ISOLATION OF PHYTOCONSTITUENTS FROM ETHYL ACETATE EXTRACT OF *lannea acida*

BY

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18/56EE172

BEING A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, FACULTY OF PHYSICAL SCIENCES, UNIVERSITY OF ILORIN, ILORIN NIGERIA.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.Hons) DEGREE IN CHEMISTRY

SEPTEMBER, 2023.

CERTIFICATION

This is to certify that this project has been read and approved as meeting the requirement of the department of chemistry, physical sciences, University of Ilorin, in partial fulfilment in the award of Bachelor of Science (B Sc.Hons) Degree in Chemistry.

..... **.....**
Dr. A.A. Hamid. **Date**
Project supervisor

..... **.....**
Prof. Modinah A. O. Abdul Raheem. **Date**
Head of Department

..... **.....**
External Examiner. **Date**

DECLARATION

This is to certify that the project work titled " isolation of phytoconstituents from ethyl acetate extract of *lannea acida*" is an original work carried out by OLANIRE AYOTOMIWA OLUWASEUN with matric number 18/56EE172 under my supervision.

..... **.....**

OLANIRE, Ayotomiwa Oluwaseun **Date**
18/56EE172

DEDICATION

This project is dedicated to GOD, the one who loves me with no restraints and to my immediate family, my mom, my brother and my sister. I did it for you.

ACKNOWLEDGEMENT

My intense gratitude goes to GOD for helping me and taking care of me. I'm super grateful that he kept me throughout my program and helped me to successfully complete the program despite so many hurdles and health complications. .I'm grateful for the gift of the Holy Ghost, a guide, my best person, and an ever present help at all times. I will forever be grateful to him.

My heartfelt gratitude goes to my incomparable supervisor, Dr A.A.Hamid for his guidance, advice, parental role and great support towards the completion of my project work I sincerely have never met any lecturer as awesome as he is throughout my undergraduate program.

My sincere gratitude goes to the H.O.D, Prof Modinah A. O. Abdul Raheem, and also my level adviser Mrs Arowona T. Baker and to all the teaching and non-teaching staff of chemistry department. I'm forever indebted to you all.

I want to say a special THANK YOU to my mom, for being my rock and support, for being a great mother and also for standing as the Dad who never saw what my university days were like. You are enough mom, no one else compares to you. I'm also super grateful to my immediate siblings, Akanb iomo ose paro and Omoeniosedibebere. I can't forget Olayinka, Ibrahim, and Precious my baby. Thank you for being my family.

I can never forget my super aunties, Mommy Precious and Mommy Ibrahim. Your love is unrivalled, thank you for letting me know what a true family feels like.

I'm also very grateful to everyone I met at RCCG Prince of Life Parish. My pastors and ministers, thank you so much for every support and care I've received from you. I also say a very big thank you to my family and Pastor at Halleluyah Church of God Mission. Thank you so much!

My acknowledgement will be incomplete without saying a huge thank you to my very dear friend Opeyemi who has been my best friend and roommate since hundred level. To all my friends, Victoria, MommyA, Joshua, Jeremiah, Olumide, Calmenon, Precious. Thank you Ade, thank you Torious, thanks for being super amazing guys.. Thank you so much for everything I can not possibly put into words. I'm also grateful to Mommy Fola and Dr Solomon for giving me a family and a place in Ilorin throughout my stay. To my loves Folakemi, Korede and Rhoda, you're amazing. Thank you.

I know it's impossible to bring back the dead, but I'm grateful for the fatherly care and love I received from my late Father Mr. Kolawole Olanire. I am making you so proud dad, and this is just the beginning.

ABSTRACT

AIM:

To isolate and study the phytochemical and biologically active compounds from the ethyl acetate extract of *Lannea acida* plant.

METHODOLOGY:

Successive extraction was employed during the course of the work. The grounded (aerial parts) was soaked in an aspirator bottle for five days using ethyl acetate to extract the polar components from the plant sample.

After five days, the ethyl acetate extract was obtained via decantation and subsequent filtration with cotton wool as a filter bed. The crude extract was exposed to air and also microwaved to remove the solvent.

These crude extract was then made into a slurry with silica gel and was packed into a column for a column chromatographic analysis. Fractions of isolates were obtained and spotted on TLC plates using capillary tubes. The TLC plates were put into a beaker containing ethyl acetate and hexane in the ratio by which the isolates were obtained respectively. The TLC plates were taken out after a while and put under UV light to identify the pure compounds in the fractions.

The isolates containing pure compounds were left to dry and packed into sample bottles to be preserved for NMR spectroscopy for further elucidiation.

The peliminary phytochemical investigation of the ethyl acetate extract of Lannea acida revealed the presence of glycoside, flavonoids, saponins, terpenoids, tanins while steroid and alkaloid were found to be absent.

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CHAPTER ONE

1.0 Medicinal plants

The use of medicinal plants dates back thousands of years, with documented evidence from ancient civilizations such as the Egyptians, Greeks, and Chinese. Traditional healers and indigenous communities worldwide have long relied on botanical knowledge passed down through generations to treat various ailments. These practices reflect the deep connection between humans and their natural environment, where plants served as primary sources of remedies. For example, the ancient Egyptians utilized aloe vera for skin conditions, while traditional Chinese medicine incorporated ginseng for vitality and longevity (Dhar et al., 2006; Tilburt & Kaptchuk, 2008).

Medicinal plants owe their healing properties to a diverse array of bioactive compounds, including alkaloids, flavonoids, terpenes, and polyphenols. These compounds often exhibit antimicrobial, anti-inflammatory, antioxidant, analgesic, and immunomodulatory activities, among others. For instance, the alkaloid morphine from the opium poppy (Papaver somniferum) remains a potent analgesic, while quercetin, a flavonoid found in various plants, exhibits antioxidant and anti-inflammatory effects. These bioactive molecules have become invaluable resources for drug discovery and the development of pharmaceuticals and nutraceuticals (Gurib-Fakim, 2006; Newman & Cragg, 2007).

Medicinal plants continue to play a pivotal role in modern healthcare, with numerous pharmaceutical drugs derived from plant sources. Examples include aspirin from willow bark (Salix spp.), and artemisinin from sweet wormwood (Artemisia annua) for malaria treatment. However, the sustainable utilization of medicinal plants faces challenges such as habitat destruction, overharvesting, and biodiversity loss. Conservation efforts, sustainable harvesting

practices, and cultivation initiatives are essential to ensure the availability of these valuable resources for future generations (Ghimire et al., 2019; World Health Organization, 2013).

Medicinal plants represent an enduring testament to the intricate relationship between humans and the natural world. Their historical and cultural significance, along with their rich reservoir of bioactive compounds, continues to drive research, innovation, and the development of therapeutic agents. As we navigate an era of increasing interest in traditional and alternative medicine, the preservation and sustainable management of medicinal plant resources are imperative to harness their full potential for human health and well-being.

1.1 Introduction to Lannea Acida as a medicinal plant

The name of the genus, “Lannea,” is based on a Latin word “lana” which translates to “wool” in reference to young plant parts which are densely hairy or possibly to the wool on the roots of some Lanneaspecies. The synonyms of L. acida are Lannea buettneri Engl.Lannea djalonica A. Chev., Lannea glaucescens Engl, Lannea lagdoensis(Engl. and K. Krause) Mildbr., Lannea oleosa A. Chev., Odinaacida(A. Chev.) Walp., and Sorindeia lagdoensis Engl. and K. Krause.The genus Lannea consists of approximately 40 species which are usually trees, shrubs, or suffrutices, occupying different habitats in Sub-Saharan Africa, with only one species recorded in Tropical Asia and several species introduced throughout the world.

Lannea acida, referred to as "African Grape" in colloquial terms, is a botanical species indigenous to various African nations, encompassing Nigeria, Sudan, Senegal, Mali, and Ghana. The tree in question is classified under the Anacardiaceae family and is characterized by its deciduous nature, complex leaves, and small, greenish-white flowers. These blooms eventually transform into berry-like fruits that possess a distinctly sour taste (Adeniji et al., 2007). The utilization of this plant by indigenous tribes for a multitude of purposes, including traditional

medicine, dietary requirements, and cultural customs, has persisted for numerous centuries. The growing interest of researchers and scientists in this subject is attributed to its possible therapeutic capabilities and nutritional worth.

It is known as “Faru” among the Hausa’s in Nigeria. It is a valuable multi-purpose tree widely used by local people. The bark is used in the treatment of stomach troubles, beriberi, schistosomiasis and haemorrhoids. The leaves and bark have been reported to be useful in the treatment and management of gout, rheumatism, wounds, swelling and burns.

Lannea acida possesses a noteworthy historical background pertaining to its extensive utilization throughout various African societies. Indigenous communities have employed various components of botanical organisms to address their therapeutic requirements. Lannea acida is a plant species that is utilized for medicinal purposes, including in the treatment of a range of maladies such as malaria, diarrhea, dysentery, and respiratory disorders. These therapeutic applications involve the utilization of several parts of the plant, including the roots, leaves, fruits, and bark (Irvine, 1961). The aforementioned customary applications underscore the plant's importance as a fundamental healthcare asset for indigenous communities.

Lannea acida has been extensively utilized for its therapeutic properties among diverse African societies, spanning a significant period of time. Various indigenous tribes have employed different components of plants in order to effectively tackle a diverse array of health concerns. Lannea acida has been utilized in traditional medicine for the treatment of several maladies such as malaria, diarrhea, dysentery, respiratory disorders, and other conditions (Irvine, 1961). The aforementioned traditional knowledge serves as a testament to the plant's efficacy in addressing prominent health issues within these communities.

A wide range of phytoconstituents inside Lannea acida has been revealed through scientific investigations. The bioactive compounds encompass a variety of chemical constituents, such as alkaloids, flavonoids, tannins, terpenoids, and triterpenoids, as reported by Adomako-Bonsu et al. (2019) and Ogunlesi et al. (2008).

The antioxidant and pharmacological capabilities of Lannea acida are very remarkable. The presence of antioxidants in plants has been observed to potentially contribute to the safeguarding of cells from oxidative damage induced by free radicals. According to Olorunnisola et al. (2011), this implies possible advantages in the prevention or reduction of diseases associated with oxidative stress.

The Effects of Anti-Inflammatory Agents: Inflammation is a prevalent characteristic observed in numerous chronic disorders. The anti-inflammatory capabilities exhibited by Lannea acida render it a promising choice for the management of disorders characterized by excessive inflammation.

