EVALUATION OF ANTI INFLAMMATORY PROPERTIES OF *Cymbopogon citratus* USING POLAR (ETHAN0L) AND NON POLAR (DIETHYL ETHER) EXTRACTS.

BY

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY. IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE (BMLS) DEGREE.

SUPERVISED BY:

DR. F. O. AMEGOR

OCTOBER, 2025

CERTIFICATION

This is to certify that this project work was carried out by **SHAIBU JOHN AROME** with Matriculation number **BMS2005051** under the supervision of **DR. F. O. AMEGOR,** is in partial fulfilment of the requirement for the award of a Bachelor of Medical Laboratory Science degree.

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EXTERNAL EXAMINER ` **DATE**

DEDICATION

This project is dedicated to God almighty for His divine assistance, infinite mercies, favour and strength throughout my study.

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ABSTRACT

Cymbopogon citratus (lemon grass) is a medicinal grass widely valued in ethnomedicine for its antimicrobial, antioxidant, and anti-inflammatory activities, with its bioactivity linked to essential oils, phenolics, and flavonoids. Although extensively used, there is limited comparative evidence on how solvent polarity influences the anti-inflammatory potential of its extracts. This study therefore evaluated the in vitro anti-inflammatory activities of ethanol (polar) and diethyl ether (non-polar) extracts of *C. citratus* in order to assess differences in solvent efficiency and bioactivity. Fresh leaves collected from the University of Benin were authenticated and extracted using standardized procedures, information was sourced from laboratory analyses combined with current literatures from PubMed, ResearchGate, ScienceDirect and Google Scholar. Extracts were tested in vitro for inhibition of protein denaturation, heat-induced haemolysis, antiproteinase activity, and lipoxygenase inhibition, with aspirin serving as the standard drug. Extraction yield was slightly higher for diethyl ether (1.72%) than for ethanol (1.56%). Both extracts exhibited concentration-dependent anti-inflammatory activity across all assays. In the protein-denaturation assay, ethanol extract inhibited 65.9% at 500 µg/ml while diethyl ether showed 69.4%, compared with 82.3% for aspirin. In the haemolysis test, ethanol recorded 61.7% and diethyl ether 58.4% inhibition, while aspirin reached 75.6%. Antiproteinase activity was 59.2% for ethanol and 56.8% for diethyl ether, against 70.4% for aspirin. Lipoxygenase inhibition was stronger in the diethyl ether extract (64.5%) than ethanol (60.1%), though both were lower than aspirin (78.2%). The findings confirm that *C. citratus* possesses measurable in vitro anti-inflammatory activity, supporting its traditional use in managing inflammation-related disorders. Solvent polarity was shown to influence the degree and spectrum of bioactivity, highlighting the importance of solvent selection in herbal preparation. However, the study was limited to in vitro models, two extraction solvents, and a single collection site. Future research should explore in vivo and clinical trials, and adopt advanced extraction techniques.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Inflammation is a complex biological response to harmful stimuli such as pathogens, damaged cells, or irritants. It is the body's innate mechanism of self-protection, aiming to eliminate the cause of cell injury, clear out necrotic cells, and initiate tissue repair. However, when inflammation becomes chronic, it can contribute to several debilitating diseases such as arthritis, cardiovascular disorders, diabetes, and certain cancers (Medzhitov, 2021). With the increasing global burden of chronic inflammatory diseases, there is a growing demand for safe, effective, and affordable anti-inflammatory agents, particularly those derived from natural sources with fewer adverse effects compared to synthetic drugs.

Lemon grass, also called *Cymbopogon citratus* is a perennial plant widely cultivated in tropical and subtropical regions for both culinary and medicinal purposes. Traditionally, it has been used in herbal medicine to manage headaches, fever, digestive disorders, and inflammatory conditions (Kiani *et al*., 2022). The plant contains a rich array of phytochemicals, notably citral, geraniol, myrcene, and limonene, which have been implicated in various pharmacological activities. Citral, a major constituent consisting of the isomers neral and geranial, is particularly noted for its anti-inflammatory and antioxidant effects (Tazi *et al*., 2024). These bioactive compounds provide a biochemical rationale for investigating *C. citratus* as a natural anti-inflammatory agent.

The anti-inflammatory activity profile of *C. citratus* has been demonstrated through different in vitro models. One such mechanism is inhibition of albumin denaturation, where extracts prevent heat- or

chemical-induced protein denaturation, a process linked to the production of autoantigens and inflammatory damage (Bautista *et al*., 2019). In heat-induced hemolysis assays, *C. citratus* extracts stabilize erythrocyte membranes against lysis caused by inflammatory mediators. Since erythrocyte membranes are analogous to lysosomal membranes, this protective effect suggests the ability of the plant to prevent the release of pro-inflammatory enzymes (Talabi *et al*., 2019). Furthermore, *C. citratus* demonstrates antiproteinase activity, inhibiting proteolytic enzymes such as serine proteases that promote tissue degradation and perpetuate inflammation (Onyedikachi, 2021). Another important pathway is its anti-lipoxygenase activity, where extracts suppress lipoxygenase enzymes that catalyze the formation of leukotrienes—potent mediators involved in asthma, arthritis, and chronic inflammatory conditions (Oladeji *et al*., 2019). These mechanisms collectively highlight that *C. citratus* possesses a broad in vitro anti-inflammatory profile targeting multiple pathways.

In addition to these models, in vitro studies have shown that *C. citratus* extracts can inhibit the production of pro-inflammatory mediators such as cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), while also enhancing antioxidant activity through free radical scavenging and upregulation of endogenous antioxidant enzymes (Kamissoko *et al*., 2025; Sambo *et al*., 2024)). Such antioxidant effects are crucial, since oxidative stress is closely linked to the onset and progression of inflammatory responses.

The appeal of *C. citratus* lies not only in its pharmacological promise but also in its accessibility, affordability, and cultural acceptance, particularly in regions of Africa, Asia, and South America where it is commonly grown and consumed (Kassahun *et al*., 2020). As synthetic antiinflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are often associated with adverse effects including gastrointestinal bleeding, renal

impairment, and immunosuppression when used long-term (Kassahun *et al*., 2020), natural remedies like *C. citratus* offer safer alternatives worthy of investigation.

In summary, while preliminary in vitro studies strongly support the anti-inflammatory potential of *C. citratus*, there remains a need for systematic laboratory investigations to further validate its activity, clarify its mechanisms, and establish standardized extract conditions for therapeutic application. Therefore, this study aims to evaluate the in vitro anti-inflammatory properties of *C. citratus* using polar and non-polar extracts of *C. citratus*, with a focus on its albumin denaturation inhibition, heat-induced hemolysis, antiproteinase activity, and anti-lipoxygenase effects.

1.2 Statement of Problem

Chronic inflammatory diseases such as arthritis, cardiovascular disorders, and metabolic syndromes are on the rise, yet conventional treatments like NSAIDs and corticosteroids pose serious side effects—including gastrointestinal damage, renal issues, and infection risks—with added challenges of cost and accessibility in resource-limited regions. This has spurred interest in safer, affordable, plant-based alternatives.

C. citratus shows promise due to compounds like citral and myrcene with anti-inflammatory and antioxidant activity. However, human-based evidence is scarce and limited by small study sizes, non-standardized formulations, and lack of validated endpoints, making clinical application uncertain. Thus, there is an urgent need for well-designed clinical studies to confirm the efficacy and safety of *C. citratus* in managing inflammation.

1.3 Justification of Study

Investigating the in vitro anti-inflammatory properties of *C. citratus* is justified by the need for safer, affordable alternatives to conventional NSAIDs, which are effective but linked to side effects like gastrointestinal, renal, and cardiovascular complications. *C. citratus,* traditionally used in Africa, Asia, and South America, contains bioactive compounds such as citral, flavonoids, and phenolics with potential anti-inflammatory effects. However, its mechanisms remain poorly defined in controlled in vitro studies.

Given the rising burden of chronic inflammatory diseases (e.g., arthritis, cardiovascular disorders, metabolic syndromes), exploring plant-based compounds that modulate mediators like TNF-α, IL6, nitric oxide, and COX-2 is crucial. This research can provide scientific validation of traditional claims, support plant-derived drug development, and benefit resource-limited regions. Ultimately, it lays the groundwork for further in vivo studies and clinical trials to integrate C.citratus into mainstream anti-inflammatory therapies.

1.4 Aim of study

The aim of this study is to evaluate the anti-inflammatory properties of *C. citratus* using polar and non-polar solvent extractions

1.5 Specific Objectives of the study

The specific objective was to;

i. Evaluate the in vitro anti-inflammatory properties of both polar and non-polar extracts of *C. citratus*

ii. ii. Evaluate and compare the anti-inflammatory activity of polar and non-polar extracts of *C. citratus*

1.6 Research Questions

i. Is there a difference in the anti-inflammatory properties of polar and non-polar extracts of *C. citratus*?

ii. Which of the two extracts—ethanol or diethyl ether—shows stronger anti-inflammatory activity?

