ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

The Chemical Profiling of *Cajanus cajan* (L.) Huth (Fabaceae) Seed Oil Extract

Abdulai Turay (M21303090)[1]

Hamza Amin Kargbo[2]

Dr. Eugene B. S. Conteh[3]

Dissertation Submitted To

The "African Centre of Excellence for Drug Research, Herbal Medicine Development and Regulatory Science"

In Partial Completion for a Degree of Master in Drug Discovery and Development Awarded

The Postgraduate School, University of Lagos.

Publication Date: 2025/05/16

How to Cite: Abdulai Turay; Hamza Amin Kargbo; Dr. Eugene B. S. Conteh (2025). The Chemical Profiling of *Cajanus cajan* (L.) Huth (Fabaceae) Seed Oil Extract. *International Journal of Innovative Science and Research Technology*, 10(4), 3827-3868. https://doi.org/10.38124/ijisrt/25apr1955

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

CERTIFICATION

This is to certify that this dissertation work entitled "**THE CHEMICAL PROFILING OF *CAJANUS CAJAN*** (L.) HUTH **(FABACEAE) SEED OIL EXTRACT**" was carried out by ABDULAI TURAY (Reg.no:M213023090). The work mentioned in the dissertation was carried out at the Faculty of Pharmacy under the guidance of **PROF. O. T. ASEKUN** as partial fulfilment for the award of the Degree, Master of Science in **Drug Discovery and Development**, University of Lagos, Idi-Araba, Lagos, Nigeria.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

DECLARATION

I, ABDULAI TURAY, do hereby declare that this dissertation entitled **"THE CHEMICAL PROFILING OF *CAJANUS CAJAN* (L.) HUTH (FABACEAE) SEED OIL EXTRACT"**, submitted to the University of Lagos, Idi-Araba, Lagos, Nigeria, in partial fulfillment for the Degree of Master of Drug Discovery and Development, was carried out by me under the guidance of **Mrs. Olayinka T. Asekun**, Professor, Department of Chemistry, University of Lagos, Arkoka, Lagos, Nigeria, during the period of **2022 – 2023**.

I would also like to acknowledge my colleague in the academic field, **Hamza Amin Kargbo**, Lecturer and IT Technician at the Department of Information Communication and Technology, Freetown Polytechnic, Kossoh Town, Jui, Freetown, Sierra Leone, for his invaluable assistance and support in the research work, which significantly contributed to the success of this dissertation.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

DEDICATION

I dedicate this work to Allah and my late parents, **Idrissa Turay and Ya Adama Conteh**. May their souls rest in perfect peace.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

ACKNOWLEDGEMENT

First and foremost, it gives me great pleasure to record my deep sense of gratitude and indebtedness to my esteemed supervisor, **Mrs. Olayinka. T. Asekun**, a Professor at the Department of Chemistry, University of Lagos, Arkoka, Lagos, Nigeria, for her constant insight, guidance, countless serenity, encouragement and painstaking efforts in ensuring that my project comes to completion. I am indebted to her kindness and never-failing co-operation. I extend my gratitude to **Dr Moshood O. Akinleye**, a Senior Lecturer at the Faculty of Pharmacy, University of Lagos, Idi- Araba, Lagos, Nigeria, for his constant encouragement, support and facilities he provided during the bench work. I thank our respected Centre Leader, **Dr Omobalanle Ade-Ademilua,** for her invaluable support and timely help during the job. I also express my heartfelt thanks to our Course Coordinator, **Dr.O.O. Shonekan,** for setting the stage before leaving for further training and to **Dr Adeyemi** for his tremendous effort as Acting Coordinator in ensuring our program was well coordinated. I owe my heartfelt thanks to my esteemed and beloved Head, Department of Pharmaceutical Chemistry, College of Medicine and Allied Health Sciences, **Dr Eugene B.S Conteh,** who encouraged me to apply for this course and constantly followed up with calls to see that things were working well with me throughout my stay in Nigeria. My sincere thanks to all other members of staff at the Faculty of Pharmacy, University of Lagos, Idi-Araba, Lagos, Nigeria, who directly or indirectly gave a helping hand to me while carrying out the study. This project would not be unique without the timely help and continuous support of my ever-loving friends and colleague MSc classmates, **Malik Dawood Kamara, Mamoud Massaquio, Prince Dehnue, and Meloria E.A Greene**. Thank you all. With immense pleasure, I express my deep gratitude to the lab technicians, Faculty of Pharmacy, University of Lagos, Idi-Araba, Lagos. Above all, I dedicate myself to the unfailing presence of **GOD** and the constant love and encouragement given to me by my beloved wife, **Haja Hawanatu Yumkella**, who deserves credit for success in whatever I do.

.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

TABLE OF CONTENTS

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

LIST OF FIGURES

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

LIST OF TABLES

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

ABSTRACT

This work investigated The Chemical Profiling of *Cajanus Cajan* (L.) Huth (Fabaceae) Seed Oil Extract using HPLC, GC-MS and FTIR analytical methods. The extraction was done with ethanol to yield a 2.40% yield crude extract. The crude extract was partitioned using hexane, ethyl acetate, dichloromethane and water. The yields of the fractions compared to the 400 g seed used for extraction are 0.30%, 0.46%, 0.36%, 0.65%. The result of the fractions compared to the crude extract (9.6 g) is 17.10%, 25.72%, 20.20% and 36.97 % of the crude extracts, respectively. HPLC analysis revealed the presence of Four (4) prominent phytochemicals: Gallic Acid, Magnoflorine, Rutin and Pinostrobin. The concentration of Gallic Acid, Magnoflorine, Rutin and Pinostrobin in

0.5 mg/mL (500 µg/mL) of *cajanus cajan* ethanol seed extract was 22.609 µg/mL, 175.236 µg/mL, 169.705 µg/mL and 90.525 µg/mL respectively. The results of the chemical composition of the n- hexane fraction of *Cajanus cajan* seed oil obtained by GC-MS analysis show nine (9) compounds with a 98% total oil content. The major compounds identified in the seed oil are octadecanoic acid (32.36%), 9, 12-octadecadienoic acid (Z, Z) (29.23%) and linoelaidic acid (25.31%). The FTIR analysis with Aqueous, ethyl acetate and dichloromethane fractions confirms the presence of the following phytochemicals: Esters, flavonoids, Alkaloids, Alcoholic or Phenolic compounds and carbonyl compounds.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

CHAPTER ONE
INTRODUCTION

➢ *Medicinal Plants*

Plants and their extracts have served as fundamental remedies in healthcare practices for centuries. This practice, known as herbalism, utilises various substances, including minerals, shells, specific animal parts, fungi, and bee products. By incorporating these natural ingredients, herbal medicine offers a holistic approach to healthcare that can contribute to overall well-being. By choosing herbal remedies, individuals can tap into the power of nature to support their overall well-being. Nature has been a reliable source of therapeutic agents for over a millennium. An impressive number of modern medicines have been derived from natural sources. Natural resources are key to discovering innovative treatments for a wide range of illnesses. (Bhushan R. Gudalwar et al., 2021). Plant compounds have potentially significant therapeutic applications against human and animal pathogens, including bacteria, fungi, and viruses (Silva et al., 2020). The use of medicinal herbs has significantly grown in recent years as a means of enhancing health and well-being. Extensive studies of the adverse effects of these herbal medicines are essential for ensuring the efficiency and quality of herbal medicine ("Prevalence and Associated Factors of Burnout Syndrome among Nurses in Public Hospitals, Southwest Ethiopia," n.d.). *Cajanus cajan* (L.) Huth. (Family*: Fabaceae),* generally known as "Pigeon pea" (English). *Cajanus cajan is a valuable, non-toxic grain recognized both as a nutritional supplement and a source of medicinal benefits*. Pigeon pea is a protein, vitamin B, and mineral wellspring for veggie-loving populations and creatures. The plant *C. cajan* contains many bioactive constituents such as stilbenes, flavones, phytosterols, coumarins, and many more that possess therapeutic applications for diabetes, hepatitis, malaria, cancer, hyperglycemia, etc. (Orni et al., n.d.)

The medicinal value of the plants lies in the bioactive compounds that produce definite physiological actions in the human body (Abdisa & Kenea, 2020). The bioactive phytoconstituents include alkaloids, flavonoids, saponins, tannins, phenols, and essential oils (Abdisa & Kenea, 2020). The chemical constituents of the plant may vary in different species, varieties, plant parts used, growth conditions, and the age of the plant (Okey-Ndeche et al., 2020). There are a variety of phytochemicals present in plants that have unique therapeutic potential. Alkaloids, flavonoids, saponins, and tannins are essential phytochemicals. In this study, efforts were made to comprehensively compile information related to these bioactive compounds in *Cajanus cajan* seed extracts.

- *Plants in Traditional Medicine*

For thousands of years, people in China, India, and many other nations have used sophisticated traditional medicine (TM) techniques based on plants (Innocent, 2016). They are a crucial source of medications, particularly in underdeveloped and developing nations where plant-based traditional medicine is still used for healthcare. It has been well established that plants continue to serve as the foundation of conventional medical practises and that over 80% of the world's population continues to receive their healthcare from plant-based systems ("Can Remittances Alleviate Energy Poverty in Developing Countries? New Evidence from Panel Data," n.d.). In Burkina Faso, 1033 species (50%) were identified for utilisation. The most crucial usage categories were traditional medicine, human sustenance, and animal feed. The 12 most frequent plant families in Burkina Faso varied greatly in their use and application. The plant families with the most utilized species were Fabaceae, Poaceae, and Malvaceae. The study selected Khaya senegalensis, Adansonia digitata, and Diospyros mespiliformis as the most helpful plants in Burkina Faso. The most prevalent health issues treated with medicinal plants are infections, digestive system diseases, and genitourinary illnesses. The most significant plant families in traditional medicine were Fabaceae, Poaceae, Asteraceae, Apocynaceae, Malvaceae, and Rubiaceae. The most important medicinal plants were Tamarindus indica, Vitellaria paradoxa, and Adansonia digitata (Abo-Zeid et al., 2018).

- *Plants in Modern Medicine*

Modern pharmaceuticals have greatly advanced by utilizing natural compounds originally used in traditional medicine (Kinghorn, 1992). These Plant-derived natural compounds have demonstrated significant potential in the creation of innovative drugs and therapies. They provide diverse chemical structures that can be utilised for various therapeutic purposes, expanding the possibilities for medical advancements. Based on estimations, most of today's best-selling medications are imitations or natural product derivatives. Active substances have been separated from plants for use as medicines since the early 19th century when scientists began isolating and studying various plant species' chemical components. This approach has led to the discovery of numerous life-saving drugs, such as aspirin derived from willow bark and taxol derived from the Pacific yew tree, highlighting the immense potential of plant-based medicine in modern healthcare. The isolation of morphine from the opium poppy was a landmark achievement in the advancement of plant-based pharmaceuticals. This breakthrough paved the way for effective pain management and opened doors to further exploration and utilisation of natural compounds found in plants for medicinal purposes (Abbas et al., 2022). Other early medications from medicinal plants were also obtained due to drug discovery research, some of which are still in use today. It was found that the painkiller codeine 2 was produced quickly by boiling crude morphine in acetic anhydride (Butler, 2004). Digitoxin 3, a cardiotonic glycoside derived from Digitalis purpurea L. (foxglove), improves cardiac conduction. Through the ages, the anti-malarial drug quinine 4, discovered in the bark of Cinchona succirubra, has been used to treat malaria. fever (DerMarderosian & Beutler, 2002).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

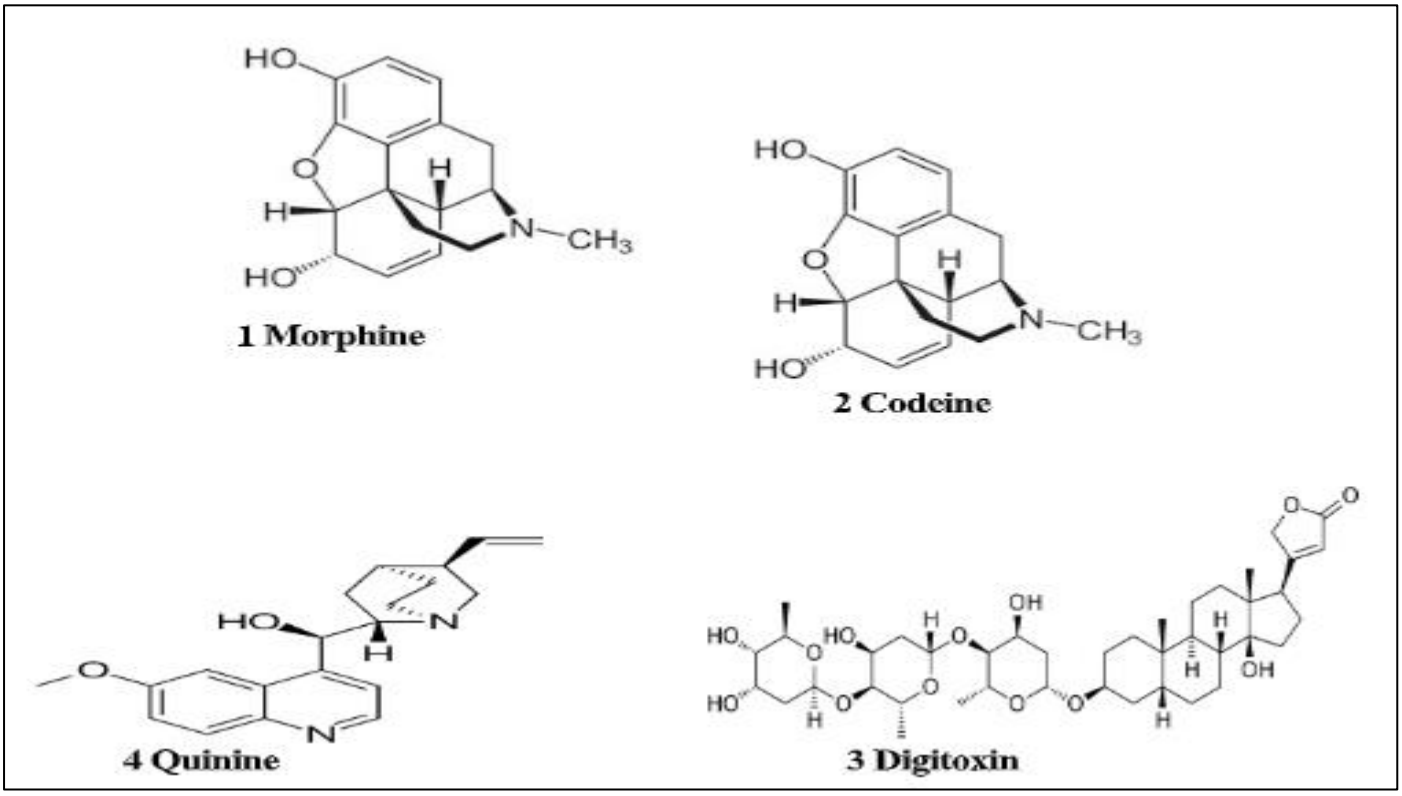

Fig 1 Components 1

Another medication introduced into Western medicine is an L-histidine-derived alkaloid, Pilocarpine 5, isolated from the Rutaceae plant Pilocarpus jaborandi. Pilocarpine 5 has been widely used for its potent effects on the parasympathetic nervous system, particularly in treating glaucoma and xerostomia. Its ability to stimulate saliva and increase tear production has made it a valuable therapeutic option for patients with these conditions. (Aniszewski, 2007)**.** Additionally, the anti- neoplastic compounds vinblastine **6** and vincristine **7** were found in the plant *Catharanthus roseus* (L.) G. Don (Apocynaceae), formerly known as Vinca rosea L. (Heijden et al., 2004), and artemisinin **8**, which was discovered in Artemisia annua L. (Asteraceae) and is currently an effective treatment for certain strains of *Plasmodium* parasites that are multidrug resistant. Alzheimer's disease is now treated with galantamine **9**, a natural substance derived from *Galanthus woronowii* Losinski (Amaryllidaceae). In modern medicine, new drugs for treating human diseases are discovered by natural products derived from medicinal plants (Koehn & Carter, 2005).

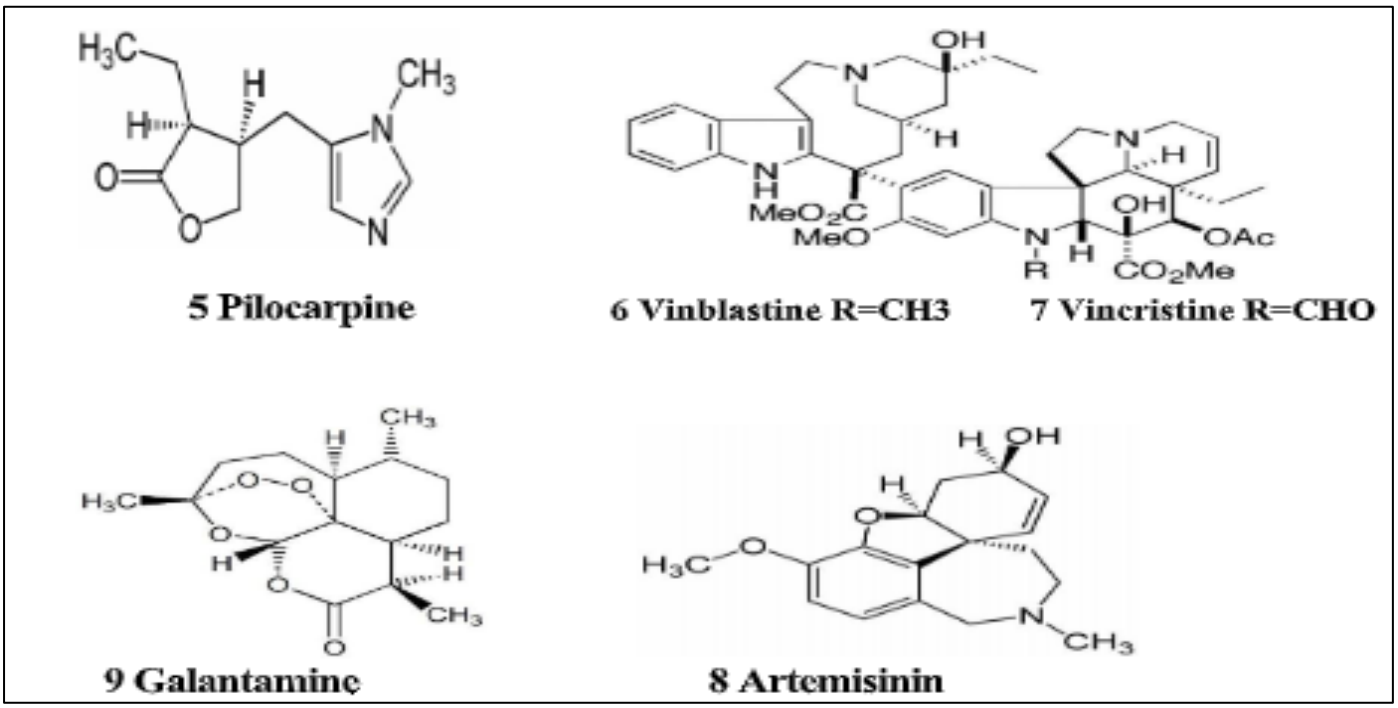

Fig 2 Components 2

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

Natural plant products have significantly impacted the spices, cosmetics, and fragrances markets. Arbutin **10** from Arctostaphylos uva-ursi Spreng, which lightens and inhibits melanin, is one of a few examples of natural plant products that have gained popularity in the cosmetics industry. Another notable example is turmeric, which contains curcumin and is known for its anti-inflammatory and antioxidant properties. These natural ingredients offer a safer and more sustainable alternative to synthetic chemicals commonly used in these industries. Rutin **11** from *Afrormosia laxiflora* is harmful as an emollient and antioxidant and is often used in skincare products for its ability to protect the skin from environmental damage. Additionally, green tea extract, rich in polyphenols, has become a popular cosmetic ingredient due to its soothing and rejuvenating properties. These natural plant products provide effective results and contribute to the growing demand for eco-friendly and ethically sourced beauty products. Terpenoid and phenolic compounds derived from plants have also shown promise as industrial sweeteners and flavour enhancers. These natural compounds offer a healthier alternative to artificial sweeteners and can be derived from various plant sources, such as stevia, vanilla, and cinnamon. With the increasing demand for clean-label and natural ingredients in the food industry, plant-derived terpenoids and phenolic compounds are being extensively researched for their potential applications in producing sweeteners, flavourings, and enhancers. These natural compounds offer a healthier alternative to artificial sweeteners and can be used in various food and beverage products. Furthermore, their sustainable sourcing and production methods align with the increasing consumer preference for environmentally conscious choices in the food industry (Kinghorn & Soejarto, 2002). A few examples include Hernandulcin [6-(1,5-dimethyl-1-hydroxy-hex-enyl)-3-methylcyclohex-2- en]. **12** from the leaves and flowers of *Lipia dulci* Trev (Verbanaceae), periandrin V (3β- O-(β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl)-25-al-olean-18(19)-en-30-oic acid) **13** from the rhizomes of *Periandra dulci L.* (Leguminosae) (Brazilian Licorice) are much sweeter than sucrose.

