut from the stem. [48] [49] [31]

1.5.5. Ethnopharmacology

Traditional uses

Various part of the tree are used for medicinal purposes [29]. In West Africa the bark, roots, leaves, flowers, fruits and seeds are commonly used in traditional medicine to treat a wide diversity of complaints, both internally and externally, sometimes in combination with other medicinal plants. The bark is most important for medicinal uses, followed by the leaves.[50].

Pharmacological Activities

Indigenous healers in Africa use different parts of the locust bean tree for health benefits. In a survey conducted on healers in Togo, Parkia biglobosa was one of the highest cited plants used for treating hypertension. [51] The tree was also one of two plants "listed as having real wound-healing properties in South-Western Nigeria, influencing the proliferation of dermal fibroblasts significantly. [52] In a similar survey conducted in Guinea relating to their use of antimalarial plants, *Parkia biglobosa* was cited among those most often successfully used [53] .In an analysis on the antibacterial properties of the plant, another study found that "these properties compare favorably with those of streptomycin, making it a potential source of compounds used in the management of bacterial infections.[54]

Aims and Objectives

Aim: phamacognostic screening on *Acanthus montanus* and *Parkia biglobosa* and their anti-dysentery properties.

To evaluate the acute toxicity of the ethanolic leaf extract of *Parkia biglobosa* and *Acanthus montanus* (50:50) on Albino Wister rats.

Also to evaluate and confirm the anti-dysentery activity of the ethanolic leaf extract of *Parkia biglobosa* (Jacq.) G. Donand *Acanthus montanus* (50:50)

2. Material and methods

2.1. Materials

2.1.1. Apparatus

Test tubes, Beakers, Conical flasks, Glass slide, Measuring cylinder, Cover slip, Spatula, Rack, Whatman NO. 1 filter paper, Glass funnel, Petri dishes, Bunsen burner

2.1.2. Equipments

Hot air oven (Gen lab thermal engine), Water bath (Gen lab thermal engine), Mechanical weighing balance, Microscope, Rotatory evaporator

2.1.3. Solvents/drugs

Ethanol, Distilled water, Tween 80, Loperamide

2.1.4. Reagents

Concentrated and diluted hydrogen chloride, Picric acid, Ferric chloride (5%), Dragendorffs reagent, Mayer's reagent, Wagner's reagent, Fehling solution (A and B), Sodium bicarbonate, Dilute ammonia solution,10% alcoholic solution.

2.2. Methods

2.2.1. Plant collection and identification

Fresh and healthy leaves of *P.biglobosa* and *A. montanus* were collected in November 2020 from a forest in Nsukka Local Goverment Area in Enugu State, Nigeria and identified in Pharmacognosy Hebarium of Madonna University. The leaves were air dried at a room temperature under shade, foreign material was removed and hygiene mantained . The dried plant sample was grinded to powder using an electronic blender.

2.3. Macroscopic and organoleptic evaluation

The fresh plant sample was examined with the naked eyes and the following plant parts were observed: Leaf type, Colour, Apex, Odour, Taste, Texture, Shape, Margin, Base, Venation

2.4. Microscopic analysis

2.4.1. Microscopic examination of fresh leaves

The fresh sample was washed, cut into smaller pieces and placed in 70% chloral hydrate solution in a test tube and heat in a water bath to clear the cells. The cleared leaf sample was then placed on a slide and viewed under the microscope.

2.4.2. Microscopic examination of powdered leaves

A small quantity of the powdered crude drug was placed on a slide and few drops of chloral hydrate solution were added to it. The mixture was passed across the flame of a Bunsen burner repeatedly until bubbles occurred and allowed to cool for proper clearing of the sample. Two drops of glycerin were added to the slide as mountants and the slide was covered with cover slip and viewed under the microscope. The microscopic characters (such as; sclereids, cork cells, calcium oxalate crystals, fibers etc.) were observed and noted.

