CHROMATOGRAPHIC ANALYSIS OF METHANOLIC LEAF EXTRACT OF EXTRACT *FICUS SUR (CAPENSIS)* (MORACEAE)

BY

DUNKWU JOHN

PHA1707015

SUPERVISED BY:

Dr. O DION. E. EMMANUEL

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

FACULTY OF PHARMACY UNIVERSITY OF BENIN

BENIN CITY

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A PROJECT WRITTEN IN THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY AND SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DOCTOR OF PHARMACY IN THE FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

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CERTIFICATION

This is to certify that this work was successfully carried out by **DUNKWU JOHN** in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

_____ _____

Dr. E.E. Odion Date

(Project Supervisor)

_____ _____

Dr E.E. Odion Date

(Head of Department)

_____ _____

Dunkwu John Date

(Student)

DEDICATION

I wholeheartedly dedicate this work to God Almighty my all sufficiency

ACKNOWLEDGEMENT

I give all the thanks to the Almighty God for seeing me through in this work.

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ABSTRACT

Since the beginning of time, specific pathological illnesses have been treated using a well-known therapeutic approach known as phytomedicine, a branch of traditional medicine. In folklore medicine, Ficus capensis (Sims) G.Don. is frequently used for the treatment of a wide range of illnesses, including tumors, inflammation, cough, rheumatism, fever, diarrhea, wounds, and in the prevention of numerous health issues. Regarding their numerous medical and pharmacological applications, however, little is known about their real phytochemical composition. Using suggested analytical techniques, the phytochemical components of ficus capensis leaf were examined in this study through preliminary and chromatographic examination. The results showed the existence of bioactive substances as phenols, alkaloids, tannins, saponins, and flavonoids. The fact that so many of these phytochemicals are present indicates that ficus capensis extract has some potential for use in medicine. Some of the bioactive substances found in plants that have been identified have documented medical and physiological advantages.

CHAPTER ONE

1.0 Introduction

A well-known branch of traditional medicine known as phytomedicine has been used all over the world as a therapeutic strategy to treat particular pathological disorders since the dawn of time (Josephine *et al*., 2019).

A renewed interest in finding phytochemicals in native and naturalized plants for pharmaceutical and dietary purposes has emerged in the last ten years (Oktay, *et al*., 2003; Wangensteen, *et al*., 2004). In recent years, there has also been an increase interest in alternative therapies and the therapeutic use of natural products. Due to its accessibility and affordability, phytomedicine has been a culturally acceptable method to treat a variety of illnesses. This entails using herbal products that may comprise the entire plant or its portions, which are known to contain certain components that may effect changes in the human body. Thus, diverse plants have been used extensively in human and animal life for a variety of reasons, specifically as food for nutritional advantages and medications for the treatment of ailments (Mensah *et al*, 2019).Numerous phytochemicals and metabolites, ranging from steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, and cardiac glycosides, have been found in plants (Ajibesin., 2011). Various organic substances that have anti-inflammatory and antibacterial properties are produced by plants. The *Ficus capensis* plant has been utilized to treat leprosy, leucodermia, other swelling afflictions with its leaves and roots (Nguyi, 1988; Dafalla, 2005; Oyeleke, et al., 2008).

Phytochemicals

Phytochemistry is a branch of science that studies the chemical components found in plants. A variety of approaches have been developed within this field, ranging from

the processing of plant tissue samples to complex procedures for revealing organic structures. Examining the chemical elements that are naturally present in plants is the main focus of the science of phytochemistry. The chemical structures of these substances, their metabolic processes (both synthesis and breakdown), their natural distribution, their biological activities, as well as methods for collecting and analyzing them quantitatively and qualitatively, are just a few of the variables that are included in this examination. The right treatment of plant material is essential before beginning any phytochemical investigation. The samples should be heated, frequently in an oven set at a constant 60°C until a constant weight is reached, as this is an efficient and simple way to ensure stability. This process guarantees ideal circumstances for the ensuing compound analysis.

Plant metabolism:

Plant metabolism, also known as plant biochemistry or plant physiology, refers to the set of chemical reactions and processes that occur within plants to sustain life, grow, and reproduce. Just like in animals and other organisms, plant metabolism encompasses various biochemical pathways and cellular activities (Rieseberg et al 2023).

Primary Metabolites

Primary plant metabolites are a group of organic compounds that play essential roles in the basic physiological processes of plants. These compounds are involved in primary metabolic pathways and are necessary for plant growth, development, and survival (Guardiola-Márquez et al 2022).

1. **Carbohydrates:**

Glucose: A simple sugar that is a central molecule in plant metabolism, serving as an energy source.

Starch: A polymer of glucose molecules, commonly stored in plant tissues such as roots, tubers, and seeds for energy reserves.

Cellulose: A polysaccharide that makes up the structural component of plant cell walls.

1. **Amino Acids:**

- Amino acids are the building blocks of proteins, which are essential for plant growth and development.
- Examples of primary amino acids in plants include glycine, glutamine, and aspartic acid.

2. **Nucleotides:**

- Nucleotides are the building blocks of nucleic acids (DNA and RNA), which carry genetic information.
- Adenosine triphosphate (ATP) is a critical nucleotide that serves as the primary energy currency in plant cells.

3. **Lipids:**
    - **Fatty acids:** Essential components of membrane lipids and a source of stored energy in the form of triglycerides.
    - **Phospholipids:** Major constituents of cell membranes.
    - **Cholesterol:** Present in plant cell membranes, playing a role in membrane fluidity and stability.
4. **Organic Acids:**

- Organic acids such as citric acid and malic acid are involved in the citric acid cycle (TCA cycle) and energy metabolism.

5. **Phenolic compounds:**

- Phenolic compounds, including phenolic acids and flavonoids, play roles in defense against pathogens, UV protection, and antioxidative processes.

6. **Vitamins and cofactors:**

- Plants produce various vitamins (e.g., vitamin C) and cofactors (e.g., coenzyme A) that are essential for enzymatic reactions and overall metabolism.

7. **Chlorophyll and pigments:**

- Chlorophyll is responsible for capturing light energy during photosynthesis.
- Other pigments, such as carotenoids and anthocyanins, have roles in photosynthesis, protection against oxidative stress, and attraction of pollinators.

8. **Hormones:**

- Plant hormones, including auxins, gibberellins, and cytokinins, regulate growth, development, and responses to environmental stimuli.

These primary plant metabolites are synthesized through various metabolic pathways and are essential for the functioning of plant cells and the overall health and vitality of plants.

They serve as the foundation for the synthesis of secondary metabolites, which are compounds that have more specialized roles in plant defense, adaptation, and interactions with the environment.

Plant secondary metabolites, also known as secondary plant compounds or specialized metabolites, are organic compounds produced by plants that are not directly involved in their growth, development, or reproduction, as opposed to primary metabolites. Secondary metabolites serve various ecological and adaptive functions, including defense against herbivores, protection against pathogens, attraction of pollinators, and adaptation to environmental stressors (Bhatla et al 2018). These compounds often have unique chemical structures and diverse biological activities. Some common categories of plant secondary metabolites include:

The ability of tannins, a type of polyphenol, to precipitate proteins has been used for ages in the production of leather from unprocessed animal hides. Proteins become more resistant to microbial and fungal attacks as a result of the crosslinks tannin molecules create with them throughout this transformation process. Modern analyses, however, show that many compounds categorized as tannins based on their structure and derivation from biosynthetic sources may have little to no potential for producing leather (Hagerman and Butler, 1981). The terms "hydrolysable tannins" and "condensed tannins" are used to classify two important types of tannins. Multiple phenolic acid molecules, including gallic and hexahydroxydiphenic acids, combine with a central glucose molecule to generate hydrolysable tannins. These ester connections allow the molecules to bind together and become stable. Notably, gallotannins and ellagitannins—each made up of units of gallic acid and ellagic acid—are the main forms of hydrolyzable tannins. Geraniin, which may be found in Geranium robertianum (Herb Robert) and Geranium maculatum (American cranesbill), is one of the ellagitannins that have been identified as having medicinal value (Catarino *et al.,* 2017), and tellimagrandins 1 and 2, which were recovered from the pomegranate, the oak bark, and the meadowsweet (Filipendula ulmaria) (Yi et al.,

2004; Evans, 2009). Condensed tannins, also known as proanthocyanidins, are derived from oligomeric precursors of flavonoids and differ in the linkage types between flavonoid units, the patterns of hydroxylation, the stereochemistry of the pyran ring's carbons 2, 3, and 4, and the presence of additional substituents. Tea from Camellia sinensis and the leaves and bark of Hamamelis virginiana are two examples of substances that contain both hydrolyzable and condensed tannins (Puneet et al., 2013). Tannin-containing medications have antidiarrheal properties and have been used as antidotes for heavy metal and alkaloid poisoning. The essential tea ingredient epigallocatechin-3-gallate has antiangiogenic effects in mice.In addition, Vaccinium oxycoccos (cranberry) juice has a long history of use as a urinary antiseptic (Jepson and Craig, 2008), a claim that was supported by a randomized, double-blind, placebo-controlled study with 153 elderly women (Avorn et al., 1994).

Flavonoids:

The largest group of phenols that are found in nature is called flavonoids. Nearly 500 of these compounds—out of the more than 2000 that have been identified so far—are still unbound (Evans, 2009). A chroman ring with an aromatic ring at positions 2, 3, or 4 makes up the basic structure of flavonoids. Based on the degree of oxidation of the central ring (ring C), flavonoids can be divided into several kinds. Anthocyanins, flavones, and flavonols are the most common of these. Flavones and their close relatives, which are found in the cell sap of young tissues and are frequently endowed with yellow colours, are abundant in nature. They are more prevalent in superior plants and juvenile tissues. They are especially prevalent in the Polygonaceae, Rutaceae, Leguminosae, Umbelliferae, and Compositae families of plants. The medicinal potential of compounds containing flavonoids, including Glycyrrhiza

glabra (liquorice root), Chamaemelum nobile (Roman chamomile), and Ginkgo biloba (gingko), has been recently discovered. Many herbal remedies high in flavonoids have been incorporated into the British Pharmacopeia, including Betula pendula (Birch Leaf), Calendula officinalis Flower, Sambucus nigra (Elder Flower), Equisetum ramosissimum (Horsetail), Tilia cordata (Lime Flower), Leonurus cardiaca (Motherwort), and Passiflora edulis (Passion Flower), this class is known for its anti-inflammatory and antiallergic qualities, potential as an antithrombotic and vasoprotective agent, capacity to block tumor promotion, and function in protecting gastric mucosa (Montanher et al 2007).

1.1 Extraction

Extraction is a method that involves treating plant material with a solvent to dissolve the components that are medicinally active while leaving inert material undisturbed. It comprises the conventional technique of using selected solvents to separate a combination of naturally active chemicals from plant tissues (Handa, 2006). Extraction is primarily used to separate soluble plant compounds from insoluble cellular residue. After the water is removed, the metabolite mixture is relatively complex and can exist in liquid, semisolid, or dried powder form, making it appropriate for both internal and exterior use. (Azwanida, 2015). The different solubilities of the solute, additional matrix components, and the stabilizing solvent are key factors in the extraction process. (Omeroglu *et al.,* 2019).

Three types of extraction are frequently used: liquid/solid, liquid/liquid, and acid/base (Chuo *et al.,* 2014). Appropriate extraction methods must be used in order to successfully extract these bioactive chemicals, taking into account elements like the plant components used, the solvent selected, the length of the extraction, the particle size, and agitation throughout the extraction process. (Jurinjak *et al.,* 2018; Visht and

Chaturvedi, 2012). These processes, which operate according to various extraction principles, include solvent extraction, distillation, pressing, and sublimation. Solvent extraction stands out among these methods as the one that is most frequently used. (Harborne, 1998). The choice of solvent, the specific plant part employed as the initial material, and the extraction procedure represent fundamental factors that have been documented to impact the extract's quality (Pandey and Tripathi, 2014). A properly completed extraction method is the first step toward isolating and identifying specific components within raw plant material. This calls for careful sample selection and preparation as well as a thorough analysis of the pertinent literature to establish acceptable techniques adapted to various compound classes or plant species. (Jones and Kinghorn, 2006).

