BIOACTIVE CHEMICAL SCREENING AND CHARACTERIZATION

OF ETHYL ACETATE EXTRACT OF DATURA METEL

(Purple thorn-apple)

BY

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CERTIFICATION

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DEDICATION

This project is dedicated to Almighty God and everyone who has contributed in one way or the other to the realization of this work.

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ABSTRACT

Throughout the world, plants are used traditionally to treat many ailments, particularly infectious diseases, such as diarrhea, fever, cold as well as for birth control and dental hygiene. This is as a result of the presence of various secondary metabolites in them. In this study, the leaves of D. metel were collected, dried and pulverized. Then, the powdered sample was extracted using ethyacetate solvent and screened for the presence of phytochemicals. After this, the extract was characterized by High Performance Liquid Chromatography (HPLC) to determine its specific constituents. The result of the phytochemical study showed the presence of five (5) out of nine (9) secondary metabolites that were investigated. These include glycosides, phenolics, alkaloids, steroids, and eugenols. However, the following metabolites such as saponins, terpenoids, flavonoids, and tannins were absent. The HPLC study showed an abundance of useful constituents with pharmacological activities such as atropine, scopolamine and quercetin. These compounds have been seen to perform health remediating functions such as antidepressant, anti-inflammation, anxiolytic, and many more. The findings of this study proves the D. metel plant is composed of useful bioactive substances. Therefore, the D. metel plant has a huge potential in performing medicinal functions to humans, so it is advisable to either consume it or utilize it in the discovery of novel drugs.

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CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Human beings have depended on nature for their simple requirements as being the sources for medicines, shelters, food stuffs, fragrances, clothing, flavours, fertilizers and means of transportation throughout the ages. For the large proportions of the world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, where herbal medicine has a continuous history of long use (Refaz *et al.,* 2017).

The foundations of typical traditional systems of medicine for thousands of years that have been in existence have formed from plants. The plants remain to offer mankind with new medicines. Some of the beneficial properties ascribed to plants have been recognised to be flawed and medicinal plant treatment is based on the experimental findings of hundreds to thousands of years (Refaz *et al.,* 2017). The healthy life process is closely related to the intricate and ingenious interactions among a large number of inherent biological molecules. On the one hand, some biological macromolecules play crucial roles in normal life activities by regulating the body's signal transduction and maintaining normal metabolism. On the other hand, pharmacological researches also revealed that the majority of drugs take

effects through the interactions with the disease-related drug targets, such as enzymes and receptors (Mulabagal and Calderon, 2010; Wu *et al.,* 2016). Taking advantage of those targeted biomolecules, it becomes possible for us to conduct fast and high-throughput screening for bioactive components from natural products targeting these enzymes and receptors, which could not only provide new ideas and approaches for new drug discovery, but also help to reveal the mechanisms of action of bioactive small natural molecules (Chen *et al.,* 2016). This study therefore seeks to determine the phytochemical profile of the ethyl acetate fraction of Datura metel.

1.1.1 BACKGROUND OF STUDY

Medicinal plants not only have the unique advantage with abundant resource bases, but are also gradually becoming promising medicinal resources with tens of thousands of natural bioactive constituents, especially for the early drug discovery and development stage. According to recent global statistics, almost 42% of all 1562 newly approved drugs from 1981 to 2014 are directly or indirectly derived from natural medicinal plants, such as the traditional Chinese medicine (TCM) (Newman and Cragg, 2010). Meanwhile, the proportion of natural bioactive compounds exceeds more than one half of the approved 1073 new types of small molecule drugs in the years from 1981 to 2010 (Atanasov *et al.,* 2015). However, due to the complicated chemical compositions, and the wide differences in

component contents, there always exist some thorny issues in screening for bioactive components, for example, the inconspicuous nonspecific adsorptions, the apparent interferences of false positive results, the undetectable trace active components and so on. Consequently, these obstacles pose serious challenges to both the identification of bioactive components and further unraveling their possible mechanisms of action (Potterat and Hamburger, 2013).

1.1.2 STATEMENT OF PROBLEM

Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The products obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. Extraction methods used pharmaceutically involve the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Ncube *et al*., 2008). The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective

solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contain a complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans (Handa *et al.,* 2008). In this regard, D. metal has been used traditionally by rural people in most communities, during parties, to make people feel a sense of euphoria without consideration of the various chemical constituents of the plant. This study is aimed at detecting constituents like alkaloids which have physiological effects.

1.1.3 SCOPE OF WORK

The design of this study is to use ethyl acetate as a solvent for the isolation and characterization of Datura metel extract.

1.1.4 JUSTIFICATION OF THE STUDY

Globally, the use of medicinal plants have gained increasing relevance especially in the aspect of serving as treatment method for numerous ailments. This relevance is attributed majorly to the presence of secondary metabolites in plants that contain certain properties and functions and with the increase in the demand of treatment methods for the several discovered ailments, it becomes imperative to have varying effectual plant materials that can serve as remedy to such ailments. This study will

provide knowledge on the health potency of Datura metel as a result of its secondary metabolites.

