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EVALUATION OF THE ANTIOXIDANT POTENTIAL, ANTIDIABETIC, AND NEUROPROTECTIVE ACTIVITIES OF THE ETHANOLIC EXTRACTS OF *COLOCASIA ESCULENTA* AND *VERNONIA AMYGDALINA* LEAVES

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ABSTRACT

Oxidative stress is linked with several diseases, including diabetes and neurodegenerative disorders. Treatment options should be developed that are focused on addressing the root causes of these conditions while also managing their symptoms. This study, therefore, aimed to appraise the in vitro antioxidant potential and enzyme inhibitory activities, as well as carry out an in-silico study on bioactive compounds from ethanolic crude extracts of Colocasia esculenta (CE) and Vernonia amygdalina (VA). In vitro, antioxidant assays were performed on both plant extracts using standard methods (DPPH and ABTS scavenging assays). The study utilized high-performance liquid chromatography (HPLC) to identify phytoconstituents, identifying sixteen compounds in CE and twelve in VA. In vitro enzyme inhibitory assays were carried out, and three target proteins were chosen for molecular docking analysis. Phytochemical analysis indicated that CE extracts had higher total flavonoid content compared to total phenolic content, while VA extracts had higher total phenols than total flavonoids. Both extracts demonstrated strong antioxidant capacities with significant inhibition of ABTS and DPPH free radicals. Additionally, they exhibited significant inhibitory activity against α-amylase, α-glucosidase, acetylcholinesterase, and butyrylcholinesterase, with VA showing greater efficacy in each case compared to CE. Therefore, the findings suggest that these compounds are mostly responsible for the enzyme-inhibitory activities exhibited by the plant extracts. Thus, the compounds can be proposed as potential therapeutic agents against both diseases.

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Keywords: *Colocasia esculenta*, *Vernonia amygdalina*, antioxidant assay, enzyme inhibitory assay, protein targets, HPLC, bioactive compounds.

1. INTRODUCTION

1.1 Background of the Study

Natural product usage has long been acknowledged as a means of managing and treating human ailments. The secondary metabolites present in marine species, plants, fungi, and microorganisms continue to be important in the development of novel medications in today's world of sophisticated chemistry. It's interesting to note that natural small molecules have been the source of approximately fifty per cent of approved pharmaceuticals in the last 20 years, underscoring the ongoing significance of natural sources in drug development. It has also been shown that natural ingredients are useful in curing illness (Rollinger *et al*., 2004).

The most effective source of leads or hit chemicals for medication development has been natural products (NPs). More than a hundred novel products, mainly anti-infectives and anti- cancer medicines, are in clinical development. Researchers have identified and isolated numerous natural compounds that possess therapeutic properties and that show promise in treating a diverse array of diseases. NPs offer significant advantages compared with their synthetic counterparts. First off, evolution has "optimized" their structures to perform particular biological tasks, including battling infections, interacting with other species, and engaging with receptors and proteins. Second, compared to traditional synthetic small-molecule libraries, natural products have a wider range of chemical properties, offering benefits like scaffold diversity and structural originality (Lachance *et al*., 2012). Furthermore, the abundance of sp3 carbon atoms and chiral centres contribute to their distinctive capacity to interact with receptors and provide specific target selectivity. These advantages make NPs a valuable source of leads and hit compounds for developing new drugs (Atanasov *et al*., 2021). The extraction, purification, and identification of secondary metabolites in medicinal plants have been enhanced by various processes such as digestion, decoction, Soxhlet extraction, and analytical techniques including Gas Chromatography (GC), High-performance Liquid Chromatography (HPLC), mass spectrometry, UV-Visible spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy. These methods are crucial in studying medicinal plants and isolating bioactive compounds with potential pharmaceutical applications. In low- and middle-income countries like Nigeria, issues such as drug availability, cost, and side effects are of significant concern when it comes to treating diseases like diabetes and neurological disorders. As a result, herbal prescriptions have garnered attention as an alternative to conventional pharmacotherapy.

Throughout history, traditional medical practices have employed medicinal plants, often known as medicinal herbs, for therapeutic purposes. Availability and affordability in comparison to

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contemporary medications make them popular in non-industrialized societies for usage as folk medicine. This is due to the fact that plants are organic biofactories that are powered by sunlight and produce a variety of bioactive substances that are harvestable and extractable. Throughout the environment, primary metabolites are widely dispersed and essential to plants' regular growth and development (Applezweig, 1980). In contrast, plants can create a restricted quantity of secondary metabolites, which are compounds formed through the biosynthesis of primary chemicals. They frequently participate in ecologically significant functions in the interactions between plants and their surroundings and support the survival of plants. Natural chemicals are typically derived from plants and are the preferred starting point for creating novel drugs (Lahlou, 2007). The process of producing high-value semisynthetic medicinal compounds requires the use of secondary metabolites instead of primary metabolites. This is because secondary metabolites are better suited for usage as intermediates due to their extremely complicated stereo structures with multiple chiral centres. These bioactive molecules are being researched for their potential medicinal properties and are seen as promising model compounds for manufacturing and enhancing artificial and partially artificial medicinal substances (Atanasov *et al*., 2015; Moses *et al*., 2014).

Examples of these secondary metabolites include alkaloids, terpenoids, flavonoids, phenolics, saponins, tannins, sterols, etc. These compounds exhibit a wide range of bioactivities that have been the focus of extensive research. Alkaloids, for example, are known for their pharmacological effects, including antibacterial and anti-inflammatory properties. Terpenoids, on the other hand, have shown potential as antimicrobial and antineoplastic drugs. The antioxidant and anti-inflammatory properties of flavonoids have been extensively researched, and phenolics have been acknowledged for their prospective uses in the management of conditions like heart disease alongside hyperglycaemia. The diverse bioactivities exhibited by these compounds make plants valuable resources for the development of new medicines and treatments (Fongang *et al*., 2021).

Diabetes mellitus (DM), commonly known as "diabetes," is a prevalent group of endocrine disorders marked by consistently high levels of sugar in the blood. This condition occurs when the pancreas produces inadequate insulin levels or when the body's cells become less susceptible to the hormone's influence (Gardner and Shoback, 2011). The World Health Organization (WHO) has reported that diabetes is impacting around 200 million individuals worldwide. Furthermore, they have projected that by 2030, the prevalence of this condition will significantly deteriorate the overall health of the affected population. Diabetes and its consequences progress more quickly as a result of oxidative stress (Asmat *et al*., 2016). Typically, diabetes is linked to a higher production of free radicals and weakened antioxidant defences.

Neurodegenerative diseases (NDs) encompass a range of disorders that gradually weaken and deteriorate various parts of the nervous system, often targeting specific areas of the brain. These diseases typically manifest slowly over time, with their effects and symptoms becoming apparent

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in later stages of life. Oxidative stress and inflammation are widely recognized as significant elements that contribute to the process of neurodegeneration (Pereira *et al*., 2021). It is estimated that over 10 million individuals with NDs will live in the top 10 most populous nations, including Nigeria by 2030 (Dorsey *et al*., 2007; Okubadejo *et al*., 2010). Acetylcholine, a neurotransmitter, is hydrolysed by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), both of which play a significant role in ND pathology. Plant extracts with AChE inhibitory activity are being studied for their potential neuroprotective effects. It is widely acknowledged that two of the most prevalent and fatal diseases among the elderly are DM and NDs.

Colocasia esculenta, commonly referred to as Cocoyam and Taro, is a versatile plant renowned for its medicinal attributes. Its healing properties have been harnessed for treating a wide range of illnesses, such as arthritis, asthma, diarrhoea, internal bleeding, neurological issues, and skin ailments. This plant has a long history of usage in herbal medicine and continues to be valued for its therapeutic potential.

Vernonia amygdalina, also known as Bitter leaf for its bitter taste, is a herb that belongs to the Asteraceae family. Its plant extracts have long been used in traditional medicine to treat bacterial, protozoal, and helminthic infections. Evidence from scientific investigations supports the efficacy of these conventional medical applications (Egedigwe and Ijeh, 2010).

For centuries, people across the globe have harnessed the healing properties of these two herbs for various medicinal purposes. These plants contain flavonoids, tannins, alkaloids, and saponins, among other bioactive compounds. It has been demonstrated that these compounds have medicinal capabilities, including antioxidant, neuroprotective, antidiabetic, and anti-inflammatory effects. Furthermore, these plants' therapeutic properties for alleviating a wide range of illnesses, including ulcers, wounds, and respiratory issues, have been the focus of in-depth research in traditional medicine.

1.2 Justification

Oxidative stress has been identified as a contributing factor to the progression of diabetes and its complications, as well as neuronal cell death, which accompanies neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Asmat *et al*., 2016). Additionally, epidemiological studies have shown that Type 2 Diabetes (T2DM) has negative effects on brain structure and function, leading to varying degrees of cognitive decline and possibly dementia. These insights highlight the importance of developing treatment options that address the underlying causes of diabetes and neurodegeneration in addition to managing their symptoms (Santiago *et al*., 2023)

Drug availability, cost, and side effects are of concern in low and middle-income countries such as Nigeria for the treatment of these diseases. Plant metabolites offer a diverse range for new drug

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discovery. Hence, herbal prescriptions have gained attention as an alternative to conventional pharmacotherapy.

Both *Colocasia esculenta* and *Vernonia amygdalina* are traditional medicinal plants with therapeutic potential. These plants have been used in herbal medicine for their supposed therapeutic properties, indicating the presence of bioactive compounds with potential pharmacological effects (Chakraborty *et al*., 2015). Investigations into the bioactive substances in both plants, their possible medicinal uses, and their capacity to alleviate diabetes and neurodegenerative diseases such as Alzheimer's and Parkinson's disease are necessary. This research will contribute to the growing body of knowledge on the pharmacological properties of traditional medicinal plants. It will provide valuable insights into the development of natural treatments for diabetes mellitus and neurodegenerative diseases.

1.3 Aim and Objectives

1.3.1 Aim

This study aims to evaluate and investigate the antioxidant potentials, antidiabetic, neuroprotective activities, and bioactive phytonutrient composition of *Colocasia esculenta* and *Vernonia amygdalina* leaf extracts.

1.3.2 Objectives

The specific objectives of this study are to:

i. carry out the quantitative and qualitative phytochemical screenings on the crude extracts of *Colocasia esculenta* and *Vernonia amygdalina*;
ii. evaluate the antioxidant potential of the extracts of *Colocasia esculenta* and *Vernonia amygdalina* using DPPH and ABTS free radical inhibitory potential assays;
iii. perform enzymatic inhibition of α-amylase, α-glucosidase, acetylcholinesterase, and butyrylcholinesterase on the extracts of *Colocasia esculenta* and *Vernonia amygdalina*;
iv. carry out compound identification on the extracts of *Colocasia esculenta* and *Vernonia amygdalina* using the HPLC-MS techniques;
v. carry out in silico studies to investigate the probable interactions of the identified chemical compounds with protein targets by considering the scoring functions of all the ligands for the estimation of binding energies using Autodock Vina.

2. METHODOLOGY

2.1 Chemicals and Kits

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In this study, a variety of kits and substances were used. All reagents and chemicals were of analytical grade and commercially available. The list includes the following: acarbose, p-nitrophenyl-α-D-glucopyranose (PNPG), intestinal α-glucosidase, pancreatic α-amylase, 1,1-diphenyl-2-picrylhydrazyl (DPPH), acetylcholine iodide, butyrylcholine iodide, Ellman's reagent (DTNB), ABTS, BHT, gallic acid, and quercetin, all of which were obtained from Sigma-Aldrich, Inc. (Saint Louis, MO). Other chemicals used were of analytical grades and prepared using sterilized distilled water in an all-glass apparatus.

2.2 Sample Collection

The plant samples were collected from a farm in Ado-Ekiti, Ekiti state. The Department of Biological Science at Ekiti State University carried out the taxonomic identification of the plant samples, assigning herbarium numbers UHAE 2024060 for *Colocasia esculenta* (L)Schott (Araceae) and UHAE 2024061 for *Vernonia amygdalina.* Del. (Asteraceae).

2.3 Preparation and Extraction of Plant Extracts

At room temperature, the plant samples were air-dried; to thoroughly extract the plant's bioactive components, dried samples were ground into a powder and soaked in 96% ethanol for 72 hours, after which sieving followed the prior concentration in a water bath.

The concentrated extracts were then stored in amber vials to prevent light-induced degradation. They were kept in a refrigerator (temperature-controlled environment) to maintain their stability and integrity for subsequent analysis and experimentation.

