MOLECULAR DOCKING AND BIOLOGICAL ACTIVITY STUDIES OF GUAVA LEAF POWDER

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ABSTRACT

CADD encompasses a diverse array of theoretical and computational techniques integral to contemporary drug discovery processes. These methods have played pivotal roles in the development of pharmaceuticals currently in clinical application or undergoing clinical trials. There are many phytochemicals found in guava leaves and have beeninvestigated for their potential health advantages. It includes kaempferol, hyperin, myricetin, gallic acid, epicatechin, catechin, epigallocatechin gallate, chlorogenic acid, and caffeic acid. The biological properties of guava leaf extracts, such as their hepatoprotective, antibacterial, anticancer, antidiabetic, antioxidant, and antidiarrheal properties, have been investigated. Critical discussion is also extended to the GL extract diverse bioactivities. Here we aim to find out the anti-inflammatory

propertiesof ethanolic extract of *Psidium gujava leaves* powder by GC-MS screening of volatileactive constituents and performing molecular docking of the constituents against various anti-inflammatory proteins using AutoDockVina. The method used to identify potential anti-inflammatory activity in plant compounds is molecular docking. Molecular docking is a molecular modelling technique used to predict how proteins (enzymes) interact with small molecules. Molecular docking in drug development is included in the SBDD group by utilizing information from the target protein structure to find the protein & active site that binds to the drug compound. The prediction of the best active site is expected to create a bond between the compound and the target protein to form expected biological activity.

INTRODUCTION

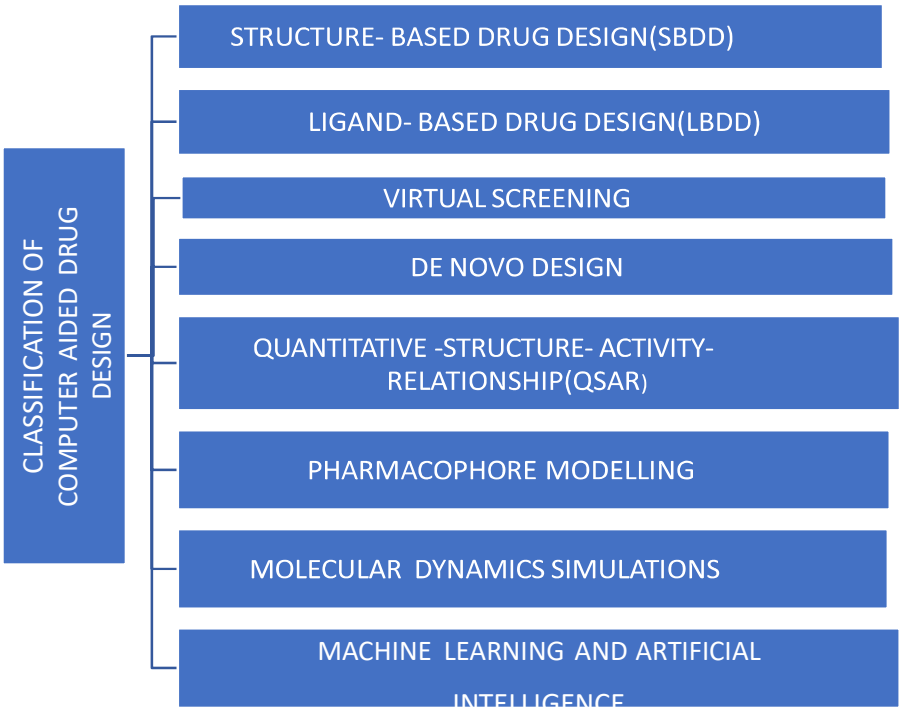
COMPUTER AIDED DRUG DESIGN

Here's a breakdown of the key components of CADD:

- Computational Chemistry: This involves the application of computational methods to understand and predict the chemical properties and behaviour of molecules. It includes techniques such as quantum mechanics, molecular mechanics, and molecular dynamics simulations.
- Molecular Modelling: It is used to generate three-dimensional models of molecules, such as proteins and ligands. These models are then analysed to understand their structure, interactions, and potential binding sites.
- Molecular Design: Molecular design involves the generation of new molecules with desired properties, such as potency, selectivity, and bioavailability. This can be achieved through techniques like de novo design, fragment-based design, and structure-based design.
- Rational Drug Design: Rational drug design is an approach that uses the knowledge of the biological target and its interactions with ligands to design new drugs. This can involve virtual screening, where large libraries of compounds are screened computationally to identify potential drug candidates.

Molecular modelling and computational chemistry are assuming an increasingly important role in understanding the basis of drug receptor interactions and assisting the medicinal agent in the design of new therapeutic agent. Computer graphics has emerged as a cost-effective tool and adequate computational power isnow available, which removes limitations that have crippled computational chemistry. This advances the development of software tools for probing the 3D aspect of specificity.

Classification



Drugs Discovered Using CADD

* Aliskiren	* Oseltamivir	* Zanamivir	* Dorzolamide
* Rupintrivir	* Saquinavir	* Nolatrexed	* Captopril

Applications

- Lead Identification and Optimization
- ADME-Tox Prediction
- Virtual Screening
- Prediction of Drug-Target Interactions
- Repurposing Existing Drugs [1]

ACTIVITIES OF GUAVA LEAF POWDER

Anti-Inflammatory Action

Traditional practices have utilized guava to address gastrointestinal and respiratory issues, as well as inflammation. Numerous studies have delved into its medicinal properties, particularly focusing on its anti-inflammatory and analgesic attributes, primarily through leaf extracts. While research predominantly examines leaf extracts, there are also investigations into the anti-inflammatory and antinociceptive effects of guava fruits. However, despite these efforts, the precise mechanisms through which P. guajava exerts its effects and the signalling pathways involved remain elusive.

Existing knowledge links guava's biological properties to its phenolic compounds, which include protocatechuic, ferulic, ascorbic, gallic, and caffeic acids, as well as quercetin. These compounds likely contribute to guava's medicinal efficacy and underscore its potential as a therapeutic agent. The anti-inflammatory action of guava leaves is attributed to several bioactive compounds present in them, particularly phenolic compounds such as flavonoids and phenolic acids.

These compounds exert their anti-inflammatory effects by inhibiting inflammatory mediators and pathways in the body. For example, flavonoids like quercetin have been shown to inhibit the production of inflammatory cytokines and enzymes, thereby reducing inflammation. Phenolic acids such as gallic acid and caffeic acid also exhibit anti-inflammatory properties by modulating signalling pathways involved in inflammation. Studies have demonstrated the effectiveness of guava leaf extracts in reducing inflammation in various experimental models. These extracts have been shown to alleviate inflammation in conditions such as arthritis, gastritis, and respiratory disorders. Additionally, guava leaf tea or extract is often used topically to soothe skin inflammation and irritation. Overall, the anti-inflammatory action of guava leaves highlights their potential therapeutic value in managing inflammatory conditions and underscores the importance of further research to elucidate their mechanisms of action and optimize their use in healthcare.

Myricetin, quercetin, and kaempferol had the strongest inhibitory effects against α-glucosidase and α-amylase; however, a clear synergistic effect was also observed. Glycosides are necessary for the inhibitory function to be performed. When rabbits were fed ethanol extracts made from guava leaves, their serum triglyceride and low-

density lipoprotein levels were significantly reduced, but their high-density lipoprotein levels were somewhat relieved.

