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Original Research Article

Assessment of the Effect of Flavonoids Biomolecules on Fat Mass and Obesity Associated (FTO) Protein as Anti-Obesity Agents: An *In-Silico* Study

Alexander Idoko[1,2]*, Elijah J. Parker [2], Obioma U. Njoku[2]

[1]*Department of Biochemistry, Faculty of Natural Sciences, Caritas University, Amorji-Nike, PMB 01784 Enugu, Nigeria*
[2]*Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria*

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ABSTRACT

Recent studies on the management of obesity are centered on the ability of small compounds to modulate fat mass and obesity-associated protein (FTO). This study is aimed at investigating the inhibitory effects of flavonoid biomolecules on fat mass and obesity-associated protein (FTO) *in silico*. The studied ligands from methanol flavonoid-rich fraction of lime juice (MFLJ) and ethylacetate flavonoid-rich fraction of honey (EAFH) include quercetin, Epigallocatechin, p-Coumarin, Caffeic acid, Naphthoresorcinol, Gallic acid, and Sinapic acid. The ligands were characterized using high-performance liquid chromatography (HPLC). Molecular docking of the ligands and the FTO protein was performed using AutoDock Vina software. Results show that Ser-229, Tyr-108, Asp-233, and Glu-234 are the catalytic sub-units of the FTO protein, which were essential in hydrogen bond formation and interactions between ligands and the FTO protein. The ΔG value of binding affinity for all ligands revealed their potential as inhibitors of FTO protein. Quercetin (-8.2 Kcal/mol), epigallocatechin (-8.0 Kcal/mol), and p-coumarin (-7.3 Kcal/mol) possessed the highest inhibitory effect on the fat mass and obesity-associated (FTO) protein compared to the standard drugs (atorvastatin: -7.5 Kcal/mol and orlistat: -6.6 Kcal/mol). In conclusion, quercetin, epigallocatechin, and p-coumarin exhibited the highest inhibitory effect against FTO protein. This reveals their potential as anti-obesity agents that could be used in the treatment of obesity.

Keywords: Anti-obesity, Binding affinity, Biomolecule, Flavonoids, Ligands, Obesity.

Introduction

Obesity is known to result from excessive consumption of food and its subsequent deposition as excess body fat and has become a global health concern.[1] Accumulation of high calories than the body can utilize result in obesity. In an obese state, the risk of cardiovascular disease, type 2 diabetes mellitus, cancer, osteoarthritis, and sleep apnea increases.[1,2] The most important risk factor for obesity is a sedentary life style, where inactivity gradually becomes a choice and excess calories are not burned, in the presence of food intake. Factors contributing to the increase in body weight and obesity may be genetic, socioeconomic status, environmental, low calcium intake,[3] and imbalance in the utility and intake of calories.[2] Obesity is therefore a concern for all classes, races, genders, and ages.[4] It has become a major public health problem. It has been reported that obesity and overweight result in decrease life span, irregularities in metabolism and hampered cellular processes that eventually result in artificial or premature aging.[5] The fat mass and obesity-associated protein (FTO) is an N^6-methyladenosine demethylase, reportedly associated with high obesity risk and other illnesses including type 2 diabetes, glioblastoma, myeloid leukemia, cervical carcinoma, and breast cancer.[6] The fat mass and obesity-associated gene (FTO) is found in chromosome 16q12.2, having 410.50 kb (total length), with 9 exons and 8 introns.[7]

*Corresponding author. E mail: alexidoko@caritasuni.edu.ng
idokoalexander1@gmail.com
Tel: +2348032354823

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Obesity is among the numerous metabolic syndromes linked to dysfunction in many biochemical mechanisms and enzymatic pathways, such as promotion of fat mass and obesity-associated protein (FTO).[8] Essentially, the impact of FTO protein in the development of obesity is associated with the accumulation of body fat in the adipose tissue, leading to increased BMI and waist circumference.[8] Up-regulation of FTO protein was said to result in improved fat metabolism, increased adipogenesis, and obesity.[9] Through molecular docking coupled with molecular dynamics simulation, it was reported that mutations influence FTO substrate binding capacity, changing the affinity of the ligand to FTO protein.[10] Increasing level of FTO protein increases obesity risk by influencing food intake, reducing satiety, and resulting in overeating.[10]

The nutritional and medicinal properties of honey depend on its composition. The source of the plant, production methods, and season are responsible for the different chemical composition of honey.[11] Composition of pure honey include minerals, volatile compounds, alkaloids, flavonoids, reducing sugars, polyphenols, glycosides, anthraquinone, and cardiac glycosides.[12,13] *Citrus aurantiifolia* is a rich source of flavonoids and vitamin C,[15] and is often used to accent the flavors of foods and beverages.[14,16] Some medicinal and health benefits associated with honey and lime juice include prevention of hypercholesterolemia in rats,[17] anti-obesity, antioxidant, and anti-hyperlipidemic effects in carbohydrate and lipid diets-obese rats,[18] renal and liver protective effects.[19] Consumption of honey enhances the body's defense mechanism against oxidative stress coupled with free radical-enabled cellular damage and aging.[13] The bioactive chemical components of fresh lime juice and honey accentuate their significant place as medicinal compounds effective as anti-obesity and anti-diabetic,[20] antimicrobial,[21] anti-hypercholesterolemic and anti-hyperglycemic agents.[17]

Flavonoids are known for their multiple medicinal functions and properties such as antioxidant, anti-obesity, free radical scavenging,

anti-inflammatory, and cytoprotective effects.[14,15] As radical scavengers, flavonoids are capable of hampering the oxidation of low-density lipoprotein (LDL) and then exert their inhibitory action against atherosclerosis.[14]

Through molecular docking and simulation studies, different flavonoids such as quercetin, exemestane, kaempferol, letrozole, rutin,[22] catechins, epicatechin, epigallocatechin and gallocatechin[10] are reported to have inhibitory interaction with FTO protein in the adipocytes, where they exert their anti-obesity effects through lipolytic mechanism by reducing adipogenesis and enhancing lipolysis.[10,22] Molecular docking studies have also reported the activity of other flavonoids such as flavonols,[23] apigenin, naringenin,[24] and taiwaniaquinoids[25] as anti-obesity agent targeting the FTO protein. This study investigated the anti-obesity potentials of flavonoids including quercetin, epigallocatechin, p-coumarin, caffeic acid, naphthoresorcinol, gallic acid, and sinapic acid, isolated from methanol flavonoid-rich fraction of fresh lime juice (MFLJ) and ethylacetate flavonoid-rich fraction of honey (EAFH) via *in silico* approach. Docking was done between these flavonoids and the target protein, FTO. The standard anti-obesity drugs (orlistat and Atorvastatin) were used as positive control ligands to check if the studied flavonoids possess anti-obesity activity that will influence their potential use as replacement or healthy alternatives or as adjuvants in the management of obesity.

Materials and Methods

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and products of Sigma Aldrich, USA, British Drug House (BDH) England, Burgoyne, Clinical Lancet Laboratory, India, Harkin and Williams, England, Qualikems India, Fluka Germany, May and Baker England.

HPLC Chemicals and materials

Formic acid (analytical grade), methanol (lichrosolv), acetonitrile (lichrosolv), rutin (95%) and quercetin (95%), and quercitrin (85%), kaempferol (90%) and isorhamnetin (99%) (analytical grades) reference standards.

Collection of Citrus Fruit and Honey

Fresh lime fruits were harvested from an orchard in Imezi-Owa in Ezeagu Local Government Area of Enugu state, latitude: 6° 22' 4" N and longitude: 7° 21' 54" E, Nigeria in March 2020. In April 2020, freshly harvested honey was collected with the comb from divine favor Bee-keeping and honey production farm, Ezikolo Abbi, Uzo-Uwani Local Government Area of Enugu state, Nigeria, with coordinates: 6°43'60" N and 7°1'0" E. the lime fruits were identified and authenticated at the Department of Plant Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria with a voucher number of UNH 622a.