2.4.3. Transverse section of the leaves

A clean sharp surgical blade was used to obtain a thin transverse section of a fresh waterleaf that was collected in a Petri dish containing 70% of ethanol. The section was mounted on slides and viewed under the microscope after being cleared with chloral hydrate.

2.5. Chemomicroscopy

2.5.1. Test for starch

Powered leaf was mounted in a few drops of iodine solution and observed under the microscope for a blue black coloration. [55].

2.5.2. Test for calcium oxalate crystals

Powered leaf was mounted in a few drops of chloral hydrate and observed under a microscope for calcium oxalate crystals present, add a few drops of Concentrated hydrochloric acid which will make the crystal to disappear. [55]

2.5.3. Test for lignin

Powdered leaf was mounted in a few drops of phloroglucinol and concentrated hydrochloric acid and observed under a microscope for pink-red fibre coloration.

2.5.4. Test for cutin

Powdered leaf was mounted in a few drops of Sudan III solution and observed under a microscope for a red coloration. [55]

2.5.5. Test for cellulose

Powered leaf was mounted in a few drops of iodine solution and 80% sulphuric acid solution and observed under a microscope for blue-black coloration.

2.6. Phytochemical analysis

The test carried out was based on procedures outlined [56]. Phytochemical screening was performed on the powered plant sample for tannins, carbohydrates, flavonoids, saponins, cardiac glycosides, reducing sugars, alkaloids and proteins.

2.6.1. Test for tannins

About 2g of the powered sample was added 20ml of water, filtered and used for the following test:

Ferric Chloride Test

To 3ml of the filtrate with few drops of ferric chlnooride were added. Formation of a greenish black precipitates indicate the presence of Tannins [56]

Lead Acetate Test

To 3ml of the filtrate was added lead acetate solution. Formation of precipitate indicates the presence of Tannins.

2.6.2. Test for carbohydrates

Moliisch's test

About 2g of the powdered sample was boiled with 10ml of distilled water and filtered. To the filtrate few drops of molisch reagent was added and concentrated sulphuric acid was then gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicates the presence of carbohydrates.

Fehling's solution test

About 5ml of a mixture of equal volume of Fehling's solutionI and II were added to 2g of powdered sample and then heated on a water bath for 5 minutes. A brick red precipitate indicates the presence of reducing sugar.

2.6.3. Test for flavonoids

About 10ml of ethyl acetate was added to 2g of the powdered sample and heated on a water bath for 3minutes. The mixture was cooled, filtered and the filtrate was used for the following tests.

Ammonium Test

4ml of the filtrate was shaken with 1ml of dilute ammonia solution. The layers were accounted to separate. A yellow color in the ammonia Cal layer indicates the presence of flavonoids.

1% Aluminium chloride solution test

Small amount of the filtrate was shaken with 2ml of 1% aluminium chloride solution and the layers were allowed to separate. The formation of yellow colour in the aluminium chloride layer indicates presence of flavonoids.

2.6.4. Test for saponins

2g of powdered sample was boiled with 20ml of distilled water for 2 minutes. The mixture was allowed to cool and filtered, the filtrate was then used for the following tests [56]

Frothing Test

5ml of the filtrate was diluted with 15ml of distilled water. The mixture was vigorously shaken and then observed on a stand for stable froth indicating the presence of Saponins.

Emulsion Test

To 10ml of the frothing solution, 2 drops of olive oil was added, the mixture was shaken and observed for the formation of emulsion indicates presence of Saponins [56].

2.6.5. Test for glycosides

About 10ml of 1% sulphuric acid was added to 2g of powered sample in a test tube and boiled for 15minutes on a water bath, filtered while hot, then cooled and neutralized with 20% potassium hydroxide solution. 10ml of a mixture of equal volume of Fehling's solution I and II was added and boiled for 5minutes. a brick red precipitate indicates the presence of glycosides.

