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In-Vitro Anticancer And *In-Silico* Evaluation Of Ethanolic Crude Extracts Of *Trigonella Foenum* (Fenugreek) Leaves On Human Breast Cancer Mcf-7 Cell Lines

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Abstract: Breast cancer is a major global health challenge, and natural products offer promising avenues for novel therapeutics. This study evaluated the *in-vitro* anticancer activity of ethanolic crude extracts of *Trigonella foenum-graecum* (fenugreek) leaves against MCF-7 human breast cancer cells, complemented by *in-silico* docking and ADMET analyses. GC-MS profiling identified key phytoconstituents including ricinoleic acid, elaidic acid, isolinoleic acid, stearic acid, azelaic acid, phytol, and squalene, most of which exhibited favorable drug-likeness and low predicted toxicity. MTT assays revealed dose-dependent inhibition of MCF-7 proliferation, with cell viability decreasing from 57.6% at 250 µg/ml to 16.8% at 500 µg/ml, corresponding to inhibition rates of 42.4% and 83.2%, respectively. In-silico docking suggested strong binding affinities of azelaic acid, elaidic acid, and ricinoleic acid to cancer-related targets, potentially mediating apoptosis and metabolic disruption. These findings indicate that fenugreek leaves possess significant anticancer potential, warranting further mechanistic and in-vivo studies for therapeutic development.

Index Terms - *Trigonella foenum-graecum*, fenugreek leaves, MCF-7 cells, GC-MS, molecular docking, ADMET, anticancer activity.

I. INTRODUCTION

Cancer is a group of diseases marked by uncontrolled cell growth and potential to invade or spread. It results from genetic and epigenetic changes disrupting cell-cycle control, apoptosis, and DNA repair. These changes drive tumor growth, immune evasion, and metastasis. Cancers are named by tissue of origin and vary greatly in behavior and treatment response, necessitating precision medicine [1]. **Breast cancer** arises mostly from ductal or lobular epithelial cells. It is heterogeneous, subtyped by histology and receptor status (ER, PR, HER2), influencing prognosis and therapy. Molecular profiling identifies intrinsic subtypes (e.g., luminal A/B, HER2+, basal-like) with distinct clinical outcomes. Advances in screening and targeted treatments have improved survival, though global disparities remain [2]. Globally, breast cancer is now the most diagnosed cancer. Incidence is highest in high-income nations due to aging, screening, and lifestyle factors, but mortality is rising faster in low- and middle-income countries due to late detection and limited care. Prevention, early diagnosis, and treatment access are key to reducing future burden. [3]

Fenugreek (*Trigonella foenum-graecum*) is a legume valued for seeds and leaves used in food and traditional medicine. It contains saponins, flavonoids, alkaloids (e.g., trigonelline), fiber, and oils, contributing to reported antidiabetic, lipid-lowering, antioxidant, and anti-inflammatory effects [4]. Traditionally used for digestion, lactation, and metabolic support, fenugreek shows preclinical anticancer potential, particularly from

saponins and flavonoids. These may induce apoptosis, cell-cycle arrest, and oxidative stress in cancer models. However, clinical validation is still needed [5].

Gas chromatography–mass spectrometry (GC-MS) is ideal for analyzing volatile compounds in plant extracts. GC separates compounds by volatility [6], while MS identifies them by mass spectra. It's widely used in phytochemical profiling, especially for essential oils and small bioactive substance [7]. ***In-vitro*** studies use isolated cells to test compounds' effects on viability, apoptosis, and related pathways. While useful for early screening, results require confirmation in animal and clinical studies due to biological complexity.[8], [9]. ***In-silico*** methods like molecular docking and virtual screening simulate how compounds interact with targets. These tools predict activity and guide drug discovery, especially when integrated with experimental validation [10], [11], [12].

Methods

In Vitro Anticancer Study – MCF-7 Cell Line

Cell Culture Maintenance MCF-7 cells were cultured in appropriate growth medium under sterile conditions using a laminar flow hood. Cells were subcultured during log-phase to maintain viability. Thawing Frozen Cells Frozen cryovials were thawed in a 37°C water bath, transferred aseptically into growth medium, centrifuged, and resuspended before being seeded into culture vessels. Subculturing Adherent Cells Adherent cells were detached using Trypsin/TrypLE™, centrifuged, resuspended in fresh medium, counted, and seeded at recommended densities.

Subculturing Suspension Cells: - Suspension cells were passaged during log-phase growth by sampling, counting, diluting to seeding density, and incubating in shaker flasks without baffles.