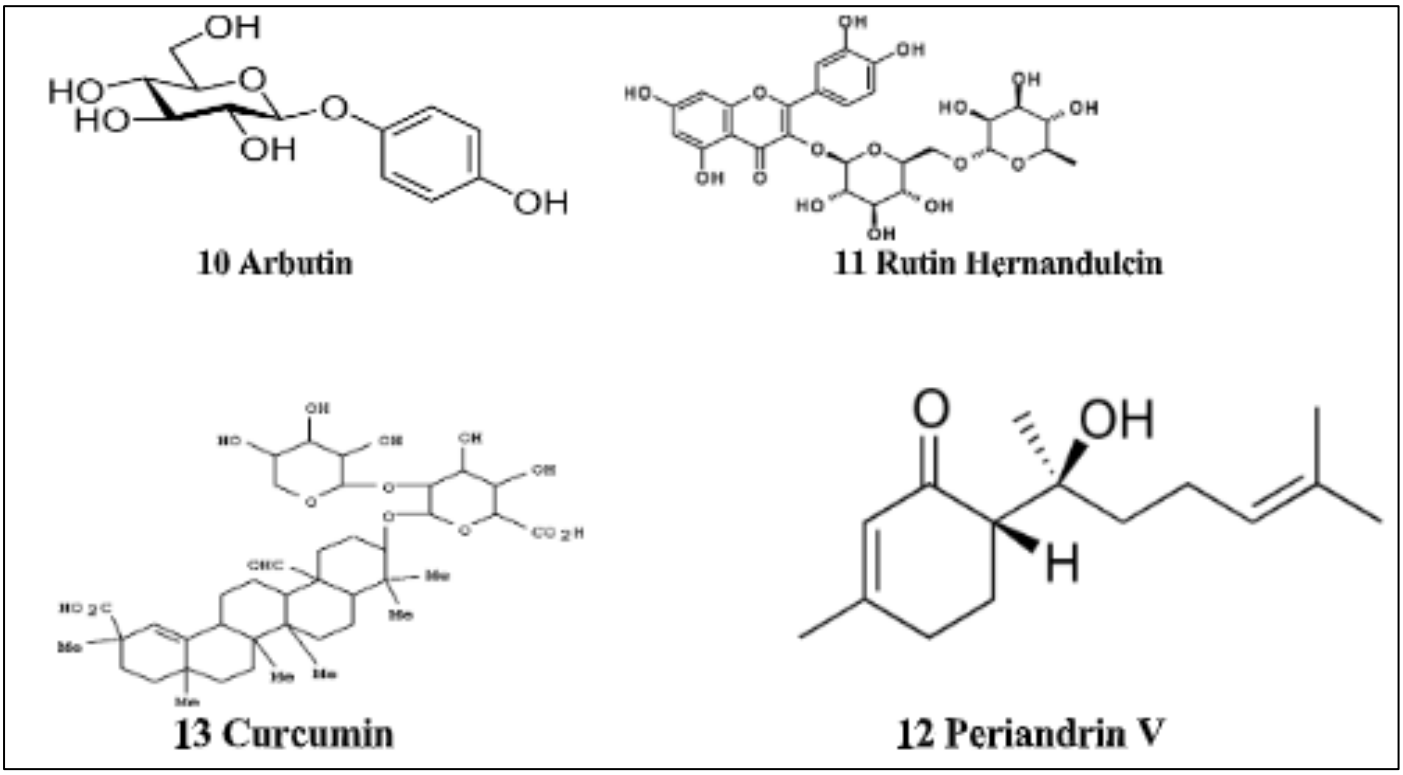

Fig 3 Components 3

➢ *How Technological Advancement Affects Research on Medicinal Plants.*

Advances in technology are revitalizing research in the field of natural products and, directly or indirectly, removing historical barriers to the development of natural products (*Journal. Pd*, n.d.). The ability to separate the chemical components of plants has been further enhanced by advancements in separation methodologies for technologies like high- performance liquid chromatography (HPLC) and countercurrent partition chromatography (McChesney et al., 2007). With the advent of high-field NMR, structure elucidation technology has advanced, enabling quick and simple structure determination. Nowadays, hyphenated techniques (GC-MS, LC-MS, LC-NMR, LC-MS-NMR, etc.) that combine gas chromatography (GC) and high-performance liquid chromatography (HPLC) with detectors allow for direct (online) identification of plant constituents before their isolation (Phillipson, 2007). Chemical research aimed at isolating the active substances from plants used in traditional medicine has led to the discovery of new drugs, even though the active principle isolated from plants may not necessarily replace the plant extract. Drugs that lack the ideal human or animal medicine biological characteristics would be subjected to structure modification to enhance them ("Hyphenated Techniques and Their Applications in Natural Products Analysis | SpringerLink," .d.).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

➢ *Obstacles to Research on Medicinal Plants*

Although plants have immensely contributed to healthcare, systematic research on them remains limited. While many plants have yet to be studied, a select few have undergone extensive research. Only 5–15% of the roughly 350,000 species of higher plants have reportedly experienced a systematic search for the presence of bioactive compounds (Cragg et al., 1997). Many valuable medicinal plants are at risk of extinction due to environmental and human-induced factors, such as agricultural development and the indiscriminate eradication of flora (Gericke, 2002). This is due to rising trade demands for cheaper healthcare products and novel plant-based therapeutic markets instead of pricier target-specific pharmaceuticals and biopharmaceuticals. The greatest immediate threat to biodiversity is habitat loss. As a result, positive legal and scientific interest has been sparked to document the potential of these plants before they vanish entirely or cause genetic diversity to be lost. Because many healers and indigenous knowledge holders are elderly and dying or being killed in ecclesiastical, religious, or political conflicts without recording their knowledge, they are more vulnerable than the medicinal plants themselves. It is common knowledge that traditional medicine is shrouded in secrecy (Amuka et al., 2014). Families with traditional knowledge often guard it closely to protect their competitive advantage to maximise the pharmaceuticals' commercial potential. Due to the development of modern education and, to some extent, modern medicine, younger generations today are less interested in learning about plants from their parents. Another significant obstacle to the development of new drugs is this. Despite the long history of success in using natural products as drugs over the past couple of decades, there has been a steady decline in natural product research globally. Combinatorial chemistry and high-throughput synthesis (HTS) may have contributed to some of this decline (Ichoron et al., 2019).

➢ *Statement of the problem*

Therapeutic failures and the rise of emerging diseases present major obstacles to global healthcare systems. These issues are further exacerbated by factors such as antimicrobial resistance and the rapid spread of infectious diseases, making it crucial for researchers and healthcare professionals to adapt and develop innovative solutions to combat these threats continuously. Phytochemicals are produced in plants as protectants, but researchers have also demonstrated their role as an alternative option in managing human diseases. The medicinal value of plants lies in the bioactive compounds that produce definite physiological actions in the human body. The bioactive phytoconstituents include alkaloids, flavonoids, saponins, tannins, phenols, and essential oils (Marcía-Fuentes et al., 2021). Phytochemicals exhibit promising capabilities in the prevention and treatment of cancers, heart diseases, and neurological disorders. They possess antioxidant, anti- inflammatory, and antimicrobial properties that can help boost the immune system and fight against pathogens.

Additionally, phytochemicals have been found to have minimal side effects compared to traditional pharmaceutical drugs, making them a promising avenue for future therapeutic interventions. Furthermore, the study of phytoconstituents can provide insights into the mechanisms of action of these natural compounds, allowing for the development of more targeted and effective drugs. This knowledge can also contribute to discovering new therapeutic targets and treatment strategies for various diseases (R.K. Singh, 2020). By understanding the structure and function of phytochemicals, scientists can modify and optimise these compounds to create more effective and targeted treatments for various ailments. Studying phytoconstituents can also shed light on traditional medicine practices and help validate their use in modern healthcare systems.

This study aimed to identify and characterise the specific phytochemicals in *Cajanus cajan* (L.) huth (fabaceae) seed extracts for their medicinal properties. They conducted various extraction and analysis techniques to determine the concentration and composition of these bioactive compounds, providing valuable information about their potential therapeutic applications.

➢ *Aim and Objectives*

- *Aim*

This study focuses on analyzing the chemical composition of seed extracts obtained from Cajanus cajan (L.) HUTH (FANACEAE)**.**

- *The specific objectives of this study are to:*

✓ Carry out the quantitative analysis on the seed extracts of *C. cajan.*
✓ Isolate and characterise the phenolic compounds present in *C. cajan* seed extracts

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

- *Significance of the study*

The exploration of phytochemicals from medicinal plants is crucial for the development of treatments for infectious diseases, especially when drug-resistant microorganisms emerge. Medicinal plants are successful natural sources for treating various infectious diseases in humans. Scientists are focusing on discovering natural compounds from medicinal plants to introduce new drugs that will be more effective than those available. Contemporary studies emphasize plant-derived natural products as viable alternatives to conventional medicines in developing regions. They have formed the basis of sophisticated traditional medicine and make an excellent lead for new drug development. They are a good choice as they have fewer side effects, are less expensive and are effective against broad-spectrum drug-resistant microorganisms. Plants used in traditional medicine contain many substances that can be used to treat chronic and infectious diseases. Traditional healers and pharmaceutical drug companies exploit these chemical constituents with great potential for medicinal use.

Medicinal plants have been used as an ancient tradition, especially where modern drugs are not affordable or inaccessible. Even today, plants are the most exclusive source of drugs for most of the world, and people in developing countries mainly use traditional medicine for their primary health care. According to the World Health Organization, approximately 80% of the global population relies on traditional medicine for primary healthcare.

Herbal remedies continue to play a role in the cure of diseases. Commercially proven drugs used in modern medicine were initially used crudely in traditional folk healing practices. When fractionated, the number of active components in crude extracts from medicinal plants, small or diluted, become concentrated and, therefore, show activity. Medicinal plants are an essential source of new chemical substances with potential therapeutic benefits. Many plants in Africa, such as *C. cajan*, have been investigated for their chemical components, and some isolated compounds have been shown to possess enjoyable biological activity.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

CHAPTER TWO
LITERATURE REVIEW

- *Medicinal Plants as Complementary and Alternative Therapeutic Options*

Phytochemicals derived from medicinal plants are receiving growing attention from researchers aiming to develop treatments for chronic and infectious diseases, especially those linked to drug-resistant pathogens (Márcia-Fuentes et al., 2021). Medicinal plants have consistently served as natural remedies for numerous human ailments. Current scientific efforts are geared towards isolating bioactive compounds from plants to formulate more effective therapeutic agents than existing pharmaceuticals. Modern research highlights the promise of plant-derived natural products as substitutes for conventional drugs, particularly in developing nations. Traditional medicinal systems, which have relied on plants for centuries, offer a valuable foundation for new drug discovery. Natural products are often preferred due to their lower cost, fewer adverse effects, and efficacy against resistant microorganisms. Plants used in indigenous healing practices are rich in chemical constituents effective against a variety of chronic and infectious diseases. These compounds are harnessed by both traditional healers and the pharmaceutical industry, particularly in areas where access to modern medicines is limited or unaffordable.

Today, plants continue to be the principal source of therapeutic agents for a significant portion of the global population. According to the World Health Organization (WHO, 1993), approximately 80% of people worldwide rely on traditional medicine for their primary healthcare needs. Herbal therapies maintain a crucial role in disease treatment, and many commercially available pharmaceuticals have their origins in traditional herbal practices. Through processes such as fractionation and dilution, the active components of crude plant extracts are concentrated, thereby enhancing their biological activity. Medicinal plants remain a vital source of novel chemical entities with therapeutic potential. Extensive research on African plants, including *Cajanus cajan*, has identified various bioactive compounds exhibiting promising biological activities.

- *General Methods of Extraction of Plants*

Extraction serves as the fundamental initial stage in the investigation of medicinal plants, facilitating the isolation of crucial chemical constituents for detailed analysis and characterization. The extraction process typically involves key steps such as pre-washing, drying (or freeze-drying) of plant material, grinding into a fine, homogeneous powder, and optimizing the interaction between the sample surface and the solvent system to enhance extraction efficiency. Maintaining the stability and integrity of bioactive compounds during this process is essential.

When a plant is selected based on its traditional medicinal applications (Fabricant & Farnsworth, 2001), the extraction method should closely replicate traditional preparation techniques to preserve its authenticity. Solvent selection is critical and is influenced by the polarity of the target bioactive constituents. Various solvents are employed to achieve efficient extraction; for example, polar solvents like methanol, ethanol, and ethyl acetate are used to isolate hydrophilic compounds, whereas dichloromethane or mixtures of dichloromethane and methanol are more effective for extracting lipophilic substances. In certain cases, hexane may be utilized to remove chlorophyll from plant extracts ("Anti-Infective Potential of Natural Products: How to Develop a Stronger in Vitro 'Proof-of-Concept' - ScienceDirect," n.d.).

Given that target molecules may vary from non-polar to polar and could be thermally sensitive, it is vital to select appropriate extraction methods. Traditional techniques such as sonication, reflux heating, and Soxhlet extraction are commonly used (United et al., 2002; People's Republic of China Pharmacopoeia, 2000; The Japanese Pharmacopoeia, 2001). Extraction can also be achieved through maceration or percolation using fresh or dried plant materials immersed in water or organic solvent systems.

Modern extraction methods—including solid-phase microextraction, supercritical fluid extraction, pressurized liquid extraction, microwave-assisted extraction, solid-phase extraction, and surfactant-mediated extraction—offer numerous advantages. These benefits include reduced consumption of organic solvents, minimized sample degradation, elimination of extensive sample preparation before chromatographic analysis, enhanced efficiency, improved selectivity, and accelerated extraction kinetics. Furthermore, the possibility of automation has made these modern techniques increasingly favorable for extracting bioactive substances from plant materials (Huie, 2002).

- *General Methods of Characterisation of Compounds of Plant Extracts*

Due to the complex composition and polarity differences among phytochemicals, the separation of plant extracts poses significant challenges during compound identification and characterization. It is usually practised to isolate these bioactive compounds to get pure molecules to utilise various separation methods such as TLC, column chromatography, flash chromatography, Sephadex chromatography, and HPLC. The pure chemicals are next analysed for structure and biological activity. Non-chromatographic methods such as immunoassay, which uses monoclonal antibodies (MAbs), phytochemical screening test, and Fourier-transform infrared spectroscopy (FTIR), can also collect and identify bioactive chemicals.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

- *Chromatographic Techniques*

✓ *Thin-layer chromatography (TLC) and Bio-autographic methods*

Thin-Layer Chromatography (TLC) is an efficient, fast, and cost-effective technique used to determine the number of components in a mixture. By comparing the Rf values of a compound with those of known substances, TLC helps identify chemicals within a sample. Other techniques such as applying phytochemical screening reagents, which cause color changes based on the compounds present in a plant extract, or observing the plate under UV light, can also be used. This method is often employed to verify the purity and authenticity of isolated compounds.

Bio-autography is a valuable technique for identifying bioactive compounds with antibacterial properties in plant extracts. It combines chromatographic separation with in situ activity assessment, enabling the identification and isolation of active compounds. Typically, bio-autography utilizes microbe growth inhibition on TLC plates to detect antimicrobial compounds. This method is regarded as one of the most effective for identifying antimicrobial agents (Shahverdi et al., 2007). There are three primary bio-autography methods to detect antimicrobial activity on a chromatogram: (i) direct bio-autography, where microorganisms grow directly on the TLC plate; (ii) contact bio-autography, where antimicrobial compounds are transferred from the TLC plate to an inoculated agar plate; and (iii) agar overlay bio-autography, in which an agar medium is applied directly to the TLC plate ("A Direct Bioautographic Tlc Assay for Compounds Possessing Antibacterial Activity | Journal of Natural Products," n.d.). The inhibition zones formed on the TLC plate by any of these techniques help visualize the position of the bioactive chemical exhibiting antimicrobial activity, as indicated by its Rf value ("A Direct Bioautographic Tlc Assay for Compounds Possessing Antibacterial Activity | Journal of Natural Products," n.d.). To isolate bioactive components, preparative TLC plates (1mm thick) are used, and the components showing antimicrobial activity are separated. After scraping the spots from the plates, the compounds are eluted using ethanol or methanol, and further purification is carried out using preparative chromatography. The isolated components are then identified using advanced techniques such as HPLC, LCMS, and GCMS. Despite its sensitivity, this method is limited to microorganisms that grow well on TLC plates. Additional challenges include the complete removal of residual low-volatile solvents, such as n-BuOH, trifluoroacetic acid, and ammonia, as well as the diffusion of active compounds from the stationary phase into the agar layer. Bio-autography is a key method for localizing the antimicrobial activity in an extract, thus aiding in the rapid discovery of novel antimicrobial agents through bioassay-guided isolation (Cos et al., 2006). The agar overlay method of bio-autography is advantageous because it uses fewer samples than traditional disc diffusion methods and is ideal for bioassay-guided chemical isolation. Moreover, since the extract is separated into its individual components, this approach simplifies the identification and isolation of bioactive compounds.

✓ *High-Performance Liquid Chromatography*

High-performance liquid chromatography (HPLC) is a versatile, robust, and widely used method for separating natural products. It is increasingly being chosen as the primary technique for fingerprinting research in herbal plant quality control (Fan et al., 2006). Natural products are typically isolated after preliminary biological testing of crude extracts to accurately identify the active constituents. These biologically active components are often minor constituents of the extract, and HPLC's excellent resolving power makes it ideal for efficiently processing multi-component samples on both analytical and preparative scales. Modern benchtop HPLC systems are often modular, consisting of a solvent pump, a sample introduction device (such as an auto-sampler or manual injection valve), an analytical column, a guard column, a detector, and a recorder or printer. Chemical separations in HPLC occur because different compounds migrate at different rates in the chosen column and mobile phase. The choice of stationary and mobile phases significantly affects the degree of separation. Phytochemical identification and separation are commonly performed using an isocratic system (a single, constant mobile phase). However, gradient elution where the percentage of organic solvent changes over time can be advantageous when examining multiple components that have significantly different retention times under the chosen conditions. The process of separating or purifying the target compound from other similar molecules or impurities using HPLC is known as purification. Each component should ideally produce a distinct peak under specific chromatographic conditions. The chromatographer can adjust the conditions, including mobile phase, flow rate, detectors, and columns, to achieve optimal separation, depending on the components being separated and their similarities. Compound identification through HPLC is essential for every test. A detector must be chosen to identify the target molecule, and once the detector is optimized, a separation assay should be designed. The assay parameters should be set so that the known sample produces a clear peak on the chromatograph, with a suitable retention time and good separation from other peaks. UV detectors are commonly used due to their high sensitivity for compound analysis (Lia et al., 2004). Most naturally occurring compounds have some level of UV absorbance at low wavelengths (190-210 nm), which is particularly useful when the compound of interest is present in low amounts. In addition to UV detection, other detection methods, such as diode array detectors (DAD) coupled with mass spectrometers (MS), are also employed for phytochemical detection (Tsao & Deng, 2004). Using tandem mass spectrometry (MSn) provides detailed structural information about molecules. The combination of HPLC and MS enables rapid and accurate identification of chemical components in medicinal plants, especially when pure standards are unavailable (Ye et al., 2007). The success of natural product isolation through HPLC depends heavily on the preparation of crude samples and the choice of solvent for reconstitution. For example, dried powdered plant material must be processed to ensure the target compound is effectively released into solution. Organic solvents, such as methanol or chloroform, are often used as the initial extractant, and after a period of maceration, solid material is removed by filtration. The resulting filtrate is then concentrated and processed through HPLC for separation. Guard columns are essential when analyzing crude extracts, as many natural products contain components like chlorophyll and other endogenous substances that may interfere

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

with the performance of analytical columns over time.

- *Non-Chromatographic Techniques*

Immunoassays that use monoclonal antibodies to target pharmaceuticals and low molecular weight bioactive compounds are increasingly important tools for analyzing bioactive molecules. These assays are highly specific and sensitive in detecting target substances. Various methods, including enzyme tests and both qualitative and quantitative analyses, are employed. Enzyme-linked immunosorbent assays (ELISA) based on monoclonal antibodies (MAbs) are often more sensitive than traditional HPLC techniques. Monoclonal antibodies are produced through hybridoma technology, a method that involves creating specialized cells (Shoyama et al., 2006). The process for generating monoclonal antibodies against plant-based compounds using hybridoma technology includes the following steps: (i) A rabbit is immunized with specific plant compounds to induce antibody production, supported by B cell activation. (ii) Tumors can be induced in mice or rabbits. (iii) Spleen cells, which are rich in B and T cells, are then isolated from these animals. These spleen cells develop antibodies against the plant compound, and myeloma cells, which cause tumors, are also used. (iv) Polyethylene glycol (PEG) is used to stimulate hybridoma formation by fusing the spleen and myeloma cells. The hybrid cells are then grown in a selective medium containing hypoxanthine, aminopterin, and thymidine (HAT). (v) The desired hybridoma is selected for cloning and antibody production against the plant compound. This step results in single-cell colonies that will grow and can be used to screen hybridomas that produce the target antibody. (vi) The chosen hybridoma cells are cultured to produce monoclonal antibodies against specific plant compounds. (vii) Finally, monoclonal antibodies are used in enzyme-linked immunosorbent assays (ELISA) to identify similar compounds within mixtures of plant extracts.

- *Phytochemical Screening Assay*

Phytochemicals are compounds derived from plants, often referring to the diverse range of secondary metabolites found in plants. The phytochemical screening assay is a straightforward, fast, and cost-effective method that quickly identifies the types of phytochemicals present in a sample. It serves as a crucial tool in the analysis of bioactive compounds.

- *Fourier-Transform Infrared Spectroscopy (FTIR)*

Fourier Transform Infrared (FTIR) spectroscopy is an essential technique for characterizing and identifying chemical compounds or functional groups (chemical bonds) within plant extracts (Sasidharan et al., 2011). FTIR spectra of pure substances are often so unique that they serve as a chemical "fingerprint." By comparing the spectrum of an unknown molecule with a database of known compounds, the identity of common plant components can often be determined. FTIR samples can be prepared using different methods. For liquid samples, the simplest approach involves placing a drop of the sample between two sodium chloride plates, forming a thin film. Solid samples may be ground with potassium bromide (KBr) to create a thin pellet for analysis. Alternatively, solid samples can be dissolved in a solvent, such as methylene chloride, and placed on a salt plate, where the solvent evaporates, leaving a thin layer of the substance for analysis.

➢ *Solvents for Plant Extraction*

The choice of solvent significantly impacts the extraction of compounds and the bioactivity of the resulting extract (Antimicrobial_activity_of_leaf_extracts_of_Senna_obtusifolia_(L). Pdf, n.d.). The polarity of solvents (non-polar, polar, and less polar) plays a crucial role in extracting bioactive compounds that influence antimicrobial properties. Optimizing the extraction process is essential to maximize the yield of active constituents. Factors such as extraction rate, quantity of compounds extracted, handling of extracts, and solvent toxicity should be carefully evaluated to assess solvent effectiveness. Various solvents are used for extracting antimicrobial compounds (Masoko et al., 2008). One study showed that methanol extracts from the rhizomes and leaves had the most significant antimicrobial activity against all tested organisms, while hexane extracts had no effect. Petroleum ether and methanol extracts of Cassia occidentalis leaves were effective against E. coli at a concentration of 400 mg/mL, with inhibition zones ranging from 5 to 11 mm, respectively. Water, being a universal solvent, is often used in traditional medicine preparation. It effectively extracts natural products such as pigments and bioactive compounds, resulting in high yields. However, some solvents selectively dissolve specific bioactive compounds. This selectivity has been linked to the solubility of agents like xanthones, benzophenones, and flavonoids, particularly biflavonoids like GB1. While water extracts typically show weaker activity (Samie et al., 2005), acetone is often favored as it extracts both polar and non-polar components efficiently. Additionally, n-hexane extracts from G. kola seeds demonstrated strong activity against five Vibrio species, while ethyl acetate extracts exhibited broad-spectrum activity against both gram-positive and gram-negative bacteria.

