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COMPUTER-AIDED ANALYSIS OF FENUGREEK PHYTOCONSTITUENTS FOR PHARMACOLOGICAL ACTIVITIES USING PHYTOCHEMICAL SCREENING AND MOLECULAR DOCKING APPROACHES

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ABSTRACT

Objective: The goal of the current study was to compare the phytochemical properties of aqueous, ethanolic, and hydroalcoholic extracts of *Trigonella foenum-graecum L*. seeds and to use computer-aided molecular docking to examine the therapeutic potential of its main phytoconstituents for anti-dandruff, anti-alopecia, and antioxidant properties.

Materials and Methods: Soxhlet extraction was used to separate the powdered seeds of *Trigonella foenum-graecum L*. using aqueous, ethanolic, and hydroalcoholic (50:50, v/v) solvent systems. The primary secondary metabolites were identified using qualitative phytochemical screening. Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) were used to further evaluate the hydroalcoholic extract in order to identify flag chemicals such as diosgenin and kaempferol. AutoDock Vina combined with PyRx was used to perform molecular docking investigations against protein targets that are anti-dandruff (PDB ID: 2XFH), anti-alopecia (PDB ID: 4K7A), and antioxidant (PDB ID: 3VXI). Discovery Studio Visualizer was used to examine protein–ligand interactions, and SwissADME and ProTox-II were used to estimate pharmacokinetics and toxicity.

Result: When compared to aqueous and ethanolic extracts, the hydroalcoholic extract showed the richest phytochemical profile. The presence of diosgenin and kaempferol was verified by TLC and HPLC analysis. Molecular docking revealed that kaempferol had the strongest binding against the anti-dandruff target, diosgenin had a promising interaction with the anti-alopecia target, and quercetin had the highest affinity toward the antioxidant target. The chosen phytoconstituents had acceptable safety profiles and good drug-likeness, according to ADME and toxicity assessments.

Conclusion: The results show that *Trigonella foenum-graecum L.* is an important source of bioactive phytochemicals with strong anti-dandruff, anti-alopecia, and antioxidant properties. Phytochemical screening, chromatographic characterisation, and molecular docking are integrated to give scientific evidence for the creation of phytoconstituents derived from fenugreek as potential therapeutic candidates.

KEYWORDS - *Trigonella foenum-graecum L*, Anti- alopecia Activity, Anti-oxidant Activity, Anti- Dandruff Activity, Molecular docking

INTRODUCTION

As a rich source of bioactive chemicals, medicinal plants have been widely used to prevent and treat a wide range of illnesses. Phytoconstituents derived from plants have a variety of pharmacological characteristics, such as anti-inflammatory, antibacterial, antioxidant, and hair growth-promoting effects[1]. Finding natural substances that can act as safer substitutes for manufactured medicinal agents has drawn more attention in recent years. The screening and assessment of plant-derived bioactive compounds for possible therapeutic uses has been expedited by the merging of phytochemical research with computer-aided drug development techniques[2].

The *Fabaceae* family includes the well-known medicinal and nutritional plant *Trigonella foenum-graecum L*. (Fenugreek), which is grown extensively in Asia, Africa, and the Mediterranean region. Alkaloids, flavonoids, saponins, phenolic compounds, tannins, steroids, amino acids, and terpenoids are among the many bioactive components found in *Trigonella foenum-graecum L* [3]. seeds. Significant antioxidant, antibacterial, anti-inflammatory and dermatological advantages have been found for major phytoconstituents such trigonelline, diosgenin, kaempferol, quercetin, rutin, and gallic acid.

Alopecia and dandruff are two of the most prevalent dermatological and cosmetic conditions impacting people globally. While dandruff is linked to scalp irritation, microbial colonization, and abnormal shedding of scalp skin, alopecia is defined by severe hair loss brought on by genetic, hormonal, inflammatory, or environmental causes[4]. Furthermore,

oxidative stress has been identified as a significant contributing factor to diseases of the scalp and damage to hair follicles. As a result, natural phytoconstituents with antioxidant, anti-dandruff, and anti-alopecia qualities have garnered a lot of interest for the creation of innovative treatment approaches[5].

Molecular docking and other computer-aided drug discovery methods have become important tools for predicting how bioactive molecules will interact with certain biological targets. Rapid phytoconstituents screening, lead molecule selection, and binding affinity evaluation toward target proteins linked to disease pathways are all made possible by molecular docking. An effective method for investigating the therapeutic potential of medicinal plants is the combination of phytochemical screening and molecular docking[6].

Thus, a comparative phytochemical analysis of aqueous, ethanolic, and hydroalcoholic extracts of Trigonella *Trigonella foenum-graecum L*. seeds was carried out in the current work. Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) were used to further evaluate the hydroalcoholic extract in order to identify the main marker chemicals. Additionally, computer-aided molecular docking experiments were performed on specific phytoconstituents to examine their possible anti-dandruff, anti-alopecia, and antioxidant properties. This provided scientific proof of the therapeutic value of *Trigonella foenum-graecum L*[7-8].

2. MATERIAL AND METHODS

2.1 Plant Material

Trigonella foenum-graecum L. seeds were gathered and verified. For additional analysis, the seeds were cleaned, shade-dried, and ground into a coarse powder.

