Evaluation of Anti-Oxidant and Anti-Obesity Potential of Methanolic Extract of Gossypium Herbaceum Plant: An In Vitro and In Silico Study

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Abstract

Obesity and overweight is mainly caused by excessive fat accumulation in adipose tissues. Given its connections to type 2 diabetes mellitus (T2DM), cardiovascular diseases, osteoarthritis, obstructive sleep apnoea, depression, and cancer, obesity is currently one of the biggest global public health concerns. Thus, the top public health priority of the day is the development of a safe and effective anti-obesity medicine. Treatments that specifically target pancreatic lipase or other lipid metabolism-related enzymes may be assessed for their potential to treat obesity in a targeted way. In order to evaluate the potential of the methanolic extract of the complete Gossypium herbaceum (MEGH) plant in silico and in vitro for regulating obesity, the current study examined the phytochemical analysis of the extract as well as its anti-oxidant and lipase-inhibiting properties. MEGH underwent a phytochemical analysis (qualitative and quantitative) using accepted methods. Phytosterols, tannins, cardiac glycosides, alkaloids, and flavonoids were identified in the qualitative phytochemical analyses of MEGH. Autodock Vina and BIOVIA discovery studio were used to perform molecular docking using certain ligands that interact with obesity associated protein (PDB ID: 3LFM) and fat mass. The Catechin displayed the highest docking score (-8.0). Better lipid peroxidation inhibition and lipase inhibition were found in the in vitro experiments that experimentally validated the anti-obesity activities, indicating their considerable potential in the management of obesity.

Key words: *Obesity, Gossypium Herbaceum, Flavonoids, Molecular docking*

1. Introduction

Obesity is an excessive build-up of body fat that poses a health concern. People of all ages and socioeconomic levels are affected by the global epidemic of obesity. Since 1975, the prevalence of obesity has almost tripled, according to the World Health Organization (WHO). Over 1.9 billion people worldwide were overweight in 2016, with over 650 million of them being classified as obese. This rise is especially noticeable in wealthy nations and metropolitan areas, but low- and middle-income nations are also seeing an increase in this tendency [1, 2]. Obesity is linked to a number of harmful health consequences, including cardiovascular diseases, type II diabetes, respiratory ailments, musculo-skeletal disorders, certain malignancies, and mental health difficulties [3]. Conventional methods of treating obesity, like medication therapies and lifestyle changes, frequently have poor results and are accompanied by adverse consequences. As a result, there is more interest in performing research on natural products and plant extracts as possible anti-obesity medicines due to their safety profile [4].

Gossypium herbaceum, commonly known as Levant cotton, belongs to the Malvaceae family and was basically used in the production of medicines and food stuffs. Traditionally, it is used after birth to remove the placenta, increase lactation, cause digestive problems, bleeding, diarrhea, diuretics, and aid wound healing. Thus, it shows pharmacological aspects such as anti-bacterial, anti-convulsant, anti-depressant, anti-diabetic, anti-fertility, ant-helmintic, anti-oxidant, toxic, anti-spermatogenic, anti-tumor, anti-ulcer, anti-epileptic, urolytic and anti-viral activity [5]. The putative anti-obesity benefits of *Gossypium herbaceum* have lately attracted the attention of researchers.

Lately, there has been a greater interest in targeting pancreatic lipase to control obesity. Pancreatic lipase is a significant lipolytic enzyme involved in fat metabolism by hydrolyzing triacylglycerol to monoacylglycerol and fatty acids. Hence, pancreatic lipase contributes significantly to the absorption of dietary triacylglycerol in humans. Therefore, lipase inhibition lowers fat absorption as it interferes with the breakdown of fat, which limits the amount of lipids that are utilized after consumption [6]. In addition the use of molecular docking as an *in silico* technique provides comprehensive information about the binding of selected phytoconstituents to the interacting amino acids of a targeted protein molecule [7].

The aim of the current study is to examine the methanolic extract of *Gossypium herbaceum* whole plant anti-oxidant and anti-obesity properties using *in-vitro* and *in silico* methods. *In vitro* anti-obesity potential was determined by using a lipase inhibition assay and a lipid peroxidation assay. However, *in silico* molecular docking was performed to assess the binding affinity of Catechin, Gossypol, Flavone-3-ol, Stigmasterol, Gamma sitosterol against fat mass and obesity-oriented (FTO) protein to determine their significant role in treating obesity.

