PHARMACOGNOSTIC STANDARDIZATION OF THE LEAVES OF *MARGARITARIA DISCOIDEA* (BAILL.) G.L.WEBSTER [FAMILY: PHYLLANTHACEAE]

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DEPARTMENT OF PHARMACOGNOSY

FACULTY OF PHARMACY

UNIVERSITY OF BENIN

BENIN CITY

JANUARY 2023

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF PHARMACOGNOSY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHARMACY (PHARMD) DEGREE

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CERTIFICATION

We the undersigned hereby agree that this work was carried out by FAVOUR AIZENOSA OSAZUWA-OJO in partial fulfillment of the requirements for the award of Doctor of Pharmacy (PharmD) degree in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City.

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Dr. O. H. Uwumarongie (Project Supervisor) — Date

_____ _____

Dr. (Mrs.) J. O. Ofeimun (Ag. Head of Department) — Date

DEDICATION

This work is dedicated to God Almighty for enabling me to get into the School of Pharmacy. I also wish to dedicate this work to my parents, Late Barr. Peter Osazuwa Ojo and Barr. (Mrs.) Faith Osazuwa-Ojo, for their relentless care and support throughout my stay in the School of Pharmacy.

ACKNOWLEDGEMENT

In the spirit of gratitude, I appreciate God Almighty for His guidance, wisdom and strength during the course of this training. My gratitude also goes to my project supervisor, Dr. Osamuyi Henry Uwumarongie, for his patience, relentless sacrifice and selfless desire for his students to succeed. I want to thank the technical staff of the Instrumentation Unit, Department of Chemistry, Faculty of Physical Sciences, University of Benin and Dr. Joshua Orizu of Bato Chemical Laboratory, Lagos; for their technical assistance in the proximate and high-performance liquid chromatographic (HPLC) analysis of the plant material.

I also want to acknowledge my mum, Barr. (Mrs.) Osazuwa-Ojo; for her love, support, advice, financial contribution and trust during my stay in the School of Pharmacy. Special thanks to my aunt, Mrs. Dorcas Rosario-Iyoha; for encouraging me in my choice of career. Of course, I cannot forget my siblings, Eseosa, Victory and Blessing; you are the best support system anyone could ever ask for. To my late dad, Barr. Peter Osazuwa Ojo; who sponsored this project, thank you for all the support you provided for me.

A special thanks to my friends and colleagues: Jane Evbuomwan, Eseosa Irorere, Eseosa Iyagbaye, Muniratu Ozigis and Janet Akande; my journey through the School of Pharmacy would not have been complete without you guys. To Ose, Sarah, Joy, Nowa, Chimdalu, the GLCO family and Refinery; thank you for the love and prayers you showered on me.

Tremendous appreciation goes to the Grandeza class, my junior colleagues and my senior colleagues, Dr. Osaigbovo and Dr. Eloghosa; who advised me when I had challenges in school. Special thanks to everyone who helped me in one way or another through these years of my life. Finally, I wish to thank the Vice Chancellor and his management team for the opportunity given to me to study Pharmacy in the University of Benin.

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ABSTRACT

Traditional herbal remedies according to World Health Organization, will have a place in the health-care system only if recommendations for their use are founded on research that establishes their credibility and acceptability. The goal of standardization is to validate herbal products in terms of their safety, efficacy, quality and reproducibility. Hence this research work, designed to study some inherent characteristics of the leaf of *Margaritaria discoidea* that determines the quality of its product.

The plant leaves were collected and identified. Pharmacognostic studies for the determination of some quality control parameters for the leaves of *M. discoidea* were conducted using standard prescribed methods.

Macroscopy of *M. discoidea* leaves revealed that the leaves were simple, petiolated, alternate with entire margins and reticulate venation. Microscopy showed the presence of straight-walled epidermal cells, unicellular trichomes, calcium oxalate crystals and paracytic stomata. The transverse cut through the mid-rib section showed isobilateral tissue arrangement. chemo-microscopy showed the presence of lignin, cellulose, mucilage, tannins, starch and fixed oils. Phytochemical screening of the powdered leaves showed the presence of glycosides, saponins, tannins, flavonoids, steroidal nucleus and alkaloids. Thin layer chromatography (TLC) revealed the presence of a number of separated constituents in the methanolic extract of the plant. Quantitative determinations for proximate analysis were moisture content (8.23%), total ash (8.62%), crude fibre (3.43%), crude fat (9.10%), crude protein (19.80%)and carbohydrates (59.05%). Vitamin C was present in minute quantity. Elemental analysis showed the presence of calcium, magnesium, manganese, iron, sodium, copper, zinc and potassium. Methanol extractive value, Foaming and Swelling indices were 25.83%, < 100 and 5 ml/g, respectively. High performance liquid chromatography (HPLC)

confirmed twelve constituents in the plant material. The compounds with the highest concentrations are kaempferol, phyllochrysine, phyllanthine and betulinic acid.

From this research work, standardization indices which could be used for the proper identification of the plant (*M. discoidea*) so as to prevent adulteration, have been provided. These details could be recorded in an official monograph.

CHAPTER ONE

1.0 INTRODUCTION

Traditional medicine has been widely used abroad and in Africa, particularly in Nigeria. Since time immemorial, herbs have been used, and cases have been noted in history such as those of the Ancient Egyptians and Chinese Empire. The WHO has estimated that at least 80% of the world's population sources the active ingredients of synthetic drugs from plants (Tilburt, 2008). Medicinal plants directly contribute to modern medicine with active substances (use of plants, after pulverization, in sachets for teas, tablets, etc.) or can be used for the preparation of extracts (raw, purified, or concentrated), or they can be used indirectly (use of the active principles as such, or as molecular models used for the design and production of synthetic drugs) (Miraldi and Baini, 2019).

According to the WHO, herbal medicines include herbs, fresh juices, gums, fixed oils, essential oils, resins, and dry powders of herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting, or stir-baking with honey, alcoholic beverages, or other materials (WHO, 2000). These products are easily accessible to humans because they are present in their surroundings.

There are certain constituents that make plants of value in therapy, and they are regarded as "secondary metabolites." They are so-called due to the lack of necessity for the plant's survival. However, they have been implicated in defensive and protective mechanisms. Plants, like animals, evolved to adapt to their surroundings through the genetic encoding of useful and diverse secondary metabolite syntheses (Kabera *et al*., 2014). These secondary metabolites include terpenoids, alkaloids, glycosides, tannins, saponins, etc. These compounds have been found to have essential pharmacological properties.

Traditional medicines have a lot of clinical relevance. There have been numerous cases of its medicinal use in Nigeria. Examples include the use of pawpaw (*Carica papaya*, Caricaceae)

fruits and seeds as purgative, watermelon (*Citrullus lanatus*, Cucurbitaceae) dried pulp in aiding digestion and palm kernel nuts (*Elaeis guineensis*, Arecaceae) in menstrual flow (Aiyeloja and Bello, 2006).

However, there are challenges encountered in the use of herbal medicines in Nigeria because of no adequate standardization of herbal medicines. The system for reporting adverse effects resulting from the use of herbal medicines is not clearly defined. Also, ethical issues have risen over the years regarding traditional medicine (Tilburt, 2008). Due to these challenges, there is a need for standardization of herbal medicinal products as it validates the safety and efficacy of herbal medicines.

1.1 STANDARDIZATION

The practice of prescribing a set of standards or intrinsic properties, constant parameters and unmistakable qualitative and quantitative values that convey an assurance of quality, efficacy, safety and repeatability is known as standardization of herbal medicines (Kunle *et al.*, 2012). One of the major issues confronting traditional medicine is standardization, which is difficult but not impossible to achieve. Traditional herbal remedies will have a place in the health care system only if recommendations for their use are based on studies that make them credible and acceptable (WHO, 2000). Standardization seeks the validation of herbal products in terms of safety, efficacy, quality and reproducibility, in a manner similar to that of synthetic drugs. However, there are drawbacks associated with the use of traditional medicines which usually contain numerous principles (active components) in a single plant and may also be hidden or not visible, in contemporary pharmacotherapy products (Kunle *et al.*, 2012).

This gives rise to methods that ensure that the ingredients are isolated from the plants with certain precision. Standardization starts with the collection of the plants and continues through the extraction of certain active principles and the storage of the extracts. Plants are vegetative complexes; even minor differences in factors such as age and origin, harvesting

period, specific parts of the plant to be processed, extraction methods used, drying and storage can change the isolated principles (Miraldi and Baini, 2019). Thus, raw materials (whole or plant parts), extraction methods, collection methods and finished herbal products should all be standardized.

1.2 STANDARDIZATION INDICES

Standardization indices include but are not limited to macroscopic, microscopic and Chemo-microscopic examinations, foreign organic matter, ash values, moisture content, extractive values, qualitative and quantitative chemical evaluations, as well as chromatographic examination (Kunle *et al*., 2012).

1.2.1 Macroscopic Examination

Macroscopic examination refers to an evaluation of the plant or plant parts by using the sensory organs such as sight, smell, touch, or taste to give information about the plant. This type of investigation is subjective due to the fact that it is based on the individual's perception of the examined plant or herb. Its findings are referred to as organoleptic properties.

This examination is used for physical identification of plants. Detailed botanical/ macroscopic descriptions of the physical characteristics of each plant can be used to ensure that both the identity and purity of plant materials are recorded in official books such as various monographs (Kunle *et al.*, 2012). These official books contain well-detailed information, including a pictorial description of the plant in question, which helps to curtail adulteration. Some of the information that could be recorded by sight are the shapes and colours of plants which vary based on the part of the plant viewed. The shape of the drug may be cylindrical (Sarsapilla), sub-cylindrical (Mesophyll) or conical (Aconite). The colour, which varies in different plants are an important diagnostic character (Kamboj, 2012). The colour of barks is usually brown but some may vary from grey to reddish-brown as in yohimbe (*Corynanthe johimbe),* which is used as an aphrodisiac in Nigeria.

The odour could be pungent, odourless or fragrant; and could also differ in plants depending on the physical state of the herb (as in dried, powdered, or crushed). Taste is a type of sensation felt by the tongue's epithelial layer. Information gotten from taste is acidic (sour), saline (like salt), saccharic (sweetish), bitter or tasteless (having no taste) (Kamboj, 2012). Texture is recorded as information received from touch, as it could be smooth or hairy. These characteristics vary from plant to plant, as well as between different parts of a particular plant.

1.2.2 Microscopic Examination

Microscopic analyses of plants are invaluable for assuring the identity of plant materials and could be used as an initial screening test for impurities (Kunle *et al*., 2012). It involves the detailed examination of herbal medicines and is used for the identification of organized drugs, based on their known histological characteristics (Bhusnure *et al*., 2019).

Organized crude drugs could be analyzed qualitatively in their entire and powdered forms, with the aid of a microscope. Every plant possesses a characteristic tissue feature. A microscope can be used to confirm the histological characteristics of drugs of plant origin, with reference to what has been recorded in official books. Samples such as thin transverse sections and longitudinal sections obtained from bark, wood or leaves are viewed under the microscope to identify crude drugs. Some of the parameters examined in microscopy are the presence or absence of stomata, calcium oxalate crystals and trichomes (Bhusnure *et al*., 2019).

1.2.3 Chemo-Microscopic Examination

Microscopic evaluation can also include the study of constituents in the powdered drug by the use of chemical reagents. This is referred to as chemo-microscopy. Starch is identified by its blue-black colour with iodine solution and all lignified tissues give a pink stain with

phloroglucinol and concentrated hydrochloric acid. Mucilage is stained pink with ruthenium red. All these could be used to distinguish cellular structures (Kamboj, 2012).

1.2.4 Phytochemical Tests

Phytochemical standardization encompasses all possible information generated with regards to the chemical constituents present in an herbal drug. Phytochemical evaluation for standardization purpose comprises preliminary testing for the presence of different chemical groups, quantification of chemical groups of interest (e.g., total alkaloids, total flavonoids and total tannins), establishment of fingerprint profiles, multiple marker-based fingerprint profiles and quantification of important chemical constituents (Calixto, 2000).