2.2 Preparation of Extract

Trigonella foenum-graecum L. seeds were cleaned, dried in the shade, and then ground into a powder. The Soxhlet extraction method was used to extract the powdered material individually using distilled water, ethanol, and a hydroalcoholic solvent mixture (ethanol:water, 50:50 v/v). Before being used again, the extracts were filtered, concentrated under low pressure, and kept at 4°C. Aqueous Extract (AE), Ethanolic Extract (EE), and Hydroalcoholic Extract (HAE) were the names given to the extracted materials. To identify the main secondary metabolites found in Trigonella foenum-graecum L. seeds, all extracts underwent qualitative phytochemical screening[9-11].

2.3 Preliminary phytochemical Screening

Standard phytochemical tests were used to qualitatively screen the aqueous, ethanolic, and hydroalcoholic extracts of *Trigonella foenum-graecum L*. for the presence of alkaloids, flavonoids, saponins, phenolic compounds, tannins, steroids, coumarins, amino acids, carbohydrates, and terpenoids. The intensity of the observed reactions was used to record the results[12].

2.4 Thin layer chromatography

The main phytoconstituents in the hydroalcoholic extract of *Trigonella foenum-graecum L*. were identified using thin-layer chromatography (TLC). Prior to analysis, the dried extract (10 mg/mL) was diluted in methanol and passed through a 0.45 µm membrane filter[13]. Diosgenin and kaempferol standard solutions (1 mg/mL) were made in methanol. Using a capillary tube, samples and standards were put as bands on silica gel 60 F254 TLC plates that had already been coated. The plates were created in a saturated TLC chamber using an appropriate mobile phase setup[14]. Following development, the plates were allowed to air dry before being exposed to UV radiation at 254 and 366 nm. Following derivatization, the isolated spots were further observed, and for identification, the Rf values of the sample constituents were compared with those of the respective standards[15].

2.5 High Performance Liquid Chromatography

The hydroalcoholic extract of *Trigonella foenum-graecum L*. was subjected to HPLC analysis to establish the presence of kaempferol and diosgenin. Before injection, the extract was dissolved in 1 mg/mL of HPLC-grade methanol, sonicated for 10 minutes, and filtered through a 0.45 µm membrane filter. Diosgenin and kaempferol standard solutions were made independently in methanol at the same concentration[16].

A Shimadzu HPLC system with a reverse-phase C18 analytical column and a UV-visible detector was used for the chromatographic analysis. HPLC-grade solvents supplied at a steady flow rate under isocratic conditions made up the mobile phase. Chromatograms were acquired at the proper detection wavelength using an injection volume of 20 µL. By comparing the retention durations of the extract's peaks with those of verified reference compounds kaempferol and diosgenin compounds were identified[17-18].

2.6 Molecular Docking

The binding potential of certain phytoconstituents of *Trigonella foenum-graecum L*. against protein targets linked to anti-alopecia, anti-dandruff, and antioxidant properties was assessed using computer-aided molecular docking experiments[19]. Major bioactive substances, including as diosgenin, kaempferol, quercetin, rutin, trigonelline, and gallic acid, were chosen as ligands for docking studies based on phytochemical screening and chromatographic analysis.

Using Open Babel software, the three-dimensional structures of the chosen ligands were extracted from the PubChem database in SDF format and transformed into PDB format. The Protein Data Bank (PDB) provided the target proteins' crystal structures. Co-crystallized ligands and water molecules were removed from protein structures before docking, and polar hydrogen atoms and Kollman charges were then added[20].

AutoDock Vina combined with PyRx virtual screening software (Version 0.8) was used to perform molecular docking. Before docking, the MMFF94 force field was used to minimize the ligands' energy[21]. The binding pocket of the native ligand found in the crystal structure was used to define the coordinates of the active site. The default AutoDock Vina parameters were used for docking simulations, and each ligand's binding affinity was reported as binding energy (kcal/mol)[22].

For additional examination, the docked conformations with the lowest binding energy and best interactions were chosen. Discovery Studio Visualizer was used to show and study protein–ligand interactions, such as hydrogen bonds, hydrophobic interactions, and π-interactions. Compounds with possible anti-dandruff, anti-alopecia, and antioxidant properties were found by comparing the docking scores and interaction profiles of the phytoconstituents[23-24].

3. RESULT AND DISCUSSION

3.1 Comparative Phytochemical Screening of *Trigonella foenum-gracum L.* extract

The phytoconstituents profile of the aqueous, ethanolic, and hydroalcoholic extracts of *Trigonella foenum-graecum L.* varied, according to qualitative phytochemical examination.

Alkaloids, flavonoids, saponins, phenolics, tannins, steroids, coumarins, amino acids, carbohydrates, and terpenoids were among the phytochemicals that were most prevalent in the hydroalcoholic extract. While aqueous extract exhibited somewhat poorer recovery (Table no – 1) of non-polar and semi-polar components, ethanolic extract displayed moderate extraction efficiency[25].

