Research Article

Molecular Docking Analysis of Secondary Metabolites of *Trigonella foenum graecum* and *Carica papaya* with FTO: An Insilico Approach

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ABSTRACT

This study deals with the evaluation of inhibitory activity of Secondary Metabolites of *Trigonella foenum graecum* and *Carica papaya* on Fat mass and obesity-associated protein (FTO) using *in silico* docking studies. In order to understand the mechanism of ligand binding and to identify potent FTO inhibitors, a study involving molecular docking and virtual screening has been performed. The hydro alcohol leaf extracts of *Carica papaya* and *Trigonella foenum graecum* contains rich sources of phytochemicals viz, total phenols, tannins and flavonoids. The secondary metabolites were determined by GC-MS analysis. Twenty seven chemical constituents have been identified. The major chemical constituent present in the hydro alcohol extracts of *Carica papaya* and *Trigonella foenum graecum* were involved in docking study using AutoDock 4.2, based on the Lamarckian principle. The results showed that all the selected compounds in the *Trigonella foenum graecum* showed binding energy ranging between -5.02 kcal/mol to -12.7 kcal/mol and *Carica papaya* showed between -4.76 to -14.6 kcal/mol. Out of ten chemical constituents, n- Hexadecanoic acid and Beta sitosterol has the highest docking score along with the highest number of hydrogen bonds formed. Analysis of the results of the Autodock software suggested that n- Hexadecanoic acid and Beta sitosterol can act as an anti-obesity agent. This molecular docking analysis could lead to the further development of potent FTO inhibitors for the prevention and treatment of obesity and related conditions.

Keywords: Autodock tool, FTO, GC-MS Analysis, Obesity, Phytochemicals

INTRODUCTION

Obesity is a serious health problem all over the world as a result of changes in lifestyle, especially in eating habits. Obesity is implicated in various diseases, including type II diabetes, hypertension, cancer and CHD.[1] Obesity is characterized by an increase in the number and size of adipocytes differentiated from fibroblastic pre-adipocytes in adipose tissue.[2] Adipose tissue plays a major role in maintaining energy homeostasis by storing tri-acylglycerol as a fuel for the body. Excessive adipose tissue formation is attributed to an imbalance between energy intake and energy expenditure.[3] *Trigonella foenum graecum*, and *Carica papaya* posess both primary and secondary metabolites which acts as therapeutic agents for many diseases. *Trigonella foenum graecum*, and *Carica papaya* were chosen for this study.

Trigonella foenum-graecum is commonly known as fenugreek (English). The plant has been scientifically used for the treatment of wounds, inflammation, gastrointestinal ailments, as cholesterol lowering agent,[4] on diabetes[5] bronchitis, inflammation, chronic cough, liver disorder[6] and as an anti-fertility agent[7]. *Trigonella foenum graecum* is reported to contain several active chemical constituents such as alkaloids, saponins, steroids, tannins, flavonoids, amino acids and trigonilline.[7]

Carica papaya is commonly known as papaya (English). *Carica papaya* leaf extracts are prescribed as a tonic for heart and also for the treatment of fever, pyrexia, diabetes, gonorrhea, syphilis, inflammation and for dressing wounds**.** Phytochemical analysis reveals the presence of flavonoids, alkaloids, carbohydrates, saponins, glycosides, phytosterols, phenolics, terpenoids and tannins in the leaves of *Carica papaya.*[8]

Synthetic compounds which are used to treat obesity may cause side effects, so plant based drugs are used as alternate therapies to reduce obesity. In this study, we estimated total phenols, flavonoids and tannins, and then the extracts were subjected to GC-MS analysis for the detection of secondary metabolites. These secondary metabolites were docked with FTO. The susceptibility to obesity of an individual is determined by combined effects of genetic and environmental factors and one of the genes for obesity that have been identified is the Fat Mass and Obesity-associated gene (FTO), encoding for the Fat mass and obesity-associated protein (FTO) or also known as alpha-ketoglutarate-dependent dioxygenase.[9] Variants in FTO are associated with BMI and increased risk of obesity.[10] FTO variations are associated with increase food intake, satiety and also with decreased lipolytic activity in adipocytes.[11] Due to this FTO protein was used as the target protein for *in silico* model to observe if the secondary metabolite of *Trigonella foenum graecum*, and *Carica papaya* could act as antagonist.

