



Targeting Bacterial DNA Gyrase-B with Fenugreek-Derived Phytochemicals: An *In Silico* Strategy for Antibacterial Lead Discovery

Daniel Bauta Musa^{1,*}, Yakubu Magaji¹, Ugochukwu Okechukwu Ozojiofor¹,
Patrick Anthony Vantsawa²

¹*Department of Biotechnology, Faculty of Science, Nigerian Defence Academy,
Kaduna 800283 Nigeria*

²*Department of Biological Science, Faculty of Science, Nigerian Defence Academy,
Kaduna 800283 Nigeria*

Received 13 June 2025; Received in revised form 20 January 2026

Accepted 3 February 2026; Available online 30 June 2026

ABSTRACT

Antibiotic resistance poses a major public health challenge globally. Fenugreek (*Trigonella foenum-graecum*) contains phytochemicals that have been studied for possible antibacterial activity. This study examines the interaction of fenugreek seed phytochemicals with DNA gyrase-B using computational methods. GC-MS was employed to identify bioactive compounds. Lipinski's Rule of Five and Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) analyses were used to evaluate drug-likeness and pharmacokinetic properties. Selected ligands were docked with bacterial DNA gyrase-B using AutoDock Vina and analyzed using BIOVIA Discovery Studio. GC-MS revealed 29 compounds from the aqueous and methanolic extracts, 12 of which passed the drug-likeness screening. ADMET profiling indicated favorable ADMET profiles for several candidates. Molecular docking revealed that cyclodocosane, ethyl exhibited the highest binding affinity (-9.6 kcal/mol) against DNA gyrase-B. Although, only one specific hydrophobic interaction was observed. This hydrophobic contact involves a nonpolar amino acid residue (Leu294) in the binding pocket of the protein, key to maintaining the complex's integrity. This compound outperformed the standard drug ciprofloxacin (-9.2 kcal/mol). The result indicate that the ligand may serve as a starting point for further optimization. Fenugreek seeds contain phytochemicals with promising antibacterial potential, especially against DNA gyrase-B.

Cyclodocosane, ethyl may serve as a lead compound for novel antibacterial drug development, pending further validation and optimization.

Keywords: ADMET; Cyclodocosane; DNA Gyrase-B; Ethyl; Fenugreek; *In silico* discovery; Phytochemicals

1. Introduction

The global rise of antimicrobial resistance is a critical public health issue, recognized by the World Health Organization as a top ten threat to human health [1]. The increasing prevalence of multidrug-resistant bacterial strains, such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, has significantly compromised the effectiveness of traditional antibiotics [2]. The current crisis calls for the identification of new antibacterials, especially those originating from natural sources that have long been the backbone of drug discovery.

Plants have served as store of bioactive compounds having great pharmacological effects. Phytochemicals, which are secondary metabolites present in plants, have been documented to possess antimicrobial, antioxidant, anticancer, and anti-inflammatory properties [3]. These compounds, which include alkaloids, flavonoids, saponins, and terpenoids, exhibit diverse structural scaffolds and mechanisms of action that make them attractive leads in drug discovery [4].

Fenugreek (*Trigonella foenum-graecum*) is a widely cultivated legume [5] used in traditional medicine and cooking globally. Its seeds, which are rich in protein, fiber, and bioactive phytochemicals, have been investigated for a range of pharmacological properties, including antibacterial, antidiabetic, and hepatoprotective effects [6, 7]. Several studies have reported that fenugreek seed extracts exhibit activity against various Gram-positive and Gram-negative bacterial strains [8, 9].

In addition to the well-characterized flavonoids and alkaloids, phytochemical screenings using Gas Chromatography-Mass

Spectrometry (GC-MS) have identified cyclodocosane, ethyl (also referred to as ethylcyclodocosane) as a significant bioactive constituent in various medicinal plant extracts, including those of the *Trigonella* genus [10]. As a long-chain macrocyclic hydrocarbon, cyclodocosane, ethyl has garnered attention for its potential role in modulating bacterial defense mechanisms. Computational studies, including molecular docking, have suggested that this compound exhibits a high binding affinity for key bacterial enzymes and inflammatory mediators, such as NADPH oxidase (NOX) and nitric oxide synthase (NOS), which are often co-opted during bacterial pathogenesis [11, 12]. Furthermore, the presence of such macrocyclic alkanes in fenugreek seed extracts is thought to contribute to the overall lipophilic profile of the plant's secondary metabolites, potentially enhancing the permeability of the bacterial cell membrane and facilitating the inhibitory action of other co-occurring antimicrobial agents like DNA Gyrase-B inhibitors [13].