2.4 Qualitative Phytochemical Analysis

Using the conventional methods outlined by Ushie *et al.* (2016) and Santhi and Sengottuvel (2016) Before further analysis, a preliminary examination of the phytochemicals present in the extract was conducted.

2.4.1 Screening for alkaloids

The alkaloid test was carried out using an adapted version of the standard protocol, as reported by Santhi and Sengottuvel (2016). In a steam bath, 10 millilitres of the plant extract were stirred with 5 millilitres of 1% HCl. The presence of alkaloids was verified by the production of a reddish-brown precipitate when Meyer's reagent was added.

2.4.2 Screening for flavonoids

A lead acetate test was carried out by modifying the standard procedure that Santhi and Sengottuvel (2016) detailed. In particular, a positive flavonoid test resulted from mixing 5 millilitres of the plant extract with 1 millilitre of 10% lead acetate. This mixture yielded a yellow-white precipitate.

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2.4.3 Screening for steroids

The steroid test was conducted according to the standard procedure as outlined by Santhi and Sengottuvel (2016). This entailed mixing 5 mg of the extract with 2 ml of acetic anhydride and 2 ml of sulfuric acid. This resulted in a colour change of the extract from violet to blue or green, indicating the presence of steroids.

2.4.4 Screening for terpenoids

Salkowski's Test was carried out using a modified version of the Evans (1997) method, in which two millilitres of the plant extract were dissolved in two millilitres of chloroform, dried, and then treated with two millilitres of concentrated sulfuric acid to produce a greenish colour that indicated that terpenoids were present.

2.4.5 Screening for phenols

According to the modified standard procedure Evans (1997) described, the ferric chloride test was employed to determine the presence of phenols. Following the application of ferric chloride solution to 10 mg of extracts, a bluish-black colouration developed, indicating the presence of phenols.

2.4.6 Screening for saponins

The saponins test was conducted utilizing an adapted method based on the standard procedure outlined by Brain and Turner (1975). To confirm the presence of saponins, 0.5 mg of the extract was agitated with 5 ml of distilled water to create a frothy look.

2.4.7 Screening for tannins

The tannin analysis was conducted following the industry standard outlined by Santhi and Sengottuvel (2016). A small sample of extract was mixed with water, heated in a water bath, and then filtered. Subsequently, ferric chloride was introduced to the filtrate, resulting in the development of a dark green colour, which verified that tannins were present.

2.4.8 Screening for glycosides

The presence of glycosides was ascertained by modifying the conventional method developed by Brain and Turner (1975). One millilitre of acetic acid, two drops of ferric chloride, and two millilitres of concentrated sulfuric acid were introduced to two millilitres of the sample to produce a reddish-brown colouration. This indicated a positive test for cardiac glycosides.

2.5 Quantitative Phytochemical Analysis

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Quantitative phytochemical screening involves the systematic analysis and measurement of the various phytochemicals present in a plant or plant-based sample.

2.5.1 Alkaloid determination

The methodology described by Harborne (1998) was used to determine the alkaloid residue. 200 ml of a solution comprised of 10% acetic acid in ethanol was added to a 250 ml beaker containing 5g of the sample. The beaker was then covered and allowed to stand for 4 hours. After filtration, the liquid extract was concentrated to a quarter of its original volume using a water bath. Throughout the process, concentrated ammonium hydroxide was slowly introduced to the extract. After the mixture was left to stand, the solid that formed was isolated and measured.

$$\%\ Alkaloid = \frac{\text{weight of alkaloid}}{weight\ of\ sample} \times 100$$

2.5.2 Total tannin determination

The method for quantifying tannin followed a standard procedure (Ejikeme *et al*, 2014), which involved mixing 10 g of phosphomolybdic acid with Folin-Denis reagent and refluxing the mixture for two hours. After cooling, 500 cm^3 of distilled water was added to the solution to dilute it. Subsequently, 100 cm^3 of distilled water was combined with 1 gram of each extract powder in a conical flask and gently heated for an hour on an electric hot plate. The mixture was then filtered through Whatman filter paper into a 100 cm^3 volumetric flask. In another 100 cm^3 conical flask, 5.0 cm^3 of Folin-Denis reagent, 10 cm^3 of saturated Na2CO3 solution, and 10 cm^3 of the diluted extract were combined with 50 cm^3 of distilled water for colour development. The solution was left to stand in a water bath at 25 °C for 30 minutes. The optical density of the solution at 700 nm was measured using a Spectrum Lab 23A spectrophotometer, and the results were compared to a standard tannic acid curve.

$$Tannic\ acid\ (mg/100g) = \frac{Concentration\ (C) \times volume\ of\ extract \times 100}{volume\ of\ aliquot \times sample\ weight}$$

where C is the tannic acid concentration as shown on the graph.

2.5.3 Total flavonoid determination

The sample's total flavonoid content was ascertained using the methods outlined by Ejikeme *et al.* (2014). In this procedure, 200 µl of distilled water is used to dilute 1 mL of the sample, 150 µL of NaNO3 (5%) solution is added, the mixture is incubated for 5 minutes, 15 µL of AlCl3 (10%) solution is added, the mixture is allowed to stand for 6 minutes, 2 ml of NaOH (4%) solution is added, and the mixture is made up to 5 mL with distilled water. After thoroughly shaking the

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mixture, allow it to stand at room temperature for 15 minutes before measuring the absorbance at 510 nm.

$$\% \, Flavonoid = \frac{Weight \, of \, flavonoid}{Weight \, of \, sample} \times 100 \, (Gallic \, acid \, eq. [mg/g])$$

2.5.4 Total phenols determination

The Folin-Ciocalteu reagent, as reported by Ferreira *et al*. (2020), was used to ascertain the total phenolic content of the crude sample. One millilitre of 7.5% Na_2CO_3 solution and 0.5 millilitres of 10% Folin-Ciocalteu reagent will be combined with a 500-millilitre extract sample. The reaction mixture's absorbance at 760 nm will be measured following 40 minutes of incubation at 45 °C using gallic acid as the standard. The outcome will be presented as milligrams of gallic equivalent per gram of dry sample (mg GAE/g dry sample).

2.5.5 Saponin determination

Each sample weighed five grams. A 250 cm^3 conical flask was filled with 100 cm^3 of 20% aqueous ethanol. The mixture was continuously stirred and heated to 55 degrees Celsius over a 4-hour period using a hot water bath. Following filtering, the mixture was heated for four hours at a constant temperature of 55 degrees Celsius while stirring constantly. The residue was then extracted again using an additional 100 cm^3 of 20% aqueous ethanol. At 90 degrees Celsius, the combined extract was boiled down to 40 cm3 over a water bath. The concentrate was poured into a 250 cm^3 separator funnel, which was then filled with 20 cm^3 of diethyl ether and given a good shake. The ether layer was thrown away, but the aqueous layer was salvaged. There were two iterations of the purifying procedure. After adding 60 cm^3 of n-butanol, 10 cm^3 of 5% sodium chloride was used twice to wash the butanol extract. After discarding the sodium chloride layer, A water bath was used to heat the remaining solution for thirty minutes. After which, it was placed inside a crucible and dried to a constant weight in an oven (Ejikeme *et al*., 2014)

2.5.6 Cyanogenic glycoside determination

One gram of the sample was put in a round-bottom 250 cm^3 flask, and about 200 cm^3 of distilled water was added. The flask was then allowed to sit for the autolysis process for two hours to determine the cyanogenic glycoside content. In a conical flask (250 cm^3), the entire distillation process was carried out using 20 cm^3 of 2.5% sodium hydroxide in the sample and the addition of tannic acid, an antifoaming agent. The resultant distillate was mixed with 2 cm^3 of 5% KI, 8 cm^3 of 6M NH4OH, and 100 cm^3 of cyanogenic glycoside. After that, the entire combination was titrated using a micro burette and 0.02 M $AgNO_3$ in front of a dark background. Sustained turbidity was used to determine the endpoint (Ejikeme et al., 2014).

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The estimated quantity of the cyanogenic glycosides present in the sample was calculated as

$$Cyanogenic\ glycoside\ = \left(\frac{mg}{100}g\right) = \frac{Titre\ value\ \times\ 1.08\ \times\ exact\ volume}{Aliquot\ volume\ \times\ sample\ weight} \times 100$$

2.6 Antioxidant Assays

2.6.1 DPPH scavenging activity assay

Employing the methodology outlined by Ilahi *et al*. (2013), the extracts' antioxidant activity was evaluated by determining the degree to which they inhibited DPPH (1,1-diphenyl-2 picrylhydrazyl) free radicals. One millilitre of carefully diluted extracts was mixed with one millilitre of a DPPH radical-containing 0.4 mM methanolic solution. A spectrophotometer was used to measure the absorbance of the mixture at 517 nm after it had been left in a dark background for 30 minutes. For every extract, the percentage inhibition of DPPH free radicals will be computed, and the outcomes will be contrasted with the control using BHT as the standard.

$$\%\ inhibition = (Abs_{control}-Abs_{sample}) / (Abs_{control})\ x\ 100$$

where Abs_{sample} is the absorbance of the DPPH radical + sample extract/standard, and $Abs_{control}$ is the absorbance of the DPPH radical + methanol. IC_{50}, or the concentration at which the DPPH radical diminished by 50%, was used to express the results. The plotted graph of the percentage inhibition vs concentration curve was used to calculate the concentration of extract that provided 50% inhibition (IC_{50}).

2.6.2 ABTS scavenging assay

Employing the techniques of Zhao *et al*. (2008), the extracts' capacity to scavenge (2,2'- azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid) free radicals was measured. An ABTS radical cation solution (7 mM ABTS dissolved in 0.01 M PBS, pH 7.4), 2.0 ml diluted, was combined with the sample (0.2 ml) in varied doses. After 20 minutes of room temperature treatment for the reaction mixture, the spectrophotometer was used to measure the absorbance at 734 nm. Next, using BHT as the standard, the ABTS free radical scavenging ability of the samples was computed and expressed as a percentage of inhibition compared to the control.

$$\%\ inhibition = (Abs_{control}-Abs_{sample}) / (Abs_{control})\ x\ 100$$

where Abs_{sample} is the absorbance of Abts radical + sample extract/standard, and $Abs_{control}$ is the absorbance of Abts radical + methanol. The results were expressed as IC_{50}, which means the concentration at which the Abts radical was quenched by 50%. The concentration of extract providing 50% inhibition (IC_{50}) was calculated from the plotted graph of % inhibition versus concentration curve.

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2.7 Antidiabetic Activity Assays - in vitro

Researchers have been conducting extensive studies to discover natural compounds that could help in managing diabetes. They are particularly interested in finding plants and compounds that possess strong anti-diabetic properties. The focus is on identifying substances that can inhibit two critical enzymes, namely α-glucosidase and α-amylase. They are responsible for breaking down carbohydrates, which can lead to elevated glucose levels after meals in individuals with diabetes. By inhibiting these enzymes, it is possible to regulate postprandial hyperglycaemia and potentially lower the risk of developing diabetes (Poovitha and Parani, 2016).

2.7.1 α-amylase inhibitory assay

To evaluate the effect of plant extracts on the inhibitory activity of α-amylase. Samples in a tube were mixed with 0.02 M sodium phosphate buffer (pH 6.9) and α-amylase solution (250µL total volume) at different concentrations (from 1 to 10 mg/ml). After a 10-minute pre-incubation period at 25 º C, the tube contents were added to a 0.02 M sodium phosphate buffer (pH 6.9) containing 1% starch solution. The addition of a dinitro salicylic acid reagent then stopped the process. Employing a Multiplate Reader, the absorbance was measured at 540 nm after the contents of each test tube were diluted with distilled water. Using the same procedure, a control was also made. The formula below was utilized to determine the inhibitory activity of α-amylase (Telagari and Hullatti, 2015)

$$\% \text{ Inhibition} = (Abs_{control} - Abs_{sample}) / (Abs_{control}) \times 100$$

The plotted graph of the percentage inhibition versus concentration curve was used to calculate the concentration of extract that provided 50% inhibition (IC_{50}).

2.7.2 α-glucosidase inhibitory assay

Kim *et al*. (2005) evaluated the impact of the plant extracts on α-glucosidase activity. Samples ranging in concentration from 1 to 10 mg/ml were mixed with 50µL of α- glucosidase-containing 0.02M sodium phosphate buffer (pH 6.9) and incubated for 10 minutes at 25°C. A 3.0 mM p-nitrophenyl glucopyranoside substrate solution in 20 mM phosphate buffer (pH 6.9) was added. After 20 minutes of incubation at 37°C, the mixture was stopped by adding 0.1M Na_2CO_3. The release of yellow-coloured para-nitrophenol from p-nitrophenyl glucopyranoside at 405 nm was used to measure the activity of α-glucosidase. The same process was used to prepare a control as well. The α-glucosidase inhibitory activity was assessed using the subsequent formula:

$$\% \text{ Inhibition} = (Abs_{control} - Abs_{sample}) / (Abs_{control}) \times 100$$

The plotted graph of the percentage inhibition versus concentration curve was used to calculate the concentration of extract that provided 50% inhibition (IC_{50}).