The antibacterial activity exhibited by the plant indicates its potential efficacy in addressing a range of infectious disorders. This phenomenon holds particular significance in areas characterized by restricted availability of contemporary medical services, where traditional treatments frequently assume a pivotal function (Adomako-Bonsu et al., 2019).

The Potential of Anti-Diabetic Agents: With the escalating global incidence of diabetes, there is growing interest in exploring the potential of Lannea acida as a novel therapeutic agent for diabetes treatment. The aforementioned characteristics render the plant a topic of significance in the pursuit of efficacious remedies (Adomako-Bonsu et al., 2019).

In addition to its immediate medical utility, the cultural value of Lannea acida is of paramount importance. The use of the plant into rituals and rites is a common practice, serving as a manifestation of its profound association with traditional belief systems. The preservation of cultural heritage and the reinforcement of indigenous community identity are significantly influenced by its role.

The traditional knowledge pertaining to Lannea acida encompasses several aspects such as its development patterns, ecological relationships, and ways for preparing therapeutic medicines. This body of knowledge holds significant cultural value. The documentation and preservation of this information are crucial for the long-term sustainability of traditional healthcare practices as well as the protection of biodiversity.

The presence of alkaloids in Lannea acida may potentially contribute to its therapeutic qualities. Alkaloids are renowned for their pharmacological properties, and their occurrence within the plant may elucidate certain traditional applications. The plant is a great example of medicinal plants.

1.2 Important constituents of medicinal plants

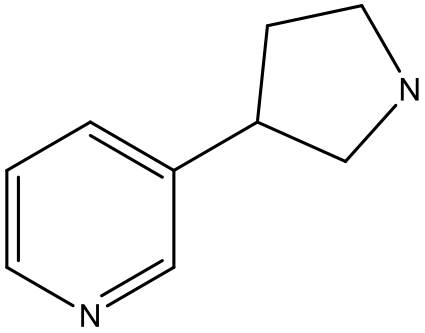
1.2.2 Akaloids: Nature's Nitrogenous Wonders

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. They are mostly naturally occurring in a wide range of plants and other organisms (secondary metabolites) and they have often pharmacological effects. This group also includes some related compounds with neutral and even weakly acidic properties. Some synthetic compounds of similar structure are also attributed to alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and more rarely other elements

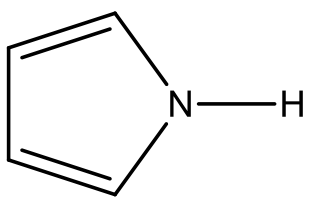
such as chlorine, bromine, and phosphorus. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine, the psychedelic psilocin, the stimulant caffeine, nicotine, the analgesic morphine, the antibacterial berberine, the anticancer compound vincristine, the antihypertension agent reserpine, the cholinomimeric galantamine, the spasmolysis agent atropine, the vasodilator vincamine, the anti-arrhythmia compound quinidine, the anti-asthma therapeutic ephedrine, and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste.

The boundary between alkaloids and other nitrogen-containing natural compounds is not clear-cut. Compounds like amino acid peptides, proteins, nucleotides, nucleic acid, amines, and antibiotics are usually not called alkaloids. Natural compounds containing nitrogen in exocyclic position (mescaline, serotonin, dopamine, etc.) are usually attributed to amines rather than alkaloids.

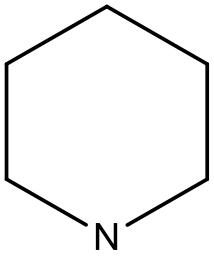
Some basic alkaloids are shown below



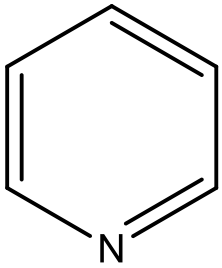
NICOTINE



PYROL



PIPERIDINE



PYRIDINE

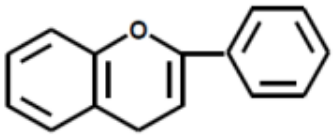
Some biologically relevant plant-derived alkaloids

Classes	Name	Biological properties	Plant family
Truealkaloid	Atropine	Anticholinergenic drug	Solanaceac
	Nicotine	Potent poison that at low doses is stimulating	Solanaceac
	Morphene	Narcotic and anesthetic properties	Papaveraceac
Protoalkaloid	Mescaline	Hallicinogen	Cactaceac
	Hordenine	Stimulant of the central nervous system	Cactaceac
	Ephedrine	Sympathetic nervous system stimulant	Ephedraceac
Pseudoalkaloid	Aconitine	Highly poisonous	Ranunculaceac
	Theobromine	Stimulating the central nervous system	Malvaceac
	Conine	Highly poisonous	Apiaceac Sarraeeniacac

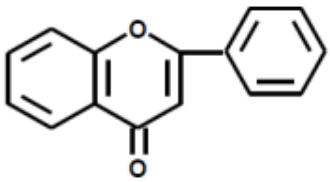
1.2.3 FLAVONOIDS

Flavonoids are the most abundant polyphenols in human diet, representing about 2/3 of all those ones ingested. Like other phytochemicals, they are the products of secondary metabolism of plants and, currently, it is not possible to determine precisely their number, even if over 4000 have been identified. In fruits and vegetables, they are usually found in the form of glycosides and sometimes as acylglycosides, while acylated, methylated and sulfate molecules are less frequent and in lower concentrations.

They are water-soluble and accumulate in cell vacuoles. Flavonoidal compounds are considered as the largest group of naturally occurring phenols. Flavonoids also constitute the majority of the yellow-colored plant pigments. hey comprise a structurally uniform group, as they are all derived from the same parent nucleus, viz., 2-phenyl-benzopyran (Flavan); thus, they have a basic C15 skeleton.



Flavan
(2-phenylbenzopyran)

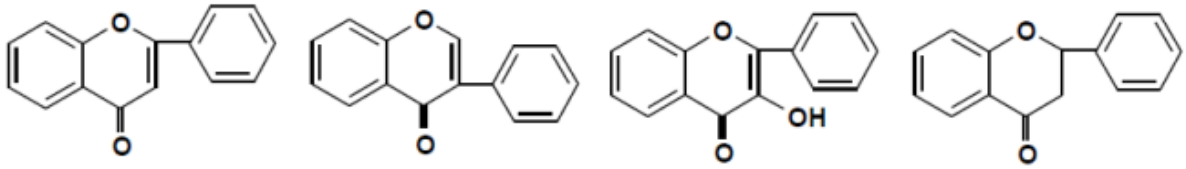


Flavone
(2-phenylbenzopyrone)

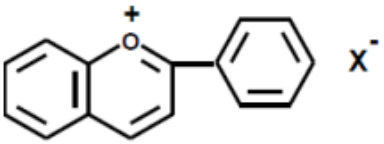
Many hundreds flavonoidal compounds are known nowadays. These are either in a glycosidic form or as free genins. The majority of flavonoids have free hydroxyl groups (phenolic groups), and these are usually at the 3,4,7,3`,4` and 5` positions of the flavone ring system.

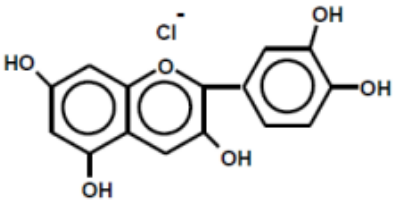
Flavonoidal compounds are classified according to the oxidation level, and/or the oxygenation of the central pyran ring, into: flavans, flavones, isoflavones, flavonols, flavanones,

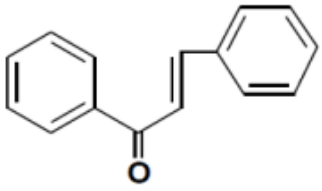
anthocyanidins, chalcones and aurones: Flavones are 2-phenyl chromones (2-phenyl benzo-pyrone). Isoflavones are 3-phenyl chromone derivatives. Flavonols are 3-hydroxyflavones. Flavanones are 2,3-dihydro-derivatives of flavones (i.e., the 2,3-double bond is lacking).

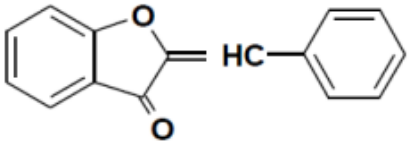


Anthocyanidins, chalcones and aurones, although included within flavonoids, they lack the typical parent flavone structure. Anthocyanidins and their glycosides (the anthocyanins) are ionic oxonium salts. This subclass is responsible for the permanent blue, purple, violet, mauve, scarlet and red colors of flowers, fruits and leaves of higher plants. Being ionic, anthocyanins and anthocyanidins are all soluble in polar solvents and insoluble and non-extractable by water immiscible, non-polar organic solvents. The color of anthocyanins and anthocyanidins is pH-dependant. An example of anthocyanidins is the widely distributed compound, cyanidin.

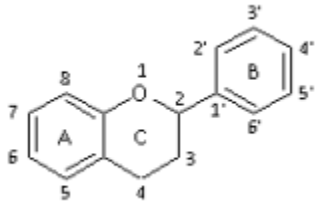

Anthocyanidin


Cyanidin chloride


Chalcone


Aurone

Chalcones, have no central α-pyrone ring, so they are not true flavonoidal compounds. The parent compound chalcone, is chemically phenyl- styryl -ketone, or benzylidene-acetophenone. Aurones on the other hand are oxidized forms that are obtained by aerial or enzymatic oxidation. Instead of the central α-pyrone ring of the normal flavonoidal structure, aurones have a five membered ring. Except for anthocyanidins and anthocyanins, almost all flavonoids are yellow in color. However, aurones, chalcones and flavonols are usually of deeper yellow colors. Chemical structure of flavonoids Chemically, flavonoids have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C). Their basic structure is a skeleton of diphenylpropane, namely, two benzene rings (ring A and B) linked by a three carbon chain that forms a closed pyran ring with benzenic A ring. Therefore, their structure is also referred to as C6-C3-C6.