The chemical nature of the active principles forms the basis for the rationale behind phytochemical test methods, for the analysis of the constituents. Chemical tests are used for the determination of specific chemical constituents that may be present in any drug to which its therapeutic activity is attributed. These chemical constituents are different active principles such as alkaloids, glycosides, tannins, volatile oils and saponins; which can be qualitatively identified by different chemical tests (Patil *et al*., 2013).

1.2.5 Thin-Layer Chromatography

Thin layer chromatography is a separation technique used in separating mixtures of compounds or constituents, present in various herbal mixtures. TLC could also be used for the qualitative determination of small amounts of impurities in a compound. The technique is used for evaluating medicinal plant materials and their preparations. The advantages of TLC for generating herbal medicine fingerprints include; its simple, adaptability, high velocity, specificity, sensitivity and easy sample preparation. TLC is a practical way to assess the quality and potential for adulteration of herbal medicines (Kamboj, 2012). The following parameters should be determined on the basis of official books, for the analysis of each individual plant material (WHO, 1998).

i. Type of adsorbent and method of activation.

ii. Method of preparation and concentration of the test and reference solutions.

iii. Volume of the solutions to be applied on the plate.

iv. Mobile phase and the distance of migration.

v. Drying conditions and method of detection.

vi. The values of the retention factors.

vii. Fluorescence and colour.

1.2.6 Moisture Content

The moisture content determines the amount of moisture present in the plant material. Drying is usually achieved by heating to 100 - 105 °C or by drying in a desiccator over phosphorus pentoxide at room temperature under atmospheric or reduced pressure, for a specified time. The desiccation method is especially useful for materials that degrade under elevated temperatures (WHO, 1998).

1.2.7Ash Content

The total ash method is designed to determine the total amount of material that is left after ignition. This includes ash, which is derived from the plant tissue itself and the residue of the extraneous matter (e.g., sand and soil) adhering to the plant surface (WHO, 1998).

1.2.8 Elemental Analysis

Heavy metals such as cadmium, lead and nickel are usually released from the soil and sediments. Contamination by heavy metals can be accidental or intentional, which may be the result of environmental pollution. These can cause harm to users of traditional medicines. There arises the need for heavy metals to be within a certain limit in herbal preparations and standardization is very necessary (Kunle *et al.*, 2012).

The potential intake of the toxic metal can be determined based on the level of its concentration in the product and the recommended dosage of the product. The determination

of heavy metals can be found in many official books and is based on colour reactions with specific reagents and the amount present is estimated by comparison with a standard (WHO, 1998).

Instrumental analyses are used to determine the presence of metals in minute amounts as well as the concentration of the metals. One of the most common methods used is atomic absorption spectroscopy (AAS) (Kunle *et al.*, 2012).

1.2.9 Extractive Values

This method determines the amount of active constituents extracted with solvents from a given amount of plant material. It is often employed for materials which have no suitable chemical or biological assay to determine their constituents (WHO, 1998).

1.2.10 Foaming Index

Saponins are secondary metabolites that have been discovered to have numerous medicinal applications. Many medicinal plant materials contain saponins which can cause a persistent foam to form, when an aqueous extract is shaken. The foaming ability of an aqueous extract of a plant material and their extracts, can be measured in terms of its foaming index (WHO, 1998).

1.2.11 Swelling Index

Many medicinal plant materials have some specific therapeutic or pharmaceutical utility because of their secondary metabolites. These metabolites have different properties, such as swelling properties. Examples of such metabolites include gums, mucilage, pectin and hemicellulose. The swelling index is the volume in milliliters taken up by the swelling of 1 g of plant material under specified conditions. Its determination is usually based on the addition of water or any specified swelling agent to each whole, cut, or pulverized individual plant material. Using a glass-stoppered measuring cylinder, the material is repeatedly shaken for 1 hour and then allowed to stand for the required period of time. The volume of the mixture (in

milliliters) is then measured. The mixing of whole plant material with the swelling agent is easy to achieve, but cut or pulverized material requires vigorous shaking at specified time intervals to ensure even distribution of the material in the swelling agent (WHO, 1998).

1.2.12 High-Performance Liquid Chromatography (HPLC)

HPLC is one application highly utilized in the separation and isolation of naturally pharmaceutically active compounds, including alkaloids and glycosides (Patil *et al*., 2013). The separation principle of HPLC is based on the distribution of the analyte between the eluent (as a mobile phase) and the packing material of the column (as a stationary phase), under high pressure through a column pump. Various detectors are used to quantify and assay the separated compounds. Examples are ultraviolet detectors (UV) and mass spectroscopy (MS) (Pratiwi *et al*., 2021).

1.3 FAMILY: Phyllanthaceae

Margaritaria discoidea is a tree in the family Phyllanthaceae. It is 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. Phyllanthaceae is a family of flowering plants in the class Dicotyledon and order Malpighiales. Phyllanthaceae are one of five families recognised by the Angiosperm Phylogeny Group. The family contains 2000 species in 59 presently accepted genera, 10 tribes, and two subfamilies. This new classification was based on molecular studies using DNA technology (Hoffman *et al.*, 2006).

The family Phyllanthaceae is further divided into two subfamilies: Antidesmatoideae and Phyllanthoideae. Antidesmatoideae is subdivided into six tribes, and Phyllanthoideae is subdivided into four (Hoffman *et al*., 2006). Phyllanthaceae contains more than 1,700 species, which range from herbs to trees and they are found in tropical and warm temperate regions. The family is especially diverse in Malaysia.

The largest genera include Phyllanthus (1270), Cleistanthus (140), Antidesma (100), Aporosa (90), Uapaca (60), Baccaurea (50) and Bridelia (50); with each of them having many species (Stevens, 2001). *Sauropus* grows from Indo-Malaysia to Australia. *Antidesma* is native to the Paleotropics and warm temperate regions. *Cleistanthus* is native to the Paleotropics. *Baccaurea* grows from Indo-Malaysia to the western Pacific. *Aporosa* is native to Indo-Malaysia. *Bridelia* grows throughout the Paleotropics. *Uapaca* is found in Africa and Madagascar (Berry, 2017).

1.3.1 Description:

Members of the family often have finely cracked bark and two-ranked leaves that are glandless. There are two seeds in each chamber of the fruits. The pantropical *Phyllanthus* has simple leaves, which appear as if they are leaflets of a compound leaf (Berry, 2017).

The family consists of trees, herbs, or shrubs, rarely climbers, succulents or aquatics. The leaves are simple, stipulated, alternate or rarely opposite; and may be absent in some *Phyllanthus species*. The margins are entire and rarely toothed. There is no sap and only axillary inflorescences, which are rarely terminal. The flowers are unisexual, occasionally bisexual, actinomorphic, bracteate and range in colour from yellow to green or pink to maroon. The sepals may be free or fused, usually imbricate or valvate. Petals are either free or absent. The ovary is superior and the carpels are fused. The fruit is usually a capsule, schizocarp, drupe or berry (Hyde *et al.*, 2022).

1.3.2. Examples of Plants in the Phyllanthaceae Family

Some examples of plants that belong to the family Phyllanthaceaeare *Phyllanthus amarus, Breynia disticha, Bridelia ovata, Margaritaria discoidea, Phyllanthus muellerians, Bridelia mollis, Bridelia micrantha and Antidesma venosum.*

1.4 THE GENUS: *Margaritaria*

It is the smallest pantropical genus of the Phyllanthaceae family (formerly of the Euphorbiaceae family) (Linnaeus, 1782). It is widely distributed in tropical and subtropical regions of Asia, Africa, Australia, North and South America and various oceanic islands (González, 2010).

1.4.1 Description:

The genus comprises of dioecious (rarely monoecious) trees or shrubs. The leaves are stipulated, alternate, simple and entire. Flowers are axillary. The seeds are hemispheric, with two (2) seeds per loculus. There is a fleshy outer seed coating that is blue (Hyde *et al.*, 2022)

1.4.2 Ecology

The *Margaritaria* genus is widely distributed in tropical and subtropical regions of Asia, Africa, Australia, North and South America; and various oceanic islands. The countries in which they can be found are Suriname, Mexico, Guyana, French Guiana, Costa Rica, the Caribbean and Bolivia (González, 2010).

Other species of *Margaritaria* are also found in Aldabra, Comoros, Madagascar, Mauritius, Sri Lanka, South Madagascar, Sub-Saharan Africa, North Queensland, South West Madagascar, Hispaniola, South-East Asia, South China, India, New Guinea, Queensland, Luzon, Mexico, Central America, South America, the West Indies, the Bahamas, Cuba and Haiti (Govaerts, 2022).

1.5 SPECIES OF *Margaritaria*

The species of *Margaritaria* are widely distributed in Asia, Africa, Australia, North and South America. They include; *M. anomala, M. cyanosperma, M. decaryana, M. discoidea, M. dubium-traceyi, M. hispidula, M. hotteana, M. indica, M. luzoniensis, M. nobilis, M. rhomboidalis, M. scandens, and M. tetracocca* (Govaerts, 2022).

1.5.1 *Margaritaria discoidea*

The scientific, common, and vernacular names are as follows:

Scientific name: *Margaritaria discoidea* Baill. G.L.Webster

Common names:pheasant-berry, egossa red pear, or bushveld peacock-berry.

Vernacular names:Bushveld peacock-berry (English) and Common pheasant-berry (English) (Hyde *et al*., 2022).

1.5.1.1 Taxonomy

Taxonomy is the science of naming, describing, and classifying organisms and includes all plants, animals, and microorganisms in the world. Using morphological, behavioural, genetic, and biochemical observations, scientists identify, describe, and arrange species into classifications, including those that are new to science. The taxonomy of *M. discoidea* is as follows:

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyte

Subphlum: Angiosperms

Class: Dicotyledonae

Order: Malpighiales

Family: Phyllanthaceae

Subfamily: Antidesmatoideae

Genus: *Margaritaria*

Species: *M. discoidea*

1.5.1.2 Description

*M. discoidea is a v*ery variable shrub or tree. The leaves are often very small, thinly textured, bright green in colour, ovate, elliptic or obovate. In forests, specimens can grow to be 10 to

13 cm long. Flowers are green and unisexual; and the sexes are found on different trees. Male flowers are seen numerously in axillary clusters while female flowers are seen in axillary pairs. The fruit is a 3 - lobed capsule that is pale green when ripe (Hyde *et al*., 2022).

Figure 1.1: *M. discoidea* tree growing in its natural habitat within the premises of University of Benin.

1.5.1.3 *M. discoidea* in Ethnomedicine

In traditional medicine, *M. discoidea* has been used in the treatment of many ailments. It is claimed to be used as an anti-allergic, anti-inflammatory and anti-arthritic (Obiri *et al*., 2014); as well as in the treatment of wounds and ulcers (Sofidiya *et al*., 2015). It has been used in the management of wounds and skin infections in Ghana (Ekuadzi *et al*., 2013;Sofidiya *et al*., 2015).

1.5.1.4 Biological Activities

A lot of research has been done on the traditional medicine applications of *M. discoidea,* which have been documented. One of the first pharmacological activities recorded for *M. discoidea* was the acaricidal activity of a water-soluble and oil-soluble hexane extract (Kaaya *et al.,* 1995). A study was done to evaluate the antibacterial, antioxidant, and anti-inflammatory effects of the leaves and stem bark of *M. discoidea,* and it was observed that 70% ethanolic extracts of the various plant parts tested for antibacterial activity using the agar well diffusion and micro dilution assays showed that the bark extract gave the highest activity against *Bacillus subtilis*, while the leaf extract showed no effect against any of the examined organisms. The leaf and bark extracts demonstrated free radical scavenging activity. Both extracts had high phenolic content, which correlated with their antioxidant activities. The extracts also showed significant anti-inflammatory activity (Dickson *et al*., 2010).