Preparation of Flavonoid-Rich Fraction from Fresh Lime Juice

Lime juice extract (150.09 g) was fractionated in a silica gel (120-200 mesh) column. The column was eluted in a stepwise gradient using 100% each of the following solvents; n-hexane, dichloromethane, methanol, and ethanol. Fractions from each solvent were collected and concentrated in an Electro-Thermostatic Water Cabinet, model: DK-8A, Shanghai Yuejin Medical Instrument. Dichloromethane and n-hexane did not elute any phytochemical in the process. The concentrated solvent fractions yielded methanol fraction (MF; 14.41 g: 9.60% w/w) and ethanol fraction (EF; 11.21 g: 6.20% w/w). The methanol fraction which gave the highest concentration of flavonoids was subjected to further separation in a silica gel (60-120 mesh) column, using varying ratios of methanol and ethanol (1:0; 9:1; 8:2; 6:4; 4:6; 5:5) for elution. The sub-fractions (100 mL each) were concentrated in a water bath at 40°C. The sub-fractions were screened for flavonoids and the methanol sub-fraction of lime juice was found to be richest in flavonoid, and was tagged MFLJ (methanol flavonoid-rich fraction of lime juice).

Preparation of Flavonoid-Rich Fraction from Honey

This was carried out according to the methods of Soria *et al*.[30] and Manyi-Loh *et al*.[31] Briefly, into a 2000 mL separating funnel was transferred 200 g of honey. The honey was diluted with 100 mL distilled water and then extracted with 200 mL of chloroform. Two layers were formed: the upper chloroform layer and the lower aqueous layer after the mixture settled. The aqueous layer was transferred into a separating funnel and n-hexane (200 mL) was added and mixed thoroughly. Three layers were formed after the mixture was left to stand for 1 hour, giving rise to the n-hexane layer with bubbles, the n-hexane dark brown layer, and the n-hexane orange-colored layer. A qualitative phytochemichemical test was done on all the layers and the second layer (n-hexane dark brown layer) was found to be richest in flavonoid. The second layer was then transferred into the separating funnel and after separation, ethylacetate and water in the ratio of 1:1 were added and mixed thoroughly and allowed to settle for 1 hour. Two layers were formed: an upper layer with viscous bubbles and a lower layer of yellowish liquid solution. The lower layer was collected and concentrated in a hot water bath at 40^0C. Qualitative phytochemical test of the concentrated fraction confirmed flavonoid to be very high, and terpenoid and glycoside, to be slightly present. Using the separating funnel, the concentrated fraction was mixed with ethylacetate and water in the ratio of 1.7:0.3 for the final extraction of flavonoid fraction, and this was tagged as the ethylacetate flavonoids rich fraction of honey (EAFH).

High-Performance Liquid Chromatography (HPLC): An HPLC Diode array (DA) detector was used to analyze the quantity and types of flavonoids in MFLJ and EAFH.[32] High-Performance Liquid Chromatography was performed on Agilent LC-8518, model: AG 5042, United States of America, having a solvent delivery LC-8518 pump with a high-pressure switching valve, low-pressure gradient, a micro syringe for sample injection and a high-sensitivity LC-8518 Diode array (DA) detector. A 40 µL sample volume and a column size of 150 x 4.6 mm were used. The system mobile phase was set up in the ratio of acetonitrile:Water:Formic acid (25:74:1). The column temperature was set at 40ºC, maximum wavelength of 254 nm, and a run time of 25 min.

Preparation of standard solutions: The reference standards (0.001 g each) were weighed into a test tube and dissolved with 10 mL of 70% methanol or ethanol to prepare stock solutions. After agitating each test tube of the standard for 10 min with the use of a vortex mixer, Model: BI-VM-2500, New Delhi, India, the mixture was filtered using a micron filter, model: AQ-78254, China, and the filtrate was collected in a sample container.

Sample Preparation and Extraction: In a closed test tube, 10 mL of 70% methanol was used to dissolve 0.1 g of MFLJ or EAFH sample and after 1 hour 30 min, the extracted sample was decanted, and centrifuged using Demon/IEC HN-5 centrifuge, model NNS, Germany and filtered into a 5 mL sample bottle using a micron filter, model: AQ-78254, China.

Protein Retrieval

The crystal structure of FTO (PDB ID: 3LFM) (Figure 3) was obtained from the Protein Data Bank (PDB). The protein structure was refined using Discovery Studio software.

Ligand Retrieval

The 3D format of Quercetin, Epigallocatechin, p-Coumarin, Caffeic acid, Naphthoresorcinol, Gallic acid, and Sinapic acid were downloaded in an SDF file from the PubChem database. The SDF files were converted to PDB using the Pymol software.[26]

Pharmacokinetic and Drug likeness properties of the compounds in MFLJ and EAFH

Table 2 shows the pharmacokinetic and drug-likeness potentials of the various compounds in the flavonoid-rich fractions of lime juice and honey. Prediction of the drug-likeness and pharmacological properties of these ligands were done following the Lipinski rule of 5 while web

servers;swissadme (http://www.swissadme.ch/index.php) and pKCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction) were used to determine the pharmacokinetic properties.

Protein and Ligand Preparation
Before molecular docking, AutoDock tools were used to add polar hydrogen, grid options, and charges to the FTO protein and the ligands. The active site investigation was performed using Molecular Operating Environment.[27]

Molecular Docking
AutoDock Vina Software was employed for the molecular docking,[28] while the protein-ligand interactions (2D diagrams and 3D (surface) views) were achieved by Discovery studio software version 2021 and Pymol software version 1.56 Sep 17_14,[26] respectively. With SwissADME Server, the solubility, physicochemical, Lipinski drug-likeness, pharmacokinetics, and lipophilicity properties of the studied ligands were ascertained.[29]

Results and Discussion

Phytochemical constituents of different solvent fractions of Lime Juice
The phytochemical analysis result of the different solvent fractions of lime juice is shown in Table 1. The result shows that flavonoids, alkaloids, and carbohydrates were slightly present in the ethanol fraction, while the methanol fraction shows high amount of flavonoids and small amount of saponins, carbohydrates, alkaloids, and proteins. In the ethyl acetate fraction, flavonoids and alkaloids were slightly present while carbohydrates and proteins were present in moderate amount. The water fraction shows slight presence of flavonoids, alkaloids, saponins, and carbohydrates. On the basis of the above result, the methanol flavonoids-rich fraction was selected for further investigation.

Compounds identified from the HPLC analysis of methanol flavonoid-rich fraction of lime juice (MFLJ)
Table 2 shows the HPLC profile of the methanol flavonoid-rich fraction of lime juice (MFLJ). Five different flavonoid compounds which include gallic acid, caffeic acid, p-coumaric acid, sinapic acid and quercetin were identified. The concentration of p-coumaric acid was highest followed by gallic acid and then caffeic acid. The HPLC chromatogram of MFLJ (Figure 1) shows that p-coumaric acid and gallic acid gave the highest peak values at concentrations of 54.23 mg/100 g and 22.16 mg/100 g, respectively.

Phytochemical constituents of different solvent fractions of honey
Table 3 shows the qualitative phytochemical screening result for the different solvent fractions of honey. Chloroform fraction showed moderate presence of polyphenol while n-hexane fraction showed polyphenol and flavonoid to be moderately present and ethylacetate fraction showed that flavonoid was highly present.

Compounds identified from the HPLC analysis of methanol flavonoid-rich fraction of honey (EAFH)
The HPLC analysis of the ethylacetate flavonoid-rich fraction of honey (EAFH) result revealed the presence of four different flavonoid compounds. These conpounds include gallic acid, epigallocathechin, napthoresorcinol and quercetin (Table 4, Figure 2). Epigallocatechin had the highest peak area, with a concentration of 73.45 mg/100 g, followed by gallic acid with a concentration of 23.98 mg/100 g .

Binding affinities of the ligands
Results showed that the lead molecules have strong binding affinities within the active site of the proteins (Table 5).

3D view of target proteins
Figure 3 shows the 3D view of the target protein (fat mass and obesity-associated (FTO) protein with the whole protein in red colour and active site region in white colour.

Structural display of Studied Phytocompounds (flavonoids) in MFLJ and EAFH
The chemical structures of the various flavonoid compounds studied are shown in Figure 4.