Hepatoprotective

Rats treated with guava leaf extract showed increased activity of both PPARα and adenosine monophosphate-activated protein kinase (AMPK), which are essential for liver lipid metabolism. Furthermore, the extracts from guava leaves may help reduce hepatic insulin resistance. The liver's ability to function is linked to the enzymes aspartate aminotransferase (AST) and alanine transaminase (ALT). Elevations in these markers are indicative of fatty liver, which guava leaf extract may help to limit. Furthermore, because the liver's major job is to stabilize blood glucose levels, it has been discovered that diabetes is closely linked to hepatic dysfunction, including liver enlargement, steatosis, and fibrosis. The bioactive substances found in guava leaves, guaijaverin and avicularin, are strong inhibitors of the glucose transporter 4 (GLUT4) and dipeptidyl-peptidase IV, respectively, which are responsible for increasing blood glucose levels. In rats with type 2 diabetes mellitus, treatment with guava leaf extract that increased flavonoid levels increased insulin resistance and inhibited the rise in glucose and fat levels.

Antimicrobial

Bioactive substances originating from plants are a promising source of antimicrobials. These substances function by preventing the growth, disruption, and lysis of microbial cell walls; they also hinder the formation of biofilms; suppress DNA replication and transcription; obstruct the production of adenosine triphosphate (ATP); suppress bacterial toxins; and produce reactive oxygen species (ROS). Strong antibacterial effects against Pseudomonas aeruginosa, Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, and Bacillus subtilis are demonstrated by the essential oils extracted from guava leaves.

Phenolic acids, flavonoids, terpenoids, glycosides, and saponins were found in both the aqueous and organic extracts of guava leaves, and their abundance is positively connected with antibacterial action. HPLC-TOF-ESI/MS examination of fermented Gallic acid, chlorogenic acid, rutin, isoquercitrin, avicularin, quercitrin, kaempferol,

morin, and quercetin were all found in guava leaves. These substances have the ability to suppress glucosamine, a marker of fungal cell development, and ergosterol, a component of the fungal cell membrane. Likewise, tannins soluble in water found in guava leaves have bacteriostatic properties. Their modes of action include inhibiting extracellular enzyme synthesis, preventing oxidative phosphorylation, and retaining substratum. They have been shown to have an inhibitory effect on Staphylococcus aureus clinical isolates resistant to antibiotics. Quercetin was shown to be one of the most abundant flavonoids in guava leaves with the highest pharmacological activity by Hirudkar et al. in another investigation. Furthermore, triterpenoids were linked to action against bacterial and fungal infections, with a minimum inhibitory concentration (MIC) of 0.79 μg/mL, a minimum bactericidal concentration of 51 μg/mL, and a respectable antifungal activity with a minimum inhibitory concentrationof 12.6 μg/mL, a methanolic extract of guava leaves showed antibacterial activity against E. coli. The function of Interleukin-7 (IL-7) has a well-established role in bolstering the immune system to combat microbial infections. Extracts from guava leaves are thought to affect intestinal mucosal cells, promote the production of IL-7, and support the growth of B and T cells.

Anti-diarrhoeal Activity

It is believed that guava leaves have antidiarrhoeal qualities. The antidiarrheal properties of ethanolic guava leaf isolates in Wistar rats were documented by Mazumdar et al. In rats fed castor oil, the authors found that extracts at dosage levels of 750 and 500 mg/kg exhibited antidiarrhoeal potential. In addition, Ojewole et al. observed comparable effects in rodents when they used aqueous extracts of guava leaves. They observed that oral administration of guava leaf extracts at doses ranging from 52 to 410 mg/kg was found to prevent diarrhoea, slow intestinal transit, and cause dilatory evacuation of undesirable gastric contents. In a dose-dependent manner, the guava leaf extracts decreased symptoms associated with diarrhoea, including interstitial fluid secretion and faecal dropping wetness. In the duodenum of rabbits given varying concentrations of guava leaves, there was concentration- dependent pulsing and pendulum retreatments. Water extracts from guava leaves havebeen shown to have antidiarrheal properties by Dewi et al. in another investigation. Green tea leaf extract (T) and guava leaf water extract (G) were combined by the authors. The extracts were applied to such as (G: T) 112.5:110.55, (G: T) 75:221.1,

and (G: T) 37.5:331.65 mg/kg body weight, among other combinations. The results, which showed improved stool weight, stool onset, stool consistency, and diarrhoea period, demonstrated that all combinations had strong antidiarrheal effects.

Antioxidant Activity

The existence of phenolic substances, including taxifolin, pyrocatechol, and gallic acid, ellagic acid, ferulic acid, and a few other compounds are in charge of guava leaves antioxidant properties. The results of guava leaf extracts high-performance liquid chromatography study showed the Quercetin, hesperetin, kaempferol, quercitrin, rutin, catchin, and apigenin are the seven primary flavonoids that are present; additional bioactive substances such kaempfertin, isoquinoline, and corilaginoline alkaloids have also been found which contributes to give them their antioxidant qualities. Further investigations on Guava Leaves extract demonstrated a scavenging effect on peroxyl radicals and a reduction in linoleic acid oxidation. The study also demonstrated a linear relationship between the phenolic content of the guava leaf extract and the antioxidant's potency and capacity to scavenge free radicals. Guava leaves extracts at 4000 ppm or more can stop fresh pork sausages from oxidizing, which suggests that they could be used as a functional food additive.

Anti Diabetic Activity

Guava Leaves flavonoids and polysaccharides have been linked to potential antidiabetic effects in a number of research. The function of β-cells in pancreatic islets and hepatocyte morphology were significantly improved by Guava Leaves extract's guaijaverin and avicularin flavonoids in mice with diabetes. Guaijaverin inhibited blood glucose homeostasis action. Avicularin prevented intracellular lipid aggregation by obstructing glucose uptake by GLUT-4 in vitro and showed no discernible toxicity,in contrast to the enzyme dipeptidyl-peptidase IV for adipose cells 3T3-L1. Guava Leaves polysaccharides (GLPs) were isolated by Luo et al., and further the antidiabetic effects were next examined in combination with streptozotocin-induced diabetic mice with a diet heavy in fat. The scientists found that GLP was linked to a significant decrease in fasting blood glucose, triglycerides, creatinine, glycated serum protein, and total cholesterol. Elevated postprandial glucose concentrations can result from suboptimal glycaemic control. According to Nair et al., α-amylase and α-

glucosidase inhibitors have the potential to reduce postprandial glucose absorption, making them viable targets for diabetes treatment.

Anti-Cancer/Anti-Tumour Activity

Triterpenoids, sesquiterpenes, tannins, psiguadials, volatile oils, flavonoids, benzophenone glycosides, and other quinones can all be found in good amounts in guava leaves. The anticancer effects of terpenoids and flavonoids found in guava leaves are demonstrated via immune system regulation, inhibition of signal transfer and tumour cell adhesion, and obstruction of tumour angiogenesis and cell proliferation imply that these leaves have a strong inhibitory effect on cancer cell lines related to colon, prostate, nasopharyngeal, breast, and cervical cancer.

The primary process by which colorectal cancers grow is angiogenesis. Long-term angiogenesis is essential to the growth of tumours towards malignancy because blood arteries effectively provide oxygen and essential chemicals to the tumour cells as they develop. Rich in vitamin E, β-caryophyllene, and flavonoids (apigenin), guava leaf extracts shown potent antiproliferative action against cell lines from human colon cancer. β-caryophyllene interacts with the transcription factor HIF-1α, which controls the biological processes linked to hypoxia, tumour metastasis, and tumour-mediated angiogenesis. Furthermore, in the presence of β-caryophyllene, HIF-1α mediates the transcription of vascular endothelial growth factor (VEGF), which explains why guava leaf extract has antiangiogenic and anti-colorectal cancer properties.