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2.8 Neuroprotective Activity Assays -in vitro

The enzymes acetylcholinesterase and butyrylcholinesterase (AChE and BChE), which break down acetylcholine, a neurotransmitter, are thought to be key factors responsible for the pathophysiology of neurodegenerative disorders. The potential neuroprotective effects of plant extracts containing compounds with AChE and BChE inhibitory activity have been studied, and bioactive compounds like alkaloids, flavonoids, terpenoids, and phenolic compounds have been looked into for potential therapeutic effects in Alzheimer's disease and other conditions characterized by cognitive decline.

2.8.1 Acetylcholinesterase and butyrylcholinesterase inhibition assay

A modified colourimetric method based on Ellman's approach was used to assess the inhibition of cholinesterase enzymes (AChE and BChE). The enzyme activities were measured in a 1 ml reaction mixture containing phosphate buffer (0.1 M, pH 8.0), DTNB (10 mM), 0.05 ml cytosol, and acetylcholine iodide or butyrylcholine iodide (150 mM). The change in absorbance at 412 nm was monitored using a UV spectrophotometer over 180 seconds at 25°C. The inhibitory activity of AChE or BChE was calculated as a percentage of inhibition relative to the control. The IC50 was calculated by analysing a graph that depicts the percentage of inhibition against varying concentrations.

2.9 Determination of IC_{50}

By employing a linear regression curve produced from a plot of the percentage inhibition caused by the extracts versus different concentrations (µg/mL) of the extract, the concentrations of the crude extract needed to achieve 50% inhibition (IC50) were ascertained (Aykul and Martinez-Hackert, 2016).

2.10 Quantification of Phytochemicals using HPLC Analysis

An adapted approach based on the linearity, accuracy, precision, and sensitivity validation criteria of the International Conference on Harmonization (ICH) was employed for HPLC analysis.

Employing high-performance liquid chromatography, a technique for identifying active chemicals in raw plant material was validated. Using a Waters XTerra® RP18, 5 µm, 250 ×4.6 mm column kept at 40 °C, the procedure successfully separated active ingredients from plant extracts. For best separation, a mobile phase made of acetonitrile (60:40) with 5 mM hexane sulfonate and 0.05 M potassium phosphate buffer, pH 6.0, was used. The mixture flowed isocratically for optimal separation. Elution was observed at 216 nm, and the mobile phase flow rate was 1.0 mL/min.

The D-7000 Merck-Hitachi HPLC system from Tokyo, Japan, manufactured by Hitachi- High Technologies Corporation and running on version 2.0 software, was the HPLC system used in the

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investigation. It consisted of an L-7200 autosampler, an L-7100 low-pressure gradient pump, and a D-7000 interface coupled with an L-7400 UV detector. An L-7350 column oven was used to control the temperature of the column. The stationary phase was a chromatography column, namely the Waters XTerra® RP18 from Waters Corp., situated in Wexford, Ireland, with dimensions of 250 × 4.6 mm ID and a particle size of 5 µm. The flow rate was kept constant at 1.0 mL/min, and the injection volume was 10 µL. Using UV detection at a precise wavelength of 216 nm, the eluents were observed. Acetonitrile and phosphate buffer/ion pairing agents were used to form the mobile phases. Equimolar (0.05 M) solutions of KH_2PO_4 and K_2HPO_4 were mixed to the appropriate pH using magnetic stirring to create buffer solutions. When required, hexane sulfonic acid (HSA) in the prescribed quantity was dissolved in buffer solutions before pH correction. Appropriate quantities of buffer/HSA were mixed with acetonitrile to create the mobile phases. The mobile phases were degassed in an ultrasonic water bath for about fifteen minutes before being used. Furthermore, 100 mg of the crude plant extract (1 mg/mL) was diluted in 100 mL of acetonitrile, filtered, and introduced into the HPLC apparatus. A standard solution was created by dissolving 20 mg of artemisinin working standard in 20 mL of acetonitrile with the assistance of sonication. This solution was utilized for method development and validation purposes.

Method validation: Method validation was meticulously conducted in compliance with the guidelines outlined by the International Conference on Harmonization (ICH). In this process, a solution containing 1.0 mg/mL of artemisinin in acetonitrile was utilized as a 100% solution. The validation parameters applied were linearity, accuracy, precision, and sensitivity.

Linearity: To assess the linearity of the method, we prepared working standard solutions at five different concentration levels, which corresponded to 120%, 100%, 75%, 50%, and 25%. These solutions were then analyzed in triplicate to determine the linearity of the method.

Accuracy: The analysis's accuracy was assessed by adding established amounts of working standards to the plant extract. This was carried out at three varying levels: 80%, 100%, and 120%. Following this, the quantity of bioactive compounds recovered was determined at each level to evaluate the accuracy of the analysis. This method is essential for guaranteeing the dependability and accuracy of the analytical technique utilized to evaluate the bioactive compounds in the plant sample.

Precision: To ensure consistency, six injections of a 100% solution were performed on the same day to assess repeatability. To evaluate inter-day precision, six replicate injections of a freshly prepared extract solution (100%) were conducted over three consecutive days. The peak areas of the analytes were adjusted, and the coefficient of variation (CV) of the adjusted areas was then determined.

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Sensitivity: The detection limit (LOD) and quantitation limit (LOQ) were established by examining dilutions of a standard solution (1 mg/ml). The LOD was identified as the lowest concentration that produced a signal-to-noise ratio (S/N) of 3:1, whereas the LOQ was determined as the concentration that resulted in a signal-to-noise ratio of 10:1.

Bioactive content: Using a technique called cold maceration, 100 grams of dried and crushed plant material was first steeped in one litre (1000 mL) of cold hexane and allowed to steep for 24 hours. After that, the extract was dried completely by evaporating it in a rotary evaporator set at 40 °C. A newly devised approach was used to assess the residue that remained after dissolving it in 1000 millilitres of acetonitrile and comparing the outcomes with a standard solution containing one milligram per millilitre. The dry weight of the plant sample was then used to calculate the percentage amount of bioactive chemicals in each extract.

2.11 In Silico and Molecular Docking Studies

2.11.1 Preparation of protein targets

The X-ray crystal structures of α-amylase (PDB ID: 4GQQ), α-glucosidase (PDB ID: 5NN5), and acetylcholinesterase (AChE) (PDB ID 4M0E). These structural data were retrieved from the Protein Data Bank (PDB) accessible at https://www.rcsb.org/. After retrieval, the structures were meticulously prepared using the University of California, San Francisco (UCSF) Chimera software, in accordance with the detailed procedures specified by Butt *et al*. (2020).

The Chimera software from UCSF (University of California, San Francisco) serves as a powerful tool for visualising and analysing a wide range of data, including density maps, 3D microscopy, molecular structures, and associated data (Goddard *et al*., 2007). It addresses the complexities associated with cutting-edge experimental methods, offering advanced options for rendering high-quality visualizations and employing professional strategies for software design and distribution. Notably, Chimera is excellent in terms of effectiveness, expandability, visualization, and user-friendliness and is freely available.

2.11.2 Preparation of Ligands

All compounds identified in HPLC analysis of *Colocasia esculenta* (Chlorogenic Acid, Gallic Acid, Oxalic Acid, Beta-Carotene, Catechin, Cinnamic Acid, Sitosterol, Anthocyanidin, Apigenin, Luteolin, Taraxerol, Iso-Orientin, Orientin, Isovitexin, Vitexin, and Vicenin), and the analysis of *Vernonia amygdalina* (Benzophenone, Garcinoic acid, Vernodalin, Vernodalol, Vernonioside A, Vernonioside B, Vernomygdin, Luteolin, Myrtenal, Garanal, Ascaridol, and Myrtenol), were obtained from PubChem ligand library and prepared using UCSF Chimera software as described by Butt *et al*. (2020).

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2.11.3 Molecular docking

To assess the docking parameters, we conducted docking studies for all the compounds using Autodock tools and Autodock Vina. The target proteins included α-amylase (PDB ID: 4GQQ), α-glucosidase (PDB ID: 5NN5), and acetylcholinesterase (AChE) (PDB ID: 4M0E) obtained from the protein database. The flexible potential function's default settings were used for the docking parameters, and no restrictions or constraints were applied during the studies. We selected co-crystallized ligands and generated grids around the active sites of 4GQQ, 5NN5, and 4M0E. Furthermore, we visualized the receptor-ligand interactions using the Biodiscovery studio software.

2.12 Drug-Likeness Assessment

Drug-likeness assessment evaluates the potential of a compound to become a drug based on its chemical structure and properties. Several rules and guidelines help determine drug likeness, the most well-known being Lipinski's Rule of Five (Benet *et al*., 2016). The Rule of Five is a set of criteria used to evaluate the drug-likeness of a compound. According to this rule, a compound is more likely to be an orally active drug in humans if it adheres to the following criteria with no more than one violation:

- Total Molweight (MW): Less than 500 Daltons.
- cLogP (octanol-water partition coefficient): Less than 5.
- Less than 5 Hydrogen Bond Donors (HBD).
- Less than 10 Hydrogen Bond Acceptors (HBA).

This study used the Osiris Data Warrior software to assess the druggable attributes of the identified compounds from both extracts and confirm their suitability for drug development.

2.13 Data Analysis

The data analysis was conducted using GraphPad Prism 8.0 (Version 8, Software Program, GraphPad Prism Inc., San Diego, CA). The findings were expressed as the average value with the standard deviation. To assess the biochemical indices, a one-way analysis of variance (ANOVA) was carried out, followed by Tukey's post hoc test for further evaluation. Statistical significance was determined at a p-value below 0.05.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Phytochemical analysis

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During the analysis of the ethanolic extracts of *Colocasia esculenta* and Vernonia *amygdalina*, comprehensive qualitative and quantitative tests were conducted to verify the presence and levels of flavonoids, saponins, alkaloids, phenols, and tannins (Table 3.1).

Table 3.2 reveals that the leaf extracts of *Colocasia esculenta* and *Vernonia amygdalina* contain substantial amounts of various bioactive compounds, such as flavonoids, alkaloids, tannins, phenols, and saponins.

The results obtained from these evaluations provided concrete evidence of the existence of these bioactive compounds in the extracts and also offered a detailed insight into their specific concentration levels. This exhaustive examination presented a thorough overview of the chemical composition and makeup of the extracts, shedding light on the rich array of bioactive compounds present in the samples.

Table 3.1: Qualitative phytochemical screening on *Colocasia esculenta* and *Vernonia amygdalina* leaf extracts

Samples	Alkaloids	Flavonoids	Phenols	Tannins	Saponins
Colocasia esculenta	+	+	+	+	+
Vernonia amygdalina	+	+	+	+	+

Key: + signifying availability

Table 3.2: Quantitative phytochemical screening on *Colocasia esculenta* and *Vernonia amygdalina* leaf extracts

Samples	Alkaloids (mg/100g)	Flavonoids (mg/100g)	Phenols (mg/100g)	Tannins (mg/100g)	Saponins (mg/100g)
Colocasia esculenta	26.35±0.02	37.58±0.06	6.62±0.2	10.19±0.08	1.67±0.2
Vernonia amygdalina	39.45±0.2	57.37±0.4	63.77±0.04	41.88±0.1	54.92±0.06

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3.1.2 HPLC analysis

The HPLC analysis of *Colocasia esculenta* and *Vernonia amygdalina* crude extracts revealed sixteen and twelve bioactive compounds, respectively.

In *Colocasia esculenta*, the bioactive compounds identified were Chlorogenic Acid, Gallic Acid, Oxalic Acid, B-carotene, Catechin, Cinnamic Acid, Sitosterol, Anthocyanidin, Apigenin, Luteolin, Taraxerol, Iso-Orientin, Orientin, Isovitexin, Vitexin, Vicenin (Table 3.3).

In *Vernonia amygdalina*, the bioactive compounds identified were Benzophenone, Garcinoic acid, Vernodalin, Vernodalol, Vernonioside A, Vernonioside B, Vernomygdin, Luteolin, Myrtenal, Garanal, Ascaridol, Myrtenol (Table 3.4).