1.1.5 AIM AND OBJECTIVES

1.1.5.1 AIM

The aim of this study is to determine the phytochemical profile of the ethylacetate extract of Datura metel.

1.1.5.2 OBJECTIVES

The specific objectives of this research are to;

(i) Collect, dry, and pulverize the leaves of D. metal.

(ii) Extract the powdered sample of the plant using ethylacetate.

(iii) Determine the physico-chemical constituents of the Datura metel leaf extract using standard methods.

1.2 LITERATURE REVIEW

1.2.1 Medicinal Plants

A medicinal plant is defined as any plant which has compounds that can be used for the therapeutic purpose or which contain precursors of chemo pharmaceutical synthesis. Throughout the world, plants are used traditionally to treat many ailments, particularly infectious diseases, such as diarrhea, fever, cold as well as for birth control and dental hygiene. Also, many psychoactive substances used in

traditional medicine are of plant origin. Traditionally used medicinal plants produce a variety of known therapeutic properties (Chopra and Ananda, 2003). Herbal Medicine is still the mainstay of health care in several developing countries. The widespread use of herbal remedies and health care preparations, as those described in ancient texts such as the Vedas, and are obtained from commonly used traditional herbs and medicinal plants, have been traced for the occurrence of natural products with medicinal properties. Some interesting outcomes have been found with the use of a mixture of natural products to treat diseases, most notably the synergistic effects and poly-pharmacological application of plant extracts (Gibbons, 2003).

1.2.2 Datura metel

Datura metel is a spreading shrub with upright branches. A perennial herbaceous plant in the Solanaceae family can grow to be 1.5m tall. Simple, alternating, dark green leaves that are roughly oval, shallowly lobed, and glabrous. Flowers are enormous, solitary, and trumpet-shaped, with a fragrant aroma that is most appreciated in the mornings and evenings, and they come in a variety of colors ranging from white to yellow and light to dark purple. Insects pollinate the flowers, which are hermaphrodite. The fruit is shaped like a capsule with tiny spines. Datura can handle medium soil but prefers rich, moist, or even very alkaline soil. It does not thrive in the shadows. It prefers a warm temperature and is distributed in warmer regions of the world (Drake *et al*., 1996). Datura is most likely of

American origin and is widely cultivated for its beautiful flowers in all tropical and subtropical regions (Glotter *et al*., 1973). Datura is most likely of American origin and is commonly planted for its gorgeous flowers in all tropical and subtropical locations (Wang *et al*., 2008). Datura is most likely of American origin and is commonly planted for its gorgeous flowers in all tropical and subtropical locations (Agra *et al*., 2007). There are various species of Datura which are now cultivated for the production of secondary metabolites.

Plate 1: Leaf of Datura metel plant.

1.2.3 Reported medicinal uses of Datura metel

Tropane alkaloids found in D. metel are utilized as sedatives, antispasmodics, and mydriatics (Nuhu, 2002). The entire plant, particularly the leaves and seed, has anaesthetic, hallucinogenic, anti-asthmatic, antispasmodic, antitussive, narcotic, bronchodilator, anodyne, hypnotic, and mydriatic properties. The leaves are used as a local application for rheumatic swellings of the joints, Lumbago, Sciatica, Neuralgia, uncomfortable Tumors, Scabies, Eczema, Allergy, and glandular Inflammations such as Mumps; they are also smoked to ease spasmodic Asthma. Externally, seeds are also utilized for piles (Yusuf *et al*., 2009). Insanity, fever with catarrh, diarrhea, skin illnesses, and brain problems are all treated with seeds, leaves, and roots.

1.2.3.1 Antibacterial Activity

Crude aqueous and ethanol extracts of D. metel's leaf, stem bark, and roots were tested against eight clinical bacterial isolates (Streptococcus betahemolytic, S. dysenteriae Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus and Salmonella typhi). The leaf and stem bark extracts were antagonistic against the test bacteria species with inhibitory zones, and the ethanol extract significantly inhibited Staph. aureus (Akharaiyi, 2011).

1.2.3.2 Insecticidal Activity

Different percentages (2.5, 5.0, 7.5, and 10.0%) of methanolic extract of Datura metel seeds were tested against Helicoverpa armigera (Hubner), a moth whose larvae feed on a wide range of plants, including several important farmed crops. The 1.5 and 2.0% methanolic extract fractions had significant negative effects on a variety of biological parameters, including larval survival, weight and duration, pupal time, percentage of pupation, and adult emergence (Singh and Singh 2008).

1.2.3.3 Arthritis Treatment

Gout is a condition caused by an excess of uric acid in the body, which results in the development of small crystals of monosodium urate monohydrate that deposit in human tissues, particularly the joints (Virsaladze *et al*., 2007). (Kamienski, and Keogh, 2006). D. metel, which is historically used to treat gout, was tested for xanthine oxidase inhibitory action (Umamaheswari, 2007). The methanolic extracts of D. metel demonstrated more than 50% xanthine oxidase inhibitory action (in vitro), which was equivalent to the standard antigout medication, allopurinol, which showed 93.21% inhibition at 100 g/mL concentration with an IC50 value of 6.75g/mL. The methanolic extract was also screened for in vivo hypouricemic activity against potassium oxonate-induced hyperuricemia in mice and the extract was found effective (Umamaheswari, 2007).