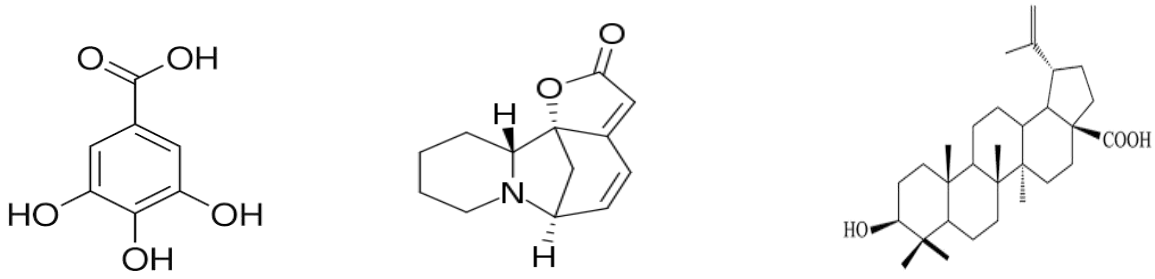
In another study, a 70 percent (v/v) aqueous ethanol stem bark extract of *M. discoidea* suppressed the in vivo degranulation of mast cells and demonstrated anti-allergic action. It also exhibits anti-inflammatory activity and attenuates Freund's adjuvant-induced arthritis through reducing the serum concentrations of Tumour necrosis factors (TNF) and Interleukin-6 (IL-6) (Obiri *et al*., 2014).

Reports have also recorded that an organic stem bark extract contains phenolic compounds (such as gallic acid) and the alkaloid – securinine; that indicated cytotoxic activity against

ovarian cancer cells (Johnson-Ajinwo *et al.*, 2015). Also, the extract of the plant has been reported to possess gastroprotective effect (Sofidiya *et al*.,2015). Report has it that the extract of the plant is used to disrupt the larval and pupal stages of a tropical disease vector (Olaleye *et al*., 2017); while according to another study, the aqueous extract of its stem bark has anti-inflammatory and analgesic properties. As a result of its activity, some communities use the plant to treat conditions such as stomachaches, pain and inflammations (Adedapo *et al.*, 2008).

1.5.1.5 Some Compounds Present in *M. discoidea*

Some chemical compounds that have been isolated from *M. discoidea* and reported in the literature are; securinine, betulinic acid and gallic acid (Johnson-Ajinwo *et al*., 2017).



Gallic Acid Securinine Betulinic Acid

Figure 1.2: Some isolated compounds in *M. discoidea* using column chromatography techniques.

1.6 JUSTIFICATION

Studies have been published concerning the biological activities of the plant. Therefore, some inherent characteristics of the leaf that helps ensure quality control of its extracts or medicinal product needs to studied due to the limited data available in literature. Hence, this study, which was designed to help determine some of inherent characteristics of the leaf of *M. discoidea.*

1.7 AIM AND OBJECTIVES

The aim of the study was to carry out pharmacognostic standardization of the leaves of *M. discoidea* (Baill). G.L.Webster [Phyllanthaceae].

Specific objectives were to;

1. collect and accurately identify the plant material;
2. carry out qualitative studies on the leaves of *M. discoidea* (macroscopic, microscopic and chemo-microscopic examinations; phytochemical screening and thin-layer chromatography);
3. carry out quantitative studies on the leaves of *M. discoidea* (determination of moisture content, total ash value, food analysis, vitamin c content, elemental analysis, methanol extractive value, foaming index, swelling index and high performance liquid chromatography); and
4. document my findings from this research for dissemination of information.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 MATERIALS

These include all materials used for this study such as the plant material, solvents and chemicals/reagents.

2.1.1 Plant Materials

This includes the leaves of *M. discoidea* used in this study.

2.1.2 Solvents/Chemicals/Reagents

This include; concentrated sulphuric acid, Fehling's solution a and b, 10% alcoholic solution of alpha-napthol, glacial acetic acid, 5% ferric chloride, Wagner's reagent, Hager's reagent, Dragendorff's reagent, Mayer's reagent, phloroglucinol solution, n/50 iodine solution, chloral hydrate solution, Sudan(IV) reagent, concentrated nitric acid, concentrated hydrochloric acid, ruthenium red, ethyl acetate, n-hexane, ethanol, glycerin, 66% sulphuric acid, perchloric acid, picric acid, strontium chloride, chloroform, methanol, 1% sulphuric acid, acetic anhydride, 0.5% ferric ammonium citrate solution, 40% formaldehyde, 5% potassium hydroxide solution, dilute hydrochloric acid, 33% acetic acid, sodium potassium tartrate and acetonitrile.

2.1.2 Laboratory Materials

These include; weighing balance, slides and cover slips, glass-wares (beakers, conical flasks, measuring cylinder, funnel, pipette, glass stirrer, thin layer chromatography stands, petri-dishes, test tubes),flame photometer, Soxhlet apparatus, Atomic Absorption Spectrophotometer, binocular microscope with camera and water bath.

2.2 METHODS

The various methods used in this study are shown below.

2.2.1 Plants Collection And Identification

The leaves of *Margaritaria discoidea* were collected on 25th day of August, 2021, from the premises of the Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. Identification was done at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City by a plant taxonomist, Prof. H. A. Akinnobosun; where the voucher specimen number (UBH-M625) was assigned .

2.2.2 Preparation of the Powdered Sample

The leaves were air-dried under shade for two (2) days and then transferred to an oven maintained at a temperature of 65^{o}C for 8 hours before it was milled using an electric milling machine. The powdered sample was stored in an airtight container, labelled and kept until required.

2.2.3 Preparation of the Plant Extract

The powdered leaves (200.76 g) of *M. discoidea* was weighed and packed into a thimble, which was transferred into the Soxhlet apparatus for extraction using two (2) litres of methanol. After exhaustive extraction, the extract obtained was placed in an evaporating dish and evaporated to dryness on a thermostatically controlled water bath. The dried extract was weighed, stored properly in a labelled container covered with aluminium foil paper and kept in the refrigerator for preservation until needed.

2.2.4 Qualitative Studies

This includes macroscopic, microscopic and chemo-microscopic examinations; phytochemical screening and Thin Layer Chromatography (TLC).

2.2.4.1 Macroscopic examination of *M. discoidea*

A leaf was placed on a white paper, and several characteristics including composition, shape, size, margin, apex, base, venation, texture, peculiarities, odour, taste, and form, were examined and recorded (Trease and Evans, 2002).

2.2.4.2 Microscopic examination of *M. discoidea*

i. **Microscopy of the powdered leaf sample**

A small portion of the powdered leaf sample was cleared with chloral hydrate solution (prepared by dissolving 50 g of chloral hydrate in 20 ml of water in a 100 ml beaker). A small quantity was mounted on the glass slide, a drop of glycerin was added to it, and was covered with a slip. Microscopic characters such as the presence and type of trichomes, fibers, and stomata, and the nature and arrangement of the cell wall were examined. Microscopic structures that were observed to be present were captured with the aid of a camera at a magnification of × 100.

ii. **Microscopy of the transverse section through the midrib and lamina of the leaf**

Several cuts were made through the midrib and lamina of a freshly collected *M. discoidea* leaf with the aid of a sharp razor blade and transverse sections were placed in a petri dish containing distilled water. The sections were mounted on a slide and examined under the microscope of a magnification of × 100. The images were captured with the aid of a camera. The slide was then removed from the stage of the microscope, and a drop of phloroglucinol was added to the cut transverse sections on the slide. This was allowed to stand for one (1) minute. A drop of concentrated hydrochloric acid was added to the slide, and it was allowed to stand for another one (1) minute. A drop of glycerin was added to the cut transverse

sections on the slide, and the slide was viewed under the microscope at a magnification of × 100.

2.2.4.3 Chemo-microscopic examination of *M. discoidea*

Chemo-microscopical examinations were carried out on the powdered leaves of *M. discoidea* using the method of Khandelwal, 2008.

i. **Test for lignin**

The powdered sample was mounted on a glass slide, and a few drops of phloroglucinol and concentrated hydrochloric acid were added. The slide was viewed with a microscope, and the results observed were recorded.

ii. **Test for cellulose**

The powdered sample was placed on a glass slide, and a few drops of N/50 iodine solution and 66% sulphuric acid were added to it. The slide was mounted and viewed under a microscope. The results observed were recorded.

iii. **Test for phenolics**

The powdered sample was mounted on a slide, and a drop of ferric chloride solution was added. The slide was viewed with a microscope, and the results observed were recorded.

iv. **Test for starch**

The powdered sample was mounted on a slide, and a few drops of N/50 iodine solution were added to the sample. The slide was viewed with a microscope, and the results observed were recorded.

v. **Test for mucilage**

The powdered sample was mounted on a slide, and a few drops of phenol red were added to the sample. The slide was viewed with a microscope, and the results observed were recorded.

vi. **Test for fixed oil**

The powdered sample was mounted on a slide, and a few drops of Sudan (IV) solution were added to the sample. The slide was viewed with a microscope, and the results observed were recorded.

2.2.4.4 Phytochemical tests

These tests were carried out on the powdered leaf sample using standard phytochemical procedure of Sofowora,1993. These test were done to confirm the absence or presence of secondary plant metabolites such as glycosides, alkaloids, tannins, etc. in the plant material.

a. General test for glycosides

A volume of 30ml of water was added to a little powdered plant material of *M. discoidea* (0.1 g) in a beaker and was heated gently heated on a boiling water bath for about five (5) minutes.The beaker was shaken vigorously, stirred with a glass rod, filtered hot and then allowed to cool.

i. **Molisch's test**

Two mililitres (2 ml) of the above filtrate was measured into a test tube. A drop of a 10% alcohol solution of naphthol was added. The tube was inclined at an angle of 45° and 2 ml of concentrated sulphuric acid was cautiously poured down the side of the test tube to form a layer below the filtrate without mixing with it.

ii. **Fehling's test**

Two mililitres (2 ml) of the above filtrate was measured into a test tube and 1 ml of Fehling's solutions A and B were added. The test tube was gently heated on a boiling water bath for five (5) minutes.

b. Test for saponins

A volume of 20ml of water was added to a 1 g of the plant material in a beaker and was heated gently heated on a boiling water bath for about fifteen (15) minutes.The beaker was shaken vigorously, stirred with a glass rod, filtered hot and then allowed to cool.

i. To 5 ml of the above filtrate in a test tube, 2 ml of distilled water was added. The contents of the test tube was shaken vigorously.

ii. To 5 ml of the above filtrate in a test tube, 2 ml of dilute sulphuric acid was added. The test tube was heated on a water bath for five (5) minutes. It was then allowed to cool and filtered. The residue was washed with water into the test tube and the filtrate was shaken vigorously.

iii. Two millilitres (2 ml) of Fehling's solution and 2 ml of sodium bicarbonate was added to 5 ml of the above filtrate in a test tube and was boiled for five (5) minutes over a boiling water bath. It was then allowed to cool and was shaken vigorously.

c. Tests for cyanogenetic compounds

i. Approximately 0.5g of the powdered leaf sample was transferred in a test tube and 5ml of distilled water was added to the powder. A sodium picrate paper was inserted into the test tube which was stoppered immediately. The test tube was placed at room temperature and observed for thirty(30) minutes for any colour change in the paper.

ii. Approximately 0.5g of the powdered leaf sample was transferred in a test tube and a sodium picrate paper was inserted into the test tube which was stoppered immediately. The test tube was placed at room temperature and observed for thirty(30) minutes for any colour change in the paper.