Active site interaction (2D diagram) of studied phytocompounds of MFLJ and EAFH against the target Protein
The active site interaction of the ligands against the target protein is shown in Figure 5. Figure 5 also revealed the molecular interactions of the compounds within the targeted active site of FTO protein receptors. Here, the interacting amino acid residues within the active sites, the bond lengths, and the bond types are displayed.[33]

3D view of ligand-protein interactions of FTO
Interactions of the studied ligand with FTO protein are shown in Figure 6.
Lipinski's rule (Table 6) was employed to determine the drug-likeness of the studied compounds (quercetin, epigallocatechin, p-coumarin, caffeic acid, naphthoresorcinol, gallic acid, and sinapic acid).[34] Following the Lipinski rule of 5, the molecular weight of a potential drug candidate must be < 500 g/mol, the H-bond donor atom must not be > 5, the H-bond acceptor atom must not be > 10 and the log P value must be < 5. In this light, quercetin, epigallocatechin, p-coumarin, caffeic acid, naphthoresorcinol, gallic acid, and sinapic acid were found to have satisfied these rules and docking analysis proceeded to evaluate their inhibitory activity against FTO protein.[32]

Table 1: Phytochemical constituents of different solvent fractions of lime Juice

Constituent	Ethanol fraction	Methanol fraction	Ethyl acetate fraction	Water fraction
Flavonoid	+	+	+	+
Saponins	-	+	-	+
Alkaloids	+	+	+	+
Carbohydrates	+	+	+	+
Proteins	-	+	+	-

Key: - = absent, + = present

Table 2: Compounds identified in methanol flavonoid-rich fraction of lime juice (MFLJ)

Peak No	Peak ID	Ret Time	Height	Area	Conc. (mg/100 g)
1	p-coumaric acid	2.915	28538.189	2468514.000	54.2333
2	Caffeic acid	1.698	18302.734	678717.000	14.9114
3	Gallic acid	1.090	53136.008	1008833.000	22.1641
4	Sinapic acid	5.465	5682.101	323112.219	7.0988
5	Quercetin	6.015	1564.842	72479.273	1.5924

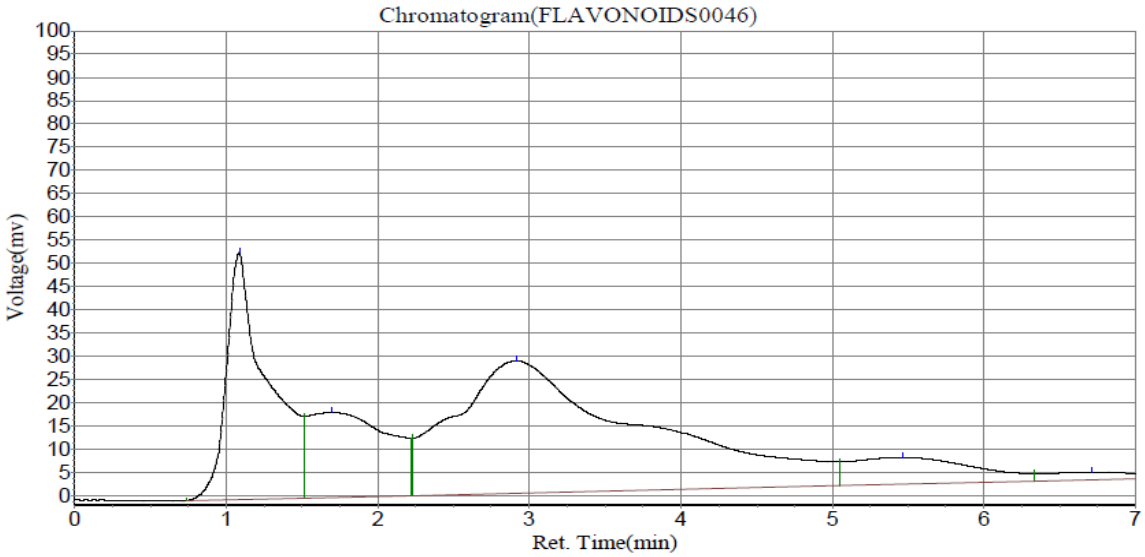


Figure 1: HPLC Chromatogram of methanol flavonoid-rich fraction of lime juice (MFLJ)

Table 3: Phytochemical constituents of different solvent fractions of honey

Constituent	Chloroform fraction	n-Hexane fraction	Ethylacetate fraction
Polyphenol	+	+	-
Tannin	-	-	-
Phenol	-	-	-
Flavonoid	-	+	+
Glycoside	-	+	+
Terpenoid	-	+	+

Key: - = absent, + = slightly present

Flavonoids were fractionated by solvents extraction as MFLJ (Table 1) and EAFH (Table 3), and then characterized from MFLJ (Table 2, Figure 1) and EAFH (Table 4, Figure 2), by high-performance liquid chromatography. Flavonoids are known for their multiple medicinal properties such as antioxidant, free radical scavenging, anti-inflammatory, and cytoprotective activities.[14, 18,35] In our *in vitro* anti-obesity study, we reported that flavonoids in MFLJ and EAFH displayed significant antioxidant and anti-inflammatory effects. They effectively increased high-density lipoprotein (HDL), reduced low-density lipoprotein (LDL), total cholesterol (TC), very low-density lipoprotein (VLDL), triacylglycerol (TAG) and adipocyte size in obese rats.[18] An underlying mechanism by which flavonoids exert their anti-obesity property is thought to involve their ability to manipulate the ability of the sympathetic nervous system to control appetite and to enhance hepatic fatty acid oxidation by enzyme regulation and improvement of energy expenditure.[15,10]

This study investigated the modulating effects of the studied flavonoids (ligands) on FTO protein, to expound the anti-obesity abilities of these ligands on this protein structure and function.[19] Molecular docking was performed to study the anti-obesity ability of the studied flavonoids through binding interaction with the structure of FTO protein, in order to modulate its structure and function.[35] FTO has been reported to be associated with enlargement of adipose tissue, upregulation and susceptibility of metabolic syndrome, BMI, and increase in waist circumference.[36] Flavonoids are believed to be beneficial for obesity and diabetes prevention due to their ability to modulate the activity of fat mass and obesity-associated (FTO) protein receptors.[35,37] Tony *et al.* (2018)[35] in their report stated that catechin and its derivatives could be used as an anti-obesity agent against FTO protein. Quercetin binds to FTO protein to exert its anti-obesity effect by forming a hydrogen bond between the Ser-229 (COO^-) residue of FTO protein and the 6-OH functional group of quercetin.[35] Quercetin binding with FTO protein produced a -8.2 kcal/mol binding affinity value. Meanwhile, the binding interaction of Atorvastatin and Orlistat with FTO protein was by hydrogen bonding to the Glu-234 and Tyr-108 (COO^-) residues of FTO protein, with binding affinities of -7.5 kcal/mol, and -6.6 kcal/mol, respectively. The stronger binding of quercetin to FTO protein than both the standard drugs and other ligands could be due to the hydrogen bond interaction between the Ser-229 (COO^-) residue of FTO protein and the 6-OH functional group of quercetin.[35] Quercetin was reported to exert its anti-obesity effect by blocking adipogenesis via stimulation of the mitogen-activated protein kinase (MAPK) signal pathway and inducing apoptosis of matured adipocytes.[38] Thus, via its hydrogen bond interaction with the FTO protein, quercetin could have modulated the protein function and exerted its anti-obesity effect.[35,38]

Both epigallocatechin and p-coumarin formed two hydrogen bonds with the FTO protein. The ability of a biomolecule with pharmacotherapeutic potential to form complex hydrogen bonds is a key factor in inhibiting the activity of the protein it binds to.[35,39] Epigallocatechin binds to FTO protein by forming two hydrogen bonds via interaction between the 10-OH functional group of epigallocatechin and a COO^- group from Ser-229 residue of FTO protein and 4-OH functional group of epigallocatechin and an NH_2 functional group of Arg-322 residue of FTO protein. FTO protein interacts with epigallocatechin through a binding affinity of -8.0 kCal/mol contributed by the two hydrogen bonds. Epigallocatechin could have exerted its anti-obesity effect on the FTO protein by inhibiting the activity of the protein.[39]

