COMPARATIVE ANALYSIS OF VITAMIN COMPOSITION IN POLAR AND NON-POLAR EXTRACTS OF *Cymbopogon citratus*

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

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BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELORS DEGREE IN MEDICAL LABORATORY SCIENCE (BMLS) UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

SUPERVISED BY

DR. O.F. AMEGOR

SEPTEMBER, 2025

CERTIFICATION

This is to certify that this seminar work was carried out by **ILUEBBEY, THEOPHILUS OSIOMEGHIE** with the matriculation number **BMS2009046** under the supervision of **DR. O.F. AMEGOR** in partial fulfillment for the award of Bachelor of Medical Laboratory Science (B.MLS) Degree.

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(Ag. HEAD OF DEPARTMENT)

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PROF. OMORUYI PIUS OMOSIGHO

(EXTERNAL EXAMINER) **DATE**

DEDICATION

I dedicate this project to God Almighty for his love, grace, wisdom and the knowledge he bestowed upon me throughout my time at the University of Benin.

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I give thanks to Almighty God, my creator who has granted me grace and strength to finish this project work within the limited time frame.

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ABSTRACT

Cymbopogon citratus is a widely used medicinal and culinary herb known to contain a variety of vitamins and bioactive compounds. Solvent-based extraction plays a crucial role in determining the yield and profile of these nutrients, particularly vitamins, which differ in polarity and solubility. This study aimed to compare the vitamin composition of polar (ethanol) and non-polar (diethyl ether) extracts of *C. citratus* to determine which solvent more effectively recovers specific vitamins. Extraction was carried out using standard maceration techniques with ethanol and diethyl ether as solvents. Vitamin analysis was conducted spectrophotometrically, and data were statistically analyzed using one-way ANOVA. Relevant literature was sourced from peer-reviewed journals indexed in scientific databases and platforms such as PubMed, ResearchGate, Google Scholar, and ScienceGate. The results showed that the polar extract contained significantly higher levels of vitamin A (51.02 ± 0.004 ppm), vitamin B6 (2.533 ± 0.010 ppm), and vitamin D (55.89 ± 0.020 ppm). In contrast, the non-polar extract yielded significantly higher concentrations of vitamin B12 (2.957 ± 0.155 ppm), vitamin C (59.71 ± 0.035 ppm), vitamin E (36.07 ± 0.2335 ppm), vitamin K (19.90 ± 0.2137 ppm), and benzoic acid (6.124 ± 0.027 ppm), all with p-values < 0.05. In conclusion, the choice of solvent significantly affects the recovery of vitamins from *C. citratus*. Ethanol is more suitable for extracting water-soluble or moderately polar vitamins, while diethyl ether is better for highly lipophilic compounds. It is recommended that future research include in vivo investigations to evaluate the biological relevance, bioavailability, and therapeutic potential of the extracted vitamins, building upon the in vitro findings of this study.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Cymbopogon citratus is a perennial aromatic grass from the Poaceae family, native to South and Southeast Asia but now widely cultivated in tropical and subtropical regions worldwide. Its characteristic lemon-like scent is primarily due to the presence of citral, a cyclic monoterpene, and its essential oil is rich in compounds such as geranial, neral, myrcene, and various flavonoids and phenolic compounds (Oladeji *et al.*, 2019). In recent years, there has been growing interest in natural, plant-based sources of nutrition due to rising concerns about the health impacts of synthetic additives and processed foods. Among such plants, *C. citratus* (commonly known as lemongrass) has attracted attention for its dual role in traditional medicine and modern nutrition. A study evaluating a lemongrass-lime mixed beverage demonstrated that high-pressure processing preserved key nutrients such as vitamin C and phenolic compounds without compromising microbiological safety or physicochemical properties. The retention of these bioactive compounds even after several weeks of storage highlights the potential of lemongrass in functional food development, particularly in beverages where nutrient preservation is essential (Kieling *et al.*, 2019). In another study exploring the application of lemongrass in craft brewing, *C. citratus* was found to enhance both the nutritional and microbial stability of wheat beers. The plant contributed essential vitamins, antioxidants, and sensory improvements, although higher concentrations were noted to influence alcohol content and acidity. This suggests a potential for lemongrass not only as a nutritional additive but also as a functional agent that enhances shelf life and consumer acceptability of fermented products (Belcar and Gorzelany, 2022).

A broader nutritional profiling study of vegetables used in Thai cuisine identified *C. citratus* as one of the plants with high dietary fiber content, contributing significantly to daily nutritional intake. Though vitamin C was found in higher concentrations in other herbs, the inclusion of lemongrass in this comprehensive analysis affirms its standing among nutrient-rich culinary plants. Such evaluations support its continued use in both traditional and modern food systems targeting improved dietary quality (Suttisansanee *et al.*, 2023).

Vitamins are essential organic compounds required in small amounts for normal metabolism, growth, and overall health, and are classified based on their solubility: fat-soluble (A, D, E, K) and water-soluble (B-complex and C). Fat-soluble vitamins are stored in body tissues and can accumulate to toxic levels if consumed excessively, while water-soluble vitamins are generally not stored and excess amounts are excreted, making regular dietary intake important. Nutritionally, vitamins play diverse and critical roles: they function as coenzymes, antioxidants, hormones, and regulators of gene expression, supporting processes such as energy metabolism, immune function, vision, bone health, and cellular growth (Ofoedu *et al.*, 2021). Deficiencies in vitamins can lead to serious health problems, including impaired development, increased risk of infections, anemia, neurological disorders, and chronic diseases, while toxicities are rare but possible, especially with fat-soluble vitamins or excessive supplementation. The B-complex vitamins, for example, are vital for energy production, nervous system function, and DNA synthesis, with specific needs varying across life stages and between sexes (Ali *et al.*, 2022).

1.2 Statement of Problem

The increasing demand for natural health-promoting ingredients in functional beverages has prompted interest in *C. citratus* derivatives. However, despite the widespread use of lemongrass in herbal medicine and its known antioxidant and antimicrobial properties, there is a significant

lack of scientific validation regarding the use of lemongrass essential oils in beverage formulations. This gap in evidence has hindered the development of standardized, high-quality lemongrass-based drinks, particularly those that retain bioactive compounds and satisfy consumer sensory expectations (Kieling and Prudencio, 2019).

In animal nutrition, oxidative stress induced by environmental factors such as heat remains a major challenge in tropical livestock systems. While commercial antioxidants are commonly used, there is a growing need for more sustainable, plant-based alternatives. Methanolic extracts of *C. citratus* have shown promise due to their high content of bioactive compounds like ascorbic acid and sinapic acid. Yet, despite their potential, questions remain about their long-term stability and comparative efficacy under field conditions, necessitating further validation of their application in ruminant health (Purba *et al.*, 2025). Though lemongrass has been incorporated as a flavoring and medicinal herb in food and beverage systems, its antibacterial efficacy, particularly against multidrug-resistant pathogens, is still under-evaluated. Recent studies have shown that lemongrass oil exhibits only moderate effectiveness when compared to other essential oils such as thyme and oregano. This limited potency raises concerns about its standalone application in food safety management and underscores the need for improved formulations or synergistic blends to enhance antimicrobial effectiveness (Yasir *et al.*, 2022).

1.3 Justification of Study

In recent years, the global shift toward plant-based nutrition has intensified the search for functional foods with both nutritional and therapeutic benefits. *C. citratus* is a well-known aromatic herb traditionally used in herbal teas and folk medicine. While its antimicrobial and antioxidant properties are well documented, there remains a notable underutilization of its full nutritional profile, particularly its vitamin content, in the development of modern functional

beverages or supplements. Furthermore, limited commercial products currently leverage lemongrass extract primarily for its sensory or antimicrobial qualities rather than its micronutrient contributions. Given the increasing prevalence of diet-related chronic diseases and micronutrient deficiencies, there is a compelling need to explore plant-based solutions that are affordable, accessible, and effective. By investigating the vitamin content and potential applications of lemongrass extract, this study not only addresses a nutritional knowledge gap but also contributes to the growing body of evidence supporting indigenous plant resources as viable alternatives to synthetic additives in food and health industries. Additionally, this research holds potential for rural development through the value-added utilization of a readily available and culturally accepted herb.

