ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327

Computational Docking and in Vitro Validation of Anti-Obesity and Antioxidant Activities of Hydroethanolic Extract of Psidium Guajava Leaf

G. Kinnera Ratna Sri[1*]; M. Ganga Raju[2]; N V L Suvarchala Reddy V[3]; Devika S.[4]; G V Raghu Vamsi[5]; G. Suprasanna[6]; T. Astha Lakshmi[7]

[1,2,3]Faculty, Department of Pharmacology, 2Student
[4,5,6,7]Gokaraju Rangaraju College of Pharmacy, Hyderabad, India

Corresponding Author: G. Kinnera Ratna Sri[1*]

Publication Date: 2025/06/12

Abstract: Obesity is a chronic health condition linked to various metabolic disorders, necessitating effective treatment strategies beyond lifestyle modifications. One promising approach involves inhibiting digestive enzymes to reduce fat and carbohydrate absorption. In this study, we evaluated the anti-obesity potential of Hydro-ethanolic extract of Psidium guajava leaves (HEPG) using in vitro and in silico methods. Phytochemical screening confirmed the presence of flavonoids, phenolic compounds, and other bioactive constituents, with quantified flavonoid and phenolic contents of 127 µg QE/g of HEPG and 168 µg GE/g of HEPG respectively. thin coating Gallic acid was identified as a phenol and quercetin as a flavonoid in HEPG by chromatographic analysis. Antioxidant assays demonstrated significant hydroxyl radical scavenging (IC_{50}: 214 µg/mL) and ferrous reducing activity (IC_{50}: 206 µg/mL). Enzyme inhibition studies revealed notable lipase (IC_{50}:155 µg/mL) and α-amylase (IC_{50}: 157 µg/mL) inhibitory activities, comparable to standard orlistat and acarbose. In silico molecular docking studies further supported these findings, showing strong binding affinities of flavonoids and gallic acid to fat mass and obesity-associated protein (PDB ID: 3LFM), with glide scores close to that of orlistat (-11.6). Hence, our findings suggest that HEPG possesses significant anti-obesity potential, likely due to its flavonoid and phenolic content, making it a promising natural alternative for obesity management.

Keywords: *Flavonoids, Phenols, A-Amylase, Lipase, Anti-Obesity, Molecular Docking.*

How to Cite: G. Kinnera Ratna Sri; M. Ganga Raju; N V L Suvarchala Reddy V; Devika S.; G V Raghu Vamsi; G. Suprasanna; T. Astha Lakshmi. (2025). Computational Docking and in Vitro Validation of Anti-Obesity and Antioxidant Activities of Hydroethanolic Extract of Psidium Guajava Leaf. *International Journal of Innovative Science and Research Technology*, 10(5), 4451-4462. https://doi.org/10.38124/ijisrt/25may2327.

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM), cardiovascular illnesses, osteoarthritis, obstructive sleep apnea, depression, and cancer are all closely associated with obesity, a major global public health issue [1]. Over 1.9 billion persons aged 18 and older were considered overweight in 2016, with an astounding 650 millions of them being classified as obese, according to the World Health Organization (WHO) [2]. According to the ICMR-INDIAB survey, the percentage of Indians who are obese ranges from 11.8% to 31.3% and 16.9% to 36.3% [3]. A complex disease, obesity is caused by a number of variables. The most alarming element is the over consumption of calories, which is mostly brought on by Western diets that are heavy in processed foods that are high in fat, carbohydrates, and fibres imbalance causes extra energy and fat accumulation when combined with insufficient physical activity. As a result, controlling and reducing weight continues to be a top priority when treating patients who are obese or overweight [4].

Synthetic drugs. They are on the other hand, are made to target certain disease symptoms in accordance with scientific knowledge of pathology. Herbal medication, on the other hand, is generally regarded as harmless and works to support the body's natural healing process. Since the beginning of human civilization, traditional medicine has been utilized for both prevention and treatment. Because of its accessibility,

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327

cost, ease of use, and shown success, traditional medicine has become increasingly popular while having little or no negative effects or toxicity. Obesity considerably increases exposure to COVID19 because of the common metabolic and inflammatory processes that connect the two illnesses. Natural sources have drawn more attention as a useful natural alternative to prevent fat and metabolic syndrome, and they present a potential basis for the creation of innovative anti-obesity products [5].

In silico The binding of molecules is one widely used technique. makes it possible to forecast when small molecules will align with specific goals. In order to ascertain their affinity and activity, pharmaceuticals are made to target particular proteins in the body. This method makes it easier to create, characterize, and alter interactions and complicated topologies, as well as the properties of molecules that depend on their Gibbs free energy and three-dimensional geometries [6].

Flavonoids, phenols, and other chemicals have been reported to be present in the leaves of Psidium guajava L. [7]. Since flavonoids have been demonstrated to have stronger anti-obesity effects, we have selected Psidium guajava L. leaves as a model system to assess their potential in the fight against obesity [8].

II. MATERIALS AND METHODS

A. Materials

HM Media Laboratories Pvt. Ltd., Research Lab Fine Chem Industries., Mumbai, Burgoyne Burbidge's & Co., Mumbai, Tokyo Chemical Industry Co., Ltd., Japan, Qualigen's fine chemicals, Mumbai, and SD Fine Chemicals Ltd., Mumbai were among the sources of the chemical compounds used in the study.

B. Preliminary Phytochemical Screening of the Extract

Alkaloids, flavonoids, tannins, phenols, proteins, triterpenoids, phytosterols, and saponins were among the phytochemical elements identified by preliminary phytochemical screening of a hydro-ethanolic extract of Psidium guajava leaves (HEPG) using recognized techniques [9,15].

C. Determination of Total Flavonoid Content

Psidium guajava (HEPG) hydroethanolic extract's total flavonoid concentration is determined using a UV-visible spectrophotometric approach based on the development of an aluminum chloride-flavonoid complex, which absorbs maximum at 420 nm. The standard ingredient is quercetin. After 5 minutes, aliquots of 25, 50, 100, 200, and 250 µg/mL of a standard solution of quercetin (1 mg/mL in methanol) are reacted with 0.3 mL of 5% sodium nitrate and 3 mL of 10% aluminum chloride. Two milliliters of 1 M sodium hydroxide are added after six more minutes, and the volume is then adjusted to ten milliliters using purified water. At 420 nm, the absorbance is measured. The sample is prepared in the same way as the standards, with 10 mg of HEPG dissolved in 10 mL of methanol (1 mg/mL). Without quercetin or sample, a blank is made. By comparing the sample absorbance to the standard calibration curve, the total flavonoid concentration is determined and represented as micrograms of quercetin equivalent per gram of dry HEPG (µg QE/g) [16].

D. Determination of Total Phenol Content

At 420 nm, the absorbance is measured. The sample is prepared in the same way as the standards, with 10 mg of HEPG dissolved in 10 mL of methanol (1 mg/mL). Without quercetin or sample, a blank is made. By comparing the sample absorbance to the standard calibration curve, the total flavonoid concentration is determined and represented as micrograms of quercetin equivalent per gram of dry HEPG (µg QE/g) [16]. A microgram of gallic acid equivalent (µg GE/g HEPG) per gram of dry extract is used as the calibration curve to determine the total phenol concentration, and absorbance is measured at 570 nm [17].

E. Identification of Flavonoids and Phenols by Thin Layer Chromatography

Thin-layer chromatography (TLC) is used to determine whether flavonoids and phenols are present in the hydroethanolic extract of Psidium guajava (HEPG). Silica gel is applied to glass slides to create TLC plates, which are then dried for 30 minutes at 37°C. To indicate the origin, a line is drawn 1.2 cm from the base. Capillary tubes are used to spot the standards (quercetin and gallic acid, 1 mg/mL each in methanol) and the sample (1 mg/mL HEPG in ethanol) onto the plate. A methanol:chloroform:hexane (7:2:1) solvent system is used to create the flavonoid plate, which is then seen at 254 nm under UV illumination. Violet spots for phenolic compounds are seen when 2% ferric chloride ($FeCl_3$) is sprayed on the mobile phase of an ethyl acetate:toluene:acetic acid (6:3:2) plate. The compound's travel distance divided by The retention factor (Rf) is calculated by dividing the travel distance of the chemical by the travel distance of the solvent front. The presence of phenols and flavonoids in HEPG can be qualitatively detected and compared to established standards using this method [10, 18].

