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Original Article

Computational Analysis of 31 Selected *Trigonella foenum-graecum* (Fenugreek) Phytochemicals as Modulating Agents of Human Lutropin and Follicle Stimulating Hormone

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ABSTRACT

Background and Objectives: *Trigonella foenum-graecum* (Fenugreek) has well known for various pharmacological activities. In the current investigation, we aimed to study 31 chosen phytoconstituents of *T. foenum-graecum* (Fenugreek) as potent modulating agents of human lutropin subunit beta (hLH beta) and human Follicle Stimulating Hormone (hFSH) using docking method. **Materials and Methods:** The 31 chosen constituents of *T. foenum-graecum* (Fenugreek) were studied on the docking behaviour of hLH beta and hFSH by using the Swiss dock method. **Results:** The docking analysis showed that Graecunin (E) of *T. foenum-graecum* (Fenugreek) has exhibited the highest binding energy (-9.98 and -10.30 kcal/mol) with the hLH beta and hFSH respectively. **Conclusion:** Thus, the current finding gives new in sight about the 31 selected ligands of *T. foenum-graecum* (Fenugreek) as potent modulating agents of human lutropin subunit beta (hLH beta) and human follicle stimulating hormone (hFSH), which will help in managing Polycystic Ovary Syndrome (PCOS) related disorders.

Keywords: *Trigonella foenum-graecum*, Fenugreek, Docking, Human Lutropin Subunit Beta, Human Follicle Stimulating Hormone, Graecunin (E).

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INTRODUCTION

Trigonella foenum-graecum (Fenugreek) belongs to Fabaceae (pea) family and which is native to Central Asia, Western Asia, Mediterranean region, Northern Africa and South-Eastern Europe (Singh *et al*., 2022). Till date 135 *Trigonella* species have been reported these include *Trigonella arcuata, Trigonella caelesyriaca, Trigonella capitata, Trigonella cancellata, Trigonella cariensis, Trigonella cassia, Trigonella cephalotes, Trigonella cilicica, Trigonella corniculata, Trigonella cretica, Trigonella cylindracea, Trigonella filipes, Trigonella foenum-graecum, Trigonella gladiata, Trigonella isthmocarpa, Trigonella kotschyi, Trigonella lycica, Trigonella macrorrhyncha, Trigonella mesopotamica, Trigonella monospeliaca, Trigonella plicata, Trigonella procumbens, Trigonella pseudocapitata, Trigonella sibthorpii, Trigonella smyrnea, Trigonella spicata, Trigonella spinosa, Trigonella spruneriana, Trigonella strangulata, Trigonella velutina, Trigonella velutinoides* (Akan *et al*., 2020).

Among above mentioned *Trigonella* species, *Trigonella foenum-graecum* (Fenugreek) is one of the well-known species used for more 100 years in the Iranian traditional medicine (Hajimehdipoor *et al*., 2010). The vernacular names for *Trigonella foenum-graecum* (Fenugreek) are "Fenugrec" in French, "Bockshornklee" in Germany, "Methi" in Hindu, "Kelabet" in Indonesia, "Fieno Greco" in Italy, "Venthiam" in Maldives, "Penantazi" in Myanmar, "Fenegriek" in Netherlands, "Fenacho" in Portuguese, "Fenugreco" in Spanish (Tewari *et al*., 2024). Different plant parts of *T. foenum-graecum* (Fenugreek) are traditional used as follows i) whole seeds and dried plants are used as pest and insect repellents for grain storage; ii) seeds are used as tonic and blood glucose lowering; iii) young seedling are consumed as vegetable; iv) aerial part of plants are used as animal feed; v) seeds are used as raw material for diosgenin (steroid) isolation; vi) whole plant is used as organic manure and also used to improve soil fertility; vii) seed powder is used as food flavor agents (Moradi kor *et al*., 2013).

Trigonella foenum-graecum (Fenugreek) has been reported to possess various biological activities such as anti-bacterial,

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anti-diabetic, anti-cancer, anti-cholesterolemic, anti-hypertensive, anti-inflammatory, anti-obesogenic, anti-oxidant, anti-tumor, anti-ulcer, carminative, emollient, expectorant, febrifuge, galactogogue, hepato-protective, immune-modulatory, laxative, parasiticide (Moradi kor *et al.*, 2013, Anand Swaroop *et al.*, 2017, Alu'datt *et al.*, 2024, Bakhtiar *et al.*, 2024).