1.2.3.4 Antioxidant Activity

D. metel leaf, stem bark, and root aqueous extracts demonstrated phytochemical and antioxidant activity. The plant's aqueous extract demonstrated antioxidant activity ranging from 49.30 to 23.82% and can be used as a natural source of antioxidants (Akharaiyi, 2011).

1.2.4 Bioactive chemical constituents in medicinal plants

A variety of active substances are found in secondary metabolites, which are biosynthesized from primary metabolites. In the plant kingdom, their range is more constrained. For a particular plant species growing in various environments, they differ in both quality and quantity. They commonly build up in smaller amounts and are typically produced at different embryonic stages by cell types with particular functions. The medicinal plants are abundant in secondary metabolites, a variety of chemicals that have been widely exploited in the pharmaceutical and medicine industries. These substances include alkaloids, glycosides, amines, insecticides, steroids, flavonoids, and related metabolites (Santosh *et al.,* 2007). Many of the plant secondary metabolites are constitutive, exist in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated in response to tissue damage or pathogen attack. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant such as alkaloids, steroids, tannins, flavonoids, resins,

fatty acids, etc. Out of the total number of secondary metabolites reported in the dictionary of natural products, 33,000 are terpenoids, 16,000 alkaloids, and 8,182 flavonoids. These being an integral part of the basic metabolism also have an ecological role and are often involved in plant protection against biotic or abiotic stresses (Weisshaar and Jenkins, 1998). Flavonoids, one type of secondary metabolite, have a role in the cell pigmentation of seeds and flowers, which draws pollinators and other seed dispersers and aids in plant reproduction. Additionally, plant secondary metabolites have medicinal qualities that are beneficial to human health. Secondary metabolites in plants are often categorized based on the metabolic pathways that they follow. Flavonoids, steroids, and alkaloids are the four major chemical families into which they fall.

1.2.4.1 Alkaloids

Heterocyclic nitrogen compounds are alkaloids. Tryptophan, tyrosine, and lysine are only a few examples of the amino acids that make up the primary metabolites from which they are generated. Many alkaloids have complex chemical structures and lengthy alkaloid biosynthesis routes. In the past three thousand years, alkaloids have been used medicinally by humans as purgatives, antitussives, and sedatives for snakebite, fever, and insanity (Jain *et al.,* 2009).

Alkaloids are a class of chemical substances that exist in nature. They make up the biggest single class of secondary plant metabolites and are extensively dispersed.

Approximately 5500 alkaloids are known about. They are used in medication, as recreational drugs, and in entheogenic rituals and are known to have pharmacological effects (Jain *et al.,* 2009).

1.2.4.2 Flavonoids

A class of polyphenolic chemicals known as flavonoids. They are present in all photosynthesizing cells and are frequently found in tea, wine, propolis, honey, fruits, vegetables, nuts, seeds, stems, and flowers. Since ancient times, their use has persisted despite being known to possess medicinal characteristics and play a significant part in effective medical treatments. They are effective free radical scavengers and water-soluble antioxidants that guard against oxidative cell damage and have powerful anti-cancer activity. They serve as anti-inflammatory, antispasmodic, anti-allergic, and antibacterial drugs in addition to being utilized to improve aquaresis (Mills and Bone, 2000). According to some reports, flavonoids may enhance blood circulation and reduce blood pressure. Additionally, flavonoids have a pharmacological action that prevents the activity of several enzymes, including cyclooxygenase, aldose reductase, xanthine oxidase, phosphodiesterase, and lipoxygenase. Additionally, they regulate other hormones like thyroid hormone, androgens, and estrogens. Both the proliferative and exudative phases of inflammation have been demonstrated to be responsive to their anti-inflammatory effects. In plants, flavonoids are known to be generated at specific locations and

are responsible for floral color, fragrance, fruit color and dispersion, seed and spore germination, seedling growth, and development. Plants are shielded from several biotic and abiotic stresses by flavonoids, which also serve as a special UV screen, function as signal molecules, allelopathic compounds, phytoalexins, detoxifying agents, and antimicrobial defensive compounds (Jain *et al.,* 2009).

1.2.4.3 Tannins

'Tannin' refers to a category of polymeric phenolic compounds capable of tanning leather or precipitating gelatin from solution, a characteristic known as astringency'. From 500 to 3,000 molecular weights are present in nearly every plant part: bark, wood, leaves. Plants can produce tannins by combining flavonoid derivatives that have been transferred to their woody tissues. A polymerization of quinone units may also produce tannins (Hussein and Anssary, 2019; Twilley *et al.*, 2020).
In humans, tannins are responsible for several physiological functions, including the activation of phagocytic cells, host-mediated tumour activity, and a variety of anti-infective effects. Hydrogen bonding and hydrophobic effects, as well as the creation of covalent bonds, are some of their chemical activities. They may be able to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. Polysaccharide is also a component of their structure. Antimicrobial relevance of this action has yet to be investigated by the scientific community. Also, low tannin

concentrations alter the shape of the germ tubes of Crinipellis perniciosa (Julsing et al., 2006).