1.4 Aim of the Study

The aim of this study is to evaluate the vitamin composition of *C. citratus* (lemongrass) as influenced by extraction with polar and non-polar solvents.

1.5 Specific Objectives

1. To quantify the vitamin content of *C. citratus* using polar (ethanol) and non-polar (diethyl ether) solvent extracts.
2. To compare the efficiency of polar and non-polar solvents in extracting vitamins from *C. citratus*.

1.6 Research Questions

1. What types and quantities of vitamins are extractable from *C. citratus* using polar and non-polar solvents?
2. How does solvent polarity influence the efficiency of vitamin extraction from lemongrass?

1.7 Research Hypotheses

Null Hypothesis (H_0):

There is no significant difference in the vitamin content of *C. citratus* extracted using polar and non-polar solvents.

Alternative Hypothesis (H_1):

There is a significant difference in the vitamin content of *C. citratus* extracted using polar and non-polar solvents.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of *C. citratus* (Lemongrass)

C. citratus is a tall, perennial, and aromatic grass species belonging to the Poaceae (Gramineae) family. It is widely recognized for its lemon-scented leaves, which owe their distinctive aroma to high concentrations of citral—a key component of its essential oil (Oladeji *et al*., 2019). Native to South and Southeast Asia, *C. citratus* is now extensively cultivated across tropical and subtropical regions globally, including India, Nigeria, Indonesia, and Brazil (Kumar *et al*., 2023).

Botanically, the plant grows in dense clumps and reaches heights of 1 to 2 meters. Its long, narrow leaves are rough-edged and deep green, and both the leaves and stems serve as the primary sources of essential oils. These oils, particularly rich in citral, are widely used in cosmetics, perfumery, food flavoring, and aromatherapy. Beyond citral, lemongrass contains diverse phytochemicals including flavonoids, terpenoids, phenolic acids, and tannins, which contribute to its bioactivity (Gaba *et al*., 2020).

Traditionally, *C. citratus* has played a significant role in herbal medicine. It is commonly prepared as a tea or infusion for managing fever, digestive disturbances, respiratory conditions, and inflammation. Its antimicrobial and antioxidant activities have also made it a subject of increasing pharmacological interest. In culinary practice, especially in Asian cuisines, lemongrass is used as a flavoring agent in soups, curries, teas, and meat dishes due to its citrus-like aroma and taste.

Agronomically, lemongrass is a hardy crop adaptable to various soil types including clay loam, sandy loam, and loam. It grows well in both rainfed and irrigated conditions, and is considered a

sustainable crop owing to its low irrigation needs and resistance to pests. India remains the largest producer of lemongrass essential oil, with cultivation concentrated in regions such as Uttar Pradesh, Assam, and Tamil Nadu (Kumar *et al*., 2023).

The versatility of *C. citratus*—ranging from its traditional therapeutic applications to its industrial uses in essential oil production—has earned it significant attention in both ethnobotanical research and commercial agriculture.

2.1.1 Scientific Classification of *C. citratus*

The scientific classification of *C. citratus*, commonly known as lemongrass, is as follows:

- Kingdom: Plantae (plants)
- Division (Phylum): Tracheophyta (vascular plants)
- Class: Liliopsida (monocotyledons)
- Order: Poales
- Family: Poaceae (Gramineae)
- Genus: *Cymbopogon*
- Species: *Cymbopogon citratus* (DC.) Stapf

(Gaba *et al*., 2020)

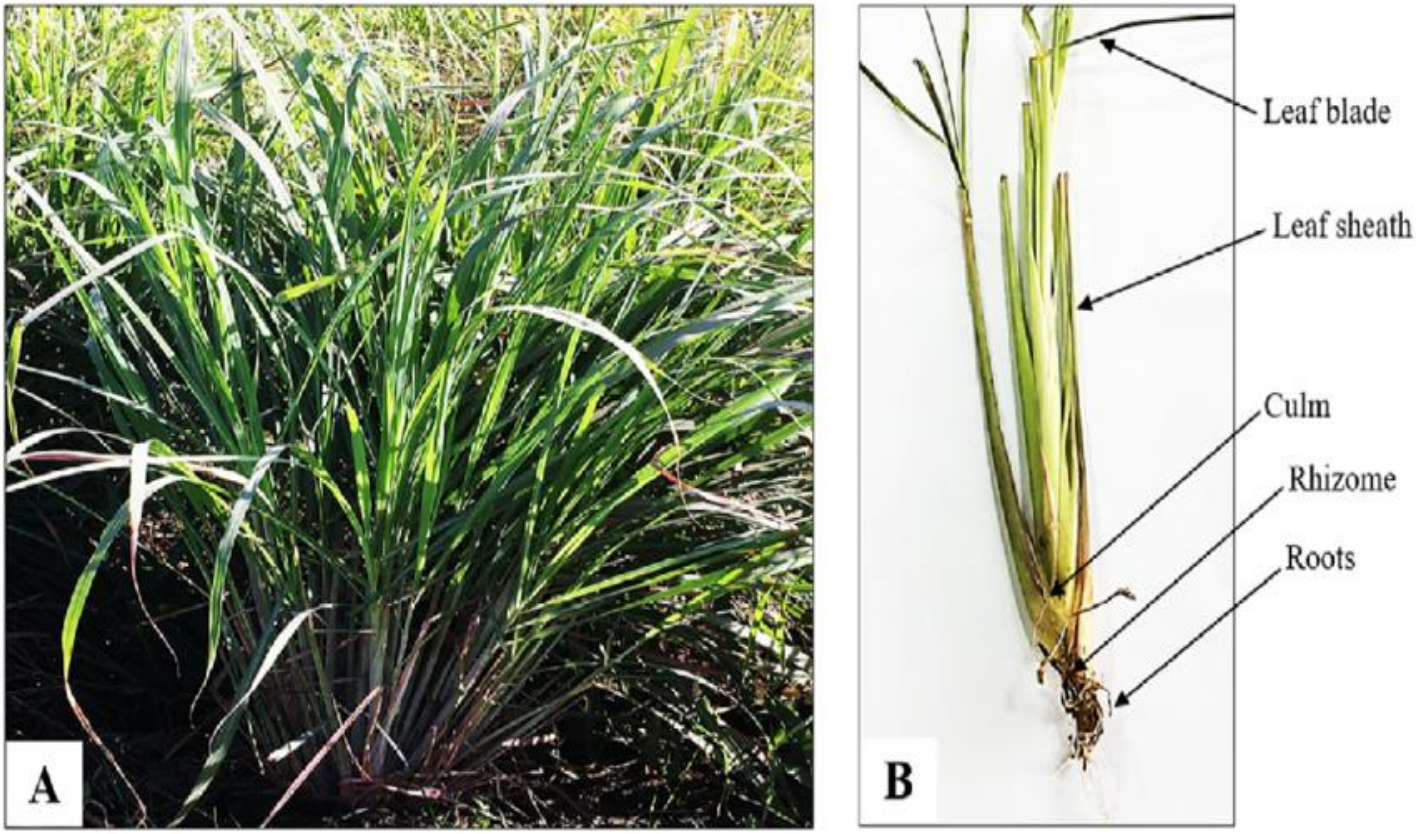

Fig. 2.1: Showing photographs of *C. citratus*— (a) Whole grass in habitat; (b)Entire plant. (Madi *et al.,* 2022).

2.2 Pharmacological and Medicinal Uses of *C. citratus*

C. citratus has demonstrated a wide range of pharmacological activities due to its rich profile of bioactive compounds, including phenolic acids, flavonoids, terpenoids, saponins, and essential oils. These compounds are responsible for its antioxidant, anti-inflammatory, antimicrobial, and immune-modulating effects, which underpin its use in traditional medicine and emerging pharmaceutical applications.

Studies have shown that aqueous and methanolic extracts of *C. citratus* exhibit strong antioxidant activity, as measured by DPPH and FRAP assays, with inhibition rates reaching up to 95.9% (Ulufan *et al*., 2022). The antioxidant potential is closely associated with phenolic and flavonoid content, which varies with the plant part, solvent used, and formulation method. For instance, the leaf tips contain higher concentrations of flavonoids, contributing to stronger radical scavenging capacity (Ranjah *et al*., 2022).