2.6.6. Test for alkaloids

About 2 g of the powdered sample was boiled with 20ml of 3% sulphuric acid in 50% ethanol on a water bath for 10minutes, cooled and filtered. 2ml each of the filtrate was tested with a few drops of Mayer's reagent (potassium mercuric iodide solution), Dragendorff's reagent (Bismuth potassium iodide solution), Wagner's reagent(iodo-potassium iodide solution) and Picric acid solution(1%). Alkaloids give reddish brown precipitate with Wagner's reagent; with Dragendroff's reagent a red precipitate was shown; with Mayer's reagent a creamy white coloured precipitate; with Picric acid a yellowish precipitate.

2.6.7. Test for proteins

About 2g of the powdered sample was extracted with 20ml of distilled water, filtered and the filtrate was used for the following test

Million's test

To a little portion of the filtrate in a test tube, two drops of million's reagent was added. A white precipitate indicates the presence of proteins.

2.7. Proximate analysis

2.7.1. Ethanol extractive value

This was done by weighing about 2g of the powdered sample and macerated with 40mls of ethanol, after 48hours the mixture was filtered and evaporated to dryness using the oven. The weight of the container was measured before the filtrate was poured in and when the filtrate was evaporated to dryness, then the weight of the extract was determined by subtracting the weight of the container from the weight of the container containing the extract. (Dept. of Pharmacognosy University of Madonna practical manual)

2.7.2. Water extractive value

About 2g of the powdered sample was weighed and macerated with 40ml of water in a closed conical flask, after 48hours the mixture was filtered and 40 ml of the filtrate was evaporated to dryness using the oven. The weight of the container was measured before the filtrate was poured in and when the filtrate was evaporated to dryness, then the weight of the

extract was determined by subtracting the weight of the container from the weight of the container containing the extract. (Dept. of Pharmacognosy University of Madonna practical manual)

2.7.3. Determination of moisture content

A preheated porcelain crucible was weighed (W_1) and 2g of the powered drug was measured into the crucible and reweighed (W_2). The sample was gradually heated in the oven up to the temperature of 105^0C for about 4hours until a constant weight was obtained. The heated sample was cooled in the desiccators and weighed (W_3) and the moisture content was calculated. [56]

2.7.4. Determination of ash values

Total ash values

A porcelain crucible was placed in muffle furnace for about 15mintues at 35^0C, cooled in a desiccator for about one hour and the crucible was weighed (W_1). 2g of the sample is accurately weighed into the preheated porcelain crucible and reweighed (W_2). The sample is ashed in a muffle furnace at 650^0C for about 6hours until the sample turns grey (white ash). The crucible is removed with crucible tong, cooled in a desiccator and reweighed (W_3). The percentage ash content is determined by the relationship. [56]

Water soluble ash value

A porcelain crucible was placed in muffle furnace and ignited to a constant weight at the temperature of 450^0C, cooled and weighed (W_1). 2g of the powdered drug was placed in the crucible and reweighed (W_2). The crucible containing the drug was incinerated at low temperature initially to burn off the carbon content. The heat was gradually increased until all the carbon was burnt off. The crucible was cooled in desiccators, reweighed and the content was transferred into a small beaker. About 5ml of water was added to the content and boiled for 5mintues, filtered with an ashless filter paper containing the residue was dried in the oven. The filter paper containing the residue was compressed into the crucible and subjected to heat until the ashless filter paper was eliminated and the crucible is reweighed [55]

Acid insoluble ash value

The total ash gotten from incinerating the powdered leaf at 450^0C was transferred into a beaker containing 25ml of dilute hydrochloric acid, boiled on a water bath for about 5mintues and filtered with an ashless filter paper. The beaker and crucible were washed repeatedly through the filter paper with hot water until they are free from acid (i.e. neutral to litmus paper). The insoluble matter and the ashless filter paper was dried in the oven, ignited in the muffle furnace at 450^0C to a constant weight and the amount of acid insoluble ash per gram of the powered drug was calculated. [56]

2.8. Plant extraction

Maceration of both plant sample was done separately using cold maceration method with ethanol for 72 hours and then filtered with Whatman No 1 filter paper. The resulting filtrate was evaporated using a rotatory evaporator to give a dried extract. The concentrated extract of both plants was weighed separately into a closed glass container and was kept at refrigerator temperature. The concentrated extract was used for different laboratory screening and test at a ratio of 50:50.