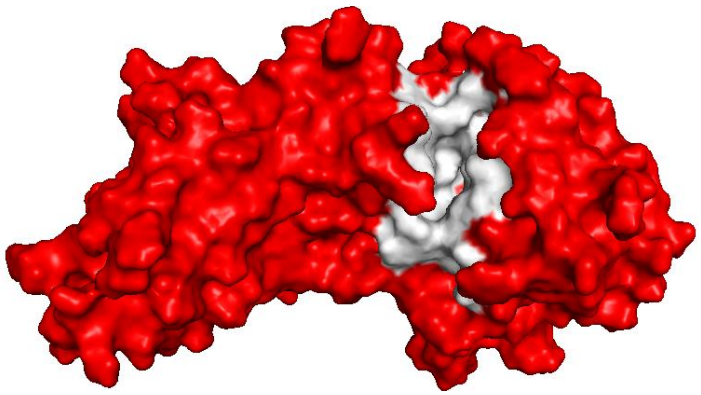
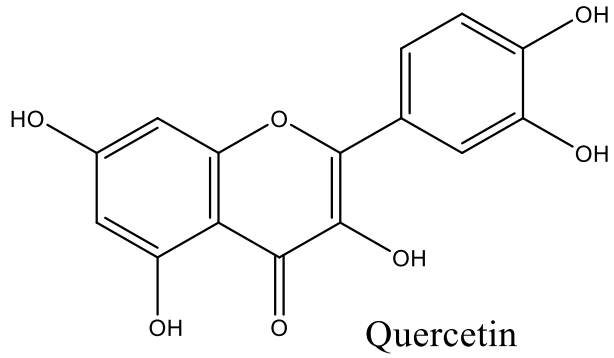
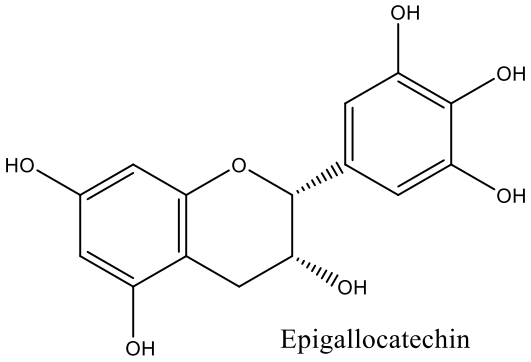


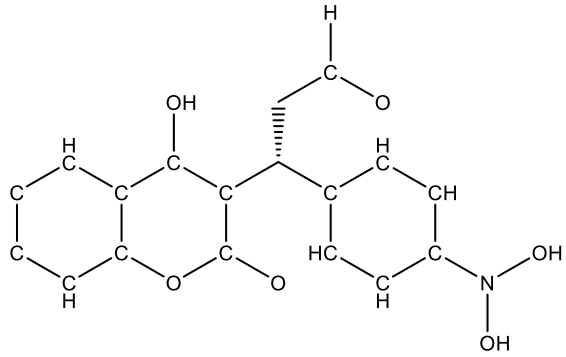
Figure 3: FTO (PDB ID: 3LFM), 3D (surface) view of the target protein with whole protein in red colour and active site region in white colour.

Table 4: Compounds identified in ethylacetate flavonoid-rich fraction of honey (EAFH)

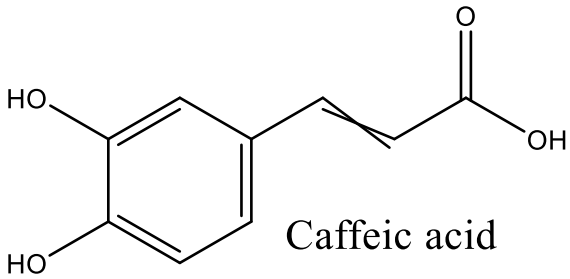
Peak No	Peak ID	Ret Time	Height	Area	Conc (mg/100g)
1	**Gallic acid**	**1.007**	**58989.625**	**1227905.375**	**23.9781**
2	**Epigallocatechin**	**1.873**	**84653.148**	**3761383.750**	**73.4511**
3	**Napthoresorcinol**	**7.615**	**761.316**	**24346.453**	**0.4754**
4	**Quercetin**	**6.032**	**2468.652**	**107303.359**	**2.0954**

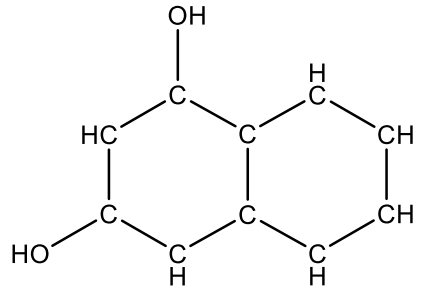




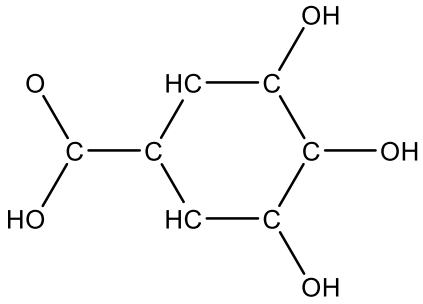


P-Coumarin





Naphthoresorcinol



Gallic acid

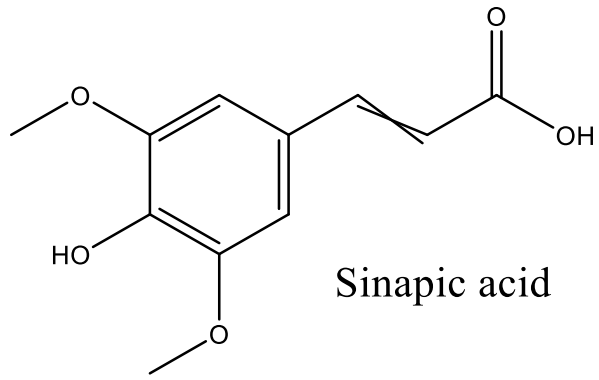


Figure 4: Chemical structures of studied compounds (flavonoids) in MFLJ and EAFH

Table 5: Binding Affinities of flavonoid compounds in MFLJ and EAFH against the target proteins (Fat Mass and Obesity Associated (FTO) Protein PDB ID: 3LFM)

Studied compounds	FTO (Kcal/mol)
Caffeic acid	-6.1
Epigallocatechin	-8.0
Garlic	-6.1
Naphthoresorcinol	-6.3
p-Coumarin	-7.3
Quercetin	-8.2
Sinapic acid	-6.3
Atorvastatin	-7.5
Orlistat	- 6.6

Epigallocatechin was also reported to inhibit the activities of pancreatic lipase and α-amylase. Its mechanism of anti-obesity effect was explained via molecular docking and dynamic simulation, to be linked to its ability to bind to these proteins, with complex hydrogen bond interaction to inhibit their activities.[39] Flavonoids such as epigallocatechins, are reported to exert their anti-obesity ability by activating the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) in the adipocytes and up-regulate lipolysis and down-regulate lipogenesis.[40]

p-Coumarin formed two hydrogen bonds when it binds to FTO protein, through the interaction of a 2-OH functional group of p-Coumarin and a COO^- group of Tyr-108 residue of FTO protein, and an 11-OH functional group of p-Coumarin and a COO^- group of Ser-229 residue of FTO protein. The binding affinity of p-Coumarin's to FTO protein is -7.3 kCal/mol, which also suggests a viable potential of inhibition of FTO protein in its anti-obesity effect.[35] p-Coumaric acid was said to prevent obesity via the activation of thermogenesis in brown adipose tissue (BAT), by up-regulating the thermogenesis–related protein in transcriptional and protein levels.[41]

Gallic acid binds to FTO protein by a hydrogen bond formation via interaction between the 7-OH functional group of gallic acid and a COO^- group of Ser-240 residue of FTO protein. Gallic acid possibly has exerted its anti-obesity effect by binding to FTO, producing ΔG = -6.1 kCal/mol binding affinity less than those of the standard anti-obesity drugs (Atorvastatin ΔG = -7.5 kcal/mol and Orlistat ΔG = -6.6 kcal/mol). The anti-obesity ability of gallic acid is said to be due to its inhibitory effect on fat formation and triacylglycerol accumulation.[42]

