Nutritional Bio-Active Compounds in *Annona Muricata* (Soursop) and its Potential Role in Promoting Health and Preventing Disease (Cancer)

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Certification

This is to certify that Meka Margaret MOSIMABALE with matriculation number LCU/PG/003550 carried out this research work titled "Nutritional Bio-Active Compounds in *Annona Muricata* (Soursop) and its Potential Role in Promoting Health and Preventing Disease (Cancer)" in the Department of Human Nutrition and Dietetics, Faculty of Public Health, Lead City University, Ibadan, Oyo, for the award of Master of Science Degree (MSc) in Human Nutrition and Dietetics and that this project work has not been previously submitted.

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Prof. Olusola Ladokun **Date**

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Dedication

This work is dedicated to God Almighty and my family members.

Acknowledgment

My profound gratitude goes to the Almighty God for His infinite mercy that I enjoyed all through the course of this research work.

I would like to acknowledge the late Dr. (Mrs). Aderonke Mosuro, the former Head of the Department, for her mentorship and guidance. Special thanks goes to my Supervisor, Prof. Olusola Ladokun, the Deputy Vice Chancellor, for dedicating her time to guiding me through the preparation of this Thesis. I am indeed grateful; I don't take any of your tutelage for granted. God in His infinite mercy will continue to bless you and grant you profound progress.

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Abstract

Non-communicable diseases (NCDs) such as cancer are leading causes of morbidity and mortality worldwide. Among various therapeutic targets, the Human Anaplastic Lymphoma Kinase (ALK) enzyme plays a crucial role in the development of certain cancers. This study investigates the nutritional bioactive compounds in *Annona Muricata* (soursop) and their potential in promoting health and preventing cancer. This study was carried out to comprehensively investigate the fruit (flesh), seeds, and skin of *Annona muricata* by analyzing their proximate composition, characterizing and screening the methanolic extracts of the seed and flesh using Gas Chromatography-Mass Spectrometry (GC-MS), and assessing the antioxidant properties of the extracts through assays including DPPH scavenging, Nitric Oxide (NO) assay, ferric reducing antioxidant power (FRAP) assay, and lipid peroxidation (LPO) assay. Additionally, computational study (*in silico*) was conducted to assess the inhibitory properties of bioactive compounds in the methanolic extracts against the Human ALK enzyme. Phytochemical screening revealed the presence of alkaloids, tannins, phlobatannins, saponins, phenols, reducing sugars, steroids, cardiac glycosides, terpenoids, and flavonoids in the seed, flesh, and bark of *Annona Muricata*. Antioxidant assays demonstrated significant total antioxidant capacities, with the bark showing the highest capacity (41.10 mg/100g), followed by the flesh (36.53 mg/100g) and the seed (35.30 mg/100g). The GC-MS analysis identified 134 compounds in the methanolic extracts, and molecular docking studies revealed that several of these compounds exhibit strong binding affinities with the ALK receptor. Notably, Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy (TTD) exhibited the highest binding affinity at -8.3 kcal/mol, outperforming the standard reference drug Ceritinib (-7.2 kcal/mol). These findings suggest that *Annona Muricata* contains potent bioactive compounds with significant antioxidant properties and potential inhibitory effects on cancer-related enzymes, highlighting its potential as a functional food in cancer prevention and health promotion.

Keywords: *Annona Muricata*, Anaplastic Lymphoma Kinase (ALK), bio-active compounds, antioxidant, phytochemicals, functional food, soursop, cancer.

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Table of Contents

List of Tables

List of Figures

Chapter One

Introduction

1.1 Background to the Study

Chronic non-communicable diseases (NCDs) are known to be the main and most driving reason for death and disability on the planet. They are a group of conditions that are not primarily brought about by any acute infection but rather can affect the long-term well-being of an individual and will frequently create a need for requirement for long haul therapy and care when signs and side effects endure or manifest[1]. Conditions of this nature include cancers, cardiovascular infection, diabetes and chronic lung illnesses, etc. Chronic, non-communicable diseases (NCDs) are consistently expanding all over the planet, and developing countries are bearing a large part of the expense with 80% mortality happening there. NCDs, for example, cardiovascular illness, cancer, diabetes and lung problems are supposed to cause 3/4 of the disease burden in low- and middle-income nations by 2030[1]. NCDs cause untold torment, suffering, inability and difficulty for a large number of individuals. Moreover, the financial expenses are huge, both as far as lost efficiency and rising medical care costs. Unless efforts are made to lessen the occurrence of NCDs, a World Bank insight forecasts NCDs will cost the worldwide economy about $35 trillion from 2005 to 2030[1]. drugs may affect the nutritional status of the body, acting on senses, appetite, resting energy expenditure, and food intake; conversely, food or one of its components may affect bioavailability and half-life, circulating plasma concentrations of drugs resulting in an increased risk of toxicity and its adverse effects, or therapeutic failure. Therefore, the knowledge of these possible interactions is fundamental for the implementation of a nutritional treatment in the presence of a pharmacological therapy [2].

Cancer and other chronic, non-communicable diseases (NCDs) are showing up at epidemic degrees all around and are adversely influencing peoples in the developing world. The survival rates of cancer are overall lower in developing countries presumably because of a combination of late diagnosis and limited access to treatment[1]. Nutrition is expected to play an immense part in hindering, treatment and managing cancers as one of the leading NCDs. According to WHO's most recent report, chronic non-communicable diseases (NCDs) are responsible for a staggering 41 million annual deaths, tending to 74% of overall mortality. This weight disproportionately impacts low-and focus pay countries (LMICs), addressing 77% of all NCD passing and 86% of awkward NCD passings (those incidents before age 70). Cardiovascular diseases are the fundamental wellspring of NCD mortality, declaring 17.9 million lives consistently, followed by cancers (9.3 million), chronic respiratory diseases (4.1 million), and diabetes (2.0 million, recalling those resulting for kidney contamination).

Together, these four disease groups address over 80% of premature NCD deaths. Modifiable risk factors, for instance, tobacco use, genuine inactivity, risky alcohol use, ill-managed weight control plans, and air pollution in a general sense add to the NCD's burden.

1.2 Cancer

Cancer is a group of diseases portrayed by the uncontrolled development and spread of malignant or benign cells. On the off chance that the spread isn't controlled, it can bring about death. Cancer can influence practically any piece of the body and is a main source of death around the world. As per the World Wellbeing Association (WHO), cancer represented almost 10 million deaths in 2022, with the most well-known types being lung, breast, colon, rectum, and

prostate cancers[1]. The WHO gauges that by 2030, there will be 23.6 million new instances of cancer every year, with a significant expansion in less monetarily regions of the world.

One huge hereditary factor in the advancement of specific cancers is the change of the Anaplastic Lymphoma Kinase (ALK) gene. The ALK gene gives guidelines to making a protein engaged with cell development and improvement. Transformations or revisions in this quality can prompt uncontrolled cell development and cancer.

1.3 Role of Dieting in Cancer Management

Diet and nutrition are important factors in cancer prevention and treatment because an unbalanced diet increases the risk of cancer onset, while malnutrition negatively impacts the efficacy of cancer treatment[3]. This highlights the vital job of dietary mediation in forestalling, Recent epidemiologic research has identified lifestyle and genetic factors associated with cancer prevention, while the identification of novel molecular agents has drastically changed treatment strategies. Since outcomes have improved for cancer patients, management of survivors has received increasing attention, especially concerning the role of diet and nutrition alleviating, or in any event, treating predominant non-communicable diseases (NCDs)[3].

Utilitarian food varieties, described by parts that present medical advantages past essential sustenance, are earning respect and acknowledgment. Research in this field has zeroed in on the effect of bioactive compounds found in food sources, especially the phenolic compounds common in products of the soil. These compounds, eminently polyphenols, show defensive impacts against different diseases, including cancer and cardiovascular diseases. Their cancer prevention agent properties battle free extremists, which can cause cell degeneration and add to

the improvement of obsessive circumstances. The developing interest in natural products that have bioactive parts mirrors a shift towards proactive wellbeing the executives through dietary decisions. Further examination investigating the particular components of activity and ideal admission levels of bioactive compounds will be vital in saddling their maximum capacity for illness anticipation and wellbeing advancement.

1.4 Nutritional Bioactive Foods

Nutritional bio-active compounds are normally happening substances in food that have potential medical advantages past essential nourishment. They are tracked down in a wide assortment of foods, including natural products, vegetables, entire grains, vegetables, nuts, and seeds. A few instances of nutritional bio-dynamic compounds incorporate polyphenols, carotenoids, flavonoids, and omega-3 fatty acids[8].

Functional foods are a class of food items that contain bioactive compounds, which are normal or processed substances that give medical advantages when eaten in successive amounts[5]. Foods with restorative properties, otherwise called functional foods, are a particular classification of food items perceived for their medical advantages past essential nutritional worth. These foods have a background marked by use in conventional medication rehearses worldwide and have been exposed to thorough logical examination to explain their remedial potential.

Nutritional bioactive compounds, derived from sources such as fruits, vegetables, and medicinal plants, exhibit diverse functionalities that render them valuable components in the development of functional foods. These compounds, including vitamins, minerals, antioxidants, fiber, and phytochemicals, can be incorporated into existing food products to augment specific attributes such as antioxidant activity, cholesterol regulation, and glycemic control. Furthermore, their

ability to reduce the risk of chronic diseases, including cardiovascular diseases, diabetes, obesity, and certain cancers, makes them ideal candidates for the creation of novel food products with targeted health benefits.

The beneficial effects of bioactive compounds are mediated through various physiological mechanisms, encompassing antioxidant activity, hormonal actions, and immune system modulation. These mechanisms facilitate processes such as nutrient absorption, substance transit through the digestive tract, colonic butyric acid production, and gut microbiome regulation [7].

Importantly, the structural modification of bioactive compounds can significantly influence their functional and nutritional properties. For instance, the interaction between polyphenols found in fruits and vegetables and proteins can lead to structural changes, impacting their overall efficacy[4]. Understanding these interactions is crucial for optimizing the functionality of bioactive compounds in functional foods [8].

While bioactive compounds can be synthesized, their natural presence in various food sources like seafood is gaining recognition due to their health-promoting properties and therapeutic potential in disease management[5,6].

Garlic, for example, contains allicin, a compound with antimicrobial, antiviral, and anticancer properties, underscoring the potential of dietary interventions in promoting public health and improving quality of life.

Turmeric, another normally utilized flavor, contains curcumin, which has strong anti-inflammatory properties. Chronic irritation has been connected to different diseases, including cancer and Alzheimer's. In this manner, integrating turmeric into dieting has been shown to

forestall or diminish inflammation and possibly decrease the gamble of fostering these immedicable diseases[12].

Nutritional bio-active compounds have been displayed to have an assortment of medical advantages which incorporate decreasing the gamble of chronic diseases like coronary illness, stroke, cancer, and diabetes, helping the resistant framework, working on mental capability, diminishing irritation, advancing solid maturing. There is proof that specific drinks and foods can safeguard against caries and gingivitis[13]. For instance, some cereals are known to be fortified with omega-3 fatty acids, while a few orange juices are fortified or reinforced with L-ascorbic acid (ascorbic corrosive) with antioxidant property.

1.5 Statement of the Problem

Chronic, non-communicable diseases (NCDs) are steadily increasing around the world, and developing countries are bearing much of the cost with 80 percent of deaths occurring there. NCDs such as cardiovascular disease, cancer, diabetes and lung disorders are expected to cause three-quarters of the disease burden in low- and middle-income countries by 2030. NCDs are usually characterized by untold pain, suffering, disability and hardship for millions of people. In addition, the economic costs are staggering, both in terms of lost productivity and rising health care expenses [5]. Unless action is taken to reduce the toll of NCDs, a World Bank study estimates NCDs will cost the global economy about $35 trillion from 2005 to 2030. A number of NIH research and research training programs are working to address these health issues[10,11]. Cancer and other chronic, non-communicable diseases (NCDs) are reaching epidemic proportions globally and are taking a particular toll on populations in the developing world. Cancer survival tends to be poorer in developing countries most likely because of a combination of late diagnosis

and limited access to treatment. Many cancers could be prevented by applying existing knowledge, implementing tobacco control programs, vaccinating against liver and cervical cancers, providing early detection and treatment, and instituting public health campaigns promoting healthy diets and exercise[10].

Bioactive compounds which can be found in a variety of fruits, vegetables, herbs, and spices, and have shown to have a range of health benefits, including reducing the risk of chronic diseases, boosting the immune system, and improving cognitive functions and development[1]. Drugs and food on the other hand, can interact mutually, since drugs can change the patient's nutritional status, body weight and nutrient availability, while foods and herbal products may significantly influence the drug's effects and efficacy in a direct or indirect manner: in the first case, a food or one of its components may modify the effect of the drug, in the second case, the food causes alterations in the absorption or metabolism of the drug. [2].

1.6 Justification of the Study

Functional foods are gaining significant attention due to their potential health benefits beyond basic nutrition. These foods contain bio-active compounds, natural or modified, that can offer numerous physiological benefits, such as improved cardiovascular health, enhanced immune system function, and reduced risk of chronic diseases. Consequently, there is a growing interest in understanding the relationship between nutritional bio-active compounds and their structural modifications to develop functional foods that can improve human health. The objective of this study is to explore and evaluate the potential of modifying the structural properties of bio-active compounds in soursop, and to create functional foods that can target specific health concerns.

Chronic diseases, such as cardiovascular diseases, diabetes, and certain cancers, are major global health concerns. In many cases, these diseases are linked to lifestyle factors, including diet. Developing functional foods enriched with bio-active compounds can offer a natural and effective way to mitigate the risk of chronic diseases, promoting overall health and well-being. Nutritional bio-active compounds have been observed to enhance the absorption of important nutrients like vitamins and minerals. They have shown immense potential in preventing and managing various health conditions. Through nutritional approaches to therapy, functional foods can be tailored to specifically target and address conditions such as inflammation, oxidative stress, and immune dysfunction, reducing the risk of diseases like arthritis, Alzheimer's, and cancer[10].

1.7 Aim and Objectives of the Study

This study aims to examine nutritional bioactive compounds present in *Annona Muricata* (soursop) that can serve as functional foods which are potent in the prevention and management of cancer.

To achieve the aim of this research, the following specific objectives will be met:

i. analysis of proximate composition of fruit (flesh), seeds and skin of *Annona Muricata*;

ii. characterization and phytochemical screening of methanol extract of seed and flesh of *Annona Muricata* using Gas Chromatography-Mass Spectrometry (GC-MS);

iii. evaluation of the antioxidant property of *Annona Muricata* methanol extract through various assays: DPPH scavenging, Nitric Oxide (NO) assay, ferric reducing antioxidant power (FRAP) assay and Lipid peroxidation (LPO) assay; and

iv. *in silico* study of the inhibitory property of bioactive compounds identified in methanolic extract of *Annona Muricata* on the Human Anaplastic Lymphoma Kinase enzyme.

1.8 Significance of the Study

The study of nutritional bio-active compounds and their primary adjustment for useful food sources holds huge significance in the field of nourishment and food science. This exploration region plans to investigate the capability of different bio-active compounds present in food sources and their alteration to upgrade their useful properties, the investigation of nutritional bio-active compounds and their underlying change for practical food sources is profoundly huge. It can possibly further develop well-being results, forestall and oversee diseases, drive advancement in the food business, add to feasible food creation, and set out monetary open doors. This exploration region will hold guarantee for working on generally human well-being and prosperity with regards to dietary decisions, sickness anticipation, therapy and administrations.

1.9 Hypothesis

Alternative Hypothesis - Ha: There is a significant relationship between cancer treatment, prevention and management and compound present in *Annona Muricata*.

Null Hypothesis - H_0: There is no significant relationship between cancer treatment, prevention and management and compound present in *Annona Muricata*.

1.10 Scope of the Study

The scope of this study is to examine the nutritional bioactive compound present in soursop bark, flesh and seed through solvent extraction, phytochemical screening, GCMS characterization and antioxidant assay.

1.11 Limitation of the Study

The study examined the potential cancer prevention and management properties of *Annona Muricata*, focusing specifically on certain bioactive compounds found in its extracts. However, this approach may overlook the role of other secondary metabolites present in the fruit, which can vary among different species.

The utilization of computational strategies and atomic docking to affirm the inhibitory capability of the recognized bioactive compounds is powerful and proficient. Nonetheless, these techniques are innately hypothetical, depending on numerical biophysical scoring capabilities. Subsequently, the outcomes can't supplant in that frame of mind of the natural product remove, in this manner restricting the immediate relevance of these discoveries to human well-being.

1.12 Definition of Terms:

Functional Foods: Foods that have been designed or modified to provide specific health benefits beyond basic nutrition. These foods contain bio-active compounds that can improve physiological functions or reduce the risk of disease.

Bioavailability: The proportion of a nutrient or bio-active compound that is absorbed and available for use by the body after consumption. Structural modifications can influence the bioavailability of these compounds by improving their absorption and utilization.

Pharmacokinetics: The study of how drugs or bio-active compounds are absorbed, distributed, metabolized, and excreted by the body. Understanding the pharmacokinetics of structurally modified bio-active compounds is crucial for determining their effectiveness and potential side effects.

Nutraceuticals: Foods or food products that contain bio-active compounds with potential health benefits. These substances are often isolated or concentrated from natural sources and used as supplementary or therapeutic agents to support health and prevent disease.

Phytochemicals: Natural bio-active compounds found in plants that are known to provide health benefits. Examples include polyphenols, carotenoids, flavonoids, and phenolic acids. Structural modifications of phytochemicals can enhance their antioxidant, anti-inflammatory, or anti-cancer properties.

Probiotics: Live microorganisms (such as certain strains of bacteria or yeast) that, when consumed in adequate amounts, provide health benefits to the host by improving the gut microbiota. Structural modifications of probiotics can enhance their survival during processing and storage, as well as their colonization and effectiveness in the human gastrointestinal tract.

Prebiotics: Non-digestible food ingredients that selectively stimulate the growth and activity of beneficial bacteria in the gut, thereby improving host health. Structural modifications of prebiotics can enhance their stability, functionality, and specificity in promoting the growth of desirable gut bacteria.

Shelf-life: The period during which a food product remains safe and maintains its desired quality attributes before it becomes unacceptable for consumption. Structural modifications can enhance the stability of bio-active compounds in functional foods, thereby extending their shelf-life and ensuring their efficacy.

Synergy: The interaction of multiple components in a food matrix to produce an effect greater than the sum of their individual effects. Structural modifications can enhance the synergy

between bio-active compounds and other food components, leading to increased health benefits in functional foods.

Clinical trial: A research study that evaluates the safety and efficacy of a new drug or treatment in humans. Clinical trials are often used to evaluate the health benefits of functional food products.

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Chapter Two

Literature Review

2.1 Bio-Active Compounds

Nutritional bio-active compounds are normally occurring substances in foods that usefully affect human well-being past fundamental nutrition. They can be found in a wide assortment of foods, including natural products, vegetables, entire grains, nuts, and seeds. Bio-active compounds have been found to work by protecting cells from damage, lowering inflammation, and supporting the immune system[1].

A comprehensive review delves into the intricate relationship between dietary plant-derived bioactive compounds, intestinal functionality, and the gut microbiome. The study encompasses a wide array of topics, including the influence of dietary choices on intestinal health, the composition of fecal microbiota, and the impact of phytochemicals found in fruits and vegetables. This is of significant relevance in the ongoing research and development of functional foods, as it elucidates the connection between bioactive compounds and their potential to enhance intestinal health[56]. Bioactive substances are those compounds that are capable of interacting with specific components of living tissues, promoting health through their diverse bioactivity profiles. Examples of naturally occurring dietary bioactive compounds encompass phenolic compounds (polyphenols), carotenoids, indoles, phytosterols, terpenes, sulfur compounds, quinones, omega-3 fatty acids, and probiotics. Additionally, certain nutrients such as vitamins, minerals, proteins,

and biopeptides that demonstrate physiological benefits beyond their primary nutritional functions are also considered bioactive compounds [4].

Bioactive compounds, including terpenoids, polyphenols, alkaloids and other nitrogen containing constituents, exert various beneficial effects arising from their antioxidant and anti-inflammatory properties. These compounds can be found in vegetables, fruits, grains, spices, and their derived foods and beverages such as tea, olive oil, fruit juices, wine, chocolate and beer[57].

Starchy root and tuber crops are regarded as fundamental components of the human diet, contributing to dietary diversity and offering various nutritional and health advantages. These benefits include antioxidant, hypoglycemic, hypocholesterolemic, antimicrobial, and immunomodulatory activities[7]. The presence of bioactive constituents such as phenolic compounds, saponins, bioactive proteins, glycoalkaloids, and phytic acids in these crops is responsible for the observed effects. Notably, many starchy tuber crops, with the exception of potatoes, sweet potatoes, and cassava, remain under-explored for their potential health benefits. In certain Asian countries, some edible tubers are also utilized in traditional medicine. Starchy roots and tubers are not only versatile ingredients in culinary applications but also hold significant potential as functional foods and nutraceutical ingredients, contributing to disease risk reduction and overall well-being[5,7].

Starchy root and tuber crops serve as functional foods due to their diverse nutritional and health benefits. These crops are rich sources of carbohydrates, playing a crucial role in human and animal diets. Furthermore, they contain a variety of bioactive compounds, including phenolic compounds, saponins, bioactive proteins, glycoalkaloids, and phytic acids, which contribute to

their observed physiological effects. These bioactive compounds are responsible for the antioxidant, hypoglycemic, hypocholesterolemic, antimicrobial, and immunomodulatory activities exhibited by starchy roots and tubers[5].

Although, a study shows that, there is possible interactions between food and medications which are relevant in clinical practices, but often unknown or overlooked. They occur more frequently with orally administered drugs. Indeed, food and beverages may alter the pharmacokinetic and pharmacodynamic profiles of a drug, leading to two different conditions: Increased concentrations in biological fluids that could enhance drug effect, up to the risk of side effects and toxicity; Reduced concentrations in biological liquids, and thus reduced effect of the drug, with the risk of total or partial ineffectiveness [2].

A recent study investigated the phenolic composition and antioxidant potential of grapefruit byproducts for application in novel antioxidant packaging. Employing Response Surface Methodology, researchers identified various phenolic compounds with potential antioxidant properties, suggesting their utility as active ingredients in sustainable packaging materials.

Further research explored the effect of incorporating natural extracts into packaging materials. One study examined the impact of orange peel essential oil on chitosan and fish skin gelatin blend films. Results indicated that the addition of the essential oil enhanced the blend films' properties, offering potential for improved functionality in packaging[6].

Additionally, the influence of green propolis extract on active pectin-based films was investigated. Findings demonstrated that incorporating green propolis extract significantly

improved the functional properties of the films, further supporting its potential as an active packaging ingredient.

Beyond packaging applications, research has delved into the bioactive compound profile and antioxidant activity of diverse frozen pulp cultivars[7]. Utilizing spectrophotometry, colorimetry, and modified extraction methods, this study quantified carotenoids, pigments, ascorbic acid, and total phenolics, revealing valuable insights into their composition and potential health benefits.

Guava plant is used as food and traditional medicine due to its pharmacologic properties. Guava leaf possesses a high content of several bioactive compounds, especially phenolic compounds, which contribute to antioxidant and anti-inflammatory activities. The most potent antioxidant found in guava leaves is known as quercetin.

The primary traditional uses of guava leaves are for the treatment of gastrointestinal illnesses (diarrhea, stomach pain, gastroenteritis, indigestion, and dysentery) and dermatological problems (skin infection, skin aging, and ulcers)[58]. A comprehensive overview provides valuable insights into the diverse array of bioactive components in guava and their potential applications.

The main bioactive compounds in mango by-products are polyphenols and carotenoids, among others. Polyphenols are known for their high antioxidant and antimicrobial activities. Carotenoids show provitamin A and antioxidant activity. Among the mango by-products, thekernelhasbeenstudiedmorethantegumentandpeelsbecauseoftheproportionandcomposition. The kernel represents 45–85% of the seed. The main bioactive components reported for the kernel are gallic, caffeic, cinnamic, tannic, and chlorogenic acids; methyl and ethyl gallates; mangiferin, rutin, hesperidin, and gallotannins; and penta-O-galloyl-glucoside and rhamnetin-3-[6-2-

butenoilhexoside] [59]. The article further looks at the medicinal properties of mango and its bio-active compound mangiferin, referring to concentrates on that recommend the potential medical advantages of this compound against way of life related messes. The physicochemical attributes of mango strip powder, including its dampness content, fat substance, and debris content, are additionally talked about.

Chrysophyllum albidum (C. albidum), also known as African star apple, is primarily a forest tree species with its occurrence in the Central, East and West Africa regions. It belongs to Sapotaceae family. In Nigeria, it is widely grown in the South Western part and locally called “agbalumo”. The fruit of C. albidum is traditionally used for nutritional purposes and to relief gastrointestinal tract disturbances[60]. The stem bark is used for the treatment of malaria and yellow fever, while the leaf is used as an emollient and for the treatment of skin eruption, stomach-ache and diarrhea The seeds, roots and leaves extracts are used to arrest bleeding from fresh wounds, inhibit microbial growth and enhance wound healing process. The root and stem bark extracts have been reported for the anti-fertility and antimicrobial effects. The anti-hyperglycemic and hypolipidemic effect of ethanol extract of the seed cotyledon and leaf have been evaluated[60].

A Research study on the nutrients composition and phytochemical items in the lyophilized consumable pieces of Chrysophyllum albidum natural product showed that the review underscores of the significance of fruits and fruit products in adding to food security and giving fundamental nutrients and micronutrients. It means to examine the nutritional worth and phytochemical items in the different palatable pieces of Chrysophyllum albidum organic product, including the natural product mash, organic product skin, and seed shell pericarp.

The article utilizes different strategies to dissect the supplement sythesis and phytochemical items in the natural product parts. This incorporates the utilization of conventional mineral investigation strategies, as well as other applicable methods. The concentrate likewise references past examination on restorative plants with potential antidiabetic action, featuring the meaning of Chrysophyllum albidum natural product in home grown medication.

The nutritional worth of *Chrysophyllum albidum* plants grown from the ground and its potential medicinal advantages give significant data on the mineral contents of the products of the soil and the significance of food examination and instrumentation in concentrating on the nutritional sythesis of food of the fruits[10,60].

Eggplant exhibits some mystical values which result in its most sort for within the region found. At some point, it is difficult to ascertain its mystical functionalities as it is widely used in the treatment of various ailments such as abdominal pain, constipation, diarrhea, and as an anti-ulcer agent. It also regulates blood pressure, enriches vitamins, boosts the immune system, reduces cholesterol, and prevents diabetes[61].

A study on eggplants also showed that the Plants produce a large number of secondary metabolites that impact human sustenance and wellbeing. Eggplants as vegetables are superb reservoirs of nutrients, dietary fiber, minerals, and phytochemicals. High intake of vegetables in the diet lessens the gamble of cardiovascular diseases and different health problems.

Eggplant is a non woody yearly plant with different fundamental supplements. It contains compounds like aspartic corrosive, tropane, flavonoids, and glycoalkaloids. Eggplant is low in calories and high in dampness content. Eggplant has been found to have potential medical

advantages, including calming, against asthmatic, and antiplatelet properties. It might likewise help in the counteraction and therapy of cancer and cardiovascular diseases. Customary home grown drugs frequently depend on the compounds tracked down in eggplant.