Table no – 1 Comparative Phytochemical Screening of *Trigonella foenum-graecum L.* Extracts

Phytochemical Class	Aqueous Extract	Ethanolic Extract	Hydroalcoholic Extract
Alkaloids	+	++	+++
Flavonoids	++	+++	+++
Saponins	++	++	+++
Phenolic Compounds	++	+++	+++
Tannins	+	++	+++
Steroids	-	++	+++
Coumarins	+	++	+++
Amino Acids	+++	++	+++
Carbohydrates	+++	++	+++
Terpenoids	+	++	+++

Table No – 2 Qualitative Phytochemical Screening of Hydroalcoholic Extract of *Trigonella foenum-graecum L.* Seeds

Phytochemical Class	Identified Constituents	Qualitative Test(s) Used	Result
Alkaloids	Trigonelline	Dragendorff's Test, Wagner's Test	Positive
Flavonoids	Quercetin, Kaempferol, Rutin	Alkaline Reagent Test, Shinoda Test	Positive
Saponins	Diosgenin, Yamogenin	Foam Test	Positive
Phenolic Compounds	Gallic Acid, Caffeic Acid, Vanillic Acid	Ferric Chloride Test	Positive
Tannins	Condensed Tannins	Gelatin Test, Lead Acetate Test	Positive
Steroids	β-Sitosterol, Stigmasterol	Salkowski Test, Liebermann–Burchard Test	Positive
Coumarins	Scopoletin*	UV Fluorescence Test	Positive
Amino Acids	Histidine, Lysine	Ninhydrin Test	Positive
Carbohydrates	Mucilage, Fiber	Molisch's Test, Benedict's Test	Positive
Terpenoids	Limonene, Carvone	Salkowski Test	Positive

The hydroalcoholic solvent system's greater extraction efficiency can be explained by its capacity to dissolve both polar and somewhat non-polar phytoconstituents, leading to increased phytochemical diversity (Table no – 2).

3.2 Thin layer chromatographic Analysis

The primary phytoconstituents in the hydroalcoholic extract of *Trigonella foenum-graecum L*. were identified using TLC analysis. The reference standards, diosgenin and kaempferol, were represented as well-resolved spots in the chromatographic profile. Kaempferol's Rf value in the extract was determined to be 0.62, which was quite similar to the standard compound's Rf value. In a similar vein, diosgenin showed an Rf value of 0.52, which was similar to the conventional diosgenin.

The existence of both chemicals in the extract was confirmed by visualization under UV light, which showed discrete spots with similar movement patterns. The hydroalcoholic extract of Trigonella foenum-graecum L. includes notable amounts of flavonoids and steroidal saponins, which may contribute to its pharmacological actions, according to the TLC results.

3.3 HPLC analysis of *Trigonella foenum-gracum L.* extract

The presence of diosgenin and kaempferol in the hydroalcoholic extract of *Trigonella foenum-graecum L*. was further verified by HPLC analysis. The retention durations of the reference standards were represented by distinctive peaks in the chromatographic profile[26]. At a retention time of 5.21 minutes, kaempferol was found, which is quite similar to the typical retention time of 5.30 minutes. In a similar vein, diosgenin showed a retention time of 6.85 minutes as opposed to the normal compound's 6.92 minutes. The little fluctuations found fell within reasonable analytical bounds. These results verified that bioactive phytoconstituents in *Trigonella foenum-graecum L*. were successfully extracted and identified. The possible anti-alopecia, anti-dandruff, and antioxidant properties examined in later molecular docking investigations are supported by the presence of kaempferol and diosgenin.

3.4 Molecular Docking analysis for Anti-oxidant activity

Molecular docking studies were conducted against the target protein (PDB ID: 3VXI) in order to examine the antioxidant capacity of the phytoconstituents of *Trigonella foenum-graecum L*. Quercetin and kaempferol, two major flavonoids found during phytochemical screening, were chosen as test chemicals and contrasted with ascorbic acid, a common antioxidant[27]. With a docking score of −6.861 kcal/mol and binding free energy (ΔG) of −51.20 kcal/mol, quercetin showed the highest binding affinity for the target protein, according to the docking data. Kaempferol came in second with a docking score of −6.533 kcal/mol and ΔG value of −50.51 kcal/mol. By contrast, the reference chemical ascorbic acid displayed a binding free energy of -17.77 kcal/mol and a docking score of -5.607 kcal/mol (Table no – 3).

These results imply promising antioxidant potential since the flavonoids in *Trigonella foenum-graecum L*. interact with the antioxidant target protein more strongly than the standard reference molecule.

Table No 3 - Docking Scores of *Trigonella foenum-graecum L*. Constituents Against Antioxidant Target (PDB ID: 3VXI)

Compound	Docking Score (kcal/mol)	ΔG Binding Energy (kcal/mol)
Quercetin	-6.861	-51.20
Kaempferol	-6.533	-50.51
Standard (Ascorbic Acid)	-5.607	-17.77

Protein ligand Interaction Analysis

The stability of ligand-protein complexes is largely dependent on hydrogen bonding interactions. HIE326, ASN290, and ASN313 formed hydrogen bonds with quercetin at bond distances of 2.24 Å, 2.08 Å, and 1.82 Å, respectively. At distances of 2.20 Å, 2.01 Å, and 2.10 Å, kaempferol interacted with HIE326, ASN290, and ASP292. At distances of 1.87 Å, 2.03 Å, and 2.12 Å, the standard ascorbic acid established hydrogen bonds with HIE326, GLY188, and ASN313. (Table no – 4)

The higher binding affinities of quercetin and kaempferol in comparison to the typical antioxidant molecule may be explained by the higher number of beneficial interactions found for these compounds.

Table No 4 Key Amino Acid Interactions of Docked Complexes

Compound	Amino Acid Interactions	Distance (Å)
Quercetin	HIE326, ASN290, ASN313	2.24, 2.08, 1.82
Kaempferol	HIE326, ASN290, ASP292	2.20, 2.01, 2.10
Ascorbic Acid	HIE326, GLY188, ASN313	1.87, 2.03, 2.12

ADME prediction

SwissADME research showed that every substance had good pharmacokinetic properties. Quercetin and kaempferol showed strong drug-likeness due to their high gastrointestinal absorption, satisfactory bioavailability ratings (0.55), and adherence to Lipinski's rule of five. It was anticipated that none of the substances would cross the blood–brain barrier (Table no – 5).