DNA gyrase, a type II topoisomerase enzyme in charge of adding negative supercoils into DNA during replication and transcription, presents an advantageous target for antibacterial therapy. Involved in ATP hydrolysis, the B subunit of DNA gyrase (GyrB) supplies the energy needed for strand passage and supercoiling [14]. GyrB is a validated target for antibacterial drugs including fluoroquinolones and aminocoumarins since inhibition of this enzyme interferes with fundamental bacterial processes such as DNA supercoiling and topology maintenance. GyrB, as a subunit of DNA gyrase, is capable of introducing negative supercoils into DNA at the expense of ATP hydrolysis [15, 16]. The enzyme is also indispensable for both the initiation and

elongation stages of DNA replication. Inhibition of GyrB leads to the stalling of replication forks and prevents the initiation of new rounds of replication, eventually causing cell death [17]. In transcription, DNA Gyrase relieves the positive supercoils ahead of the transcription bubble; accumulation of positive supercoils stalls RNA polymerase, leading to a decrease in mRNA synthesis and disrupting gene expression [18].

Amaro et al. [19] explained that molecular docking provides crucial details on the interactions between drugs and receptors. This allows scientists to predict how a drug will bind to and impact the target protein. Being able to picture and study these interactions helps a lot when learning how drugs act and finding suitable candidates. This *in silico* technique reduces the initial costs of hit detection and raises the possibility of finding the intended potential therapies by enabling a virtual examination of millions of molecules in a manageable length of time [20].

The antibacterial potential of fenugreek seeds (*Trigonella foenum-graecum*) has been increasingly recognized in recent pharmacological research, with its efficacy attributed to a diverse profile of secondary metabolites, including flavonoids, alkaloids, and saponins [13]. *In silico* and *in vitro* studies have elucidated that the primary mechanism underlying this antibacterial activity involves the targeted inhibition of the GyrB subunit. Molecular docking analyses have demonstrated that fenugreek-derived compounds, particularly its flavonoid constituents and alkaloid derivatives, exhibit high binding affinity for the ATP-binding site of the GyrB subunit, thereby disrupting the energy-dependent catalytic cycle of the enzyme [21-23]. This inhibition effectively halts proliferation in bacterial strains such as *S. aureus* and *E. coli*, positioning fenugreek as a promising source for the development of novel GyrB-targeted antimicrobial agents [24].

This study aims to assess fenugreek seed-derived phytochemicals as potential inhibitors of bacterial DNA gyrase-B using *in silico* molecular docking studies.

2. Materials and Methods

2.1 Plant material collection and authentication

Dried fenugreek seeds (*Trigonella foenum-graecum*) were purchased from Kawo Market in Kaduna Metropolis, Nigeria. Botanical identification and authentication were carried out by a taxonomist at the Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria, and a voucher specimen was deposited with the accession number NDA/BIOH/2023/12.

2.2 Preparation of plant extracts

Dried fenugreek seeds were washed, shade-dried, and pulverized into a fine powder using a laboratory blender. Then, two separate extractions were performed, each using 100 g of powdered seed: one with 700 mL of distilled water and one with 700 mL of methanol. Each mixture was macerated at room temperature for 48 hours with occasional stirring. The mixtures were filtered with Whatman No. 1 paper, then concentrated by rotary evaporation at 50°C to obtain crude aqueous and methanolic extracts, which were stored at 4°C until analysis.

2.3 Qualitative phytochemical screening

Preliminary phytochemical screening was performed on the extracts to identify key secondary metabolites including alkaloids, flavonoids, phenols, tannins, saponins, glycosides, and terpenoids. Standard qualitative methods were employed as described by Gul et al. [25] with minor modifications. Colorimetric and precipitation-based tests were used to detect the presence of each phytochemical group.

2.3.1 Test for flavonoids

The Shinoda Test was used to test for flavonoids. After mixing pieces of magnesium ribbon and concentrated HCl with aqueous crude extract for a few minutes, the appearance of a pink tint indicated the presence of flavonoid.

2.3.2 Test for Phenols

After dissolving 50 mg of the extract in 5 ml of distilled water and adding a few drops of neutral 5% ferric chloride solution, the development of a dark green color indicated the presence phenolic compounds.

2.3.3 Test for Alkaloids

Acidified alcohol (10 ml) was added to 0.1 g of extract before boiling and filtering. The filtrate was gently mixed with 0.4 ml of dilute ammonia and 1 ml of chloroform. To extract the chloroform layer, 2 ml of acetic acid was used. Mayer's reagent was then added. A cream-colored precipitate indicated the presence of alkaloids.

2.3.4 Test for Tannins

The seed extract was treated with 15% ferric chloride solution. The resultant color was noted. A blue color indicated the presence of hydrolysable tannin.

2.3.5 Test for Saponins

In a test tube, 5.0 ml of distilled water was combined with aqueous crude seed extract and vigorously shaken. The froth was forcefully combined with a few drops of olive oil; the appearance of foam indicated the presence of saponins.

2.3.6 Test for Terpenoids

To 5.0 ml of aqueous plant extract, 2.0 ml of chloroform was added and evaporated in a water bath before being heated with 3.0 ml of concentrated H₂SO₄. The change in coloration to a grey color indicated the presence of terpenoids.