The plant material is normally submerged in a solvent throughout the extraction process for a specific amount of time. The interaction between a solid substance and a solvent, which results in the dissolution and migration of soluble components from the solid to the solvent phase, is the basis for the solid-liquid extraction process. In solvent extraction, soluble constituents are transferred along a gradient of concentration, and the rate of mass transfer depends on the concentration of the ingredient until equilibrium is reached. Mass transfer from the plant material to the solvent then comes to an end. Additionally, by increasing solubility, increasing the solvent's temperature can intensify mass transfer. (Pandey and Tripathi, 2014; Jones and Kinghorn, 2006). Additionally, when a new solvent is added to replace the solvent equilibrium with the plant material, changes in the concentration gradient take place. (Handa, 2006). Essential qualities of an ideal extraction solvent, or solvent blend, include characteristics such as being quickly removable, inert, non-toxic, free of plasticizers, not easily prone to combustion, and exhibiting little to no chemical

reactivity (Visht and Chaturvedi, 2012). As a result, in the field of solvent extraction, the solvent selection process has utmost significance.

When choosing a solvent, factors including solubility, selectivity, cost-effectiveness, and safety should be taken into account. (Harborne, 1998).

The phytochemical quality desired for extraction, extraction rates, the variety of metabolites to be extracted, the solvent's toxicity within the bioassay process, potential health risks associated with the extractants, and ease of subsequent handling of the extract are some of the factors that influence the choice of solvent. Getting the best yields and greatest quality of the desired compounds is the main goal of the extraction process (Omeroglu *et al.,* 2019).

1.2 Methods of Extraction

1.2.1 Maceration:

Maceration is a versatile process that involves soaking a substance in a liquid to achieve various objectives, such as softening, favor infusion, or extraction of specific components (Prado and Rostagno, 2022). The specific context and purpose of maceration determine the techniques and substances involved in the process. This method is often used to prepare herbal extracts or tinctures for medicinal purposes (Alamgir, and Alamgir, 2017).

It is a practice that is well-known and frequently used. It entails putting plant materials, whether they are in a powdered form or are coarse, inside a container that is well sealed. A solvent is added inside of this container, and the combination is then allowed to rest at room temperature for two to three days while being frequently

stirred to aid in the extraction of plant components. Under normal atmospheric pressure, the aim of using a hermetically sealed extractor is to prevent solvent evaporation. This procedure's main goal is to cause the softening and disruption of the plant material's cell walls, which will result in the release of soluble phytoconstituents. When the specified amount of time has passed, the resulting mixture is put through pressing, filtration, or decantation to separate the extract from the plant matrix. (Azwanida, 2015; Handa *et al.,* 2008).

Maceration is also employed in pharmaceutical and chemical industries. It is used to extract active compounds or chemicals from plant materials by soaking them in a suitable solvent (Seidel, 2005).

1.2.2 Percolation

Percolation techniques of extraction are a method used to extract solutes, compounds, or substances from a solid matrix or material by passing a solvent or extraction fluid through it. This method is commonly used in various industries, including pharmaceuticals, food processing, and herbal medicine, to obtain desired components from raw materials (Naviglio, et al 2019).

The choice of solvent is crucial in percolation extraction. The solvent should be compatible with the matrix and should have the ability to dissolve the target compounds efficiently. Common solvents include water, ethanol, methanol, and various organic solvents, depending on the nature of the extraction (Bubalo, et al 2018).

The solid matrix containing the target compounds is typically prepared beforehand. This may involve grinding, cutting, or breaking down the matrix into smaller particles

or pieces to increase the surface area and enhance the extraction process (Thomas et al 2020).

The solvent is slowly passed through the matrix by gravity or with the assistance of a pump. It is essential to control the flow rate and maintain it at a steady pace. The solvent percolates through the matrix, dissolving the target compounds as it moves through (Raaman, 2006).

Percolation extraction techniques are versatile and widely used in industries where selective extraction of compounds from solid matrices is required. The method allows for efficient extraction of specific components while leaving undesirable components behind. However, the success of percolation extraction depends on factors like the choice of solvent, particle size of the matrix, flow rate, and extraction time, all of which need to be optimized for each specific application (Manousi, and Samanidou, , 2019).

1.2.3 Decoction

Decoction is a traditional technique of extraction that involves simmering or boiling plant material (such as roots, bark, seeds, or herbs) in water to extract the active compounds and flavors. (Shaw and Charters, 2016). It's a common method used in herbal medicine and traditional cooking to prepare teas, infusions, and herbal remedies (Kosalec, et al 2009).

Choose the plant material (usually dried) that contains the compounds you want to extract. Different parts of plants, such as roots, barks, leaves, seeds, and flowers, may be used depending on the desired properties (Alamgir,. and Alamgir, 2017). The procedure involves bringing the sample to a boil in a predetermined amount of water for a predetermined amount of time (between 15 and 60 minutes). After boiling, the mixture is allowed to cool before being strained, filtered, and supplemented with

enough water through the medication to reach the necessary final volume. This approach works particularly well for extracting hard plant materials, water-soluble chemicals, and thermostable molecules. In comparison to maceration, this method frequently produces a higher percentage of oil-soluble chemicals.

1.2.4 Reflux Extraction

Reflux extraction is a widely used laboratory technique for extracting compounds from solid or liquid samples using a solvent. It is commonly employed in chemistry and analytical chemistry to extract, purify, or isolate specific compounds from a sample. (Romanik, et al 2007). The process involves continuously boiling the solvent and then condensing the vapor back into the original container, ensuring that the solvent remains in contact with the sample for an extended period. (Handa, 2008).

Reflux extraction is particularly useful when extracting heat-sensitive compounds or when a more prolonged contact time between the sample and solvent is required. It allows for efficient and controlled extraction while minimizing solvent loss through evaporation (Jha, 2022).

1.2.5 Soxhlet Extraction

Soxhlet extraction is a widely used technique in chemistry and analytical chemistry for the extraction of compounds from solid samples using a continuous cycle of solvent heating and cooling (De Castro, and Garcıa-Ayuso, 1998.). It is often employed when you need to extract compounds that are either heat-sensitive or present in trace amounts. (Uwineza, And Waśkiewicz, 2020).

Soxhlet extraction is an efficient and automated method for extracting compounds from solid samples. It allows for prolonged contact between the sample and solvent, making it suitable for extracting compounds that are less soluble or require extended

extraction times. Additionally, it minimizes solvent consumption compared to other extraction methods (Jha and Sit, 2022)

1.2.6 Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE) or high-pressure solvent extraction (HPSE), is an advanced extraction technique used to efficiently and rapidly extract analytes from solid or semi-solid samples. PLE utilizes elevated temperatures and pressures to enhance the extraction process, making it faster and more effective compared to traditional extraction methods. (Subedi, et al 2015).

Pressurized liquid extraction offers several advantages, including faster extraction times, reduced solvent consumption, and improved extraction efficiency. It is widely used in various fields, including environmental analysis, food testing, and pharmaceutical research, where efficient and rapid extraction of analytes from solid samples is essential.

1.2.7 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is a specialized extraction technique that uses supercritical fluids, such as carbon dioxide (CO2), as the solvent to extract target compounds from solid or liquid samples. Supercritical fluids exhibit properties of both gases and liquids at specific temperature and pressure conditions, making them effective solvents for a wide range of applications (Lopez-Hortas, et al 2022).

SFE is commonly used in various industries, including pharmaceuticals, food and beverage, natural product extraction, and environmental analysis. Its versatility and ability to provide clean and selective extractions make it a valuable technique in analytical chemistry and research.

1.2.8 Ultrasound assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) is a modern extraction technique that utilizes ultrasonic waves to enhance the extraction of compounds from solid or liquid samples. It is widely used in various fields, including chemistry, food science, environmental science, and herbal medicine. UAE is particularly effective for extracting thermally labile compounds, such as bioactive compounds from plants or heat-sensitive molecules from pharmaceuticals (Chemat, et al 2017).

Ultrasound-assisted extraction is a versatile and efficient method for extracting a wide range of compounds from various samples, making it a valuable tool in research and industry (Yusoff, et al 2022).

1.2.9 Microwave Assisted Extraction (Mae)

Microwave-assisted extraction (MAE) is a modern extraction technique that uses microwave energy to enhance the extraction of compounds from solid or liquid samples. It is a rapid and efficient method that is widely used in various fields, including chemistry, food science, environmental science, and pharmaceuticals. MAE is particularly useful for extracting heat-sensitive compounds and can significantly reduce extraction times compared to traditional methods (Eskilsson, and Björklund, 2000).

Microwave-assisted extraction is a valuable and versatile technique that offers many advantages in terms of speed and efficiency, making it a preferred choice in both research and industry for various applications (Pico, 2013).

1.2.10 Pulsed electric field (PEF) extraction

Pulsed Electric Field (PEF) extraction is a non-thermal food processing technology that has gained popularity in recent years for various applications, including the extraction of bioactive compounds from plant materials, the improvement of juice yield from fruits, and the enhancement of oil extraction efficiency from seeds(Kate, et

al 2016). PEF extraction relies on the application of short, high-voltage electrical pulses to disrupt cell membranes and facilitate the release of intracellular compounds (Martínez, 2020).

Benefits of PEF extraction techniques include reduced energy consumption compared to traditional thermal methods, preservation of heat-sensitive compounds, and improved extraction efficiency. However, the optimization of PEF parameters and equipment design is essential to achieve the desired results for specific applications.

PEF extraction has been used in the food industry for applications like fruit juice extraction, vegetable oil extraction, and the extraction of bioactive compounds from plant materials for the production of functional foods and nutraceuticals. It also has potential applications in waste water treatment, biotechnology, and pharmaceutical industries (Kumar, et al 2017).

1.2.11 Enzyme Assisted Extraction (EAE)

Enzyme-assisted extraction techniques are methods that utilize enzymes to enhance the extraction of specific compounds or bioactive substances from various raw materials. These techniques are commonly used in the food, pharmaceutical, and biotechnology industries, as enzymes can improve the efficiency, selectivity, and sustainability of extraction processes (Puri, et al 2012).

Enzyme-assisted extraction techniques are widely used in the extraction of various compounds, including flavors, colors, antioxidants, essential oils, proteins, and bioactive compounds from plant materials, microbial biomass, and other sources. These techniques play a crucial role in the development of sustainable and efficient extraction processes in various industries. (Marathe, et al 2017).

1.2.12 Hydro Distillation and Steam Distillation

Hydrodistillation and steam distillation are both methods used to extract essential oils and other volatile compounds from aromatic plants, herbs, and spices. These techniques are based on the principle of separating the volatile compounds from plant materials by heating and vaporization, followed by condensation (Charles, and Simon, 1990).

In summary, the main difference between hydrodistillation and steam distillation is the use of water. Hydrodistillation involves direct contact between water and the plant material, while steam distillation uses steam generated separately from the plant material. Steam distillation is generally preferred when a higher concentration of essential oil and better quality are desired, while hydro-distillation may be more suitable for certain plant materials or when water contact is not an issue. The choice between these techniques depends on the specific plant material, the desired end product, and the available equipment and resources.

1.3 Chromatography

Chromatography is a popular method of dissolving mixtures of substances into their constituent components according to their chemical or physical characteristics. It is a crucial instrument in a number of scientific fields, such as chemistry, biochemistry, biology, and environmental research. Chromatography relies on the idea that distinct components of a mixture will bind to stationary and mobile phases with differing affinities, allowing for separation (Bidlingmeyer, 1993).