Naphthoresorcinol binds to FTO protein by hydrogen bond formation through an interaction between the 1-OH group of naphthoresorcinol and a COO^- group of Arg-322 residue of FTO protein. To the best of the authors' knowledge, naphthoresorcinol has no published or reported molecular docking interaction with FTO protein nor anti-obesity effect yet, this is the first time it has been attempted, and with its binding affinity of ΔG = -6.3 kcal/mol, which is close to that of the standard drug Orlistat (ΔG = -6.6 kcal/mol), it anti-obesity potential might be exploited.[35] Naphthoresorcinol is an inhibitor of the enzyme, prostaglandin synthase (also called cyclooxygenase (COX).[43] Prostaglandin is biosynthesized by prostaglandin synthase. Inhibition of prostaglandin synthase by the inhibitors, indomethacin, or vitamin C in obese patients with vascular dysfunction improved the patients' vascular function.[44] Hypercholesterolemia-induced vasoconstriction and oxidized LDL-induced impairment of vascular function were stopped by the use of prostaglandin inhibitors.[45]

Sinapic acid binds to FTO protein by a hydrogen bond formation, interacting between the 1-OH group of Sinapic acid and an NH_2 group of Glu-234 residue of FTO protein. Both Sinapic acid and naphthoresorcinol exhibited similar binding affinity of ΔG = -6.3 kCal/mol but displayed different amino acid residual interactions with the FTO protein. Data supporting the inhibitory effect of sinapic acid against FTO protein as an anti-obesity agent was lacking at the time this study was conducted. This is the first study to report the molecular docking mechanism of sinapic acid as an anti-obesity agent against FTO. Sinapic acid binding affinity (ΔG = -6.3 kCal/mol) to FTO protein, could have modulated this protein to exert its anti-obesity effect by preventing adipogenesis, reduced serum concentration of LDL, TC, and free fatty acid accumulation.[46] As an anti-obesity agent, sinapic acid was said to reduce organ and body weight, serum glucose, LDL, and TC concentrations, and increased HDL concentration in obese rats.[47]

Caffeic acid was found to possess binding affinity of -6.1 kCal/mol and binds to FTO protein by the formation of two hydrogen bonds via the interaction of the 3-OH functional group of caffeic acid and a COO^- group of Asp-233 residue of FTO protein, and 9-OH functional group of caffeic acid and a COO^- group of Ser-229 residue of FTO protein. In both *in vivo* and *in vitro* studies on teleost fish models, caffeic acid was reported to possess anti-obesogenic properties by decreasing fat accumulation in the adipose tissue.[48]

Table 6: Pharmacokinetic and Drug likeness properties of flavonoid compounds in MFLJ and EAFH

Parameters	Quercetin	Epigallo catechin	p-coumarin	Caffeic acid	Naphtho resorcinol	Gallic acid	Sinapic acid
Molecular weight (g/mol)	302.24	458.37	342.77	180.16	160.17	170.12	224.21
Num. H-bond acceptor	7	11	4	4	2	5	5
Num. H-bond donor	5	8	4	3	2	4	2
Lipophilicity $CLogP_{o/w}$ value	1.23	0.95	3.55	0.93	1.91	0.21	1.31
Water solubility	Soluble	Soluble	Soluble	Very Soluble	Soluble	Very Soluble	Soluble
GI absorption	High	Low	High	High	High	High	High
Druglikeness:	Yes	No; 2 violations:	Yes.	Yes.	Yes.	Yes.	Yes.
Obeys Lipinski rule?	0-violation	NorO>10, NHorOH>5	0-violation	0-violation	0-violation	0-violation	0-violation
Toxicity class	3	6	3	5	4	4	4
Hepato-toxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Immuno-toxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active
Cytotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Carcinogenicity	Active	Inactive	Active	Active	Inactive	Active	Inactive
Mutagenicity	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive

This could support the anti-obesity property of caffeic acid and its inhibitory effect on FTO protein, modulating the protein's reduced expression and function in the adipose tissue. By binding to FTO protein, caffeic acid could exert its anti-obesity effect by improving energy expenditure and activating the brown adipose tissue for thermogenic response, resulting in weight loss.[49]

Gallic acid, quercetin, naphthoresorcinol, caffeic acid, p-coumaric acid, and sinapic acid bind to the active site of FTO receptors, with each compound exerting its anti-obesity effect by modulating this protein. In this study, we propose that these ligands function as agonists when bound to any of the active sites to activate the receptor. By activating the FTO receptor, they can help to decrease food intake and reduce body weight, as well as improve lipid and glucose metabolism.[50] Binding affinity is a significant determinant of the effectiveness of protein-ligand interactions.[51] It can be suggested that ligands binding affinities to fat mass and obesity-associated (FTO) protein may have a significant effect on obesity,[52] as observed from our *in vivo* study.[18] The interaction of these ligands with the FTO protein could inhibit its production[36] and hence result in a reduced level of FTO, which ultimately could be responsible for EAFH and MFLJ anti-obesity abilities.[18,53]

Conclusion

This study revealed the potential of quercetin, epigallocatechin, p-coumarin, caffeic acid, naphthoresorcinol, gallic acid, and sinapic acid (flavonoid bioactive compounds) and the standard anti-obesity drugs atorvastatin and orlistat as inhibitors of FTO protein. The studied compounds especially quercetin and epigallocatechin have greater binding affinity to FTO protein than the standard anti-obesity drugs (atorvastatin and orlistat). This property could be harnessed in producing anti-obesity drugs from these compounds. Essentially, this study showed that the molecular interaction between the studied ligands and FTO protein was due to hydrogen bond formation by the Ser-229, Tyr-108, Asp-233, and Glu-234 amino acids residue from the FTO protein. However, further investigation should exploit and target other obesity-associated proteins for promising anti-obesity drug development.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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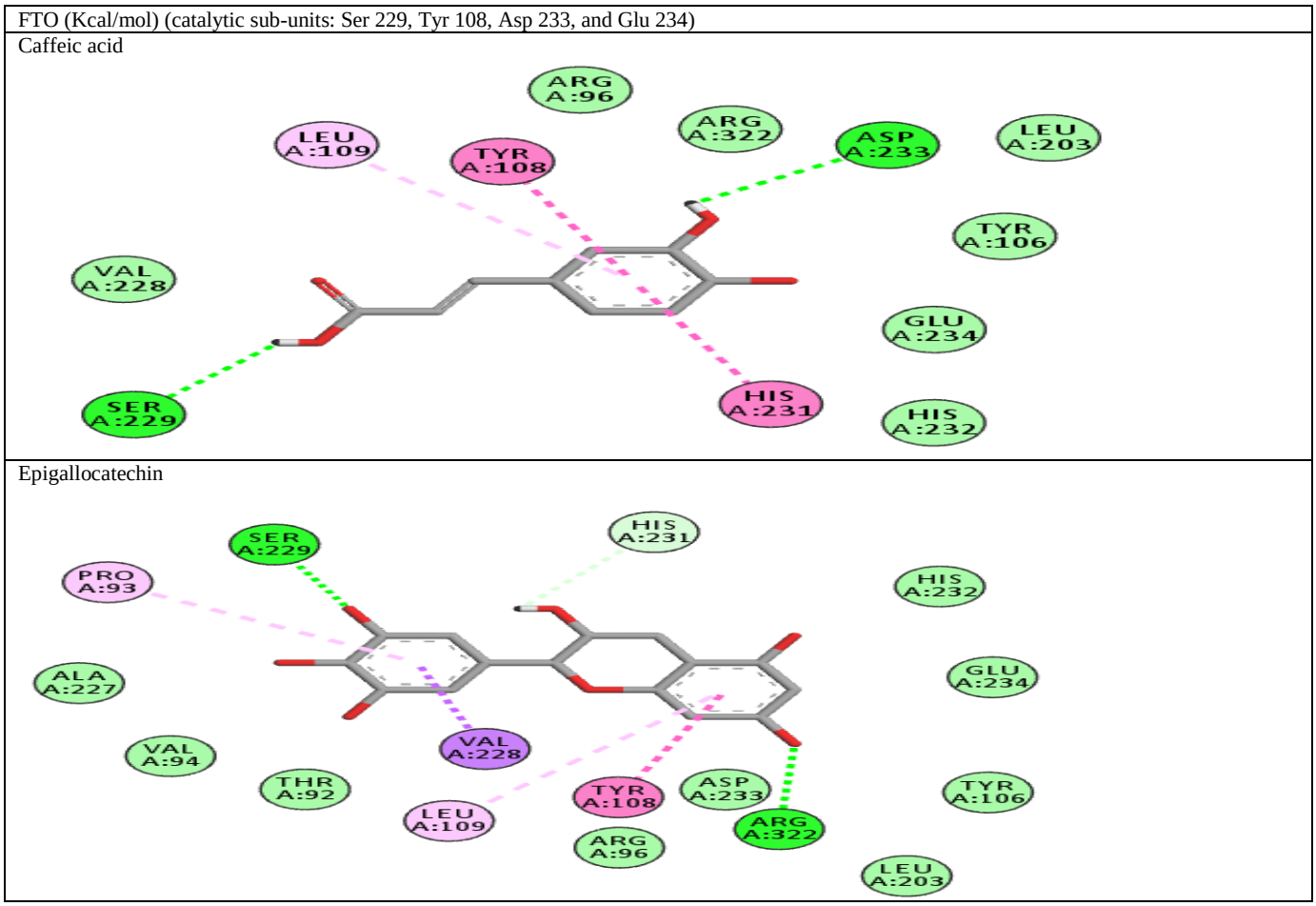

