INSILICO IDENTIFICATIONOF BIOACTIVE COMPOUNDS IN ETHANOLIC OKRA

BY

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CERTIFICATION

This is to certify that this project work was carried out by OBAFEMI WINNER JOSHUA, ADEGBOYE ABDULMUEES AREMU, ADENIYI ROFIAT ADETOMIWA, SOLIU KAOSARA OMOTOYOSI, OLOKOOBI KABIRAT KEHINDE with HND/23/SLT/FT/0294, HND/23/SLT/FT/0833, HND/23/SLT/FT/0890, HND/23/SLT/FT/0965, HND/23/SLT/FT/0966 in Science Laboratory Technology Department of Kwara State Polytechnic. It has been read and approved as meeting part of the requirements in partial fulfillment for the Award of Higher National Diploma (HND) in Science Laboratory Technology (Biochemistry Unit), Institute of Applied Sciences (IAS), Kwara State Polytechnic, Ilorin.

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DEDICATION

This report is dedicated to Almighty God, the beginning and last who has given me this great opportunity to cover this programme.

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ABSTRACT

The increasing demand for natural therapeutic agents has intensified the search for bioactive compounds in medicinal plants using computational methods. This study aimed to identify potential bioactive compounds present in the ethanolic extract of Abelmoschus esculentus (okra) through in silico analysis. Phytochemical screening was conducted using relevant literature and databases to determine the major compounds commonly found in ethanolic okra extracts. The selected compounds were subjected to molecular docking studies against key biological targets associated with antioxidant, anti-inflammatory, and antidiabetic activities. Drug-likeness and pharmacokinetic properties were assessed using ADMET prediction tools to evaluate their suitability as drug candidates. The results revealed that compounds such as quercetin, catechin, and rutin exhibited strong binding affinities with target proteins, suggesting potential biological activity. Additionally, these compounds met Lipinski's rule of five, indicating favorable oral bioavailability. This study demonstrates that in silico screening is a reliable and cost-effective approach for the preliminary identification of therapeutic compounds in okra, thereby supporting its potential application in drug development and nutraceutical formulation.

CHAPTER ONE

1.0 INTRODUCTION

1.1 INTRODUCTION TO IN SILICO IDENTIFICATION OF BIOACTIVE COMPOUNDS IN ETHANOLIC OKRA

Inflammation is a helpful recovery process that cells utilize to stop the progression of harm or injury to tissues caused by foreign invaders and start the healing process. It's a complicated process involving white blood cells, macrophages, and inflammatory cytokines such as prostaglandins, TNF- (Tumor Necrosis Factor), interleukin IL-6, and IL-8, to mention a few. The mobilization of arachidonic acid for prostaglandin production is a hallmark of inflammation. Cyclooxygenases such as COX-1 and COX-2 enzymes convert arachidonic acid to prostaglandins. COX-1 is required for the body's homeostatic activities, such as platelet synthesis for blood, kidney development and function, gastric mucosa maintenance, and so on. Increased inflammation, angiogenesis, metastatic and proliferative invasion, decreased apoptosis, and the establishment of an immunosuppressive microenvironment are all linked to COX-2- derived prostaglandin PGE2 (Durazzo *et al.,* 2019). Non-steroidal anti-inflammatory medications (NSAIDs) work as COX inhibitors and are a useful tool for treating inflammation, but they have drawbacks and adverse effects. Hence, organic COX-2 inhibitors should be investigated. Deregulation of the inflammatory response is an issue, and chronic inflammation has been linked to cancer, diabetes, Alzheimer's and other diseases (Durazzo *et al.,* 2019).

Inflammation regulation is a complicated process that can be simplified by focusing on the causes of inflammation. COX-II and NF-κB are two genes that have been linked to cancer, rheumatoid arthritis (RA), inflammatory bowel disease (IBD),

multiple sclerosis, atherosclerosis, systemic lupus erythematosus, type I diabetes, chronic obstructive pulmonary disease, and asthma (Islam, 2019). NSAIDS such as ibuprofen, a currently available therapeutic agent, have several drawbacks, including ulcerative perforations of the stomach lining (Gemede *et al.,* 2015), Severe stomach cramps, and hepatotoxicity (Zhu *et al.,* 2020). Targeting inflammationis currently a therapeutic means to cure ailments. The use of topical corticosteroids, hydrocortisone, and prednisone to treat eczema is a good example (Al-Shawi *et al.,* 2021). Tumor necrosis factor (TNF) inhibitors are also commonly used in the treatment of rheumatoid arthritis (Barcellos and Lionello, 2011). As a result, safer and more natural ways of reducing inflammation are required. Abelmoschus esculentus L. (Moench), commonly known as okra, is a flowering plant belonging to the Malvaceae family which produces tasty green pods with a slimy inside filled with seeds arranged unevenly and is native to Africa's tropics (Bonciu, 2020). A. esculentus has been demonstrated to contain a wide range of photochemical and nutritional value, which explains its widespread use in traditional medicine. The seeds and pods are high in minerals and vitamins, all of which contribute to the health advantages (Nilesh Jain, 2012). Syphilis is treated with an infusion made from the roots (Nilesh Jain, 2012). In Nigeria, the root juice is applied externally to cure cuts, wounds, and boils.8 Catarrhal infections, dysuria, and gonorrhoea are all treated with it (Lowry *et al.,* 2015). The roasted seed infusion has sudorific effects (Lowry *et al.,* 2015). A. esculentus is used to treat dysentery, catarrhal infections, plasma replacement, and gonorrhea. spermatorrhoea, bronchitis, pneumonia, diarrhoea, acute inflammation and irritation of the stomach and intestines (Roy *et al.,* 2014). Several studies on the antioxidant activity of various portions of the plant have been undertaken (Roy *et al.,* 2014). In vitro antioxidant assay of methanol extract of okra fruits was reported by Atawodi

and colleagues (Jain *et al.,* 2012). They used the xanthine oxidase and 2-deoxyguanosine techniques to demonstrate antioxidant and radical scavenging activity, with 50 percent inhibitory concentration values of 25 and 43 mg/ml. Khomsug and his colleagues discovered that procycanidin B2 was the most common phenolic compound. Procycanidin B2, epicatechin, and rutin have been found in pulped seeds (Gemede *et al.,* 2015). Pre-treatment (soaking and blanching) boosted nutritional composition but decreased antioxidant activity, whereas roasting (1600 °C for 10-60 minutes) increased nutrient composition but decreased antioxidant activity (Petropoulos *et al.,* 2018). Ansari and colleagues found okra extract to be a non-enzymatic inhibitor of lipid peroxidation in liposomes (Ying *et al.,* 2014). Total phenolics, total flavonoids, and antioxidant activity of different parts (flower, fruit, leaf, and seed) and different enrichment fractions of water extracts of the A. esculentus plant were compared by Liao and co-workers (Andras *et al.,* 2015). They found total phenolics and total flavonoids, both of which are antioxidants, in all of the plant extracts, though the percentages differed. The maximum levels of total phenolics and total flavonoids were identified in the okra leaves (Sabitha *et al.,* 2010). Molecular docking, an in silico method, is extremely beneficial since it lowers the cost of wet-lab research, saves animals, time, and resources, and properly guides medication selection and production. The goal of molecular docking simulation is to anticipate a ligand's binding affinity with a protein and the most stable complex; the lower or more negative the binding affinity, the better. Molecular docking simulation has simplified and verified in vivo and in vitro studies, as well as drug modeling and design for pharmaceutical researchers (Khomsug *et al.,* 2010). Drug-likeness is a qualitative assessment of a molecule's potential as an oral drug in terms of bioavailability. It was determined through structural or physicochemical inspections

of research compounds that they had progressed enough to be deemed oral drug candidates, using five separate rule-based filters with varying ranges of attributes within which the molecule is defined as drug-like. These filters include; Lipinski set of 5, Ghose, Veber, Egan, and Muegge methods. Several researchers have used chromatographic methods to uncover phytocompounds in plants (Khomsug *et al.*, 2010). Only a few A. esculentus pod reports have been documented. The structural formula of bioactive chemicals found in A. esculentus pods has not been adequately characterized by HPLC and molecular docking studies of its bioactive phytocompounds. To the best of our knowledge, this is the first study of A. esculentus pod compounds using HPLC analysis and in silico molecular docking methods. The work aims to use HPLC and molecular docking to uncover possible anti-inflammatory inhibitors in A. esculentus pods.

Spices and herbs have been an essential part in human life for centuries both for culinary and medicinal purposes, they have been used at a domestic and industrial level as flavoring, preservation, and coloring agent in nutraceutical, pharmaceutical, and cosmetics products (Doreddula *et al.*, 2014). Spices not only enhance the flavor, aroma, and color of food and beverages, butthey can also give protection from acute and chronic diseases. More Americans are considering the use of spices and herbs for medicinal and therapeutic/remedy use, especially for various chronic conditions. There is now ample evidence that spices and herbs possess antioxidant, anti-inflammatory, antitumorigenic, anticarcinogenic, and glucose and cholesterol lowering activities as well as properties that affect cognition and mood (Al-Shawi *et al.*, 2020).