F. In Vitro Antioxidant Activity

- *Hydroxyl Radical Scavenging Assay*

The Hydroxyl Radical Scavenging Assay, which measures the sample's capacity to neutralize hydroxyl radicals, is used to assess the antioxidant activity and IC50 of the hydroethanolic extract of Psidium guajava (HEPG). Gallic acid is used as a reference in the test to compare the activity of HEPG. Phosphate buffer (pH 7.4), EDTA, ferric chloride, 2-deoxy-D-ribose, hydrogen peroxide, ascorbic acid, thio barbituric acid, and hydrochloric acid are among the reagents that are manufactured. A 1 mg/mL solution of HEPG in hydroethanol and standard gallic acid solutions (50–300 µg/mL) are employed. Phosphate buffer, EDTA, ferric chloride, and 2-deoxy-D-ribose are mixed together for each reaction combination, and they are then incubated for an hour at 37°C. After adding hydrogen peroxide and ascorbic acid, the mixture is incubated for an additional hour. Following the addition of HCl and Thio barbituric acid, the mixture is heated for 15 minutes at 100°C before being cooled. A UV-visible spectrophotometer is used to measure the absorbance at 532

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327

nm [11]. The following formula is used to determine the percentage of hydroxyl radical scavenging activity:

$$\% \text{ Inhibition} = [(Abs_C - Abs_S) / Abs_C] \times 100$$

Where, Abs_S is the absorbance of the test sample and Abs_C is that of the control.

➢ *Ferrous Reducing Antioxidant Capacity Assay*

The Ferric Reducing Antioxidant Power (FRAP) assay, which gauges the antioxidants' capability to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), is used to evaluate the antioxidant capacity of the hydroethanolic extract of Psidium guajava (HEPG). Standard solutions of HEPG (1 mg/mL in hydroethanol) and ascorbic acid (50–300 µg/mL) are made. The sample or standard, 2 mL of 0.2 M phosphate buffer (pH 6.6), and 2 mL of 1% potassium ferrocyanide make up the reaction mixture, which is then incubated for 20 minutes at 50°C. Two milliliters of 10% trichloroacetic acid are added after incubation, and the mixture is centrifuged for ten minutes at 1000 rpm. Two milliliters of the supernatant are taken out and combined with one milliliter of 0.1% ferric chloride and two milliliters of distilled water. After that, a UV-Visible spectrophotometer is used to detect the absorbance at 593 nm. IC50 and % inhibition are two ways to express antioxidant activity [19].

G. In vitro Anti-Obesity Activity

➢ *In vitro Lipase Inhibitory Activity:*

An enzyme called porcine pancreatic lipase breaks down dietary fats into free fatty acids and monoglycerides. The ability of a test substance to inhibit this enzyme is assessed using the pancreatic lipase inhibition assay. This inhibition raises the possibility of lowering calorie intake and fat absorption. Porcine pancreatic lipase (0.5 g in 15 mL buffer) is mixed with a triolein emulsion (1% v/v triolein and Tween 40) and a 0.1 M phosphate buffer (pH 8). HEPG and regular orlistat are made at 50–300 µg/mL. The reaction mixture is first read at 450 nm (T1), then incubated at 37°C for 30 minutes, and finally read again (T2). It contains 800 µL of emulsion, 200 µL of enzyme, and different amounts of test or standard. Enzyme activity is reflected in the change in absorbance. The corresponding IC50 and percentage inhibition were computed [12].

➢ *In Vitro Aamylase Inhibitory Activity*

The in vitro α-amylase inhibition assay assesses a sample's capacity to prevent the breakdown of carbohydrates, which may help with weight control by lowering calorie intake. The assay relies on α-amylase hydrolyzing starch, which is identified by a color shift that occurs when iodine combines with unhydrolyzed starch to produce a blue-black complex. A starch solution made by boiling soluble starch in NaOH and correcting pH with HCl is one of the reagents, along with 0.4 M NaOH, 2 M and 0.1 M HCl, and 1 mM iodine (made by dissolving iodine and KI in water with two drops of concentrated HCl). The standard, acarbose (10 mg in DMSO), and the test extract, HEPG (10 mg in methanol), are both diluted to 50–300 µg/mL. For the experiment, 20 µL of sample and 40 µL of starch are preincubated for 3 minutes at 37°C. Next, 20 µL of α-amylase is added, and the mixture is incubated for an additional 15 minutes. 200 µL of iodine solution and 80 µL of 0.1 M HCl are used to terminate the reaction. At 650 nm, absorbance is measured. By computing the corresponding IC50 and the percentage of inhibition, inhibitory activity was ascertained [13].

H. Molecular Docking Studies

Accessing the target protein, choosing ligands, preparing the protein and ligands, locating the active binding site, carrying out docking studies, and visualizing the outcomes are some of the stages involved in molecular docking. The RCSB Protein Data Bank provided the 3D structure of the fat mass and obesity-associated (FTO) protein (PDB: 3LFM). Ligands such as tannin (gallic acid), flavonoids (Reynoutrin, Guaijaverin, Quercetin, and Catechin), and the The ZINC and PubChem databases provided standard Orlistat, which was converted from SDF to PDB format using OpenBabel and stored in MOL. In order to prepare the protein, polar hydrogens were added, water molecules were eliminated, and the BIOVIA (Discovery Studio 2021) client was used to determine the active binding site. For site-specific docking, the binding site's characteristics were downloaded. Ligands were made by identifying the molecule's root in Autodock 1.5.7 and storing the results in PDBQT format.

The prepared protein's PDB format was processed to allocate charges and atoms before being saved in PDBQT format for docking computations using Autodock Vina, which was used for the docking study. Vina.exe was used to do docking with a configuration file. A lower glide score indicates a more favorable binding interaction. The data were evaluated, including the glide score, which represents binding affinity and negative Gibbs free energy. BIOVIA was used to illustrate the optimal docking pose, showing the distances between the interaction groups as well as the protein's interacting amino acids with the ligand in 2D and 3D diagrams. Better binding is indicated by a larger negative glide score, which measures binding strength [6, 14].

III. RESULTS

A. Qualitative Analysis

The presence of different phytochemical constituents such as flavonoids, alkaloids, tannins, proteins and amino acids, phenolic compounds, triterpinoides, and phytosterols, respectively, was revealed by the qualitative phytochemical screening of hydro ethanolic extract of Psidium guajava leaves (HEPG) in figures 1 and 1.

Table 1: Qualitative Analysis of Hydro-Ethanolic Extract of Psidium Guajava

Phytochemicals	Results
Flavonoids	+
Alkaloids	+
Tannins	+
Proteins	+
Phenolic compounds	+
Triterpenoid's	+
Phytosterols	+
Saponins	+

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327

Carbohydrates	-
Anthocyanins	-

Note: + indicates Presence; - indicates Absence

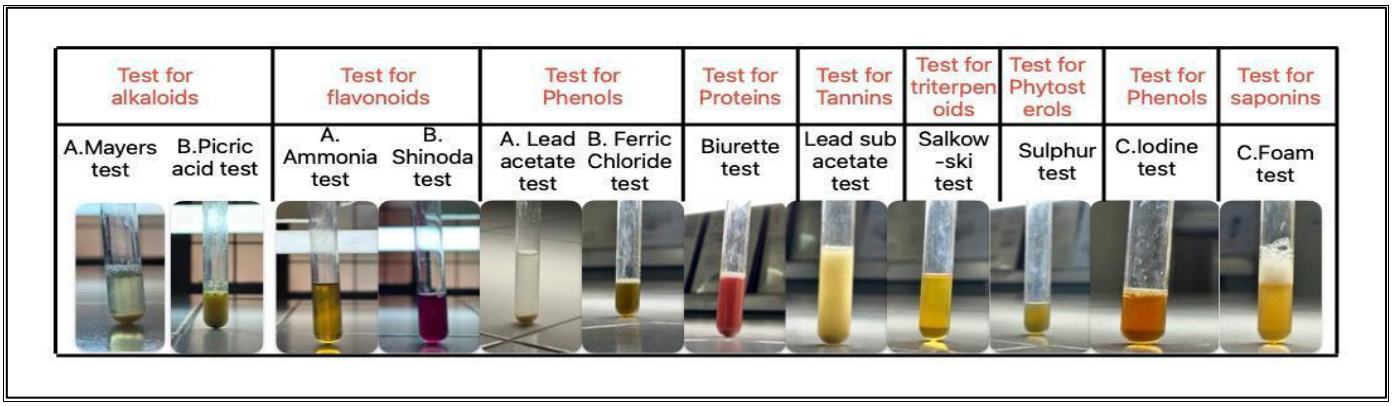


Fig 1: Qualitative Analysis of Phytochemical Constituents in HEPG

B. Estimating the Total Amount of Flavonoids

The concentration of flavonoids in HEPG leaf extract was found to be 127 µg QE/g of HEPG. The results were depicted in figure 2 and table 2.