In terms of anti-inflammatory properties, in vitro and in vivo experiments reveal that *C. citratus* extracts can inhibit key pro-inflammatory mediators like IL-1β and cyclooxygenase enzymes, while sometimes upregulating beneficial cytokines such as IL-6 (Dwivedi, 2024). These modulatory effects have potential applications in managing chronic inflammation and inflammatory diseases.

The plant's antimicrobial effects are attributed largely to its essential oils, particularly citral, neral, and geranial. These compounds have demonstrated activity against a broad spectrum of pathogens, including Staphylococcus aureus and Candida albicans, suggesting potential use in natural antiseptics and food preservation (Rahim *et al*., 2020).

Further, animal studies support its immune-enhancing effects, where supplementation with lemongrass extracts improved immune biomarkers and resistance to infections, likely through its antioxidant and anti-inflammatory mechanisms (De Oliveira E Silva *et al*., 2021).

Although preclinical findings are promising, clinical validation in humans remains limited, and more robust in vivo and human trials are required to establish therapeutic efficacy and dosage guidelines (Kiani *et al*., 2022; Adhikary *et al*., 2023).

2.3 Nutritional and Phytochemical Composition

C. citratus contains a variety of micronutrients and phytochemicals that contribute to its nutritional and therapeutic value. Vitamins such as A, C, and E have been identified in its leaves, with some studies reporting concentrations higher than those found in other common herbs (Yusuf *et al*., 2023). These vitamins, particularly C and E, have frequently been used as standard antioxidants in research involving lemongrass, highlighting their functional relevance (Alabi *et al*., 2021).

Beyond vitamins, *C. citratus* is rich in flavonoids, phenolic compounds, and essential oils. Notable flavonoids like apigenin are associated with antioxidant, anti-inflammatory, and antimicrobial activities (Fathima Thasrin *et al*., 2023). The phenolic content of the plant is also substantial, and often correlates with enhanced antioxidant potential, supporting its role in reducing oxidative stress-related illnesses (Fathima Thasrin *et al*., 2023).

The essential oil of *C. citratus* is dominated by citral, composed of both E-citral and Z-citral, along with other constituents like β-myrcene, geranyl acetate, limonene, and terpineol (Mukarram *et al*., 2022). These volatile compounds are largely responsible for its aroma and

documented antibacterial, antifungal, and anti-inflammatory effects (Mukarram *et al*., 2022; Kiełtyka-Dadasiewicz *et al*., 2024).

Several factors influence the phytochemical composition of *C. citratus*, including plant variety, age, season, and environmental conditions. Geographic differences have been shown to affect the concentration of citral and other essential oil components (Shamsheer *et al*., 2022). Additionally, interactions with soil microbes such as Azospirillum brasilense and arbuscular mycorrhizal fungi can alter levels of phenolics and flavonoids (Sete da Cruz *et al*., 2024). Post-harvest processing methods—such as oven-drying versus shade-drying—also affect the final phytochemical yield, with some treatments enhancing phenolic content while reducing others (Urama *et al*., 2024).

2.4 Extraction Techniques and Solvent Polarity

Solvent-based extraction is one of the most common approaches for isolating vitamins and bioactives from plants such as *C. citratus*. Conventional methods like maceration, Soxhlet extraction, and hydrodistillation remain widely used due to their simplicity, although they often require longer durations and large volumes of solvents (Chuo *et al*., 2020).

The choice of solvent plays a crucial role in determining extraction efficiency, with solvent polarity being the key factor. Polar solvents such as water, ethanol, and methanol are effective for extracting hydrophilic compounds like vitamin C, B-complex vitamins, and phenolic acids. Non-polar solvents such as hexane and diethyl ether are more suitable for extracting fat-soluble vitamins such as A, D, E, and K (Awotedu *et al*., 2020; Yesufu *et al*., 2022).

Recent innovations have introduced greener and more efficient solvent systems, including natural deep eutectic solvents (NADES) and ethyl lactate. These offer tunable polarity, lower

toxicity, and better extraction yields for both polar and non-polar compounds when used either singly or in binary combinations (Nawaz *et al*., 2022). Studies have shown that combining solvents of different polarities can enhance the simultaneous extraction of diverse bio actives in a single step, making the process more sustainable and comprehensive (Ghaffar and Perveen, 2024).

2.5 Historical Uses of *C. citratus*

C. citratus has a longstanding history of use in traditional medicine and cultural practices across Asia, Africa, and South America. For centuries, it has been employed as a herbal remedy for fever, digestive ailments, respiratory infections, and inflammation. In Ayurveda and traditional Thai medicine, lemongrass tea and decoctions are consumed to relieve symptoms of colds, headaches, stomach discomfort, and menstrual irregularities (Israr *et al*., 2023).

In West African folk medicine, *C. citratus* is commonly used for its analgesic and antipyretic effects. Decoctions of the leaves or stalks are prepared to manage malaria-related fever, cough, and general body pain. The plant's essential oil, particularly citral, is traditionally applied topically for muscle soreness, skin conditions, and as a natural insect repellent (Gaba *et al*., 2020).

Culinary applications of lemongrass are also deeply rooted in historical traditions. In Southeast Asian cuisine, it has long served as a key aromatic in soups, curries, and teas. Beyond flavoring, its use in food was often intertwined with its presumed medicinal value, believed to enhance digestion and prevent infection.

In various indigenous practices, the plant has also held symbolic and ritual significance — burned as incense for spiritual cleansing or used in bath preparations for its calming effects. These historical uses continue to inform modern herbalism and functional food industries, bridging ancestral knowledge with emerging pharmacological interest (Oladeji *et al*., 2019).

2.6 Vitamins

Vitamins as micronutrients play vital roles in human health by supporting immune function, preventing metabolic disorders, and mitigating oxidative stress (Asuku *et al*., 2020).

2.6.1 Types of Vitamins

Vitamins are classified into two main categories based on their solubility: fat-soluble and water-soluble. This classification affects their absorption, transport, storage, and excretion in the human body (Akram *et al*., 2020).

A. Fat-Soluble Vitamins

Fat-soluble vitamins include vitamins A, D, E, and K. These vitamins are absorbed along with dietary fats in the intestine and are stored in the liver and adipose tissue, which allows for long-term storage. Because of this, excess intake of fat-soluble vitamins can lead to toxicity (hypervitaminosis).

i. Vitamin A (retinoids and carotenoids) is crucial for vision, immune function, and cellular differentiation. Deficiency can lead to night blindness and increased susceptibility to infections (Brown and Noelle, 2015).

ii. Vitamin D (cholecalciferol and ergocalciferol) is essential for calcium homeostasis and bone health. It can be synthesized in the skin through sunlight exposure, and deficiency is associated with rickets in children and osteomalacia in adults.

iii. Vitamin E (tocopherols and tocotrienols) acts as a lipid-soluble antioxidant, protecting cell membranes from oxidative damage. Deficiency is rare but may occur in individuals with fat malabsorption disorders.

iv. Vitamin K (phylloquinone and menaquinone) is necessary for blood coagulation and bone metabolism. Deficiency can lead to impaired blood clotting and increased bleeding risk (Booth, 2017).

B. Water-Soluble Vitamins

Water-soluble vitamins include the B-complex group and vitamin C. These vitamins are not stored in large amounts in the body and are excreted in urine, requiring more frequent dietary intake (Gropper and Smith, 2021).