Skeleton of Diphenylpropane

1.2.4 SAPONINS

Saponins are a diverse group of natural compounds found in various plants. They are characterized by their amphiphilic nature, possessing both hydrophilic and hydrophobic properties. Saponins derive their name from their ability to produce a soapy lather when mixed with water, owing to their surfactant properties. Saponins are widely distributed in the plant kingdom and can be found in various plant parts, including roots, stems, leaves, and seeds. They play important roles in plants, serving as defense compounds against pathogens, pests, and herbivores. Saponins are also involved in plant adaptation and have been found to have valuable medicinal properties, making them useful in pharmaceutical, agrochemical, flavor, and aroma industries. The chemical structure of saponins consists of a hydrophilic sugar moiety (glycone) attached to a hydrophobic aglycone or sapogenin. The glycone portion is typically composed of one or more sugar units, such as glucose, galactose, or xylose. The aglycone portion can vary widely and includes steroidal, triterpenoid, or other types of structures (Upadhyay et al., 2018).

Saponins exhibit a range of biological activities, including antimicrobial, antifungal, antiviral, anti-inflammatory, antioxidant, and anticancer properties. These activities make saponins valuable in traditional medicine and have led to their investigation for potential therapeutic applications (Upadhyay et al., 2018). The extraction of saponins from plant materials can be achieved using various techniques. Traditional methods, such as maceration, Soxhlet extraction, and reflux extraction, have been employed for saponin extraction. These methods involve the use of solvents, such as water, ethanol, or methanol, to dissolve and extract the saponins from the plant material (Mohaddes-Kamranshahi et al., 2019).

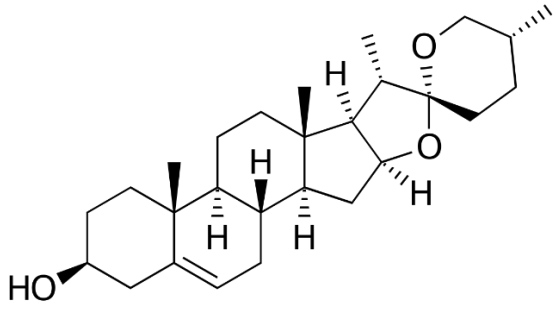
Modern extraction techniques, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE), have also been applied to enhance the

efficiency and yield of saponin extraction. These techniques utilize physical forces, such as ultrasound waves or microwave energy, to disrupt the plant cell walls and facilitate the release of saponins (Mohaddes-Kamranshahi et al., 2019; Deng et al., 2019).

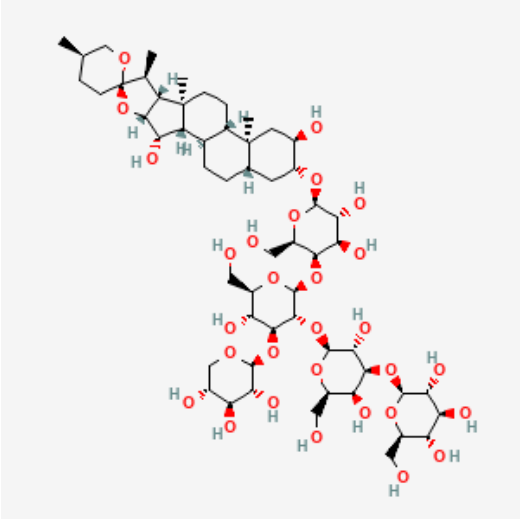
Analytical techniques, such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS), are commonly employed for the identification, quantification, and structural characterization of saponins. These techniques allow for the separation and analysis of individual saponin compounds present in complex mixtures (Savarino et al., 2021; Jin et al., 2018).

Saponins possess a wide range of biological activities and have been used in traditional medicine for centuries. The extraction and analysis of saponins from plant materials involve a combination of traditional and modern techniques, enabling the isolation and characterization of these valuable compounds. Further research on saponins continues to explore their potential applications in various fields, including medicine, agriculture, and industry.

Some common saponins



Diogensin



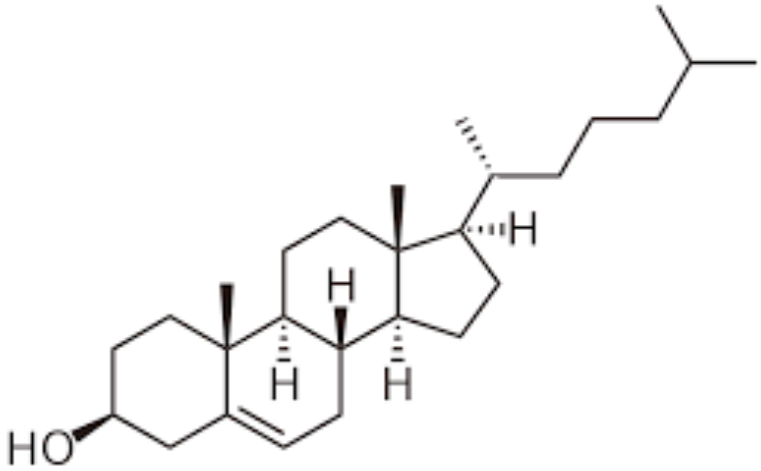
Digitonin

1.2.6 STEROIDS

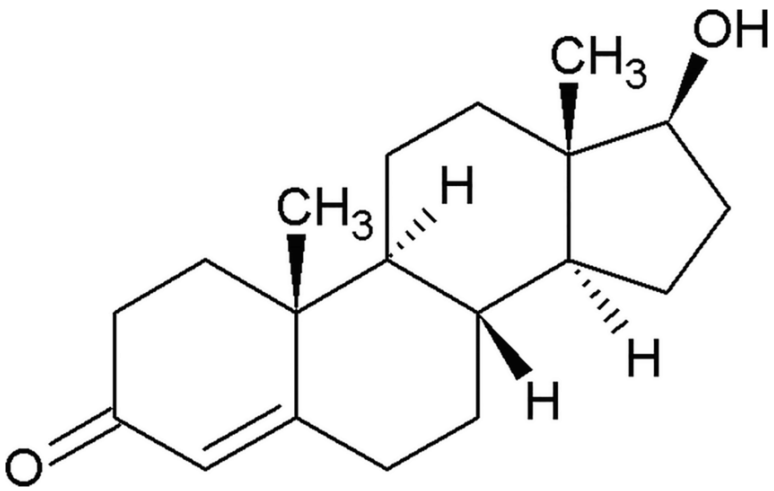
Steroids are a class of organic compounds that have a characteristic structure consisting of four fused rings. They are found in both plants and animals and play essential roles in various biological processes. Steroids are classified into different types based on their structure and function, including corticosteroids, sex steroids, anabolic steroids, and steroidal saponins. Corticosteroids are a group of steroid hormones produced by the adrenal glands. They are involved in regulating immune responses, inflammation, metabolism, and stress responses. Synthetic corticosteroids, such as prednisone and dexamethasone, are commonly used in medicine for their anti-inflammatory and immunosuppressive properties (Park et al., 2017). Sex steroids, including estrogen, progesterone, and testosterone, are primarily involved in the development and regulation of sexual characteristics and reproductive functions. They play crucial roles in the growth and maintenance of reproductive tissues, bone health, and the regulation of secondary sexual characteristics (Park et al., 2017). Anabolic steroids are synthetic derivatives of testosterone that are used to promote muscle growth and enhance athletic performance. They are commonly abused by athletes and bodybuilders, leading to potential health risks and adverse effects on the cardiovascular system, liver, and endocrine system (Griffin et al., 2021). Steroidal saponins are a type of steroid compound found in various plants. They are characterized by their amphiphilic nature, possessing both hydrophilic and hydrophobic properties. Steroidal saponins have been studied for their potential pharmacological activities, including anti-inflammatory, antimicrobial, antitumor, and hepatoprotective effects (Sharma et al., 2021; Tian et al., 2020). The extraction and analysis of steroids from plant and animal sources involve various techniques, including liquid chromatography, mass spectrometry, and spectroscopic methods. These techniques allow for the identification, quantification, and

structural characterization of different steroid compounds (Boggs et al., 2017; Tallo-Parra et al., 2017; Qin et al., 2012).

Some common steroids



Cholesterol



Testosterone

1.2.7 PROSTAGLANDINS

Prostaglandins are a group of lipid compounds derived from polyunsaturated fatty acids, such as arachidonic acid . They play crucial roles in various physiological processes and are involved in inflammation, pain, fever, blood clotting, and reproductive functions .

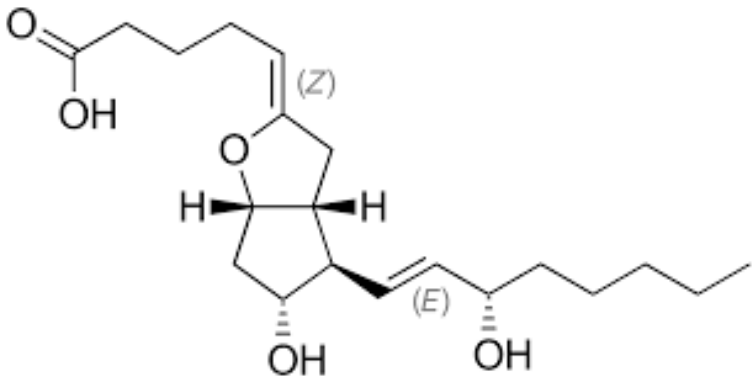
The biosynthesis of prostaglandins involves a series of enzymatic reactions. The key enzyme involved in prostaglandin synthesis is cyclooxygenase (COX), also known as prostaglandin-endoperoxide synthase. COX catalyzes the conversion of arachidonic acid into prostaglandin G2 (PGG2) and prostaglandin H2 (PGH2), which are then converted into specific prostaglandins by various tissue-specific enzymes . Prostaglandins exert their effects by binding to specific cell surface receptors, known as prostaglandin receptors. There are multiple types of prostaglandin receptors, each with distinct functions and tissue distributions.

Upon binding to their receptors, prostaglandins initiate intracellular signaling pathways that regulate various physiological processes . The role of prostaglandins in inflammation is well-established. They are involved in the initiation and maintenance of the inflammatory response by promoting vasodilation, increasing vascular permeability, and recruiting immune cells to the site of inflammation. Prostaglandins also contribute to the development of pain and fever by sensitizing pain receptors and affecting the hypothalamic thermoregulatory center .