iii. Approximately 0.5g of the powdered leaf sample was transferred in a test tube, 5ml of distilled water was added to the powder. A sodium picrate paper was inserted into the test tube which was stoppered immediately. The mixture was heated for five (5) minutes

on a boiling water bath. The mixture was allowed to stand for thirty(30) minutes for any colour change in the paper

d. Tests for anthracene derivatives

i. Borntrager's test

A little quantity (0.5 g) of the powdered leaf of *M. discoidea* was extracted with 10ml of chloroform in a test tube by heating gently for two (2) minutes. It was then filtered with cotton wool, and to 2 ml of the filtrate, 1ml of dilute ammonia solution was added. The mixture was shaken vigorously and allowed to stand.

ii. Modified Borntrager's test 1

A little quantity (0.5 g) of the powdered leaf of *M. discoidea* was extracted with 2.5 ml of 10% sulphuric acid and 2.5 ml of hydrogen peroxide in a test tube by heating gently for five (5) minutes. It was then filtered with cotton wool, and the filtrate was shaken vigorously with 5 ml of chloroform. Three millilitres (3 ml) of the chloroform layer of the filtrate was transferred into a clean test tube and was shaken with 1.5 ml dilute ammonia solution.

iii. Modified Borntrager's test 2

A little quantity (0.5 g) of the powdered leaf of *M. discoidea* was extracted with 2.5 ml of 15% ferric chloride and 1 ml of concentrated hydrochloric acid in a test tube by heating gently for five (5) minutes. It was then filtered with cotton wool, and the filtrate was shaken vigorously with 5 ml of chloroform. Three millilitres (3 ml) of the chloroform layer of the filtrate was transferred into a clean test tube and was shaken with 1.5 ml dilute ammonia solution.

e. Tests for phenolic compounds

About 1 g of the powdered plant material of *M. discoidea* was extracted with 50 ml of distilled water in a beaker by heating on a boiling water bath for fifteen (15) minutes. It was filtered and allowed to cool for ten (10) minutes.

i. **General tests for tannins**

A drop of aqueous 15% ferric chloride solution was measured into 2 ml of the above filtrate in a test tube.

ii. **Iron complex tes**t

To 2 ml of the above filtrate in a test tube, 5 ml of 0.5% ferric ammonium citrate solution and 1 g of sodium acetate were added. The mixture was heated on a boiling water bath, cooled and boiled to obtain a precipitate.

iii. Formaldehyde test

To 5 ml of the above filtrate in a test tube, 3 drops of 40% formaldehyde solution and 6 drops of dilute hydrochloric acid were added. The mixture was boiled til bubbling and cooled. The precipitates obtained were washed with hot water, warm alcohol and 5% potassium hydroxide solution.

iv. Modified iron complex test

To 5 ml of the above filtrate in a test tube, 1 drop of 33% acetic acid and 1 g of sodium potassium tartrate were added. The mixture was heated on a boiled, cooled and filtered to obtain a precipitate.The precipitates obtained were washed with hot water into the filtrate. To the filtrate, 3ml of 0.5% ferric ammonium citrate solution was added in the test tube and the mixture was heated on a boiling water bath.

v. **Tests for flavonoid**

To 3ml of the above filtrate in a test tube, 2 ml of dilute sodium hydroxide was added and followed by 2 ml of concentrated hydrochloric acid.

f. Test for steroids

A volume of 10ml of methanol was added to a little powder in a beaker and was heated gently heated on a boiling water bath for about five (5) minutes.The mixture was filtered and

the filtrate was evaporated to dryness in an evaporating dish. The residue was used for the test of the steroidal nucleus.

i. Salkwoski's test for steroidal nucleus

A small quantity (0.5 g) of the extract was dissolved in 2 ml of chloroform. Two millilitres (2 ml) of concentrated sulphuric acid was cautiously poured down the side of the test tube to form a layer.

g. Test for alkaloids

i. Using water for the extraction of alkaloids

Five grams (5 g) of the powdered sample was extracted with 50 ml of distilled water in a beaker by boiling gently for thirty (30) minutes on a water bath. It was then filtered and allowed to cool for ten (10) minutes. Two millilitres (2 ml) each of the filtrate was transferred into four different test tubes, which were labelled A, B, C, and D into which few drops of Dragendorff's, Mayer's, Wagner's and Hager's alkaloidal reagents were added respectively.

ii. Using acid for the extraction of alkaloids

Five grams (5 g) of the powdered sample was extracted with 50 ml of 1% sulphuric acid in a beaker by boiling gently for thirty (30) minutes on a water bath. It was then filtered and allowed to cool for ten (10) minutes. Two millilitres (2 ml) each of the filtrate was transferred into four different test tubes, which were labelled A, B, C, and D into which few drops of Dragendorff's, Mayer's, Wagner's and Hager's alkaloidal reagents were added respectively.

iii. Using alcohol for the extraction of alkaloids

A little quantity (0.5 g) of the methanol extract was dissolved in 1% sulphuric acid. It was then filtered and the filtrate was transferred into four different test tubes, which were labelled A, B, C, and D into which few drops of Dragendorff's, Mayer's, Wagner's and Hager's alkaloidal reagents were added respectively.

iv. **Using chloroform for the extraction of alkaloids**

Five grams (5 g) of the powdered sample was extracted with 50 ml of chloroform in a beaker by boiling gently for thirty (30) minutes on a water bath. It was then filtered and the filtrate was reduced to dryness by heating over a water bath and the residue was dissolved in 1% sulphuric acid. It was then filtered and allowed to cool for ten (10) minutes. Two millilitres (2 ml) each of the filtrate was transferred into four different test tubes, which were labelled A, B, C, and D into which few drops of Dragendorff's, Mayer's, Wagner's and Hager's alkaloidal reagents were added respectively.

v. **Base-line test**

To 1 g of the powdered leaf sample in an evaporating dish, 2 g of calcium hydroxide and a sufficient amount of distilled water were added to make the powdered plant material into a paste. The paste was allowed to stand for five (5) minutes and was dried on a boiling water bath. Extraction of the dried paste was done with 50 ml of chloroform, the mixture was heated gently on a water bath. The extract was filtered and the filtrate was reduced to dryness by heating on a water bath. The residue was dissolved with 1% sulphuric acid and then filtered. Two millilitres (2 ml) each of the filtrate was transferred into four different test tubes, which were labelled A, B, C, and D into which few drops of Dragendorff's, Mayer's, Wagner's and Hager's alkaloidal reagents were added respectively.

2.2.4.5 Preliminary thin layer chromatography

A little quantity (0.2 g) of the methanol extract of the leaves of *M. discoidea* was dissolved in 10 ml of methanol in a beaker. Various mobile phases consisting of different ratios of mixtures of different solvents (ethyl acetate : n-hexane, chloroform : methanol and chloroform : n-hexane) were prepared in chromatographic tanks, well mixed and allowed to saturate the tanks at room temperature.

Commercial aluminium TLC plates measuring 20 cm by 20 cm were cut into sizes of 13.5 cm by 2 cm. The plates were carefully handled. The activated TLC plates were measured and 1 cm from the base was marked as the position of the origin where the spot will be placed. The solvent front was marked at 12 cm away from the origin.The TLC plates were activated at 120°C oven for thirty (30) minutes before use. The dissolved extract was streaked on the plate along the point of origin with a capillary tube with a distance of about 0.5 cm from each side of the plate. The plates were carefully placed in a vertical position in the chromatographic tanks covered with lids. The solvent systems moved in an ascending motion toward the solvent front. After an upward movement of 12 cm, the plates were quickly removed from the stands and allowed to dry. The colours of the resolved compounds were viewed under daylight and by spraying with 12% sulphuric acid prepared in methanol, followed by activation in the oven at 107^{o}C for three (3) minutes. Colours observed were recorded and the retention factor (R_f) was calculated for each component observed.

2.2.5 Quantitative Studies

This includes determination of moisture content and total ash content; food analysis (crude fibre, fat, protein and carbohydrates); vitamin c and elemental analysis; methanol extractive value, swelling index, foaming index and HPLC analysis.

2.2.5.1 Determination of moisture content

The powdered drug (2 g) was accurately weighed into the clean crucible. It was then placed in the oven at 105°C, heated for one (1) hour, cooled in a desiccator and weighed. The procedure was repeated until there was no further loss in weight. The average percentage weight loss in relation to the air-dried powdered drug was determined for three replicates.

2.2.5.2 Total ash content

Two grams (2 g) of the powdered sample were accurately weighed into a previously ignited and tared crucible. The sample was spread in an even layer and ignited by gradually increasing the temperature to 500 - 600 °C until the powder turned white, indicating the absence of carbon.This ash was weighed after cooling in the desiccator. The total ash content was calculated in mg per gram of air-dried powdered drug (WHO, 1998). The total ash was calculated in the following equation:

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of the original powder}} \times 100$$

2.2.5.3 Crude fibre determination

The method of Onwuka, 2005 was used to determine the crude fibre of the plant material. Two grams (2 g) of the powdered drug were boiled under reflux for 30 minutes with 200 ml of a solution containing 1.25 g of sulphuric acid per 100 ml of solution. The resultant solution was filtered, and the residue was washed with boiling water until the washings were not acidic.

The residue was transferred to a beaker and boiled with 200 ml of a solution containing 1.25 g of carbonate-free NaOH per 100 ml for 30 minutes. In a Gooch crucible, the final residue was filtered through a thin but close pad of washed and ignited asbestos. The residue was incinerated, cooled, and weighed. This procedure was done for three determinations.

$$\% \text{ Crude Fibre} = \frac{\text{The loss of weight after incineration}}{\text{Weight of the original powder}} \times 100$$

2.2.5.4 Crude fat determination

The method of Onwuka, 2005 was used in the determination of crude fat of the plant material. Clean boiling flasks (250 ml) were dried in an oven at 105°C for about 30 minutes. They were transferred into a desiccator and allowed to cool.Two grams (2 g) of the powdered drug were accurately weighed into labelled thimbles, and the corresponding labelled, cooled boiling flasks were weighed. The flasks were filled with 300 ml of petroleum ether, and the extraction thimble was plugged with cotton wool.The Soxhlet apparatus was assembled, and reflux was allowed for six (6) hours.The thimble was removed carefully and the petroleum ether was collected in the tap container of the apparatus and drained into a clean container for re-use.When the flask was almost free of petroleum ether, it was removed and dried at 105 °C for one (1) hour. The flask was then transferred into the desiccator, allowed to cool, and then weighed. This procedure was done for three determinations. The percentage of the crude fat was calculated as follows:

$$\% \text{ Fat} = \frac{\text{Weight of fat}}{\text{Weight of the original powder}} \times 100$$

2.2.5.5 Crude protein determination

The method of Onwuka, 2005 was employed in the determination of crude protein of the plant material. This determination was done for three replicates.

i. Protein digestion:

Two grams (2 g) of the powdered sample was weighed into a Kjeldahl flask, and 5 g of anhydrous sodium was added. One gram (1 g) of copper sulphate and a speck of selenium were added to the mixture. A volume of 25 ml concentrated sulphuric acid and 5 glass beads were introduced into the mixture. The mixture was transferred into a fume cupboard and heated very gently, and the temperature was gradually increased with occasional shacking until the solution appeared green.The black particles at the mouth and neck of the flask were collected, filtered, cooled, and washed with distilled water.The mixture was re-heated gently at first until the green colour disappeared, then allowed to cool. The digest was washed several times with distilled water before being transferred into a 250-ml volumetric flask, and distilled water was used to make up the mark. Distillation of the digest was done using the Markham distillation apparatus.

ii. Protein distillation

The Markham distillation apparatus was steamed for 15 minutes before use. A 100-ml conical flask containing 5 ml of boric indicator was placed under the condenser such that the condenser tip was under the liquid.The digest (5 ml) was pipetted into the body of the apparatus through the small funnel aperture and was weighed down with distilled water followed by 5 ml of 60 % NaOH solution. Steam was applied through the flask for five (5) minutes to collect sufficient ammonium sulphate. The receiving flask was removed, the tip of the condenser was washed with distilled water and the condensed water was then removed. The solution was titrated using 0.01Nhydrochloric acid into the receiving flask and the nitrogen content and the crude protein was calculated with the formula:

% Crude protein = %N × Conversion factor

Conversion factor is usually 6.25 and %N represents nitrogen content.

$$\% \text{ N} = \frac{(\text{Vs} - \text{Vb}) \times \text{Nacid} \times 0.01401}{\text{Weight of the original powder}} \times 100$$

Where

V_s is volume of acid requires to titrate the sample.