2.9. Thin layer chromatography

A thin layer chromatography tank covered with aluminum foil and a developing solvent covering the bottom of the chamber to a depth of approximately 0.5cm. It is covered so that the solvent is not affected by evaporation. The plates were first activated by heating laboratory oven at temperature of 105 ^{o}c for 1 hour.A starting line was drawn 2cm from the base and one drop of the extract was spotted at the starting line using a capillary tube.The TLC plate was carefully placed into the tank and allowed to develop. The TLC plate was then removed from the tank and the solvent allowed to evaporate. It was then placed under a UV lamp and the sample spots were noted. The distance travelled by the solvent and solute noted and the Retardation factor ($R_f$value) was calculated.The $R_f$was calculated using this formula:

$$R_f = \frac{distance\ traveled\ by\ the\ spots}{\text{distance traveled by the solvent}}.$$

2.10. Acute toxicity method (lorke's method)

Healthy albino rats were subjected to acute oral toxicity studies based on lorke's method, with ethanolic extract of both P. biglobosa leaf and A. motanus in a ratio of 50:50. This method has two phases which are phase 1 and phase 2.[57].

2.10.1. Phase one

This phase required nine rats, which were divided into three groups of three animals each. Each animal were administered different doses (10,100,1000mg/kg) of ethanol extract of*P. biglobosa* leaf and *A. motanus* in a ratio of 50:50. The animals were placed under observation for 24hours to monitor their behavior as well as if mortality will occur.

2.10.2. Phase two

This phase required the use of three rats, which were distributed into three groups of one animal each. The animals were administered higher doses (1500, 3000, 5000mg/kg) of *P. biglobosa* leaf and *A. motanus* in a ratio of 50:50 and then observed for 24hours for behaviour as well as mortality. The LD_{50} was calculated by formula: $LD_{50} = \sqrt{(D_0 \times D_{100})}$

D_0 = Highest dose that gave no mortality.

D_{100}= Lowest dose that produced mortality of three animal each (n=6)p

2.11. **Preparation of the micro-organism /***Entamoeba histolytica*,

Castor oil was tried but could not induce dysentery for one week, A method was adopted where some meat sauce was then obtained by parboiling meat and allowed to stay for 48hours. The meat sauce was then cultured and tested to confirm the presence of *Entamoeba histolytica*, this made the cultured sauce stayed for 72hours with a foul odour, it was then used for induction of dysentery [58]

2.12. Experiment animal grouping and procedure

Wister albino rat weighing 96grams to 152grams were obtained from a commercial source and were kept in a well ventilated cage in the animal farm of the faculty of pharmacy Madonna University, the animals had free access to food and water. The induction of the dysentery was done by single administration of 5ml/kg body weight of the meat sauce containing *Entamoeba histolytica* to each of the animals.

The rats were divided into 3 groups, 2 rats were kept in group 1 and group 2, group 3 comprises of 6 rats which were subdivided into three groups comprising of two rats each. The rats were weighed individually using electrical weighing balance before the induction of dysentery, weight ranging from 96grams to 152grams.

Group 1 serves as the control which receives plain water at 5ml/kg body weight, Group 2 as the standard which receive standard treatment of Imodium (Loperamide) at 5mg/kg body weight. Group 3,4 and 5 were given 250mg/kg, 500mg/kg, 1000mg/kg of the ethanol extract mixture. The changes in fecal consistency were observed for 8hrs and result recorded after every 4hrs. All dosage forms were given orally.