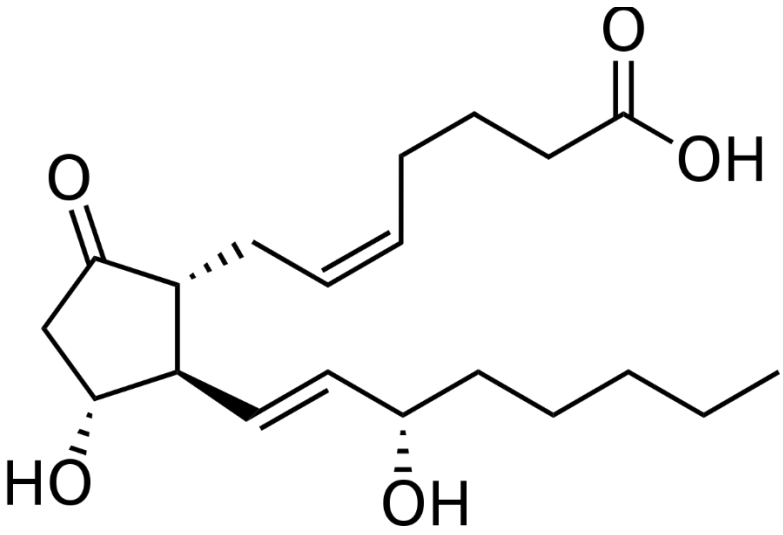
In addition to their role in inflammation, prostaglandins play important roles in reproductive functions. They are involved in the regulation of ovulation, uterine contraction during labor, and maintenance of pregnancy Dong et al. (2019). Prostaglandins are also implicated in the regulation of blood clotting and platelet aggregation . The pharmacological modulation of prostaglandin synthesis and activity has been a target for the development of therapeutic interventions. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen,

inhibit the activity of COX enzymes, thereby reducing prostaglandin synthesis and providing relief from pain and inflammation. Selective COX-2 inhibitors have been developed to specifically target the COX-2 enzyme, which is primarily involved in inflammation .

Prostaglandins play diverse roles in various physiological processes. They are involved in inflammation, pain, fever, blood clotting, and reproductive functions. Understanding the biosynthesis, signaling, and functions of prostaglandins has important implications for the development of therapeutic interventions targeting these pathways.



Prostacyclin



prostaglandinE2

1.3 ETHYL ACETATE AS A PROMISING SOLVENT.

The choice of ethyl acetate as the extraction solvent for the isolation of phytoconstituents from Lannea acida is a crucial element in this study. This decision is supported by numerous fundamental factors some of which are:

The effectiveness of a solvent: Ethyl acetate, which is a polar aprotic solvent, has gained significant attention in the field of phytochemical research due to its proven ability to extract a wide range of phytoconstituents. The mild polarity of the substance is very beneficial in the process of capturing a wide range of bioactive plant chemicals, including as phenolics, terpenoids, and flavonoids.

Safety and Environmental Considerations: Ethyl acetate is widely recognized for its comparatively low toxicity and the implementation of safe handling protocols. This is consistent with established laboratory safety protocols. In addition, the environmental ramifications of its usage are commonly seen as less significant when compared to other organic solvents that are routinely employed in phytochemical extractions.

The selection of ethyl acetate is influenced significantly by its high extraction efficiency. The utilization of this method enables the efficient dissolution and extraction of phytoconstituents from plant matrices, hence ensuring a thorough representation of the bioactive chemicals that are contained in Lannea acida.

Analytical compatibility is a critical factor to consider when assessing the compatibility of substances extracted using ethyl acetate with subsequent analytical procedures. Numerous phytochemical substances that have been extracted by the method of ethyl acetate extraction

exhibit favorable characteristics for comprehensive examination through spectroscopic and chromatographic techniques, hence enabling accurate identification of these compounds.

1.4 Research Objectives

1. To isolate and characterize phytoconstituents from the ethyl acetate extract of Lannea acida.

2. To evaluate the potential biological activities of isolated phytoconstituents.

1.5 Significance of the Study

1. Contribution to the understanding of Lannea acida as a medicinal plant.

2. Discovery of potential therapeutic agents.

CHAPTER TWO

2.0 Literature review

2.1 Lannea acida leaves

2.2 Buds of Lannea Acida plant

2.3 Scientific classification

Kingdom:Plantae

Clade:Tracheophytes

Clade:Angiosperms

Clade:Eudicots

Clade:Rosids

Order:Sapindales

Family:Anacardiaceae

Genus:Lannea

Species:L. acida

2.4 Description of Lannea Acida

Lannea acida, also known as Kikié, is a medicinal plant that belongs to the Anacardiaceae family. It is widely used in traditional medicine in West Africa for various purposes (Maroyi, 2018). The bark of Lannea acida has been traditionally used to treat infertility, gynecological complaints, and rheumatism (Oumarou et al., 2017). The plant has gained attention in scientific research due to its potential medicinal properties. The species in question is mostly found within the Guinea and Sudan savannas of West and Central Africa. The tree in question is of diminutive stature and possesses the potential to reach a height of 12 meters. Its bark exhibits a scaly texture and is characterized by a brown-black hue. Moreover, when the bark is incised, it reveals a fibrous

inside that displays shades of red and yellow. The leaves of this plant exhibit an alternating arrangement and are imparipinnate in nature. When young, the leaves possess a red rachis. The leaflets are devoid of hair (glabrous) and the leaf-blade is narrowly oval, measuring 4-12 cm in length and 1.5-5 cm in width. The leaf-blade is characterized by an apex that is either acute or acuminate, and a base that is cuneate in shape. The fruit of this plant is classified as an ellipsoid drupe, exhibiting a yellowish to red-hue when it reaches its ripe state. The process of fruiting often commences towards the conclusion of the dry season or the commencement of the rainy season, spanning a duration of several months. It is noteworthy that the plant tends to be devoid of leaves during the fruiting phase. The tree is characterized by a height of up to 10 meters and a trunk circumference ranging from 2 to 3 meters. It is commonly found in savanna ecosystems, especially in rocky environments, and thrives in regions with an average annual precipitation of 635mm. This tree species is distributed throughout a wide geographical range spanning from Senegal to Nigeria, as documented by Michel et al. in 2004.

The presence of thick, fissured bark contributes to the tree's ability to withstand and mitigate the impact of bushfires. In the context of Ivory Coast Upper Volta, it is customary to refrain from cutting down the bush during the process of land clearance for agricultural purposes. The tree in question is widely recognized for its utility and holds a significant place in the pharmacopoeia of Senegal. The wood exhibits a pale hue and possesses a relatively low density. The task of crafting little stools, boards, and other utensils is widely acknowledged as challenging. The material in question has a high degree of flexibility and is commonly employed in the construction of bows. The bark has fibrous characteristics, rendering it suitable for the production of cordage; nonetheless, the resulting quality is generally subpar. Additionally, it produces an edible gum. In Senegal, the internal usage of bark is employed for the treatment of

beriberi, schistosomiasis, and haemorrhoids, while its external application is utilized for eye-related ailments. Additionally, in combination with other medicinal plants, the bark is used to address dysentery and sterility. In Northern Nigeria, individuals use a bark-infusion as a remedy for gastrointestinal ailments. In the Casamance region of Senegal, individuals engage in the practice of inhaling vapour derived from a decoction of bark as a remedy for tooth caries and buccal infections. The utilization of root-bark is regarded as beneficial for the treatment of skin infections. In the region of Ivory Coast-Upper Volta, the application of this substance is observed in bathing practices and the formulation of lotions for the treatment of skin conditions such as blotches and herpes, among others. An analogous internal preparation is administered following a fermentation period of 4-5 hours for the treatment of gonorrhoea. In the region of Casamance, a traditional remedy for curing orchitis involves the preparation of a tampon by combining powdered root with salt, which is then applied to the scrotum. In Upper Volta, the Moore people administer a root-bark decoction as an enema to children suffering from rickets. However, they acknowledge that this practice poses significant risks to infants.

The bark possesses a broad range of medical properties, making it a popular commodity in Dakar markets where it is marketed as a remedy specifically for women, aiding in facilitating childbirth and addressing issues of infertility when combined with other botanical substances. The utilization of powdered bark has been acknowledged as a therapeutic intervention for the management of danévéle, which is a symptomatic manifestation commonly associated with beriberi. In Angola, the utilization of the bark of L. anti scorbutica Engl. has been documented as a remedy for scorbutic ulcers affecting the oral cavity, as well as other manifestations associated with scurvy. In Ivory Coast, the sap derived from the process of crushing the bark is administered to those suffering from epilepsy, as well as those prone to experiencing dizziness and fainting

episodes. In West Africa, the consumption of young leaves is observed. The leaf study conducted on material sourced from Ivory Coast revealed the following composition: carbohydrates accounted for 67% of the total content, protein constituted 18%, minerals comprised 5%, and so on. According to Alfred et al. (2018), the cattle in Senegal engage in browsing activities on the vegetation.

A research study was conducted to examine the estrogenic properties of the ethanolic extract derived from the bark of Lannea acida. The study also aimed to evaluate the extract's potential in mitigating bone loss in a rat model of osteoporosis induced by ovariectomy. According to Oumarou et al. (2017), the research discovered that the extract demonstrated estrogenic activity and displayed promise in the prevention of bone loss. This finding implies that Lannea acida possesses promising attributes as a viable natural substitute in the realm of postmenopausal osteoporosis prevention and treatment.

An in vitro study was conducted to explore the uterotonic effects of Lannea acida, utilizing aqueous and methanolic extracts. The research conducted by Ngadjui et al. (2021) demonstrated that the extracts derived from Lannea acida displayed uterotonic properties, indicating its possible application in the facilitation of childbirth. This discovery provides empirical evidence that substantiates the historical utilization of Lannea acida bark for the resolution of challenging birthing situations.

Moreover, much research has been conducted on Lannea acida in order to investigate its antioxidant qualities. According to Tetsatsi et al. (2019), an investigation revealed that the aqueous extract derived from Lannea acida shown antioxidant properties and effectively restored the oxidant status in rats. These findings suggest that Lannea acida extract holds promise as an

agent with antioxidant capabilities. This observation implies that Lannea acida possesses potential preventive properties against situations associated with oxidative stress.

A comprehensive investigation was also conducted to assess the safety profile of methanol leaf extract derived from Lannea acida in Wistar albino rats, with a specific focus on toxicological consequences. According to the findings, it was determined that the methanol leaf extract derived from Lannea acida has a comparatively low level of toxicity, suggesting its potential suitability for incorporation into therapeutic preparations. This report presents significant data concerning the safety profile of Lannea acida. (Nwaogu et al. (2021).