1.2.4.5 Phenols

A single phenolic ring is required for some of the most potent phytochemicals on the planet. The phenylpropane-derived chemicals cinnamic and caffeic acids are typical examples. There are two popular plants that contain caffeic acid-- Tarragon and Thyme. Microorganisms have been found to be poisoned by catechol and pyrogallol, which are both hydroxylated phenols. Catechol and pyrogallol both have two 2OH groups (Jain et al., 2019). There is evidence that the number of hydroxyl groups on the phenol group and the number of sites on the phenol group are connected to their relative toxicity to microorganisms. According to other research, higher levels of oxidised phenols are more inhibiting (Namedo, 2007).

1.2.4.6 Phytosterols

The hydroxyl group at C-3, which is often in the beta configuration, and branching side chains from eight to ten or more carbon atoms at C-17 define sterols, one of several major classes of steroids. They are prevalent across the entire animal and, in especially, plant kingdoms. They play a crucial part in the biosynthetic pathways that result in the steroidal species as well as structural roles as components of membranes. Sterols serve as the building blocks for plant biosynthesis. The

building blocks for the manufacture of plant steroids are the phytosterols, which are widely acknowledged to be present in all higher plants. Since humans are unable to produce them, they must be obtained from food (Jain *et al.,* 2009). Several studies have indicated that they have reduced serum cholesterol levels. Plant sterols are industrially important chemical sources for steroid compounds, insecticides, antioxidant and anticancer drugs. A summary of some of the sterols, isolated from various plants has been reported. The most often isolated sterols from higher plants is β-sitosterol, β-stigmasterol, lanosterol and campesterol are also quite common. A similar finding was also reported by some other workers with βsitosterol, campesterol and stigmasterol as most often isolated compounds from higher plants. Evidence suggests that phytosterols possess antioxidant activity, anti-inflammatory activity, anti-cancer activity against cancer of lungs, stomach, ovary, and estrogen-dependent human breast cancer (Ju *et al.,* 2004).

1.2.5 Techniques of Isolation and Purification of Bioactive Molecules from Plants

Purification and separation of bioactive chemicals from plants is a technology that has shown considerable advancement (Altemimi *et al*., 2015). On the one hand, this new technique allows for the parallel creation and availability of numerous complex bioassays, while on the other hand, it enables precision isolation, separation, and purification techniques. When looking for bioactive chemicals, the goal is to design a method that can screen the source material for bioactivity such

as antioxidant, antibacterial, or cytotoxicity while also being simple, specific, and fast (Mulinacci *et al*., 2004). Animal experiments are more expensive, require more time, and are prone to ethical problems, hence in vitro methods are frequently used. Finding definitive methodologies or protocols to extract and identify certain bioactive compounds is difficult due to a number of variables. This might be because a plant has several components (tissues), many of which create relatively distinct molecules, and because the bioactive phytochemicals have a variety of chemical structures and physicochemical characteristics (Sarajlija *et al*., 2012). The selection and collecting of plant materials are regarded as the first steps in the process of isolating and identifying a bioactive phytochemical. The last stage entails retrieving ethnobotanical data to identify potential bioactive compounds. The active substances that are responsible for the bioactivity can subsequently be isolated and purified by creating extracts using a variety of solvents. Column chromatographic techniques can be used for the isolation and purification of the bioactive compounds. Developed instruments such as High Pressure Liquid Chromatography (HPLC) accelerate the process of purification of the bioactive molecule. Different varieties of spectroscopic techniques like UV-visible, Infrared (IR), Nuclear Magnetic Resonance (NMR), and mass spectroscopy can identify the purified compounds (Popova *et al.,* 2009).

1.2.6 Solvents used in Extraction

The various solvents that are used in the extraction procedures are:

Ether

Ether is commonly used selectively for the extraction of coumarins and fatty acids Cowan, 1999).

Dichloromethanol

It is another solvent used for carrying out the extraction procedures. It is specially used for the selective extraction of only terpenoids (Cowan, 1999)

Water

Water is an all-purpose solvent that can be used to extract plant materials with antibacterial properties. Although traditional healers usually utilize water, it has been discovered that plant extracts from organic solvents provide more reliable antibacterial action than water extract. Additionally, water soluble phenolics (mainly anthocyanins) are only significant as antioxidant chemicals while water soluble flavonoids (primarily anthocyanins) have little antibacterial importance (Das *et al*., 2010).

Acetone

Acetone dissolves many hydrophilic and lipophilic components from the two plants used, is miscible with water, is volatile and has a low toxicity to the bioassay used, it is a very useful extractant, especially for antimicrobial studies where more phenolic compounds are required to be extracted. A study reported that extraction

of tannins and other phenolics was better in aqueous acetone than in aqueous methanol (Das *et al*., 2010; Eloff, 1998). Both acetone and methanol were found to extract saponins which have antimicrobial activity (Ncube *et al.,* 2008).