Cryopreservation Protocol Cells were harvested, counted, and resuspended in freezing medium. Aliquots were frozen at a controlled rate, stored at –80°C overnight, then transferred to liquid nitrogen [13].

MTT Assay for Cell Proliferation

MTT dye is reduced by cellular enzymes to formazan, indicating metabolic activity. MCF-7 cells were seeded in 96-well plates (5,000–10,000 cells/well), treated, incubated with MTT, solubilized in DMSO, and absorbance measured at 570 nm to assess proliferation or cytotoxicity [14].

In Silico Docking Studies

Software & Hardware

- **Hardware:** Intel i9 CPU, 16 GB RAM, NVIDIA RTX 4050 GPU
- **Software: PyRx and Discovery Studio**

Ligand Preparation 3D ligand structures were downloaded from PubChem (SDF format) and imported into PyRx for docking.

Protein Preparation Protein structures were obtained from the Protein Data Bank (PDB format). Non-amino acid residues (e.g., HETATM, CONECT) were removed using '**Notepad ++**', and cleaned files were used for AutoDock. Docking Docking simulations were performed to identify ligand-protein interactions, evaluating binding affinity and compatibility [15]

ADMET Predictions

SwissADME is a free tool predicting ADME, drug-likeness, and pharmacokinetics, aiding compound selection and minimizing development failures [16]. ADMET Lab 3.0 Assesses pharmacokinetic and toxicity profiles to prioritize compounds with favorable ADMET and binding properties, streamlining drug discovery [18]. Lipinski's Rule of Five Used to evaluate oral bioavailability based on molecular weight, lipophilicity, and hydrogen bonding potential. Solubility is prioritized during optimization [17].

IV. RESULTS AND DISCUSSION

In this study, one plant extract and the solvent fractions were evaluated for their possible Anticancer activities alongside Doxorubicin as a positive control. The Phytochemical analysis of Fenugreek leaves by using GC-MS indicated presence of different constituents like Squalene, Phytol, Azelaic acid, Stearic acid, 2-ethyl-Heptanoic acid, 9,12 Octadecadienoic acid (Z,Z), 9E,11E)-Octadecadienoic acid, Quinoline, (Z)-18-Octadec-9-enolide, 2,3-dihydroxypropyl ester, Caryophyllene, Linoleic acid ethyl ester (Ethyl Linoleate), Ricinoleic acid, 13-Hexyloxacyclotridec-10-en-2-one; 1,8,11-Heptadecatriene, Elaidic Acid (9-Octadecenoic acid), 9-Tetradecenal (Table 1).

Table 1 – Constituents of Fenugreek Leaf Extract

Sl no.	Name	Molecular Formula	CAS	Retention Time	Similarity	Base m/z	Properties
1	Squalene	$C_{30}H_{50}$	111-02-4	17.50	95	410.00	Hydration, Anti-inflammatory, Antioxidant
2	Phytol	$C_{20}H_{40}O$	150-86-7	30.14	93	123.00	Anti Inflammatory, Antioxidant
3	Azelaic acid	$C_9H_{16}O_4$	123-99-9	13.76	98	187.00	Insulin Sensitivity, Antioxidant, Antidiabetic
4	Stearic acid	$C_{18}H_{36}O_2$	57-11-4	16.60	97	129.00	Anti diabetic, Cardiovascular health, Metabolic function
5	2-ethyl-Heptanoic acid	$C_8H_{16}O_2$	106-76-3	13.74	96	129.00	Metabolic function
6	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	60-33-3	22.97	91	67.05	Glucose Metabolism, Cholesterol Regulation
7	9E,11E)-Octadecadienoic acid	$C_{18}H_{32}O_2$	544-71-8	24.32	93	67.05	Metabolic function
8	Quinoline	C_9H_7N	92-22-5	14.50	97	129.00	Antimicrobial, Antioxidant
9	(Z)-18-Octadec-9-enolide	$C_{18}H_{32}O_2$	80060-76-0	26.02	88	55.05	Antioxidant
10	2,3-dihydroxypropyl ester	$C_5H_{10}O_4$	2277-28-3	28.68	88	67.05	Lipid Metabolism
11	Caryophyllene	$C_{15}H_{24}$	87-44-5	19.63	96	204.00	Anti-inflammatory
12	Linoleic acid ethyl ester (Ethyl Linoleate)	$C_{20}H_{36}O_2$	544-35-4	32.28	89	67.05	Glucose Metabolism, Cardiovascular Health
13	Ricinoleic acid	$C_{18}H_{34}O_3$	141-22-0	34.09	82	98.05	Anti-inflammatory, Metabolic stability
14	1,8,11-Heptadecatriene	$C_{17}H_{30}$	56134-03-3	14.5	87	234.00	Cellular metabolism
15	Elaidic Acid (9-Octadecenoic acid)	$C_{18}H_{34}O_2$	112-79-8	18.5	98	282.00	Antidiabetic, Lipid metabolism
16	9-Tetradecenal	$C_{14}H_{26}O$	1937-62-8	16.5	79	210.00	Biochemical process