The previous reports engaged us to carry out the present investigation on 31 chosen constituents which includes 1) 4-amino benzoic acid; 2) Carpaine; 3) Chlorogenic acid; 4) Coumarin; 5) Daidazein; 6) 3, 7-o-dimethylquercetin; 7) Diosgenin; 8) Ellagic acid; 9) Fenugreekine; 10) Fenugrin B; 11) Ferulic acid; 12) Gentianine; 13) Graecunin (E); 14) Hesperidin; 15) Hydroxy benzoic acid; 16) Hydroxy tyrosol; 17) 4-Hydroxyisoleucine; 18) Isoquercetin; 19) Isorhamnetin; 20) Kaempferol; 21) Kaempferol 3-(2-p- coumarylglucoside); 22) Luteolin; 23) 3-o-Methylquercetin; 24) Naringenin; 25) Naringin; 26) Neurin; 27) Prunin; 28) Quercetin; 29) Rosmarinic; 30) Standard drug Orlistat; 31) Trigonelline and 32) Trimethylamine.

These above said *T. foenum-graecum* (Fenugreek) phytoconstituents were aimed to investigate on the docking analysis of human lutropin subunit beta (hLH beta) and human Follicle Stimulating Hormone (hFSH) by using the swissdock method, which helps in developing anti-PCOS agents for managing PCOS related disorders.

MATERIALS AND METHODS

Ligand preparation

The chemical structures of 31 *T. foenum-graecum* (Fenugreek) ligands were selected based on earlier reports (Wani and Kumar, 2018, Syed *et al.*, 2020, Salam *et al.*, 2023, Mekky *et al.*, 2024, Zhao *et al.*, 2024), which includes 1) 4-amino benzoic acid (CID 978); 2) Carpaine (CID 442630); 3) Chlorogenic acid (CID 1794427); 4) Coumarin (CID 323); 5) Daidzein (CID 5281708); 6) 3, 7-o-dimethylquercetin (CID 5316900); 7) Diosgenin (CID 99474); 8) Ellagic acid (CID 5281855); 9) Fenugreekine (CID 444170); 10) Fenugrin B (CID 5280704); 11) Ferulic acid (CID 445858); 12) Gentianine (CID 354616); 13) Graecunin (E) (CID 156783); 14) Hesperidin (CID 10621); 15) Hydroxy benzoic acid (CID 135); 16) Hydroxy tyrosol (CID 82755); 17) 4-Hydroxyisoleucine (CID 2773624); 18) Isoquercetin (CID 5280804); 19) Isorhamnetin (CID 5281654); 20) Kaempferol (CID 5280863); 21) Kaempferol 3-(2-p- coumarylglucoside) (CID 44258861); 22) Luteolin (CID 5280445); 23) 3- o-Methylquercetin (CID 5280681); 24) Naringenin (CID 439246); 25) Naringin (CID 442428); 26) Neurin (CID 10042); 27) Prunin (CID 92794); 28) Quercetin (CID 5280343); 29) Rosmarinic (CID 5315615); 30) Standard drug Orlistat (CID 3034010); 31) Trigonelline (CID 5570) and 32) Trimethylamine (CID 1146) were downloaded from PubChem compound database. These 31 selected *T. foenum-graecum* (Fenugreek) structures were drawn and prepared by using ChemDraw 2D and 3D software tools (Kumaraswamy *et al.*, 2023). Thus, these prepared three-dimensional structures were used for further studies (swissdock).

Preparation of target enzymes

The 3-D [three-dimensional] structure of human lutropin subunit beta [hLH beta] (UniProt▼ AF- P01229) and human follicle stimulating hormone [hFSH] (PDB▲▲ ID: 1XWD with a resolution of 2.92 A°) was downloaded from UniProt▼ and ▲▲Protein Data Bank (PDB) databases respectively. 'B' chain of hFSH was prepared independently by removing other chains, ligands, and even the crystallographically observed "water" [H_2O] molecules by using UCSF Chimera software tool (Arulselvan *et al.*, 2024).

ADMET analysis

'ADMET' [absorption, distribution, metabolism, excretion and toxicity] analysis was performed for 31 selected *T. foenum-graecum* (Fenugreek) phytoconstituents through the "pkCSM" (freely available) web server (Prakash *et al.*, 2023).

Docking study

A docking studies was performed for 31 selected phytoconstituents of *T. foenum-graecum* (Fenugreek) with two target proteins (hLH and hFSH) using the Swissdock free web server.[16] Finally, PLIP (Protein-Ligand Interaction Profiler) free online server was utilized to analysis the binding site of best-docked pose for each ligand (Srinivasan *et al.*, 2023).