According to the authors, guajadial works as an anticancer agent. via inhibiting DNA synthesis, suppressing the cell cycle during the G1 phase, and activating estrogenic receptors to induce apoptosis. Benzophenones, guavinoside B and guavinoside E, derived from guava leaves were shown in a related investigation to suppress the development of HCT116 human colon cancer cells. These substances changed the expression of important proteins involved in apoptotic signalling and cell proliferation in addition to potently inducing apoptosis in cancer cells.[2]

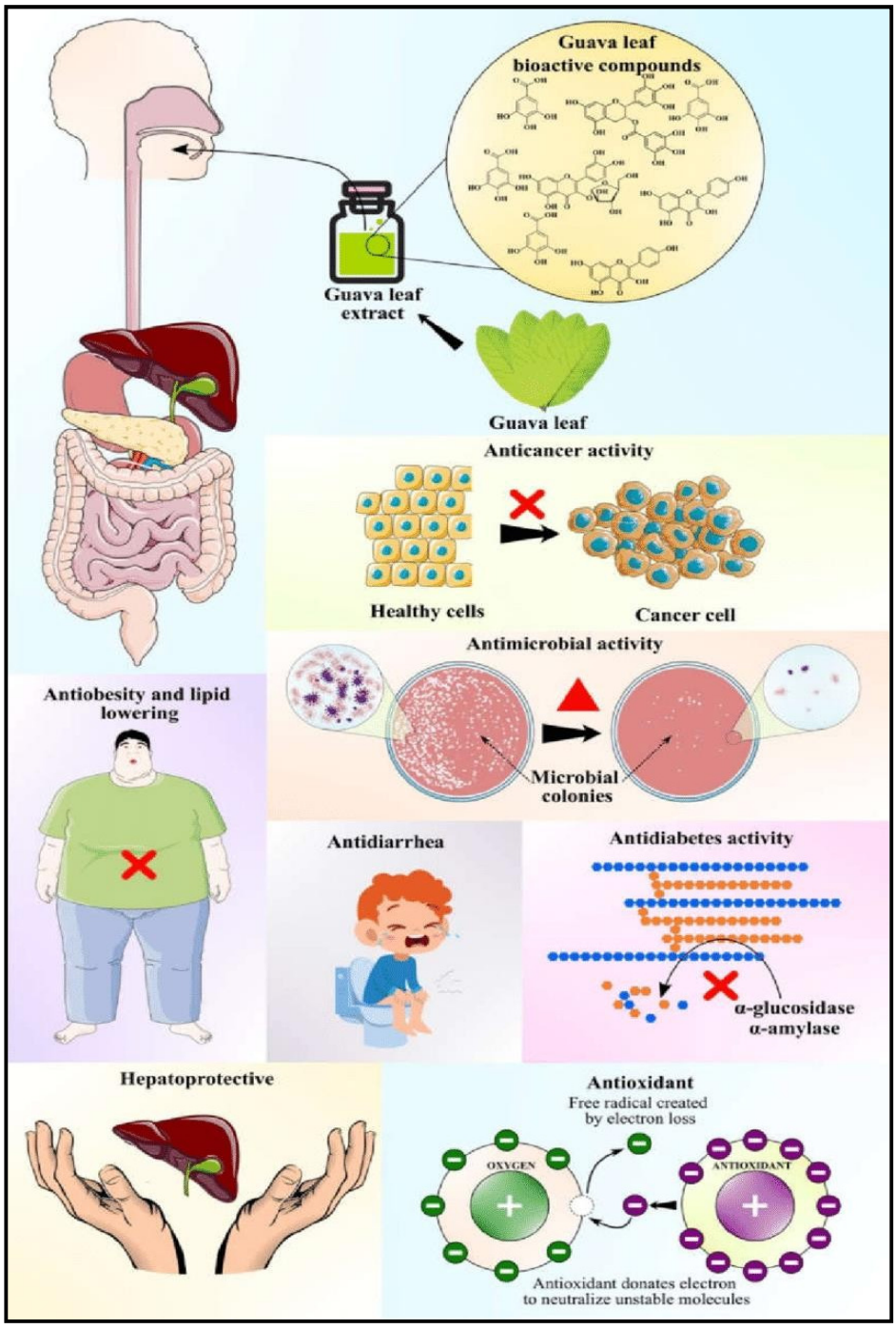


Fig.No. 1: Activities of Guava Leaves Powder

GC-MS ANALYSIS

The powerful analytical method known as gas chromatography-mass spectrometry (GC-MS) is used to detect and quantify a wide range of chemical substances. Gas chromatography's great separation power and mass spectrometry's sensitive and focused detection powers are combined in GC-MS.

Principle

The two separate procedures of gas chromatography (GC) and mass spectrometry (MS) are key to the principles of GC-MS.

Gas Chromatography (GC): Using the principle of gas chromatography, components of a mixture can be separated according to how they are distributed between a stationary phase and a mobile phase. An inert gas, such as nitrogen or helium, serves as the mobile phase in GC, transporting the sample along a stationary phase-filled column. A liquid or solid that is immobilized on a solid support can be the stationary phase. Different components elute at different times in the sample due to interactions between the components and the stationary phase as it moves through the column. The retention time is the point in time at which a component elutes from the column.

Mass spectrometry (MS): One method for determining the mass-to-charge ratio (m/z) of ions in a sample is mass spectrometry. A mass analyser is then used to separate the ionized sample according to their m/z ratio. In GC-MS, quadrupole, ion trap, and time-of-flight (TOF) mass analysers are the most often utilized mass analysers. After the ions have been separated, they are found and recorded, resulting in a mass spectrum that shows the relative abundance of each ion as well as the mass-to-charge ratio.

GC-MS Instrumentation: The mass spectrometer, data system, and gas chromatograph make up the three primary parts of the GC-MS apparatus.

Gas Chromatograph: The components of a gas chromatograph are a column, a detector, and an injection port. The sample is introduced into the column via the injection port. Usually, a fused silica capillary covered in a stationary phase makes up the column.

Flame ionization detectors (FID), thermal conductivity detectors (TCD), and electron capture detectors (ECD) are the most often utilized detectors in GC.

Mass spectrometer: The mass spectrometer is made up of a detector, a mass analyser, and an ion source. As the separated components leave the GC column, they are ionized using the ion source. Electrospray ionization (ESI), chemical ionization (CI), and electron impact (EI) are the most often utilized ionization techniques in GC-MS. After that, the ionized components are added to the mass analyser and sorted according to their m/z ratio. The quadrupole, ion trap, and time-of-flight (TOF) mass analysers are the most often utilized mass analysers in GC-MS. A mass spectrum is produced by measuring each ion's abundance using the detector.

Sample Preparation

The general procedures for GC-MS sample preparation are as follows:

- **Sample collection:** Gather the sample and store it in a hygienic, airtight container suitable for the type of sample. Make that the container is stored in accordance with the recommended criteria and is appropriately labelled.
- **Sample extraction:** Use an appropriate extraction technique to remove the desired analytes from the sample matrix. Solid-phase extraction (SPE), liquid-liquid extraction (LLE), and solid-phase microextraction (SPME) are examples of common extraction procedures.
- **Sample cleanup**: The sample may require cleaning to get rid of any impurities that could interfere with the extraction process, depending on the sample matrix. This could entail methods like centrifugation, solid-phase extraction, or filtration.
- **Sample derivatization**: A large number of organic chemicals are too volatile for GC-MS analysis directly. To change them into more volatile forms, they must be derivatized. Trimethylsilyl (TMS) derivatives, methoxymethyl (MOM) derivatives, are common derivatization reagents.
- **Sample concentration**: To raise the concentration of the desired analytes, concentrate the sample. This could entail methods like solid-phase extraction, freeze-drying, or evaporation.