Research over the past decade has reported on the diverse range of health properties possessed by herbs and spices via their bioactive constituents, including

sulfur-containing compounds, tannins, alkaloids, phenolic diterpenoids, and vitamins, especially flavonoids and polyphenols. Spices and herbs such as clove, rosemary, sage, oregano, and cinnamon are excellent sources of antioxidants with their high content of phenolic compounds (Barcellos and Lionello, 2011). It is evident that frequent consumption of spicy foods was also linked to a lower risk of death from cancer and ischemic heart and respiratory system diseases (Bonciu, 2020). Among these spices is the Country Onions, scientifically known asAfrostyrax lepidophyllus from Huaceae family, a culinary treasure from the vibrant rainforests of Central and West Africa which is perfect for adding depth and richness to a variety of dishes.

This onion stands out with its unique garlic-like flavor profile, infusing a distinctive pungency into traditional dishes. It is oval in shape with a brown bark, the seed is widely used as a flavoring ingredient in many traditional dishes because of its strong and pungent aroma. Country Onion is a bulbous plant that belongs to the Alliumfamily, which also includes garlic, onions, and chives. It is native to Africa where it is an important traditional food source for local communities, Country onions is a flavoural and nutritive ingredient, widely used in different parts of Africa, including Nigeria, Cameroon and, Ghana for centuries as a staple ingredient in their cuisine. People love this charming spice for their unique flavors and aroma, which resemble garlic more than traditional onions. But what truly sparks curiosity is their hidden health benefits (Nilesh Jain, 2012). They have a long history as cherished ingredients in African cuisines and, beyond their culinary charm, offer notable health benefits. Country onions has been reported as a rich source of antioxidants containing compounds like allicin and alliin, which have been shown to have antibacterial, antiviral, and antifungal properties.

Studies have also suggested that Country Onion can help lower blood pressure and cholesterol, reduce inflammation, boost immune system, possessed anticancer properties and improve cardiovascular health. It is available in Cameroon, Gabon,Nigeria and Ghana. Country onions is currently listed by the International Union for Conservation of Nature as “vulnerable”, giving it high conservation value (Lowry *et al.,* 2015). Afrostyrax lepidophyllusis also known as Olum or Bombimbi. This work is aimed at identifying the bioactive component of this treasured spice. Insilico methods was used to study the anti-inflammatory potentials of the plant phytocompounds and other commercial drug to ascertain its efficacy as acclaimed ethnomedically and by previous researchers.

Recently, the world was shaken by an acute respiratory disease caused by the coronavirus or SARS-CoV-2. Coronavirus, which belongs to the Coronaviridae family, can infect the respiratory system in humans and animals. The SARS-CoV-2 genome is 96.2% identical to the bat CoV RaTG13, while 79.5% is identical to SARS-CoV (Khanal *et al.,* 2021; Kumar *et al.,* 2020; Peretto *et al.,* 2020). Based on the viral genome sequencing and evolutionary analysis results, bats are the original host of the virus, and SARS-CoV-2 from bats infects humans (Chen *et al.,* 2020). Tang et al. (2020) analyzed the COVID-19 genotype in different patients from several provinces and found that SARS-CoV-2 had mutations in different patients in China. They carried out a population genetic analysis of 103 SARS-CoV-2 genomes and classified two types of SARS- CoV-2 progression: L type (≈70%) and S type (≈30%). Strains in type L, derived from type S, are evolutionary, more aggressive, and infectious. However, the level of difference in SARS-CoV-2 is smaller than the H7N9 avian influenza mutation (Wu *et al.,* 2015).

The SARS-CoV cell receptor is found in the respiratory tract, a pathogenic replica of the coronavirus Angiotensin Converting Enzyme (ACE2), and regulates species transmission and human-to-human transmission. The S-glycoprotein on the surface of the coronavirus can attach to the receptor, ACE2, on the surface of human cells. The S-glycoprotein includes two subunits: S1 and S2. S1 determines the virus-host range and cellular tropism with the main functional domain, Reseptor Binding Domain (RBD), whereas S2 mediates cell-virus membrane fusion with two main gates, heptad repeats 1 (Chen *et al.,* 2009, 2020; Lung *et al.,* 2020).

Currently, considerable research on potential antiviral and drug candidates is being conducted. One of the screenings of the chemical structure that has the potential as an active RNA-dependent RNA polymerase (RdRp) inhibitor in SARS-CoV-2 includes theaflavin compounds (ZINC3978446) tea leaves. Lung et al. (2020), regarding a molecular docking study on the RdRp of SARS-CoV-2, SARS-CoV, and mer-CoV, showed that theaflavins had a relatively lower iDock score than the RdRp of SARS-CoV-2, namely −9.11 kcal/mol, with a low binding energy of −8.8 kcal/mol. The hydrophobic interactions contributed significantly to the additional hydrogen bonding and bonding between theaflavins and RdRp and indicated that one of the π cation interactions was formed between theaflavins and Arg553 (Lung *et al.,* 2020). CoV-matched drugs included ACE2 receptor entry, major protease (Mpro), and RdRp (Kumar *et al.,* 2020). However, targeted drugs show significant side effects and lower potency. Thus far, Mpro is one of the best characterized and most promising drug targets in CoVs. Mpro can process polyproteins from viral RNA transcription. In addition, target Mpro cleaves up to 11 moieties on protein replication (1ab, ≈790 kDa), and Mpro inhibition basically blocks virus replication (Bhardwaj *et al.,* 2020; Kumar *et al.,* 2020).

Tea leaf (Camellia sinensis) has many benefits because it has a high antioxidant content. Currently, several studies are being conducted and have confirmed that the content of tea is more active for anti-SARS-CoV-2 therapy treatment than remdesivir and chloroquine. One of the screenings of the chemical structure that has the potential as an active RdRp inhibitor in SARS-CoV-2 is bioactive compounds, such as oolonghomobisflavan-A, theaflavin- 3-O-gallate, and theaflavin (TF) (Bhardwaj *et al.,* 2020). These bioactive compounds are components of catechin oxidation, which contribute color, taste, and aroma to black tea. Enzymatic oxidation events in black tea processing have started at the beginning of the mill. This event is a process of oxidation of catechin compounds with the help of polyphenol oxidase enzymes. Oxidation of catechin compounds, especially epigalocatechins and their errors, will produce quinones that will further condense into bisflavanols, theaflavins, oolonghomobisflavan-A, and theaflavin-3-O-gallates.

The condensation and polymerization processes proceed to form insoluble substances (Bhardwaj *et al.,* 2020; Chandini *et al.,* 2013; Lung *et al.,* 2020). Many bioactive molecules include a polymerized polyphenol from the tea plant (C. sinensis L.) that acts as an effective SARS-CoV-2 Mpro inhibitor (Bhardwaj *et al.,* 2020). According to Kanbarkar and Misha (2020), the bioactive compound theaflavin is similar to SARS-CoV-2 drugs and has no side effects. Therefore, this study aimed to analyze in silico and find the value of molecular docking simulations for the bioactive compounds oolonghomobisflavan-A, theaflavin-3-O-gallate, and theaflavin (TF). It also aimed to find the levels of selected compounds for analysis using a spectrophotometer and simulating the response surface methodology (RSM).

1.2 Aim of the Study

The aim of this study is to identify and evaluate "In Silico Identification of Bioactive Compounds in Ethanolic Okra (Abelmoschus esculentus)".

1.2.1 Specific Objectives

- To extract Okra with ethanol
- To carry out phytochemical screening on the extract
- To carryout antioxidant screening
- To identify the bioactive compounds in the extract using HPLC
- To perform molecular docking on the identification bioactive compounds

1.3 Scope of the Study

This study focuses on the *in silico* (computer-aided) identification and analysis of bioactive compounds present in ethanolic extracts of okra (Abelmoschus esculentus). It aims to identify potential therapeutic compounds using computational approaches, particularly molecular docking, ADMET profiling, and drug-likeness prediction tools. The study covers the following specific areas:

1. **Phytochemical Selection**: Identification and selection of known bioactive phytochemicals reported in ethanolic extracts of okra based on literature review and phytochemical databases.
2. **Ligand Preparation**: Structural retrieval and optimization of selected compounds from chemical databases such as PubChem, ChEMBL, or ZINC.
3. **Target Protein Selection**: Identification of relevant disease-related protein targets (e.g., enzymes, receptors) for docking, particularly those linked to antioxidant, antidiabetic, antimicrobial, or anti-inflammatory activities.
4. **Molecular Docking Analysis**: Use of molecular docking tools (e.g., AutoDock Vina, PyRx, or SwissDock) to simulate interactions between the

selected okra compounds and target proteins, predicting binding affinity and possible modes of interaction.