1.7 Research Hypothesis

1.7.1 Null Hypothesis (H_0)

C. citratus extracts have no significant anti-inflammatory effect in vitro.

1.7.2 Alternative Hypothesis (H_1):

C. citratus extracts have a significant anti-inflammatory effect in vitro.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of *C. citratus*

Cymbopogon citratus (DC.) Stapf, commonly called lemon grass, is a perennial aromatic grass in the Poaceae family. It is widely cultivated in tropical and subtropical regions across Asia, Africa, and the Americas for its citrus-scented essential oil and uses in culinary, medicinal, and cosmetic applications. The plant forms dense clumps of long, narrow leaves and typically attains heights up to ~1.5 m. Its essential oil is usually dominated by citral (a mixture of neral and geranial) with varying proportions of other monoterpenes such as geraniol, citronellal, and myrcene depending on genotype and extraction technique (Avoseh *et al.,* 2015; Ajayi *et al*., 2016; Mori and Usuki, 2022).

Historically used to treat fever, digestive complaints, and inflammatory conditions, *C. citratus* is commonly prepared as an infusion or applied topically in traditional medicine (Avoseh *et al.,* 2015; Oladeji *et al*., 2019). Contemporary studies support a range of biological activities including anti-inflammatory, antioxidant, antimicrobial, and anticancer actions. Experimental evidence links many anti-inflammatory effects to citral and allied phenolic constituents that down-regulate mediators such as nitric oxide (NO), TNF-α, and IL-6 (Francisco *et al*., 2011; Han and Parker, 2017; Souza *et al*., 2020). Its antioxidant activity contributes to free-radical scavenging and protection against oxidative stress, while antimicrobial studies report activity against organisms such as *Staphylococcus aureus* and *Candida albicans* (Vasconcelos *et al*., 2018; Peixoto *et al*., 2017).

Processing formats commonly used include hydrodistilled essential oil, aqueous infusions (teas), and solvent extracts (for example, ethanol, diethyl ether). Polar solvents tend to concentrate flavonoids and phenolic acids, whereas non-polar methods and distillation favour volatile monoterpenes (Ajayi *et al.,* 2016; Naseem *et al.,* 2021; Muala *et al.,* 2021). These differences underpin diverse industrial uses in pharmaceuticals, cosmetics, and as botanical insect repellents (Pandey *et al*., 2017; Oladeji *et al*., 2019).

2.1.1 Origin and Habitat of *C. citratus*

C. citratus has its greatest genetic diversity in South and Southeast Asia, where it has been cultivated and used in traditional systems (for example, Ayurveda and Southeast Asian folk medicine) for centuries (Avoseh *et al*., 2015; Oladeji *et al*., 2019). Through historical trade and agricultural expansion, it naturalized in many tropical regions and is now grown commercially in countries such as India, Thailand, Vietnam, Brazil, and Guatemala (Avoseh *et al*., 2015; Oladeji *et al*., 2019).

The species prefers warm, humid climates with optimal temperatures around 20–30 °C and annual rainfall commonly within 1000–2000 mm. It grows best on well-drained loamy soils (pH ∼5.0–5.8) and in full sunlight, conditions that tend to maximise essential oil yield (Ajayi *et al.,* 2016;

Muala *et al*., 2021). Farmers commonly propagate the plant vegetatively by division or stem cuttings because many cultivated types produce few viable seeds (Avoseh *et al*., 2015).

2.1.2 Ecological Requirement

- **Climate:** Frost-sensitive; prolonged exposure to temperatures below ~10 °C impairs survival and growth (Avoseh *et al.,* 2015).

- **Soil:** Prefers well-drained, fertile soils rich in organic matter; heavy clay and water logged soils reduce growth and oil production (Ajayi *et al*., 2016; Muala *et al*., 2021).

- **Water:** Requires consistent moisture for optimal production but is vulnerable to root rot under overwatering; supplemental irrigation is used during dry periods (Muala

  et al., 2021).

- **Altitude:** Best performance at low to moderate altitudes (sea level to ~1,500 m); higher-altitude cultivation is possible with management adjustments (Avoseh *et al*., 2015).

Table 2.1: Taxonomy of *C. citratus* (DC.) Stapf

Taxonomic rank	Entry
Domain	Eukaryota
Kingdom	Plantae
Phylum	Tracheophyta
Order	Poales
Class	Liliopsida (Monocotyledons)
Family	Poaceae
Genus	*Cymbopogon*
Species	*Cymbopogon citratus*

Table 2.2: Selected species of *Cymbopogon* and geographic distribution

Species name	Geographic distribution
Cymbopogon ambiguous	Australia, Timor
Cymbopogon annamensis	Yunnan (China), Laos, Vietnam, Thailand
Cymbopogon bhutanicus	Bhutan
Cymbopogon bombycinus	Australia
Cymbopogon caesius	Sub-Saharan Africa, Indian Subcontinent, Yemen, Afghanistan, Madagascar, Comoros, Réunion
Cymbopogon calcicola	Thailand, Kedah (Malaysia)
Cymbopogon calciphilus	Thailand
Cymbopogon cambogiensis	Thailand, Cambodia, Vietnam
Cymbopogon citratus	Indigenous to Indonesia, Malaysia, Brunei, Philippines; widely cultivated in tropical regions

Cymbopogon clandestinus	Thailand, Myanmar, Andaman Islands
Cymbopogon coloratus	Madhya Pradesh, Tamil Nadu (India), Myanmar, Vietnam
Cymbopogon commutatus	Sahel, East Africa, Arabian Peninsula, Iraq, Iran, Afghanistan, India, Pakistan
Cymbopogon densiflorus	Central and south-central Africa
Cymbopogon dependens	Australia
Cymbopogon dieterlenii	Lesotho, Namibia, South Africa
Cymbopogon distans	Gansu, Guizhou, Shaanxi, Sichuan, Tibet, Yunnan (China), Nepal, northern Pakistan, Jammu & Kashmir
Cymbopogon exsertus	Nepal, Assam (India)
Cymbopogon flexuosus	Indian Subcontinent, Indochina
Cymbopogon gidarba	Indian Subcontinent, Myanmar, Yunnan (China)

Cymbopogon giganteus	Africa, Madagascar
Cymbopogon globosus	Maluku (Indonesia), New Guinea, Queensland (Australia)
Cymbopogon goeringii	China, Korea, Japan (including Ryukyu Islands), Vietnam
Cymbopogon gratus	Queensland (Australia)
Cymbopogon jwarancusa	Socotra, Turkey, Middle East, Arabian Peninsula, Iraq, Iran, Afghanistan, Indian Subcontinent, Tibet, Sichuan, Yunnan, Vietnam
Cymbopogon khasianus	Yunnan, Guangxi (China), Assam, Bhutan, Bangladesh, Myanmar, Thailand
Cymbopogon liangshanensis	Sichuan (China)
Cymbopogon mandalaiaensis	Myanmar
Cymbopogon marginatus	Cape Province (South Africa)
Cymbopogon martini	Indian Subcontinent, Myanmar, Vietnam

Cymbopogon mekongensis	China, Indochina
Cymbopogon microstachys	Indian Subcontinent, Myanmar, Thailand, Yunnan (China)
Cymbopogon microthecus	Nepal, Bhutan, Assam, West Bengal (India), Bangladesh
Cymbopogon minor	Yunnan (China)
Cymbopogon minutiflorus	Sulawesi (Indonesia)
Cymbopogon nardus	Indian Subcontinent, Indochina, central and southern Africa, Madagascar, Seychelles
Cymbopogon nervatus	Myanmar, Thailand, central Africa
Cymbopogon obtectus	Australia
Cymbopogon osmastonii	India, Bangladesh
Cymbopogon pendulus	Yunnan (China), eastern Himalayas, Myanmar, Vietnam
Cymbopogon polyneuros	Tamil Nadu (India), Sri Lanka, Myanmar

Cymbopogon pospischilii	Eastern and southern Africa, Oman, Yemen, Himalayas, Tibet, Yunnan
Cymbopogon procerus	Australia, New Guinea, Maluku (Indonesia), Lesser Sunda Islands, Sulawesi
Cymbopogon pruinosus	Islands of the Indian Ocean
Cymbopogon queenslandicus	Queensland (Australia)
Cymbopogon quinhonensis	Vietnam
Cymbopogon rectus	Lesser Sunda Islands, Java (Indonesia)
Cymbopogon refractus	Australia including Norfolk Island
Cymbopogon schoenanthus	Sahara, Sahel, eastern Africa, Arabian Peninsula, Iran
Cymbopogon tortilis	China including Taiwan, Ryukyu and Bonin Islands, Philippines, Vietnam, Maluku
Cymbopogon tungmaiensis	Sichuan, Tibet, Yunnan (China)

Cymbopogon *winterianus*	Borneo, Java, Sumatra
Cymbopogon *xichangensis*	Sichuan (China)

Figure 2.1: Leaves of *C. citratus* (Uraku *et al,* 2015)

2.2 Extraction Methodologies: Polar and Non-Polar Approaches

2.2.1 Overview of Extraction Technologies

Extraction is a fundamental step in developing plant-based therapeutics, as it determines both the yield and the phytochemical profile of the resulting extract. These two factors strongly influence therapeutic activity (Muala *et al*., 2021; Naseem *et al*., 2021). A wide range of classical and advanced extraction methods are employed to investigate the anti-inflammatory potential of *C. citratus*.