2. Materials and Methods

2.1 Materials

Solvents and acids used in this study are analytical grade and purchased from SD Fine Chemicals Ltd. and Research Lab Fine Chem Industries., Mumbai. The reference drugs Quercetin, Atropine and Gallic acid were procured from Research Lab Fine Chem Industries and Tarapur M.I.D.C., Boisar, Mumbai. Materials were weighed using a Shimadzu Corporation, Japan electronic weighing balance. The samples were centrifuged using REMI, India centrifuge. The egg yolk was homogenized using REMI, Indian homogenizer. The absorbance was measured using Merck Prove 300 Spectroquant® UV Spectrophotometer.

2.2 Plant collection and drying

The aerial parts of *Gossypium herbaceum* were collected from several locations in and around Hyderabad during the month of June 2022. The Plant was identified and authenticated by a Botanist in Hyderabad. The aerial parts were shade-dried for a week and grinded into a coarse powder using an electrical grinder. The coarse powder was utilized for the extraction procedure, and the remaining powder was stored.

2.3 Preparation of Methanolic Extract of *Gossypium herbaceum* (MEGH) whole plant

The powdered material of *Gossypium herbaceum* aerial parts were dried and extracted with methanol using the soxhlation process. The methanolic extract obtained was evaporated to dryness using a condenser. Large volumes of medication can be extracted with a considerably lower amount of solvent. This extraction method is cost effective in terms of time, energy, and financial investments.

2.4 Preliminary phytochemical screening of the extract

The methanolic extract of *Gossypium herbaceum* (MEGH) was subjected to preliminary phytochemical screening to identify various phytochemical constituents such as alkaloids, flavonoids, tannins, phytosterols, cardiac Glycosides, anthocyanins and saponins using well-established methods [8].

2.5 Determination of total flavonoid content

The Total flavonoid content of the methanolic extract of *Gossypium herbaceum* was determined according to a modified aluminium chloride colorimetric method [9]. Briefly, 4 ml of distilled water was added to 200 µL of MEGH in methanol (1 mg/ mL). After mixing the contents, the mixture was left to rest for five minutes at room temperature. Post 5 min, 2 mL of 1M sodium hydroxide, 3 mL of 10% aluminum chloride, and 3 mL of 5% sodium nitrite solution were added and made up to 10 milliliters by adding distilled water. The reaction mixture's absorbance was measured at 510 nm using the UV-visible spectrophotometer and compared to the reagent blank. Hence, the total amount of flavonoid content in MEGH was determined by measuring the absorbance of a standard quercetin (1 mg/mL) at different doses and utilizing the results to generate a calibration curve. The entire amount of flavonoids was expressed as microgram equivalents per gram dry weight of MEGH (µg/g MEGH).

2.6 Determination of total tannin content

Total tannins were determined using the modified Folin-Ciocalteu technique [10]. Briefly, 7.5 ml of distilled water was added to aproximately 200 µL of MEGH in methanol (1 mg/ mL). The mixture was mixed properly, and then 0.5 ml of Folin-Ciocalteu phenol reagent and 1 ml of 35% sodium carbonate solution was added. The mixture was shaken well and allowed to sit at room temperature for half an hour. Post 30 minutes, the mixture was made up to 10 mL with distilled water. The Same procedure as previously described was used to generate a standard calibration curve for gallic acid. An UV/Visible spectrophotometer was used to measure the absorbance of the test and standard solutions at 725 nm against the blank solution. The total tannin concentration was expressed as microgram equivalent per gram of extract (µg/g MEGH).

2.7 Determination of total alkaloid content

Total alkaloids were determined using the UV Spectrophotometric method [11]. Briefly, 2 mL of 2N hydrochloric acid was added to approximately 200 µL of MEGH in methanol (1 mg/ mL) and filtered. The filtrate was then transferred to a seperating funnel and 5mL of bromocresol green solution and 5 mL of phosphate buffer was added. The mixture was shaken vigorously and extracted with chloroform. Chloroform was added to dilute the extract in a 10 mL volumetric flask. The same procedure was used to generate a standard calibration curve for ascorbic acid. UV/Visible spectrophotometer was used to measure the absorbance of the test and standard solutions at 470 nm against the blank solution. The total alkaloid concentration was expressed as microgram equivalent per gram of extract (µg/g MEGH).

2.8 *In vitro* anti-oxidant activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical Scavenging assay: Using the approach of Boly [23]. A Vortex shaker was used to mix the mixture, which was then permitted to stand at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-spectrophotometer (Phoenix-2000V UV-VIS, Biotech Engineering Management Co. Ltd. (UK), Nicosia, Cyprus). The percentage inhibition was estimated using the equation.