The basic operating principle of chromatography as an instrumental technique is revealed when a heterogeneous mixture of molecules is introduced to the surface of a solid or liquid stationary phase, or inside its limits. The carefully designed migration of these molecules, facilitated by the action of a mobile phase, is the dynamic

component of this process. The basic principles of this molecular separation depend on intricate factors affected by the molecules' special affinities or disparities included within their molecular weights, as well as their adsorption (liquid-solid), partition (liquid-solid), and liquid-solid properties (Cuatrecasas et al., 1968; Porath, 1997). The manner in which the mobile phase, stationary phase, and molecular components of the mixture interact has a significant impact on how chromatography is carried out. Partition-based chromatography, which achieves astounding performance in the separation and characterisation of tiny molecules like amino acids, carbohydrates, and fatty acids, provides a powerful illustration. The isolation of macromolecules like proteins and nucleic acids, however, benefits greatly from affinity chromatography, as demonstrated by ion-exchange chromatography. Different chromatographic variations each have a particular function: Gas-liquid chromatography successfully separates alcohol, esters, lipids, and amino groups while also providing insights into enzymatic interactions. Paper chromatography effectively separates proteins and adds to studies into protein synthesis. Parallel to this, molecular-sieve chromatography establishes itself, especially in the evaluation of protein molecular weights. In the molecular world, the specialist field of agarose-gel chromatography is used to filter RNA, DNA particles, and viral organisms (Gerberding and Byers, 1998).

The stationary phase, which can either be a liquid medium encasing a solid substrate or a solid matrix, is the key component of chromatography. Concurrently, the mobile phase, a dynamic entity, follows its own trajectory and takes on the identities of either a fluidic "liquid" (resulting in liquid chromatography, or LC), or a fluidic "gas" (resulting in gas chromatography, or GC). In addition to solid materials, gas chromatography also finds strategic use in the investigation of gaseous substances and

volatile liquid mixtures. When working with thermally sensitive and non-volatile materials, liquid chromatography shines in a pleasing contrast, directing its skill toward samples that show resistance to evaporation (Donald et al., 2006).

The achievement of a nuanced equilibrium within a carefully chosen time frame is the overall goal driving chromatography, going beyond its special ability for molecule separation. In order to achieve this goal, a complex web of chromatographic techniques has been developed, each of which draws on particular principles to rewrite the history of efficient separation. Salient variations that each articulate a unique harmony of theory and practice emerge from this vast library of techniques. They include:

- High-pressure liquid chromatography (HPLC) chromatography
- Gas chromatography
    - Thin-layer chromatography (TLC)
    - Gel permeation chromatography
    - Paper chromatography
    - Column chromatography
    - Ion-exchange chromatography and
    - Affinity chromatography (Harwood & Moody, 1989).

1.3.1 Gas Chromatography

Gas chromatography utilizes a meticulously planned procedure that takes advantage of a column acting as the stationary phase. A vital part of the chromatographic apparatus, an inert solid, is meticulously coated on the surface of this column by a

liquid stationary phase. This procedure falls under the category of "gas-liquid" chromatography, where the carrier phase is made up of carefully selected gases like helium or nitrogen. A tremendous force of high pressure is used by the mobile phase, which is made up of an inert gas, to move through the column (Guiochon, & Guillemin, 1988).

The sample that is designated for examination vapourizes and effortlessly changes into a gaseous form within the mobile phase. As a result, the numerous components that make up the sample spread themselves between the stationary and mobile phases, which coexist on the. Gas chromatography has a well-deserved reputation for being a simple method that is also incredibly versatile and capable of producing results with a variety of facets. It is a key tool in the field of analytical chemistry due to its enhanced sensitivity and quick applicability. Gas chromatography stands out as the equipment of choice for achieving the very difficult task of segregating and distinguishing even the smallest of molecular species (McNair, et al 2019). This is especially notable. Because of its analytical skill, it is a valuable asset, especially when it comes to isolating and characterizing trace amounts of analytes, a task that is of utmost importance in many scientific and industrial endeavors.

1.3.2 High-Pressure Liquid Chromatography (HPLC)

High-pressure liquid chromatography (HPLC) within the field of chromatographic techniques emerges as a dynamically developing field, revealing its potential as a potent technique that expertly facilitates the quick and effective conduct of comprehensive structural and functional analyses, as well as the meticulous purification of a wide range of molecules. The wide range of applications for HPLC is astonishing and includes the challenging separation and identification procedures for

numerous important biomolecules (Veit, J., 2019). These involve the intricate coordination of proteins, steroid hormones, nucleic acids, lipids, carbohydrates, amino acids, and a wide range of other bioactive chemicals that are essential to biological systems.

In HPLC, a dynamic interaction occurs as the mobile phase is forced through painstakingly constructed columns by strong pressure pressures that vary between 10 and 400 atmospheres. The flow rate of this dynamic trajectory is accelerated, with speeds ranging from 0.1 to 5 centimeters per second. Particularly, the addition of minute particles to this choreographed dance and the application of increased pressure to the cascading solvent stream have a transformational impact on the chromatographic separation process, bestowing a remarkably accelerated and efficient potential to produce rapid analytical results of the utmost importance (Regnier, 1983). The key elements that come together to form the intricate tapestry of an HPLC configuration include a reservoir brimming with solvents, a high-pressure pump equipped with the ability to carry out precise and controlled manipulations, a meticulously calibrated commercially prepared column that assumes a key role in the chromatographic process, and an astute and discerning detector that captures and deciphers the nuanced signals inherent in the chromatographic lan A well-organized computerized system serves as the precise conductor for the chronological aspects of the separation story, harmonizing the many components into a well-balanced symphony of ideal material accrual and perceptive data collection. (Regnier, 1983), of dye-ligand chromatography hinges upon precise control over environmental variables, such as maintaining optimal pH conditions. This strategic orchestration is further augmented through the employment of elution methodologies that harness the

potential of high-ionic strength solutions. A harmonious alignment between the intrinsic ion-exchange properties of the adsorbent material and the judicious use of high-ionic strength elution solutions results in this symbiotic interaction. This complex interaction results in the successful separation of adsorbed proteins in the chromatographic column, offering a crucial development in the field of protein separation and purification. (Scopes, 1984; Cutler, 2004).

Column Chromatography

Chromatographic techniques can successfully isolate different parts of proteins because of changes in features like size, shape, net charge, stationary phase, and binding capacity. Column chromatography is the main method used for this, especially for biomolecule purification. In this procedure, the sample is loaded into a stationary phase inside a column before a wash buffer is used as the mobile phase. The sample moves through the fiberglass-supported column material before eventually accumulating at the device's base in a volume- and time-dependent manner (Das & Dasgupta, 1998).

Thin-layer chromatography

A chromatographic separation technique known as "thin-layer chromatography" utilizes the concepts of "solid-liquid adsorption." This process involves carefully coating glass plates with a stationary phase made of a solid adsorbent substance. Similar to column chromatography, the choice of adsorbent material includes materials like cellulose, silica gel, and alumina. The capillary action phenomenon aids in the mobile phase's ascension through the stationary phase in the operational dynamics. The combination that was initially pipette-deposited at the bottom parts of

the plate is mobilized as the solvent rises, pushing these components upwards at different flow rates. The separation of analytes results from this carefully planned approach.The relative polarities of the substance in issue, the solid phase, and the solvent all affect how quickly this upward migration proceeds (Sherman et al., 1991). Fluorescence, radioactivity, or certain chemical reactions are sometimes used to create recognizable, visually unique reactive products when the sample molecules lack natural coloring. These goods help the chromatogram's molecules' locations to be clearly defined. Under both ambient room lighting and ultraviolet (UV) illumination, the formation of visible coloring can be seen. Each molecule's location within the mixture is determined by a calculation that compares the distance traveled by the molecule to that of the solvent. The symbol Rf stands for this quantitative value, which is known as the "relative mobility". For the molecules present, the Rf value acts as a qualitative description (Donald et al., 2006).

Gel-permeation chromatography:

Gel-permeation chromatography is based on a fundamental principle that exudes elegance and simplicity: it uses the properties of dextran-infused materials to orchestrate the careful separation of macromolecules based on their inherent differences in molecular dimensions. This method, a model of multifunctionality, serves two purposes in the complex field of molecular analysis. It is mostly used as a skillful tool for calculating the molecular weights assigned to proteins, which provides an insightful look into their complex structural makeup. At the same time, it assumes the role of an expert agent, successfully orchestrating the gradual reduction of salt concentrations found in protein solutions. This important endeavor results in the

refinement of these solutions, elevating them to states conducive to further research and analysis (Walls and Sinéad, 2011). A synchronized interplay of parts unfolds inside the gel-permeation column's inner sanctuary. The stationary phase, a collection of inert molecules dotted with microscopic pores, prepares the way for the complex molecular procession. A solution full of molecules with different sizes runs through the column in a repetitive cadence, creating an orchestrated symphony of constant speed. The problem is that molecules whose size surpasses the pore's dimensional restrictions find themselves stymied, their attempts to pierce the gel's particle walls crushed. As a result of their confinement inside a small area, they instead come face to face with their fate while being caught within the interstitial voids that punctuate the intervals between particles. For those molecules of even greater size, however, a different course is in store since they are able to maneuver through the pores in the porous particles and overcome these molecular obstacles quickly, gracefully, and nimbly. Those who are small in stature and have dimensions that modestly fit within miniscule pores sneak into these conduits and make their way via their confusing passageways at the same time. Their egress from the column occurs in a manner characterized by extended retention durations, revealing a particular temporal pattern that replicates their size-based hierarchy as their diameters gradually diminish (Helmut, 1969).

The Sephadeks G type stands out among the myriad of available column materials as the genuine standard-bearer, enjoying a dominant position as the material that is most usually used to build these chromatographic structures. The repertoire, however, goes beyond this lone champion, as a variety of substitute materials, such as dextran, agarose, and polyacrylamide, collectively form an eclectic ensemble that is wisely

utilized in the creation of these columns, each material endowed with its own distinctive qualities and contributions (Determann, 2012).

Paper Chromatography

The supporting material in paper chromatography is a layer of cellulose that has been deeply saturated with water. This approach uses a thick filter paper as the support, with water droplets saturating its pores to create the stationary "liquid phase." A suitable liquid ingredient is enclosed within a developing tank during the mobile phase. Notably, "liquid-liquid" chromatography is categorized as a type of paper chromatography (Stoddard et al., 2007).

Ion-exchange chromatograph

Ion-exchange chromatography is a method of chromatography that is firmly based on the controlled regulation of electrostatic interactions between protein moieties that are embellished with discernable electric charges and a structurally sound, solid support matrix. In this situation, the matrix is endowed with a particular ion charge that is diametrically opposing to the polarity of the target protein that is expected to be isolated. The intriguing phenomenon wherein the protein entity exhibits a strong propensity, or a marked affinity, if you will, towards forging a cohesive connection with the very confines of the chromatographic column itself is made possible by this intriguing ion charge dichotomy, which creates a finely balanced milieu that fosters the orchestrated assembly of ionic bonds. The deft orchestration of carefully calibrated modulations in parameters, ranging from pH variations and the careful manipulation of ion salt concentrations to the astute manipulation of the ionic strength inherent in the buffer solution, allows for the strategic emancipation of proteins from

this chromatographic stage. Such artistic control results in the delicate dance organized with precision that is the separation of proteins from their chromatographic home, creating a ritualized separation process (Karlsson et al., 1998). The anion-exchange matrices, which are endowed with a positive ion charge by nature, capture negatively charged proteins with an almost magnetic attractiveness. These matrices function as a true haven for the adhesion and adsorption of proteins that exhibit a distinctive negative charge profile. In striking contrast, cation-exchange matrices gracefully manifest themselves as groupings of a negatively charged disposition. These matrices have an intriguing ability to catch, efficiently trap, and persistently retain proteins that are positively charged to a high degree, beautifully sealing their fate within their structured embrace (Amercham Biosciences, 2002).

Affinity Chromatography

It is possible to purify enzymes, hormones, antibodies, nucleic acids, and certain proteins using this chromatographic method (Wilchek and Chaiken, 2000). In this method, a ligand (such as dextran, polyacrylamide, cellulose, and others) that may form a compound with a specific protein is used to bind to the packing material of the column. In contrast to unbound proteins, which are allowed to leave the column, the specific protein that forms a complex with the ligand becomes attached to the solid support and is consequently kept inside the column. The bound protein is then released from the column by causing changes to its ionic strength, which can be accomplished by modifying pH or by adding a salt solution (Firer, 2001).