RESULTS

Table 1 shows the results of the Absorption and Distribution (AD) analysis of 31 selected *T. foenum-graecum* (Fenugreek) ligands, in which three ligands (Fenugreekine, Graecunin (E) and Naringin) were predicted to have poor intestinal absorption property. Similarly, two ligands (Diosgenin and Kaempferol 3-(2''-p-coumarylglucoside)) of *T. foenum-graecum* (Fenugreek) were predicted to have both plasma-glycoprotein- I and II inhibitory effect (as shown in Table 1).

Table 2 shows the Metabolism, Excretion and Toxicity (MET) analysis of 31 selected *T. foenum-graecum* (Fenugreek) ligands, in which two ligands (Carpaine and Diosgenin) were predicted to have cytochrome P450 3A4 substrate binding effect. Interestingly, eleven ligands of *T. foenum-graecum* (Fenugreek) namely i) Coumarin, ii) Daidazein, iii) 3,7-*O*-dimethylquercetin, iv) Ellagic acid, v) Gentianine, vi) Isorhamnetin, vii) Kaempferol, viii) Luteolin, ix) 3-*O*-methylquercetin, x) Naringenin and xi) Quercetin were predicted to inhibit cytochrome P450 1A2 activity (as shown in Table 2).

Table2 shows the toxicity analysis of 31 selected *T. foenum-graecum* (Fenugreek) ligands, in which one ligand (Hydroxy tyrosol) was predicted to possess AMES toxicity or mutagenicity.

The current docking analysis showed that Graecunin (E) has the Highest Binding Energy (HBE) (-9.98 kcal/mol) with the human lutropin subunit beta (hLH beta) protein. In contrast, Trimethylamine had the Lowest Binding Energy (LBE) (-5.08 kcal/mol) with the human lutropin subunit beta (hLH beta) protein (as shown in Table 3).

Eight ligands (Diosgenin, Fenugreekine, Graecunin (E), Hesperidin, Isoquercetin, Kaempferol 3-(2-p-coumarylglucoside), Naringin and Rosmarinic) have shown interactions with CYS 77 amino acid residue of human lutropin subunit beta (hLH beta) protein. Similarly, six ligands (Chlorogenic acid, 3, 7-*O*-Dimethylquercetin, Fenugreekine,

Table 1: Absorption and Distribution analysis (AD) of 31 chosen *Trigonella foenum-graecum* (Fenugreek) ligands using the pkCSM online server.

Ligands	WS*	IA♦	SP♦	P-gp[1]	P-gpI[2]	P-gpII[3]	VDss***	FU*	BBB**
4- amino benzoic acid	-1.907	81.966	-2.731	No	No	No	-1.608	0.59	-0.389
Carpaine	-4.724	91.891	-2.782	Yes	No	No	0.812	0.378	-0.351
Chlorogenic acid	-2.449	36.377	-2.735	Yes	No	No	0.581	0.658	-1.407
Coumarin	-1.517	97.344	-1.921	No	No	No	-0.143	0.367	-0.007
Daidzein	-3.793	94.839	-2.748	Yes	No	No	-0.172	0.107	-0.064
3,7-O-dimethylquercetin	-3.241	80.216	-2.735	Yes	No	No	0.644	0.061	-1.15
Diosgenin	-5.539	96.565	-3.39	No	Yes	Yes	0.426	0	0.2
Ellagic acid	-3.181	86.684	-2.735	Yes	No	No	0.375	0.083	-1.272
Fenugreekine	-2.891	7.386	-2.735	Yes	No	No	0.33	0.486	-2.804
Fenugrin B (Apigenin 7-O-beta-D-glucopyranoside)	-2.559	37.609	-2.735	Yes	No	No	0.342	0.218	-1.391
Ferulic acid	-2.817	93.685	-2.72	No	No	No	-1.367	0.343	-0.239
Gentianine	-1.27	98.525	-2.733	No	No	No	-0.086	0.476	-0.202
Graecunin (E)	-2.846	22.793	-2.735	Yes	Yes	No	-0.353	0.437	-2.227
Hesperidin	-3.014	31.481	-2.735	Yes	No	No	0.996	0.101	-1.715
Hydroxy tyrosol	-1.139	72.809	-2.893	No	No	No	-0.084	0.593	-0.39
4-hydroxyisoleucine	-2.887	62.88	-2.737	No	No	No	-0.517	0.478	-0.564
Hydroxyl benzoic acid	-1.877	83.961	-2.723	No	No	No	-1.557	0.592	-0.334
Isoquercetin	-2.925	47.999	-2.735	Yes	No	No	1.846	0.228	-1.688
Isorhamnetin	-3	76.014	-2.735	Yes	No	No	1.123	0.091	-1.135
Kaempferol	-3.04	74.29	-2.735	Yes	No	No	1.274	0.178	-0.939
Kaempferol 3-(2''-p-coumarylglucoside)	-2.941	45.927	-2.735	Yes	Yes	Yes	0.422	0.05	-1.688
Luteolin	-3.094	81.13	-2.735	Yes	No	No	1.153	0.168	-0.907
3-O-methylquercetin	-3.16	76.069	-2.735	Yes	No	No	0.217	0.067	-1.16
Naringenin	-3.224	91.31	-2.742	Yes	No	No	-0.015	0.064	-0.578
Naringin	-2.919	25.796	-2.735	Yes	No	No	0.619	0.159	-1.6
Neurin	-0.301	100	-2.819	Yes	No	No	0.267	0.791	-0.009
Prunin	-2.889	36.035	-2.735	Yes	No	No	0.035	0.19	-1.261
Quercetin	-2.925	77.207	-2.735	Yes	No	No	1.559	0.206	-1.098
Rosmarinic (Rosmarinic acid)	-3.059	32.516	-2.735	Yes	No	No	0.393	0.348	-1.378
Trigonelline	-1.931	96.44	-2.736	Yes	No	No	-0.758	0.857	-0.234
Trimethylamine	0.439	100	-2.76	Yes	No	No	0.321	0.061	-2.609