C. Determination of total Phenol content

The concentration of Phenols in HEPG leaf extract was found to be 168 µg GA/g of HEPG. The results were depicted in figure 3 and table 3.

Table 2: Quantitative Estimation of Flavonoids in HEPG

S.no	Concentration (µg/ml)	Absorbance
1.	25	0.011
2.	50	0.019
3	100	0.034
4.	200	0.049
5.	250	0.061
6.	Test sample (HEPG)	0.035

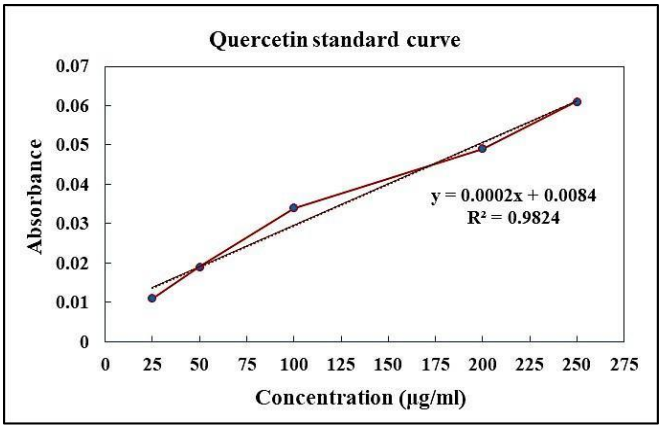


Fig 2: Determination of Flavonoids Quantitatively in HEPG

Table 3: Quantitative Estimation of Phenols in HEPG

S.no	Concentration (µg/ml)	Absorbance
1.	25	0.081
2.	50	0.140
3	100	0.223
4.	200	0.319
5.	250	0.398
6.	Test sample (HEPG)	0.291

D. Flavonoids and Phenols Identification by Thin Layer Chromatography (TLC)

Retention factor (R_f) values of HEPG extract and standard quercetin and gallic acid were found to be similar as 0.96 (Quercetin) and 0.32 (Gallic acid). Hence, one of the flavonoid and phenol compounds in HEPG leaf extract was found to be quercetin and gallic acid. Results were represented in figure 4 and table 4.

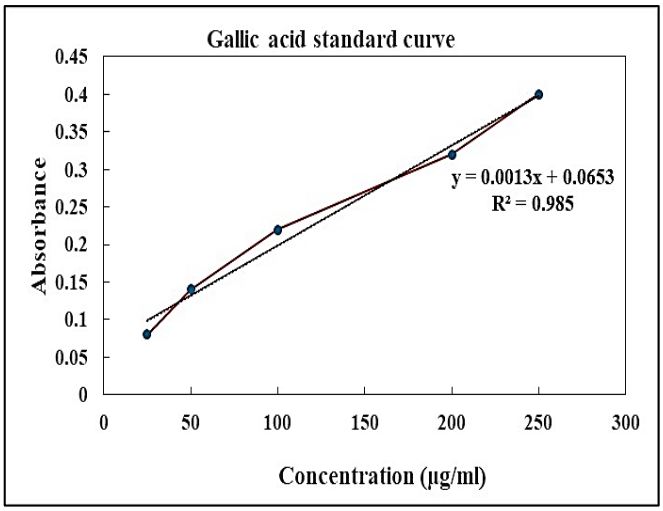


Fig 3: Quantitative Estimation of Phenols in HEPG

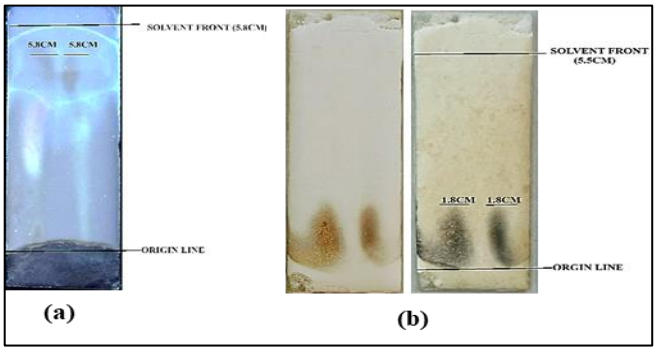


Fig 4: TLC Plate of a) Flavonoids; b) Phenols

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327

Table 4: R_f Value for Flavonoids and Phenols

S.no	Sample spot	Distance travelled (cm)	Solvent Distance (cm)	R_f value
1.	Standard (Quercetin)	5.6	5.8	0.96
2.	HEPG extract	5.6	5.8	0.96
3.	Standard (Gallic acid)	1.8	5.5	0.32
4.	HEPG extract	1.8	5.5	0.32

E. In Vitro Anti-Oxidant Activity

➢ *Hydroxyl Radical Scavenging Assay*

The antioxidant capacity was examined using Hydroxyl Radical Scavenging Assay. HEPG extract showed dose dependent inhibition. The IC_{50} value of HEPG & Gallic acid was found to be 214 µg/mL and 202 µg/mL respectively. Results were represented in figure 5 and table 5.

➢ *Ferrous Reducing Antioxidant Capacity Assay*

The antioxidant capacity was examined using ferrous reducing capacity assay. HEPG showed dose dependant inhibition. The IC_{50} value of HEPG and Ascorbic acid was found to be 206 µg/mL, 186 µg/mL respectively. Results were represented in figure 6 and table 6.

Table 5: Antioxidant Capacity of HEPG using Hydroxyl Radical Scavenging Assay

S.no	Compounds	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1	HEPG	50	03.40±0.85	214
		100	18.35±0.78	
		150	26.85±0.21	
		200	46.45±1.20	
		250	58.80±1.41	
		300	78.10±0.28	
2	Gallic acid	50	5.45±0.21	202
		100	19.9±0.14	
		150	28.75±1.20	
		200	47.95±0.49	
		250	64.40±0.70	
		300	82.85±1.63	

Values are Expressed as Mean ± SD (n=2)