Eggplant contains different phytochemicals like nasunin, caffeine, chlorogenic corrosive, and delphinidin 3-coumaroyl rutinoside 5-glucoside. These phytochemicals add to the cancer prevention agent and searching exercises of eggplant. Eggplant is a rich wellspring of minerals, nutrients, dietary fiber, protein, and cell reinforcements. It contains significant supplements like iron, calcium, potassium, and magnesium[61].

Soursop (*Annona muricata*) is a plant belonging to the Annonaceae family that has been widely used globally as a traditional medicine for many diseases. *Anonna muricata's* activities were shown to include anticancer (25%), antiulcer (17%), antidiabetic (14%), antiprotozoal (10%), antidiarrhea (8%), antibacterial (8%), antiviral (8%), antihypertensive (6%), and wound healing (4%). Several biological activities and the general mechanisms underlying the effects of *Anonna muricata* have been tested both in vitro and in vivo. *Anonna muricata* contains chemicals such as acetogenins (annomuricins and annonacin), alkaloids (coreximine and reticuline), flavonoids (quercetin), and vitamins, which are predicted to be responsible for the biological activity of A. muricata[62].

A study of the Pharmacological Exercises of Soursop (*Annona Muricata*), a plant having a place with the *Annona Muricataceae* family that has been generally utilized universally as a customary medication for some diseases. A study showed the traditional use, synthetic use, and pharmacological activities of Anonna muricata which were examined. From 49 exploration

articles that were gotten from 1981 to 2021, *Anonna muricata's* exercises were displayed to incorporate anticancer (25%), antiulcer (17%), antidiabetic (14%), antiprotozoal (10%), antidiarrhea (8%), antibacterial (8%), Antiviral (8%), antihypertensive (6%), and wound mending (4%). A few biological exercises and the overall instruments hidden the impacts of Anonna muricata have been tried both in vitro and *in vivo*. Anonna muricata contains synthetic compounds, for example, acetogenins (annomuricins and Annona Muricatacin), alkaloids (corex-imine and reticuline), flavonoids (quercetin), and nutrients, which are anticipated to be liable for the biological action of *A. muricata*[11,61].

In an investigation of Apple phytochemicals and their medical advantages, Jeanell brought up that Proof recommends that a diet high in leafy foods might diminish the gamble of chronic diseases, like cardiovascular illness and cancer, and phytochemicals including phenolics, flavonoids and carotenoids from products of the soil might assume a key part in lessening chronic diseases risk. Apples are fiercely consumed, rich wellspring of phytochemicals and epidemiological investigations have connected the utilization of apples with diminished chance of certain cancers, cardiovascular illness, asthma, and diabetes. In the research center, apples have been found to have serious areas of strength for exceptionally action, restrain cancer cell expansion, decline lipid oxidation, and lower cholesterol. Apples contain various phytochemicals including quercetin, cetechin, phloridzin and chlorogenic corrosive, which are serious areas of strength for all. The phytochemical structure of apples shifts extraordinarily between various assortments of apples, and there are additionally little changes in phytochemicals during the development and aging of the natural product. Capacity meaningfully affects apple phytochemicals, yet handling can significantly influence apple phytochemicals[12].

Wolffia globosa, or watermeal, is a sea-going plant having a place with the Lemnaceae family that is polished off as food and sold in neighborhood markets of Tailand. This concentrate in view was finished to measure chosen active compounds and minerals in Wolffia globosa ethanolic separate and assess its cancer prevention agent action. All out phenolic, avonoid, and anthocyanin contents were dissected. Superior execution fluid chromatography was utilized for the assurance of beta-carotene, ferulic corrosive, luteolin-7-O-β-D-glucoside, and kaempferol. Mineral items (iron, potassium, calcium, magnesium, zinc, and) not entirely set in stone by nuclear retention spectroscopy. Antioxidative action was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azobis (3-ethylbenzothiazoline-6-sulfonic corrosive)) extremist rummaging examines. The beta-carotene, ferulic corrosive, luteolin-7-O-β-D-glucoside, and kaempferol items in the concentrate were 2.52 ± 0.10, 1.40 ± 0.10, 2.42 ± 0.50, and 1.57 ± 0.14 mg/g extricate, separately. The most noteworthy mineral substance in the Wolffia globosa remove was magnesium. The wet concentrate of Wolffia globosa showed higher measures of all minerals than the dry concentrate. Newly ready and bubbled Wolffia globosa separates showed revolutionary rummaging action at 1000 µg/milliliter with 75.77 ± 0.93% and 67.10 ± 0.20% restraint of DPPH and 70.40 ± 7.20% and 59.78 ± 3.16% hindrance of ABTS, individually. Wolffia globosa as plant is a promising novel wellspring of normal phytochemical constituents and cell reinforcements and has potential for improvement as a plant-based nutraceutical item for the treatment of diseases brought about by free radicals[13].

Taxus brevifolia, commonly known as the Pacific Yew, is one of the most important medicinal plants for anticancer research. It became widely known after the discovery of paclitaxel (Taxol), a highly effective chemotherapeutic agent. Paclitaxel has been extensively used to treat a wide

variety of cancers, including ovarian, breast, lung, and prostate cancers. Paclitaxel works by stabilizing microtubules during cell division, which prevents the successful completion of mitosis (cell division). As a result, cancer cells are unable to multiply, leading to apoptosis (programmed cell death).

Numerous *in vivo* studies have been conducted using paclitaxel in animal models and human clinical trials. In ovarian cancer models, paclitaxel demonstrated significant tumour reduction, leading to increased survival rates in patients. In breast cancer patients, paclitaxel is widely used in combination with other chemotherapeutics, and *in vivo* studies in mice have shown its effectiveness in reducing metastasis and enhancing immune responses.

Computational modeling has been applied to study the binding affinity of paclitaxel to tubulin, the protein it targets to stabilize microtubules. These studies have confirmed that paclitaxel has a high affinity for β-tubulin, which is crucial for its anticancer activity. *In silico* docking experiments have also explored potential paclitaxel analogs with improved solubility and reduced side effects, enhancing the drug's pharmacokinetics. Paclitaxel is a standard treatment in oncology, particularly in taxane-based chemotherapy regimens. It has proven effective not only in solid tumours but also in some hematologic malignancies. However, the harvesting of paclitaxel from natural sources like Taxus brevifolia has environmental consequences, leading to synthetic production alternatives.

While effective, paclitaxel can cause severe side effects, including neurotoxicity and hypersensitivity reactions. To mitigate these, researchers have investigated nanoparticle delivery systems to improve its targeting and reduce toxicity.

Catharanthus roseus, also known as Madagascar Periwinkle, is another significant plant in anticancer treatment due to its production of the alkaloids vincristine and vinblastine. These compounds are widely used in chemotherapy, especially in the treatment of leukemia, lymphoma, and breast cancer. Both vincristine and vinblastine are mitotic inhibitors, disrupting the formation of microtubules required for cell division. By binding to tubulin, they prevent polymerization, halting the mitotic process and inducing apoptosis in rapidly dividing cancer cells. In studies involving human cancer cell lines and animal models, vincristine and vinblastine have shown potent anticancer activity. For example, *in vivo* trials in mice with leukemia and lymphoma demonstrated a significant reduction in tumor size. Vincristine has also been used in clinical trials to treat pediatric leukemia, showing high efficacy in inducing remission.

In silico studies have been employed to model the interaction of vincristine and vinblastine with their molecular targets, such as tubulin. These simulations have helped in understanding the binding dynamics and optimizing drug dosages for better therapeutic outcomes. Virtual screening approaches have also been used to identify new derivatives of these alkaloids with improved activity and fewer side effects.

Vincristine is primarily used for hematological malignancies like acute lymphoblastic leukemia (ALL) and Hodgkin's lymphoma. Vinblastine, on the other hand, is commonly used for breast cancer, testicular cancer, and Kaposi’s sarcoma. These compounds have dramatically improved survival rates in cancers that were once considered fatal.

Like paclitaxel, vincristine and vinblastine are associated with dose-limiting side effects, such as peripheral neuropathy and myelo suppression. Overcoming these toxicities remains a key research focus, including the use of targeted delivery systems and combination therapies.

Curcuma longa, commonly known as turmeric, contains the bioactive compound curcumin, which has shown significant potential in cancer prevention and treatment. Curcumin's anticancer activity has been documented against various types of cancers, including colon, breast, pancreatic, and prostate cancers. Its effects are mediated by multiple mechanisms, making it a multi-targeted anticancer agent.

Curcumin exerts its anticancer effects through various pathways: It inhibits cell proliferation by blocking transcription factors like NF-κB, which are involved in inflammation and tumorigenesis. Curcumin induces apoptosis by modulating pro- and anti-apoptotic proteins. It also inhibits angiogenesis (the formation of new blood vessels) by downregulating vascular endothelial growth factor (VEGF). Additionally, curcumin modulates epigenetic changes, such as DNA methylation, which are crucial in cancer development. Multiple *in vivo* studies in animal models have shown that curcumin effectively suppresses tumour growth. For instance, in colorectal cancer models in mice, curcumin has demonstrated inhibition of tumour growth by suppressing inflammatory markers and inducing apoptosis. *In vivo* studies in pancreatic cancer models have also shown that curcumin can reduce tumour size and increase survival rates when combined with standard chemotherapeutics.

In silico studies have been instrumental in understanding curcumin's interactions with various cancer-related targets. Molecular docking studies have revealed strong binding affinities between

curcumin and proteins involved in cancer cell survival, such as Akt, Bcl-2, and COX-2. These computational approaches have also aided in the design of curcumin analogs with better bioavailability, as the poor solubility of curcumin in water limits its therapeutic use.

Although curcumin has shown potent anticancer effects in preclinical models, its clinical application has been hampered by low bioavailability. However, clinical trials are ongoing to explore the potential of curcumin in combination therapies or in nanoparticle formulations that enhance its delivery to tumors. Curcumin is also being explored as a chemopreventive agent due to its ability to reduce inflammation and oxidative stress, both of which are associated with cancer development.

The primary challenge with curcumin is its poor systemic bioavailability due to rapid metabolism and excretion. To overcome this, researchers are developing curcumin derivatives, liposomal formulations, and conjugates to improve its solubility and effectiveness in cancer therapy.

The disclosure that plants produce hormonally active phytochemicals has adjusted how we might interpret the association among diet and human wellbeing. It is deeply grounded that natural product or plant removes are a mind-boggling combination of different constituents and, in the vast majority of the occurrences, it isn't evident whether a solitary compound or a combination of compounds is liable for the revealed effects[14]. The prospect of the entire spice or multi-spice readiness tends to different targets, however conceivably will mitigate the harmfulness and results of a solitary, disconnected compound from the plant. Numerous in vitro and in vivo examinations called attention to high nutritional and potential tissue explicit activity of

concentrate of Punica granatum. Evidences are collecting that compounds present in a natural product or spice separate expand each other's biological impact. For instance, it has been accounted for that quercetin and ellagic corrosive (both are likewise present in pomegranate) together utilize a more noticeable inhibitory impact against cancer cell development than either compound alone[15]. We had found that PME has antiestrogenic impact in the mammary organ, without compromising the useful impacts of estrogen in the cardiovascular and skeletal framework and had no estrogenicity in the uterus[14]. PME might actually be considered as an ideal SERM and further examinations could exhibit its appropriateness and conceivable application in estrogen subordinate bosom cancers with helpful impacts in other chemical ward tissues[16].

2.2 *Annona Muricata* (Soursop)

2.2.1 Taxonomy

It is known by many common names such as; Soursop, Graviola, Guanabana, or thorny custard apple. Annona is a genus of tropical fruit trees belonging to the family Annonaceae. The number of genera and species in the family Annonaceae has been a long term debate among researchers. Annonaceae family is one of the earliest angiosperms. Most Annona species have South America and the Antilles as their origin, with Mexico as its center of origin. The term Annona etymologically is derived from the Latin word Annona which means 'yearly produced[63]. In the genus Annona, edible fruits are produced by Annona cherimola, Annona muricata, Annona reticulata, Annona squamosa, Annona atemoya (a natural hybrid of Annona cherimola and Annona squamosa), and Annona diversifolia. The Annona genus is usually composite fruits made up of scaly sections that grow together in fir-cone fashion. Annona muricata is the most

tropical semi-deciduous tree with the largest fruits of the Annona genus. It is widespread in the tropic area of Asia, Central and South America, including the Amazon basin. It is known as "guanabana" in Spanish, "graviola" in Portuguese, "soursop" in English and "ebo" in Yoruba.

Soursop (Annona muricata L.) belongs to the family Annonaceae and is reported to have the largest fruit in the genus, Annona. It is best known as "sawasop" in Nigerian vernacular. It is a fruit tree that was in almost every home garden in time past. The need for space, urbanization, inadequate knowledge about the fruit tree potentials, economic relevance and/or preference, has led to its fast eroding out of the urban and semi-urban areas, with relatively fewer stands, compared to decades ago. Soursop has been reported to originate from the American tropics but has been successfully introduced and domesticated around the world. Several past and current researches have been aimed at revealing the medicinal potential and other properties of soursop. Hitherto, the reported ethnomedicinal properties of soursop include but not restricted to analgesic, antibacterial, anticancer, antifungal, antioxidant, antitumor, antiulcer, antiviral, anti-arthritic, anti-diabetic, anti-hypertensive, anti-inflammatory, anti-insomnia, anti-rheumatic, anti-stress, immune enhancing, as well as wound healing capacity[61,63].

Ecology and Physiology

Annona Muricata is the one of the most tropical semi deciduous fruit-tree with the biggest produce of the *Annona Muricata* genus. It is a common place tropical areas and local to SubSaharan Africa nations.

Table 2.1: *Annona Muricata* Plant Profile

Kingdom:	Plantae
Division:	Angiosperms
Class:	Magnoliids
Order:	Magnoliales
Family:	*Annona Muricata*ceae
Genus:	*Annona Muricata*
Species:	*Annona Muricata*

Source: [46]

Recently, it is found in numerous areas of Tropical Africa and there are various names for Soursop in various nations. It is low-fanning and thick yet a direct result of its improved appendages and arrives at a level of 25 or 30 feet (7.5-9 m).

The foul leaves, regularly evergreen, are substitute, smooth, gleaming, dim green on the upper surface, lighter underneath; oval, lanceolate, pointed at the two finishes. The little trees bear their natural product aimlessly on twigs, branches and trunks.

These natural products (Figure 2.1) territory in size from four to twelve crawls long, and up to six creeps in width. They can be oval or unpredictably formed as one side for the most part becomes quicker than the other. The natural product is oval or heart-formed, in some cases unpredictable, unbalanced or bended, because of ill-advised carper advancement or bug injury.

Most species under Annona Muricata genus have a place with the tropical America, aside from A. senegalensis, which has a place with tropical Africa[17, 18].

The Annona Muricata species are deciduous bushes or trees of little level, which range from 5 to 11 meters. This fluctuation is a direct result of a few elements including animal groups, soil conditions, environment changes, and yield the executives. The Annona Muricata are corroded to grayish and are shaggy when youthful however become glabrous in later phases of development[19].

Annona Muricata plant is a wellspring of sweet and fragrant flavor natural product (Figure 2.1), which is polished off straightforwardly or as handled items, for example, juices, frozen yogurt, and fermented beverages[21,22]. Aside from this, it is an expected wellspring of bioactive synthetics and supplements to further develop human health[23].

(a) **(b)**

(c)

(d)

Figure 2.1: Fruit (a,b), Leaves (c) and Seed (d) of *Annona Muricata*

Source: [47]

Annona Muricata contains different phytochemicals in roots, leaves, twigs, bark, seeds, and organic product's mash. The major bioactive compound incorporates acetogenins, alkaloids, phenolic compounds, Flavanoids, tannins, carotenoids, terpenoids, anthocyanins, phenols, and phlobatannins. As far as nutritional organization, the soursop natural product is a genuine force to be reckoned with of fundamental nutrients, minerals, and cell reinforcements. It is a brilliant wellspring of L-ascorbic acid, giving a huge part of the suggested day to day consumption in only one serving.

Additionally, it contains vitamins B1, B2, and B3, as well as minerals like potassium, magnesium, and iron. The natural product is likewise wealthy in dietary fiber, which supports processing and advances a sound stomach *microbiome*. The culinary flexibility of the *Annona Muricata* natural product is genuinely exceptional, with many culinary applications across different societies. In tropical districts where it is developed, the natural product is in many cases delighted in new, either consumed all alone or utilized as a critical fixing in natural product servings of mixed greens, smoothies, and treats. Its velvety surface makes it an ideal base for milkshakes, frozen yogurts, and sorbets, while its tart flavor adds profundity to appetizing dishes like salsas, marinades, and sauces[22].

Past its culinary allure, the soursop organic product is venerated for its heap medical advantages and restorative properties. In customary medication, different pieces of the *Annona Muricata* tree, including the organic product, leaves, seeds, and bark, have been utilized to treat many sicknesses, including stomach related messes, respiratory diseases, and parasitic pervasions. Moreover, starter research recommends that specific compounds found in soursop might have calming, antimicrobial, and anticancer properties, making it a subject of interest in the field of normal medication and drug research[2]. The *Annona Muricata* natural product, or soursop, is a captivating plant treasure with a rich history and many ideals. From its thorny outside to its smooth tissue, this tropical delicacy spellbinds the faculties with its tempting fragrance, enticing flavor, and noteworthy nutritional profile.

The organic product, bark, leaves and underlying foundations of A. muricata are known to be wealthy in *flavonoids*, *isoquinoline* alkaloids and *Annona Muricataceous acetogenins* (ACGs).

The piece of *Annona Muricata phytochemicals* fluctuates extraordinarily between its various species. Research proof propose that concentrates from organic product, leaves, seeds, and bark of different *Annona Muricata* species show gainful impacts including *antitumoral*, antiprotozoal *antidiabetic*, mitigating, *hepatoprotective*, pain relieving, and *anxiolytic* effects[24,25,28]. Nonetheless, being a particularly significant wellspring of therapeutic worth, impact of dosages, bioavailability, and toxicological and clinical examinations concerning human application remained Unexplored[34,35].

Recognizable proof of plant-based drugs has fundamentally added to anticancer medication revelations and has brought about 33% of anticancer medications endorsed by the US Food and Medication Organization (USFDA) by and large, for example *paclitaxel*, *camptothecin*, *vincristine* and their analogs. Considering this set of experiences, it is basic to proceed to investigate and distinguish new restorative plants and to decide their true capacity as a wellspring of new medications. Notwithstanding, a large number of these plant-determined unadulterated compound medications were chosen in light of high strength, target-explicit cell killing and, in that capacity, show extensive potential for unfavorable impacts. In any case, past these wellsprings of new medication revelation, plant-based prescriptions have likewise been utilized for quite a long time to treat numerous sicknesses and diseases, and their phytochemical constituents are known to have various pharmacologically valuable properties. A portion of these phytochemicals are very much endured in view of their verifiable use; they show restricted strength communications with a wide scope of cell targets and restricted unmistakable harmfulness.

In the past 50 years, natural compounds including plant products, have played a major role in drug discovery and have provided value to the pharmaceutical industry. For instance, therapeutics for various infectious diseases, cancer, and other debilitation diseases caused by metabolic disorders have all benefitted from many drug classes that were initially developed based on active compounds from plant sources[39].

Annona Muricata has broad conventional use, and extensive proof has been fostered that it very well might be valuable helpful specialists in the fight against certain cancers[20].

2.2.2 Ethnomedicinal Uses

Similar to other *Annona* species such as *A. squamosa* and *A. reticulata*, various parts of the *A. muricata* tree have been extensively used in traditional medicine across diverse cultures. Remarkably, the natural product tracks down application in dealing with diseases like ligament torment, loose bowels, diarrhea, fever, intestinal sickness, parasitic contaminations, stiffness, skin rashes, and worms. It's additionally accepted to increment bosom milk creation in moms post-childbirth[19,21]. The leaves are utilized to address conditions like cystitis, diabetes, cerebral pains, and sleep deprivation. Moreover, interior utilization of leaf decoctions is accepted to have rheumatic and neuralgic properties, while cooked leaves are applied topically for treating abscesses and rheumatism. Squashed seeds are customarily utilized for their anthelmintic action against different worms and parasites[23].

Annona cherimola Miller fruit has been used in Mexican traditional medicine as natural remedy against diabetes. The in vitro anti-glycation and anti-α-glucosidase roles of Annona cherimola Miller pulp extract (CE) were investigated, Moreover, healthy and diabetic subjects were

enrolled in a cross-over design intervention study aimed at investigating the effects of pulp intake on postprandial glycemia. The study shows that CE was able to inhibit albumin glycation in vitro and to inhibit α-glucosidase enzyme. Furthermore, the pulp intake did not contribute to an increase in postprandial glycemia, making it a suitable source of health-promoting phytonutrients and a potential functional food in diabetics and pre-diabetics diet [21].

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines to meet their primary health care needs. The primary characteristics of these medicines are medicinal plant chemical substances that exert a physiologic action on the human body. The most important plant bioactive compounds are thought to be alkaloids, flavonoids, tannins, and phenolic compounds. New anti-infective drugs can be discovered from higher plants using the plant chemical phytochemical ethnopharmacological approach[26].

Ethnomedicine, the investigation of conventional restorative practices, offers an abundance of information about the mending properties of different plants. *Annona Muricata*, normally known as soursop or *graviola*, is one such plant that has been used for quite a long time in customary medication across many societies. This exposition expects to investigate the *ethnomedicinal* uses of *Annona Muricata*, featuring its importance, conventional applications, and possible current ramifications. *Annona Muricata* is a tropical natural product bearing tree local to the Americas, especially tracked down in the Caribbean, Focal America, and portions of South America. It has a place with the *Annona Muricataceae* family and is portrayed by its spiky green organic product with an extraordinary flavor profile that mixes notes of strawberry and pineapple. Over the entire course of time, different native networks have analysed *Annona Muricata* for its restorative

properties. The organic product, leaves, seeds, and bark of the tree are totally used for their helpful advantages. These conventional purposes have been gone down through ages and keep on being polished in numerous districts today [22].

Past its restorative purposes, A. muricata additionally tracks down application in different locales. In tropical Africa, the plant fills in as an astringent, bug spray, and piscicide. It's likewise used to treat hacks, agony, and skin diseases. In India, the leafy foods are utilized for treating catarrh, while the root-bark and leaves are accepted to have mitigating and anthelmintic properties[24,25]. In Malaysia, a squashed leaf combination of A. muricata joined with A. squamosa and Hibiscus rosa-sinensis is utilized as a head juice accepted to forestall fainting[27]. Quite, in South America and tropical Africa (counting Nigeria), A. muricata leaves are utilized as an ethnomedicine against growths and cancer[24]. Moreover, the leaves, barks, and roots are credited with calming, hypoglycemic, soothing, smooth muscle relaxant, hypotensive, and antispasmodic effects[19,21].

One of the most conspicuous ethnomedicinal uses of *Annona Muricata* is its part in battling cancer. Native people group in the Amazon rainforest have long utilized various pieces of the plant to treat cancerous growths. Research directed lately has shown that concentrates from *Annona Muricata* have strong anticancer properties, with studies demonstrating its viability against different sorts of cancer cells, including those of the bosom, colon, prostate, and lung. Past its anticancer impacts, *Annona Muricata* is likewise utilized in the therapy of various different illnesses. In customary medication, the leaves of the soursop tree are frequently prepared into teas or decoctions to mitigate side effects of fever, hacks, and respiratory contaminations. The organic product itself is consumed to support invulnerability and advance stomach related wellbeing, while the seeds and bark are utilized for their antimicrobial properties.

Furthermore, *Annona Muricata* is esteemed for its calming and mitigating properties. Native people group frequently use it to quiet the nerves, assuage pressure, and advance unwinding. Its mitigating impacts make it valuable in overseeing conditions like joint pain and rheumatism [24].

It means a lot to take note of that while A. *muricata* has a rich history of customary therapeutic use, logical approval for many of these applications is as yet progressing. Besides, *Annona Muricata* has been read up for its likely cardiovascular advantages. Research recommends that compounds found in the natural product might assist with bringing down circulatory strain and cholesterol levels, hence decreasing the gamble of coronary illness and stroke. Its high cell reinforcement content is additionally accepted to add to its cardioprotective impacts. Notwithstanding its long history of customary use and promising logical examination, *Annona Muricata* faces difficulties in earning boundless respect and acknowledgment in present day medication. While it holds incredible potential as a wellspring of novel therapeutics, further examinations are expected to approve its viability and security for clinical use. Also, issues like manageability, normalization of concentrates, and moral contemplations encompassing its commercialization should be addressed [26].

Notwithstanding its conventional restorative applications, the product of A. *muricata* is broadly utilized in food items like refreshments, confections, frozen yogurts, shakes, and syrups. *Annona Muricata* fills in as a captivating illustration of the crossing point between customary information and current science in the field of ethnomedicine. Its different exhibit of ethnomedicinal utilizes, going from cancer therapy to invulnerable help, features the significance of safeguarding and concentrating on customary mending rehearses. By overcoming any issues

between conventional insight and contemporary examination, *Annona Muricata* holds guarantee as a significant wellspring of elective medication for the future[27,28].

2.2.3 Cultural Significance and Culinary Uses

Soursop holds significant cultural importance in regions where it grows, featuring prominently in traditional medicine, folklore, and cuisine. Indigenous communities have long revered the fruit for its purported medicinal properties, attributing it with anti-inflammatory, antioxidant, and anti-cancer effects. In addition to its alleged health benefits, soursop is cherished for its versatility in culinary applications. It's consumed fresh, juiced, blended into smoothies, or incorporated into jams, ice creams, and savory dishes. Moreover, its leaves are brewed into herbal teas and tinctures, believed to alleviate various ailments ranging from digestive issues to hypertension[23]. *Annona Muricata*, commonly known as soursop or graviola, holds significant cultural value across various regions of the world due to its diverse uses in traditional medicine, culinary practices, and folklore. This essay will explore the cultural significance of *Annona Muricata* and its impact on society globally[23].

Firstly, in traditional medicine, *Annona Muricata* has been utilized for centuries for its purported medicinal properties. In many cultures, various parts of the soursop tree, including its leaves, fruit, seeds, and bark, are believed to possess therapeutic benefits. For example, in parts of South America and the Caribbean, soursop tea made from the leaves is often consumed as a remedy for ailments such as fever, cough, and inflammation. Additionally, extracts from the fruit and seeds are thought to have anticancer properties and are used in alternative cancer treatments. The widespread use of *Annona Muricata* in traditional medicine reflects its cultural importance as a natural remedy deeply rooted in indigenous knowledge systems[23,24].

Secondly, *Annona Muricata* holds cultural significance in culinary traditions around the world. The fruit, with its sweet and tangy flavor, is a popular ingredient in various dishes and beverages. In tropical regions where the soursop tree is cultivated, such as in Southeast Asia, Latin America, and the Caribbean, soursop is commonly used in smoothies, desserts, jams, and ice creams. Its unique taste and versatility in cooking have led to its integration into local cuisines, becoming a symbol of cultural identity and culinary heritage[22,23].