Note: WS*- Water solubility expressed in log mol/L; IA♦ - Intestinal absorption expressed in percentage absorbed; SP♦- Skin permeability expressed in log Kp; P-gp[1] - P-glycoprotein substrate; P-gpI[2] - P-glycoprotein-I inhibitor; P-gpII[3] - P-glycoprotein-II inhibitor; VDss*** - Volume of distribution (VD) at steady state (SS) in humans expressed in log L/kg; FU*- Fraction unbound in humans; BBB**- Blood brain barrier permeability expressed in log BB.

Table 2: Metabolism, Excretion and Toxicity (MET) analysis of 31 chosen *Trigonella foenum-graecum* (Fenugreek) ligands using the pkCSM web server.

Ligands	Cytochrome P450 (CYP)							AMES**	HT***
	2D6*	3A4◆	1A2■	2C19[a]	2C9[b]	2D6[c]	3A4[d]		
4- amino benzoic acid	No	No	No	No	No	No	No	No	No
Carpaine	No	Yes	No	No	No	No	No	No	No
Chlorogenic acid	No	No	No	No	No	No	No	No	No
Coumarin	No	No	Yes	No	No	No	No	No	No
Daidzein	No	No	Yes	Yes	Yes	No	No	No	No
3,7-O-dimethylquercetin	No	No	Yes	Yes	Yes	No	No	No	No
Diosgenin	No	Yes	No	No	No	No	No	No	No
Ellagic acid	No	No	Yes	No	No	No	No	No	No
Fenugreekine	No	No	No	No	No	No	No	No	No
Fenugrin B (Apigenin 7-O-beta-D-glucopyranoside)	No	No	No	No	No	No	No	No	No
Ferulic acid	No	No	No	No	No	No	No	No	No
Gentianine	No	No	Yes	No	No	No	No	No	Yes
Graecunin (E)	No	No	No	No	No	No	No	No	No
Hesperidin	No	No	No	No	No	No	No	No	No
Hydroxy tyrosol	No	No	No	No	No	No	No	Yes	No
4- hydroxyisoleucine	No	No	No	No	No	No	No	No	No
Hydroxyl benzoic acid	No	No	No	No	No	No	No	No	No
Isoquercetin	No	No	No	No	No	No	No	No	No
Isorhamnetin	No	No	Yes	No	No	No	No	No	No
Kaempferol	No	No	Yes	No	No	No	No	No	No
Kaempferol 3-(2"-p-coumarylglucoside)	No	No	No	No	No	No	No	No	No
Luteolin	No	No	Yes	No	Yes	No	No	No	No
3-O-methylquercetin	No	No	Yes	No	No	No	No	No	No
Naringenin	No	No	Yes	No	No	No	No	No	No
Naringin	No	No	No	No	No	No	No	No	No
Neurin	No	No	No	No	No	No	No	No	No
Prunin	No	No	No	No	No	No	No	No	No
Quercetin	No	No	Yes	No	No	No	No	No	No
Rosmarinic (Rosmarinic acid)	No	No	No	No	No	No	No	No	No
Trigonelline	No	No	No	No	No	No	No	No	No
Trimethylamine	No	No	No	No	No	No	No	No	No

Note: 2D6* - Cytochrome (CYP) P_{450} 2D6 substrate; 3A4◆ - Cytochrome (CYP) P_{450} 3A4 substrate; 1A2 ▪ - Cytochrome (CYP) P_{450} 1A2 inhibitor; 2C19 [a] – Cytochrome (CYP) P_{450} 2C19 inhibitor; 2C9[b]- Cytochrome (CYP) P_{450} 2C9 inhibitor; 2D6[c]- Cytochrome (CYP) P_{450} 2D6 inhibitor; 3A4[d]- Cytochrome (CYP) P_{450} 3A4 inhibitor; AMES**–AMES toxicity; HT*** - Hepato (liver) toxicity.