MATERIALS AND METHODS

Collection of plant materials

Plant specimen for the proposed study was collected from medicinal plant vendor. Care was taken to select healthy leaves. *Trigonella foenum graecum*, and *Carica papaya* was authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal science, Plant Anatomy Research Centre, Chennai.

Preparation of extract

Trigonella foenum graecum, and *Carica papaya* leaves were taken and washed with distilled water and then shade dried. The dried leaf was powdered and extracted with alcohol (70%), Ethyl acetate and ethanol using soxhlet apparatus. The extract was stored in a glass bottle under refrigerated condition throughout the period of the experiment.

Phytochemical Analysis

Hydroalcohol, Ethyl acetate and Ethanol extracts were subjected to standard phytochemical analysis to find the presence of the following phyto constituents that are phenols, flavonoids, sterols, tannins,[12] terpenoids, cardiac glycosides,[13] carbohydrates, amino acids, phylobatannins, and phytosterols.[14]

Estimation of Total phenols

Total phenolic content was estimated using the Folin-Ciocalteu method of Mc Donald.[15] 5ml of Folin ciocalteu reagent and 4ml of aqueous Na_2Co_3 were added to 0.5ml of the extract. After 15 min incubation at room temperature, the absorbance was read at 765nm. The standard curve was prepared using chlorogenic acid. The phenols were expressed in terms of chlorogenic acid equivalents.

Estimation of Total Flavonoids

The determination of flavonoids was performed according to the colorimetric assay of Chang.[16] To 1ml of extract, 3 ml of methanol, 0.2ml of 1 M potassium acetate, 0.2ml of 10% aluminium chloride and 5.6ml of distilled water was added and left at room temperature for 30 minutes. Absorbance of the mixture was read at 415 nm using UV spectrophotometer. Calibration curve was prepared using quercitin as standard.

Estimation of Tannin-phenolics

Tannin - phenolics were determined by the method of Peri and Pompi.[17] 1ml of extracts was taken in test tube and the volume was made up to 1ml with distilled water and 1ml of water serves as the blank. To this 0.5ml of folins phenol reagent (1:2) followed by 5ml of 35% sodium carbonate was added and kept at room temperature for 5 mins. Blue color developed was read at 640nm. The standard curve was prepared using chlorogenic acid.

Gas chromatography-mass spectrometry analysis:

The phytochemicals were analyzed by GC-MS (GC Clarus 500 perkin Elmer) employing the electron impact (EI) mode (ionizing potential 70eV) and a capillary column (Resteck-624 ms) (30 m × 0.25 mm, film thickness 1.8µm) packed with 5% dimethyl poly silixone) and the ion source temperature was monitored at 200°C. The inert gas helium (99.9995%) was used as carrier gas, at flow rate of 1.491 ml/min. Split ratio 10:1 sample size, 1µl injected using the split less injection technique; fused capillary silica column HP-5. Temperatures: injector: 260°C, detector: 30°C, column: 70°C, 10°C min-1, 260°C (10min). The total GC running time was 35 min. Mass detector turbo mass gold- Perkin Elmer detector was used. Turbo mass 3.2 software was used for the identification of compounds via the data bases of WILEY8, NIST08s and FAME Library. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database libraries. The name, molecular weight and structure of the components of the test materials were ascertained.

Docking

To study the nature of interactions, binding mode and selectivity of FTO with secondary metabolites of *Trigonella foenum graecum*, and *Carica papaya* docking was carried out with, Autodock 4.2. AutoDockTool was used for creating PDBQT files from traditional PDB files.[18] The sequence of FTO (swissprot ID: Q9C0B1) was retrieved from swissprot database. The three dimensional structure of FTO (PDM ID: 3LFM) was downloaded from PDB database. The domain FTO catalytic Domain and C-terminal domain (region 35-327 and 329-500) belongs to FTO NTD and FTO CTD family. The active sites of FTO were identified using Q-site finder. The drug compound structures were drawn using ACD chemsketch and converted into PDB format using open Babel tool. The 3D structures of FTO were docked with various inhibitors using autodock software. The docking results were analyzed using Discovery studio visualize tool.