In vitro DPPH radical scavenging activity was determined using Blois's (1958) method to determine the free radical scavenging activity of the extract against the synthetic DPPH (2,2-diphenyl-1-picrylhydrazyl) [12]. Briefly, 3 mL of DPPH in methanol (0.1 mM/L) was combined with 1 mL of MEGH at various concentrations (10 to 320 µg/mL). The mixture was shaken well and then permitted to stand at room temperature for 30 minutes. Post 30 minutes, the absorbance was measured at 517 nm using a UV-spectrophotometer. The same procedure is repeated using ascorbic acid as the standard. Inhibitory activity was calculated as % inhibition, along with the IC_{50} values.

Ferrous Reducing Antioxidant Capacity Assay: *In vitro* ferrous reducing antioxidant activity was determined using modified the Oyaizu (1986) method [13]. Briefly, 2 mL of 1% potassium ferricyanide and 2 mL of 0.2 M phosphate buffer of pH 6.6 were added to 1 mL of MEGH at various concentrations (10 to 320 µg/mL). All the mixtures were incubated at 50^0C for 20 min. Post incubation, 2 mL of 10% trichloroacetic acid was added and then centrifuged for 10 min at 1000 rpm. Post centrifugation, the supernatant was collected and dissolved in equivalent amount of distilled water and then 1mL of 0.1% ferric chloride was added to it. The same procedure is repeated using ascorbic acid as the standard.

UV/Visible spectrophotometer was used to measure the absorbance of the test and standard solutions at 700 nm against the blank solution. Inhibitory activity was calculated as % inhibition, along with the IC_{50} values.

***In vitro* Lipid peroxidation assay:** To assess lipid peroxidation, homogenate extracted from egg yolk was utilized as a lipid-rich medium in the modified thiobarbituric acid-reactive species (TBARS) method [14]. The egg yolk homogenate was prepared by combining one gram of egg yolk with 100 milliliters of Tris-HCl (100 mM, pH 7.4) using the Remi homogenizer. Briefly, 500 µL of egg homogenate was added to a test tube along with 1 mL of various MEGH concentrations. The mixture was shaken well and homogenized. Post homogenization, 25 µL of 0.07 M ferrous sulphate was added to the mixture and incubated for thirty minutes to promote lipid peroxidation. Thirty minutes later, 750 µL of 0.8% thiobarbituric acid (w/v) dissolved in 1.1% sodium dodecyl sulphate (SDS) and 20% acetic acid (pH 3.5) were added, vortexed, and heated for sixty minutes. Post 60 min, 3.0 mL of n-butanol was added to the reaction mixture once it had cooled, and the mixture was centrifuged for 10 minutes at 3000 rpm to separate the supernatant organic layer. Post addition of 3 mL of n-butanol solution to the upper organic layer, the absorbance was measured at 532 nm in relation to the blank. The same procedure is carried out with ascorbic acid serving as the standard. Inhibitory activity as % inhibition and IC_{50} values were computed.

2.9 In vitro anti-obesity activity

In vitro lipase inhibitory activity: *In vitro* lipase inhibitory activity of MEGH was assessed using a modified method using triolein as a substrate [15]. The rate of oleic acid release from triolein was evaluated to assess lipase inhibitory activity. A suspension of 1% triolein and 1% tween 40 (v/v) in 0.1 M phosphate buffer of pH 8 was prepared and emulsified. Further, 0.5 g of porcine pancreatic lipase was prepared by dissolving it in 15 mL of 0.1 M phosphate buffer. Briefly, 800 µL of triolein emulsion and 200 µL of porcine pancreatic lipase were added to a test tube along with 1 mL of various MEGH concentrations. Orlistat, a strong pancreatic lipase inhibitor, was used as the reference standard. The test tubes were incubated at 37°C for 30 minutes, after which the absorbance at 450 nm was measured using UV-Visible spectrophotometer. Inhibitory activity was calculated and expressed as % inhibition along with the representation of respective IC_{50} values.

2.10 Molecular Docking studies

Accession of target Protein: Fat mass and obesity associated (FTO) protein's three-dimensional structure (PDB: 3LFM) was retrieved from the RCSB protein Data Bank.

Selection of Ligand: The chemical structures of the commercial drug orlistat and ligands (Catechin, Gossypol, Flavone-3-ol, Stigmasterol, Gamma sitosterol) were obtained using the PubChem and ZINC compound database. To produce atomic coordinates, it was first downloaded in MOL SDF format and then translated into a PDB file using Open Babel software.

Protein Preparation and identifying the proteins active binding site: In order to prepare the protein for site-specific docking, water molecules were removed, polar hydrogens were added, and the binding site's properties (XYZ values) were downloaded using BIOVIA (Discovery Studio 2021 client).

Ligand Preparation: Autodock 1.5.7 was used to open the PDB format of the corresponding ligands, identify the root, and save the file in PDBQT format.