- **Sample reconstitution**: Put the sample back into a solvent that works well for the GC-MS equipment and the sample itself. The selection of the solvent ought to
  optimize the concentration of the target analytes in the sample while reducing the impact of other constituents.
- **Sample injection:** A suitable injection technique, such as split, split less, or on-column injection, should be used to inject the sample. The particularsample setup, the sample matrix, the relevant analytes, and the GC-MS instrument may all affect the steps.
- **Data system:** In GC-MS, the term "data system" refers to the hardware and software that operate the device, process, and evaluate the mass spectrometer data. Software for instrument control, data collecting, processing, and analysis is usually included in the data system.
- **Instrument Management:** The temperature profile of the GC column and the mass spectrometer's ionization mode are two examples of the experimental parameters that are set up using the instrument control software
- **Obtaining Data:** The mass spectrometer is managed by software to get mass spectra at predetermined intervals, usually milliseconds. The binary format used to save the raw data from the mass spectrometer is unique to the manufacturer of the device.
- **Data processing:** It is necessary to process the mass spectrometer's raw data in order to get meaningful details about the chemical components of the sample. The raw data is subjected to a number of processes by the data processing software, including peak integration, deconvolution, and baseline correction.
- **Data Analysis:** Following processing, the data can be examined to determine the concentration of each component of the sample. To determine the components of the sample, the data analysis program compares the mass spectra acquired from the sample with a database of mass spectra of recognized chemicals.[3]

MOLECULAR DOCKING

Molecular docking is a computational technique used in structure-based drug design to predict the preferred orientation and conformation of a small molecule (ligand) within the binding site of a biological target, typically a protein. The goal of molecular docking is to predict the binding mode and affinity of a ligand to its target, which can provide valuable insights for drug discovery and optimization. Here's how molecular docking works:

The construction of the ligand and receptor structures, the use of a suitable docking algorithm to search for potential binding modes, and the scoring of the resulting poses to predict binding affinity are the three basic phases in the molecular docking process.

Molecular Docking Method Types:

- Rigid-body docking: In this method, the receptor and the ligand are regarded as rigid entities. In order to find the ideal orientation and location, the ligand is docked onto the receptor site by rotating and translating it in three dimensions.
- Flexible docking: Both the ligand and the receptor are viewed as flexible entities in flexible docking. Allowing both the ligand and the receptor to go through conformational changes throughout the docking process allows the ligand to be docked into the receptor site.

Following steps are involved in molecular docking:

1. Target protein preparation: The target protein must be ready before molecular docking may begin. After obtaining the protein structure from the Protein Data Bank (PDB), it is ready for docking by eliminating any water molecules, co-crystallized ligands, or other heteroatoms. After that, any steric collisions or other undesirable interactions are eliminated from the protein structure by optimizing it using energy minimization techniques.

2. Ligand preparation: The method used to generate the target protein, is also used to prepare the ligand structure. Chemical databases are used to download the ligand, or chemical sketching software is used to prepare it. After that, any steric collisions or other undesirable interactions are eliminated from the ligand structure by optimizing it using energy minimization techniques.

3. Grid creation: Using the dimensions of the binding site and the size of the ligand, a grid is created around the protein's active site. This grid reduces the amount of time needed for computation during the docking process by providing a framework for the docking computations.

4. Docking calculations: Flexible docking and stiff body docking are the two primary categories of docking methods. Both the ligand and the protein are regarded as rigid bodies in rigid body docking; only the orientation is maximized for the ligand. Both the ligand and the protein are viewed as flexible entities in flexible docking, and the conformational changes in the ligand brought about by protein binding are also taken into consideration.

5. Analysis of docking results: To make sure the ligand establishes the right interactions with the protein active site and the top-ranked poses are chemically acceptable, they are visually examined. Additionally, the docking score is used to assess the ligand's binding affinity. The results are compared to available experimental data.[4]

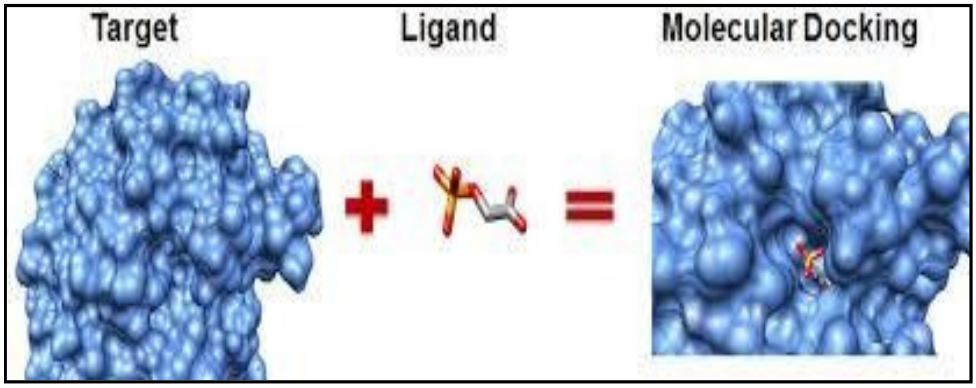


Fig.No.2: Molecular Docking

AIM & OBJECTIVES

Psidium guajava, commonly known as guava, indeed possesses a wide range of pharmacological properties attributed to its leaves such as antioxidant, anticancer, antidiabetic, and anti-inflammatory properties, further aiding its medicinal applications. There are several scientific reports on molecular docking studies and GC-MS screening of guava leaf powder.Here we aims to find out the anti- inflammatory properties of ethanolic extract of *Psidium gujava* leaves powder by GC-MS screening of volatile active constituents and performing molecular docking of theconstituents against various anti-inflammatory proteins using AutoDockVina.

Objectives

- To prepare the ethanolic extract of guava leaf powder by maceration.
- To determine the active constituents of ethanolic extract of the powder by phytochemical screening.
- To evaluate the physico-chemical parameters of the ethanolic extract.
- To determine the volatile active constituents using GC-MS analysis.
- To evaluate anti inflammatory activity of guava leaf powder by performing molecular docking using AutoDockVina.

DRUG PROFILE

The *Myrtaceae* family includes the guava tree, which is a highly special and traditional plant that is grown for its many nutritional and therapeutic uses. A variety of guava tree parts, including the roots, leaves, bark, stem, and fruits, have been used to treat a variety of illnesses, including diabetes, diarrhoea, and stomach-aches. The dark green, elliptical, oval leaves of the guava plant are distinguished by their obtuse-style apex. In addition to being used to treat gastrointestinal and respiratory conditions, guava leaves are also utilized to boost platelets in dengue fever patients. The antispasmodic, cough sedative, anti-inflammatory, antidiarrheal, antihypertensive, anti-obesity, and antidiabetic qualities of guava leaves are also commonly utilized.

Fig.No.3: Guava Leaves Powder

Fig.No. 4: Guava Leaves

Standardisation

It is the procedure used to guarantee the uniformity of potency, effectiveness, and quality in every batch. The main goal of standardization is to guarantee uniformity in quality. In order to achieve standardization, the chemical composition of the herbal constituents must be tested to make sure the proper proportions are present.

a)Moisture content: Moisture content is a critical factor influencing the stability, microbial activity, and shelf-life of guava leaf powder. Determining moisture content involves heating a measured quantity of the powder at a set temperature until a constant weight is achieved. This process helps in assessing the amount of water present in the powder which is essential for ensuring product quality and longevity.

a) Total ash: Total ash refers to the residue remaining after the complete incineration of guava leaf powder at high temperatures. Analysing total ash content provides insights into the purity of the product, as contaminants and foreign matter typically burn away during the incineration process. Higher total ash content may indicate a higher concentration of inorganic minerals in the leaf powder, which can be beneficial or detrimental depending on the intended use and quality standards.

b) pH: The pH of guava leaf powder is a significant parameter affecting both its stability and efficacy. Determining the pH involves dissolving a measured quantity of the leaf powder in distilled water and then using a pH meter to measure the acidity or alkalinity of the solution. The pH value provides valuable information about the chemical nature of the leaf powder, influencing its solubility, compatibility with other substances, and potential interactions with biological systems.[2]

MATERIALS AND METHODS

Apparatus and Reagents

200ml Beaker, Watch glass, Spatula, Glass rod, Ethanol

Preparation of Extract

The coarse powder is extracted using pure ethanol.