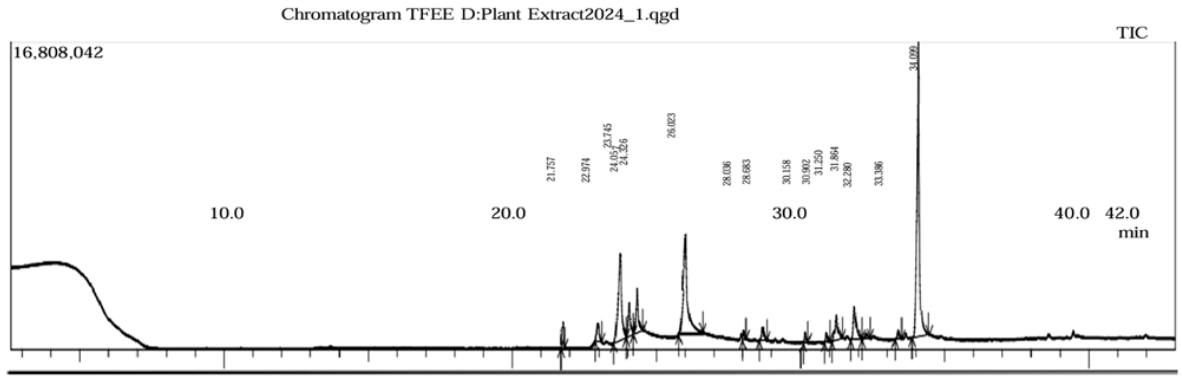


Fig: Chromatogram plant extract

Table – 2: ADME Selected Compound - SWISS ADME

Sl no.	Name	Molecular Formula	SMILES	Golden	$F_{20\%}$	Volume Distribution	Metabolism	Excretion Clearance	Acute Toxicity
1	Ricinoleic acid	$C_{18}H_{34}O_3$	CCCCCC[C@H](C/C=C\CCCCCCCC(=O)O)O	Accepted	Low	0.5.8 Good	4.13	3.872 Low	0 Alert
2	Elaidic Acid.(9-Octadecenoic acid)	$C_{18}H_{34}O_2$	CCCCCCCC/C=C/CCCCCCCC(=O)O	Accepted	Low	0.858 Good	3.43	2.414 Low	0 Alert
3	Isolinoleic Acid (9E,11E-Octadecadienoic acid)	$C_{18}H_{32}O_2$	CCCCCC/C=C/C=C/CCCCCCCC(=O)O	Accepted	Moderate	0.608 Good	3.34	1.652 Low	0 Alert
4	Stearic Acid	$C_{18}H_{36}O_2$	CCCCCCCCCCCCCCCCCC(=O)O	Accepted	Moderate	0.752 Good	3.81	2.425 Low	0 Alert
5	Azelaic acid	$C_9H_{16}O_4$	C(CCCC(=O)O)CCCC(=O)O	Rejected	High	0.26 Good	2.29	1.727 Low	0 Alert