***In silico* Molecular docking analysis:** A computer ligand-target docking technique was used to examine the structural basis of this protein target selectivity [16]. The generated protein's PDB format was read using Autodock 1.5.7 software. Kollaman and Gasteiger charges have been added. AD4 type atoms were assigned and recorded in PDBQT format for docking. For molecular docking, a grid box was chosen, and a macromolecule in protein PDBQT format was picked, along with the appropriate PDBQT ligand from the set map types, and docking was performed using Autodock Vina. The configuration file is produced in text format and contains details of protein active site specific properties, which is a required step for Autodock Vina. The command prompt used vina.exe and the appropriate commands to execute molecular docking. Finally, the molecular docking results were presented as a glide score.

Visualization of the best docking pose: The generated docking poses were visualized using BIOVIA (Discovery Studio 2021 client). The amino acids of the protein that interacted with the ligand were shown in 2D and 3D figures, as well as the distance between the interaction groups. The best docked score is regarded as the greater negative glide score; the more negative the glide score, the more favourable the binding.

3. Results

Percentage yield and physical appearance of MEGH

The soxhlation method was used to prepare a methanolic extract of *Gossypium herbaceum* whole plant (MEGH). The MEGH yield was found to be approximately 7.8% w/w. The physical appearance of the methanolic extract of Gossypium herbaceum is deep green colour as seen in figure-1.

Figure 1. Methanolic Extract of *Gossypium herbaceum* Whole Plant

Preliminary phytochemical screening of MEGH

The preliminary phytochemical screening of the methanolic extract of *Gossypium herbaceum* shows the presence of alkaloids, flavonoids, phytosterols, tannins and cardiac glycosides which was represented in table 1 and figure 2.

Table 1. Preliminary Phytochemical Screening of MEGH

Phytochemical	Results
Alkaloids	+
Flavonoids	+
Tannins	+
Phytosterols	+
Cardiac glycosides	+
Anthocyanins	-
Saponins	-

Note: + indicates Presence; - indicates absence

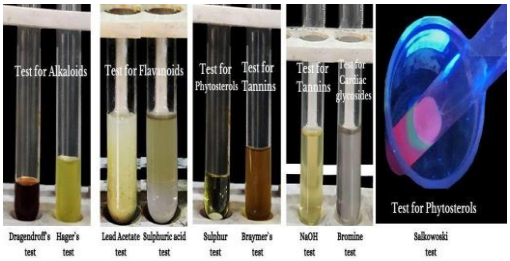


Figure 2. Qualitative Phytochemical Screening of MEGH

Determination of total flavonoid content

The quantitative phytochemical analysis of MEGH showed that the concentration of flavonoids was found to be 124 µg/g of MEGH. The results were depicted in figure-3 and table-2.

Table 2. Total Flavonoids Content in MEGH Against Quercetin

S.no.	Concentration (µg/mL)	Absorbance
1.	10	0.008
2.	40	0.011
3.	80	0.017
4.	160	0.027
5.	320	0.041
6.	MEGH	**0.021**

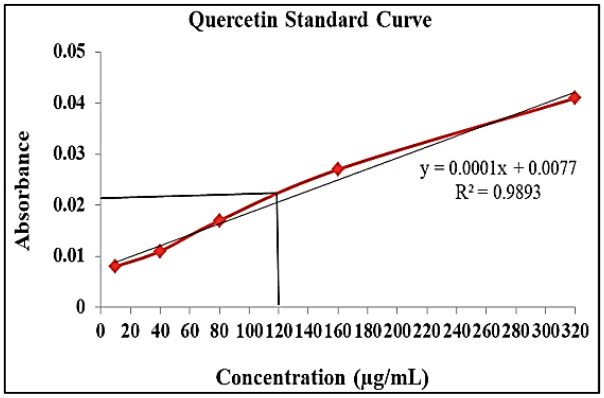


Figure 3. Total Flavonoids Content in MEGH Against Quercetin

Determination of total tannin content

The concentration of total tannins in MEGH was found to be 48 µg/g of MEGH. The results were depicted in figure-4 and table-3.