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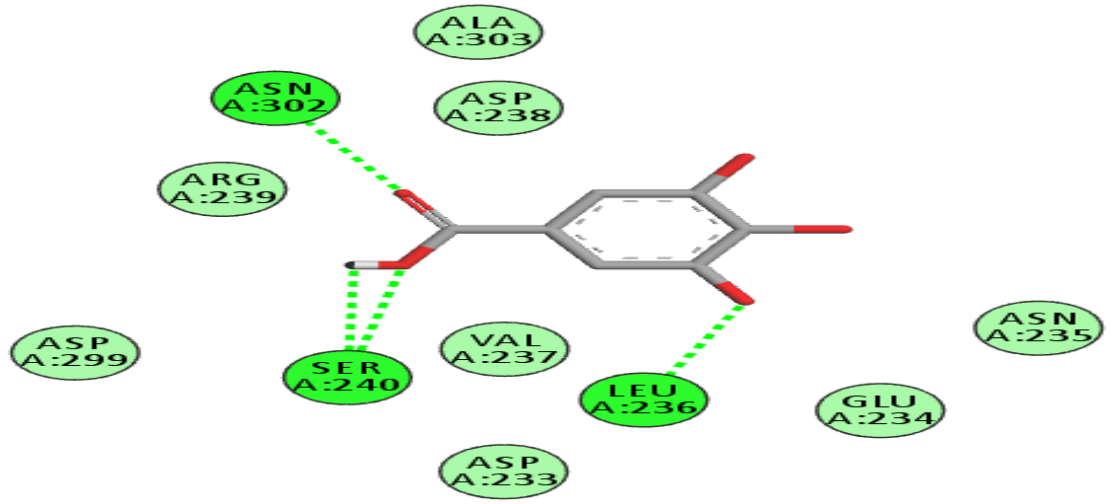
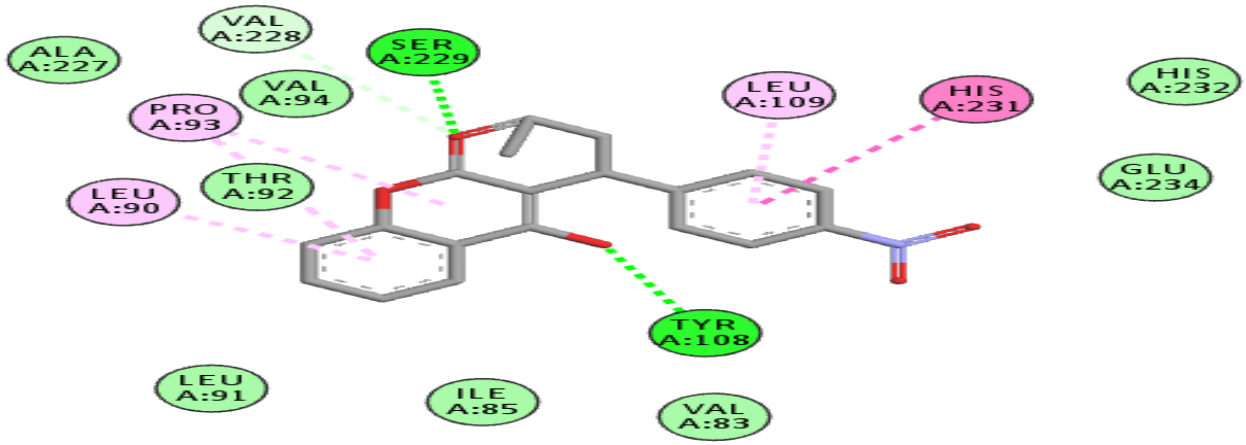
Garlic



p. Coumarin



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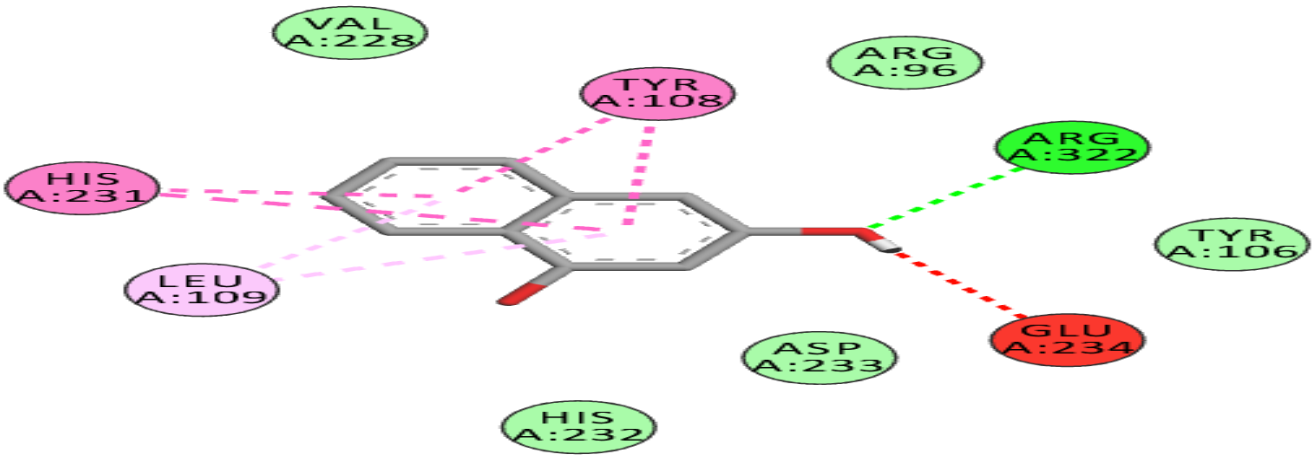

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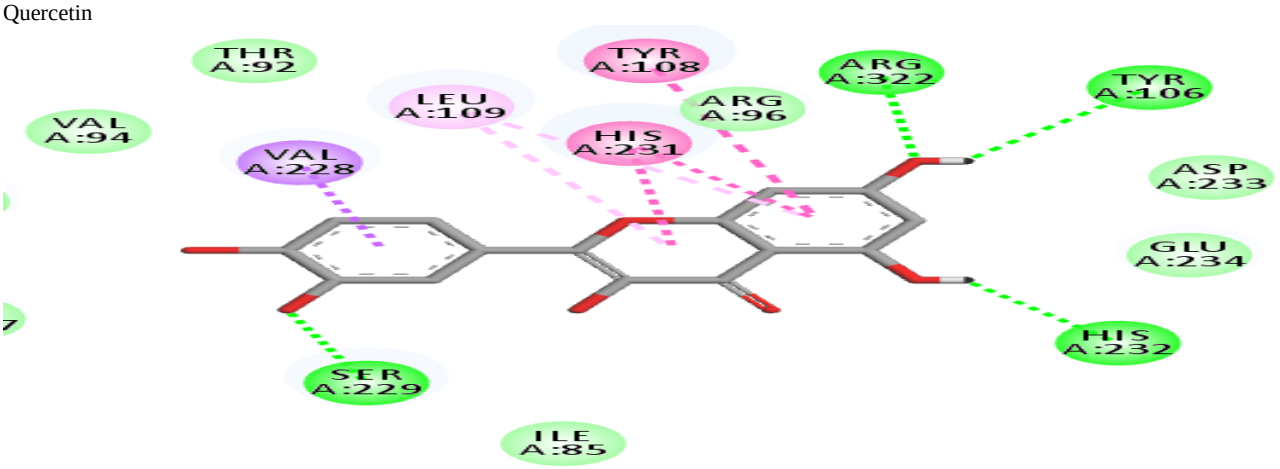
Quercetin