5. **Pharmacokinetics and Drug-Likeness Evaluation**: In silico evaluation of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles and Lipinski's Rule of Five to assess the drug-likeness of the identified compounds.
6. **Data Interpretation**: Analysis and interpretation of docking scores, interaction patterns, and pharmacokinetic data to determine potential bioactive candidates for further in vitro or in vivo validation.

1.4 Justification of the Study

The search for novel, effective, and safe therapeutic agents from natural sources has gained increasing global attention, especially in the context of rising drug resistance, chronic diseases, and adverse drug reactions. Okra (*Abelmoschus esculentus*), a widely consumed vegetable in many tropical and subtropical regions, is known to be rich in various bioactive compounds such as flavonoids, phenolic acids, polysaccharides, and alkaloids. These compounds have been associated with significant antioxidant, antidiabetic, antimicrobial, and anti-inflammatory properties.

However, the process of isolating and experimentally validating each compound is often costly, time-consuming, and resource-intensive. This creates the need for in silico (computational) approaches that allow rapid and accurate prediction of bioactivity, drug-likeness, and interaction potential of natural compounds against biological targets. Using molecular docking and ADMET profiling tools enables researchers to screen and prioritize the most promising compounds for further laboratory validation.

This study is therefore justified on the following grounds:

1. **Scientific Relevance**: It contributes to the identification of natural therapeutic candidates from okra, supporting its ethnomedicinal relevance and offering a foundation for drug development research.
2. **Innovation and Efficiency**: It applies modern in silico techniques to speed up the drug discovery process, making it less expensive and more efficient compared to traditional screening methods.
3. **Public Health Impact**: The findings may lead to the discovery of plant-based compounds with potential to treat diseases such as diabetes, infections, and inflammation, which are of major concern in both developed and developing countries.
4. **Support for Future Experimental Work**: By narrowing down potential lead compounds, this study helps reduce trial-and-error in experimental research, guiding future in vitro and in vivo investigations on ethanolic okra extracts.

CHAPTER TWO

LITERATURE REVIEW

2.1 INSILICO IDENTIFICATION OF BIOACTIVE COMPOUNDS IN ETHANOLIC OKRA

The plant Abelmoschus esculentus L. is commonly referred to as lady finger or okra. It is native to Africa, a member of the Malvaceae family, and is grown in Africa, Asia, Southern Europe, and America in tropical, subtropical, and warm temperate temperatures.1-2 Okra is a crop with significant economic significance in the world's subtropical regions. It originated in Ethiopia and has since spread to North Africa, the Mediterranean, Arabia, and India. Okra is a food that can be added to soups, salads, and stews and consumed either raw or cooked. Okra fruit is high in moisture, nutrient-dense, and a fantastic source of vitamins and minerals.3 Okra contains carbohydrates primarily in the form of mucilage, which is used in many industrial sectors and for therapeutic purposes.

The fruits, seeds, and leaves of okra have applications due to its composition and qualities. Numerous research have examined the bioactive potential of okra mucilage and its rheological properties; these findings are documented in current bibliographic surveys. Still, it is important to emphasize the value of okra as a low-cost functional food. Functional foods are a significant area for innovation since they aim to improve consumers' physical and mental health in addition to preventing nutrition-related diseases and satisfying hunger and giving humans the nutrients they need. Vegetables are the richest sources of chemicals with positive health effects because they are high in polyphenols, which are an extremely effective form of antioxidants (Zhu *et al.*, 2020).

In this regard, the purpose of this paper is to emphasize the advantages of this fruit as an inexpensive raw material and a potential natural substitute in a number of contexts, such as its application as a functional food. Of particular interest is the use of okra mucilage as a technological resource with intriguing properties for industrial application.

2.1.1 Plant Description:

Biological name: Hibiscus esculentus, Abelmoschus esculentus.

Scientific Classification:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

(Unranked): Rosids

Order: Malvales

Genus: Abelmoschus

Species: A.Esculentus

Binomial name: Abelmoschus esculentus

Other Names: Kacang Bendi, qiu kui, Okra, okura, Okro, Quiabos, Ochro, Quiabo, Gumbo, Quimgombo, Bamieh, Bamya,

Quingumbo, Bamia, Ladies Fingers, Bendi, , Bhindi, Kopi Arab (Nilesh, 2012).

Whole plant

Flower

2.2 BOTANICAL DESCRIPTION AND GEOGRAPHIC DISTRIBUTION

It is predicted that 6 million t of fresh fruit and vegetables are produced worldwide each year from okra (both species). 95% of this amount is composed of common okra. Common okra and West African okra are only used in West and Central Africa, which makes for around 10% of global production. About fifty percent of the market is shared by them. Abelmoschus esculentus (typically 2n = 130) is probably an amphidiploids (allotetraploid), developed from Abelmoschus tuberculatus Pal & H.B.Singh (2n = 58), a wild species from India, and a species with 2n = 72 chromosomes (perhaps Abelmoschus ficulneus (L.). Abelmoschus caillei Stevels, another tasty okra species, grows in humid regions of West and Central Africa. Abelmoschus esculentus is one of the parental species of Abelmoschus caillei, and there are strong evidence that this species is also amphidiploid. The common and West African okra are frequently combined because there don't seem to be any functional distinctions between them (Lowry *et al.,* 2015). Abelmoschus caillei and Abelmoschus esculentus differ morphologically in a number of ways, but the epicalyx provides the most useful distinguishing feature: the epicalyx segments' widths are 4–13 mm in Abelmoschus caillei and 0.5–3 mm in Abelmoschus esculentus. The fruit

morphology of the two okra species can be used to distinguish them quite accurately, though not 100%. The most dangerous okra fungal diseases in Africa are powdery mildew (Erysiphe cichoracearum, Oidium abelmoschi), vascular wilt (Fusarium oxysporum), Cercospora blight (Cercospora Abelmoschus, Cercospora malayensis), and damping-off (Macrophomina phaseolina, Pythium aphanidermatum, and Rhizoctonia solani). Okra mosaic virus (OkMV), transmitted by flea beetles (Podagrica), is ubiquitous in Africa although damage is far less important than that caused by okra leaf curl disease (OLCV), transmitted by whitefly. Yellow vein mosaic virus (BYVMV), a major contributor to crop loss in Asia, is also carried by whiteflies. The vectors are the only thing that can be used to control these viruses. A significant issue is the genus Meloidogyne nematodes. Crop rotation, such as using cereals, and heavy applications of organic manure can prevent nematode damage. Important pests are fruit and stem borers (Earias spp. and Heliothis spp., Pectinophora gossypiella), flea beetles (Podagrica spp.) and jassids (Empoasca spp.). Because crops are harvested often, chemical control is risky. Common okra is in general more seriously affected by diseases and pests than West African okra (Miller, 2015).

2.3 BIOACTIVE COMPONENTS OF ABELMOSCHUS ESCULENTUS.

The profile of the bioactive components in different parts of okra is well documented: for okra pod polyphenolic compounds, carotene, folic acid, thiamine, riboflavin, niacin, vitamin C, oxalic acid, and amino acids ; for okra seed polyphenolic compounds, like oligomeric catechins and flavonol derivatives, protein and oil fraction. For roots, mostly minerals, tannins, and flavonol glycosides; for leaves, primarily carbohydrates and flavonol glycosides. the existence in varying percentages of the total phenolics and total flavonoids and antioxidant qualities in

different portion of plant i.e., leaf, flower, fruit, and seed (Roy *et al.*, 2014). Numerous constituents of okra, including as flavonoids, polysaccharides, and vitamins, have demonstrated noteworthy biological activity. Understanding the biological activities and advantageous qualities of bioactive components begins with evaluating their interactions during the measurement of antioxidant properties. The following can be used to identify the current primary methodologies used in studies of bioactivities interactions in food matrices:

(1) Development of the model system of interactions.

(2) Separating substances that can be extracted from those that cannot

(3) Research on extracts rich in physiologically active chemicals.

Screening revealed the presence of Tannins, Steroids, Flavonoids, Saponins, Alkaloids, Anthraquinones, Phenol, Terpenoids, Cardiac Glycosides and Cardenoids.Methods of extraction and screening for bioactive substances As alternatives to traditional extraction procedures, a number of cutting-edge approaches have been created that offer benefits in terms of reproducibility, extraction yields, solvent consumption, and extraction time.

Screening and extraction techniques for bioactive compounds

1. Ying et al. (2014) reported the successful application of an ethyl acetate–n-butanol gradient solvent system consisting of n-hexane– ethyl acetate–n-butanol–water for the screening of antiproliferative drugs against cancer cells in okra. Elution stages could be used to separate components with significant polarity differences. The antiproliferative activity of the fractions obtained from High-Speed Counter-Current Chromatography separation using the gradient solvent system was evaluated. Two bioactive components were

found: 4-hydroxy phenethyl trans-ferulate, a large anticancer molecule with intermediate activity, and carolignan, a minor anticancer compound with strong action. Supercritical fluid extraction of okra seeds was carried out at a pilot scale using carbon dioxide as solvent, at temperatures of 40, 50, and 60°C and pressures of 150, 300, and 450 bar. The yields from supercritical fluid extraction and Soxhlet extractions in n-hexane were similar. The fatty acid profiles of the extracts revealed a high unsaturated/saturated ratio (Ying *et al.,* 2014).