Table 3. Total Tannin Content in MEGH against Gallic acid

S.no.	Concentration (µg/mL)	Absorbance
1.	20	0.068
2.	40	0.125
3.	60	0.180
4.	80	0.226
5.	100	0.309
6.	Test sample (Extract)	**0.146**

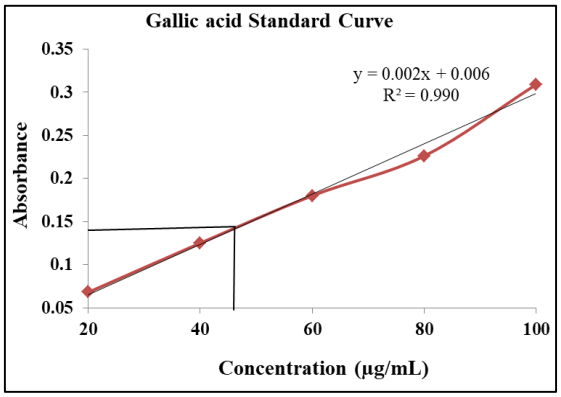


Fig. 4: Total Tannin Content in MEGH Against Gallic acid

Determination of total alkaloid content

The concentration of total alkaloids was found to be 58 µg/g of MEGH. The results were depicted in figure-5 and table-4.

Table 4. Total Alkaloid Content in MEGH against Atropine

S.no.	Concentration (µg/mL)	Absorbance
1.	20	0.132
2.	40	0.185
3.	60	0.235
4.	80	0.271.
5.	100	0.308
6.	Test sample (Extract)	**0.221**

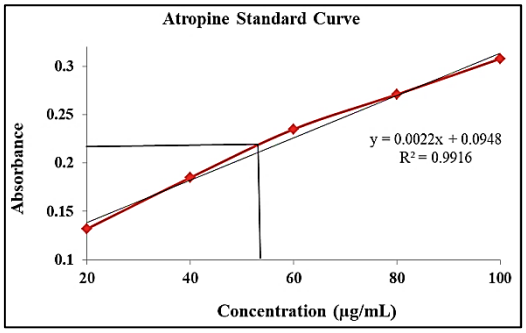


Figure 5. Total Alkaloid Content in MEGH Against Atropine

***In vitro* anti-oxidant activity**

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Free Radical Scavenging Assay

The assay revealed that MEGH dose-dependently reduced the free radicals released by DPPH, as seen in figure 6 and table 5. The ascorbic acid & MEGH showed 50% inhibitory concentrations (IC_{50}) at 179 & 142 µg/mL respectively.

Table 5. DPPH Free Radical Scavenging Assay

S.no	Compou-nds	Concentration (µg/ml)	% Inhibition
1	MEGH	10	17.50±0.71
		20	19.50±0.71
		40	25.00±1.41
		80	28.50±0.71
		160	46.50±2.12
		320	77.50±0.71
2	Ascorbic acid	10	20.50±2.12
		20	23.50±3.54
		40	30.00±2.83
		80	35.50±2.12
		160	55.00±4.24
		320	87.50±6.36

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

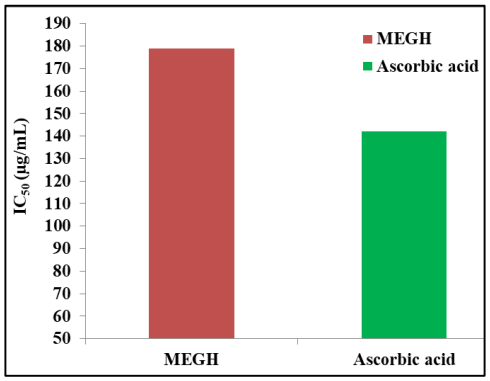


Figure 6. DPPH Free Radical Scavenging Assay: IC_{50} of MEGH and ascorbic acid

Ferrous Reducing Capacity Assay:

The assay revealed that MEGH dose-dependently reduced the free radicals, as seen in figure 7 and table 6. The ascorbic acid and the MEGH were found to have 50% inhibitory concentrations (IC_{50}) of 157 µg/mL and 139 µg/mL respectively.

Table 6. Ferrous Reducing Antioxidant assay of MEGH and ascorbic acid

S.no	Compou-nds	Concentration (µg/ml)	% Inhibition
1	MEGH	10	16.00±2.83
		20	20.00±1.41
		40	24.00±2.83
		80	30.00±1.41
		160	50.50±0.71
		320	88.00±1.41
2	Ascorbic acid	10	21.50±2.12
		20	25.00±4.24
		40	31.50±3.54
		80	41.50±0.71
		160	55.00±1.41
		320	84.50±3.54

Values expressed as Mean ± SD (n=2)

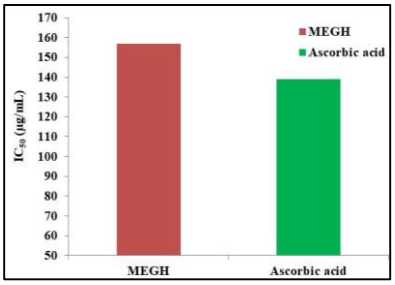


Figure 7. Ferrous Reducing Assay: IC_{50} of MEGH and Ascorbic Acid

***In- vitro* Lipid Peroxidation Assay (TBARS assay)**

The assay revealed that MEGH dose-dependently inhibited lipid peroxidation capacity as seen in figure 8 and table 7. The ascorbic acid and the MEGH were found to have 50% inhibitory concentrations (IC_{50}) of 171 µg/mL and 129 µg/mL respectively

