MINERAL ANALYSIS AND COMPARATIVE PHYTOCHEMICAL INVESTIGATION OF THREE EXTRACT OF *Tetrapleura Tetraptera*

BY

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FACULTY OF PHYSICAL SCIENCES

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UGBOWO, BENIN CITY

EDO STATE

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A PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, FACULTY OF PHYSICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FUFILLMENT OF THE REQUIREMNTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.SC HONOURS) IN CHEMISTRY

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CERTIFICATION

This is to certify that this research project was carried out by BOLUWAJI VICTOR OLUWATOBI with the matriculation number PSC2105249 in the Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This research is dedicated to God Almighty who gave me the strength to finish this work successfully, and to my parents Mr. and Mrs. Boluwaji as well as my siblings and friends for their immense and unwavering support.

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My sincere gratitude goes to God Almighty for His guidance, protection, and provision to the end of this project work.

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I feel greatly indebted to my parents, Mr. and Mrs. Boluwaji for their relentless efforts, financial support and unconditional love throughout the period of this undergraduate programmed. Also, to my lovely siblings for their words of encouragement.

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I sincerely remain grateful to my friends; Goodness, Bext, Destiny, Itohan, Emma, Eunice and to my course mates.

ABSRACT

This study examined the mineral composition and phytochemical profile of *Tetrapleura tetraptera* fruit pods. The fruit pod was purchased from Uselu market, dried and extracted by maceration method using aqueous, methanol and hexane solvents. The elemental composition was analyzed with a portion of the powdered sample using g X-ray fluorescence spectroscopy (XRF) while phytochemical screening of the three extracts was done via standard methods. Mineral analysis revealed potassium (28.317%) as the most abundant element, followed by calcium (12.962%), magnesium (7.656%), and aluminum (6.413%). Moderate amounts of Sulphur (1.652%) and chlorine (2.412%) were also detected, while iron was present at a relatively low concentration (1.502%). The dominance of potassium and calcium highlights the fruit's nutritional potential for maintaining electrolyte balance, bone development, and muscle function. Magnesium and aluminum further contribute to enzymatic activities and possible medicinal value, though the low iron content indicates limited benefit for hemoglobin synthesis and oxygen transport. Phytochemical screening showed that alkaloids, eugenol, and reducing sugars were present across all extracts. Glycosides, flavonoids, tannins, phenolics, and terpenoids were prominent in methanol and aqueous extracts, while hexane extracts yielded fewer polar compounds. Steroids were absent in all extracts. These results confirm that solvent polarity strongly influences phytochemical extraction, with polar solvents yielding a broader spectrum of bioactive constituents.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The plant material, *Tetrapleura tetraptera* (TT) was chosen to be used as a reliable source of polyphenols because it has been underutilized and limited to usage in traditional medicine and food production with minimal research focus currently (Kemigisha et al., 2018). *T. tetraptera* is a flowering plant common in Western and Central Africa. The plant is called *prekese* and *uhio* in Ghana and Nigeria, respectively, and has been adored for its numerous uses. The fruit is also commonly known as *aidan fruit* in many parts of the world. It is noted for its sweet fragrance and is used as spice in dishes and beverages and its bark, leaf and fruits have been used for medicinal purposes. *T. tetraptera* fruits have been recognized for their high nutritional value (~64 % carbohydrates, 289 mg g^{-1} potassium, 200 mg g^{-1} calcium, 342 mg g^{-1} manganese, and 5 mg g^{-1} vitamins) and phytochemical composition (phenolic acids, flavonoids, saponins, tannins and alkaloids) (Mensah et al., 2024). It has been reported to have significant medicinal value due to its biological significance as it possesses antioxidant, anti-proliferative, anti-inflammatory, antimicrobial, antidiabetic, and anti-parasitic properties (Dzah, 2022, Dzah, 2023). It is therefore commonly used to treat diseases such as diabetes, cancer, hypertension, immune disorders, malaria and many other diseases. Earlier studies have also extracted polyphenols from TT, tested its biological activity and its phenolic composition (Dzah, 2022, Dzah, 2023). Given its rich phytochemical content, it is important to further explore other different solvents for extraction and identification of the medicinal phytochemicals in the plant. Thus, the study is aimed at investigating the mineral analysis and comparative phytochemical screening of three extracts of Tetrapleura Tetraptera.

1.1.1 BACKGROUND OF STUDY

Medicinal plants have long been central to healthcare systems, especially in many parts of Africa where they are relied upon as affordable and accessible alternatives to orthodox medicine. Their therapeutic value is largely attributed to the presence of phytochemicals—naturally occurring compounds such as alkaloids, flavonoids, tannins, saponins, and terpenoids—which are known to exhibit diverse biological activities including antioxidant, antimicrobial, anti-inflammatory and antidiabetic effects (Anyamele et al., 2023; Babalola, 2023).

One plant that has attracted considerable attention is *Tetrapleura tetraptera*, commonly referred to as Aidan fruit. Beyond its widespread use as a culinary spice, the plant holds significant cultural and medicinal importance in West Africa. It has been reported to have significant medicinal value due to its biological significance as it possesses antioxidant, anti-proliferative, anti-inflammatory, antimicrobial, antidiabetic, and anti-parasitic properties (Dzah, 2022, Dzah, 2023). It is therefore commonly used to treat diseases such as diabetes, cancer, hypertension, immune disorders, malaria and many other diseases.

Similarly, investigations of different plant parts such as the fruit, leaves and stem bark reveal distinct phytochemical compositions, with some studies suggesting the stem bark yields stronger antioxidant activity than other parts (Babalola, 2023).

These variations highlight the need for comparative phytochemical investigations. By evaluating how different solvents and plant parts influence the type and number of phytochemicals obtained, researchers can determine the most effective extraction strategies for specific therapeutic purposes. This study seeks to provide a comprehensive analysis of the phytochemical composition of methanol, hexane and aqueous extracts, thereby contributing to a better understanding of solvent selection for maximizing the medicinal and nutritional benefits of *Tetrapleura Tetraptera*

1.1.2 STATEMENT OF PROBLEM

For centuries, the fruit of *Tetrapleura tetraptera* has been valued across West Africa not only as a spice but also as a medicinal resource for conditions such as hypertension, diabetes, infections and inflammatory diseases. Ethnobotanical accounts attribute these benefits to the plant's rich phytochemical composition, yet the scientific evidence remains fragmented and often contradictory (Mensah et al., 2024; Anyamele et al., 2023).

The first issue is that findings on the phytochemical profile of *T. tetraptera* lack consistency. While some studies report abundant flavonoids and phenolics in polar extracts, others show these compounds in much lower amounts, with saponins and terpenoids dominating instead (Gberikon et al., 2015; Ouattara et al., 2024). Such inconsistencies make it difficult to establish a reliable understanding of which compounds are truly responsible for the plant's medicinal activity. This problem ought to be resolved through a systematic comparison of solvents with different polarities so that the influence of solvent type on phytochemical yield becomes clearer.

Finally, the absence of standardized comparative data has limited the translation of *T. tetraptera* from traditional use to formal pharmacological applications. At present, neither dosage forms nor therapeutic guidelines can be developed with confidence because the solvent system that best concentrates bioactive compounds has not been established (Ekeanyanwu et al., 2024). This problem ought to be resolved by generating robust comparative evidence that can guide both traditional practitioners and pharmaceutical researchers in maximizing the plant’s therapeutic potential.

In light of these issues, this study seeks to fill the gap by conducting a comparative phytochemical investigation of *T. tetraptera* extracts obtained using hexane, water and methanol.