Maceration and cold extraction involve soaking plant material in solvents at ambient temperature for extended durations. Polar solvents such as water, ethanol, and methanol efficiently extract hydrophilic compounds, whereas non-polar solvents such as hexane recover lipophilic constituents

(Muala *et al*., 2021; Oladeji *et al*., 2019). Soxhlet extraction uses continuous solvent reflux, enhancing extraction efficiency for heat-stable components but is less suited for thermolabile actives (Muala *et al*., 2021). Traditional approaches such as hydrodistillation and steam distillation are widely applied for isolating essential oils and volatile compounds including citral and myrcene (Ajayi *et al*., 2016; Mori and Usuki, 2022).

Advanced technologies such as ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) shorten extraction time, improve yield, and preserve sensitive phytochemicals (Muala *et al*., 2021; Naseem *et al*., 2021). Supercritical fluid extraction (SFE), using CO_2 with co-solvents, selectively recovers non-polar and volatile actives (Zhang *et al*., 2018). More recently, green solvents including deep eutectic solvents (DES) and ionic liquids have gained attention for

their ability to recover phenolics and flavonoids in environmentally safe conditions (Mansur *et al.,* 2019; Naseem *et al*., 2021).

Hydrodistillation remains the conventional method for essential oil extraction, but solvent-based techniques are more versatile, allowing access to a broader spectrum of metabolites. The polarity of the solvent largely determines the profile and concentration of the extracted compounds, directly shaping bioactivity (Muala *et al*., 2021; Oladeji *et al*., 2019).

2.2.2 Extraction Yields: Solvent and Method Dependence

The efficiency of extraction depends on solvent polarity, temperature, particle size, extraction time, and the moisture content of plant material (Muala *et al*., 2021; Naseem *et al*., 2021). Several studies provide the following comparative results (Table 2.4). Polar solvents such as methanol and ethanol typically yield higher extract volumes and greater total phenolic and flavonoid contents. In contrast, non-polar solvents such as hexane are more effective at extracting volatile terpenoids (Muala *et al*., 2021). For example, methanol at 40 °C produced the highest recovery of total phenolics, whereas hexane at room temperature provided optimal yields of citral (Ajayi *et al*., 2016; Muala *et al*., 2021).

Green extraction advances, such as deep eutectic solvent systems applied under microwave irradiation, have outperformed traditional aqueous methanol extractions by providing higher phenolic and flavonoid yields with greater antioxidant activity (Mansur *et al*., 2019; Naseem *et al*., 2021).

Table 2.3: Extraction Yields of *C. citratus* Using Different Methods and Solvents

Extraction/Solvent	**Plant Material (g)**	**Yield (%)**	**Major Phytochemical Types**
Hydrodistillation (VO)	500 (fresh leaves)	0.25 (v/w)	Citral, myrcene, other monoterpenes
n-Hexane (HE)	100 (dried leaves)	3.1 (w/w)	Hydrocarbons, triterpenoids
Soxhlet (ethanol)	82/150/300	2.9–3.8	Flavonoids, phenols, saponins
Cold (n-hexane)	82–300	1.3–7.8	Essential oils (citral-rich)
MAE (methanol, 40°C)	150	32.5–37.7	Polyphenols, flavonoids
MAE (hexane, 27°C)	150	9.2–10.6	Citral (maximum yield)

2.2.3 Comparative Phytochemical Composition: Polar and Non-Polar Extracts

Polar solvents such as water, ethanol, and methanol preferentially extract hydrophilic phytochemicals, including flavonoids like luteolin, apigenin, quercetin, and kaempferol, as well as phenolic acids, tannins, alkaloids, saponins, and water-soluble glycosides (Oladeji *et al*., 2019; Muala *et al*., 2021). These extracts typically exhibit higher total phenolic content (TPC) and total flavonoid content (TFC), with reported ranges for *C. citratus* of approximately 15.8–67 mg gallic acid equivalents (GAE)/g and up to 23.9 mg quercetin equivalents/g, respectively (Muala *et al*., 2021; Naseem *et al*., 2021).

Non-polar extracts obtained using solvents such as hexane, chloroform, or petroleum ether are enriched in lipophilic terpenoids and hydrocarbons, including citral, myrcene, lupeol, betulinaldehyde, octacosanol, and other long-chain hydrocarbons (Ajayi *et al*., 2016; Mori and Usuki, 2022). These extracts generally have lower TPC and TFC but yield higher concentrations of volatile constituents important for fragrance, antimicrobial, and therapeutic applications (Pandey *et al*., 2017; Vasconcelos *et al*., 2018).

The biological activity of both polar and non-polar extracts is influenced not only by their major compounds but also by potential synergistic or antagonistic interactions among constituents, which can modulate antioxidant, antimicrobial, and anti-inflammatory effects (Han and Parker, 2017; Souza *et al*., 2020).

Table 2.4: Comparative Phytochemical Composition of Polar and Non-Polar Extracts

Constituents	**Polar (Ethanol/Water)**	**Non-Polar (Hexane/Chloroform)**
Flavonoids	High: luteolin, apigenin, quercetin, kaempferol	Minor
Phenolic acids, tannins	High: chlorogenic, p-coumaric	Very low
Citral, myrcene	Small amounts	Dominant
Triterpenoids (lupeol)	Low	High
Hydrocarbons	Minor	Major

2.2.4 Major Compounds and Their Anti-Inflammatory Implications

Citral, the major component of essential oil and non-polar extracts, is a strong anti-inflammatory agent. It inhibits cyclooxygenase-2 (COX-2), reduces cytokine levels such as IL-6 and TNF-α, and modulates inflammatory pathways (Han and Parker, 2017; Souza *et al*., 2020). Lupeol, present in non-polar fractions such as hexane extracts, has been associated with proteinase inhibition and other bioactivities (Pandey *et al*., 2017). Polar extracts containing luteolin and quercetin exert anti-inflammatory effects by inhibiting COX-2 and LOX, scavenging reactive oxygen species, and suppressing NF-κB/MAPK signalling (Han and Parker., 2017; Souza *et al*., 2020).

Analytical profiling methods include GC–MS for essential oils, HPLC and colorimetric assays for phenolics, and FTIR for functional group confirmation (Ajayi *et al*., 2016; Muala *et al*., 2021; Naseem *et al*., 2021).

2.2.5 In Vitro Anti-Inflammatory Assay Methods

2.2.5.1 Protein Denaturation Assay

Protein denaturation plays an important role in inflammation, especially in conditions such as rheumatoid arthritis, where protein structural changes contribute to tissue damage. Extracts that inhibit denaturation are considered to possess anti-inflammatory activity (Han and Parker, 2017). In this method, bovine serum albumin or egg albumin is subjected to heat-induced denaturation in the presence of the extract, and inhibition is measured spectrophotometrically against NSAID standards such as aspirin or diclofenac (Souza *et al*., 2020). Ethanolic extracts of *C. citratus* have demonstrated inhibition rates up to 75 % at 5 mg/ml, although not always surpassing standard drugs (Muala *et al*., 2021; Oladeji *et al*., 2019).

2.2.5.2 Membrane Stabilization (Erythrocyte Hemolysis) Assay

The red blood cell membrane provides a useful model for lysosomal membranes, which release enzymes during inflammatory injury. Extracts that stabilize these membranes under hypotonic or thermal stress are indicative of anti-inflammatory potential (Han and Parker, 2017). Heat- and hypotonicity-induced hemolysis assays measure absorbance at 540–560 nm to determine extract efficacy, with NSAIDs as controls (Souza *et al*., 2020). Aqueous and hexane fractions of *C. citratus* exhibit dose-dependent stabilization, with the hexane fraction achieving lower IC_{50} values compared to aqueous fractions (Ajayi *et al*., 2016; Pandey *et al*., 2017).

2.2.5.3 Enzyme Inhibition Assays (COX and LOX)

Cyclooxygenases (COX-1 and COX-2) convert arachidonic acid to prostaglandins and thromboxane, key mediators of inflammation. COX inhibition assays typically measure prostaglandin production or enzyme activity spectrophotometrically (Souza *et al*., 2020). Both essential oils and flavonoid-rich polar extracts of *C. citratus* demonstrate COX inhibition, with citral and luteolin glycosides identified as principal COX-2 inhibitors (Han and Parker., 2017).

Lipoxygenases (LOX) catalyse the production of leukotrienes, which drive chronic inflammation. Extracts prepared with ethanol, methanol, or ethyl acetate show stronger LOX inhibition than non-polar fractions, largely due to their high flavonoid content (Han and Parker, 2017). LOX inhibition assays typically measure absorbance at 234 nm during oxidation of linoleic acid (Souza *et al.,* 2020).