V_b is volume of the acid required to titrate the blank.

N_{acid} is the normality of acid (0.1N).

2.2.5.6 Carbohydrates determination

The carbohydrate content was obtained by calculation after all the other crude fractions have been estimated by proximate analysis(Onwuka, 2005).

$$\%\text{Available carbohydrates} = 100 - (\%\text{Moisture} + \%\text{Ash} + \%\text{Protein} + \%\text{Fibre})$$

2.2.5.7 Vitamin c determination

The method of Onwuka, 2005 was employed in the determination of vitamin c content of the plant material. About 2 grams of the powdered leaf sample of *M. discoidea* was weighed and 100 ml of distilled water was added to the powder to turn it into a paste in a volumetric flask. The paste was filtered, and a clear solution was obtained.About 10 ml of the solution was pipetted into small flasks, to which 2.5 ml of acetone was added. The mixture was titrated with indophenol solution (2,6-dichlorophenolindophenol) to a faint pink colour that persisted for fifteen (15) seconds.In the standardization of indophenol solution, 10 ml of standard ascorbic acid solution was pipetted into a small flask and titrated with indophenol solution until a faint pink colour persisted for fifteen (15) seconds.

The formula for a calculation of one milligram (mg) of vitamin C (ascorbic acid) is:

Vitamin C = mg/100ml = 20(v) × (c)

Where

v = ml indophenol solution in titration

c = mg vitamin C/ml indophenol

Ascorbic acid concentration was expressed as mg of ascorbic acid is equivalent to 1 ml of the dye solution(i.e., 10 ml of ascorbic acid solution = 0.002 g ascorbic acid).

If v ml dye solution required 0.002 g of ascorbic acid to neutralise it, then 1 ml of dye solution would require X g of ascorbic acid to neutralise it.

$$X\ g = \frac{0.002g\ ascorbic\ acid}{v\ ml}$$

2.2.5.8 Elemental analysis

In the elemental analysis, Sodium (Na) and Potassium (K) were analysed using a flame photometer; while Magnesium (Mg), Calcium (Ca), Copper (Cu), Zinc (Zn), Cadmium(Cd), Nickel (Ni), Lead (Pb), Manganese (Mn), Chromium (Cr), and Iron (Fe) were analysed using Atomic Absorption Spectroscopy (AAS) (Onwuka, 2005).

2.2.5.9 Determination of methanol extractive value

About 2 g of the air-dried drug powder was weighed into a 250-ml stoppered conical flask, 100 ml of methanol was added and stoppered firmly. The flask was shaken on a mechanical shaker for 5 to 6 hours, the extract was filtered by suction (using a Buchner funnel and flask); the process was repeated until the powdered drug was exhaustively extracted.

A bulking agent was added to the filtrates on an evaporating dish of known weight and the filtrates were evaporated to dryness on a water bath maintained at 60 ^{o}C. The residue was dried to a constant weight in an oven at 105 ^{o}C and the final weight was recorded.

2.2.5.10 Determination of the foaming index

The method of WHO, 1998 was done for the determination of the foaming index. Approximately 1 g of the powdered plant material was weighed into a 500-ml conical flask containing 100 ml of boiling distilled water. This was allowed to boil for 30 minutes on a water bath, and then allowed to cool. The mixture was filtered into a 100-ml measuring cylinder. The filtrate was prepared using distilled water in a 100-ml volumetric flask.

The extract obtained was dispensed into ten (10) stoppered test tubes of height (15 cm) and diameter (15 mm), in volumes of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml.The volume of the extract in each tube was made up to 10 ml with distilled water and then shaken vigorously for fifteen

(15) seconds. Each tube was allowed to stand for 15 minutes, and the height of the foam obtained was measured and recorded.

A height of foam less than 1 cm in every test tube was assigned a foaming index of less than 100, and where the height of foam was 1 cm in a test tube, the volume of the extract in that test tube (A) was used to calculate the foaming index, using the formulae shown below.

$$\text{Foaming Index} = \frac{1000}{A}$$

where, A = the volume, in ml, of the extract used for preparing the dilution in the tube having a height of 1 cm.

2.2.5.11 Determination of the swelling index

The method of WHO, 1998 was done for the determination of the swelling index of the plant material. Triplicate determinations were done for the plant *M. discoidea.* About 1 g of the powdered plant material was accurately weighed into a 50-ml measuring cylinder with internal diameter of 23mm, and 10 ml of distilled water was added.The mixture was then shaken thoroughly at 10-minute intervals for one (1) hour. The mixture was allowed to stand for three (3) hours at room temperature (27 ^{o}C). The volume in ml occupied by 1 g of the plant material was measured, and the mean value of the individual determinations was calculated relative to 1 g of the powdered plant material.

2.2.5.12 Hplc analysis

HPLC analysis was performed using a Shimadzu (Nexera Mx) type HPLC with a column (UBondpak C-18, 100 mm in length, 4.6 mm internal diameter, and 7 µm in thickness), a flow rate of 1ml/minute, a UV wavelength detector of 254nm, and a pump pressure of 15mPa. Ten grams (10 g) of the sample was extracted with acetonitrile, the extract was stabilized with ethyl acetate, and it was introduced into a 25-ml standard flask and made up to the mark. A volume of 5 ul of sample was injected at a flow rate of 2 ml/min and acetonitrile/water (70:30) was used as the carrier/mobile phase.

CHAPTER THREE

3.0 RESULTS

3.1 QUALITATIVE STUDIES

Results for the qualitative studies include the following:

3. 1.1 Macroscopical or Organoleptic Features of the Leaf of *M. discoidea*

The results of the macroscopical characteristics of the leaf are as shown in Table 3.1 and Figure 3.1

Table 3.1: Macroscopical characteristics of the leaves *M. discoidea*

S/N	DESCRIPTION	ATTRIBUTES
1.	Colour	
	i. Upper Surface	Dark Green
	ii. Lower Surface	Light Green
2.	Condition	Fresh
		Whole
3.	Leaf	
	i. Base	Exstipulate
	ii. Shape	Elliptical
	iii. Sheath	Absent
	iv. Petiole	Short, grooved and cylindrical petiole
4.	Lamina	
	i. Composition	Simple (Pinnate)
	ii. Shape	Elliptical
	iii. Size	Average Length = 8.7 cm (5.6 - 12.1cm)
		Average Breadth = 3.7cm (1.8 - 5.4cm)
	iv. Margin	Entire
	v. Apex	Acuminate
	vi. Base	Symmetric (Cuneate)
	vii. Venation	Reticulate
	viii. Surface	Glabrous
	ix. Wet Texture	Smooth
	x. Dry Texture	Smooth and papery
	xi. Odour	Before Crushing : Fruity
		After Crushing : Green odour
	xii. Taste	Without Chewing : Tasteless
		With Chewing: Bitter

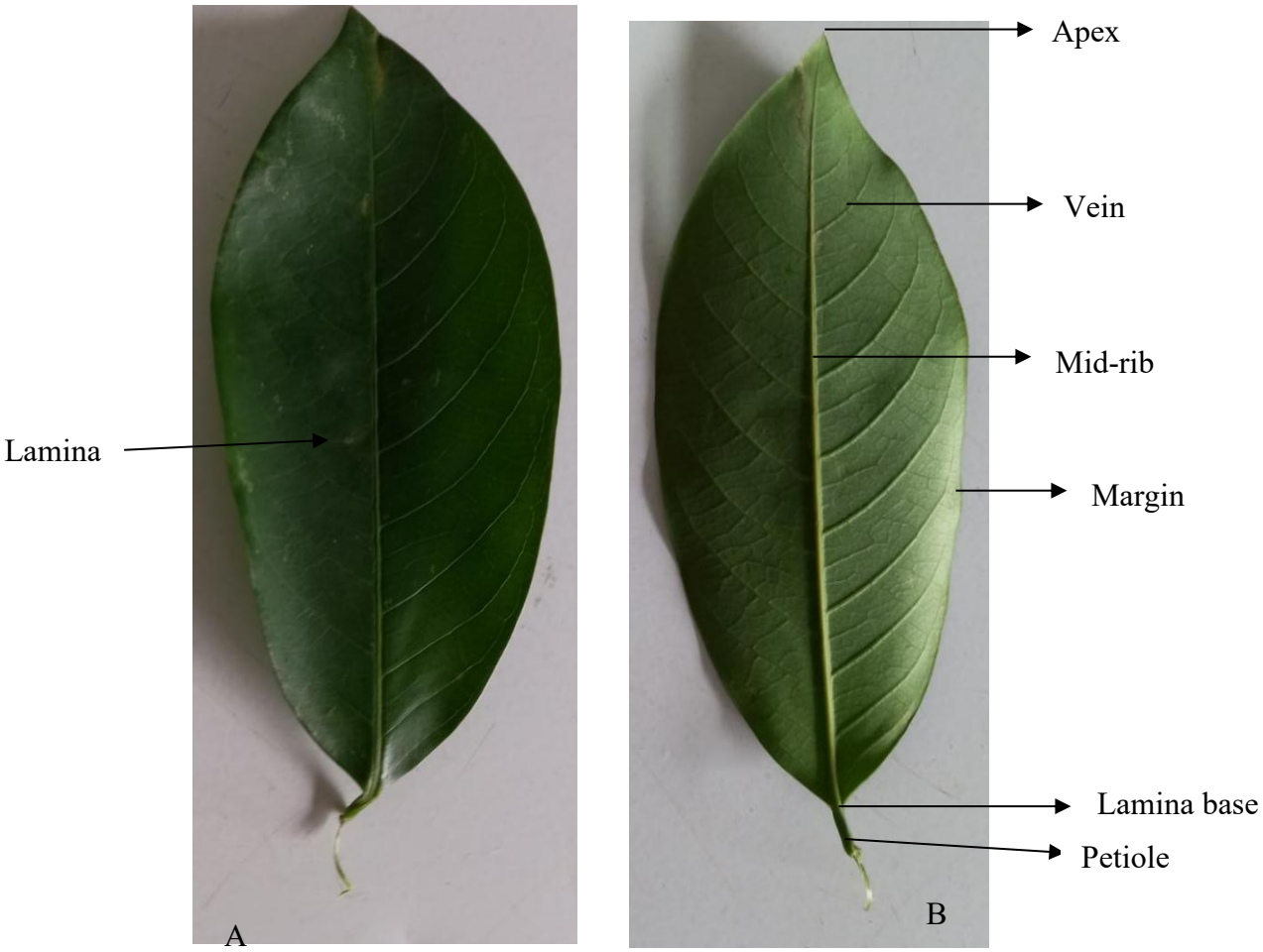


Figure 3.1: Macroscopical features of the upper (A) and lower (B) surface of the leaf of *M. discoidea.*

3. 1.2 Microscopical Features

The microscopical examination of the upper epidermal layer of the *M. discoidea* leaf showed the presence of straight walled epidermal cells. The lower epidermal layer of the *M. discoidea* leaf showed the presence of unicellular trichomes, calcium oxalate crystals and paracytic stomata (Figures 3.3 and 3.4). The transverse section through the midrib and lamina showed isobilateral arrangement of tissues in the leaf. When the transverse section was stained with phloroglucinol and Conc. HCl, the xylem and pericycle were stained red (Figure 3.2).