1.1.3 RELEVANCE OF STUDY

This study holds particular relevance because it addresses a gap in the current understanding of *Tetrapleura tetraptera* by directly comparing extracts prepared with hexane, water and methanol. Much of the existing literature focuses on either aqueous or ethanol-based extractions, leaving the role of non-polar solvents such as hexane largely unexplored. By conducting a systematic comparison across solvents of different polarities, the research will provide clearer evidence on how solvent choice influences the yield and diversity of phytochemicals. This not only resolves earlier inconsistencies in findings but also enriches the scientific knowledge base on medicinal plants used in African ethnomedicine (Ouattara et al., 2024; Gberikon et al., 2015).

The study is also relevant in the context of traditional medicine. Communities that use *T. tetraptera* typically prepare it by boiling the fruit in water, yet there is little evidence to show whether such preparations capture the same compounds identified in laboratory studies that rely on methanol or other organic solvents. By including water alongside methanol and hexane, the research creates a bridge between traditional practices and modern scientific investigation, allowing for a more balanced evaluation of the plant's therapeutic potential (Ekeanyanwu et al., 2024).

In addition, the work is of value to pharmaceutical and nutraceutical development. Identifying the solvent system that yields the most therapeutically relevant phytochemicals is essential for standardizing herbal remedies and isolating compounds with potential for drug discovery. By comparing polar, intermediate and non-polar solvents, this research will highlight the extraction approach most suitable for maximizing bioactive compound recovery (Babalola, 2023).

The significance of the study also extends to public health and the economy. With the rising global interest in natural remedies and functional foods, a stronger scientific foundation for *T. tetraptera* could encourage its wider application, creating opportunities for local cultivation, trade and value-added production. At the same time, it offers communities a culturally familiar and affordable alternative to synthetic medicines (Mensah et al., 2024; Anyamele et al., 2023).

Overall, the relevance of this research lies in its potential to resolve uncertainties in phytochemical reports, support traditional knowledge with scientific validation, guide pharmaceutical exploration, and contribute to both healthcare and socioeconomic development.

1.1.4 SCOPE OF THE STUDY

This study focuses on mineral analysis and the comparative phytochemical analysis of *Tetrapleura Teraptera* using methanol, aqueous and hexane as extraction solvents. The research aims to identify the bioactive compounds present in the fruit pod and evaluate the efficiency of these three solvents in extracting phytochemicals. The study provides foundational knowledge of the phytochemical composition of *Tetrapleura Tetraptera* and the comparative effectiveness of methanol, aqueous and hexane in solvent extraction.

1.1.5 AIMS AND OBJECTIVES

The aim of this project is to conduct mineral analysis and comparative phytochemical screening of *TerapleuraTeraptera*.

SPECIFIC OBJECTIVES:

To achieve the aim, the following specific objectives are stated to;

1. Collect, dry and pulverize *TerapleuraTerapptera*the fruit pod.
2. Determine the mineral analysis by XRF using the powdered sample
3. Extract separately the powdered sample by maceration method using methanol, aqueous and hexane solvents
4. Determine the phytochemicals in the three solvents extract using standard methods

1.2 LITERATURE REVIEW

1.2.1 MEDICINAL PLANTS

Medicinal plants represent the most ancient form of medication, used for thousands of years in traditional medicine in many countries around the world. The empirical knowledge about their beneficial effects was transmitted over the centuries within human communities. Natural products play a pivotal role as a source of drug compounds and, currently, a number of modern drugs which are derived from traditional herbal medicine are used in modern pharmacotherapy.

Medicinal plants have long been used for healing and health maintenance across cultures. This integrated note blends historical context, definitions, and usage patterns into a concise overview suitable for quick reference or teaching, without separating into subtopics.

Medicinal plants originate from ancient civilizations such as Mesopotamia, Egypt, India, and China. Texts like the Ebers Papyrus, Ayurvedic treatises, and Traditional Chinese Medicine (TCM) chronicles document early herbal therapies. Cultural diversity shaped herbal traditions, often linked to local flora, ecological knowledge, and spiritual or holistic worldviews.

Medicinal plants are defined as plants used for healing, preventive health, or to support bodily functions; they contain bioactive compounds that can modulate physiological processes. Common parts used include herbs (leaves, flowers), roots, bark, stems, seeds, and essential oils, and they can be prepared as teas, decoctions, tinctures, extracts, powders, capsules, ointments, and various topical applications. The key chemical classes often involved are phenolics, flavonoids, terpenoids, alkaloids, saponins, and essential oils.

Historically, traditional medicine systems such as Ayurveda (India) and Unani (Middle East/Asia) used plant-based formulations for balance, inflammation, infections, and vitality. Traditional Chinese Medicine (TCM) emphasizes herb formulas designed to address pathways like Qi, blood, and organ harmony. Indigenous and folk practices rely on locally available species for wound healing, respiratory issues, digestive problems, and more. In modern practice, there is a move toward standardization, phytochemical research to identify active constituents and mechanisms, clinical research to assess safety and efficacy, and regulatory oversight to ensure labeling, safety monitoring, and consideration of sustainable harvesting and potential drug interactions.

1.2.2 AIDAN PLANT (TETRAPLEURA TETRAPTERA)

The plant material, *Tetrapleura tetraptera* (TT) was chosen to be used as a reliable source of polyphenols because it has been underutilized and limited to usage in traditional medicine and food production with minimal research focus currently (Kemigisha et al., 2018). *T. tetraptera* is a flowering plant common in Western and Central Africa. The plant is called *prekese* and *uhio* in Ghana and Nigeria, respectively, and has been adored for its numerous uses. The fruit is also commonly known as *aidan fruit* in many parts of the world. It is noted for its sweet fragrance and is used as spice in dishes and beverages and its bark, leaf and fruits have been used for medicinal purposes.

T. tetraptera is deciduous, grows on the fringe of the West and Central African rainforest zone (found in Uganda, Mali, Burkina Faso, Mauritania and countries from Gambia to Nigeria) and is at its best growing most luxuriantly in the rain forest, reaching 20-25 m in height, with a girth of about 1.2-3 m.

PLANT SAMPLE

Fig 1.1: Tetrapleura tetraptera

1.2.3 TAXONOMY/CLASSIFICATION OF TETRAPLEURA TETRAPTERA

Kingdom	**Plantae**
Division	**Tracheophyta**
Subdivision	**Angiosperm**
Class	**Magnoliopsida**
Order	**Fabales**
Family	**Fabaceae**
Genus	**Tetrapleura**
Species	**Tetraptera**

Common Name (English)	**Aidan fruit**
Igbo	**Oshosho and Obogolo**
Ghana	**Prekese**
Yoruba	**Aridan**
Bini	**Ighimiakia**

1.2.4 CULTIVATION AND DISTRIBUTION OF TETRAPLEURA TETRAPTERA.

Tetrapleura tetraptera is distributed across tropical Africa, from Senegal to Tanzania, and thrives in rainforests, secondary forests, and riverine forests located in moist lowland tropical regions. While ideal daytime temperatures are approximately 25–30 °C and soils are moist and well-aerated (Mohammed et al., 2023), its widespread distribution also reflects its adaptability across habitats [adapted]. The fruit is a glossy dark-brown pod featuring four longitudinal wing-like ridgestwo of which are woody, while the other two house soft, oily pulpand contain small, hard, black, round seeds that ripen between September and December. Seed dormancy can be effectively overcome by scarification followed by soaking in sulfuric acid (Mohammed et al., 2023; Usman et al., 2019). When planted at a two-centimeter depth in forest soil, germination begins within about 7–10 days, reaching roughly 84% under suitable nursery conditions (Usman et al., 2019). The seedlings display rapid growth under full shade and are ready for transplanting approximately six weeks after germination. To minimize shock and defoliation, gradual acclimatization to brighter light is recommended before field planting (Usman et al., 2019).

This plant is a tropical moist lowland species found up to an elevation of 600 meters. Temperature: performs best at daytime temperatures of 25–30°C; tolerates 20–35°C. Rainfall: prefers mean annual rainfall of 1,700–3,000 mm but tolerates 1,200–5,000 mm. Light: requires a sunny position. Soil: grows best in light to medium, well-drained soil. Soil pH: prefers pH 4.5–6.5; tolerates 4–7.