Moreover, Annona Muricata includes conspicuously in old stories and social practices in numerous districts. In certain societies, the soursop tree is accepted to have magical properties and is related with fantasies and legends. For instance, in specific Caribbean nations, it is said that establishing a soursop tree in one's yard can avert detestable spirits and carry best of luck to the family. Additionally, in native Amazonian societies, the organic product is in some cases utilized in otherworldly services and ceremonies to advance recuperating and refinement. These social convictions and practices feature the well-established association between Annona Muricata and the social texture of networks where it is developed. Annona Muricata, or soursop, holds critical social worth across the world, as proven by its boundless use in customary medication, culinary practices, and legends. From its restorative properties to its part in neighborhood cooking styles and social customs, soursop assumes a diverse part in forming the social personality of networks where it is worshipped. As social orders proceed to embrace and protect their social legacy, Annona Muricata will without a doubt stay a valued image of custom and essentialness for ages to come[24, 25].

2.2.4 Health Benefits and Research

While anecdotal evidence extols soursop's medicinal virtues, scientific research has yielded mixed findings regarding its efficacy in treating specific health conditions. Compounds found within soursop, such as acetogenins and antioxidants, have been studied for their potential anti-cancer properties, with some preliminary studies suggesting inhibitory effects on cancer cell growth. However, further research is needed to validate these claims and determine the fruit's optimal therapeutic applications. *Annona Muricata*, commonly known as soursop or graviola, is a tropical fruit that offers numerous health benefits. From its potent antioxidant properties to its potential cancer-fighting abilities, *Annona Muricata* has gained attention for its therapeutic properties. Here's a comprehensive essay on its health benefits[25].

Annona Muricata, a fruit native to the tropical regions of the Americas, has been revered for centuries by indigenous communities for its medicinal properties. Its popularity has grown in recent years due to scientific research highlighting its health benefits.

Annona Muricata is rich in essential nutrients, including vitamin C, vitamin B_6, folate, potassium, and dietary fiber. These nutrients play crucial roles in maintaining overall health and well-being. One of the key health benefits of *Annona Muricata* is its potent antioxidant properties. Antioxidants help neutralize harmful free radicals in the body, which can cause oxidative stress and damage to cells. By scavenging free radicals, *Annona Muricata* helps protect against chronic diseases such as heart disease, diabetes, and cancer[27].

Anti-inflammatory Effects: *Annona Muricata* contains compounds that exhibit anti-inflammatory effects. Chronic inflammation is linked to various health problems, including

arthritis, asthma, and heart disease. Consuming *Annona Muricata* may help reduce inflammation and alleviate symptoms associated with inflammatory conditions[27].

Immune Support: The vitamin C content of *Annona Muricata* plays a crucial role in supporting the immune system. Vitamin C is essential for the production and function of white blood cells, which are the body's primary defense against infections and illnesses. Including *Annona Muricata* in the diet can help strengthen the immune system and reduce the risk of infections[27].

Digestive Health: *Annona Muricata* is a good source of dietary fiber, which is important for digestive health. Fiber helps regulate bowel movements, prevent constipation, and promote the growth of beneficial bacteria in the gut. A healthy digestive system is essential for nutrient absorption and overall well-being[41].

Cardiovascular Benefits: Potassium, a mineral found in *Annona Muricata*, plays a key role in maintaining healthy blood pressure levels. Adequate potassium intake is associated with a reduced risk of hypertension and cardiovascular disease. Including potassium-rich foods like *Annona Muricata* in the diet can help support heart health and lower the risk of stroke and heart attack[27]. A wide array of chemical compounds from various parts of Annonaceae plants have been discovered, isolated and characterized. The results of both phytochemical investigations and biological studies on various plants from this family have led to the identification of a wide diversity of compounds such as annonaceous acetogenins, flavonoids, alkaloids and essential oils. These phytochemical constituents have been found to exhibit a broad range of biological activities such as immunosuppressive, antineoplastic, cytotoxic, antimicrobial, anti-inflammatory effects[41].

Cancer-Fighting Potential: Perhaps one of the most intriguing health benefits of *Annona Muricata* is its potential cancer-fighting properties. Several studies have investigated the anticancer effects of *Annona Muricata* extract, particularly against various types of cancer cells. While more research is needed to fully understand its mechanisms of action, *Annona Muricata* shows promising potential as a natural cancer therapy[27].

Annona Muricata is a tropical fruit that offers a wide range of health benefits. From its antioxidant and anti-inflammatory properties to its potential cancer-fighting abilities, *Annona Muricata* has earned its reputation as a superfood. By including this nutritious fruit in your diet, you can support your overall health and well-being.

2.2.5 Cultivation and Harvesting

Developing *Annona Muricata* requires a tropical or subtropical environment with very much depleted, prolific soil and more than adequate daylight. Engendering is normally accomplished through seeds or vegetative strategies like joining and stem cuttings. The trees commonly start fruiting inside 3 to 5 years of planting, with top creation happening from 5 to 8 years onwards. Collecting is ordinarily finished by hand when the natural products arrive at development, demonstrated by a slight respect delicate strain and an adjustment of variety. Development of *Annona Muricata*, generally known as soursop or graviola, includes different perspectives from planting to collecting and post-reap the board. Soursop is a tropical organic product tree local to the Americas, valued for its tasty foods grown from the ground properties [29].

Annona Muricata is a little, evergreen tree that has a place with the *Annona Muricataceae* family. It is local to the tropical areas of the Americas however is currently developed in many regions

of the planet with reasonable environments, including Southeast Asia, Africa, and the Pacific Islands. The product of the soursop tree is enormous, prickly, and green in variety, with a white, sinewy, and succulent inside containing dark seeds. It is known for its sweet and tart flavor, frequently utilized in drinks, treats, and customary medication. Soursop flourishes in warm, heat and humidities with temperatures between 25°C to 35°C (77°F to 95°F) and yearly precipitation going from 1000mm to 2000mm. It can endure brief times of dry spell however requires ordinary watering, particularly during the blooming and fruiting stages. The tree favors very much depleted, rich soil with a pH level of 5.5 to 6.5. It is delicate to ice and can't endure temperatures beneath 5°C (41°F) [30].

Soursop can be engendered from seeds, cuttings, or unions. Seeds are normally utilized for proliferation because of their simple accessibility and minimal expense. New seeds extricated from ready organic products ought to be planted promptly to keep them from drying out. Seeds grow inside 2 to 3 weeks under ideal circumstances. On the other hand, stem cuttings taken from sound, mature trees can be established in a nursery climate. Joining onto rootstocks of related Annona Muricata species is one more strategy used to engender chosen cultivars. Soursop trees ought to be established in good to go soil with sufficient waste. Dividing between trees relies upon the assortment and wanted shelter size however regularly goes from 5 to 8 meters. Prior to planting, it is fitting to integrate natural matter into the dirt to further develop ripeness and dampness maintenance. Youthful trees ought to be furnished with shade and assurance from solid breezes until they become established[29].

Watering: Soursop trees require regular watering, especially during dry periods and flowering/fruiting stages. However, waterlogged conditions should be avoided to prevent root rot.

Fertilization: Apply balanced fertilizers containing nitrogen, phosphorus, and potassium regularly to promote healthy growth and fruit development. Additional micronutrients such as magnesium, calcium, and zinc may also be required, depending on soil conditions.

Pruning: Prune soursop trees annually to remove dead or diseased branches, promote airflow, and maintain desired shape and size. Pruning also encourages the development of new growth and improves fruit quality.

Pollination: Soursop flowers are typically pollinated by beetles, flies, and other insects attracted to their strong scent. However, hand pollination may be necessary in areas with limited insect activity to ensure adequate fruit set.

Pest and Disease Control: Common pests of soursop include fruit flies, aphids, scales, and mites, which can be controlled using biological, cultural, or chemical methods. Diseases such as anthracnose, powdery mildew, and leaf spot can be managed through proper sanitation, fungicide applications, and resistant cultivars.

Soursop fruits usually mature within 2 to 3 months after flowering. Mature fruits are firm to the touch and emit a strong, sweet fragrance. They should be harvested carefully using pruning shears or a sharp knife to avoid damaging the delicate skin. Fruits can be stored at room temperature for a few days or refrigerated for longer shelf life. However, soursop is best consumed fresh as it tends to lose flavor and texture upon prolonged storage. The fruit can also be processed into juice, puree, or other value-added products for extended use. Cultivation of *Annona Muricata*, or soursop, offers numerous benefits including a sustainable source of nutritious fruit, potential income generation, and ecosystem services such as carbon sequestration

and soil conservation. By following proper cultural practices and management techniques, farmers can maximize yields and quality while minimizing environmental impact. Soursop cultivation not only contributes to food security and economic development but also promotes biodiversity conservation and sustainable agriculture practices[29].

2.2.6 Challenges and Conservation

Notwithstanding its prevalence, *Annona Muricata* faces different difficulties, including vulnerability to vermin and diseases, for example, anthracnose and organic product flies. Moreover, living space misfortune and deforestation compromise wild populaces, requiring protection endeavors to safeguard hereditary variety and biological system flexibility. Annona Muricata, or soursop, is an enrapturing herbal example eminent for its particular appearance, delicious flavor, and potential medical advantages. Whether appreciated new or integrated into different culinary manifestations, this tropical fortune proceeds to spellbind and move the two fans and scientists alike[27].

Annona Muricata has a long history of customary therapeutic use, with its leaves, seeds, and organic product accepted to have strong mitigating, cell reinforcement, and anticancer properties. In any case, in spite of its social and monetary significance, Annona Muricata faces various protection challenges. One of the essential difficulties is environment misfortune and discontinuity. Deforestation, agrarian extension, urbanization, and foundation improvement are contributing variables to the deficiency of regular territory for Annona Muricata. As backwoods are cleared for farming or logging, the regular environments of soursop trees are obliterated, prompting a decrease in populace numbers and hereditary diversity[28,29].

One more test is overexploitation because of the popularity for *Annona Muricata* foods grown from the ground subsidiaries. Reaping of soursop organic products for business purposes frequently happens without legitimate administration works on, prompting impractical abuse of wild populaces. Also, the restorative properties of *Annona Muricata* have prompted expanded interest for its leaves, seeds, and concentrates, further intensifying the strain on wild populaces. Obtrusive species and bugs likewise represent a danger to the protection of Annona Muricata. Presented species like obtrusive plants, bugs, and diseases can outcompete local soursop trees for assets or straightforwardly harm them, prompting populace declines. Environmental change adds one more layer of intricacy to the preservation endeavors, as movements in temperature and precipitation examples can adjust the appropriate natural surroundings for Annona Muricata and increment the recurrence and power of outrageous climate events[29].

To address these difficulties and ration *Annona Muricata* really, a diverse methodology is required. Right off the bat, there is a requirement for environment preservation and rebuilding endeavors to safeguard the excess regular living spaces of soursop trees and make halls for quality stream and movement. This might include laying out safeguarded regions, carrying out maintainable land the board rehearses, and advancing agroforestry frameworks that integrate Annona Muricata into differentiated cultivating scenes. Furthermore, feasible collecting rehearses should be executed to guarantee that the double-dealing of *Annona Muricata* is directed in a way that doesn't think twice about long haul practicality of wild populaces. This might incorporate the foundation of collect standards, occasional terminations, and confirmation projects to advance dependable reaping practices and fair exchange. Thirdly, intrusive species the board and biosecurity measures are fundamental to forestall the spread of obtrusive bugs and

diseases that undermine Annona Muricata populaces. This might include the evacuation of intrusive species, quarantine measures for imported plant material, and examination into biological control methods[27].

Besides, investigation into the development, spread, and hereditary variety of *Annona Muricata* is pivotal for creating protection techniques and rearing projects pointed toward further developing protection from irritations, diseases, and natural burdens.

Training and mindfulness raising endeavors are additionally imperative to draw in nearby networks, policymakers, and partners in the preservation of *Annona Muricata*. By featuring the environmental, social, and financial significance of soursop trees, and advancing practical work valuable open doors, for example, eco-the travel industry and non-lumber timberland items, we can collect help for protection drives and cultivate a feeling of stewardship towards *Annona Muricata* and its living spaces. The protection of Annona Muricata faces various difficulties, including environment misfortune, overexploitation, obtrusive species, and environmental change. Nonetheless, by taking on an all-encompassing methodology that coordinates living space protection, feasible reaping rehearses, intrusive species the board, examination, and training, we can make progress toward guaranteeing the drawn out endurance of this significant species and the environments it inhabits[25].

2.3 Cancer

Cancer is a non-communicable disease characterized by uncontrolled cell proliferation and metastatic potential and is predominantly influenced by modifiable factors such as diet, environment, and lifestyle, with only a minor fraction attributed to genetic defects[28,29]. Despite

this knowledge and substantial investments in advanced treatments, cancer remains a leading cause of mortality globally, necessitating the exploration of complementary therapies like dietary interventions and lifestyle modifications to enhance disease control and improve survival rates[30,31].

While numerous observational studies have demonstrated the potential of healthy diets in reducing cancer risk and improving treatment outcomes, research emphasis has traditionally centered on detection and medical interventions, relegating the role of nutrition to the side. Consequently, the efficacy and safety of diet-based approaches remain ambiguous, and current standards of care often neglect to integrate dietary considerations effectively[33,34].

The global cancer burden exhibits substantial geographical variation in terms of prevalent cancer types. Lower-income countries experience a higher incidence of infection-related cancers such as cervical, liver, and stomach cancers, while prostate cancer is more prevalent in higher-income countries. Breast cancer is the most common cancer among women globally, with cervical cancer disproportionately affecting lower-income regions.

Lung cancer, despite its global predominance in men, is declining in higher-income countries due to changing smoking patterns. However, it continues to rise in some lower-income countries, particularly among women. Similar variations are observed in the incidence trends of prostate, breast, and colorectal cancers between higher and lower-income regions.

Notably, these global cancer patterns are not static but evolve over time and across populations. Migration studies reveal that cancer patterns among migrant populations shift within two generations to align with those of the host country. Furthermore, significant intra-country

changes in cancer incidence have been documented over the past half-century, exemplified by the dramatic rise and subsequent stabilization of colorectal cancer incidence in Japanese men since the late 1970s.

2.4 Nutritional Approaches to Cancer Therapy

The intricate relationship between nutrition and cancer has garnered increasing attention in recent years, with research highlighting the potential of dietary interventions as adjunctive therapies to conventional cancer treatments. Nutritional approaches to cancer therapy encompass a wide range of strategies, including dietary modifications, targeted nutrient supplementation, and the use of functional foods.

2.4.1 Alkalizing Diet

The Warburg speculation places that lacking cell oxygenation (hypoxia) is a major driver of cancer improvement, prompting anaerobic breath, lactic acidosis, and ensuing irritation. The gamble of acidosis and other pH irregular characteristics might be more huge in more established individuals since they continuously lose some renal corrosive base administrative capability, lessening the viability of the buffering components in the body.

Alternately, keeping up with ideal cell pH and oxygen levels through sufficient nourishment might hinder cancer movement while defending solid cells. A diet rich in alkalizing foods, as proposed by the Warburg speculation, might actually moderate cancer risk. Be that as it may, elective methodologies, for example, antacid water or bicarbonate supplements need logical approval and may try and posture unfavorable impacts.

A reasonable diet integrating crude foods with a negative dietary corrosive burden (alkalizing at the cell level) may give different medical advantages, including expanded cell oxygen immersion, possibly decreasing cancer risk. Table 2.2 gives a functional food graph to dietary decisions for accomplishing an ideal corrosive base equilibrium. Coordinating satisfactory measures of alkalizing foods into feasts containing acidifying foods is suggested for a balanced dietary methodology[36,37].

The alkalizing diet, also known as the alkaline diet, is based on the idea that certain foods can influence the pH balance of the body, promoting a more alkaline (less acidic) environment. Proponents of this diet claim that an alkaline environment in the body can help prevent or even treat cancer, as they believe cancer thrives in acidic conditions. However, the concept of an alkalizing diet in cancer therapy has been met with both support and significant criticism. Below is an extensive review of the alkalizing diet in the context of cancer therapy, covering both sides[37].

The alkalizing diet classifies foods as either acid-forming or alkaline-forming based on their potential to affect the pH of the body. Foods like meat, dairy, processed foods, and refined sugars are considered acid-forming, while fruits, vegetables, nuts, and legumes are considered alkaline-forming. The goal of this diet is to reduce the intake of acid-forming foods and increase the consumption of alkaline-forming foods, thereby supposedly promoting a more alkaline internal environment.

The underlying hypothesis is that chronic acidity in the body can lead to a range of health problems, including cancer. Advocates of the alkaline diet argue that maintaining an alkaline pH in the blood and tissues may discourage cancer development and improve overall health.

Proponents of the alkaline diet often claim that cancer cells thrive in acidic environments and that an alkaline body state inhibits cancer growth. They cite research showing that tumors tend to produce acidic by-products due to their metabolic processes, leading to an acidic microenvironment in the tissues where they grow.

The idea that altering your diet can change the pH of your blood is central to the diet's claims. Advocates suggest that by eating alkaline-forming foods, you can make your blood less acidic, thereby creating an unfavourable environment for cancer to grow.

The diet also claims to reduce systemic inflammation, which has been linked to cancer and other chronic diseases. Alkaline-forming foods, especially fruits and vegetables, are thought to help counter inflammation by supplying antioxidants, vitamins, and minerals.

Another claim is that an alkaline diet helps the body's detoxification processes, especially through the liver and kidneys, by reducing the overall acid load. This is believed to improve the body's ability to eliminate waste and toxins, theoretically decreasing cancer risk.

While the alkalizing diet has gained popularity, particularly in alternative health circles, many experts in the medical and scientific community criticize it for oversimplifying the relationship between diet, body pH, and cancer. Several key points refute the diet's claims:

One of the strongest arguments against the alkalizing diet is the body's natural ability to regulate its pH levels. The human body maintains a stable blood pH (around 7.35 to 7.45) through various mechanisms, such as respiration and kidney function. It is nearly impossible to change the blood's pH significantly through diet alone. While certain foods can temporarily alter the pH of urine, this does not translate to systemic changes in blood pH or tissue pH.

The idea that cancer thrives in acidic environments is based on a misunderstanding of how tumors function. While cancer cells do produce acidic by-products as a result of their altered metabolism (Warburg effect), the acidity is localized around the tumour, not a reflection of the overall blood pH. This localized acidity does not mean that the entire body is acidic, nor does it suggest that altering diet will directly affect the tumour environment.

There is little to no scientific evidence supporting the claim that an alkaline diet can prevent or treat cancer. A 2021 study published in *BMJ Open* found that there is no reliable data showing that dietary changes can significantly affect cancer outcomes by altering pH levels. Mainstream cancer research has not identified pH modulation as a viable treatment strategy.

The classification of foods as "acid-forming" or "alkaline-forming" is based on their ash content after combustion in a lab, not on how they are metabolized in the body. Foods like citrus fruits, which are acidic in nature, are classified as alkaline-forming because their metabolic by-products are alkaline. This distinction is often misunderstood by those following the alkaline diet.

The health benefits attributed to the alkaline diet are likely due to its emphasis on whole, plant-based foods such as fruits, vegetables, and nuts, which are rich in antioxidants, vitamins, and minerals. These components can support overall health and may reduce cancer risk, but not

because of their effect on body pH. The reduction of processed foods, sugars, and red meat in the diet may also contribute to better health outcomes, but not due to their "acidic" properties.

Though the alkalizing diet lacks scientific backing, some legitimate research is being conducted into how pH modulation might play a role in cancer treatment:

Researchers are studying how the acidic microenvironment of tumours can be targeted to enhance cancer therapies. Tumours create an acidic environment that helps them resist traditional treatments like chemotherapy and radiation. Some experimental treatments aim to disrupt this microenvironment, potentially making tumours more vulnerable to treatment. For example, buffering agents like sodium bicarbonate have been tested in animals to see if they can neutralize tumor acidity and improve treatment efficacy, but these are far from being dietary solutions.

There is some interest in metabolic therapies that seek to alter the metabolism of cancer cells. However, this research is still in its early stages, and it does not directly support the principles of the alkaline diet. These therapies focus more on disrupting the metabolic pathways of cancer cells rather than altering the body's pH.

2.4.2 Anti-Inflammatory Diet

Chronic inflammation, a strong cell stressor, worsens metabolic issues and adds to the improvement of different non-communicable diseases, prominently cancer. An orderly survey and meta-investigation confirm this connection, uncovering an elevated cancer risk related with

favorable to provocative diets. This highlights the capability of calming diets as a dietary mediation to relieve cancer risk by controlling the admission of inflammation-initiating foods. Indeed, even poor quality chronic inflammation, frequently set off by the utilization of provocative foods, can considerably hoist cancer risk.

Table 2.2: Food Characteristics Related to Acidogenic or Alkalizing Effect for Certain Foods

Category	**Acidifier (Positive Dietary Acid Load)**			**Alkalizer (Negative Dietary Acid Load)**		
	Least healthy	Medium	Weak	Weak	Medium	Healthiest
Fruits	Prunes, canned fruits, industrial juices	Sour cherry, sour plum	Sweet plums, unripe fruits	Oranges, bananas, peaches, pomegranates	Grapes, apples, pears, melons	Lemons, dates, figs, mangoes
Vegetables, Beans, Legumes	Fried potatoes, chips, pickled vegetables	Lima beans, peas, lentils, boiled potatoes	Kidney beans, some cooked vegetables	Cucumbers, carrots, tomatoes, mushrooms, cabbage	Olives, green beans, okra, bell peppers	Garlic, onions, spinach
Nuts and Seeds	Peanuts, cashews	Walnuts, pistachios	Seeds of pumpkin, sunflower, sesame	-	-	Raw almonds
Meats	Pork, shellfish, rabbit	Beef, lamb, turkey, veal	Fish, chicken	-	-	-
Eggs and Dairy	Cream, ice cream	milk, cheese, industrial eggs	Yogurt, raw milk, farm eggs	-	-	-

Grains and Cereals	Pastries, pasta, cereals	White rice, white flour, oats, bread	Whole wheat, brown rice	-	-	-
Oils	Frying oils	Sunflower oil, sesame oil	-	-	-	Virgin olive oil
Other foods	Chocolate, mayonnaise, protein shakes	Jam, sugar, vinegar	Processed honey	-	Ginger, natural honey	-

Source: [37]

Therefore, incorporating anti-inflammatory foods like tomatoes, olive oil, green leafy vegetables, oranges, and almonds into one's diet is recommended. Conversely, limiting the intake of inflammatory foods such as refined carbohydrates, alcohol, fried potatoes, soda, and red meat is advised to reduce cancer risk and enhance disease management.

2.4.3 Plant Based Diet

Plant-based diets incorporate assorted dietary examples described by low creature item utilization and high plant item admission. While certain examinations on Western veggie lover diets have not shown massive contrasts in cancer occurrence contrasted with non-vegans, possibly because of the utilization of specific plant-based foods with unfriendly wellbeing impacts, an even plant-based diet, for example, the Hovannessian live, crude plant-based diet, has been demonstrated to be helpful in cancer counteraction and by and large wellbeing.

A critical figure the viability of plant-based diets for cancer counteraction lies in suitable food decisions and feast arranging. Various examinations have connected lacks and unfavorable wellbeing results in plant-based diets to unfortunate dietary decisions and an absence of understanding with respect to ideal food determinations. An even plant-based diet, integrating

adequate measures of reasonable crude and cooked vegetables, organic products, nuts, seeds, and entire grains, can furnish fundamental supplements and bioactive compounds with defensive impacts against cancer.

Proof proposes that a fair, alkalizing plant-based diet might diminish the gamble of different cancers, including bosom, prostate, colorectal, and lung cancer. For example, concentrates on contrasting the dietary propensities for African Americans consuming a Western diet high in greasy meats to those consuming a rustic African diet wealthy in beans and vegetables have shown an essentially higher gamble of colorectal cancer in the previous gathering. Hence, while plant-based diets are not innately defensive against cancer, a very much arranged and adjusted plant-based diet that consolidates suitable food decisions and readiness strategies can be a significant device in cancer counteraction and in general wellbeing advancement.

2.4.4 Nutrient Supplementation:

Vitamin D: Vitamin D deficiency has been linked to increased cancer risk and poor prognosis in several cancer types. Supplementation with vitamin D has shown potential in enhancing the response to chemotherapy and radiotherapy, possibly through its immunomodulatory and antiproliferative effects.

Omega-3 Fatty Acids: Omega-3 fatty acids, found in fatty fish and some plant sources, possess anti-inflammatory and antiangiogenic properties, making them a promising adjunct to cancer therapy. Studies suggest that omega-3 supplementation may improve quality of life, reduce treatment-related side effects, and potentially enhance survival in cancer patients.

Antioxidants: Antioxidants like vitamins C and E, selenium, and carotenoids have been investigated for their potential to protect against oxidative stress and DNA damage induced by cancer treatments. However, the evidence for their efficacy in improving cancer outcomes remains inconclusive.

Cruciferous Vegetables: Cruciferous vegetables, such as broccoli, cauliflower, and kale, contain glucosinolates, which are metabolized into isothiocyanates. Isothiocyanates have been shown to inhibit the growth of cancer cells and induce apoptosis in preclinical models.

Berries: Berries are rich in polyphenols, flavonoids, and other phytochemicals with antioxidant and anti-inflammatory properties. Some studies suggest that berry consumption may reduce the risk of certain cancers and improve outcomes in cancer patients.

Turmeric: Turmeric contains curcumin, a polyphenol with potent anti-inflammatory and anticancer effects. Curcumin has been shown to inhibit tumor growth, induce apoptosis, and sensitize cancer cells to chemotherapy and radiotherapy in preclinical studies.