2.3.7 Test for Glycosides

To detect glycosides, Liebermann's Test was used. To the aqueous seed extract, 2.0 ml of acetic acid and 2 ml of chloroform was added. After allowing the mixture to cool, concentrated H₂SO₄ was added. The appearance of green coloration indicated the presence of aglycone, the steroidal part of glycosides.

2.4 Gas Chromatography-mass spectrometry analysis

GC-MS was used to identify the bioactive compounds present in fenugreek extract. This was performed as described by Ralte et al. [26] with minor adjustments using the Aligent 7890A gas chromatography equipped with 5977C mass selective detector (Aligent Technologies, USA). In a nutshell, 100 µl of the seed extract was dissolved in 1 ml of the extraction solvents (i.e., water and methanol). The solution was aggressively agitated for 10 seconds with a vortex stirrer before being filtered through a 0.2-micron membrane filter and then the clear extract was analyzed. The heating program used was as follows: initial temperature of 40°C, ramp 15°C/min to 115°C, hold 2 min; ramp 5°C/min to 140°C, hold 5 min; ramp 2°C/min to 210°C, hold 8 min; final temperature of 210°C maintained an additional 3 min. At the end of this period, the oven temperature was increased up to 250°C, at the rate of 5°C/min, and maintained for 9 min. The carrier gas used was helium (99.9%), with a constant flow rate of 1 mL/min. The ionization voltage used was 70 eV. The sample was injected in a 5:1 split mode. The mass spectral scan range was 50-800 (m/z). The ion source temperature was kept at 230°C, and the interface temperature at 240°C. The MS start time was 5 min, and the end time was 75 min, with a 5 min solvent cut time. The GC-MS spectra of volatile chemicals was evaluated using Mass Hunter and matched with the

NIST 14 (National Institute of Standards and Technology) online library Version.

2.5 Drug-likeness screening

The identified phytochemical compounds were subjected to *in silico* drug-likeness evaluation using Lipinski's Rule of Five via the Supercomputing Facility for Bioinformatics and Computational Biology server (<https://scfbio-iitd.res.in/>). Parameters such as molecular weight, logP, hydrogen bond donors and acceptors, and molar refractivity were assessed to determine suitability for oral bioavailability. Lipinski's Rule of Five describes a set of physicochemical criteria used to assess the likelihood of good oral bioavailability in drug candidates, suggesting that compounds are more likely to show poor absorption when they exceed 5 hydrogen bond donors, 10 hydrogen bond acceptors, a molecular weight of 500 Da, or a logP value of 5 [27].

2.6 ADMET prediction

Pharmacokinetic profiling and toxicity prediction of the drug-like compounds were conducted using ADMETlab 3.0 (<https://admetlab3.scbdd.com/>) and SwissADME (<http://www.swissadme.ch/>). Properties such as absorption (Caco-2 permeability, HIA), distribution (blood-brain barrier permeability, volume of distribution), metabolism (CYP450 interactions), excretion (plasma clearance, half-life), and toxicity (hERG inhibition, AMES test, hepatotoxicity) were analyzed to identify compounds with favorable drug profiles.

2.7 Molecular docking analysis

The selected bioactive compounds were docked against bacterial DNA gyrase-B (PDB ID: 4DUH) (Fig. 1) using AutoDock Vina. Protein and ligand preparation were carried out using AutoDock Vina, and docking visualization was done with BIOVIA Discovery Studio Visualizer 2021. Grid box parameters were defined based on the active site of GyrB (Table 1). Molecular

docking was performed by defining a grid box centered at $x = 20.88807 \text{ \AA}$, $y = 37.54363 \text{ \AA}$, and $z = 59.359 \text{ \AA}$, which encompasses the active site of the target protein. The grid box dimensions were set to $50 \text{ \AA} \times 50 \text{ \AA} \times 50 \text{ \AA}$ along the x-, y-, and z-axes, respectively, to allow sufficient space for ligand flexibility during docking. An exhaustiveness value of 30 was applied to ensure a thorough conformational search and improve the reliability of the predicted binding poses. Binding affinities were recorded in kcal/mol, and molecular interactions were analyzed to evaluate the binding mechanisms.

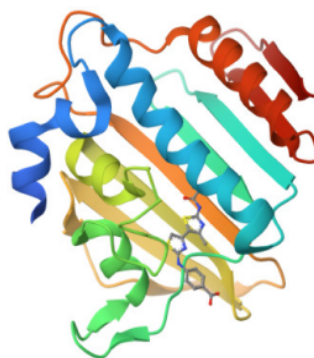


Fig. 1. The Target Protein Used in Docking Analysis (GyrB).

Table 1. Docking Parameters Used in Molecular Docking.