Habitat: tropical moist lowlands; up to 600 m elevation. Climate: optimal 25–30°C; tolerates 20–35°C; 1,700–3,000 mm annual rainfall (tolerates 1,200–5,000 mm). Light & Soil: full sun; light to medium, well-drained soil. pH: prefers slightly acidic to neutral (4.5–6.5; tolerates 4–7).

1.2.5 REPORTED MEDICINAL USES OF TETRAPLEURA TETRAPTERA.

Tetrapleura tetraptera has been used traditionally for a number of medicinal purposes. Some of the reported health benefits include:

1.2.5.1 Antimicrobial properties

The use of medicinal plants like Tetrapleura tetraptera in the treatment of various human ailments such as respiratory infections, convulsions, and rheumatism has gained medical concern for the past few years especially in Africa, owing to the increasing cost and resistance of pharmaceutical antibiotics. Parts of the plant, including the leaf, stem bark, and fruits, play crucial roles in antimicrobial activity due to phytochemicals.Leaf extract of *Tetrapleura tetraptera* has been shown to exhibit antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albicans* (Ajiboye et al., 2021). The stem bark extract has demonstrated comparatively stronger antimicrobial activity, particularly against *S. aureus*, *Streptococcus pneumoniae* and *Candida* species (Enyi-Idoh et al., 2017). This suggests that the use of different parts of the plant may be strategically employed in treating a range of bacterial and fungal infections due to their antimicrobial properties (Olasehinde et al., 2019). Several studies further support the antibacterial potential of *T. tetraptera* extracts against various bacterial strains, though the efficacy often depends on both the solvent used for extraction and the bacterial species tested (Adesina et al., 2020). For instance, aqueous extracts of the fruit and seed have been reported to inhibit *Klebsiella pneumoniae* and *Staphylococcus aureus*, while organic extracts also exhibit inhibitory effects against fungi such as *Candida albicans* (Ekeanyanwu et al., 2024).

1.2.5.2 Anti-inflammatory properties

Tetrapleura tetraptera exhibits notable anti-inflammatory activity, which has underlined its traditional use in managing arthritis, inflammatory conditions, and rheumatic pain. Leaf fractions obtained with different solvents show varying degrees of anti-inflammatory effects. The fruit also contributes to anti-arthritic properties, likely through its anti-inflammatory actions. The plant’s anti-inflammatory potential is partly attributed to carvacrol, a monoterpene present in its composition and in essential oils from various plants. Carvacrol has documented therapeutic relevance and, in studies, reduces pro-inflammatory cytokines such as IL-1β and IL-8 in a dose-dependent manner, and it can inhibit the expression of cyclooxygenase-2 (COX-2) and nitric oxide synthase (NOS).Other constituents of *Tetrapleura tetraptera*including menthol, methyl eugenol, α-copaene, and octadecanoic acidhave well-documented anti-inflammatory activities (Doe et al., 2024). Studies involving the fruit extract demonstrated a clear, dose-dependent reduction of inflammation in a rat model using egg albumin-induced hind-paw edema, with doses ranging from 50 to 800 mg/kg (Ojewole& Adewunmi, 2017). These collective findings reinforce the potential of *T. tetraptera* as a valuable source for ethnomedicinal anti-inflammatory applications.

1.2.5.3 Anti-diabetic properties

Diabetes mellitus (DM) is a metabolic disorder arising from insufficient insulin activity. The World Health Organization (WHO) estimates that approximately 180 million people worldwide live with diabetes, a figure projected to rise by 2030. While conventional allopathic therapies are commonly used to manage DM, recent evidence suggests that Tetrapleura tetraptera may contribute to glycemic control.

Diabetes is often accompanied by elevated oxidative stress and a decline in antioxidant capacity, and phytochemicals particularly flavonoids are recognized for their free-radical scavenging and antioxidant activities (Famobuwa et al., 2015). Since *T. tetraptera* contains notable levels of flavonoids, these compounds may underpin its potential anti-diabetic effects (Famobuwa et al., 2015). The plant's stem and leaf contain limonene, a cyclic monoterpene with reported antihyperglycemic and antioxidant properties that could help mitigate diabetic complications (Adesina et al., 2020). Some studies report that fruit extracts of *T. tetraptera* exhibit glucose-lowering activity, with one study showing effects comparable to standard antidiabetic drugs like glibenclamide (Famobuwa et al., 2015). Additionally, aqueous root-bark extract has demonstrated significant antidiabetic effects in diabetic rats, supporting its potential use in diabetes management (Omonkhua et al., 2014).

1.2.5.4 Anti-proliferative (anti-cancer) properties

Medicinal plants have contributed to cancer treatment, and Tetrapleura tetraptera is among them. Plant-based compounds are sought after because some cancer therapies cause harsh side effects like hair loss, toxicity, and resistance. T. tetraptera has been reported to be cytotoxic to carcinoma cells and to reduce tumor burden. Specifically, the fruit's methanolic extract has shown anti-proliferative activity against leukemia and breast cancer cells. Studies on HepG2 liver cancer cells found that T. tetraptera extract reduced cell viability in a dose-dependent manner, with a minimum inhibitory concentration around 168 µg/mL, and often performed comparably to certain reference compounds.

Polyphenols in *Tetrapleura tetraptera* may contribute to its anticancer potential. These compounds can trigger apoptosis in cancer cells and, under oxidative stress, may act as pro-oxidants that promote cell death. For example, aqueous extracts of *T. tetraptera* have been observed to disrupt mitochondrial membrane potential in cancer cells, a critical event in the apoptotic cascade. Moreover, polyphenols are known to activate pathways associated with mitochondrial biogenesis and mitophagy, contributing to their antiproliferative and antitumor actions (Dzah et al., 2024; Dhiman et al., 2020).

1.2.6 REPORTED SCIENTIFIC USES OF TETRAPLEURA TETRAPTERA

Beverages and Preservative Potential of *Tetrapleura tetraptera*

In addition to its role in traditional alcoholic fermentation, *Tetrapleura tetraptera* has been developed into non-alcoholic functional beverages. One such product is the "Natu Prekese drink," formulated with *T. tetraptera* and honey. It is popularly claimed to alleviate menstrual discomfort, enhance immune function, and improve blood circulation; however, these claims are not yet substantiated by regulatory bodies such as the FDA (Adesina et al., 2016; Kemigisha et al., 2018). A related innovation involves producing a prekese beverage syrup through fruit milling and boiling, followed by filtrate concentration into syrup that can be reconstituted with sugar and water. The antioxidant levels of this drink were found to be high, at times surpassing those of polyphenol-rich juices like pomegranate, blueberry, and black cherry (Adusei et al., 2019). Consumer acceptance trials showed favorable results, and the Ghana Standards Authority deemed the product acceptable for market distribution (Derkyi et al., 2018).

Beyond beverage formulations, *T. tetraptera* extracts have been investigated as natural preservatives. For example, watermelon juice supplemented with 100 mg/mL of *T. tetraptera* extract showed reduced fungal and bacterial contamination over seven days compared with unpreserved controls (Anumudu, 2020; Enwereuzoh et al., 2015). These findings align with growing demand for chemical-free, microbially safe, and nutritious beverages. Still, a key challenge remains in controlling spore-forming bacteria such as *Bacillus* species (Lin et al., 2019).