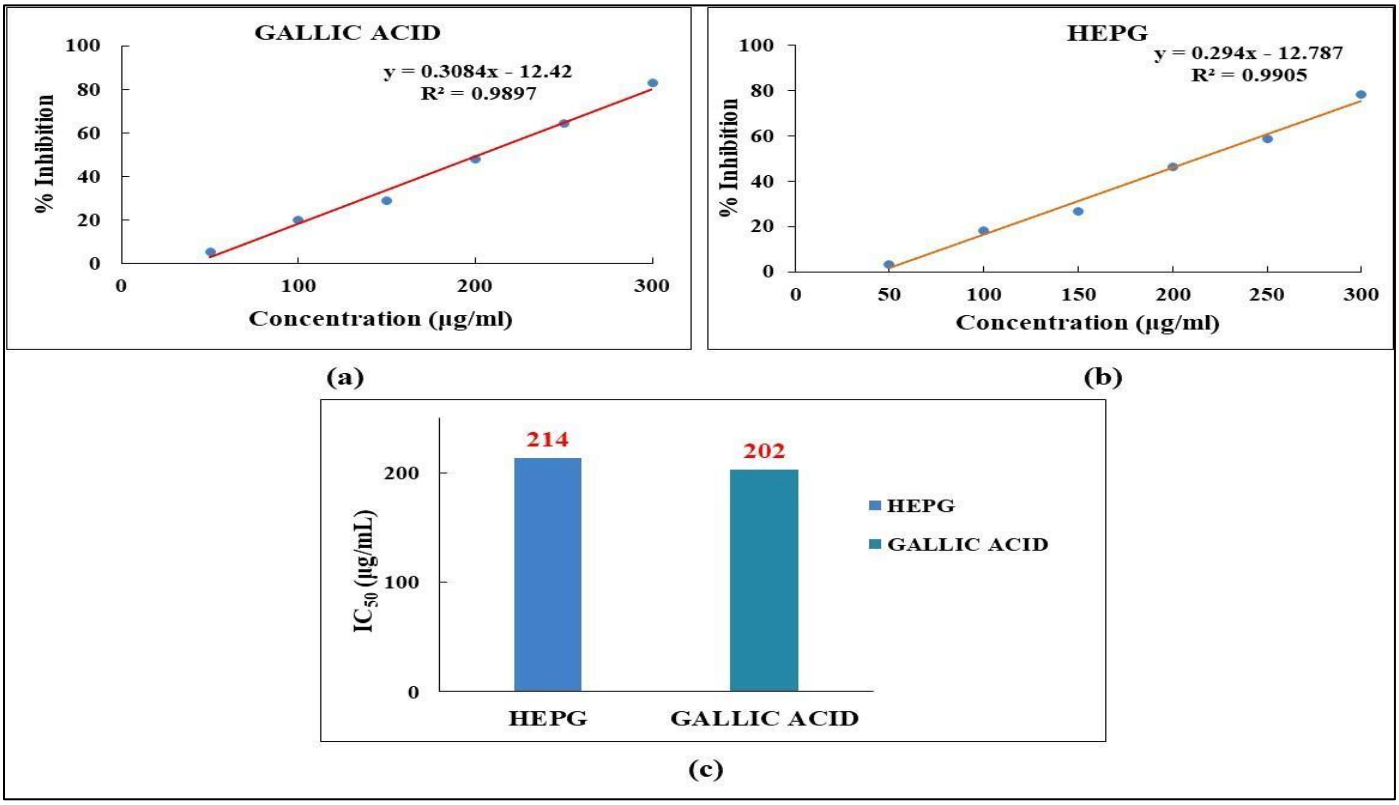


Fig 5: Hydroxyl Radical Scavenging Assay a) % Inhibition of HEPG; b) % Inhibition of Gallic acid; and c) IC_{50} of HEPG and Gallic Acid

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327

Table 6: Antioxidant Capacity of HEPG using Ferrous Reducing Capacity Assay

S.no	Compounds	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1	HEPG	50	16.00±1.41	206
		100	19.15±0.21	
		150	27.50±0.56	
		200	46.35±0.49	
		250	64.25±0.63	
		300	77.60±1.69	
2	Ascorbic acid	50	21.50±0.14	186
		100	27.15±1.20	
		150	32.80±0.42	
		200	52.00±1.31	
		250	68.75±0.91	
		300	80.95±1.48	

Values are Expressed as Mean ± SD (n=2)

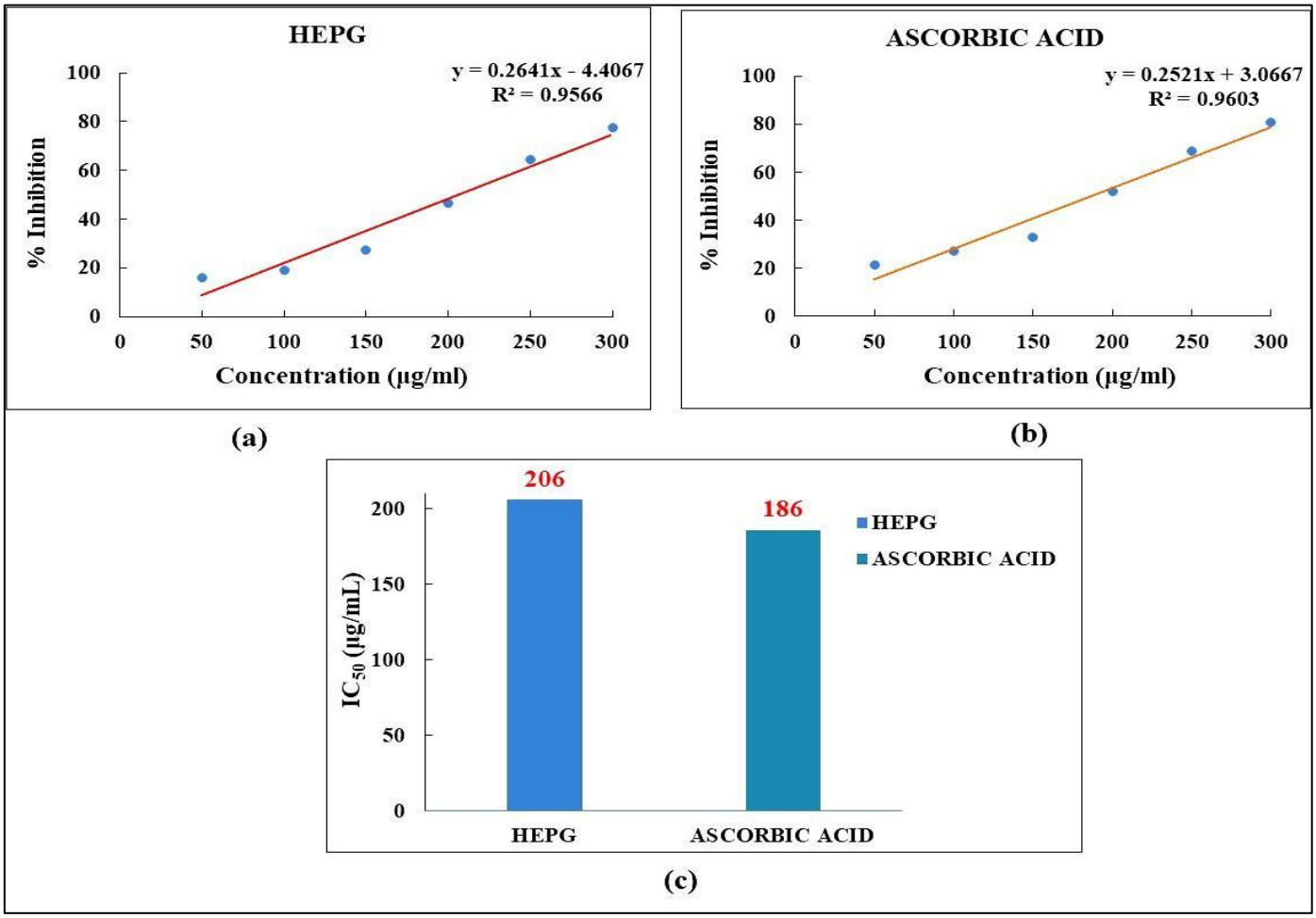


Fig 6: Ferrous Reducing Capacity Assay: a) % inhibition of HEPG; b) % inhibition of Ascorbic acid; and c) IC_{50} of ascorbic acid and HEPG

F. In Vitro Anti-Obesity Activity

➢ *In Vitro Lipase Inhibitory Activity*

HEPG showed dose dependant inhibition of lipase activity. The IC_{50} value of HEPG and Orlistat was found to be 155 µg/mL and 138 µg/mL. Results were represented in figure 7 and table 7.

➢ *In Vitro A- Amylase Inhibitory Activity*

HEPG showed dose dependant inhibition of α- amylase activity. The IC50 value of HEPG and Acarbose was found to be 157 µg/mL and 142 µg/mL. The results were represented in figure 8 and table 8.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327

Table 7: Inhibition of Lipase Activity of HEPG and Orlistat

S.no	Compounds	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1	HEPG	50	20.00±1.41	155
		100	31.50±0.71	
		150	52.20±0.14	
		200	65.40±0.42	
		250	79.25±0.35	
		300	84.45±0.63	
2	Orlistat	50	25.80±1.27	138
		100	51.55±0.64	
		150	74.40±0.28	
		200	84.45±0.64	
		250	91.95±0.07	
		300	80.95±1.48	

Values are expressed as Mean ± SD (n=2)