Lannea acida has also been the subject of scientific investigation due to its antibacterial capabilities. The present work aimed to examine the antibacterial properties of essential oils rich in sesquiterpenes derived from Lannea egregia, a species closely related to Lannea acida. The research demonstrated that the essential oil displayed antibacterial properties hence indicting it's potential as a natural antimicrobial agent. (Ogundajo et al. (2021)

Historically, West Africa has employed this particular substance for a range of purposes, encompassing the treatment of infertility, gynecological ailments, and rheumatism. Scientific investigations have yielded empirical support for the traditional use of the subject under study, as well as delved into its phytochemical composition, pharmacological properties, and safety characteristics. Lannea acida exhibits potential in various domains, including estrogenic effects, mitigating bone loss, uterotonic effects, antioxidant capabilities, and antibacterial activities. Additional investigation is required in order to comprehensively comprehend the mechanisms of action and possible applications of Lannea acida within the context of contemporary medicine.

2.5 Importance of studying phytoconstituents in Lannea Acida

Phytoconstituents are the biologically active compounds present in plants that contribute to their medicinal properties. Studying the phytoconstituents in Lannea acida is of great importance for several reasons. Firstly, Lannea acida has been traditionally used in herbal medicine for various purposes, including treating infertility, gynecological complaints, and rheumatism (Ngadjui et al., 2020).

By studying the phytoconstituents of Lannea acida, we can identify the specific compounds responsible for its medicinal properties. This knowledge can help in the development of new drugs or therapeutic interventions based on the active compounds present in the plant. For example, one study found that the ethanol extract of Lannea acida bark exhibited estrogenic effects and prevented bone loss in an ovariectomized rat model of osteoporosis (Oumarou et al., 2017).

This suggests that the phytoconstituents in Lannea acida may have potential in the treatment of postmenopausal osteoporosis. By identifying and studying these phytoconstituents, we can gain a better understanding of their mechanisms of action and potential therapeutic applications.

Secondly, studying the phytoconstituents in Lannea acida can provide insights into its phytochemistry and pharmacological properties. A review article on Lannea acida highlighted the importance of studying its phytochemistry and pharmacological properties to evaluate its therapeutic potential (Maroyi, 2018).

By analyzing the phytoconstituents, we can determine the chemical composition of Lannea acida and identify the bioactive compounds responsible for its medicinal properties. Furthermore, studying the phytoconstituents can help in the quality control and standardization of herbal medicines derived from Lannea acida. By identifying and quantifying the phytoconstituents, we

can ensure the consistency and efficacy of herbal preparations made from Lannea acida. This is particularly important for the safe and effective use of herbal medicines in healthcare. In addition, studying the phytoconstituents in Lannea acida can contribute to our understanding of its mode of action and potential interactions with other drugs. For example, one study found that tannin-rich extracts from Lannea stuhlmannii and Lannea humilis exhibited hepatoprotective activities by enhancing the anti-apoptotic protein Bcl-2 (Sobeh et al., 2018).
Understanding the specific phytoconstituents responsible for these effects can provide valuable information for the development of new therapeutic strategies for liver diseases. Overall, studying the phytoconstituents in Lannea acida is crucial for understanding its medicinal properties, identifying potential therapeutic applications, ensuring the quality of herbal medicines, and exploring its interactions with other drugs. This research can contribute to the development of new drugs and therapeutic interventions based on the active compounds present in Lannea acida, ultimately benefiting human health.

2.6 Previous work done on Lannea Acida and some of its medicinal uses

Previous research on Lannea acida has explored its medicinal uses and pharmacological properties. One study by Oumarou et al. (2017) investigated the estrogenic effects and antiosteoporotic potential of Lannea acida bark ethanolic extract in an ovariectomized rat model of osteoporosis. The study found that the extract exhibited estrogenic activity and prevented bone loss in the rats. Another review by Maroyi (2018) critically examined the medicinal uses, phytochemistry, and pharmacological properties of Lannea acida.

The review highlighted the plant's wide use as herbal medicine in West Africa and discussed its potential therapeutic applications. Lannea microcarpa, a related species, has also been studied for its medicinal properties. Nitiéma et al. (2019) investigated the ethyl acetate fraction of Lannea

microcarpa trunk barks and its effects on angiotensin II-induced hypertension and endothelial dysfunction in mice. The study found that the fraction corrected hypertension and endothelial dysfunction in the mice. Additionally, Ouédraogo et al. (2021) conducted an overview of ethnopharmacological studies on Lannea microcarpa, focusing on its traditional use in Burkina Faso for the treatment of hypertension. The review provided information on the plant's ethnobotanical and ethnopharmacological studies, as well as its phytochemical makeup and effects on human health. Furthermore, Lannea acida has been investigated for its potential medicinal properties in various contexts. Tetsatsi et al. (2019) explored the alleviating effects of Lannea acida on the reproductive toxicity of an insecticide in rats. The study found that Lannea acida mitigated the reproductive toxicity induced by the insecticide. Solomon et al. (2018) evaluated the antiulcerogenic activity of Lannea acida stem bark extract in albino rats with ethanol-induced gastric mucosal injury. The study demonstrated the protective effects of the extract against gastric mucosal injury. In addition to its medicinal uses, Lannea acida has been studied for its anti-inflammatory and analgesic effects. Owusu & Ofori-Amoah (2017) investigated the aqueous extract of Lannea acida stem bark and its anti-inflammatory and analgesic effects. The study found that the extract exhibited significant anti-inflammatory and analgesic activities.

2.7 Aim and objectives of the study

To evaluate the phytoconstituents and biological activities of ethyl acetate extract of Lannea Acida through isolation.

2.8 Overview of Extraction of Medicinal Plants.

Extraction is a crucial step in obtaining bioactive compounds from medicinal plants for various applications, including pharmaceuticals, nutraceuticals, and herbal products. Different extraction methods and solvents can significantly impact the yield and composition of the extracted compounds. Traditional and modern techniques are employed in the extraction of medicinal plants.

2.8.2 Traditional methods of extraction.

Traditional methods of extraction of medicinal plants have been practiced for centuries and vary across different cultures and regions. These methods have been passed down through generations and are often based on empirical knowledge and observations of the local communities. While modern extraction techniques have gained popularity, traditional methods still hold value and are used in many parts of the world.

1. **Maceration**:

   Maceration is one of the simplest and most widely used traditional methods of extraction. It involves soaking the plant material in a solvent, such as water or alcohol, for an extended period. This allows the solvent to dissolve the bioactive compounds present in the plant material. Maceration is commonly used for extracting compounds that are soluble in water or alcohol Abubakar & Haque (2020).

The process of preparing plant material for analysis.

Prior to maceration, it is customary to cleanse, desiccate, and finely grind the botanical matter in order to enhance the available surface area for solvent infiltration. This stage is crucial in ensuring the extraction process is conducted with optimal efficiency.

The selection of a suitable solvent:

The selection of a solvent is contingent upon the specific phytoconstituents that are of interest. Water is frequently employed as a solvent for polar substances, whereas ethanol or methanol are favored for constituents with lower polarity. In certain instances, the utilization of a combination of solvents may be employed with the intention of targeting a more extensive array of chemicals.

The process of soaking and stirring:

The plant material, which has been ground into a coarse powder, is thereafter immersed in the chosen solvent within an appropriate vessel. In order to inhibit the evaporation of the solvent, it is common practice to seal the container. The extraction process may be enhanced with the application of gentle stirring or shaking.

Maceration is commonly characterized as a protracted and gradual procedure, involving extraction durations that span from several days to several weeks. The prolonged duration facilitates the progressive dispersion of phytoconstituents from the plant material into the solvent.

Filtration is a process that involves the separation of solid particles from a liquid or gas. Following the maceration stage, the concoction undergoes filtration in order to partition the

liquid extract from the solid plant matter. The process of filtration effectively eliminates particle debris from a substance, resulting in the production of a raw extract.

The crude extract acquired by the process of maceration is frequently subjected to concentration techniques in order to enhance the concentration of phytoconstituents. This objective can be accomplished using a range of techniques, including as evaporation conducted at lower pressure or lyophilization. (Luthria, D. L. (2008)

2. **Decoction**:

Decoction is a method of extraction that involves boiling the plant material in water for a specific period. This method is commonly used for extracting compounds from hard plant parts, such as roots, bark, or seeds. The boiling process helps to release the bioactive compounds into the water, resulting in a concentrated extract (Kachmar et al., 2021).

Decoction is a time-honored and fundamental extraction technique employed in herbal medicine and traditional healing practices. The preparation of a decoction typically involves several key steps: first, the plant material is cleaned and cut or crushed to enhance surface area and facilitate efficient extraction. Next, it is placed in a container with an appropriate amount of water, and the mixture is brought to a boil. Once boiling is achieved, the heat is reduced, and the mixture is allowed to simmer for a predetermined duration, often ranging from 15 minutes to several hours, depending on the plant material and its intended use. After this simmering period, the liquid is strained to separate it from the solid plant material, resulting in the decoction. The resulting liquid extract may be consumed as an herbal remedy, medicinal tea, or incorporated into various formulations for therapeutic purposes. Decoction is highly regarded for its ability to extract a

wide range of bioactive compounds, including water-soluble polysaccharides, alkaloids, phenolic compounds, and other phytochemicals (Dhiman et al., 2014).

3. **Infusion**:

Infusion is similar to decoction but is used for extracting compounds from more delicate plant parts, such as leaves or flowers. It involves pouring hot water over the plant material and allowing it to steep for a certain period. The hot water helps to extract the bioactive compounds without the need for boiling (Kachmar et al., 2021).

Infusion is a widely practiced extraction technique with applications in various domains, particularly herbal medicine and culinary arts. In this method, the plant material is placed in a container, and boiling water is poured over it. The hot water allows for the efficient dissolution of bioactive constituents, including essential oils, flavonoids, and other phytochemicals, from the plant material. The steeping duration can vary depending on the type of plant material and the desired strength of the infusion but typically ranges from a few minutes to several minutes. After steeping, the liquid is strained to separate it from the solid plant material, resulting in the infusion, which can be consumed as a beverage or used as a base for various culinary or medicinal preparations (Dhiman et al., 2013).