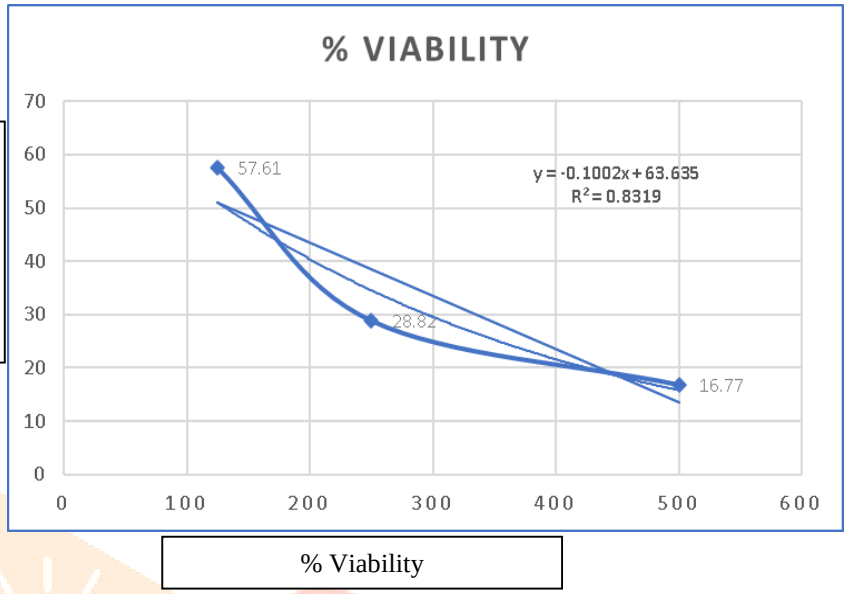
Table 3: - UV absorption of Cell line solution at 570 nm

Parameter	Blank	Negative Control (Untreated)	Standard Control	Concentration, 125 µg/ml	Concentration, 250 µg/ml	Concentration, 500 µg/ml
OD 1	0	1.69	0.163	0.974	0.487	0.283
OD 2	0	1.689	0.163	0.974	0.485	0.284
OD 3	0	1.691	0.164	0.973	0.489	0.283
Average	0	1.69	0.163333333	0.973666667	0.487	0.283333333
Average - Blank	0	1.69	0.163333333	0.973666667	0.487	0.283333333
Std Deviation	0	0.001	0.00057735	0.00057735	0.002	0.00057735
Std error	0	0.00057735	0.000333333	0.000333333	0.001154701	0.000333333
% Cell Viability		100	9.66469428	57.61341223	28.81656805	16.765286
% Inhibition		0	90.33530572	42.38658777	71.18343195	83.234714

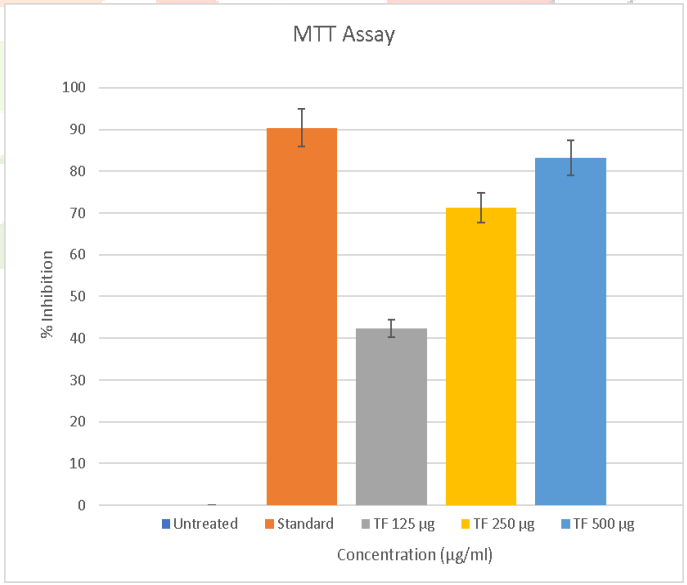
Table – 4: ADME Selected Compound - ADMET LAB 2