2.5 Human Anaplastic Lymphoma Kinase Receptor

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK) encoded by the ALK gene. Initially identified in anaplastic large cell lymphoma (ALCL), ALK has emerged as a critical oncogenic driver in various human cancers. ALK is a transmembrane protein with an extracellular ligand-binding domain, a transmembrane helix, and an intracellular tyrosine kinase domain. In its normal state, ALK is activated by binding to its ligand, leading to phosphorylation of downstream signaling molecules involved in cell growth, proliferation, and survival. However, aberrant activation of ALK through genetic alterations can lead to uncontrolled cell growth and

tumorigenesis. The discovery of ALK rearrangements and mutations has revolutionized cancer treatment, leading to the development of targeted therapies that have significantly improved patient outcomes. ALK alterations are found in various cancers, including:

Non-small cell lung cancer (NSCLC): ALK rearrangements, most commonly with the EML4 gene, are present in approximately 3-7% of NSCLC cases. The extensive molecular investigation of non-small cell lung cancer (NSCLC) has facilitated the identification of pivotal genes

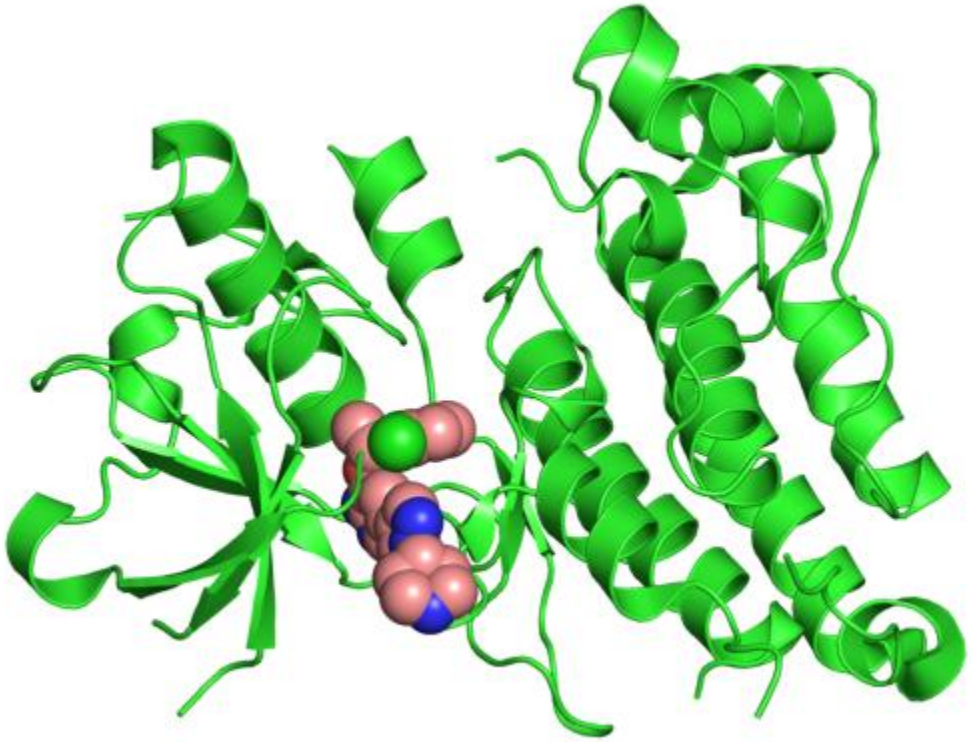


Figure 2.2: Human Anaplastic Lymphoma Kinase

Source: [49]

involved in the carcinogenesis process. Significant genes reported include epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), echinoderm microtubule-associated protein-like anaplastic lymphoma kinase (EML4-ALK), and phospholipase A2 group IIA (PLA2G2A)[38,3]. Among these, EGFR and HER2 are crucial receptors that contain an intracellular protein kinase domain with tyrosine kinase activity, which is frequently expressed in lung cancer. The amplification or overexpression of both genes has been implicated in the clinical progression of various cancers, including NSCLC. These rearrangements result in the formation of fusion proteins with constitutive kinase activity, driving tumor growth. ALK rearrangements are most associated with non-small cell lung cancer (NSCLC). Approximately 3-7% of NSCLC patients have ALK gene rearrangements, leading to the formation of an abnormal ALK protein that promotes cancer cell growth[38].

Anaplastic Large Cell Lymphoma (ALCL): ALK rearrangements, typically with the NPM1 gene, are found in a subset of ALCL cases, particularly in the systemic form of the disease.

Neuroblastoma: ALK mutations and amplifications occur in a subset of neuroblastoma cases, often associated with aggressive disease and poor prognosis.

Inflammatory Myofibroblastic Tumors (IMTs): ALK rearrangements are frequently found in IMTs, contributing to their pathogenesis.

Other Cancers: ALK alterations have also been reported in a smaller number of cases of breast cancer, colorectal cancer, ovarian cancer, and other malignancies.

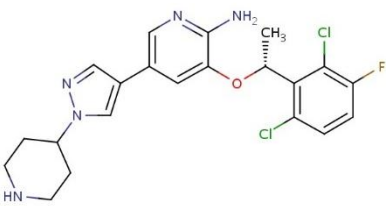
The identification of ALK as an oncogenic driver has led to the development of ALK tyrosine kinase inhibitors (TKIs) that specifically target ALK activity. These targeted therapies have revolutionized the treatment of ALK-positive cancers, resulting in significant improvements in response rates, progression-free survival, and overall survival.

2.5.1 ALK Tyrosine Kinase Inhibitors (TKIs)

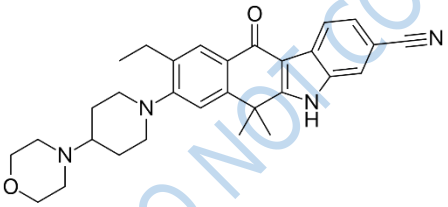
Several commercially available ALK TKIs (Figure 2.3) have been approved for the treatment of ALK-positive cancers, including:

Crizotinib: The first-generation ALK TKI, crizotinib, was approved for the treatment of ALK-positive NSCLC in 2011. It has since shown efficacy in other ALK-positive cancers, such as ALCL and IMTs.

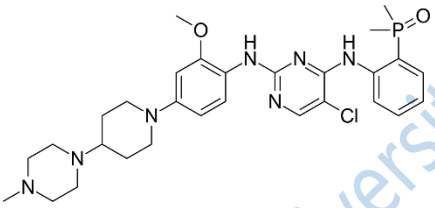
Alectinib, Brigatinib, Ceritinib, and Lorlatinib: These second- and third-generation ALK TKIs have been developed to overcome resistance to crizotinib and provide more potent and durable responses.



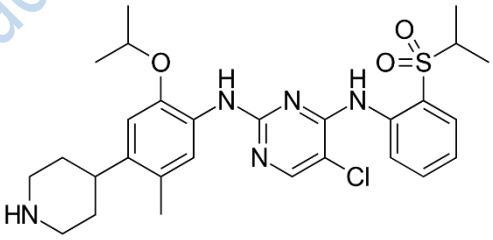
Crizotinib



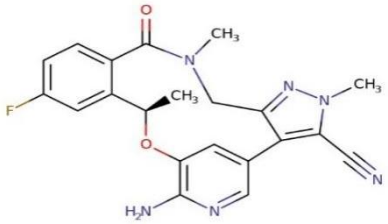
Alectinib



Brigatinib



Ceritinib



Lorlatinib

Figure 2.3: Selected Tyrosine Kinase Inhibitors

Source: [40]

2.6 Antioxidants

Antioxidants are a group of substances that are useful for fighting cancer and other processes that potentially lead to diseases such as atherosclerosis, Alzheimer's, Parkinson's, diabetes, and heart disease[29,32]. Antioxidants are molecules that inhibit oxidation, a chemical reaction that can produce free radicals and lead to chain reactions that may damage cells. Free radicals are highly reactive molecules with unpaired electrons, which can cause oxidative stress in the body and contribute to various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. Dissimilar to cytotoxic specialists that harm growth cells, antioxidants act by forestalling the beginning of cancer during carcinogenesis, and they are by and large advantageous to cells. Oxidants harm macromolecules, like proteins, lipids, chemicals, and DNA and to battle these revolutionaries, living organic entities produce catalysts or depend on non-enzymatic particles like cysteine, ascorbic corrosive, flavonoids, and vitamin K for assurance. Annona Muricata, generally known as soursop or graviola, is a tropical natural product famous for its particular flavor and potential medical advantages. One of the key parts adding to its wellbeing advancing properties is its rich cancer prevention agent content. In this paper, we will investigate the antioxidants found in Annona Muricata, their medical advantages, and their possible applications in medication and sustenance[31,32].

There has been interest in the commitment of free extremists response partaking in reactive oxygen species to the by and large metabolic annoyance that outcome in tissue injury and illness.

Reactive oxygen species, for example, superoxide anion, hydrogen peroxide, and hydroxyl extremist are created in unambiguous organelles of cells (Mitochondria and Microsomes) under ordinary physiological condition. These reactive oxygen species can harm DNA, to cause change and chromosomal harm, oxidize cell thiols and conceptual hydrogen iotas from unsaturated fats to start the peroxidation of layer lipids. Annona Muricata contains different antioxidants, including flavonoids, phenolic compounds, and L-ascorbic acid. Flavonoids are a gathering of polyphenolic compounds areas of strength for with properties. They rummage free revolutionaries, decrease inflammation, and may help safeguard against chronic diseases. Phenolic compounds, like Annona Muricatacin, acetogenins, and alkaloids, additionally display strong cancer prevention agent movement and have been read up for their possible anticancer and neuroprotective impacts. L-ascorbic acid, one more remarkable cancer prevention agent tracked down in Annona Muricata, assumes an essential part in supporting the resistant framework, advancing collagen union, and safeguarding cells from oxidative damage[33].

Antioxidants might offer obstruction against the oxidative pressure by searching the free revolutionaries, restraining lipid peroxidation and by different instruments and hence forestall disease[33]. As of late, different phytochemicals and their impact on wellbeing, particularly the concealment of active oxygen species by normal cell reinforcement from tea, flavors and spices, have been seriously studied[43]. Phenolic compounds assume a significant part in the oxidative properties of many plant-determined antioxidants[30]. Phenolic substances were likewise answered to have many biological impacts, including cell reinforcement, antimicrobial, calming and anticancer. A few examinations have explored the medical advantages of Annona Muricata antioxidants. Research proposes that the antioxidants present in soursop might have anticancer

properties, as they can hinder the development of cancer cells and prompt apoptosis (customized cell demise) in vitro and in creature studies. Moreover, the cell reinforcement and mitigating properties of Annona Muricata might assist with decreasing the gamble of chronic diseases, like cardiovascular diseases, diabetes, and joint inflammation. Also, the antioxidants found in Annona Muricata might make neuroprotective impacts, possibly relieving the movement of neurodegenerative issues like Alzheimer's and Parkinson's sickness. These antioxidants can assist with combatting oxidative pressure in the mind, shield neurons from harm, and work on mental function[28].

A few strategies have been created to gauge the proficiency of antioxidants as unadulterated compounds or in extricate. These strategies center around various components of the oxidant protection framework that is rummaging active oxygen species and hydroxyle revolutionaries, restraining of lipid peroxidation, or chelating of metal ions[31].

The DPPH free revolutionary searching examine assesses the cancer prevention agent movement of concentrates by estimating their ability to rummage free extremists. DPPH, a stable free revolutionary, changes tone from purple to yellow upon decrease by a cell reinforcement, showing a reduction in absorbance at 517 nm. This measure uncovers the capacity of compounds to give hydrogen molecules or electrons, and the component of cancer prevention agent action[32].

Chemicals keeping up with cell REDOX states require non-enzymatic atoms for help. These particles, either endogenous (e.g., glutathione) or exogenous (e.g., ascorbate, tocopherols, carotenes, retinols, polyphenols), kill free extremists alone or with enzymatic frameworks. Ascorbate (L-ascorbic acid), got from dietary sources like apples, papaya, mango, guava, and

oranges30, is an intense free extreme scrounger. It works close by glutathione and is definitely not a favorable to oxidant under ordinary conditions[29,32]. Ascorbate shows expected restorative impacts in ischemic stroke, Alzheimer's, Parkinson's, and Huntington's diseases and is utilized by competitors to check ROS delivered during exercise[33]. It likewise kills protein revolutionaries and works on endothelial capability, a forerunner to atherogenesis.

Vitamin E incorporates eight related particles: four tocopherols and four tocotrienols. Because of their non-polar nature, tocopherols essentially shield cell and organelle films from free extremists by settling these designs. They likewise alleviate oxidative pressure connected with metabolic condition and other sources[28]. Notwithstanding its medical advantages, Annona Muricata antioxidants have viable applications in medication and sustenance. Removes from soursop organic product, leaves, and seeds are being read up for their expected use in creating normal cures and drug drugs for different afflictions. Soursop enhancements and concentrates are likewise accessible as cases, powders, and teas, offering a helpful method for integrating the wellbeing advancing properties of Annona Muricata into one's diet. Annona Muricata is a rich wellspring of antioxidants, including flavonoids, phenolic compounds, and L-ascorbic acid, which add to its potential medical advantages. These antioxidants have solid cell reinforcement, calming, anticancer, and neuroprotective properties, making Annona Muricata an important expansion to a sound diet and a promising contender for future clinical exploration and remedial applications[32].

Carotenoids, a different gathering of north of 700 hydrophobic plant shades got from isoprenoid units, assume a vital part in plant physiology however are not synthesizable by people. These shades, with up to 15 formed twofold securities, go about as antioxidants as well as regulate safe

capability and possibly restrain tumorigenesis, Creatures like birds, fish, and spineless creatures get carotenoids from their diet to produce energetic varieties in skin and feathers[28,31,32].

Retinol (vitamin A), a particle primarily connected with carotenoids, is another significant exogenous cell reinforcement. Prominently, β-carotene can be switched over completely to retinol, which further changes into retinal and retinoic corrosive, all with particular biological capabilities in different tissues. Retinol and its metabolites impact crucial cycles like resistance, multiplication, development, and improvement, with vision being the most popular capability. In spite of the fact that β-carotene goes about as a cancer prevention agent, it can display favorable to oxidant action at high portions or in unambiguous cell societies. Alternately, its cell reinforcement capability has been connected to beneficial outcomes on different oxidative pressure related illnesses like diabetes, weight, low sperm motility, and hearing misfortune.

Past "cancer prevention agent vitamins," leafy foods are rich in polyphenols, one more different gathering of plant optional metabolites with different subgroups based on their sub-atomic design. Moden ls incorporate quercetin, coumaric corrosive, proanthocyanidins, and resveratrol. While customarily thought about antioxidants, polyphenols likewise have antimicrobial, antiviral, and mitigating properties, collecting critical consideration for their potential wellbeing benefits [22,29]. The overflow of these exogenous cell reinforcement particles in foods grown from the ground has driven researchers to connect a diet wealthy in these plant items with positive wellbeing results. Notwithstanding, it's memorable's critical that a solitary cell reinforcement or enhance can't supplant the mind-boggling exchange of supplements tracked down normally in products of the soil underlines that eating disconnected vitamins or polyphenols can't repeat the synergistic

impacts accomplished when these bioactive particles are ingested together in their regular food source.

2.7 Dietary Antioxidants

The significant role of dietary antioxidants in maintaining the integrity of living organisms is increasingly recognized. Emerging data continually highlight the role of oxidative stress and the involvement of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the pathogenesis of degenerative diseases. These diseases are linked to disturbances in the critical balance between oxidation and reduction status in blood and tissues, leading to the oxidation of lipids, proteins, and nucleic acids. Such oxidative damage results in alterations to the structure and function of macromolecules and the manifestation of clinical disorders, including cardiovascular diseases and cancer. Consequently, extensive research is being conducted to explore the potential effects and mechanisms of action of dietary antioxidants in these diseases.

Lycopene, a natural antioxidant found predominantly in tomatoes, exhibits a negative correlation with cardiovascular disease (CVD) incidence, as observed in studies analyzing tomato and lycopene consumption). This inverse relationship is attributed to lycopene's protective effects against low-density lipoprotein (LDL) oxidation, achieved through the inhibition of cholesterol synthesis and enhancement of LDL degradation [50]. A seminal population-based study further solidified this observation, revealing that among carotenoids like α-carotene and β-carotene, only lycopene demonstrated significant protective effects against myocardial infarction. These

findings suggest that lycopene may be a key factor underlying the cardioprotective benefits associated with vegetable consumption.

In addition to lycopene, polyphenols, the most abundant antioxidants in the human diet, derived from fruits, vegetables, coffee, tea, and cereals, have also been linked to a reduced risk of CVD [50]. Notably, flavonoid-rich beverages like tea demonstrate marked improvement in endothelial function, although their impact on blood oxidative markers remains unclear. Emerging evidence suggests that polyphenols' protective effects extend beyond their antioxidant capacity, potentially involving anti-inflammatory mechanisms and regulation of vasodilation and endothelial cell apoptosis [51].

2.8 Oxidative stress and Nutritional Cancer Status

Reactive Oxygen Species (ROS) play a significant role in all three stages of cancer development: initiation, promotion, and progression [52]. During initiation, ROS-induced DNA mutations can accumulate in cancerous tissues if unrepaired, potentially leading to oncogenic mutations and cancer onset. Cancer cells, with their altered metabolism and increased energy demand, produce more ROS than normal cells [52]. This oxidative stress (OS) promotes cancer growth by triggering growth signaling, enhancing therapeutic resistance, increasing tumor blood supply, and facilitating metastasis. ROS also influence cancer progression by modifying genes involved in apoptosis, cell proliferation, and transcription factors, upregulating antiapoptotic genes, and downregulating proapoptotic proteins via PI3K/AKT and ERK/MEK pathways. In the final stage, ROS contribute to metastasis by upregulating matrix metalloproteinases, inhibiting anti-proteases, and promoting angiogenesis [53].

A disruption of redox equilibrium or depletion of endogenous antioxidants can contribute to cancer development. Diets rich in antioxidant-rich fruits and vegetables have demonstrated protective effects against various cancers [53]. Plant-based foods containing polyphenols, potent antioxidants, exhibit anti-cancer activity against various cancers. This activity is attributed to their ability to induce apoptosis, inhibit proliferation, reduce cyclooxygenase-2 (COX-2) production, and downregulate cancer gene expression. Certain vitamins and minerals also play a role in cancer risk reduction through antioxidant action, proliferation inhibition, DNA methylation maintenance, and cell-cycle arrest. Post-treatment, a healthy diet can modify biomarkers of cancer progression, with evidence suggesting reduced mortality rates for breast, head and neck, and rectal cancers [53,54].

While vitamins like A and E have preventive effects against oral cancer, and micronutrients like vitamin D, carnitine, and selenium improve treatment outcomes, the evidence supporting their effectiveness in cancer prevention is limited. Currently, there are no specific recommendations for their use in healthy individuals. Due to methodological challenges and ethical considerations, randomized control trials on diet and cancer are scarce [51]. Therefore, current recommendations emphasize a healthy diet and lifestyle to mitigate cancer risk. Therapeutic strategies targeting reactive oxygen species (ROS) in cancer treatment employ two distinct mechanisms. The first approach involves mitigating ROS levels through antioxidant supplementation, aiming to disrupt ROS-signaling pathways and impede cancer progression. However, recent research suggests that conventional antioxidant doses may not effectively counter the elevated ROS metabolites in cancer cells. Consequently, the current rationale for antioxidant use during chemotherapy is to

replenish the total antioxidant loss caused by the depletion of antioxidant enzymes in cancerous cells [54].

The second approach focuses on amplifying ROS generation to induce cancer cell death via senescence or apoptosis. Several chemotherapeutic agents elevate intracellular ROS levels, disrupting the delicate ROS balance within cancer cells and surpassing their tolerance threshold, thereby activating various cell death pathways [55]. Moreover, exposure to anticancer drugs can trigger biomolecule oxidation or initiate a mitochondrial-dependent ROS response, significantly enhancing their cytotoxic effects on cancer cells [55,56].

2.9 Computational Techniques (*In Silico*)

Silicon, a fundamental element in modern electronics, has revolutionized various industries through its versatile properties and applications. Computational techniques play a pivotal role in harnessing the full potential of silicon, enabling advancements in semiconductor manufacturing, device design, and material science. This essay explores the evolution, current state, and future prospects of computational techniques in silicon-related fields. The evolution of computational techniques in silicon can be traced back to the early days of semiconductor research. With the advent of computers, researchers began employing numerical simulations to understand the behavior of silicon materials and devices. Initially, these simulations were limited by computational power and modeling capabilities. However, rapid advancements in hardware and software technologies have enabled increasingly accurate and complex simulations over the years. *In silico* techniques involves the use of computational techniques in drug discovery. The approach has been introduced to accelerate the process of drug discovery as it has been proven to reduce the number of compounds to be synthesized and tested *in-vivo* or *in-vitro* [19,20].

Today, computational techniques in silicon encompass a wide range of methods, including quantum mechanical simulations, finite element analysis, molecular dynamics, and Monte Carlo simulations. These techniques allow researchers to model the behavior of silicon at various length and time scales, from atomic interactions to device-level performance. Computational techniques in silicon have found applications across multiple industries and disciplines. In semiconductor manufacturing, process simulations are used to optimize fabrication processes, improve yield, and reduce production costs. Device simulations aid in the design and optimization of transistors, diodes, and other electronic components, leading to faster, more efficient devices [22].

In material science, computational techniques are used to study the properties of silicon-based materials, such as silicon wafers, nanoparticles, and thin films. These simulations provide insights into the structural, electronic, and optical properties of silicon materials, guiding the development of new materials for photovoltaics, sensors, and other applications. In addition to traditional silicon-based electronics, computational techniques are also being applied to emerging fields such as silicon photonics, quantum computing, and neuromorphic computing. Quantum mechanical simulations enable the design and optimization of silicon-based quantum devices, while machine learning algorithms are used to model complex neural networks on silicon substrates [23].

Looking ahead, computational techniques are expected to play an even larger role in silicon-related research and development. With the continued advancement of hardware technologies, including high-performance computing and parallel processing, researchers will have access to unprecedented computational resources for modeling and simulation. Moreover, the integration

of artificial intelligence and machine learning techniques promises to further enhance the capabilities of computational simulations. By leveraging large datasets and advanced algorithms, researchers can accelerate the discovery and optimization of silicon materials and devices, leading to breakthroughs in energy efficiency, computing performance, and sensing applications[36].

Computational techniques have become indispensable tools in the study and development of silicon materials and devices. From semiconductor manufacturing to material science and beyond, these techniques enable researchers to explore the vast potential of silicon and its applications in electronics, photonics, and quantum technologies. As computational capabilities continue to evolve, the future of silicon-based innovation looks brighter than ever before[20].

2.9.1 Molecular Docking

In silico docking simulations predict the preferred three-dimensional orientation of one molecule (ligand) relative to another (receptor) when they form a complex[20]. This predicted orientation provides valuable insights into the binding affinity between the two molecules, influencing their potential biological interactions (Figure 2.17). Docking studies offer a powerful tool to identify and characterize substrate binding sites, particularly the active sites of enzymes. A molecule's ability to interact with a specific protein and exert a desired biological effect hinges on its capacity to bind favorably within a particular pocket or cavity on the protein's surface.

The foundation of in silico docking studies relies on the availability of a high-resolution structure for the target protein. The success of virtual screening, a computational technique for identifying

potential drug candidates, depends heavily on the quality and quantity of structural data for both the target protein and the small molecules being docked (ligands).

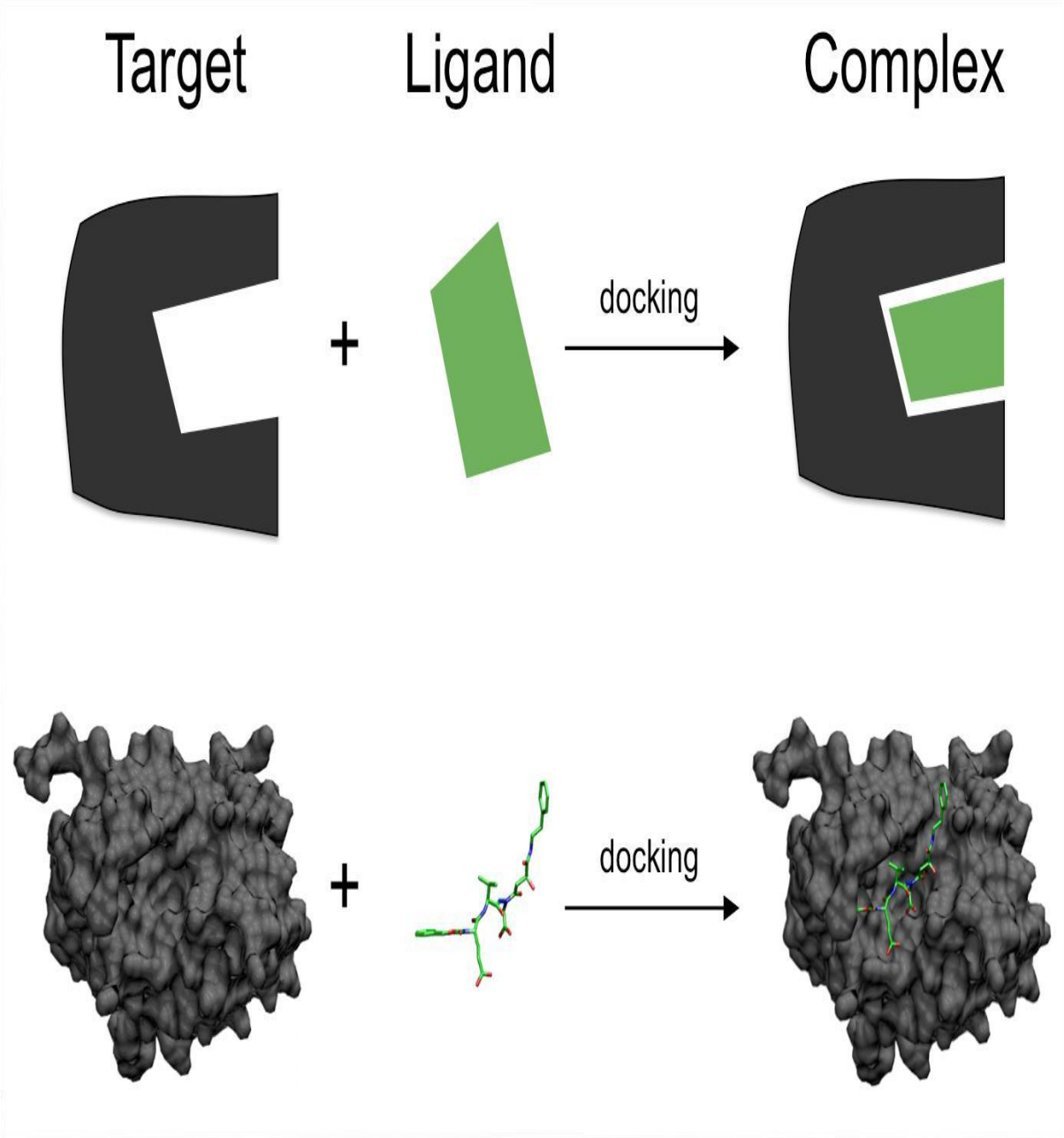


Figure 2.4: Pose of Target - Ligand Complex

Source: [48]

The initial step involves defining an appropriate binding site on the target protein. This can be achieved through two main approaches:

(1). analyzing existing crystal structures where the target protein is bound to a known ligand[18]. (2) utilizing in silico methods to predict novel binding sites on the protein surface[21].

Two key components influence the success of docking simulations: the search algorithm and the scoring function. The search algorithm explores the vast search space, encompassing all possible orientations and conformations of the ligand-protein pair. Each unique snapshot of the ligand interacting with the protein at a specific orientation is referred to as a "pose" (Figure 2.17). The scoring function, often based on physics-based molecular mechanics force fields, estimates the potential energy of each pose. These energy contributions are then summed to provide a score reflecting the predicted binding affinity between the ligand and the protein.

$$\Delta G_{bind} = \Delta G_{solvent} + \Delta G_{conf} + \Delta G_{int} + \Delta G_{rot} + \Delta G_{t/t} + \Delta G_{vib}$$

Solvent effects, changes in protein ligand conformation, free energy as a result of interactions between protein and ligand, internal rotations, association energy of ligand and receptor to form a single complex and free energy due to changes in vibrational modes are the main components of the binding energy[38]. A low (negative) energy indicates a stable system and thus a likely binding interaction. Molecular docking in silicon, a cornerstone of computational drug discovery, revolutionizes the pharmaceutical industry by simulating the interaction between small molecules and target proteins. This essay explores the principles, methods, applications, and

challenges of molecular docking in silicon. Molecular docking predicts the preferred orientation of a small molecule within a binding site of a target protein to form a stable complex. It relies on the principles of molecular recognition, where complementary shapes, electrostatics, and other physicochemical properties facilitate binding. The process typically involves three main steps: ligand preparation, receptor preparation, and docking algorithm execution.