4. **Steam Distillation**:

Steam distillation is a traditional method used specifically for extracting essential oils from aromatic plants. It involves passing steam through the plant material, causing the volatile compounds to vaporize. The vapor is then condensed and collected, resulting in the extraction of essential oils (Jazani et al., 2018).

The process begins with the introduction of steam into a flask containing the plant material. The steam heats the plant material and volatilizes the target compounds, typically essential oils or aromatic components. As the steam carries these volatile compounds, it passes through a cooling system, often a condenser, where it is condensed back into a liquid state. The condensed liquid, known as the distillate, collects in a separate container. The immiscibility of water and essential oils allows for the separation of the two phases, with the essential oils floating on top of the water. This separation is facilitated by the use of a separatory funnel or a similar device. Steam distillation is preferred for compounds that are heat-sensitive and prone to decomposition, making it a valuable technique in the isolation of essential oils and other volatile constituents (Hussain et al., 2012).

5. **Cold Pressing**:

Cold pressing is a traditional method used for extracting oils from seeds or fruits. It involves mechanically pressing the plant material to release the oil. This method is commonly used for extracting oils from plants such as olives, coconut, or citrus fruits (Fonmboh et al., 2020).

This method involves pressing or crushing plant materials, such as seeds, nuts, or fruits, without the application of heat, which distinguishes it from traditional oil extraction methods. The process begins with the preparation of the raw material, which is cleaned and dehulled if necessary. Subsequently, the material is fed into a hydraulic press or expeller press, which exerts mechanical pressure to extract the oil or juice. The absence of heat in cold pressing helps preserve the natural flavors, colors, and nutritional properties of the product, making it particularly suitable for producing high-quality edible oils, like extra virgin olive oil or cold-pressed juices. Cold pressing has gained popularity due to its minimal processing and potential

health benefits associated with retaining the bioactive compounds in the final product (Siger et al., 2008).

6. **Fermentation**:

Fermentation is a traditional method used for extracting compounds from certain plant materials. It involves allowing the plant material to undergo a natural fermentation process, often with the help of microorganisms. This process can help break down complex compounds and enhance the extraction of bioactive compounds (Essoh et al., 2023).

The fermentation process is initiated by introducing specific microorganisms into a substrate, along with suitable conditions for their growth and metabolic activity, including temperature and pH control. These microorganisms consume the available sugars and release enzymes that catalyze the conversion of the sugars into desirable end-products, which can include organic acids, alcohol, and gases. Fermentation is a critical step in the production of a wide range of food and beverage products, such as bread, beer, yogurt, cheese, and pickles, and it contributes to the development of unique flavors, textures, and preservation characteristics. The selection of specific microorganisms and fermentation conditions can significantly impact the final product's quality and properties (Steinkraus, 1996).

7. **Grinding and Powdering**:

Grinding and powdering are traditional methods used to increase the surface area of the plant material, facilitating the extraction of bioactive compounds. The plant material is typically dried and then ground or powdered using traditional tools such as mortar and pestle (Senouci et al., 2019). It is important to note that traditional extraction methods may vary depending on the specific plant material and cultural practices.

Grinding typically involves using mechanical forces to break down solid materials into smaller fragments, which can range from coarse particles to fine powders. It is achieved through the application of mechanical energy, either by manual methods like mortar and pestle or through machinery such as grinders or mills. In contrast, powdering is the final step in the process, where the ground material is further refined into a fine powder, often through sieving or milling. These processes find widespread applications in various industries, including food processing, pharmaceuticals, and materials science. The choice of grinding and powdering techniques, along with the equipment used, depends on factors such as the desired particle size, material properties, and the intended application (Paul et al., 2011).

The choice of extraction method is often based on the properties of the plant material, the desired compounds to be extracted, and the intended use of the extract. While traditional methods have their limitations, they continue to be used due to their simplicity, accessibility, and cultural significance.

2.8.3 Modern Techniques of Extraction of Medicinal Plants

The extraction of bioactive compounds from medicinal plants has been revolutionized by the development of modern extraction techniques. These techniques aim to improve extraction efficiency, reduce solvent consumption, minimize environmental impact, and enhance the quality and yield of extracted compounds.

1. **Supercritical Fluid Extraction (SFE):**

Supercritical fluid extraction utilizes supercritical fluids, such as carbon dioxide (CO2), as the extraction solvent. Under specific temperature and pressure conditions, CO2 reaches a supercritical state where it exhibits properties of both a liquid and a gas. SFE offers several

advantages, including high selectivity, low toxicity, and the ability to extract a wide range of compounds. It is particularly suitable for extracting heat-sensitive compounds. However, SFE requires specialized equipment and can be costly (Zaghdoudi et al., 2016).

2. **Microwave-Assisted Extraction (MAE):**

Microwave-assisted extraction involves the use of microwave energy to heat the solvent and accelerate the extraction process. The rapid heating and internal heating effects of microwaves enhance the mass transfer and extraction efficiency. MAE is known for its shorter extraction times, higher yields, and reduced solvent consumption compared to traditional methods. However, optimization of parameters is crucial to prevent degradation of heat-sensitive compounds (Savic, 2020).

3. **Ultrasound-Assisted Extraction (UAE):**

Ultrasound-assisted extraction utilizes high-frequency sound waves to enhance the extraction process. The mechanical effects of ultrasound, such as cavitation and microstreaming, disrupt the plant cell walls and facilitate the release of bioactive compounds. UAE is known for its shorter extraction times, higher yields, and improved extraction efficiency. It is a versatile technique that can be applied to a wide range of plant materials. However, optimization of parameters is necessary to prevent degradation of compounds and ensure reproducibility (Kopp et al., 2020).

4. **Pressurized Liquid Extraction (PLE):**

Pressurized liquid extraction, also known as accelerated solvent extraction, involves the use of elevated temperature and pressure to enhance the extraction process. PLE utilizes a solvent to extract bioactive compounds from the plant material. The high temperature and pressure increase the solubility and diffusion rates, resulting in faster and more efficient extraction. PLE offers

advantages such as reduced extraction time, increased extraction yields, and lower solvent consumption. It is suitable for a wide range of compounds and plant materials. However, the high cost of equipment and the need for specialized solvents are considerations (Radojković et al., 2016).

5. **Solid-Phase Microextraction (SPME):**

Solid-phase microextraction is a solvent-free extraction technique that utilizes a coated fiber to extract volatile and semi-volatile compounds from the plant material. The fiber is exposed to the sample, and the analytes are adsorbed onto the coating. After extraction, the fiber is desorbed, and the compounds are transferred to an analytical instrument for analysis. SPME offers advantages such as simplicity, rapid extraction, and minimal sample preparation. It is particularly useful for analyzing volatile compounds in medicinal plants (Zhang et al., 2018).

6. **Green Solvent Extraction**:

Green solvent extraction techniques aim to replace traditional organic solvents with environmentally friendly alternatives. Examples of green solvents include water, ethanol, and natural deep eutectic solvents (NADES). Water is a widely used green solvent due to its safety, availability, and low cost. Ethanol is another commonly used green solvent that offers good solubility for a wide range of compounds. NADES are a new class of green solvents composed of naturally occurring compounds. They offer advantages such as low toxicity, biodegradability, and high extraction efficiency (Abubakar & Haque, 2020; Espino et al., 2018).

7. **Soxhlet extraction**:

This process is otherwise known as continuous hot extraction. The apparatus is called Soxhlet extractor made up of glass. It consists of a round bottom flask, extraction chamber, siphon tube,

and condenser at the top. A dried, grinded, and finely powdered plant material is placed inside porous bag (thimble) made up of a clean cloth or strong filter paper and tightly closed. The extraction solvent is poured into the bottom flask, followed by the thimble into the extraction chamber. The solvent is then heated from the bottom flask, evaporates, and passes through the condenser where it condenses and flow down to the extraction chamber and extracts the drug by coming in contact. Consequently, when the level of solvent in the extraction chamber reaches the top of the siphon, the solvent and the extracted plant material flow back to the flask. The entire process continues repeatedly until the drug is completely extracted, a point when a solvent flowing from extraction chamber does not leave any residue behind. This method is suitable for plant material that is partially soluble in the chosen solvent and for plant materials with insoluble impurities. However, it is not a suitable method for thermo labile plant materials. Advantages. Large amount of drug can be extracted with smaller amount of solvent. It is also applicable to plant materials that are heat stable. No filtration is required, and high amount of heat could be applied. Disadvantages. Regular shaking is not possible, and the method is not suitable for thermo labile materials (Yoga et al., 2011).

8. **High-Performance Liquid Chromatography (HPLC)-Based Fractionation**:

HPLC-based fractionation involves the separation of extracted compounds using chromatographic techniques. HPLC allows for the separation, identification, and quantification of individual compounds in complex mixtures. It is commonly used to fractionate crude extracts into individual compounds or groups of compounds based on their physicochemical properties. HPLC-based fractionation enables the isolation of specific bioactive compounds for further analysis or application (Drevinskas et al., 2018). These modern extraction techniques offer numerous advantages over traditional methods, including improved extraction efficiency,

reduced extraction time, enhanced selectivity, and reduced environmental impact. However, it is important to consider the specific characteristics of the plant material and the target compounds when selecting the most appropriate extraction technique. Optimization of parameters and validation of the extraction process are crucial to ensure reproducibility and consistency in the extraction of bioactive compounds from medicinal plants.

2.9 Chromatography

Chromatography is a powerful analytical technique widely employed in chemistry, biochemistry, and various scientific disciplines for separating, identifying, and quantifying chemical components within complex mixtures. The word "chromatography" originates from the Greek words "chroma" (color) and "grapho" (to write), reflecting its initial use in separating plant pigments based on their color. Since its inception, chromatography has evolved into a versatile tool with numerous applications, including pharmaceuticals, environmental analysis, and biochemistry.

Chromatographic separation is based on the differential distribution of analytes between two phases: a stationary phase and a mobile phase. The sample mixture is introduced into the mobile phase, which carries it through the stationary phase. Components in the mixture interact differently with the stationary phase, leading to differential migration rates and separation. The choice of stationary phase and mobile phase, along with the separation mechanism, determines the overall separation efficiency and selectivity (Poddar et al., 2021).