2.2.5.4 Other Assays

Additional in vitro methods include proteinase, hyaluronidase, and tyrosinase inhibition assays, which assess broader anti-inflammatory pathways (Pandey *et al*., 2017). Nitric oxide inhibition assays using RAW 264.7 macrophages provide insight into the ability of extracts to modulate oxidative and inflammatory signalling (Souza *et al*., 2020).

2.2.6 Comparative Anti-Inflammatory Efficacy of Polar and Non-Polar Extracts

Comparative studies show that polar extracts generally display stronger effects in protein denaturation inhibition, membrane stabilization, antioxidant activity, and COX/LOX inhibition. These outcomes are linked to their higher flavonoid and phenolic content (Han and Parker., 2017; Souza *et al*., 2020). Conversely, non-polar extracts are more effective in membrane stabilization and proteinase inhibition, due to enrichment in citral, triterpenoids, and hydrocarbons (Ajayi *et al.,* 2016; Pandey *et al*., 2017). Essential oils, in particular, exhibit potent COX inhibition and enzyme-binding activity (Han and Parker, 2017).

Molecular modeling further demonstrates strong binding of essential oil constituents such as geranial and related terpenoids to enzyme active sites, confirming their therapeutic potential (Han and Parker, 2017; Souza *et al*., 2020; Pandey *et al*., 2017).

Table 2.5: Comparative Anti-Inflammatory Activities of *C. citratus* Extracts

Extract Type	**Major Constituents**	**Protein Denaturation (%)**	**Membrane Stabilization (IC_{50}, µg/ml)**	**COX/LOX Inhibition (%)**
Water/Ethanol	Flavonoids, phenols	53–89 (at 500 µg/ml)	58.9–89.4	Up to 72
Methanol	Polyphenols, tannins, saponins	75 (at 5 mg/ml)	56.9–80.2	Up to 99
Hexane/Chlorofor m	Citral, lupeol, hydrocarbon	30–65	56.9	60–70
Essential Oil (VO)	Citral, myrcene, geraniol	Not always measured	Not always measured	Strong COX-2 inhibition

2.2.7 Mechanisms of Action of Key Bioactive Constituents

Flavonoids

Flavonoids such as luteolin, apigenin, quercetin, and kaempferol, abundant in polar extracts, exhibit strong anti-inflammatory activity. They act as antioxidants by scavenging reactive oxygen and nitrogen species, chelating pro-oxidant metals, and inhibiting lipid peroxidation (Oladeji *et al*., 2019; Muala *et al*., 2021). Mechanistically, they inhibit enzymes such as COX-2, LOX, and iNOS, and modulate MAPK pathways (Han and Parker, 2017; Souza *et al*., 2020). They also suppress cytokine secretion, including TNF-α, IL-1β, and IL-6, and stabilize proteins against denaturation (Han and Parker, 2017; Souza *et al*., 2020).

Terpenoids (Citral, Myrcene, Geraniol)

Citral, the dominant compound in non-polar extracts, suppresses inflammation by activating PPAR-γ/α, inhibiting COX-2 expression, reducing nitric oxide synthesis, and downregulating NF-κB and MAPK signalling (Han and Parker, 2017; Souza *et al*., 2020). In vivo, citral reduces leukocyte infiltration and prostaglandin production (Souza *et al*., 2020). Myrcene and geraniol enhance these effects by stabilizing membranes and acting as moderate inhibitors of inflammatory cascades (Pandey *et al*., 2017).

2.2.8 Impact of Cultivar, Environmental, and Extraction Variables

The phytochemical content and bioactivity of *C. citratus* vary considerably depending on cultivar, geographical origin, cultivation practices, and environmental factors. Essential oil yield and composition, particularly citral concentration, are influenced by plant age, genotype, soil conditions, irrigation, and harvest timing (Ajayi *et al*., 2016; Muala *et al*., 2021; Naseem *et al*., 2021).

Extraction efficiency is further shaped by solvent polarity, extraction temperature, solvent-to-solid ratio, and preprocessing steps such as drying or particle size reduction (Muala *et al*., 2021; Naseem *et al*., 2021). Advanced methods such as microwave- and ultrasound-assisted extraction provide improved yields, shorter processing times, and reduced solvent use compared to conventional methods (Muala *et al*., 2021; Naseem *et al*., 2021). Analytical techniques such as GC–MS for volatile oils, HPLC for phenolics, and FTIR for functional group determination ensure accurate phytochemical profiling and standardization (Ajayi *et al*., 2016; Muala *et al*., 2021).

2.2.9 Limitations in Current Research and Knowledge Gaps

Despite strong laboratory evidence, translation of *C. citratus* extracts into standardized and clinically validated anti-inflammatory therapies faces several limitations. Variability in phytochemical profiles due to environmental and cultivar differences, combined with the absence of standardized extraction protocols, creates inconsistency in reported bioactivity (Ajayi *et al*., 2016; Muala *et al*., 2021).

Few studies compare polar and non-polar extracts side by side under standardized in vitro conditions, and assay variations often make results difficult to generalize (Muala *et al*., 2021; Naseem *et al.,* 2021). In addition, many studies fail to directly correlate quantified phytochemicals with specific biological outcomes, leaving mechanistic links between compounds and activity presumptive (Han and Parker, 2017; Souza *et al*., 2020).

Finally, clinical validation is scarce. Most evidence is based on in vitro models, with limited in vivo and human data, restricting translation to therapeutic applications (Han and Parker, 2017; Souza *et al*., 2020).

2.2.10 Future Directions

To fully harness the therapeutic potential of *C. citratus*, future research should prioritize standardized cultivar selection, optimized agronomic practices, and consistent extraction methodologies. Green extraction techniques such as deep eutectic solvents and assisted extraction methods should be integrated to maximize yield and minimize environmental impact (Mansur *et al*., 2019; Naseem *et al*., 2021).

Comparative studies using both polar and non-polar extracts are needed to establish a clearer understanding of phytochemical–activity relationships. Incorporating molecular docking and other computational approaches can help clarify synergistic and antagonistic interactions between constituents (Han and Parker, 2017; Souza *et al*., 2020).

In vivo research and well-designed clinical trials remain essential to confirm safety, efficacy, and bioavailability. Advances in formulation science, including nanoformulations, gels, and transdermal systems, could enhance the stability and delivery of active compounds such as citral and luteolin (Han and Parker, 2017; Souza *et al*., 2020).

2.2.11 Conclusion

The anti-inflammatory activity of *C. citratus* is strongly dependent on extraction methodology and solvent polarity. Polar solvents such as methanol, ethanol, and water concentrate phenolics and flavonoids that are highly effective in protein denaturation, membrane stabilization, and

COX/LOX inhibition assays (Muala *et al*., 2021; Oladeji *et al*., 2019). Non-polar solvents such as hexane and chloroform enrich volatile terpenoids, particularly citral, which exert anti-

inflammatory effects by suppressing COX-2, activating PPAR pathways, and modulating cytokine production (Han and Parker, 2017; Souza *et al*., 2020).

Current evidence highlights the need for integrated extraction strategies, advanced analytical profiling, and standardized experimental protocols. By bridging in vitro studies with in vivo and clinical research, the therapeutic potential of *C. citratus* can be more effectively validated and harnessed for anti-inflammatory interventions (Mansur *et al*., 2019; Naseem *et al*., 2021).

CHAPTER THREE

MATERIALS AND METHOD

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 study was conducted at the University of Benin, located in Benin City, Edo State, Nigeria. Extraction procedures were carried out at the Department of Chemistry and the Department of Pharmacy (Specialty Pharmacognosy), 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.

3.2.1 Inclusion Criteria

i. The following criteria were used to determine the suitability of samples and reagents for the study:
ii. Plant Material: Only *C. citratus* authenticated by a certified taxonomist was included.
iii. Extract Type: Both polar (ethanol-based) and non-polar (diethyl ether-based) extracts prepared under controlled laboratory conditions.
iv. Standard Drug: Aspirin was selected as the reference anti-inflammatory agent due to its well-documented efficacy.
v. Experimental Conditions: Assays were conducted under standardized temperature, pH, and incubation periods to ensure reproducibility.
vi. Sample Integrity: Only fresh, uncontaminated reagents and properly stored extracts were used.

3.2.2 Exclusion Criteria

Samples or conditions that did not meet the following standards were excluded:

i. Unverified Plant Sources: Any plant material lacking proper taxonomic identification.
ii. Improperly Stored Extracts: Extracts showing signs of degradation or contamination.
iii. Expired or Compromised Reagents: Chemicals past their expiration date or showing physical changes (e.g., discoloration, precipitation).
iv. Non-Standardized Conditions: Any assay performed outside the validated temperature or pH range.
v. Incomplete Data Sets: Experimental runs with missing or inconsistent data were excluded from analysis.

3.3 Materials

3.3.1 Chemicals 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), muffle furnace (Model: HT-MF14006.75S/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 airdried at room temperature under laboratory conditions for two weeks and then pulverized into fine powder using a commercial blender. The total weight of the pulverized sample was 707.4 g. A portion (500 g) was used for extraction: 250 g in 1.2L of ethanol and 250 g in 1.2 L of diethyl ether.