3. Results

3.1. Result of macroscopic analysis

Table 1 Organoleptic properties of *P. biglobosa*

Organoleptic properties	Fresh	Powder
Colour	Green	Green
Texture	Smooth	Coarse
Odour	Odourless	Pungent
Taste	Bitter	Bitter

Table 2 Macroscopic characteristics of *P. biglobosa*

Macroscopic properties	**Observation**
Type of leaf	Alternate
Shape	Oval
Margin	Entire
Apex	Cuspidate
Base	Rounded
Venation	Pinnate
Arrangement	Bipinnate

Table 3 Organoleptic properties of *A. motanus*

Organoleptic properties	**Fresh**	**Powder**
Colour	Green	Pale green
Texture	Smooth	Glossy and papery
Odour	Odourless	Astringent
Taste	Bitter	Bitter

Table 4 Macroscopic characteristics of *A. motanus*

Macroscopic properties	**Observation**
Type of leaf	Whorled
Shape	Runcinate
Margin	Lobed
Apex	Acuminate to cuspidate
Base	Attenuate to truncate
Venation	Pinnate
Arrangement	Pinnately

3.2. Result for proximate analysis

Table 5 Proximate analysis of *P. biglobosa*and *A. motanus*

Analysis	***P. biglobosa***	***A. motanus***
Water extractive value	23.0 %	25.0%
Ethanol extractive value	30.0%	32.7%
Moisture content	5.34%	4.51%
Total ash value	2.04%	1.59%
Water soluble ash value	0.20%	0.14%
Acid insoluble ash value	1.05%	0.91%

3.3. The phytochemical analysis

Table 6 Phytochemical analysis of *P. biglobosa and Acanthus montanus*leaf crude extract

Test	***P.biglobosa***	***A.motanus***
Tannins	+	+
Carbohydrates	+	+
Flavonoids	+	+
Saponins	+	+
Glycosides	+	+
Alkaloids	+	+
Proteins	+	+

Key: + means present, - means absent.

3.4. Chemomicroscopy

Table 7 Chemomicroscopy of powdered leaf of *P. biglobosa*.

Test	**Observation**	**Inference**
STARCH Powder+Iodine	Blue black	Present
CALCIUM OXALATE Powder+Chloral hydate+Conc. HCl	Block shaped crystals which disapper on addition of Conc. HCl	Present
CUTIN Powder+Sudan III solution	Red	Present
CELLULOSE Powder+Iodine+80% sulphuric acid	Blue black	Present
LIGNIN Powder+phloroglucinol+HCl	Pink fiber	Present

Table 8 Chemomicroscopy of powdered leaf of *A. motanus*.

Test	**Observation**	**Inference**
STARCH Powder+Iodine	Blue black	Present
CALCIUM OXALATE POWDER+CHLORALHYDATE+CONC. HCL	Prism shaped crystals which disappear on addition of Conc. HCl	Present
CUTIN Powder+Sudan III solution	Red	Present
CELLULOSE Powder+Iodine+80% sulphuric acid	Blue black	Present
LIGNIN Powder+phloroglucinol+HCl	Pink fiber	Present

3.5. Thin layer chromatograph

Table 9 Thin Layer Chromatograph of *P. biglobosa* and *A. montanus*

Plant extract	Solvent systems	Number of spots	Colour in daylight	Colour in uv	R_f values
P. biglobosa	Methanol: chloroform 13:7	6	Colourless	Light green	0.87
A. montanus	Chloroform: ethyl acetate 6:4	4	Colourless	Light green	0.86

3.6. Result of acute toxicity test (LD50)

Using Lorke's method the extract mixture in 50:50 ratio is safe for acute administration and was well tolerated even at the dose up to 5000mg/kg.

Table 10 Number of animals that died at each phase

Phase A	Dose of extract administrated (mg/kg)	Number of death recorded
GROUP 1	10	0/3
GROUP 2	100	0/3
GROUP 3	1000	0/3
PHASE B		
GROUP 1	1500	0/1
GROUP 2	3000	0/1
GROUP 3	5000	0/1

3.6.1. LD50 = $\sqrt{a \times b}$

3.6.2. Where a= highest dose that did not kill any animal

3.6.3. b= lowest dose that killed the animal.

