PHYTOCHEMICAL SCREENING AND SPLENO AND HEPATO SOMATIC INDICES OF METHANOL EXTRACT OF *TETRAPLEURA TETRAPTERA* IN CANCER INDUCED WISTER RAT

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CERTIFICATION

This is to certify that the research project was carried out by ONOSE PAMELA MOMODU with the matriculation number PSC2105225 under the supervision of Dr. Iyekowa, Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City, Edo State.

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DEDICATION

This project is dedicated to God Almighty, in whom I derive my strength and wisdom to scale through this academic phase till the end and to me, as a reward for my hard work and due diligence.

ACKNOWEDGEMENT

So many people have made this journey easy and very smooth. But I want to express my gratitude firstly to God Almighty, who has blessed me with the gift of people.

I owe a truckload of gratitude to my wonderful project supervisor, whose guidance, wisdom and high intelligence has made this thesis an easy one to conclude.

To my parents, Mr. Alloysius Momodu and Mrs. Beauty Momodu, the ones who God has blessed me with, I'm very grateful, for their love, care and support towards and through my academic journey.

To the entirety of my immediate family and extended family, friends and loved ones, I extend my heartfelt appreciation for their unending patience, support and presence throughout this journey.

ABSTRACT

This research project investigates the phytochemical profile of methanol extract of *Tetrapleura tetraptera* fruit and its effect on the spleno- and hepato-somatic indices in cancer-induced Wistar rats. Dried fruit pods of *Tetrapleura tetraptera* were extracted using methanol via Soxhlet extraction, and preliminary phytochemical screening was conducted to identify major bioactive compounds. A portion of the concentrated crude extract was subjected to a detailed phytochemical screening, which identified bioactive constituents such as flavonoids, alkaloids, saponins, tannins, and glycosides, indicating the plant's rich chemical composition. GC-MS analysis further revealed major compounds including nonadecane, octacosane, and squalene. The spleno- and hepato-somatic indices were calculated by analyzing spleen and liver weight relative to the total body weight after induction with phenyl hydrazine and treatment with the extract. The results show that the extract did not ameliorate phenyl hydrazine-induced splenomegaly. However, it exhibited a significant, dose-dependent hepatoprotective effect, preventing liver weight loss. The study highlights the *Tetrapleura tetraptera* fruit extract contains diverse phytochemicals and demonstrate a protective influence on liver morphology in Wistar rats.

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CHAPTER ONE

1.1 INTRODUCTION

Plants have long played a key role in traditional medicine, often offering healing benefits passed down through generations. Before these natural remedies can be fully understood or safely used in modern medicine, it's important to study the chemical compounds they contain- a process known as phytochemical screening. This helps identify active substances like alkaloids, flavonoids, tannins, and saponins, which are believed to be responsible for many therapeutic effects (Gills, 1992).

One such plant is *Tetrapleura tetraptera*, commonly used in parts of west Africa for managing health conditions ranging from inflammation to hypertension. While its traditional uses are well known, scientific research is still needed to support its safety and effectiveness.

An important part of evaluating a plant's safety involves looking at how it affects vital organs like the liver and spleen. This is where spleno-hepato somatic indices come in – they measure changes in organ size relative to body weight and can give clues about possible toxicity or protective effects. In this study, we explore both the phytochemical makeup of *Tetrapleura tetraptera* methanol extracts and how these extracts ameliorate the liver and spleen in cancer induced Wistar rats.

1.1.1 BACKGROUND OF STUDY

Medicinal plants have been an essential source of therapeutic compounds for centuries, offering a wide range of pharmacological benefits such as antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective effects (Akinmoladun *et al*., 2023, Egharevba et al., 2016). One of those plants is *Tetrapleura tetraptera* (Schum. & Thonn.), commonly known as Aidan or prekese, which belongs to the Fabacae family. This tropical species is native to West Africa and is traditionally utilized both as a spice and a remedy for health conditions like hypertension, diabetes, inflammation, and liver ailments (Okokon et al., 2020, Olas et al., 2018).

Phytochemical screening of plant extracts is important because it identifies bioactive constituent responsible for those observed therapeutic effects. Methanol is often used as a solvent for extraction process due to its ability to dissolve both polar and non-polar compounds efficiently, making it very suitable for isolating a wide range of secondary metabolites (Adewale et al., 2022).

Alongside phytochemical analysis, organ weight indices such as the hepato-somatic index and spleno-somatic index provides insight into the physiological and pathological status of the liver and spleen. These indices are important biomarkers in toxicological and pharmacological studies since alterations in organ weight often indicate responses to treatment, metabolic disturbances, or organ injury (Ihedioha et al., 2018). The liver and spleen play key roles in metabolism, detoxification, and hematopoiesis; therefore, changes in their relative weights can reflect the effects of experimental treatments on systemic health.

Having a vast knowledge of the effect of *Tetrapleura tetraptera* methanol extract on these indices in Wistar rats will help validate its safety, healing potential and potential anticancer properties. This research is particularly relevant because, despite the extensive traditional use of the plant, scientific evidence of its effect on organ weights and hematological status is still limited.

1.1.2 STATEMENT OF THE PROBLEM

The liver and spleen are essential organs that play key roles in metabolism, detoxification, and blood cell formation. Any change in their size or weight can signal underlying health issues, drug reactions, or the impact of bioactive substances (Ihedioha et al., 2018). Although medicinal plants are widely used to manage different health problems, there is still a little scientific evidence confirming their safety and how they affect these vital organs in cancer induced animal models.

While some studies have reported the antioxidant and liver-protective effects, there is very little information on how it influences the liver and spleen weights in animals. Similarly, although phytochemical compounds such as flavonoids, saponins, and tannins have been found in this plant, their direct effect on organ health in living systems is not well understood (Adewale et al., 2022).

The lack of detailed information creates a gap in our understanding of whether T.tetraptera extract is truly safe and effective in ameliorating blood cancer in most studies. Investigating how its methanol extract affects the liver and spleen will provide important insights into its potential benefits or risks, helping to support or challenge its traditional use.

1.1.3 JUSTIFICATION OF THE STUDY

The development of safe and cost-effective hepatoprotective agents is crucial, as many conventional drugs are expensive, difficult to access, and may produce adverse effects (Akinmoladun et al., 2023). Medicinal plants provide a promising alternative because they are naturally abundant, affordable, and contain a wide range of bioactive compounds with proven therapeutic potential. *Tetrapleura tetraptera*, widely used in African traditional medicine, has shown antioxidant, anti-inflammatory, and liver-protective properties in preliminary studies (Okokon et al., 2020, Ajayi et al., 2022) and these effects often justify the plant as an anticancer agent.

While numerous phytochemicals have been identified in *Tetrapleura tetraptera*, there is limited research that examines its methanol extract in relation to organ weight indices using sensitive biomarkers such as the Hepatosomatic Index (HSI) and Spleno-somatic index (SSI). These indices are essential indicators of organ health and systemic response to treatment in relation to blood cancer disease.

The study is therefore justified because it seeks to identify the phytochemical constituents of the methanol extract of *Tetrapleura tetraptera* and assess its impact in HSI and SSI in cancer induced Wistar rats, thereby generating essential data to confirm its safety and potential role as a natural hepatoprotective agent.

1.1.4 SCOPE OF THE STUDY

This research is limited to the phytochemical screening of the methanol extract of *Tetrapleura tetraptera* and the evaluation of its effect on the hepatosomatic index (HSI) and splenosomatic index (SSI) in cancer induced Wistar rats. This study does not extend to histopathological examination, biochemical analysis of liver enzymes, but it is strictly focused on phytochemical characterization and the influence of the methanol extract on HSI and SSI.

1.1.5 AIM AND OBJECTIVES OF THE STUDY

The aim of this study is to evaluate the phytochemical composition and the effect of methanol extract of *Tetrapleura tetraptera* on the Hepatosomatic Index (HSI) and Splenosomatic Index (SSI) in cancer induced Wistar rats.

SPECIFIC OBJECTIVES OF THE STUDY

To achieve this aim, the following specific objectives are set:

1. collect, dry and pulverize the Tetrapluera tetraptera fruit pod.
2. to extract the powdered sample using Soxhlet extractor with methanol as solvent.
3. to perform qualitative phytochemical screening of the methanol extract of *Tetrapleura tetraptera*.
4. to administer varying doses of the methanol extract of *Tetrapleura tetraptera* to cancer induced Wistar rats over a defined experimental period of 14 days.
5. to determine the hepatosomatic index (HSI) and Splenosomatic Index (SSI) of Wistar rats after administration of the methanol extract.
6. to statistically analyze the effect of the extract on HSI and SSI to identify any significant changes between treated and control groups.

1.2 LITERATURE REVIEW

1.2.1 MEDICINAL PLANTS

Medicinal plants have been a cornerstone of human health for centuries and continue to play a vital role in modern healthcare systems. Recent studies from 2015 to 2025 affirm that over 17,800 plant species are used medicinally worldwide, and nearly 25% of pharmaceutical drugs are directly derived from plant sources. These plants are valued for their rich diversity of bioactive compounds-including alkaloids, flavonoids, terpenoids, saponins, glycosides, phenolics, and essential oils-which exhibit a broad spectrum of therapeutic properties. They are commonly used to manage conditions such as inflammation, infections, diabetes, hypertension, and even cancer. The global resurgence in natural and herbal medicine has led to increased scientific scrutiny, revealing mechanisms by which these compounds act-such as modulating oxidative stress, inhibiting inflammatory enzymes, and interfering with pathogen replication or metabolism.

Aloe vera is one of the most widely researched medicinal plants in this period. Its gel contains aloesin, glucomannan, and acemannan, which contribute to its anti-inflammatory, antimicrobial, and wound-healing properties. A 2017 study demonstrated that aloesin accelerates wound healing by modulating MAPK and Smad signaling pathways (Wahedi *et al*., 2017). Further studies using hydrogel formulations of Aloe vera have shown enhanced wound closure and tissue regeneration (Meza-Valle *et al*., 2021). These effects are due not only to improved hydration but also to bioactive sterols and enzymes that promote cell proliferation and reduce inflammation.

Similarly, Allium sativum (garlic) has shown consistent therapeutic potential. Rich in sulfur-containing compounds like allicin and flavonoids like quercetin, garlic exhibits antidiabetic, cardioprotective, and antimicrobial effects. Although garlics benefits have been recognized long before 2015, recent studies continue to support its role in managing metabolic disorders by improving insulin sensitivity and reducing oxidative damage. It remains a popular functional food with therapeutic relevance.

Tetrapleura tetraptera, a plant used traditionally in west African medicine, has also been subject to renewed scientific interest. Studies confirm its antimicrobial, antidiabetic, antioxidant and fertility-boosting properties, primarily due to the content of flavonoids, terpenoids, tannins, and saponins (Adesina et al., 2016, Okechukwu *et al*., 2022). Ethanolic and aqueous extracts of the fruit have shown activity against several strains of bacteria and fungi, as well as anti-inflammatory potential through COX-2 inhibition and nitric oxide suppression.

Other notable medicinal plants include Catharanthus roseus, which produces the anticancer alkaloids vincristine and vinblastine, Artemisia annua, the source of artemisinin, a globally essential antimalarial drug, and prunella vulgaris, traditionally used for wound healing and immunity enhancement. Moringa oleifera has gained recognition for its rich nutritional profile and has been studied for its ability to reduce blood sugar, cholesterol, and inflammation, although large scale human trials remain limited. Justicia gendarussa has shown potential as a contraceptive and antiviral, with one of its compounds, patentiflorin A, demonstrating anti-HIV activity in vitro.

Scientific validation has also emerged from ethological studies. Between 2022 and 2024, researchers observed wild chimpanzees in Uganda self-medicating with plant materials when injured or ill. One chimpanzee with a wounded hand was seen consuming the fern Christella parasitica, which laboratory tests later confirmed has potent anti-inflammatory properties (Freymann et al., 2024). Another chimpanzee ingested the bark of Scutia myrtina, traditionally used in East African herbal medicine. Tests showed that 88% of plant extracts selected by chimpanzees displayed antibacterial effects, and 33% showed significant COX-2 inhibition, supporting their medicinal use. This cross-species use of medicinal plants underscores a biological and evolutionary basis for phytotherapy.

Despite these promising findings, challenges remain in ensuring the safe and standardized use of medicinal plants. Some contain compounds that may be toxic in high doses or interact negatively with prescription drugs. For instance, Aloe vera latex, which contains alon, has been flagged for potential carcinogenicity if consumed improperly (Allure, 2018). Therefore, clinical validation, dose determination, and public education are necessary for integrating plant-based therapies into evidence-based healthcare.

Recent reviews from 2015-2025 reinforce the importance of sustainable harvesting, biodiversity conservation, and the documentation of indigenous knowledge. Climate change, deforestation, and overharvesting threaten many medicinal species before their pharmacological benefits are fully understood. As such, both conservation biology, and pharmacognosy are key to preserving and advancing the therapeutic potential of medicinal plants for future generations.

1.2.2 *TETRAPLEURA TETRAPTERA*

Tetrapleura tetraptera, belonging to the Fabaceae family, is a medium to large-sized tree commonly found in West and Central Africa. It is well known in countries like Ghana, where it is called prekese, and Nigeria, where it goes by names such as aridan or uhio. The tree produces distinctive aromatic fruit pods with four wings that are widely used both as a spice in traditional cooking and for medicinal purposes (Kuate *et al*., 2015, Larbie *et al*., 2020).