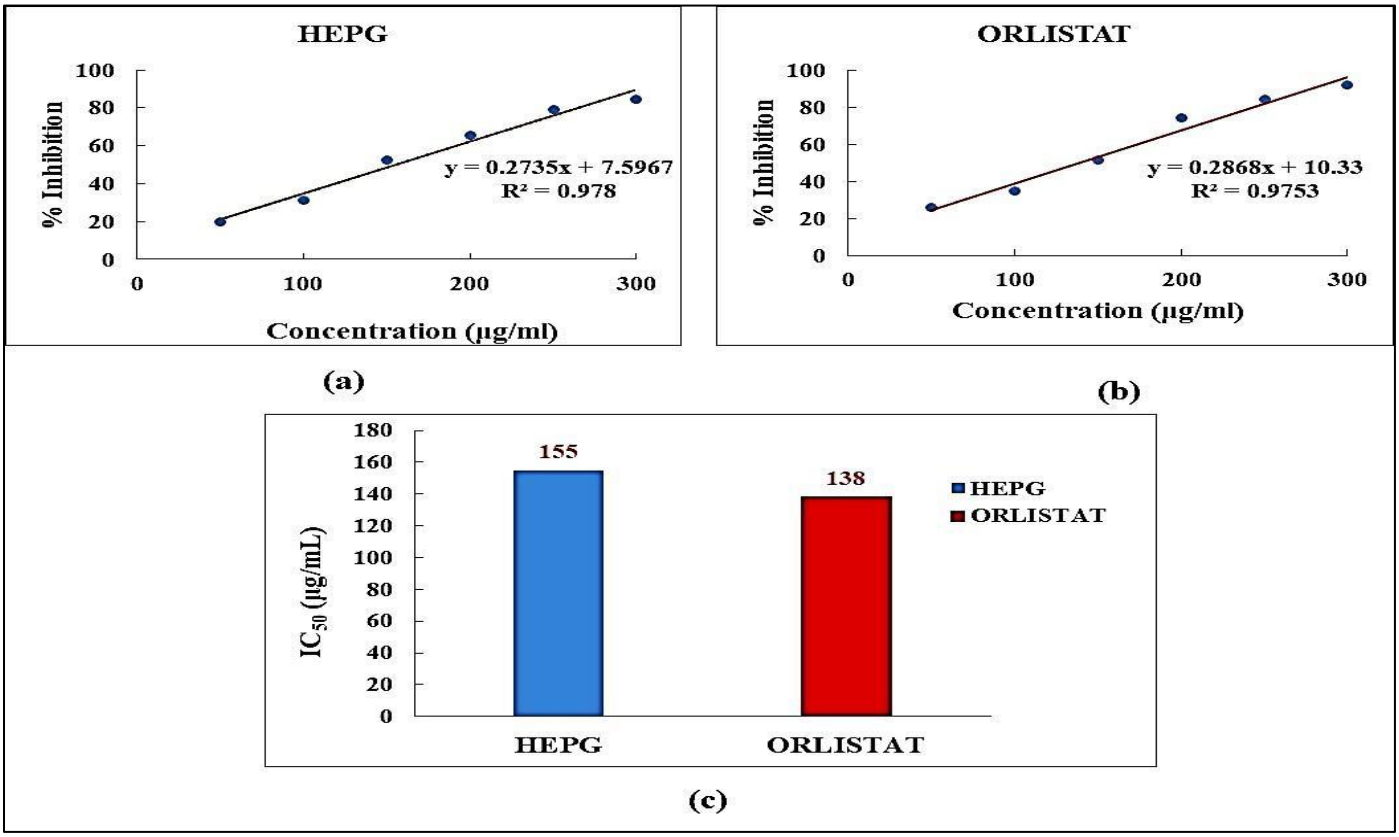


Fig 7: Lipase inhibitory Activity Assay: a) % Inhibition of HEPG b) % Inhibition of Orlistat and c) IC_{50} of HEPG and Orlistat

Table 8: Inhibition of α- Amylase Activity of HEPG and Acarbose

S.no	Compounds	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1	HEPG	50	22.80±0.14	157
		100	32.95±0.64	
		150	48.70±0.71	
		200	65.80±1.41	
		250	74.15±0.35	
		300	81.95±1.34	
2	Acarbose	50	24.95±0.64	142
		100	36.35±0.63	
		150	53.45±0.49	
		200	68.60±1.69	
		250	80.40±0.84	
		300	89.45±1.48	

Values are Expressed as Mean ± SD (n=2)

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327

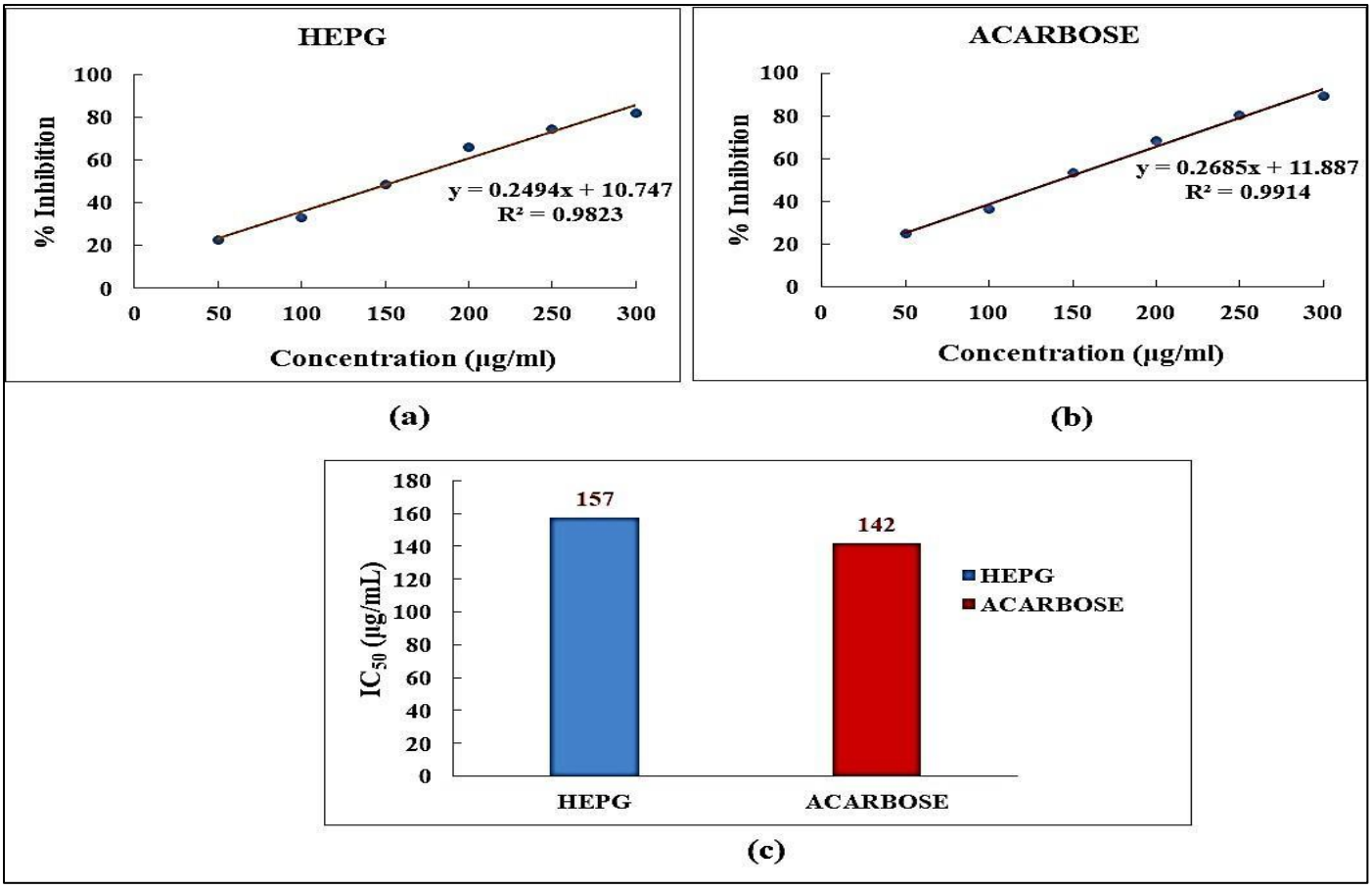
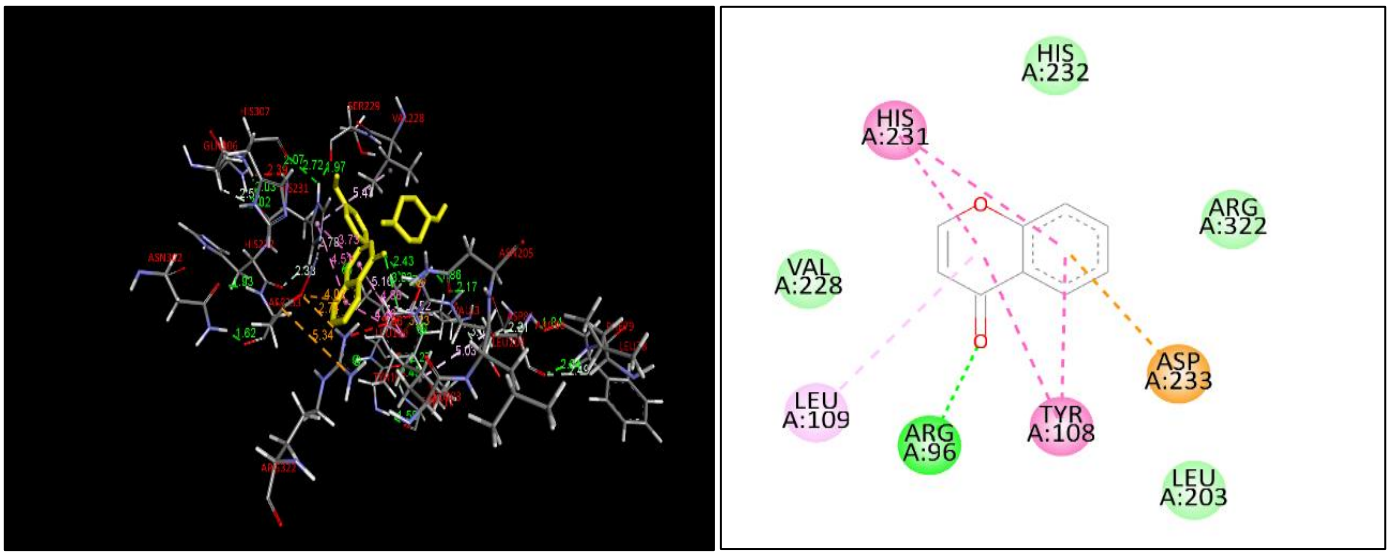