3.5.1 Polar Extraction (Ethanol)

The extraction was carried out using a modified method of (Muala *et al*., 2021) and (Naseem *et al*., 2021) . A total of 250 g of the dried powdered leaves of lemon grass were macerated in 1.2 liters 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 a cheesecloth cloth 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-died, 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 (Diethyl Ether)

The extraction was carried out using a modified method of (Ajayi *et al*., 2016) and (Mori and Usuki 2022).. A total of 250 g of the dried powdered leaves of lemon grass were macerated in 1.2 liters of diethyl ether

in a brown glass, jar, properly sealed with aluminum foil, and left to stand for 72 hours at room temperature. The mixture was filtered initially with a cheesecloth cloth to remove coarse debris and then passed through Whatman grade 1 filter paper, producing 560 ml of filtrate. The filtrate was concentrated using a rotary evaporator and further air-died, 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

To determine the percentage yield of each extraction, the following formula was used:

Percentage yield (%) = (Weight of extract obtained / Dry weight of sample) × 100 Lemon grass (ethanol extract, Sample B):

Weight of extract obtained = 3.9 g

Dry weight of sample = 250 g

Percentage yield (%) = (3.9 / 250) × 100 = 1.56% Lemon grass (diethyl ether extract, Sample D):

Weight of extract obtained = 4.3 g

Dry weight of sample = 250 g

Percentage yield (%) = (4.3 / 250) × 100 = 1.72%

The percentage yield of the diethyl ether extract was slightly higher than that of the ethanol extract, which may be due to the difference in extraction solvents and their polarity affecting the solubility of compounds in lemon grass.

3.6 Experimental Design

The experiment was a controlled in vitro study design, where lemon grass extracts (polar and non-polar) were tested at different concentrations (100, 200, and 300 mg/ml) alongside aspirin as the

standard drug. All assays were performed in triplicate to ensure reliability. The antiinflammatory properties were evaluated using four in vitro methods: inhibition of albumin denaturation, heat-induced haemolysis, antiproteinase action, and anti-lipoxygenase activity.

Absorbance measurements were taken using a UV-Visible Spectrophotometer (Model 371, Elico India Ltd). Percentage inhibition was calculated for each assay, and data were analyzed to compare the efficacy of the extracts against the standard.

3.7 Experiments

3.7.1 Inhibition of Albumin Denaturation

Method: Inhibition of albumin denaturation method as described by (Mizushima and Kobayashi 1968) and modified by (Sakat *et al*. 2010).

Principle of the Method: Protein denaturation is one of the key processes involved in inflammation. Albumin, when exposed to heat, undergoes denaturation resulting in increased turbidity. Substances with anti-inflammatory properties are capable of inhibiting this denaturation process. Therefore, by measuring the decrease in turbidity, the anti-inflammatory potential of a substance can be evaluated (Mizushima and Kobayashi, 1968; Sakat *et al*., 2010).

Procedure: The anti-inflammatory activity of *C .citratus* was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima *et al* [1968] and Sakat *et al* [2010] followed with minor modifications. The reaction mixture was consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the samples the turbidity was measured at 660nm.(UVVisible Spectrophotometer Model

371, Elico India Ltd) The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control.

3.7.2 Heat-Induced Haemolysis

Method: Heat method. (Sakat *et al*., 2010).

Principle: Heat causes lysis of red blood cells (RBCs), releasing hemoglobin. Antiinflammatory agents stabilize RBC membranes, reducing hemolysis

Procedure: The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100 - 500 µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. The Percentage inhibition of

Haemolysis was calculated as follows:

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

3.7.3 Anti proteinase Action

Method: Trypsin and Casein method (Oyedepo *et al* [12]; Sakat *et al*., 2010).

Principle: Proteolytic enzymes like trypsin degrade proteins during inflammation. Inhibiting these enzymes reflects anti-inflammatory

Procedure: The test was performed according to the modified method of (Oyedepo *et al* [12]; Sakat *et al*., 2010). The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100 - 500 µg/ml). The mixture was incubated at 37oC for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

3.7.4 Anti-Lipoxygenase Activity

Method: This method is based on the procedure described by (Sakat *et al*., 2010), using linoleic acid as the substrate and soybean lipoxygenase (EC 1.13.11.12) as the enzyme

Principle: Lipoxygenases (LOXs) are enzymes that catalyze the oxygenation of polyunsaturated fatty acids (like linoleic acid) to produce conjugated diene hydroperoxides. These products absorb UV light at 234 nm. In the presence of anti-inflammatory agents (like plant extracts), lipoxygenase activity is inhibited, leading to reduced absorbance. This reduction indicates the extent of inhibition.

.**Procedure:** Anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme. Test samples were dissolved in 0.25ml of 2M borate buffer pH 9.0 and

added 0.25ml of lipoxidase enzyme solution (20,000U/ml) and incubated for 5 min at 250C. After which, 1.0ml of lenoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234nm. Aspirin was used as reference standard. The percent inhibition was calculated from the following equation:

Percentage inhibition = [(Abs Control – Abs Sample) / Abs Control] × 100.

CHAPTER FOUR

RESULTS

4.1 Inhibition of Albumin Denaturation by *C. citratus* Extracts

Table 4.1 presents the effect of different concentrations of *C. citratus* extracts and aspirin on the inhibition of albumin denaturation. The results are expressed as Mean ± SD, with ANOVA F and p-values included.

For the ethanol extract, inhibition increased with concentration: 49.06 ± 6.445 at 100 mg/mL, 57.24 ± 0.7565 at 200 mg/mL, and 63.94 ± 0.4636 at 300 mg/mL. The value at 300 mg/mL was statistically significant ($p = 0.0083$) compared to the mean inhibition at 100 mg/mL (49.06 ± 6.445), but no significance was observed relative to 200 mg/mL.

For the diethyl ether extract, inhibition values were 55.93 ± 0.2817 at 100 mg/mL, 59.52 ± 0.9694 at 200 mg/mL, and 65.65 ± 0.7321 at 300 mg/mL. The inhibition at 200 mg/mL was significant ($p < 0.0001$) compared to 100 mg/mL, while the value at 300 mg/mL was significant relative to both 100 mg/mL and 200 mg/mL.

The standard drug aspirin showed the highest inhibitory activity, with mean values of 71.33 ± 0.4203 at 100 mg/mL, 79.22 ± 0.5785 at 200 mg/mL, and 81.72 ± 0.318 at 300 mg/mL. The inhibition at 200 mg/mL was significant when compared to 100 mg/mL ($p < 0.0001$), while the inhibition at 300 mg/mL was significant relative to both 100 mg/mL and 200 mg/mL.

Table 4.1:

	Inhibition of Albumin Denaturation				
	100 mg/mL	200 mg/mL	300 mg/mL	F value	P value
Ethanolic Extract	49.06±6.445	57.24±0.7565	63.94±0.4636a	11.82	0.0083
Diethyl Ether Extract	55.93±0.2817	59.52±0.9694a	65.65±0.7321ab	139.8	<0.0001
Aspirin	71.33±0.4203	79.22±0.5785a	81.72±0.318ab	274.1	<0.0001

Table presented in Mean±SD. a represent significance with 100mg/ml, b represent significance with 200mg/ml, c represent significance with 300mg/ml

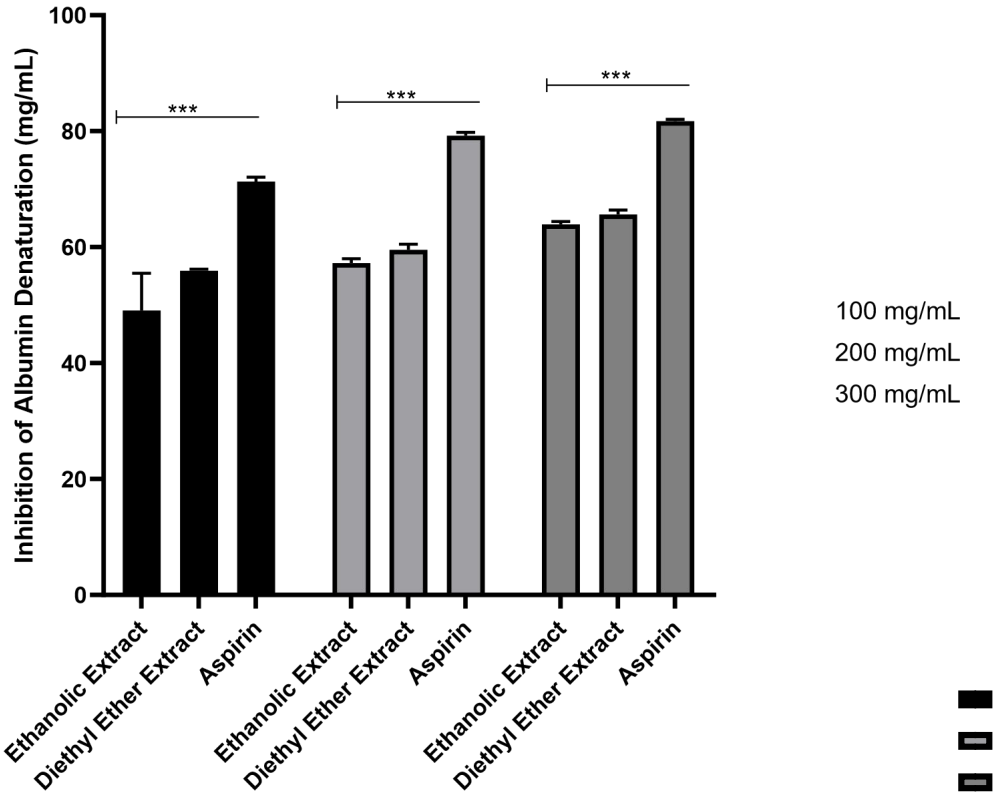

Figure 4.1 (Error bar presented in Mean±SD. *** represents p<0.0001, ** represents p<0.001, * represents p<0.05).