Scientific research conducted in recent years has validated many of the traditional health claims associated with *tetrapleura tetraptera*. For example, Kuate and colleagues (2015) showed that extracts from the fruit helped improve several symptoms related to metabolic syndrome in rats fed a high-fat, high carbohydrate diet combined with a diabetic agent. The extract reduced body weight gain, lowered fasting blood sugar, improved insulin sensitivity, and decreased oxidative stress, high blood pressure, and liver damage. These effects were found to be similar to those produced by metformin, a drug commonly used to treat diabetes.

The fruit of *tetrapleura tetraptera* contains many biologically active compounds such as saponins, tannins, steroids, terpenoids, flavonoids, flavonoids, coumarins, alkaloids, and amino acids, which contribute to its medicinal properties (Nwafor *et al*., 2024; Enabulele & Ugha, 2019). Saponins, for example, are known for their anti-inflammatory and antimicrobial effects, while flavonoids and coumarins act as antioxidants and help with blood vessel relaxation.

Toxicological studies have found that the fruit extract has a low toxicity level when given acutely, with the lethal dose (LD_{50}) being higher than 5000mg/kg in animal models. However, when taken repeatedly at very high doses, mild damage to the liver and kidneys were observed, indicating the importance of dosing and safety assessment (Bello *et al*., 2022).

Kadiri et al. (2020) further demonstrated that extracts of *tetrapleura tetraptera* have protective effects on the liver and kidneys against cyanide poisoning in rats, showing that the plant could be useful in neutralizing certain toxins. Additionally, Udobre and colleagues (2020) identified several fatty acid esters in the fruit with notable antioxidant and antimicrobial activities, which support its traditional use in treating skin infections and inflammatory conditions. Enabulele and Ugha (2019) also found that water-based extracts from the fruit could inhibit harmful bacteria like Klebsiella pneumoniae and Staphylococcus aureus, which are often responsible for respiratory and wound infections.

Beyond medicinal uses, *tetrapleura tetraptera* is gaining attention for its application in food science. For instance, Adadi and Kanwugu (2020) successfully enhanced a traditional beverage called pito by adding *tetrapleura tetraptera* and hibiscus sabdariffa, improving both its taste and health benefits.

A detailed review by Adesina, Iwalewa, and Johnny (2016) summarized the wide range of pharmacological effects linked to specific compounds in *tetrapleura tetraptera*. These include anticonvulsant and blood pressure lowering effects from 7-hydroxy-6-methoxy coumarin, anti-inflammatory effects from hentriacontane, and molluscicidal properties from saponins. Other compounds such as chalcones and flavanones contribute antioxidant activity, while the plant overall exhibits benefit in blood sugar regulation, wound healing, contraception, pain relief, and anxiety reduction.

Nutritionally, the fruit is rich in proteins, carbohydrates, fats, vitamins (A, C, E, and B-complex), and very important minerals such as calcium, potassium, magnesium, iron, and zinc (Enabuele & Ugha, 2019, Nwafor *et al*., 2024). This nutrient content makes it a valuable food supplement and health tonic in local diets.

In conclusion, *tetrapleura tetraptera* shows significant potential as a multifunctional plant with both medicinal and nutritional value. Its bioactive compounds offer promising benefits for managing metabolic diseases, infections, inflammation, and protecting organs from toxins. However, more clinical research and safety evaluations are necessary to confirm these effects and ensure safe human use.

FIG. 1 *TETRAPLEURA TETRAPTERA* (AIDAN) FRUIT

1.2.3 TAXONOMY/CLASSIFICATION OF *TETRAPLEURA TETRAPTERA*

The taxonomy of *tetrapleura tetraptera* is as follows;

- Kingdom: Plantae
- Phylum (Division): Magnoliophyta (Angiosperms)
- Class: Magnoliopsida (Dicotyledons)
- Order: Fabales
- Family: Fabaceae (Legume family)
- Genus: Tetrapleura
- Species: *Tetrapleura tetraptera*

Some common names for *tetrapleura tetraptera* are;

- English: Aidan tree, Four-angled fruit
- Ghana (Twi): Prekese
- Nigeria (Igbo): Oshosho / Uhiokrihio
- Nigeria (Yoruba): Aidan
- Nigeria (Hausa): Danidanee
- Cameroon: Essank

1.2.4 CULTIVATION AND DISTRIBUTION OF *TETRAPLEURA TETRAPTERA*

Tetrapleura tetraptera is a leguminous tree native to tropical regions of West and Central Africa, including countries such as Nigeria, Ghana, Cameroon, ivory coast, and Uganda (Kemigisha et al., 2018, World Flora Online, 2024). It naturally grows in humid tropical rainforests and moist lowland forests, favoring well-drained, fertile soils with ample rainfall. The species commonly inhabits secondary forests and forest margins, where it contributes to biodiversity and enhances soil fertility through nitrogen fixation, a characteristic feature of plants in the Fabaceae family (Adesina, Iwalewa & Johnny, 2016, Nwafor, Eze, & Ogah, 2024).

For cultivation, *tetrapleura tetraptera* is primarily grown from seeds, which have a hard outer coat that can hinder germination. Pretreatment methods such as scarification or soaking in concentrated sulfuric acid are used to improve germination rates and speed (Usman, Uleh, & Onyeri, 2023). Specifically, soaking seeds in 50% sulfuric acid for about 15 minutes has been shown to significantly enhance seed germination and seedling vigor compared to untreated seeds. Seeds are typically sown in nursery beds composed of topsoil mixed with river sand to ensure good aeration and moisture retention. After about 3 to 4 months, seedlings are transplanted to the field, ideally during the rainy season to support successful establishment (Kemigisha *et al*., 2018).

The tree prefers tropical climates with temperature ranging from 22^0 C to 30^0C and annual rainfall between 1200 and 2000 mm. it thrives best in well-drained sandy loam or loamy soils enriched with organic matter. While young plants tolerate some shade, mature trees grow optimally under full sunlight (Adesina *et al.,* 2016).

In addition to its ecological role, *tetrapleura tetraptera* is valued in rural communities for its medicinal and culinary uses. The fruit pods are widely used in traditional medicine to treat conditions like diabetes, hypertension, and inflammation, and they are also used as aromatic spices in local dishes (Kemigisha *et al*., 2018, Enabulele & Ugha, 2019).

In countries such as Ghana, Nigeria and Uganda, the collection and sale of *tetrapleura tetraptera* products contribute to local livelihoods. However, most harvesting is from wild populations due to limited domestication efforts, which has led to calls for sustainable cultivation practices to preserve natural resources and maintain the species' availability (Kemigishal *et al*., 2018).

1.2.5 REPORTED MEDICINAL USES OF *TETRAPLEURA TETRAPTERA*

Tetrapleura tetraptera is extensively used in traditional African medicine due to its wide range of health benefits. Various parts of the plant-especially the fruit pods, bark, and leaves-are commonly utilized to treat multiple ailments (Adesina, Iwalewa, & Johnny, 2016).

The fruit pods are particularly known for their effectiveness in managing diseases such as diabetes, high blood pressure, asthma, and inflammatory conditions. Research has shown that the pods contain compounds with antioxidant, anti-inflammatory, antimicrobial, and blood sugar-lowering effects, which support their traditional medicinal uses (Kuate *et al*., 2015, Enabulele & Ugha, 2019).

Studies on diabetic models indicate that extracts from *tetrapleura tetraptera* can help lower blood sugar levels, confirming its antidiabetic potential. Its anti-inflammatory properties are mainly due to the presence of phytochemicals like flavonoids and saponins, which help reduce inflammation and pain (Kuate *et al*., 2015, Nwafor, Eze, & Ogah, 2024).

The bark and leaves are traditionally used to treat respiratory ailments such as asthma and bronchitis. The plant exhibits properties that relax airways and help clear mucus, providing relief in respiratory distress (Adesina *et al*., 2019).

Additionally, *tetrapleura tetraptera* has been found to possess antimicrobial effects against a variety of bacteria and fungi, supporting its use in treating infections and promoting healing of wounds (Enabulele & Ugha, 2019).

The plant is also used to address digestive problems like diarrhea and stomach cramps. Its antispasmodic effect helps to ease intestinal discomfort and aid digestion (Adesina *et al*., 2016).

Overall, pharmacological research supports the traditional use of *tetrapleura tetraptera*, highlighting its bioactive compounds as key contributors to its health benefits. This underscores its significance not only in folk medicine but also as a promising source for new therapeutic agents (Kuate *et al*., 2024).

1.2.6 REPORTED SCIENTIFIC USES OF *TETRAPLEURA TETRAPTERA*

Scientific studies have highlighted the significant pharmacological properties of *tetrapleura tetraptera*, which are linked to its rich content of bioactive compounds such as flavonoids, saponins, tannins, alkaloids, and essential oils (Adesina, Iwalewa, & Johnny, 2016). These constituents contribute to the plant's wide ranging therapeutic effects.

Research has confirmed the antioxidant capacity of *tetrapleura tetraptera*, largely due to its phenolic compounds found in the fruit and bark extracts. These antioxidants help neutralize harmful free radicals and reduce oxidative stress, which is involved in many chronic illnesses (Nwafor, Eze, & Ogah, 2024).

Studies have also demonstrated that *tetrapleura tetraptera* extracts exhibit antimicrobial activity against a variety of bacterial and fungal pathogens. This supports its traditional use in fighting infections and indicates its potential for the development of new antimicrobial drugs (Enabulele & Ugha, 2019).

The plant's anti-inflammatory and pain-relieving effects have been validated in animal models, reinforcing its traditional application intreating inflammation and pain. These effects are attributed to bioactive molecules that influence inflammatory processes (Kuate *et al*., 2015).

In addition, scientific investigations have revealed that *tetrapleura tetraptera* can reduce blood sugar levels and enhance insulin sensitivity in diabetic animals supporting its use in natural antidiabetic treatments (Kuate *et al*., 2015).

Moreover, the plant has been reported to possess blood pressure-lowering (antihypertensive), muscle-relaxing (antispasmodic), and liver-protective (hepatoprotective) properties, further extending its medicinal value (Adesina *et al*., 2016).

Given these scientifically verified bioactivities, *tetrapleura tetraptera* is considered a promising source for developing new drugs, particularly for managing metabolic diseases, infections, and inflammatory conditions.

1.2.7 CHEMICAL CONSTITUENTS IN MEDICINAL PLANTS

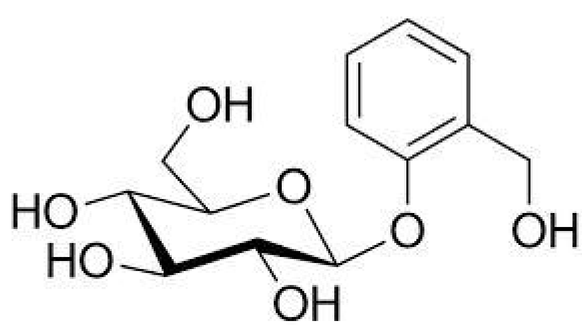
Medicinal plants contain a wide range of bioactive chemical constituents that contribute to their therapeutic properties. These compounds are classified into primary and secondary metabolites (carbohydrates, proteins, lipids) and secondary metabolites which are mainly responsible for medicinal effects. The key classes include:

1.2.7.1 GLYCOSIDES

Glycosides are naturally occurring compounds composed of a sugar moiety (glycone) bonded to a non-sugar component (aglycone or Genin) through a glycoside bond. They are widely distributed in plants and play diverse roles in plant metabolism and defense (Harborne & Williams, 2015). The sugar part can be glucose, rhamnose, and galactose, or other sugars, while the aglycone varies widely, giving rise to different classes of glycosides with distinct biological activities (Evans, 2016).

Glycosides are classified based on their aglycone structure into groups such as cardiac glycosides, cyanogenic glycosides, saponin glycosides, and flavonoid glycosides. Cardiac glycosides, like digoxin, affect heart function and are used medically to treat heart-failure and arrhythmias (Hostettmann & Marston, 2017). Cyanogenic glycosides release toxic hydrogen cyanide when hydrolyzed, serving as defense compounds against herbivores (Jones, 2018).

In plants, glycosides can contribute to coloration, taste, and resistance to pests and diseases. They also serve as storage forms of biologically active substances, which can be activated upon hydrolysis (Harborne & Williams, 2015). In pharmacology, many glycosides are important therapeutic agents due to their wide range of biological effects, including anti-inflammatory, antimicrobial, and anticancer properties (Evans, 2016).



Structure of glycosides

1.2.7.2 SAPONINS

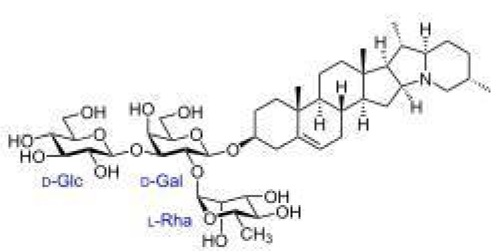
Saponins are naturally occurring compounds found in many medicinal plants, recognized for their ability to produce foam when mixed with water. They consist of a sugar portion attached to a non-sugar component called sapogenin, which may be either steroidal or triterpenoid in structure (Hostettmann & Marston, 2017). These compounds are widespread and play an important role in the therapeutic effects of various plants.