The B-complex vitamins include:

i. Vitamin B1 (thiamine) – involved in energy metabolism; deficiency leads to beriberi.

ii. Vitamin B2 (riboflavin) – a coenzyme in redox reactions; deficiency causes ariboflavinosis.

iii. Vitamin B3 (niacin) – used in NAD/NADP coenzymes; deficiency leads to pellagra.

iv. Vitamin B5 (pantothenic acid) – essential for CoA synthesis.

v. Vitamin B6 (pyridoxine) – important for amino acid metabolism.

vi. Vitamin B7 (biotin) – involved in carboxylation reactions.

vii. Vitamin B9 (folate) – crucial for DNA synthesis and cell division; deficiency causes megaloblastic anemia and neural tube defects.

viii. Vitamin B12 (cobalamin) – vital for nerve function and red blood cell formation; deficiency leads to pernicious anemia and neurological issues (Kennedy, 2016).

ix. Vitamin C (ascorbic acid) functions as an antioxidant and is required for collagen synthesis and immune support. Its deficiency causes scurvy, characterized by bleeding gums, joint pain, and fatigue (Carr and Maggini, 2017).

2.6.2 Benzoic Acid in Plant Systems

Benzoic acid plays several important biological roles in plants, acting as a secondary metabolite involved in growth regulation, stress response, and defense. It can promote plant biomass and nutrient content at optimal concentrations by enhancing metabolic pathways, such as carbon metabolism and the biosynthesis of cofactors and antioxidants, but may inhibit growth at high concentrations (Shang *et al*., 2024). Benzoic acid is also a precursor to salicylic acid, a key hormone in plant defense, and can induce the activation of antioxidant enzymes and pathogenesis-related proteins, thereby increasing resistance to diseases like early blight in tomato and common blight in beans (Abo-Elyousr *et al*., 2022). Additionally, benzoic acid functions as an allelochemical, influencing root growth and development by modulating auxin, ethylene, and reactive oxygen species signaling pathways, often resulting in root growth inhibition in neighboring plants (Zhang *et al*., 2018). In soil, benzoic acid released from roots can shift microbial community composition and stimulate soil organic matter decomposition, affecting nutrient cycling and plant-microbe interactions (Zwetsloot *et al*., 2020). It also helps plants tolerate abiotic stresses, such as excess boron, by regulating nutrient uptake and enhancing cell

wall components, further supporting plant growth under adverse conditions (Farghaly *et al*., 2022).

2.7 Gaps in Existing Literature

Although *C. citratus* has been widely studied for its essential oils and phenolics, there remains a notable lack of comparative vitamin extraction studies using both polar and non-polar solvents. Some investigations have evaluated non-polar solvents like hexane and hexane–toluene mixtures for essential oil recovery, but did not assess vitamin content (Usharani, 2021). Similarly, polar solvents such as acetone, ethanol, and methanol have been studied for phenolic extraction, yet without vitamin quantification (Aouadi *et al*., 2024). Other studies using hydrodistillation or microwave-assisted extraction focus on essential oil profiles and pH effects rather than micronutrients (Ajayi *et al*., 2016). Although some literature acknowledges the utility of both water and organic solvents, direct comparative evaluation of vitamin extraction efficiency by solvent polarity remains scarce (Widiputri *et al*., 2019).

In Nigeria, various studies have examined *C. citratus* from regions such as Jos, Kaduna, and Benin City, confirming the presence of essential oils, vitamins A, C, and E, and minerals like calcium and iron (Magotra *et al*., 2021). However, most of these studies rely on broad compositional analysis through standard methods such as hydrodistillation, titration, or proximate analysis, without detailed quantification of individual vitamins or exploration of solvent variation (Gaba *et al*., 2020). Additionally, bioavailability and pharmacological validation are rarely addressed in depth.

Another critical gap is the limited availability of data on vitamin stability during extraction and processing. While vitamins A, C, and E are frequently assessed, other micronutrients such as B-complex vitamins, D, and K are often underreported. Vitamin C, for instance, is known to degrade under heat, oxygen, and light, especially during thermal processing and storage (Giannakourou and Taoukis, 2021). However, comprehensive side-by-side comparisons of stability for other vitamins across different solvents or processing parameters remain rare (Sachdeva *et al*., 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Area of study

The study was carried out at the University of Benin, situated in Benin City, Edo State, Nigeria.

3.2 Study Location

The extraction procedures were conducted in the Department of Chemistry and the Department of Pharmacy (Pharmacognosy Unit), both within the University of Benin, which are equipped with the necessary laboratory facilities for solvent extraction and related analyses. Subsequent phytochemical and proximate analyses were performed at Docchy Laboratories, Awka, Anambra State, Nigeria, a specialized laboratory facility furnished with modern equipment for biochemical and phytochemical investigations.

3.3 Materials

3.3.1 Chemical and Reagents

Ethanol (analytical grade) was obtained from Sigma-Aldrich, Germany, while Diethyl ether (LR grade, stabilized) was procured from Molychem, India. All solvents and reagents used were of high analytical grade.

3.3.2 Equipment

The major equipment used in this study included an electronic weighing balance (Model: TS200, OHAUS), rotary evaporator (Model: Julabo F10), UV-Visible spectrophotometer (Model: 752N, Hinotek), hot air oven (Model: DHG-9023A, Memmert), and muffle furnace (Model: HT-MF1400-6.75S/G).

3.4 Collection and Identification of *C. citratus*

Fresh samples of *C. citratus* were collected within the University of Benin premises around the Faculty of Basic Medical Sciences, Benin City, Edo State, Nigeria. The plant was identified and

authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, and its identity was confirmed under the voucher number UBH-C451 at the Herbarium Unit.

3.5 Preparation of plant extract

The plant extract was prepared at the Department of Chemistry and the Department of Pharmacy (Specialty Pharmacognosy), University of Benin. The leaves of *C. citratus* were separated from the stems, cleaned to remove debris, and thoroughly washed with clean tap water. They were air-dried at room temperature under laboratory conditions for two weeks and then pulverized into fine powder using a commercial blender. The total weight of the dried pulverized sample was 707.4g.

3.5.1 Polar Extraction

The extraction was carried out using a modified method of Onyeukwu *et al*. (2024). A total of 250g of the powdered leaf was macerated in 1.2L of ethanol in a brown glass jar, properly sealed with aluminum foil, and left to stand for 72 hours at room temperature. The mixture was first filtered with cheesecloth to remove coarse debris and then passed through Whatman Grade 1 filter paper, yielding 565ml of filtrate. The filtrate was concentrated using a rotary evaporator and further air-dried, producing 3.9g of dry extract. The extract was subsequently stored at 4°C in a refrigerator until required for use.

3.5.2 Non polar Extraction

The extraction was carried out using a modified method of Njideaka *et al*. (2024). A total of 250g of the powdered leaf was macerated in 1.2L of diethyl ether in a brown glass jar, sealed with aluminum foil, and allowed to stand for 72 hours at room temperature. The mixture was filtered initially with cheesecloth and subsequently with Whatman Grade 1 filter paper, producing 520ml

of filtrate. The filtrate was concentrated using a rotary evaporator and air-dried, yielding 4.3g of dry extract. The extract was subsequently stored at 4°C in a refrigerator until required for use.

3.5.3 Percentage Yield

The percentage yield for each extraction was determined using the formula:
Yield (%) = (Dry weight of extract / Dry weight of plant material) × 100

Ethanol extraction:

Yield = (3.9 g / 250 g) × 100 = 1.56 %

Diethyl ether extraction:

Yield = (4.3 g / 250 g) × 100 = 1.72 %

The diethyl ether (non-polar) extraction yielded slightly more extract (1.72%) compared to the ethanol (polar) extraction (1.56%). This may be attributed to the ability of diethyl ether to solubilize non-polar phytochemicals more efficiently, suggesting that *C. citratus* contains a significant proportion of non-polar constituents. In contrast, ethanol, being a polar solvent, primarily extracted polar compounds, resulting in a marginally lower yield.

3.6 Biochemical Analysis

3.6.1 Fat-soluble vitamins

1. Vitamin A content

Method: Colorimetric determination (Bayfield and Cole, 1980)

PRINCIPLE: The assay determines vitamin A by measuring, with a spectrophotometer, the color produced when its acetate or palmitate form reacts with trichloroacetic acid.

REAGENTS

Saponifying solution: 2 N KOH in 90% alcohol

Petroleum ether (40–60 °C)

Anhydrous sodium sulphate

Chloroform

Vitamin A palmitate standard

60% TCA in chloroform, prepared freshly before use

Procedure:

The procedures were carried out in the absence of light to avoid interference. A 1 g portion of the sample was mixed with 1.0 ml of the saponification mixture and refluxed at 60 °C for 20 minutes in the dark.