Table No 5 ADME Profile of Antioxidant Compounds

Parameter	Quercetin	Kaempferol	Ascorbic acid
Molecular Weight (g/mol)	302.24	286.24	176.12
H-Bond Acceptors	7	6	6
H-Bond Donors	5	4	4
Log P	1.06	1.70	-0.31
GI Absorption	High	High	High
BBB Permeability	No	No	No
Bioavailability Score	0.55	0.55	0.56
Lipinski Violations	0	0	0
Synthetic Accessibility	3.23	3.14	3.47

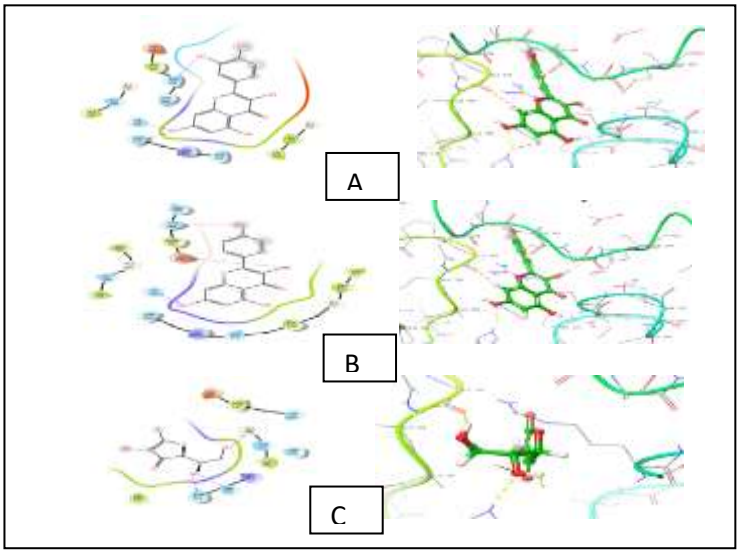

Figure No 1 - 2D and 3D images of Docked complex of *Trigonella foenum* seed Chemical Constituents for Anti-oxidant activity; A – Quercetin , B – Kaempferol, C – Ascorbic acid

Toxicity Prediction

Quercetin was assigned to toxicity class 3 by ProTox-II analysis, while kaempferol and ascorbic acid were assigned to toxicity class 5, indicating much lower toxicity profiles. While kaempferol showed an overall more favorable toxicity profile, all substances were anticipated to be inactive for immunotoxicity.

Table No - 6 Toxicity Prediction of Antioxidant Compounds

Parameter	Quercetin	Kaempferol	Ascorbic Acid
ProTox-II Class	3	5	5
Hepatotoxicity	Less Inactive	Less Inactive	Inactive
Carcinogenicity	Less Inactive	Inactive	Inactive
Immunotoxicity	Inactive	Inactive	Inactive
Mutagenicity	Less Inactive	Less Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Less Inactive

If the conference has page restrictions, you may keep only the quercetin figures and state that the other interaction diagrams are available upon request or in supplemental data because quercetin had the best docking score (Table no – 6).
This format is small, focused on publications, and ideally fits the title of the conference you have chosen. The anti-dandruff/antifungal (PDB ID: 2XFH) and anti-alopecia (PDB ID: 4K7A) sections can now have the same structure.

3.5 Molecular docking analysis for Anti – Alopecia Activity

The anti-alopecia potential of the phytoconstituents of *Trigonella foenum-graecum L.* was assessed using molecular docking studies against the target protein (PDB ID: 4K7A). Diosgenin was chosen as the test ligand among the bioactive substances found, and it was contrasted with minoxidil, a common anti-alopecia medication.
The docking data showed that the conventional compound minoxidil had a docking score of −4.925 kcal/mol and a binding free energy of −25.91 kcal/mol, while diosgenin had a docking score of −2.609 kcal/mol and a binding free energy (ΔG) of −46.01 kcal/mol. Diosgenin showed a much reduced binding free energy, indicating a durable association with the

target protein, but minoxidil showed a somewhat superior docking score (Table no – 7). According to these results, diosgenin may be a natural lead molecule that promotes hair growth and has promising anti-alopecia potential.

Table No 7 – Comparative Phytochemical Screening of *Trigonella foenum-graecum L.* Extracts for Anti – Alopecia Activity

Compound	Docking Score (kcal/mol)	ΔG Binding Energy (kcal/mol)
Diosgenin	-2.609	-46.01
Standard (Minoxidil)	-4.925	-25.91

Protein ligand Interaction Analysis

Diosgenin and GLN858 formed a sustained hydrogen bond at a bond distance of 1.93 Å, according to protein–ligand interaction studies, indicating favorable binding inside the target protein's active region. By contrast, the common medication minoxidil showed several stabilizing contacts, such as a salt-bridge interaction with ARG786 (3.78 Å), a hydrogen bond with ARG786 (1.90 Å), and a π-cation interaction with HIS789 (4.98 Å). These extra interactions could account for minoxidil's higher docking score. However, diosgenin's potential as a naturally occurring (Table no – 8) anti-alopecia chemical is supported by its significant binding free energy.