Fig 8: α- Amylase Inhibitory Activity Assay: a) % Inhibition of HEPG; b) % Inhibition of Acarbose c) IC_{50} of HEPG & Acarbose

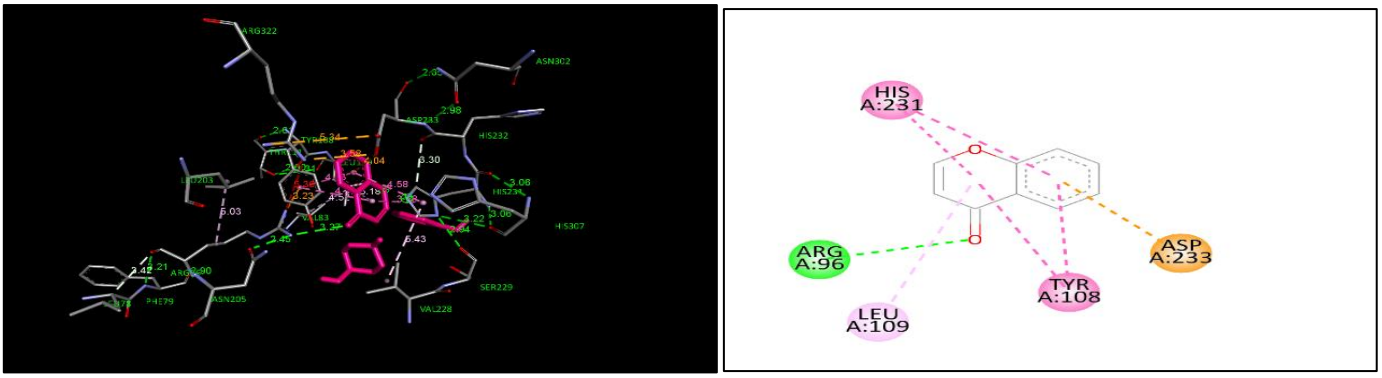
G. In Silico Molecular Docking Studies

According to docking research, selected ligands (Reynoutrin, Guaijaverin, Gallic acid, Quercetin and catechin) displayed better binding affinity with protein 3LFM by negative gliding scores nearer to that of standard anti-obese drug orlistat. The results were depicted in figure 9 & table 9. The results showed that Reynoutrin is having the highest glide score (-9.2) closer to the Orlistat (-11.6). The order of the gliding scores: Reynoutrin (-9.2) > Guaijaverin (-8.8) > Catechin (-8.0) > Quercetin (-7.8) > Gallic acid (-5.9).

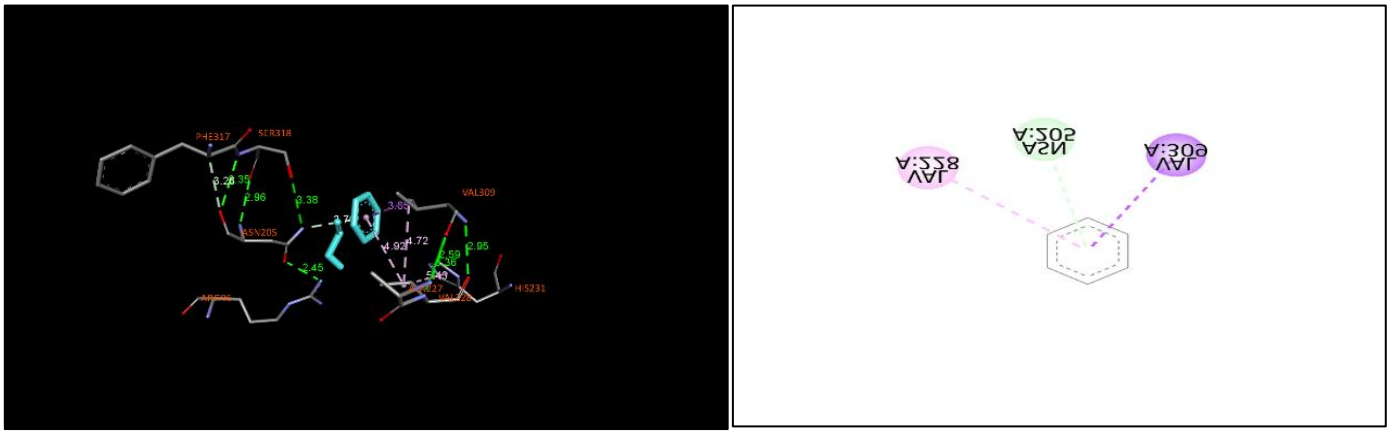


a) Reynoutrin

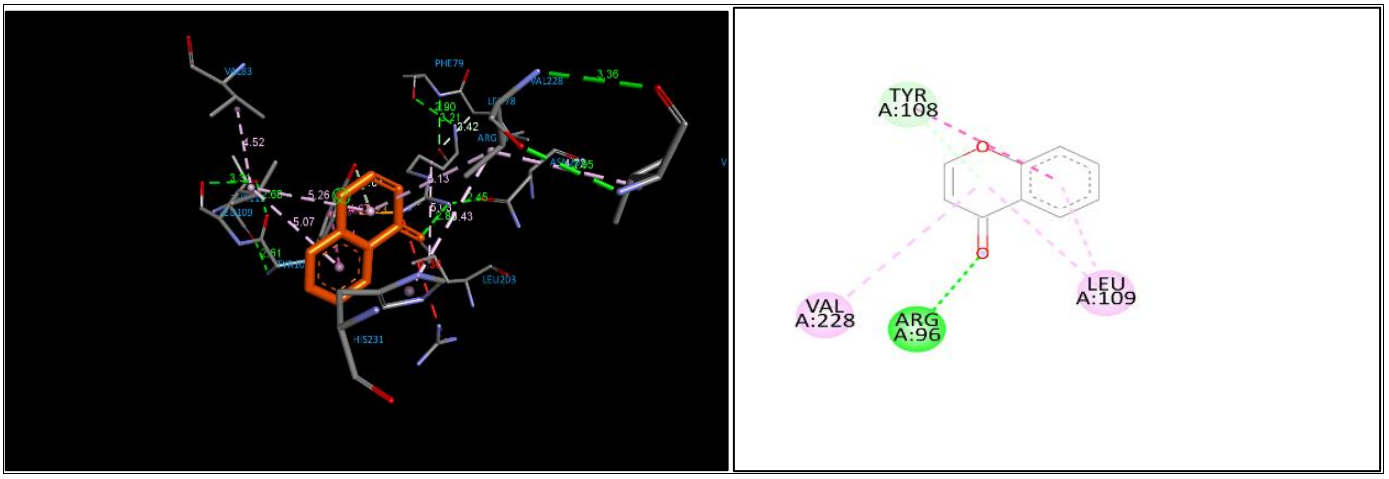
ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327