Fenugrin B, Hesperidin and Naringin) have shown interactions with TYR 79 amino acid residue of human lutropin subunit beta (hLH beta) protein (as shown in Table 3). However, three ligands (Coumarin, Ferulic acid and Trimethylamine) did not show any hydrogen bond interactions with human lutropin subunit beta (hLH beta) protein.

The present swissdock analysis showed that Graecunin (E) has the Maximum Binding Energy (MBE) (-10.30 kcal/mol) with the

human Follicle Stimulating Hormone (hFSH) protein. On the other hand, Trimethylamine had the Least Binding Energy (LBE) (-4.99 kcal/mol) with the human follicle stimulating hormone (hFSH) (as shown in Table 4).

Interestingly, all *T. foenum-graecum* (Fenugreek) ligands have shown interactions with CYS 28 amino acid residue of human Follicle Stimulating Hormone (hFSH) protein (as shown in Table 4).

DISCUSSION

According to Magdy Mohamady *et al.*, (2018) had demonstrated that *Trigonella foenum-graecum* (fenugreek) seed supplementation as exhibited therapeutic potential in the letrozole induced Polycystic Ovary Syndrome (PCOS) female albino rats. On the other hand, Abbasi and Abbasi (2019) had reported good impact on menstrual cycle, maturation of eggs, reducing ovarian volume and infertility in *Trigonella foenum-graecum* (fenugreek) seeds treated women's for three months duration. Similarly, Mirgaloybayat *et al.*, (2024) had demonstrated better glycemic status, lipid profile and decreased hair loss in

Table 3: The Swissdock binding energy analysis of 31 chosen *Trigonella foenum-graecum* (Fenugreek) ligands with the human lutropin subunit beta (hLH beta) protein using Swissdock method.

Ligand name	Swissdock binding energy (-kcal/mol)	Interactions of amino acid residues	Bond distance (H-A) in A°	Bond distance (D-A) in A°
4-Amino benzoic acid	-5.78	ARG 26 THR 60	2.26 2.81	3.12 3.72
Carpaine	-7.81	ASP 119	2.46	3.34
Chlorogenic acid	-6.90	TYR 79	3.23	3.73
Coumarin	-6.13	NI▪		
Daidzein	-6.58	ARG 114	2.61	3.30
3, 7-*O*-Dimethylquercetin	-7.06	CYS 54 TYR 79 ASP 119 HIS 126	3.07 3.26 and 2.94 2.20 1.97	3.85 4.02 and 3.41 3.11 2.81
Diosgenin	-7.81	CYS 77	1.99	2.92
Ellagic acid	-6.91	GLU 39 HIS 132 PRO 133	1.92 2.83 2.44	2.89 3.56 3.07
Fenugreekine	-9.21	CYS 54 CYS 77 THR 78 TYR 79 ASP 119 GLY 121 GLY 122 LYS 124 HIS 126	2.84 and 1.98 2.93 2.75 2.49 and 3.27 2.39 2.18 2.08 3.17 2.39	3.58 and 2.89 3.75 3.64 3.25 and 3.75 3.29 3.16 3.01 4.06 3.20
Fenugrin B (Apigenin 7-O-beta-D-glucopyranoside)	-7.80	GLU 39 TYR 79 SER 101 LYS 124	3.36, 3.34, 2.47 and 2.10 2.91 and 3.34 2.01 2.43 and 2.19	4.09, 4.09, 3.36 and 3.02 3.69 and 3.93 2.97 3.17 and 3.04
Ferulic acid	-6.21	NI▪		
Gentianine	-6.09	ARG 63 LEU 72	2.27 2.49	3.23 3.46