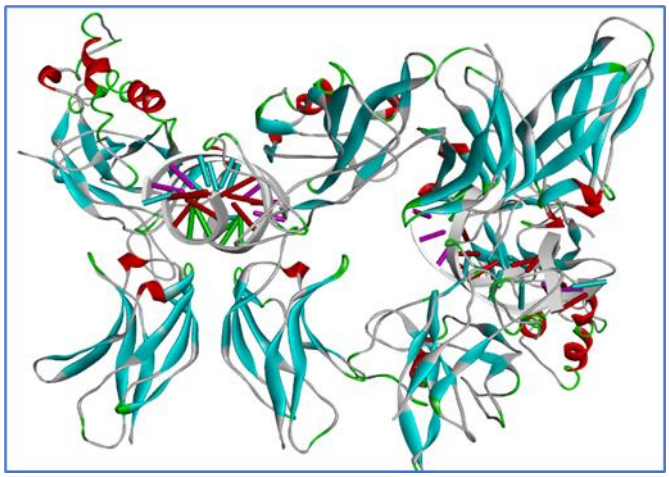
Sl no.	Name	Molecular Formula	SMILES	Golden	$F_{20\%}$	Volume Distribution	Metabolism	Excretion Clearance	Acute Toxicity
1	Ricinoleic acid	$C_{18}H_{34}O_3$	CCCCCC[C@H](C/C=C\CCCCCCCC(=O)O)O	Accepted	Low	0.5.8 Good	4.13	3.872 Low	0 Alert
2	Elaidic Acid.(9-Octadecenoic acid)	$C_{18}H_{34}O_2$	CCCCCCCC/C=C/CCCCCCCC(=O)O	Accepted	Low	0.858 Good	3.43	2.414 Low	0 Alert
3	Isolinoleic Acid (9E,11E-Octadecadienoic acid)	$C_{18}H_{32}O_2$	CCCCCC/C=C/C=C/CCCCCCCC(=O)O	Accepted	Moderate	0.608 Good	3.34	1.652 Low	0 Alert
4	Stearic Acid	$C_{18}H_{36}O_2$	CCCCCCCCCCCCCCCCCC(=O)O	Accepted	Moderate	0.752 Good	3.81	2.425 Low	0 Alert
5	Azelaic acid	$C_9H_{16}O_4$	C(CCCC(=O)O)CCCC(=O)O	Rejected	High	0.26 Good	2.29	1.727 Low	0 Alert

Concentration of Drug (µg)	% Viability
125	57.61
250	28.82
500	16.77

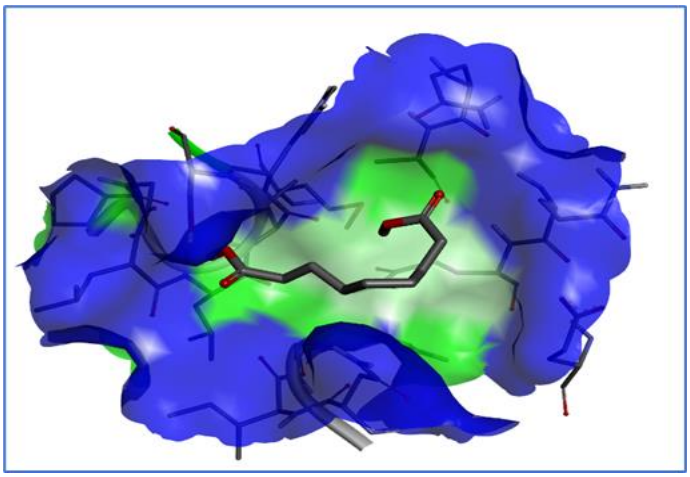


MTT ASSAY SUMMARY		
Culture Condition	% Inhibition	y = m x + c
Untreated	0	x = y - c / m
Standard	90.34	= 50 - 63.635 / - 0.1002
TF 125 µg	42.39	= - 13.635 / -0.1002
TF 250 µg	71.18	x = 136.077
TF 500 µg	83.23	
IC 50 Value (µg)	136.08	