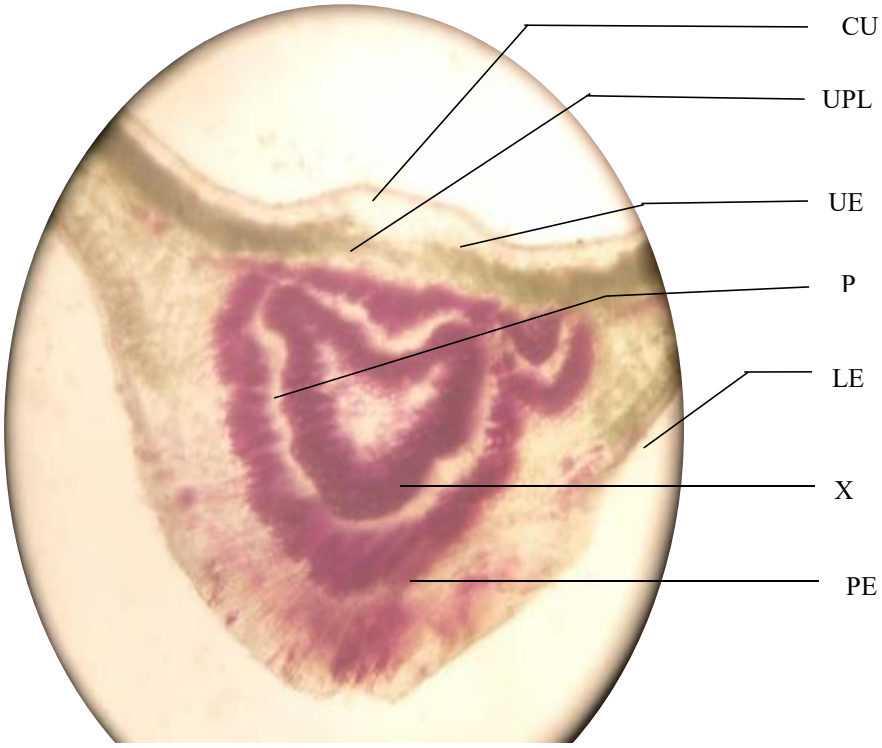


Figure 3.2: Transverse section of the isobilateral arrangement of the leaf of *M. discoidea* showing: upper cuticle (CU), upper palisade layer (UPL), upper epidermis (UE), lower epidermis (LE), pholem (P), xylem (X), and pericycle (PE) (Mag × 100).

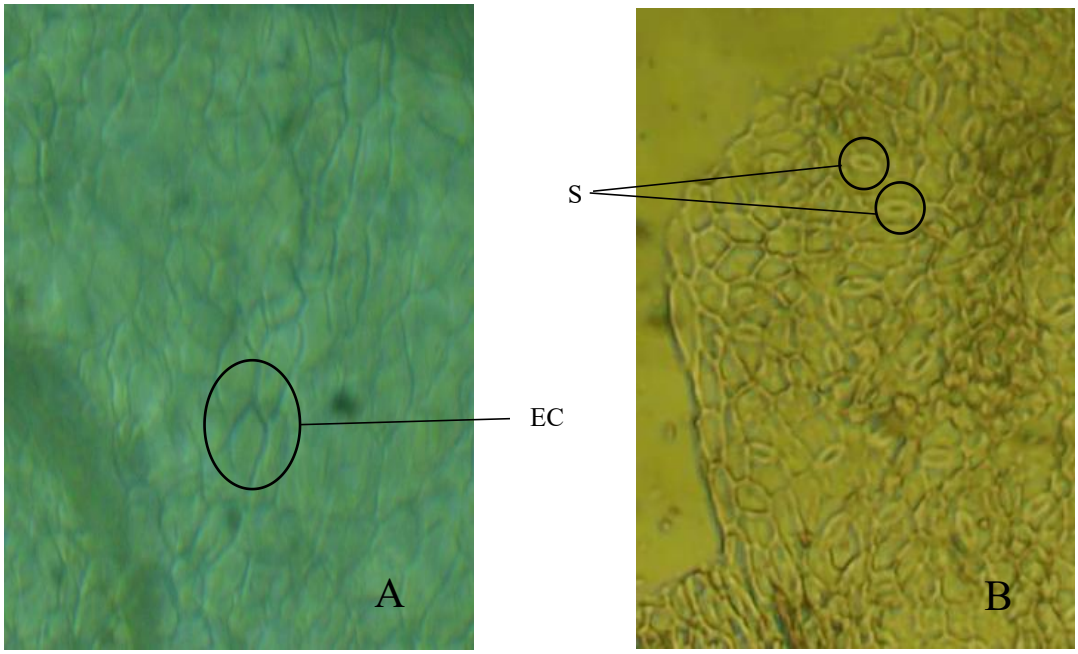


Figure 3.3: Microscopical examination of the cleared upper (A) and lower (B) epidermis of the leaf of *M. discoidea* showing; straight walled epidermal cells (EC) and paracytic stomata (S)(Mag × 100).

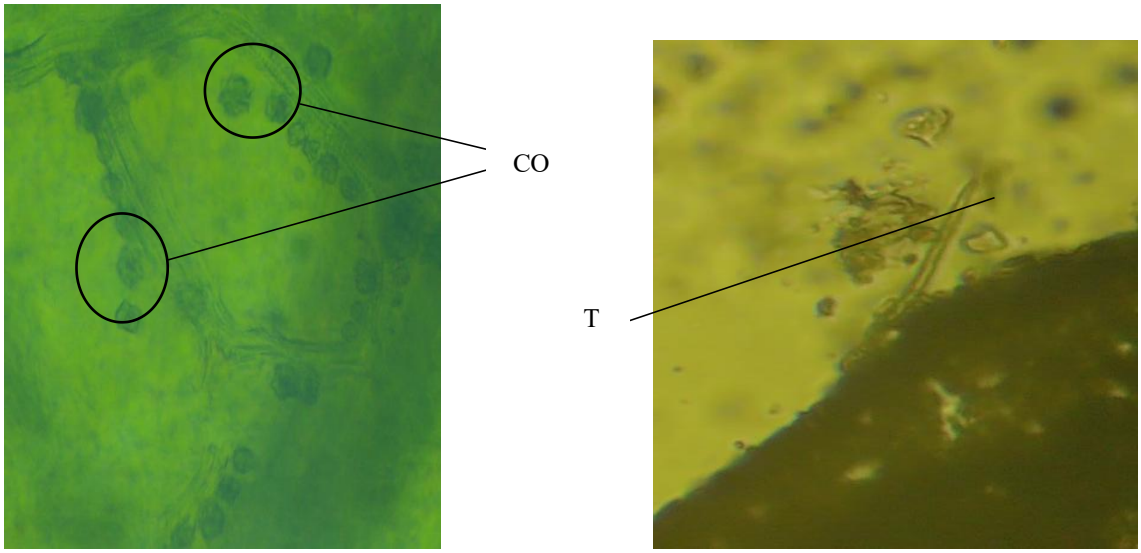


Figure 3.4: Microscopical examination of the lower epidermal layer of the leaf of *M. discoidea* showing; prismatic calcium oxalate crystals (CO) and unicellular trichome (T)(Mag × 100).

3. 1.3 chemo-microscopy

chemo-microscopy test of *M. discoidea* showed the presence of lignin, cellulose, mucilage, tannins, starch and fatty oils. A summary of the chemomicroscopical test results are shown in Table 3.2

Table 3.2: Chemo-microscopic tests and results for the leaves of *M. discoidea*

S/N	TEST	OBSERVATION	INFERENCE
1.	Test for Lignin	Red colour observed	Lignin present
2.	Test for Cellulose	Blue black colour observed	Cellulose present
3.	Test for Mucilage	Pink colour observed	Mucilage present
4.	Test for Phenols	Blue-green colour present	Tannins present
5.	Test for Starch	Blue black colour observed	Starch present
6.	Test for Fixed oils	Dark red colour observed	Fixed oils present

3.1.4 Phytochemical Test Results

The results obtained from the phytochemical screening carried out on the powdered sample is as shown below (Table 3.3). Glycosides, tannins, flavonoids, steroids and alkaloids were shown to be present.

Table 3.3: Phytochemical tests and results for the leaves of *M. discoidea*

S/N	TEST	OBSERVATION	INFERENCE
1.	Molisch's Test	A purple ring was formed at the interface	Carbohydrate present
2.	Fehling's Test	A reddish-brown precipitate was observed	Reducing sugar present
3.	Test for Saponins	Frothing persisted for more than one (1) minute.	Saponins present
4.	Test for Cyanogentic compounds	No colour change	Cyanogenetic compounds absent
5.	Test for Anthracene		
	a. Borntrager's test	Two separate layers with cloudy precipitate at the interface	Anthracene derivatives absent
	b. Modified Borntrager's test	A cloudy solution observed	Anthracene derivatives absent
6.	Test for Phenolics:		
	a. General Test for Tannins	Blackish precipitate observed	Tannins present
	b. Iron Complex Test	Reddish precipitate observed	Pseudo-tannins present
	c. Formaldehyde Test	No precipitate observed	Condensed tannins absent
	d. Modified Iron Complex Test	Reddish pink precipitate insoluble in water, ethanol and ammonia.	Hydrolysable tannin present
	e. Tests for Flavonoid	Yellow coloured solution which become colourless after addition of Conc. HCl	Flavonoid present
7.	Test for Steroids		
	Salkwoski's Test	Upper green layer with brown ring at the interface observed	Steroidal nucleus present

Table 3.3 Continued: Phytochemical tests and results for the leaves of *M. discoidea*

S/N	TEST	OBSERVATION	INFERENCES
8.	Test for Alkaloids		
a.	Extracted with Water		
	i. Dragendorff's reagent	Reddish brown precipitate observed	Alkaloid salt suspected
	ii. Mayer's reagent	Cream coloured precipitate observed	Alkaloid salt suspected
	iii. Wagner's reagent	Reddish brown precipitate observed	Alkaloid salt suspected
	iv. Piciric acid	Yellowish precipitate observed	Alkaloid salt suspected
b.	Extracted with Acid		
	i. Dragendorff's reagent	Reddish brown precipitate observed	Alkaloid base and/or salt suspected
	ii. Mayer's reagent	Cream coloured precipitate observed	Alkaloid base and/or salt suspected
	iii. Wagner's reagent	Reddish brown precipitate observed	Alkaloid base and/or salt suspected
	iv. Piciric acid	Yellowish precipitate observed	Alkaloid base and/or salt suspected
c.	Extracted with Alcohol		
	i. Dragendorff's reagent	Reddish brown precipitate observed	Alkaloid base and/or salt suspected
	ii. Mayer's reagent	Cream coloured precipitate observed	Alkaloid base and/or salt suspected
	iii. Wagner's reagent	Reddish brown precipitate observed	Alkaloid base and/or salt suspected
	iv. Piciric acid	Yellowish precipitate observed	Alkaloid base and/or salt suspected
d.	Extracted with Chloroform		
	i. Dragendorff's reagent	A clear red solution observed	Alkaloid base absent
	ii. Mayer's reagent	Clear solution observed	Alkaloid base absent
	iii. Wagner's reagent	Dark amber colour clear solution observed	Alkaloid base absent
	iv. Piciric acid	Lemon green colour clear solution observed	Alkaloid base absent
e.	Baseline Test for Alkaloids		
	i. Dragendorff's reagent	Reddish brown precipitate observed	Alkaloid base confirmed
	ii. Mayer's reagent	Cream coloured precipitate observed	Alkaloid base confirmed
	iii. Wagner's reagent	Reddish brown precipitate observed	Alkaloid base confirmed
	iv. Piciric acid	Yellowish precipitate observed	Alkaloid base confirmed

3. 1.5 Thin Layer Chromatography

Results from the thin layer chromatography (TLC) that was done with different solvent systems showed that the solvent system ethyl acetate and n-hexane (7:3) gave the best resolution of the different constituents in the methanol extract.

The constituents were viewed under daylight and after being sprayed with 12% H_2SO_4 in methanol. The results of the TLC plates viewed under daylight and after being sprayed with 12% H_2SO_4 are presented in Table 3.4.