Graecunin (E)	-9.98	CYS 77	1.88	2.84
		ASP 119	2.72 and 3.60	3.17 and 4.04
		GLY 121	2.35 and 2.38	3.28 and 3.36
Hesperidin	-8.34	CYS 54	2.62	3.42
		CYS 77	3.30	3.78
		TYR 79	2.41 and 3.20	3.00 and 3.95
		GLY 121	2.40	3.35
Hydroxy benzoic acid	-6.10	GLU 39	1.80	2.76
		PRO 44	2.70	3.08
		ASP 131	2.84	3.35
		HIS 132	2.68	3.47
Hydroxy tyrosol	-6.14	ARG 26	2.07	2.98
		CYS 58	1.93	2.89
4-Hydroxyisoleucine	-6.08	ARG 26	1.99	2.94
		TYR 57	2.96	3.58
		CYS 58	2.86	3.32
Isoquercetin	-8.25	CYS 54	1.72 and 1.95	2.67 and 2.89
		GLY 56	2.89	3.32
		CYS 58	3.30	3.84
		CYS 77	2.53	3.41
		THR 78	3.03 and 3.58	3.93 and 3.93
		SER 118	3.14 and 3.35	3.77 and 3.77
		ASP 119	1.95	2.92
		GLY 121	3.35	3.98
		LYS 124	2.99	3.68
Isorhamnetin	-6.89	GLY 56	2.49 and 2.29	3.38 and 3.00
		THR 78	2.64	3.24
		SER 118	2.83	3.56
Kaempferol	-6.79	GLU 39	1.98	2.89
		GLY 42	2.96	3.70
		PRO 133	2.08	2.99
Kaempferol 3-(2-p-coumarylglucoside)	-8.02	CYS 77	2.75	3.26
		LYS 124	2.74	3.71
		HIS 126	1.84 and 2.14	2.79 and 2.94
Luteolin	-7.11	THR 78	1.96	2.89
		ARG 109	3.10	3.99
		ARG 114	3.11	3.68
		THR 117	3.16	3.51
3-O-Methylquercetin	-6.67	ASP 119	2.48	3.35
Naringenin	-6.66	ARG 114	2.61	3.30
Naringin	-8.31	CYS 54	2.74 and 2.04	3.44 and 2.94
		CYS 58	2.57	3.54
		CYS 77	2.72	3.37
		TYR 79	3.12	3.91
		ASP 119	2.25	2.94
		GLY 121	2.90	3.23
Neurin	-6.63	ARG 22	2.14	2.96

Prunin	-7.72	CYS 54 CYS 58 VAL 75 THR 78 SER 118	3.05 3.00 3.09 3.13 3.37 and 2.53	3.79 3.83 3.96 4.10 4.02 and 3.32
Quercetin	-6.98	GLU 39 ASP 131 HIS 132 PRO 133	1.85 2.82 3.28 2.03	2.79 3.51 4.09 2.79
Rosmarinic (Rosmarinic acid)	-7.02	CYS 54 CYS 77 ASP 119	3.39 and 2.00 3.26 2.15	3.85 and 2.84 3.26 3.11
Trigonelline	-6.42	GLY 42	1.96	2.92
Trimethylamine	-5.08	NI•		

Note: NI• – No hydrogen bond interactions.

Table 4: The Swissdock binding energy analysis of 31 chosen *Trigonella foenum-graecum* (Fenugreek) ligands with the human Follicle Stimulating Hormone (hFSH) protein using Swissdock method.

Ligand name	Swissdock binding energy (-kcal/mol)	Interactions of amino acids residues	Bond distance (H-A) in A°	Bond distance (D-A) in A°
4-amino benzoic acid	-5.97	ALA 11 TRP 27 CYS 28 TYR 74	3.32 and 1.99 2.39 1.95 2.65	3.87 and 2.88 3.39 2.85 3.27
Carpaine	-8.51	CYS 28 TYR 74	1.95 2.65	2.85 3.27
Chlorogenic acid	-7.30	CYS 28 THR 52 TYR 74	1.95 and 2.15 2.15 3.08	2.85 and 2.96 3.02 3.27
Coumarin	-6.09	CYS 28 TYR 74	1.95 2.65	2.85 3.27
Daidzein	-6.86	ALA 11 CYS 28 TYR 74	1.99 1.95 2.99	2.91 2.85 3.27
3,7-o-dimethylquercetin	-7.36	ALA 11 CYS 28 TYR 74 THR 92	2.84 1.95 2.65 2.75	3.44 2.85 3.27 3.63
Diosgenin	-8.34	CYS 28 CYS 51	1.95 1.97	2.85 2.94
Ellagic acid	-6.75	CYS 28 CYS 51 THR 52 TYR 74	1.95 2.37 2.55 2.65	2.85 3.15 3.30 3.27