The binding energy of a protein-ligand complex is influenced by several factors, including solvent effects, changes in protein-ligand conformation, free energy from protein-ligand interactions, internal rotations, association energy of ligand and receptor to form a single complex, and free energy changes due to alterations in vibrational modes. A low (negative) binding energy indicates a stable system, suggesting a likely binding interaction.

Molecular docking in silico, a cornerstone of computational drug discovery, has revolutionized the pharmaceutical industry by simulating the interaction between small molecules and target proteins. This essay delves into the principles, methods, applications, and challenges of molecular docking in silico.

Molecular docking aims to predict the preferred orientation of a small molecule within a binding site of a target protein to form a stable complex. It is based on the principles of molecular recognition, where complementary shapes, electrostatics, and other physicochemical properties facilitate binding. The docking process typically involves three main steps: ligand preparation, receptor preparation, and the execution of docking algorithms.

In ligand preparation, small molecules are optimized for docking by ensuring correct protonation states, tautomerization, and energy minimization. Receptor preparation involves refining the

target protein structure, adding missing atoms or residues, and identifying the binding site. The docking algorithm then predicts the optimal orientation and conformation of the ligand within the receptor's binding site, evaluating potential interactions and scoring them based on binding affinity.

The applications of molecular docking are vast, ranging from drug discovery and virtual screening to understanding biochemical pathways and enzyme mechanisms. However, challenges remain, including accurately modeling flexible proteins and accounting for solvent effects and entropy changes.

The ideal starting point for docking is having a 3D structure of the target, experimentally determined by X-ray crystallography or NMR techniques and deposited in the protein data bank (PDB). The rate of target structure determination has been greatly accelerated by structural genomics as over 81,000 protein structures have been determined and deposited in the protein data bank (PDB). However, in the absence of experimentally determined 3D structure of target protein, the use of computers can be employed as an alternative for predicting the 3Dstructure of such target proteins.

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Chapter Three

Materials and Methods

3.1 Research Design

This study aims to ascertain the nutritional bioactive compounds found in *Annona Muricata* (Soursop). It comprises four stages, commencing with a comprehensive literature review to ascertain optimal methodologies and conditions. Subsequently, proximate analysis was undertaken to determine the percentage composition of nutrients. Gas chromatography-mass spectrometry (GCMS) analysis was employed to obtain phytochemical information, and an antioxidant assay was conducted. Furthermore, computational analysis was utilized to identify prominent phytochemicals and determine the bioactive compounds present in both the flesh and seeds of the fruit, particularly those exhibiting pronounced activity against breast cancer. Figure 3.1 illustrates the sequential process of the research methodology.

3.2 Materials

3.2.1 Plant Material

Samples of *Annona Muricata* (Soursop) were purchased from Sabo Market in Ibadan, Oyo State, Nigeria, and transported to the study area. The fruit was then separated into three components: flesh, seed, and bark. Flesh, bark, flesh, and seed of *Annona Muricata* were washed separately in fresh water thoroughly 2–3 times and once finally with sterile water to remove adhering dust. The three parts were dried on a sterile blotter under shade and then powdered in a mixture grinder and prepared for solvent extraction.

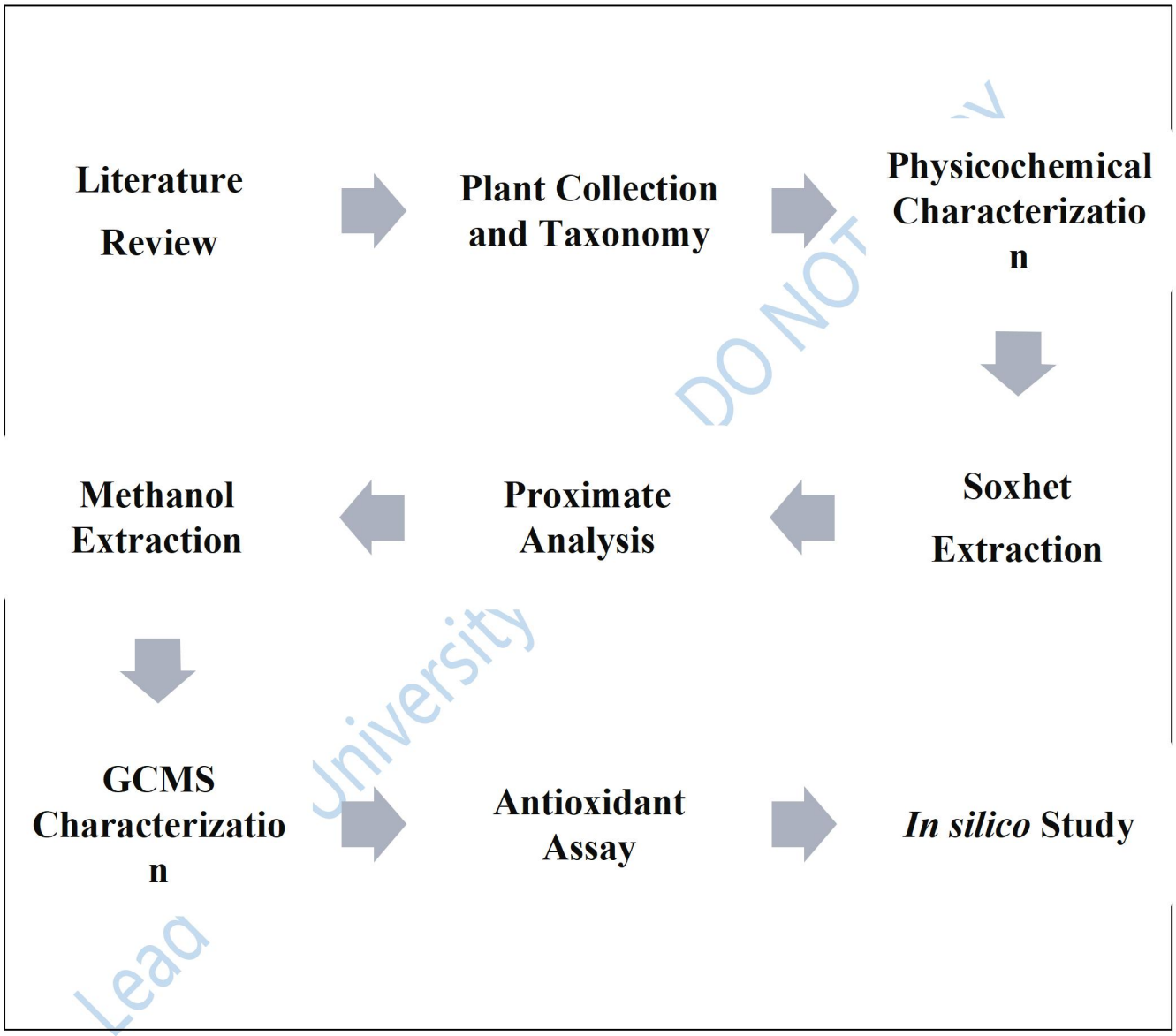

Figure 3.1: Research Design Process Flow.

Source: Researcher's Field work, 2024

3.2.2 Reagents

Table 3.1 contains the list of reagents used and their suppliers.

3.2.3 Equipment Used and Model

Equipment employed for this study include Analytical Balance, Digestion tubes, Digestion Block Heaters, Fume Cupboard, Soxhlet apparatus, oven, Desiccator, GallenKamp Furnace, Gas Chromatography-Mass Spectrophotometer (Modal; Agilent technologies 7890A, VF – 5ms fused silica capillary column of 30m x 0.25mm x 0.25mm).

3.3 Physicochemical Analysis

The methods specified by ISO 3961 (1989) and the Association of Official Analytical Chemist (A.O.A.C., 18TH EDITION, 2005) were used as seminal reference for the physicochemical analysis.

3.3.1 Acid Value

25ml of diethyl ether and 25ml of ethanol were mixed in 250ml beaker. The resulting mixture was added to 10g of oil in a 25ml conical flask and a few drops of phenolphthalein was added to the mixture. The mixture was titrated with 0.1M NaOH to the end point with consistent shaking for which a dark pink color was observed and the volume of 0.1M NaOH (Vo) was noted. Free fatty acid (FFA) was calculated as

$$V_0 - W_0$$

Where: V_0= volume of the titrant

W_0= is the weight of sample

Where 100ml of 0.1M NaOH = 2.83g of oleic acid, Wo = sample weight , then acid value = FFA - 2 (Laboratory Handbook, 1997).

Table 3.1: List of Reagents and Suppliers

S/N	Reagents	Supplier
1.	5M H_2SO_4	BDH Laboratories
2.	Hydrochloric Acid	BDH Laboratories
3.	Sodium Hydroxide	BDH Laboratories
4.	2% Boric Acid Solution	BDH Laboratories
5.	Methyl Red – Bromocresol green mixed indicator	BDH Laboratories
6.	Kjeldahl Catalyst tablet	BDH Laboratories
7.	Ether (40° – 60°C b.pt).	BDH Laboratories
8.	Methanol	BDH Laboratories
9.	Acetone	BDH Laboratories
10.	Potassium iodide (KI)	BDH Laboratories
11.	Chloroform	BDH Laboratories
12.	Glacial acetic acid	BDH Laboratories
13.	Distilled Water	University Laboratory

Source: Researcher's Field work, 2024

3.3.2 Peroxide Value

To 1g of the oil sample, add 1g of potassium iodide and 20ml of solvent mixture (glacial acetic acid /chloroform, 2/1 by volume) were added and the mixture was boiled for one minute. The hot solution was poured into a flask containing 20ml of 5% potassium iodide.

A few drops of starch solution were added to the mixture and the latter was titrated with 0.025 N sodium thiosulphate and the peroxide value was determined as follows.

$$PV = \frac{SN103}{W}$$

Where: PV= peroxide value

S= sample titration

N= Normality of sodium thiosulphate

W= weight of sample (g)

3.3.3 Saponification Value

2 g of the example was weighed into a conical flask; 25 ml of 0.1Nethanoic potassium hydroxide was then added. The substance which was continually blended was permitted to bubble tenderly for 60 min. A reflux condenser was put on the cup containing the combination and a couple of drops of phenolphthalein pointer was added to the warm arrangement and afterward titrated with 0.5 M Hcl to the end point until the pink shade of the inculcator recently vanished. A similar method was utilized for different examples and a clear. The articulation for saponification esteem (SV) is given by:

$$SV = 56.1N\frac{(Vo - Vi)}{m}$$

Where: SV= Saponification value

N= Normality of the HCL

Vo= Volume of 0.5N of HCL consumed in the blank test

Vi= Volume of 0.5N of HCL consumed in the actual test

M= molar weight of potassium hydroxide

3.3.4 Iodine value

0.4 g of the sample was weighed into a conical flask and 20 ml of carbon tetra chloride was added to dissolve the oil. Then 25ml of Dam reagent was added to the flask using a safety pipette influenced chamber. A stopper was then inserted, and the content of the flask was vigorously swirled. The flask was then placed in the dark for 2 h and 30 min. At the end of this period, 20 ml of 10% aqueous potassium iodide and 125 ml of water were added using a measuring cylinder. The content was titrated with 0.1 M sodium thiosulphate solution until the yellow colour almost disappeared. A few drops of 1% starch indicator were added and the titration continued by adding thiosulphate drop-wise until blue coloration disappeared after vigorous shaking. The same procedure was used for the blank test and for other samples. The iodine value (IV) is given by the expression:

$$IV = 12.69c(V1 - V2)m$$

Where: IV = Iodine value

$V1$ = The volume of sodium thiosulfate needed for the sample titration

$V2$= The volume of sodium thiosulfate needed for the blank titration

C= thiosulphate equivalent of one mL of bromine solution

m= mass of iodine absorbed by the sample (grams)

3.3.5 % Yield of Oil

30 g of the sample was placed in the thimble and about 150 ml of petroleum ether was poured into the round bottom flask. The apparatus was heated at 40-60% and allowed for 3 h of continuous extraction using soxhlet apparatus. The experiment was repeated for different weights of the sample, 35, 40 and 50 g. At the end the solvent was distilled, and the percentage of oil extracted was determined.

3.4 Proximate Analysis

All analysis was carried out in triplicate. The parameters studied on Soursop seed and Soursop flesh include percentage moisture content, percentage crude protein, percentage crude fat, percentage crude fiber, percentage ash content, percentage Nitrogen free extract, percentage energy, and percentage carbohydrate.

3.4.1 Crude Protein Determination (AOAC Official Method 988.05)

The crude protein in the sample were determined by the routine semi-micro Kjeldahl, procedure/technique. This consists of three techniques of analysis namely Digestion, Distillation and Titration.

Digestion

0.5g of each finely ground dried sample was weighed carefully into the Kjeldahl digestion tubes to ensure that all sample materials got to the bottom of the tubes. To this were added one Kjeldahl catalyst tablet and 10ml of Conc. H_2SO_4. These were set in the appropriate hole of the Digestion Block Heaters in a fume cupboard. The digestion was left on for 4 hours, after which a clear colourless solution was left in the tube. The digest was cooled and carefully transferred into

100 ml volumetric flask, thoroughly rinsing the digestion tube with distilled water and the flask was made up to mark with distilled water.

Distillation

The distillation was finished with Markham Distillation Apparatus which permits unpredictable substances, for example, smelling salts to be steam refined with complete assortment of the distillate. The apparatus steamed out for around ten minutes. The steam generator is then taken out from the intensity source to all the creating vacuum to eliminate condensed water. The steam generator is then put on the intensity source (for example warming mantle) and every part of the apparatus was repaired suitably. 5ml piece of the overview above was pipetted into the body of the apparatus by means of the little channel opening. To this was added 5ml of 40% (W/V) NaOH through a similar opening with the 5ml pipette. The blend was steam-refined for 2 minutes into a 50ml conical flask containing 10ml of 2% Boric Corrosive in addition to blended indicator arrangement set at the getting tip of the condenser.

Titration

The green color solution obtained was then titrated against 0.01N HCL contained in a 50ml Burette. At the end point or equivalent point, the green colour turns to wine colour which indicates that all the Nitrogen trapped as Ammonium Borate [(NH 4)2BO3] have been removed as Ammonium chloride (NH4CL). The percentage nitrogen in this analysis was calculated using the formula:

$$\%N = \frac{TitreValue X Nitrogen(amu) X Molarity of HCl X Volume of Flask X 100}{Weight of sample digested \in milligram x Vol. of digest for steam distillation}$$

The crude protein content is determined by multiplying percentage Nitrogen by a constant factor of 6.25.

3.4.2 Crude Fat / Ether Extract Determination (AOAC Official Method 2003.06)

1g of each dried example was weighed into fat free extraction thimble and attachment gently with cotton fleece. The thimble was put in the extractor and fitted up with reflux condenser and a 250ml soxhlet flask which has been recently dried in the broiler, cooled in the desicator and gauged. The soxhlet flask is then filled to 3/4 of its volume with petrol ether (b.pt. 40º - 60ºC), and the soxhlet flask. Extractor in addition to condenser set was put on the radiator. The radiator was placed on for six hours with steady running water from the tap for buildup of ether fume. The set is continually looked for ether spills and the intensity source is changed properly for the ether to tenderly bubble. The Ether is passed on to direct north of a few times express over no less than 10 - multiple times until it is shy of siphoning. It is after this is seen that any ether content of the extractor is painstakingly depleted into the ether stock container. The thimble containing test is then eliminated and dried on a clock glass on the seat top. The extractor, flask and condenser is supplanted and the distillation go on until the flask is essentially dry. The flask which currently contains the fat or oil is separated, its outside cleaned and dried to a steady weight in the broiler. If the underlying load of dry soxhlet flask is Weighed and the last weight of stove dried flask + oil/fat is W1, crude fat/oil is acquired by the equation:

$$\frac{W_1 - W_0}{WeightofSample} X100$$

Where: W_1= weight of oil/fat

W_0= weight of empty crucible

3.4.3 Dry Matter and Moisture Determination (AOAC Official Method 967.08)

2g of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into the oven set at 100^{O}C to dry to a constant weight for 24 hours overnight. At the end of the 24 hours, the crucible plus sample was removed from the oven and transferred to desiccator, cooled for ten minutes and weighed. If the weight of empty crucible is W_0, Weight of crucible plus sample is W_1, Weight of crucible plus oven-dried sample W_3.

$$\%DryMatter = \frac{W_3 - W_0}{W_1 - W_0} X100$$

$$\%Moisture = \frac{W_1 - W_3}{W_1 - W_0} X100$$

$$\%Moisture = 100 - \%DryMoisture$$

Where: W_1= weight of oil/fat

W_3 = Weight of crucible plus oven-dried sample

W_0= weight of empty crucible

3.4.4 Determination of Ash (AOAC Official Method 942.05)

2.0gm of the sample were weighed into a porcelain crucible. This was transferred into the muffle furnace set at 550°C and left for about 4 hours. About this time it had turned to white ash. The crucible and its content were cooled to about 100ºC in air, then room temperature in a desiccator and weighed. This was done in duplicate. The percentage ash was calculated from the formula below:

$$\%DryMatter = \frac{WeightofAsh}{WeightofSample} X100$$

Where: Wa = $WeightofAsh$

Ws = $WeightofSample$

3.4.5 Fibre Determination (AOAC 958.06)

2.0 g of the sample was accurately weighed into the fibre flask and 100 ml of 0.25M H_2SO_4 was added. The mixture was heated under reflux for 1 hour with the heating mantle. The hot mixture was filtered through a fibre sieve cloth. The filtrate obtained was thrown off and the residue was returned to the fiber flask to which 100 ml of 0.313N NaOH was added and heated under reflux for another 1 hour. The mixture was filtered through a fiber sieve cloth and 10ml of acetone added to dissolve any organic constituent. The residue was washed with about 50ml hot water on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue were oven-dried at 105°C overnight to drive off moisture. The oven-dried crucible containing the residue was cooled in a desiccator and later weighed to obtain the weight W_1. The crucible with weight W1 was transferred to the muffle furnace for ashing at 550°C for 4 hours.

The crucible containing white or grey ash (free of carbonaceous material) was cooled in the desiccator and weight to obtain W_2. The difference $W_1 - W_2$ gives the weight of fiber. The percentage fiber was obtained by the formula:

$$\%Fibre = \frac{W_1 - W_2}{WeightofSample} X100$$

Where: W_1= oven-dried crucible containing the residue

W_2= crucible containing white or grey ash

3.4.6 Nitrogen-Free Extract (NFE) or Carbohydrate by Difference Determination

NFE was determined by difference. This was done by subtracting Sum of (Moisture % +

% Crude Protein + % Ether Extract + % Crude Fibre + % Ash) from 100 i.e. (100 – (% M + % CP + % EE + % CF + % Ash).

3.4.7 Refractive index

A refractometer determined the oils' refractive index. Oil samples were placed on the lower prism, the prism box was adjusted, and the index was read from the scale with ±0.0002 accuracy.

3.4.8 Solvent Extraction (Methanol Extract)

The seed was separated from the flesh and bark and pulverized using a grinding engine. A 300g sample was weighed and placed in a 3-liter plastic container. Methanol (1.5 liters) was added, and the mixture was stirred intermittently for 48 hours. After sieving, the filtrate was transferred to a beaker and evaporated using a water bath.

3.5 Antioxidant Assay of Flesh, Bark, And Seeds Of *Annona Muricata*

Plants with secondary metabolites showing significant antioxidant activity often possess anticancer activity[48]All quantitative assays were performed in triplicate and results were expressed as mean ± standard deviation.

Scavenging activity will be calculated as:

$$\% \text{ Scavenging activity} = \frac{A_{control} - A_{sample}}{A_{control}} \; x \; 100\%$$

3.5.1 DPPH radical scavenging

Various concentrations of ascorbic acid (reference antioxidant) were prepared as standard. The sample extract was diluted to different concentrations. Each standard and sample solution were

mixed with 1, 1-diphenyl-2-picrylhydrazyl (0.04% DPPH) solution.They were incubated in the dark at room temperature. Absorbance was measured at 517 nm after 30 minutes.

3.5.2 FRAP Assay

A working FRAP reagent was prepared by mixing acetate buffer, TPTZ solution, and ferric chloride solution. Ascorbic acid standards and sample extract were diluted to different concentrations. FRAP reagent were added to each standard and sample solution. They were incubated at 37°C for 30 minutes. Absorbance was measured at 593 nm.

3.5.3 Nitric Acid Assay

Ascorbic acid standards and sample extract were diluted to different concentrations. Griess reagents were mixed with each standard and sample solution. They were incubated at room temperature for 30 minutes. Absorbance was measured at 540 nm.

3.5.4 Lipid Peroxidation Scavenging Activity (%Inhibition)

Flesh, bark, and seed samples of *Annona Muricata* were dried, powdered, and extracted using methanol. The extracts were concentrated using a rotary evaporator. Lipid peroxidation was induced in a linoleic acid emulsion system by incubating the samples with ferrous sulfate and ascorbic acid at 37°C for 1 hour. The reaction mixture contained 0.2 mL of extract, 0.2 mL of linoleic acid emulsion, 0.2 mL of ferrous sulfate (10 mM), and 0.2 mL of ascorbic acid (10 mM). Lipid peroxidation was measured by the thiobarbituric acid reactive substances (TBARS) assay. After incubation, 1 mL of 20% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid were added and heated at 95°C for 30 minutes. The mixture was cooled and centrifuged at 3000 rpm

for 10 minutes. The absorbance of the supernatant was measured at 532 nm. The percentage inhibition of lipid peroxidation was calculated using a control without the extract.

3.6 Phytochemical Screening (Qualitative Analysis)

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by[1,2].The methanolic extracts of the bark, seed and flesh of *Annona Muricata* were screened for the following phyto constituents; alkaloids, saponins, terpenoids, flavonoids, Cardiac glycosides, phenols, tannins and phlobatannins.

3.6.1 Screening for Tannins

0.5 g of the sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black colouration.

3.6.2 Screening for Phlobatannins

0.5g extract of each plant sample was boiled with 2mL of 1% aqueous hydrochloric acid for 10 minutes. Deposition of a red precipitate indicated the presence of phlobatannin.

3.6.3 Screening for Saponin

2 g of the sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and observed for formation of emulsion.

3.6.4 Screening for Flavonoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4.

3.6.5 Screening for Steroids

2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. Colour changed from violet to blue or green indicates the presence of steroids.

3.6.6 Screening for Terpenoids (Salkowski Test)

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish-brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

3.6.7 Screening for Cardiac Glycosides (Keller-Killani Test)

5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

3.6.8 Screening for alkaloid

A 5 mg sample of the extract dissolved in 3 ml of acidified ethanol was warmed slightly and then filtered. Few drops of Mayer's reagent and 1 ml of Dragendroff's reagent were added to 1 ml of the filtrate and turbidity was observed.

3.6.9 Screening for Phenols

2 g powder sample was carried out for 2 hours in 100 ml of diethyl ether using a soxhlet apparatus. The defatted sample (0.50 g) was boiled for 15 minutes with 50 ml of ether for the extraction of the phenolic components. Exactly 10 ml of distilled water, with 2 ml of 0.1 N ammonium hydroxide solution, and 5 ml of concentrated amyl alcohol were also added to 5 ml of the extract and left to react for 30 minutes for color development. The optical density was measured at 505 nm. 0.20 g of tannic acid was dissolving in distilled water and diluted to 250 ml mark (1 mg / ml) in preparation for phenol standard curve. Varying concentrations (0.2–1.0 mg/ml) of the standard tannic acid solution were pipetted into five different test tubes to which 2 cm3 of NH_3OH, 5 ml of amyl alcohol, and 10 ml of water were added. The solution was made up to 100 ml volume and left to react for 30 minutes for color development. The optical density was determined at 505 nm with UV/VIS TG 50 spectrophotometer.

$$\text{Phenolic acid (mg /100 g)} = \frac{C \times \text{extract volume}}{\text{Aliquot volume} \times \text{weight of sample}} \times 100$$

where C is concentration of tannic acid read off the graph.

3.7 GC-MS Characterization

For GC-MS detection, an electron ionization system with an ionization energy of 70eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1 ml/min. Injection and mass transfer line temperatures were set at 200 and 240 °C respectively. The oven temperature was programmed from 80 °C to hold for 2 mins@ 10 °C/min to 240 °C to hold for 6 mins. 2 ml of water solution of the samples was manually inserted in the split less mode, with a split ratio of 1:40 and with a mass scan of 50–600amu. The total running time of the GC-MS was 35min. The relative percentage of each extract constituent was expressed as a percentage with peak area

normalization. Interpretation of the mass spectrum of the plant extracts was conducted using the database of the National Institute of Standard and Technology (NIST) library, having more than 62,000 spectral patterns. The spectra of the compounds were compared with the spectra of the National Institute of Standard and Technology (NIST) library database.

3.8 *In Silico* Study

3.8.1 Protein preparation

The 3D structure of the Human Anaplastic Lymphoma Kinase (ALK) protein (PDB ID: 2XBA) was acquired from the Protein Data Bank (PDB) website (http://www.pdb.org/pdb/home/home.do). Receptor preparation was carried out using the "Prepare protein" method in BIOVIA Discovery Studio 2024[1]. To prepare the receptor, native ligands and water molecules were removed from the crystal structure under physiological pH 7.4 conditions. Following this, coordinates for 2XBA (X= 6.907829 Y= 19.051805 Z=7. RADIUS= 7.822268) were determined, and polar hydrogen was added to the structure. The resulting 2XBA structure was extracted in PDB format and saved for use as targets in the virtual screening process.

3.8.2 Ligand preparation

The control for targeting ALK consisted of One hundred and thirty-four (134) bioactive compounds detected in *Annona Muricata* and the FDA-approved drug Ceritinib. The 3D structures of these compounds, including the standard ligands (Ceritinib), were retrieved in simple data format (SDF) from the PubChem server using Open Babel in PyRx (version 0.8).

PyRx utilized the Merck molecular force field 94 (MMFF94) to energetically transform the ligands into their most stable structures[1].

3.8.3 Molecular docking

Flexible docking approach was applied with minor modifications[1]. Using PyRx (version 0.8) incorporating Auto Dock Vina, molecular docking analysis of the selected ligands and the target protein was performed. Protein data bank (.pdb), partial charges, and atom type (.pdbqt) files for the proteins were generated based on their existing PDB files. To stabilize the receptor, the ligand's bonds were set to rotate freely and the grid box was adjusted according to the protein's active sites[3] After completing the molecular docking studies, text files containing scoring results were produced for manual comparison. Additionally, 10 poses for each protein-ligand complex of all phytocompounds, focusing on minimum binding energy (BE, kcal/mol) was generated.

3.8.4 ADMET Profiling

To evaluate the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the chosen test compounds, *in silico* predictive models were used. The SwissADME server played a pivotal role in assessing various ADME properties, including lipophilicity (Log P), water solubility (ESOL Log S), drug-likeness based on the Lipinski rule, bioavailability score, and pharmacokinetics-related factors such as gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, permeability glycoprotein (Pgp) binding, and Cytochrome P450 (CYP) inhibition. Furthermore, the ProTox-II online server was employed to predict the acute toxicity class, LD50, as well as the potential for hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, and immunotoxicity associated with the compounds

3.9 Result Analysis

3.9.1 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The relative percentage of each extract constituents were expressed as percentage with peak area normalization. Interpretation of mass spectrum of plant extracts were conducted using the data base of National Institute of Standard and Technology (NIST) library having more than 62, 000 spectral patterns. The spectrum of the compounds was compared with the spectrum of the National Institute of Standard and Technology (NIST) library data base.