➢ *Cajanus cajan*

Cajanus cajan (L.) Huth., commonly known as "Pigeon pea," is a significant leguminous crop from the Fabaceae family, widely grown across Asia, Africa, and certain regions of South America (Chinecherem, n.d.). It is distributed throughout tropical and subtropical regions worldwide and plays a crucial role in agriculture and nutrition (Three Stilbenes from Pigeon Pea with Promising Anti-MRSA Biofilm Formation Activities, 2023). Pigeon peas are a valuable, non-toxic grain with numerous medicinal applications in addition to their use as a food supplement (Krishna, Anitha, & Ezhilarasan, 2020). They are a rich source of protein, vitamin B, and minerals, benefiting both humans and animals that consume vegetables. The plant contains various bioactive compounds, such as stilbenes, flavones, phytosterols, and coumarins, which have been linked to the treatment of conditions like diabetes, hepatitis, malaria, cancer, and hyperglycemia ("The Antiplasmodial Potential of Medicinal Plants Used in the Cameroonian Pharmacopoeia: An Updated Systematic Review and Meta-Analysis," n.d.).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

Moreover, Cajanus cajan is believed to possess antioxidant, anti-tumor, anti-malarial, anti-cancer, and antibacterial properties. The leaves of the plant are traditionally used for a range of ailments, including diabetes, bronchitis, bladder stones, wounds, coughing, and jaundice. In Chinese traditional medicine, pigeon pea leaves are utilized to stop bleeding, relieve pain, and treat parasitic infections. Fresh leaves are chewed to treat aphthae, while a leaf decoction has been found effective for treating coughs and diarrhea. Recently, pigeon pea leaves have been used in treating burns, bedsores, and other wounds. Additionally, pigeon pea leaves demonstrate significant anti-inflammatory, antibacterial, and anti-irritant effects, as well as the ability to reduce capillary permeability (Dutta, Halder, & Khaled, 2023). In traditional Chinese medicine (TCM), the plant is particularly valued for treating ischemic necrosis of the femoral head. This dissertation offers a comprehensive and up-to-date review of Cajanus cajan extracts, focusing on phytochemical screening and chemical analysis for potential therapeutic applications in the future.

- *Scientific Classification*

Kingdom: *Plantae* Class: *Magnoliopsida* Subclass: *Rosidae* Order: *Rosidae* Family: *Fabaceae* Genus: *Cajanus* Species: *Cajanus cajan* (L.) Huth.

➢ *Role of pigeon pea (Cajanus cajan L.) in human nutrition and health*

Legumes and grains serve as affordable sources of protein and energy in developing countries, including India. Pigeon pea (Cajanus cajan L.) is frequently consumed as a low-cost protein source, often in the form of dhal. While it is a rich nutrient source, certain anti-nutritional components, which are reduced through various processing methods, can mask its full nutritional potential. Studies have highlighted the role of pigeon pea's non-nutritive compounds in boosting antioxidant and anti-carcinogenic activities. Recent findings suggest that the bioactive compounds in pigeon peas play a significant role in modifying gut microbiota, potentially reducing inflammation (Talari & Shakappa, 2018).

The seeds of C. cajan are also consumed as a vegetable, with young pods harvested before seed formation and used in curries or relishes. The dry seeds are processed into tempe (a traditional Indonesian dish made by fermenting C. cajan with *Aspergillus oryzae*, followed by soaking, dehulling, and cooking the seeds) and ketchup (a fermented pigeon pea sauce, used as a soy sauce alternative in Indonesia). C. cajan flour, often blended with wheat to enhance protein content in baked goods, and transparent noodles of higher quality than mung bean noodles, are made from dehulled seeds. Fresh seeds are rich in vitamins, particularly provitamin A and the B complex vitamins. For every 100 g of edible portion, dry pigeon pea seeds typically contain 7-10.3 g of water, 14-30 g of protein, 1-9 g of fat, 36-65.8 g of carbohydrates, and 5-9.4 g of fiber (Orwa *et al*., 2009).

➢ *Phytochemistry*

Phytochemistry focuses on the study of plant compounds, particularly secondary metabolites, which plants produce to protect themselves from threats such as insects, pests, diseases, herbivores, UV radiation, and environmental stress. This field explores the structural makeup of these metabolites, their biosynthesis, biological roles, and mechanisms of action, as well as their potential medical, industrial, and commercial applications (Phillipson, 2007). A thorough understanding of phytochemicals is vital for drug discovery and the development of new treatments for significant diseases. Phytochemicals are found in a variety of plant-based foods, including fruits, vegetables, whole grains, spices, legumes, herbs, shrubs, and trees. These compounds accumulate in different parts of the plant, such as leaves, fruits, bark, stems, roots, seeds, and flowers. While certain phytochemicals can also be synthesized by other organisms like fungi, their synthesis processes may vary. Many phytochemical-rich foods, excluding highly processed foods like sugar, are already staples in our diet or beverages. A simple way to increase phytochemical intake is by consuming at least five to nine servings of colorful fruits and vegetables daily (Phillipson, 2007)

➢ *Chemistry of Cajanus cajan*

- *Phytochemical reports*

Phytochemicals are naturally occurring biologically active compounds found in plants that offer numerous health benefits, serving as both therapeutic agents and nutritional components. They help protect plants from diseases, pests, and environmental stress while contributing to the plant's color, scent, and flavor.

The methods used by Trease and Evans (1989) and Sofowora (1993) were applied to identify phytochemicals in Cajanus cajan. The phytochemical screening revealed the presence of saponins, tannins, alkaloids, flavonoids, anthraquinones, and reducing sugars. However, terpenoids and cardiac glycosides were not present in several components of the plant (Sahu, Verma, & Haris, 2014). The plant's significant medicinal effects may be linked to the high levels of flavonoids found in its leaves and seeds. Flavonoids, being potent biological antioxidants, contribute to the management of cardiovascular diseases and oxidative stress. They play a key role in supporting the body’s antioxidant defense system, working synergistically with other antioxidants, vitamins, and enzymes to protect against these health conditions. Because of their antioxidant and anti-inflammatory capabilities, flavonoids have significant anti-mutagenic and anti-carcinogenic qualities (Aja,Alum, Ezeani, Nwali, & Edwin, 2015). The phytochemical tests revealed that Cajanus cajan had a significant quantity of tannins. This suggests that the leaves and seeds of Cajanus cajan contain anti-bacterial capabilities. According to Carson and Riley, tannic acid is thought to have anti- bacterial and astringent effects that act on mucosal tissue—tannic indigestion on the tongue and within the mouth.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

Constipation is caused by health acids, which may also be used to treat diarrhoea. Tannins are polyphenols derived from diverse portions of plants belonging to various species. Tannins can also help reduce haemorrhages and limit naked swellings (Amaral et al., 2017). High levels of saponins indicate that the leaf of *Cajanus cajan* is an excellent source of saponins. Saponins, which bind to bile salts, have been linked to regulating excessive cholesterol levels. Bile salts combine with cholesterol to produce tiny micelles that aid in its absorption. Saponins lower blood cholesterol levels by blocking their reabsorption. According to Schneider and Woliling, saponins limit sodium ion (Na) efflux by blocking the entry of Na+ out of the cell (IDOSR-JAS-21-59-75.Pdf, n.d.). This increases the concentration of Na+ in the cells by activating the Na+ Ca2+ anti-porter in cardiac muscles, which enhances heart muscle contraction, according to Rausch et al. Saponins contain antioxidant, anti-inflammatory, anti-apoptosis, and immunostimulant activities. (Amaral et al., 2017)

- *Flavonoids from Canjanus cajan*

Flavonoids are a vast collection of polyphenolic chemicals generated by plants that serve critical functions in plant health, development, and growth (Falcone Ferreyra, Rius, & Casati, 2012). Flavonoids can operate as phytoalexins, photoprotectors, and nod inducers for nitrogen-fixing bacteria in Leguminosae. Flavonoids have been demonstrated to impact invertebrate pest species' eating behavior (Green, Stevenson, Simmonds, & Sharma, 2003), and flavonoid profiles have also been utilised in plant chemotaxonomy to unravel evolutionary connections. In the literature, 27 flavonoids representing seven flavonoid groups have been reported. There was no evidence of flavanols (catechins/flavan-3-ols) or aurones reported from pigeon pea, nor was there any investigation of flavonoids from pigeon pea flowers (Nix, Paull, & Colgrave, 2015).

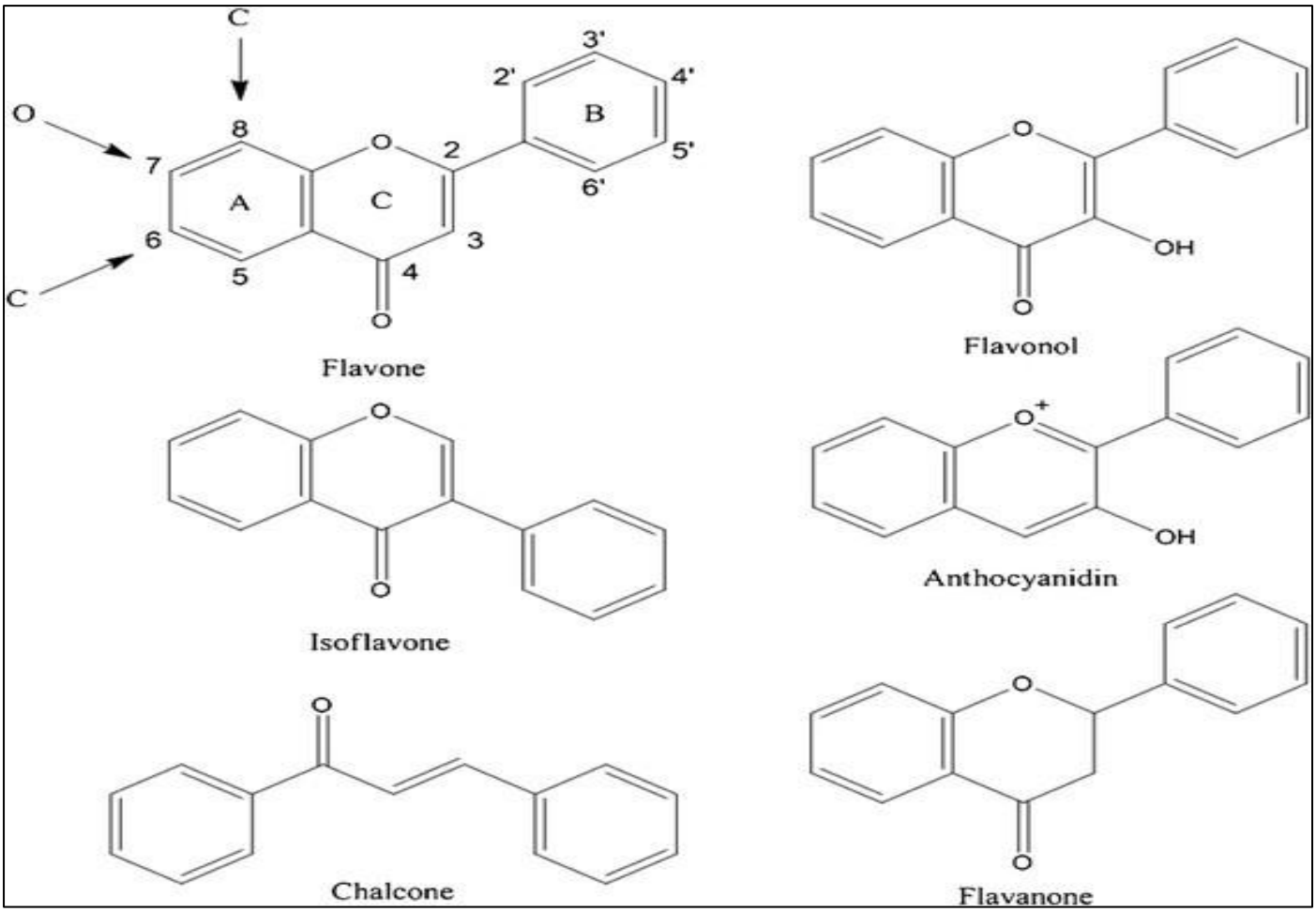

Fig 4 Flavonoids Compounds from *Canjanus cajan*

- *Compound reported Isolated from cajanus cajan*

Studies on the chemical components of *C. cajan* leaves revealed that they are abundant in flavonoids and stilbenes. Additionally, they have saponins, a notable amount of tannins, and modest amounts of reducing sugars, resins, and terpenoids (Pal, Mishra, Sachan, & Ghosh, 2011). According to chemical investigations, compounds with antioxidant capabilities include 2'-2' methyl cajanone, 2'-hydroxy genistein, isoflavones, cajanin [Figure 1], cahanones, etc. Genistein and genistin have also been discovered in roots (Kong et al., 2010). It also contains substances that have anticancer activity, such as hexadecanoic acid, amyrin, -sitosterol, pinostrobin [Figure 2], longistylin A [Figure 3], and longistylin C [Figure 4]. Cajanuslactone, a coumarin that is present, contributes to antibacterial activity. Antispasmodic activity is caused by cajaninstilbene Acid

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

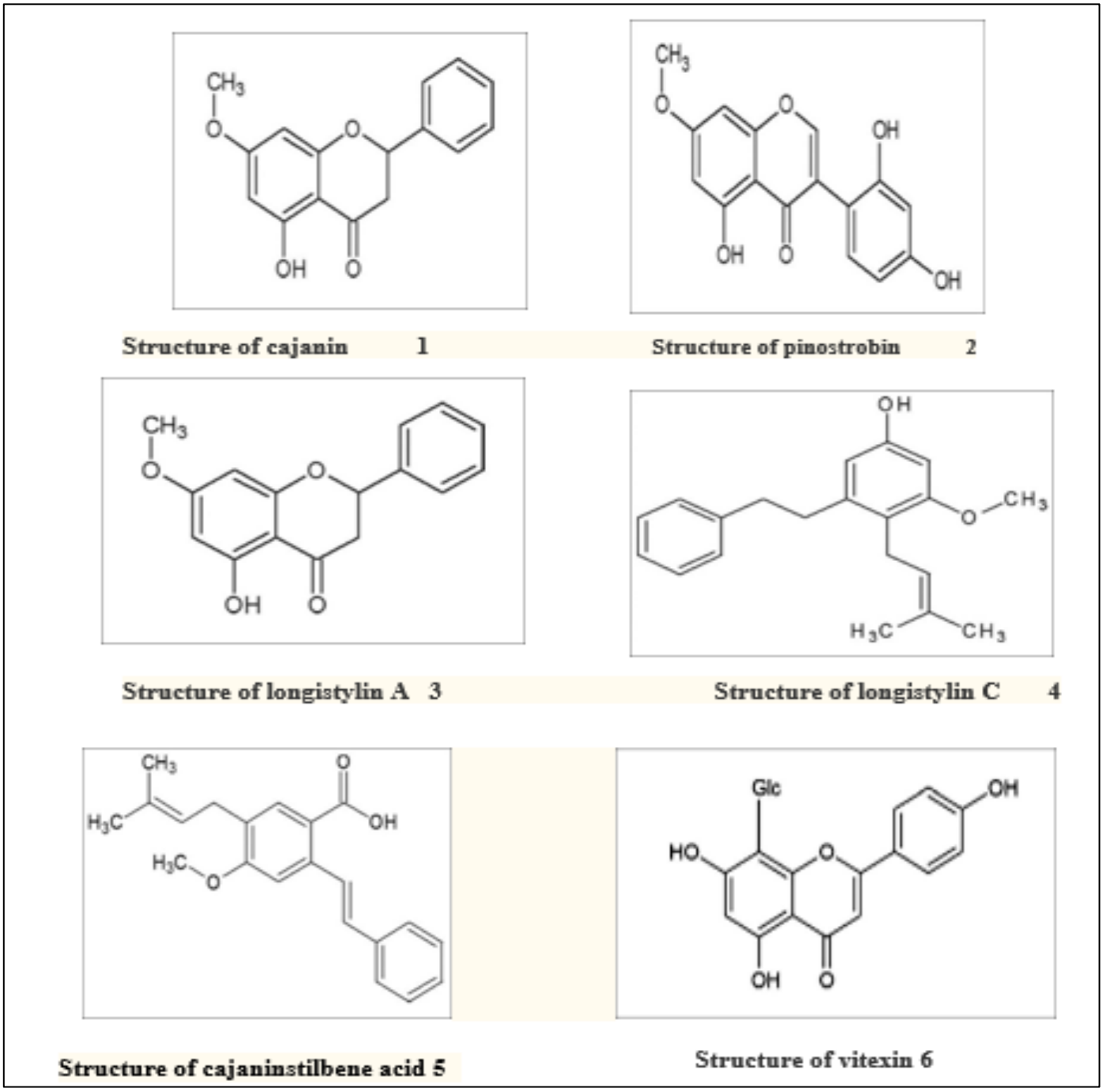

Fig 5 Components 5

- *Volatile Compounds from The Seed Oil of Cajanus Cajan*

The essential oils of four Nigerian plant species, including Cajanus cajan, were extracted using hydrodistillation and analyzed with Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). The oils from Cajanus cajan were found to be rich in sesquiterpenes, accounting for 92.5%, 81.2%, and 94.3% in the leaves, stem, and seeds, respectively (Ogunbinu, Flamini, Cioni, Adebayo, & Ogunwande, 2009). Bioactivity-guided fractionation of Cajanus cajan extracts led to the identification of eight compounds, including betulinic acid, biochanin A, cajanol, genistein, 2′-hydroxy genistein, longistylin A and C, and pinostrobin (Duker-Eshun, Jaroszewski, Asomaning, Oppong-Boachie, & Brøgger Christensen, 2004). Additionally, Solvent-Free Microwave Extraction (SFME) of essential oil from pigeon pea leaves revealed that the main constituents were sesquiterpenes (72.89%), with notable components such as α-copaene (5.89%), β-caryophyllene (7.46%), α-himachalene (12.97%), α-humulene (17.43%), alloaromadendrene (8.45%), and α-bisabolene (12.64%) (Qi et al., 2014).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

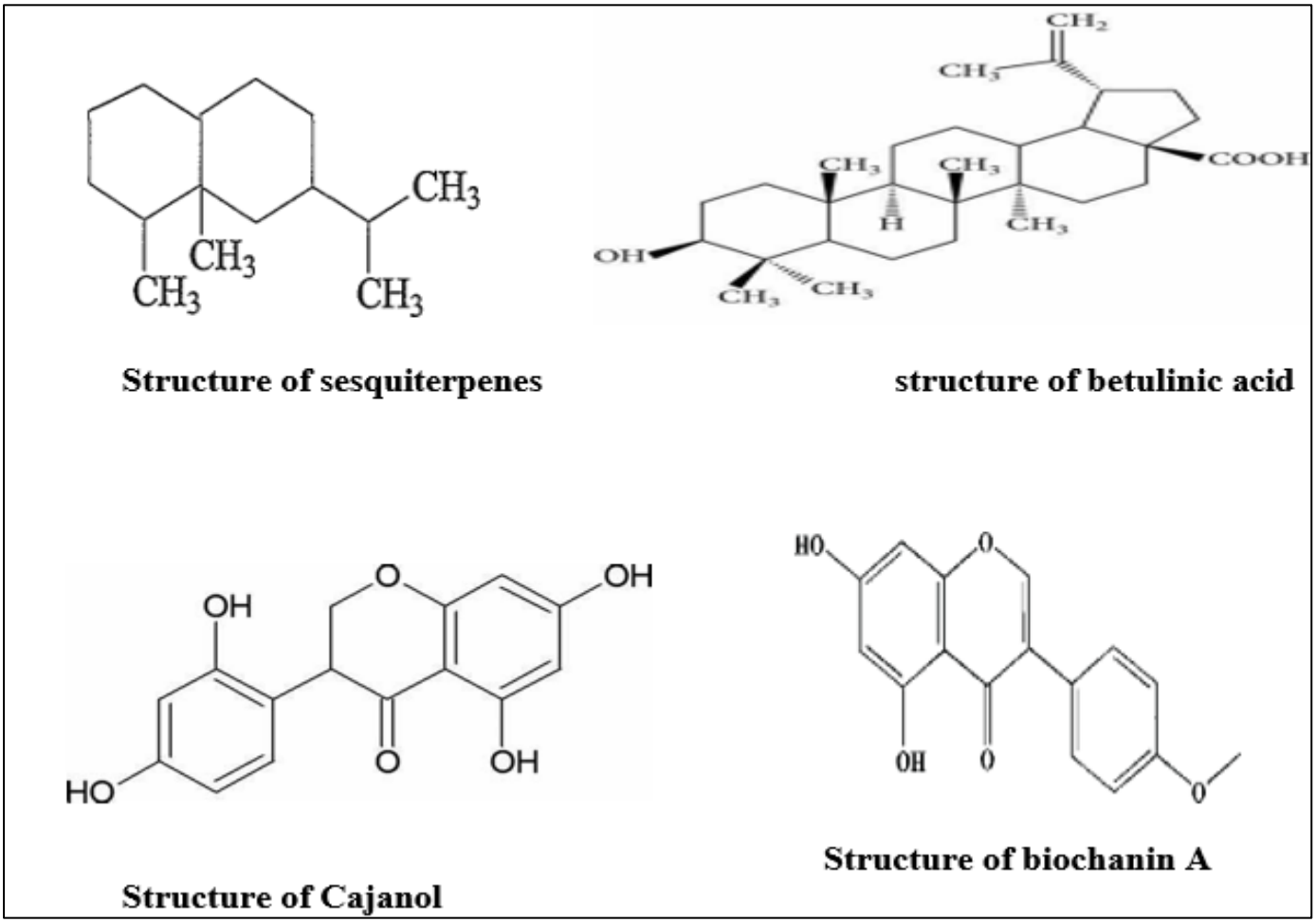

Fig 6 Components 6

➢ *Biological activities of some compounds of Cajanus cajan*

Various compounds have been identified in *C. cajan* over the past few decades, and some have excellent biological activities. From the leaves of C. *cajan,* a brand-new natural coumarin, cajanuslactone, a potential antibacterial agent against Gram-positive microorganisms, has been discovered. It has been discovered that the three stilbenes, cajanin, longistylin C, and longistylin A, have hypocholesterolemic effects (Luo, Sun, Si, & Chen, 2008). Betulinic acid derived from roots and longistylin A and C taken from leaves have both been proven to have anti-plasmodial properties. Pinostrobin, a leaf-derived substituted flavanone, has anti-inflammatory properties and prevents sodium channel-activated depolarization of synaptoneurosomes in the mouse brain (Nicholson, David, Pan, & Liu, 2010). Genistein and genistin, two isoflavonoids extracted from the roots, were discovered to have antioxidant action. Cajanol, an isoflavone discovered in the roots, has been demonstrated to have anticancer properties. Major antioxidant activities were discovered in four significant components, including pinostrobin, cajaninstilbene acid, vitexin, and orientin, which were extracted from ethanolic preparations of leaves. Significant antibacterial activity was also demonstrated by isoflavonoids that were extracted from the ethanolic extract of leaves (Zu et al., 2010). A portion of the protein fraction isolated from leaves also showed hepatoprotective effects, and flavonoids and tannins, which are phenolic compounds, give rise to anthelmintic activity.