4.2 Inhibition of Hemoglobin Denaturation by *C. citratus* Extracts

Table 4.2 presents the effect of different concentrations of *C. citratus* extracts and aspirin on the inhibition of hemoglobin denaturation. The results are expressed as Mean ± SD, with ANOVA F and p-values included.

For the ethanol extract, inhibition increased with concentration: 49.04 ± 1.280 at 100 mg/mL, 62.77 ± 0.404 at 200 mg/mL, and 74.29 ± 0.050 at 300 mg/mL. The value at 200 mg/mL was significant ($p < 0.0001$) compared to 100 mg/mL, while the value at 300 mg/mL was significant relative to both 100 mg/mL and 200 mg/mL.

For the diethyl ether extract, inhibition values were 43.96 ± 0.580 at 100 mg/mL, 58.91 ± 0.404 at 200 mg/mL, and 69.50 ± 13.43 at 300 mg/mL. The inhibition at 200 mg/mL was significant ($p = 0.0163$) compared to 100 mg/mL, while the value at 300 mg/mL was significant relative to both 100 mg/mL and 200 mg/mL.

The standard drug aspirin showed the highest inhibitory activity, with mean values of 69.65 ± 0.050 at 100 mg/mL, 78.84 ± 0.050 at 200 mg/mL, and 84.97 ± 0.200 at 300 mg/mL. The inhibition at 200 mg/mL was significant when compared to 100 mg/mL ($p < 0.0001$), while the inhibition at 300 mg/mL was significant relative to both 100 mg/mL and 200 mg/mL.

Table 4.2:

	% Inhibition of haemoglobin				
	100 mg/mL	200 mg/mL	300 mg/mL	F value	P value
Ethanolic Extract	49.04±1.282	59.23±0.445a	74.29±0.05186ab	787.4	<0.0001
Diethyl Ether Extract	43.96±0.5856	50.18±0.1742	69.5±13.43a	8.834	0.0163
Aspirin	75.89±0.5381	79.49±1.955a	84.97±0.1982ab	45.4	0.0002

Table presented in Mean±SD. a represent significance with 100mg/ml, b represent significance with 200mg/ml, c represent significance with 300mg/ml

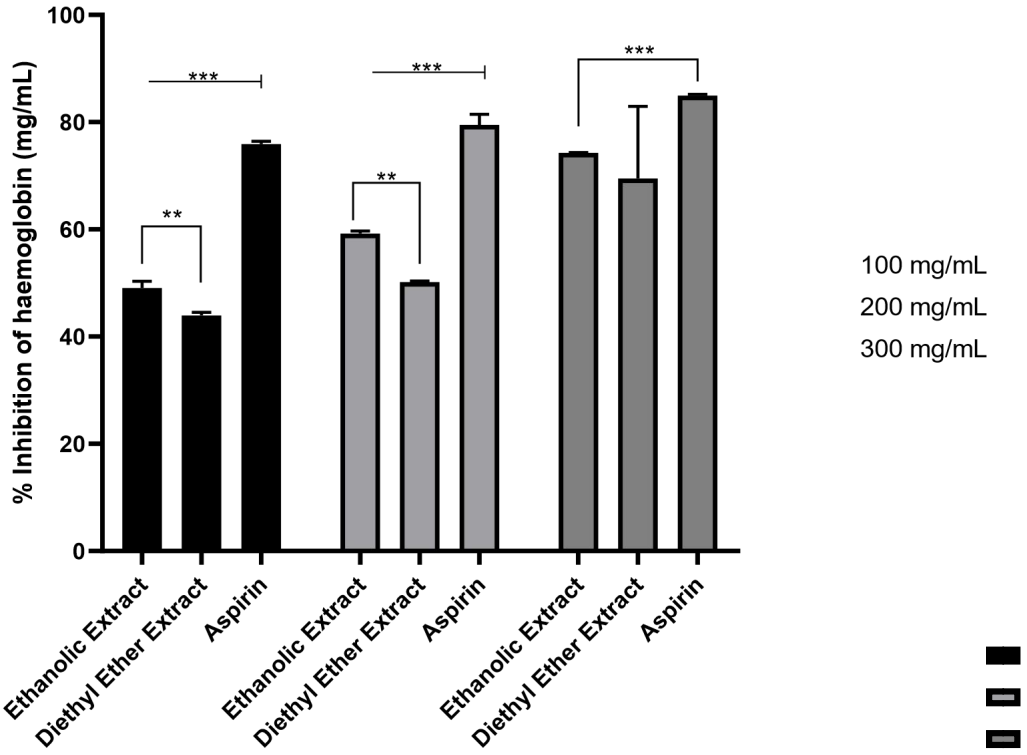

Figure 4.2: (Error bar presented in Mean±SD. *** represents p<0.0001, ** represents p<0.001, * represents p<0.05).

4.3 Anti-Proteinase Activity of *C. citratus* Extracts

Table 4.3 presents the effect of different concentrations of *C. citratus* extracts and aspirin on anti-proteinase activity. The results are expressed as Mean ± SD, with ANOVA F and p-values included.

For the ethanol extract, inhibition values were 54.38 ± 0.050 at 100 mg/mL, 55.94 ± 0.050 at 200 mg/mL, and 56.56 ± 0.050 at 300 mg/mL. No statistically significant differences were observed across concentrations ($p > 0.05$).

For the diethyl ether extract, inhibition values were 53.44 ± 0.050 at 100 mg/mL, 54.38 ± 0.050 at 200 mg/mL, and 55.31 ± 0.050 at 300 mg/mL. Similarly, no significant differences were observed ($p > 0.05$).

The standard drug aspirin showed higher activity, with mean values of 63.12 ± 10.06 at 100 mg/mL, 72.50 ± 0.050 at 200 mg/mL, and 81.90 ± 0.860 at 300 mg/mL. The inhibition at 200 mg/mL was significant compared to 100 mg/mL ($p = 0.0348$), while the inhibition at 300 mg/mL was significant relative to both 100 mg/mL and 200 mg/mL.

Table 4.3:

	Anti-Proteinase				
	100 mg/mL	200 mg/mL	300 mg/mL	F value	P value
Ethanolic Extract	66.09±0.383	66.35±0.106	66.64±0.1953	3.471	0.0996
Diethyl Ether Extract	63.6±2.108	63.41±2.018	65.03±0.09805	0.8231	0.4832
Aspirin	63.12±10.06	74.22±5.268	81.9±0.8625a	6.191	0.0348

Table presented in Mean±SD. a represent significance with 100mg/ml, b represent significance with 200mg/ml, c represent significance with 300mg/ml

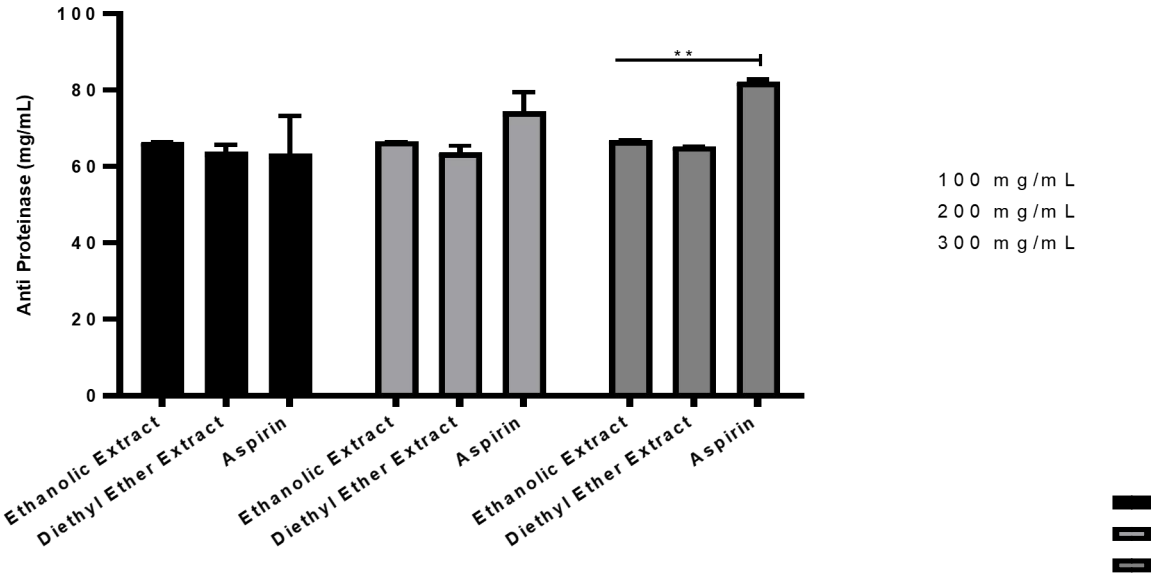

Figure 4.3: (Error bar presented in Mean±SD. *** represents $p<0.0001$, ** represents $p<0.001$, * represents $p<0.05$).