2. (András *et al.,* 2005). The concentration of biologically active chemicals (β-sitosterol, sitosterol esters, and tocopherols) from okra seeds was found to be feasible using SFE with carbon dioxide. SFE yields of oil and sterols were generally quite comparable to those produced with n-hexane as the solvent; however, SFE requires a lower extraction temperature. The response surface approach was used to establish the optimal process parameters, which were P = 450bar and T = 60°C. However, it is advised to utilize the intermediate temperature, T = 50°C, to prevent the potential of thermal deterioration of valuable components, as the yield drop in this case is negligible (Ying *et al.,* 2014).
3. SFE with carbon dioxide was found to be m nnnna suitable technique for concentration of biologically active compounds (β-sitosterol, sitosterol esters, and tocopherols) from okra seeds. The overall yields of oil and sterols by SFE were very similar to those obtained using n-hexane as solvent, but the extraction temperature in the case of SFE is lower. The best process parameters were determined with the response surface method as P = 450bar

and T = 60°C, but, to avoid the possibility of thermal degradation of valuable components, it is recommended to use the intermediate temperature T = 50°C, because the yield decrease in this case is unimportant (András *et al.*, 2015).

Table 1. The proximate value per 100g edible portion of okra

Item	Quantity
Water	90.17 g
Energy	31 kcal(129 kJ)
Protein	2.00 g
Total lipid	0.10 g
Ash	0.70 g
Carbohydrate	7.03g
Total dietary fibre	3.2 g
Total sugars	1.2 g
Sucrose	0.40 g
Glucose	0.13 g
Fructose	0.21 g
Starch	0.34 g

Minerals

Ca	81 mg
Fe	0.8 mg
Mg	57 mg
P	63 mg
K	303 mg
Na	8 g
Zn	0.60 mg
Cu	0.094 mg
Mn	0.990 mg
Se	0.7 m g

Lipid

Total Saturated fatty acids	0.026 g
Palmitic acid(16:0)	0.022 g
Stearic acid(18:0)	0.003 g
Total mono-unsaturated fatty acids	0.017 g
Oleic acid(18:1)	0.016 g
Total polyunsaturated fatty acids	0.027 g
Undifferentiated (linoleic acid; 18:2)	0.026 g
Undifferentiated (linolenic acid; 18:3)	0.001 g
Phytosterols	24 mg

Amino acids

Tryptophan	0.017 g
Threonine	0.065 g
Isoleucine	0.069 g
leucine	0.105 g
lysine	0.081 g
methionine	0.021 g
Cystine	0.019 g
Phenylalanine acid	0.065 g
Tyrosine	0.087 g
Valine	0.091 g
Histidine	0.031 g
alanine	0.073 g
Aspartic	0.145 g
Glutamic acid	0.271 g
Glycine	0.044 g
Proline	0.045 g
Serine	0.044 g
Lutein + zeaxanthin	516 m g
Arginine	0.084 g

2.4 VALIDATED PHARMACOLOGICAL ACTIVITIES OF THE OKRA

2.4.1 Antidiabetic and hyperlipedemic activity

Rats with streptozotocin-induced diabetes were used to test the antidiabetic and antihyperlipidemic properties of A. esculentus peel and seed powder (AEPP and AESP). In an acute toxicity study, AEPP and AESP showed no toxicity or fatality up to a dose of 2 g kg−1. Thus, in order to assess the antidiabetic effect, one by fifth and one by tenth doses of both powders were selected. Glibenclamide (5 mg kg−1) was taken orally as the usual medication. In comparison to diabetic control rats (306 mg kg−1), blood glucose levels significantly decreased in diabetic rats given oral doses of AEPP and AESP (89.50–109.67 mg kg−1). Following the treatment, there was also a notable rise in the levels of total protein and hemoglobin, as well as a drop in the levels of glycosylated hemoglobin and serum glutamate-pyruvate transferase. It's interesting to note that after receiving AEPP and AESP, the raised lipid profile levels in diabetic rats returned to normal (Sahitha *et al.,* 2011).

The in vitro antidiabetic activity of A. esculentus was examined through detection of α-glucosidase and α-amylase inhibitory activity of the aqueous extracts of A. esculentus peel (AAPP) and seed (AASP). The hypoglycemic effect of A. esculentus aqueous extracts was supported by the significant concentration-dependent inhibition of α-glucosidase (IC50 = 142.69 ± 0.32 g mL−1 and 150.47 ± 0.28 g mL−1) and α-amylase (IC50 = 132.63 ± 0.16 g mL−1 and 147.23 ± 0.21) g mL−1 by the AAPP and AASP. Fan et al. have reported that A. esculentus pods exhibit hypolipidemic and hypoglycemic properties. Six female mice were used in the in vivo experiment, and they were fed a high-fat diet for six weeks. Measurements were made of blood glucose, total cholesterol, high-density lipoprotein (LDL), low-density

lipoprotein (LDL), and serum triglycerides. The findings showed that although the total cholesterol level has not altered, the blood glucose and triglycerides have returned to normal levels (Saha and Jain, 2011).

2.4.2 Antioxidant Activity

By using DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging and ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization tests, the antioxidant activity of A. esculentus seeds and pulp was assessed. Methanol extraction produced 1.97 and 3.53% of the pulp and seeds, respectively. In both techniques, the extracts demonstrated a strong antioxidant activity with a scavenging activity more than 50%. Using the DPPH technique, the IC50 values for pulp and seeds were determined to be 55.73 mg mL−1 and 44.1 mg mL−1, respectively. In the meantime, the IC50 values for pulp and seeds in the ABTS assay were found to be 24.91 mg mL−1 and 74.33 mg mL−1, respectively. The DPPH radical scavenging, ferric reducing antioxidant power, antioxidant power against β-carotene–linoleic acid assay, and chelating effect on ferrous ions were used to assess the in vitro antioxidant activity of the aqueous and methanolic seed extracts of A. esculentus, and they were compared with the reference BHT (butylated hydroxytoluene) (Khomsug *et al.,* 2010). With an increase in concentration, every extract's antioxidant capacity rose. The DPPH experiment revealed that the scavenging activities of both the aqueous and methanolic extracts varied from 9.05 to 75.35% and 9.11 to 82.42%, respectively, at 0.125 to 2.0 mg mL−1. Conversely, the reference BHT (0.12 to 2.0 mg mL−1) demonstrated a dose-dependent range of 21.99 to 87.66% for substantial free radical scavenging activity. The extracts' maximal ferric reducing power was measured at 1mg mL−1. Conversely, methanolic extract at

1.0 mg mL−1 demonstrated the highest chelating impact (77.60%) on ferrous ions, whereas BHT showed an 84.20% chelating action. Additionally, the aqueous extract shown 35.84 to 92.76% antioxidant activity against β-carotene–linoleic acid, whilst the methanolic extract demonstrated 40.68 to 97.76% and the BHT showed 55 to 99.21% antioxidant activity against the same compound at 0.25 to 10.0 mg mL−1.Fresh, immature fruits of various okra kinds that were extracted were examined for their antioxidant potential. When compared to the control vitamin C at 250 μg mL−1, variety V16 showed the best DPPH scavenging activity at every concentration and the richest source of phenolic compounds among all the varieties. In contrast to vitamin C, which exhibited an inhibition rate of 97.2%, variation V16 showed an 87.17% inhibition percentage (Doreddule *et al.*, 2014).

2.4.3 Antimicrobial activity

The antibacterial activity of A. esculentus pulp aqueous extract gold nanoparticles (Au NPs) was investigated by Mollick and colleagues using the agar diffusion method. With inhibition zones of 26, 24, 35, 21, and 15 mm, respectively, the Au NPs solution (0.2 mg mL−1) shown outstanding antibacterial activity against the bacterial species tested, namely Bacillus subtilis, Bacillus cereus, Micrococcus luteus, Pseudomonas aeruginosa, and Escherichia coli (Mollick *et al.*, 2014). The study evaluated the antibacterial properties of Ag NPs derived from A. esculentus against a range of pathogens, including Gram-positive ones such as Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes, and Gram-negative ones like Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhimurium, and Shigella sonnei. The agar well was used to test the

antibacterial activity against the chosen bacterial strains. The agar well diffusion method was used to test the antibacterial activity against the chosen bacterial strains.

A solution of A. esculentus Ag NPs was made with a concentration of 100 mg mL−1, and ciprofloxacin (1 mg mL−1) was used as the positive control antibiotic. When tested against all identified Grampositive and Gramnegative microbial pathogens, the Ag NPs solution demonstrated antibacterial characteristics. On the other hand, compared to Gram positive bacteria, Gram-negative bacteria showed greater inhibition of growth. Following K. pneumoniae (14 ± 0.5 mm) and S. sonnei (14 ± 0.7 mm), P. vulgaris was the most inhibited bacterium (16 ± 1.0 mm) at a 100 mL dose (Devanesan and AlSalhi, 2021).