Table 3.4: Results of TLC showing retention values (R_f) of the resolved constituents for the methanolic extract using solvent system ethyl acetate: n-hexane (7:3).

S/N	Spots (Colour in Daylight)	Spots (Colour in 12% H_2SO_4)	R_f Values
1.	Colourless	Purple	0.09
2.	Green	Green	0.14
3.	Colourless	Purple	0.16
4.	Orange	Brown	0.18
5.	Green	Green	0.21
6.	Green	Green	0.29
7.	Colourless	Purple	0.34
8.	Orange	Blue	0.38
9.	Orange	Green	0.46
10.	Orange	Brown	0.50
11.	Colourless	Blue	0.54
12.	Green	Green	0.63
13.	Orange	Green	0.77
14.	Colourless	Purple	0.83
15.	Orange	Brown	0.93
16.	Orange	Purple	0.98
17.	Green	Blue	0.99

Solvent front = 12cm.

3.2 QUANTITATIVE STUDIES

The results of proximate analysis, elemental content determination, methanol extractive value, foaming and swelling indices; as well as that of the high performance liquid chromatography are as shown below. Proximate analysis involved the determination of moisture content, total ash value, crude fibre, fat, protein and carbohydrate.

3. 2.1 Moisture Content

The result of the moisture content of the leaves of *M. discoidea* is shown in Table 3.5 below.

3. 2.2 Total Ash Value

The result of total ash content is shown in Table 3.5 below.

3. 2.3 Food Analysis

The results for the crude fibre, crude fat, crude protein and crude carbohydrate contents of the leaves of *M. discoidea* are shown in Table 3.5 below.

Table 3.5: Results of proximate analysis for the powdered leaves of *M. discoidea*

S/N	Parameters	Values (%)
1.	Moisture content	8.23 ± 0.01
2.	Ash content	8.62 ± 0.01
3.	Crude fibre	3.43 ± 0.20
4.	Crude fat	9.10 ± 0.14
5.	Crude protein	19.80 ± 0.25
6.	Carbohydrate	59.05 ± 0.26

Key: Values are expressed as Mean ± SEM. n = 3.

3. 2.4 Vitamin C and Elemental contents

The vitamin C and elemental contents of the leaves of *M. discoidea* is shown in Table 3.6 below. Potassium and calcium elements were the most abundant of all the elements shown to be present in the powdered leaf.

Table 3.6: Results of the vitamin C and elemental contents for the leaves of *M. discoidea*

S/N	Parameters	Values(mg/kg)
1.	Vitamin c	0.01
2.	Calcium	45.93
3.	Magnesium	1.01
4.	Sodium	3.21
5.	Potassium	131.97
6.	Copper	0.02
7.	Iron	0.80
8.	Manganese	0.46
9.	Zinc	0.23
10.	Lead	BDL
11.	Chromium	BDL
12.	Cadmium	BDL
13.	Nickel	BDL

Key: BDL means below detectable level. Values are expressed as mean values. n = 3.

3. 2.5 Methanol Extractive Value

The methanol extractive value of the leaves of *M. discoidea* was calculated to be 25.83%.

3. 2.6 Foaming Index

The Foaming index of the leaves of *M. discoidea* was to found to be <100.

3. 2.7 Swelling Index

Swelling index of the leaves of *M. discoidea* was found to be 5 ml/g.

3. 2.8 High-Performance Liquid Chromatography

Table 3.7: Results of HPLC analysis showing the various components in the acetonitrile extract of the leaf, their retention times, areas and heights.

Components	Retention	Area	Height
1. Phyllochrysine	3.70	2484.80	51.83
2. Gallic acid	5.88	598.90	11.58
3. Phyllanthine	7.97	691.20	15.03
4. Epiphyllanthine	9.12	59.04	3.32
5. Phyllanthidine	10.50	60.50	2.07
6. Betulinic acid	15.50	662.54	12.84
7. Kaempferol	17.23	2621.94	36.36
8. Securinol	19.40	139.79	5.27
9. Securinine	19.95	57.16	4.05
10. Allosecurinine	20.5	102.91	3.42
11. Viroallosecurinine	21.42	96.76	3.03
12. Corilagin	23.08	51.02	2.22

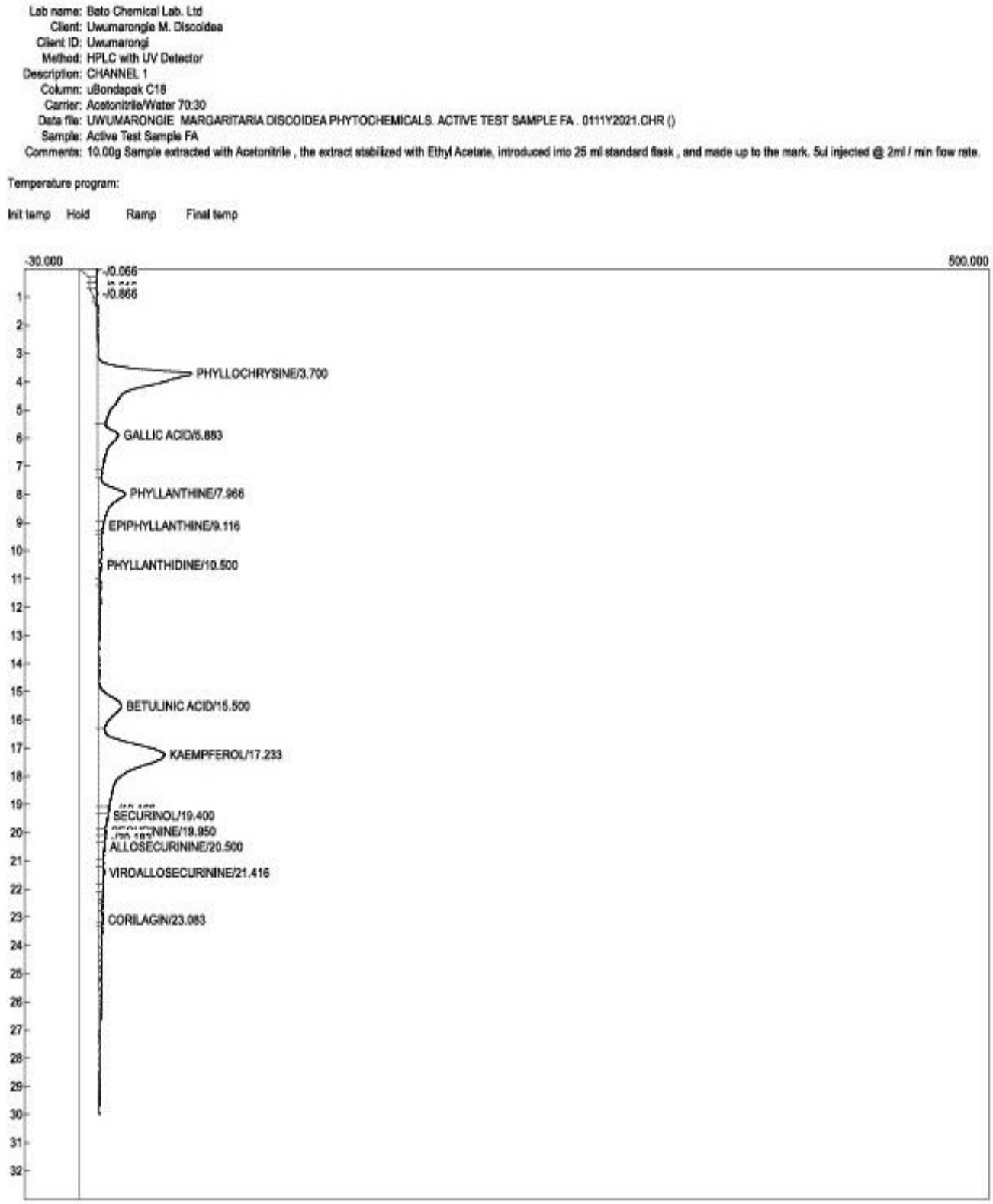


Figure 3.5: HPLC chromatogram showing the separated components in the acetonitrile extract of the powdered leaves of *M. discoidea*.

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1 DISCUSSION

The *M. discoidea* plant was accurately identified at the Department Of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State. This is to ensure the proper identification of the plant and also give room for further research studies on the plant material.

Macroscopic examination of the plants helps in the identification of the plant and also prevents adulteration of the plant materials. Detailed botanical and macroscopic descriptions of each plant's physical characteristics can be used to ensure both identity and purity (Kunle *et al*, 2012). The macroscopic attributes confirmed the plant was from the family Phyllanthaceae showing that the leaves were simple, petiolated, alternate with entire margins and reticulate venation which was described in the study of Hyde *et al*., 2022. The plant was also confirmed to be from the genus *Margaritaria* as the leaves were stipulated as being green in colour, elliptical in shape, and within the range of 10 to 13 cm in length (Hyde *et al.*, 2022).

The microscopic examination is used to ensure the identification of organized drugs based on their known histological characteristics (Bhusnure *et al*., 2019). These histological characteristics can be used to differentiate between species of the same genus and avoid adulteration of the plant material. The microscopic examination revealed the presence of a straight-walled epidermal cell, unicellular trichomes, prismatic calcium oxalate crystals, and paracytic stomata. And the transverse section showed the isobilateral arrangement of the leaf. Isobilateral leaf arrangement has been seen in dicot trees such as those in the genus *Margaritaria.* Staining the transverse section with phloroglucinol highlights the lignified

tissues, such as the xylem. Lignins are complex phenylpropane polymers that serve as plant cell wall strengthening materials and vary in composition depending on their source. It frequently impregnates the cell walls of vascular plant tracheids, vessels, fibres, and sclereids (Trease and Evans, 2002). These histological characteristics must be present, if a powdered sample is claimed to be *M. discoidea.*

Chemo-microscopic examination shows structures that cannot be seen without staining with reagents. The chemo-microscopy test of *M. discoidea* showed the presence of lignin, cellulose, mucilage, tannins, starch, and fixed oils. Cellulose is one of the main components of the cell wall of the plant. Tannins occur in solution in the cell sap and are widely distributed in plants. frequently in individual vacuoles. There are many different types of fixed oils and fats, and they can be found in both vegetative and reproductive systems. They frequently exist in seeds, where they occasionally take the place of carbohydrates as a supplementary source of food and are frequently linked to protein reserves. Lipids and fats are crucial components of cellular membranes. Starch is found in granules of various sizes in all plant organs, but it is most abundant in roots, rhizomes, fruits, and seeds. It normally occurs in larger grains than are discovered in chlorophyll-containing tissues within the same plant (Trease and Evans, 2002). Mucilages are polysaccharide complexes made of units of uronic acid and sugar. While they dissolve or swell in water, they are insoluble in alcohol. They are typically deposited on it in thick layers of the cell wall. These all have pharmaceutical uses; mucilage and starches are used as suspending and binding agents in drug formulation.

The significance of phytochemical screening is in its use to identify particular chemical components that might be present in any medicine and are responsible for its therapeutic effect. These chemical components are several active principles that can be qualitatively

recognised using various chemical assays, including total alkaloids, glycosides, tannins, flavonoids, and saponins. Phytochemical screening of the aqueous extract of the dried powdered leaves of *M. discoidea* showed the presence of carbohydrates, reducing sugar, saponins, tannins, flavonoids, steroidal nuclei, and alkaloids.

Carbohydrates are linked to the transport of certain pharmacologically active substances (such as alkaloids) in the bloodstream, making it easier for them to bind to specific receptors. It has also been documented to have certain immunomodulating and anti-inflammatory activities as well (Weymouth-Wilson, 1997). Reducing sugars shows the presence of monosaccharides and disaccharides (except sucrose). Carbohydrates have the potential to significantly influence cell cytotoxicity. The presence of carbohydrates has a significant effect in cancer cell assays. Sugar chains containing monosaccharides surpassed sugar chains containing disaccharides and polysaccharides in cytotoxicity activity. These findings demonstrated the significance of a carbohydrate moiety in influencing biological activities (Juang and Liang, 2020). These sugars have the ability to function as reducing agents.