Table No – 8 Key Amino Acid Interactions of Docked Complexes

Compound	Amino Acid Interactions	Distance (Å)
Diosgenin	H-Bond: GLN858	1.93
Minoxidil	H-Bond: ARG786 π-Cation: HIS789 Salt-Bridge: ARG786	1.90 4.98 3.78

ADME prediction

The SwissADME web server was used to assess the pharmacokinetic characteristics of minoxidil and diosgenin. Diosgenin has a Log P value of 4.49, three hydrogen bond acceptors, one hydrogen bond donor, and a molecular weight of 414.62 g/mol. It was anticipated to be blood–brain barrier (BBB) permeable and showed high gastrointestinal (GI) absorption, suggesting potential exposure to the central nervous system. Diosgenin has a bioavailability score of 0.55 and one violation of Lipinski's rule of five, mostly because of its lipophilicity. With a synthetic accessibility score of 6.94, it appears to have a more complex structure.

The standard medication minoxidil, on the other hand, has a molecular weight of 209.25 g/mol, high GI absorption, no BBB permeability, no breaches of the Lipinski rule, and a bioavailability score of 0.55. Minoxidil is relatively easier to manufacture, as indicated by the lower synthetic accessibility score (2.51).

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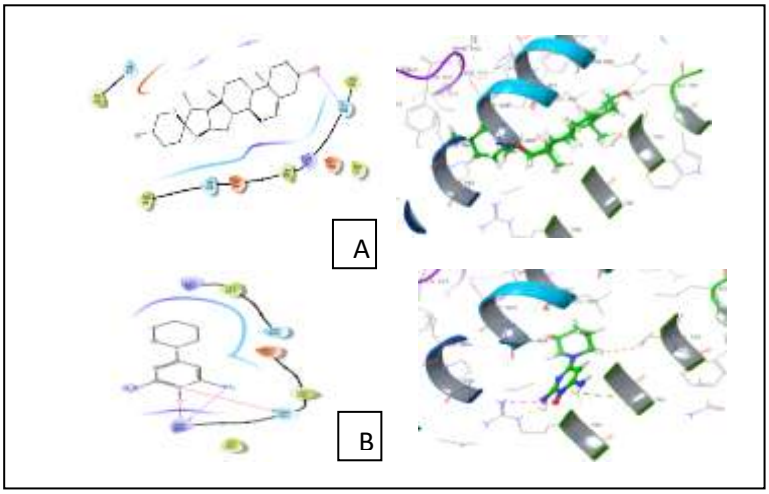

Figure No 2 - 2D and 3D images of Docked complex of TF seed Constituents for Anti-Alopecia activity; A – Diosgenin, B – Minoxidil

Table No – 9 ADME Profile of Antioxidant Compounds

Parameter	Diosgenin	Minoxidil
Molecular Weight (g/mol)	414.62	209.25
H-Bond Acceptors	3	3
H-Bond Donors	1	1
Log P	4.49	1.23
GI Absorption	High	High
BBB Permeability	Yes	No
Bioavailability Score	0.55	0.55
Lipinski Violations	1	0

Toxicity Prediction

The ProTox-II platform was used to predict the toxicity profiles of minoxidil and diosgenin. Diosgenin's comparatively low acute toxicity was shown by its classification under toxicity class 6. With the exception of a possible immunotoxic impact, it was expected to be less inactive for hepatotoxicity and carcinogenicity, active for immunotoxicity, and (Table no – 10) inactive for mutagenicity and cytotoxicity, indicating an overall acceptable safety profile.

Table No – 10 Toxicity Prediction of Antioxidant Compounds

Parameter	Quercetin	Kaempferol
ProTox-II Class	6	4
Hepatotoxicity	Less Inactive	Less Inactive
Carcinogenicity	Less Inactive	Less Inactive
Immunotoxicity	Active	Inactive
Mutagenicity	Inactive	Less Inactive
Cytotoxicity	Inactive	Less Inactive

Compared to diosgenin, the reference medication minoxidil was classified as toxicity class 4. It was projected to stay inactive for immunotoxicity but to be less inactive for hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity.

3.6 Molecular docking analysis for Anti – Dandruff Activity

The anti-dandruff potential of the phytoconstituents of Trigonella foenum-graecum L. was assessed using molecular docking studies against the target protein (PDB ID: 2XFH). Kaempferol and trigonelline, two of the bioactive substances found, were chosen as test ligands and contrasted with clotrimazole, a common antifungal medication used to treat fungal infections linked to dandruff.

According to the docking data, kaempferol had a binding free energy (ΔG) of −46.62 kcal/mol and the highest docking score (−6.759 kcal/mol) among the phytoconstituents. With a docking score of −6.064 kcal/mol and a far lower binding free energy of −73.26 kcal/mol, the common medication clotrimazole demonstrated a highly stable ligand–protein complex. With a docking score of −4.310 kcal/mol and a ΔG value of −26.88 kcal/mol, trigonelline showed the lowest binding affinity (Table no – 11).

According to these results, kaempferol may be a viable natural substance for the treatment of fungal scalp problems because it has a substantial binding affinity for the target protein linked to dandruff.

Table No – 11 Docking Scores of *Trigonella foenum-graecum L.* Constituents Against Anti-Dandruff Target (PDB ID: 2XFH)

Compound	Docking Score (kcal/mol)	ΔG Binding Energy (kcal/mol)
Kaempferol	-6.759	-46.62
Standard (Clotrimazole)	-6.064	-73.26
Trigonelline	-4.310	-26.88

Protein–Ligand Interaction Analysis

Kaempferol established many hydrogen bonds with GLN292, SER345, HID351, and HID88 at bond lengths of 2.08 Å, 2.02 Å, 1.89 Å, and 1.83 Å, respectively, according to protein–ligand interaction studies. The ligand–protein combination was further stabilized by a π–π stacking interaction with PHE288 at 5.07 Å.