3.7. Results of anti-dysentry activity

The physical nature of the faeces was loose with mucus and blood this was apparent in all groups after 8hrs of oral administration of *E. histolytical* suspension. The nature of the faeces changed in all groups after 8hrs, except for group 1 (control). There was also change in colour of faeces, the colour changes from cream to dark green, which is the colour of the mixed extract.

Table 11 Anti-dysentery activity of the ratsfor Day 1

Groups	Treatment	Faecal consistency at 0hrs	Faecal consistency within 8hrs	Faecal consistency within 16hrs
Group 1 (control)	Water 5ml/kg body weight	Loose stool with mucus and blood	Loose stool with mucus and blood	Loose stool with mucus and blood
Group 2 (standard)	Loperamide 5mg/kg body weight	Loose stool with mucus and blood	Loose stool with mucus and blood	Semisolid stool with mucus and blood
Group 3 (mixed ethanol extract of *P.*	250mg/kg body weight	Loose stool with mucus and blood	Loose stool with mucus and blood	Semisolid stool with mucus and blood

biglobosa and A. montanus)A				
B	500mg/kg body weight	Loose stool with mucus and blood	Loose stool with mucus and blood	Semisolid stool with mucus and blood
C	1000mg/kg body weight	Loose stool with mucus and blood	Semisolid stool with mucus and blood	solid stool with mucus on top

Table 12 Anti-dysentery activity of the rats for Day 2

Groups	**Treatment**	**Faecal consistency within24hrs**	**Faecal consistency within 32hrs**
Group 1 (control)	Water 5ml/kg body weight	Loose stool with mucus and blood	Loose stool with mucus and blood
Group 2 (standard)	Loperamide 5mg/kg body weight	Partially semisolid stool with mucus on top	Hard palate stool without mucus or blood
Group3(mixed ethanol extract of *P. biglobosa and A. montanus*)A	250mg/kg body weight	Semisolid stool with mucus	Hard palate stool without mucus or blood
B	500mg/kg body weight	solid stool with mucus on top	Hard palate stool without mucus or blood
C	1000mg/kg body weight	Hard palate stool with mucus on top	Hard palate stool without mucus or blood

4. Discussion

The phytochemistry of leaves of both plants showedthe presence of some medicinally important secondary metabolites including; tannins, carbohydrates, flavonoids, alkaloids, proteins, glycosides and saponins, this is a strong indication that the plants have potential medicinal values [35]

Chemomicroscopy of both plants showed the presence of starch, calcium oxalate, lignin, cellulose. While the proximate analysis gave 25.0% and 32.7% for water extractive value and ethanol extractive value respectively (for *Acanthus montanus*) and 23.0% and 30.0% for water extractive value and ethanol extractive value respectively (for *Parkia biglobosa*).

The absence of death at 5000mg/kg of the extract shows that the lethal dose of the ethanol extract of the plant mixture is higher than 5000mg/kg which may be an indication of safety of the mixture.

The anti-dysentery activity of the mixture of both plants with different concentration of 250mg/kg, 500mg/kg, 1000 mg/kg body weight of ethanol extract showed a significant change in the faecal consistency of the rats. The effect obtained on the administration of 500mg/kg body weight of the extract mixture is more comparable to the standard(loperamide).The 1000mg/kg of the combined extract produced a result faster than that of loperamide.

The change in faeces colour shows that the mixed extract was highly metabolized in the liver and excreted through the faeces.

Animals treated with the drug were alive and the negative control animals infected with the bacteria died after few days of the experiment. But when the induction of dysentery was done with castor oil, the animals were able to recover on their own, this is because castor oil is a vegetable product, it can be use as purgative in mammals and to induce labour in pregnant women.

5. Conclusion

In conclusion, the synergistic use of the extract contains the secondary metabolites glycosides, alkaloids, flavonoids, which may be responsible for their anti-dysentery synergistic activity. Mixed extracts from both leaves of *P. biglobosa* and *A. montanus* can be recommended as an anti-dysentery agent and can serve as a lead to formation of anti dysentery drugs.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest among the authors.