Saponins were shown to be present in the aqueous extract of the plant. Saponins are a large family of plant-derived amphiphilic glycosides such as steroids and triterpenes. Saponins exhibit a wide range of biological and pharmacological properties and serve as major active principles in traditional medicines by expressing a large diversity of structures on both sugar chains and aglycones. Saponin isolation from natural sources is typically complicated due to the micro-heterogeneity of saponins in nature. Large amounts of naturally occurring saponins and congeners could be obtained through chemical synthesis (Yang *et al.*, 2021). The biological assays of synthetic saponin that have been recently published focused on several disease models, including anticancer, anti-infection, immunomodulatory, antiglucosidase, and cytoprotective effects (Juang and Liang, 2020).

The aqueous extract of *M. discoidea* leaves shows the presence of pseudo-tannins and hydrolyzable tannins. Tannins are a heterogeneous group of high-molecular-weight, water-soluble polyphenolic compounds found in plants. Tannins are chemically reactive phenolic compounds that form inter- and intramolecular hydrogen bonds that can interact with and precipitate macromolecules such as proteins and carbohydrates. Tannins can be divided into two categories: condensed tannins and hydrolyzable tannins. Two further categories of hydrolyzable tannins include gallotannins, which produce sugar and gallic acid upon hydrolysis, and ellagitannins, which produce not only sugar and gallic acid upon hydrolysis but also ellagic acid. Weak acids hydrolyze these molecules, and then high temperatures break them down to produce pyrogallol. The most prevalent polyphenols that come from plants are condensed tannins. These substances are not easily hydrolyzed; instead, they break down under acidic, alcoholic conditions to produce phlobaphenes, which are red pigments. Tannins have a variety of pharmacological actions, such as anti-inflammatory, anti-cancer, anti-nutritional, antioxidant, and free radical scavenging activities. Additionally, they appear to have positive effects on metabolic diseases and delay the start of a number of oxidative stress-related illnesses (Smeriglio *et al*., 2016).

Flavonoids were found to be present in the aqueous extract of the leaves of *M. discoidea.* All plants contain flavonoids, which are a large category of polyphenolic substances with a benzopyrone structure. The phenylpropanoid pathway is responsible for their production. Plants are known to synthesise flavonoids, which are hydroxylated phenolic compounds, in response to microbial infection. Studies that address the potential health advantages associated with the antioxidant properties of these polyphenolic substances have been conducted. Flavonoids' functional hydroxyl groups scavenge free radicals or chelate metal ions to exert their antioxidant properties. Metal chelation may be essential for preventing the production of free radicals, which can harm target biomolecules. Due to their strong

antioxidant activity in both in vivo and in vitro systems, flavonoids are regarded as having health-promoting characteristics as dietary components (Kumar and Pandey, 2013).

Flavonoids have the power to activate the human body's defence mechanisms. Studies have shown that flavonoids may protect against a wide range of bacterial and viral infections as well as degenerative illnesses like cancer, heart disease, and other age-related illnesses. When plant tissues are exposed to various biotic and abiotic stressors, flavonoids also function as a secondary antioxidant defence system (Kumar and Pandey, 2013). The result in this study corresponds with the study of flavonoid glycosides from the stem bark of *Margaritaria discoidea,* which demonstrate antibacterial and free radical scavenging activities (Ekuadzi *et al*., 2013). The plant *M. discoidea* was shown to have flavonoids, which may account for its use in the promotion of wound healing.

Possession of the steroidal nucleus shows the presence of cardiac glycoside. The cardiac muscle is specifically and significantly impacted by cardiac glycosides. A sick heart can benefit from a very small dose of medication. These substances are most beneficial when treating congestive heart failure. They intensify heartbeats without correspondingly increasing oxygen intake. Cardiac glycosides are a family of substances that are divided into two primary types based on the structure of their aglycone. Cardiac glycosides are steroids with the basic nucleus of cyclopentanoperhydrophenanthrene replaced at C17. They can be C23 or C24 steroids. In contrast to bufadienolides, which were first identified as toad skin poisons, cardenolides have a five-membered lactone group in the C17 with an unsaturated lactone ring (butenolide). the C17 substituent with a six-membered lactone ring that is doubly unsaturated (-pyrone). Cardenolides and bufadienolides can both be produced by plants. The double bond of the butenolid ring is located at position 21 or position 22 in another group

called isocardenolides, as opposed to position 20. Cardenolides in plants seem to be restricted to angiosperms (Morsy, 2017).

Both human treatment and an organism's natural defence depend heavily on alkaloids. About 20% of the known secondary metabolites discovered in plants are alkaloids. Alkaloids in plants guard against predators and control growth. Alkaloids are particularly well known for their medicinal uses as anaesthetics, cardioprotectants, and anti-inflammatory drugs. Among the well-known alkaloids used in clinical settings are nicotine, ephedrine, strychnine, quinine, and quinine. Alkaloid compounds are known due to both their potential for drug discovery and a very aggressive development in the study of traditional remedies (ethnopharmacology) (Heinrich *et al*., 2021). Alkaloids have been shown to be present in the plant *M. discoidea,* as seen in the study of alkaloids from the leaves of *Phyllanthus discoideus* (Mensah *et al*., 1988).

The thin layer chromatography revealed the presence of twelve (12) constituents in the methanolic fraction of the plant when viewed under daylight. A total of seventeen (17) constituents were elucidated when the TLC plates were sprayed with 12% H_2SO_4. Thin-layer chromatography is used for the qualitative determination of small amounts of impurities in a compound. It is used to identify primary compounds, which can be expanded on in further studies.

Quantitative evaluation is an important parameter in evaluating parameters and adulterants in powder drugs. These values are to be obtained by exercising discretion in determining the purity and safety of these crude drugs. Moisture content determines the amount of water present. A low moisture content is preferable to prevent the growth of microorganisms. The moisture content of the plant was 8.23%; given that it is less than the maximum allowed moisture content (8 to 14%) for vegetable medications (African Pharmacopoeia, 1986), this

indicates a low moisture content. The total ash showed 8.62%; although the readings are largely dependent on the kind of soil and mineral composition of the soil used to nurture the plant, the results are suggestive of low inorganic levels. From the qualitative results, the plant material was shown to have fixed oils and carbohydrates, with the carbohydrate material having the largest percentage (59.05%). The quantitative analysis confirmed the presence of carbohydrates (59.05%), fibre (9.10%), protein (19.80%), and crude fat (3.43%). The proximate analysis described the nutritional status of the leaves of *M. discoidea.*

Vitamin C has been known to have antioxidant activities and has been implicated in wound healing, and it was found in the leaves of *M. discoidea.* Elements such as calcium, magnesium, manganese, iron, sodium, copper, zinc, and potassium are found in the elemental analysis. The metals have significance in the metabolic processes of the human body. Sodium, calcium, and potassium are involved in the generation and sustenance of action potential along the nerves in the body system. Trace elements such as copper and manganese are also vital as minerals in the body. Iron is the major factor in blood formation. Heavy metals such as lead, cadmium, chromium, and nickel were below detectable levels, which indicated the safe use of the plant (WHO,1996).

The methanol extractive value was 25. 83% indicating that large amount of compounds were soluble in methanol. This therefore means that there is a large quantity of polar constituents in the leaves *of M. discoidea.* Swelling index confirmed the presence of mucilage as previously shown in the chemo-microscopical test. The result showed that the swelling index was found to be 5 ml/g. This indicated a large amount of mucilage to be present in the leaves of *M. discoidea.* Mucilages are used as suspending and binding agents in drug formulation. Foaming index confirmed the presence of saponins; the results showed that the foaming

index was found to be <100. The saponins were in small quantities, and saponins have been associated with cytoprotective activity.

High-Performance Liquid Chromatography (HPLC) has a higher specification and resolution than TLC and can be used for both qualitative and quantitative determination of the constituents in the sample. C-18, a column used for analysing substances with strong hydrophobicity, was the column used. Carbon chain length affects the sorbent phase's hydrophobicity, which improves the retention of ligands. Due to its high level of hydrophobicity, C18 is frequently referred to as the "classic reverse phase matrix." Because the carbon chains in C-18 are longer, it is more hydrophobic than other reverse phases (Shen, 2019).

HPLC results showed the presence of compounds such as phyllochrysine, gallic acid, phyllanthine, epiphyllanthine, phyllanthidine, betulinic acid, kaempferol, securinol, securinine, allosecurinine, viroallosecurinine, and corilagin. The area is directly proportional to the concentration they have in the sample. Kaempferol has the largest area, followed by phyllochrysine and phyllanthine. A tiny number of Phyllanthaceae (Euphorbiaceae) plants contain a class of natural compounds known as securinega alkaloids (or securinane-type alkaloids). With structural variations, these alkaloids have a tetracyclic structure composed of a piperidine or pyrrolidine, a 6-azabicyclic [3.2.1]-octane, and an isobutenolide. These alkaloids have a variety of important biological properties, including the ability to stimulate the central nervous system and anticancer, antimalarial, and antibacterial properties. Securinega alkaloids, such as securinine, allosecurinine, phyllanthine, epiphyllanthine, phyllochrysine, securinol, viroallosecurinine,phyllanthidine, and dihydroallosecurinine from *M. discoidea*, have been isolated by chemical analyses of *Margaritaria* species (Moraes *et al.*, 2015). Studies have shown that *Pseudomonas aeruginosa* and *Staphylococcus aureus* are

susceptible to the antibacterial effects of the cytotoxic pyridine alkaloid viroallosecurinine (Mensah *et al*., 1990).

In a study, phyllanthine was shown to decrease the mRNA expression of adipogenic genes, reduce the accumulation of liver triglycerides, and raise the expression of lipolytic genes in the adipose tissues and the liver of mice and rats (Jagtap *et al*., 2016). Gallic acid has been linked to a number of positive effects, including anti-inflammatory, antioxidant, and anti-cancer characteristics. According to studies, this substance has therapeutic effects on cardiovascular, metabolic, cognitive, and gastrointestinal diseases (Kahkeshani *et al*., 2019). Betulinic acid is a natural product that has a variety of biological effects, including potent anti-tumor activity. This anticancer characteristic is related to its capacity to activate the mitochondrial apoptosis pathway in cancer cells, causing apoptotic cell death. Studies reveal that normal cells and tissues are generally resistant to betulinic acid, in contrast to the cytotoxicity it exhibits against a number of cancer forms, suggesting a therapeutic window (Fulda, 2008). Kaempferol, a flavonoid, has structural similarities to the hormone oestrogen. Because of its estrogen-like properties, kaempferol can be used to treat hormone-regulated cancers such as ovarian, breast, cervical, hepatocellular carcinoma, and leukaemia (Singh *et al*., 2022). Phyllanthaceae plant species have a significant amount of corilagin. Previous research has shown that corilagin has a wide range of therapeutic advantages, including thrombolytic, anticancer, antihyperalgesic, and hepatoprotective effects (Liu *et al.*, 2017). From previous studies of chromatographic technique using ultra-HPLC(UHPLC), several constituents such as gallic acids and kaempferol were found in genus *Margaritaria* (Santiago *et al.,* 2022).

4.2 CONCLUSION

The pharmacognostic standards for the leaves of *Margaritaria discoidea* (Baill.) G.L. Webster [Family: Phyllanthaceae] that may contribute to the said therapeutic effects of the plant when used in the treatment of various diseases in herbal homes have been investigated, reported and documented in this study. These standards will help check against adulteration of the plant material; especially for plant materials collected from same region.

4.3 RECOMMENDATIONS

Further studies should be carried out on the plant to confirm the ethnomedicinal uses of the leaves and other parts of *M. discoidea* and to determine the feasibility of using the plant extract as a nutritional supplement. The results of the study can be used to create a monograph.

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