In medicinal plants, saponins are known for a wide range of biological activities, including anti-inflammatory, antimicrobial, antioxidant, antiviral, and immune-boosting properties. These effects make saponins crucial in both traditional remedies and contemporary medical research (Sparg, Light, & van Staden, 2016). For instance, saponins help reduce inflammation by blocking inflammatory chemicals and neutralizing harmful free radicals that contribute to tissue damage (Kuate *et al*., 2015).

Moreover, saponins can improve the absorption of other active plant compounds by increasing the permeability of cell membranes, which enhances the effectiveness of herbal treatments (Hostettmann & Marston, 2017). Some saponins also exhibit cholesterol-lowering capabilities and have been explored for their benefits in heart health (Madan, Tiwari, & Mishra, 2018).

Plants rich in saponins, such as Glycyrrhiza glabra (licorice), Panax ginseng, and *Tetraptera Tetraptera*, are commonly used to address infections, inflammation, and metabolic conditions (Adesina, Iwalewa, & Johnny, 2016).

Overall, saponins are key phytochemicals in medicinal plants that contribute significantly to their



healing effects and hold promise for pharmaceutical development.

Structure of saponins

1.2.7.3 PHENOLICS

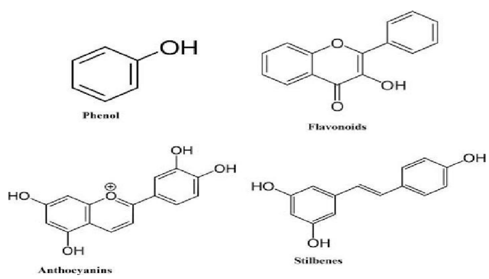
Phenolics, also known as phenolic compounds or polyphenols, are a diverse group of secondary metabolites widely distributed in plants. They are characterized by the presence of one or more hydroxyl groups attached to aromatic rings (Rice-Evans, Miller, & Paganga, 2015). Phenolic compounds include flavonoids, tannins, phenolic acids, and lignans, among others, and play crucial roles in plant defense and human health.

In medicinal plants, phenolics contribute significantly to antioxidant activity by neutralizing free radicals and reducing oxidative stress, which is a key factor in the development of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders (Shahidi & Ambigaipalan, 2015). Their ability to scavenge reactive oxygen species protects cells from damage and supports the body's natural defense systems.

Apart from antioxidant effects, phenolics exhibit anti-inflammatory, antimicrobial, antiviral, and anticancer activities. They can inhibit enzymes involved in inflammation and modulate signaling pathways related to immune responses (Balasundram, Sundram, & Samman, 2016). These properties explain the widespread use of phenolic-rich plants in traditional medicine.

Medicinal plants such as *tetrapleura tetraptera* are known to contain significant levels of phenolic compounds, which contribute to their therapeutic effects, including anti-diabetic, anti-inflammatory, and antimicrobial actions (Adesina, Iwalewa, & Johnny, 2016).

Phenolic compounds are also responsible for the color, flavor, and astringency of many plants and influence their interactions with the environment.



Structure of phenolics

1.2.7.4 TANNINS

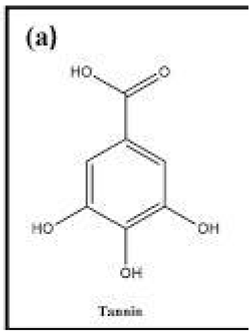
Tannins are a class of polyphenolic compounds commonly found in various plant species, particularly in barks, leaves, seeds, and fruits. They are known for their ability to bind and precipitate proteins, a property that gives them astringent qualities (Haslam, 2016). Chemically, tannins are divided into two main groups: hydrolysable tannins (which yield gallic or ellagic acid upon hydrolysis) and condensed tannins (also known as proanthocyanidins), which are polymers of flavonoid units (Chung *et al*., 2020).

In medicinal plants, tannins contribute significantly to therapeutic activities. One of their primary roles is antimicrobial action, where they inhibit the growth of bacteria, fungi, and viruses by forming complexes with microbial enzymes and proteins (Okuda, 2017). This has made tannin-rich plants widely used in traditional medicine for treating wounds, diarrhea, sore throats, and infections.

Tannins also exhibit antioxidant and anti-inflammatory effects. They help scavenge free radicals and reduce oxidative damage in tissues, while also modulating inflammatory responses by interfering with pro-inflammatory cytokines and enzymes (Serrano *et al*., 2009).

Plants like *tetrapleura tetraptera* are reported to contain tannins as part of their phytochemical profile. These tannins contribute to the plant's astringent and antimicrobial properties, justifying its use in traditional African medicine for gastrointestinal issues, wound healing, and inflammatory conditions (Adesina, Iwalewa, & Johnny, 2016).

In addition to therapeutic benefits, tannins also help protect plants from herbivores and pathogens, acting as natural defense chemicals.



Structure of tannins

1.2.7.5 EUGENOLS

Eugenols is a naturally occurring phenolic compound classified under the group of aromatic essential oils. It is most famously found in clove oil (Syzygium aromaticum), but it also appears in smaller amounts in other medicinal plants such as basil (Ocimum spp.), cinnamon (Cinnamomum spp.), and *tetrapleura tetraptera* (Adesina, Iwalewa, & Johnny, 2016).

Chemically, eugenol ($C_{10}H_{12}O_2$) is known for its spicy aroma and it is characterized by a methoxyphenol structure. It is widely studied for its antimicrobial, analgesic, anti-inflammatory, antioxidant, and local anesthetic properties (Marchese *et al*., 2017).

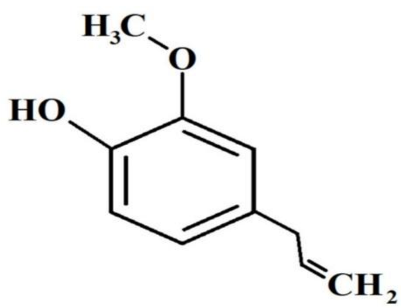
In medicinal plants, eugenol plays a protective role by deterring herbivores and pathogens. Pharmacologically, it has shown strong bactericidal and fungicidal activity, which supports its use in traditional medicine for treating infections and oral health issues. Eugenol disrupts microbial membranes and inhibits enzymes involved in microbial metabolism, making it effective against resistant strains (Kamatou *et al*., 2012).

Eugenol is also a potent anti-inflammatory agent. It works by inhibiting the synthesis of prostaglandins and suppressing the activity of cyclooxygenase (COX) enzymes, which are responsible for pain and inflammation (Pramod *et al*., 2010). Because of this, it is used in managing toothaches, muscle pain, and arthritis.

As an antioxidant, eugenol scavenges free radicals and helps protect cells from oxidative damage, a process linked to aging and chronic diseases such as cancer and cardiovascular disorders (Marchese *et al*., 2017).

Although eugenol is generally considered safe at therapeutic doses, excessive or prolonged use may lead to toxicity, especially in liver and mucosal tissues (Kamatou *et al*., 2012).

In the context of *tetrapleura tetraptera*, eugenol and related compounds contribute to the plant's microbial and anti-inflammatory activities, supporting its traditional use in managing infections and inflammatory conditions (Adesina *et al*., 2016).



Structure of eugenols

1.2.7.6 FLAVONOIDS

Flavonoids are a large group of naturally occurring polyphenolic compounds found abundantly in fruits, vegetables, leaves, seeds, and medicinal plants. Structurally, they are based on a 15-carbon skeleton arranged in a C6-C3-C6 configuration, forming subclasses such as flavones, flavonols, flavanones, isoflavones, anthocyanidins, and chalcones (Harborne & Williams, 2015).

In medicinal plants, flavonoids play vital roles in plant physiology, including UV filtration, pigmentation, and protection against pathogens. More importantly, they exhibit a wide range of therapeutic properties beneficial to human health, including antioxidant, anti-inflammatory, antimicrobial, antiviral, anticancer, and cardioprotective effects (Panche, Diwan, & Chandra, 2016).

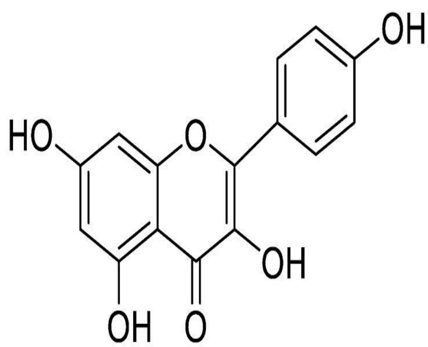
Their antioxidant activity is perhaps the most well-documented. Flavonoids neutralize free radicals and reactive oxygen species (ROS), thereby preventing oxidative stress-induced cell damage linked to chronic diseases such as cancer, diabetes, and neurodegenerative disorders (Shahidi & Ambigaipalan, 2015).

Flavonoids also exert anti-inflammatory effects by inhibiting enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), and by modulating the release of inflammatory cytokines

(Middleton *et al*., 2015). This supports their traditional use in treating inflammatory conditions such as arthritis, fevers, and wounds.

Plants like *tetrapleura tetraptera* are known to contain significant levels of flavonoids, contributing to their use in traditional medicine for managing infections, metabolic disorders, and inflammatory diseases (Adesina, Iwalewa, & Johnny, 2016). These flavonoids may also enhance the activity of other bioactive compounds through synergistic effects.

In addition, flavonoids are believed to improve vascular health by strengthening capillaries and reducing blood pressure, further underlining their importance in herbal medicine (Kumar & Pandey, 2013).



Structure of flavonoids

1.2.7.7 TERPENES

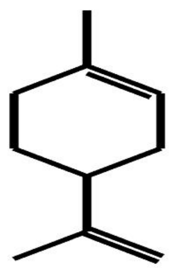
Terpenes are a diverse class of naturally occurring organic compounds composed of repeating isoprene (C_5H_8) units. They are the largest group of plant secondary metabolites and are responsible for the aromatic and flavor characteristics of many herbs, flowers, and essential oils (Croteau *et al*., 2015). Based on the number of isoprene units, terpenes are classified into monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}), among others.

In medicinal plants, terpenes play crucial ecological roles such as attracting pollinators, deterring herbivores, and protecting against pathogens. They are also responsible for many therapeutic properties and are found in the essential oils of plants like Mentha spp., Cannabis sativa, Boswellia spp., and *tetrapleura tetraptera* (Adesina, Iwalewa, & Johnny, 2016).

Pharmacologically, terpenes exhibit a wide range of bioactivities, including anti-inflammatory, antiviral, antibacterial, analgesic, antioxidant, and anticancer effects (Thoppil & Bishayee, 2016). For example, limonene and pinene, both monoterpenes, are known for their anti-inflammatory and bronchodilator effects. Boswellic acids, which are triterpenes, are widely used for their anti-arthritic and anti-inflammatory activities.

Terpenes act through multiple mechanisms such as modulation of enzyme activity, interference with cell signaling pathways, and disruption of microbial membranes. This makes them useful in the treatment of conditions like infections, respiratory diseases, inflammation, and even certain cancers (Mahato & Sen, 2014).

In the context of *tetrapleura tetraptera*, terpenes contribute to its aromatic profile and medicinal uses. Studies have suggested that the essential oil of its fruit contains monoterpenes and sesquiterpenes with antimicrobial and anti-inflammatory potential (Adesina *et al*., 2016).



Limonene

Structure of terpenes

1.2.7.8 STEROIDS

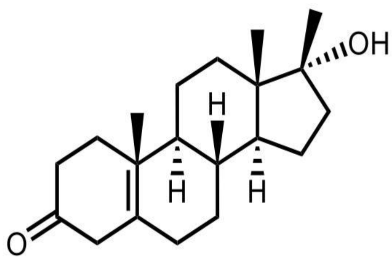
Steroids are a class of lipophilic organic compounds with a characteristic four-ring core structure known as the cyclopentanoperhydrophenanthrene ring. In medicinal plants, they occur mainly as phytosterols and steroidal saponins, which are structurally similar to cholesterol and other animal-derived steroids (Goad & Akihisa, 2013).

Phytosterols such as β-sitosterol, stigmasterol, and campesterol are commonly found in the seeds, roots, fruits, and leaves of various medicinal plants. These plant-based steroids exhibit several pharmacological activities, including anti-inflammatory, antioxidant, immunomodulatory, anticancer, and cholesterol-lowering properties (Sharma *et al*., 2016). By competing with dietary cholesterol for absorption in the intestines, phytosterols help reduce serum cholesterol levels, thus supporting cardiovascular health.

Steroidal saponins, another important subgroup, are glycosylated steroids known for their hormone-like effects. They are found in plants like Dioscorea spp., Tribulus terrestris, Fenugreek (Trigonella foenum-graecum), and *Tetrapleura tetraptera*. These compounds are believed to stimulate endogenous production of steroid hormones such as testosterone and estrogen and have been investigated for use in treating hormonal imbalances, sexual dysfunction, and menopausal symptoms (Ghosal, 2015).

In the case of *tetrapleura tetraptera*, steroidal compounds have been identified as part of its phytochemical profile and are linked to its anti-inflammatory and fertility-enhancing properties, supporting its traditional use intreating reproductive and metabolic disorders (Adesina, Iwalewa, & Johnny, 2016).