Clotrimazole, a common medication, exhibited a significant binding affinity via forming a halogen bond with HID88 (3.09 Å) and a salt-bridge interaction with CYS353 (3.11 Å). Although trigonelline formed hydrogen bonds with ILE87 and HID88 at 1.84 Å and 2.15 Å, respectively, its relatively low docking score could be explained by the lack of further stabilizing contacts (Table no – 12).

Table No – 12 Key Amino Acid Interactions of Docked Complexes

Compound	Amino Acid Interactions	Distance (Å)
Kaempferol	H-Bond: GLN292, SER345, HID351, HID88 π–π Stacking: PHE288	2.08, 2.02, 1.89, 1.83 5.07
Trigonelline	H-Bond: ILE87, HID88	1.84, 2.15
Clotrimazole	Halogen Bond: HID88 Salt-Bridge: CYS353	3.09 3.11

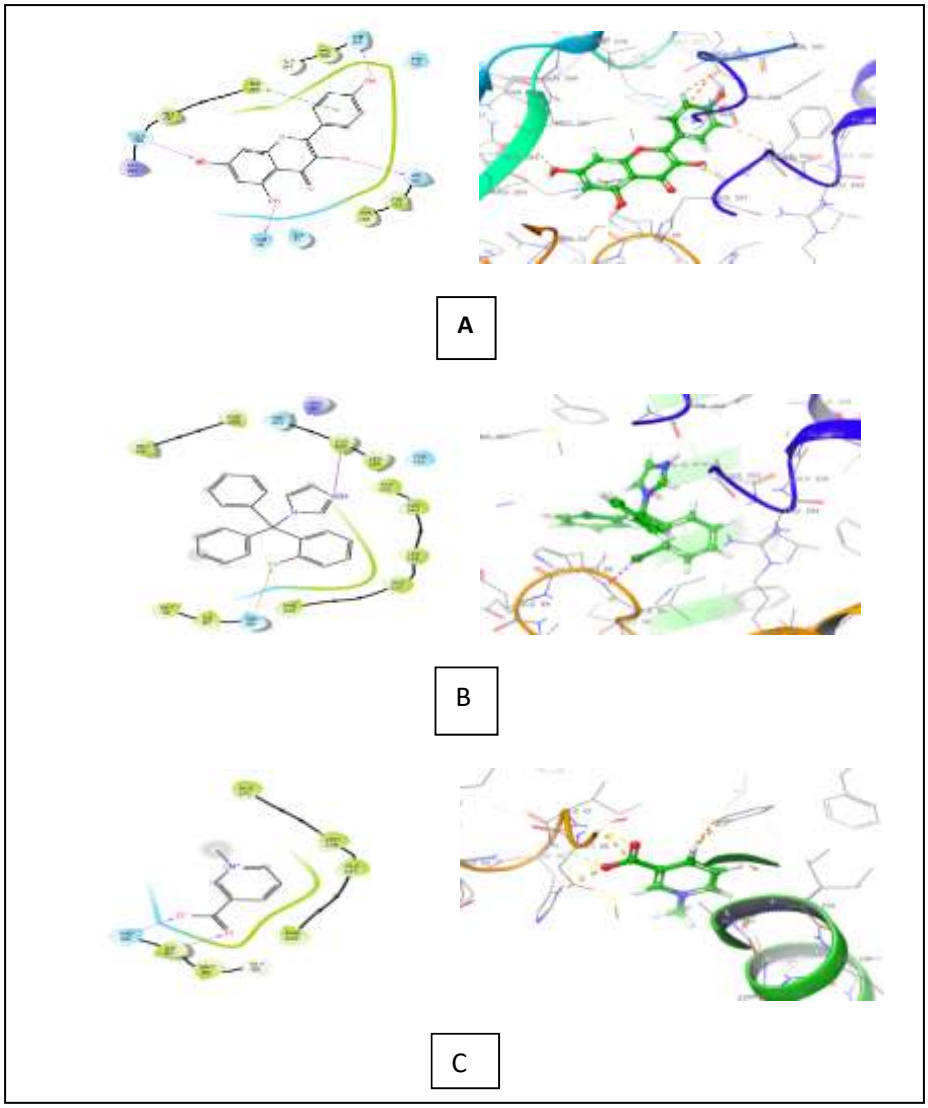

Figure No 3 - 2D and 3D images of Docked complex of TF seed Chemical Constituents for Anti-Fungal activity; A – Kaempferol, B – Clotrimazole, C - Trigonelline

ADME Prediction

The SwissADME web server was used to assess the pharmacokinetic characteristics of the chosen drugs. High gastrointestinal absorption, no anticipated blood–brain barrier (BBB) permeability, adherence to Lipinski's rule of five, and a bioavailability score of 0.55 were all desirable drug-like features of kaempferol.

Additionally, trigonelline showed no BBB permeability, high GI absorption, and an outstanding synthetic accessibility score (1.04), indicating ease of synthesis (Table no – 13). Due to its physicochemical characteristics, the conventional medication clotrimazole demonstrated one Lipinski rule violation while having significant GI absorption and BBB permeability.