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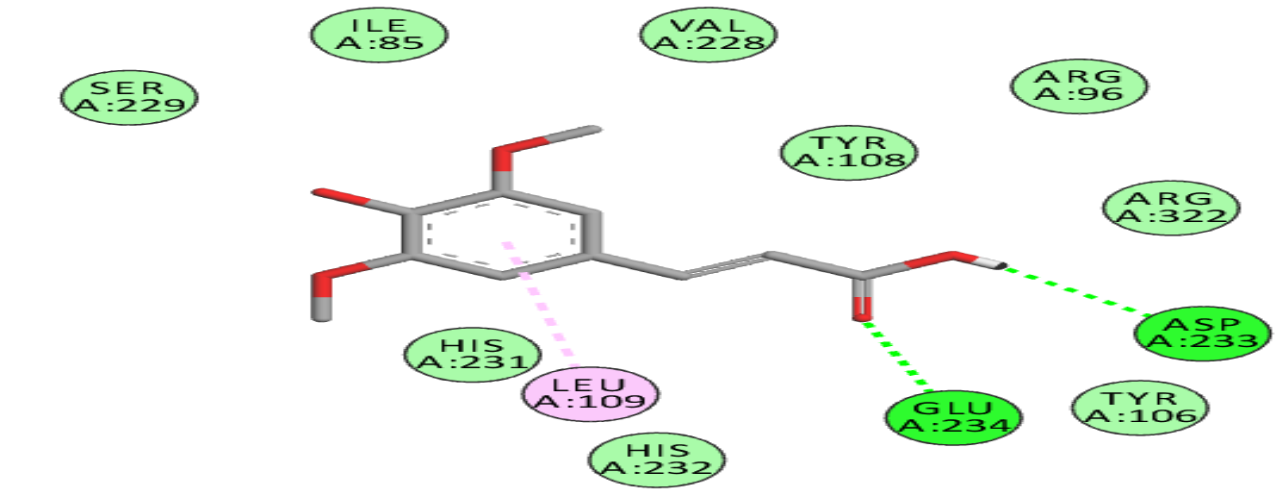

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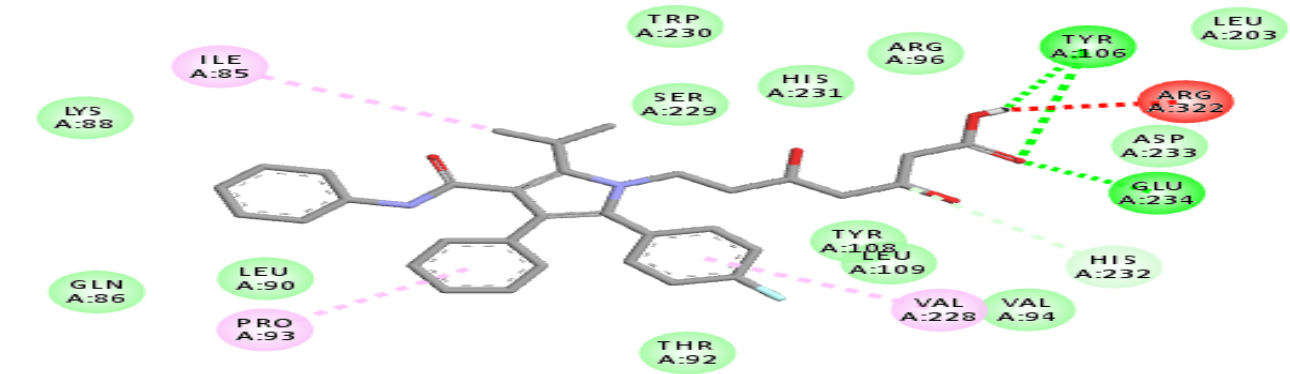

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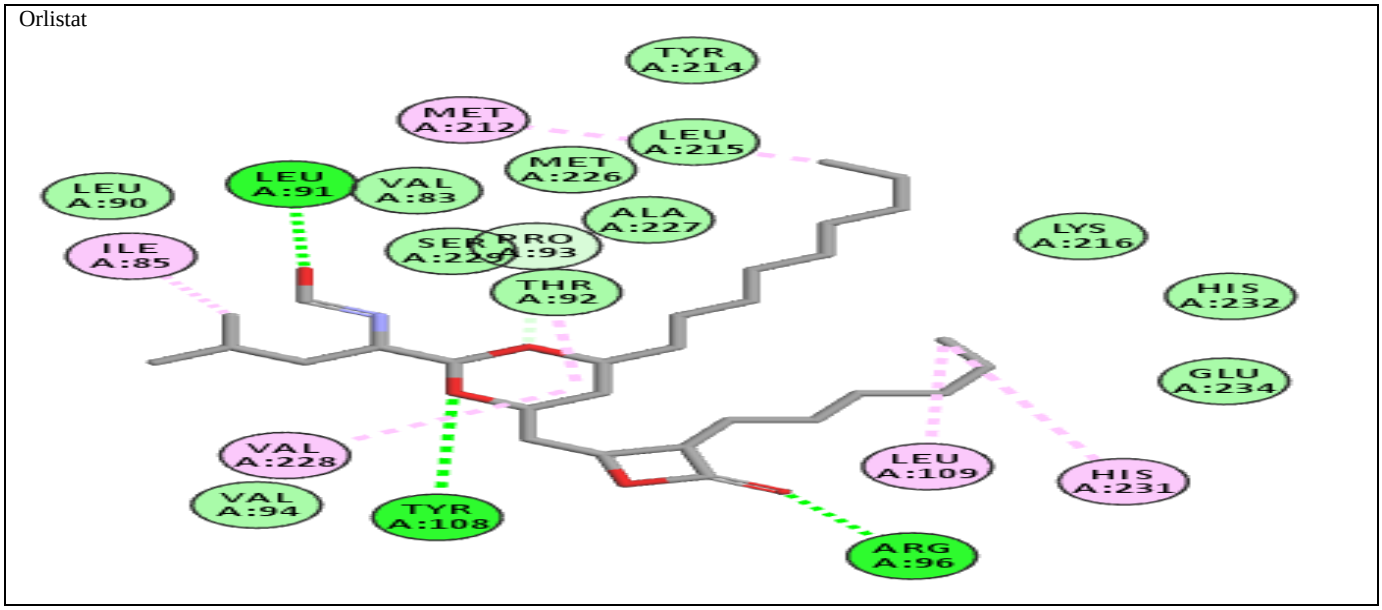
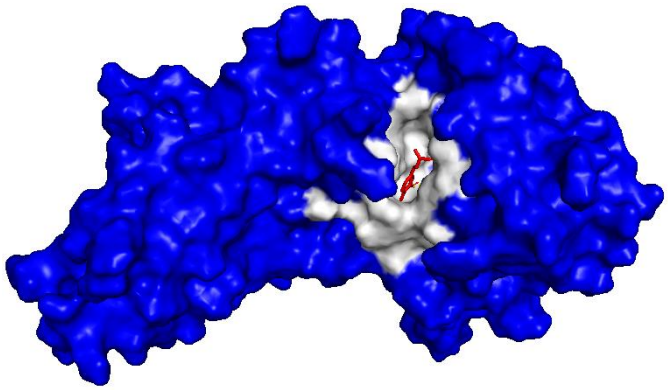
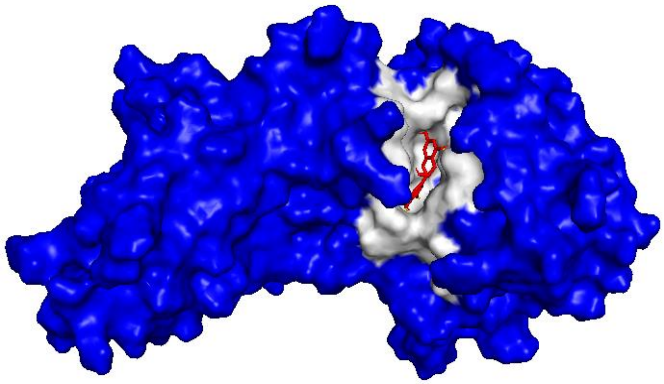