Table 7. Lipid Peroxidation inhibition by MEGH and ascorbic acid

S.no	Compo-unds	Concentration (µg/ml)	% Inhibition
1	MEGH	10	16.50±2.12
		20	20.00±1.41
		40	22.50±2.12
		80	28.00±1.41
		160	51.00±2.83
		320	80.00±1.41
2	Ascorbic acid	10	23.00±2.83
		20	26.50±3.54
		40	32.00±1.41
		80	43.50±0.71
		160	58.00±1.41
		320	87.50±2.12

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

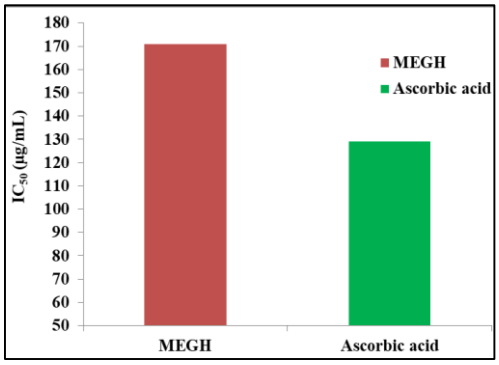


Figure 8. Lipid Peroxidation Assay: IC_{50} of MEGH and Ascorbic Acid

***In vitro* anti-obesity activity**

***In vitro* lipase inhibitory activity:**

The *in vitro* assay revealed that MEGH dose-dependently inhibited lipase activity as seen in figure 9 and table 8. Standard orlistat and MEGH were found to have 50% inhibitory concentrations (IC_{50}) of 168 µg/mL and 115 µg/mL respectively.

Table 8. Inhibition of lipase activity by MEGH and Orlistat

S.no	Compounds	Concentration (µg/mL)	% Inhibition
1	MEGH	10	16.00±1.41
		20	21.50±0.71
		40	28.50±2.12
		80	32.00±1.41
		160	52.00±1.41
		320	77.50±2.12
2	Orlistat	10	26.00±1.41
		20	31.50±0.71
		40	36.50±2.12
		80	46.00±1.41
		160	58.50±0.71
		320	89.50±2.12

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

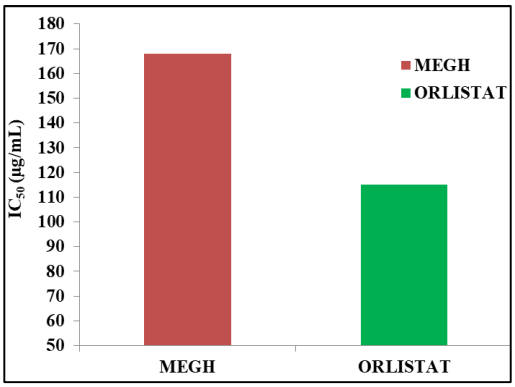
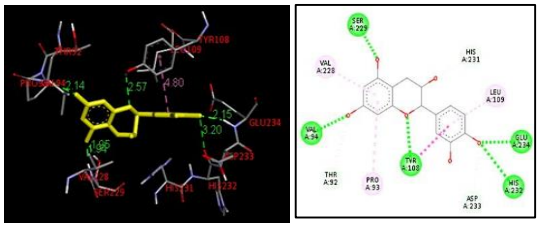


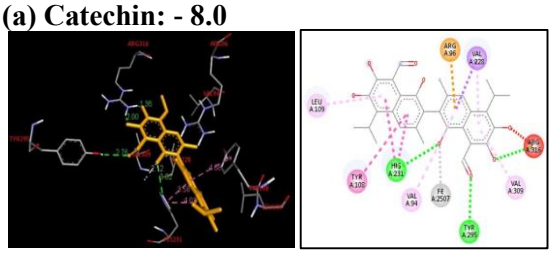
Figure 9. Lipase Inhibitory Activity Assay: IC_{50} of MEGH and Orlistat