Pure ethanol extract

The 5gm powder is placed in 200ml beaker and poured 20ml pure ethanol and kept for 6 hours (Maceration method). The extract was filtered.

Preliminary Phytochemical Screening of Extract:

Chemical tests for alkaloids

A small portion of dried ethanolic extract was shaken (acidified) with dilute hydrochloric acid and filtered. The acidified filtrate was tested with the following reagents, to detect the presence of alkaloids.

Table No 1: Chemical tests for alkaloids

Test	Procedure
Mayer's test	The acidified extract (two ml) was treated with 1 ml of Mayer's reagent (potassium mercuric iodide), shaken and noted for the presence of a creamy precipitate.

Hager's Test	The acidified extract (two ml) was treated with 1 ml of Hager's reagent (saturated picric acid solution) and observed for the presence of yellow precipitate.
Dragendorff's test	The acidified extract was treated with a few ml of Dragendorff's reagent (Potassium bismuth iodide) and observed for the presence of orange precipitate.

Chemical tests for Glycosides

Table No.2: Chemical test for Anthraquinone glycosides

Test	Procedure
Borntrager's Test	Add to 1gm of drug, 5-10ml of Dil.HCl and boil on water bath for 10 min and filter.Extract the filtrate with CCl4/Benzene mixture and add an equal amount of NH4OH solution to the filtrate and shake. Formation of pink or red colour in the ammoniacal layer. It is due to the presence of Anthraquinone moiety.
Modified Bontrager's Test	To 1gm of drug add 5ml of Dil.HCl followed by 5ml of Ferric chloride solution. Boil for 10 min on a water bath, cool and filter and the resulting filtrate was extracted with CCl4 or Benzene and add to it an equal volume NH4OH.The appearance of pink to red colour is due to the presence of Anthraquinone moiety.

Table No.3: Chemical test for Saponin Glycosides

Test	Procedure
Foam Test	To 1gm of the crude drug add 10 to 20 ml of water (DW) and shake for few minutes. The critical appearance of a distinct frothing that eventually persists for 60 to 120 sec shows the presence of Saponins.

Table No.4: Chemical test for Steroidal and Triterpenoid Glycosides

Test	Procedure
Salkowaski Test	The alcoholic extract of the drug was duly evaporated to dryness and extracted with chloroform and add slowly with CON. H2SO4 along the sidewall of the test tube to the respective chloroform extract. Appearance of distinct yellow colored ring at the interface of 2 liquid that gradually becomes red after a lapse of 2 mins. The presence of Steroidal moiety confirmed.
Trichloroacetic acid Test [Cl3.COOH]	The triterpenes on the incorporation of a saturated solution of trichloroacetic acid results in to the formation of a coloured ppt.

Table No.5: Chemical test for Cardiac Glycosides

Test	Procedure
Keller KillianiTest	Approximately 5 ml of each plant extract was evaporated at 40°C and the residue

	was collected. A few mg of residue was suspended in 5 ml water. 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added to it. This solution was underplayed with 1 ml of conc. sulphuric acid. A brown ring at the interface indicated the presence of deoxy sugar, a characteristic of cardiac glycosides

Chemical tests for Carbohydrates

Extracts were dissolved individually in 5ml of distilled water and filtered. The filtrate was used for the following test.

Table No.6: Chemical tests for Carbohydrates

Test	Procedure
Molisch's Test	The filtrate 2 ml was treated with a few drops of Molisch'sreagent and 2 ml of concentrated sulphuric acid was addedthrough the sides of the test tube without shaking. Observed forthe presence of violet ring at the junction of two solutions.
Fehling's Test	The filtrate 1 ml treated with 1 ml each of Fehling's solutionA and B and boiled on a water bath for half an hour, thenobserved for the presence of red residue at the bottom of testtube.
Benedict'sTest	The filtrate was treated with 2 ml of Benedict's reagent. Thenthe mixture was heated on a boiling water bath for two

	min andthe presence of red precipitate was noted.

Chemical tests for Tannins

Table No.7: Chemical tests for Tannins

Test	Procedure
Lead acetate test	To the filtrate, 5ml of 10% lead acetatesolution was added. Formation of white ppt indicates the presence of tannin.
Ferric chloride test	A small quantity of the extract is taken in a test tube and diluted with water then treated with dilute ferric chloride solution (5%) and observed for the presence of blue color.

Chemical tests for Flavanoids

Table No.8: Chemical tests for Flavanoids

Test	Procedure
Aqueous sodiumhydroxide test	Aqueous sodium hydroxide solution was added to thefew ml of the extract and the presence of yellowcoloration of the solution was noted
Filter paper test	The filter paper was wetted with small quantity ofalcoholic solution of the extract. That filter paper wasexposed to ammonia vapours and noted the yellow colour.

Chemical tests for Proteins

Table No.9: Chemical tests for Proteins

Test	Procedure
Biuret Test	The extract (two ml) was treated with one drop of 2% copper sulphate solution. To this 1ml of 95% ethanol was added followed by excess of potassium hydroxide solution and Observed for the presence of violet colored solution.
Ninhydrin Test	The extract (few ml) was treated with two drops of ninhydrin solution and heated on a water bath and then the presence of violet colour was noted.
Xanthoprotic test	Extract was treated with few drops of conc. HNO3. Formation of yellow indicates the presence of proteins.

Physico-Chemical Parameters

ASHValues

Ash values in crude drugs serve several important purposes, including:

- Quality Assessment: They help to assess the presence of impurities, such as soil, sand, or other inorganic matter, which can affect the purity and efficacy of the material.
- Purity Evaluation: By measuring the amount of ash left after incineration, ash values help determine the purity of crude drugs. Higher ash content may indicate the presence of contaminants or adulterants, while lower ash content may suggest a higher degree of purity.

- Identity Verification: Ash values are used as one of the parameters to establish the identity of crude drugs. Different plant species or plant parts have characteristic ash values, and comparing the obtained values with standard references helps confirm the identity of the material.

Total Ash Value

It is the entire amount of material that is still present after burning. This comprises both "non-physiological" ash, which is the leftover material from the extraneous matter, and "physiological ash," which is formed from the plant tissue itself.

Procedure: Weigh the crucible and note the result to four decimal places. Put around 2 g of weight into the crucible to four decimal places, record the weight. Ash sample for two hours at 600°C, Once room temperature is reached, cool in a desiccator and weigh soon after. Analyze the ashed sample's weight and record it to four decimal places.[11]

GC-MS Analysis

A GC Clarus 500 Perkin Elmer system with an AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer equipment was used for the GC-MS analysis using the following guidelines:

- This column, an Elite-1 fused silica capillary column was operated in electron impact mode at 70 eV.
- The injector temperature was 250^0C, and the ion-source temperature was 280ºC
- Helium gas was used as the carrier gas at a constant flow of 1 ml/min, with an injection volume of 0.5 µI.
- Programed temperatures for the oven were 110^0C(isothermal for 2 minutes), 200^0C (10 ^{0}C/min), 280 ^{0}C (5 ^{0}C/min), and a 9-min isothermal at280 degrees Celsius.
- Mass spectra were obtained at 70 eV with pieces ranging from 40 to 450 Da and a scan interval of 0.5 seconds.