Dye-Ligand Chromatography

The finding that many enzymes have a strong propensity to form partnerships with purine nucleotides had a significant impact on the trajectory of dye-ligand chromatography. Empirical evidence of interactions between enzymes and the Cibacron Blue F3GA dye supported this discovery (Amicon, 1989). The planar ring shape with negatively charged moieties that characterizes this dye's structural motif has an intriguing resemblance to the three-dimensional structure of the Nicotinamide Adenine Dinucloetide (NAD) molecule. This fundamental structural connection is supported by the dye's demonstrated affinity for attaching to certain locations, specifically the adenine and ribose binding areas. This parallelism reflects the chemical interactions seen in the context of NAD. The dye exhibits an impressive ability to mimic the functional role of ADP-ribose, demonstrating a binding efficacy that greatly outperforms that of alternative adsorbents by a noticeable margin, with a magnitude approaching 10 to 20 times greater. The successful application of dye-ligand chromatography depends on exact control of environmental factors, such as maintaining ideal pH levels. The use of elution procedures that maximize the potential of high-ionic strength solutions further enhances this strategic orchestration. A harmonious alignment between the intrinsic ion-exchange properties of the adsorbent material and the judicious use of high-ionic strength elution solutions results in this symbiotic interaction. The successful separation of adsorbed proteins within the chromatographic column is the result of this complex interaction, and it represents a significant improvement in the field of protein separation and purification (Scopes, 1984; Cutler, 2004).

Hydrophobic Interaction Chromatography (HIC)

The chromatographic method known as Hydrophobic Interaction Chromatography (HIC) cleverly makes use of the adsorbent components that were created initially with the goal of ligand binding within the context of affinity chromatography. The hydrophobic contacts between side chains that are stably fixed to the chromatographic matrix are at the core of HIC (Mahn and Asenjo, 2005; Queiroz et al., 2001). These interactions dynamically unfold among the side chains.

Pseudoaffinity Chromatography

The diverse field of chromatographic methods includes pseudoaffinity chromatography as a distinctive and noteworthy element. The fascinating use of carefully chosen substances, particularly azodyes and anthraquinone dyes, which appear as ligands of unmatched significance, is the basis of this specialized method. Due to their distinctive chemical characteristics, these substances exhibit an outstanding and notable affinity, a trait that is especially noticeable in their interactions with enzymes from various classes, such as dehydrogenases, kinases, transferases, and reductases. Due to their increased ability to bind, these substances are particularly well suited to enhancing ligand-based interactions when used in chromatography (Porath, 1992). Immobilized Metal Affinity Chromatography (IMAC), a well-known and well-recognized manifestation of this novel technology, serves as an example embodiment that highlights the power of pseudoaffinity chromatography. IMAC essentially represents the use of pseudo affinity principles, successfully utilizing the remarkable affinity demonstrated by these specific chemicals to orchestrate accurate and targeted separations inside the chromatographic framework.

1.4 Ethnomedicinal Uses

The leaves of Ficus sur capensis, also known as the Broom Cluster Fig or Cape Fig, have been traditionally used by indigenous communities in South Africa for various ethnomedicinal purposes. (Ogunlaja, 2017). Infusions or decoctions made from Ficus sur capensis leaves have been used as a traditional remedy for respiratory ailments (Krief, et al 2005).

In some traditional medicinal practices, Ficus sur capensis leaves have been used to address gastrointestinal issues. Herbal preparations made from the leaves, such as teas or infusions, have been consumed to relieve stomachaches, indigestion, and diarrhea (Owolabi, et al 2022).

In addition to wound healing, Ficus sur capensis leaves may be applied topically to the skin to address various skin conditions, including irritations, rashes, and fungal infections (Mabona, et al., 2013).

Ficus sur capensis leaves have been used topically as poultices or dressings to promote the healing of wounds, cuts, and sores (Mabona, 2014).

Nearly every component of the F. capensis plant has been discovered to be helpful. Chewing the leaves can treat peptic ulcers, drinking leaf maceration can heal chest issues, and drinking leaf infusions can treat tonsillitis and stomach ache. The sap of young shoots is taken against gonorrhea, and preparations are used to treat infertility, tuberculosis, abscesses and sores, as well as being lactogenic, purgative, and aphrodisiac. Leaf decoctions are used as a disinfectant wash and in the treatment of ophthalmia. Leprosy, epilepsy, rickets, infertility, gonorrhoea, oedema, respiratory problems, and as an emollient have all been treated with the plant extensively . The Igede people of Benue state in Nigeria have used F. capensis as a cure for dysentery

and as a bandage for wounds. Pharmacological activities of F. capensis include antibacterial, relaxing of the gastrointestinal tract, antioxidant, immune system booster , and tocolytic activity (Esievo, et al 2018).

Traditionally, different parts of the plant such as the leaves, stem, bark, and root are believed by the Hausa communities in Northern Nigeria to be efficacious in the treatment of different ailments such as psychosis, inflammation, epilepsy, and depression. The antioxidant, anti-inflammatory, anti-insomnia, hepatoprotective, wound-healing, and analgesic properties of Ficus Sur have been described.

1.5 Pharmacological Properties of Ficus Capensis.

Ficus capensis, commonly known as the Cape fig or the banyan fig, is a species of fig tree native to southern Africa. While it is primarily known for its use in landscaping and as a shade tree, it may have some pharmacological properties as well. However, it's important to note that the pharmacological properties of Ficus capensis are not as well-studied as those of other medicinal plants, and more research is needed to fully understand its potential benefits and risks. Here are some of the reported pharmacological properties of Ficus capensis:

Anti-Inflammatory Properties: Some species of figs, including Ficus capensis, contain compounds with potential anti-inflammatory properties. These compounds may help reduce inflammation, which is associated with various health conditions (Ondua et al 2019).

Antioxidant Activity: Ficus capensis fruits contain antioxidants, which can help protect cells from oxidative damage caused by free radicals. Antioxidants have been linked to various health benefits (Akomolafe et al 2016).

Traditional medicine: In traditional African medicine, various parts of Ficus capensis, including the leaves and roots, have been used for their potential medicinal

properties. It has been used to treat ailments such as diarrhea, dysentery (Madikizela et al 2012).

Anti-microbial activity: Some research suggests that extracts from Ficus capensis leaves and other parts of the plant may have antimicrobial properties (Oyeleke et al 2008).

Wound Healing: In traditional medicine, Ficus capensis has been used topically to aid in wound healing. Some studies suggest that certain compounds in figs may promote wound healing.

Anti-Diabetic Potential: Some research has explored the potential of Ficus capensis extracts in managing diabetes. It is thought that certain compounds in figs may help regulate blood sugar levels (Akomolafe et al 2016).

Gastrointestinal Effects: In traditional medicine, Ficus capensis has been used to treat gastrointestinal disorders such as diarrhea and dysentery. It may have mild astringent properties that could help alleviate these symptoms. (Wickens 2008).

It's important to note that while Ficus capensis has shown potential in various pharmacological areas, more rigorous scientific research is needed to establish its safety and efficacy for specific medicinal uses. Additionally, the use of any plant or herbal remedy should be done under the guidance of a qualified healthcare professional, as there can be risks and interactions with other medications or health conditions.

1.6 Literature Review

Ficus capensis, also called the bush fig or the fig of heaven, is a quickly expanding deciduous or evergreen tree (Berg and Allg. 1990). It typically grows to a height of between 5 and 12 meters (16 and 39 feet), but it can grow as high as 35 to 40 meters (115 to 131 feet). The big, alternating, and spirally arranged leaves have unevenly

serrated edges and range in shape from ovate to elliptic. Fresh foliage has an obvious crimson color and is paper-like. In contrast to F. sycomorus's flaky, yellow back, young trees' bark is smooth and a light shade of greyish-white; as the tree ages, the bark darkens and becomes more rough. The figs are carried on short or long drooping spurs (or fascicles) which may emerge from surface roots, the trunk or especially from lower main branches. The figs are 2 to 4 cm in diameter and acquire a rosy, speckled exterior when ripe. The fig seeds are dispersed after passing through the intestinal tracts of birds, bats and primates. The tree is found from Cape Verde and Senegambia, across tropical West Africa to Cameroon and the Central Africa Republic, then eastwards to Eritrea, Northern Somalia and Yemen, and southwards through all tropical eastern and southern African countries. The tree is of variable height depending on location. In Senegal, the plant can be 4-6 m tall, while in Nigeria, it can be 6-9 m tall or up to 20 m or more. The trunk is 1.2m or over in girth, with spherical crown, often low-branching. The tree is of open and wooded Saudano-Guinean savanna and secondary jungle, by watercourses from Senegal to West Cameroon, in lowlands to high altitudes, and widespread in tropical Africa (Burkill 1997)

The tree is grown across Nigeria, however it is more prevalent in the Middle Belt (North Central) of the country. The plant is known as Ogbaikolo among the Igalas, Opoto in Yoruba (Fadimu and Mohammed 2014). Akokoro in the Nsukka region of Enugu State, and Uwaryara in Hausa. The Obada and Fulani in Edo State refer to it as Rimabichehi (Otitoju et al 2014).

1.7 Objectives of the Study

The objectives of the study include;

- To evaluate the phytochemical constituents present in the methanolic extract of *Ficus Capensis* using Gas chromatography Mass spectrometry(GC-MS) and High performance liquid chromatography mass spectrometry(HPLC-MS)
- To Evaluate the bioactive components in the crude extract of *Ficus Capensis.*

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Apparatus and Equipment

The experimental set-up and tools used in this investigation included a variety of material. These included a beaker, separating funnel, milling machine (Galiham), distilled water, test tube, water bath, glass bottle, Vagen cotton wool (100g), analytical weighing balance (Kerro P7 BL-2204), test-tube holder, universal bottle,

foil paper, hexane, dichloromethane, mesh silica gel, anhydrous sodium sulfate, and an Agilent 6890N Gas Chromatography system (Apparatus and Equipment section). Analytical-grade methanol (with a purity of 99.99%, ME0322500 Methanol, ExpertQ, ACS, ISO, Reag. Ph Eur) is the reagent utilized in this experiment.

2.2 Methods

Collection and identifications of plants

Ficus Sur Capensis plant was gotten from the campus of university of Benin-city Nigeria, between 5pm – 6pm in January 2023. The plants where selected with care, the plants leaves where identified by Dr. Akinnibosun Henry Adewale, an expert in the Department of Plant and Plant Biotechnology within the Faculty of Life Science at the University of Benin. As a testament to this process, a unique sample voucher number (UBH-F331) was assigned, and a representative specimen was carefully preserved in the herbarium for future reference.

The leaves of *Ficus Sur Capensis* was air dried for sixteen days before been ground to a fine powder with aid of mechanical grounded (British milling machine) and kept in a tight container until it was ready for used.

2.3 Extraction

Ficus Sur (Capensis) finely powdered plant material was extracted using a methanolic technique. A glass jar was used for this treatment, and the maceration method was used. Specifically, 750 mL of methanol was added to a glass jar along with 105.4g of the powdered plant material. The jar's contents were shaken ferociously for five minutes, then allowed to rest for thirty minutes. Three times they went through this shaking and resting cycle. The resultant solution was left undisturbed at room temperature for a total of 7 days. The solution was then put through filtration using

cotton wool and a glass funnel. In order to facilitate concentration, the filtrate was then air dried at room temperature. The final concentrate was kept within a refrigerator at a temperature of 4°C.

2.4 Phytochemical Screening Process

The powdered plant material was carefully weighed out to a quantity of 10.0000g, and then it was put into a clean, dry conical flask. Next, a thoughtfully calculated amount of water was added to completely cover the collected material inside the conical flask. Then, for the next 10 minutes, this combined concoction was placed on a water bath and gently heated. The finished composite was then put through a filtration process using cotton wool and a plastic funnel after this thermal treatment. The filtrate, or obtained solution, was used as the starting point for a variety of phytochemical assays, as described in the following explanation.