1.2.7 CHEMICAL CONSTITUENTS IN MEDICINAL PLANT

Medicinal plants contain a wide variety of bioactive chemical constituents that contribute to their therapeutic effect. Secondary metabolites, such as phenolics, flavonoids, alkaloids, saponins, and terpenoids produced by plants, are the constituents of plant's defense system but they have remarkable potential to treat various diseases (Tungmunnithum*et al*., 2018).These compounds can be classified into different groups based on their chemical structure and biological activity.Some major classes include:

1.2.7.1 Glycosides

Glycosides are naturally occurring organic compounds found in plants, consisting of a sugar (glycone) and a non-sugar (aglycone or genin) portion. They are secondary metabolites that play a crucial role in plant defense, metabolism, and communication. In medicinal plants, glycosides are of immense pharmacological importance due to their diverse bioactivities, including cardiotonic, anti-inflammatory, antimicrobial, and anticancer effects. Glycosides are widely used in modern and traditional medicine for the treatment of various ailments. Their activity is largely determined by the structure of the aglycone component, which can belong to various chemical classes such as steroids, flavonoids, anthraquinones, and phenolics.

Glycosides are composed of two primary components:

Glycone (Sugar Moiety) – Typically consists of monosaccharides such as glucose, rhamnose, arabinose, or galactose. The sugar enhances the solubility and bioavailability of the glycoside.

Aglycone (Non-Sugar Moiety) – This determines the biological activity and therapeutic properties of the glycoside. It can be a steroid, flavonoid, anthraquinone, or other bioactive structure.

The glycosidic bond, which links the sugar and aglycone, can be hydrolyzed by enzymes or acidic conditions, releasing the active aglycone, which exerts pharmacological effects in the body. Examples include salicin, cardiac glycosides (digoxin from Digitalis spp.)

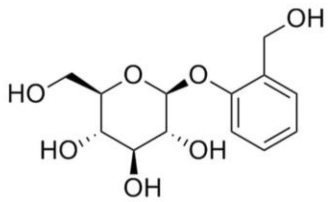

Fig 2.2: salicin

1.2.7.2 Saponins

Saponins are glycosides of triterpenes and steroids e.g. dioscin, they are important bioactive component which plays an important role in plant survival and also serve as a vital resource in pharmaceuticals as its underexplored biodiversity could lead to new drug discovery. A characteristic feature of saponins is their ability to foam when extract using an aqueous solution. As at recent plant derived saponins are used in the treatment of a range of diseases in convectional and traditional medicine.

Saponins are known to have a wide range of importance, example include: anti-bacterial, antiviral, anti-inflammatory, anti-fungal, anti-cancer, and anti-parasitic. Additionally, some saponins are also known to be used as flavoring due to their intense sweetness or bitterness.

1.2.7.3 Phenolic compounds

Phenolic phytochemicals represent the largest and most prevalent category of phytochemicals found within the plant kingdom. Among dietary phenolics, the three most significant groups are flavonoids, phenolic acids, and polyphenols. Flavonoids stand as the most extensive group of plant phenols and have been the subject of extensive study. Phenolic acids constitute a diverse group, encompassing widely distributed hydroxybenzoic and hydroxycinnamic acids.

Natural phenolic compounds are crucially important as they play a vital role in cancer prevention and treatment. The diverse bioactivities exhibited by phenolic compounds account for their chemo preventive properties, including antioxidant, anti-carcinogenic, anti-mutagenic, and anti-inflammatory effects. Additionally, these compounds contribute to inducing apoptosis by halting the cell cycle, regulating carcinogen metabolism and oncogenesis expression, inhibiting DNA binding and cell adhesion, as well as impeding migration, proliferation, or differentiation, and blocking signaling pathway.An example of phenolic compound is gallic acid

Fig 3.3: gallic acid

1.2.7.4 Tannins

Tannins are large polyphenolic compounds that are soluble in water and contains an ample number of hydroxyl and other appropriate groups, such as carboxyl's, enabling them to form strong complexes with protein and other macromolecules (Praveen and Kumud, 2012). Tannin is present in high concentration in nearly every part of a plant such as the bark, leaves, fruits, roots, and seeds, an increase in the production of tannin in plant usually arise as a result of sickness, therefore it is assumed that tannin is involved in the biological role of the plant to fight against infection, insects or animal herbivory. Tannis are typically found in form of light white amorphous powders or shiny, nearly colorless loose masses, characterized by a distinctive odor and an astringent taste.

These compounds have a wide-ranging application, from ancient tanning practices (dating back to ca. 1500BC in the Mediterranean) to medical and food industry uses. In medicine, particularly in traditional Asian healing practice such as Japanese and chines natural remedies, plant extract containing tannins are utilized as astringents for addressing diarrhea, as diuretic, against stomach and duodenal tumors, as well as in the production of anti-inflammatory, antiseptic, and hemostatic pharmaceuticals. Example is ellagic acid

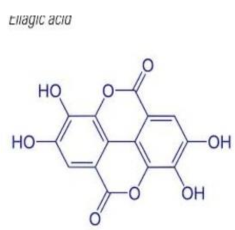

Fig 4.4: ellagic acid

1.2.7.5 Eugenols

Eugenolsin Medicinal Plants (with Reference to *Tetrapleura tetraptera*) Eugenol, a phenoliccompound commonly found in medicinal plants, is valued for its antimicrobial, antioxidant, anti-inflammatory, and analgesic activities. In *Tetrapleura tetraptera*, eugenol has been detected as part of its phytochemical profile, and it is thought to contribute significantly to the plant's traditional use in treating infections, pain, and inflammation, as well as its role in natural food preservation. Mechanistically, eugenol acts by disrupting microbial cell membranes, interfering with enzyme activity, and scavenging reactive oxygen species, thereby reducing microbial growth and oxidative stress in biological and food systems. Its presence in *T. tetraptera* thus strengthens the scientific basis for the plant's ethnomedicinal applications and its potential as a source of natural preservative agents [Adesina et al., 2016; Anyamele et al., 2022].

Example is eugenol (a phenylpropanoid compound)

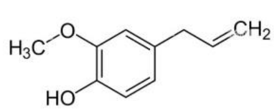

Fig 5.5: eugenol

1.2.7.6 Flavonoids

Flavonoids, as secondary metabolites, primarily consisting of a benzopyrone ring that carries phenolic or polyphenolic groups at various positions (Cavalcente*et al.*,2018). Over 10,000 flavonoid compounds have been isolated and identified as at recent. Most flavonoids are widely accepted as therapeutic agents (Shkondrov*et al.*,2017). Flavonoids have various range of application as it is used in the production of natural dye (Villela *et al.*,2019; Paramita *et al.*, 2018), in the production of skin care products and also in the production of anti-wrinkle skin agent. Flavonoid has also been seen to be used extensively as anticancer, (Zhao *et al.,* 2019) antimicrobial, antiviral, antiangiogenetic (Zhao *et al.,* 2019; Camero *et al.,* 2018), antimalarial, antioxidant, antitumor, and anti-proliferative agents (Patel *et al.,* 2018).

Flavonoids possess significant antioxidant capabilities, providing protection to the human body against free radicals and reactive oxygen species. The arrangement of hydroxyl groups and other attributes within the chemical structure of flavonoids plays a crucial role in determining their antioxidant and free radical-scavenging actions. A good example of flavonoid is catechin.

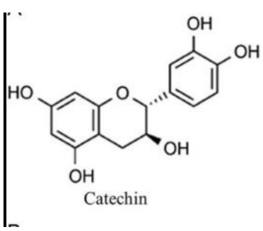

Fig 6.6: catechin

1.2.7.7 Terpenoid

Terpenoids, also known as isoprenoids or terpenes, are a substantial class of natural compounds present in most living organisms (Oldfield and Lin, 2012). They are widely distributed in nature, particularly in plants, where they serve as a component of essential oils. Their fundamental unit is hydrocarbon isoprene CH2=C(CH3)-CH=CH2. Many plants produce volatile terpenes either to attract insects for pollination or to deter certain animals that consume them.