- The GC runs for a total of thirty-six minutes. Each component's relative percentage amount was determined by comparing its average peak area to the overall areas.
- TurboMass software was used to manage chromatograms and mass spectra.

Identification of components

Analysis of the mass spectrum GC-MS was performed using the National Institute of Standards and Technology's database (NIST). A comparison was made between the spectrum of the unknown component and the known components kept in the NIST collection. The names, molecular weights, and structure of the component of the test material can be determined.[12]

Table No.10: GC MS analysis of *Psidium guajava* leaves extract

Peak#	R. Time	Area %	Name	Molecular Formula	Molecular weight
1	3.582	0.37	Butanoic acid, 2-methyl-, methyl este	C6 H12 O2	116
2	8.436	0.57	dl-Limonene $$ Cyclohexene, 1-me	C10 H16	136
3	8.534	1.37	1,8-Cineole $$ 2-Oxabicyclo[2.2.2]	C10 H18 O	154
4	11.783	0.20	(E)-2,6-Dimethyl-5,7-octadien-2-ol	C10 H18 O	154
5	14.013	0.34	Cyclohexasiloxane, dodecamethyl-	C12 H36 O6 SI6	444
6	15.55	0.22	Alpha.-Copaene	C15 H24	204

	5				
7	16.776	19.76	Trans-Caryophyllene	C15 H24	204
8	17.793	2.44	Alpha.-Humulene	C15 H24	204
9	18.438	1.12	Germacrene D	C15 H24	204
10	19.115	7.48	Trans-alpha.-bisabolene	C15 H24	204
11	19.233	0.44	Aromadendrene 2 $$	C15 H24	204
12	19.351	9.75	Beta.-Bisabolene	C15 H24	204
13	19.949	1.19	Delta.-Cadinene	C15 H24	204
14	20.124	0.36	(-)-Endo-2,6-dimethyl-6-(4-methyl-	C15 H24	204
15	20.381	1.09	CIS-.alpha.-bisabolene $$	C15 H24	204
16	20.923	21.87	Nerolidol B (CIS OR TRANS	C15 H26O	222
17	21.803	10.55	(-)-Caryophyllene oxide	C15 H24O	220
18	22.174	0.35	Trans-Caryophyllene	C15 H24	204
19	22.44	0.59	Humulene oxide $$	C15	220

	4			H24O	
20	22.807	0.69	Germacrene D	C15 H24	204
21	22.917	0.68	Tricyclo[3.3.1.13,7]decane, 2-brom	C10 H15	214
22	23.099	3.29	(+)-Aromadendrene	C15 H24	204
23	23.202	4.93	Torreyol $$ 1-Naphthalenol	C15 H26O	222
24	23.441	1.91	Globulol $$ (-)-Globulol	C15 H26O	222
25	23.631	1.46	Beta.-Bisabolol	C15 H26O	222
26	23.963	1.36	Alpha.-bisabolol	C15 H26O	222
27	25.711	0.20	2-Methyl-6-(trimethylsilyl)benzophe	C17 H20 O	268
28	26.512	0.92	8-Acetyl-3,3-epoxymethano-6,6,7-t	C14 H20 O3	236
29	27.223	0.35	1,2-Benzenedicarboxylic acid, dibut	C16 H22 O4	278
30	28.671	1.89	1,2-Benzenedicarboxylic acid, buty	C20 H30 O4	334
31	32.600	0.71	Propionic acid, 2-isopropo	C7 H14 O3	146
32	38.26	0.82	Bis(2-ethylhexyl)	C24H38	390

	8		phthalate	O4	
33	48.521	0.72	Hexadeca-2,6,10,14-tetraen	C20 H34 O	290

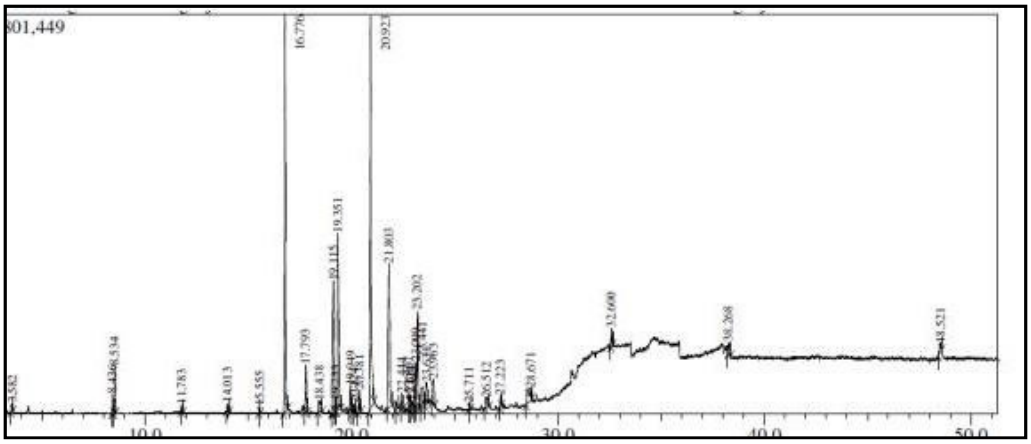


Fig.No.5: Chromatogram obtained from the GC/MS with the extract of *Psidium guajava* leaves[3]

Molecular Docking of Bioactive Compounds of Drug

One popular software technique for predicting the binding affinities and modalities of small compounds to target proteins is molecular docking with AutoDock Vina. The proteins utilized in this instance are 6-COX. Using AutoDock Vina for molecular docking usually requires the following steps:

Step 1: Preparation of Protein Structures

Obtain the protein structures by downloading the anti-inflammatory target protein from the RCSB PDB (Protein Data Bank). Any water molecules, co-crystallized ligands, or heteroatoms that are not part of the binding site should be eliminated. including nonpolar hydrogens into the protein structure to accurately depict nonpolar interactions, and giving partial charges to the atoms of the protein and characterizing side chain flexibility to record conformational changes. By following these procedures, AutoDock Vina is able to precisely assess interactions between proteins and ligands.

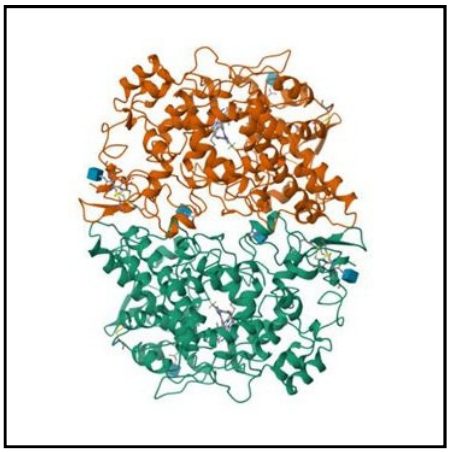
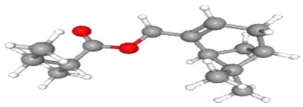
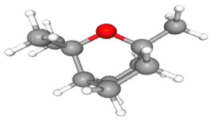
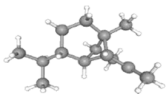


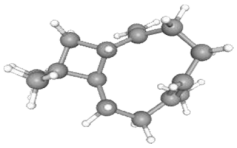
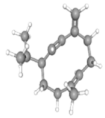
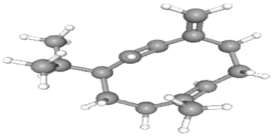
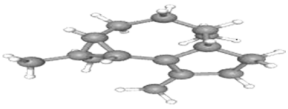
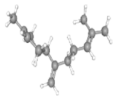
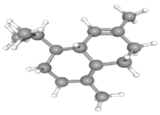
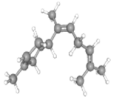
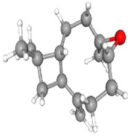
Fig.No.6: cyclooxygenase-2 (prostaglandin synthase-2) complexed with a selective inhibitor, sc-558 in i222 space group