Molecular Docking studies

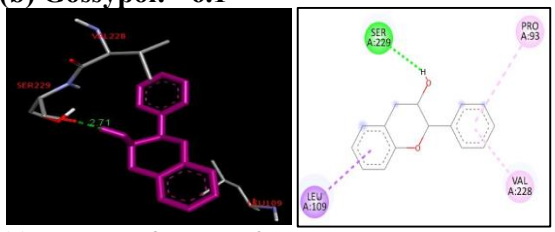
The docking studies were carried out by Autodock Vina. Docking experiments revealed that several ligands from our study (Catechin, Gossypol, Flavone-3-ol, Stigmasterol and Gamma sitosterol) had a better binding affinity with the FTO protein with the PDB id 3LFM, as evidenced by negative gliding scores and glide scores closer to orlistat, the commonly prescribed anti-obesity medication. Figure 10 and table 9 presented the results. The results showed that Catechin has the greatest glide score (-8.0), which is closer to the orlistat glide score (-10.3) than Flavone-3-ol (-7.0), Gossypol (-6.1), Stigmasterol (-3.1) and Gamma sitosterol (-3.1).



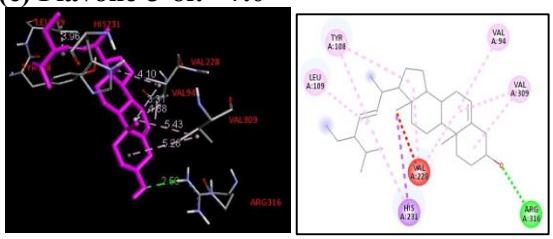
(a) Catechin: - 8.0



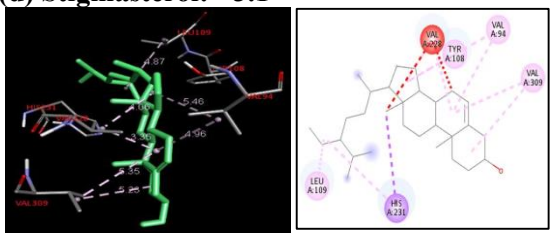
(b) Gossypol: - 6.1



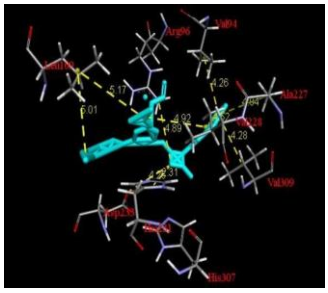
(c) Flavone-3-ol: - 7.0



(d) Stigmasterol: - 3.1



(e) Gamma sitosterol: - 3.1



(f) Orlistat: - 3.1

Figure 10. Docking Modes of Respective Ligand with FTO

Table 9. Docking Results of Flavonoids Against Fat Mass and Obesity Associated (FTO) Protein (PDB: 3LFM)

S.no.	Name of the ligand	Glide Score
1.	Catechin	-8.0
2.	Gossypol	-6.1
3.	Flavone-3-ol	-7.0
4.	Stigmasterol	-3.1
5.	Gamma sitosterol	-3.1
6.	Orlistat	-10.3

The more negative glide score indicates the better binding affinity

4. Discussion

Obesity rates in low- and middle-income countries are rapidly increasing, particularly in cities. It is now recognized as a chronic illness with pandemic proportions in the industrialized world. According to a survey conducted in US hospitals, the majority of younger patients treated for COVID-19 complications were obese. Obesity is well known to limit breathing, lower immunity, raise inflammation, and have a detrimental impact on heart health, making a person more susceptible to infectious diseases. Obesity has also been linked to other metabolic disorders such as type 2 diabetes, high blood pressure, cancer, liver, kidney, heart, and gallbladder disease. Obesity is a severe issue that must be addressed immediately because it can lead to a variety of other health problems. Aside from lifestyle changes and physical activity, additional therapeutic options are required to effectively reduce obesity.

Diet-induced increases in blood lipids, cholesterol, and glucose levels are strongly linked to obesity. As a result, one strategy to prevent obesity is to delay the digestion and absorption of fat and carbohydrates from the diet. The development of nutrition, digestion and absorption inhibitors is one of the most important methods to obesity management. Orlistat is the best allopathic drug that slows lipase metabolism; nonetheless, it might damage the liver and cause gastrointestinal issues. Hence, extract from natural herbs were assumed to be harmless and additional investigation is required [17].

One of the most extensively researched *in vitro* techniques for determining the potential efficacy of natural items as anti-obesity medicines is the suppression of digestive enzymes. In this study, we evaluated the possible anti-obesity effects of methanolic extract of *Gossypium herbaceum* extract using *in vitro* and *in silico* methods.