Table 3.3: HPLC Identified bioactive compounds from ethanolic extracts of *Colocasia esculenta*

Compounds	Retention Time (min)	Area (m^2)	Height (m)	Class of Phytochemicals
Chlorogenic Acid	1.266	1319.74	35.63	Phenol
Gallic Acid	2.750	2648.98	25.74	Phenol
Oxalic Acid	4.450	799.50	13.32	Dicarboxylic
B-carotene	5.466	353.35	8.66	Terpenoid
Catechin	6.483	241.14	7.07	Flavonoid
Cinnamic Acid	7.333	81.99	5.39	Phenol
Sitosterol	7.950	102.30	5.09	Sterol/Steroid
Anthocyanidin	8.416	82.30	5.05	Flavonoid
Apigenin	11.050	8719.26	154.96	Flavonoid
Luteolin	12.166	2915.10	56.79	Flavonoid
Taraxerol	13.700	1335.69	29.03	Terpenoid
Iso-orientin	14.816	175.34	0.44	Flavonoid
Orientin	15.716	164.74	6.46	Flavonoid
Iso-vitexin	16.250	103.27	5.27	Flavonoid
Vitexin	17.233	119.02	6.40	Flavonoid
Vicenin	17.616	581.32	8.54	Flavonoid

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Table 3.4: HPLC Identified bioactive compounds from ethanolic extracts of *Vernonia amygdalina*

Compounds	Retention Time (min)	Area (m^2)	Height (m)	Class of Phytochemicals
Benzophenone	1.300	206.42	15.69	Phenol Terpenoid
Garcinoic acid	1.850	99.27	12.19	Sesquiterpene
Vernodalin	2.183	78.51	8.97	Sesquiterpene
Vernodalol	3.050	80.88	5.32	Steroidal Saponins
Vernonioside A	4.000	130.05	3.77	Steroidal Saponins
Vernonioside B	5.316	97.05	4.32	Sesquiterpene
Vernomygdin	6.200	322.94	14.83	Flavonoid
Luteolin	8.166	39.97	1.94	Terpenoid
Myrtenal	10.616	30.99	2.55	Sesquiterpenoid
Garanal	11.283	45.50	2.42	Terpenoid
Ascaridol	12.750	36.12	2.69	Terpenoid
Myrtenol	13.433	65.14	2.64	

3.1.3 Antioxidant screening

As illustrated in the graphs of Figures 3.1 and 3.2, the ethanolic leaf extracts of *Colocasia esculenta* and *Vernonia amygdalina* exhibited significant DPPH and ABTS percentage inhibitions. The DPPH and ABTS percentage inhibitions for the ethanolic extracts of both plants were 77.5% and 75.3% for *C. esculenta* and 87.2% and 83.9% for *V amygdalina,* respectively, operated at a concentration of 1000 µg/mL. The DPPH and ABTS scavenging activity were assessed at varied concentrations (15.63, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL).

Figure 3.1 reveals the free radical scavenging capacity of *Colocasia esculenta* and *Vernonia amygdalina* leaf extracts based on their capacity to suppress DPPH radicals. Both extracts exhibited high antioxidant capacity.

Figure 3.2 reveals the free radical scavenging capacity of *Colocasia esculenta* and *Vernonia amygdalina* leaf extracts based on their capacity to suppress ABTS radicals. Both extracts exhibited high antioxidant capacity.

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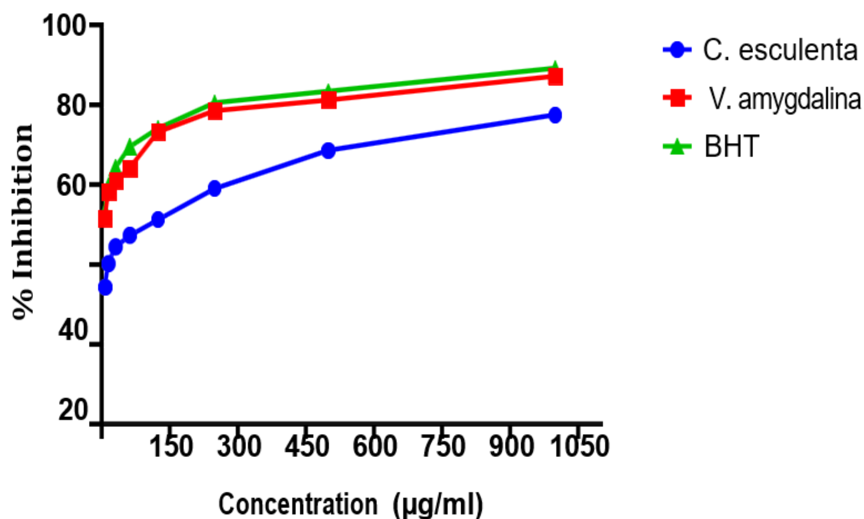

Figure 3.1: DPPH Scavenging Activity of both plant extracts

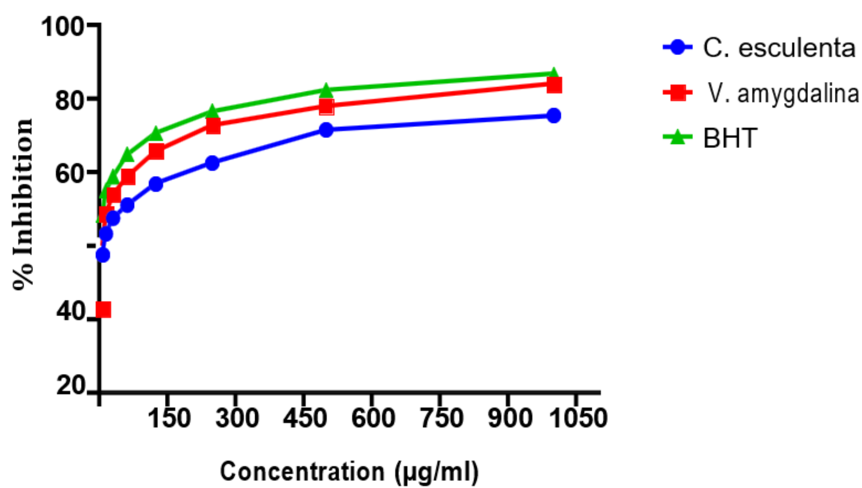

Figure 3.2: ABTS Scavenging Activity of both plant extracts

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Table 3.5: The IC_{50} values of the Antioxidant Assay of the extracts of *Colocasia esculenta*, *Vernonia amygdalina* and BHT.

	Colocasia esculenta (µg/ml)	*Vernonia amygdalina* (µg/ml)	BHT (µg/ml)
DPPH	123.35 ± 0.48	6.80±0.35	6.05±0.46
ABTS	61.5±0.62	19.07±0.65	14.25±0.10

Results are computed as mean ± SD (n=2)

3.1.4 Antidiabetic activity assays - in vitro

As illustrated in the graphs of Figures 3.3 and 3.4, the ethanolic leaf extracts of *Colocasia esculenta* and *Vernonia amygdalina* were evaluated against α-amylase and α-glucosidase activity. For α-amylase and α-glucosidase inhibition, the extracts from both plants showed maximum inhibition at a concentration of 1000 µg/mL. The percentage inhibition for *Colocasia esculenta* was 59.8% (α-amylase) and 56.5% (α-glucosidase). The percentage inhibition for *Vernonia amygdalina* was 69.2% (α-amylase) and 70.5% (α-glucosidase).

Figure 3.3 reveals that *Colocasia esculenta* and *Vernonia amygdalina* demonstrated considerable inhibitory activity against the enzyme α-amylase.

Figure 3.4 reveals that *Colocasia esculenta* and *Vernonia amygdalina* demonstrated considerable inhibitory activity against the enzyme α-glucosidase.

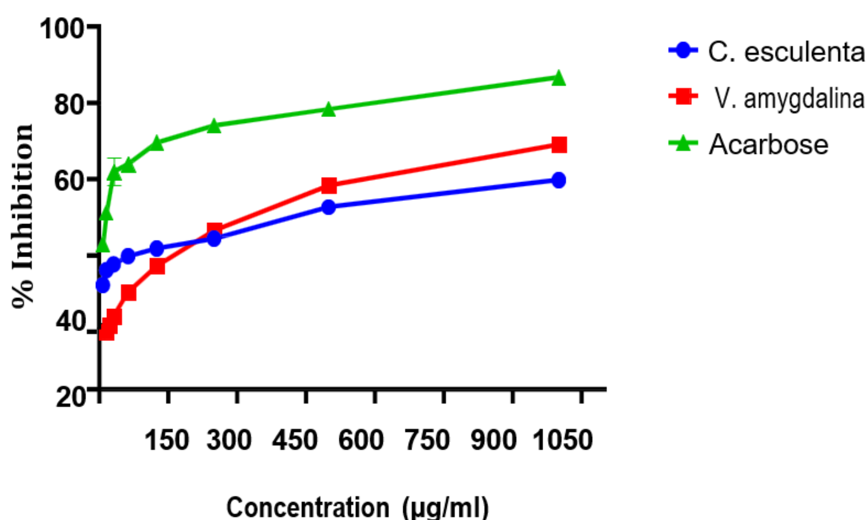

Figure 3.3: α-amylase inhibition

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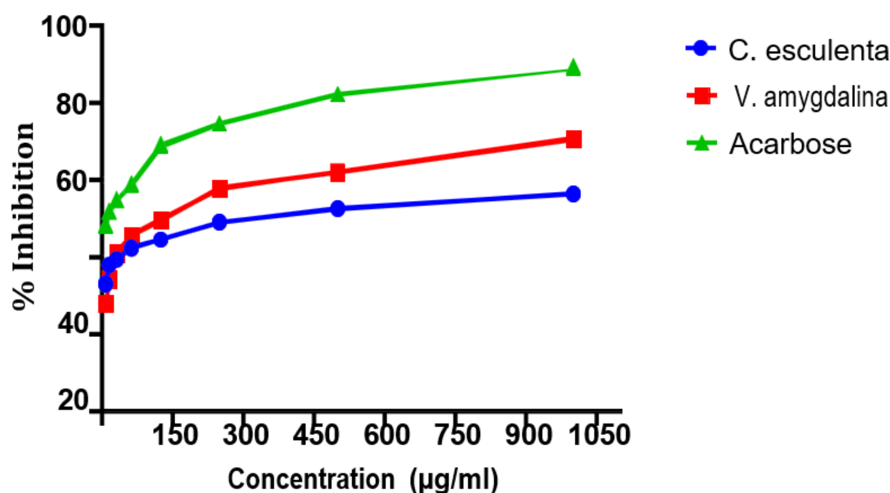

Figure 3.4: α-glucosidase inhibition

Table 3.6: The IC50 values of the Antidiabetic Activity Assays of the extracts of *Colocasia esculenta*, *Vernonia amygdalina* and Acarbose.

	Colocasia esculenta **(µg/ml)**	***Vernonia amygdalina*** **(µg/ml)**	**Acarbose (µg/ml)**
α-amylase	260.34±0.91	205.10±0.89	14.87±0.34
α-glucosidase	257.03±0.23	195.94±0.87	15.12±0.51

Results are expressed as mean ± SD (n=2)

3.1.5 Neuroprotective activity assays -in vitro

As illustrated in the graphs of Figures 3.5 and 3.6, the ethanolic leaf extracts of *Colocasia esculenta* and *Vernonia amygdalina* were evaluated against acetylcholinesterase and butyrylcholinesterase activity. For acetylcholinesterase and butyrylcholinesterase inhibition, the extracts from both plants showed maximum inhibition at a concentration of 1000 µg/mL. The percentage inhibition for *Colocasia esculenta* was 63.4% (acetylcholinesterase) and 68.6% (butyrylcholinesterase). The percentage inhibition for *Vernonia amygdalina* was 70.9% (acetylcholinesterase) and 74.2% (butyrylcholinesterase).

Figure 3.3 reveals that *Colocasia esculenta* and *Vernonia amygdalina* demonstrated substantial inhibitory activity against the enzyme acetylcholinesterase.

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Figure 3.4 reveals that *Colocasia esculenta* and *Vernonia amygdalina* exhibited significant inhibitory activity against the enzyme butyrylcholinesterase.