Alcohol

The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. It means that they are more efficient in cell walls and seed degradation which have nonpolar character and cause polyphenols to be released from cells. More useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrades polyphenols in water extracts, whereas in methanol and ethanol they are inactive. Moreover, water is a better medium for the occurrence of the micro-organisms as compared to ethanol (Lapornik *et al.,* 2005). The higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent was increased (Bimakr *et al.,* 2010). Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material (Wang , 2010). Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often

obtained through initial ethanol or methanol extraction (Cowan, 1999). Methanol is more polar than ethanol but due to its cytotoxic nature, it is unsuitable for extraction in certain kinds of studies as it may lead to incorrect results.

Chloroform

Terpenoid lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents (Cowan, 1999).

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1.1 MATERIALS

Soxhlet extractor
Hand gloves

Weighing balance (digital)

Separatory funnel

Thin layer chromatography (TLC) plates (0.2 mm thick: E.merck)

Stirrer

Retort stand and clamp

Whatmann's filter paper

Beakers

Funnels

Test tubes

Measuring cylinder

Silica gel (60 - 120 mesh) for column chromatography

Mortar and pestle

Datura metel plant

2.1.2 REAGENTS

All reagents used are of analytical grade.
Ethyl Acetate (BDH, England)

Distilled water

2.2 METHODS

2.2.1 Collection of Sample

Datura metel fresh leaves were collected from their natural habitat in Oluku environment in Ovia North-East Local Government Area of Edo state, and were identified by Prof. E.I. Aigbokhan of the Department of Plant BIology and Biotechnology, University of Benin.

2.2.2 Sample treatment

The D. metel leaves were air dried at room temperature in the laboratory for twenty eight (28) days. They were then pulverized into fine powder in preparation for extraction.

2.2.3 Extraction

167 g of D. metel powdered leaves were packed in thimble and exhaustively extracted in soxhlet extraction using 600 ml of ethyl acetate for an 8-hourly period. The extract was dried over sodium sulphate (Na_2SO_4) and concentrated using a rotary evaporator (RE, 200) at 50^0C. The remnants were then extracted with increasing polarity using chloroform, ethyl acetate and methanol solvents respectively in the soxhlet extractor to afford four extracts of hexane, chloroform, ethyl acetate and methanol.

2.2.4 Phytochemical screening of D. metel extracts

The phytochemical screening of the D. metel plant was performed using standard methods and procedures described by Sofowora (1993) and Trease and Evans (1987).

2.2.4.1 Test for steroids

2 ml of acetic anhydride was added to 0.5 g planet extract in 2 ml of dilute sulphuric acid (H_2SO_4). A colour change from violet to blue green indicates the presence of steroids

.

2.2.4.2 Test for Terpenoids

5 ml of each extract was mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid (H_2SO_4) was carefully added down the side of the inner wall to the test tube to form a layer. A reddish-brown colouration indicates the presence of terpenoids.

2.2.4.3 Test for Alkaloids

Drangedoff's reagent, Wagner's reagent and picric acid were used to test for alkaloids. About 1 ml each of the plant extract was transferred from three different test tubes A, B, and C.

For portion A: 2 ml of Drangedoff's reagent and a mixture of potassium Bismuth iodine salt was added. A reddish-brown precipitate indicates a positive test. To

portion B: 2 ml of Wagner's reagent was added. Reddish brown precipitate indicates a positive test.

To portion C: 2 ml of picric acid was added. A yellow precipitate indicates a positive test.

2.2.4.4 Test for Flavonoids

1 ml of the extract was measured and a few drops of dilute NaOH solution was added. An intense yellow colour appears in the test tube. It becomes colourless on addition of a few drops of dilute acid, this indicates the presence of flavonoids.

2.2.4.5 Test for Saponins

1 ml of the plant extract was shaken with water in a test tube and observed for frothing. Saponin rein swiss (supplied by Merck) was used as the standard.

2.2.4.6 Test for Glycosides

1 ml of the extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) was added. A pale-yellow colouration indicates the presence of phenolic compounds.

2.2.4.7 Test for Eugenols

2 ml of the extract mixed with 5 ml of 5 % potassium hydroxide (KOH) solution. The aqueous layer was separated and filtered. Few drops of the dilute HCl were added to the filtrate. A pale-yellow precipitate indicates a positive test.

2.2.4.8 Test for Tannins

To 2 ml of the extract, 10 ml of distilled water was added and boiled for 5 minutes and then filtered into halves;

(i) To 2 drops of the filtrate, ferric chloride solution was added. Formation of a bluish precipitate is required to indicate the presence of hydrolysable tannin. (ii) To about 2 drops of the filtrate, 2 ml of dilute HCl was added and boiled for 5 mins. A red precipitate is required to indicate the presence of condensed tannin.

2.2.5 Determination of the retention factor (Rf) by Thin Layer Chromatography

Percolated TLC plates were cut to size of 9 cm length by 1 cm width and used in varying solvent systems of n-hexane, ethylacetate, chloroform, and methanol. The Rf values were calculated from the distance moved by the compound against the solvent front.