Fenugreekine	-9.37	CYS 28	1.95	2.85
		THR 52	3.03 and 2.31	3.91 and 3.27
		PHE 53	3.43	4.03
		TYR 74	2.65	3.27
		ASP 93	2.63	3.46
		THR 95	3.22	4.05
		LEU 99	2.83	3.64
		GLY 100	2.74	3.55
Fenugrin B (Apigenin 7-O-beta-D-glucopyranoside	-8.05	CYS 28	1.95	2.85
		THR 50	3.05	3.38
		CYS 51	2.05	2.94
		TYR 58	2.94	3.39
		TYR 74	2.65	3.27
		THR 95	1.95 and 3.00	2.91 and 3.53
Ferulic acid	-6.47	CYS 28	1.95	2.85
Gentianine	-6.19	CYS 28	1.95	2.85
		TYR 74	2.65	3.27
Graecunin (E)	-10.30	ALA 11	2.68	3.39
		CYS 28	1.95	2.85
		TYR 74	2.65	3.27
		ASP 93	2.02 and 3.30	2.95 and 3.86
		THR 95	3.24 and 2.95	4.09 and 3.72
		VAL 96	2.32	3.02
		GLY 98	3.27	4.05
		GLY 100	2.16	2.98
Hesperidin	-9.27	CYS 28	1.95	2.85
		TYR 74	2.65	3.27
		ASP 93	3.16	3.52
		LEU 99	2.33	3.26
		GLY 100	2.35	3.18
		TYR 103	2.07	2.80
Hydroxy benzoic acid	-5.98	ALA 11	3.34 and 2.02	3.89 and 2.90
		TRP 27	2.42	3.32
		CYS 28	1.95	2.85
		TYR 74	2.65	3.27
Hydroxy tyrosol	-6.03	TRP 27	3.10	4.09
		CYS 28	1.95	2.85
		TYR 74	2.40 and 2.65	3.27 and 3.27
4-Hydroxyisoleucine	-7.15	CYS 28	1.95	2.85
		TYR 74	2.65	3.27
Isoquercetin	-7.97	CYS 28	1.95	2.85
		TYR 74	2.65	3.27
		PRO 77	2.47	3.43
		ASP 93	1.95	2.92
		GLY 98	3.56	4.09
		LEU 99	2.48	3.45
		GLY 100	2.95	3.66
		PRO 101	3.68	3.97

Isorhamnetin	-6.98	CYS 28 TYR 74 SER 91	1.95, 3.23 and 2.18 2.65 3.51	2.85, 3.99 and 3.03 3.27 4.09
Kaempferol	-6.94	ALA 11 CYS 28 TYR 74 SER 91	2.04 1.95 2.99 3.05	2.92 2.85 3.27 3.43
Kaempferol 3-(2-p-coumarylglucoside)	-8.74	ALA 11 CYS 28 TYR 74 ASP 93 GLY 98	3.07 1.95 2.65 3.11 2.50	3.78 2.86 3.27 4.01 3.47
Luteolin	-6.77	CYS 28 TYR 58 TYR 74	1.95 3.22 2.65	2.85 3.86 3.27
3- *O*-Methylquercetin	-7.29	CYS 28 CYS 51 TYR 58 TYR 74	1.95, 2.94 and 2.11 2.14 3.23 2.65	2.85, 3.73 and 2.94 3.10 3.86 3.27
Naringenin	-6.94	ALA 11 CYS 28 THR 52 TYR 74	3.41 1.95, 3.21 and 2.63 2.95 and 2.91 2.65	4.02 2.85, 3.96 and 3.04 3.79 and 3.79 3.27
Naringin	-8.59	CYS 28 TYR 74 PRO 77 GLY 98 LEU 99 GLY 100	1.95 2.65 2.23 3.77 2.32 2.25	2.85 3.27 3.19 4.09 3.10 3.09
Neurin	-6.41	CYS 28 ARG 44 TYR 74	1.95 1.95 and 2.25 2.65	2.85 2.83 and 2.93 2.27
Prunin	-7.47	CYS 28 CYS 51 TYR 74 THR 75 ASP 93	1.95 and 2.65 2.21 2.65 2.38 2.36	2.85 and 3.23 3.18 3.27 3.11 3.31
Quercetin	-7.03	CYS 28 TYR 74	1.95 and 1.95 2.65	2.85 and 2.853.27
Rosmarinic (Rosmarinic acid)	-7.43	ALA 11 CYS 28 TYR 74 GLY 98 GLY 100	2.94 and 2.06 1.95 and 3.40 2.59 and 1.99 2.752.65	3.55 and 2.87 2.85 and 4.09 3.27 and 2.94 3.55 3.39
Trigonelline	-5.81	TRP 27 CYS 28 TYR 74	2.63 1.95 2.65	3.57 2.85 3.27

Trimethylamine	-4.99	CYS 28TYR 74	1.952.65	2.853.27

Note: NI• – No hydrogen bond interactions.