Center-x	20.88807 Å
Center-y	37.54363 Å
Center-z	59.359 Å
Size-x	50 Å
Size-y	50 Å
Size-z	50 Å
Exhaustiveness	30

3. Results and Discussion

The crude seed extracts (aqueous and methanol) were subjected to preliminary qualitative phytochemical screening to determine the presence of selected major groups of phytochemicals (flavonoids, phenols, alkaloids, tannins, saponins, terpenoids, and glycosides) with the results recorded in Table 2. Analysis indicated that the aqueous extract of fenugreek seed contains flavonoids, phenols, alkaloids,

tannins, saponins, terpenoids, and glycosides. However, the methanolic seed extract showed the presence of phenols, alkaloids, saponins, and glycosides only; flavonoids, tannins, and terpenoids were absent. Phytochemical screening revealed the presence of secondary metabolites such as flavonoids, alkaloids, phenols, and saponins—compounds well documented for their antimicrobial properties. These findings align with previous reports which attribute the antimicrobial activity of fenugreek to its diverse bioactive constituents [7, 28]. The absence of certain phytochemicals in the methanol extract does not necessarily mean they are missing from the plant material; rather, it indicates that their solubility limit in methanol was not reached or that the solvent was unable to break their molecular bonds with the plant matrix [29-30]. Conversely, the detection of compounds in aqueous extracts that are "better dissolved in methanol" may occur if those compounds are present as water-soluble salts or glycoside

conjugates in their natural state within the seed [31-33].

Table 2. Qualitative Phytochemical Analysis of Fenugreek Seed.

Plant constituents	Aqueous	Methanolic
Flavonoids	+	-
Phenols	+	+
Alkaloids	+	+
Tannins	+	-
Saponins	+	+
Terpenoids	+	-
Glycosides	+	+

+ = present, - = absent

The crude aqueous and methanolic extracts of fenugreek seed were analyzed using GC-MS. The aqueous extract had the maximum phytochemicals of 18 bioactive compounds whereas the methanolic extract had 11 bioactive compounds (Table 3).

Table 3. GC-MS of Fenugreek Seed Extracts

Peak Number	Name	Mol. Formula	Mol. Weight (g/mol)	Area	RT (min)
Aqueous Extract					
1	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]	C ₁₂ H ₁₇ NO ₂	207.27	1.3482	12.21
2	Thiocyanic acid, ethyl ester	C ₃ H ₅ NS	87.15	4.8788	12.85
3	Thiophene, tetrahydro-2-methyl	C ₅ H ₁₀ S	102.20	0.6345	13.34
4	Methyl 4-O-methyl-D-arabinopyranoside	C ₇ H ₁₄ O ₅	178.18	4.0856	14.19
5	1-Docosene	C ₂₂ H ₄₄	308.60	6.3697	20.77
6	Octacosyl heptafluorobutyrate	C ₃₂ H ₅₇ F ₇ O ₂	606.80	0.9789	22.23
7	Cyclodocosane, ethyl	C ₂₄ H ₄₈	336.60	8.3667	23.08
8	Octatriacontyl pentafluoropropionate	C ₄₁ H ₇₇ F ₅ O ₂	697.00	8.1747	23.99
9	Tetatriacontyl pentafluoropropionate	C ₃₇ H ₆₉ F ₅ O ₂	640.90	0.8282	24.67
10	1-Dodecanol, 2-octyl	C ₂₀ H ₄₂ O	298.50	0.6934	25.02
11	2-Piperidinone, N-[4-bromo-n-butyl]	C ₉ H ₁₆ BrNO	234.13	1.0821	26.15
12	Dotriacontyl heptafluorobutyrate	C ₃₆ H ₆₅ F ₇ O ₂	662.90	0.8039	27.20
13	Silane, trichlorooctadecyl	C ₁₈ H ₃₇ Cl ₃ Si	387.90	9.3664	28.02
14	Erucic acid	C ₂₂ H ₄₂ O ₂	338.60	6.5859	30.21
15	Oleic acid (two positions for Aqueous and Methanolic Extracts)	C ₁₈ H ₃₄ O ₂	282.50	0.444	31.36
16	Cyclododecanone, 2-(6-chloro-1-oxohexyl)	C ₁₈ H ₃₁ ClO ₂	314.89	8.061	32.12
17	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₄₀ O ₄	356.50	7.1352	34.98
18	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	C ₂₁ H ₄₀ O ₄	356.50	5.9041	36.73
Methanolic Extract					
1	1-Cyclohexylnonene	C ₁₅ H ₂₈	208.38	1.4317	6.01
2	Decanamide, N-(2-hydroxyethyl)	C ₁₂ H ₂₅ NO ₂	215.33	4.9662	16.92
3	Oxacyclotetradecane-2,11-dione, 13-methyl	C ₁₄ H ₂₄ O ₃	240.34	1.4694	17.20
4	cis,cis-7,10,-Hexadecadienal	C ₁₆ H ₂₈ O	236.39	1.7809	20.17