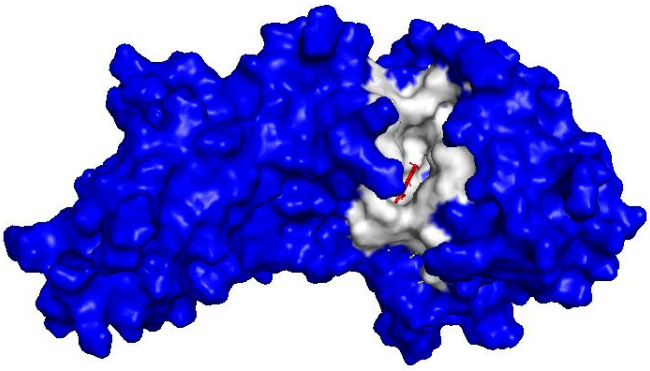
Figure 5: Molecular interactions of the flavonoid compounds and standard drugs within the targeted active site of fat mass and obesity-associated (FTO) protein



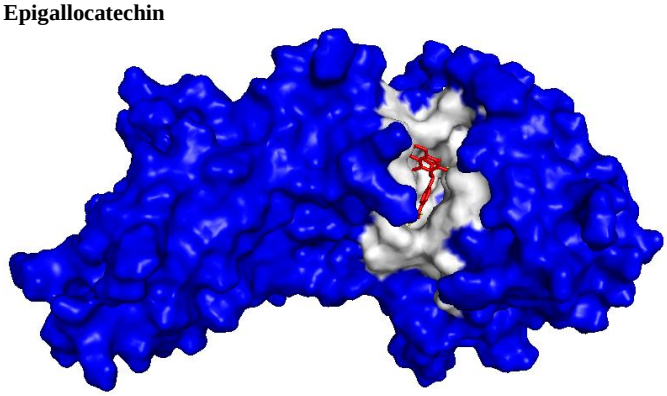
Caffeic acid



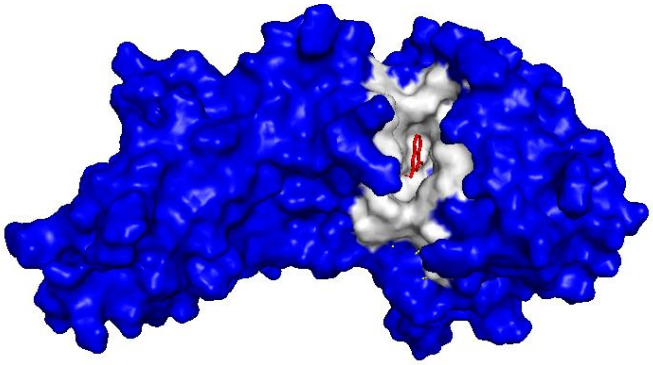
Epigallocatechin

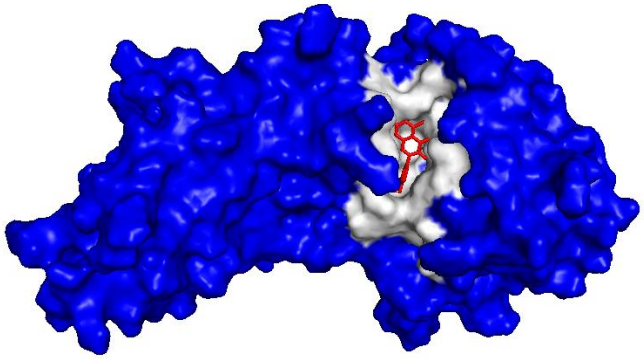


Gallic acid



p. Coumarin





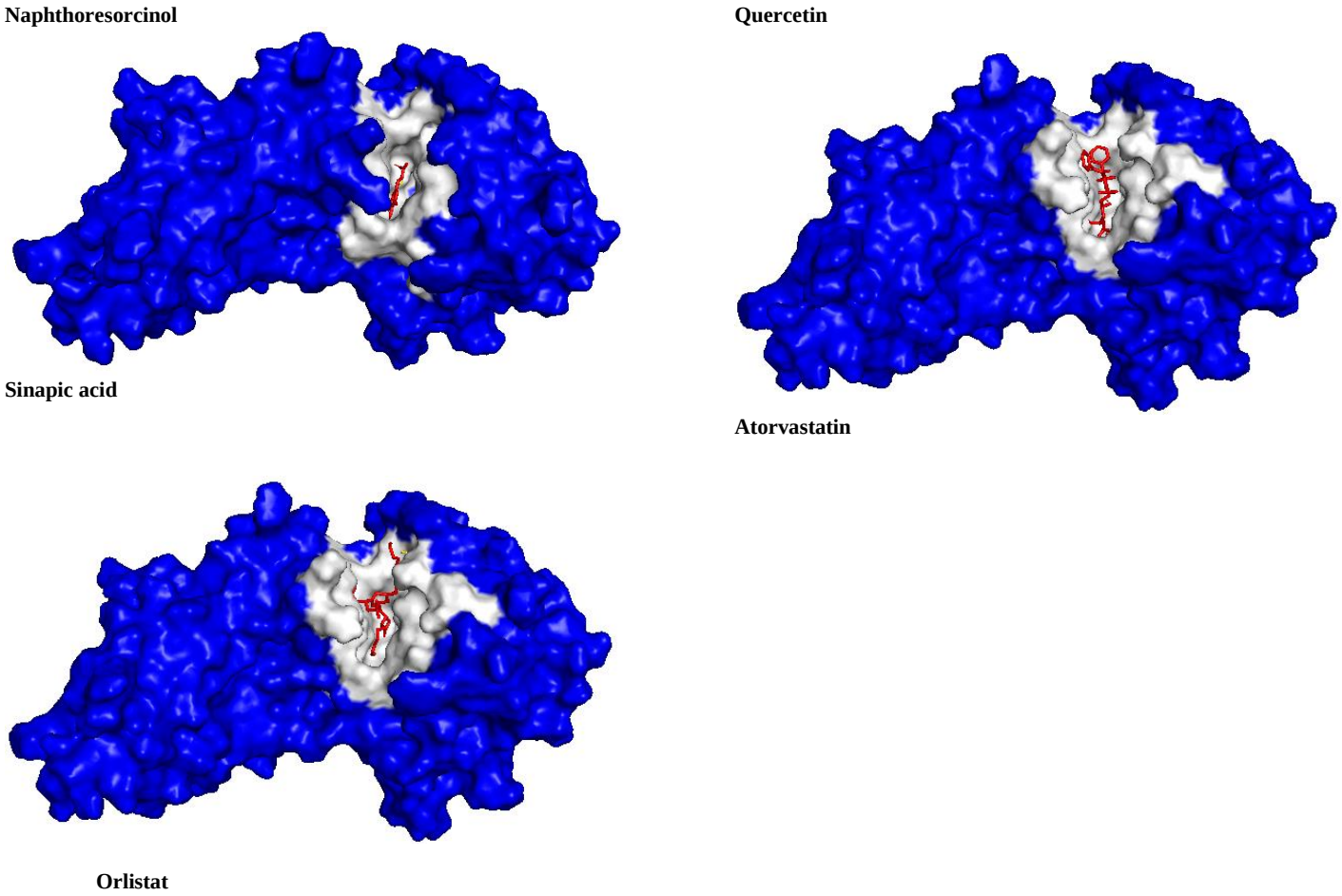


Figure 6: 3D (surface) view of FTO-ligand interactions; protein (blue), catalytic site (white), ligands (red)

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