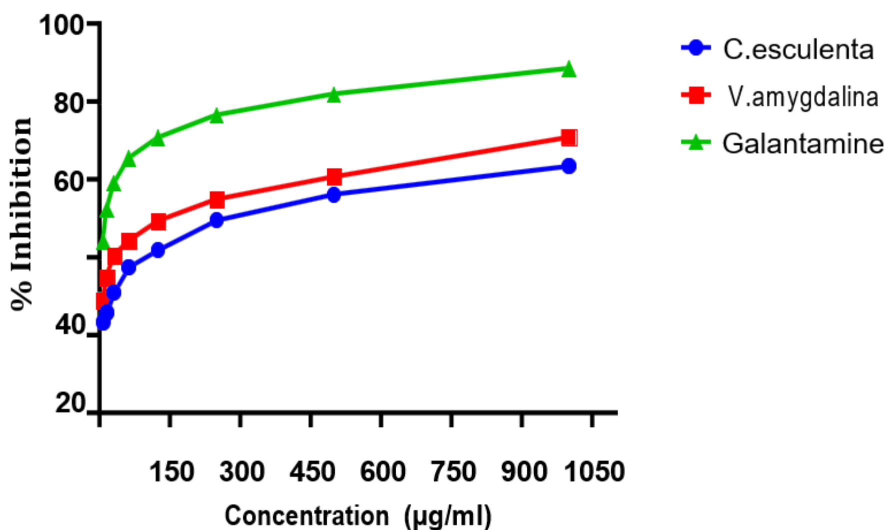

Figure 3.5: Acetylcholinesterase inhibition

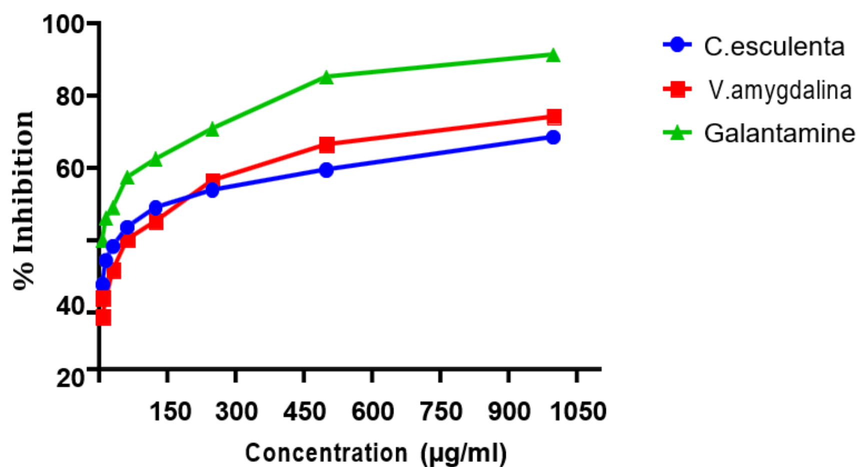

Figure 3.6: Butyrylcholinesterase inhibition

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Table 3.7: The IC50 values of the Neuroprotective Activity Assays of the extracts of *Colocasia esculenta*, *Vernonia amygdalina* and Galantamine.

	Colocasia esculenta **(µg/ml)**	***Vernonia amygdalina*** **(µg/ml)**	**Galantamine (µg/ml)**
Acetylcholinesterase	262.95±0.97	214.48±0.95	14.95±0.5
Butyrylcholinesterase	246.2±0.82	226.98±0.94	6.76±0.35

Results are expressed as mean ± SD (n=2)

3.1.6 In silico and molecular docking analysis

Table 3.8 reveals the post-docking analyses of the identified compounds from *Colocasia esculenta* showed that Chlorogenic acid had the highest binding affinity when docked at the binding site of α-amylase (PDB ID: 4GQQ).

Table 3.9 reveals that chlorogenic acid had the highest binding affinity when docked at the binding site of α-glucosidase (PDB ID: 5NN5).

Table 3.10 reveals that orientin had the highest binding affinity for acetylcholinesterase (PDB ID: 4M0E).

Tables 3.11, 3.12 and 3.13 show the post-docking analyses of the identified compounds from *Vernonia amygdalina,* which revealed that Luteolin and Vernomygdin had the highest binding affinity when docked at the binding site of α-amylase (PDB ID: 4GQQ), α- glucosidase (PDB ID: 5NN5), and acetylcholinesterase (PDB ID: 4M0E).

Figure 3.7 shows the post-docking analysis, which revealed the formation of six hydrogen bonds represented by the green dotted lines in the interaction between chlorogenic acid and α-amylase.

Figure 3.8 shows the post-docking analysis, which revealed the formation of four hydrogen bonds, an amide- π stacked, and one π-alkyl bond represented by the pink and light pink dotted lines, respectively, in the interaction between chlorogenic acid and α-glucosidase.

Figure 3.9 shows the post-docking analysis which revealed the formation of four hydrogen bonds, an amide- π stacked, and one π-alkyl bond represented by the pink and light pink dotted lines respectively in the interaction between orientin and acetylcholinesterase.

Figure 3.10 shows the post-docking analysis which revealed the formation of one π- π stacking, one π-alkyl bond, and an unfavourable donor-donor represented by the pink, light pink and deep red dashed lines respectively in the interaction between luteolin and α- amylase.

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Figure 3.11 shows the post-docking analysis which revealed the formation of four hydrogen bonds one π- π interaction (stacking) and one π-alkyl bond depicted by the green, pink, and light pink dashed lines respectively in the interaction between luteolin and α-glucosidase.

Figure 3.12 shows the post-docking analysis which revealed the formation of three hydrogen bonds, one π- π stacking and one π-alkyl bond represented by the green, pink, and light pink dashed lines respectively in the interaction between luteolin and acetylcholinesterase.

Figure 3.13 shows the post-docking analysis which revealed the formation of one hydrogen bond and two π-alkyl bonds represented by green, and light pink dashed lines respectively in the interaction of vernomygdin and α-amylase.

Figure 3.14 shows the post-docking analysis which revealed the formation of three hydrogen bonds, and two π-alkyl bonds in the interaction between vernomygdin and α-glucosidase.

Figure 3.15 shows the post-docking analysis which revealed the formation of one hydrogen bond represented by the green dashed line, one π-σ bond represented by the violet dashed line and two π-alkyl bonds represented by light violet dashed lines in the interaction between vernomygdin and acetylcholinesterase.

Table 3.8: The binding energies of some identified compounds from *Colocasia esculenta* extracts with α-amylase.

Name	Binding Energy(kcal/mol)
Chlorogenic acid	-6.5
Orientin	-6.3
Luteolin	-6.1
Taraxerol	-6.0
Vitexin	-5.9
Apigenin	-5.8
Catechin	-5.7
Sitosterol	-5.6
Anthocyanidin	-5.4
Gallic acid	-4.6
Vicenine	-4.5

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Beta-carotene	-4.4
Cinnamic acid	-4.2
Oxalic acid	-2.7
Iso-orientin	3.1
Isovitexin	13.1

Table 3.9: The binding energies of some identified compounds from *Colocasia esculenta* extracts with α-glucosidase.

Name	Binding Energy(kcal/mol)
Chlorogenic acid	-6.5
Luteolin	-6.2
Orientin	-6.0
Taraxerol	-5.9
Catechin	-5.8
Apigenin	-5.7
Vitexin	-5.6
Anthocyanidin	-5.4
Beta-carotene	-4.8
Gallic acid	-4.5
Vicenine	-4.3
Cinnamic acid	-4.2
Sitosterol	-4.2
Oxalic acid	-2.7
Isovitexin	2.3
Iso-orientin	3.3

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Table 3.10: The binding energies of some identified compounds from *Colocasia esculenta* extracts with acetylcholinesterase.

Name	Binding Energy(kcal/mol)
Orientin	-6.6
Luteolin	-6.3
Chlorogenic acid	-6.2
Taraxerol	-6.1
Apigenin	-6.0
Catechin	-5.9
Anthocyanidin	-5.5
Sitosterol	-5.4
Vitexin	-5.3
Beta-carotene	-4.4
Gallic acid	-4.3
Vicenine	-4.1
Cinnamic acid	-3.9
Oxalic acid	-2.6
Isovitexin	-2.4
Iso-orientin	-2.0

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Table 3.11: The binding energies of some identified compounds from *Vernonia amygdalina* extracts with α-amylase.

Name	Binding Energy(kcal/mol)
Luteolin	-6.3
Vernomygdin	-5.9
Vernodalin	-5.8
Garcinoic acid	-5.5
Vernodalol	-5.1
Benzophenone	-4.8
Ascaridol	-4
Myrtenol	-3.9
Myrtenal	-3.8

Table 3.12: The binding energies of some identified compounds from *Vernonia amygdalina* extracts with α-glucosidase.

Name	Binding Energy(kcal/mol)
Luteolin	-6.3
Vernomygdin	-6
Vernodalin	-5.8
Garcinoic acid	-5.5
Vernodalol	-5
Benzophenone	-4.8

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Ascaridol	-4.1
Myrtenol	-4
Myrtenal	-3.8

Table 3.13: The binding energies of some identified compounds from *Vernonia amygdalina* extracts with acetylcholinesterase.

Name	Binding Energy(kcal/mol)
Luteolin	-6.4
Vernomygdin	-6.1
Vernodalin	-5.8
Garcinoic acid	-5.4
Vernodalol	-5.2
Benzophenone	-4.8
Ascaridol	-4.5
Myrtenol	-4.2
Myrtenal	-4

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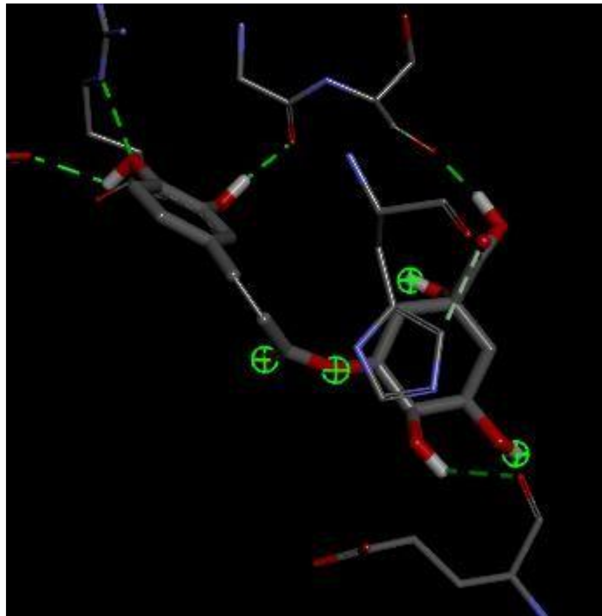

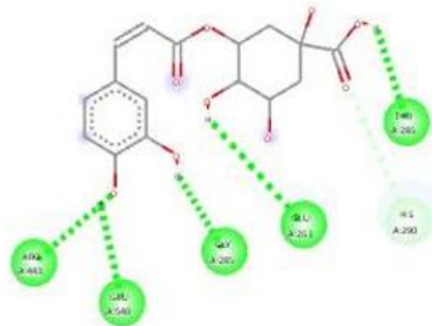

Figure 3.7: 2D receptor-ligand interaction of Chlorogenic acid (*Colocasia esculenta*) in the binding site of the receptor of α-amylase (PDB ID: 4GQQ).

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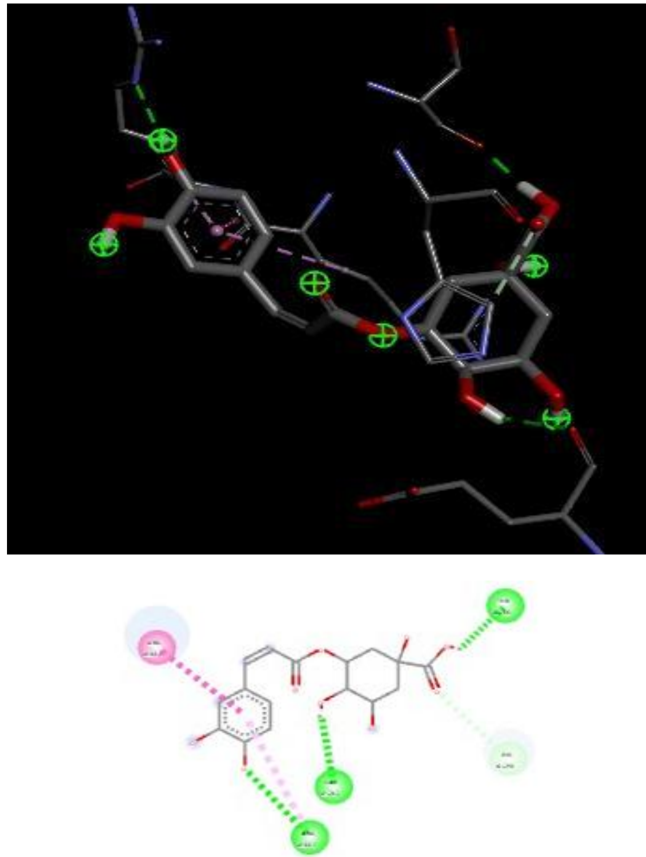

Figure 3.8: 2D receptor-ligand interaction of Chlorogenic acid (Colocasia esculenta) in the binding site of the receptor of α-glucosidase (PDB ID: 5NN5).