The major chemical constituents present in the *Trigonella foenum graecum*, and *Carica papaya* extracts were drawn using chemsketch and optimized. Ligands were prepared in the autodock 4.2 for docking studies. The optimized ligands were docked into FTO using "Ligand fit" model in auto dock 4.2.[19] The energy interaction between protein and ligand can be calculated.[20]

Auto Dock calculation

Docking can be carried out by various methods. But, the most efficient method is Lamarckian genetic algorithm. AutoDock was run several times to get various docked conformations, and used to analyze the predicted docking energy. The binding sites for these molecules were selected based on the ligand-binding pocket of the templates.[21] Auto dock tools provide various methods to analyze the results of docking simulations such as, conformational similarity, visualizing the binding site and

its energy and other parameters like intermolecular energy and inhibition constant.[22]

Statistical Analysis

All statistical analysis was performed using SPSS 20.0 statistical software (IBM, USA). Significant differences among the groups was studied using analysis of variance (ANOVA) followed by least significant difference (LSD) test. Results were considered to be statistically significant at $P < 0.001$.

RESULTS AND DISCUSSION

Obesity is considered an emergency health problem in all industrialized countries and in spite of the number of studies to prevent or treat obesity, its prevalence continues to rise.[23] Genetic predisposition, changes in life style and diet are among the various factors which lead to increase in incidence of obesity and related consequences such as cancer, aging, cardiovascular diseases and number of other pathological conditions including type 2 diabetes.[24, 25]

Table 1: The phytochemicals present in the plant extract of *Trigonella foenum graecum*

Compounds	Hydro alcoholic extract	Ethyl acetate extract	Ethanolic extract
Flavonoids	++	+	++
Terpenoids	+++	++	++
Tannins	++	++	++
Phlobatannins	+	~	~
Phenols	++	~	+
Cardiac glycosides	++	+	++
Amino acids	+++	~	++
Carbohydrates	+++	+	++
Sterols	+++	++	+++
Phytosterols	+++	++	+++

+ = present; +++ = present in higher amounts; ~ =absent; ++ = moderately present.

The presence of carbohydrates, flavonoids, tannins, terpenoids, alkaloids, steroids, phlobatannins, cardiac glycosides, phenols, aminoacids, phytosterols were noted in hydroalcoholic, ethyl acetate and ethanolic leaf extracts of *Trigonella foenum graecum* and *Carica papaya.* Table 1 and 2 shows the presence of various phytochemicals present in Trigonella foenum graecum and *Carica papaya.* It was observed that the preliminary phytochemicals are present in higher amounts in hydroalcoholic extract followed by ethyl acetate and ethanolic extract.

From the preliminary results hydro alcoholic leaf extracts of *Trigonella foenum graecum* and *Carica papaya* shows higher concentration of phytochemicals. Table 3 shows the total phenols, tannins and flavonoid content in hydro alcoholic leaf extracts of *Trigonella foenum graecum* and *Carica papaya.* It is noted that hydro alcoholic leaf extracts of *Carica papaya* contains high amount of phenolic compounds (2180±50mg CAE/100g) than that of *Trigonella foenum graecum* (1260±30mg CAE/100g). Phenols are proved to have hypotensive effects[26] and antioxidant properties.[27]

Table 2: The phytochemicals present in the leaf extract of *Carica papaya*

Compounds	Hydro alcoholic extract	Ethyl acetate extract	Ethanolic extract
Flavonoids	+++	+	++
Terpenoids	++	+	+
Tannins	++	++	++
Phlobatannins	~	~	~
Phenols	++	+	++
Cardiac glycosides	++	+	+
Amino acids	++	+	+++
Carbohydrates	++	+	++
Sterols	++	+	++
Phytosterols	+	+	+

+ = present; +++ = present in higher amounts; ~ =absent; ++ = moderately present.