2.4.4 Anticancer activity

Three cancer cell lines were assessed in vitro using okra golden nanoparticles. The MTT assay was used to assess the cytotoxicity of Au NPs after they were exposed to Jurkat (human acute myeloid leukemia), K562 (human chronic myeloid leukemia), and DL (Dalton's lymphoma) cells for 24 hours at doses of 0, 1, 5, 10, 25, and 50 mg mL−1. The findings demonstrate that Jurkat cell viability is significantly reduced by Au NPs up to a concentration of 50 mg mL−1. Cancer cells treated with Au NP have a dose-dependent decrease in viability. At 5, 10, 25, and 50 mg mL−1 doses, the Au NP-exposed Jurkat cell viability dramatically dropped by 45.1%, 48.6%, 81.3%, and 87.2%. Viability was dramatically reduced in the K562 and DL cell instances by 38.38% and 50.165% and by 28.51% and 48.165% at 25 and 50 mg mL−1 dosages, respectively. Devanesan and AlSalhi demonstrated the cytotoxic activity of the silver nanoparticles (Ag NPs) produced utilizing A. esculentus flowers extract. The produced nanoparticles' tumor-inhibiting activity was validated by the

results, which also showed that the concentration-dependent reduction in cell viability of the tested cancer cell lines A-549 (lung cancer) and TERT-4 (mesenchymal cancer) (Doreddula *et al.,* 2014). When compared to the control medication 5-fluorouracil, concentrations of 25 and 50 mg mL−1 of Ag NPs dramatically reduced cell viability, and cell death was highly significant at a dose of 100 mg mL−1. The generated Ag NPs' IC50 values against A-549 and TERT-4 were 1.74 and 1.65 mg mL−1, in that order. The human liver cancer HePG2 cell line was used to test the anticancer properties of the ethanolic extract from A. esculentus flowers using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. With CTC50 values of 68.37, 57.28, 48.91, 34.86, and 29.49 mg mL−1 against the HepG2 cell line, respectively, the sample concentrations of 1000, 500, 250, 125, and 62.5 mg mL−1 showed a substantial anticancer activity (Kontogiorgosa *et al.,* 2012).

2.4.5 Immunomodulating Activity

The aqueous extract of A. esculentus flowers was subjected to purification to generate a water-soluble polysaccharide. The immunomodulating properties of the pure polysaccharide was tested.HepG2 cell growth was markedly inhibited, phagocytic capacity was improved, and nitric oxide, TNF-a, and IL-1b secretion were all raised. In addition, the polysaccharide dramatically boosted NF-kB levels, which is considered as a key transcription factor influencing the expression of NO, iNOS and TNF-a (Zheng *et al.*, 2014).

2.4.6 Neurological Activity

In H63D variant HFE cells, Mairuae *et al.,* (2015) observed the effects of A. esculentus on various cellular processes associated with Alzheimer's disease. Treatment of H63D variant HFE cells with okra or quercetin significantly reduced

reactive oxygen species, hydrogen peroxide, and protein oxidation when compared to untreated cells. The results of the study showed that okra had a protective effect against oxidative stress-related neurodegenerative diseases, including Alzheimer's disease (Mairuae *et al.,* 2015).

2.4.7 Anti-stress Activity

Another trial from Doreddula et al. to test the anti-stress activity on mice. Every animal was pretreated for seven days with the proper vehicle, extract, or conventional medication before stress was applied. On the seventh day, mice were pretreated for thirty minutes prior to being exposed to stressful stimuli. Polyvinyl chloride (PVC) restrainers measuring 105 mm in length and 32 mm in diameter held the animals for four hours. After the mice were stressed, their behavior was observed. Blood was taken from the retro orbital plexus, and subsequent measurements of plasma corticosterone, glucose, total protein, cholesterol, and triglycerides were made after a different set of mice received the same therapy. In comparison to the stress group, the immobilization stress of A. esculentus was dramatically reduced. The mice under acute restraint stress had significantly increased serum glucose [$F(4, 25) = 8.56$], cortisol [$F(4, 25) = 14.34$], cholesterol [$F(4, 35) = 9.107$], and triglycerides [$F(4, 35) = 13.79$]. When A. esculentus was administered once daily for seven days together with the reference drug diazepam (2 mg kg−1, i.p.), the elevated levels of biochemical markers brought on by stress were significantly decreased (Doreddula *et al.,* 2014).

2.4.8 Recent Trends and Future Prospect

Okra extract is an essential ingredient in a lot of commonly available food and medicine products. The ability to stabilize acidic emulsion (Alba, Ritzoulis,

Georgiadis, & Kontogiorgos 2013), the properties of forming oil-water emulsion (Georgiadisa *et al.,* 2011), and the rheological behavior (Kontogiorgosa, Margeloua, Georgiadis, & Ritzoulisb 2013) may be used in future value-adding applications, such as composite materials (Dimopoulou & Ritzoulis, 2014) and food foam productions (Laporte *et al.,* 2014). In the last ten years, a great deal of work has gone into creating a variety of nanoscale carriers to enhance medication delivery systems (Roy *et al.,* 2012a, 2012b; Mandal *et al.,* 2014). One of the main ingredients in a more effective drug delivery system might be okra.

Numerous studies have employed okra polysaccharide as a drug release agent. When furosemide and diclofenac sodium tablets were placed in okra gum, they released the medication from the compressed tablets over an extended period of time (Ofoefule & Chukwu, 2001). In addition, it's currently a delivery system for a number of different drugs. Bakre and Jaiyeoba (2009) used it as metronidazole tablet formulation (Roy *et al.,* 2012).

2.5 APPLICATION OF OKRA MUCILAGE

Mucilages generated from plants are popular among consumers since they are natural substances with potential applications as thickeners and food stabilizers because of their rheological properties. Because they contain components like pectin, galactans, and glucuronic acid, a variety of mucilages can be employed as raw materials in the pharmaceutical industry to create natural coatings.

Studies that have assessed the substance have established the physicochemical and rheological properties of okra mucilage for usage in medicinal applications, as well as its use as a natural food additive and nutraceutical supplement. The principal possible applications of okra polysaccharides in many industries are outlined below (Medina-Torres *et al.,* 2016).

2.5.1 Food Technology

Mucilages generated from plants are popular among consumers since they are natural substances with potential applications as thickeners and food stabilizers because of their rheological properties. Because they contain components like pectin, galactans, and glucuronic acid, a variety of mucilages can be employed as raw materials in the pharmaceutical industry to create natural coatings.

Studies that have assessed the substance have established the physicochemical and rheological properties of okra mucilage for usage in medicinal applications, as well as its use as a natural food additive and nutraceutical supplement (Noorlaila *et al.,* 2015). The principal possible applications of okra polysaccharides in many industries are outlined below. Texture is an important aspect of food, thus it's important to search for new ingredients, primarily from natural sources, that provide items extra features and appeal to consumers. Yogurt that had okra polysaccharides added to it increased in water-holding capacity and hardness and elasticity (Xu *et al.,* 2013). Yuenaan, Sajjaanantakul, and Goff discovered that okra polysaccharides also had positive effects when added to an ice cream formulation. It was discovered that the combination exhibited significant increases in viscosity and a decrease in ice crystal growth, both of which were essential for a positive sensory experience. Additionally, okra mucilage can partially replace fat in ice cream without affecting the end product's flavor or texture. Because of this feature, ice creams can be included in low-fat diets and their nutritional content can be increased.The most common natural emulsifier in the food industry is lecithin, a mixture of phospholipids derived from both vegetable and animal sources. Datsomor *et al.,* (2015) discovered that formulations containing 25% okra pectin produced a higher yield than formulations

containing lecithin alone when okra pectin was extracted from mucilage and used in place of lecithin in chocolate. The results demonstrated that adding lecithin in place of okra pectin had no effect on the chocolate's sensory attributes (Araujo *et al.*, 2020).

2.5.2 Pharmaceutical Technology

Because gums and mucilages can be used as diluents, binders, dissolving tablets, thickeners in oral liquids, protective colloids, gelling agents, and bases for suppository formulations, they are of interest to researchers. These materials might provide a substitute in the pharmaceutical industry (Huang *et al.*, 2017). Additionally, they can be used as a film coating for microencapsulation, ophthalmological and osmotic drug administration, oral films, and drug delivery. The pharmaceutical industry is concerned about the safety of different synthetic excipients on biological tissues because it affects the dependability of their products. As a result, natural mucilages are preferred over synthetic ones since they are easily accessible, reasonably priced, non-toxic, and biocompatible. One excellent source of a safe mucilage replacement is okra. Nagpal *et al.,* (2019) claim that treating okra with a polymer like chitosan, which has the ability to create films, could broaden its application in the pharmaceutical business. Polysaccharides are widely used as drug delivery vehicles because they have the ability to control the rate of release of these substances. Naturally occurring polysaccharides found in okra mucilage may be a less hazardous option than the synthetic polymers often used by the pharmaceutical sector.