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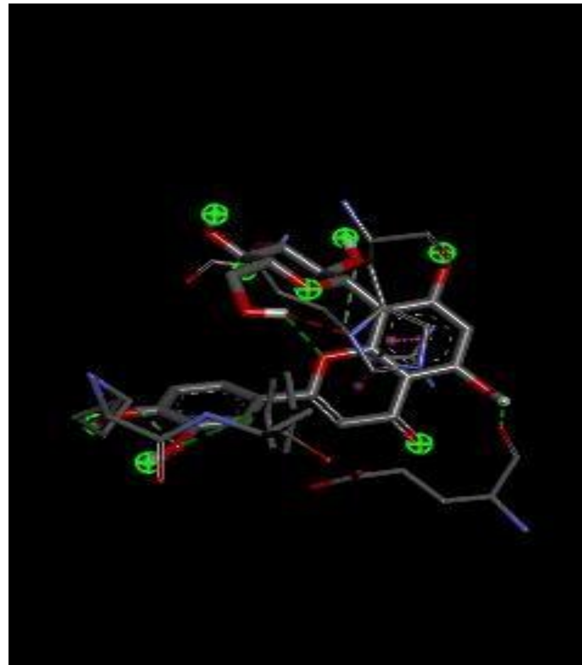

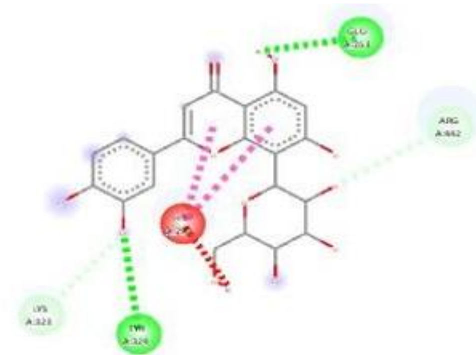

Figure 3.9: 2D receptor-ligand interaction of Orientin (*Colocasia esculenta*) in the binding site of the receptor of acetylcholinesterase (PDB ID: 4M0E)

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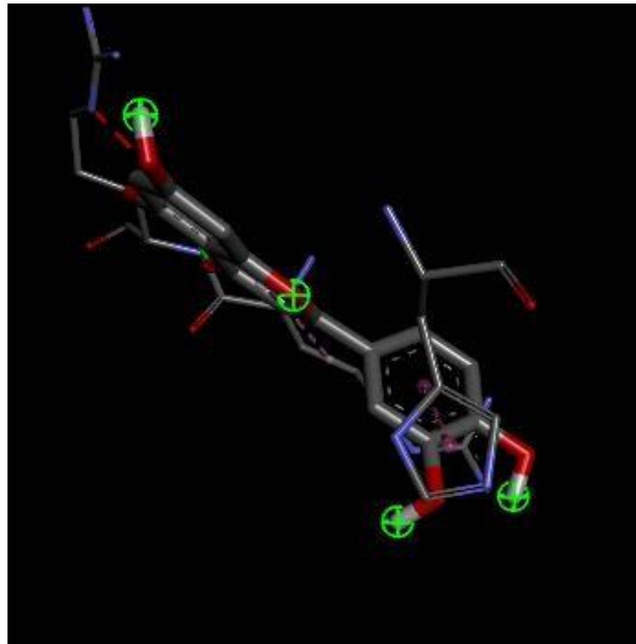

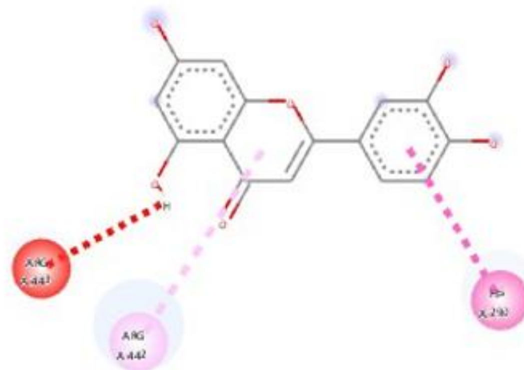

Figure 3.10: 2D receptor-ligand interaction of luteolin (*Vernonia amygdalina*) in the receptor's binding site of α-amylase (PDB ID: 4GQQ)

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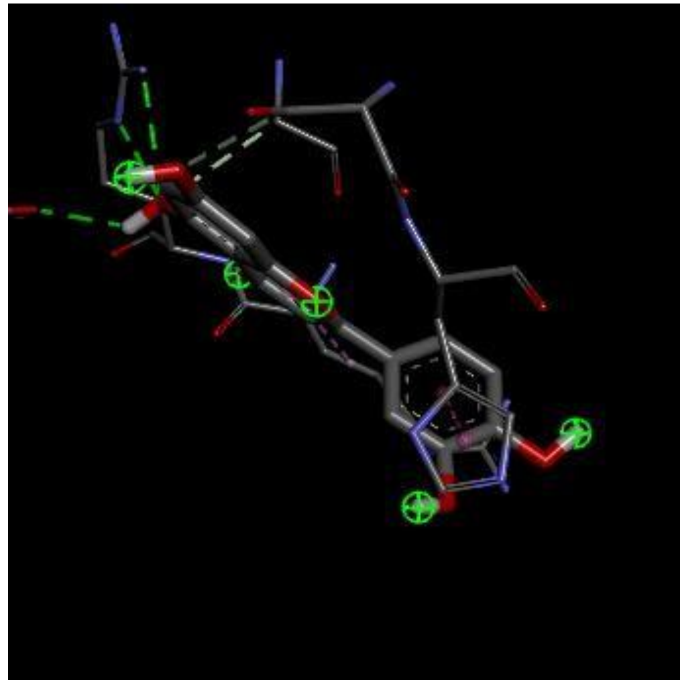

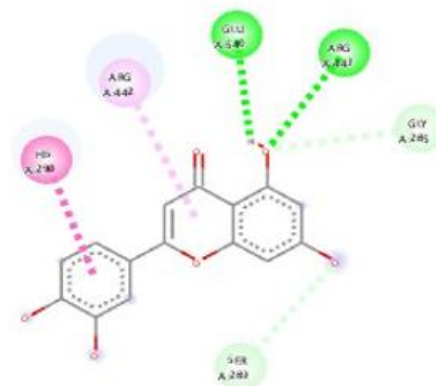

Figure 3.11: 2D receptor-ligand interaction of luteolin (*Vernonia amygdalina*) in the receptor's binding site of α-glucosidase (PDB ID: 5NN5)

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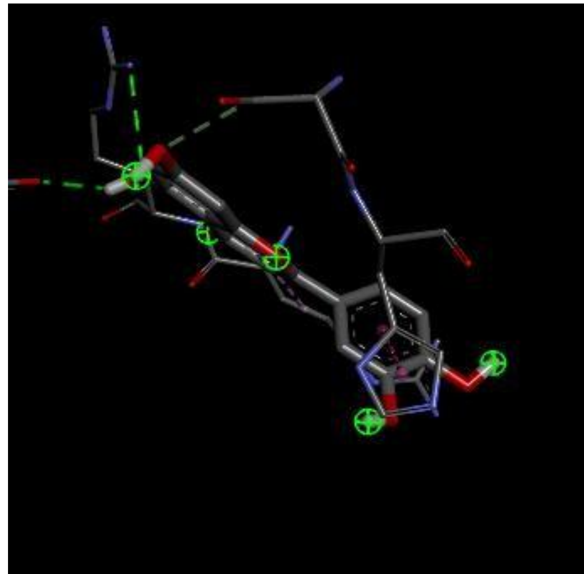

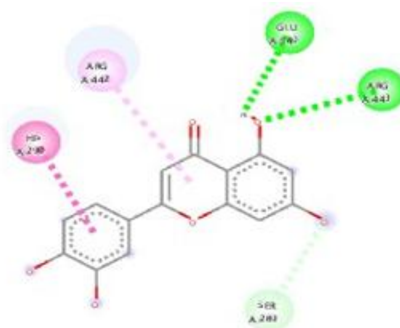

Figure 3.12: 2D receptor-ligand interaction of luteolin (*Vernonia amygdalina*) in the binding site of the receptor of acetylcholinesterase (PDB ID: 4M0E)

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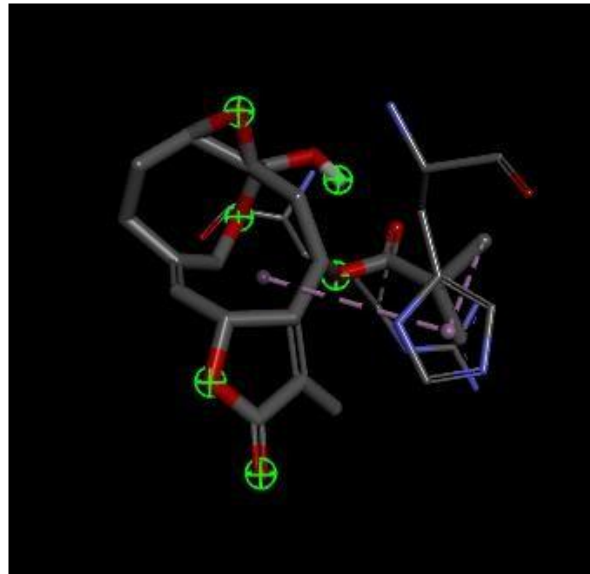

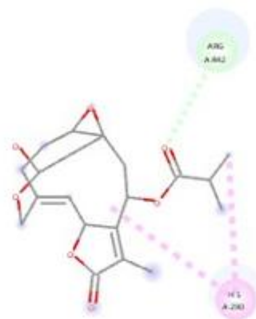

Figure 3.13: 2D receptor-ligand interaction of vernomygdin (*Vernonia amygdalina*) in the receptor's binding site of α-amylase (PDB ID: 4GQQ)

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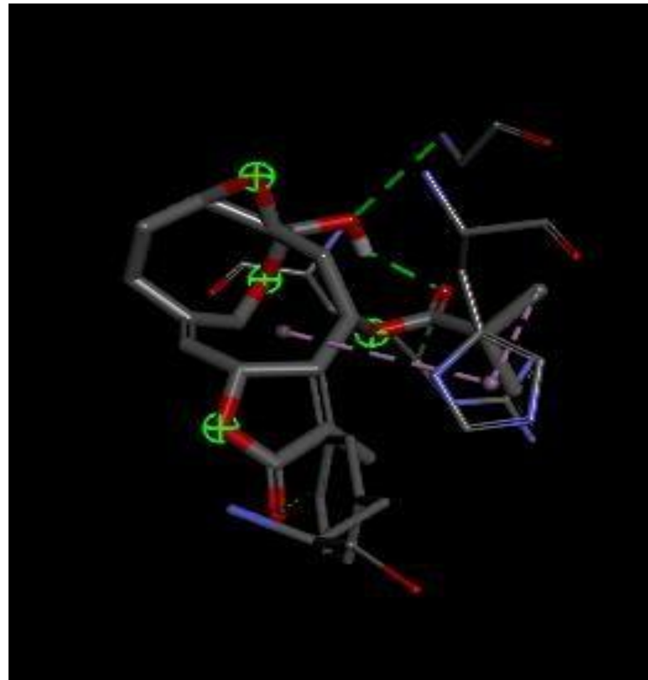

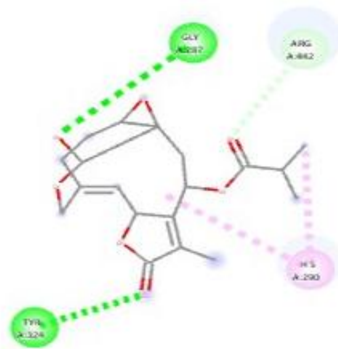

Figure 3.14: 2D receptor-ligand interaction of vernomygdin (*Vernonia amygdalina*) in the receptor's binding site of α-glucosidase (PDB ID: 5NN5)

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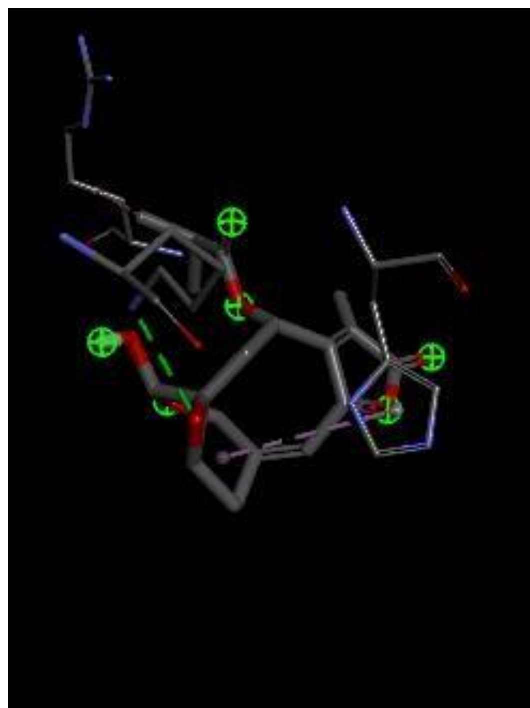

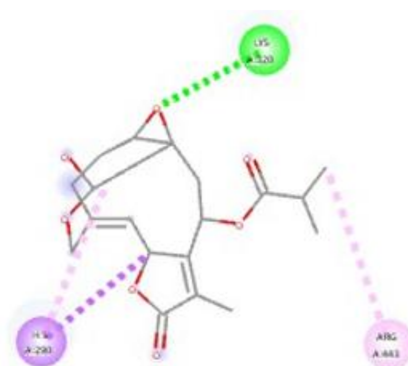

Figure 3.15: 2D receptor-ligand interaction of vernomygdin (*Vernonia amygdalina*) in the receptor's binding site of acetylcholinesterase (PDB ID: 4M0E)

3.1.7 Drug likeness assessment

The druggable attributes of some of the identified compounds from *Colocasia esculenta* and *Vernonia amygdalina* were evaluated using the Data Warrior software.