- *Antimicrobial activities reported for Cajanus cajan.*

Cajanus Cajan extracts were found to have antibacterial action against eight different microbial species, including *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger,* and *Candida albicans* (Abo-Zeid, Abdel-Samie, Farghaly, & Hassan, 2018). The SFE extracts of *C. cajan* had a strong inhibitory impact on *S. epidermidis, S. aureus*, and *B. subtilis*. In vivo, antibacterial activity was shown in mice infected with *Staphylococcus aureus,* and histopathology was used to study the mechanism by which the plant extract operates on these germs. The antimicrobial activities of *Cajanus Cajan* ethanol extracts and supercritical fluid extraction extracts were evaluated, and the plant extracts demonstrated significant antimicrobial activities both *in vivo* and *in vitro*, suggesting that this could be a potential candidate for the treatment of *S. aureus* and may also work against MRSA. (Mona *et al*., 2018). In a study conducted by Qi et al.., The essential oil obtained using solvent-free microwave extraction and hydrodistillation showed remarkable antibacterial activity against gram-positive and gram-negative bacteria, *B. subtilus*, and *P. acnes*(Ijpsr, H., & Mathad, 2017).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

- *Anti-plasmodial and Anti-malarial activity of Cajanus cajan*

The pigeon pea plant (*Cajanus Cajan*) contains two key compounds: logistic A, C, and betulinic acid, all of which have anti-plasmodial properties and perform effectively against Plasmodium falciparum. Extracts from the roots and leaves of the pea plant have shown fairly strong *in vitro* activity against P. falciparum, the principal causative agent of malaria("Therapeutic Molecules for Multiple Human Diseases Identified from Pigeon Pea (Cajanus Cajan L. Millsp.) through GC–MS and Molecular Docking - ScienceDirect," n.d.).

- *Antibacterial Activity of Cajanus cajan*

Extracts of C.cajan leaves were used in bioassay-guided chloroform fractionation to isolate novel natural coumarins: Cajanuslactone as well as two phytoalexins: Cajaninstilbene acid and pinostrobin(Sarkar, Hazra, Mandal, Biswas, & Mandal, 2009a). Cajanuslactone was shown to have strong antibacterial action against the pathogen *S. aureus*. Pigeon pea leaf extracts have been demonstrated to be beneficial against certain pathogenic bacteria, with test results indicating that the extract may efficiently limit the multiplication of the bacterium Salmonella Thypi. Typhoid is an infectious illness caused by the gram (-ve) bacterium *Salmonella thypi*, and it is a major health problem in many impoverished nations. *C. cajan's* main constituents are divided into two groups: stillbene and flavonoids, and the extract of this plant may effectively suppress the development of *S. aureus, S. thypi,* and *E. coli* (Zhang et al., 2010).

- *Antihelmintic Activity of Cajanus cajan*

The HA (hydro-alcoholic) extraction from the aerial sections of *Cajanus cajan* was studied for anthelmintic capabilities using the adult earthworm (Pheretima posthuma) found in India since their morphological and physiological traits are similar to intestinal parasites and roundworms (Pal, Sahoo, & Mishra, 2005). This feature was considered to exist due to the presence of phenolic compounds such as flavonoids and tannins, which have anti-helmintic properties. *C.cajan* seed extracts have a high concentration of bioactive chemicals with anti-helminthic properties(Das et al., 2018)

- *Anticancer Activity of Cajanus cajan*

Cajanol, an isoflavone found in *C. cajan* roots, is an important phytoalexin. Cajanol's anticancer activities in MCF-7 human breast cancer cells were investigated and tested (Nicholson et al., 2010). Other parameters such as DNA fragmentation assay, cell cycle distribution and morphological assessment of nuclear change, mitochondrial membrane potential disruption, reactive oxygen species (ROS) generation and caspase-3 and caspase-9 expression, Bcl-2, PARP, and cytochrome- C expression were quantified to determine the mechanism of cajanol cell growth inhibition (Nicholson *et al.*, 2010).

Cajanol has been shown to decrease MCF-7 cell proliferation in a time- and dose-dependent manner. Cajanol has been shown to stop the cell cycle in the G2/M phase and to trigger apoptosis via a mitochondria-dependent reactive oxygen species (ROS) route. Cajaninstilbene acid, derived from Pigeon Pea (*Cajanus cajan*), is structurally similar to estrogens and possesses anti-estrogenic activities, suggesting therapeutic actions against breast cancer 12 cells. *Cajanus Cajan* methanol extract has strong cytotoxicity against various cancer cell lines (Qi et al., 2014)

- *Anti-Mutagenic properties of Cajanus cajan*

Cajanus cajan contains a variety of phytochemical compounds, including tannins, reducing sugars, anthraquinones, triterpenoids, alkaloids, phenols, saponins, and flavonoids. Notably, the flavonoid fraction of Cajanus cajan has shown enhanced cytotoxic and genetic effects in animals induced with mutagenic agents, highlighting its potential therapeutic properties (Titanji et al., 2008). One of the key flavonoids derived from C. cajan is quercetin, which has been found to be a potent agent against mutagen-induced cells. It provides protection against DNA damage and chromosomal alterations in both germ and somatic cells, offering potential as a protective agent in mutagenic conditions, such as in rat hepatic cells (Titanji et al., 2008).

- *Hepatoprotective Effects of Cajanus cajan.*

The hepatoprotective efficacy of *Cajanus cajan* methanol extracts has been evaluated in Swiss albino mice with liver damage induced by carbon tetrachloride (CCl4). The extract exhibited a mild protective effect by significantly reducing serum levels of liver enzymes, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as cholesterol levels (Chen et al., 1985). Additionally, the methanol-aqueous fraction (MAF2) of the leaf extract demonstrated protective effects against alcohol-induced liver damage in rats. Co-administration of MAF2 led to a reduction in liver enzyme activity while enhancing the activity of antioxidant enzymes, indicating its potential in the treatment of alcohol-induced liver damage (Chen et al., 1985).

- *Antioxidant activities reported for Cajanus cajan*

The antioxidant properties of *C. cajan* leaves were determined using the 2, 2-diphenyl-1- picrylhydrazyl (DPPH) radical-scavenging assay and the -carotene-linoleic acid test on water, ethanol, ethyl acetate, and petroleum ether extracts. Those tests were also performed on cajaninstilbene acid (3-hydroxy-4-prenylmethoxystilbene-2-carboxylic acid), Pinostrobin, vitexin,and orientin, which are the four major components of *C. cajan* ethanol extract. Following the completion of the experiments, it was discovered that the leaf extracts of C.cajan can be useful natural antioxidants that are likely useable as medication and can be employed in the food or health industries(Zhang et al., 2011). Negative pressure cavitation extraction (NCPE), a novel technique, was proposed, as was the extraction of the principal isoflavonoids of *C. cajan,* genistein and genistin. This technique demonstrated significant

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

concentration-dependent antioxidant activity. Syringol, also known as 2, 6-dimethoxyphenol, appears to have anti-oxidative effects and has been demonstrated to decrease oxidative stress (Sarkar, Hazra, Mandal, Biswas, & Mandal, 2009b).

Previous research has discovered stilbenes, flavones, coumarins, and phytosterols in *C. cajan* leaves, which appear to have anti-oxidant characteristics. Organic compounds, phenolics, fatty acids, amino pyrimidines, tripeptides, and phytohormones are some of the major metabolites found in *C. cajan* plants that exhibit anti-oxidant and iron-chelating action. In the study, Wang et al. (2015) extracted a huge quantity of pure flavonoids and stilbenes from *C. cajan* using several extraction strategies to discover whether the methodology offers more robust anti-oxidant capabilities. Negative-pressure cavitations and aqueous two-phase extraction tend to maximise the production of flavonoids and stilbenes, resulting in relatively significant anti-oxidant activity(Aggarwal, Nautiyal, & Negi, 2015).

- *Anti-Diabetic Activities Reported for Cajanus cajan*

In alloxan-diabetic and oral glucose-loaded rats, the anti-diabetic effect of methanol leaf extract of *Cajanus cajan* (L.) (Fabaceae) was investigated. The extract's acute toxicity and lethality (LD50) and phytochemical analyses were also assessed. The extract dramatically lowered fasting blood sugar levels in alloxan diabetic rats in a dose-dependent manner, with the most significant hypoglycemic impact occurring between 4 and 6 hours. The extract also reduced the peak postprandial rise in blood glucose levels in normal rats by 101.8 and 57.40%, respectively (Ezike, Akah, Okoli, & Okpala, 2010). The data suggest that the leaves of *C. cajan* might be helpful as an anti-diabetic medication. The extract induced significant and dose-related hypoglycemia effects in diabetic rats, implying a beneficial effect in DM. Alloxan monohydrate causes diabetes by destroying the cells of the Islets of Langerhans, resulting in impaired insulin production and hyperglycemia. As a result, the extract may function via mechanism(s) that enhance/boost insulin secretion. Furthermore, the extract's effective and prolonged decrease in blood glucose levels in diabetic rats suggests that it may be therapeutic in overt instances of DM ("Animal Models to Test Drugs with Potential Anti-diabetic Activity - ScienceDirect," n.d.).

- *Anticancer Activities Reported for Cajanus cajan*

Cajanol, an isoflavone found in the roots of *C. cajan*, is a significant phytoalexin. Cajanol's anticancer activities in MCF-7 human breast cancer cells were examined and tested (Nicholson et al., 2010). Other parameters such as DNA fragmentation assay, cell cycle distribution and morphological assessment of nuclear change, mitochondrial membrane potential disruption, reactive oxygen species (ROS) generation and caspase-3 and caspase-9 expression, Bcl-2, PARP, and cytochrome-C expression were quantified to determine the mechanism of cajanol cell growth inhibition. (Nicholson et al., 2010). Cajanol has been shown to decrease MCF-7 cell proliferation in a time and dose-dependent manner (Nicholson et al., 2010). Cajanol has been shown to stop the cell cycle in the G2/M phase and to trigger apoptosis via a mitochondria-dependent reactive oxygen species (ROS) route (Nicholson et al., 2010). Cajaninstilbene acid, derived from Pigeon Pea (*Cajanus cajan*), is structurally similar to oestrogens and has cytotoxic effects on the oestrogen alpha receptor. It also has anti-estrogenic activities, making it a possible treatment for breast cancer.

- *Antihypertensive Reported for Cajanus cajan*

Oxidative stress is a critical mechanism in many chronic illnesses and can be managed to slow disease development. Pigeon pea protein hydrolysates' free radical scavenging, lipid peroxidation inhibitory and ferric-reducing properties imply that they can reduce tissue oxidative stress. Furthermore, ACE and renin inhibitors can be studied further as natural medicines for treating hypertension, a significant risk factor for stroke. The multifunctionality of pigeon pea peptides allows for the development of functional foods and nutraceuticals with potential applications in the prevention and treatment of chronic illnesses (Olagunju, Omoba, Enujiugha, Alashi, & Aluko, 2018).

Legumes are high-protein sources in the human diet, and their consumption has been linked to preventing chronic illnesses due to their bioactive components. *Cajanus cajan* (pigeon pea) is an underutilised legume with a high protein content (24%). Enzymatic hydrolysis using pepsin and pancreatin produced protein hydrolysates from pea isolate. Hydrolysates were tested for amino acid content, antioxidant capabilities, and antihypertensive characteristics in vitro and in vivo. The hydrolysates had a significant concentration of hydrophobic amino acids, particularly isoleucine and phenylalanine (Bravo, Angelia, Uy, Garcia, & Torio, 2022)

- *Herbal formulations from Cajanus cajan*

The global healthcare business is likewise shifting towards herbal medications. The study's primary goal was to create a stable and functionally effective toothpaste without using any synthetic ingredients commonly included in such formulations. Based on their findings, they concluded that the formulated toothpaste by *Cajanus cajan* leaf extract possesses all of the desirable characteristics of an ideal toothpaste compared to marketed close-up toothpaste compared to all evaluation parameters. Toothpaste from *Cajanus cajan* leaf extract, such as moisture, volatile matter, cleaning ability, spreading, PH, abrasiveness, and gritty matter, was found harmless, more effective, and economical than synthetic close-up marketed toothpaste. (Malgi, Mane, Kumar, Paramshetty, & Kobanna, n.d.)

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

CHAPTER THREE
MATERIALS AND METHODS

➢ *Source of C. Cajan Seed Extract.*

The *Cajanus cajan* seed (400 g) was extracted using ethanol, resulting in the collection of 9.58 g of crude ethanol extract. This extract was sourced from the African Centre of Excellence for Drug Research, Herbal Medicine Development, and Regulatory Science (ACEDHARS) at the University of Lagos. This ethanol extract may have various bioactive components, which could potentially contribute to its medicinal properties.

➢ *Solvent-Solvent Partitioning of C. Cajan Seed Extract.*

The oily *C. cajan* seed ethanol extract was partitioned into various solvents using the Modified Kupchan Partition (Rashid, Amran, & Hossain, 2017) mentioned below.

- *Preparation of Aqueous Solution*

The oily *Cajanus cajan* seed ethanol extract was dispersed in 100 mL of distilled water to form an aqueous solution. This solution was then partitioned consecutively with three solvents of varying polarities. Following this partitioning process, each of the fractions obtained was analyzed individually for the detection and identification of pharmacologically active medicinal phytochemicals. This method allows for the separation of bioactive compounds based on their solubility and polarity, which can aid in pinpointing specific compounds responsible for therapeutic effects.

- *Partitioning with n-hexane*

The aqueous solution was extracted with 100 mL of n-hexane using a separatory funnel. The solvent eventually formed two layers in the separatory funnel in which the aqueous part was at the bottom and the non-aqueous solvent formed the upper layer. The procedure was done three times (100 mL x 3) until the upper layer became clear.

- *Partitioning with ethyl acetate.*

The aqueous solution was extracted with 100 mL of ethyl acetate using a separatory funnel. The solvent eventually formed two layers in the separatory funnel in which the aqueous part was at the bottom and the non-aqueous solvent formed the upper layer. The procedure was done three times (100 mL x 3) until the upper layer became clear.

- *Partitioning with dichloromethane*

The aqueous solution was extracted with 100 mL of dichloromethane using a separatory funnel. The solvent eventually formed two layers in the separatory funnel in which the aqueous part was at the bottom and the non-aqueous solvent formed the upper layer. The procedure was done three times (100 mL x 3) until the upper layer became clear.

➢ *Thin Layer Chromatography (TLC) Analysis*

Layer Chromatography was used to separate the chemical constituents of the *C. cajan* seed extract using aluminium-backed TLC plates. The ethanol crude extract of *C. cajan* was dissolved in a volatile solvent and fractionated into different fractions by the separatory funnel. A thin start line of about 1-2 cm was drawn from the bottom of the paper with a soft pencil. The plates were spotted with each of the fractions including the crude extracts of *C. cajan* (N-hexane, ethyl acetate, aqueous and the crude) using a micro-syringe and air-dried. The TLC plates were developed with two eluent systems, chloroform/methanol (9:1) and hexane/chloroform (9:1). Development of the chromatograms was done in a closed chromatographic tank containing eluent. The tank was covered for saturation of the eluent to take place in the developing tank. The edge of the plate bearing the sample spot was dipped in the mobile phase in the tank. The mobile phase travels up the paper by capillary action; reaches the sample spots and moves the components of the samples along. The sample components partition themselves between the two (2) phases depending on their polarity difference. The solvent front was marked and visualization was done with UV light at 256 and 266 nm. The formula below was used to measure the retention factor (R*f*) which is the distance the compound travelled to the distance the solvent travelled.

$$R\,f = \frac{\text{distance moved by the compound}}{\text{Distance moved by the solvent}}$$

➢ *Fourier-Transformed Infrared Spectroscopy (FTIR) of C. cajan seed extracts*

The equipment used was Agilent Cary 630 ATR-FTIR. A sample was loaded and a spectrum was collected using the Diamond ATR accessory. The procedure: The sample press knob was turned in a counterclockwise direction to open the sample press so that the tip is elevated slightly from the diamond sampling window. A small amount of material to be measured was placed on the diamond crystal. The crystal is the clear, circular-shaped material held in place by the surrounding metal disk. It was ensured that the sample covered the entire surface area of the diamond crystal.

➢ *Gas Chromatography-Mass Spectrometry (GC-MS) Analysis*

The GC-MS analysis of the hexane extract of *Cajanus cajan* was carried out using an Agilent 5977B GC/MSD system coupled with an Agilent 8860 auto-sampler. The Gas Chromatograph was interfaced with a Mass Spectrometer, equipped with an Elite-5MS

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

fused silica capillary column (30 × 0.25µm ID × 0.25 µm df) composed of 5% diphenyl/95% dimethyl polysiloxane. For GC-MS detection, an electron ionization system was utilized in electron impact mode, with an ionization energy of 70 eV. Helium gas (99.999%) served as the carrier gas, maintained at a constant flow rate of 1 mL/min, and a 1µL injection volume was employed with a split ratio of 10:1.

The injector temperature was set to 300 °C, while the ion-source temperature was maintained at 250 °C. The oven temperature was programmed starting at 100 °C (isothermal for 0.5 min) and then increasing at a rate of 20 °C/min up to 280°C, which was held for 2.5 minutes. Mass spectra were recorded at 70 eV, with a scanning interval of 0.5 seconds and mass fragments ranging from 45 to 450 Da. The solvent delay was set to 0 to 3 minutes. This method allows for detailed identification and characterization of the bioactive compounds present in the hexane extract based on their retention time and mass spectra.

➢ *High-Performance Liquid Chromatography (HPLC) Analysis*

- *Preparation of Standard Solutions*

✓ **Method A.**

Standards: Formononetin, Rutin, Magnoflorine, Luteolin and Quercetin.

A 5 mg of each of the above standards was weighed and dissolved in 5 mL of methanol to get 1000 µg/mL separately. Exactly 2 mL was taken from each standard and added together to get 10 mL; this gave 1:5 dilutions for each standard. This gave us 200 µg/mL for each standard in the Mixed Standard. Graded concentrations were prepared in 20, 40, 60, 80 and 100 µg/mL from the stock concentration of 200 µg/mL.

Each of the Mixed Standard Concentrations was poured into vials and arranged in Autosampler trays where 10 µL was injected in duplicate into the HPLC.

✓ **Method B.**

Standards: Gallic Acid, Magnoflorine, Luteolin and Pinostrobin.

The method A procedure was also used for B. The only difference is method B has four components, so 2 ml was taken from each of the four standards (1000 µ g/mL) to get 8 ml and 2 mL of methanol was added to make up to 10 ml. This also gave 200 µg/mL. Graded concentrations were also prepared in 20, 40, 60, 80 and 100 µg/ml from the stock concentration of 200 µg/mL.

- *Preparation of Sample Solutions*

To prepare the sample for further analysis, a total of 0.5 mL of the sample was added to 4.5 mL of a 40% methanol solution, achieving a serial dilution of 1:10. The mixture was then subjected to ultrasonic extraction for 20 minutes to ensure efficient extraction of the bioactive compounds. After ultrasonic extraction, the sample was cooled to room temperature. To remove any solid particles or impurities, the sample solution was filtered through a 0.45 µm microporous membrane, ensuring that only the liquid phase, containing the desired compounds, was collected for subsequent analysis. This process enhances the clarity of the sample and prepares it for precise chromatographic or spectroscopic measurements.

- *HPLC Conditions*

✓ **METHOD A**

Mobile phase - Methanol: Acetonitrile: Water (40: 15: 45) containing 1% Acetic acid. Column - C18 150 × 4.6mm 5 µm. Wavelength – 257 nm Temperature - Ambient Flow rate – 1 mL/min Injection – 10 µL

▪ *Note: Method a is Isocratic.*

✓ **METHOD B**

Mobile phase - Acetonitrile: TFA (100: 0.5) MOBILE PHASE A. Acetonitrile: Water: TFA (20: 80: 0.01) MOBILE PHASE B.Column - C18 150 × 4.6 mm 5 µm. Wavelength – 260 nm

Temperature - Ambient Flow rate – 1 mL/min Injection – 10 µL

▪ *Note: Method B is the Gradient Method.*

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

CHAPTER FOUR
RESULTS

➢ *Percentage Yield of C. cajan*

The percentage yield of the ethanol extract of *C. cajan* was calculated from the weight of the plant seed used for extraction (400 g) and the crude extract obtained (9.58 g) gave a 2.40 % yield.

➢ *Solvent-solvent partitioning of C. cajan Seed Extract*

After the evaporation of the solvents, the weights of the different fractions obtained are given in Table 1.

Table 1 Weight of Fractions Obtained from the Crude Extract of Oily *Cajanus cajan* Seed

Fractions	Weight	%Yield compared to seed used (400 g)
n-Hexane fraction	1.2115g	0.30%
Ethyl acetate fraction	1.8224g	0.46%
Dichloromethane fraction	1.4314g	0.36%
Aqueous fraction	2.6188g	0.65%

➢ *Thin Layer Chromatography Analysis of Cajans Cajan Seed Extracts*

The Thin Layer Chromatography (TLC) analysis of Cajanus cajan seed extracts revealed interesting results for the different fractions. Here's a summary of the findings based on the TLC chromatogram:

- Ethyl Acetate Fraction: The TLC plate developed in chloroform/methanol (9:1) showed four distinct spots with Retention Factors (Rf) values of 0.640, 0.681, 0.855, and 0.913. This indicates that the ethyl acetate extract contains multiple compounds with varying polarities, as evidenced by the number and position of the spots.
- Hexane Fraction: The hexane fraction showed two spots with Rf values of 0.860 and 0.887. These spots suggest that non-polar or less polar compounds are present in the hexane extract, which is consistent with the use of hexane as a solvent for extracting non-polar compounds.
- Crude Extract: The crude extract displayed only one spot with an Rf value of 0.905. This suggests that the crude extract may contain a dominant compound, or the other compounds may have been less detectable or had similar properties under the experimental conditions.
- Aqueous Fraction: No spots were observed in the aqueous fraction, indicating that the polar compounds in the extract were likely not retained in the aqueous phase under the conditions used.

The elution with chloroform/methanol (9:1) worked well for separating compounds in the ethyl acetate fraction, while the hexane/chloroform (9:1) eluent did not result in any visible spots, possibly due to the low polarity of the compounds present or the conditions not being suitable for their separation.

These results highlight the diversity of compounds extracted from C. cajan seeds and provide insight into the potential bioactive substances present in different solvent fractions. The ethyl acetate fraction, with its multiple spots, may be of particular interest for further bioactivity testing and compound isolation.

Fig 7 TLC plates showing partitioning of phytochemicals for chloroform/methanol (9:1) viewed under 256 nm (UV).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

➢ *FTIR Analysis Results*

The FTIR results for the Aqueous, ethyl acetate and dichloromethane are presented in the sub- sections below:

- *FTIR Results for the Aqueous Fraction of Cajanus Cajan*

The FTIR result of *Cajanus cajan* aqueous extract gave a total of seven (7) peaks between 3332.24 cm-1 to 1085.6 cm^{-1}. The Peak at 3332.24cm^{-1} which is broad and intense indicates the presence of O-H stretch which is hydrogen bonded. The peak at 2180.49cm^{-1} suggests the presence of C≡C stretch for alkyne or C≡N for nitriles. The sharp and intense peak at 1636.30cm^{-1} suggests the presence of C=O stretch from an ester. This is confirmed by the presence of a C-O peak at 1058.56 cm-1. A peak at 1401.47 suggests the presence of a C-H bend of methylene (Table and Figure 2). The spectrum suggests the presence of carbonyl compounds and nitriles.