Endnotes

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Chapter Four

Results and Discussion of Findings

4.1 Results

Table 4.1 presents the proximate composition of Annona muricata leaf, flesh, and seed samples, including moisture, crude protein, total fat, total carbohydrate, dietary fiber, and ash content. Notably, moisture content is crucial for preservation due to its impact on microbial growth, while variations in protein, fat, and carbohydrate content reflect differences in food composition and energy contribution.

Table 4.2 shows the phytochemical screening results of methanolic extracts from the seed, flesh, and bark of Annona muricata. It highlights the presence of various compounds like alkaloids, tannins, saponins, phenols, and flavonoids. The table indicates that all three parts of the plant have similar phytochemical profiles.

Table 4.3 provides the binding affinity of the top four compounds from Annona muricata leaves with Human Anaplastic Lymphoma Kinase (ALK). It includes compounds such as Tricyclo[20.8.0.0(7,16)]triacontane and Aspidospermidin-17-ol. The binding affinities range from -7.2 to -8.3 kcal/mol, indicating varying degrees of interaction strength.

Table 4.1: Proximate Composition of Bark, Flesh, and Seed of Annona Muricata

PARAMETER	Bark	Flesh	Seed
Moisture (%)	8.4	12.42	6.36
Ash Content(%)	1.04	0.73	0.87
Total Fatty Acid (%)	6.47	8.84	12.64
Nitrogen Content(%)	2.54	2.97	2.64
Crude Protein(%)[%N XF(6.25)]	15.88	18.56	16.50
Crude Fibre (%)	5. 2	1.6	1.3
Carbohydrate Content (100 –X)	60.47	54.88	59.69

Source: Researcher's Field work, 2024

Table 4.2: Phytochemical Screening Results of Methanolic Extract of *Annona Muricata*

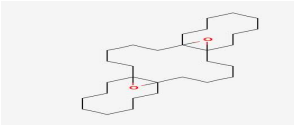
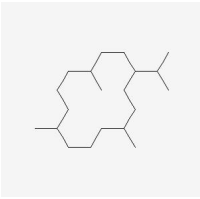
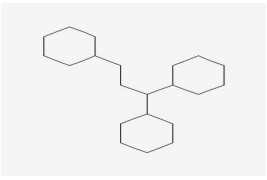
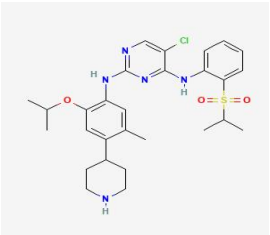
Code	Seed	Flesh	Bark
Alkaloid	+	+	+
Tannin	+	+	+
Phlobatannin	+	+	+
Saponin	++	++	++
Phenol	+	+	+
Reducing Sugar	++	++	++
Steroid	+	+	+
Cardiac Glycoside	+	+	+
Terpenoid	+	+	+
Flavonoid	+	+	+

Code

+ =Present **++ = Much Present** **++ += Mostly Present** **-= Absent**

Source: Researcher's Field work, 2024

Table 4.3: Binding Affinity of the Top Four Phytocompounds in *Annona Muricata* with Human Anaplastic Lymphoma Kinase (ALK)

S/N	Compounds	PUBCHEM CID	Structure	Binding affinity (Kcal/mol)
1.	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy TTD	543764		-8.3
2	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy- ACED	550059		-7.7
3	Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)- Cembrane	15702		-7.2
4	1,1,3-Tricyclohexylpropane THP	143294		-7.2
	Ceritinib	57379345		-7.2

Source: Researcher's Field work, 2024

Table 4.4 records the buildups inside a 4 Å range that communicate with the ligands and the protein receptor ALK. Its subtleties collaborations for Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy (TTD), Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy (ACED), Cembrane, and 113-Tricyclohexylpropane. The buildups incorporate normal amino acids like LEU, VAL, and ASP, showing the particular restricting locales for each compound.

Table 4.5 portrays the ADME (Assimilation, Appropriation, Digestion, and Discharge) properties of the top compounds from Annona muricata. It incorporates atomic weight, solvency, log P values, and bioavailability scores. The table assists with foreseeing the pharmacokinetic profiles of these compounds.

Table 4.6 subtleties the poisonousness properties of compounds present in concentrates of Annona muricata and the standard medication Ceritinib. It incorporates LD50 values, hepatotoxicity, cancer-causing nature, immunotoxicity, mutagenicity, and cytotoxicity. This extensive poisonousness profile is vital for surveying the security of these compounds.

Table 4.4: Residues Within 4A Interacting With the Ligands and the Protein Receptor

S/N	Ligand	Residues within 4A
1.	TTD	LUE 1122, VAL 1130, ALA 1148, LYS 1150, LUE 1196, LUE 1198, MET 1199, GLY 1202, ASP 1203, and LEU 1256.
2.	ACED	LEU 1122, VAL 1130, VAL 1148, LEU 1196, GLU 1197, MET 1199, ALA 1200, GLY 1202, ASP 1203, and LEU 1256.
3.	Cembrane	LEU 1122, VAL 1130, ALA 1148, VAL 1180, LEU 1196, GLU 1197, LEU 1198, MET 1199, ASP 1203, LYS 1205, SER 1206, ARG 1253, LEU 1256, and ASP 1270
4.	1,1,3-Tricyclohexylpropane	LUE 1122, VAL 1130, ALA 1148, LYS 1150, LUE 1196, MET 1199, GLY 1202, ASP 1203, ARG 1253, ASN 1254, LEU 1256, and ASP 1270
5.	Standard Ligand: Ceritinib	LEU 1122, VAL 1130, ALA 1148, LYS 1150, LEU 1198, MET 1199, GLY 1202, ASP 1203, ARG 1253, ASN 254, LEU 1256, GLY 1269, and ASP 1270.

Source: Researcher's Field work, 2024

Table 4.5: Residues Within 4A Interacting With the Ligands and the Protein Receptor

S/N	TTD	ACED	Cembrane	THP	Ceritinib
Molecular weight	444.73	414.49	280.53	290.53	558.14
ESOL log S	-8.56	-3.62	-8.07	-7.47	-7.1
Solubility class	Insoluble	Soluble	Insoluble	Insoluble	Insoluble
Mean log P	6.14	2	6.99	7.21	3.91
Lipinski violations	1	0	1	1	1
Bioavailability score	0.55	0.55	0.55	0.55	0.55
GI absorption	Low	High	Low	Low	Low
BBB permeant	No	Yes	No	No	No
Pgp substrate	Yes	No	No	Yes	Yes
CYP1A2 inhibitor	No	No	No	Yes	No
CYP2C19 inhibitor	No	Yes	No	No	Yes
CYP2C9 inhibitor	No	No	Yes	No	No
CYP2D6 inhibitor	No	Yes	No	No	Yes
CYP3A4 inhibitor	No	No	No	No	No

Table 4.6: Toxicity and Properties of TTD, ACED, TPH Present in Extracts of *A. muricata* and Ceritinib

S/N	**Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy**	**Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-**	Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)-	**1,1,3-Tricyclohexylpropane**	**Ceritinib**
LD50 (mg/kg)	5000	325	750	15380	**3550**
Toxicity	5	4	3	6	**5**
Hepatotoxicity	Inactive	Inactive	Inactive	Inactive	**Inactive**
Carcinogenicity	Inactive	Inactive	Inactive	Inactive	**Inactive**
Immunotoxicity	Inactive	Active	Inactive	Inactive	**Active**
Mutagenicity	Inactive	Inactive	Inactive	Inactive	**Inactive**
Cytotoxicity	Inactive	Inactive	Inactive	Inactive	**Inactive**

Source: Researcher's Field work, 2024

Figure 4.1: Illustrates the quantitative screening of various phytochemicals in the methanolic extracts of Annona muricata. This figure helps visualize the concentration levels of key phytochemicals like flavonoids, phenols, and alkaloids in different parts of the plant.

Figure 4.2: Displays the DPPH scavenging activity (% inhibition) of the bark, flesh, and seed of methanolic extracts from Annona muricata. It demonstrates the antioxidant capacity of each plant part, with variations in their ability to neutralize free radicals.

Figure 4.3: Shows the ferric reducing antioxidant power (FRAP) of the bark, flesh, and seed of methanolic extracts of Annona muricata. This figure highlights the reducing power of the extracts, indicating their potential as natural antioxidants.

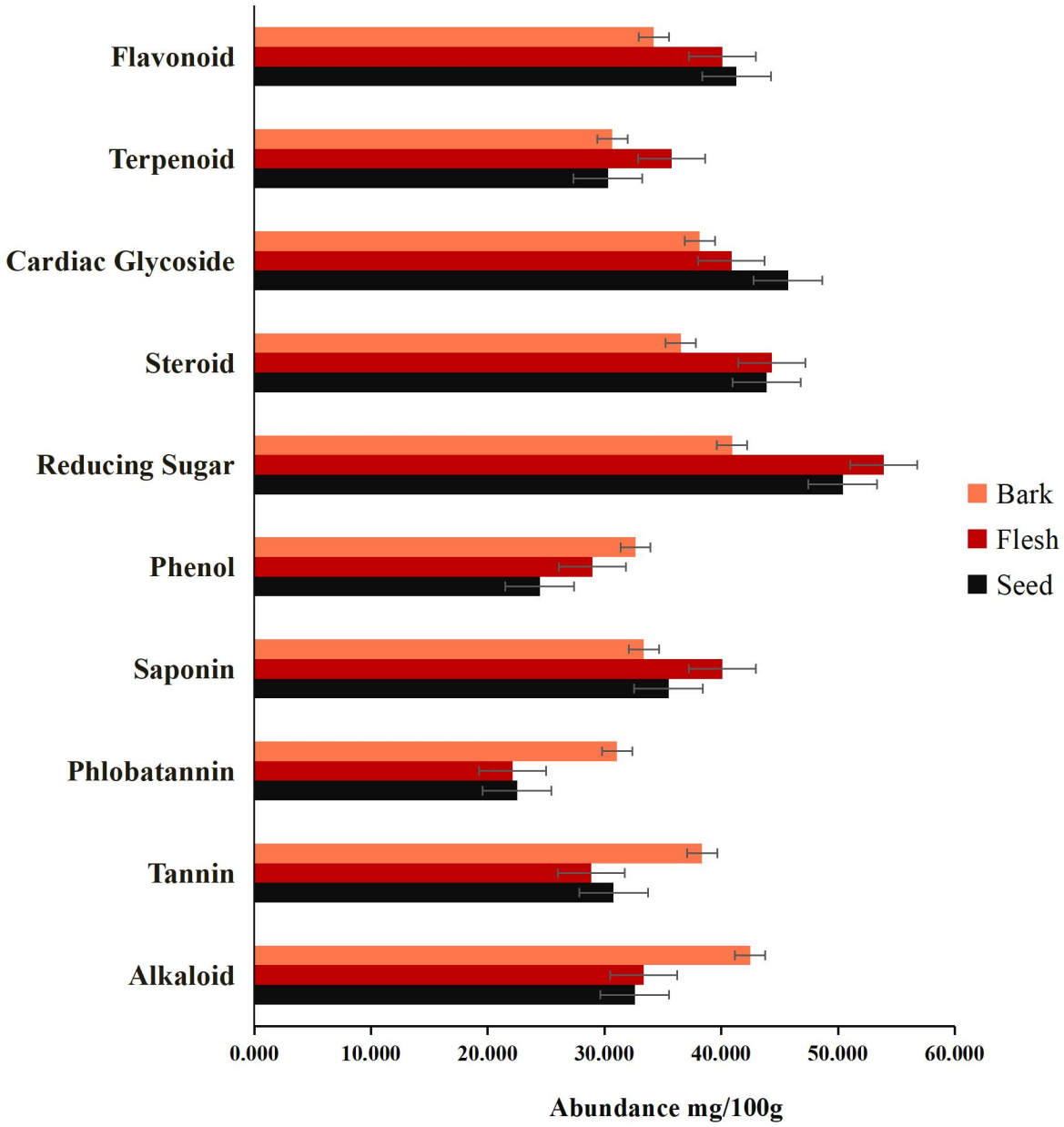


Figure 4.1: Phytochemical Quantitative Screening

Source: Researcher's Field work, 2024

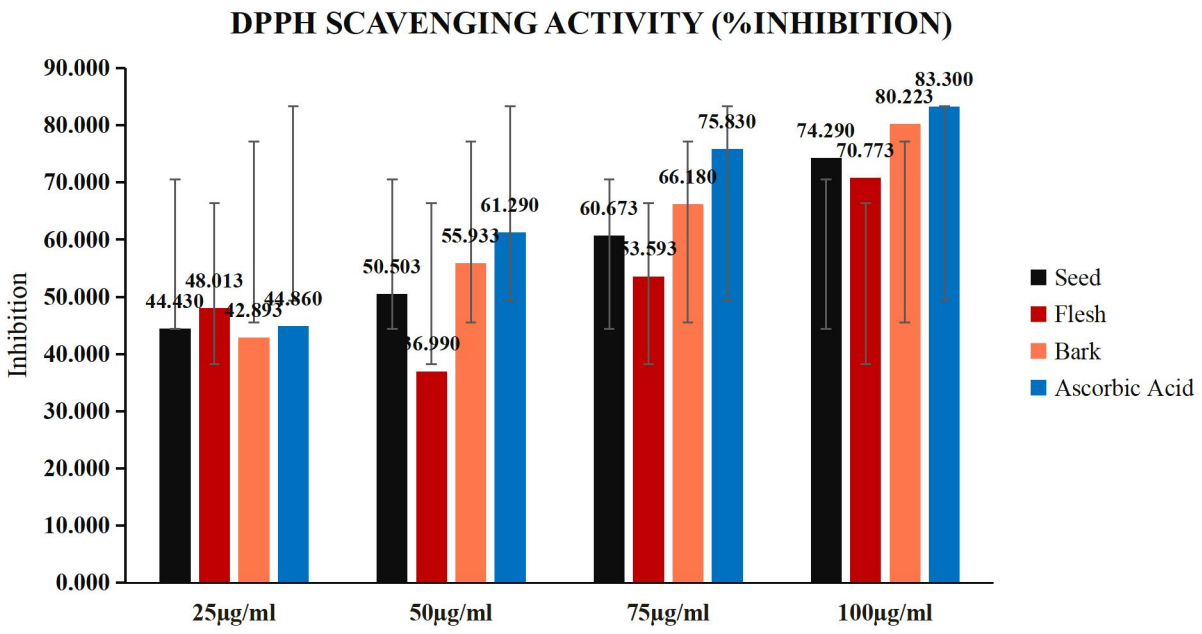


Figure 4.2: DPPH Scavenging Activity (%Inhibition) of Bark, Flesh and Seed of Methanolic Extracts of *Annona Muricata*

Source: Researcher's Field work, 2024

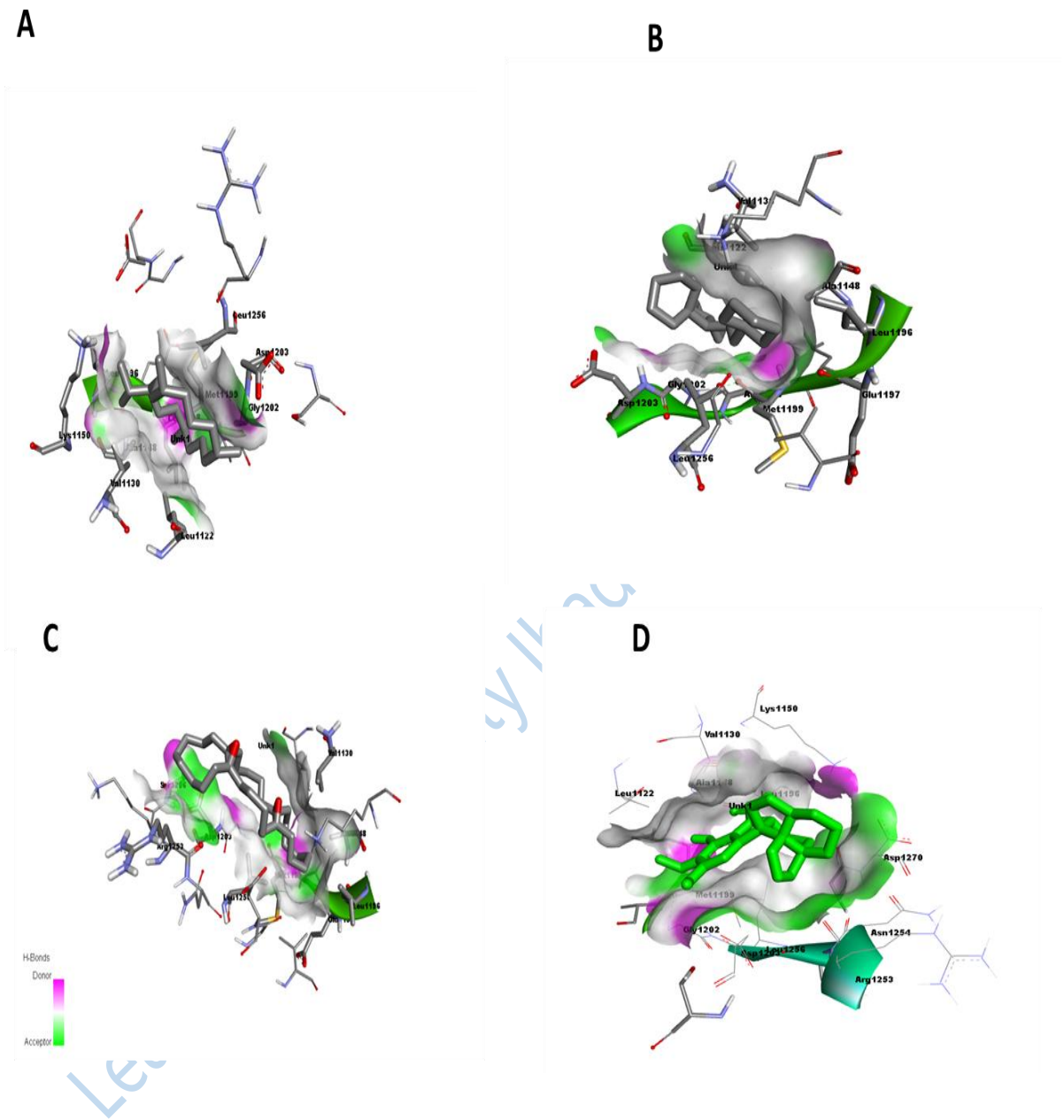


Figure 4.3: The 3D View of the Molecular Interaction of the Top Four Compounds of *A. muricata* Samples: (A) TTD, (B) ACED, (C) Cembrane, and (D) 1,1,3-Tricyclohexylpropane, with ALK

Source: Researcher's Field work, 2024

Figure 4.4: Depicts the lipid peroxidation scavenging activity (% inhibition) of the bark, flesh, and seed of methanolic extracts from Annona muricata. This figure indicates how effectively each extract can inhibit lipid peroxidation, a marker of oxidative stress.

Figure 4.5: Illustrates the nitric oxide scavenging activity (% inhibition) of the bark, flesh, and seed of methanolic extracts from Annona muricata. It shows the capacity of these extracts to scavenge nitric oxide radicals, contributing to their overall antioxidant activity.

Figure 4.6: This table provides the toxicity properties of TTD, ACED, Cembrane, THP, and Ceritinib. Toxicity parameters such as hepatotoxicity, mutagenicity, and carcinogenicity are included. Understanding these properties helps assess the safety profile of these compounds for potential use in medical treatments.

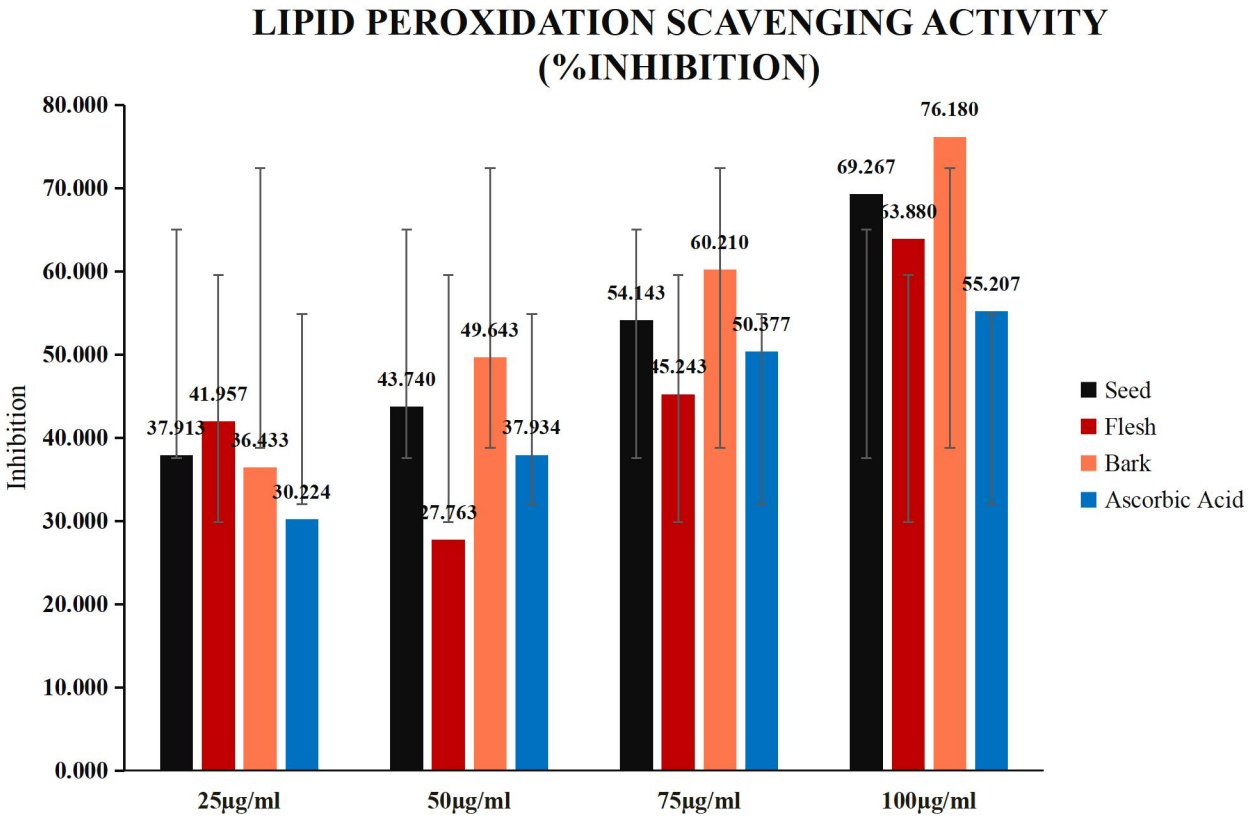


Figure 4.4: Lipid Peroxidation Scavenging Activity (%Inhibition) of Bark, Flesh and Seed of Methanolic Extracts of *Annona Muricata*

Source: Researcher's Field work, 2024

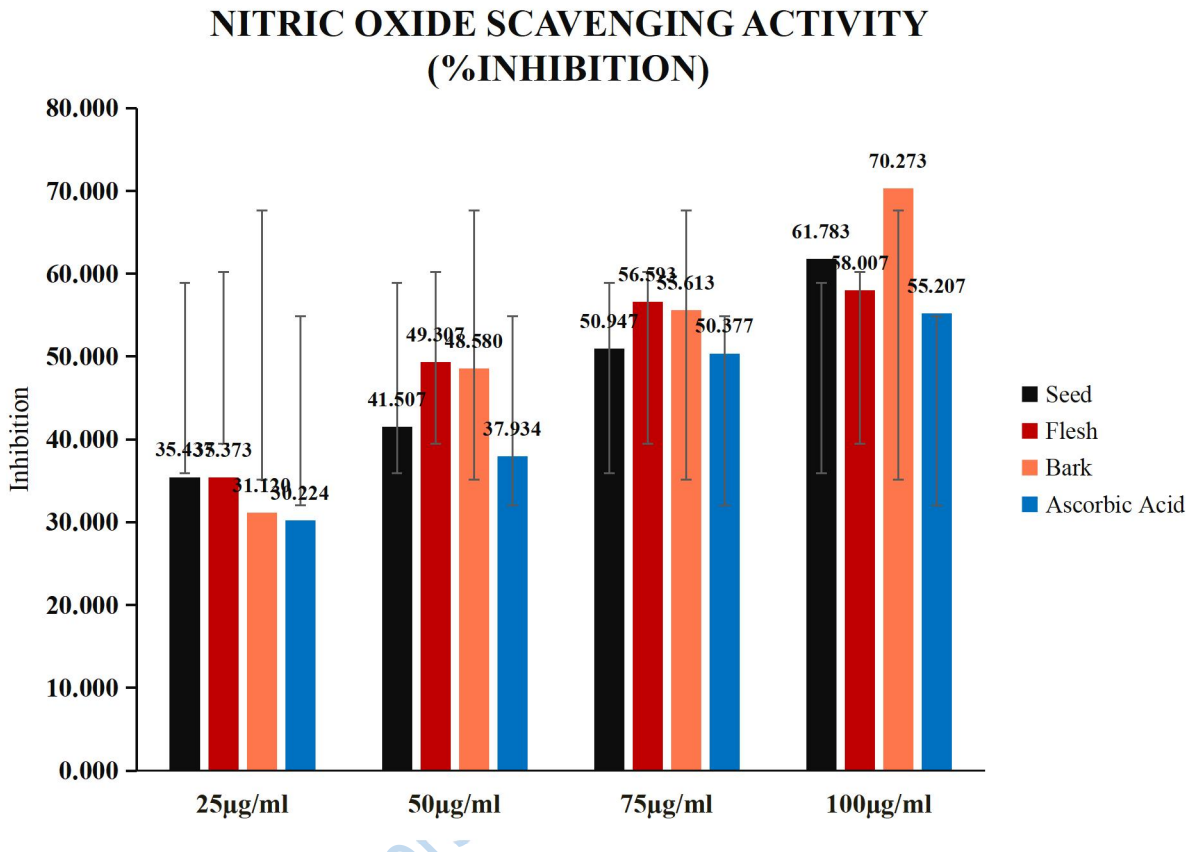


Figure 4.5: Nitric Oxide Scavenging Activity (%Inhibition) of Bark, Flesh and Seed of Methanolic Extracts of *Annona Muricata*