Table 3.14 shows the identified compounds from *Colocasia esculenta*: Chlorogenic acid, Orientin, Oxalic acid, Beta-carotene and Catechin all possess physicochemical properties that would make them orally active drugs in humans.

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Table 3.15 shows the identified compounds from *Vernonia amygdalina*: Luteolin, Vernomygdin, Vernodalin, Garcinoic acid, and Vernodalol all possess physicochemical properties that would make them orally active drugs in humans.

Table 3.14: Some druggable compounds identified from *Colocasia esculenta* extracts according to Lipinski's rule of five

Name	Total Molweight (Daltons)	cLogP	Stereo Centers	Rotatable Bonds	H-Acceptors	H-Donors
Chlorogenic acid	354.31	-0.7685	4	5	9	6
Orientin	448.379	-0.4237	5	3	11	8
Luteolin	286.238	1.99	0	1	6	4
Taraxerol	426.726	7.3406	8	0	1	1
Vitexin	432.38	-0.078	5	3	10	7
Apigenin	270.239	2.3357	0	1	5	3
Catechin	290.27	1.5087	2	1	6	5
Sitosterol	414.715	7.8552	9	6	1	1
Anthocyanidin	207.251	3.3665	0	1	1	0
Gallic acid	424.451	6.1512	0	6	5	1

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Table 3.15: Some druggable compounds identified from *Vernonia amygdalina* extracts according to Lipinski's rule of five

Name	Total Molweight (Daltons)	cLogP	Stereo Centers	Rotatable Bonds	H-Acceptors	H-Donors
Luteolin	286.238	1.99	0	1	6	4
Vernomygdin	364.393	1.1106	6	3	7	1
Vernodalin	360.361	0.8315	5	5	7	1
Garcinoic acid	426.595	8.0874	1	10	4	2
Vernodalol	392.403	1.1199	5	8	8	2
Benzophenone	182.221	2.7896	0	2	1	0
Ascaridol	168.235	2.3961	2	1	2	0
Myrtenol	152.236	1.7943	2	1	1	1
Myrtenal	286.238	1.99	0	1	6	4

3.2 Discussion

3.2.1 Qualitative and quantitative phytochemical screening of *Colocasia esculenta* and *Vernonia amygdalina* leaf extracts

The research study aimed to analyze the presence and concentrations of phytochemicals in the extracts obtained from leaves of *Colocasia esculenta* (cocoyam) and *Vernonia amygdalina* (bitter leaf). Both of these plants are recognized for their potential therapeutic effects in managing chronic diseases. The phytochemical quantification of the extracts (Table 3.1) revealed higher total flavonoid contents in CE (37.58±0.06 mg GAE /100 g) than total phenolic contents (6.62±0.2 mg GAE/100 g) and higher total phenols in VA (63.77±0.04 mg GAE/100 g) than total flavonoid contents (57.37±0.4 mg GAE /100 g).

These bioactive compounds, such as alkaloids, flavonoids, saponins, phenols, and tannins, have been extensively studied for their medicinal properties. These compounds have shown potential in developing herbal formulations with minimal side effects compared to conventional synthetic methods for treating various diseases, including cancer, diabetes mellitus and neurodegenerative diseases (Ali *et al*., 2022). These phytochemicals have been reported to possess pharmacological benefits and applications.

3.2.2 HPLC analysis

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The HPLC analysis of *Colocasia esculenta* and *Vernonia amygdalina* crude extracts revealed sixteen and twelve bioactive compounds, respectively.

In *Colocasia esculenta*, the bioactive compounds identified were Chlorogenic Acid, Gallic Acid, Oxalic Acid, B-carotene, Catechin, Cinnamic Acid, Sitosterol, Anthocyanidin, Apigenin, Luteolin, Taraxerol, Iso-Orientin, Orientin, Isovitexin, Vitexin, and Vicenin (Table 3.3).

In *Vernonia amygdalina*, the bioactive compounds identified were Benzophenone, Garcinoic acid, Vernodalin, Vernodalol, Vernonioside A, Vernonioside B, Vernomygdin, Luteolin, Myrtenal, Garanal, Ascaridol, Myrtenol (Table 3.4)

The Phytochemical investigations on the *C. esculenta* leaf extracts by Khare (2007) showed that they contained flavones, apigenin, luteolin, and anthocyanins. The leaves of the plant also contain fibers, calcium oxalate, minerals and starch, Vitamin A, B, C, etc. (Pawar *et al*., 2007)

In this investigation, compounds from *C. esculenta,* such as Catechin, Iso-orientin, Orientin, Vitexin, and Vicenine, were recognized as flavonoids, while Chlorogenic acid and Gallic acid are categorised as phenolic compounds.

The bioactive compounds in *V. amygdalina,* such as Garcinoic acid, Myrtenal, Myrtenol, and Ascaridol, are generally classified as terpenoids. While Vernodalin, Vernodalol, and Vernomygdin are generally classified as Sesquiterpenes.

Several research studies have emphasized the pharmacological advantages of both plants, including their antioxidant, antitumor, anti-inflammatory, anti-diabetic, and antihypertensive properties. The evaluation of bioactive compounds in medicinal plants was initiated by the World Health Organization's endorsement of traditional plant-based treatments for different illnesses, considering their easy accessibility, cost-effectiveness in cultivation, and minimal side effects (W.H.O., 2023). Understanding the composition of these compounds can help in establishing the correct dosages by leveraging the known effects of similar compounds. This knowledge can also aid in predicting the toxicity levels of the bioactive compounds.

3.2.3 Antioxidant screening of the *Colocasia esculenta* and *Vernonia amygdalina* leaf extracts

The free radical scavenging capacity of *Colocasia esculenta* and *Vernonia amygdalina* leaf extracts was evaluated based on their ability to scavenge DPPH free radicals. The results (Figure 3.1) of the DPPH free radical scavenging assay showed that *Vernonia amygdalina* had a higher antioxidant capacity than *Colocasia esculenta* compared with BHT, a synthetic antioxidant used as a control. This could be due to higher amounts of phenol and flavonoid content present in VA than in CE (Table 3.2), which contain more OH groups and unsaturated centres in their structural components. This structural feature enables them to donate a proton to the DPPH radical, thereby counteracting

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its effects (Chakraborty *et al*., 2015). The free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to a colourless ethanol solution. The use of DPPH assay provides an easy and rapid way to evaluate the antioxidant capacity of the extracts by spectrophotometry.

ABTS percentage inhibitory activity of both plant extracts showed that *Vernonia amygdalina* had a higher antioxidant capacity than *Colocasia esculenta,* compared with BHT, a synthetic antioxidant used as a control (Figure 3.2). This could be due to higher amounts of phenol and flavonoid content present in VA than in CE. Flavonoids are good at ABTS radical scavenging due to several key structural and chemical properties such as hydroxyl groups, conjugation and double bonds, planarity, chelation ability and redox properties. The presence of hydroxyl groups on the flavonoid structure, particularly in the B-ring, enhances their ability to provide hydrogen atoms or electrons, which are essential for neutralizing free radicals like ABTS (2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid).

Vernonia amygdalina's low IC50 values (Table 3.5) indicate its effective inhibitory activity at low concentrations, which is relatively close to the standard BHT.

3.2.4 Antidiabetic activity Assays - in vitro

Colocasia esculenta and *Vernonia amygdalina* exhibited significant inhibitory activity against α-amylase (Figure 3.3), with VA showing a higher significant inhibition when compared to CE, but both extracts showed lower inhibitory activity compared to the standard control acarbose.

Colocasia esculenta and *Vernonia amygdalina* exhibited significant inhibitory activity against α-glucosidase (Figure 3.4), with VA showing a higher significant inhibition when compared to CE, but both extracts showed lower inhibitory activity compared to the standard control acarbose.

Phytochemicals that are known to inhibit α -amylase and α-glucosidase, enzymes involved in carbohydrate digestion, include flavonoids such as luteolin; tannins, phenolic acids such as chlorogenic acid, anthocyanins such as cyanidin, alkaloids, and terpenoids such as beta- carotene, and taraxerol. Furthermore, research has linked polyphenols and their ability to donate hydrogen atoms (H^+) to their significant role in inhibiting the enzymes α-glucosidase and α-amylase. (Kalita *et al*., 2018). These phytochemicals inhibit α-amylase and α- glucosidase by binding to the active sites of the enzymes, thereby reducing their ability to hydrolyse starches and disaccharides into glucose. This can help manage postprandial blood glucose levels, making these compounds of interest for diabetes management (Poovitha and Parani, 2016).

3.2.5 Neuroprotective activity assays -in vitro

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Colocasia esculenta and *Vernonia amygdalina* exhibited significant inhibitory activity against acetylcholinesterase (Figure 3.5), with VA showing a significantly higher inhibition

when compared to CE, both extracts showed lower inhibitory activity compared to the standard control galantamine.

Colocasia esculenta and *Vernonia amygdalina* exhibited significant inhibitory activity against butyrylcholinesterase (Figure 3.6), with VA showing a higher significant inhibition when compared to CE, but both extracts showed lower inhibitory activity compared to the standard control galantamine.

Phytochemicals that inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) include alkaloids, flavonoids, terpenoids, tannins, and phenols (Gray *et al*., 2018). Acetylcholinesterase is the primary cholinesterase found in the brain, responsible for breaking down acetylcholine. It exhibits a greater level of specificity towards acetylcholine during the hydrolysis process. These phytochemicals inhibit AChE and BChE by binding to the active sites of these enzymes, preventing the breakdown of acetylcholine and butyrylcholine, which can help enhance cholinergic transmission and have potential therapeutic effects for conditions like Alzheimer's disease.

3.2.6 In silico and molecular docking analysis

Table 3.8 shows post-docking analyses of α-amylase protein in complex with chlorogenic acid, which had the highest binding energy of all the compounds identified in CE.

Figure 3.7 indicates the interaction of chlorogenic acid with the protein target (α-amylase). This revealed the formation of six hydrogen bonds: ARG443, GLU540, GLY285, GLU261, THR286 and HIS290.

Table 3.9 shows post-docking analyses of α-glucosidase protein in complex with chlorogenic acid, which had the highest binding energy.

Figure 3.8 indicates the interaction of chlorogenic acid with the protein target (α- glucosidase). This revealed four hydrogen bonds: ARG443, THR286, GLU261, HIS290 and amino acid interactions with one Amide-Pi stacked ARG442.

Table 3.10 shows post-docking analyses of acetylcholinesterase in complex with orientin, which had the highest binding energy.

Figure 3.9 indicates the receptor-ligand interaction of orientin with the protein target (acetylcholinesterase). This revealed the formation of four hydrogen bonds: GLU261, ARG442,

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LYS323, TYR324, and amino acid interactions Pi-Pi stacked HIS290, including an unfavourable donor-donor (Two hydrogen bond donors being in proximity and do not facilitate hydrogen bonding)

Tables 3.11 and 3.12 show the post-docking analyses of α-amylase and α-glucosidase proteins in complex with luteolin, which had the highest binding energy with both proteins, respectively identified in VA.

Figure 3.10 indicates the receptor-ligand interaction of luteolin with protein target (α- amylase), revealing the formation of one Pi-Pi stacked HIS290, one Pi-alkyl bond ARG442 and an unfavourable donor-donor.