2.2.6 Characterization of bioactive constituents

High Performance Liquid Chromatography (HPLC) was used to carry out the characterization of the bioactive constituents.

2.2.7 HPLC analysis

2.2.7.1 Definition

High performance liquid chromatography (HPLC) is a method used to separate a mixture of complex samples. HPLC is an active process in which materials are

pumped at high pressure through a separation column, which contains a stationary phase, usually a chemically functional bends that separates the compound mixtures. Samples are infused through the injector, and carried via the mobile phase across the stationary phase to effect the separations. After separation through the column, the samples are exposed to a detector system that identify and quantify the individual compounds.

Extraction

167 g of sample was measured into the amber bottle. 20 ml of ethyl acetate was added to it, which was shaken vigorously for 30 mins. After the shaking, the aqueous end was run off while the organic solvent was collected into 25 ml standard flask, made up to the mark and ready for analysis.

Analysis

The standard form of (analytes) profile were first injected into the HPLC and this created a chromatogram, with a given peak and their peak profile. These were used to create a window in the HPLC in preparation of the test sample analysis. Then, aliquots of the extracted test sample were injected into the HPLC also, to obtain a corresponding peak tree and peak profile in a chromatogram. Then, the peak area of the sample is compared with that of the standard relative to the concentration of the standard to obtain the concentration of the sample.

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

3.1 Results

Percentage yield of extraction

The percentage yield of the ethylacetate extract of the was calculated using the formula;

$$\text{Percentage yield} = \frac{mass\ of\ extract}{mass\ of\ sample}\ x\ 100$$

Mass of extract = 26.7 g

Mass of powdered plant = 167 g

$$\text{Therefore, \% yield} = \frac{26.7}{167}\ x\ 100$$

$$= 10.15\%$$

The phytochemical screening of the ethylacetate extract of Datura metel identified the presence of various secondary metabolites as shown in Table 3.1.

Table 3.1: Phytochemical screening of the ethylacetate extract of Datura metal.

Phytochemical	Tests	Ethylacetate constituent
Glycosides	General test	+
Saponins	Frothing	-

Phenolics	Ethanol/Ferric chloride	+
Alkaloids	Picric acid	++
Terpenoids	Salkowski test	-
Steroids	Acetic acid/H_2SO_4	+
Eugenols	Ethanol/Ferric chloride	+
Flavonoids	Lead acetate	-
Tannins	Ferric chloride	-

+ = Present

- = Absent

++ = Largely present

3.2 Discussion

3.2.1 Phytochemical profile of the ethylacetate extract of Datura metel The ethylacetate extract showed the presence of glycosides, phenolics, alkaloids, steroids, and eugenols. However, the following metabolites such as saponins, terpenoids, flavonoids, and tannins were absent. This result was compared to the the study of the ethylacetate fraction of *Citrullus lanatus* carried out by

Alebiosu and Yusuf, (2015) who both discovered the presence of all the constituents alkaloids, tannins, phenols, and glycosides but saponins, steroids, terpenes, carbohydrates, anthraquinones and carbohydrates were all absent. These constituents are responsible for most pharmacological activities of plants (Sofowora, 1993).

3.2.2 Thin Layer Chromatography (TLC)

TLC is a chromatographic tool used to monitor the separation of the constituents of an extract. To do this, the retention factor (Rf) is calculated and this gives direction to how best a particular component or constituents can be isolated.

$$Rf = \frac{distance\ moved\ by\ the\ compound}{distance\ moved\ by\ the\ solvent}$$

Table 3.2 Retention factor and colour reaction of the ethylacetate extract of Datura metel

Spot	Distance moved by compound (cm)	Nature of spot		
		Colour under white light	Retention factor (Rf)	
Origin	0.00	-	Dark green	0.00

1	3.5	Sharp	Light yellow	0.44
2	4.7	Sharp	Light green	0.59
3	5.2	Sharp	Grey	0.65
4	6.5	Sharp	Dark green	0.81

Solvent system: 100% Ethyl acetate

Solvent front: 8 cm

Table 3.3: Retention factor and colour reaction of the ethylacetate extract of Datura metel.

Spot	Distance moved by compound (cm)	Nature of spot	Colour under white light	Retention factor (R_f)
Origin	0.00	-	Dark green	0.00
1	5.5	blurred	Dark green	0.69

Solvent system: 100% Methanol

Solvent front: 8 cm

Table 3.4: Retention factor and colour reaction of the ethylacetate extract of Datura metel.

Spot	Distance moved by compound (cm)	Nature of spot	Colour under white light	Retention factor (R_f)
Origin	0.00	-	Dark green	0.00
1	0.5	blurred	Dark green	0.0625

Solvent system: 100% hexane

Solvent front: 8 cm

Table 3.5: Retention factor and colour reaction of the ethylacetate extract of Datura metel.