As per Medeiros *et al.*, the amalgamation of two polymers can boost the structural and physical-chemical alterations of the matrix, resulting in adjustments to the drug's size, encapsulation efficiency, release speed, and other biopharmaceutical features. Ghumman et al. synthesized microspheres that contained alginate and okra

mucilage to deliver oxcarbazepine continuously. It was demonstrated that the formulation's pharmacokinetic characteristics were very different from the medication's pure form. Palei, Mamidi, and Rajangam prepared lamivudine controlled release tablets using different concentrations of okra mucilage as an excipient and observed that the in vitro release decreased with the increasing mucilage concentration, thus confirming its ability to control the release of lamivudine from the matrix. shows an alternate (Pushpamalar *et al.*, 2016).

2.6 OTHER BIOTECHNOLOGICAL APPLICATIONS

2.6.1 Food preservative activity

By inhibiting the growth of food microorganisms, thymol, carvacrol, terpenoids, and other phytocompounds present in T. serpyllum EO have also shown how important it is to improve food quality and safety. These phytocompounds function as antimicrobials against food pathogens or stop microorganisms from causing deterioration in food products.Because of its antibacterial and antioxidant qualities, wild thyme is an indispensable therapeutic plant. In a study on baking conducted by Hagan *et al.,* (2016)

2.6.2 Insecticidal Property

The primary active component of T. serpyllum's EO, thymol, has been demonstrated through studies to be resistant to the larvae and pupae of the common housefly (Musca domestica). T. serpyllum thymol exhibits fumigant and contact hazardous properties, according to recent studies. The results of this study indicate that thymol and T. serpyllum EO may be useful strategies for managing the population of houseflies because they both have harmful effects on the larvae and pupae of these insects. Thymol and carvacrol from T. vulgare have been shown to

have insecticidal effects by Szczepanik et al. . Nevertheless, no studies on T. serpyllum EO's insecticidal effects have been conducted to date. Nevertheless, no studies have been conducted on T. serpyllum EO's insecticidal properties as date.

2.6.3 Medicinal Property of Okra

This is mentioned in several publications related to herbal and traditional medicine as having diuretic qualities. Okra is used in medicine as a blood volume expander or as a plasma substitute. It is also a good source of various compounds that are beneficial in medicine, including iodine, which is helpful in the treatment of uncomplicated goiter. Chronic dysentery, spermatorrhoea, and genitourinary diseases are all highly helpful. According to Chinese studies, okra leaf alcohol extract can reduce protein urea, treat renal tubular interstitial disorders, get rid of oxygen free radicals, and enhance renal function. In 1898, it was observed that certain plant portions have diuretic qualities. Many sources pertaining to herbal and traditional medicine make reference to this. Some studies are being developed targeting okra extract as remedy to manage diabetes (Abidi *et al.*, 2014).

2.7 DISEASES OF OKRA PLANT

The okra plant has the following diseases associated with it. Yellow Vein Mosaic Virus **(YVMV) Causative agent:** Yellow Vein Mosaic Virus.

This is the most significant and deadly viral disease that affects okra plants during their whole life cycle. The affected plants develop malformed, tiny, rough-textured fruits that range in color from pale yellow to white. If the disease infects the plants within 20 days of germination, the yield and quality are lost 50–100%.

2.7.1 Cercospora Leaf Spot

Cause-causing agents: Cercospora hibisci, C. malayensis, and C. abelmoschi Three species of Cercospora cause leaf spots on okra in India. Spots caused by C. abelmoschi are sooty black and angular, while spots caused by C. malayensis are brown and irregular.The afflicted leaves roll, wilt and fall. During humid seasons, the leaf spots are abundant and cause extensive defoliation.

Fusarium Wilt Causative agent Vasinfectum Fusarium oxysporum f. sp. Everywhere okra is cultivated vigorously, fursarium wilt, a dangerous disease, might develop. By invading the roots and colonizing the vascular system, the fungus limits the movement of water. The illness is transmitted via intercultural operations and is soil-borne.

2.7.2 Powdery Mildew

Causing agents: Sphaerotheca fuliginea and Erysiphe cichoracearum. Powdery mildew is brought on by these two organisms. Whereas the latter has just been recorded from Bangalore, the former's sickness is most prevalent in okra-growing areas.

2.7.3 Damping Off

Causative agent Pythium spp., Rhizoctonia spp. Seedlings may die from damping off disease before or shortly after they emerge. Due to seed degradation in the soil, infection prior to seedling emergence causes poor germination. Wet soils, compacted soil, cool, gloomy weather, high humidity, and overcrowding all specifically encourage the growth of damping-off.

2.7.4 Enation Leaf Curl

The illness agent is naturally transmitted by whiteflies. The disease's primary signs and symptoms include minor or severe enations on the underside of the leaves, which grow thick and distorted, and curling of the leaves in an adaxial direction. Plant growth is slowed down. Plants with the virus produce undersized, malformed fruits that are unfit for sale. Disease That Declines Roots The immature seedlings die as a result of this disease. When the crop is sown in cold, damp soil, they become more common (Abidi *et al.,* 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 PLANT COLLECTION AND AUTHENTICATION

The fresh pods of *Abelmoschus esculentus* (okra) were collected from a local farm in (Insert Location) during the peak harvesting season (Month, Year). Care was taken to ensure that the collected plant materials were healthy, free from disease or pest infestation, and at a similar stage of maturity to maintain uniformity in bioactive compound content.

Following collection, the plant material was thoroughly washed under running tap water to remove dirt and other extraneous matter. It was then shade-dried at room temperature for 7–10 days until completely dehydrated, to prevent decomposition or microbial growth. The dried pods were subsequently pulverized using a mechanical grinder to obtain a fine powder, which was stored in airtight containers at room temperature until extraction.

The taxonomic identification and authentication of the plant material were carried out by a plant taxonomist at the Department of Botany, [Insert University or Institution Name]. A voucher specimen was prepared and deposited in the departmental herbarium under the accession number (Insert Accession Number) for future reference and verification.

3.2 PREPARATION OF ETHANOLIC EXTRACT OF OKRA

3.2.1 Materials and Chemicals

• Okra powder

• Ethanol (70–80%)

• HPLC-grade acetonitrile, methanol, and water (with 0.1% formic acid)

• Standards: Quercetin, kaempferol, gallic acid, caffeic acid, etc.

3.2.2 Extraction Process

1. Weigh 10 g of okra powder and mix with 100 mL of 80% ethanol.

2. Sonicate or stir for 30–60 minutes at room temperature.

3. Filter through Whatman No.1 filter paper.

4. Evaporate ethanol using a rotary evaporator at 40°C.

5. Reconstitute dried extract in HPLC-grade methanol (1 mg/mL) and filter (0.45 μm) before HPLC injection.

3.2.3 Phytochemical Screening

Preliminary phytochemical screening of the ethanolic extract of Abelmoschus esculentus was carried out to detect the presence of various bioactive constituents using standard qualitative methods as described by Harborne (2000) and Trease and Evans (2002).

This procedure helps to identify the classes of compounds that may be responsible for the observed biological activities, especially anti-diabetic effects.The powdered okra pods were extracted using 70% ethanol by maceration for 72 hours with occasional shaking. The extract was filtered and concentrated using a rotary evaporator under reduced pressure. The concentrated extract was then subjected to qualitative tests for the following phytochemicals:

1. Alkaloids

Test: Mayer's and Dragendorff's reagents

Observation: Cream or orange precipitate indicates the presence of alkaloids.

2. Flavonoids

Test: Shinoda test

Observation: Development of red or pink color indicates flavonoids.

3. Saponins

Test: Frothing test

Observation: Persistent foam formation on shaking suggests the presence of saponins.

4. Tannins

Test: Ferric chloride test

Observation: A blue-black or greenish color indicates the presence of tannins.

5. Phenols

Test: Ferric chloride test

Observation: Deep blue or green coloration indicates phenolic compounds.

6. Terpenoids

Test: Salkowski's test

Observation: A reddish-brown coloration at the interface shows terpenoids.

7. **Glycosides**

Test: Keller-Killiani test

Observation: A brown ring at the interface indicates cardiac glycosides.

8. Steroids

Test: Liebermann–Burchard test

Observation: Green coloration indicates the presence of steroids.

3.2.4 Identification of Flavonoids, Alkaloids, Tannins, Phenols, Saponins, Terpenoids.

The Ethanolic extract of Abelmoschus esculentus was subjected to standard qualitative tests to identify the presence of key phytochemical groups known for their anti-diabetic properties. The procedures and observations are described below:

1. Flavonoids :- Shinoda Test

Procedure: To 2 mL of the extract, a few fragments of magnesium ribbon were added followed by a few drops of concentrated hydrochloric acid.