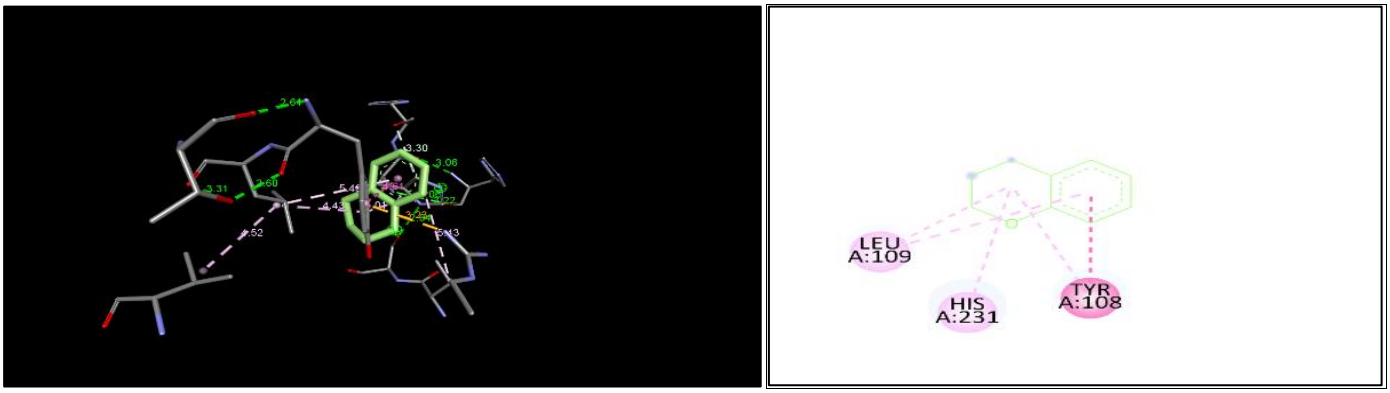
b) Guaijaverin



c) Gallic Acid

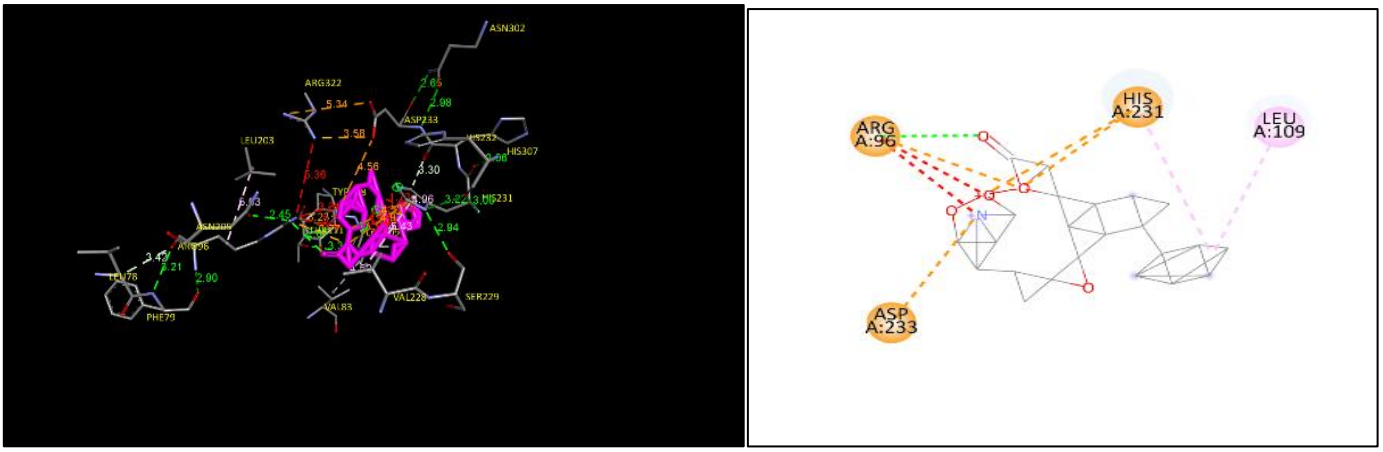


d) Quercetin



e) Catechin

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327



f) Orlistat

Fig 9: Docking Modes for the Respective Ligands (a, b, c, d, e, f) with 3LFM Protein

Table 9: Docking Results of Ligands against Fat Mass Obesity Protein (PDB: 3LFM)

S.no.	Name of the ligand	Glide Score
1.	Reynoutrin	-9.2
2.	Guaijaverin	-8.8
3.	Gallic acid	-5.9
4.	Quercetin	-7.8
5.	Catechin	-8.0
6.	Orlistat	-11.6

- Note: More negative glide score indicates the better binding affinity.

IV. DISCUSSION

Nowadays, most people agree that obesity is a chronic illness that has spread to dangerous, epidemic proportions around the globe. with example, hospital surveys conducted in the United States revealed that many younger patients treated with COVID-19 problems also struggled with weight. It should come as no surprise that obesity frequently contributes to other major health issues including diabetes, high cholesterol, and heart disease. Regular exercise and a change in lifestyle are important, but they are frequently insufficient on their own. The hunt for more secure treatment alternatives has been fueled by this [20].

By slowing down the digestion and absorption of carbohydrates and fats, we may specifically target the way our systems metabolize food, which is a potential way to help manage obesity. This method aids in lowering total caloric consumption and fat accumulation. At the moment, the only commonly used medication that inhibits lipase and other fat-digesting enzymes is orlistat. Nevertheless, it has been linked to unpleasant adverse effects, such as gastrointestinal distress and liver damage [21]. As a result, research is being done on natural substitutes that have less adverse effects. Testing a natural substance's capacity to block digestive enzymes is one of the most popular ways to assess its potential to combat obesity. Using both lab-based (in vitro) and computational (in silico) methods, we examined in this work whether a hydro-ethanolic extract of Psidium guajava leaves (HEPG) could function as a natural anti-obesity treatment.

The HEPG extract contains a range of bioactive compounds, including as proteins, triterpenoids, alkaloids, flavonoids, phenolics, and tannins, according to preliminary testing. saponins and phytosterols. Flavonoids have been shown to have long-term effectiveness in preventing and treating obesity by influencing all known obesogenic pathways, according to Kawser Hossain M [22]. This is consistent with the current study's estimation of the quantitative amount of flavonoids, which was around 127 µg QE/g of HEPG. Because HEPG extract quantitatively contains flavonoids with anti-obesity action, this led to the notion that it might have anti-obesity potential.

Bioactive substances can be identified and analyzed using a semi-quantitative method called Thin Layer Chromatography (TLC). The findings demonstrated a clear distinction between phenolic and flavonoid molecules, corroborating One can ascertain whether quercetin and gallic acid are present in HEPG extract by contrasting the reported Rf values of HEPG spots with those of conventional quercetin and gallic acid.

According to R. N. Venkatachalam, Psidium Guajava extracts have the ability to scavenge hydroxyl radicals and reduce ferrous metals [23]. In support of this, HEPG at varying concentrations demonstrated ferrous reducing antioxidant capacity and hydroxyl radical scavenging activity by displaying IC50 values of 206 µg/mL and 214 µg/mL, which are closer to the IC50 values of ascorbic acid and standard gallic acid.

According to a scientific study by S. L. Ong, a significant factor in lowering calorie intake is a measure of inhibition of lipid metabolism [12]. The IC50 value of 155 µg/mL, which is closer to the IC50 value of regular Orlistat, indicates that HEPG at various concentrations produced the lipase inhibitory effect.

According to M. Singh and his colleagues, α-amylase inhibitory action in vitro is a key factor in lowering calorie absorption [24]. HEPG had an α-amylase inhibitory activity at varying concentrations, as seen by its IC50 value of 157 µg/mL, which is closer to the IC50 value of normal acarbose.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327

Flavonoids (Reynoutrin, Guaijaverin, Quercetin, and catechin) and Gallic acid have higher glide scores (-9.2, -8.8, -7.8, and -8.0) and Gallic acid (-5.9) than the standard drug orlistat (-11.6) against fat mass and obesity-associated (FTO) protein (PDB id: 3LFM) for gastric and pancreatic lipase inhibition activity, according to an in silico molecular docking study. These results were found to be comparable to a study by D. Elebeedy [25] where flavonoids demonstrated the greatest glide score as an anti-obesity drug. Therefore, it can be concluded that the presence of gallic acid and flavonoids in HEPG has the best chance of acting as pancreatic lipase inhibitors for their corresponding anti-obesity properties.