Table 3: Total Phenols, Tannins and Flavonoids of the hydroalcoholic leaf extract of *Trigonella foenum graecum & Carica papaya*

Hydroalcoholic Extract	Total phenolic content (mg CAE/100g)	Total tannin content (mg CAE/ 100g)	Total flavonoid content (mg quercitin/100g)
Trigonella foenum graecum	1260±30	280±4	53.5±1.2
Carica papaya	2180±50 [a]*	240±9 [a]*	91.5±3.5 [a]*

Values are expressed as mean ± SD. Significant at $P^* < 0.001$. a-Values are compared between *Trigonella foenum graecum & Carica papaya.*

Flavonoids are the class of plant secondary metabolites that are widely distributed in variety of plants. Flavonoids are important group of polyphenolic compounds which include flavones, flavonones, isoflavones, flavonols, flavon-3-ols and anthocyanins.[28] Flavonoids have inherent ability to modify the body's reaction to allergens, viruses and carcinogens.[29] It was noted that leaf extract of *Carica papaya* contained higher amounts of flavonoids (91.5±3.5 mg quercitin/100g) when compared to *Trigonella foenum* graecum (53.5±1.2 mg quercitin/100g). Tannins have been shown to possess anti inflammatory effect and it helps in controlling gastritis, oesophagitis, enteritis and bowel disorders. Tannins have shown to possess antiviral[30] and antibacterial activity.[31, 32] Hydro alcoholic leaf extracts of *Trigonella foenum graecum* and *Carica papaya* contains tannin content was 280 $\pm$ 4 mg CAE/ 100g and 240 $\pm$ 9 mg CAE/ 100g.

GC-MS analysis was performed for the hydro alcoholic leaf extracts of *Trigonella foenum graecum* and *Carica papaya* to assess their phytochemical constituents. GC-MS chromatogram of the hydro alcoholic leaf extracts of *Trigonella foenum graecum* and *Carica papaya* showed many peaks indicating the presence of totally 27 phytochemical constituents. Nineteen compounds were identified in hydro alcoholic extract of *Trigonella foenum graecum* by GC-MS analysis as shown in Table 4. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and Concentration (%) were presented for both the extracts (Table 4 and 5). The compounds present in the hydro alcoholic extract of *Carica papaya* were identified by GC-MS analysis and presented in Table 5. Eight compounds were present in the leaf extract of *Carica papaya* by GC-MS analysis.

Table 4: Secondary metabolites of *Trigonella foenum graecum* by GC-MS Analysis

RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
3.32	Levoglucosenone	$C_6H_6O_3$	126	6.08
4.44	Dianhydromannitol	$C_6H_{10}O_4$	146	5.26
10.75	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.49
11.00	Tetradecanoic acid,ethyl ester	$C_{16}H_{32}O_2$	256	0.33
11.90	3,7,11,15-Tetramethyl-2-hexadecen-l-ol	$C_{20}H_{40}O$	296	2.38
12.69	Isophytol	$C_{20}H_{40}O$	296	0.66
13.01	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.72
13.11	Hexadecanoic acid, anhydride	$C_{12}H_{22}O_3$	214	0.66
13.26	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	13.89
14.74	Phytol	$C_{20}H_{40}O$	296	3.62
15.39	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	5.18
15.50	9,12,15- Octadecatrienic acid,methyl ester, (ZZZ) [synonyms: linolenic acid, methyl ester]	$C_{19}H_{32}O_2$	292	43.06
15.82	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	3.86
18.59	Eicosanoic acid,ethyl ester	$C_{22}H_{44}O_2$	340	0.41
21.40	Pentadecanoic acid, 2,6,10,14-tetramethyl-methyl ester	$C_{20}H_{40}O_2$	312	0.41`
24.16	Docasanoic acid	$C_{24}H_{48}O_2$	368	0.33
25.60	1-Docosene	$C_{22}H_{44}$	308	1.81
27.44	Spirost-5-en-3-ol, acetate ,(3a,25R)	$C_{29}H_{44}O_4$	456	2.38
27.92	Cholest-5-ene, 3-bromo (3a)	$C_{27}H_{45}Br$	448	1.48

Table 5: Secondary metabolites of *Carica papaya* by GC-MS Analysis

RT	Name of the compound	Molecular compound	Molecular weight	Peak area %
4.46	Dianhydromannitol	$C_6H_{10}O_4$	146	15.34
11.45	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	24.54
12.97	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.13
13.25	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.45
14.74	Phytol	$C_{20}H_{40}O$	296	29.45
24.34	Squalene	$C_{30}H_{50}$	410	7.36
25.45	Propanoic acid,anhydride	$C_{60}H_{10}O_3$	130	9.20
31.79	B-sitosterol	$C_{29}H_{50}O$	414	5.52