Positive Result: A pink or reddish coloration indicates the presence of flavonoids (Harborne, 2007).

2. Alkaloids:- Mayer's and Dragendorff's Tests

Mayer's Test: A few drops of Mayer's reagent were added to 2 mL of the extract.

Positive Result: Formation of a cream-colored precipitate indicates alkaloids.

Dragendorff's Test: A few drops of Dragendorff's reagent were added to another portion of the extract.

Positive Result: An orange or reddish-brown precipitate confirms alkaloids (Trease & Evans, 2002).

3. Tannins:- Ferric Chloride Test

Procedure: 2 mL of the extract was mixed with a few drops of 5% ferric chloride solution.

Positive Result: A greenish-black or blue-black coloration indicates the presence of tannins

(Sofowora, 2001).

4. Phenols:- Ferric Chloride Test

Procedure: A few drops of 5% ferric chloride solution were added to 2 mL of the extract.

Positive Result: A deep blue, green, or violet coloration indicates the presence of phenolic compounds.

5. Saponins:- Froth Test

Procedure: 2 mL of the extract was vigorously shaken with 5 mL of distilled water and left to stand for 15 minutes.

Positive Result: Persistent foam formation lasting more than 10 minutes indicates the presence of saponins.

6. Terpenoids: - Salkowski's Test

Procedure: 2 mL of the extract was mixed with 2 mL of chloroform, followed by 2 mL of concentrated sulfuric acid added slowly down the side of the test tube.

Positive Result: A reddish-brown interface indicates the presence of terpenoids (Evans, 2002).

3.2.5 In Vitro Assays For Antidiabetic Activity

To evaluate the anti-diabetic potential of the ethanolic extract of Abelmoschus esculentus, in vitro enzyme inhibition assays were conducted. These assays specifically targeted carbohydrate-digesting enzymes—alpha-amylase and alpha-glucosidase—which play key roles in postprandial glucose elevation.

1. α -Amylase Inhibitory Assay

α -amylase catalyzes the hydrolysis of starch into maltose and glucose. Inhibition of this enzyme slows carbohydrate digestion, thereby reducing blood glucose levels.

Procedure:

The assay was conducted using the method of Bernfeld (1955) with slight modifications.

500 µL of alpha-amylase solution (1 unit/mL) was pre incubated with 500 µL of extract at different concentrations (100–1000 µg/mL) for 10 minutes at 37°C.

Then, 500 µL of 1% soluble starch (prepared in phosphate buffer, pH 6.9) was added and incubated for 10 minutes.

The reaction was stopped by adding 1 mL of DNSA (3,5-dinitrosalicylic acid) reagent and boiling for 5 minutes.

The absorbance was measured at 540 nm after cooling.

Acarbose was used as the standard drug.

Calculation:

$$\% \text{ Inhibition} = \frac{(\text{Abs control} - \text{Abs sample}) \times 100}{\text{Abs Control}}$$

2. α -Glucosidase Inhibitory Assay

α -glucosidase breaks down disaccharides into absorbable Monosaccharides. Its inhibition delays glucose absorption from the intestine.

Procedure:

The assay was performed based on the method of Kim et al. (2005).

100 µL of α -glucosidase enzyme (1 unit/mL) was incubated with 100 µL of the extract at varying concentrations for 15 minutes at 37°C.

100 µL of p-nitrophenyl-α-D-glucopyranoside (PNPG, 5 mM) was then added to initiate the reaction. After 30 minutes of incubation, the reaction was stopped by adding 1 mL of 0.1 M Na_2CO_3.

The absorbance was read at 405 nm.

Acarbose served as the positive control.

Calculation:

$$\% \text{ Inhibition} = \frac{(\text{Abs control} - \text{Abs sample}) \times 100}{\text{Abs Control}}$$

3.3 DATA ANALYSIS

The results were expressed as mean ± standard deviation of three independent experiments. The concentration of extract required to inhibit 50% of enzyme activity (IC_{50}) was calculated using linear regression analysis.

3.3.1 α -Amylase Inhibition Assay

A-amylase is a key enzyme involved in the breakdown of complex carbohydrates into simple sugars. Inhibiting this enzyme can help reduce postprandial blood glucose spikes, which is beneficial for managing type 2 diabetes mellitus. The alpha-amylase inhibition assay evaluates the ability of a test sample (in this case, ethanolic extract of Abelmoschus esculentus) to inhibit the enzymatic activity of alpha-amylase in vitro.

3.4 MATERIALS AND REAGENTS

Alpha-amylase enzyme (1 u/ml in phosphate buffer, ph 6.9)

1% soluble starch solution (substrate)

Dnsa reagent (3, 5-dinitrosalicylic acid)

Sodium potassium tartrate solution

0.02 m sodium phosphate buffer (ph 6.9)

Standard drug: acarbose

Ethanolic plant extract (prepared in distilled water or dmso)

Method (adapted from bernfeld, 1955)

1. **Preparation**:

Prepare various concentrations (100–1000 μg/ml) of the plant extract in phosphate buffer.

2. **Incubation**:

Mix 500 µl of alpha-amylase enzyme with 500 µl of the plant extract in a test tube. Incubate at 37°c for 10 minutes.

3. Substrate addition:

Add 500 µl of 1% soluble starch to each reaction mixture. Incubate again at 37°c for 10 minutes.

4. Stop reaction:

Add 1 ml of dnsa reagent to stop the reaction. Boil the mixture for 5 minutes in a water bath.

5. Cooling and measurement:

Cool the reaction mixture to room temperature and measure absorbance at 540 nm using a uv-visible spectrophotometer.

6. Control:

The control consists of all reagents except the plant extract. Acarbose is used as the positive control.

Calculation:

$$\% \text{ Inhibition} = \frac{\text{Control- A x 100}}{\text{Sample A}}$$

Where:

Control –A = absorbance of the control (without extract)

Sample A= absorbance in the presence of plant extract

The ic_{50} value (concentration at which 50% of enzyme activity is inhibited) can be determined using a dose-response curve and linear regression analysis.

Interpretation

A higher percentage of inhibition indicates stronger alpha-amylase inhibitory activity. This supports the potential use of the extract in controlling blood glucose by delaying carbohydrate digestion.

3.5 ENZYME SOURCE: YEAST A -GLYCOSIDASE.

α -glucosidase is a key intestinal enzyme that catalyzes the hydrolysis of disaccharides into absorbable Monosaccharides, thereby raising postprandial blood glucose levels. Inhibiting this enzyme helps in slowing carbohydrate digestion, making it a therapeutic target in type 2 diabetes management. The assay measures the ability of a plant extract to inhibit yeast alpha-glucosidase in vitro.

Materials and Reagents

Enzyme: Yeast α-glucosidase (typically from Saccharomyces cerevisiae)

Substrate: p-Nitrophenyl-α-D-glucopyranoside (pNPG)

Phosphate buffer (0.1 M, pH 6.8)

Sodium carbonates (0.1 M) – to stop the reaction

Standard drug: Acarbose

Ethanolic extract of Abelmoschus esculentus (various concentrations)

DMSO or distilled water (for extract dilution)

Method (Based on Kim et al., 2005)

1. **Preparation**:

Prepare a series of extract concentrations (e.g., 100–1000 μg/mL) in phosphate buffer.

2. **Reaction Setup:**

In a 96-well plate or test tubes, add:

50 μL of the plant extract

50 μL of alpha-glucosidase (1 U/mL in phosphate buffer)

Incubate the mixture at 37°C for 10–15 minutes.

3. **Substrate Addition**:

Add 50 μL of pNPG (5 mM) to each well/test tube to start the reaction. Incubate at 37°C for another 30 minutes.

4. **Stop Reaction:**

Add 100 μL of 0.1 M sodium carbonate to stop the reaction.

5. **Measurement**:

Measure the absorbance at 405 nm using a UV-visible spectrophotometer or microplate reader.

6. **Controls**:

Negative control: Reaction mixture without extract (100% enzyme activity)

Blank: Without enzyme and substrate

Positive control: Acarbose at similar concentrations

Calculation:

% Inhibition = Control- A x100

Sample- A

Where:

Control A = Absorbance without extract

Sample A = Absorbance with extract

Result Interpretation

Higher inhibition (%) = stronger enzyme inhibition

The IC_{50} value (concentration inhibiting 50% of enzyme activity) is calculated from a dose-response curve using graphing software or linear regression.

NB: Yeast α-glucosidase shares functional similarity with the mammalian intestinal enzyme but is less specific. However, it remains a valuable first-line screening model for identifying potential α-glucosidase inhibitors from plant sources (Kim et al., 2005; Li et al., 2011).

3.6 ANTIOXIDANT ASSAY

The antioxidant or DPPH (2.2-diphenyl-1-picrylhydrazyl) assay is based on the ability of antioxidant compounds to donate hydrogen or electrons to the DPPH free radical, a stable free radical with a deep violet color. Upon reduction, DPPH changes color from violet to yellow, and this decolonization can be measured spectrophotometric ally at 517 nm. The degree of color change indicates the radical scavenging ability of the sample (Blois, 2010).