2.9.2 Techniques and Variations

Chromatography encompasses several techniques, each tailored to specific separation requirements. Some common chromatographic methods include:

1. Column Chromatography.

2. Thin-Layer chromatography.

3. Gas chromatography.

2.9.3 Column Chromatography

Column chromatography is a widely used technique in separation science that allows for the purification and isolation of compounds based on their physical and chemical properties (Sharma et al., 2020). It operates on the principle of differential partitioning of analytes between a stationary phase and a mobile phase. The stationary phase is typically a solid material packed into a column, while the mobile phase is a liquid or gas that flows through the column. As the mobile phase passes through the column, the analytes interact with the stationary phase and are separated based on their affinity for the stationary phase and their solubility in the mobile phase. There are several types of column chromatography, including affinity chromatography, ion-exchange chromatography, gel filtration chromatography, and size-exclusion chromatography (Sharma et al., 2020).

Affinity chromatography utilizes specific interactions between a target analyte and an immobilized ligand on the stationary phase to selectively bind and separate the analyte from a complex mixture (Li et al., 2017). Ion-exchange chromatography separates analytes based on their charge properties, with positively charged analytes binding to negatively charged stationary phases and vice versa (Zajickova & Špánik, 2019). Gel filtration chromatography separates analytes based on their size, with larger analytes eluting first as they are excluded from the pores of the stationary phase (Sharma et al., 2020).

Size-exclusion chromatography is a similar technique that separates analytes based on their size, but it utilizes a porous stationary phase instead of a gel matrix (Yamamoto et al., 2021). Column chromatography is applied in various fields, including protein purification, pharmaceutical analysis, natural product isolation, and environmental analysis. In protein purification, affinity chromatography columns with immobilized ligands specific to the target protein are commonly used to achieve high selectivity and purity (Li et al., 2017).

In pharmaceutical analysis, column chromatography is used for the separation and quantification of drug compounds in complex matrices (Sandesh et al., 2021). Natural product isolation often involves the use of column chromatography to separate and purify bioactive compounds from plant extracts (Kanagavalli, 2018). Environmental analysis can benefit from column chromatography techniques for the separation and quantification of pollutants in water and soil samples (Langford & Lurie, 2021).

Advancements in column chromatography techniques have led to improved efficiency and selectivity. For example, the development of monolithic columns has allowed for higher permeability and lower backpressure, resulting in faster separations and increased sample throughput (Salmean & Dimartino, 2018). Monolithic columns have been used in various chromatographic techniques, including gas chromatography and supercritical fluid chromatography (Zajickova & Špánik, 2019). The use of 3D printing technology has also enabled the creation of bespoke monolithic structures, allowing for the design of column formats and cartridge designs tailored to specific applications (Salmean & Dimartino, 2018).

This development has opened up new possibilities for the optimization of separation conditions and the development of novel column configurations. In recent years, there has also been a focus on the development of continuous chromatography systems, which offer advantages such as

higher productivity, reduced solvent consumption, and improved process efficiency (Steinebach et al., 2016).

Continuous chromatography systems, such as multicolumn chromatography and counter-current chromatography, utilize multiple columns in parallel or in series to achieve continuous separation and purification of target compounds (Brämer et al., 2019). These systems have been applied in biopharmaceutical production, where they can streamline the purification process and increase overall productivity (Steinebach et al., 2016).

Column chromatography is widely used due to its versatility. It is a technique that allows for the separation and purification of compounds based on their physical and chemical properties.

It encompasses various types of chromatography, including affinity chromatography, ion-exchange chromatography, gel filtration chromatography, and size-exclusion chromatography. Column chromatography is applied in protein purification, pharmaceutical analysis, natural product isolation, and environmental analysis. Advancements in column chromatography techniques, such as the development of monolithic columns and continuous chromatography systems, have further improved the efficiency and selectivity of the technique.

2.9.4 Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a widely used technique in analytical chemistry for the separation and identification of compounds in a mixture (Sherma & Morlock, 2008). It is a planar chromatographic method that involves the separation of components based on their differential migration on a thin layer of stationary phase coated on a solid support, such as a glass or plastic plate (Sherma & Morlock, 2008).

TLC is a versatile and cost-effective technique that offers several advantages, including simplicity, rapid analysis, and the ability to analyze multiple samples simultaneously. In TLC, a small amount of the sample mixture is spotted near the bottom of the TLC plate, and the plate is then placed in a developing chamber containing a solvent system (Sherma & Morlock, 2008). As the solvent moves up the plate by capillary action, it carries the sample components with it. The different components of the mixture interact differently with the stationary phase, resulting in their separation along the plate. The separation is visualized by the use of a suitable detection method, such as UV light, staining reagents, or chemical reactions.

TLC is widely used in various fields, including pharmaceutical analysis, natural product chemistry, food analysis, and environmental monitoring. In pharmaceutical analysis, TLC is often used for the identification and quantification of active pharmaceutical ingredients (APIs) in drug formulations (Reis et al., 2009). It is also used for the analysis of impurities, degradation products, and stability studies of pharmaceutical compounds.

In natural product chemistry, TLC is commonly employed for the isolation and identification of bioactive compounds from plant extracts (Chaerunisaa et al., 2020). It allows for the rapid screening of large numbers of samples and the detection of compounds with specific biological activities. TLC is also utilized in food analysis for the determination of food additives, contaminants, and the identification of natural products in food matrices (Gilli et al., 2014). It is a valuable tool for quality control and authenticity testing of food products.

In environmental monitoring, TLC has been used for the analysis of pollutants, such as pesticides, herbicides, and heavy metals, in water, soil, and air samples (Raeisi et al., 2019). It provides a quick and cost-effective method for screening and monitoring environmental contaminants.

Advancements in TLC techniques have further enhanced its capabilities and applications. For example, high-performance thin-layer chromatography (HPTLC) is an improved version of TLC that offers higher resolution and sensitivity (Jug et al., 2018). HPTLC utilizes specialized plates and optimized mobile phases to achieve better separation and detection of compounds. Additionally, the combination of TLC with other analytical techniques, such as mass spectrometry (MS), allows for the identification and structural characterization of separated compounds (Klingelhöfer & Morlock, 2014). TLC-MS hyphenation provides complementary information on the chemical composition of the separated compounds.

Thin-Layer chromatography offers simplicity, rapid analysis, and cost-effectiveness, making it a valuable tool in various fields, including pharmaceutical analysis, natural product chemistry, food analysis, and environmental monitoring. Advancements in TLC techniques, such as high-performance thin-layer chromatography (HPTLC) and the combination with mass spectrometry (MS), have further expanded its capabilities and improved its resolution and sensitivity. TLC continues to be an essential analytical tool for qualitative and quantitative analysis in diverse applications.

2.9.5 Gas Chromatography

Gas chromatography (GC) is an analytical technique used for the separation and analysis of volatile and semi-volatile compounds in a mixture (Zajickova & Špánik, 2019). It operates on the principle of differential partitioning of analytes between a stationary phase (typically a high-boiling liquid or a solid adsorbent) and a mobile phase (an inert gas) (Zajickova & Špánik, 2019).

GC offers high resolution, sensitivity, and selectivity, making it a valuable tool in various fields, including chemistry, bioanalysis, environmental monitoring, and forensic sciences. In GC, the

sample is injected into a heated injection port, where it vaporizes and enters the column. The column is packed with a stationary phase or consists of a thin film coated on the inner surface of the column. As the carrier gas flows through the column, the analytes interact with the stationary phase and are separated based on their affinity for the stationary phase and their volatility.

The separated analytes are then detected by a suitable detector, such as a flame ionization detector (FID), thermal conductivity detector (TCD), or mass spectrometer (MS). GC has a wide range of applications. In the field of chemistry, it is used for the analysis of organic compounds, such as hydrocarbons, alcohols, acids, esters, and pesticides (Zhang et al., 2021). It is also employed in the analysis of polymers, pharmaceuticals, and natural products. In bioanalysis, GC is used for the quantification of drugs, metabolites, and biomarkers in biological samples (Jain et al., 2019). It is particularly useful for the analysis of volatile compounds, such as volatile fatty acids, in fermentation processes (Aramrueang et al., 2022).

Environmental monitoring is another important application of GC. It is used for the analysis of air, water, and soil samples to detect and quantify pollutants, such as volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and pesticides (Wachełko et al., 2021). GC is also utilized in the analysis of food and beverages to determine the presence of contaminants, such as pesticide residues, mycotoxins, and food additives (Truong et al., 2021).

In forensic sciences, GC plays a crucial role in the identification and quantification of drugs of abuse, such as cocaine, amphetamines, opioids, and cannabinoids (Lanzarotta et al., 2018). It is used in toxicology laboratories for the analysis of biological samples, such as blood, urine, and hair, to detect and confirm the presence of drugs and their metabolites. GC is also employed in forensic fire investigation to analyze accelerants and volatile compounds associated with arson cases (Ireland et al., 2018).

Advancements in GC technology have further improved its capabilities and efficiency. For example, the development of capillary columns with a thin film coating has increased the resolution and sensitivity of GC analysis (Pei et al., 2023).

The use of multidimensional GC systems, where two or more columns with different selectivities are connected in series, allows for enhanced separation of complex mixtures (Pei et al., 2023). Additionally, the coupling of GC with mass spectrometry (GC-MS) provides additional information on the identity and structure of the separated compounds (Silinski et al., 2019).

Gas Chromatography (GC) is applied in various fields, including chemistry, bioanalysis, environmental monitoring, and forensic sciences. GC provides high resolution, sensitivity, and selectivity, making it a valuable tool for the identification, quantification, and characterization of compounds in complex mixtures. Advancements in GC technology have further improved its capabilities, allowing for enhanced separation and detection of analytes. GC

CHAPTER THREE

3.0 Materials and Methods

Sample collection

3.1. Collection and preliminary preparation of the plants

The plant was collected and identified from Ibadan, Oyo state by Dr. Odewo Akiniyi Samuel of Forestry Research Institute of Nigeria, Jericho Ibadan.

3.2. Apparatus and reagents used

Extraction: Aspirator bottle, cotton wool, Aluminium foil, paper tape, funnel, Conical flask, Test tube, measuring cylinders, weighing balance, distillation set, UV light, column, capillary tubes, beakers, retort stand with clamp.

Reagent: Hexane, chloroform, ethylacetate, and methanol were used

3.3. Extraction of "Lannea acida"

Successive extraction was employed during the course of the work. The grounded air-dried (aerial parts) was soaked in an aspirator bottle for five days using ethyl acetate and then water to extract the less polar and polar bioactive components from the plant sample.