Figure 3.11 indicates the receptor-ligand interaction of luteolin with protein target (α- glucosidase). This revealed the formation of four hydrogen bonds, GLU540, ARG443, GLY285, SER283, one Pi-alkyl bond ARG442, and one Pi-Pi stacked HIS290.

Table 3.13 shows the post-docking analyses of acetylcholinesterase protein in complex with luteolin, which had the highest binding energy of all the compounds identified in VA, compared to all the compounds identified in VA when docked with acetylcholinesterase.

Figure 3.12 indicates the receptor-ligand interaction of luteolin with a protein target (acetylcholinesterase). This revealed the formation of three hydrogen bonds: GLU540, ARG443, SER289; one Pi-Pi stacked HIS290, and one Pi-alkyl bond ARG442.

Figure 3.13 shows the receptor-ligand interaction of vernomygdin with α-amylase. This revealed the formation of one hydrogen bond, ARG442 and two pi-alkyl bonds, HIS290 and HIS290.

Figure 3.14 shows the receptor-ligand interaction of vernomygdin with α-glucosidase. This revealed the formation of three hydrogen bonds, TYR324, GLY287, ARG442 and two pi-alkyl bonds, HIS290 and HIS290.

Figure 3.15 shows the receptor-ligand interactions of vernomygdin with acetylcholinesterase. This revealed the formation of one hydrogen bond, LYS320, pi-sigma bond HIS290 and two pi-alkyl bonds ARG443 and HIS290.

Molecular docking plays a crucial role in creating and refining new medications. It involves the precise prediction of the way a potential drug molecule will bind to its target protein, as well as estimating the strength of this interaction. This process is essential for developing effective and targeted drugs (Pinzi and Rastelli, 2019).

With the recent developments in computer science and the exponential growth of structural, chemical, and biological data available on an ever-increasing number of therapeutic targets, the use

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of in silico techniques such as chemo-informatics, molecular modelling, and artificial intelligence (AI) has increased dramatically over the past few decades (Jorgensen, 2004; Macalino *et al*., 2015).

3.2.7 Drug Likeness Assessment

As shown in Tables 3.14 and 3.15, compounds from *Colocasia esculenta*: Chlorogenic acid, Orientin, Oxalic acid, Beta-carotene, Catechin, and Compounds from *Vernonia amygdalina*: Luteolin, Vernomygdin, Vernodalin, Garcinoic acid, Vernodalol all possess chemical and physical properties predicting their potential as orally active drugs in humans indicating their potential as leads for drug development. For a drug or compound to be considered as orally active in humans it must have no more than one violation of the following: one more than five hydrogen donors, ten hydrogen acceptors, molecular weight less than 500 Daltons, and cLogP less than five. Using molecular descriptors and physicochemical properties, straightforward rules and predictive models can help prioritize the oral activity of molecules from chemical libraries (Di and Kerns, 2003).

The first qualitative method used to direct the creation of "orally accessible" compounds is Lipinski's "rule of five," which is predicated on the limits on characteristics (clogP, molecular weight, and number of hydrogen-bond donors and acceptors) below which oral activity is expected to be subpar (Lipinski *et al*., 1997).

4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

The HPLC analysis affirms that *Colocasia esculenta* leaf extracts contain sixteen phytochemicals, which include: chlorogenic acid, gallic acid, apigenin, oxalic acid, luteolin, taraxerol, catechin, beta-carotene, sitosterol, cinnamic acid, vitexin, isovitexin, orientin, isoorientin, and anthocyanidin, with apigenin, a flavonoid compound, having the highest concentration with peak height at 154.96m. *Vernonia amygdalina* leaf extracts contain twelve phytochemicals, which include: benzophenone, garcinoic acid, vernodalin, vernodalol, vernomygdin, vernonioside A and B, luteolin, myrtenal, myrtenol, ascaridol and garanal, with a phenolic compound having the highest concentration with peak height at 15.69m.

The study evaluated the in vitro antioxidant potential and antidiabetic and neuroprotective activities of both plant extracts. Results confirm that the extracts possess antioxidant properties when subjected to DPPH and ABTS scavenging assays, with *V. amygdalina* exhibiting a higher antioxidant potential between the two samples when compared with BHT, a synthetic antioxidant used as control.

The plant extracts have been found to have antidiabetic and neuroprotective properties. This is demonstrated by their ability to inhibit the enzymes α-amylase, α-glucosidase,

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acetylcholinesterase, and butyrylcholinesterase. *V. amygdalina* exhibits higher inhibitory activity than C. esculenta, and both plant extracts exhibited lower inhibitory activity when compared to the known standard drugs acarbose and galantamine.

Furthermore, the study also reveals that chlorogenic acid and orientin were identified as the most druggable compounds from *C. esculenta,* and luteolin was identified as the most druggable compound isolated from *V. amygdalina*. Therefore, the findings suggest that these compounds are mostly responsible for the inhibitory activities exhibited by the plant extracts, indicating their potential for drug development.

4.2 Recommendation

The identified compounds from both extracts, chlorogenic acid, orientin, (CE) and luteolin and vernomygdin (VA), which possessed the most druggable attributes, and had the highest binding energies with the target proteins, can be further studied as target hits against these proteins α-amylase and α-glucosidase in diabetes and acetylcholinesterase and butyrylcholinesterase in neurodegenerative diseases.

The isolation of the compounds chlorogenic acid and orientin from *Colocasia esculenta* leaf extracts and luteolin and vernomygdin from *Vernonia amygdalina* should be carried out

Further studies should be conducted to confirm the plant extracts' antidiabetic and neuroprotective effects in vivo.

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APPENDICES

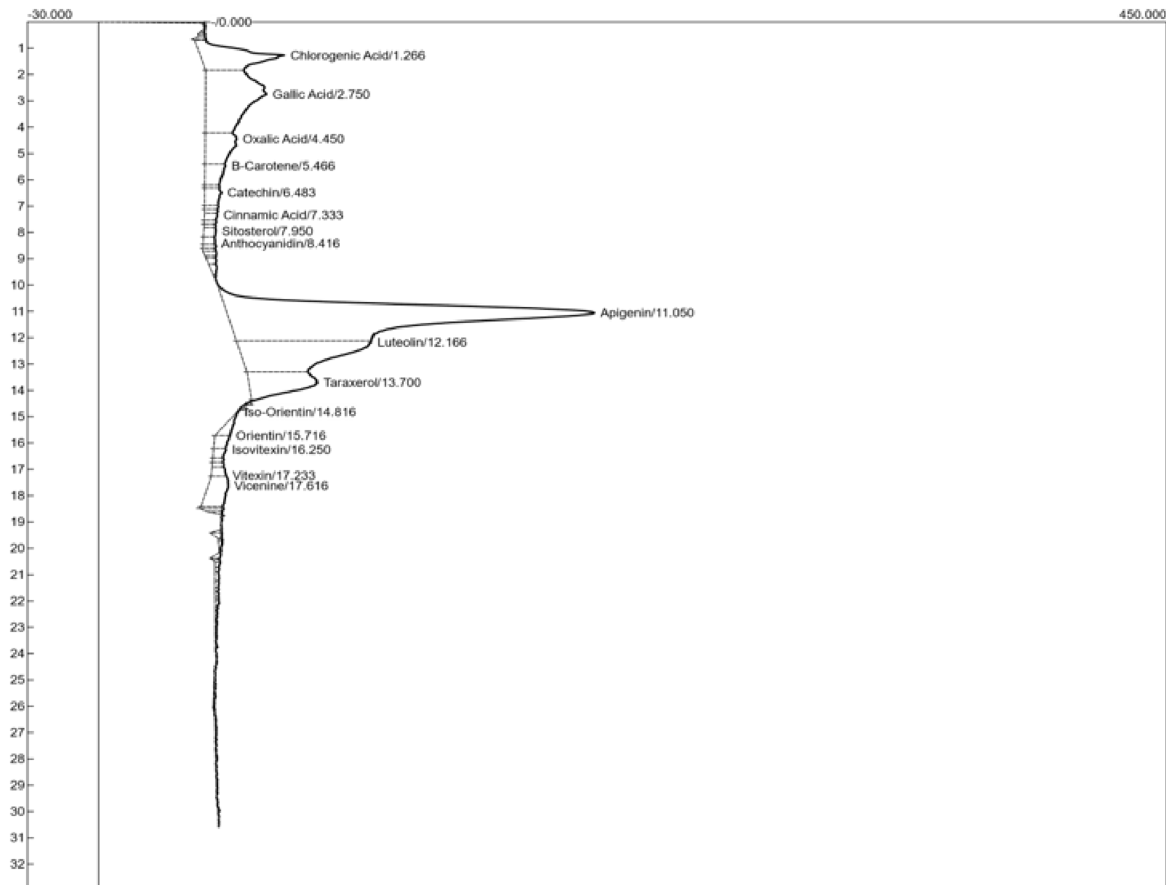

Figure 1: HPLC Chromatogram of The Ethanolic Extracts of *Colocasia Esculenta*

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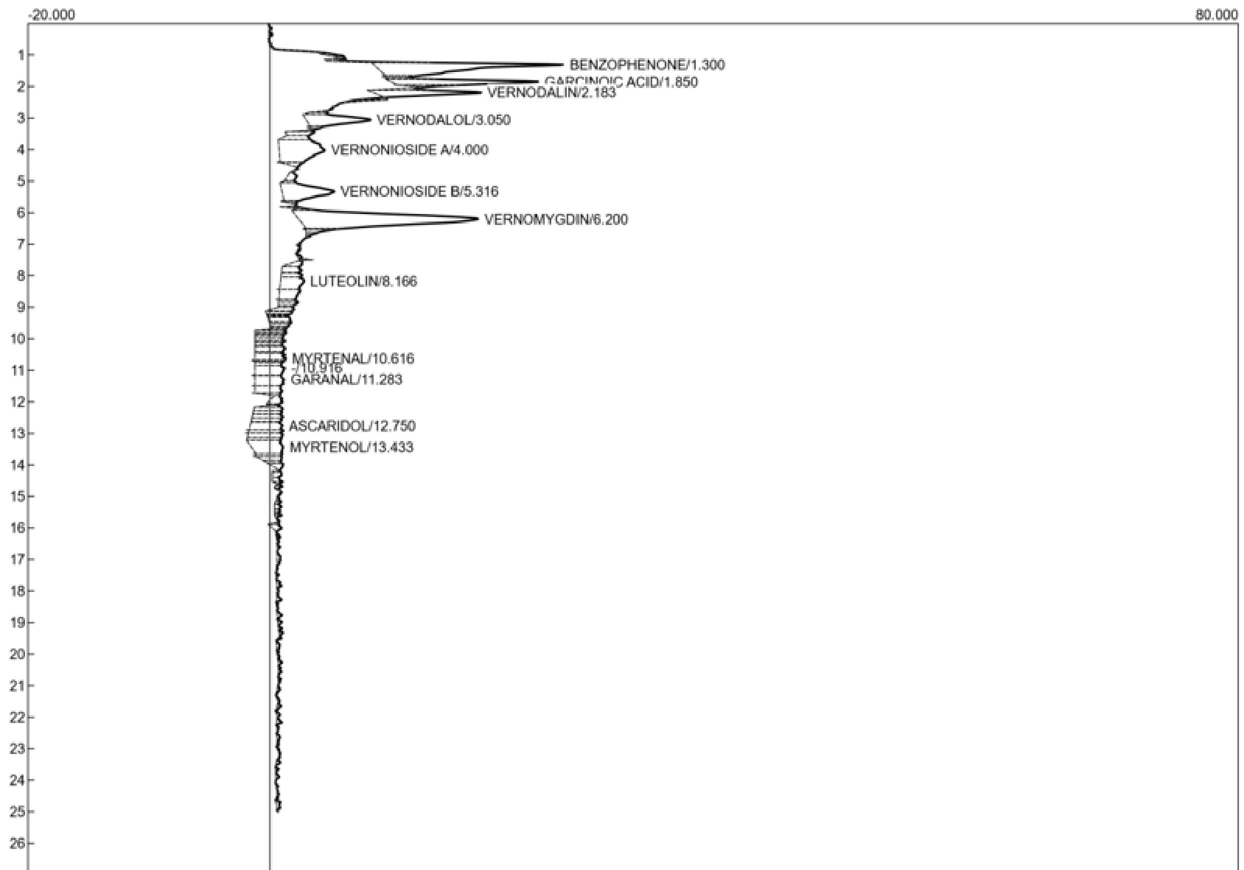

Figure 2: HPLC Chromatogram of The Ethanolic Extracts of *Vernonia amygdalina.*

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