Table 2 The IR Results for the Aqueous Fraction of *Cajanus cajan* Seed Crude Extract

Characteristic absorption range (cm^{-1})	**Functional Group**	**Functional Group Name**	**Phytochemical Class suggested**
1058.56	C-O stretch	Ester/ether	
1401.47	C-H bend	Methylene	
1636.30	C=O stretch	Carbonyl	Esters, flavonoids
1938.22	……..	……..	
2113.40	……..	……..	
2180.49	C≡C stretch or C≡NStretch	Substituted Alkyne Substituted Nitrile	Alkaloids
3332.24	O-H stretch	Alcohol	Alcoholic / Phenolic compounds

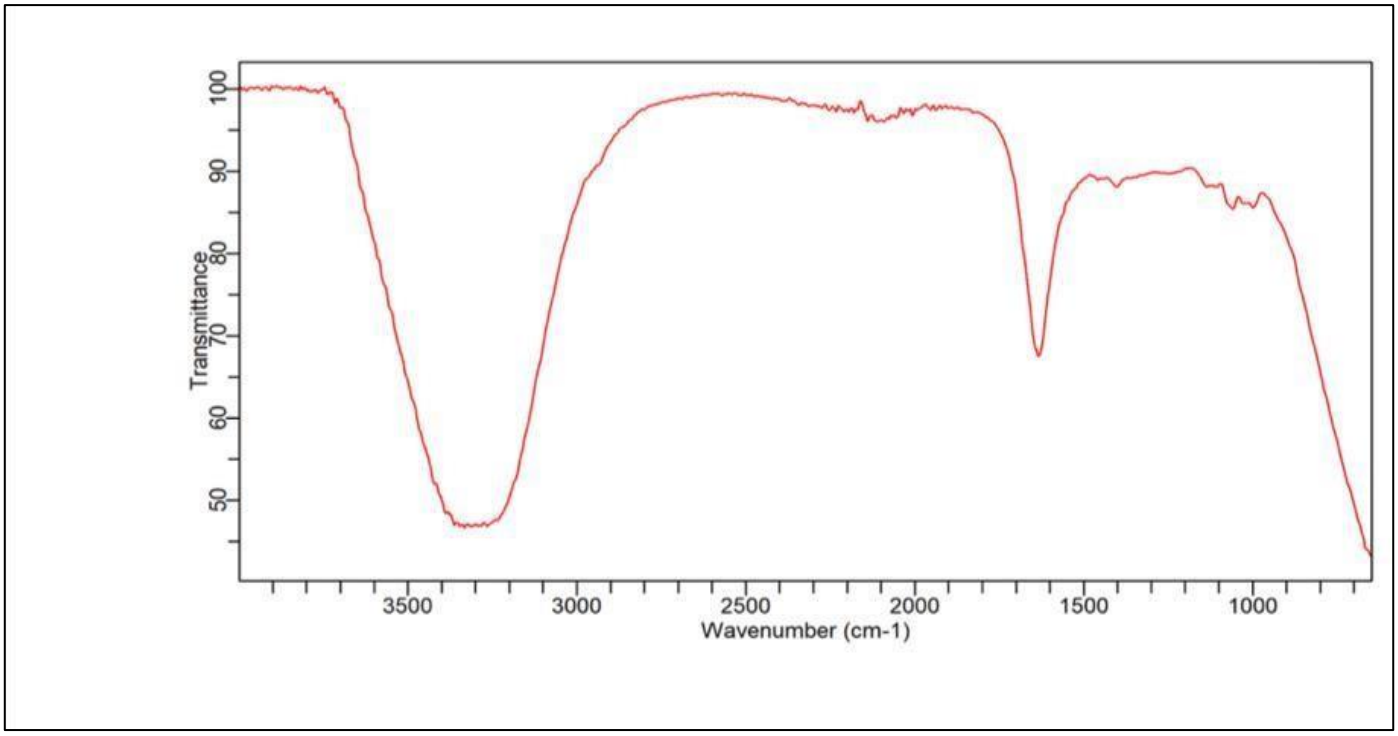

Fig 8 IR Spectrum of *Cajanus Cajan* Aqueous Extract

- *FTIR Results for the Ethyl Acetate Fraction of Cajanus cajan*

The FTIR result of *Cajanus cajan* ethyl acetate extract gave a total of 16 peaks between 3526.06 cm-1 to 723.10 cm^{-1}. The broad but non-intense peak at 3470.15cm^{-1} suggests the presence of a primary N-H stretch hydrogen bonded which is confirmed by a C-N stretch at 1162.92cm^{-1}. A sharp and intense peak at 2855.14 cm^{-1} suggests the presence of C-H stretch aldehyde, which is confirmed by a weak overtone C=O and sharp carbonyl C=O peak at 3440.33cm^{-1} and 1729.48 cm^{-1}. A very sharp and intense Peak at 2922.23 cm^{-1} shows the presence of a C-H stretch of alkane which is confirmed by the C-H bend of a methyl and methylene peak at 1379.11cm^{-1} and 1461.11cm^{-1}. Peak 1271.02cm^{-1} suggests the presence of the C-O stretch of ester. The peak at 1073.47cm^{-1} shows an S=O stretch of sulfonic acid. The spectrum of IR data in Table 3 is shown in Figure 3. The spectrum suggests the presence of alkaloids and probably some carbonyl compounds.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

Table 3 The IR Results for The Ethyl Acetate Fraction of *Cajanus Cajan* Seed Crude Extract

Characteristic absorption range cm-1	Functional Group	Functional Group Name	Phytochemical Class suggested
1073.47	S=O stretch	Sulfoxide	
1121.92	C-O stretch	Ether	
1162.92	C-N bend	Amine	Alkaloids
1271.02	C-O stretch		
1379.11	C-H bend	Methyl	
1461.11	C-H bend	Methylene	
1729.48	C=O stretch	Ketone	
2855.14	C-H stretch	Aldehyde	
2922.23	C-H Stretch	Alkane	
3440.33	C=O overtone	Ketone	
3470.15	N-H stretch	primary amine	Alkaloid
3526.06	Weak N-H	2º or 3º amine	Alkaloid

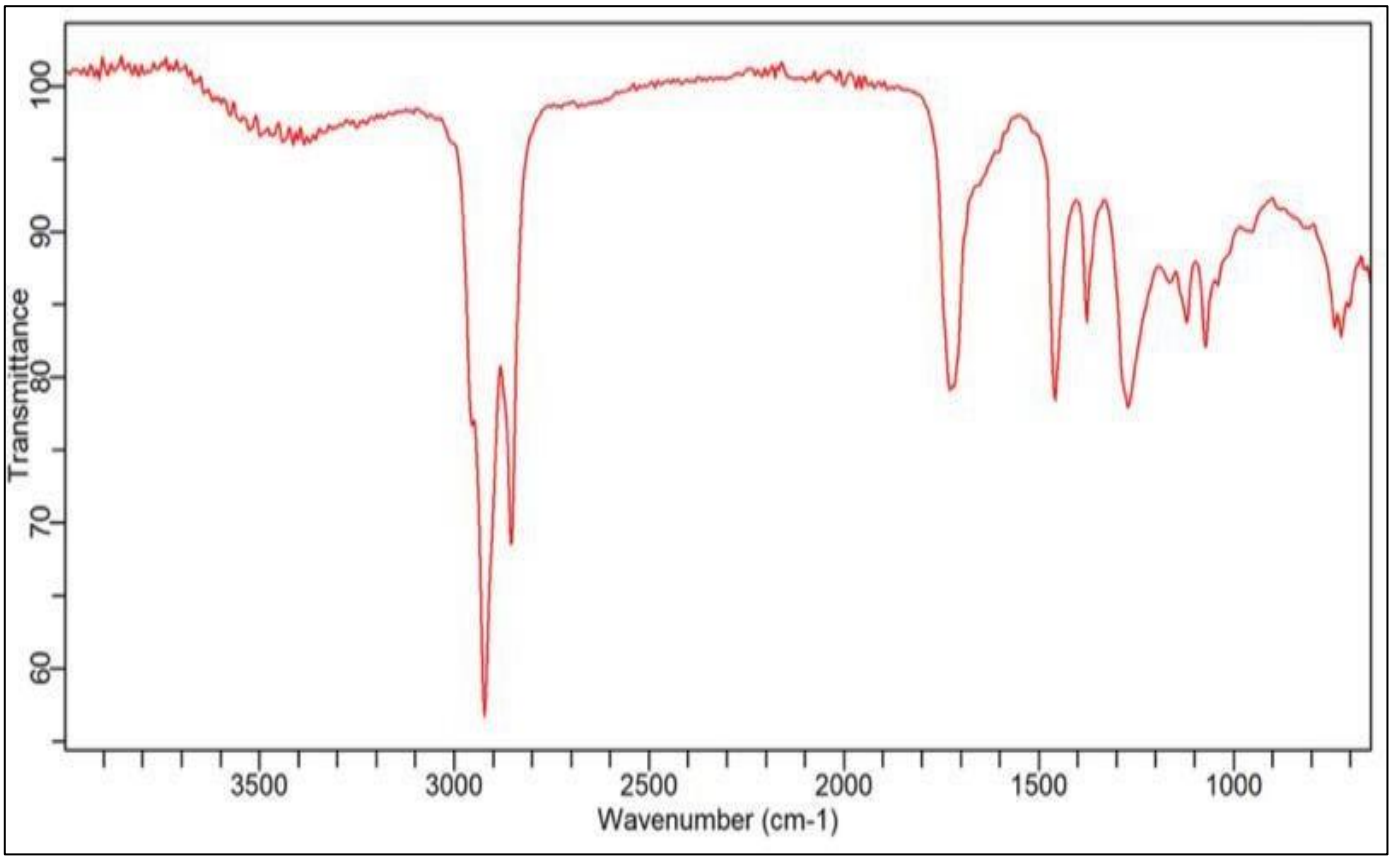

Fig 9 IR Spectrum of *Cajanus cajan* Ethyl Acetate Seed Extract

- *FTIR Results for The Dichloromethane Fraction of Cajanus Cajan*

The FTIR result of *Cajanus cajan* dichloromethane extract gave a total of 18 peaks between 3332.24cm^{-1} to 708.19cm^{-1}. The broad but non-intense peak at 3332.24cm^{-1} suggests the presence of alcohol O-H stretch which is hydrogen bonded. The peak at 2925.96cm^{-1} suggests the presence of a C-H stretch of alkane, which is confirmed by the C-H stretch of methyl and methylene bend at 1371.66cm^{-1} and 1446.20 cm^{-1}. The peak at 2855.14cm^{-1} suggests the presence of a C-H stretch of alkene which is confirmed by a C=C stretch at 1688.48cm^{-1}. The peak at 2109.67cm^{-1} shows a C-N Stretch substituted nitrile. The peak at 1252.38 cm^{-1} shows the presence of C-O phenol, which is confirmed by a C=C stretch at 1688.48 cm-1 and an O-H stretch at 3332.24 cm-1. The IR spectrum also showed the bands typical for aromatic compounds in the region of 1500-1600 cm^{-1}. The spectrum of IR data in Table 4 is shown in Figure 4 below. The spectrum indicates the presence of phenolic compounds

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

Table 4 The FTIR Results for the Dichloromethane Fraction of *Cajanus cajan* Seed Crude Extract

Characteristic absorption range cm^{-1}	Functional Group	Functional Group Name	Phytochemical Class suggested
826 to 761	Aromatic rings	Benzene/phenol	Phenolic compounds
1252.38	C-O stretch	Phenol	Phenolic compounds
1371.66	C-H bend	Methyl	
1446.20	C-H bend	Methylene	
1513.29	C=C	Aromatic band	Phenolic compounds
1602.75	C=C	Aromatic band	
1688.48	C=C stretch	Alkene	
1990.39	……..	……..	
2109.67	C-N Stretch	Substituted nitrile	
2855.14	C-H	Alkene	
2925.96	C-H Stretch	Alkane	
3332.24	O-H stretch	Alcohol	
3500	O-H	Phenol	Phenolic compounds

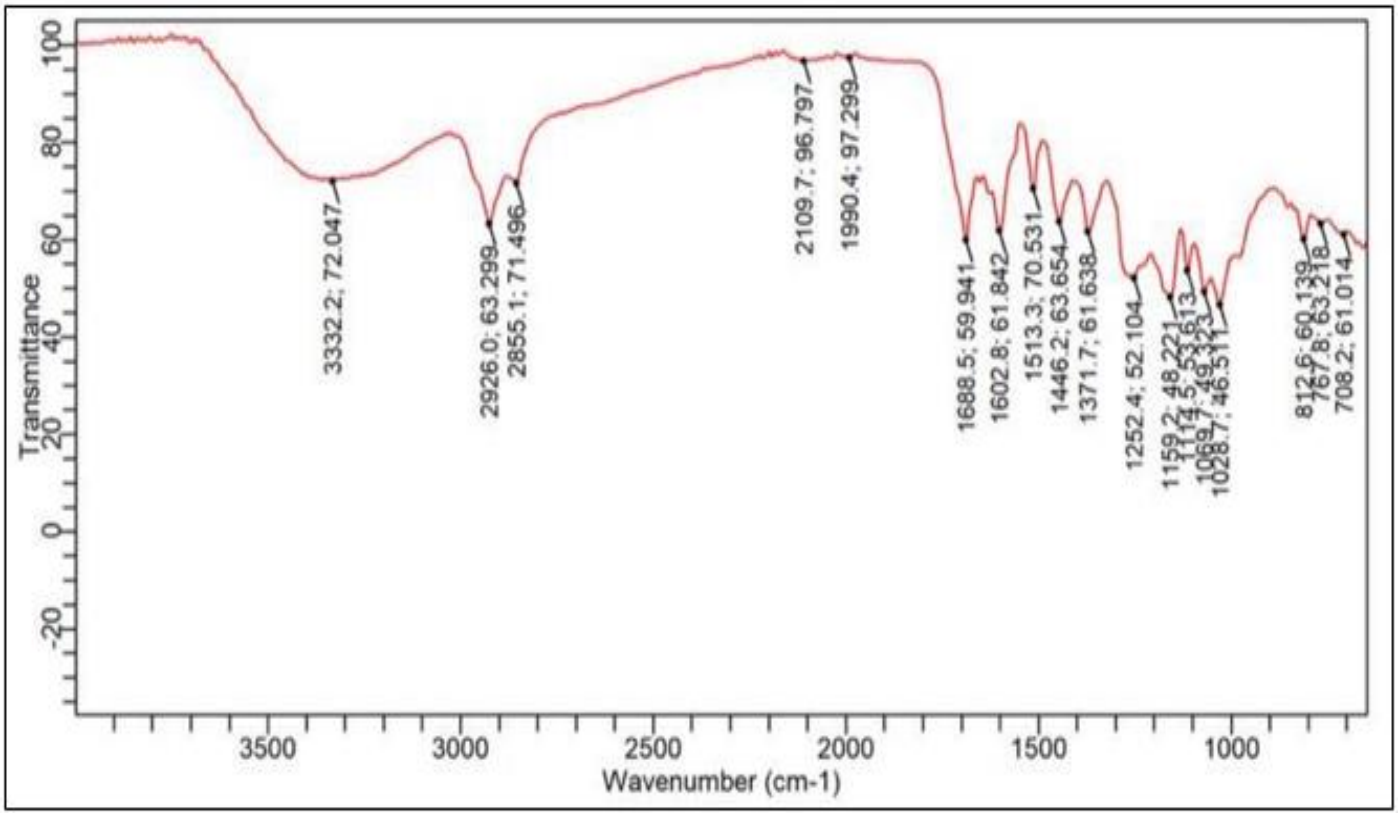

Fig 10 IR Spectrum of *Cajanus cajan* Dichloromethane Seed Extract

➢ *GC-MS Analysis Results*

The results of the chemical composition of the n-hexane fraction of *Cajanus cajan* seed oil obtained by GC-MS analysis are shown in Table 5. The Total Ion Chromatogram for the hexane fraction of the seed oil is shown in Figure 5. The hexane fraction of *Cajanus cajan* seed oil yield is 17.10% from the crude oil. The total number of compounds identified in the seed oils is nine(9). The total oil content of the seed oil is 98%. The major compounds identified in the seed oil are octadecanoic acid (32.36%), 9, 12-octadecadienoic acid (Z, Z) (29.23%) and linoelaidic acid (25.31%). The mass spectra and structures of the major compounds identified in the seed oil are shown in Figures 7 to 15.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

Table 5 Chemical Composition of Essential Oils of *Cajanus cajan* N-Hexane Seed Extract

PEAK NO	COMPOUND	RETENTION TIME (MIN)	COMPOSITION (%)	%Quality
1	1-hexadecyne	12.57	0.42	70
2	Octadecanoic acid	13.56	32.36	99
3	9,12-Octadecadienoic acid, methyl	14.31	0.53	99
4	Linoelaidic acid	14.87	25.31	99
5	9,12-Octadecadienoic acid (Z, Z)	14.90	29.23	99
6	Eicosanoic acid	15.08	6.69	97
7	Hexadecanoic acid, ethyl ester	16.68	0.47	89
8	Bis(2-ethylhexyl) phthalate	18.57	3.48	91
9	Docosanoic acid, ethyl ester	19.14	0.36	98
Total oil Content			= 98.85%	

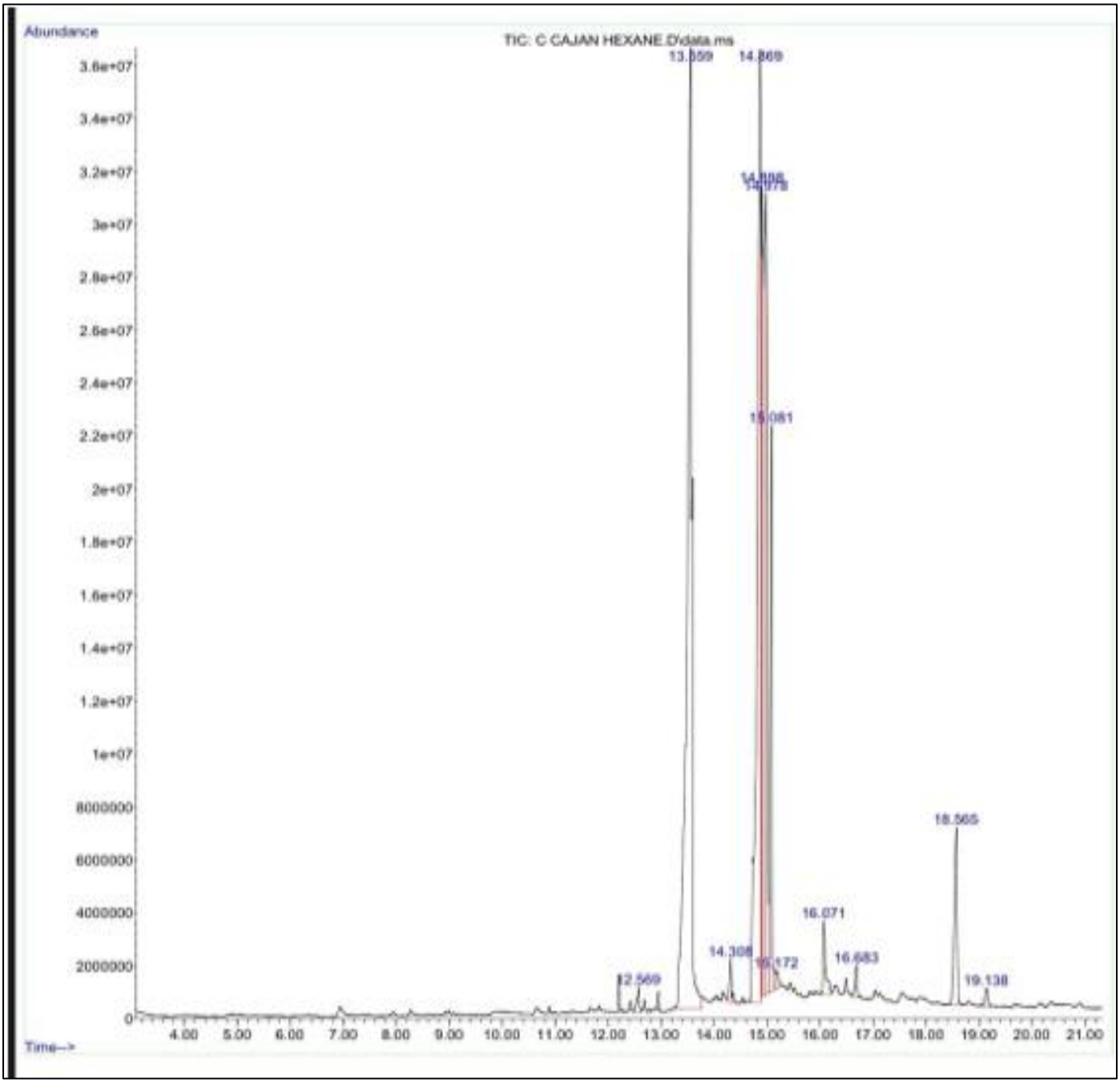

Fig 11 Total Ion Chromatogram of *Cajanus cajan* N-Hexane Seed Extracts

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

- *Mass Spectra of Some Major Compounds from Total Ion Chromatogram of Cajanus cajan n-Hexane Seed Extracts*

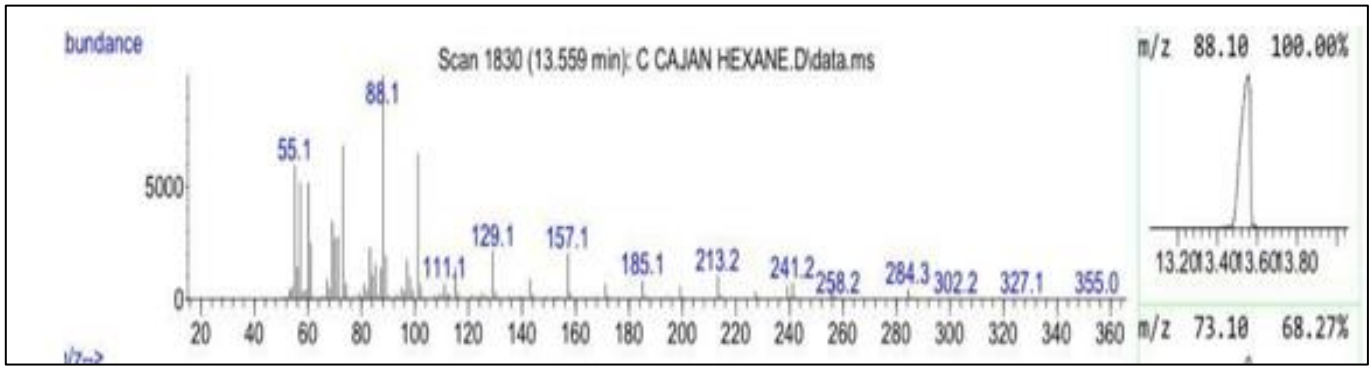

Fig 12 Mass spectrum of Octadecanoic acid, 32.36 % (Peak 2)