Additionally, terpenoids plays a significant role in the cosmetic and flavor industries due to their volatile nature and the diverse array of scent and flavors they offer. Beyond their aromatic and flavor uses, terpenes also exhibit medicinal properties such as anti-carcinogenic, anti-malaria, anti-ulcer, anti-microbial and others. A good example of this is camphor

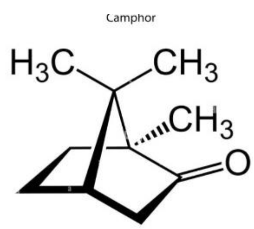

Fig 7.7: camphor

1.2.7.8 Steroids

Steroidal compounds are important secondary metabolites in medicinal plants, often recognized for their anti-inflammatory, cardioprotective, and immunomodulatory effects. In *Tetrapleura tetraptera*, several phytochemical analyses have confirmed the presence of steroids among other bioactive constituents (Adesina et al., 2016; Anyamele et al., 2022). Both qualitative and quantitative screenings of fruit, pulp, seed, and bark extracts have repeatedly demonstrated the occurrence of steroids alongside alkaloids, phenols, saponins, flavonoids, and terpenoids (Enwereuzoh et al., 2015; Orji et al., 2019).

These steroidal compounds likely contribute to the plant's traditional applications—such as treating inflammation, hypertension, and other ailments—by modulating prostaglandin synthesis, stabilizing cell membranes, and influencing lipid metabolism (Adesina et al., 2016; Iwu). Such mechanisms are consistent with known steroid activities observed in other medicinal plants and provide a plausible biochemical basis for some of *T. tetraptera*'s therapeutic effects (Anyamele et al., 2022; Orji et al., 2019).

1.2.7.9 Alkaloids

Alkaloids are a group of naturally existing chemical compounds, usually characterized by the presence of basic nitrogen atoms, and occasionally including neutral or weakly acidic compounds. Some synthetic compounds are also categorized as alkaloid. In addition to carbon, nitrogen, or hydrogen, alkaloids can also contain sulfur and infrequently, bromine, phosphorus, or chlorine. Alkaloid are seen to be produced by a large variety of organisms which includes fungi, bacteria, plants and animals. The biological precursors of the majority of alkaloids are amino acids like tyrosine, histidine, aspartic acid and others. Many of these compounds are seen to have a low solubility in water but are readily dissolved in organic solvents if consumed. Alkaloids are secondary metabolite hence they play a crucial role in plant protection and its survival against microorganism, exhibiting antibacterial and antifungal activities.

An example of well-known alkaloid includes morphine, strychnine, quinine, ephedrine, and nicotine. Alkaloids which are contained in a ring system are known as "indole". Alkaloids exhibit a wide array of medicinal properties. Morphine, a potent narcotic, is utilized for pain relief, but its addictive nature restricts its practicality. On the other hand, codine, which is a methyl ether derivative from opium poppy, serves as an effective analgesic with relatively lower addictive potential.

Example of this is nicotine

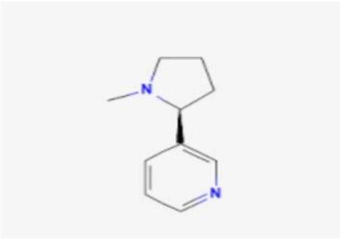

Fig 8.8: Nicotine

1.2.7.10 Essential Oils

In Tetrapleura tetraptera, essential oils have been identified as key bioactive fractions contributing to both therapeutic and preservative effects. Studies report that the essential oil of T. tetraptera exhibits strong antimicrobial activity against pathogens such as Staphylococcus aureus, Bacillus cereus, and Saccharomyces cerevisiae, with minimum inhibitory concentrations (MICs) of approximately 42, 46, and 36 mg/mL, respectively (Moses, 2018; Orji et al., 2019).

These bioactivities are largely attributed to the rich composition of volatile phytochemicals such as eugenol, monoterpenes, and sesquiterpenes, which act by disrupting microbial cell membranes and scavenging free radicals (Anyamele et al., 2022). The essential oil profile of T. tetraptera thus supports its dual role in traditional medicine and in modern applications as a natural preservative for food and beverages (Adesina et al., 2016).

1.2.8 CHEMICAL COMPOUNDS ISOLATED FROM TETRAPLEURA TETRAPTERA

Several phytochemical investigations have led to the isolation of saponins, triterpenoid glycosides, phenolics, coumarins, amino acid derivatives, and volatile oils from Tetrapleura tetraptera.

1. Triterpenoid saponins (Fruit pulp and bark)

i. Aridanin – a novel triterpenoid glycoside identified as 3-O-[β-D-glucopyranosyl-(2′-acetamido-2′-deoxy)] oleanolic acid. It is the characteristic compound of the fruit pulp and is considered responsible for several pharmacological activities.

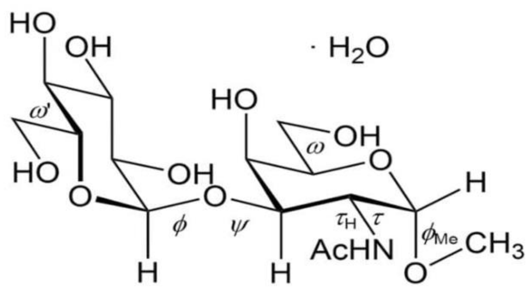

Fig 9.9:Aridanin

ii. Tetrapterosides A and B – two new oleanane-type saponins isolated from the stem bark, each with defined sugar chains and acyl substituents.

iii. Additional oleanolic acid and echinocystic acid saponins have also been reported, many with N-acetylglucosamine residues in their glycosidic chains.

2. Phenolics and coumarins

i. Scopoletin (7-hydroxy-6-methoxycoumarin) – a coumarin reported in extracts of the fruit, contributing to antioxidant and antimicrobial properties.
ii. Ferulic acid and related derivatives have been isolated from branches and leaves, linked to anti-inflammatory activity.

3. Amino acid derivatives (Seeds)

Rare amino acids such as L-γ-methyleneglutamic acid and L-γ-ethylideneglutamic acid were isolated from the seeds, expanding the plant's chemical profile (Aladesanmi, 2006).

4. Volatile oils (Leaves)

GC–MS analysis of Nigerian leaf essential oil identified some compounds, representing ~89.5% of the oil. Major constituents include 1,8-cineole (19.4%), 6,10,14-trimethyl-2-pentadecanone (13.6%), phytol (9.1%), α-pinene (8.1%), and geranylacetone (6.7%).

1.2.9 EXTRACTION

Extraction in chemistry is the process of separating bioactive compounds from plant materials using solvents or mechanical methods. This technique isolates valuable phytochemicals, such as alkaloids, flavonoids, saponins, and phenolics, which can be used for medicinal, nutritional, or industrial purposes.

1.2.9.1 TYPES OF EXTRACTION

i. **Maceration Extraction**

Maceration is a simple and traditional extraction method where plant materials are soaked in a solvent (water, oil and alcohol) at room temperature(typically 20-25^{o}C) to dissolve and extract bioactive compounds. Maceration can take from less than one hour to even a few weeks. This process allows the solvent to penetrate plant cells, breaking them down and releasing phytochemicals such as alkaloids, flavonoids, tannins, and essential oils**.**

Working principle of maceration extraction

The working principle of the maceration method is based on diffusion and osmosis, where soluble phytochemicals move from the plant material into the solvent due to a concentration gradient. When the powdered fruit of *Tetrapleura tetraptera* is immersed in solvents such as hexane, methanol, or water, the solvent penetrates the plant cell walls and dissolves intracellular compounds.

The process begins with solvent molecules diffusing into the plant matrix, causing the cell walls to swell and soften. This increases permeability and allows phytochemicalsincluding flavonoids, tannins, alkaloids, saponins, terpenoids, and phenolic compoundsto dissolve into the solvent. Continuous agitation enhances this process by maintaining a fresh concentration gradient between the solvent and the plant tissue, ensuring maximum transfer of bioactive compounds.