Statement of ethical approval

The protocol of this study was approved by the Faculty of Pharmacy Ethical committee Madonna University. Nigeria.

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Appendix

1. Appendix A: percentage yield (%)

1.1. Yield of the extraction

150g each of both plants gave 18.93g of *Acanthus montanus* and 15.14g for *Parkia biglobosa* yield on successive cold maceration with ethanol.

Weight of sample = 150g

Weight of *A. montanus*extract = 18.93g

Weight of *P. biglobosa* extract = 15.14g

% yield = weight of extract / weight of sample x 100

% yield of *A. montanus* =18.93g /150g x 100

= 12.62% w/w

%yield of *P. biglobosa* = 15.14g / 150g x 100

= 10.09% w/w

1.2. Appendix B: proximate analysis

1.2.1. Water extractive value for P. biglobosa

2g of sample was macerated in 40ml of water for 48hours.

40ml of filtrate was used

Weight of beaker (A) = 38.6g

Weight of empty beaker + extract (B) = 39.06g

Weight of extract =B-A

Weight of *P. biglobosa* extract = 0.46g

% water extractive value = weight of extract / weight of sample x 100

% water extractive value of *P. biglobosa* = 0.46g / 2g x 100 = 23.0% w/w

1.2.2. Water extractive value for A. montanus

2g of sample was macerated in 40ml of water for 48hours.

Volume obtained after maceration = 40 ml

40ml of filtrate was used

Weight of beaker (A) = 48.35g

Weight of empty beaker + extract (B) = 48.80g

Weight of extract = B-A

Weight of *A. montanus* extract = 0.45g

% water extractive value = weight of extract / weight of sample x 100

% water extractive value of *A. montanus* = 0.45g / 2g x 100 = 22.50%

1.2.3. Ethanol extractive value for P. biglobosa

2g of sample was macerated in 40ml of ethanol for 48hours.

40ml of filtrate was used

Weight of empty beaker (A) = 38.121g

Weight of beaker + extract (B) = 38.721g

Weight of extract = B-A

Weight of *P. biglobosa* extract = 0.60g

% water extractive value = $\frac{\text{Weight of extract}}{\text{Weight of sample powder}}$ x 100

% water extractive value of *P. biglobosa* = $\frac{0.60\text{g}}{2\text{g}}$ x 100 = 30.0% w/w

1.2.4. Ethanol extractive value for A. montanus

2g of sample was macerated in 40ml of water for 48hours.

40ml of filtrate was used

Weight of empty beaker (A) = 108.87g

Weight of beaker + extract (B) = 109.524g

Weight of extract = B-A

Weight of *A. montanus* extract = 0.654g

% water extractive value = $\frac{\text{Weight of extract}}{\text{Weight of sample powder}}$ x 100

% water extractive value of *A. montanus* = $\frac{0.654\text{g}}{2\text{g}}$x 100 = 32.7% w/w

1.2.5. Total ash value for P. biglobosa

Average Weight of empty crucible A= 58.069g

Average Weight of crucible + ash B= 58.110g

Weight of ash = B - A = 0.041g

Average weight of sample= 2.001g

% Total ash value = 2.04%

Acid insouluble ash

Crucible +Acid + ash A= 58.571g

Crucible + Acid filterate B = 58.550g

Acid insoluble ash= A - B

= 0.021

Average Weight of sample= 2.001g

% Acid insoluble ash=$\frac{0.021\ x\ 100}{2.001}$ = 1.05%

Water souluble ash

Crucible + ash A = 57.7410g

Crucible +Water + ash B= 57.745g

Water soluble ash= B - A

= 0.004g

Average Weight of sample= 2.00g

% Water soluble ash= 0.2%

1.2.6. Moisture Content

Weight of crucible A = 54.91g

Weight of crucible B = 60.59g

Weight of crucible C = 52.06g

Weight of powered plant A =2.00g

Weight of powered plant B =2.01g

Weight of powered plant C =2.00g

Mean weight of powered sample =2.00g

Weight of crucible + powered plant of A,B,C = 56.91g, 62.60g, 54.06g respectively (before drying)