Overall, the presence of steroids in medicinal plants enhances their therapeutic value, and many plant steroids serve as precursors for the synthesis of pharmaceutical steroids used in modern medicine.

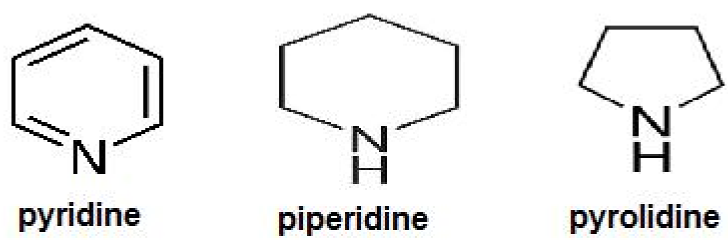


Structure of Steroids

1.2.7.9 ALKALOIDS

Alkaloids are a diverse group of nitrogen-containing organic compounds found in many medicinal plants. They are typically derived from amino acids and often possess strong physiological effects on humans and animals. These compounds usually have a bitter taste and can be highly bioactive even at low concentrations (Roberts & Wink, 2018).

Structurally, alkaloids vary widely and are categorized into several classes, including pyrrolidine, pyridine, indole, quinoline, and isoquinoline alkaloids. Well-known examples include morphine, quinine, caffeine, nicotine, and berberine, which are extracted from plants such as Papaver somniferum, Cinchona officinalis, Camellia sinensis, and Berberis spp., respectively (Dewick, 2015).



Structure of alkaloids

1.2.7.10 ESSENTIAL OILS

Essential oils are aromatic, volatile compounds distilled from plant parts and consist of a complex blend of monoterpenes, sesquiterpenes, and their oxygenated derivatives such as phenols and esters. These oils contribute to plant defense, signaling, and have diverse therapeutic applications (e.g., antimicrobial, anti-inflammatory, sedative) (Verywell Health, 2021; EatingWell, 2023).

A 2015-2022 systematic review highlights their antimicrobial, antioxidant, and immunomodulatory properties, reinforcing their relevance in minimizing pathogen resistance and inflammation (especially relevant in natural product research).

For *tetrapleura tetraptera*, a key 2022 study examined the essential oil and oleoresin extracted from its fruits. Using GC-MS analysis, researchers identified multiple volatile compounds. The essential oil demonstrated concentration-dependent antibacterial activity against Staphylococcus aureus and Escherichia coli as well as antioxidant activity using DPPH radical scavenging and ferric-reducing assays-suggesting potential for food preservation and microbial spoilage inhibition.

Previous work focused on the leaf essential oil of *tetrapleura tetraptera*, identifying dominant compounds such as 1,8-cineole, α-pinene, and phytol. The oil exhibited moderate toxicity in brine shrimp assays and modest antibacterial activity against Bacillus subtilis.

These findings position *tetrapleura tetraptera* essential oils as promising agents for antibacterial, antioxidant, and possibly pharmacological use-supporting its traditional medicinal usage and potential development into natural preservatives or therapeutic agents.

1.2.8 CHEMICAL COMPOUNDS ISOLATED IN *TETRAPLEURA TETRAPTERA*

Research has shown that *Tetrapleura tetraptera*, often used as a spice and medicine in West Africa, is packed with different natural compounds that help explain why people have trusted it for generations. One of the important groups of chemicals present are the flavonoids, such as luteolin, which are well known for their role in protecting the body against stress and damage caused by free radicals (Okokon et al, 2020).

The plant also contains sterols like stigmasterol and β-sitosterol. These plant-based steroids are not harmful kind people fear, but rather natural substances that may help support heart health (Adewale et al., 2022). Alongside these, researchers have identified triterpenoids, including lupeol and related saponins, which are often linked with anti-inflammatory and antimicrobial effects – adding to the plant's healing reputation (Egharevba et al., 2016).

In addition to these, *T. tetraptera* provides glycosides, which are sugar-linked compounds with many biological roles, and some special pigments like pheophytin a, plus long-chain alcohols such as n-tetracosanol (Adewale et al., 2022). Interestingly, a unique compound called aridanin, named after the local name of the plant "Aridan", has been reported and is considered one of its signature chemicals (Freymann et al., 2024).

Beyond these specific compounds, several screenings confirm the presence of alkaloids, tannins, coumarins, and terpenoids – all of which contribute to the flavor, medicinal value, and preservative qualities that make the fruit so valuable both in the kitchen and in traditional healing (Ajayi et al., 2022).

Altogether, these findings show that *Tetrapleura tetraptera* is more than just a spice; it is a rich storehouse of natural compounds that support its use in traditional African medicine and highlight its potential for modern applications.

1.2.9 EXTRACTION

Extraction is the process of separating bioactive compounds (phytochemicals) from the plant material (*Tetrapleura tetraptera* fruits, leaves, bark, or seeds) using suitable solvents or techniques.

PURPOSE OF EXTRACTION

To obtain essential oils, flavonoids, saponins, tannins, alkaloids, steroids, etc, for medicinal, nutritional, and industrial use.

Types of Extraction Methods

1. Solvent Extraction (Maceration/ Soxhlet Extraction)
   i. Plant parts are soaked in solvents like ethanol, methanol, water, acetone, chloroform, or hexane.
   ii. Heat or continuous solvent circulation (Soxhlet) improves yield.
   iii. Commonly used for flavonoids, tannins, saponins, and alkaloids.
2. Aqueous Extraction (Water Extraction / Decoction / Infusion)
   i. Boiling (decoction) or steeping (infusion) of the fruit in water.
   ii. Traditional method for preparing herbal teas and medicinal remedies.
3. Steam Distillation / Hydrodistillation
   i. Used mainly for essential oil extraction.
   ii. Steam passes through the crushed fruits, releasing volatile oils (e.g., limonene, myrcene, linalool).
4. Cold Pressing (Mechanical Expression)
   i. Mainly for seed oil extraction.
   ii. Seeds are pressed mechanically without heat to preserve fatty acids (oleic, linoleic acids).
5. Supercritical Fluid Extraction (SFE) (advanced method)
   i. Uses supercritical CO_2 as solvent.
   ii. Highly efficient, solvent-free, and eco-friendly.
   iii. Extracts essential oils and lipophilic compounds in pure form.
6. Ultrasound and Microwave-Assisted Extraction (UAE, MAE) (modern techniques)
   i. Sound waves or microwaves break cell walls and release phytochemicals faster.
   ii. More efficient and eco-friendly than traditional solvent methods.

1.2.10 DISTILLATION METHODS FOR *TETRAPLEURA TETRAPTERA*

Tetrapleura tetraptera contains numerous volatile aromatic compounds, essential oils, and other bioactive chemicals. These compounds are often extracted for medicinal, cosmetic, and flavoring applications. Distillation is a key technique for this extraction because boiling point differences to separate volatile oils from non-volatile plant matter.

Some of the major distillation techniques used for *tetrapleura tetraptera*:

1) Steam distillation

Principle: Steam is passed through the plant material, causing the volatile compounds to vaporize at temperatures lower than their normal boiling points.

Process:

I. Crushed or sliced tetrapleura pods are placed in a distillation chamber.
II. Steam from a separate boiler passes through the plant material, volatilizing the oils.
III. The mixture of steam and oil vapors travels through a condenser, where it cools and liquefies.
IV. Oil and water separate by density, essential oil floats or sinks depending on composition.

Advantages:

I. Prevents decomposition of heat-sensitive compounds.
II. Produces high-quality essential oils with strong aroma.

Applications: Used to extract terpenoids, aromatic aldehydes, esters, and phenolic compounds for perfumes, medicinal syrups, and flavoring agents.

2) Hydro distillation

Principle: Plant material is immersed in water and boiled; the volatile components vaporize with steam and are condensed back into liquid.

Process:

I. Tetrapleura pods are submerged in water and heated until boiling.
II. Volatile components evaporate along with water vapor.
III. Condensation occurs in a cooling system, sparating oils from water.

Advantages:

Simple and cost-effective method.

Disadvantages:

I. Long extraction time.
II. Risk of hydrolysis and thermal degradation of some components.

Applications: Suitable for small-scale extraction of essential oils for traditional medicine and culinary use.

3) Fractional Distillation

Principle: Separation of volatile compounds based on their different boiling points.

Process:

I. Essential oils obtained from initial distillation are further distilled using a fractionating column.
II. Different fractions such as monoterpenes, sesquiterpenes, and oxygenated compounds are collected separately.

Advantages:

Produces highly purified components for specialized applications.

Applications: Used when isolating specific compounds for pharmaceutical formulations or research purposes.

4) Vacuum Distillation

Principle: Distillation carried out under reduced pressure to lower boiling points.

Process:

I. The pressure inside the distillation apparatus is reduced using a vacuum pump.
II. This allows volatile compounds to vaporize at lower temperatures, protecting them from heat damage.

Advantages:

I. Prevents thermal decomposition of sensitive molecules.
II. Produces oils with high purity and retained bioactivity.

Applications: Industrial-scale production of high-quality essential oils and fragrance ingredients.

5) Water-Steam (Hybrid) Distillation

Principle: Combination of direct water boiling and steam passage through plant material.

Process:

I. Plant material rests above water while steam passes through.
II. Reduces water contact while still benefiting from steam penetration.

Advantages:

I. Faster than hydrodistillation.
II. Less hydrolysis compared to full immersion.

Applications: Extraction of fragrant oils for perfumery and natural food flavoring.

1.2.11 CHROMATOGRAPHY

What is Chromatography?

Chromatography is a laboratory procedure for separating a mixture into its constituent parts, relying on their distinct differences in interactions with a stationary phase and a mobile phase. It remains highly preferred in chemistry, biology, pharmacy and environmental sciences for the purpose of analysis, purification or even identification of compounds.

This process works on the principle of differential distribution of components between two phases

i. Stationary Phase – A solid or liquid supported on a solid.
ii. Mobile Phase – A liquid or gas that moves over or through the stationary phase.

Components in the mixture interact differently with these phases, leading to their separation.

Types of Chromatography

Chromatography has several types based on the physical state of the mobile phase and the mobile phase and the mechanism of separation. The main types include:

1) Paper Chromatography
   i. Uses paper as the stationary phase and a solvent as the mobile phase.
   ii. Commonly used for analyzing pigments and amino acids.
2) Thin-Layer Chromatography (TLC)
   i. Involves a thin layer of adsorbent (e.g., silica gel) on a plate as the stationary phase.
   ii. Used for checking the purity of compounds, monitoring reactions.
3) Column Chromatography
   i. Uses a column packed with adsorbent material as the stationary phase and a liquid as the mobile phase.
   ii. Ideal for separating large quantities of substances.
4) Gas Chromatography (GC)
   i. The mobile phase is an inert gas, and the stationary phase is a liquid or solid on a column.
   ii. Used for volatile compounds analysis like essential oils and fuels.
5) High-Performance Liquid Chromatography (HPLC)
   i. An advanced form of column chromatography using high pressure for faster and more efficient separation.
   ii. Widely used in pharmaceutical and biochemical analysis.
6) Ion-Exchange Chromatography
   i. Separates molecules based on charge differences using a charged stationary phase.
   ii. Common for protein and amino acid separation.
7) Affinity Chromatography

i. Uses specific binding interactions between molecules for separation (e.g., antigen-antibody).
ii. Applied in purifying enzymes and antibodies.

<u>Uses of Chromatography</u>

Chromatography is widely applied in various fields, including:

I. Pharmaceutical industry – Purification of drugs and quality control.
II. Food industry – Purification of drugs and quality control.
III. Food industry – Detecting additives, contaminants, and nutritional components.
IV. Environmental Analysis – Monitoring pollutants in water and air.
V. Clinical Applications – identifying metabolites, drugs, and biomarkers.
VI. Forensic science – Detecting toxins, drugs, and explosives.
VII. Biochemical Research – isolation of proteins, nucleic acids, and enzymes.

1.2.12 BLOOD CANCER

Blood cancer is a type of cancer that affects the blood, bone marrow, or lymphatic system, where abnormal blood cells grow uncontrollably and interfere with the production and function of normal blood cells.

Normally, the bone marrow makes blood stem cells that develop into red blood cells (carry oxygen), white blood cells (fight infection), and platelets (help clotting).

In blood cancer, this process goes wrong: immature or abnormal cells multiply, crowding out healthy cells, weakening immunity, reducing oxygen supply, and increasing bleeding risk.

Major Types of Blood Cancer

1. Leukemia:
   i. Cancer of the bone marrow and blood.
   ii. Leads to uncontrolled production of abnormal white blood cells.
   iii. Can be acute (fast-growing) or chronic (slow-growing).

Examples: Acute lymphoblastic leukemia (ALL), Acute myeloid leukemia (AML), Chronic lymphocytic leukemia (CLL), Chronic myeloid leukemia (CML).

2. Lymphoma
   i. Cancer of the lymphatic system (lymph nodes, spleen, thymus, bone marrow).
   ii. Abnormal lymphocytes (a type of white blood cell) build up and impair the immune system

Two main types:

a) Hodgkin lymphoma (characterized by Reed-Sternberg cells).

b) Non-Hodgkin lymphoma (many subtypes).