After refluxing, the tubes were allowed to cool, and 20 ml of distilled water was added, followed by thorough mixing. Two successive extractions of vitamin A were performed with 10 ml of

petroleum ether (40–60 °C) per extraction. The resulting petroleum ether layers were pooled and washed repeatedly with water to remove contaminants.

Residual moisture in the combined extract was removed by adding anhydrous sodium sulphate. A 1 ml portion of the petroleum ether extract was then evaporated at 60 °C until dry, and the resulting residue was re-dissolved in 1 ml of chloroform.

Vitamin A standards (vitamin A palmitate) ranging from 0 to 7.5 µg were prepared in a series of test tubes, and the volume in each was adjusted to 1.0 ml with chloroform.

To each tube, including both standards and samples, 2.0 ml of freshly prepared TCA reagent was added rapidly and mixed.

The absorbance was then measured immediately at 620 nm using a Genesys 10UV spectrophotometer. The same procedure was applied to the sample extract, and the vitamin A content was expressed in mg/kg.

2. **Vitamin D Content**

Method: Colorimetric determination (Brockmann *et al*., 1974)

PRINCIPLE: The assay is based on the spectrophotometric estimation of the yellow color formed by the reaction of vitamin D with a chloroform solution of trichloroacetic acid (TCA).

REAGENTS

1. Chloroform
2. Methanol

3. Vitamin D_3 working standard
4. 0.25 N Hydrochloric acid (HCl)
5. 15.0% Trichloroacetic acid (TCA) solution
6. 0.375% Thiobarbituric acid (TBA) solution

Procedure

The vitamin D working standard was prepared by accurately weighing 25 mg of vitamin D_3 and dissolving it in a 25 ml volumetric flask using a chloroform–methanol solution mixed in a 1:9 ratio. The mixture was topped up to the mark with the same solvent mixture and shaken thoroughly. Then, 0.1 ml of the test sample was precisely measured and placed into a new 25 ml volumetric flask to prepare the sample extract.

The same chloroform–methanol (1:9) mixture was used to dissolve and dilute the sample to the mark, followed by thorough mixing.

Measured portions of both the standard and sample solutions were placed into different test tubes. To each tube, 1.6 ml of 0.25 N hydrochloric acid, 0.5 ml of 15% trichloroacetic acid, and 0.5 ml of 0.375% thiobarbituric acid were subsequently added.

The mixtures were then vortexed or gently shaken to ensure uniform reaction. The absorbance of the yellow-colored solution was promptly measured at 464 nm against a reagent blank using a Genesys 10UV spectrophotometer. Vitamin D concentration was then obtained from the standard calibration curve and reported in mg/kg.

3 Vitamin E Content

Method: Colorimetric determination using the Emmerie–Engel reaction (Rosenberg, 1992)

PRINCIPLE: The Emmerie–Engel reaction is based on the reduction of ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions by vitamin E. The resulting ferrous ions form a red-colored complex with 2,2'-dipyridyl, which can be measured spectrophotometrically. Since both vitamin E and carotenes are extractable in xylene, the initial absorbance is taken at 460 nm to estimate carotene content. A correction is then made by adding ferric chloride and taking a second reading at 520 nm, which is specific to the vitamin E–dipyridyl complex.

REAGENTS

1. Absolute ethanol
2. Xylene
3. 2,2'-Dipyridyl solution (1.2 g/L prepared in *n*-propanol)
4. Ferric chloride solution (1.2 g/L in ethanol)
5. Standard vitamin E solution (D,L-α-tocopherol, 10 mg/L in absolute alcohol)
6. 0.1 N Sulphuric acid

Procedure

Extraction of Vitamin E

A 2.5 g portion of the sample was blended with 50 ml of 0.1 N sulfuric acid and left to stand overnight at room temperature. The mixture was subsequently agitated thoroughly and filtered

using Whatman No. 1 filter paper, and the clear filtrate obtained served for the estimation of vitamin E.

Estimation of Vitamin E

Into three clean, stoppered centrifuge tubes, 1.5 ml of the sample extract was pipetted into tube 1, 1.5 ml of the standard solution into tube 2, and 1.5 ml of distilled water into tube 3 to serve as the blank Each tube was treated with 1.5 ml of ethanol and 1.5 ml of xylene, mixed well, and centrifuged to separate layers. One millilitre of the xylene phase was collected into a stoppered tube, mixed with 1.0 ml of 2,2'-dipyridyl reagent, and 1.5 ml of this mixture was placed in a cuvette for absorbance reading at 460 nm to adjust for carotene content.

Afterward, 1.0 ml of ferric chloride solution was added to each tube, and the tubes were allowed to stand for 15 minutes at room temperature to allow color development.

The absorbance was recorded at 520 nm using a spectrophotometer to determine the vitamin E concentration. The vitamin E content was then calculated from the standard calibration curve and expressed in mg/kg of the sample.

4 Vitamin K Content

Method: Spectrophotometric determination (AOAC, 2000)

PRINCIPLE: The estimation of vitamin K is based on the saponification of the sample to release fat-soluble vitamins, followed by extraction with petroleum ether. The extracted vitamin K forms a yellow-colored compound whose absorbance can be measured spectrophotometrically at 450 nm.

Procedure

A 0.5 g portion of the sample was homogenized and saponified with 2.5 ml of 12% alcoholic potassium hydroxide in a water bath at 60 °C for 30 minutes.

The saponified mixture was then transferred to a separating funnel containing 10–15 ml of petroleum ether and mixed thoroughly.

The lower aqueous layer was separated and transferred to a second funnel, while the upper petroleum ether layer, which contained vitamin K, was collected. The extraction process was repeated until the aqueous layer became colorless, ensuring complete removal of vitamin K.

Anhydrous sodium sulphate was added in small amount to the petroleum ether extract to absorb remaining moisture, after which the final volume of the extract was measured.

After color development, absorbance was taken at 450 nm on a Genesys 10-S spectrophotometer.

The vitamin K content was then determined from a standard calibration curve and expressed in mg/kg of the sample.

3.6.2 Water-soluble vitamins

1. Estimation of vitamin b1

Method: UV–spectrophotometry (Al-Shaalan, 2015).

PRINCIPLE: Vitamin B1 (thiamine) exhibits characteristic ultraviolet absorption at 261 nm in aqueous solution. The concentration is estimated using Beer–Lambert's law, with absorbance directly proportional to concentration. A known extinction coefficient (E = 25) and dilution factor are applied in the final calculation.

PROCEDURE:

A 1.0 g portion of the sample was weighed into a clean conical flask and dissolved in 100 ml of deionized water. The solution was shaken thoroughly and then heated for 5 minutes.

After heating, it was allowed to cool and filtered through Whatman No. 1 filter paper.

A portion of the clear filtrate was placed in a cuvette, and absorbance was recorded at 242 nm with a UV–Vis spectrophotometer.

CALCULATION:

$$\text{Concentration (mg\%)} = \frac{A * DF * 5}{25}$$

Where:

- A = Absorbance reading
- DF = Dilution factor
- E = Extinction coefficient (25)
- 5 = Volume of cuvette (ml)

2. Estimation of vitamin b2

Method: UV–spectrophotometry (Al-Shaalan, 2015).

PRINCIPLE: Vitamin B2 (riboflavin) exhibits characteristic ultraviolet absorption at 242 nm in aqueous solution. The concentration is determined using Beer–Lambert's law, with absorbance being directly proportional to the concentration. A known extinction coefficient (E = 25) and dilution factor are applied in the final calculation.

PROCEDURE:

A 1.0 g portion of the sample was weighed into a clean conical flask and dissolved in 100 ml of deionized water. The solution was shaken thoroughly and then heated for 5 minutes.

After heating, it was allowed to cool and filtered through Whatman No. 1 filter paper.

The clear filtrate was then transferred into a spectrophotometric cuvette, and the absorbance was measured at 242 nm using a UV-visible spectrophotometer.