2.4.1 Cardiac Glycoside Test

A single drop of FeCl3 solution was added to a test tube containing 5 mL of the filtrate together with 2 mL of glacial acetic acid. When a brownish solution was observed as a result, it was determined that cardiac glycosides were absent (Talukdar and Chaudhary, 2010).

2.4.2 Saponin Test

In a test tube with 5 mL of the filtrate in it, 2 mL of distilled water was added, and the amalgam was violently stirred. According to Alamzeb et al. (2018), froth at the solution's surface functioned as a visual signal of saponin content.

2.4.3 Flavonoid Test

3 mL of the aqueous filtrate and 3 drops of sodium hydroxide (NaOH) solution were added to another test tube. Flavonoids were indicated by the emergence of a

prominent and bright yellow hue that turned colorless upon the addition of a few drops of diluted hydrochloric acid (HCl) (Khandelwal, 2008).

2.4.4 Steroid Test

A further 5 mL of the aqueous filtrate was added to a test tube, and then 2 mL of concentrated sulfuric acid (H2SO4) and chloroform were added. An obvious dark solution was found, indicating the lack of steroids.

2.4.5 Tannin Test (Braymer's Test)

3 mL of the filtrate and 3 drops of a 0.1% FeCl3 solution were put together in a different test tube. Following this, a potential color change was observed, with a blue-black colour signifying the presence of tannins (Sofowora, 1993).

2.5 Preparation of Plant Extract for GC-MS Analysis

An electronic balance was used to precisely weigh 50.0000 mg of the concentrated extract, which was then put into a beaker with care. To help the extract dissolve and become homogeneous, 10 mL of the solvent mixture (1:1 - Hexane: Dichloromethane) was carefully measured and added to the beaker flask. A column filled with 100-200 mm mesh silica gel and 3.0000 g of anhydrous sodium sulfate was used to purify the homogenized extract. Hexane was used to create a dense slurry during the pre-conditioning of this column. Thus, the resulting extract was prepared for later phytochemical screening. A 1 mL sample aliquot was added through the injection port for the subsequent GC-MS analysis.

2.6 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

An Agilent 6890N gas chromatograph with an autosampler and an Agilent mass spectrometer were used to perform the GC-MS study. Using the pulsed splitless mode, a 1 ml volume of the sample was injected onto a 30 m x 0.25 mm ID DB 5MS coated fused silica column with a film thickness of 0.15 mm. Helium was used as the carrier

gas, and a 20 psi column head pressure was maintained while the flow rate was kept constant at 1 ml/min. The operational parameters were predetermined, and the column temperature was first set at 55 °C for 0.4 minutes. Then the temperature was raised to 200 °C at a rate of 25 °C/min, then to 280 °C at a rate of 8 °C/min, and then maintaining the temperature for 2 minutes while increasing it by 25 °C per minute to its final temperature of 300 °C. Based on their retention times, the components were identified, with lower retention time components eluting before higher retention time components.

2.7 HPLC-MS Analysis of Methanol Extract from Ficus Capensis

2.7.0 Standard Solution Preparation

Nineteen alkaloidal standards were separately dissolved in methanol to create stock solutions with a concentration of 1000 g/ml, which served as the basis for the creation of a standard reference. Quinolinamine, Benzenesulfonamide, Allylamine, Benzamide, Indolizine, Pyrazoline, Imidazole, Propargylamine, Ethylenimine, Difluoramine, Simulansamide, Norethindrone, Androstane, Methanamine, Isoxazolidine, Isobutylamine, and Amphetamine were among the compounds on the list of standards used in this investigation. In order to maintain their integrity until use, these painstakingly created solutions were carefully maintained at 4°C in a refrigerator.

2.7.1 Sample Preparation for HPLC

200 mg of the homogenized methanolic leaf extract was carefully combined with 200 ml of deionized water. This combination then underwent a reflux process for an hour, after which it was allowed to cool to room temperature. The final extract was painstakingly run through 125 mm Whatman filter paper. The resultant filtrate was diluted using a 2% ammonia solution to achieve a 1:3 (v/v) ratio. The pH of the

solution was carefully adjusted to a neutral value of 7 using a 0.01 M HCl solution to assure analytical precision, creating the ideal circumstances for future HPLC analysis.

2.7.2 Separation and Clean-up

In the HPLC system, more precisely the 1260 Ultra High-Pressure Liquid Chromatography, a volume of five microliters carefully extracted from the diluted stock solution (80 g/ml) was introduced (injected). Methodically optimizing the chromatogram's peak separation while keeping close watch on the stock standards at a wavelength of 242 nm. The tabulated format shown below offers a thorough summary of the operational parameters for carrying out the HPLC analysis.

Table 2: HPLC conditions

Parameter	Conditions
Column	Agilent Lichrospher 100-5 RP8 (250x4.6 mm) (C18)
Flow rate	1.00 ml/min

Injection Volume		5 µL	
Column Temperature		35 ºC	
Mobile A		0.1 % phosphoric acid	
Mobile phase B		Methanol	
Run time		6 min.	
Gradient time			
Time (min.)	0	2.5	6
% B	25	25	50

CHAPTER THREE

3.1 RESULTS

Table 1. Organoleptic properties of the leaf of *Ficus sur capensis* plant. The appearance, aroma, texture and taste of the leaf of *Ficus sur capensis* were evaluated

and found to be elliptically-shaped, possess a characteristic herbal scent and a bitter taste.

Organoleptic property	Description
Appearance	Oval to elliptic in shape with irregularly serrated margins, the big, alternating, and spirally arranged leaves.
Aroma	Sweet scent
Texture	Shiny
Taste	Bitter to taste

Table 3.0. Methanolic extraction of *Ficus Sur (Capensis)* leaf

Solvent	Weight of Powdered sample (g)	Weight of extract (g)	Percentage yield (%)
Methanol	105.4	17.23	16.35

TABLE 3.1: Preliminary Analysis of Phytochemical Analysis of Ficus Sur

Phytochemicals	Result
Flavonoids	+ve

Saponins	-ve
Tannins	+ve
Cardiac glycosides	-ve
Steroids	+ve
Alkaloid	+ve

Where +ve: Present; -ve: Absent.

GCMS RESULT

S/N	RT	AREA%	COMPOUND
1	3.391	0.23	Pyridine

2	5.016	0.03	Propargylamine
3	5.216	3.10	Colchicine
4	5.502	0.62	N-Methoxy-2-carbomethoxyazetidine
5	5.611	0.19	3-Methylpentan-2, 4-dione dioxime
6	5.880	1.14	Maleimide
7	6.017	1.00	Chlorophenyl
8	6.274	0.52	Cis-2 3-Bis (2,4,5- trimethyl-3- thienyl
9	9.452	2.13	1,2-benzenedicarboxylic acid
10	6.681	0.30	8H-1, 3-Dioxolo isoindolo
11	6.887	0.96	Acetamide
12	7.047	0.84	Methyl-3-phenoxybenzoate methane
13	7.299	0.33	Sarcosine
14	7.447	1.10	Propenamide
15	7.550	1.15	Pyrimidinedione
16	7.917	3.76	1H-[yrazole-5-carboxamide, 3-cyclopropyl-N-(Dimethylamino) Carbonly phenyl-1-methyl
17	8.146	0.38	Estra-1
18	8.374	1.87	Pyrrolidine

19	8.661	0.61	Oxazolone
20	9.467	2.65	19-Norethindrone
21	9.610	1.82	Androstane
22	9.931	0.81	Benzocyclohepta lene
23	10.131	1.23	thiazolidine-4-one
24	10.280	1.92	Butanoic acid
25	10.543	1.17	2-phenoxyphenylacetonitrile
26	10.783	1.19	Benzamide,
27	11.024	1.97	2,20-Cycloaspidospermidine
28	11.201	1.78	Dimethoxypheny1
29	11.556	1.65	Sarpagan-16-carboxylix acid
30	11.785	1.41	2-Docosenoic Acid
31	12.174	0.43	Cobalt
32	12.483	0.37	Phosphonic acid
33	12.614	1.12	3-Methoxy-4-nitrobenzoic acid
34	12.901	1.24	Teicuclos
35	13.267	1.38	Silane
36	13.416	0.55	1H-pyrazole-3-carboxaldehyde

37	13.667	0.53	Phthalic acid
38	14.005	1.09	Cobalt
39	14.846	0.62	6-dicarboxylic acid, diethyl ester
40	15.149	0.54	Difluorobenzamide
41	15.424	2.18	6-)4-Methoxy-pheny1)-3-trifluoromethyl
42	15.596	1.75	Ethyl 4- (2-ethoxycarbony 1-7-methox butanoate
44	16.305	5.62	3Alpha-methoxy-3beta-(methanesulfo namido)
45	17.472	1.84	Propenoic acid
46	17.822	0.66	A-friedooleanane-1, 3-dione,
47	17.993	1.28	Fumarix acid, 4-isopropylpheny pentadecyl esther
48	19.853	5.19	4-(Beta-bromo-4- cloro-phenylstryryl)-N, N-dimethylaniline
49	21.054	0.93	Methyl p-[3-[,6-diamino-5-nitropyrimidin-4-ylamino] acetonyl] methyl amino] benzoate 2-oxime
50	21.266	0.08	4, 25-secoobscurinervan-4-ol,22-etyl-15, 16-dimethoxy-, diacetate(ester, (4. Beta., 22. Alpha)

51	21.678	2.27	2-methyl-7-acetoxy-2,4,50trimethoxy-isoflavone
52	22.044	0.03	Methanamine
53	22.691	0.96	Dimethyl 2-(1-methyl-2thioxo-3-indolinylidene)-1, 3-dithiole-4,5-dicarboxylate
54	23.984	0.93	1,3,4-Thiadiazol-2-amine, 5-methyl 1, 1-Diethylguanidine
55	24.293	0.18	Indolizine
56	24.436	0.25	Loxynil
57	24.802	0.20	Isoxazolidine
58	25.008	0.09	1-Methyl-3, 5-dinitro-1H-(1,2,4) triazole
59	25.260	0.35	Propargylamine
60	25.575	0.70	Propiolonitrile
61	25.712	0.76	Isobytylamine
62	26.508	3.83	N-Desmethyltapentadol
63	26.576	0.83	2-1-Phenyl ethylidene-hydrazone-3-methyl-2,3-dihydrobenzothiazole
64	26.954	6.65	1-(2-Amadantylidene) semicarbazie MDMA methylene homolog

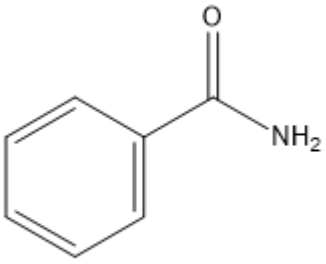
65	27.372	1.03	4-morpholine
66	27.955	5.00	Tricyclo(11.1.0.0(4,6)]tetradic-9-ene-5, 14-decarboxylix acid, dimethyl ester
67	28.424	1.05	Preg-4-en-3-one,

HPLCS RESULT

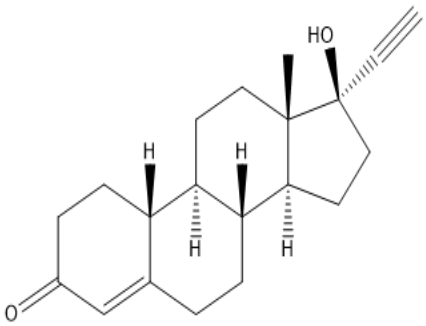
Table 3.3. Activity of Bioactive Compound Identified via HPLC-MS in methanolic extract of *Ficus Sur* leaf

Name	Retention Time (min)	Amount (ng/ul)
Benzenenesulfoname	1.781	63.41790
Norethindrone	3.054	16.01069
Benzamide	3.618	15.57004
Indolizine	4.035	13.97320
Pyrazoline	4.271	21.7457
Isobutylamine	5.426	15.0967
Colchicine	6.434	8.99242
Methanamine	6.877	8.16549
Simulansamide	7.161	4.42832
Androstone	7.804	27.18517
Propargylamine	8.159	9.40588