5	Oleic acid (two positions for Aqueous and Methanolic Extracts)	$C_{18}H_{34}O_2$	282.50	5.558	20.74
6	cis-9-Hexadecenal	$C_{16}H_{30}O$	238.41	6.6202	24.16
7	9,12-Octadecadienoyl chloride, (Z,Z)	$C_{18}H_{31}ClO$	298.90	6.4194	28.73
8	n-Propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324.50	11.7804	30.71
9	Trans-traumatic acid	$C_{12}H_{20}O_4$	228.28	0.7750	31.36
10	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.50	9.989	34.75
11	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356.50	15.1669	36.46

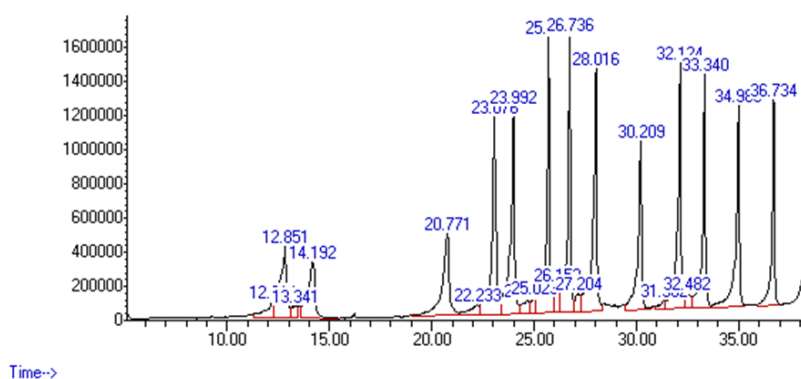
RT=Retention Time, Mol. = Molecular

The GC-MS analysis identified 29 bioactive compounds. The compounds identified are classified into several categories of secondary metabolites, including saponins, alkaloids, phenolics, and flavonoids. Each chromatographic peak

corresponds to a specific bioactive compound (Fig. 2), as determined by matching its retention time, molecular weight, and molecular formula with those of reference compounds in the NIST 14 Online Library.

A: Chromatogram of Aqueous Extract

Abundance



B: Chromatogram of Methanolic Extract

Abundance

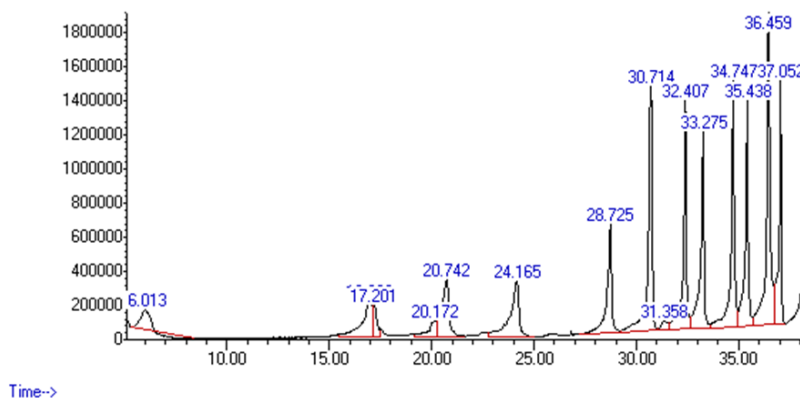


Fig. 2. GC-MS Chromatogram of Fenugreek Seed Extract (A: Aqueous and B: Methanolic).

Among these 29 compounds, 12 met the drug-likeness criteria based on Lipinski's Rule of Five (Table 4). Compounds that violate more than one of these limits are less likely to have good oral bioavailability and are therefore not considered drug-like. In this

case, 12 of the compounds fell within all these limits predicted to have better absorption, distribution, and permeability properties, while the other compounds exceeded with more than one violation (e.g., too large, too polar, or too lipophilic).

Table 4. Drug-Likeliness Properties of Bioactive Compounds from Fenugreek Seed Extracts.