Source: Researcher's Field work, 2024

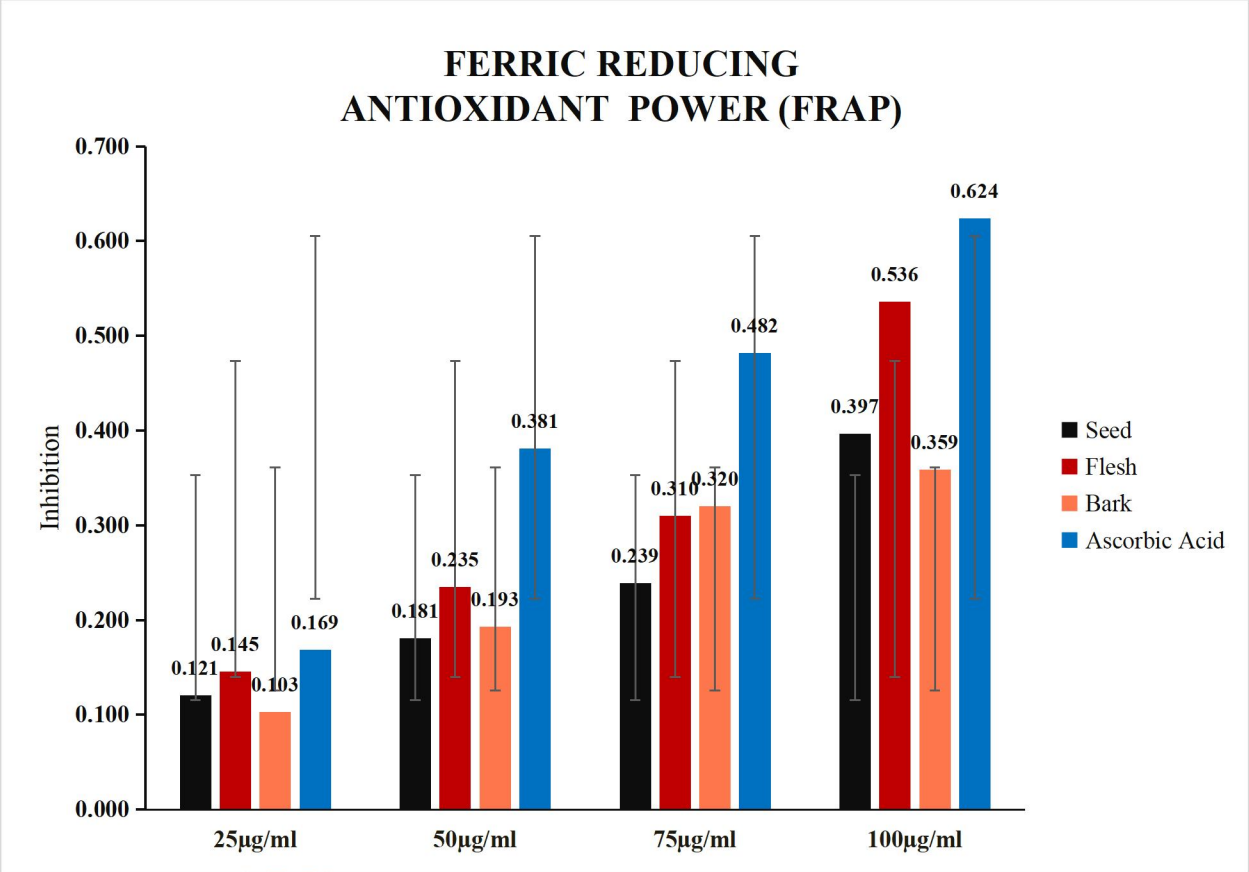


Figure 4.6: Ferric Reducing Antioxidant Power Activity (%Inhibition) of Bark, Flesh and Seed of Methanolic Extracts of *Annona Muricata*

Source: Researcher's Field work, 2024

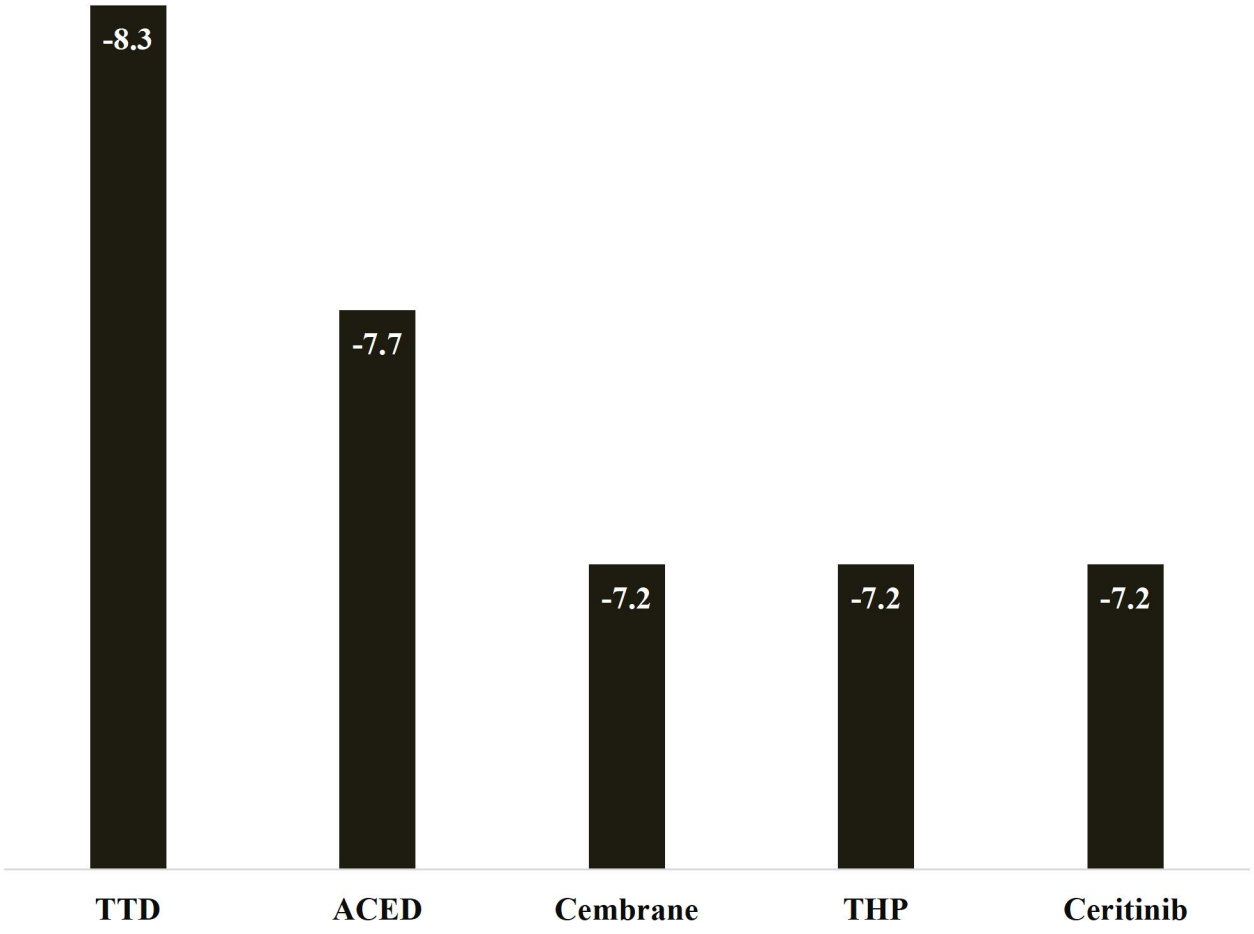


Figure 4.7: Binding Energy (-kcal/mol)

Source: Researcher's Field work, 2024

Figure 4.7: This figure shows the 2D view of the molecular interaction of the top four compounds from Annona muricata (TTD, ACED, Cembrane, and 1,13-Tricyclohexylpropane) with ALK. Each panel (A-D) details the specific interactions between the compounds and the active sites of the ALK protein, highlighting critical binding interactions that contribute to their potential efficacy as inhibitors.

Figure 4.8: The figure presents the 3D view of the molecular interactions between the top four compounds of Annona muricata and ALK. The 3D visualization provides a spatial perspective on how these compounds fit within the binding pocket of ALK, emphasizing the threedimensional arrangement and potential points of contact that are vital for their binding affinity .

Figure 4.9: This figure illustrates the 2D and 3D views of the molecular interaction of Ceritinib with ALK. Panels (E) and (F) depict the detailed binding interactions, showing how Ceritinib aligns with the binding sites of ALK. These visualizations are essential for comparing the standard drug Ceritinib with the compounds derived from Annona muricata in terms of binding efficiency and interaction profile.

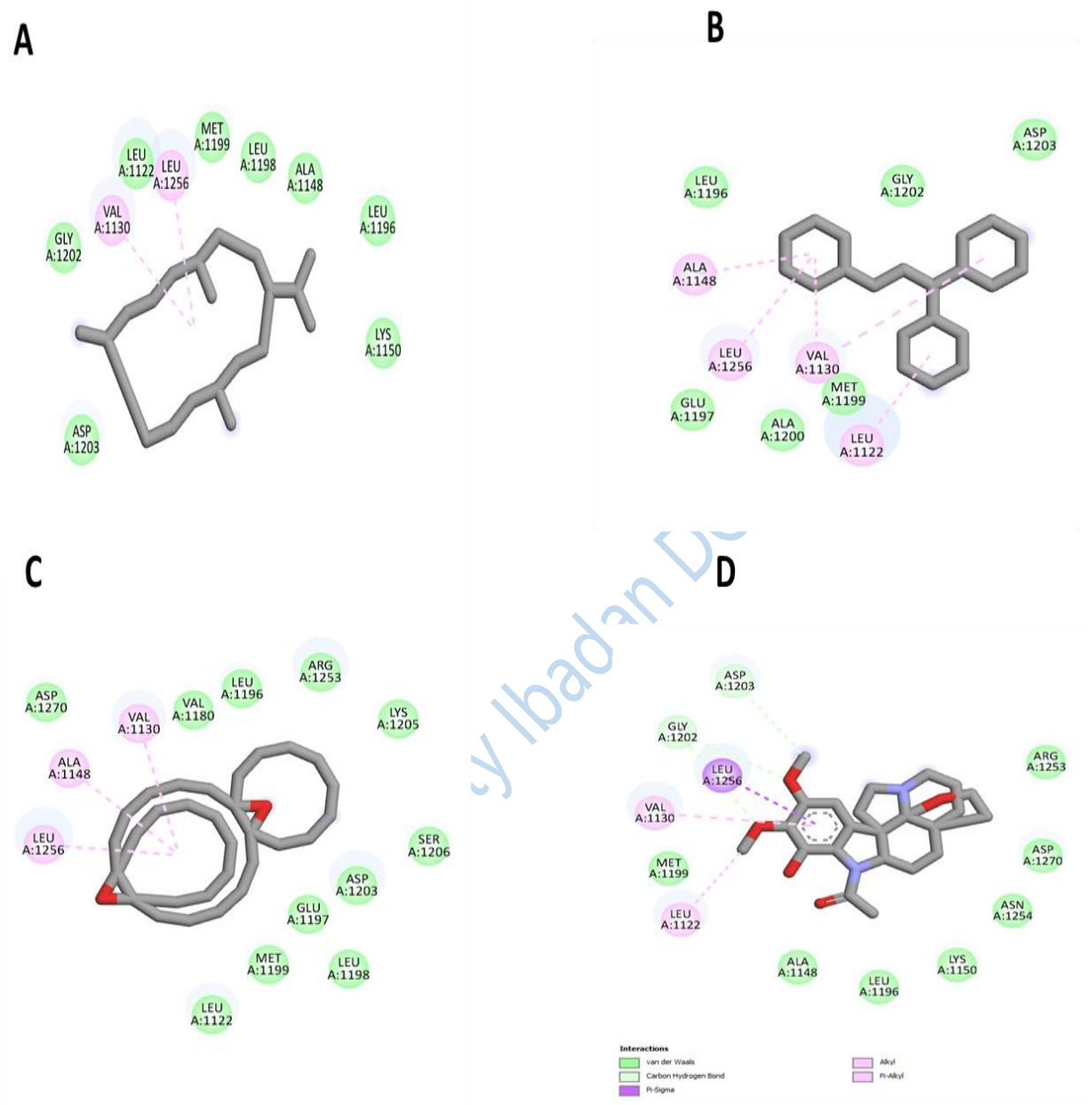


Figure 4.8: The 2D View of the Molecular Interaction of the Top Four Compounds Present in *A. muricata*: (A) TTD, (B) ACED, (C) Cembrane, and (D) 1,1,3-Tricyclohexylpropane, with ALK

Source: Researcher's Field work, 2024

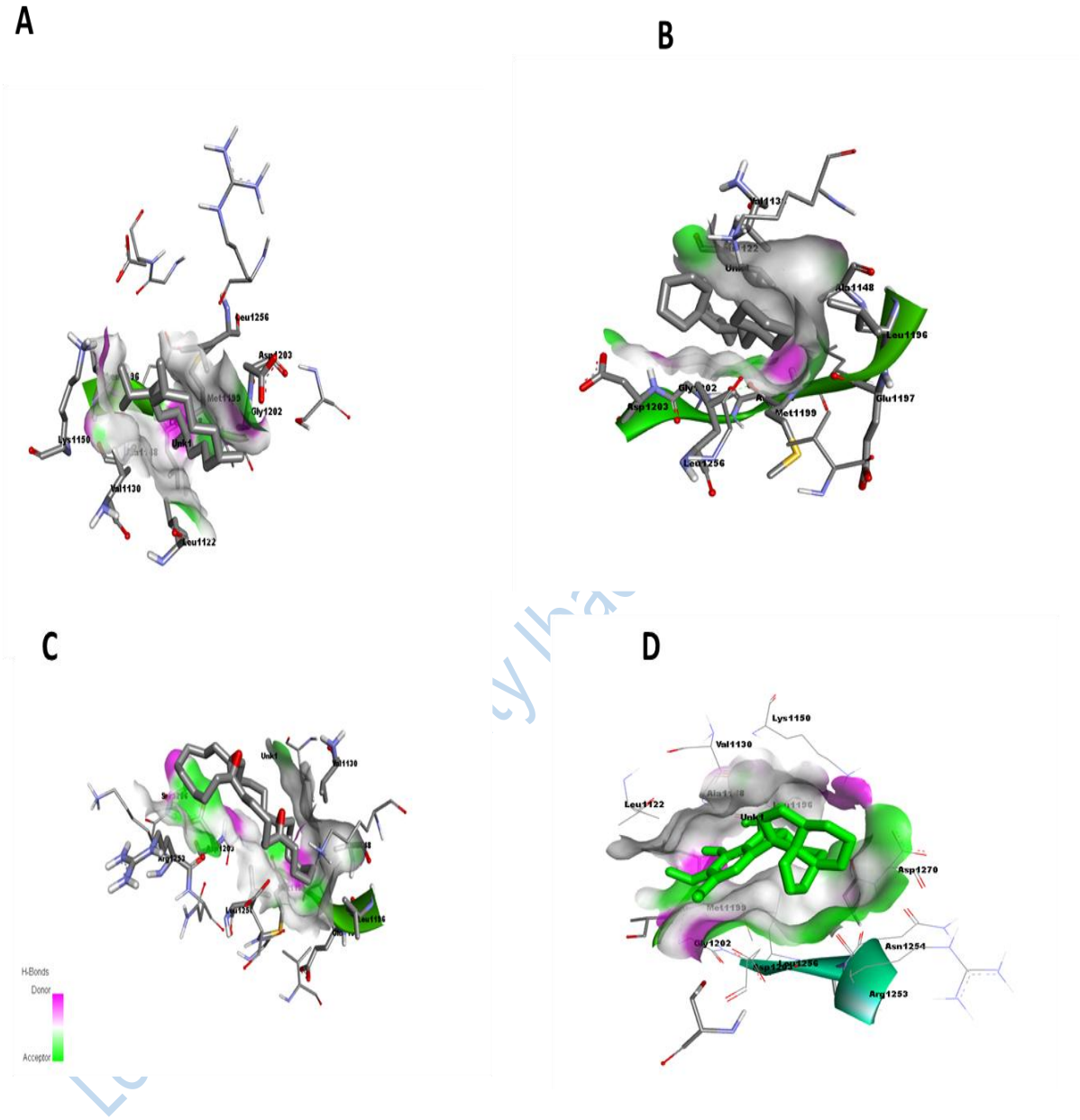


Figure 4.9: The 3D View of the Molecular Interaction of the Top Four Compounds Present in *A. muricata*: (A) TTD, (B) ACED, (C) Cembrane, and (D) 1,1,3-Tricyclohexylpropane, with ALK

Source: Researcher's Field work, 2024

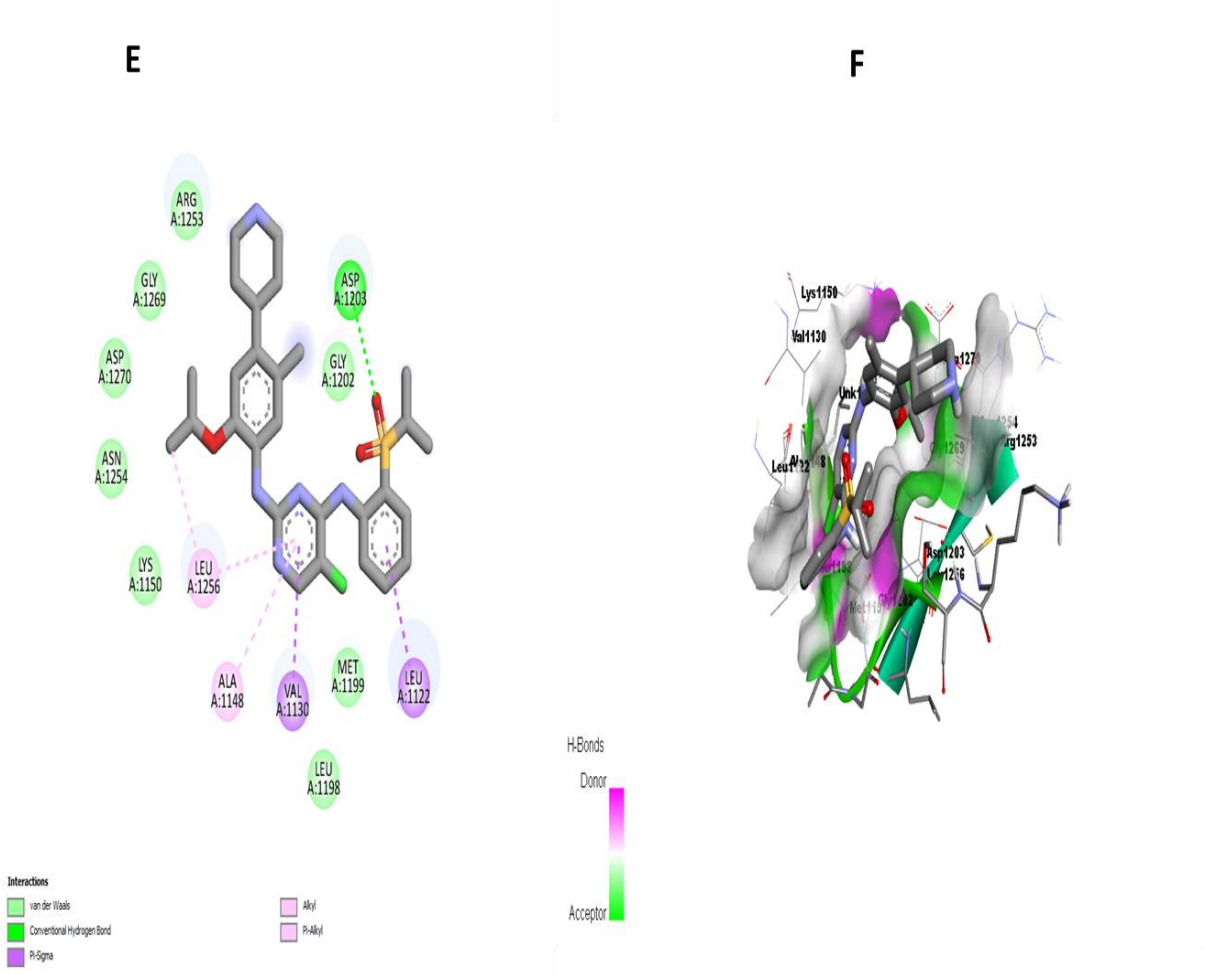


Figure 4.10: (E) 2D View of the Molecular Interaction of Ceritinib, (F) 3D View of the Molecular Interaction of Ceritinib with ALK

Source: Researcher's Field work, 2024

4.2 Discussion of Findings

This section shows the results that were obtained from all comprehensive analysis done on the study plant. The following assays: Physico-chemical analysis, proximate analysis, phytochemical qualitative and quantitative analysis, anti-oxidant assay and computational analysis were evaluated to determine the bioactive compounds present in *Annona Muricata*.

Food and food substance quantitative estimation, including moisture, crude protein, total fat, total carbohydrate, and dietary fiber, is done using proximate analysis. The results of proximate composition of *Annona Muricata* leaf, flesh and seed samples are shown in Table 4.1. Sometimes the quality of food is estimated using the moisture content. However, because microorganisms like mold and fungus thrive in damp environments, moisture content is one of the most important elements in preservation[1].

Fresh *Annona Muricata* Bark, flesh and seed samples dried until constant weight, moisture content was found to be 8.4, 12.42, and 6.36 g/100 g sample. The quantity of total nitrogen multiplied by protein factors equals crude protein. Protein nitrogen and a few nonprotein nitrogens made up the total nitrogen. The protein content of different foods varied[1,2]. Fresh *Annona Muricata* Bark, flesh and seed Nitrogen content was found to be 2.54, 2.97, and 2.64 g/100 g sample.

The amount of fat that includes fatty acids, oil-soluble colors, fat-soluble vitamins, and steroids is known as total fat, or ether extract[1,2]. Total fat of *Annona Muricata* Bark, flesh and seed samples were found to be 6.47, 8.84, 12.64, g/100 g sample.

Total carbohydrates are the quantity of carbohydrates, which are one of the primary building blocks of plant structural materials[1,2].

Proximate analysis showed that the total carbohydrate of *Annona Muricata* Bark, flesh and seed samples to be based on difference approach, whereby CarbohydrateContent was found to be 60.4, 54.88, 59.69, g/100 g sample.

Dietary fiber is the amount of total dietary fiber[1,3]. Dietary fiber of *Annona Muricata* Bark, flesh and seed samples were found to be 5. 2, 1.6, 1.3g/100 g sample.

The total quantity of mineral residue that remains after burning leaf samples to a consistent weight is known as the ash content[3]. Ash content of *Annona Muricata* Bark, flesh and seed samples was found to be 1.04, 0.73**,** 0.87g/100 g sample. Carbohydrate, fat, and protein all contribute to the overall composition of energy.

Essential phytochemicals, a range of primary and secondary plant metabolites with known biological activities and effects such as anti-hyperglycemic, anti-inflammatory, anti-diabetic, and anti-microbial properties, are abundant in medicinal plants and herbs[3,4] . Classes of compounds identified from the methanolic extracts of bark, flesh and seed of *Annona Muricata* using the different qualitative methods of analysis and their relative abundance are alkaloids, flavonoids, saponins, tannins, phenols, steroids, phlobatannins, cardiac glycosides, reducing sugar and terpenoids and are shown in table 4.1.

The qualitative screening of phytochemicals in *Annona Muricata* (Soursop) reveals a consistent presence of all the classes of bioactive compounds across different parts of the plant: seed, flesh, and bark suggesting that the entire fruit can be utilized in developing therapeutic strategies. Saponnins and Reducing sugars are much more present that the other classes of compounds. Saponins have been noted to possess anticancer activities. They have been shown to inhibit cancer cell growth and metastasis by inducing apoptosis and enhancing the immune response

against cancer cells. While primarily noted for their nutritional contributions, reducing sugars in *Annona Muricata* may also contribute to its overall bioactive profile by supporting other phytochemical actions.

It has been demonstrated that flavonoids are very effective scavengers of the majority of oxidizing molecules, including single oxygen and different free radicals[6] linked to several illnesses. Flavonoids protect mucous membranes and have anti-oxidant properties[6,7]. Vegetables high in flavonoids are popular functional foods because they can be utilized to treat heart conditions[8].

Due to their high bioavailability, flavonoids have been shown to provide pharmacologically meaningful plasma concentrations in humans when consumed consistently through diet[9]. Furthermore, flavonoids may have cardioprotective properties against ischemia reperfusion, according to several studies[8,9,10]. While tannins lessen the mucosa's permeability to chemical irritation, saponins have the capacity to activate factors that protect mucous membranes. As a result, they lessen inflammation, protect and astringe the stomach mucosa, and control excessive acidity.

According to reports, terpenoids can relax cardiovascular smooth muscle by stimulating the production of nitric oxide (NO) and quenching reactive oxygen species (ROS) or by inhibiting Ca^{2+} influx in vascular smooth muscle[10]. These phytochemicals may reveal *Annona Muricata* many therapeutic qualities, including its anti-inflammatory, anti-ulcer, and anti-oxidative qualities, as seen by their presence in the methanol fraction of the leaf, flesh and seed.

Figure 4.1 shows the phytochemical determination of the bark, flesh and seed components of the *Annona Muricata*. The quantitative phytochemical analysis of *Annona Muricata* reveals

variations in alkaloid, tannin, phlobatannin, saponin, phenol, reducing sugar, steroid, cardiac glycoside, terpenoid, and flavonoid content between the seed and flesh components. The seed extract generally exhibited higher concentrations of most phytochemicals compared to the flesh. For instance, the mean alkaloid content in the seed (32.60 mg/g) was nearly double that of the flesh (16.35 mg/g), with similar trends observed for tannins (seed: 30.78 mg/g, flesh: 24.46 mg/g), phenols (seed: 50.41 mg/g, flesh: 24.3 mg/g), and terpenoids (seed: 30.31 mg/g, flesh: 41.25 mg/g). Interestingly, the flesh extract contained a higher concentration of reducing sugars (53.91 mg/g) compared to the seeds (33.35 mg/g).

Antioxidants are added to food as food additives to stabilize foods whose composition would cause them to significantly degrade in the presence of oxygen and other reactive oxygen species. Examples of this include the development of rancidity from the oxidation of unsaturated fats, which results in off-odors and flavors, and discoloration from the oxidation of pigments or other food components.

The Bark, flesh and seed of *Annona Muricata* methanolic extract showed similar range of antioxidant potential when compared to standard ascorbic acid by DPPH scavenging assay method, FRAP, Lipid peroxidation and Nitric Oxide assay and the IC_{50} values obtained as shown in Figures 4.2, 4.3, 4.4 & 4.5 respectively.

The TBARS method has been widely used to determine the degree of lipid oxidation which results from lipid peroxidation of polyunsaturated fatty acids shown in figure 4.4. The values obtained by FRAP show that the lowest antioxidant activity was that of the Bark (0.360 at conc. 100µg/ml), and that the highest antioxidant activity was that of the Flesh(0.537 at conc. 100µg/ml) , as shown in Figure 4.2. The ferric reducing power of the extract at 100 µg/ml gave

0.398 µg/ml for seed, 0.537 µg/ml for flesh, 0.360 µg/ml for bark and 0.624 µg/ml for ascorbic at 100µg/ml extract and that of inhibition of lipid peroxidation (LPO) was 69.29 µg/ml for seed, 63.79 µg/ml for flesh, 76.20 µg/ml for bark and 82.81µg/ml for ascorbic at 100µg/ml extract respectively.

The methanol fractions of the bark, flesh, and seed of *Annona Muricata* were characterized using GC-MS, which revealed the presence of 134 phytochemicals.

To explore the detailed intermolecular interactions between the ligand and the target protein Human Anaplastic Lymphoma Kinase (ALK), an automated docking program, AutoDock Vina, packaged in PyRx 8.0, was employed. The three-dimensional structure of the target protein was obtained from the Protein Data Bank (PDB) entry 2XBA.