3. Myeloma (Multiple Myeloma)
   i. Cancer of plasma cells (a type of white blood cells that produces antibodies).
   ii. Abnormal plasma cells build up in bone marrow, weaken bones, damage kidneys, and reduce normal antibody production.

<u>Causes & Risk Factors</u>

I. Blood cancers don't usually have a single cause, but several factors increase risk:
II. Genetic mutations: DNA changes in blood cell development.
III. Family history: Having relatives with blood cancer may raise risk.
IV. Age: More common in older adults, though some types affect children
V. Radiation exposure: High levels (e.g., radiation therapy, nuclear accidents).
VI. Chemical exposure: Benzene and some chemotherapy drugs.
VII. Weakened immune system: HIV, organ transplants, autoimmune diseases.
VIII. Infections: Certain viruses (e.g., Epstein-Barr virus, HTLV-1).

1.2.13 CHEMICALS INDUCING CANCER

Chemicals that induce cancer are called carcinogens. These are substances (natural or synthetic) that can cause changes in DNA or interfere with normal cellular processes, leading to uncontrolled cell growth and eventually cancer.

<u>Major Types of Carcinogenic Chemicals</u>

1. Polycyclic Aromatic Hydrocarbons (PAHs)

Found in: tobacco smoke, charred/grilled meats, coal tar, exhaust fumes.

Example: Benzo[a]pyrene.

2. Aromatic Amines & Azo Dyes

Found in: dyes, rubber, plastics, cigarette smoke.

Example: Benzidine, 2-naphthylamine.

3. Nitrosamines & Nitrates

Formed from nitrates/nitrites (used as preservatives in processed meats) under acidic stomach conditions.

Example: N-nitrosodimethylamine (NDMA).

4. Halogenated Compounds

Found in: industrial chemicals, pesticides.

Example: Vinyl chloride (used in plastics, linked to liver cancer).

5. Asbestos (fibrous silicate minerals)

Causes lung cancer and mesothelioma.

6. Alcohol (Ethanol)

Metabolized into acetaldehyde, which damages DNA and proteins.

7. Heavy Metals

Arsenic (contaminated water, pesticides) – skin, bladder, lung cancer.

Cadmium (batteries, tobacco smoke) – lung cancer.

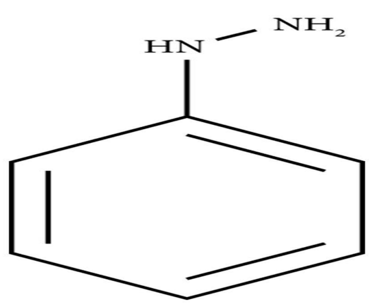
Chromium (VI) (dyes, leather, paints) – lung cancer.

8. Aflatoxins (natural toxins)

Produced by Aspergillus fungi on stored grains/peanuts.

Potent cause of liver cancer.

9. Phenyl Hydrazine



Causes hemolytic anemia by destroying red blood cells.

Leads to Heinz body formation and methemoglobinemia.

Induces splenomegaly and affects the liver (hepatotoxicity, extramedullary hematopoiesis).

Can damage the kidneys due to hemoglobin breakdown products.

Generate oxidative stress (ROS) leading to cell and tissue damage.

Has mutagenic and carcinogenic potential with long-term exposure.

May cause gastrointestinal irritation and neurological symptoms.

How Chemicals Induce Cancer

i. Genotoxic effects: Directly damage DNA (mutations, chromosomal breaks).
ii. Epigenetic effects: Alter gene expression without changing DNA sequence.
iii. Promotion: Stimulates abnormal cell growth once mutation is present.

1.2.14 MEDICINAL PLANTS WITH ANTICANCER POTENTIAL

Cancer remains one of the leading causes of mortality worldwide, and the search for safer, natural therapies has placed medicinal plants in the spotlight. These plants contain bioactive phytochemicals – such as alkaloids, flavonoids, terpenes, phenolics, and saponins---that exhibit anticancer activities by scavenging free radicals, inhibiting tumor growth, blocking angiogenesis, and triggering programmed cell death (apoptosis). Below are some important medicinal plants with proven or potential anticancer effects.

1, *Tetrapleura tetraptera* (Aidan fruit, Prekese)

Phytochemicals: Flavonoids, saponins, alkaloids, tannins, essential oils, phenolic acids.

Anticancer potential: Preliminary studies suggest that *T. tetraptera*'s strong antioxidant activity may help protect against oxidative DNA damage, a key step in cancer development. Its flavonoids and phenolic compounds show potential in suppressing tumor growth, while its saponins may induce apoptosis in abnormal cells.

Additional medicinal uses: Traditionally used in West Africa for managing hypertension, inflammation, infections, diabetes, and as a postpartum recovery tonic.

2. *Curcuma longa* (Turmeric)

Bioactive compound: Curcumin.

Mechanism: Curcumin targets multiple molecular pathways, reducing oxidative stress, blocking pro-inflammatory enzymes, and inhibiting angiogenesis and metastasis.

Evidence: Numerous lab and clinical studies show promise against cancers of the breast, colon, pancreas, and prostate.

3. Catharanthus roseus (Madagascar periwinkle)

Compounds: Vincristine, vinblastine (alkaloids).

Mechanism: These compounds disrupt microtubule formation, effectively halting cell division and leading to cancer cell death.

Medical use: Already established in modern chemotherapy protocols, especially for leukemia and lymphoma.

4. Camellia sinensis (Green tea)

Key compounds: Epigallocatechin-3-gallate (EGCG), catechins.

Mechanism: Acts as a potent antioxidant, regulates gene expression, suppresses proliferation, and sensitizes cancer cells to chemotherapy.

Evidence: Linked with reduced risk of breast, prostate, liver, and colorectal cancers in population studies.

5. Withania somnifea (Ashwagandha)

Active ingredients: Withanolides.

Mechanism: Enhances apoptosis, inhibits NF-KB (a cancer-promoting transcription factor), and reduces stress hormones that may fuel cancer progression.

6. Zingiber officinale (Ginger)

Phytochemicals: Gingerols, shogaols, paradols.

Mechanism: Induces apoptosis, suppresses tumor-promoting inflammation, and interferes with multiple signaling pathways associated with cancer spread.

Evidence: Promising results against ovarian, pancreatic, and colorectal cancers.

7. Nigella sativa (Black seed, “Seed of Blessing”)

Active compound: Thymoquinone.

Mechanism: Known to suppress angiogenesis (formation of new blood vessels that feed tumors), modulate immune responses, and induce apoptosis.

Evidence: Tested against breast, colon lung, and prostate cancers with significant anticancer activity in preclinical models.

8. Garcinia kola (Bitter kola)

Phytochemicals: Exhibits strong antioxidant activity, DNA protection, and suppression of carcinogen-induced tumor growth.

These medicinal plants provide a rich source of natural compounds that may complement or inspire conventional anticancer therapies.

Their mechanisms include:

i. Antioxidant activity (neutralizing free radicals that damage DNA).
ii. Apoptosis induction (forcing cancer cells to self-destruct)

iii. Anti-angiogenesis (preventing blood supply to tumors).
iv. Anti-inflammatory effects (since chronic inflammation is linked to cancer).

While plants like Catharanthus roseus have already produced modern chemotherapy drugs, others like *Tetrapleura Tetraptera* are still under exploration, showing great promise for future drug development.

1.2.15 ORTHODOX ANTICANCER DRUGS

Orthodox anticancer drugs are conventional medicines that have been developed through rigorous scientific research and clinical trials. They are approved by regulatory authorities such as the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA), and they form the backbone of cancer treatment in modern medicine. Unlike traditional or herbal remedies, these drugs are standardized, precisely dosed, and have well-defined mechanisms of action.

Main Classes of Orthodox Anticancer Drugs

1. Alkylating Agents
   i. Mechanism: They damage DNA by adding alkyl groups, preventing cancer cell replication.
   ii. Examples: Cyclophosphamide, Ifosfamide, Melphalan.

2. Antimetabolites
   i. Mechanism: Interfere with DNA and RNA synthesis by mimicking natural metabolites.
   ii. Examples: Methotrexate, 5-Fluorouracil (5-FU), Cytarabine.

3. Plant Alkaloids (Mitotic Inhibitors)
   i. Mechanism: Inhibit microtubule formation, blocking cell division.
   ii. Examples: Vincristine, Vinblastine, Paclitaxel.

4. Topoisomerase Inhibitor
   i. Mechanism: Block topoisomerase enzymes, leading to DNA breaks.
   ii. Examples: Etoposide (Topoisomerase II inhibitor), Irinotecan (Topoisomerase I inhibitor).

5. Antitumor Antibiotics
   i. Mechanism: Bind to DNA and prevent replication and transcription.
   ii. Examples: Doxorubicin, Bleomycin, Actinomycin D.

6. Hormonal Agents
   i. Mechanism: Block hormone receptors or decrease hormone production in hormone-sensitive cancers.
   ii. Examples: Tamoxifen (anti-estrogen), Letrozole (aromatase inhibitor), Flutamide (anti-androgen).

7. Platinum-Based Compounds
   i. Mechanism: Form DNA cross-links that prevent replication.
   ii. Examples: Cisplatin, Carboplatin, Oxaliplatin.

8. Targeted Therapy & Immunotherapy (Modern Orthodox Drugs)
   i. Mechanism: Target specific molecules in cancer growth pathways or stimulate immune system.
   ii. Examples: Imatinib (TKI), Trastuzumab (monoclonal antibody), Pembrolizumab (immune checkpoint inhibitor).

1.2.16 THE LIVER: YOUR BODY'S METABOLIC POWERHOUSE

The liver is the largest internal organ and one of the most vital, performing over 500 essential tasks to keep the bod functioning smoothly. Its not just an organ; it's a complex chemical processing plant, a storage facility, and a filtration system all in one.

1.2.16.1 ANATOMY AND LOCATION

1. Location: The liver is located in the upper right quadrant of the abdomen, just below the diaphragm and protected by the rib cage.
2. Structure: It is a large, meaty, wedge-shaped organ, weighing about 1.4 kilograms (3 pounds) in the average adult.
3. Lobes: it is divided into two main lobes (right and left) and two smaller lobes (caudate and quadrate).
4. Dual blood supply: this is a unique and critical feature:
   i. Hepatic Artery: Supplies oxygen-rich blood from the heart.
   ii. Portal Vein: Supplies nutrient-rich blood from the digestive organs (stomach, intestines, spleen, pancreas). This allows the liver to process nutrients and toxins absorbed from blood.

1.2.16.2 KEY FUNCTIONS OF THE LIVER

The liver's function can be grouped into several major categories:

1. Metabolism

   i. Carbohydrate Metabolism: Stores glucose as glycogen and converts it back to glucose when the body needs energy. It helps maintain stable blood sugar levels.
   ii. Fat Metabolism: Breaks down fats to produce energy and creates cholesterol and triglycerides, which are essential for building cells.
   iii. Protein Metabolism: Synthesizes most of the plasma proteins (like albumin) and breaks down amino acids from protein digestion.

2. Detoxification

   i. Filters Blood: Processes and neutralizes harmful substances like alcohol, drugs (medications), and metabolic waste products (like ammonia, which converts to urea to be excreted by the kidneys).
   ii. Bile Production: Produces bile, a greenish-yellow flid is stored in the gallbladder and released into the intestines to:
      a) Emulsify fats, making them easier to digest.
      b) Carry away waste products (like bilirubin from broken-down red blood cells) out of the body through feces.

3. Synthesis

   i. Produces blood-clotting factors: Creates proteins necessary for blood to clot, preventing excessive bleeding.
   ii. Produces Albumin: The main protein in blood plasma, which helps maintain blood volume and pressure.

4. Storage

   i. Stores vitamins (A, D, E, K and B12) and minerals (like iron and copper), releasing them when needed.

5. Immunity

   i. Contains immune cells (Kupffer cells) that act as a filter, removing bacteria and other foreign particles from the blood.

1.2.16.3 COMMON LIVER DISEASES AND CONDITIONS

Condition	Description	Primary Causes
Hepatitis	Inflammation of the liver. Can be acute (short-term) or chronic (long-term).	Viruses (Hepatitis A, B, C, D, E), alcohol, toxins, autoimmune diseases.

Fatty Liver Disease	Buildup of excess fat in the liver cells.	Alcoholic (AFLD): Due to heavy drinking. Non-Alcoholic(NAFLD): Linked to obesity, type 2 diabetes, metabolic syndrome.
Cirrhosis	Late-stage scarring (fibrosis) of the liver caused by long-term damage. Scar tissue replaces healthy tissue, impairing function.	Chronic alcohol abuse, hepatitis B/C, NAFLD.
Liver Cancer	Primary liver cancer (hepatocellular carcinoma) often occurs in a liver already damaged by cirrhosis.	Chronic hepatitis B/C infection, cirrhosis, NAFLD, aflatoxins.
Liver Failure	A life-threatening condition where a large part of the liver becomes damaged and can no longer function.	Can be acute (e.g., from acetaminophen overdose) or chronic (from cirrhosis).
Hemochromatosis	A genetic disorder causing the body to absorb and store too much iron, which damages the liver.	Hereditary genetic mutation.
Autoimmune Liver Diseases	The body's immune system attacks liver cells.	Autoimmune Hepatitis, Primary Biliary Cholangitis (PBC), Primary Screening Cholangitis (PSC).