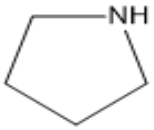
STRUCTURES OF SOME OF THE PHYTOCHEMICAL CONSTITUENTS



benzamide



Norethindrone



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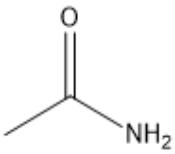

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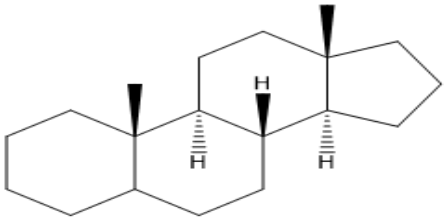

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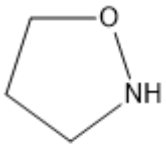
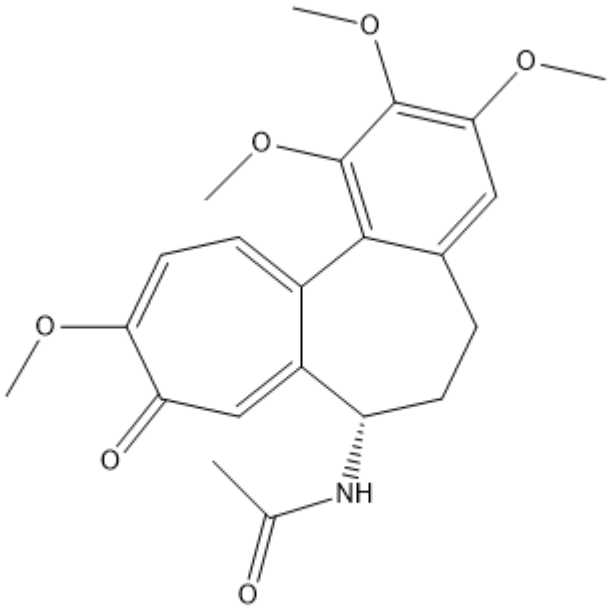
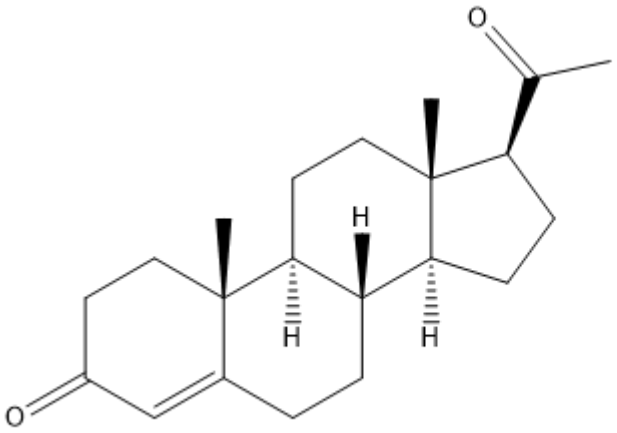


Isoxazolidine



N-[(7S)-1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide



Pregn-4-ene-3,20-dione

CHAPTER FOUR

4.0 Discussion

In the pursuit of exploring the potential therapeutic and bioactive properties of natural compounds, plant extracts have become a focal point of scientific research. *Ficus Sur*, commonly known as Capensis, has a history of traditional medicinal use (Krief, et al 2005).

Table 1 provides information on the organoleptic properties of *Ficus sur capensis* leaves. These properties, which include appearance, aroma, texture, and taste, offer initial sensory insights into the plant. The leaves were observed to be oval to elliptic in shape with serrated margins, featuring alternating and spirally arranged leaves. They possessed a sweet scent, a shiny texture, and a bitter taste.

This study delves into the chemical composition of *Ficus Sur* leaves, specifically focusing on a methanolic extraction process. The results of this extraction, alongside preliminary phytochemical analysis, Gas Chromatography-Mass Spectrometry (GC-MS) data, and High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) findings, provide valuable insights into the potential bioactive compounds present in *Ficus Sur* leaves.

The first set of results reveals the efficiency of the methanolic extraction process. From 105.4 grams of powdered *Ficus Sur* leaves, 17.23 grams of extract were obtained, yielding a percentage of 16.35%. This indicates that the chosen solvent, methanol, was effective in extracting a substantial portion of the plant's chemical constituents.

Table 3.1 presents a preliminary phytochemical analysis, where the presence or absence of various phytochemicals in the *Ficus Sur* leaf extract is determined. This analysis is crucial in understanding the potential medicinal or nutritional properties of

the extract. Notable findings include the presence of flavonoids, tannins, and alkaloids, while saponins and cardiac glycosides were absent. These results suggest the presence of compounds associated with antioxidant and medicinal properties, which are often found in plant extracts.

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis provides a comprehensive list of compounds identified in the *Ficus Sur* leaf extract. Some significant compounds include Colchicine, Maleimide, Androstane, and 1,2-benzenedicarboxylic acid. These compounds span a range of chemical classes and may have various biological activities. Colchicine, for instance, is known for its anti-inflammatory properties, making it a potential candidate for further investigation.

The High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) analysis focuses on quantifying specific bioactive compounds in the extract. Noteworthy compounds include Benzenenesulfoname, Norethindrone, Androstone,4-morphine and others.

Phytochemical constituents of pharmacological importance are discussed below: Norethindrone is a synthetic progestin, which is a type of female sex hormone. It has several pharmacological uses in the field of gynecology and reproductive health (Rivera et al 1999).

Norethindrone is commonly used as a progestin component in combination oral contraceptives (birth control pills). It is often combined with an estrogen hormone to provide effective contraception by suppressing ovulation, thickening cervical mucus to inhibit sperm penetration, and altering the endometrial lining to prevent embryo implantation(Rivera et al 1999).

Norethindrone may be prescribed as part of hormone replacement therapy for postmenopausal women. It can help alleviate menopausal symptoms such as hot

flashes and vaginal dryness by providing a progestin component to balance estrogen therapy. Norethindrone is typically used in combination with estrogen in these cases (Vestergaard et al 2003).

Norethindrone may be used to treat abnormal uterine bleeding, including heavy menstrual bleeding (menorrhagia) and irregular menstrual cycles. It can help regulate the menstrual cycle and reduce excessive bleeding by stabilizing the uterine lining (Gray 2007).

Norethindrone is sometimes prescribed to manage endometriosis, a condition where endometrial tissue grows outside the uterus. It can suppress the growth of endometrial tissue, reduce pain associated with endometriosis, and help control symptoms of the condition (O'Reilly et al 2012).

It's important to note that the specific formulation, dosage, and duration of norethindrone treatment can vary depending on the medical condition being addressed and individual patient factors. Norethindrone should only be used under the guidance and prescription of a healthcare provider, and potential risks and side effects should be discussed with a medical professional before starting any treatment.

Pyridine is an organic compound with a ring structure containing a nitrogen atom. It has a variety of industrial and laboratory applications, but it is not commonly used as a medication or therapeutic agent for pharmacological purposes in human medicine (Zarenezhad et al 2021).

Pyridine is often used as a chemical intermediate in the synthesis of various pharmaceutical compounds and drugs. It can be part of the structural framework of certain drugs (Horton et al 2003).

Pyridine is used as a solvent in pharmaceutical research and development, particularly in the synthesis and analysis of drugs and drug formulations (Lockley et al 2012).

Pyridine derivatives are sometimes used in the flavor and fragrance industry to create specific scents or tastes (Hammond, 2013).

While pyridine itself is not directly used as a medication, its derivatives and related compounds may have pharmaceutical applications. However, the use of such compounds is typically limited to specific medical conditions and is guided by regulatory agencies and healthcare professionals. It is important to handle pyridine and its derivatives with care and in accordance with safety guidelines due to their chemical properties. Benzene-sulfonamide, also known as sulfanilamide, is a sulfonamide antibiotic that has been historically used in pharmacology and medicine. Its pharmacological uses have evolved over time, and it has been mostly replaced by other antibiotics due to the development of antibiotic resistance and the availability of safer and more effective options (Tačić, *et al* 2017).

Sulfanilamide was one of the first synthetic antibiotics developed in the mid-20th century. It works by inhibiting the growth of bacteria, particularly gram-positive and some gram-negative bacteria, by interfering with the synthesis of folic acid, a crucial component for bacterial DNA and RNA synthesis. However, it is less effective against many modern antibiotic-resistant strains of bacteria (Tačić, et al 2017).

Sulfanilamide and related sulfonamide drugs were used to treat urinary tract infections caused by susceptible bacteria. However, due to the development of more effective and less toxic antibiotics, sulfonamides are now rarely used for this purpose. (Petri 2006).Sulfanilamide was historically used topically as a powder or ointment to prevent or treat bacterial infections in burns and wounds. It was applied to the affected area to inhibit bacterial growth. (Dai et al 2010).Sulfanilamide and other sulfonamide drugs were used as part of early treatment regimens for malaria caused by Plasmodium species. However, they are no longer recommended for this purpose

due to widespread resistance and the availability of more effective antimalarial medications (Schlitzer 2007).It's important to note that the use of sulfanilamide and related sulfonamides has significantly declined over the years due to concerns about drug resistance, side effects, and the availability of safer and more effective antibiotics and antimicrobial agents. In many cases, they have been replaced by other classes of antibiotics, such as penicillins, cephalosporins, and fluoroquinolones, which are more selective and have a lower risk of resistance.

Peg-4-En-3-One, Also Known As Pregnenolone, Is a Natural Steroid Hormone and a precursor to various other hormones in the body. While it is not typically used as a pharmacological agent itself, pregnenolone plays a vital role in the biosynthesis of other important hormones, and its derivatives may have certain pharmacological uses. Here's an overview; (Söderström, 2001).

Androstane is a naturally occurring steroid compound with a molecular structure based on the androstane skeleton. It is a fundamental structure in the biosynthesis of various important steroid hormones, including androgens (male sex hormones) and certain other hormones produced by the adrenal glands. (Báthori et al 2008).Androstane represents a critical component of the endocrine system and is central to the development and maintenance of male sexual characteristics and other physiological processes. Its role in hormone biosynthesis underscores its significance in both basic biology and medical applications. (Gracia, 2018).

4-Morpholine is a chemical compound with a morpholine ring structure. While it is not typically used as a medication on its own, morpholine-based compounds may have various pharmacological uses (El Newahie 2016).Morpholine is often used as an intermediate in the synthesis of various pharmaceutical compounds and drugs. It can be incorporated into the chemical structure of drugs to impart specific properties

or functions (Ahmed 2023).Some morpholine derivatives have been studied for their potential antiviral properties. These compounds may be explored for their ability to inhibit viral replication or infection (De Castro et al 2022).It's important to note that the specific pharmacological applications of morpholine derivatives can vary widely depending on the chemical structure of the compound and its intended purpose. The use of such compounds in medicine is guided by regulatory agencies and healthcare professionals to ensure safety and efficacy. Additionally, the handling of chemical compounds like 4-morpholine should always be done in accordance with safety guidelines and regulations.

benzamide derivatives have therapeutic uses, the pharmacological properties and safety profiles of specific compounds may vary significantly. As with any medication, the use of benzamide derivatives should be under the supervision of a healthcare professional, and the choice of a particular drug should depend on the specific medical condition being treated and individual patient considerations. Additionally, regulatory agencies like the FDA (U.S. Food and Drug Administration) evaluate and approve drugs based on their safety and efficacy before they can be prescribed for clinical use (Dinarello, 2010).

Pyrrolidine itself is not typically used as a pharmacological agent in medicine, but it can serve as a structural element or a part of the chemical composition in various pharmaceutical drugs. Pyrrolidine-containing compounds or derivatives have a wide range of pharmacological applications. Here are some examples: antipsychotic medications, antiarrhythmias, antidepressants, analgesic, antiviral drugs (Łowicki,. and Przybylski, 2022).