S/N	Name	Molecular weight (g/mol)	logP (<5)	H-bond acceptor (<10)	H-bond donor (<5)	Rotatable bonds (<10)	Violations
1	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]	207.27	1.91	3	2	1	No
2	Thiocyanic acid, ethyl ester	87.15	1.22	2	0	1	No
3	Thiophene, tetrahydro-2-methyl	102.20	1.90	1	0	0	No
4	Methyl 4-O-methyl-D-arabinopyranoside	178.18	1.27	5	2	2	No
5	1-Docosene	308.60	8.60	0	0	19	Yes (2)
6	Octacosyl heptafluorobutyrate	606.80	12.52	2	0	29	Yes (3)
7	Cyclodocosane, ethyl	336.60	9.22	0	0	1	Yes (1)
8	Octatriacontyl pentafluoropropionate	697.00	15.79	2	0	38	Yes (3)
9	Tetraatriacontyl pentafluoropropionate	640.90	14.23	2	0	34	Yes (3)
10	1-Dodecanol, 2-octyl	298.50	6.88	1	1	17	Yes (2)
11	2-Piperidinone, N-[4-bromo-n-butyl]	234.13	2.17	1	0	4	No
12	Dotriacontyl heptafluorobutyrate	662.90	14.09	2	0	33	Yes (3)
13	Silane, trichlorooctadecyl	387.90	8.90	0	0	17	Yes (2)
14	Erucic acid	338.60	7.67	1	1	19	Yes (2)
15	Oleic acid	282.50	6.11	1	1	15	Yes (2)
16	Cyclododecanone, 2-(6-chloro-1-oxohexyl)	314.89	5.45	2	0	6	Yes (1)
17	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	356.50	4.92	4	2	18	Yes (1)
18	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	356.50	4.92	4	2	18	Yes (1)
19	1-Cyclohexylnonene	208.38	5.48	0	0	7	Yes (2)
20	Decanamide, N-(2-hydroxyethyl)	215.33	2.24	2	2	10	Yes (1)
21	Oxacyclotetradecane-2,11-dione, 13-methyl	240.34	3.26	3	0	0	No
22	cis,cis-7,10,-Hexadecadienal	236.39	5.22	1	0	12	Yes (2)
23	cis-9-Hexadecenal	238.41	5.44	1	0	13	Yes (2)
24	9,12-Octadecadienoyl chloride, (Z,Z)	298.90	6.57	1	0	14	Yes (2)
25	n-Propyl 11-octadecenoate	324.50	6.98	2	0	17	Yes (2)
26	trans-traumatic acid	228.28	2.83	2	2	10	Yes (1)
	Ciprofloxacin**	331.34	1.58	5	2	3	No

**Standard drugs

These selected compounds (Table 5) were further evaluated for their pharmacokinetic and toxicity profiles using ADMET prediction tools. Notably, several compounds demonstrated favorable human intestinal absorption, low blood-brain barrier permeability, and low predicted toxicity—key parameters that underscore their

potential as orally active, safe antimicrobial candidates (Table 6).

Molecular docking analysis against bacterial DNA gyrase-B (Fig. 3a-n) revealed that cyclodocosane, ethyl exhibited the highest binding affinity (-9.6 kcal/mol), surpassing the standard antibiotic ciprofloxacin (-9.2 kcal/mol) (Fig. 3n), (Table 7). This compound interacted with

Leu294 (Fig. 3e), (Table 7), a residue located within the GyrB ATP-binding site, suggesting its potential to interfere with bacterial DNA supercoiling processes as well as its potential to act as a scaffold for further optimization. Other compounds, including oxacyclotetradecane-2,11-dione, 13-methyl and hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl], also showed appreciable binding energies, further supporting the antibacterial potential of fenugreek-derived phytochemicals.

Despite forming limited specific interactions, cyclodocosane, ethyl consistently exhibited a favorable docking score, which can be attributed to its strong hydrophobic complementarity with the binding pocket. The extensive aliphatic framework allows efficient van der Waals packing within the active site, resulting in a stable docking pose. Although the interaction is largely non-specific, these results suggest that cyclodocosane, ethyl may represent a hydrophobic core scaffold that could be further optimized through the introduction of polar functional groups to enhance specific interactions and binding selectivity [34]. The binding mechanisms of the molecules differ significantly. Compounds containing polar functional groups (such as hydroxyl, amine, or carbonyl moieties) form multiple hydrogen bonds with key active-site residues, which substantially enhances binding stability. Molecules with aromatic rings additionally engage in π - π or π -alkyl interactions, while hydrophobic regions contribute to van der Waals interactions that help anchor the ligand within the pocket.

The reliability of the docking protocol was confirmed by observing reproducible binding orientations across multiple docking runs, stable positioning of ligands within the active site, and consistent interactions with critical amino acid residues known to be important for biological activity.

These computational findings corroborate earlier studies that have reported the antimicrobial properties of fenugreek

seed extracts against common pathogenic bacteria [8]. However, while docking scores provide useful insights into binding affinity and pose, they do not alone confirm biological activity. Thus, experimental validation through *in vitro* and *in vivo* antimicrobial assays is recommended to substantiate these results and determine minimum inhibitory concentrations and pharmacodynamic properties.

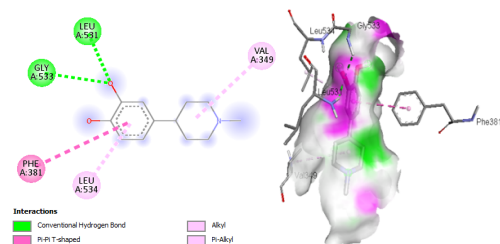


Fig. 3(a). 2D and 3D Complex of Hexahydropyridine, 1-Methyl-4-[4,5-Dihydroxyphenyl] with Gyrase-B.