Spot	Distance moved by compound (cm)	Nature of spot	Colour under white light	Retention factor (R_f)
Origin	0.00	-	Dark green	0.00

1	4.5	blurred	Dark green	0.56

Spot	Distance moved by compound (cm)	Nature of spot	Colour under white light	Retention factor (R_f)
Origin	0.00	-	Dark green	0.00
2	6.5	blurred	Light green	0.81

Solvent system: 1:1 of methanol/ethylacetate (50%/50%)

Solvent front: 8 cm

Table 3.6: Retention factor and colour reaction of the ethylacetate extract of Datura metel.

1	3.8	blurred	Dark green	0.48
2	5.0	blurred	Light green	0.63

Solvent system: 3:1 of methanol/ethylacetate

Solvent front: 8 cm

In this study, the results of the TLC displayed that the Rf value of the extract favours reasonably with 100% ethylacetate. Phytochemical constituents give different Rf values in different solvent systems. This variation in Rf values provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent systems for separation of pure compounds incorporated in different fractions by column chromatography. Mixtures of solvents with variable polarity in different ratios can be used for separation of pure compounds from plant extract. The selection of an appropriate solvent system for a particular plant extract can only be achieved by analyzing the Rf values of compounds in different solvent systems (Trease and Evans, 1996).

3.2.3 Characterisation of Datura metel plant

As shown in Table 3.7 below, The HPLC analysis of D. metel leaves displayed the presence of 19 different compounds with the three compounds such as scopolamine, atropine, and quercetin being the most concentrated compounds due to their observed high peak areas. These compunds have gained relevance in the phytotherapy of several ailments. The quantification of atropine and scopolamine

from D. metel by HPLC-MS has also been reported by Sambasivam, (2016). Quercetin (3,3',4',5,7-pentahydroxyflavone) belongs to the class called flavonoids that cannot be produced in the human body (Lakhanpal, 2007). It is yellow in color and is poorly soluble in hot water, quite soluble in alcohol and lipids and is insoluble in cold water. Quercetin is said to be one of the most widely used bioflavonoids for the treatment of metabolic and inflammatory disorders, has been used as a nutritional supplement and may be beneficial against a variety of diseases. Some of the beneficial effects of Quercetin include cardiovascular protection, anticancer, antitumor, anti-ulcer, anti-allergy, anti-viral, antiinflammatory activity, anti-diabetic, gastroprotective effects, antihypertensive, immunomodulatory, and anti-infective (Lakhanpal, 2007).

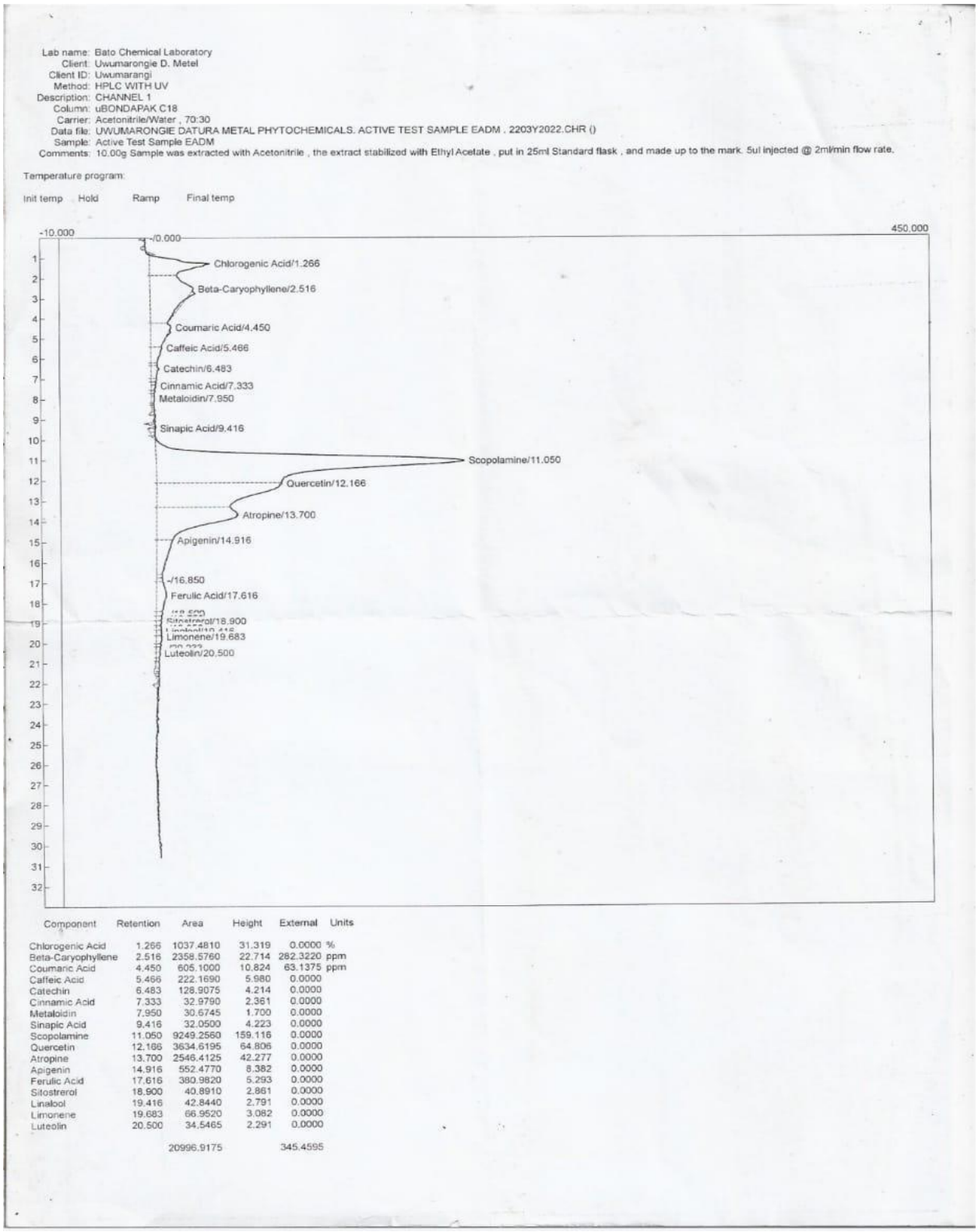
As an antidote for cholinergic medication overdose or mushroom poisoning, Atropine is a prescription pharmaceutical used to treat the symptoms of decreased heart rate (bradycardia). Atropine is also used to suppress salivation and bronchial secretions before surgery (Stephenson, 1969). Atropine can be used on its own or in combination with other drugs. Anticholinergic, Antispasmodic Agents are the class of medications that include Atropine (Sidell *et al.,* 1969). Scopolamine is a centrally acting competitive inhibitor of the muscarinic cholinergic receptor site. It is nonselective among muscarinic subtypes and exerts minimal effects on nicotinic receptors. Effects occur 15 to 30 minutes after oral, intramuscular, or