At equilibrium, the solvent becomes saturated with phytochemicals. After sufficient time, the liquid extract is separated from the solid plant residue by filtration. The filtrate contains the crude extract, which is then concentrated to remove excess solvent.Importantly, the choice of solvent plays a central role in the efficiency of maceration. Non-polar solvents like hexane extract oils, terpenes, and fatty acids; polar solvents like water extract tannins, glycosides, and sugars; while methanol, with intermediate polarity, extracts a wide spectrum including flavonoids, alkaloids, and phenolic compounds. Thus, by applying hexane, methanol, and water in parallel, this study can comparatively assess which solvent system extracts the most pharmacologically active constituents of *T. tetraptera*.

ii. Infusion Extraction

This is a method of extracting bioactive compounds from plant materials by soaking them in hot or cold water(room temperatures) for a short period. It is commonly used for delicate plant parts like leaves, flowers, and seeds to preserve heat-sensitive compounds. In addition, it is an appropriate method for preparation of fresh extract before use.

iii. Percolation Extraction

Percolation is a continuous extraction method where a solvent passes through a column of finely ground plant material to extract bioactive compounds.The apparatus used in this process is called percolator. It is a narrow cone-shaped glass vessel with opening at both ends. This technique is commonly used in pharmaceutical and herbal industries to obtain concentrated liquid extracts (tinctures or fluid extracts).

iv. Digestion Extraction

This is an extraction method that involves the use of moderate heat during extraction process, where plant materials are soaked in a solvent at moderate temperatures (40-60°C) for a prolonged period. This gentle heating accelerates the extraction process without degrading heat-sensitive compounds. This method is suitable for plant materials that are readily soluble.

v. **Decoction Extraction**

Decoction is a traditional extraction method where plant materials (usually tough parts like roots, bark, or seeds) is placed in a clean container and boiled in water to extract bioactive compounds. This process is commonly used in herbal medicine to obtain water-soluble compounds that are less affected by heat. It is used for extraction of water soluble and heat stable plant material**.**

vi. **Soxhlet Extraction**

A **Soxhlet extractor** is a laboratory apparatus used for the continuous extraction of a specific compound from solid materials using a solvent. It is particularly useful when the desired compound has limited solubility in the solvent and requires repeated washing for efficient extraction. The Soxhlet method allows the solvent to be recycled multiple times, ensuring maximum extraction efficiency.

1.2.10 USES OF EXTRACTION

Extraction is a fundamental process in the field of chemistry and other related field. It involves the separation of specific components (solutes) from a mixture, usually solid, using a solvent. The primary objective of extraction is to isolate the desired bioactive compounds from raw materials, such as plants, for further analysis or use to obtain natural products, essential oils, bioactive compounds, and pollutants.

- **Pharmaceutical Industry**

In the pharmaceutical industry, extraction is used to isolate active pharmaceutical ingredients (APIs) from medicinal plants, marine organisms, fungi, or microorganisms. The bioactive compounds obtained through extraction, such as alkaloids, flavonoids, terpenoids, glycosides, and essential oils, are further developed into drugs, herbal supplements, or alternative medicines. For instance, the extraction of *Hibiscus sabdariffa* stem provides polyphenols and flavonoids that possess antioxidant, anti-inflammatory, and antimicrobial properties, leading to the development of natural remedies and treatments for conditions like hypertension, diabetes, and infections. Solvent extraction, especially using ethanol and methanol, is commonly used to obtain plant-based extracts for use in pharmaceutical formulations.

- **Cosmetic and Personal Care Industry**

The cosmetic industry makes extensive use of plant-based extracts, essential oils, and fatty acids obtained through extraction methods. These extracts, such as aloe vera gel, lavender oil, and rose oil, are incorporated into skincare products, shampoos, and lotions due to their beneficial properties like moisturizing, soothing, anti-aging, and antimicrobial effects. For example, essential oils extracted from lavender or chamomile are used for their calming and anti-inflammatory effects in skin care formulations.

- **Food and Beverage Industry**

Extraction is crucial in the food and beverage industry for obtaining flavors, fragrances, colors, and nutrients. For example, lemon or orange oils are extracted for use in beverages, and natural colorants such as carotenoids and anthocyanins are extracted from plants to add natural coloring to food products. Additionally, extraction is used to obtain compounds like antioxidants (vitamin

C, flavonoids) and essential fatty acids (omega-3) from seeds, nuts, and fruits for use in food fortification and supplements.

- **Environmental Science**

Extraction plays a vital role in environmental science for the analysis and remediation of pollutants. Methods such as Soxhlet extraction is employed to extract toxic substances like heavy metals, pesticides, and organic pollutants from soil, water, or sediments for subsequent analysis and disposal. Environmental agencies and laboratories often use extraction techniques to monitor pollution levels and assess the contamination of natural resources. Supercritical fluid extraction, for instance, has gained attention for its ability to efficiently extract pollutants from environmental samples without leaving harmful solvent residues.

- **Agriculture**

In agriculture, extraction methods are employed to obtain natural pesticides, insect repellents, and growth promoters from plants. Plant extracts, such as those from garlic or citronella, are also used in organic farming to protect crops from pests. Also, plant extracts containing alkaloids or terpenoids are investigated for their potential use in controlling plant diseases or boosting plant growth.

1.2.11 MINERAL ANALYSIS

The mineral analysis is the process of determining the chemical composition and structure of minerals in medicinal plants.

1.2.11.1 MINERAL ELEMENTS

Studies have confirmed that *Tetrapleura tetraptera* contains a range of macro- and micro-minerals including calcium, potassium, magnesium, sodium, phosphorus, iron, zinc, manganese, copper, aluminium, silver, lead, and silicon (Akinmoladun et al., 2020; Okwu &Ekeke, 2022). These minerals complement its phytochemicals, making the plant both nutritionally rich and pharmacologically valuable.

I. **Calcium (Ca):** Essential for bone and teeth formation; also regulates muscle contraction and blood clotting. Its presence in *T. tetraptera* supports its nutritional use as a soup spice for nursing mothers, who require higher calcium intake.

II. **Potassium (K):** Helps maintain fluid and electrolyte balance, regulates heartbeat, and lowers blood pressure, making it valuable in cardiovascular health.

III. **Magnesium (Mg):** A cofactor in over 300 enzymatic reactions, aiding in energy production and muscle relaxation; its presence may contribute to the plant's reported anti-fatigue properties.

IV. **Sodium (Na):** Supports fluid balance and nerve signaling, but in moderation; small amounts in the plant contribute to metabolic balance.

V. **Phosphorus (P):** Vital for bone health and energy transfer through ATP, aligning with the plant's use as a restorative food.

VI. **Iron (Fe):** Critical for hemoglobin formation and oxygen transport; supports the traditional use of the fruit in boosting vitality and preventing anemia.

VII. **Zinc (Zn):** Enhances immunity, wound healing, and reproductive health, linking with the plant's ethnomedicinal reputation for general body strengthening.

VIII. **Manganese (Mn):** Supports antioxidant defense enzymes like superoxide dismutase, which aligns with the plant's antioxidant claims.

IX. **Copper (Cu):** Assists in red blood cell formation and connective tissue development, contributing to tissue repair and cardiovascular support.

X. **Aluminum (Al):** Found in trace amounts, has no direct benefit, but excessive accumulation may be harmful—its detection highlights the importance of dosage regulation.

XI. **Silver (Ag):** Known for antimicrobial activity; though present in trace levels, it may add to the overall antimicrobial profile of the extracts.

XII. **Lead (Pb):** A non-essential heavy metal; its presence suggests possible environmental contamination, emphasizing the need for quality control to ensure safety.

XIII. **Silicon (Si):** Improves structural integrity in plants and may aid collagen formation in humans, supporting skin, hair, and bone health.

Overall, the mineral composition of *T. tetraptera* enhances its nutritional and therapeutic value, complementing its phytochemicals to justify its broad application in ethnomedicine from strengthening diets to treating infections and managing oxidative stress.