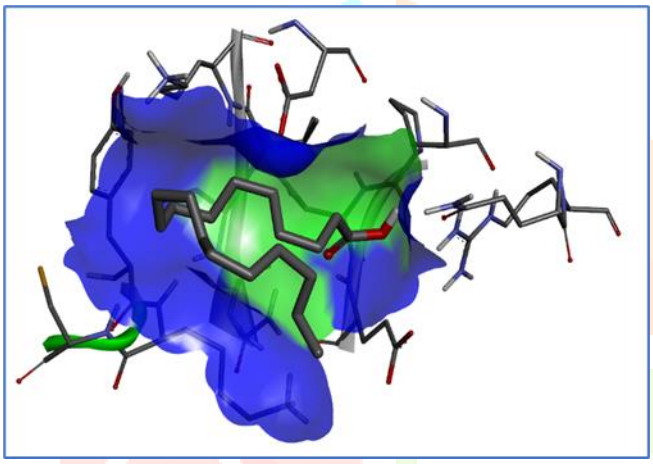




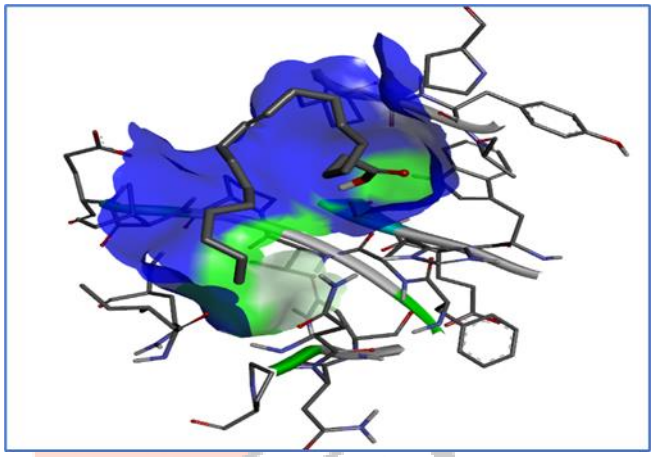
Docking Protein 1le5



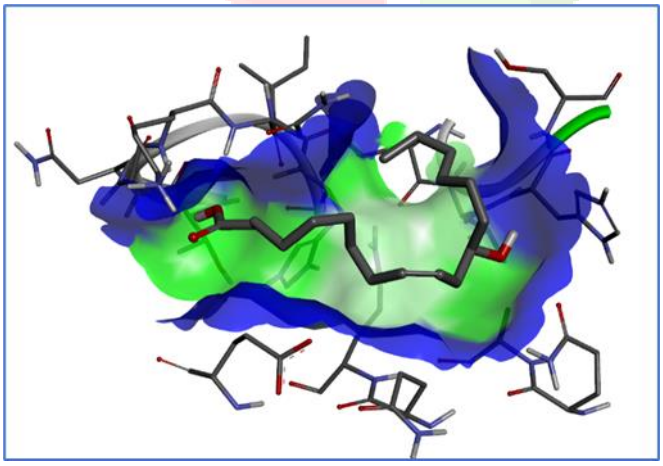
1. Ricinoleic Acid



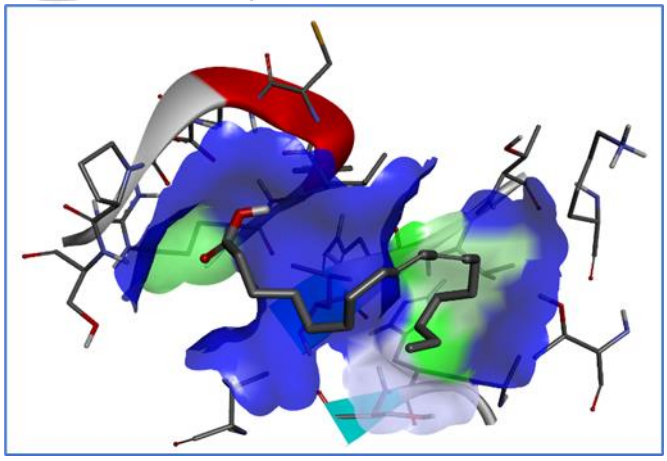
2. Elaidic Acid



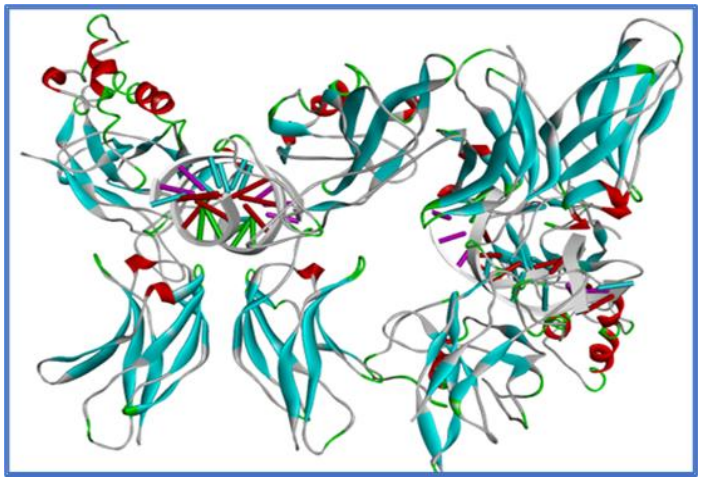
3. Isolinoleic Acid



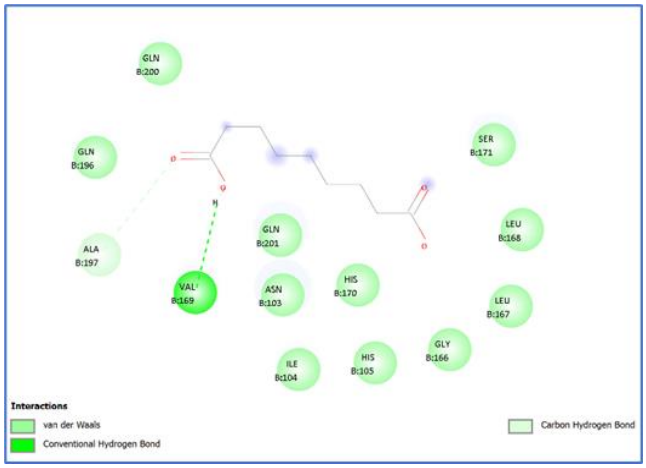
4. Stearic Acid



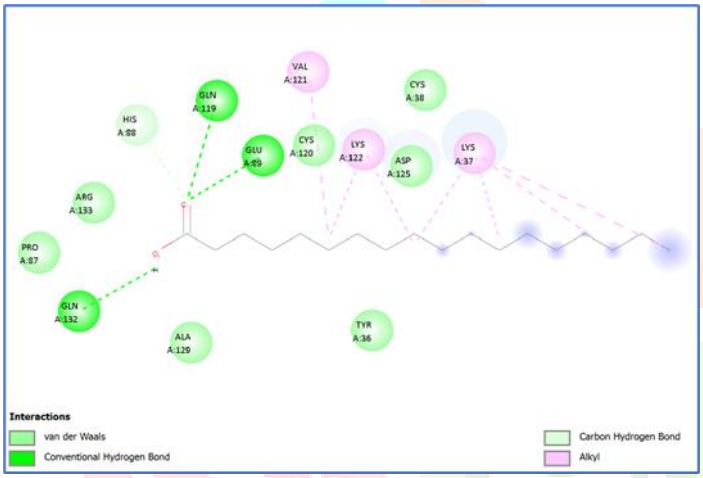
5. Azelaic Acid



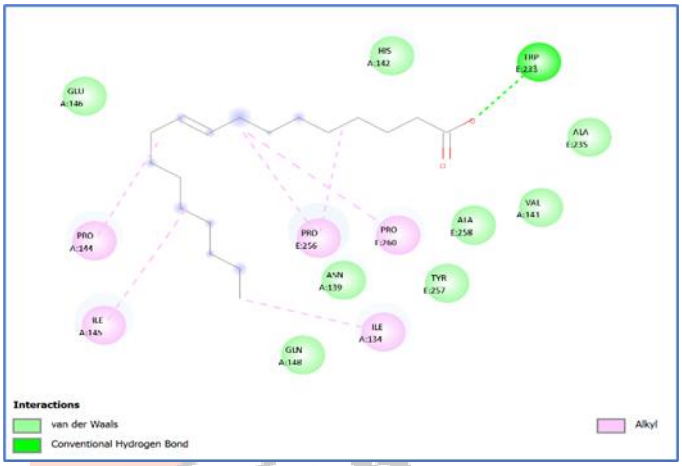
Docking Protein 1le5



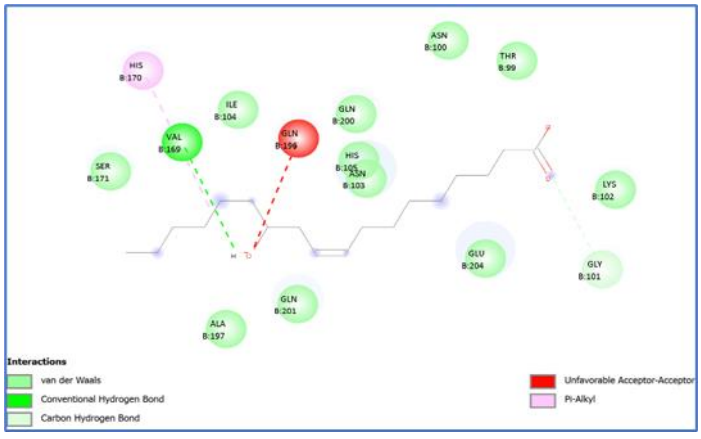
1. Ricinoleic Acid



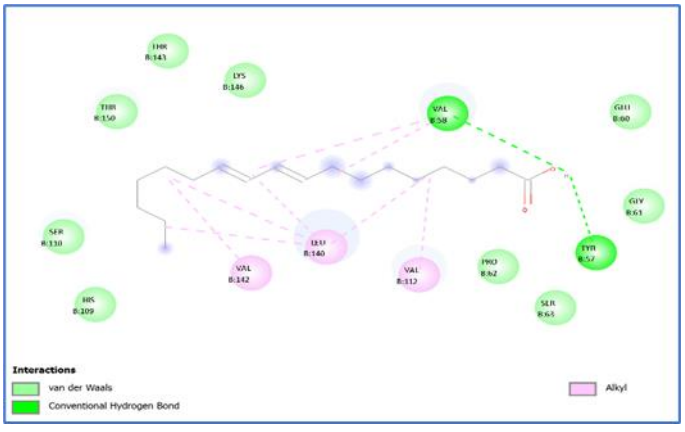
2. Elaidic Acid



3. Isolinoleic Acid



4. Stearic Acid



5. Azelaic Acid

Discussion

The present in vitro study demonstrates that a fenugreek (Trigonella foenum-graecum) leaf extract—whose GC/MS profile included ricinoleic acid, elaidic (9-octadecenoic) acid, isolinoleic (9E,11E-octadecadienoic) acid, stearic acid and azelaic acid—exerts a reproducible, dose-dependent reduction in MCF-7 cell viability as assessed by the MTT assay (absorbance read at 570 nm). These data are consistent with earlier reports that fenugreek extracts and isolated fatty-acid components produce cytotoxicity in breast and other cancer cell lines, indicating that the plant matrix is a plausible source of antiproliferative agents. [19]

Fatty acids and related metabolites can impair cancer cell viability through several convergent mechanisms. Unsaturated long-chain fatty acids (including elaidic and linoelaidic isomers) have been shown to reduce MCF-7 viability in a concentration-dependent manner and to modulate inflammatory cytokines and apoptotic signalling—effects that parallel the concentration-dependent MTT reductions observed here. [20]