Table No – 13 ADME Profile of Anti-Dandruff Compounds

Parameter	Quercetin	Kaempferol	Ascorbic acid
Molecular Weight (g/mol)	137.14	286.24	344.84
H-Bond Acceptors	2	6	1
H-Bond Donors	0	4	0
Log P	-3.11	1.70	-3.07
GI Absorption	High	High	High
BBB Permeability	No	No	yes
Bioavailability Score	0.55	0.55	0.55
Lipinski Violations	0	0	1
Synthetic Accessibility	1.04	3.14	2.25

Toxicity Prediction

The ProTox-II platform was used to predict the toxicity profiles of the chosen chemicals. The typical medication clotrimazole was allocated to toxicity class 4, suggesting somewhat higher toxicity, while trigonelline and kaempferol were categorized under toxicity class 5.

It was anticipated that all three substances would be less hepatotoxic and immunotoxic. Compared to the reference medication, kaempferol showed an inactive (Table no – 14) prediction for cytotoxicity and carcinogenicity, indicating a relatively safer toxicity profile.

Table No – 14 Toxicity Prediction of Anti-Dandruff Compounds

Parameter	Trigonelline	Kaempferol	Clotrimazole
ProTox-II Class	5	5	4
Hepatotoxicity	Less Inactive	Less Inactive	Less Inactive
Carcinogenicity	Less Inactive	Inactive	Less Inactive
Immunotoxicity	Inactive	Inactive	Inactive
Mutagenicity	Inactive	Less Inactive	Less Inactive

Because of its favorable docking score, multiple stabilizing interactions with the target protein, desirable pharmacokinetic properties, and relatively safe toxicity profile, kaempferol has a promising anti-dandruff potential overall, according to molecular docking, interaction analysis, ADME profiling, and toxicity prediction. The promise of Trigonella foenum-graecum L. as a natural source of bioactive chemicals for the creation of anti-dandruff treatments is supported by these findings. However, more in vivo and in vitro research is needed to confirm its safety and effectiveness.

CONCLUSION

Trigonella foenum-graecum L. seeds are a rich source of physiologically active phytoconstituents with intriguing therapeutic potential, as the current investigation showed. The hydroalcoholic extract (50:50, v/v) has a wider range of secondary metabolites than the ethanolic and aqueous extracts, demonstrating its higher extraction efficiency, according to comparative phytochemical screening. The presence of the two main bioactive markers, kaempferol and diosgenin, was further validated by chromatographic studies using TLC and HPLC, confirming the hydroalcoholic extract's phytochemical composition.

Quercetin showed the best antioxidant activity, diosgenin showed good anti-alopecia potential, and kaempferol showed positive interactions against the anti-dandruff target protein, according to computer-aided molecular docking. These phytoconstituents may successfully regulate the chosen therapeutic targets, according to the observed binding affinities, protein–ligand interactions, and molecular interaction patterns. Additionally, SwissADME and ProTox-II tests supported the drug-likeness of the chosen compounds by showing excellent safety profiles and acceptable pharmacokinetic characteristics.

Overall, the combination of chromatographic characterisation, molecular docking, and phytochemical screening provide scientific proof of Trigonella foenum-graecum L.'s therapeutic efficacy. The results demonstrate its potential as a natural source of bioactive chemicals for the creation of anti-dandruff, anti-alopecia, and antioxidant medications. To verify these computational predictions and determine their therapeutic efficacy and safety for upcoming pharmaceutical applications, more in vitro, in vivo, and clinical studies are necessary.

REFERENCE

1. Alu'datt M, Rababah T, Al-Ali S, Tranchant C, Gammoh S, Alrosan M, et al. Current perspectives on fenugreek bioactive compounds and their potential impact on human health: a review of recent insights into functional foods and other high value applications. J Food Sci. 2024. Alu'datt2024
2. Hafeez J, Naeem M, Ali T, Sultan B, Hussain F, Rashid HU, Nadeem M, Shirzad I. Comparative study of antioxidant, antidiabetic, cytotoxic potentials, and phytochemicals of fenugreek (Trigonella foenum-graecum) and ginger (Zingiber officinale). J Chem. 2023. Hafeez2023
3. Kadam V. Phytochemical profile of Trigonella foenum graecum Linn. 2018. Kadam2018