CALCULATION:

$$\text{Concentration (mg\%)} = \frac{A * DF * 5}{25}$$

Where:

- A = Absorbance reading
- DF = Dilution factor
- E = Extinction coefficient (25)
- 5 = Volume of cuvette (ml)

3. Estimation of vitamin b3 (nicotinamide)

Method: Nonaqueous titrimetric method (Beckett and Stenlake, 2002a).

PRINCIPLE: Nicotinamide (Vitamin B3) reacts with perchloric acid in the presence of crystal violet as an indicator. The titration results in a visible colour change from purple to greenish-blue, indicating the endpoint. The amount of perchloric acid consumed is stoichiometrically related to the vitamin B3 content.

PROCEDURE:

Five grams of the sample were dissolved in 20 ml of anhydrous glacial acetic acid and gently warmed. Then, 5 ml of acetic anhydride was added and mixed well. After adding 2–3 drops of crystal violet indicator, the solution was titrated with 0.1 M perchloric acid until a greenish-blue color was observed.

CALCULATION:

Vitamin B_3 (mg)= Titre (mL) × 0.122

4. Estimation of vitamin b6 (pyridoxine)

Method: Nonaqueous titrimetric method (Beckett & Stenlake, 2002b)

PRINCIPLE: Vitamin B_6 (pyridoxine) forms a complex with mercury(II) acetate and reacts with perchloric acid in an acetic medium. Crystal violet acts as an indicator. The titration proceeds until a green-colored endpoint is observed, and the volume of perchloric acid consumed is stoichiometrically related to the vitamin B_6 content.

PROCEDURE:

The sample (5.0 g) was dissolved in 5 ml of glacial acetic acid combined with 6 ml of 0.1 M mercury(II) acetate. After adding two drops of crystal violet as an indicator, the mixture was titrated against 0.1 M perchloric acid to a green endpoint.

CALCULATION:

Vitamin B_6 (mg) = (Titre × 2.056) / Sample weight

5. Estimation of vitamin b12 (cyanocobalamin)

Method: Adapted from standard spectrophotometric approaches described for cyanocobalamin in pharmacopeial and analytical literature (European Pharmacopoeia, 2014)

PRINCIPLE: Vitamin B12 (cyanocobalamin) reacts with phenylhydrazine under acidic alcoholic conditions, forming a derivative that further couples with pyridine to produce a measurable chromogen. The absorbance of the resulting complex is recorded spectrophotometrically at 635 nm. Quantification is achieved by comparing the sample absorbance with a standard calibration curve.

SAMPLE PREPARATION:

A 0.1 ml portion of the sample was measured into a separating funnel, after which 5 ml of distilled water was added.

The mixture was then shaken thoroughly and extracted with 5 ml of chloroform.

The mixture was shaken well and extracted with 5 ml of chloroform. The upper aqueous layer was discarded, and the chloroform layer was dried over anhydrous sodium sulphate and collected in a 50 ml volumetric flask.

The volume was then made up to 50 ml with chloroform.

PROCEDURE:

Aliquots of 2 ml each from the sample and blank solutions were introduced into separate test tubes, followed by 2.0 ml of 0.2% phenylhydrazine solution prepared in a 1:5 v/v hydrochloric acid–alcohol mixture. After thorough mixing, the tubes were heated in a water bath to near dryness and cooled. Subsequently, 2.0 ml of an equal mixture of ammonia and alcohol together with 1.0 ml of pyridine were added to each.

The absorbance of the resulting solution was measured at 635 nm using a spectrophotometer, with the blank serving as the reference. A standard cyanocobalamin solution was prepared and treated following the same procedure. A calibration curve was plotted using the absorbance values of the standards, and the concentration of the sample was extrapolated from this curve.

6. Estimation of vitamin C

Method: Colorimetric determination (Roe and Keuther, 1943)

PRINCIPLE: Ascorbate is converted into dehydroascorbate upon treatment with activated charcoal, that reacts with 2,4-dinitrophenylhydrazine (DNPH) to generate osazones. Dissolution of these osazones in concentrated sulfuric acid results in an orange solution, the absorbance of which is determined at 540 nm using a spectrophotometer.

REAGENTS

1. 4% Trichloroacetic acid (TCA)
2. 2% 2,4-Dinitrophenylhydrazine (DNPH) in 9 N H_2SO_4
3. 10% Thiourea

4. 85% Sulphuric acid

5. Standard ascorbic acid solution (100 µg/ml in 4% TCA)

Procedure

Extraction of Vitamin C

Ascorbate was extracted from 1 g of the sample using 4% trichloroacetic acid (TCA), and the volume was made up to 10 ml with the same reagent to ensure consistency.The extract was then centrifuged at 2000 rpm for 10 minutes to separate any solid residues.

Activated charcoal was added to the supernatant and mixed vigorously with a cyclomixer. The mixture was then allowed to stand for 5 minutes to facilitate impurity adsorption.

The charcoal was subsequently removed by centrifugation, and the clear supernatant obtained was used for further analysis.

Estimation of Vitamin C

Into separate test tubes, 0.2–1.0 ml of standard ascorbic acid and 0.5–1.0 ml of sample supernatant were added, and the total volume was brought to 2.0 ml with 4% TCA. Each tube received 0.5 ml of DNPH reagent and two drops of 10% thiourea, after which the contents were mixed and incubated at 37 °C for 3 hours for osazone crystal formation.

After incubation, 2.5 ml of pre-cooled 85% sulphuric acid was added carefully to each tube to dissolve the crystals formed. In the blank test, DNPH and thiourea were added only after sulphuric acid to act as a control. The tubes were cooled in ice to stabilize the mixtures, and absorbance was read at 540 nm with a spectrophotometer. The vitamin C content was obtained from a standard calibration curve computed using linear regression and expressed as mg/kg.

3.6.3 Other bioactive compounds

Estimation of Benzoic Acid

Method: UV–spectrophotometry at 503 nm (Zakaria *et al*., 1979)

PRINCIPLE:

Benzoic acid is extracted into petroleum ether after saponification and its absorbance is measured at 503 nm. The absorbance is proportional to benzoic acid concentration and is used with the sample volume and weight to calculate mg/g of sample.

REAGENTS

1. Petroleum ether (40–60 °C)
2. Anhydrous sodium sulphate
3. Calcium carbonate
4. Alcoholic potassium hydroxide (12%)

Procedure

A 0.5 g portion of the sample was homogenized and saponified with 2.5 ml of 12% alcoholic potassium hydroxide in a water bath at 60 °C for 30 minutes. The saponified mixture was then transferred into a separating funnel containing 10–15 ml of petroleum ether and mixed thoroughly. The lower aqueous layer was separated, while the upper petroleum ether layer containing benzoic acid was collected.

The extraction process was repeated until the aqueous layer became colourless, confirming complete removal of benzoic acid. The combined petroleum ether extracts were then treated

with a small quantity of anhydrous sodium sulphate to remove any residual moisture, and the final volume of the extract was recorded. The absorbance of the resulting extract was measured at 503 nm against a petroleum ether blank using a Genesys 10-S spectrophotometer.

CALCULATION

Amount of benzoic acid (mg/g) = (A503 ×Volume of extract (mL)×100×4 / Weight of Sample (mg))

Results expressed as**:** mg/g of sample.

CHAPTER FOUR

RESULTS

Table 4.1 presents the comparative concentrations of selected vitamins and benzoic acid in polar and non-polar extracts of *C. citratus*, expressed as Mean ± Standard Deviation (SD). Significant differences between the extracts were evaluated using t-tests, and p-values are reported. The polar extract yielded significantly higher concentrations of vitamin A (51.02 ± 0.004 ppm), vitamin B6 (2.533 ± 0.010 ppm), and vitamin D (55.89 ± 0.020 ppm), all with p-values less than 0.05. In contrast, the non-polar extract recorded significantly higher levels of vitamin B12 (2.957 ± 0.155 ppm), vitamin C (59.71 ± 0.035 ppm), vitamin E (36.07 ± 0.2335 ppm), vitamin K (19.90 ± 0.2137 ppm), and benzoic acid (6.124 ± 0.027 ppm), also with p-values below 0.05. These findings underscore the influence of solvent polarity on the extractability of specific vitamins and bioactive compounds from plant matrices.