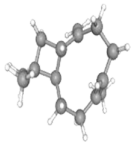
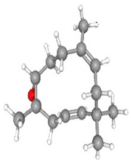
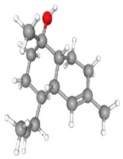
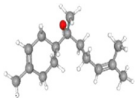
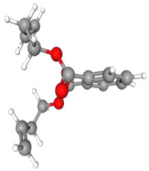
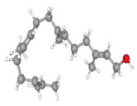
Step 2: Preparation of ligand Structures

Get the ligands' three-dimensional structure from Pubchem. Pymol is used to convert SDF files to PDBQT, which are then saved in a designated folder.[13]

Table No.11: Ligands and their structures

SL.No	LIGAND	STRUCTURE
1.	Butanoic acid, 2-methyl-, methyl este	
2.	1,8-Cineole 2-Oxabicyclo [2.2.2]	
3.	Alpha.-Copaene	

4.	Trans-Caryophyllene	
5.	Germacrene D	
6.	Trans-alpha.-bisabolene	
7.	Aromadendrene 2	
8.	Beta.-Bisabolene	
9.	Delta.-Cadinene	
10.	CIS-.alpha.-bisabolene	
11.	(-)-Caryophyllene oxide	

12.	Trans-Caryophyllene	
13.	Humulene oxide	
14.	Torreyol 1-Naphthalenol	
15.	Alpha.-bisabolol	
16.	1,2-Benzenedicarboxylic acid, dibut	
17.	Hexadeca-2,6,10,14-tetraen	

Finding ligands with a high propensity to bind to a particular target of interest is the aim. Use AutoDock Vina to get the ligand structures ready.

Step 3: Preparation of Input Files

The input files needed for AutoDock Vina should be ready. This usually consists of a ligand file in an appropriate format, such as SDF or PDB, and a protein file in PDB format (.pdb).

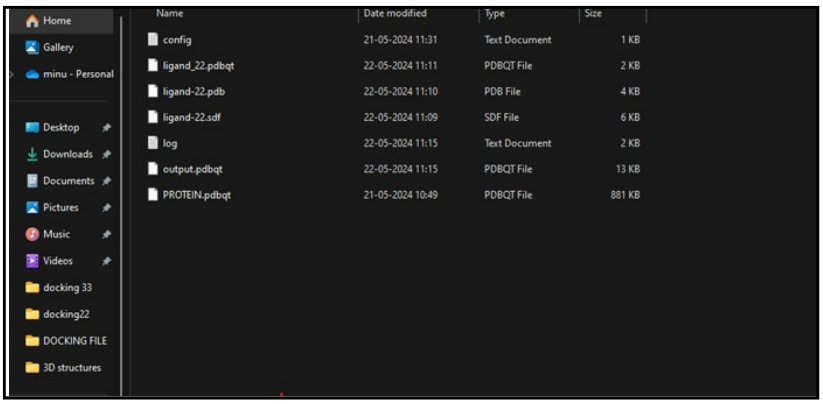


Fig.No.7:Input Files

Step 4:Define the Binding Site

Identify and define the binding site within the protein where the ligand is expected to bind. You can visually inspect the protein structure or use additional tools like cavity detection algorithms to identify potential binding pockets or active sites.[14]

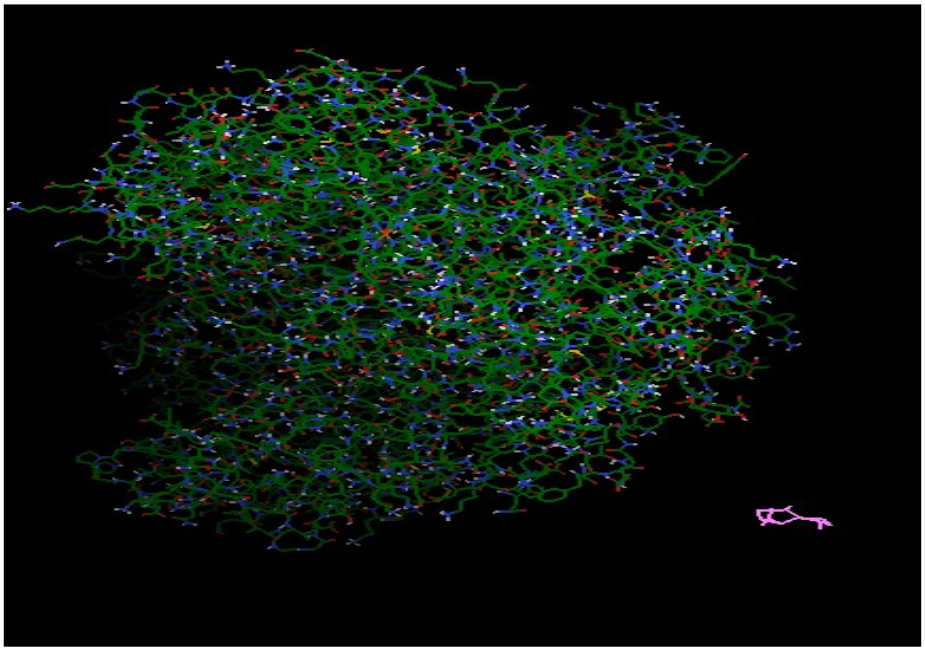


Fig.No.8: Defining the Binding Site

Step 5: Set up the Docking Parameters

Make a configuration file that details AutoDock Vina's docking parameters. This entails establishing the number of docking poses to be generated, defining the binding site, and configuring the search method.

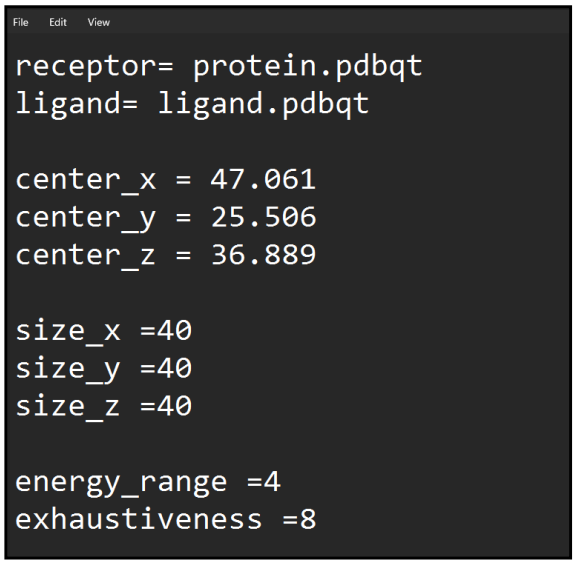


Fig.No.9: Config File

Step 6: Perform Molecular Docking

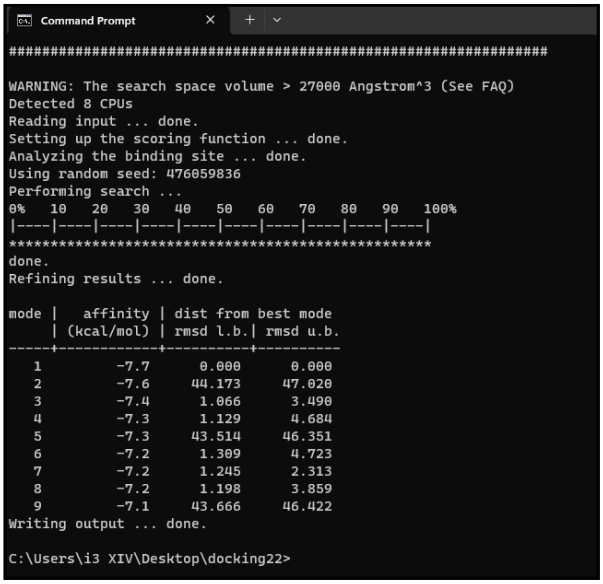


Fig.No.10: Performing Molecular Docking

Using the docking parameters configuration file and the produced protein and ligand files, launch AutoDock Vina, which will investigate various ligand conformations and

orientations within the binding site. Based on scoring functions that take into account will calculate the binding energy of each pose.