Propargylamine, also known as selegiline, is a pharmaceutical compound used for various pharmacological purposes, primarily in the treatment of Parkinson's disease and depression. Here are its main pharmacological uses; parkison, depression, neuro protective and for cognitive enhancement (Youdim, and Bakhle, 2006).

Indolizine is a heterocyclic compound that contains an indole and pyridine ring fused together. It is not as extensively studied as some other heterocyclic compounds like indole or pyridine, but it does have some potential pharmacological uses (Jia, et al 2012).Here are a few potential pharmacological applications of indolizine:Some indolizine derivatives have demonstrated antimicrobial activity. Researchers have explored their potential as antimicrobial agents, particularly in the context of drug-resistant bacterial strains(Rahman, et al 2021). Indolizines have been studied for their anti-inflammatory properties. They may have potential in the development of drugs for treating inflammatory conditions (Shrivastava, et al 2017).Some indolizine derivatives have shown antioxidant activity. Antioxidants help protect cells from oxidative stress and are important in various health conditions.(Ringel, et al, 2001).

Oxazolone is a chemical compound with potential pharmacological uses, primarily in research settings. It is not typically used as a therapeutic drug in clinical medicine. Instead, it is often employed in laboratory studies and experiments, particularly in the fields of immunology and dermatology(Recio et al 2012).

Methanamine, also known as hexamethylenetetramine or urotropin, is a chemical compound that has pharmacological uses in the treatment of urinary tract infections. When ingested, methanamine is converted in the urine into formaldehyde and ammonia, which create an environment that is inhospitable for the growth of bacteria in the urinary tract. Methenamine is used as a urinary antiseptic for the prevention and treatment of urinary tract infections. It is often prescribed for individuals who have

recurrent UTIs. When methenamine is excreted in the urine, it breaks down into formaldehyde, which is bactericidal (kills bacteria), and ammonia, which makes the urine more alkaline. This combination helps to prevent the growth and multiplication of bacteria in the urinary tract (Grayson and Whitby, 2010).

Sarcosine, also known as N-methylglycine, is a naturally occurring amino acid derivative that has been investigated for various potential pharmacological uses, primarily in the context of neuropsychiatric and neurological conditions. However, it's important to note that the clinical evidence for many of these potential uses is limited, and more research is needed to establish their efficacy and safety(Murrough, et al, 2017)

Acetamide itself does not have significant pharmacological uses as a standalone medication. However, it is used as a starting material or intermediate compound in the synthesis of various pharmaceuticals and chemicals. Acetamide's primary pharmacological relevance lies in its role as a chemical precursor in drug development and the synthesis of other compounds rather than as a direct therapeutic agent (Lednicer 2007). Some pharmaceutical compounds that contain acetamide as a structural component include;

Acetaminophen, also known as paracetamol, is a widely used over-the-counter pain reliever and fever reducer (Blough, and Wu 2011).

Chloramphenicol is an antibiotic that was once commonly used to treat a variety of bacterial infections. Acetamide is one of the structural elements in the chloramphenicol molecule.Thiacetazone is an antimicrobial agent used in the treatment of tuberculosis. It is a derivative of acetamide and has been used in combination therapy for tuberculosis treatment.It's important to note that while acetamide itself may not have direct pharmacological uses, its derivatives and

compounds that contain acetamide as part of their chemical structure can have therapeutic applications. These compounds are typically synthesized and formulated to optimize their pharmacological properties and effectiveness in treating specific medical conditions.

4.1 CONCLUSION

In conclusion, this study has shed light on the chemical composition of *Ficus Sur (Capensis)* leaf extract. The methanolic extraction yielded a substantial amount of extract, suggesting its potential for further investigation. Preliminary phytochemical analysis indicates the presence of flavonoids, tannins, and alkaloids, which are known for their beneficial properties. The GC-MS and HPLC-MS results revealed a diverse array of compounds, some of which have known pharmacological significance.

These findings provide a solid foundation for future research into the medicinal and nutritional potential of *Ficus Sur* leaves.Overall, this research contributes to the growing body of knowledge on the chemical composition of natural plant extracts and their potential health benefits.

REFERENCES

Ahmed, K., Chotana, G.A., Faisal, A. and Zaib Saleem, R.S., 2023. Chemical Synthesis of Selenium-containing Peptides. *Mini Reviews in Medicinal Chemistry*, *23*(10), pp.1090-1117.

Aisen, P.S. and Davis, K.L., 1997. The search for disease-modifying treatment for Alzheimer's disease. *Neurology*, *48*(5 Suppl 6), pp.35S-41S.

Ajibesin K. K. (2011). Dacryodes edulis (G. Don) H. J. Lam: a review on its medicinal, phytochemical and economic properties. Research Journal of Medicinal Plant 5 (1): 32 - 41.

Akomolafe, S.F., Oboh, G., Oyeleye, S.I. and Boligon, A.A., 2016. Aqueous extract from Ficus capensis leaves inhibits key enzymes linked to erectile dysfunction and prevent oxidative stress in rats' penile tissue. *NFS journal*, *4*, pp.15-21.

Alamgir, A.N.M. and Alamgir, A.N.M., 2017. Herbal drugs: their collection, preservation, and preparation; evaluation, quality control, and standardization of herbal drugs. *Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1: Pharmacognosy*, pp.453-495.

Alamgir, A.N.M. and Alamgir, A.N.M., 2017. Herbal drugs: their collection, preservation, and preparation; evaluation, quality control, and standardization of herbal drugs. *Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1: Pharmacognosy*, pp.453-495.

Alfadda, A.A. and Sallam, R.M., 2012. Reactive oxygen species in health and disease. *BioMed research international*, *2012*.

Ali, A.H., Abdelrahman, M. and El-Sayed, M.A., 2019. Alkaloid role in plant defense response to growth and stress. *Bioactive molecules in plant defense: signaling in growth and stress*, pp.145-158.

Asif, M., 2015. Chemistry and antioxidant activity of plants containing some phenolic compounds. *Chemistry international*, *1*(1), pp.35-52.

Barman, A., Marak, C.M., Barman, R.M. and Sangma, C.S., 2018. Nutraceutical properties of legume seeds and their impact on human health. In *Legume seed nutraceutical research*. IntechOpen.

Báthori, M., Tóth, N., Hunyadi, A., Márki, Á. and Zador, E., 2008. Phytoecdysteroids and anabolic-androgenic steroids-structure and effects on humans. *Current medicinal chemistry*, *15*(1), pp.75-91.

Berg CC. Distribution of African taxa of Ficus (Moraceae). [Proc. 12th AETFAT]. Mitt. Inst. Allg. Bot. Hamburg. 1990;23: 401-405.

Bhatla, S.C., A. Lal, M. and Bhatla, S.C., 2018. Secondary metabolites. *Plant physiology, development and metabolism*, pp.1099-1166.

Bidlingmeyer, B.A., 1993. *Practical HPLC methodology and applications*. John Wiley & Sons.

Blough, E.R. and Wu, M., 2011. Acetaminophen: beyond pain and fever-relieving. *Frontiers in pharmacology*, *2*, p.72.

Bubalo, M.C., Vidović, S., Redovniković, I.R. and Jokić, S., 2018. New perspective in extraction of plant biologically active compounds by green solvents. *Food and Bioproducts Processing*, *109*, pp.52-73.

Burkill HM. The useful plants of West Tropical Africa. 1997;4:194-197.

C Recio, M., Andujar, I. and L Rios, J., 2012. Anti-inflammatory agents from plants: progress and potential. *Current medicinal chemistry*, *19*(14), pp.2088-2103.

Chemat, F., Rombaut, N., Sicaire, A.G., Meullemiestre, A., Fabiano-Tixier, A.S. and Abert-Vian, M., 2017. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics sonochemistry*, *34*, pp.540-560.

Dai, T., Huang, Y.Y., K Sharma, S., T Hashmi, J., B Kurup, D. and R Hamblin, M., 2010. Topical antimicrobials for burn wound infections. *Recent patents on anti-infective drug discovery*, *5*(2), pp.124-151.

De Castro, F., De Luca, E., Benedetti, M. and Fanizzi, F.P., 2022. Platinum compounds as potential antiviral agents. *Coordination Chemistry Reviews*, *451*, p.214276.

De Castro, M.L. and Garcıa-Ayuso, L.E., 1998. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. *Analytica chimica acta*, *369*(1-2), pp.1-10.

Dinarello, C.A., 2010. Anti-inflammatory agents: present and future. *Cell*, *140*(6), pp.935-950.

El Newahie, A.M., Ismail, N.S., Abou El Ella, D.A. and Abouzid, K.A., 2016. Quinoxaline-Based Scaffolds Targeting Tyrosine Kinases and Their Potential Anticancer Activity. *Archiv der Pharmazie*, *349*(5), pp.309-326.

Esievo, K., Anthony, S., Fatokun, O. and Kunle, O., 2018. Ficus capensis Thumb.(Moraceae): review of its ethnomedicinal uses, pharmacological activities and phytochemical constituents. *Archives of Current Research International*, *12*(3), pp.1-7.

Eskilsson, C.S. and Björklund, E., 2000. Analytical-scale microwave-assisted extraction. *Journal of chromatography A*, *902*(1), pp.227-250.

Fadimu Y, Mohammed Z. Ethnomedicinal survey of anti-typhoid plants in Ijebu Ode Local Government Area of Ogun State, Nigeria. Int. J Sci Nat. 2014;5(2):332-336.

Garcia, M., Thirouard, L., Sedès, L., Monrose, M., Holota, H., Caira, F., Volle, D.H. and Beaudoin, C., 2018. Nuclear receptor metabolism of bile acids and xenobiotics: a coordinated detoxification system with impact on health and diseases. *International journal of molecular sciences*, *19*(11), p.3630.

Gray, S.H. and Emans, S.J., 2007. Abnormal vaginal bleeding in adolescents. *Pediatrics in Review*, *28*(5), pp.175-182.

Grayson, M.L. and Whitby, M., 2010. 89 Methenamine Mandelate and Methenamine Hippurate. *THE USE OF ANTIBIOTICS*, p.1205.

Guardiola-Márquez, C.E., Santos-Ramírez, M.T., Segura-Jiménez, M.E., Figueroa-Montes, M.L. and Jacobo-Velázquez, D.A., 2022. Fighting Obesity-Related Micronutrient Deficiencies through Biofortification of Agri-Food Crops with Sustainable Fertilization Practices. *Plants*, *11*(24), p.3477.

Guiochon, G. and Guillemin, C.L., 1988. *Quantitative gas chromatography for laboratory analyses and on-line process control*. Elsevier.

Hammond, C.J., 2013. Chemical composition of household malodours–an overview. *Flavour and Fragrance Journal*, *28*(4), pp.251-261.

Handa, S.S., 2008. An overview of extraction techniques for medicinal and aromatic plants. *Extraction technologies for medicinal and aromatic plants*, *1*(1), pp.21-40.

igoli JO, Ogaji OG, Tor-Aryiin A, Igoli NP (2005).Traditional Medicinal Practices Amongst the Igede people of Nigeria. Part 11 Afr. J. Tradit. Complement. Alternat. Med. 2(2): 134-152.

Jha, A.K. and Sit, N., 2022. Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends in Food Science & Technology*, *119*, pp.579-591.

Jha, A.K. and Sit, N., 2022. Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends in Food Science & Technology*, *119*, pp.579-591.

Jia-Jie, W., Zhu, Y. and Zhan, Z.P., 2012. The synthesis of aromatic heterocycles from propargylic compounds. *Asian Journal of Organic Chemistry*, *1*(2), pp.108-129.

Josephine Ozioma E. O., Antoinette N., Chinwe O. J., Chinwe O. A. N. Herbal medicines in african traditional medicine. *Herbal Medicine,* 2019;10:190–212. doi: 10.5772/intechopen.80348. [CrossRef] [Google Scholar].

Joshua K (2006). Conservation of Indigenous Medicinal Botanicals in Ekiti. State, Nigeria. Research paper.

Kate, A.E., Singh, A., Shahi, N.C., Pandey, J.P., Prakash, O. and Singh, T.P., 2016. Novel eco-friendly techniques for extraction of food based lipophilic compounds from biological materials. *Nat. Prod. Chem. Res*, *4*(5), p.1000231.