1.2.16.4 SIGNS AND SYMPTOMS OF LIVER PROBLEMS

Early liver disease often has no obvious symptoms. As it progresses, symptoms may include:

i. Jaundice (yellowing of the skin and eyes).
ii. Abdominal pain and swelling (especially on the right side)
iii. Swelling in the legs and ankles (edema)
iv. Itchy skin
v. Dark urine color
vi. Pale stool color, or bloody/tar-colored stool
vii. Chronic fatigue

viii. Nausea or vomiting
ix. Loss of appetite
x. Tendency to bruise early

1.2.16.5 HOW TO KEEP YOUR LIVER HEALTHY

The liver has a remarkable ability to regenerate, but it's not invincible. You can protect it by:

1. Drink alcohol in moderation (or not at all): this is the single most important thing you can do to prevent alcohol-related liver damage.

2. Avoid Risky Behaviors: Get vaccinated for Hepatitis A and B. practice safe sex and avoid sharing needles, which can transmit Hepatitis C.

3. Use Medications Wisely: Take prescription and over-the-counter drugs only as directed. Never mix alcohol and medication. Be extremely cautious with acetaminophen (Tylenol) and always follow dosage instructions.

4. Maintain a Healthy Weight: This helps prevent Non-Alcoholic Fatty Liver Diseases (NAFLD).

5. Eat a Balanced Diet: Focus on fruits, vegetables, whole grains, and lean proteins. Limit high-fat and high-sugar foods

6. Exercise regularly

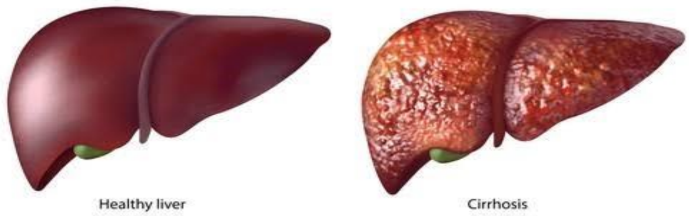
7. Practice Good Hygiene: Wash your hands regularly to avoid infections that can affect the liver.

8. Be cautious with toxins: Aerosols, insecticides, and chemicals can damage the liver. Use them in well-ventilated areas and wear protective gear.

1.2.16.6 REGENERATION

The liver is the only internal organ that can regenerate it can regrow to its normal size even after up to 90% of it has been removed. The process, however, has limits and cannot occur in the presence of continuous scarring (cirrhosis).

Structure of a liver



1.2.17 THE SPLEEN

The spleen is a vital organ located in the upper left part of the abdomen, beneath the rib cage. It is part of both the lymphatic and immune systems and plays critical roles in filtering blood, recycling red blood cells, producing white blood cells, storing platelets, and fighting infections. Although a person can survive without a spleen, losing its function increases susceptibility to infections and affects blood regulation.

1.2.17.1 FUNCTIONS OF THE SPLEEN

i. **Blood filtration:** Removes damaged or old red blood cells and recycles iron.
ii. **Immune defense:** Produces lymphocytes and antibodies to combat infections.
iii. **Blood storage:** Stores blood and platelets that can be released in emergencies.
iv. **Pathogen removal:** Eliminates bacteria and other foreign particles from the blood.
v. **Hematopoiesis:** Supports blood cell production in fetal life or under certain disease conditions.

1.2.17.2 COMMON DISEASES AND DISORDERS OF THE SPLEEN

i. **Splenomegaly (Enlarged Spleen):** Caused by infections (malaria, mononucleosis), liver diseases (cirrhosis), blood cancers (leukemia, lymphoma), or autoimmune disorders.
ii. **Hypersplenism:** Overactive spleen destroys blood cells to quickly, leading to anemia, low platelet count, or low white blood cells.
iii. **Ruptured spleen:** Typically, due to trauma, leading to internal bleeding and potentially life-threatening conditions.
iv. **Infections:** Malaria, bacterial sepsis, and viral infections can impair spleen function.
v. **Cysts and Tumors:** Rare growths that may affect spleen function.
vi. **Functional Asplenia/Hyposplenia:** Reduced or absent spleen activity, as seen in sickle cell disease, making individuals prone to infections.

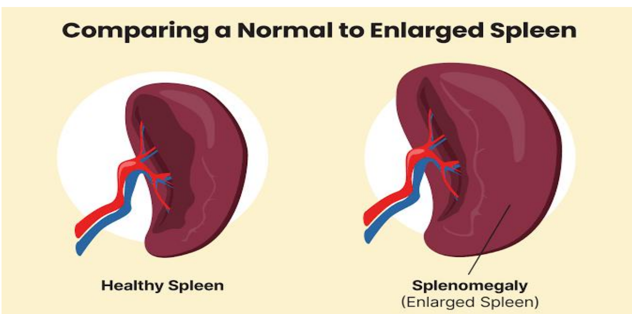
1.2.17.3 SIGNS AND SYMPTOMS OF SPLEEN PROBLEMS

i. Pain or fullness in the upper abdomen, sometimes radiating to the left shoulder
ii. Feeling full without eating much
iii. Fatigue or weakness due to anemia
iv. Frequent infections (due to impaired immune function)
v. Easy bruising or bleeding (low platelet count)
vi. Unexplained weight loss or fever (in cases of tumors or infection)

1.2.17.4 TIPS TO KEEP THE SPLEEN HEALTHY

i. Maintain a balanced diet: Include fruits, vegetables, and foods rich in antioxidants to support immune function.
ii. Exercise regularly: Enhances blood circulation and overall immune health.
iii. Prevent infections: Practice good hygiene, get vaccinations, and avoid exposure to infectious diseases.
iv. Avoid trauma: Protect the abdomen from injuries that may damage the spleen.
v. Monitor chronic conditions: Manage diseases such as liver disorders or blood disorders that can affect spleen health.
vi. Limit alcohol and toxins: Excessive alcohol can affect liver and spleen function indirectly.

STRUCTURE OF THE SPLEEN



CHAPTER TWO

MATERIALS AND METHODS

2.1.1 MATERIALS

a) Conical flask
b) Round bottom flask
c) Filter paper
d) Beakers
e) Test tubes
f) Measuring cylinder
g) Soxhlet extractor
h) Condenser
i) Pencil
j) Ruler
k) Capillary tubes
l) Scissors
m) Extraction thimbles
n) Sample bottles
o) Funnels

2.1.2 MEDICINAL PLANT

- *Tetrapleura Tetraptera*

2.1.3 SOLVENT AND REAGENT

1. Methanol
2. n-Hexane
3. Ethyl Acetate
4. Picric Acid
5. Lead Acetate
6. Iron (III) Chloride Solution
7. Ethanol
8. Acetic Anhydride
9. Tetraoxosulphate (IV) Acid
10. Potassium Hydroxide
11. Hydrochloric Acid

12. Fehlings Solution

2.1.4 Laboratory Equipment

I. Weighing balance
II. Heating mantle
III. Water bath
IV. Rotary evaporator
V. Electric Blender

2.1.5 Safety Equipment

I. Lab coat
II. Nose Mask
III. Gloves
IV. Hand Gloves

2.2 PLANT COLLECTION, IDENTIFICATION AND PREPARATION

Tetrapleura tetraptera stems were purchased at Uselu Market, Egor local Government Area, edo State and were identified by a Taxonomist, Prof. H. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. A voucher number of UBH-T472 was deposited at the herbarium. The respective plant parts were dried in the laboratory for twenty-eight days pulverized by electric blender.

2.3 METHODS

2.3.1 Extraction of Bioactive Phytochemical

Two hundred and thirty-four grams of the dried sample were extracted using Soxhlet extractor for eight hours with methanol solvent. The crude extract was then concentrated using a rotary evaporator (model, RE, 200) at 50°C. The extract was weighed and a portion was kept for phytochemical analysis and isolation while the other was used for hematological analysis.

2.3.2 Phytochemical Screening of The *Tetrapleura Tetraptera* Plant

The phytochemical screening of *Tetrapleura Tetraptera* were performed using standard methods and procedures by Sofowora (1993), Trease and Evans (1987).

Test for Glycosides: 1ml of each plant extracts were dissolved in 1ml glacial acetic acid containing 1 drop of ferric chloride solution. This was under-layered with 1ml concentrated sulphuric acid. A brown ring at the interface is indicative of the presence of glycoside.

Test for Saponins: 1ml of each plant extracts were shaken with water in a test tube and observed for the frothing. Saponins rein swiss (supplied by Merck) were used as a standard.

Test for Phenolics: 1ml of the plant extracts were added to 5ml of 90% ethanol. in addition, 1 drop of 10% ferric chloride ($FeCl_3$) were added. A pale-yellow coloration indicates the presence of phenolic compounds.

Test for Flavonoids: 2ml of the extract was boiled in 10ml distilled water and filtered. 1ml of the filtrate were measured and a few drops of dilute Lead acetate were added. An intense yellow color appears in the test tube to indicate the presence of flavonoids.

Test for Tannins: To 2ml of the plant extract, 10ml of distilled water was added and boiled for 3 minutes and then filtered into a test tube, about 2 drops of the filtrate, Ferric chloride ($FeCl_3$) solution was added; formation of a bluish precipitate is required for tannin.

Test for Eugenols: 2ml of the plant extract were mixed with 5ml of 5% potassium hydroxide solution. The aqueous layer was separated and filtered. Few drops of dilute HCl were added to the filtrate, a pale-yellow precipitate indicate a positive test.

Test for Terpenoids: 5ml of each extract were mixed in 2ml chloroform, and 3ml concentrated sulphuric acid were carefully added down the side of the inner wall of the test tube to form a layer. A reddish-brown coloration indicates the presence of terpenoids.

Test for Steroids: 2ml of acetic anhydride were added to 0.5g plant extract in 2ml dilute sulphuric acid. A color change from violet to blue - green indicate the presence of steroids.

Test for Alkaloids: Picric acid was used to test for alkaloid. About 1ml each of the plant extract was added to 2ml of picric acid. A yellow precipitate indicates a positive test.

Test for Reducing Sugars: Equal volume of Fehling's solution A and B was boiled for one minute and 1ml of the plant extract added and boiled for 5 minutes. A brick- red precipitate is required for a positive test.

2.3.3 FRACTIONATION AND ISOLATION OF CRUDE EXTRACT

Identification of pure compound from the crude methanol extract was done by combination of Vacuum Liquid chromatography (VLC) and thin layer chromatography

2.3.3.1 Vacuum Liquid Chromatographic (VLC) Analysis

The crude extract was combined uniformly with silica gel (50:50) and subjected to VLC using silica gel (60-120 mesh) as the stationary phase. The sample was eluted with the aid of a vacuum pump using combination of solvents consisting of 100% hexane, hexane: ethylacetate(1:1) and methanol (100%) solvents as mobile phase. Each of the three eluted fractions was respectively concentrated and monitored using thin layer chromatography (TLC). The three fractions eluted from the VLC were examined by precoated TLC plates in few suitable solvent systems to obtain the retention factor (Rf) of any components that appeared as spots. Fractions with similar Rf values were combined, dried and coded.

Isolated light yellow oil coded sample 1 (R_f 0.85, solvent system: hexane: acetate 3:7) was obtained from hexane: acetate (50:50) fraction while hexane and methanol VLC fractions indicated blurred separations from TLC.

2.3.3.2 THIN LAYER CHROMATOGRAPHY

(Determination of Retention Factor (R_f) By Thin Layer Chromatography)

A precoated TLC plates were cut to size of 9cm length by lcm width and used at varying solvent systems of, N- hexane, ethyl acetate, methanol in order of increasing polarity. The three fractions eluted from the VLC were examined by preparatory TLC plates in a few suitable solvent systems to obtain the retention factor (Rf) of any components that appeared as spots. The R_f value were calculated from the distance moved by the compound against the solvent front

$$\text{Retention Factor} = \frac{distance\ moved\ by\ the\ compound}{distance\ moved\ by\ the\ solvent}$$

2.3.3.3 GC-MS analysis

The GC-MS analysis of the light-yellow oil (sample 1) of the methanol extract of *Tetrapleura tetraptera* was done on a Shimadzu, GCMS-QP2010SE. Separation of the oil was carried out on a HP-5 MS (5% phenylsiloxane) column with nitrogen as the carrier gas with a flow rate, 1.80 ml/min. The oven programme was set at a temperature of 70 ^{o} C and held for 2 minutes, then it was ramped at a rate of 10^{o}C/min to 280^{o}C and held for 7 minutes. The oil sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min-1. The identification of the oil chemical constituents was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS. The retention indices, peak area percentage and mass spectra fragmentation pattern of the chromatogram of the oil sample was compared with the database of National Institute of Standards and Technology (NIST), NIST08.LIB [19] for possible identification of name, molecular weight, formula and structure of the chemical constituents in the oil sample.

2.3.4 EXPERIMENTAL ANALYSIS

Mature Wistar rats were obtained from the animal holding facility of the Anatomy Department of the University of Benin, Nigeria. These animals were used for the research study. Prior to the experiment, a 21-day acclimatization period was observed. Throughout this time, the rats had unrestricted access to a standard commercial diet and fresh water.