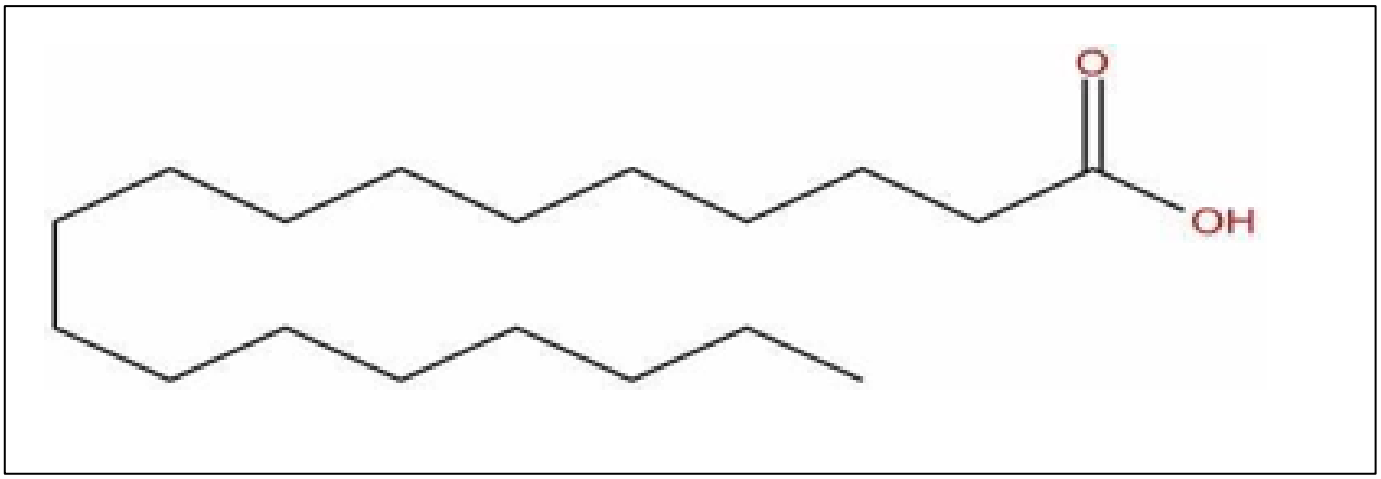

Fig 13 Structure of Octadecanoic Acid

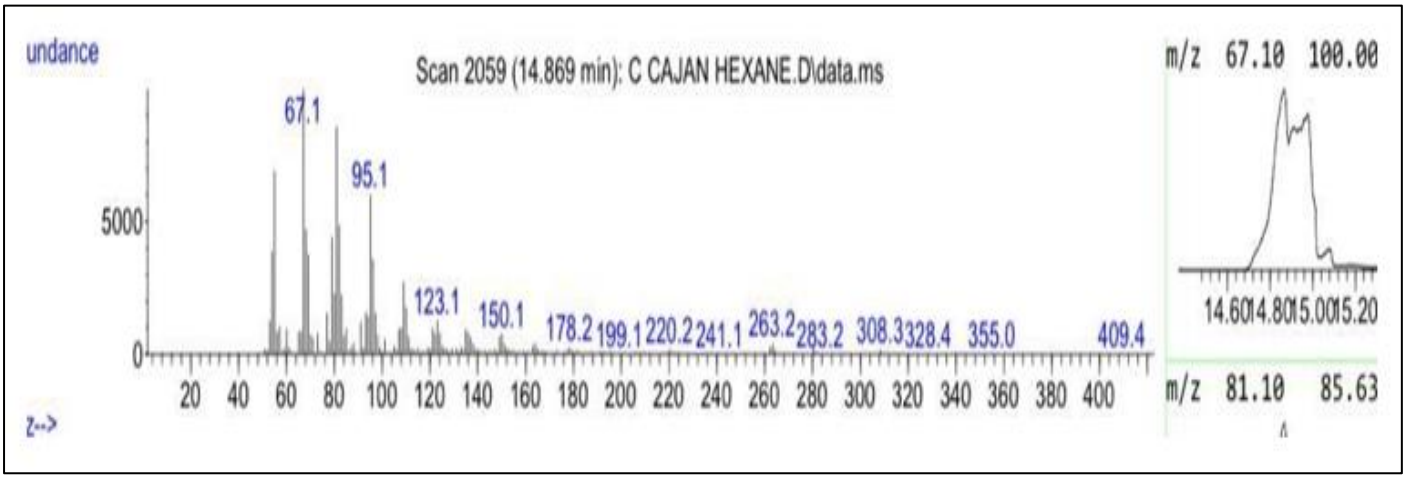

Fig 14 Mass Spectrum of Linoelaidic Acid, 25.31% (Peak 4)

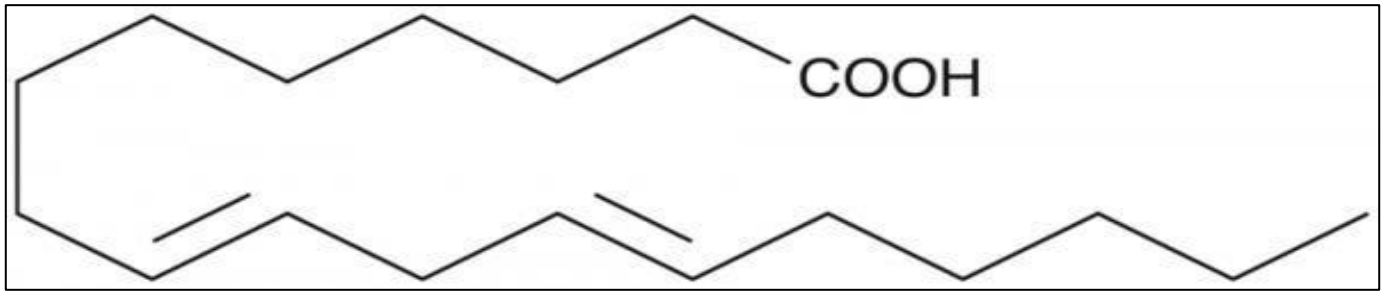

Fig 15 Structure of Linoelaidic acid

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

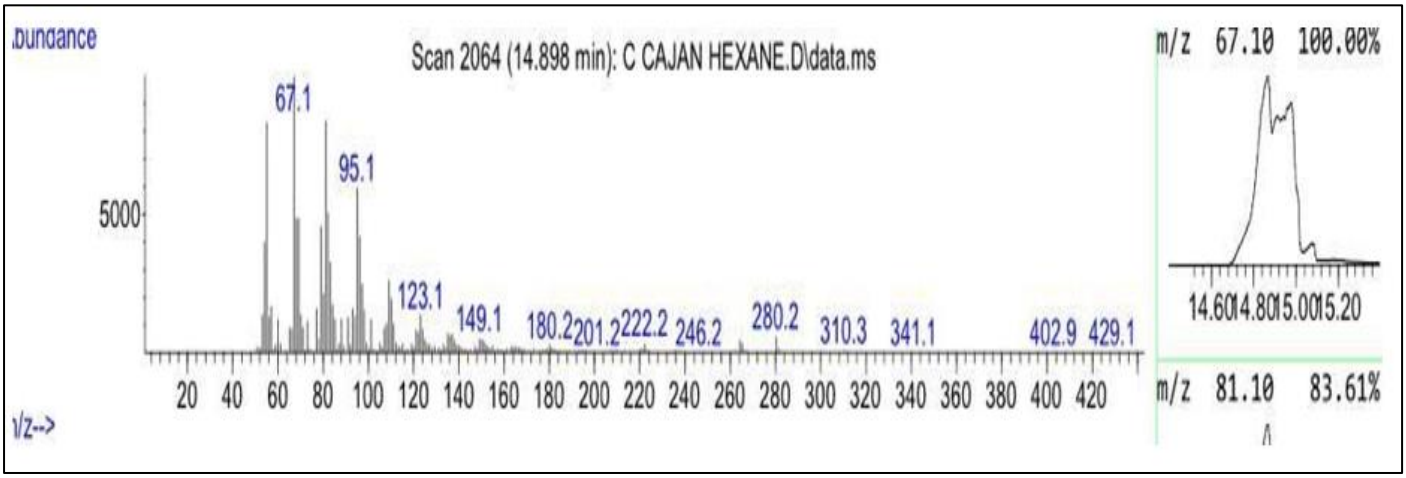

Fig 16 Mass spectrum of 9, 12-Octadecadienoic acid, 14.78% (Peak 5)

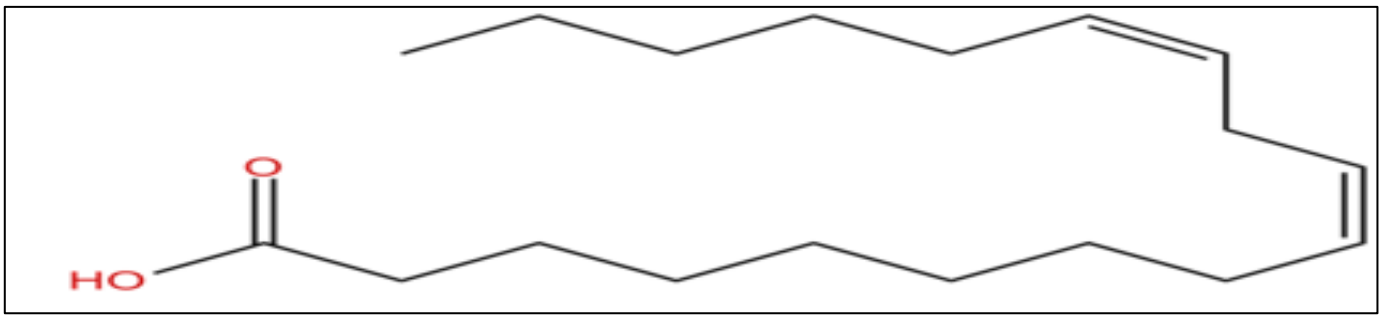

Fig 17 Structure of 9, 12-Octadecadienoic acid

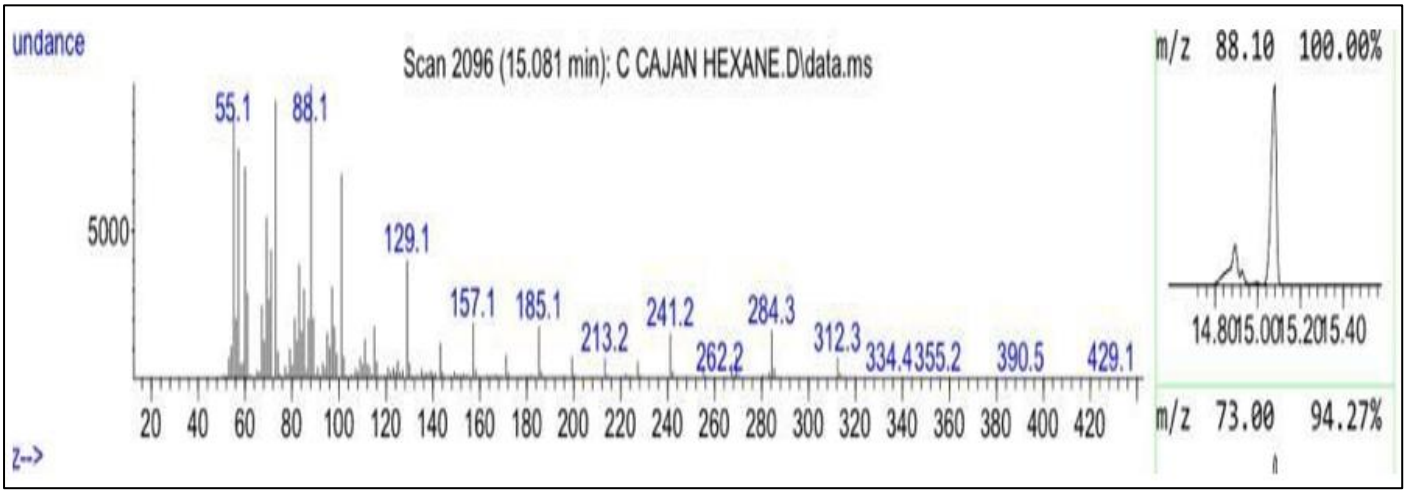

Fig 18 Mass spectrum of Eicosanoic acid, 6.69% (Peak 6)

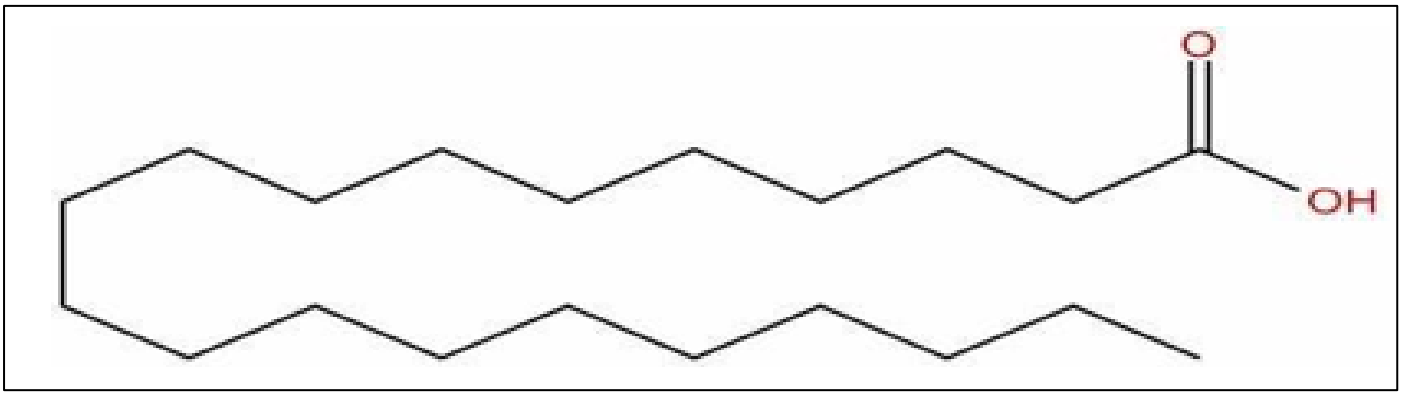

Fig 19 Structure of Eicosanoic acid

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

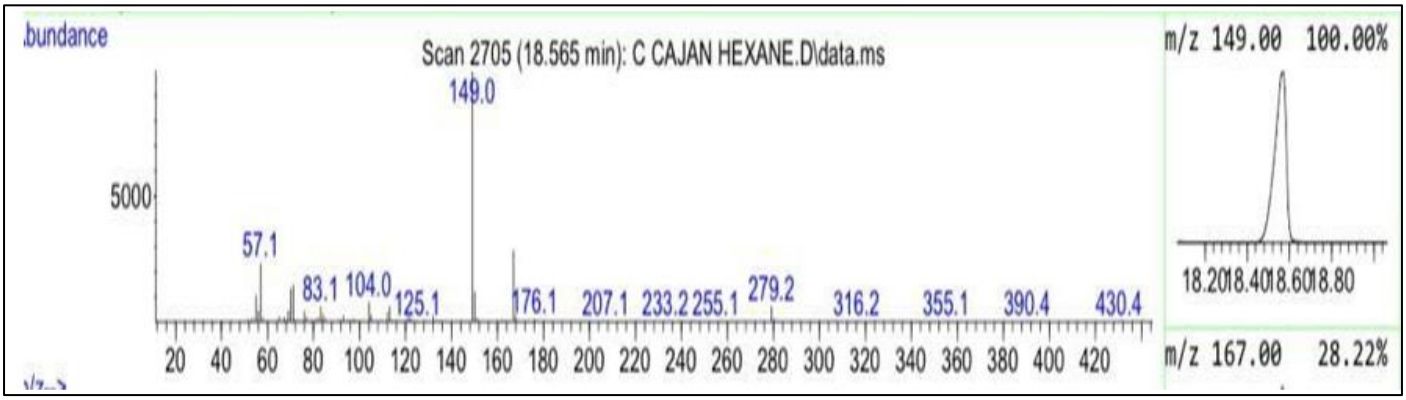

Fig 20 Mass Spectrum of Bis (2-Ethylhexyl) Phthalate, 3.48% (Peak 8)

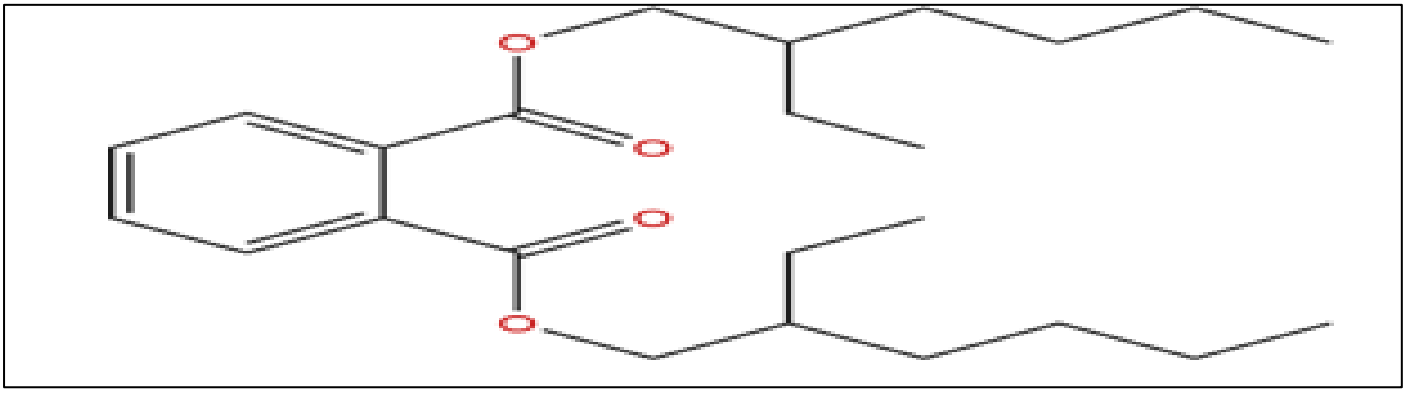

Fig 21 Structure of Bis (2-Ethylhexyl) Phthalate

➢ *HPLC analysis results*

Four (4) prominent phytochemicals were identified and quantified based on their respective areas. The accompanying chromatograms in Figure 16-17 visually show the results of the ethanol extract profiling for *C. cajan* seed across two runs.

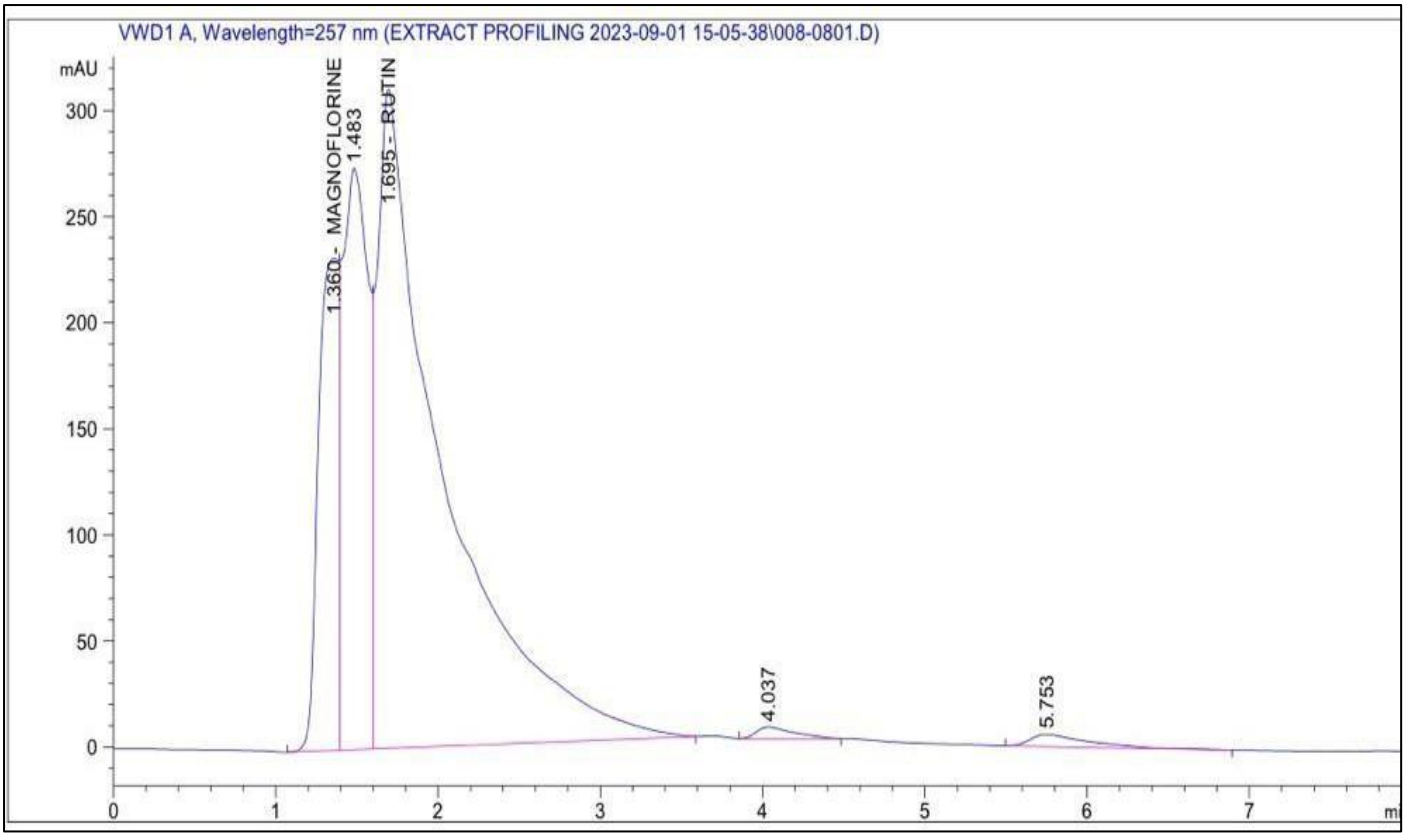

Fig 22 The Chromatogram of Magnoflorine and Rutin Versus their Retention Time.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

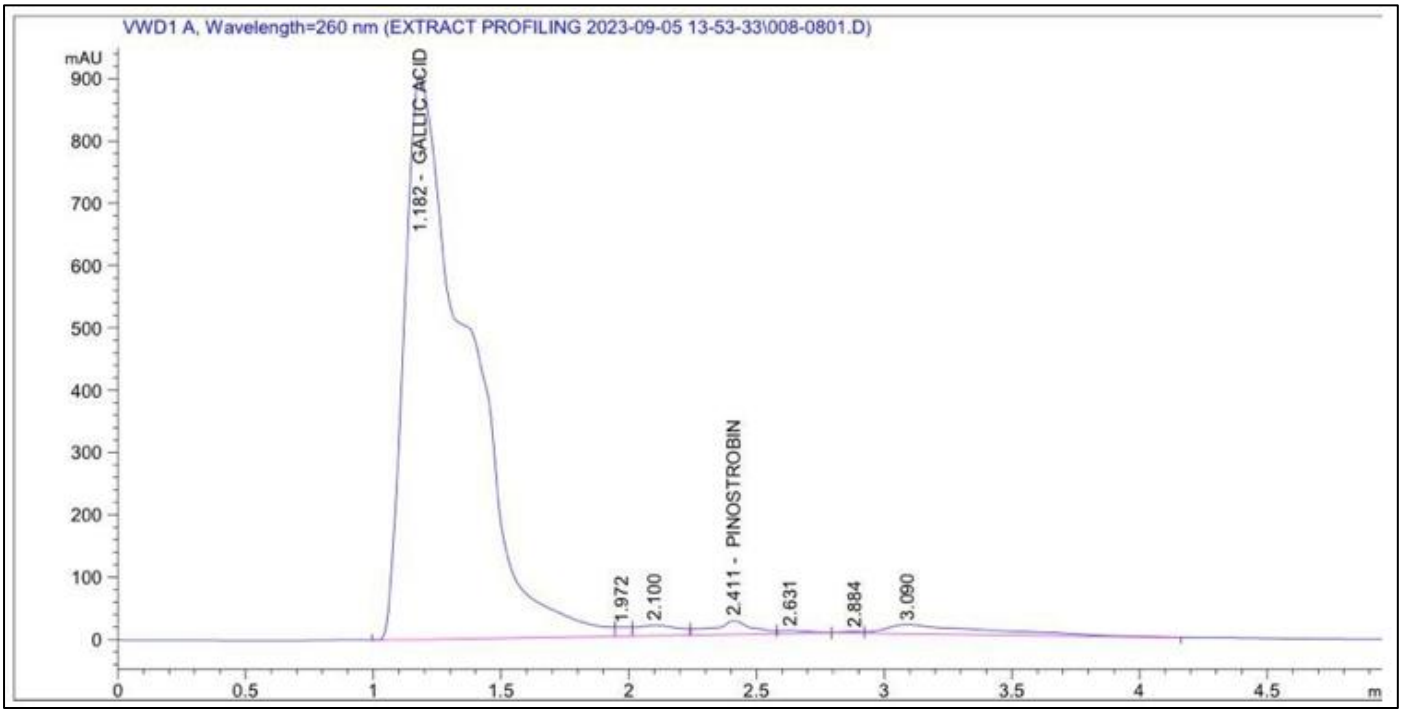

Fig 23 The Chromatogram of Gallic Acid and Pinostrobin Versus their Retention Time.