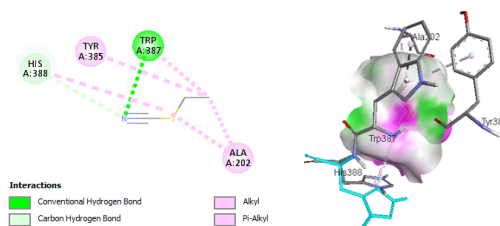


Fig. 3(b). 2D and 3D Complex of Thiocyanic Acid, Ethyl Ester with Gyrase-B.

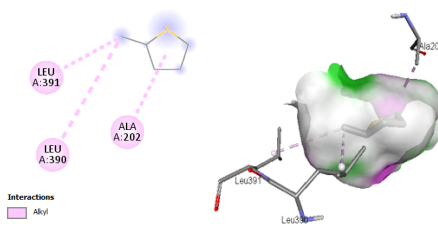


Fig. 3(c). 2D and 3D Complex of Thiophene, Tetrahydro-2-Methyl with Gyrase-B.

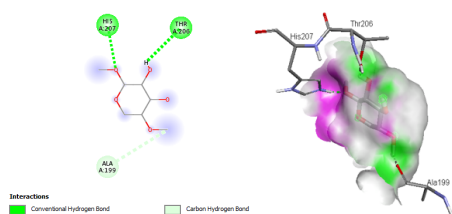


Fig. 3(d). 2D and 3D Complex of Methyl 4-O-Methyl-D-Arabinopyranoside with Gyrase-B.

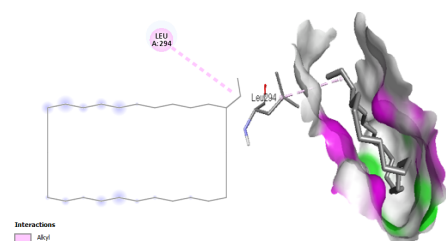


Fig. 3(e). 2D and 3D Complex of Cyclodocosane, Ethyl with Gyrase-B.

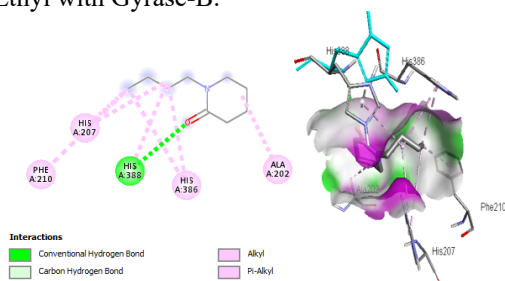


Fig. 3(f). 2D and 3D Complex of 2-Piperidinone, N-[4-Bromo-n-Butyl] with Gyrase-B.

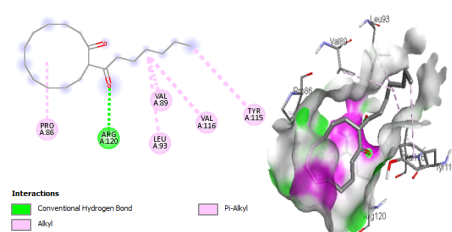


Fig. 3(g). 2D and 3D Complex of Cyclododecanone, 2-(6-Chloro-1-Oxohexyl) with Gyrase-B.

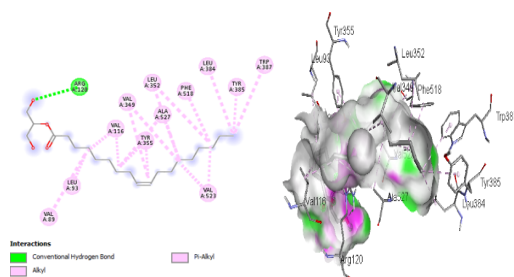
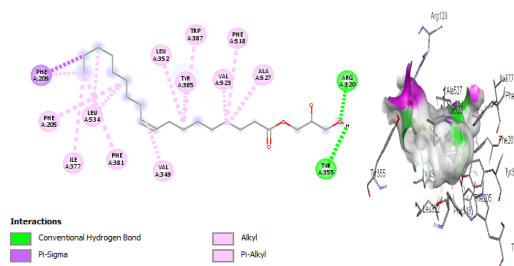


Fig. 3(h). 2D and 3D Complex of 9-Octadecenoic Acid (Z)-, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester with Gyrase-B.



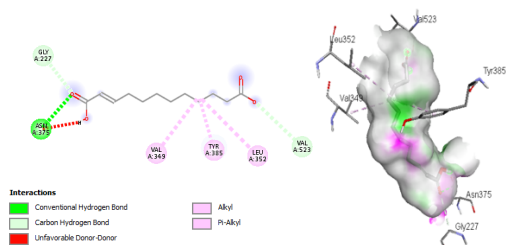


Fig. 3(l). 2D and 3D Complex of Trans-Traumatic Acid with Gyrase-B.