Table 4.1: Comparative analysis of Vitamins and Benzoic acid concentrations in Ethanol and Diethyl ether extracts of *C. citratus.*

Vitamins	**Lemongrass + ethanol (polar)**	**Lemongrass + diethyl ether(non-polar)**	**t value**	**p value**
Vitamin A(ppm)	51.02±0.004	41.45±0.039	81.27	<0.0001
Vitamin B1 (ppm)	0.1287±0.001	0.1287±0.001	0	>0.9999
Vitamin B2 (ppm)	0.06733±0.002	0.06733±0.002	0	>0.9999
Vitamin B3 (ppm)	1.385±0.006	1.312±0.006	0.6227	0.9998
Vitamin B6 (ppm)	2.533±0.01	2.128±0.01	3.442	0.014
Vitamin B12 (ppm)	2.358±0.07	2.957±0.155	5.092	<0.0001
Vitamin C (ppm)	53.08±0.317	59.71±0.035	56.3	<0.0001
Vitamin D (ppm)	55.89±0.02	50.59±0.4118	44.99	<0.0001
Vitamin E (ppm)	28.57±0.196	36.07±0.2335	63.65	<0.0001
Vitamin K (ppm)	14.99±0.118	19.9±0.2137	41.64	<0.0001
Benzoic acid (ppm)	4.291±0.016	6.124±0.027	15.57	<0.0001

Table presented in Mean±SD

CHAPTER FIVE

DISCUSSION CONCLUSSION, AND RECOMMENDTIONS.

5.1 Discussion

The ethanol extract of *C. citratus* contained a significantly higher concentration of vitamin A (51.02 ± 0.004 ppm) compared to the diethyl ether extract (41.45 ± 0.039 ppm), with a p-value of < 0.0001. Although vitamin A is typically classified as a fat-soluble compound, its enhanced extractability in ethanol suggests that it may possess sufficient amphipathic properties or matrix interactions that allow partial solubility in polar solvents under certain extraction conditions. Ethanol, in particular, has been shown to effectively disrupt plant cell walls and facilitate the release of lipophilic compounds, especially when applied in warm or agitated systems. Several studies have demonstrated that polar solvents like ethanol and methanol are not only efficient in extracting polyphenols and flavonoids but also highly effective in recovering carotenoids such as β-carotene, a key vitamin A precursor, from various plant materials (Zhang *et al.*, 2018; Wakeel *et al.*, 2019). This trend supports the hypothesis that the structural and chemical behavior of vitamin A derivatives enables their partial solubility in polar organic solvents, particularly in plant matrices rich in emulsifying or surface-active phytochemicals. Thus, the higher yield of vitamin A in the ethanol extract of *C. citratus* may be attributed to both solvent polarity and the specific interactions between the solvent and plant cellular components.

The ethanol extract of *C. citratus* also showed a significantly higher concentration of vitamin B6 (2.533 ± 0.010 ppm) compared to the diethyl ether extract (2.128 ± 0.010 ppm), with a p-value of 0.014. This result aligns with the known polarity of vitamin B6, which is a water-soluble compound typically favoring extraction in polar solvents. Ethanol, being a polar protic solvent, is capable of efficiently dissolving hydrophilic vitamins and disrupting hydrogen bonding within

plant tissues, making it suitable for extracting B6 from plant matrices. Research has shown that aqueous two-phase systems incorporating ethanol, water, and salts such as sodium carbonate (Na_2CO_3) achieve high efficiency in vitamin B6 recovery, particularly under mildly acidic conditions and moderate temperatures (Lu *et al.*, 2020). Furthermore, alternative green extraction techniques, such as ultrasound-assisted methods and the use of hydrophilic molecularly imprinted biopolymers in water-based media, have been reported to significantly enhance the yield and selectivity of vitamin B6 extraction from complex plant materials (Ostovan *et al.*, 2018; Mannaï *et al*., 2019). These findings collectively support the observation that ethanol, as a polar solvent, offers a favorable environment for extracting vitamin B6 from *C. citratus* under standard laboratory conditions.

The diethyl ether extract of *C. citratus* yielded a significantly higher concentration of vitamin B12 (2.957 ± 0.155 ppm) compared to the ethanol extract (2.358 ± 0.070 ppm), with a p-value of < 0.0001. This result is surprising, given that vitamin B12 is a highly polar, water-soluble compound, and the scientific literature overwhelmingly supports its extraction using polar solvents such as methanol-water mixtures, often under ultrasound-assisted conditions. For instance, studies on *Ulva lactuca* (green seaweed) showed optimal vitamin B12 recovery using methanol-water, with no evidence supporting non-polar solvents like diethyl ether as effective extraction media (Susanti *et al*., 2022). One possible explanation for this unexpected result is that vitamin B12 in the plant matrix may be loosely associated with hydrophobic compounds or cell wall components that are more accessible to non-polar solvents. Alternatively, interactions during the extraction process may have led to the co-extraction of B12 derivatives or interfering compounds that mimic vitamin B12 in spectrophotometric analysis. While this finding contrasts with known solubility behavior, it may reflect complex matrix effects unique to *C. citratus* or

limitations of the extraction and detection method used. Further analysis using more selective techniques such as HPLC may be necessary to confirm the identity and true solubility behavior of the extracted compound.

The diethyl ether extract of *C. citratus* contained a significantly higher concentration of vitamin C (59.71 ± 0.035 ppm) compared to the ethanol extract (53.08 ± 0.317 ppm), with a p-value of < 0.0001. This result is unexpected, as vitamin C is a highly polar, water-soluble compound with poor solubility in non-polar solvents like diethyl ether. However, recent studies have shown that spectrophotometric analysis can be affected by matrix interference, where co-extracted compounds such as pigments or phenolics produce spectral overlap, leading to potentially inflated absorbance readings. Such interference can cause overestimation of vitamin C, especially in complex plant matrices. To address this, researchers have proposed techniques like chemical modifiers or selective separation to minimize interference and improve accuracy (Tariq *et al.*, 2025). The elevated vitamin C level in the non-polar extract may therefore reflect analytical limitations rather than true solubility behavior, underscoring the need for matrix-specific method validation or chromatographic confirmation

The ethanol extract of *C. citratus* recorded a significantly higher concentration of vitamin D (55.89 ± 0.020 ppm) compared to the diethyl ether extract (50.59 ± 0.4118 ppm), with a p-value of < 0.0001. This finding is consistent with current research, which reports that ethanol, due to its polar nature, is more efficient than non-polar solvents like diethyl ether in extracting vitamin D and its precursors from plant materials. Ethanol facilitates both the disruption of plant cell walls and the solubilization of conjugated or partially polar vitamin D metabolites, thereby increasing extraction yield (Nzekoue *et al*., 2022). In particular, studies on mushrooms and other plant matrices have shown that ethanol outperforms diethyl ether in recovering ergosterol and vitamin

D2, even under basic extraction conditions such as ethanolic KOH (Morales *et al.,* 2017; Nzekoue *et al.,* 2022). Although extraction efficiency may be further enhanced by methods like ultrasound-assisted extraction (UAE), the fundamental role of solvent polarity remains a determining factor. The observed higher vitamin D concentration in the ethanol extract of *C. citratus* confirms the suitability of polar solvents for isolating fat-soluble but partially polar vitamins from botanical sources.

The diethyl ether extract of *C. citratus* contained a significantly higher concentration of vitamin E (36.07 ± 0.2335 ppm) compared to the ethanol extract (28.57 ± 0.196 ppm), with a p-value of < 0.0001. This result is well-aligned with the known chemical behavior of vitamin E (tocopherols), which are non-polar, fat-soluble compounds. The scientific literature confirms that non-polar solvents such as hexane and diethyl ether are generally more effective in extracting vitamin E from plant and oil-based materials than polar solvents like ethanol or isopropanol (Bansal *et al*., 2020; Lestari *et al*., 2022). For example, studies on palm fatty acid distillates and soymeal have demonstrated higher recovery rates of vitamin E when non-polar solvents are used, especially in oil-rich matrices (Bansal *et al*., 2020; Lestari *et al*., 2022). Furthermore, computational and experimental studies have shown that vitamin E has a higher distribution coefficient and selectivity in non-polar solvents, reinforcing the effectiveness of diethyl ether as an extraction medium (Qin *et al*., 2022). This observation supports the finding that vitamin E is more extractable in diethyl ether and confirms that solvent polarity plays a critical role in determining extraction efficiency for lipophilic bioactive compounds.