Kaur, N., 2023. *Synthesis of Azetidines from Imines by Cycloaddition Reactions*. Elsevier.

Kosalec, I., Cvek, J. and Tomic, S., 2009. Contaminants of medicinal herbs and herbal products. *Arhiv za higijenu rada i toksikologiju*, *60*(4), p.485.

Krief, S., Hladik, C.M. and Haxaire, C., 2005. Ethnomedicinal and bioactive properties of plants ingested by wild chimpanzees in Uganda. *Journal of ethnopharmacology*, *101*(1-3), pp.1-15.

Krief, S., Hladik, C.M. and Haxaire, C., 2005. Ethnomedicinal and bioactive properties of plants ingested by wild chimpanzees in Uganda. *Journal of ethnopharmacology*, *101*(1-3), pp.1-15.

Kumar, K., Yadav, A.N., Kumar, V., Vyas, P. and Dhaliwal, H.S., 2017. Food waste: A potential bioresource for extraction of nutraceuticals and bioactive compounds. *Bioresources and Bioprocessing*, *4*, pp.1-14.

Lednicer, D., 2007. *The Organic Chemistry of Drug Synthesis, Volume 7* (Vol. 7). John Wiley & Sons.

Lockley, W.J.S., McEwen, A. and Cooke, R.A.C.H.A.E.L., 2012. Tritium: a coming of age for drug discovery and development ADME studies. *Journal of Labelled Compounds and Radiopharmaceuticals*, *55*(7), pp.235-257.

Lopez-Hortas, L., Rodriguez, P., Diaz-Reinoso, B., Gaspar, M.C., de Sousa, H.C., Braga, M.E. and Dominguez, H., 2022. Supercritical fluid extraction as a suitable technology to recover bioactive compounds from flowers. *The Journal of Supercritical Fluids*, *188*, p.105652.

Łowicki, D. and Przybylski, P., 2022. Tandem construction of biological relevant aliphatic 5-membered N-heterocycles. *European Journal of Medicinal Chemistry*, *235*, p.114303.

Mabona, U., 2014. *Antimicrobial activity of southern African medicinal plants with dermatological relevance* (Doctoral dissertation).

Mabona, U., Viljoen, A., Shikanga, E., Marston, A. and Van Vuuren, S., 2013. Antimicrobial activity of southern African medicinal plants with dermatological

relevance: from an ethnopharmacological screening approach, to combination studies and the isolation of a bioactive compound. *Journal of Ethnopharmacology*, *148*(1), pp.45-55.

Madikizela, B., Ndhlala, A.R., Finnie, J.F. and Van Staden, J., 2012. Ethnopharmacological study of plants from Pondoland used against diarrhoea. *Journal of Ethnopharmacology*, *141*(1), pp.61-71.

Manousi, N., Sarakatsianos, I. and Samanidou, V., 2019. Extraction techniques of phenolic compounds and other bioactive compounds from medicinal and aromatic plants. In *Engineering tools in the beverage industry* (pp. 283-314). Woodhead Publishing.

Marathe, S.J., Jadhav, S.B., Bankar, S.B. and Singhal, R.S., 2017. Enzyme-assisted extraction of bioactives. *Food bioactives: Extraction and biotechnology applications*, pp.171-201.

McKenna, D.J., 2004. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. *Pharmacology & therapeutics*, *102*(2), pp.111-129.

McNair, H.M., Miller, J.M. and Snow, N.H., 2019. *Basic gas chromatography*. John Wiley & Sons.

Mennen, L.I., Sapinho, D., de Bree, A., Arnault, N., Bertrais, S., Galan, P. and Hercberg, S., 2004. Consumption of foods rich in flavonoids is related to a decreased cardiovascular risk in apparently healthy French women. *The Journal of nutrition*, *134*(4), pp.923-926.

Mensah K. G., Forkuo A. D., Firempong C., Anning A. K., Dickson R. A. Toxicity and safety implications of herbal medicines used in africa. *Herbal*

Medicine . 2019;10:63–84. doi: 10.5772/intechopen.72437. [CrossRef] [Google Scholar].

Murrough, J.W., Abdallah, C.G. and Mathew, S.J., 2017. Targeting glutamate signalling in depression: progress and prospects. *Nature Reviews Drug Discovery*, *16*(7), pp.472-486.

Naviglio, D., Scarano, P., Ciaravolo, M. and Gallo, M., 2019. Rapid Solid-Liquid Dynamic Extraction (RSLDE): A powerful and greener alternative to the latest solid-liquid extraction techniques. *Foods*, *8*(7), p.245.

Ogunlaja, O.O., 2017. *Phytochemical, elemental and biological studies of three Ficus species (Moraceae) found in KwaZulu-Natal, South Africa* (Doctoral dissertation).

Ondua, M., Njoya, E.M., Abdalla, M.A. and McGaw, L.J., 2019. Anti-inflammatory and antioxidant properties of leaf extracts of eleven South African medicinal plants used traditionally to treat inflammation. *Journal of ethnopharmacology*, *234*, pp.27-35.

O'Reilly, B., Bottomley, C. and Rymer, J., 2012. *Essentials of Obstetrics and Gynaecology E-Book*. Elsevier Health Sciences.

Owolabi, A.O., Ndako, J.A., Owa, S.O., Oluyori, A.P., Oludipe, E.O. and Akinsanola, B.A., 2022. Antibacterial and Phytochemical Potentials of Ficus capensis Leaf Extracts Against Some Pathogenic Bacteria. *Tropical Journal of Natural Product Research*, *6*(3).

Oyeleke, S. B., Dauda, B. E. N., Boye, O. A., (2008): Antibacterial activity of Ficus capensis. Afr. J. Biotechnol., 7: 1414 - 1417.

Oyeleke, S.B., Dauda, B.E.N. and Boye, O.A., 2008. Antibacterial activity of Ficus capensis. *African Journal of Biotechnology*, *7*(10).

Petri, W.A., 2006. Sulfonamides, trimethoprim, sulfamethoxazole, quinolones, and agents for urinary tract infections. *Goodman & Gilman's the pharmacological basis of therapeutics. New York (NY): McGraw Hill*, pp.1111-1125.

Pico, Y., 2013. Ultrasound-assisted extraction for food and environmental samples. *TrAC Trends in Analytical Chemistry*, *43*, pp.84-99.

Prado, J. and Rostagno, M. eds., 2022. *Natural product extraction: principles and applications*. Royal Society of Chemistry.

Puri, M., Sharma, D. and Barrow, C.J., 2012. Enzyme-assisted extraction of bioactives from plants. *Trends in biotechnology*, *30*(1), pp.37-44.

Raaman, N., 2006. *Phytochemical techniques*. New India Publishing.

Rahman, M.M., Rahaman, M.S., Islam, M.R., Hossain, M.E., Mannan Mithi, F., Ahmed, M., Saldías, M., Akkol, E.K. and Sobarzo-Sánchez, E., 2021. Multifunctional therapeutic potential of phytocomplexes and natural extracts for antimicrobial properties. *Antibiotics*, *10*(9), p.1076.

Rieseberg, T.P., Dadras, A., Fürst-Jansen, J.M., Ashok, A.D., Darienko, T., De Vries, S., Irisarri, I. and De Vries, J., 2023, January. Crossroads in the evolution of plant specialized metabolism. In *Seminars in cell & developmental biology* (Vol. 134, pp. 37-58). Academic Press.

Ringel, F., Schmid-Elsaesser, R. and Liang, A.C., 2001. Antioxidants for CNS ischaemia and trauma. *Expert Opinion on Therapeutic Patents*, *11*(6), pp.987-997.

Rivera, R., Yacobson, I. and Grimes, D., 1999. The mechanism of action of hormonal contraceptives and intrauterine contraceptive devices. *American journal of obstetrics and gynecology*, *181*(5), pp.1263-1269.

Rivera, R., Yacobson, I. and Grimes, D., 1999. The mechanism of action of hormonal contraceptives and intrauterine contraceptive devices. *American journal of obstetrics and gynecology*, *181*(5), pp.1263-1269.

Romanik, G., Gilgenast, E., Przyjazny, A. and Kamiński, M., 2007. Techniques of preparing plant material for chromatographic separation and analysis. *Journal of biochemical and biophysical methods*, *70*(2), pp.253-261.

Schlitzer, M., 2007. Malaria chemotherapeutics part I: History of antimalarial drug development, currently used therapeutics, and drugs in clinical development. *ChemMedChem: Chemistry Enabling Drug Discovery*, *2*(7), pp.944-986.

Seidel, V., 2005. Initial and bulk extraction. *Natural products isolation*, pp.27-46.

Shaw, E.F. and Charters, S., 2016. Functional drinks containing herbal extracts. *Chemistry and Technology of Soft Drinks and Fruit Juices*, pp.310-355.

Shrivastava, S.K., Srivastava, P., Bandresh, R., Tripathi, P.N. and Tripathi, A., 2017. Design, synthesis, and biological evaluation of some novel indolizine derivatives as dual cyclooxygenase and lipoxygenase inhibitor for anti-inflammatory activity. *Bioorganic & medicinal chemistry*, *25*(16), pp.4424-4432.

Söderström, T., 2001. *Molecular endocrinology of target enzymes in androgen metabolism: implications for prostate cancer*. Institutionen för medicinsk laboratorievetenskap och teknik/Department of Laboratory Sciences and Technology.

Sofowora A (1982) Medicinal Plants and Traditional Medicine in Africa: John Willy. New York, p. 289.

Subedi, B., Aguilar, L., Robinson, E.M., Hageman, K.J., Björklund, E., Sheesley, R.J. and Usenko, S., 2015. Selective pressurized liquid extraction as a sample-preparation

technique for persistent organic pollutants and contaminants of emerging concern. *TrAC Trends in Analytical Chemistry*, *68*, pp.119-132.

Tačić, A., Nikolić, V., Nikolić, L. and Savić, I., 2017. Antimicrobial sulfonamide drugs. *Advanced technologies*, *6*(1), pp.58-71.

Thomas, D., Schütze, B., Heinze, W.M. and Steinmetz, Z., 2020. Sample preparation techniques for the analysis of microplastics in soil—a review. *Sustainability*, *12*(21), p.9074.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A. and Yangsabai, A., 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, *5*(3), p.93.

Uwineza, P.A. and Waśkiewicz, A., 2020. Recent advances in supercritical fluid extraction of natural bioactive compounds from natural plant materials. *Molecules*, *25*(17), p.3847.

Veit, J., 2019. *Efficient Workflows for Analyzing High-Performance Liquid Chromatography Mass Spectrometry-Based Proteomics Data* (Doctoral dissertation, Universität Tübingen).

Vestergaard, P., Hermann, A.P., Stilgren, L., Tofteng, C.L., Sørensen, O.H., Eiken, P., Nielsen, S.P. and Mosekilde, L., 2003. Effects of 5 years of hormonal replacement therapy on menopausal symptoms and blood pressure—a randomised controlled study. *Maturitas*, *46*(2), pp.123-132.

Wangensteen, H., Samuelsen, A. B., Malterud, K. E., (2004): Antioxidant activity in extracts from coriander. Food Chem., 88: 293 - 297.

Wickens, G.E., 2008. Human and veterinary medicine. *The Baobabs: Pachycauls of Africa, Madagascar and Australia*, pp.81-100.

Yasir, M., Goyal, A. and Sonthalia, S., 2018. Corticosteroid adverse effects.

Youdim, M.B. and Bakhle, Y.S., 2006. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. *British journal of pharmacology*, *147*(S1), pp.S287-S296.

Yusoff, I.M., Taher, Z.M., Rahmat, Z. and Chua, L.S., 2022. A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. *Food research international*, *157*, p.111268.

Zarenezhad, E., Farjam, M. and Iraji, A., 2021. Synthesis and biological activity of pyrimidines-containing hybrids: Focusing on pharmacological application. *Journal of Molecular Structure*, *1230*, p.129833.