Drug discovery is a complex process that involves the identification of active constituents from traditional medicines. Auto dock is a ligand docking program for predicting protein- ligand binding modes and for virtual screening. Auto Dock is an automated procedure for predicting the interactions of ligands with Macro molecular targets. Table 6 shows the molecular docking score of the secondary metabolites of leaf extract of *Trigonella foenum graecum*. Out of the nineteen compounds found in GC-MS analysis, only 6 compounds were docked with FTO. FTO produced several binding points are ARG 96(HH12), ARG 96(HH22), ASN 205(HD22), THR 320(OG1), THR 320(HG1), and ARG 322(HH21) and involved in docking, the distance 2.0975, 1.90999,

1.98078, 2.39392, 3.19083 and 2.16949 were covered. Out of the 6 components, n- Hexadecanoic acid has highest docking score with highest number of six hydrogen bonds obtained. Figure 1(a) shows the molecular docking of n- Hexadecanoic acid with fat mass and obesity associated protein (FTO).

Table 6: Molecular docking score of Secondary metabolites of *Trigonella foenum graecum* with FTO

Ligands	Docking score (kcal/mol)	No. of hydrogen bonds formed
9,12,15 Octadecatrienoic acid, methyl ester	-12.6	1
Hexadecanoic acid ethyl ester	-11.1	1
Levoglucasenone	-5.02	2
Linoleic acid	-11.5	3
n- Hexadecanoic acid	-12.2	6
Octadecanoic acid ethyl ester	-12.7	3

Table 7 and Figure 1(b) shows the ligand used, the docking score and number of hydrogen bonds formed by the compounds with FTO. Out of the eight compounds found in GC-MS analysis, only four were docked. Beta sitosterol has the highest docking score along with highest number of hydrogen bonds formed, whereas squalene and propionic acid had good docking score but there is no hydrogen bond interaction. From the results, the different binding sites of the FTO were TYR 106(OH) and GLU 234(HN) and the distance was 2.91173, 1.97964, respectively.

Table 7: Molecular docking score of Secondary metabolites of *Carica papaya* with FTO

Ligands	Docking score (kcal/mol)	No. of hydrogen bonds
Dianhydromannitol	-4.76	3
Propanoic acid anhydride	-5.35	No bonds
Squalene	-14.6	No bonds
Beta sitosterol	-12.8	2

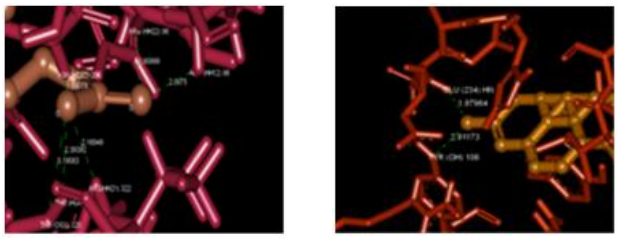

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Figure 1: The molecular docking of (a) n- Hexadecanoic acid and (b) Beta sitosterol with Fat mass and Obesity associated protein

Medicinal plants appear to be rich source of secondary metabolites, widely used in traditional medicine to combat and cure various ailments. In this study, we determined the docking scores between 10 compounds presented in the *Trigonella foenum graecum* and *Carica papaya* with FTO using automated docking model. B-sitosterol reduce blood cholesterol and is used to treat hypercholesterolemia. It inhibits cholesterol absorption in the intestine. When the sterol is absorbed in the intestine, it is transported by lipoproteins and incorporated into the cellular membranes.[33] n-Hexadecanoic acid used as antioxidant, pesticide, anti andrognic flavor, hemolytic and 5- alpha reductase inhibitor.[34] From the docking study and receptor-oriented pharmacophore based *in silico* screening, it may be proposed that n- Hexadecanoic acid and Beta sitosterol can be potent inhibitors of FTO. This may pave a way for new therapeutic inventions for obesity.

CONCLUSION

Finally we conclude from the docking pattern that it may be observed that β- sitosterol and n- Hexadecanoic acid have high docking score and hence may be used for the treatment of obesity. Comparative docking analysis of commonly used drugs for treatment of obesity also suggests that n- Hexadecanoic acid and Beta sitosterol can be an alternative remedy for obesity. However, further, work can be extended to experimental animals to study the effect of n- Hexadecanoic acid and Beta sitosterol on obesity.

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