The diethyl ether extract of *C. citratus* yielded a significantly higher concentration of vitamin K (19.90 ± 0.2137 ppm) compared to the ethanol extract (14.99 ± 0.118 ppm), with a p-value of < 0.0001. This result is consistent with the fat-soluble nature of vitamin K, which includes

analogs such as K1 (phylloquinone), K2 (menaquinones), and K4 (menadiol diacetate). Scientific literature confirms that vitamin K and its derivatives exhibit good solubility in non-polar organic solvents such as cyclohexane and methylcyclohexane, and their extraction efficiency improves at elevated temperatures or when co-solvents are present (Motati *et al*., 2023; Bryshten *et al*., 2024).Moreover, extraction techniques utilizing non-polar systems, including supercritical CO_2, have been successfully employed to isolate vitamin K from plant and microbial sources, reinforcing the compound's non-polar characteristics (Chronopoulou *et al.,* 2019). These findings support the use of diethyl ether, a non-polar solvent, for the efficient extraction of vitamin K from *C. citratus*, as demonstrated in the present study.

The diethyl ether extract of *C. citratus* contained a significantly higher concentration of benzoic acid (6.124 ± 0.027 ppm) compared to the ethanol extract (4.291 ± 0.016 ppm), with a p-value of < 0.0001. Although benzoic acid contains a polar carboxylic group, its solubility in non-polar solvents such as diethyl ether is explained by its physicochemical behavior. The compound's large aromatic ring allows for favorable van der Waals and π–π interactions with non-polar solvents, while its tendency to form dimers in such media reduces its effective polarity. This dimerization—stabilized by intermolecular hydrogen bonding—enhances its compatibility with non-polar environments. Thermodynamic and solubility parameter studies further confirm that solubility is governed by a complex interplay of dispersion, polarity, and hydrogen bonding, rather than polarity alone (Li *et al*., 2024). These findings support the higher extraction efficiency of benzoic acid in the diethyl ether fraction observed in this study.

5.2 Conclusion

This study investigated the vitamin compositions of *C. citratus* extracts using polar (ethanol) and non-polar (diethyl ether) solvents. The results revealed that solvent polarity significantly influenced the extraction efficiency of different vitamins and benzoic acid. Ethanol proved more effective for extracting vitamin A, vitamin B6, and vitamin D, while diethyl ether yielded higher concentrations of vitamin B12, vitamin C, vitamin E, vitamin K, and benzoic acid. These findings highlight the solubility behavior of individual compounds and the crucial role of solvent selection in plant-based extraction protocols. Overall, the study provides insight into the suitability of polar and non-polar solvents for targeted recovery of bioactive compounds from lemongrass and supports the importance of polarity matching for optimizing nutritional or phytochemical extraction outcomes.

5.3 Recommendations

1. Future studies should include in vivo investigations to assess the biological relevance, bioavailability, and therapeutic effects of the extracted vitamins, complementing the in vitro findings.

2. Advanced analytical methods such as HPLC, LC-MS, or NMR should be employed to improve quantification accuracy.

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APPENDIX I

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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: *Cymbopogon citratus* (DC.) Stapf.

Family: Poaceae

Local Name: West Indian Lemon grass, Lemon grass

Voucher Number: UBH-C451

Student Name: Theophilus Illuebbey *et al.*

Plant Identification and Voucher Number Issued by:

28/05/2025

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

Plant Identification and Voucher Number Certificate

APPENDIX II

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Weighing of pulverized *C. citratus* leaves

APPENDIX III

Pulverized *C. citratus* leaves in Ethanol and Diethyl ether

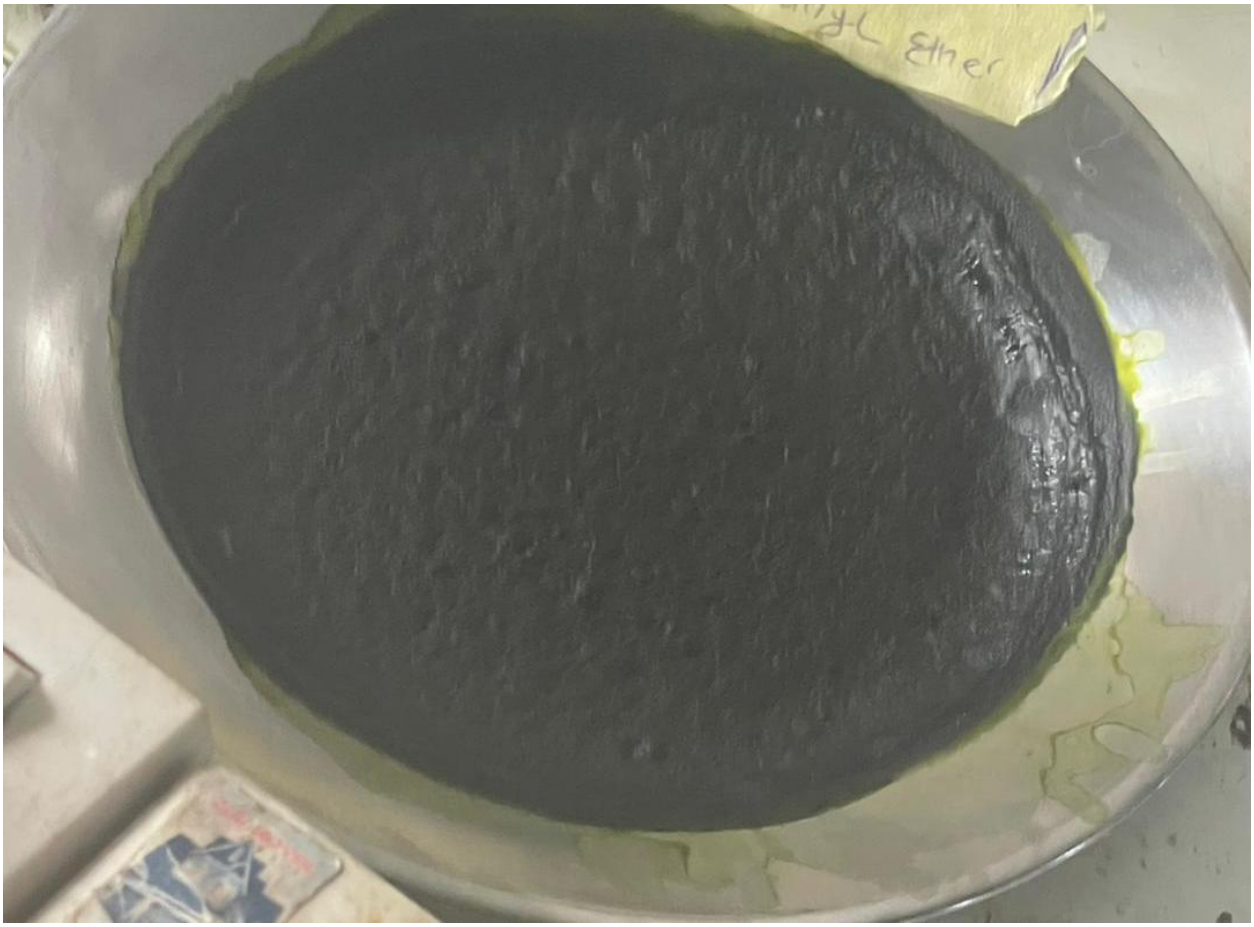
APPENDIX IV

Filtration (after 72 hours of maceration) using Whatman Grade 1 filter paper.

APPENDIX V

Concentration and solvent extraction using rotary Evaporator

APPENDIX VI



Diethyl ether extract of *C. citratus*

APPENDIX VII

Samples for Vitamin Analysis