Methanolic extracts of Trigonella foenum-graecum (fenugreek) seeds and sprouts contain multiple bioactive flavonoids (apigenin, vicenin-2, vitexin, luteolin, orientin) and isoflavones (daidzein, formononetin), as identified by high-resolution LC-MS. In MCF-7 breast cancer cells, these extracts produced dose- and time-dependent reductions in cell viability (MTT assay), increased mitochondrial DNA damage, and suppressed proliferation and metastatic potential. Overall, the results highlight fenugreek—particularly the sprouts—as a rich source of compounds with promising anticancer activity. [21]

The study found that fenugreek seed extract exhibited strong antibacterial activity, particularly against Staphylococcus aureus and Pseudomonas aeruginosa. In MCF-7 breast cancer cells, the extract inhibited proliferation at 400 µg/ml after 72 hours without significant apoptosis or necrosis, while showing no anticancer effect on liver cancer or normal Vero cell lines. These results suggest fenugreek seed extract as a potential antibacterial and selective anticancer agent. [22]

Azelaic acid and its derivatives have documented antiproliferative activity in multiple tumour cell systems and were previously reported to reduce proliferation when formulated for delivery; their presence in the fenugreek extract provides an additional plausible contributor to the anti-MCF-7 effect. [23]

Stearic acid and other saturated acids may exert subtler modulatory effects on membrane composition and signalling that, in combination with unsaturated fatty acids, produce additive or synergistic cytotoxicity—an interaction that cannot be resolved by MTT alone. [24]