Weight of crucible + powered plant of A,B,C = 55.82g, 62.35g, 53.96g respectively (after drying)

Moisture content = 5.34%

1.3. Appendix C: retardation factor

(1) Retardation factor of *Parkia biglobosa*

$$R_f = \frac{\text{distance traveled by spot}}{\text{distance traveled by the solvent}} = \frac{7.2\ \text{cm}}{8.2\ \text{cm}} = 0.87$$

(2) Retardation factor of *Acanthus montanus*

$$R_f = R_f = \frac{\text{distance traveled by spot}}{\text{distance traveled by the solvent}} = \frac{7.5\ \text{cm}}{8.4\ \text{cm}} = 0.86$$

1.4. Appendix D: acute toxicity study (LD50)

PHASE A

Preparation of stock solution: 1g of mixed extract in 1ml of water to make a stock concentration of 1000mg/ml

GROUP 1: to be given dose of 10mg/kg

Average weight of rat= (0.132kg+ 0.134kg+ 0.130kg)/ 3 = 0.132kg

Volume of extract administered = dose x average weight/ stock solution

= 10mg/kg x 0.132kg / 1000mg/ml

= 0.00132ml

GROUP 2: to be given dose of 100mg/kg

Average weight of rat = 0.134kg

Volume of extract administered = dose x average weight/ stock solution

= 100mg/kg x 0.134kg / 1000mg/ml

= 0.0134ml

GROUP 3: to be given dose of 1000mg/kg

Average weight of rat = 0.122kg

Volume of extract administered = dose x average weight/ stock solution

= 1000mg/kg x 0.122kg / 1000mg/ml

= 0.122ml

1.4.1. PHASE B

Preparation of stock solution: 1g of mixed extract in 1ml of water to make a stock concentration of 1000mg/ml

GROUP 1: to be given dose of 1500mg/kg

Weight of rat= 0.136kg

Volume of extract administered = dose x average weight/ stock solution

= 1500mg/kg x 0.136kg / 1000mg/ml

= 0.204ml

GROUP 2: to be given dose of 3000mg/kg

Weight of rat = 0.129kg

Volume of extract administered = dose x average weight/ stock solution

= 3000mg/kg x 0.129kg / 1000mg/ml

= 0.387ml

GROUP 3: to be given dose of 5000mg/kg

Weight of rat = 0.132kg

Volume of extract administered = dose x average weight/ stock solution

= 5000mg/kg x 0.122kg / 1000mg/ml

= 0.61ml

1.5. Appendix E: anti-dysentery study

(i) Preparation of stock solution: 1000mg of mixed extract in 5ml of water

stock concentration= 200mg/ml

(ii) Preparation of stock solution for standard drug (loperamide): 4mg of mixed extract in 2ml of water

stock concentration of loperamide = 2mg/ml

GROUP 1: to be given dose of 10ml of plain water

Average weight of rat= (0.147kg+ 0.149kg)/ 2 = 0.148kg

GROUP 2: to be given dose of 5mg/kg of loperamide

Average weight of rat = 0.146kg

Volume of extract administered = dose x average weight/ stock solution

= 5mg/kg x 0.146kg / 2mg/ml

= 0.365ml

GROUP 3: to be given dose of 250mg/kg of mixed ethanol extract

Average weight of rat = 0.132kg

Volume of extract administered = dose x average weight/ stock solution

= 250mg/kg x 0.132kg / 200mg/ml

= 0.165ml

GROUP 4: to be given dose of 500mg/kg of mixed ethanol extract

Average weight of rat = 0.122kg

Volume of extract administered = dose x average weight/ stock solution

= 500mg/kg x 0.122kg / 200mg/ml

= 0.305ml

GROUP 5: to be given dose of 1000mg/kg of mixed ethanol extract

Average weight of rat = 0.104kg

Volume of extract administered = dose x average weight/ stock solution

= 1000mg/kg x 0.104kg / 200mg/ml

= 0.52ml