Ethical considerations:

Permission to conduct the current study was obtained from the Faculty of Pharmacy, Animal Use and Ethics Committee of the University of Benin with a permit reference number CMS/REC/2023/240.

2.3.5 EXPERIMENTAL GROUPING AND DOSAGE OF METHANOL EXTRACT OF T. TETRAPTERA FRUIT

Group A-Control received 1 mL of distilled water

Group B: 150 mg/kg of phenyl hydrazine

Group C: 150mg/kg of phenyl hydrazine + 200mg/kg *Tetrapleura Tetraptera*

Group D: 150mg/kg phenyl hydrazine + 400 mg/kg *Tetrapleura tetraptera*

Duration of studies: 21 days

2.3.6 BODY WEIGHT DETERMINATION

At the start of the experiment and at the end of the 21-day period, the rats were weighed to the nearest grams using the Metler PE 6000® digital weighing balance. At the end of the 21-day period of administration, the animals were sacrificed and the liver and spleen were respectively isolated, washed with distilled water and weighed. Then the spleno- and hepato-somatic indices were assessed respectively for the spleen and liver of the rats.

CHAPTER THREE

RESULT, DISCUSSION AND CONCLUSION

3.1 EXTRACTION

The result of the percentage yield and physical characteristics of the extract is shown in Table 1

Table 1: Percentage Yield and Physical Characteristics of ethanol extracts of leaves, stem and root of *Tetrapleura tetraptera*

Mass of plant parts	Mass of crude extract	Yield (%)	Colour	State
Tetrapleura tetraptera (234g)	**46**	**19.658**	**Red**	**Solid**

3.2 PHYTOCHEMICAL SCREENING OF METHANOL EXTRACT OF *TETRAPLEURA TETRAPTERA* FRUIT

S/N	PHYTOCHEMICALS	*T.TETRAPTERA FRUIT*
1	Glycosides	+
2	Saponins	+
3	Phenolics	-
4	Tannins	+
5	Terpenoids	+
6	Steroids	-
7	Flavonoids	+
8	Eugenols	+

9	Alkaloids	+
10	Reducing Sugars	+

Table 2: Phytochemical screening of *Tetrapleura Tetraptera* Fruit

+ = present   - = absent

Glycosides, saponins, terpenoids, eugenols, flavonoids, tannins, alkaloids and reducing sugar were all present in stem of *T.tetraptera* while phenolics and steroids was completely not detected in the methanol extract.

3.3.1 THIN LAYER CHROMATOGRAPHY

$$\text{Retention Factor (RF) value} = \frac{\text{Distance moved by a compound}}{\text{Distance moved by a solvent (solvent front)}}$$

The precoated thin layer chromatographic results of the VLC fractions using selected solvents are shown in the following tables below.

Table 3: Retention factor and color reaction of methanol extract of *Tetrapleura tetraptera Fruit*

Solvent systems: 7:3 (Hexane: Acetate)

Solvent front: 9cm

Spot	**Distance moved by compound**	**Colour under white light**	**Retention factor**
Origin	0.0	Dark Green	0.00
1.	4.00	Dark Yellow	0.44
2.	3.80	Light Green	0.42
3.	3.40	Light Yellow	0.38

Table 4: Retention factor and color reaction of Methanol extract of *T.tetraptera* fruit using

Solvent systems: 9:1 (Methanol: Hexane)

Solvent font: 9.0cm

Spot	**Distance moved by compound**	**Color under white light**	**Retention factor**
Origin	0.00	Dark Brown	0.00

1	3.1	Greenish-Yellow	0.34

Table 5: Retention factor and color reaction of methanol extract of Methanol extract of *T.tetraptera* fruit using

Solvent system: 1:1 (3:3) (Hexane: Acetate)

Solvent font: 9.0 cm

Spot	**Distance moved by compound**	**Color under white light**	**Retention factor**
Origin	0.00	Dark Brown	0.00
1	4.4	Light Yellow	0.48
2	2.7	Dark Brown	0.3

Table 6: Retention factor and color reaction of methanol extract of Methanol extract of *T.tetraptera* fruit using

Solvent system: 9:1 (Methanol: Acetate)

Solvent font: 8.8 cm

Spot	**Distance moved by compound**	**Color under white light**	**Retention factor**
Origin	0.00	Dark Brown	0.00
1	4.00	Dark Brown	0.45

Table 7: Retention factor and color reaction of methanol extract of Methanol extract of *T.tetraptera* fruit using

Solvent system: 1:1 (Hexane: Acetate)

Solvent font: 8.8 cm

Spot	**Distance moved by compound**	**Color under white light**	**Retention factor**
Origin	0.00	Dark Brown	0.00
1	2.20	Light Green	0.25

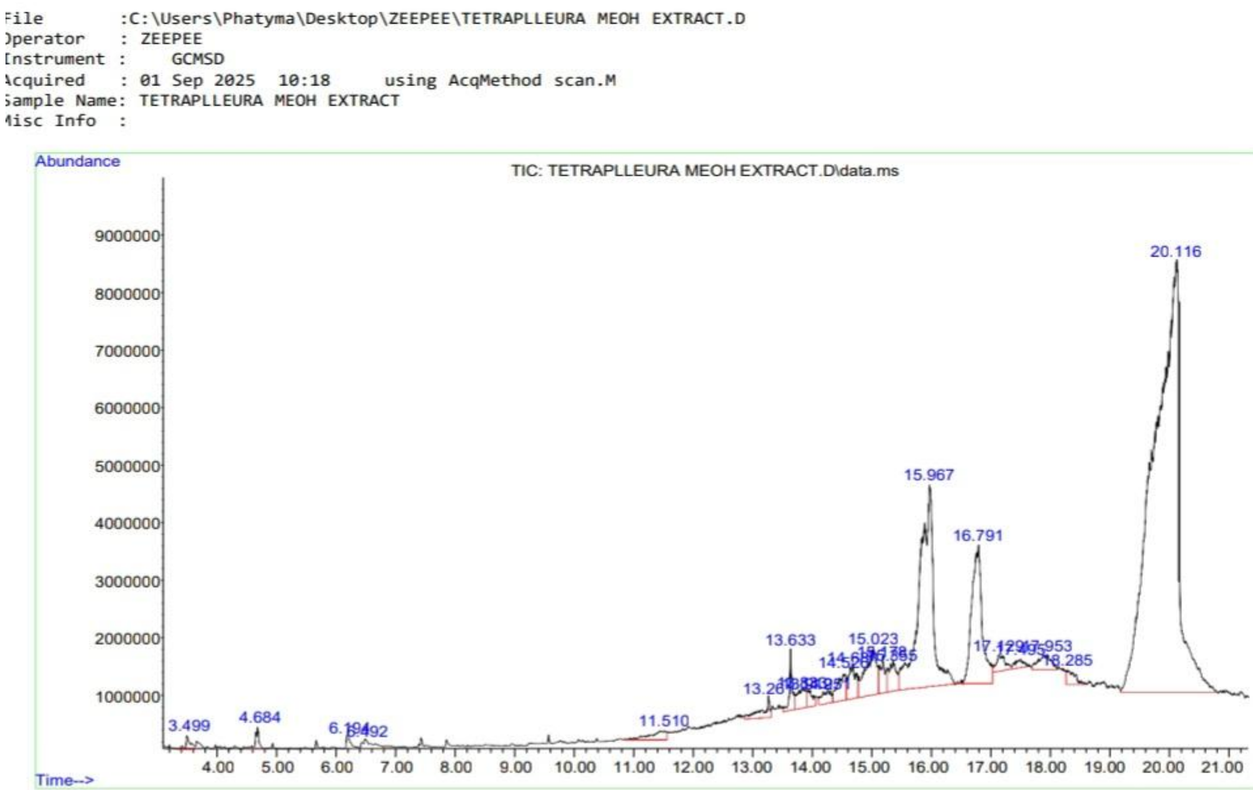
3.3.2 GC-MS ANALYSIS RESULT

The GC-MS chromatogram of the isolated light-yellow oil given in Appendix 8, showed twenty-two (22) peaks indicating from the search list of the chemical abstract service twenty-two compounds. The chemical compounds identified (according to NIST Library) in the oil fraction are presented in Table below

Peak	Retention Time	Organic compound	Area (%)
1	3.499	Phenol	0.34
2	4.684	2-methoxy-, Phenol	0.42
3	6.194	N-Aminopyrrolidine	0.33
4	6.492	4-Vinylphenol	0.45
5	11.510	1-Tetracosene	0.80
6	13.261	Methyl ester, Hexadecanoic acid	0.79
7	13.633	n-Hexadecanoic acid	0.91
8	13.833	1-Hexacosene	0.93
9	13.919	1-Tetracosene	0.48
10	14.251	Octatriacontyl pentafluoropropionate	0.62
11	14.526	2-(octadecyloxy)-, Ethanol	1.20
12	14.680	1-Nonadecene	1.40
13	15.023	Dotriacontyl heptafluorobutyrate	2.76
14	15.178	Triacontyl trifluoroacetate	1.00
15	15.355	1-Hexacosene	1.27
16	15.967	Octacosane	15.25

17	16.791	Squalene	7.85
18	17.129	2-Decyl-1-tetradecanol	0.78
19	17.495	Octatriacontyl pentafluoropropionate	0.37
20	17.953	1-Hexacosene	1.06
21	18.285	Triacontyl pentafluoropropionate	0.66
22	20.116	Nonadecane	60.32
TOTAL			100

Appendix 8: *GC-MS Analysis of Methane fraction (Yellow oil) of Tetrapleura Tetraptera*



The oil constituents of *Tetrapleura tetraptera* fruit indicates the presence of twenty-two compounds. Detected compounds include nonadecane (Retention time (RT): 20.116, 60.32%), octacosene (Retention Time (RT): 15.967, 15.25%) as the two major compounds while squalene (Retention Time (RT): 16.791, 7.85%) was also indicated in the oil fraction. Nonadecane has been reported to exhibit antimicrobial, antioxidant, and insecticidal properties. It also serves as a hydrocarbon marker in natural products and may synergize with other phytochemicals to enhance therapeutic effects (Akinmoladun et al, 2020: Elaiyaraja & Chandramohan, 2022). Octacosene, a straight chain alkane known for anti-inflammatory, antimicrobial and anticancer activities (Ajayi et al, 2022). While Squalene is a triterpenoid that has been widely recognized for its antioxidant, anticancer, hepatoprotective, immune-boosting effects. It also plays a role in cholesterol and steroid biosynthesis, making it a key bioactive in medicinal plants (Wahedi et al., 2017; Adesina et al., 2016).

3.4 SPLENO-SOMATIC INDEX

The spleno-somatic index (SSI) is a ratio used to determine the relative size of the spleen compared to the body weight of an animal. It is used to assess the spleen health and physiological status.

$$SSI = \frac{\textbf{Spleen weight}}{\textbf{Body weight}} \textbf{ x 100}$$

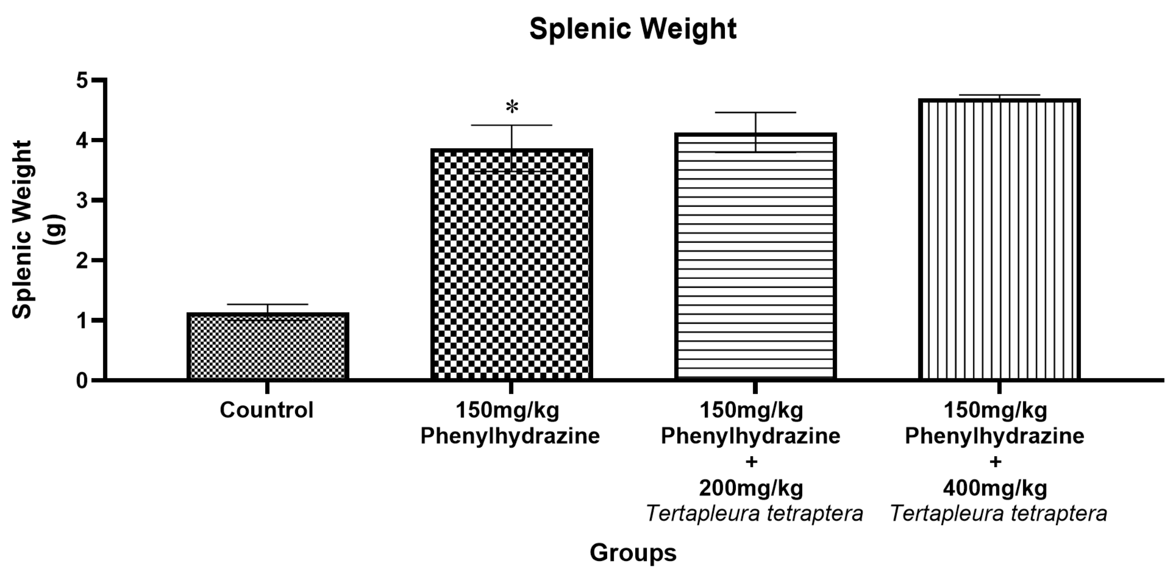


Chart 1: Splenic weight after administration. Values are given as mean ± SEM. * p<0.05 compared with control.