1.2.11.2 XRF INSTRUMENT

X-ray fluorescence spectroscopy (XRF) was employed for the mineral analysis of *Tetrapleura tetraptera*. XRF is a non-destructive analytical technique used to determine the elemental composition of a sample. The working principle is based on the interaction between X-rays and the atoms in the material. When a sample is irradiated with high-energy primary X-rays, the inner-shell electrons of the atoms are excited and displaced. As electrons from higher energy levels fall back to fill these vacancies, they emit secondary (fluorescent) X-rays with energies that are characteristic of the specific elements present in the sample.

Fig 10.10:X-ray fluorescence

The instrument detects and quantifies these emitted X-rays, thereby providing both qualitative (which elements are present) and quantitative (how much of each element is present) data. This makes XRF highly suitable for identifying macro-minerals such as calcium, potassium, and magnesium, as well as trace elements like zinc, copper, aluminum, and even heavy metals such as lead.

XRF is particularly advantageous because it is rapid, requires minimal sample preparation, and preserves the integrity of the plant material. For this study, powdered samples of *T. tetraptera* were analyzed, allowing the detection of nutritionally important minerals (e.g., Fe, Zn, Ca, K, Mg) alongside trace and non-essential metals (e.g., Al, Pb, Ag, Si). The results provide a comprehensive mineral profile that supports both the nutritional and pharmacological evaluation of the plant.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

Volumetry flask

Spatula

Retort stand and clamp

Hand gloves

Funnel

Whatman`s filter paper

Beakers

Stirrers

Test tubes

Measuring cylinder

Soxhlet extractor

Electrical weighing balance (OHAUS Model 2610),

Aluminum sheets (20 cm x 20 cm) plates (Merck, Germany)

2.1.1 EQUIPMENTS

Rotary evaporator

Mortar and pestle

Refrigerator (LG)

Digital water bath (Sanfa-Model No DK420)

Fume cupboard

electric blender.

X-ray fluorescence

2.1.2 REAGENTS/ CHEMICALS

All reagents used are of analytical grade.

Distilled water

n-Hexane (C6H14)

Ethyl acetate ($C_4H_8O_2$)

Lead acetate solution (PbC_4H_6O)

Picric acid (C6H3N3O7)

Tetraoxosulphate (VI) acid (H_2SO_4)

Chloroform (CH3CL)

Dilute hydrochloric acid (HCl)

Potassium chloride solution (KOH)

Iron (III) chloride solution ($FeCl_3$)

Glacial acetic acid (CH_3COOH)

Acetic anhydride ($C_4H_6O_3$)

2.2 METHODS

2.2.1 PLANT COLLECTION, IDENTIFICATION AND PREPARATION

Tetrapleura Tetraptera stems were purchased at Uselu Market, Egor local Government Area, Edo State. The respective plant parts were dried in the laboratory for twenty-eight days pulverized by electric blender. The plant Samples were authenticated by Prof Henry Adewale Akinnibosun, a taxonomist from the Department of Plant Biology and Biotechnology of Herbarium Unit, Faculty of Life Sciences University of Benin, Edo State. A voucher number UBH-T472 of the plant was deposited at the herbarium. After authentication, the samples were transported to the laboratory in a clean polythene bag to prevent contamination and degradation.

2.2.2 EXTRACTION OF PLANT EXTRACTS

Maceration Method

The dried fruits of *Tetrapleura tetraptera* were first pulverized into fine powder using a mechanical grinder to increase the surface area for solvent interaction. 50g of the powdered sample was then weighed and transferred into three separate conical flasks, each containing 500ml of hexane, methanol, and water. The powder was completely immersed in the respective solvents, ensuring adequate solvent-to-sample ratio to allow maximum penetration.

Each flask was covered and left to stand at room temperature for 24hours, with occasional shaking and stirring to facilitate effective solute–solvent interaction. During this period, the solvent diffused into the plant matrix, dissolving and extracting the phytochemicals present in the sample. After maceration, the mixtures were filtered using Whatman filter paper to remove plant residues, and the filtrates were collected. The crude extracts were obtained by sieving each filtrate, and dried in a water bath respectively.

2.2.3 PHYTOCHEMICAL SCREENING OF *TETRAPLEURA* TETRAPTERA EXTRACT

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

2.2.3.1 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.

2.2.3.2 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.

2.2.3.3 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.

2.2.3.4 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.

2.2.3.5 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.

2.2.3.6 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.

2.2.3.7 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.

2.2.3.8 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.

2.2.3.9 TEST FOR ALKALOIDS

Picric acid was used to test for alkaloid. About 1ml each of the plant extract was added to2ml of picric acid. A yellow precipitate indicates a positive test.

2.2.3.10 TEST FOR REDUCING SUGAR

Equal volume of Fehling's solution A and B was boiled for one minute and 1ml of plant extract was added and boiled for five minutes. A bricked-red precipitate was required for positive test.

2.2.4 MINERAL ANALYSIS OF TETRAPLEURA TETRAPTERA

The Mineral Analysis of T.tetraptera extract were performed using standard operating procedures of **X-Ray Fluorescence (XRF) Spectrometry.**

Procedures;

1. The sample cup was properly cleaned and placed rightly in the cup.

2. The sample was put into the sample cup to cover all filters at the bottom to at least 3mm thickness.
3. The instrument was turned on for 30 minutes to warm up.
4. Elemental composition was utilized.
5. The sample identity was leveled in the position of the selected sample, and the reading analysis was accepted.
6. The start button was clicked, prompting the instrument to rotate to the position of the sample in the tray at the X-ray position and measure it.
7. The process was completed within 6-7 minutes, and the X-ray was shut down. The intensities were then converted into concentrations in weight percentage.
8. The result was printed out after each analysis

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 DETERMINATION OF PERCENTAGE YIELD OF EXTRACT

FOR HEXANE EXTRACT:

$$\%\text{Yield} = \frac{Mass\ of\ crude\ extract}{Mass\ of\ powdered\ plant} \times 100$$

$$\%\text{Yield} = \frac{1.05}{50} \times 100 \quad = 2.1\%$$

FOR METHANOL EXTRACT:

$$\%\text{Yield} = \frac{Mass\ of\ crude\ extract}{Mass\ of\ powdered\ plant} \times 100$$

$$\%\text{Yield} = \frac{7.65}{50} \times 100 \quad = 15.3\%$$

FOR AQUEOUS EXTRACT:

$$\%\text{Yield} = \frac{Mass\ of\ crude\ extract}{Mass\ of\ powdered\ plant} \times 100$$

$$\%\text{Yield} = \frac{2.5}{50} \times 100 \quad = 5\%$$

3.2 PHYTOCHEMICAL SCREENING:

The result of phytochemical constituents is shown in Table 3.1 below

Table 3.1: Phytochemical Screening of Methanol, Aqueous and Hexane Extracts interpleural Tetraptera

S/N	CONSTITUENTS	METHANOL	HEXANE	AQUEOUS
1.	ALKALOIDS	+	+	+
2.	GLYCOSIDES	++	-	+
3.	SAPONINS	+	+	++
4.	PHENOLICS	-	+	+
5.	EUGENOL	+	+	+
6.	TANNINS	+	-	+
7.	FLAVONOIDS	+	-	+
8.	STEROIDS	-	-	-
9.	TERPENOIDS	++	+	++
10.	REDUCING SUGAR	+	+	+

KEYS:

+ = Present

- =Absent

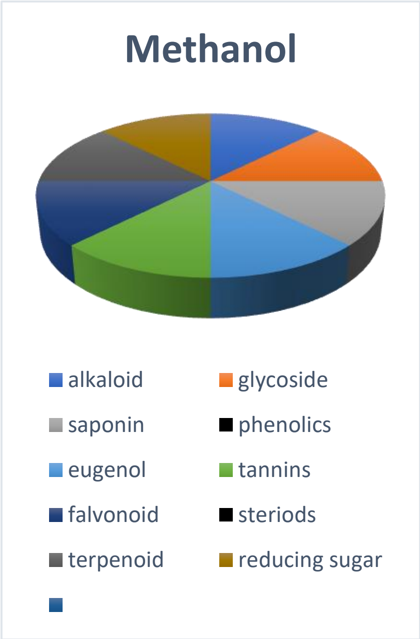

Fig 3.1: Bioactive molecules present in methanol extract

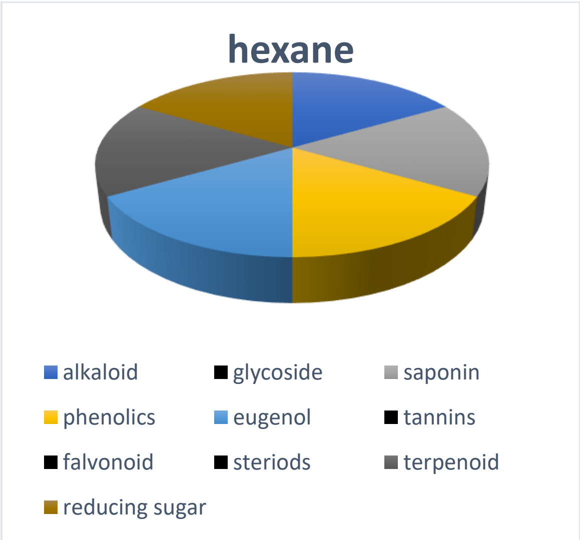

Fig 3.2: Bioactive molecules present in hexane extract

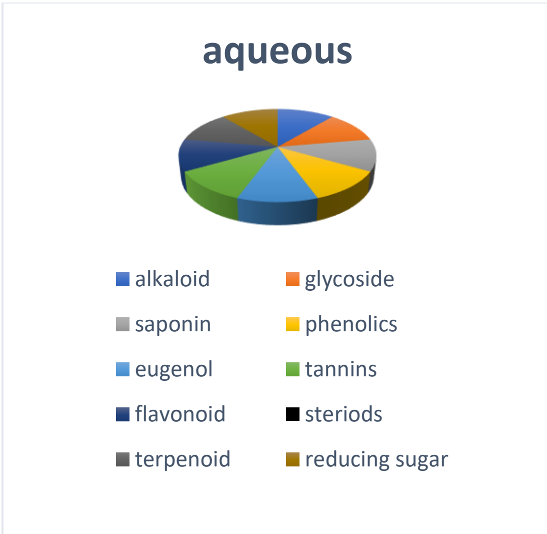

Fig 3.3: Bioactive molecules present in aqueous extract

3.3 RESULT OF ELEMENTAL ANALYSIS USING XRF SPECTROMETRY

TABLE 3.2: Mineral elements contained in fruit pod of Tetrapleura Teraptera

MINERAL ELEMENTS	**CONCENTRATION IN FRUIT POD OF TETRAPLEURA TETRAPTERA %**
Calcium	12.962
Potassium	28.317
Magnesium	7.656

Iron	1.502
Phosphorus	0.486
Manganese	0.210
Zinc	0.096
Aluminum	6.413
Sulphur	1.652
Chlorine	2.412
Titanium	0.152
Vanadium	0.022
Chromium	0.017
Cobalt	0.044
Nickel	0.012
Copper	0.437
Rubidium	0.094
Strontium	0.074
Zirconium	0.017
Niobium	0.087

Silver	0.069
Tin	0.679
Barium	0.263
Tantalum	0.009
Tungsten	0.026
Lead	0.161

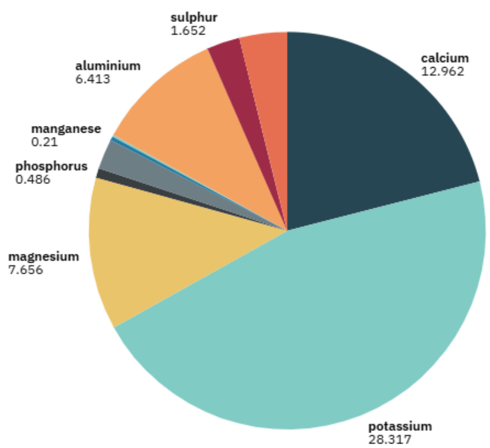

Fig 3.4 Pie chart of mineral composition of fruit pod

DISCUSSION

The mineral composition of *Tetrapleura tetraptera* fruit in this study based on the table 3.2, revealed potassium as the most abundant element, followed by calcium and magnesium. Moderate levels of aluminum and chlorine were also detected, while iron and zinc occurred at low concentrations. These results are comparable to earlier findings where potassium and calcium were consistently reported as dominant minerals in *T. tetraptera* (N'zebo et al., 2019; Mensah et al., 2024). The high potassium content suggests potential benefits in maintaining electrolyte balance and regulating blood pressure, while calcium and magnesium support bone development, muscle function, and energy metabolism. The relatively low iron concentration indicates that the fruit may not be a strong dietary source of this mineral, though iron remains crucial for hemoglobin formation and oxygen transport.

The presence of aluminum at moderate levels may reflect environmental and soil influences, since similar patterns have been reported in fruits from specific ecological regions (Babalola et al., 2023). While aluminum is not nutritionally essential, its detection highlights the importance of monitoring safe consumption levels. Trace minerals such as copper, manganese, and zinc, though present in small amounts, add nutritional significance because of their roles in enzymatic activity, antioxidant defense, and immune function.

Phytochemical screening showed that methanol and aqueous extracts contained a wide spectrum of secondary metabolites including alkaloids, flavonoids, glycosides, tannins, saponins, and terpenoids. In contrast, the hexane extract yielded a narrower profile, with notable presence of terpenoids, saponins, and phenolics but absence of some polar constituents. This observation supports earlier reports (Dzah et al., 2022; Ekeanyanwu et al., 2024), where polar solvents such as methanol and water were found to extract more flavonoids, tannins, and phenolic compounds, while non-polar solvents like hexane were less efficient for these phytochemicals.

Overall, the combined mineral and phytochemical results suggest that *T. tetraptera* fruit is both nutritionally and pharmacologically valuable. Its richness in potassium, calcium, and magnesium complements its diverse phytochemical profile, which underpins its antioxidant, antimicrobial, and therapeutic potential. Although iron is low in content, limiting its role as a major source of this mineral, the overall findings justify the traditional use of the plant in managing oxidative stress, infections, and for nutritional supplementation.

The analysis of *Tetrapleura tetraptera* fruits revealed important nutritional and phytochemical characteristics that highlight both the strengths and limitations of the plant as a medicinal and dietary resource.

CONCLUSION

This study on the comparative investigation of hexane, methanol, and aqueous extracts of *Tetrapleura tetraptera* has demonstrated that both the mineral composition and phytochemical profile of the plant are strongly influenced by the choice of extraction solvent. Calcium, potassium, and magnesium were the most abundant minerals in the fruit, underscoring the nutritional importance of the plant, while iron and other trace minerals were present in much lower concentrations, indicating a limitation in its ability to serve as a major source of dietary iron.

Phytochemical screening revealed that solvent polarity significantly determined the distribution of bioactive compounds, with methanol favoring the extraction of polar constituents such as flavonoids and alkaloids, hexane extracting non-polar compounds such as terpenoids and steroids, and water supporting the presence of tannins, glycosides, and saponins. This variation highlights the importance of solvent selection in phytochemical research and medicinal applications.

Taken together, the results affirm that *Tetrapleura tetraptera* is a nutritionally valuable and pharmacologically versatile plant, with potential applications as a functional food and as a source of therapeutic agents. However, its limitations in iron content suggest that it should be used in combination with other nutrient-rich foods for a balanced diet. The findings also validate the traditional uses of the plant while providing scientific evidence that supports its relevance in modern medicine and nutrition.

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