subcutaneous administration, and its elimination half-life is approximately 8 hours. Scopolamine is able to penetrate the brain more readily than other cholinergic antagonists because it has a large unionized presence, which it owes to its epoxide group and weaker base strength (ecker *et al.,* 2009). The theoretical framework for scopolamine's antidepressant mechanism of action was first outlined by Janowsky *et al*., (1972), when they postulated the cholinergicadrenergic hypothesis of mania and depression.

Table 3.7: Constituents of D. metel and their retention times

Component	Retention	Area	Height
Chlorogenic Acid	1.27	1037.48	31.32
	2.52	2358.58	22.71
Beta-Caryophyllene			
Coumaric Acid	4.45	605.10	10.82
Caffeic Acid	5.47	222.17	5.98
Catechin	6.48	128.90	4.21
Cinnamic Acid	7.33	32.98	2.36
Metaloidin	7.95	30.67	1.70
Sinapic Acid	9.42	32.05	4.22
Scopolamine	**11.05**	**9249.26**	**159.12**
Quercetin	**12.17**	**3634.62**	**64.81**
Atropine	**13.70**	**2546.41**	**42.28**
Apigenin	14.92	552.48	8.38
Ferulic Acid	17.62	380.98	5.29
Sitosterol	18.90	40.89	2.86

Linalool	19.42	42.84	2.79
Limonene	19.68	66.95	3.08
Luteolin	20.50	34.54	2.29



Component	Retention	Area	Height	External	Units
Chlorogenic Acid	1.266	1037.4810	31.319	0.0000	%
Beta-Caryophyllene	2.516	2358.5760	22.714	282.3220	ppm
Coumaric Acid	4.450	605.1000	10.824	63.1375	ppm
Caffeic Acid	5.466	222.1690	5.980	0.0000	
Catechin	6.483	128.9075	4.214	0.0000	
Cinnamic Acid	7.333	32.9790	2.361	0.0000	
Metaloidin	7.950	30.6745	1.700	0.0000	
Sinapic Acid	9.416	32.0500	4.223	0.0000	
Scopolamine	11.050	9249.2560	159.116	0.0000	
Quercetin	12.166	3634.6195	64.806	0.0000	
Atropine	13.700	2546.4125	42.277	0.0000	
Apigenin	14.916	552.4770	8.382	0.0000	
Ferulic Acid	17.616	380.9820	5.293	0.0000	
Sitostrerol	18.900	40.8910	2.861	0.0000	
Linalool	19.416	42.8440	2.791	0.0000	
Limonene	19.683	66.9520	3.082	0.0000	
Luteolin	20.500	34.5465	2.291	0.0000	
		20996.9175		345.4595	

Plate 3.1 : HPLC profile of Datura metel plant.

CONCLUSION

The ethylacetate extract of D. metel leaves showed the presence of various secondary metabolites in the plant which is an indication of its usefulness in phytotherapy when consumed or in the development of novel drugs. Also, the HPLC profile of the plant has seen the presence of useful compounds such as scopolamine, quercetin, and atropine. These compounds are known to be effective in treating several medical ailments such as anxiety, inflammation, and many more. Therefore, Datura metel exhibits numerous pharmacological effects.

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