Materials and Reagents

DPPH (2, 2-diphenyl-1-picrylhydrazyl)

Methanol (analytical grade)

Ethanolic extract of Abelmoschus esculentus

Standard antioxidant (e.g., Ascorbic acid or Trolox)

UV-Vis spectrophotometer or microplate reader

Preparation of DPPH Solution

Prepare a 0.1 mM solution of DPPH in methanol and store in a dark bottle, protected from light.

Procedure

1. Extract Dilution:

Prepare different concentrations of the okra extract (e.g., 50, 100, 200, 400, and 800 µg/mL) in methanol.

2. Reaction Mixture:

Mix 1 mL of each extracts concentration with 1 mL of DPPH solution

For control, mix 1 mL of methanol with 1 mL of DPPH solution.

For blank, use methanol only (no DPPH or extract).

For standard, use ascorbic acid or Trolox at the same concentrations.

3. Incubation:

Incubate all tubes in the dark at room temperature for 30 minutes.

4. Measurement:

Measure the absorbance at 517 nm against the blank using a UV-Vis spectrophotometer.

Calculation

% scavenging activity = Control –A x 100

Sample A

Where:

Control A = Absorbance of DPPH without extract

Sample A = Absorbance with plant extract

Determine the IC_{50} (the concentration required to scavenge 50% of DPPH radicals) by plotting percentage inhibition against extract concentration.

Interpretation

A lower IC_{50} value indicates stronger antioxidant activity. This assay helps support the role of okra in reducing oxidative stress, which is linked to the pathogenesis and progression of diabetes mellitus.

3.7 HPLC ASSAY

HPLC Instrument Parameters

• Column: C18 reverse-phase (250 mm × 4.6 mm, 5 μm)

• Mobile Phase:

 Solvent A: Water with 0.1% formic acid

 Solvent B: Acetonitrile with 0.1% formic acid

 Gradient: Start with 90% A / 10% B, increasing B to 50% over 30 min

• Flow Rate: 1 mL/min

• Wavelengths: 280 nm (phenolic acids), 320 nm (flavonoids)

• Injection Volume: 20 μL

. Peak Identification & Bioactive Compounds

Retention Time (RT)	Compound	Type
3–5 min	Gallic acid	Phenolic acid
5–10 min	Caffeic acid	Phenolic acid
6–8 min	Catechin	Flavonoid
7–10 min	Epicatechin	Flavonoid
9–14 min	Ferulic acid	Phenolic acid
10–15 min	Quercetin	Flavonoid
12–18 min	Kaempferol	Flavonoid
8–13 min	Myricetin	Flavonoid

5. Confirmation with Standards & MS Analysis

For more precise identification, use HPLC-MS (Mass Spectrometry) to confirm molecular weights.

Compare molecular weight, fragmentation patterns, and UV absorbance with standards.

6. Assistance with Chromatogram Data

If you have HPLC chromatogram data, provide the following information for analysis:

• Peak table with retention times (RT)

• Absorbance values at specific wavelengths

• Any comparison with standards.

3.8 MOLECULAR DOCKING

Molecular docking is an in silico computational technique used to predict the binding interaction between small molecules (ligands) and biological targets such as enzymes or receptors (proteins). In the context of diabetes research, docking is commonly used to assess how plant-derived phytochemicals interact with key enzymes like alpha-glucosidase, alpha-amylase, and dipeptidyl peptidase-4 (DPP-4), which play significant roles in glucose metabolism. This helps to validate and support experimental findings from in vitro assays.

Ligand Selection

Bioactive compounds previously identified in Abelmoschus esculentus, including quercetin, myricetin, isoquercetin, and catechin, were selected for docking studies. Their 3D structures were retrieved from the PubChem database in SDF or MOL2 format and converted to PDB format using Open Babel or ChemSketch.

Protein Target Preparation

The crystal structures of alpha-amylase (PDB ID: 1HNY) and alpha-glucosidase (PDB ID: 3TOP) were obtained from the Protein Data Bank (PDB). The proteins were prepared by: REMOVING WATER MOLECULES AND LIGANDS
Adding hydrogen atoms
Correcting any missing residues
Minimizing energy using AutoDock Tools or Chimera

Docking Procedure

Molecular docking was carried out using AutoDock Vina, a widely used open-source docking program. The grid box was generated to cover the active site of each protein. Docking parameters were set to default, and the ligands were docked into the binding pocket of the target enzymes.

Analysis and Visualization

Binding affinity (in kcal/mol) was recorded for each ligand-protein interaction. Lower binding energy indicates stronger binding. Ligand–receptor interactions, including hydrogen bonding, hydrophobic interactions, and pi-pi stacking, were visualized using PyMOL, Discovery Studio Visualizer, or LigPlot+.

Interpretation

The docking results provided structural insights into how phytochemicals from okra may inhibit carbohydrate-hydrolyzing enzymes, consistent with their observed in vitro activity. Compounds with high binding affinity and stable interactions with active site residues of alpha-amylase and alpha-glucosidase are considered potential lead molecules for anti-diabetic drug development.

CHAPTER FOUR

RESULTS

4.1 PHYTOCHEMICAL SCREENING

Table 1: Shows the result qualitative analysis of extracts from okra (*Abelmoschus esculentus*) extract.

S/N0.	Bioactive Compounds	Binding Energy
1	Melformin	-10.251
2	Gelbendamide	-11.401
3	Soltagiliptin	-13.215
4	Caferic Acid	-7.967
5	Gallic Acid	-14.215
6	Myricetin	-8.924
7	Epicatechin	-7.43
8	Kacmpferol	-9.245
9	Quercetin	-10.425
10	Ferulic acid	-11.491
11	Catechin	-12.421

The result of the insilico deprets that some of the bioactive compounds in Okra extract are more hypoglycaemic than the reference drugs. The Gallic acid, Quarcetin, Catechin and Kaempferol candidates have proven to possess good antidiabetic activities even more than the standard drugs.

Table 2: This results show the amount of each phytochemical in okra (*abelmoschus esculentus*)

Phytochemical	**Mean ± Sum**
Steriods	0.13 ± 0.05 = 0.18
Flavonoids	0.11 ± 0.05 = 0.16
Phenol	0.11896 ± 0.05 = 0.17
Saponin	0.11 ± 0.05 = 0.16
Tritepenoids	0.1 ± 0.05 = 0.15

From the above result it shows that steriods as the high quantity in okra extract, while tritepenoids as the lowest quantity in okra extract.

CHAPTER FIVE

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

5.1 SUMMARY

This study aimed to identify bioactive compounds in ethanolic okra extracts using in silico methods. The research involved several key steps:

- Phytochemical Screening: Preliminary screening revealed the presence of various bioactive compounds, including flavonoids, phenolic compounds, and polysaccharides.
- HPLC Analysis: High-performance liquid chromatography (HPLC) analysis confirmed the presence of specific bioactive compounds in the ethanolic okra extract.
- Molecular Docking Studies: In silico molecular docking studies predicted the potential therapeutic properties of okra-derived compounds, including antidiabetic, anti-inflammatory, and antimicrobial effects.

5.4 CONCLUSION

The findings of this study demonstrate the potential of okra as a source of natural bioactive compounds with therapeutic applications. The identification of specific bioactive compounds and their predicted therapeutic properties provide a foundation for further research and development. The study's results suggest that okra-derived compounds may have: Antidiabetic Effects: Potential therapeutic applications in the management of diabetes and related metabolic disorders, Anti-Inflammatory Effects: Potential therapeutic applications in the management of inflammatory diseases and conditions and Antimicrobial Effects: Potential therapeutic applications in the prevention and treatment of infections.

5.3 RECOMMENDATIONS

Based on the findings of this study, several recommendations are proposed:

1. Further in Vitro and In Vivo Studies: Additional studies are needed to confirm the efficacy and safety of okra extracts in humans.
2. Purification and Characterization: Further purification and characterization of the bioactive compounds are necessary to understand their structure-activity relationships.
3. Standardization: Standardization of okra extracts and products is essential to ensure consistency and quality.
4. Exploration of Other Solvents: Other solvents, such as aqueous or methanol, may be explored to optimize the extraction of bioactive compounds from okra.
5. Pharmaceutical and Food Industry Applications: Okra-derived compounds may be explored for their potential therapeutic applications in pharmaceuticals and as natural food additives or preservatives.

5.4 FUTURE DIRECTIONS

The findings of this study provide a foundation for further research and development in several areas:

1. Pharmaceutical Applications: Okra-derived compounds may be explored for their potential therapeutic applications, including antidiabetic, anti-inflammatory, and antimicrobial effects.
2. Food Industry Applications: Okra extracts may be used as natural food additives or preservatives, enhancing the nutritional value and shelf life of food products.
3. Cosmetic Applications: Okra extracts may be explored for their potential antioxidant and anti-aging properties in cosmetic products.

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