After five days, the ethyl acetate extract was obtained through subsequent filtration with cotton wool as a filter bed. The crude extract was exposed to air and was left to dry off to remove the solvent. These crude extract was then weighed and isolated using column chromatography.

Isolation of Phytoconstituents Using Chromatographic Techniques

1. Column Chromatography

Silica gel was packed into the column using wet packing and it was used as the mobile stationary phase. The crude extract was added at the top of the column and hexane was added. After collecting two fractions from the column, the polarity of the solvent used was changed in ratios using ethyl acetate as the second solvent.

2. Thin-Layer Chromatography

After collecting the fractions from the column, the fractions were spotted on a TLC plate using capillary tubes. The TLC plate is then put into a beaker containing a mixture of hexane and ethyl acetate in the same ratio by which the fraction being analysed was run in the column. The TLC plate is then removed from the beaker and put in a UV light to identify pure compound.

3.4 Antimicrobial activity of *Lannea acida*

1000ug/ml of the sample was weighed and dissolved into the 10ml of the solvent(DMSO50%) of the extraction for proper dissolution, from which 2.5ml was taken into another 2.5ml of the solvent, this was taken to the 6th test tube which was the last tube for the extract. The 7th and 8th test tube were negative and positive control (solvent and gentamycine) for bacteria; tioconazole for fungi control of the experiment

3.4.2 Anti-inflammatory

Anti-inflammatory activity study of lannea acida whole plant extract was performed using membrane stabilization assay. The plant extract showed promising in vitro anti-inflammatory activity in a concentration dependent order. The result of the study revealed the dose concentration have close percentage inhibition. This result has made us understand that the whole plant of lannea

acida extract has membrane stabilizing properties and it offered significant protection against Healthy Red Blood Cell (HRBC) membrane lysis and reduce protein denaturation.

Test for Secondary Metabolites

Test for Saponins

The most common qualitative test for saponins involves the Foam Test. In this procedure, a sample is mixed with water to create a solution or extract. The solution is then vigorously shaken or agitated. The presence of saponins is indicated by the formation of a stable, persistent foam or froth that lasts for several minutes. This foam test is widely used to qualitatively detect the presence of saponins in various plant extracts and natural products, relying on their characteristic ability to produce foam due to their surfactant properties.

Test for Alkaloids

The most common qualitative test for alkaloids involves adding a few drops of Dragendorff's reagent or Mayer's reagent to the sample. The procedure includes observing the formation of an orange or cream-colored precipitate, which indicates the presence of alkaloids in the sample.

Test for Flavonoid

The most common qualitative test for flavonoids is the Shinoda test. In this procedure, a small amount of the sample is mixed with a few drops of concentrated hydrochloric acid (HCl) and then diluted with water. The formation of an intense red, orange, or pink coloration indicates the presence of flavonoids. This test exploits the characteristic color changes that occur when flavonoids react with acid and is widely used for their qualitative identification in various plant extracts and natural products.

Test for Terpenoid

The most common qualitative test for terpenoids is the Salkowski test. In this procedure, a small amount of the sample is mixed with chloroform and concentrated sulfuric acid is carefully layered on top of the mixture. A reddish-brown coloration at the interface between the acid and the chloroform layer indicates the presence of terpenoids. This test relies on the reaction of terpenoids with sulfuric acid, leading to the development of this distinct coloration, which serves as a qualitative marker for the presence of terpenoids in various natural extracts and compounds.

Test for Glycoside

The most common qualitative test for glycosides is the Legal test. In this procedure, a small amount of the sample is mixed with a few drops of ferric chloride (FeCl3) solution. A reddish-brown or violet coloration that develops upon the addition of FeCl3 indicates the presence of glycosides. This test relies on the ability of glycosides to form colored complexes with ferric ions, making it a valuable method for their qualitative identification in various plant extracts and natural products.

Test for Steroid

The most common qualitative test for steroids is the Liebermann-Burchard test. In this procedure, a small amount of the sample is dissolved in acetic anhydride, followed by the addition of a few drops of concentrated sulfuric acid (H2SO4). The development of a green or blue-green coloration indicates the presence of steroids. This reaction is due to the formation of complex intermediates and is a characteristic test for identifying steroids in various natural compounds and extracts.

Test for Tannin

The most common qualitative test for tannins is the ferric chloride test. In this procedure, a small amount of the sample is mixed with a few drops of ferric chloride ($FeCl_3$) solution. The formation of a dark blue-black or greenish-black coloration indicates the presence of tannins. This color change is a result of the complexation of tannins with ferric ions, making it a widely used method for the qualitative identification of tannins in various natural products and extracts.

CHAPTER FOUR

4.1. Weight of the plant

The weight of the plant used is presented in the table below.

	THE PL	WEIGHT(g)
1	Whole leaf	1.2kg

4.2. Phytochemical Screening Result of ethyl acetate extract of *Lannea acida*

Table 4.2: Phytochemicals screening result of ethyl acetate extract of *Lannea acida*

S/N	**TEST**	**RESULT**
1	FLAVONOID	POSITIVE
2	TERPENOID	POSITIVE
3	SAPONIN	POSITIVE
4	GLYCOSIDE	POSITIVE
5	STEROID	NEGATIVE
6	TANNIN	POSITIVE
7	ALKALOID	NEGATIVE

The qualitative phytochemical screening of the Lannea acida shows that it possesses metabolites like Flavonoid, Terpernoid, Glycoside, Saponin, and Tannin while steroid and alkaloid are absent.

4.3 Anti-bacterial result of ethylacetate extract of *Lannea acida* after 24hrs

Table 4.3 Anti-bacterial result of ethylacetate extract of *Lannea acida* after 24hrs

Conc (ug/ml)	**Staphylococcus Aureus**	**Escherichia coli**	**Bacillus subtilus**	**Pseudomonas Aeruginosa**	**Salmonellae typhi**	**Klebsiellae pneumoniae**
100	**20**	16	20	16	**18**	**16**
50	**18**	**14**	**18**	**14**	**14**	**14**
25	**14**	**12**	**14**	**12**	**12**	**12**
12.5	**12**	**10**	**12**	**10**	**10**	**10**
6.25	**10**	___	**10**	___	___	**—**

As shown in table 4.3 above, at high concentration of 100 µg /ml, the extract exhibits high antibacterial activity on all six test bacterial, it shows moderate anti-bacterial activity at 50 ug/ml and it shows low anti-bacterial activity at lower concentrations of 25ug/ml, 12.5ug/ml and 6.25ug/ml.

4.7 Anti-inflammatory analysis of ethyl acetate extracts of *Lannea acida*

Table 4.7 percentage inhibition of ethyl acetate extracts of *Lannea acida* using membrane stabilization assay

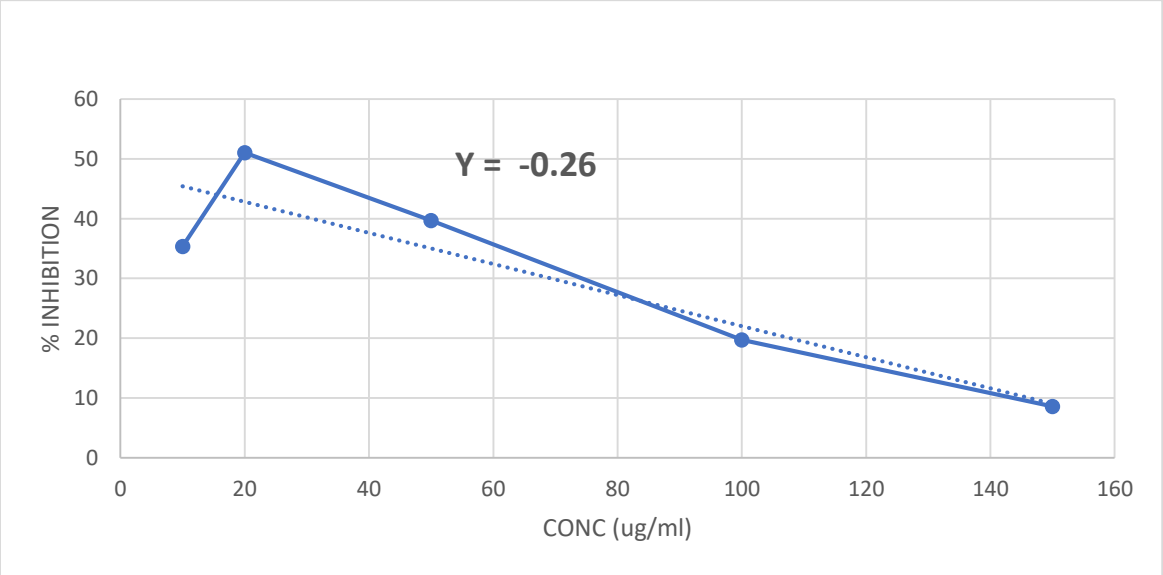
SAMPLE	%INHIBITION OF DENATURATION@100 MG/ML	%INHIBITION OF DENATURATION@200MG/ML	%INHIBITION OF DENATURATION @300MG/ML	%INHIBITION OF DENATURATION@ 400MG/ML	%INHIBITION OF DENATURATION @500MG/ML
L.A (E A)	86.36364	87.87879	87.12121	85.60606	85.60606

Anti-inflammatory activity study of *Lannea acida* extract was performed using membrane stabilization assay. The plant extract showed promising in vitro anti-inflammatory activity in a concentration dependent manner. The percentages of inhibition as shown in table 4.7 above are 86.36364% at the dose of 100µg/ml, 87.87879% at the dose 200µg/ml, 87.12121% at the dose 300 µg/ml, 85.60606% at the dose 400 µg/ml, 85.60606% at the dose 500 µg/ml. At the dose of 200µg/ml, the percentages of inhibition is significantly higher than at other doses. The result of the study revealed that *Lannea acida* extract possess membrane-stabilizing property, as it offered significant protection against Healthy Red Blood cell (HRBC) membrane analysis and reduction of protein denaturation

4.8 Antioxidant activity

Table 4.8: Antioxidant activity of ethyl acetate extract of *Lannea acida*

CONCENTRATION (ug/mL)	Absorbance	Absorbance (Ascorbic acid)	% Inhibition
10	36.9834385	57.1797299	35.32
20	43.4917063	88.7913165	51.01
50	54.1838606	89.8243749	39.68
100	70.557836	87.861564	19.70
150	79.9070144	87.3966877	8.57



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