- *Calibration Curve Determination*

✓ *Magnoflorine Standard*

Table 6 The Concentration and Peak Area of Magnoflorine Standard

Concentration µg/mL	Peak Area mAU
20	218.6
40	435.5
60	631.8
80	932.8
100	1103.9

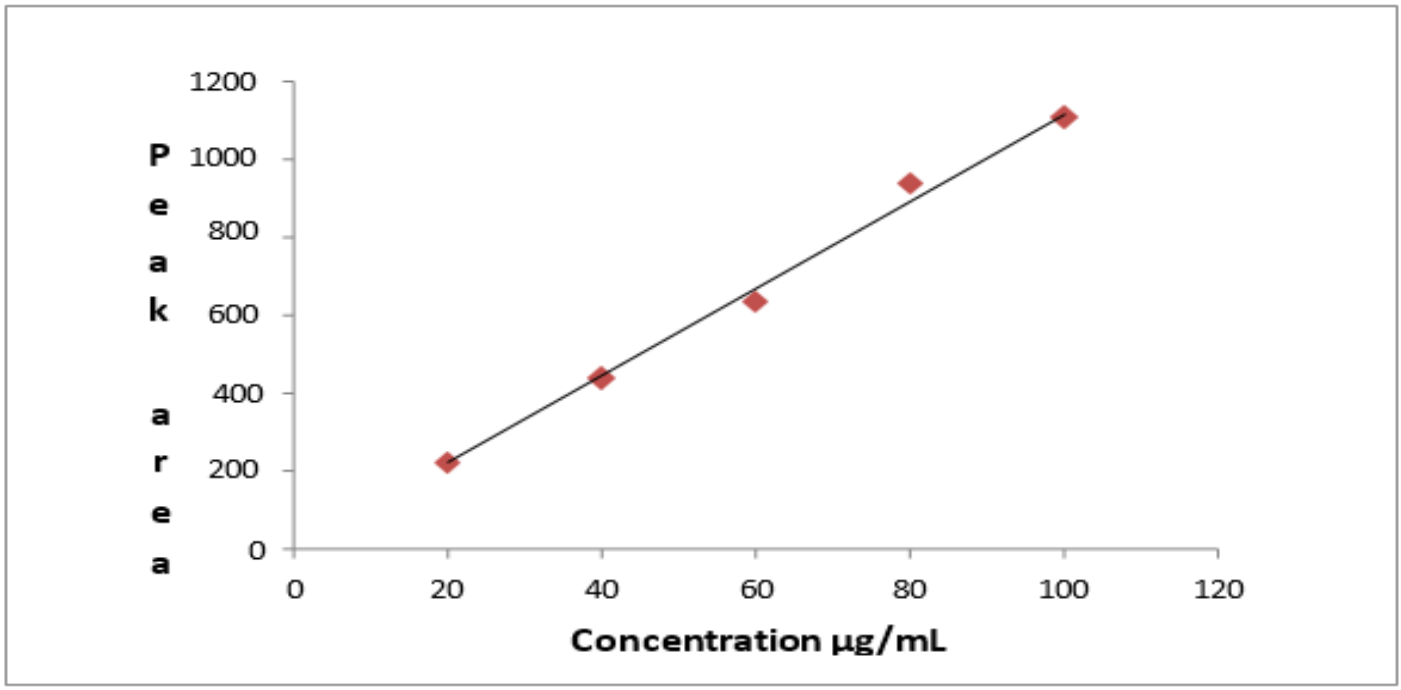

Fig 24 The Calibration Curve of Magnoflorine Standard

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

- *Rutin Standard*

Table 7 The Concentration and Peak Area of Rutin Standard

Concentration µg/mL	Peak Area
20	842.7
40	1839.9
60	2697.4
80	4355.9
100	5436.4

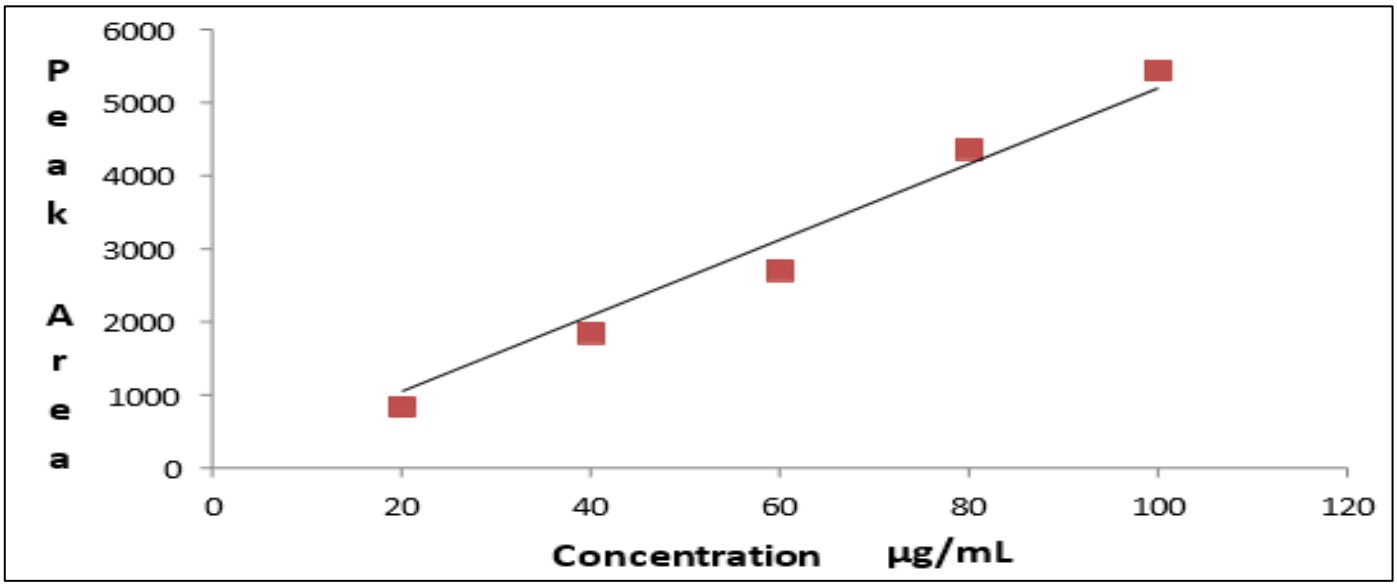

Fig 25 The Calibration Curve of Rutin Standard

- *Gallic Acid Standard*

Table 8 The Concentration and Peak Area of Gallic Acid Standard

Concentration µg/mL	Peak Area mAU
20	134.6
40	311.1
60	483.1
80	599.7
100	622.1

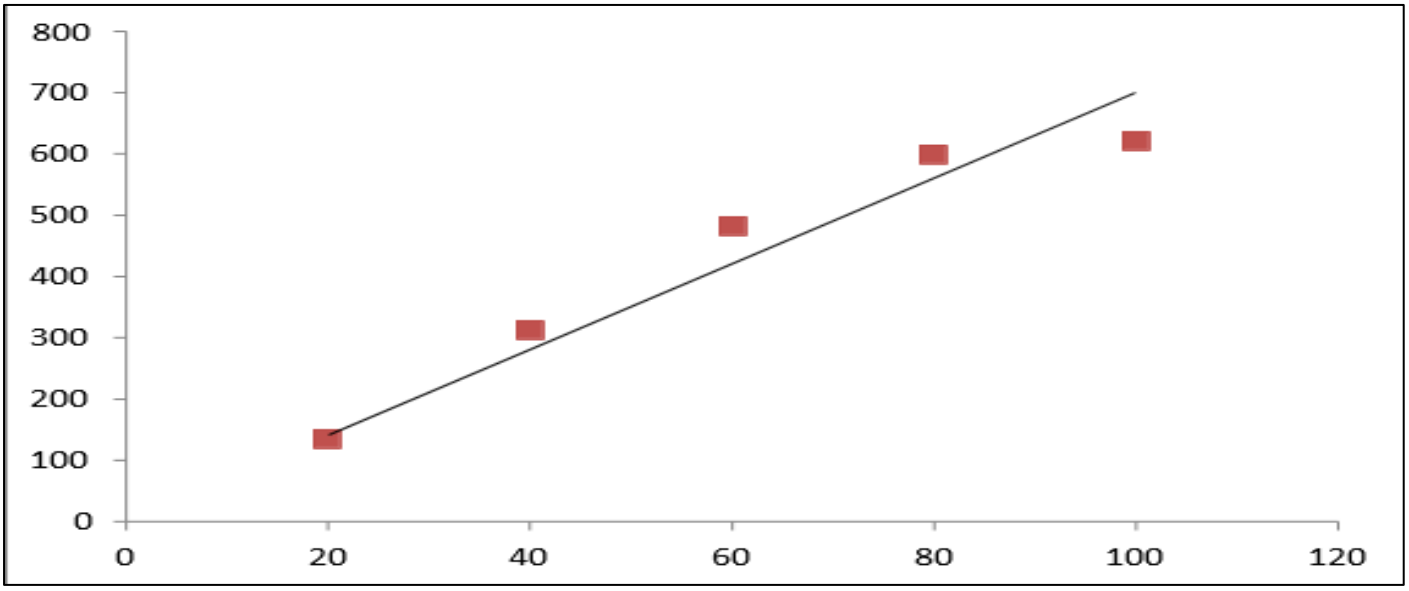

Fig 26 The Calibration Curve of Gallic Acid Standard

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

- *Pinostrobin Standard*

Table 9 The Concentration and Peak Area of Pinostrobin Standard

Concentration µg/mL	Peak Area
20	51.5
40	120.8
60	170.2
80	221.4
100	237.1

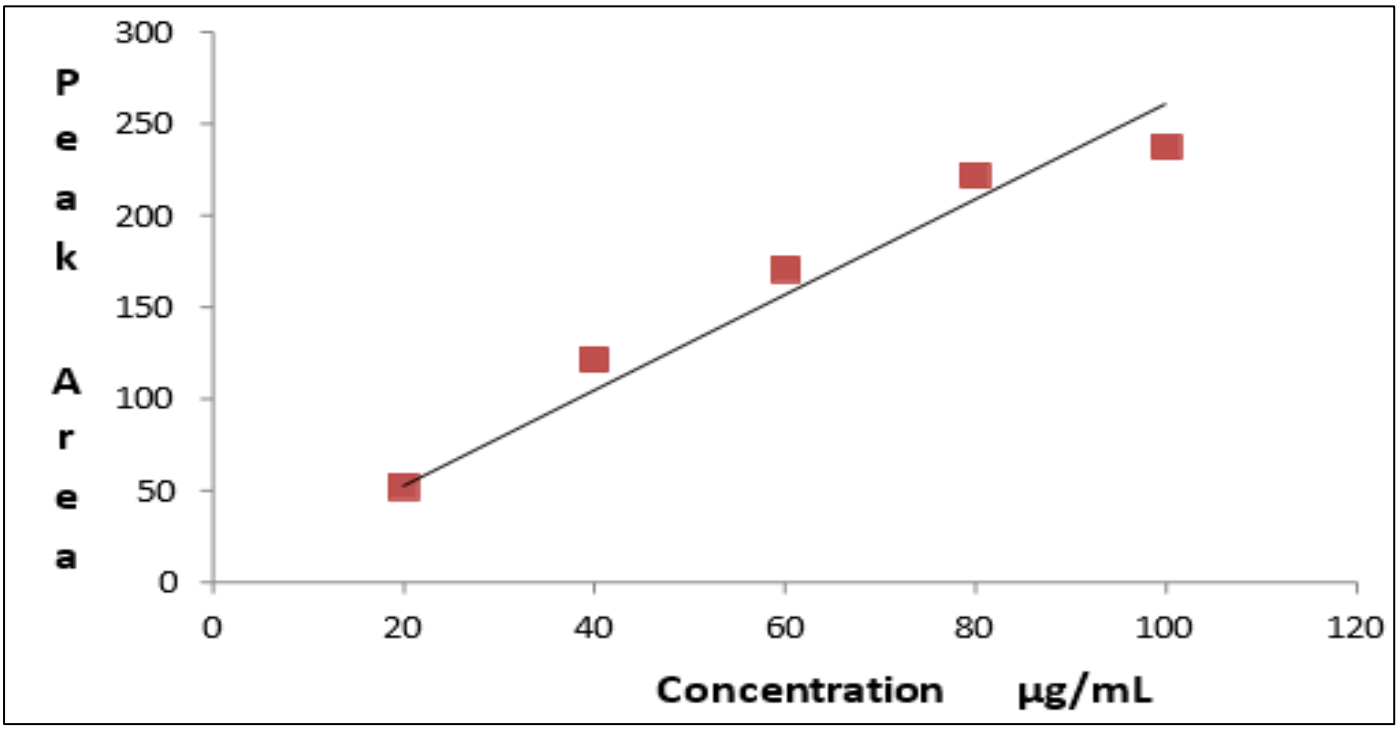

Fig 27 The Calibration Curve of the Pinostrobin Standard

Correlation coefficient (R^2) values of 0.9 or greater. Statistical summaries of linear response of Concentration µg/mL vs. peak area mAU for the four (4) phytochemicals are shown in Table 10

Table 10 Concentration µg/mL vs. Peak Area mAU for the four (4) Phytochemicals

Phytochemicals	Retention time (min)	Regression Equation (Y)	Correlation coefficient (R^2)	Peak area mAU	Concentration µg/m
Gallic Acid	1.182	7.014x	0.9257	1554.37	221.609
Magnoflorine	1.338	11.123x	0.9935	1949.15	175.236
Rutin	1.695	52.018x	0.9733	8827.75	169.705
Pinostrobin	2.411	2.613x	0.9488	236.58	90.525

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

CHAPTER FIVE
DISCUSSION AND CONCLUSION

The phytochemical analysis of *Cajanus cajan* was successfully carried out. The extraction was done with ethanol to yield a 2.40% yield crude extract. The partitioning of the crude extract was achieved using hexane, ethyl acetate, dichloromethane and water. The yields of the fractions compared to the 400 g seed used for extraction are 0.30%, 0.46%, 0.36%, 0.65%. While, the yield of the fractions compared to the crude extract (9.6 g) is 17.10%, 25.72%, 20.20% and 36.97 % of the crude extracts respectively. From the literature, the yield of seed extracts of *Cajanus cajan* varies significantly, the difference in the yield of these fractions might be due to temperate or tropical conditions, extraction, and methods of drying. (Maneechai & Rinthong, 2018)

The thin layer chromatography chromatogram of *C. cajan* seed ethyl acetate fraction developed in chloroform/methanol (9:1) showed four (4) spots with R*f* values of 0.640, 0.681, 0.855, and 0.913.

C. cajan seed n-hexane fraction showed two (2) spots with R*f* values of 0.860, 0.887 and *C. cajan* seed crude fraction showed only one (1) spot with an R*f* value of 0.905 but was not conclusive, so I proceeded to carry out the FTIR analysis.The FTIR analysis was carried out with Aqueous, ethyl acetate and dichloromethane fractions. The FTIR result of *Cajanus cajan* aqueous extract (Figure 2) gave a total of seven (7) peaks between 3332.24 cm-1 to 1085.6 cm^{-1}. The peak at 3332.24cm^{-1} which is broad and intense indicates the presence of O-H stretch which is hydrogen bonded. Hydroxyl (-OH) bending vibration is an important characteristic band in the spectra of dimeric carboxylic acid resulting from the out-of-plane bending of the bonded –OH (Silverstein, Webster, & Kiemle, 2005). The peak at 2180.49cm^{-1} suggests the presence of C≡C stretch for alkyne or C≡N for nitriles. The sharp and intense peak at 1636.30cm^{-1} suggests the presence of C=O stretch from an ester. This is confirmed by the presence of a C-O peak at 1058.56 cm-1. The peak at 1401.47 suggests the presence of a C-H bend of methylene (Table and Figure 2). The spectrum suggests the presence of carbonyl compounds and nitriles. The Phytochemical Class suggested was Esters, flavonoids, Alkaloids, and Alcoholic or Phenolic compounds. These findings agree with the report of (Anadebe, Okafor, Ezeugo, Amanjide, & Ogide, 2017)) on the presence of these compounds in the extracts.

The FTIR result of *Cajanus cajan* ethyl acetate extract gave a total of 16 peaks between 3526.06 cm-1 to 723.10 cm^{-1}. The broad but non-intense peak at 3470.15cm^{-1} suggests the presence of a primary N-H stretch hydrogen bonded which is confirmed by a C-N stretch at 1162.92cm^{-1}. The sharp and intense peak at 2855.14 cm^{-1} suggests the presence of C-H stretch aldehyde, which is confirmed by a weak overtone C=O and sharp carbonyl C=O peak at 3440.33cm^{-1} and 1729.48 cm^{-1}. A very sharp and intense Peak at 2922.23 cm^{-1} shows the presence of the C-H stretch of alkane which is confirmed by the C-H bend of a methyl and methylene peak at 1379.11cm^{-1} and 1461.11cm^{-1}. Peak 1271.02cm^{-1} suggests the presence of a C-O stretch of ester, The FTIR analysis of Cajanus cajan dichloromethane extract showed 18 peaks ranging from 708.19cm-1 to 3332.24cm-1.

The peak at 1073.47cm^{-1} shows an S=O stretch of sulfonic acid. The spectrum of IR data in Table 3 is shown in Figure 3. The spectrum suggests the presence of alkaloids and probably some carbonyl compounds. The FTIR analysis of *Cajanus cajan* dichloromethane extract revealed a remarkable 18 peaks ranging from 3332.24cm^{-1} to 708.19cm^{-1}, indicating the presence of multiple active compounds. This underscores the potential of *Cajanus cajan* as a rich source of natural products with diverse therapeutic properties. The broad but non-intense peak at 3332.24cm^{-1} suggests the presence of alcohol O-H stretch which is hydrogen bonded. The peak at 2925.96cm^{-1} suggests the presence of a C-H stretch of alkane, which is confirmed by the C-H stretch of methyl and methylene bend at 1371.66cm^{-1} and 1446.20 cm^{-1}. The peak at 2855.14cm^{-1} suggests the presence of a C-H stretch of alkene which is confirmed by a C=C stretch at 1688.48cm^{-1}. The peak at 2109.67cm^{-1} shows a C- N Stretch substituted nitrile. The peak at 1252.38 cm^{-1} shows the presence of C-O phenol, which is confirmed by a C=C stretch at 1688.48 cm-1 and an O-H stretch at 3332.24 cm-1. The IR spectrum also showed the bands typical for aromatic compounds in the region of 1500-1600 cm^{-1}. The spectrum of IR data in Table 4 is shown in Figure 4 below. The spectrum indicates the presence of phenolic compounds. The presence of phenolic compounds in the dichloromethane extract may be due to the ability of non-polar solvents to attract low molecular weight phenolics. The results of the chemical composition of the n-hexane fraction of *Cajanus cajan* seed oil obtained by GC-MS analysis are shown in Table 5. The Total Ion Chromatogram for the hexane fraction of the seed oil is shown in Figure 5. The hexane fraction of *Cajanus cajan* seed oil yield is 17.10% from the crude oil. The total number of compounds identified in the seed oils is nine (9). The total oil content of the seed oil is 98%. The major compounds identified in the seed oil are octadecanoic acid (32.36%), 9, 12-octadecadienoic acid (Z, Z) (29.23%) and linoelaidic acid (25.31%) The results compared to the literature show that *Cajanus cajan* oils were high in sesquiterpenes (92.5%, 81.2%, and 94.3% in the leaves, stem, and seeds, respectively.(Ogunbinu et al., 2009). However, in this study, sesquiterpenes was absent. This could be attributed to the difference in geographical locations of the plant source and or environmental effects. Geographical locations and environmental effects (time and period of plant collection) have been known to affect the accumulation and diversity of plant active constituents. (“Trease and Evans’ Pharmacognosy - Edition 16,” n.d.) Stearic acid is a vital fatty acid that can be found in both plants and animals. It is crucial for maintaining a healthy body and has numerous benefits. With its long-chain structure, stearic acid plays a significant role in regulating cholesterol levels and supporting cardiovascular health. Make sure you're getting enough of this essential fatty acid in your diet(“What Is Stearic Acid?” 2022). Linoelaidic acid is an unsaturated fatty acid and a carboxylic acid that belongs to an essential fatty acid. The presence of fatty acid compounds in the n-hexane extract may be due to the ability of non- polar solvents to attract non-polar compounds.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

HPLC analysis was done and revealed the presence of Four (4) prominent phytochemicals, Gallic Acid, Magnoflorine, Rutin and Pinostrobin. Correlation coefficient (R^2) values of 0.9 or greater show a Statistical summary of the linear response of Concentration µg/mL vs. peak area mAU for the four (4) phytochemicals. The concentration of Gallic Acid, Magnoflorine, Rutin and Pinostrobin in 0.5 mg/mL (500 µg/mL) of *cajanus cajan* ethanol seed extract was 22.609 µg/mL,175.236 µg/mL, 169.705 µg/mL and 90.525 µg/mL respectively. Gallic acid is indeed a notable polyphenolic compound, widely found in nature, particularly in plants such as grapes, berries, tea, and certain seeds. Its biological activities, particularly as a **direct thrombin inhibitor**, make it an essential compound for cardiovascular health research.