V. CONCLUSION

According to a recent study, Psidium guajava (HEPG) leaf hydroethanolic extract has anti-obesity properties. The existence of a number of bioactive substances, such as flavonoids and phenolics, was established by preliminary screening. The presence of quercetin and gallic acid was further confirmed by TLC analysis. The quantity of flavonoids and phenols in HEPG was determined by quantitative analysis.

By reducing ferrous ions and scavenging hydroxyl radicals, HEPG showed potent antioxidant activity. Additionally, it demonstrated notable in vitro suppression of digestive enzymes such as α-amylase and pancreatic lipase, indicating that it may lessen the absorption of fat and carbohydrates. These results were corroborated by in silico docking studies, which demonstrated that the extract's flavonoids exhibited high binding affinities to the fat mass and obesity-associated (FTO) protein and glide scores that were comparable to those of the prescription medication orlistat.

These results offer encouraging first support for the possible application of HEPG as a natural anti-obesity drug in pharmaceutical or dietary applications. To identify the active ingredients and elucidate their actions, more research is required.

ACKNOWLEDGEMENT

I sincerely thank Dr. M. Ganga Raju, Professor and Principal and the Management of Gokaraju Rangaraju College of Pharmacy for constant support in the conduct of research.

REFERENCES

[1]. C. J. Smith, T. A. Perfetti, A. W. Hayes, and S. C. Berry, “Obesity as a source of endogenous compounds associated with chronic disease: a review,” Toxicol. Sci., vol. 175, no. 2, pp. 149–155, Jun 2020.

[2]. World Health Organization, “Obesity and overweight,” WHO,2016.[Online].https://www.who.int/newsroom/factsheets/detail/obesity-and-overweight

[3]. R. Ahirwar and P. R. Mondal, “Prevalence of obesity in India: A systematic review,” Diabetes Metab. Syndr. Clin. Res. Rev., vol. 13, no. 1, pp. 318–321, Jan 2019.

[4]. Mechanick, R. Pessah-Pollack, P. Camacho, R. Correa, M. K. Figaro, J. R. Garber, S. Jasim, K. M. Pantalone, D. Trence, and S. Upala, “American Association of Clinical Endocrinologists and American College of Endocrinology protocol for standardized production of clinical practice guidelines, algorithms, and checklists–2017 update,” Endocr. Pract., vol. 23, no. 8, pp. 1006–1021, Aug 2017.

[5]. W. Sun, M. H. Shahrajabian, and Q. Cheng, “Natural dietary and medicinal plants with anti-obesity therapeutics activities for treatment and prevention of obesity during lock down and in post COVID-19 era,” Appl. Sci., vol. 11, no. 17, p. 7889, Aug 2021.

[6]. Raval and T. Ganatra, “Basics, types and applications of molecular docking: a review,” Int. J. Compr. Adv. Pharmacol., vol. 7, no. 1, pp. 12–16, Mar 2022.

[7]. P. Bulugahapitiya, S. Kokilananthan, H. Manawadu, and C. S. Gangabadage, “Phytochemistry and medicinal properties of Psidium guajava L. leaves: A review,” Plant Sci. Today, vol. 8, no. 4, pp. 963–971, Dec 2021.

[8]. T. Rufino, V. M. Costa, F. Carvalho, and E. Fernandes, “Flavonoids as anti-obesity agents: A review,” Med. Res. Rev., vol. 41, no. 1, pp. 556–585, Jan 2021.

[9]. D. M. Joshi, S. S. Pathak, S. Banmare, and S. S. Bhaisare, “Review of phytochemicals present in Psidium guajava plant and its mechanism of action on medicinal activities,” Cureus, vol. 15, no. 10, Oct 2023.

[10]. T. Agbor-Egbe and J. E. Rickard, “Identification of phenolic compounds in edible aroids,” J. Sci. Food Agric., vol. 51, no. 2, pp. 215–221, 1990.

[11]. Samak, R. P. Shenoy, S. M. Manjunatha, and K. S. Vinayak, “Superoxide and hydroxyl radical scavenging actions of botanical extracts of Wagatea spicata,” Food Chem., vol. 115, no. 2, pp. 631–634, Jul 2009.

[12]. S. L. Ong, S. U. Paneerchelvan, H. Y. Lai, and N. K. Rao, “In vitro lipase inhibitory effect of thirty two selected plants in Malaysia,” Asian J. Pharm. Clin. Res., vol. 7, no. 2, pp. 19–24, 2014.

[13]. O. Unuofin, G. A. Otunola, and A. J. Afolayan, “In vitro α-amylase, α-glucosidase, lipase inhibitory and cytotoxic activities of tuber extracts of Kedrostis africana (L.) Cogn,” Heliyon, vol. 4, no. 9, Sep 2018.

[14]. S. A. Muhammad and N. Fatima, “In silico analysis and molecular docking studies of potential angiotensin-converting enzyme inhibitor using quercetin glycosides,” Pharmacogn. Mag., vol. 11, Suppl. 1, pp. S123–S123, May 2015.

[15]. R. Shaikh and M. Patil, “Qualitative tests for preliminary phytochemical screening: An overview,” Int. J. Chem. Stud., vol. 8, no. 2, pp. 603–608, Mar 2020.

[16]. Sowmya and D. Anandhi, “Quantification of total phenolics, flavonoids and evaluation of in vitro free radical scavenging activities in Psidium guajava L.,” Indian J. Pharm. Sci., vol. 82, no. 4, Jun 2020.

[17]. S. A. Patil, P. S. Salve, R. S. Phatak, and N. D. Chivate, “Quantitative estimation of total phenolic, total flavonoid content and assessment of in-vitro antioxidant capacity of Psidium guajava leaves extracts,” Res. J. Pharm. Technol., vol. 16, no. 3, pp. 1028–1032, 2023.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25may2327

[18]. T. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavonoids, Springer Sci. & Bus. Media, Dec. 2012.
[19]. Berker, K. Güçlü, B. Demirata, and R. Apak, "A novel antioxidant assay of ferric reducing capacity measurement using ferrozine as the colour forming complexation reagent," Anal. Methods, vol. 2, no. 11, pp. 1770–1778, 2010.
[20]. Dalamaga, G. S. Christodoulatos, I. Karampela, N. Vallianou, and C. M. Apovian, "Understanding the co-epidemic of obesity and COVID-19: current evidence, comparison with previous epidemics, mechanisms, and preventive and therapeutic perspectives," Curr. Obes. Rep., vol. 10, no. 3, pp. 214–243, Sep 2021.
[21]. S. A. Tucci, E. J. Boyland, and J. C. Halford, "The role of lipid and carbohydrate digestive enzyme inhibitors in the management of obesity: a review of current and emerging therapeutic agents," Diabetes Metab. Syndr. Obes. Targets Ther., vol. 3, pp. 125–143, May 2010.
[22]. Hossain, A. Abdal Dayem, J. Han, Y. Yin, K. Kim, S. Kumar Saha, G. M. Yang, H. Y. Choi, and S. G. Cho, "Molecular mechanisms of the anti-obesity and anti-diabetic properties of flavonoids," Int. J. Mol. Sci., vol. 17, no. 4, p. 569, Apr 2016.
[23]. R. N. Venkatachalam, K. Singh, and T. Marar, "Phytochemical screening in vitro antioxidant activity of Psidium guajava," Free Radic. Antioxid., vol. 2, no. 1, pp. 31–36, Jan 2012.
[24]. Singh, T. Thrimawithana, R. Shukla, and B. Adhikari, "Inhibition of enzymes associated with obesity by the polyphenol-rich extracts of Hibiscus sabdariffa," Food Biosci., vol. 50, p. 101992, Dec 2022.
[25]. Elebeedy, A. Ghanem, A. Saleh, M. H. Ibrahim, O. A. Kamaly, M. A. Abourehab, M. A. Ali, A. I. Abd El Maksoud, M. A. El Hassab, and W. M. Eldehna, "In vivo and in silico investigation of the anti-obesity effects of Lactiplantibacillus plantarum combined with chia seeds, green tea, and chitosan in alleviating hyperlipidemia and inflammation," Int. J. Mol. Sci., vol. 23, no. 20, p. 12200, Oct 2022.