4. Chikezie AC, Stanley O, Ijeoma N, Aaron, Chinomso F, Edom CV, et al. Phytochemical composition and in vitro antioxidant potential of the ethanolic extract of Trigonella foenum-graecum (fenugreek) seeds. Asian J Res Biochem. 2025. Chikezie2025
5. Bakhtiar Z, Hassandokht M, Naghavi MR, Mirjalili MH. Nutritional value, phytochemical composition, and antioxidant potential of Iranian fenugreeks for food applications. Sci Rep. 2024. Bakhtiar2024
6. Ahmad R, Alqathama A, Al-Maimani R, Al-Said HM, Ashgar SS, Althubiti M, et al. Exploring the role of phytochemical classes in the biological activities of fenugreek (Trigonella feonum graecum): a comprehensive analysis based on statistical evaluation. Foods. 2025. Ahmad2025
7. Abdel Salam SG, Rashed M, Ibrahim N, Abdel Rahim EA, Aly T, Al-Farga A. Phytochemical screening and in-vitro biological properties of unprocessed and household processed fenugreek (Trigonella foenum-graecum Linn.) seeds and leaves. Scientific Reports. 2023 Apr 29. Salam2023
8. Cristea S, Perisoara A, Tihauan B, Ene MD, Constantin M, Florea A, Ivan E, Zala RC, Purcăreanu B, Mihaiescu D, Pirvu L. In vitro evaluation of the antifungal activity of Trigonella foenum-graecum seed extract and its potential application in plant protection. Plants. 2025 Oct 31. Cristea2025
9. Chakraborty A, Saha P. Comparative overview of phytoconstituents and pharmacological potential of Trigonella foenum-graecum leaves and seeds. International Journal of Ayurvedic Medicine. 2025 Dec 31. Chakraborty2025
10. Haouala R, Hawala S, El-Ayeb A, Khanfir R, Boughanmi N. Aqueous and organic extracts of Trigonella foenum-graecum L. inhibit the mycelia growth of fungi. Journal of Environmental Sciences. 2008 Jan 1. Haouala2008
11. Kamil SS, Razaq RA, Radhi SH, Hussein HJ. Fenugreek [Trigonella graecum] methanolic seeds extract for antioxidant (superoxide and singlet oxygen radical scavenging), antifungal effect and screening of its phytochemical constitutes using GC-MS. SAR Journal of Medical Biochemistry. 2025 Aug 30. Kamil2025
12. Sudan P, Goswami M, Singh J. Antifungal potential of fenugreek seeds (Trigonella foenum-graecum) crude extracts against Microsporum gypseum. International Journal of Research in Pharmaceutical Sciences. 2020 Jan 21. Sudan2020
13. Yaldiz G, Çamlıca M. Assessment of secondary metabolites with different uses of fenugreek. Legumes [Working Title]. 2021 Aug 10. Yaldiz2021
14. Kobarne HP, Zaware SS, Tagad S, Kobarne YR, Kasar JS, Students S, Wani M. A review on fenugreek seeds. International Journal of Advanced Research in Science, Communication and Technology. 2025 Feb 6. Kobarne2025
15. Rebey IB, Ben Abdennebi A, Chaabani E, Yeddes W, Hammami M, Tounsi M, Merah O. Fenugreek as a versatile cosmetic ingredient: phytochemical profile, skin-hair benefits and formulation opportunities. Cosmetics. 2026 Feb 16. Rebey2026
16. Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha CV, Priyadarsini KI. In vitro studies on antiradical and antioxidant activities of fenugreek (Trigonella foenum graecum) seeds. Food Chemistry. 2006 Oct 3. Kaviarasan2006
17. Bukhari SB, Bhanger MI, Memon S. Antioxidative activity of extracts from fenugreek seeds (Trigonella foenum-graecum). 2008 Dec 1. Bukhari2008
18. Khenifi ML, Serseg T, Migas P, Krauze-Baranowska M, Özdemir S, Bensouici C, et al. HPLC-DAD-MS characterization, antioxidant activity, α-amylase inhibition, molecular docking, and ADMET of flavonoids from fenugreek seeds. Molecules. 2023 Nov 27. Khenifi2023
19. Benayad Z, Gómez-Cordovés C, Es-Safi NE. Characterization of flavonoid glycosides from fenugreek (Trigonella foenum-graecum) crude seeds by HPLC-DAD-ESI/MS analysis. Int J Mol Sci. 2014 Nov 1. Benayad2014
20. Hazel A, Moumen O, Ould Ali O, Ouadjed H, Mechrour A. Exploring the antioxidant potential and HPLC profile of fenugreek (Trigonella foenum-graecum L.) seed extracts. North Afr J Food Nutr Res. 2025 May 26. Hazel2025
21. Keskes H, Belhadj S, Jlail L, El Feki AF, Sayadi S, Allouche N. LC-MS-MS and GC-MS analyses of biologically active extracts of Tunisian fenugreek (Trigonella foenum-graecum L.) seeds. J Food Meas Charact. 2018 Mar 1. Keskes201
22. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, Ugwuja EI, Aja PM. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep. 2023 Aug 17. Agu2023
23. Asiamah I, Obiri S, Tamekloe W, Armah FA, Borquaye LS. Applications of molecular docking in natural products-based drug discovery. Sci Afr. 2023 Feb 1. Asiamah2023
24. Babu V, Ahamed JI, Paul A, Ali S, Rather I, Sabir JS. Assessing spectral analysis of phytoconstituents and their in silico interactions with target proteins in plant seed extracts. Plants. 2023 Sep 22. Babu2023
25. Kaneria M, Rakholiya K, Bavaliya KR, Pandya MH, Sipai TN, Vadher SA, Patel M, Yadav VK, Solanki R, Patel S, Sahoo DK, Patel A. Untargeted metabolomics-based identification of bioactive compounds from Mangifera indica L. seed extracts in drug discovery through molecular docking and assessment of their anticancer potential. J Sci Food Agric. 2024 Feb 28. Kaneria2024
26. Tyagi S, Shukla A, Ram H, Panwar A, Kumar R, Tripathi R. In silico investigations of the multi-targeted antiviral potential of small molecule phytochemicals of Nelumbo nucifera Gaertn. seed extracts against SARS-CoV-2 for therapeutics of COVID-19. Food Front. 2023 Jan 13. Tyagi2023
27. Ashfaq F, Barkat M, Ahmad T, Hassan MZ, Ahmad R, Barkat H, Khan MI, Alhodieb FS, Asiri Y, Siddiqui S. Phytocompound screening, antioxidant activity and molecular docking studies of pomegranate seed: a preventive approach for SARS-CoV-2 pathogenesis. Sci Rep. 2023 Oct 10.