As a **thrombin inhibitor**, gallic acid can help prevent the formation of blood clots by inhibiting thrombin, an enzyme crucial for blood coagulation. This makes it potentially beneficial for conditions like **atherosclerosis** and other cardiovascular diseases (CVD), where excessive clotting and inflammation are common concerns. Additionally, the **platelet aggregation inhibitory effect** of gallic acid suggests that it can reduce the risk of thrombosis (clot formation in the bloodstream), a significant factor in cardiovascular disease progression. Recent studies, such as the one by **Kahkeshani et al. (2019)**, also highlight gallic acid's **role in gut microbiota modulation**. The alterations in gut microbiota composition are linked to the development of various cardiovascular diseases. By enhancing beneficial gut microbes, gallic acid may help reduce the risk of CVD. This has significant implications for therapeutic strategies targeting **gut health** to improve heart health, especially in males with **atherosclerosis**, as suggested by these studies.

Thus, gallic acid's multiple mechanisms of action make it a promising candidate for **cardiovascular disease prevention** and as a dietary supplement. However, further clinical studies would be needed to fully establish its effectiveness and safety, especially in humans, for managing atherosclerosis and other CVDs.

Magnoflorine is a quaternary aporphine alkaloid found in members of various plant groups, including Berberidaceae, Magnolilaceae, Papaveraceae, and Menispermaceae. Several scientific findings mention its use in the treatment of a wide range of diseases, including inflammatory ones, allergies, hypertension, osteoporosis, bacterial, viral, and fungal infections, and some civilization diseases like cancer, obesity, diabetes, dementia, or depression (Okon et al., 2020).

Rutin, also known as vitamin P or rutoside, has been studied for a variety of medicinal purposes. Rutin is an active ingredient in tea leaves, apples, and many other foods. Nowadays, rutin its nutraceutical impact has been found in the human system (Ganeshpurkar & Saluja, 2016). Pinostrobin, once established as an adipogenic suppressor, could be employed as a single ingredient or formulated with other herbs in food products for obesity prevention and management(Nicholson et al., 2010)

- *Conclusion*

From the results of this study, the following conclusions can be drawn.

- The FTIR analysis with Aqueous, ethyl acetate and dichloromethane fractions suggest the presence of the following phytochemicals: Esters, flavonoids, Alkaloids, Alcoholic or Phenolic compounds and carbonyl compounds.
- The results of the chemical composition of the n-hexane fraction of *Cajanus cajan* seed oil obtained by GC-MS analysis show a total of nine (9) compounds with a 98% total oil content of the seed. The major compounds identified in the seed oil are octadecanoic acid (32.36%), 9, 12-octadecadienoic acid (Z, Z) (29.23%) and linoelaidic acid (25.31%).
- HPLC analysis was done and revealed the presence of Four (4) prominent phytochemicals, Gallic Acid, Magnoflorine, Rutin and Pinostrobin. The concentration of Gallic Acid, Magnoflorine, Rutin and Pinostrobin in 0.5 mg/mL (500 µg/mL) of *cajanus cajan* ethanol seed extract was 22.609 µg/mL, 175.236 µg/mL, 169.705 µg/mL and 90.525 µg/mL respectively.

- *Recommendations*

Based on the results of this study, the following recommendations are suggested:

- Further analysis should be done on the extract by Nuclear Magnetic Resonance (NMR).
- The antioxidant activity of *Cajanus Cajan* seed extract since the plant has been reported to have diverse bioactivity.
- It is also important to evaluate the toxicity of the plant crude extracts to determine their safety parameters.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

REFERENCES

[1]. A Direct Bioautographic Tlc Assay for Compounds Possessing Antibacterial Activity | Journal of Natural Products. (n.d.). Retrieved July 31, 2023, from https://pubs.acs.org/doi/pdf/10.1021/np50049a003

[2]. Abo-Zeid, M. A. M., Abdel-Samie, N. S., Farghaly, A. A., & Hassan, E. M. (2018). Flavonoid fraction of Cajanus cajan prohibited the mutagenic properties of cyclophosphamide in mice in vivo. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *826*, 1–5. https://doi.org/10.1016/j.mrgentox.2017.12.004

[3]. Aggarwal, A., Nautiyal, U., & Negi, D. (2015). Characterization and evaluation of the antioxidant activity of Cajanus cajan and Pisum sativum. *International Journal of Recent Advances in Science and Technology*, *2*(1). https://doi.org/10.30750/ijrast.214

[4]. Aja, P. M., Alum, E. U., Ezeani, N. N., Nwali, B. U., & Edwin, N. (2015). *Comparative Phytochemical Composition of Cajanus cajan Leaf and Seed*. Amaral, T. Y., Padilha, I. G., Presídio, G. A., Silveira, E. A. A. S. da, Duarte, A. W. F., Barbosa, A. P. F., … López, A. M. Q. (2017). Antimicrobial and anti-inflammatory activities of *Apis mellifera* honey on the *Helicobacter pylori* infection of Wistar rats gastric mucosa. *Food Science and Technology*, *37*, 34–41. https://doi.org/10.1590/1678-457X.31016

[5]. Anadebe, V., Okafor, N., Ezeugo, J., Amanjide, I., & Ogide, B. (2017). *GC-MS Analysis of Phytochemical Compounds in Cajanus Cajan Leaf*.

[6]. Animal models to test drugs with potential antidiabetic activity—ScienceDirect. (n.d.). Retrieved August 2, 2023, from https://www.sciencedirect.com/science/article/pii/S0378874107005867

[7]. The anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'— ScienceDirect. (n.d.). Retrieved July 31, 2023, from https://www.sciencedirect.com/science/article/pii/S0378874106001851

[8]. *Antimicrobial_activity_of_leaf_extracts_of_Senna_obtusifolia_(L).pdf*. (n.d.). Retrieved from https://indexmedicus.afro.who.int/iah/fulltext/Antimicrobial_activity_of_leaf_extracts_of_Senna_ obtusifolia_(L).pdf

[9]. Bhushan R. Gudalwar, Wrushali A. Panchale, Jagdish V. Manwar, Minakshee G. Nimbalwar, Neha A. Badukale, & Ravindra L. Bakal. (2021). Pharmacognosy, phytochemistry and clinical applications of traditional medicinal plants as a memory booster. *GSC Advanced Research and Reviews*, *8*(2), 019–029. https://doi.org/10.30574/gscarr.2021.8.2.0155

[10]. Bravo, R. K. D., Angelia, M. R. N., Uy, L. Y. C., Garcia, R. N., & Torio, M. A. O. (2022). Isolation, purification and characterization of the antibacterial, antihypertensive and antioxidative properties of the bioactive peptides in the purified and proteolyzed major storage protein of pigeon pea (Cajanus cajan) seeds. *Food Chemistry: Molecular Sciences*, *4*, 100062. https://doi.org/10.1016/j.fochms.2021.100062 Can remittances alleviate energy poverty in developing countries? New evidence from panel data. (n.d.). Retrieved October 12, 2023, from https://www.researchgate.net/publication/367503867_Can_remittances_alleviate_energy_poverty_in_developing_countries_ New_evidence_from_panel_data Chinecherem, K. (n.d.). *FIO-FIO (Cajanus cajan) LEAVES.*

[11]. da Silva, R. N., Brandão, M. A. G., & Ferreira, M. de A. (2020). Integrative Review as a Method to Generate or to Test Nursing Theory. *Nursing Science Quarterly*, *33*(3), 258–263. https://doi.org/10.1177/0894318420920602

[12]. Das, S., Teja, K. C., Mukherjee, S., Seal, S., Sah, R. K., Duary, B., … Bhattacharya, S. S. (2018). Impact of edaphic factors and nutrient management on the hepatoprotective efficiency of Carlinoside purified from pigeon pea leaves: An evaluation of UGT1A1 activity in hepatitis induced organelles. *Environmental Research*, *161*, 512–523. https://doi.org/10.1016/j.envres.2017.11.054

[13]. Duker-Eshun, G., Jaroszewski, J. W., Asomaning, W. A., Oppong-Boachie, F., & Brøgger Christensen, S. (2004). Antiplasmodial constituents of Cajanus cajan. *Phytotherapy Research*, *18*(2), 128–130. https://doi.org/10.1002/ptr.1375

[14]. Dutta, S., Halder, S., & Khaled, K. (2023). *PHYTOCHEMICAL INVESTIGATION AND IN VITRO ANTIOXIDANT ACTIVITY OF SYZYGIUM JAMBOS FRUIT AND ITS SEED*. https://doi.org/10.22159/ajpcr.2023v16i2.46496

[15]. Ezike, A. C., Akah, P. A., Okoli, C. C., & Okpala, C. B. (2010). EXPERIMENTAL EVIDENCE FOR THE ANTIDIABETIC ACTIVITY OF CAJANUS CAJAN LEAVES IN RATS. *Journal of Basic and Clinical Pharmacy*, *1*(2), 81–84.

[16]. Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*, *109*(suppl 1), 69–75. https://doi.org/10.1289/ehp.01109s169

[17]. Falcone Ferreyra, M. L., Rius, S., & Casati, P. (2012). Flavonoids: Biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science*, *3*. Retrieved from https://www.frontiersin.org/articles/10.3389/fpls.2012.00222

[18]. Ganeshpurkar, A., & Saluja, A. K. (2016). The Pharmacological Potential of Rutin. *Saudi Pharmaceutical Journal*, *25*. https://doi.org/10.1016/j.jsps.2016.04.025

[19]. Green, P. W. C., Stevenson, P. C., Simmonds, M. S. J., & Sharma, H. C. (2003). Phenolic Compounds on the Pod-Surface of Pigeonpea, Cajanus cajan, Mediate Feeding Behavior of Helicoverpa armigera Larvae. *Journal of Chemical Ecology*, *29*(4), 811–821. https://doi.org/10.1023/A:1022971430463

[20]. Huie, C. W. (2002). A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. *Analytical and Bioanalytical Chemistry*, *373*(1), 23–30. https://doi.org/10.1007/s00216-002-1265-3

[21]. *IDOSR-JAS-21-59-75.pdf*. (n.d.). Retrieved from https://www.idosr.org/wp- content/uploads/2017/01/IDOSR-JAS-21-59-75.pdf

[22]. Ijpsr, H., P., & Mathad, P. (2017). *COMPARATIVE STUDY ON PHARMACOGNOSTIC AND PHYTOCHEMICAL COMPOSITION OF SEED COAT AND COTYLEDON OF CAJANUS CAJAN L.* *8*, 1000–1007. https://doi.org/10.13040/IJPSR.0975-8232.8(4).1000-07

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

[23]. Kahkeshani, N., Farzaei, F., Fotouhi, M., Alavi, S. S., Bahramsoltani, R., Naseri, R., … Bishayee, A. (2019). Pharmacological effects of gallic acid in health and diseases: A mechanistic review. *Iranian Journal of Basic Medical Sciences*, *22*(3), 225–237. https://doi.org/10.22038/ijbms.2019.32806.7897 Kong, Y., Fu, Y.-J., Zu, Y.-G., Chang, F.-R., Chen, Y.-H., Liu, X.-L., … Schiebel, H.-M. (2010). Cajanuslactone, a new coumarin with anti-bacterial activity from pigeon pea [Cajanus cajan (L.) Millsp.] leaves. *Food Chemistry*, *121*(4), 1150–1155. https://doi.org/10.1016/j.foodchem.2010.01.062

[24]. Krishna, R. N., Anitha, R., & Ezhilarasan, D. (2020). Aqueous extract of Tamarindus indica fruit pulp exhibits antihyperglycaemic activity. *Avicenna Journal of Phytomedicine*, *10*(5), 440–447.

[25]. Luo, Q.-F., Sun, L., Si, J.-Y., & Chen, D.-H. (2008). Hypocholesterolemic effect of stilbenes containing extract-fraction from Cajanus cajan L. on diet-induced hypercholesterolemia in mice. *Phytomedicine*, *15*(11), 932–939. https://doi.org/10.1016/j.phymed.2008.03.002

[26]. Malgi, R., Mane, D. V., Kumar, D. D. N., Paramshetty, V., & Kobanna, S. (n.d.). *Formulation and evaluation of herbal toothpaste by Cajanus cajan (L.) Leaf extract*.

[27]. Maneechai, S., & Rinthong, P. (2018). Total Phenolic Content and Tyrosinase Inhibitory Potential of Extracts from Cajanus cajan (L.) Millsp. *Pharmacognosy Journal*, *10*, s109–s112. https://doi.org/10.5530/pj.2018.6s.21

[28]. Marcía-Fuentes, J., Santos-Aleman, R., Borrás-Linares, I., & Sánchez, J. L. (2021). The Carano (Cassia grandis L.): Its Potential Usage in Pharmacological, Nutritional, and Medicinal Applications. In N. R. Maddela & L. C. García (Eds.), *Innovations in Biotechnology for a Sustainable Future* (pp.403–427). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-80108- 3_19

[29]. Masoko, P., Mokgotho, M. P., Mbazima, V. G., & Mampuru, L. J. (2008). Biological activities of Typha capensis (Typhaceae) from Limpopo Province (South Africa). *African Journal of Biotechnology*, *7*(20). https://doi.org/10.4314/ajb.v7i20.59423

[30]. Nicholson, R. A., David, L. S., Pan, R. L., & Liu, X. M. (2010). Pinostrobin from Cajanus cajan (L.) Millsp. Inhibits sodium channel-activated depolarization of mouse brain synaptoneurosomes. *Fitoterapia*, *81*(7), 826–829. https://doi.org/10.1016/j.fitote.2010.05.005

[31]. Nix, A., Paull, C. A., & Colgrave, M. (2015). The flavonoid profile of pigeonpea, Cajanus cajan: A review. *SpringerPlus*, *4*(1), 125. https://doi.org/10.1186/s40064-015-0906-x

[32]. Ogoda Onah, J., Akubue, P. I., & Okide, G. B. (2002). The kinetics of reversal of pre-sickled erythrocytes by the aqueous extract of Cajanus cajan seeds. *Phytotherapy Research*, *16*(8), 748–750. https://doi.org/10.1002/ptr.1026

[33]. Ogunbinu, A. O., Flamini, G., Cioni, P. L., Adebayo, M. A., & Ogunwande, I. A. (2009). Constituents of Cajanus Cajan (L.) Millsp., Moringa Oleifera Lam., Heliotropium Indicum L. and Bidens Pilosa L. from Nigeria. *Natural Product Communications*, *4*(4), 1934578X0900400427. https://doi.org/10.1177/1934578X0900400427

[34]. Okey-Ndeche, N., Pius, E., Unegbu, V., & Ndidi, O.-N. (2020). Phytochemical and Antibacterial Properties of Garcinia kola seeds (bitter kola) on Escherichia coli and Staphylococcus aureus. Global Science Independent Journal. *Global Science Education Journal*.

[35]. Okon, E., Kukula-Koch, W., Jarząb, A., Hałasa, M., Stepulak, A., & Wawruszak, A. (2020). Advances in Chemistry and Bioactivity of Magnoflorine and Magnoflorine-Containing Extracts. *International Journal of Molecular Sciences*, *21*. https://doi.org/10.3390/ijms21041330

[36]. Olagunju, A. I., Omoba, O. S., Enujiugha, V. N., Alashi, A. M., & Aluko, R. E. (2018). Antioxidant properties, ACE/renin inhibitory activities of pigeon pea hydrolysates and effects on systolic blood pressure of spontaneously hypertensive rats. *Food Science & Nutrition*, *6*(7), 1879–1889. https://doi.org/10.1002/fsn3.740

[37]. Orni, P. R., Ahmed, S. Z., Monefa, M., Khan, T., & Dash, P. R. (n.d.). *Pharmacological and phytochemical properties of Cajanus cajan (L.) Huth. (Fabaceae): A review*.

[38]. Pal, D., Mishra, P., Sachan, N., & Ghosh, A. K. (2011). Biological activities and medicinal properties of Cajanus cajan (L) Millsp. *Journal of Advanced Pharmaceutical Technology & Research*, *2*(4), 207–214. https://doi.org/10.4103/2231-4040.90874

[39]. Pal, D., Sahoo, M., & Mishra, A. K. (2005). Analgesic and anticonvulsant effects of saponin isolated from the stems of Opuntia vulgaris Mill in mice. *Eur Bull Drug Res*, *13*, 91–97.

[40]. Phillipson, J. D. (2007). Phytochemistry and pharmacognosy. *Phytochemistry*, *68*(22), 2960–2972. https://doi.org/10.1016/j.phytochem.2007.06.028

[41]. Prevalence and Associated Factors of Burnout Syndrome among Nurses in Public Hospitals, Southwest Ethiopia. (n.d.). Retrieved October 12, 2023, from https://www.researchgate.net/publication/351764652_Prevalence_and_Associated_Factors_of_Burnout_syndrome_among_Nurses_in_Public_Hospitals_Southwest_Ethiopia

[42]. Qi, X.-L., Li, T.-T., Wei, Z.-F., Guo, N., Luo, M., Wang, W., … Peng, X. (2014). Solvent-free microwave extraction of essential oil from pigeon pea leaves [Cajanus cajan (L.) Millsp.] and evaluation of its antimicrobial activity. *Industrial Crops and Products*, *58*, 322–328. https://doi.org/10.1016/j.indcrop.2014.04.038

[43]. Rashid, Md. M., Amran, M., & Hossain, M. (2017). Evaluation of Analgesic Activity by Acetic Acid Induced Writhing Method of Crude Extracts of Acacia nilotica. *Scholars Academic Journal of Pharmacy (SAJP)*.

[44]. Sahu, M., Verma, D., & Haris, K. (2014). Phytochemical analysis of the leaf, stem and seed extracts of cajanus cajan L (dicotyledonous: Fabaceae). *WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES*, *3*, 694–733.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr1955

[45]. Samie, A., Obi, C. L., Bessong, P. O., & Namrita, L. (2005). Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species. *African Journal of Biotechnology*, *4*(12). https://doi.org/10.4314/ajb.v4i12.71495

[46]. Sarkar, R., Hazra, B., Mandal, S., Biswas, S., & Mandal, N. (2009a). Assessment of in Vitro Antioxidant and Free Radical Scavenging Activity of Cajanus cajan. *Journal of Complementary and Integrative Medicine*, *6*(1). https://doi.org/10.2202/1553-3840.1248

[47]. Sarkar, R., Hazra, B., Mandal, S., Biswas, S., & Mandal, N. (2009b). Assessment of in Vitro Antioxidant and Free Radical Scavenging Activity of Cajanus cajan. *Journal of Complementary and Integrative Medicine*, *6*(1). https://doi.org/10.2202/1553-3840.1248

[48]. Sasidharan, S., Chen, Y., Saravanan, D., Sundaram, K. M., & Yoga Latha, L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary, and Alternative Medicines*, *8*(1), 1–10. https://doi.org/10.1625/jcam.8.1

[49]. Shahverdi, A. R., Abdolpour, F., Monsef-Esfahani, H. R., & Farsam, H. (2007). A TLC bioautographic assay for the detection of nitrofurantoin resistance reversal compound. *Journal of Chromatography B*, *850*(1), 528–530. https://doi.org/10.1016/j.jchromb.2006.11.011

[50]. Silverstein, R. M., Webster, F. X., & Kiemle, D. (2005). *Spectrometric Identification of Organic Compounds, 7th Edition*. Wiley.

[51]. Talari, A., & Shakappa, D. (2018). Role of pigeon pea (Cajanus cajan L.) in human nutrition and health: A review. *Asian Journal of Dairy and Food Research*, (of). https://doi.org/10.18805/ajdfr.DR- 1379

[52]. The Antiplasmodial Potential of Medicinal Plants Used in the Cameroonian Pharmacopoeia: An Updated Systematic Review and Meta-Analysis. (n.d.). Retrieved July 24, 2023, from https://www.hindawi.com/journals/ecam/2022/4661753/

[53]. Therapeutic molecules for multiple human diseases identified from pigeon pea (Cajanus cajan L. Millsp.) through GC–MS and molecular docking—ScienceDirect. (n.d.). Retrieved August 1, 2023, from https://www.sciencedirect.com/science/article/pii/S2213453017300344

[54]. Three stilbenes from pigeon peas with promising anti-MRSA biofilm formation activities. (2023, May 25). https://doi.org/10.21203/rs.3.rs-2962278/v1

[55]. Trease and Evans' Pharmacognosy—Edition 16—By William Charles Evans, BPharm, BSc, PhD, DSc, FIBiol, FLS, FRPharmS Elsevier Inspection Copies. (n.d.). Retrieved October 2, 2023, from https://educate.elsevier.com/book/details/9780702029332

[56]. What is stearic acid? Benefits, side effects, and use. (2022, April 19). Retrieved October 12, 2023, from https://www.medicalnewstoday.com/articles/stearic-acid

[57]. Zhang, D.-Y., Zhang, S., Zu, Y.-G., Fu, Y.-J., Kong, Y., Gao, Y., … Efferth, T. (2010). Negative pressure cavitation extraction and antioxidant activity of genistein and genistin from the roots of pigeon pea [Cajanus cajan (L.) Millsp.]. *Separation and Purification Technology*, *74*(2), 261–270. https://doi.org/10.1016/j.seppur.2010.06.015 Zhang, D.-Y., Zu, Y.-G., Fu, Y.-J., Luo, M., Gu, C.-B., Wang, W., & Yao, X.-H. (2011). Negative pressure cavitation extraction and antioxidant activity of biochanin A and genistein from the leaves of Dalbergia odorifera T. Chen. *Separation and Purification Technology*, *83*, 91–99. https://doi.org/10.1016/j.seppur.2011.09.017

[58]. Zu, Y., Liu, X., Fu, Y., Wu, N., Kong, Y., & Wink, M. (2010). Chemical composition of the SFE-CO2 extracts from Cajanus cajan (L.) Huth and their antimicrobial activity in vitro and in vivo. *Phytomedicine*, *17*(14), 1095–1101. https://doi.org/10.1016/j.phymed.2010.04.005