Trigonella foenum-graecum (fenugreek) seed supplemented women's. Furthermore Shukla *et al.*, (2024) had reported the insulin-sensitizing, glucose regulating, anti-obesity and anti-hyper-lipidemic effects of Furocyst (standardized *Trigonella foenum-graecum* seed extract) in women's with Polycystic Ovary Syndrome (PCOS).

Prior to docking ADMET analysis was carried out in the present study, where Gentianine has been predicated to exhibit hepatotoxicity effect (as shown in Table 3). This finding was on par with previous report, where higher dose of Gentianine showed low toxicity to experimental rats (Arsala Mansoor, 2003). Similarly, Hydroxy tyrosol has been predicated to be positive for AMES toxicity analysis. This result was in excellent correlation with recent report, where Hydroxy tyrosol showed low toxicity at physiologically relevant concentrations (Wang *et al.*, 2025).

In the current docking analysis, two ligands (Isoquercetin and Isorhamnetin) of fenugreek have exhibited interaction with GLY 56 amino acid residue of human lutropin subunit beta (hLH beta) protein. This result was in excellent agreement with earlier report (Essa *et al.*, 2019). Similarly, four ligands (4-Hydroxyisoleucine, Isoquercetin, Naringin and Prunin) of fenugreek have shown interaction with CYS 58 amino acid residue of human lutropin subunit beta (hLH beta) protein. This finding was in good correlation with previous report (Essa *et al.*, 2019). Furthermore, five ligands (Fenugreekine, Isoquercetin, Isorhamnetin, Luteolin and Prunin) of fenugreek have exhibited interaction with THR 78 amino acid residue of human lutropin subunit beta (hLH beta) protein. This result was in good agreement with earlier report (Bhatnager *et al.*, 2024).

In the present investigation, six ligands (Fenugreekine, Graecunin (E), Hesperidin, Isoquercetin, Kaempferol 3-(2-p-coumarylglucoside) and Prunin) of fenugreek have exhibited interaction with ASP 93 amino acid residue of human Follicle Stimulating Hormone (hFSH). This result was in good correlation with previous report (Fox *et al.*, 2001). Similarly, three ligands (Fenugreekine, Fenugrin B and Graecunin (E)) of fenugreek have shown interaction with THR 95 amino acid residue of human Follicle Stimulating Hormone (hFSH). The current finding was in good agreement with earlier report (Sonawani *et al.*, 2013). Furthermore, Graecunin (E) and Hesperidin ligands have shown interaction with VAL 96 and TYR 103 amino acid residue of human Follicle Stimulating Hormone (hFSH) respectively. This result was in excellent correlation with previous report (Sonawani *et al.*, 2013).

The present investigation is purely based on *in silico* (docking) approach which gives new understanding about the 31 chosen *T. foenum-graecum* (Fenugreek) phytochemicals and their potential interactions with two target [i) human lutropin subunit beta (hLH beta) and ii) human Follicle Stimulating Hormone (hFSH)] proteins. In addition, *in vitro* (hormonal) experimentally assays are needed to confirm their modulating activities of chosen Fenugreek phytochemicals.

CONCLUSION

In the current investigation, the 31 chosen *Trigonella foenum-graecum* (fenugreek) phytoconstituents have shown the potential to dock with two targeted human proteins (hLH beta and hFSH). Moreover, three ligands of fenugreek (Coumarin, Ferulic acid and Trimethylamine) do not exhibit any hydrogen bond interaction with hLH beta protein. Thus, the present finding provide new insight about the 31 chosen ligands of *Trigonella foenum-graecum* (fenugreek) as potent modulating agents of hLH beta and hFSH, which will aid in managing Polycystic Ovary Syndrome (PCOS) related disorders.

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ABBREVIATIONS

hLH beta: Human lutropin subunit beta; **hFSH:** Human follicle stimulating hormone; **PCOS:** Polycystic ovary syndrome; **CID:** Compound Identifier; **2D:** Two dimensional; **3D:** Three dimensional; **ADMET:** Absorption, Distribution, Metabolism, Excretion and Toxicity; **UniProt:** Universal Protein Resource; **PDB:** Protein Data Bank; HBE: Highest binding energy; **MBE:** Maximum binding energy; **LBE:** Least binding energy.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

SUMMARY

In the present study, 31 chosen *Trigonella foenum-graecum* (fenugreek) phytochemicals were studied on the docking behaviour of human lutropin subunit beta (hLH beta) and ii) human follicle stimulating hormone (hFSH) by using the Swiss dock method. The present docking analysis showed that Graecunin (E) of *T. foenum-graecum* (Fenugreek) has exhibited the highest binding energy with the hLH beta and hFSH respectively.

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