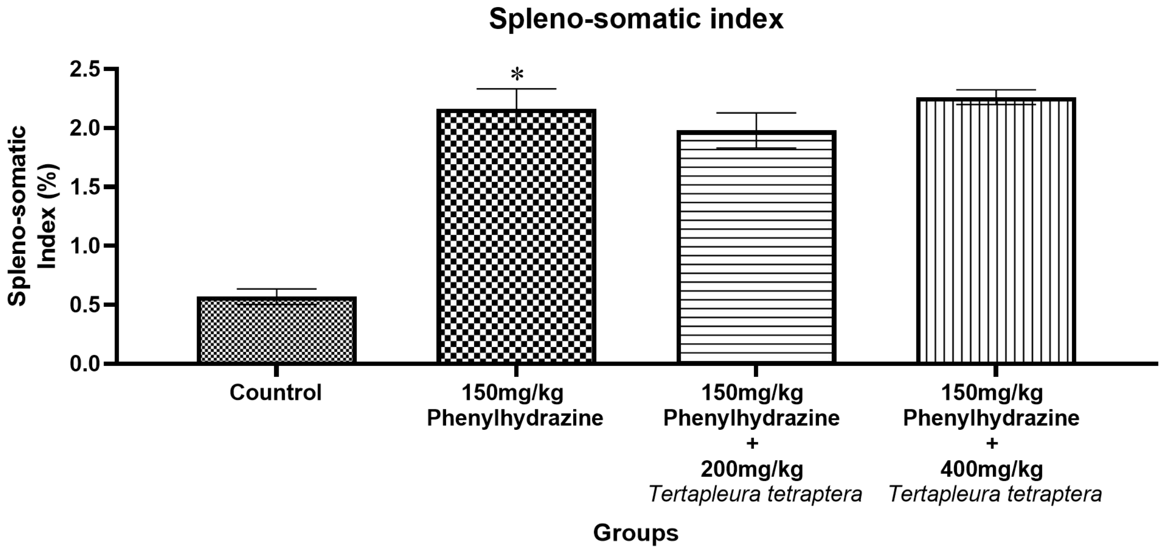


Chart 2: Spleno-somatic index after administration. Values are given as mean ± SEM. * $p<0.05$ compared with control.

Normal range SSI – 0.2-0.3% / body weight

3.5 HEPATO-SOMATIC INDEX

This is the measure used to assess the relative size of the liver compared to the body weight of an organism. It is used to assess the liver health and metabolic status.

$$\text{HSI} = \frac{\textbf{Liver weight}}{\textbf{Body weight}} \ \mathbf{x\ 100}$$

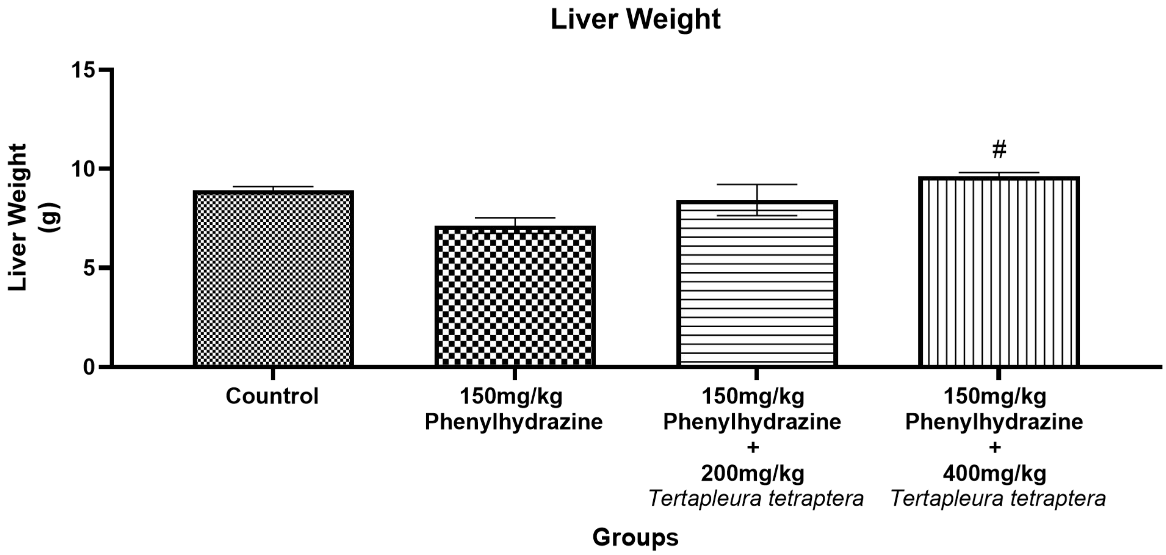


Chart 3: Liver weight after administration. Values are given as mean ± SEM. #p<0.05 compared with 150mg/kg of Phenyl hydrazine only.

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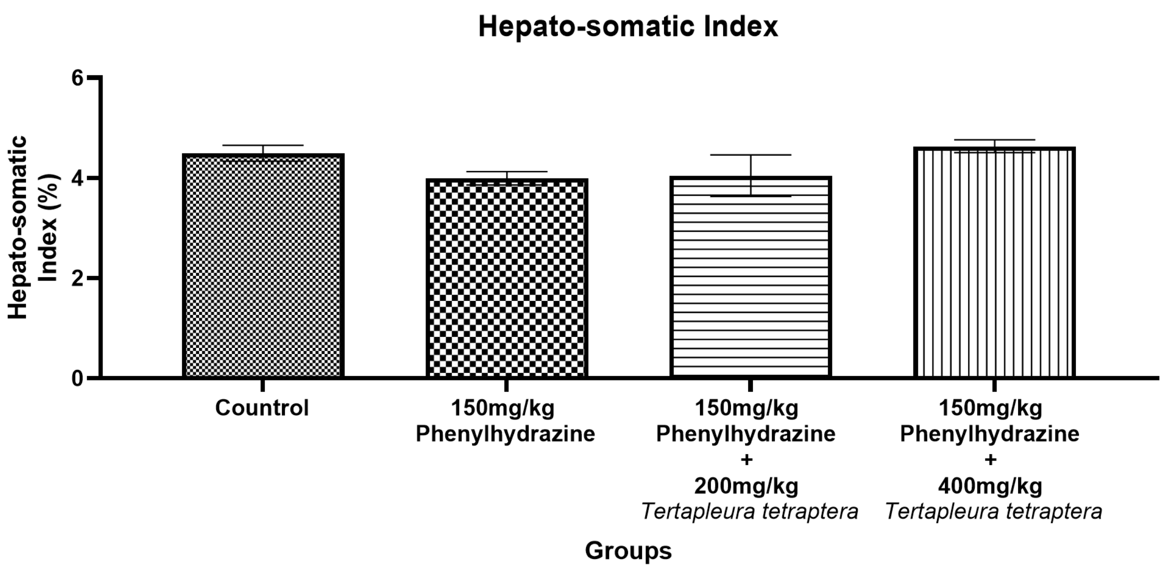

Chart 4: Hepato-somatic index after administration. Values are given as mean ± SEM.

Normal Range HSI – 3.5 – 5% / body weight

3.6 DISCUSSION

Table 2: Highlights the different phytochemicals of *Tetrapleura tetraptera* plant. From the analysis, it was observed that glycosides, saponins, terpenoids, eugenols, flavonoids, tannins, alkaloids and reducing sugar were all present in noticeable amounts, enough to be easily detected during testing. This suggests that the stem contains a rich variety of beneficial compounds. On the other hand, components such as steroids and phenolics were either completely absent or present in such tiny quantities that they could not be identified in comparison with the findings of Antiproliferative activities of methanolic extract and fractions of *Tetrapleura tetraptera* fruit (Anning, A., et al., 2021).

Table 8: The GC-MS analysis of Tetrapleura tetraptera methanol extract revealed that nonadecane, octacosene, and squalene are the major constituents. These compounds, known for their antimicrobial, antioxidant, and anti-inflammatory activities, likely contribute to the plant's traditional medicinal value and support its potential use in drug development.

Chart 1: Spleen Weight

The data illustrates the effects of absolute spleen weight across the experimental groups. The control group established a baseline spleen weight of 1.13 ± 0.12 g. administration of 150mg/kg phenylhydrazine cause a severe and statistically significant increase in spleen weight to 3.87±0.38 g, confirming the successful induction of splenomegaly. Treatment with *Tetrapleura tetraptera* extract did not counteract this effect. The group receiving pheylhydrazine plus 200mg/kg of extract showed a spleen weight of 4.13±0.29g, while the group receiving the high dose of 400mg/kg extract exhibited the highest mean weight of 4.70±0.06g. the analysis concludes that the plant extract, at the tested doses, did not prevent phenylhydrazine-induced increases in absolute spleen weight.

Chart 2: Splenosomatic Index

The chart presents the spleno-somatic index (SSI), which normalizes spleen weight to the animal's total body weight. The control group showed a normal SSI of 0.57±0.06%. induction with phenylhydrazine caused a drastic increase in the SSI to 2.17 ±0.17%, indicating a pathological enlargement of the spleen relative to body size. The efficacy of *tetrapleura tetraptera* extract was evaluated at two doses. The group treated with phenylhydrazine and 200mg/kg extract had an SSI of 1.98±0.14%, and the group treated with phenylhydrazine and 400mg/kg extract had an SSI of 2.26±0.05%. These results demonstrate that the extract did not produce a meaningful reduction in the relative spleen enlargement cause by phenylhydrazine.

Chart 3: Liver Weight

The data here tells a clear and positive story. The control group gives us our baseline for a healthy liver at 8.93g. as expected, the phenylhydrazine toxin caused significant damage, which is clearly seen in the sharp drop in liver weight down to 7.13g-the organ was clearly struggling. The low dose (200mg/kg) showed a promising trend towards recovery (8.43g) but the high dose really stood out, not just reversing the damage but bringing the liver weight to a robust 9.63g.

This strong, dose-dependent result is clear indicator that the *Tetrapleura tetraptera*'s extract effectively protected the liver from the toxin's harmful effects.

Chart 4: Hepatosomatic Index

While the absolute liver weight changed dramatically, the hepato-somatic index - which compares the liver's size to the animal's overall body weight – showed no statistically significant change across the groups. The values all hovered around 4.5%. this tells us that the changes we saw in chart 3 were proportional; the toxin caused overall weight loss that included the liver, and the extract's protection helped the entire body recover, not just the liver in isolation. So, the protective effect was not holistic, not causing any abnormal or disproportionate swelling of the organ itself.

3.7 CONCLUSION

The phytochemical screening ang organosomatic indices (spleno-somatic and hepato-somatic) of methanol extracts of *Tetrapleura tetraptera* fruit in cancer-induced Wistar rats reveal several significant organ-specific findings. Glycosides, saponins, terpenoids, eugenols, flavonoids, tannins, alkaloids and reducing sugar are among the bioactive substances that show the fruit of *Tetrapleura tetraptera* has a rich phytochemical profile.

The organosomatic index analysis suggests that the administration of *Tetrapleura tetraptera* fruit extract has a distinct, organ-dependent effect. The effect demonstrated a potent, dose-dependent hepatoprotective effect on the liver, as shown by the significant prevention of phenylhydrazine-induced liver weight loss and the restoration of normal liver mass. On the other hand, the extract showed no protective effect on the spleen, as it failed to reduce phenylhydrazine-induced splenomegaly, instead, splenic weight and spleno-somatic index remained highly elevated even at higher doses of the extract, indicating no reduction of splenic stress under the experimental conditions.

The presence of antioxidant compounds, especially flavonoids and phenolics, is suggested to be responsible for the observed liver protection, while their inability to counteract splenic enlargement highlights the organ-specific nature of phyto-therapeutic interventions. However, further research should be done to clarify the specific mechanisms of action of individual phytochemical compounds, establish precise dosage ranges for therapeutic application, investigate the reasons for the organ-specific effects, and examine potential interactions with other medications.

In summary, the findings partially support the traditional use of *Tetrapleura tetraptera* in herbal medicine for liver-related ailments while highlighting the complexity of its effects, the ineffectiveness against splenic stress, and the critical importance of appropriate dosing and target organ consideration.

RECOMMENDATION

The intake of *Tetrapleura tetraptera*-based natural remedies in traditional medicine should be encouraged for supporting liver health, as the extract demonstrated significant hepatoprotective properties. However, its use should be mindful of organ specific effects, as it did not show protective benefits under the conditions of this study.

Furthermore, quantitative phytochemical analysis should be carried out to determine the exact considerations of active compounds- such as flavonoids and phenolic compounds- responsible for the observed liver protection. This will help establish safe and effective dosage guidelines for traditional and potential clinical applications.

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University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Tetrapleura tetraptera* (Schumach & Thonn.) Taub.

Family: Fabaceae

Common Name: Soup Perfume, "Perekese"

Voucher Number: UBH-T472

Staff Name: Iyekowa, O.

Plant Identification and Voucher Number Issued by:

11/09/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, MSWS; USA, MECOSON, MBOSON, MAEIAN, MFBAN; Nigeria).

ZEEPEE Library Search Report

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PHY.M Fri Sep 12 07:54:00 2025 Page: 2

ZEEPEE Library Search Report

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			Propionic acid, 3-iodo-, octadecyl ester	365866	1000406-24-9	89
			Hexatriacontyl pentafluoropropionate	391707	1000351-89-0	81
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