4.4 Anti-Lipoxygenase Activity of *C. citratus* Extracts

Table 4.4 presents the effect of different concentrations of *C. citratus* extracts and aspirin on anti-lipoxygenase activity. The results are expressed as Mean ± SD, with ANOVA F and p-values included.

For the ethanol extract, inhibition increased from 54.04 ± 1.180 at 100 mg/mL to 60.38 ± 0.050 at 200 mg/mL, and 65.04 ± 0.560 at 300 mg/mL. The inhibition at 200 mg/mL was significant ($p < 0.0001$) compared to 100 mg/mL, while the value at 300 mg/mL was significant relative to both 100 mg/mL and 200 mg/mL.

For the diethyl ether extract, inhibition values were 47.00 ± 0.250 at 100 mg/mL, 58.50 ± 0.050 at 200 mg/mL, and 69.23 ± 0.370 at 300 mg/mL. The inhibition at 200 mg/mL was significant ($p < 0.0001$) compared to 100 mg/mL, while the value at 300 mg/mL was significant relative to both 100 mg/mL and 200 mg/mL.

The standard drug aspirin showed the highest activity, with mean values of 64.62 ± 0.120 at 100 mg/mL, 72.50 ± 0.050 at 200 mg/mL, and 78.31 ± 0.310 at 300 mg/mL. The inhibition at 200 mg/mL was significant compared to 100 mg/mL ($p < 0.0001$), while the inhibition at 300 mg/mL was significant relative to both 100 mg/mL and 200 mg/mL.

Table 4.4:

	Anti Lipogenase				
	100 mg/mL	200 mg/mL	300 mg/mL	F value	P value
Ethanolic Extract	54.04±1.188	55.69±0.3126	65.04±0.5637ab	173.4	<0.0001
Diethyl Ether Extract	47±0.25	52.12±0.2504a	69.23±0.3761ab	4576	<0.0001
Asprin	64.62±0.1258	70.81±0.06292a	78.31±0.3126ab	3601	<0.0001

Table presented in Mean±SD. a represent significance with 100mg/ml, b represent significance with 200mg/ml, c represent significance with 300mg/ml

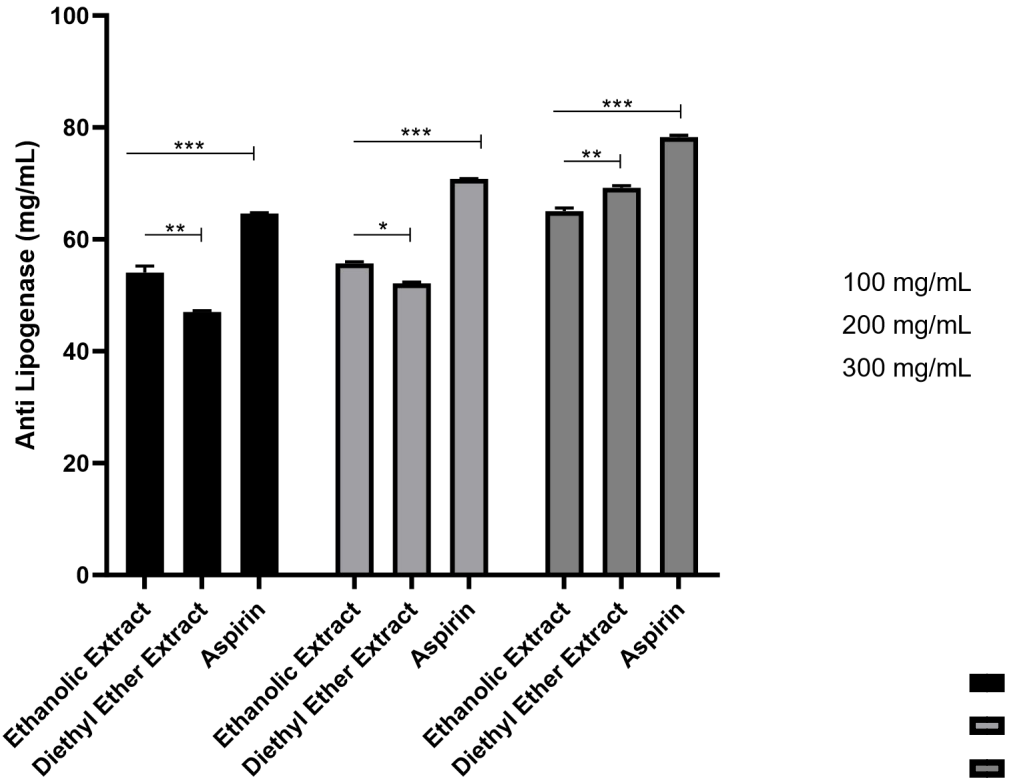

Figure 4.4: (Error bar presented in Mean±SD. *** represents p<0.0001, ** represents p<0.001, * represents p<0.05).

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The results of this study revealed that both the polar solvent and non- polar solvent extracts of *C. citratus* exhibited measurable anti-inflammatory activity across the assays conducted. Inhibition of albumin denaturation, haemoglobin denaturation, anti-proteinase, and anti-lipoxygenase activities all showed varying degrees of response to increasing extract concentrations. This aligns with recent reports that *C. citratus* contains bioactive compounds such as flavonoids, tannins, and essential oils, which are known to modulate inflammatory pathways (Oladeji *et al*., 2019; Han and Parker, 2017; Souza *et al*., 2020).

In the albumin denaturation assay, both extracts (polar and non-polar) demonstrated a concentration-dependent increase in inhibitory activity, with the diethyl ether extract generally outperforming the ethanol extract at equivalent concentrations. This suggests that non-polar phytochemicals, is more soluble in diethyl ether, may play a significant role in stabilizing protein structures under inflammatory stress (Ajayi *et al*., 2016; Pandey *et al*., 2017). However, aspirin consistently produced the highest inhibition values, confirming its superior potency as a standard anti-inflammatory drug (Souza *et al*., 2020).

The haemoglobin denaturation assay showed a slightly different trend, with the ethanol extract performing better at higher concentrations than the diethyl ether extract. This could be attributed to polar compounds in the ethanol extract that may interact more effectively with erythrocyte membranes, thereby preventing oxidative damage and membrane destabilization during inflammation (Muala *et al*., 2021; Naseem *et al*., 2021). The marked increase in inhibition at the

highest tested concentration for both extracts indicates that higher doses may be necessary to achieve clinically relevant effects (Souza *et al*., 2020).

In the anti-proteinase assay, both extracts exhibited relatively low activity compared to aspirin, with minimal variation across concentrations. This suggests that the phytochemicals present in *C. citratus* may not strongly target proteolytic enzymes involved in inflammation (Pandey *et al*., 2017; Oladeji *et al*., 2019). The lack of statistical significance in this assay highlights a potential limitation in the extracts' spectrum of anti-inflammatory action, indicating that their primary mechanisms may lie elsewhere (Han and Parker, 2017).

The anti-lipoxygenase assay produced some of the most promising results, with both extracts showing strong, concentration-dependent inhibition. The diethyl ether extract, in particular, demonstrated a sharp increase in activity at higher concentrations. Since lipoxygenase enzymes are key mediators in leukotriene biosynthesis, these findings suggest that *C. citratus* extracts could be particularly useful in managing conditions where leukotriene-mediated inflammation is prominent (Souza *et al*., 2020).

When comparing the two extracts across all assays, the diethyl ether extract generally showed higher potency in albumin denaturation and lipoxygenase inhibition, while the ethanol extract excelled in haemoglobin denaturation. This indicates that the choice of extraction solvent significantly influences the phytochemical profile and, consequently, the biological activity of the extracts (Muala *et al*., 2021; Naseem *et al*., 2021). Such differences underscore the importance of solvent selection in herbal drug development (Ajayi *et al*., 2016; Pandey *et al*., 2017).

The overall pattern of results supports the ethnomedicinal use of *C. citratus* in managing inflammatory conditions (Oladeji *et al*., 2019; Han and Parker, 2017). While the extracts did not match the potency of aspirin, their activity profiles suggest they could serve as complementary agents in anti-inflammatory therapy, particularly for individuals seeking plant-based remedies with potentially fewer side effects (Souza *et al*., 2020). The concentration-dependent trends observed also suggest that dosage optimization could enhance therapeutic efficacy (Muala *et al*., 2021).