While the MTT assay (570 nm readout) reliably reports mitochondrial dehydrogenase activity and thus relative cell viability, it does not distinguish apoptosis from necrosis nor reveal molecular mechanism. Therefore, the promising MTT results reported here should be followed by mechanistic assays: Annexin-V/PI flow cytometry, caspase activity and PARP cleavage for apoptosis, ROS and mitochondrial membrane potential measures, and cell-cycle analysis. Time-course and fractionation (isolating individual fatty acid fractions) will clarify which constituents are most active and whether effects are additive or synergistic. Finally, orthogonal in vivo testing and pharmacokinetic/toxicity profiling are required before translating these observations toward therapeutic applications. []

Conclusion

In conclusion, the *in-vitro* and *in-silico* docking studies strongly indicate the positive anticancer potential of fenugreek leaves on MCF-7 cell lines. Through a combination of biochemical assays and computational modelling, these studies highlight the efficacy of fenugreek's phytoconstituents, particularly Azelaic acid, in regulating glucose metabolism and mitigating postprandial breast cancer. The inhibition of MCF-7 breast cancer cell proliferation highlights the potential of fenugreek leaves in suppressing tumor growth and combating breast cancer effectively. These findings reinforce the therapeutic value of fenugreek leaves as a natural anticancer agent and provide a strong basis for further research into their clinical applicability in cancer management.

Conflict of Interest: -

Authors declare that there is no conflict of interest.

Acknowledgement: -

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