Step 7: Analyze the Docking Results

Graph the outcomes using molecular Pymol visualization software to investigate the relationships between the ligand and protein.[15]

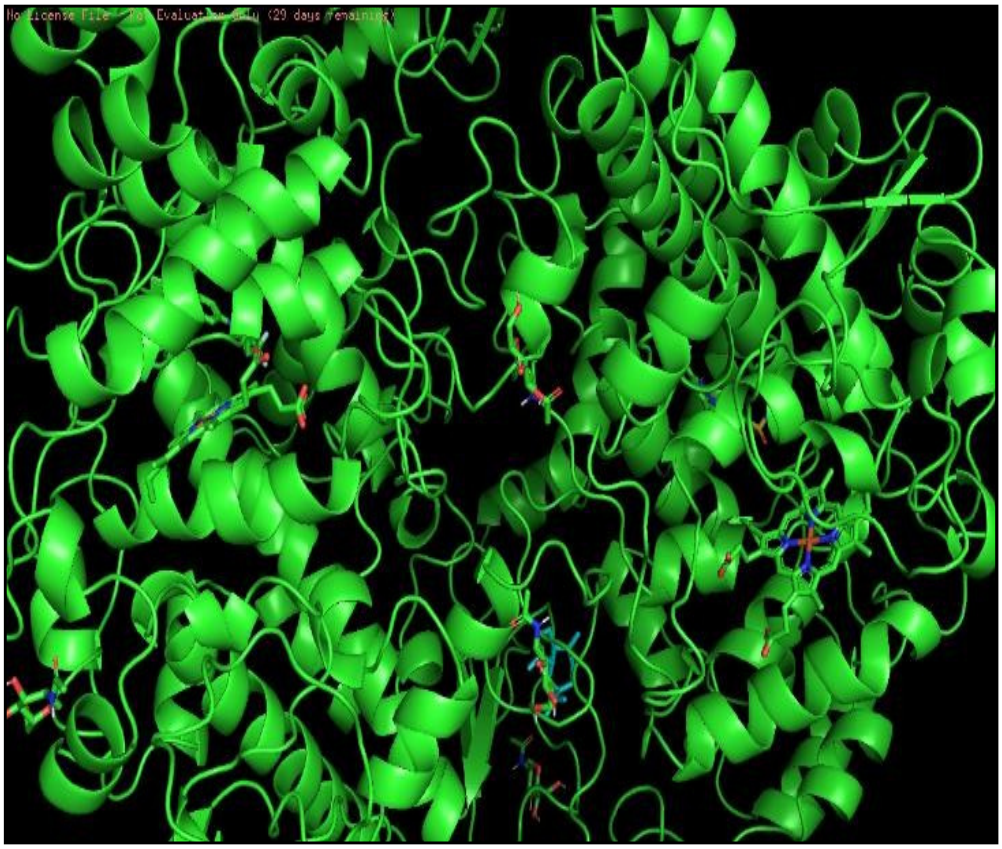


Fig.No.11: Visualization using Pymol

RESULTS & DISCUSSION

Preliminary Phytochemical Screening of Extract

Table No.12**:** Phytochemical Screening of Extract

Sl.NO:	CHEMICAL CONSTITUTE	ETHANOL EXTRACT
1	Alkaloids	+
2	Glycosides	+
3	Flavonoids	+
4	Tannins	+
5	Steroids	+
6	Carbohydrates	+
7	Proteins	+

Fig.No.12: Chemical Tests

Ash Value - Total ash value

Table No.13: Ash Value

Sl. NO:	Wt. Of empty crucible(g)	Wt. of crucible + sample (g)	Wt. of crucible + ash (g)	Wt. of ash (g)	Percentage yield (%w/w)
1.	22.46	24.46	28.46	6	9.29

Fig.No.13: Ash

Molecular Docking of Bioactive Compounds of Drug

Table No.14: Binding Affinity

SL.NO:	LIGANDS	BINDING AFFINITY
1	Butanoic acid, 2-methyl-, methyl este	-6.7
2	1,8-Cineole $$ 2-Oxabicyclo[2.2.2]	-6.0
3	Alpha.-Copaene	-7.1
4	Trans-Caryophyllene	-7.0
5	Alpha.-Humulene	-7.1
6	Germacrene D	-7.3

7	Trans-alpha.-bisabolene	-7.0
8	Aromadendrene 2 $$	-7.1
9	Beta.-Bisabolene	-6.1
10	Delta.-Cadinene	-7.4
11	CIS-.alpha.-bisabolene $$	-6.5
12	(-)-Caryophyllene oxide	-7.2
13	Trans-Caryophyllene	-7.0
14	Humulene oxide $$	-2.2
15	Germacrene D	-7.3
16	(+)-Aromadendrene	-7.7
17	Torreyol $$ 1-Naphthalenol	-7.3
18	Beta.-Bisabolol	-7.0
19	Alpha.-bisabolol	-7.1
20	1,2-Benzenedicarboxylic acid, dibut	-6.3
21	Hexadeca-2,6,10,14-tetraen	-6.9

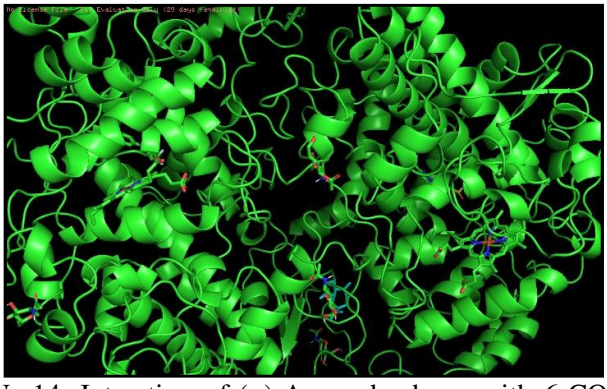


Fig.No.14: Interation of (+)-Aromadendrene with 6-COX

From the docking results, (+)-Aromadendrene was found to be the best ligand with greater binding affinity against the selected antiinflammatory proteins.

CONCLUSION

- Extract of guava leaf powder was prepared using ethanol as solvent by the process of Maceration.
- From preliminary phytochemical studies, extract shows presence of glycosides as major secondary metabolite.
- Physico-chemical parameters like ash value was performed.
- GC MS analysis data of the drug extract was collected from the journal and obtained 33 volatile substituents.
- Molecular docking of obtained volatile substituents was performed using Auto Dock Vina.
- From the results, (+)-Aromadendrene was found to be the best ligand with selected antiinflammatory protein and can be screened using highly computational method like molecular dynamic simulation for further investigation.

This study revealed the anti-inflammatory potential of highlighting that (+)-Aromadendrene exhibited the highest binding affinity against target protein. These findings contribute to the understanding of the therapeutic potential of the formulated drug and suggest (+)-Aromadendrene as a promising compound that can screened using highly computational method like molecular dynamic simulation for further investigation.

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