The MEGH contained alkaloids, flavonoids, tannins, phytosterols, and cardiac glycosides, according to preliminary phytochemical screening. A study by Mahboob A [18] revealed that flavonoids operate on all known obesogenic pathways to prevent and treat obesity with long-term efficacy. Consistent with this, the present study's quantitative estimation of flavonoid content was 124 µg/g of MEGH. In addition a review by Ma Z [19] revealed that alkaloids act on all known obesogenic pathways to control hyperlipidemia and obesity. Consistent with this, the present study's alkaloid content was found to be 58 µg/g of MEGH. However, a study by Manzoor F [20] revealed that supplementation with tannins can reduce dietary intake, and their anti-nutritional qualities may be utilized as a treatment for controlling body weight and obesity. Consistent with this, the present study's total tannin content was found to be 48 µg/g of MEGH. Hence, these observations gave rise to the theory that MEGH may have anti-obesity properties as it includes a significant amount of flavonoids, alkaloids and tannins which have anti-obesity properties.

A study by Sharma [21] reported that presence of large amounts of phytoconstituents can exhibit high scavenging and reducing power activities and acts as a potent antioxidant. In agreement with this, MEGH has profound antioxidant activity by dose-dependent inhibition of DPPH activity and ferrous reducing capacity along with IC_{50} values of 179 µg/mL and 157 µg/mL nearer to the standard ascorbic acid.

Lipid peroxidation in fat tissue is a result of oxidative stress, which produces by products like oxysterols and 4-hydroxynonenal that influence precursor cell adipogenic differentiation. It has been observed that obese patients who took orlistat had significantly lower MDA levels of lipid peroxidation in correlation with weight loss [22]. In support of this, MEGH prevented lipid peroxidation dose-dependently by exhibiting IC_{50} 171 µg/mL, which is closer to conventional ascorbic acid led to one of the possible anti- obesity potential.

In vitro lipase inhibitory activity measures the capacity of the MEGH extract to inhibit the activity of the porcine pancreatic lipase enzyme. Lipase enzymes mainly hydrolyze dietary fat molecules; therefore the inhibition of lipase enzyme provides a measure of inhibition of lipid metabolism, which plays an important role in calorie absorption according to Ong SL [23]. In agreement with this, it has been shown that MEGH dose dependently inhibited lipase activity, with IC_{50} 168 µg/mL, similar to conventional orlistat. This inhibitory effect leads to the one of the possible anti-obesity potential. According to the results of the quantitative phytochemical study, considerable amount of flavonoids, alkaloids and tannins in MEGH could account for the larger percentage of lipase inhibition.

Drug discovery is a well-known process that uses molecular docking to analyze ligand-binding interactions. *In silico* molecular docking studies can identify new compounds with increased potency and selectivity, as well as provide information about the pharmacological action of natural molecules in therapeutic contexts. This study examined phytosterols including Gamma sitosterol and Stigmasterol, flavonoids like Catechin and Flavone-3-ol, and cotton plant pigment known as gossypol. According to the findings of our investigation, flavonoids (Catechin and Flavone-3-ol); phytosterols (Stigmasterol and Gamma sitosterol); and gossypol have glide scores nearer to standard drug orlistat (-10.3) against fat mass and obesity-associated (FTO) protein (PDB id: 3LFM) for gastric and pancreatic lipase inhibition activity. These results were found to be comparable to the study where Catechin demonstrated the greatest glide score as an anti-obesity drug [24].
Therefore, it can be predicted that presence of flavonoids, gossypol and phytosterols in MEGH possesses the best potential anti-obesity activity.

5. Conclusion

In this study, we assessed the anti-obesity potential of a methanolic extract of *Gossypium herbaceum* whole plant. The present study's qualitative analysis revealed the presence of alkaloids, flavonoids, tannins, phytosterols, and cardiac glycosides in MEGH. However, MEGH showed around 124 µg Quercetin equivalent/g of MEGH, 48 µg gallic acid equivalent/g of MEGH and 58 µg of atropine equivalent/g of MEGH. MEGH can reduce obesity by inhibiting lipid peroxidation and lipase activity *in vitro*.

Furthermore, the flavonoids, tannins and alkaloids in MEGH demonstrated anti-obesity efficacy by achieving the better glide score in *in silico* molecular docking tests, similar to that of the conventional pancreatic lipase inhibitor orlistat, via binding to fat mass and obesity-associated (FTO) protein.

Overall, this study clearly shows that the flavonoids, alkaloids and tannins in the methanolic extract of *Gossypium herbaceum* whole plant may be responsible for obesity treatment. There has been no known study on the *in vitro* anti-obesity effect of methanolic extracts of *Gossypium herbaceum* whole plant.

The present study offered valuable early data by demonstrating its potential usage in weight reduction therapy. Thus, these MEGH extracts could be used as natural agents in the food and pharmaceutical industries. Further research is needed to isolate and identify the beneficial chemicals found in plant extracts, as well as to elucidate their molecular pathways in regulating obesity.

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