These findings contribute to the growing body of evidence supporting the pharmacological potential of *C. citratus*. However, the in vitro nature of the assays means that the observed effects may not fully translate to in vivo systems, where factors such as metabolism, bioavailability, and systemic interactions come into play (Han and Parker, 2017; Souza *et al*., 2020). Further research is therefore necessary to validate these results in animal models and eventually in human clinical trials (Muala *et al*., 2021; Naseem *et al*., 2021).

5.2 Conclusion

This study has demonstrated that *C. citratus* ethanol and diethyl ether extracts possess significant in vitro anti-inflammatory activity, with effects that vary depending on the assay and extraction solvent used. While aspirin remains more potent across all parameters, the extracts' activity profiles suggest potential for development as complementary or alternative anti-inflammatory agents. These results provide a scientific basis for the traditional use of *C.citratus* in managing inflammatory conditions.

5.4 Recommendations

i Use *C. citratus* extracts as supportive therapy for mild inflammatory conditions.

ii Standardize herbal formulations for consistent active compound levels.

iii Conduct in vivo studies to confirm anti-inflammatory effects and safety.

iv Isolate and identify the specific active phytochemicals.

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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).

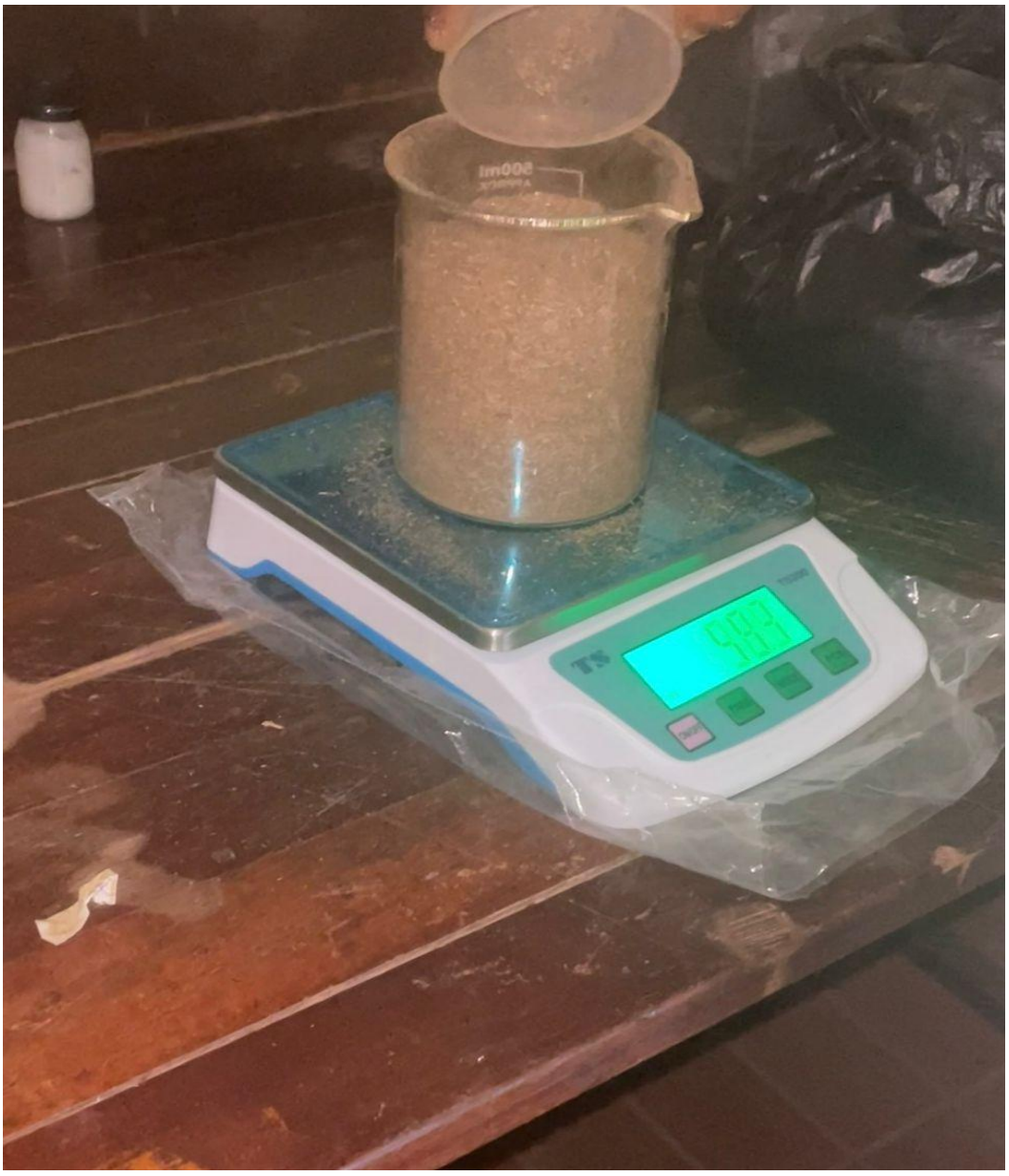
APPENDIX II

Concentration and solvent extraction using rotary Evaporator

APPENDIX III

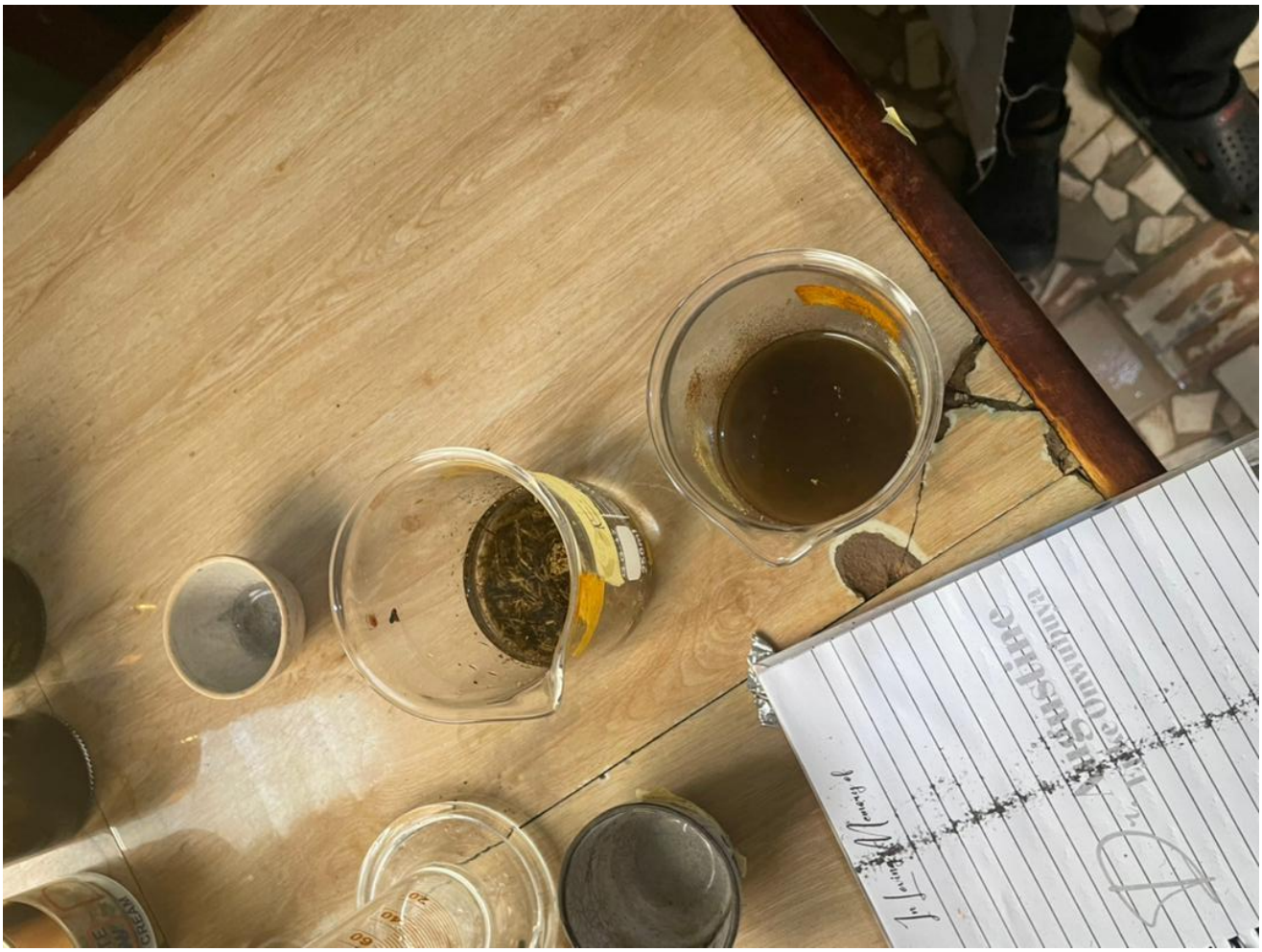
Filtration using Grade 1 whatmann filter paper

APPENDIX IV



Weighing of pulverized plant (*C. citratus*)

APPENDIX V



Analysis using pulverized samples

APPENDIX VI

Extraction using Ethanol and Diethyl ether in Browm glass jars