Receptor preparation involved removing water molecules not associated with active sites, regenerating the native state, and adding hydrogen atoms. Compounds extracted from *Annona Muricata*, identified through GC-MS analysis, were docked into the active site of ALK. The most accurate method for evaluating docking procedures is to assess how closely the lowest energy pose (binding conformation) aligns with the scoring function. Three parameters are typically considered: G-score, H-bond energy, and residual interaction. These parameters form the basis for discussing the ligand's binding affinity toward the receptor. A more negative standard free energy value indicates stronger binding affinity between the ligand and the receptor. Residual interaction indicates the specific amino acid to which the ligand binds within the protein[5].

Molecular docking analysis revealed that compounds from *Annona Muricata* exhibit varying levels of binding affinity for ALK, with the top four being Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy(TTD), Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-(ACED), 1,1,3-Tricyclohexylpropane and Cembrane (Table 4.3).

Despite the inhibitory potential against ALK exhibited by the *Annona Muricata* compounds, it is crucial to consider their ADMET properties. In silico ADMET prediction is a fast and low-cost approach to determine the compounds' therapeutic effectiveness[6]. The Lipinski filter, based on molecular weights, hydrogen bond acceptors and donors, and lipophilicity, was applied [7].

The inhibition of TBARS a measure of the oxidative stress was high suggesting that *Annona Muricata* is a good antioxidant source. Therefore, the radical scavenging assays in the cell-free systems for antioxidant studies are often considered by researchers before for their studies in cell lines and/or animal models[11]. The results of this study suggested that the free radical scavenging capacity of extracts could contribute either moderately or strongly to their antiproliferative activity, which is supported by the fact that antioxidants known as “free radical scavengers” act by preventing and repairing damage caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS), and thus can lower the risk of cancer[7,8]. Other authors have reported a positive linear relationship between antioxidant activity and anticancer effect of five herbal water extracts by comparing their percentage free radical scavenging capacity and percentage growth inhibition on A549 and MCF-7 cells[10].

These compounds demonstrate higher binding affinities than the standard ligand Ceritinib, which has a binding affinity of −7.7 kcal/mol. TTD achieved the highest docking score of −8.3

kcal/mol, followed by ACED with −7.7 kcal/mol, and Cembrane with −7.2 kcal/mol, while 1,1,3-Tricyclohexylpropane also exhibited a binding score of −7.2 kcal/mol (Figure 4.6).

Analysis of the 3D and 2D structures of the complexes formed by the three top-scoring compounds, TTD, ACED, Cembrane, and 1,1,3-Tricyclohexylpropane with ALK, reveals that the compounds occupy the enzyme's active site (Figs. 5, 6, and 7). Both the compounds and the standard ligand Ceritinib establish contacts with crucial active site amino acid residues. Table 4.4 shows the amino acid interactions between the ligands and the receptor site.

Compounds like TTD, ACED, Cembrane and 1,1,3-Tricyclohexylpropane with zero and one Lipinski violations, are likely to be orally active. This is supported by their oral bioavailability scores (Table 2). The top four compounds with a 0.55% bioavailability score have about a 55% probability of a minimum of 10% oral absorption in rat or human colon carcinoma absorptivity. Compounds with 2 or more Lipinski violations and lower bioavailability scores are less likely to be orally active. This is also reflected in the GI absorption potential of the compounds, which is high for the top four compounds that passed. Three of the top four compounds, show no blood-brain barrier (BBB) permeability. Furthermore, the inhibitory potentials of the four compounds on cytochrome P450 imply potential interactions with other drugs. This speculation arises from the fact that specific CYP isoforms are responsible for metabolizing over half of all medications. Inhibiting these isoforms, a common occurrence, may hinder the metabolism of other pharmaceuticals, leading to drug-drug interactions related to pharmacokinetics[9].

Toxicity predictions suggest favourable outcomes for the four compounds (Table 3). Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy (TTD) and 1,1,3-Tricyclohexylpropane fall

into oral toxicity classes 5 and 6, with LD50 values of 5000 and 15380 mg/kg, respectively. On the other hand, Cembrane and ACED belong to oral toxicity classes 3 and 4, with LD50 values of 750 and 325 mg/kg, respectively. These findings indicate safe usage within the specified dosage limits. None of the compounds exhibit hepatotoxic, carcinogenic, mutagenic, or cytotoxic tendencies, although ACED shows a probability of immunotoxicity. Further modifications may improve their safety as potential ALK inhibitors. Hence, these four *Annona Muricata* compounds merit consideration for experimental studies and subsequent drug development aimed at inhibiting ALK. It is important to note that this in silico investigation does not perfectly replicate cellular circumstances or the normal functioning of an entire organism. Therefore, the selected *Annona Muricata* compounds require further evaluation using in vitro and/or in vivo methodologies. In conclusion, among the 134 compounds screened for potential inhibitory activity against ALK, Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy (TTD), Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy- (ACED), Cembrane, and 1,1,3-Tricyclohexylpropane exhibited the highest binding affinity, respectively.

Endnotes

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Chapter Five

Conclusion

5.1 Summary of Findings

This study aimed to examine the nutritional bioactive compounds present in *Annona Muricata* (soursop) that can serve as functional foods potent in the prevention and management of cancer. The proximate composition of the fruit (flesh), seeds, and skin of Annona Muricata was analyzed. The results showed that the flesh is rich in carbohydrates, dietary fibers, and essential vitamins, while the seeds and skin contain significant amounts of proteins and lipids. The flesh of Annona Muricata stands out due to its high moisture (12.42%), protein (18.56%), and fatty acid content (8.84%), making it highly nutritious and beneficial for direct consumption. The seeds are particularly notable for their high fatty acid content (12.64%), which could be leveraged in oil extraction for nutritional supplements. The bark, with its high fibre (5.2%) and mineral content, could be used in health supplements aimed at improving digestion and providing essential minerals. The high crude protein and fatty acid content in the flesh and seeds suggest potential anti-cancer and anti-inflammatory properties, aligning with the study's goal of managing and preventing cancer. Methanol extracts of the seed and flesh of Annona Muricata were

characterized and screened for phytochemicals using Gas Chromatography-Mass Spectrometry (GC-MS).

The analysis identified numerous bioactive compounds, including Alkaloids, Tannins, Phlobatannins, Saponins, Phenols, Reducing Sugars, Steroids, Cardiac Glycosides, Terpenoids and Flavonoids. Saponins and reducing sugars were observed to be more abundant in composition than other bioactive compounds.

The antioxidant properties of *Annona Muricata* methanol extract of seed, flesh and bark were evaluated through various assays. The methanol extract exhibited strong antioxidant activities in all assays, indicating its potential in neutralizing free radicals and preventing oxidative stress-related diseases, including cancer. An *in-silico* study was conducted to assess the inhibitory properties of the bioactive compounds identified in the methanolic extract of *Annona Muricata* on the Human Anaplastic Lymphoma Kinase (ALK) enzyme. The molecular docking studies revealed that of the several compounds present in the extract, three compound analogs including Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy TTD which showed the strongest binding affinity (-8.3kcal) to the ALK enzyme, outperformed the reference drug ceritinib and suggesting their potential role in cancer inhibition and management.

5.2 Conclusion

The findings of this study highlight the significant presence of nutritional bioactive compounds in *Annona Muricata*, particularly in the flesh and seeds, which exhibit potent antioxidant and anticancer properties. The identified compounds, especially through in silico studies, show immense promise in the prevention and management of cancer.

5.3 Recommendation

The study aims to employ higher resolution analyses to identify some of the currently unidentified compounds, which may be absent in the existing library. Further chemical structure elucidation will be conducted on the most potent fractions isolated in this study, which will serve as lead compounds for in silico modeling and subsequent drug development. Additionally, in vitro investigations will be extended to other parts of *Annona muricata*—such as the fruits, seeds, bark, and roots—both individually and in combination, using various cell lines. Comprehensive studies will be undertaken to elucidate the molecular mechanisms of action of the plant extracts, alongside in vivo studies to explore their therapeutic potential.

5.4 Contribution to Knowledge

This study on the functionality and inhibition of bioactive compounds in *Annona Muricata* (soursop) extracts highlights key contributions to knowledge. Identified and characterization of a range of bioactive compounds in the methanolic extracts of *Annona Muricata*, particularly from the flesh and seeds. Using Gas Chromatography-Mass Spectrometry (GC-MS), this study has expanded the understanding of the phytochemical composition of soursop, revealing the presence of potent compounds such as acetogenins, alkaloids, flavonoids, and phenolic acids. By employing various assays (DPPH scavenging, Nitric Oxide assay, Ferric Reducing Antioxidant Power assay, and Lipid Peroxidation assay), this study has demonstrated the strong antioxidant properties of Annona Muricata extracts. These findings contribute to the growing body of evidence that supports the use of natural antioxidants in preventing oxidative stress-related diseases, including cancer.

The investigation of bioactive compounds in natural products has gained significant attention in recent years due to their potential therapeutic benefits. Annona muricata, commonly known as soursop, is a tropical fruit renowned for its diverse medicinal properties. This study focuses on the significance of bioactive compounds found in Annona muricata for cancer management.

Annona muricata is rich in antioxidants, which play a crucial role in neutralizing free radicals and reducing oxidative stress. Oxidative stress is a known contributor to cancer development, and antioxidants help in mitigating this risk by protecting cells from DNA damage. One of the most studied groups of bioactive compounds in Annona muricata is acetogenins. These compounds have shown promising anticancer properties, including the ability to inhibit the growth of cancer cells and induce apoptosis (programmed cell death). Acetogenins selectively target cancer cells without harming healthy cells, making them an attractive option for cancer therapy. In addition to acetogenins, Annona muricata contains flavonoids and alkaloids, which have been documented to exhibit antitumor activities. These compounds interfere with various cancer cell signaling pathways, inhibit metastasis, and enhance the efficacy of conventional chemotherapy. Bioactive compounds in Annona muricata can trigger apoptosis in cancer cells by activating intrinsic and extrinsic apoptotic pathways. This process involves the regulation of pro-apoptotic and anti-apoptotic proteins, leading to the death of cancer cells. These compounds have been shown to inhibit the proliferation of cancer cells by arresting the cell cycle at various phases. This halts the uncontrolled division of cancer cells, thereby reducing tumor growth. Chronic inflammation is a known risk factor for cancer. Annona muricata's bioactive compounds exhibit anti-inflammatory properties, reducing inflammation and potentially lowering the risk of cancer development and progression.

The in-silico study of the inhibitory properties of the identified bioactive compounds on the Human Anaplastic Lymphoma Kinase enzyme has provided insights into the potential mechanisms through which these compounds may exert anticancer effects. This computational approach adds a valuable dimension to natural product research by predicting interactions at the molecular level, guiding future in vitro and in vivo studies.

Overall, the research underscores the potential of Annona Muricata as a functional food with health-promoting properties. The comprehensive analysis of its nutritional and bioactive profiles supports its inclusion in diets aimed at disease prevention and management, particularly in cancer. The study's findings contribute to the broader field of natural product drug development. By identifying compounds with significant biological activities, this research lays the groundwork for the development of new, natural therapeutics derived from Annona Muricata.

The study showcases a robust methodological framework combining phytochemical analysis, antioxidant assays, and in silico modelling. This integrated approach can be applied to other natural products, enhancing the efficiency and comprehensiveness of natural product research.

5.5 Suggested Areas for Further Research

The following are suggested areas for further research.

1. Investigation of bioavailability and mechanism of action of the selective potent phytochemicals in the fruit
2. Processing the fruit extracts as nutritional supplements to individuals at risk of cancer.

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Appendix

A. PHYTOCHEMICAL SCREENING (QUANTITATIVE)

CODE	Alkaloid mg/100 g	Tannin mg/100 g	Phlobatannin mg/100 g	Saponin mg/100 g	Phenol mg/100 g	Reducing Sugar mg/100 g	Steroid mg/100 g	Cardiac Glycoside mg/100 g	Terpenoid mg/100 g	Flavonoid mg/100 g
Seed	32.57 32.68 32.56	30.87 30.68 30.78	22.45 22.5 22.56	35.5 35.48 35.46	24.68 24.32 24.38	50.14 50.98 50.12	43.88 43.9 43.88	45.78 45.68 45.72	30.36 30.24 30.32	41.25 41.34 41.34
Mean	32.603	30.777	22.503	35.480	24.460	50.413	43.887	45.727	30.307	41.310
Standard Deviation	0.067	0.095	0.055	0.020	0.193	0.491	0.012	0.050	0.061	0.052
Flesh	32.52 33.76	29.34 28.63	22.08 22.12	40.13 40.07	28.96 28.96	53.92 53.89	44.36 44.34	40.98 40.8	35.86 35.68	40.12 40.12

	33.76	28.64	22.18	40.07	28.98	53.92	44.36	40.82	35.7	40.1
Mean	33.347	28.870	22.127	40.090	28.967	53.910	44.353	40.867	35.747	40.113
Standard Deviation	0.716	0.407	0.050	0.035	0.012	0.017	0.012	0.099	0.099	0.012
Bark	42.44	38.43	31.04	33.8	32.46	40.6	36.62	38.08	30.7	34.21
	42.54	38.34	31.08	33.12	32.76	40.54	36.54	38.21	30.68	34.22
	42.45	38.34	31.12	33.16	32.76	41.65	36.42	38.22	30.64	34.21
Mean	42.477	38.370	31.080	33.360	32.660	40.930	36.527	38.170	30.673	34.213
Standard Deviation	0.055	0.052	0.040	0.382	0.173	0.624	0.101	0.078	0.031	0.006

B. ANTIOXIDANTS ASSAY

CODE	**FERRIC REDUCING ANTIOXIDANT POWER (FRAP)**			
	25µg/ml	50µg/ml	75µg/ml	100µg/ml
Seed	0.12	0.181	0.239	0.396
	0.122	0.182	0.238	0.398
	0.12	0.18	0.239	0.396
Mean	0.121	0.181	0.239	0.397
Standard Deviation	0.001	0.001	0.001	0.001
Flesh	0.147	0.236	0.308	0.537
	0.145	0.234	0.31	0.535
	0.144	0.235	0.312	0.535
Mean	0.145	0.235	0.310	0.536
Standard Deviation	0.002	0.001	0.002	0.001
Bark	0.102	0.192	0.319	0.358
	0.104	0.194	0.32	0.358
	0.102	0.192	0.322	0.36
Mean	0.103	0.193	0.320	0.359

Standard Deviation	0.001	0.001	0.002	0.001
ASCORBIC ACID	0.169 0.168	0.382 0.38	0.481 0.482	0.624 0.624
Mean	0.169	0.381	0.482	0.624
Standard Deviation	0.001	0.001	0.001	0.000

CODE	**DPPH SCAVENGING ACTIVITY (%INHIBITION)**			
	25µg/ml	50µg/ml	75µg/ml	100µg/ml
Seed	44.49 44.34 44.46	50.95 49.89 50.67	61.03 61.03 59.96	74.52 74.48 73.87
Mean	44.430	50.503	60.673	74.290
Standard Deviation	0.079	0.549	0.618	0.364
Flesh	48.1 47.98 47.96	37.07 36.98 36.92	53.61 53.61 53.56	70.91 70.56 70.85
Mean	48.013	36.990	53.593	70.773
Standard Deviation	0.076	0.075	0.029	0.187
Bark	43.16 42.67 42.85	56.08 55.85 55.87	66.16 66.2 66.18	80.23 80.26 80.18
Mean	42.893	55.933	66.180	80.223
Standard Deviation	0.248	0.127	0.020	0.040
ASCORBIC ACID	46.36 43.36	61.28 61.3	75.84 75.82	83.3 83.3
Mean	44.860	61.290	75.830	83.300
Standard Deviation	2.121	0.014	0.014	0.000

CODE	**LIPID PEROXIDATION SCAVENGING ACTIVITY (%INHIBITION)**			
	25µg/ml	50µg/ml	75µg/ml	100µg/ml
Seed	37.93 37.92 37.89	43.73 43.75 43.74	54.15 54.16 54.12	69.29 69.25 69.26
Mean	37.913	43.740	54.143	69.267
Standard Deviation	0.021	0.010	0.021	0.021
Flesh	41.97 41.95 41.95	27.8 27.85 27.64	45.43 45.44 44.86	63.79 64.01 63.84
Mean	41.957	27.763	45.243	63.880
Standard Deviation	0.012	0.110	0.332	0.115
Bark	36.44 36.42	49.62 49.65	60.19 60.23	76.16 76.2

	36.44	49.66	60.21	76.18
Mean	36.433	49.643	60.210	76.180
Standard Deviation	0.012	0.021	0.020	0.020
ASCORBIC ACID	45.33 45.33	56.88 56.9	75.55 75.56	82.79 82.81
Mean	30.224	37.934	50.377	55.207
Standard Deviation	0.000	0.014	0.007	0.014

CODE	**NITRIC OXIDE SCAVENGING ACTIVITY (%INHIBITION)**			
	25µg/ml	50µg/ml	75µg/ml	100µg/ml
Seed	35.85 34.68 35.78	41.51 41.48 41.53	50.94 50.94 50.96	61.79 61.8 61.76
Mean	35.437	41.507	50.947	61.783
Standard Deviation	0.656	0.025	0.012	0.021
Flesh	35.38 35.34 35.4	50 48.96 48.96	56.6 56.58 56.6	58.02 58 58
Mean	35.373	49.307	56.593	58.007
Standard Deviation	0.031	0.600	0.012	0.012
Bark	31.13 31.1 31.13	48.58 48.6 48.56	55.66 55.56 55.62	70.28 70.27 70.27
Mean	31.120	48.580	55.613	70.273
Standard Deviation	0.017	0.020	0.050	0.006
ASCORBIC ACID	45.33 45.31	56.88 56.88	75.55 75.54	85.4 85.42
Mean	45.320	56.880	75.545	85.410
Standard Deviation	0.014	0.000	0.007	0.014

C. **GCMS**

TOTAL ION CHROMATOGRAM - BARK

TOTAL ION CHROMATOGRAM – FLESH

```
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Operator   : NIMR
Acquired   : 14 Nov 2023  14:52     using AcqMethod scan.M
Instrument :    GCMSD
Sample Name: FLESH
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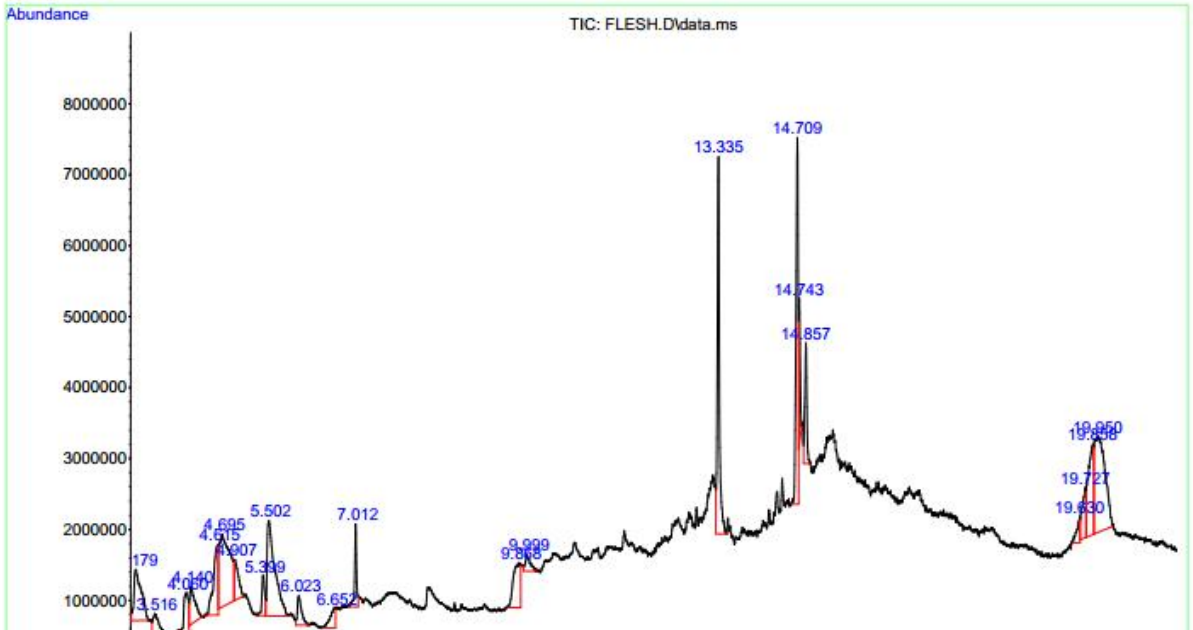

TOTAL ION CHROMATOGRAM – SEED

```
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Operator   : NIMR
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Instrument :    GCMSD
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Misc Info  :
Vial Number: 3
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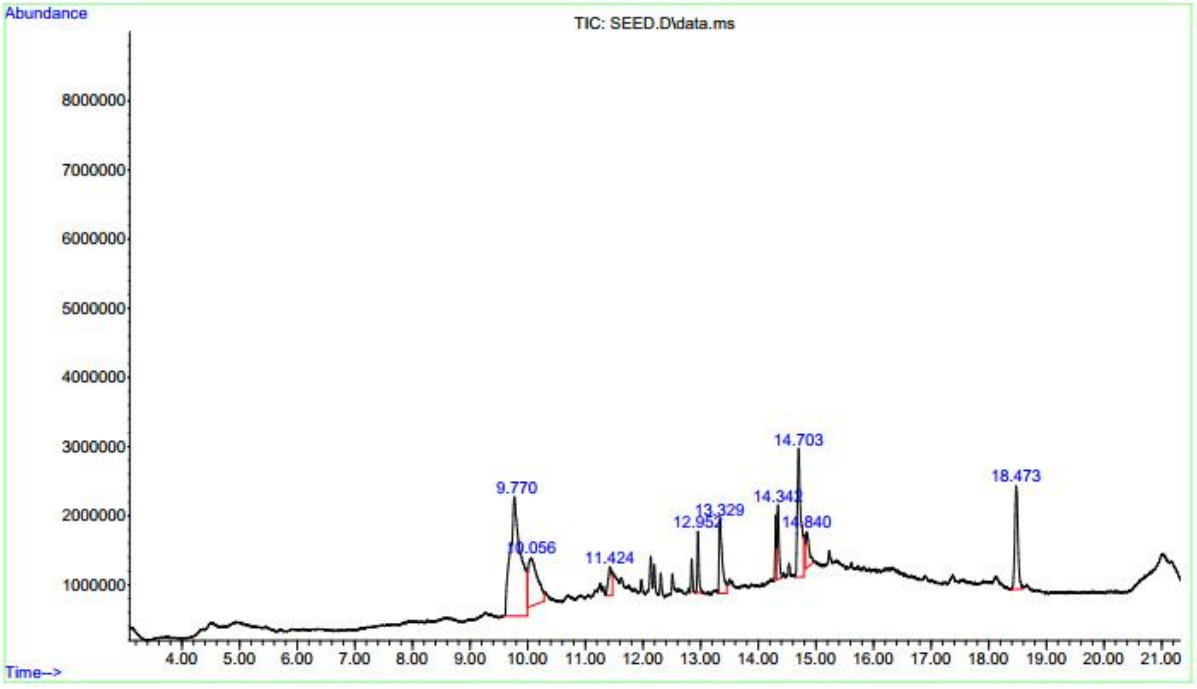

Bio-data

A. Personal Data

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B. Educational Background

Educational Institutions Attended with Dates and Qualifications

Lead City University Ibadan, Oyo **2023**
M.Sc. Nutrition and Dietetics (in-view)
University of Ibadan, Ibadan, Oyo **2014**
M.Sc. Human Nutrition
University of Nigeria, Nsukka, Enugu **2007**
B.Sc. Nutrition and Dietetics
The Federal Polytechnic Ede, Osun **2002**
HND (Diploma) Hotel and Catering
The Federal Polytechnic Ede, Osun **1999**
ND (Diploma) Hotel and Catering
Community Comprehensive High School, Ogori, Kogi **1991**
W.A.S.S.C.E, 9 Credits, O'Level

C. Work Experience with Dates

The Federal Polytechnic Ede, Osun State **2011 – Till date**

D. Awards and Fellowship Nil

E. Membership of Academic Professional Bodies

1. Institute of Dietetics in Nigeria (MIDN)
2. Nutrition Society of Nigeria (MNSN)

F. Publications

A.A. Adeola; A.A. Akindamola; G.D. Abata; O.O. Daniel; M.M. Mosimabale, *Proximate and Organoleptic Evaluation of Milk Blends Produced from Coconut (Cocos nucifera) and Tiger Nut (Cyperus exculentus L.)*, **International Journal of Sciences, Engineering & Environmental Technology** (IJOSEET), 2022, 7(5), 50-55p. ISSN 0794-9650.

J.O. Igbaro; O.F. Akinmoladun; G.D. Abata; A.O. Babalola; M.M. Mosimabale, *Breastfeeding Practices, Dietary Intake, and the Use of Contraceptives Among Some Selected Market Women in Osogbo, Osun State*, Nigeria, **International Journal of Bioinformatics and Biomedical Engineering**, 2021, Vol. 6, No. 1, 15-18p. ISSN 2381-7399 (Print); ISSN 2381-7402 (Online).

D.E. Enwerem; A.A. Akinyele; Y.O. Akande; G.D. Abata; A.O. Babalola; M.M. Mosimabale, *Prevalence of Overweight and Obesity among Market Women in Ede Osun State*, **AJMPCP**, X(X), xxx-xxx, 20YY, Article ID AJMPCP.53928.

O.O. Akinola; O.O. Oyinloye; D.E. Enwerem; I.G. Orji; M.M. Mosimabale; R.A. Mustapha, *Food hygiene practices among selected food vendors in Osun State, Nigeria*, **IOSR Journal of Nursing and Health Science**, 2018, Vol. 7(5), 80-83p.

G. Major Conferences Attended with Dates

Akinola O.O, [1]Oyinloye O.D, [2]Agbona A.A, [3]Oguntade O.I **[1]Mosimabale M.M** [1]Orji I.G., [1]Abimbola N.O **(2022)**. Paper presented at School Applied Sciences, Federal Polytechnic Ede. Titled 'The Awareness and Attitude of Consumers (Educated): Information on food product labels

Hammed I.A., [2]Akinola O.O., [3]Abata G. D., [4]Oladosu G.S., [5]Oguntade O.I., [6]Akinyemi A.O., **[7]Mosimabale M.M.** (2021). Prevalence of hypertension among adults aged 40years and above in rural and urban of Osun State, Nigeria. 51st Annual Conference Institute of Dietetics in Nigeria (IDN). Pg 55.

Akinola O.O., [2]Hammed I.A., [3]Oguntade O.I, [4]Oyinloye O.D., [5]**Mosimabale M.M.,** [6]Orji I.G., [7]Agbona A.A., [8]Enwerem D.E. (2021) Knowledge and attitude of elite on food labels in Osun State, Nigeria. 51st Annual Conference of Institute of Dietetics in Nigeria (IDN). Pg 36

H. References

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The University Compliance Certification

This is to certify that this Thesis was written by **Margaret Meka MOSIMABALE** with Matric Number **LCU/PG/003550** of the Department of Human Nutrition and Dietetics, Faculty of Public Health, Lead City University, Ibadan and it is in full compliance with the approved University format and style.

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Signature **Date**