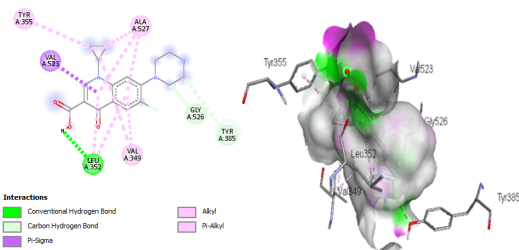


Fig. 3(m). 2D and 3D Complex of Ciprofloxacin (Standard Drug) with Gyrase-B.

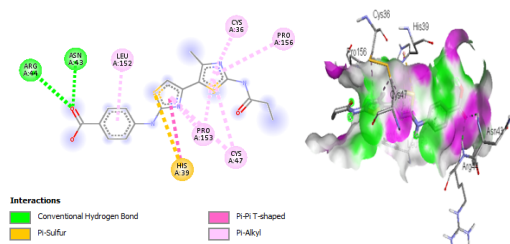
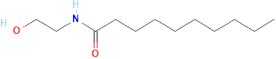
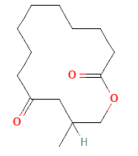
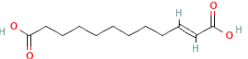
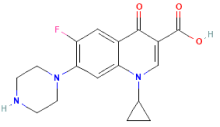
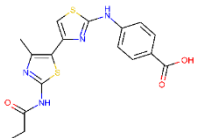


Fig. 3(n). 2D and 3D Complex of 4DUH Co-Crystallized Ligand (RLI) with Gyrase-B.

Table 5. Bioactive Compounds of Fenugreek Seed Selected for Molecular Docking Analysis.

S/N	Name	Molecular formula	Chemical structure	Molecular weight
1	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]	C ₁₂ H ₁₇ NO ₂		207.27
2	Thiocyanic acid, ethyl ester	C ₃ H ₅ NS		87.15
3	Thiophene, tetrahydro-2-methyl	C ₅ H ₁₀ S		102.20
4	Methyl 4-O-methyl-D-arabinopyranoside	C ₇ H ₁₄ O ₅		178.18
5	Cyclodocosane, ethyl	C ₂₄ H ₄₈		336.60
6	2-Piperidinone, N-[4-bromo-n-butyl]	C ₉ H ₁₆ BrNO		234.13
7	Cyclododecanone, 2-(6-chloro-1-oxohexyl)	C ₁₈ H ₃₁ ClO ₂		314.89
8	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₄₀ O ₄		356.50
9	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	C ₂₁ H ₄₀ O ₄		356.50

10	Decanamide, N-(2-hydroxyethyl)	C ₁₂ H ₂₅ NO ₂		215.33
11	Oxacyclotetradecane-2,11-dione, 13-methyl	C ₁₄ H ₂₄ O ₃		240.34
12	Trans-traumatic acid	C ₁₂ H ₂₀ O ₄		228.28
14	Ciprofloxacin (standard drug)	C ₁₇ H ₁₈ FN ₃ O ₃		331.34
15	4DUH co-crystallized ligand (RLI)***	C ₁₇ H ₁₆ N ₄ O ₃ S ₂		

***Co-crystallised ligands

Table 6. ADMET Predictions on Selected Bioactive Compounds from Fenugreek Seeds.

	Caco-2	Pgp-sub	HIA	BBB	PPB (%)	VDss	CYP2 D6-inh	CYP3 A4-sub	cl-plasma	t0.5	hERG	AMES	Carcinogenicity	H-HT
1	Low	Yes	High	Yes	38.12	Low	No	Yes	17.962	High	Low	Negative	Medium	Yes
2	High	Yes	Low	Yes	68.307	Low	No	Yes	9.24	Moderate	High	Negative	No	No
3	High	Yes	Low	Yes	79.026	Moderate	No	No	12.398	Moderate	High	Negative	Medium	Yes
4	Low	No	Low	No	21.387	Anomalous	No	No	5.801	High	High	Moderate	Medium	Medium
5	High	Yes	High	Yes	79.112	Moderate	No	No	4.947	Short	Low	Negative	No	No
6	High	Yes	High	Yes	72.267	Moderate	No	Yes	7.699	Short	High	Positive	Yes	No
7	High	Yes	High	No	92.154	Low	No	No	5.682	Short	Low	Negative	No	Medium
8	High	Yes	Moderate	Yes	97.246	Moderate	No	No	4.563	Moderate	High	Negative	No	No
9	High	Yes	Low	No	92.066	Anomalous	No	No	5.532	Short	High	Negative	No	No
10	Low	Yes	High	No	95.992	Low	No	No	5.686	Short	High	Negative	Medium	Medium
11	High	Yes	High	Yes	61.406	Low	No	No	5.698	Short	High	Negative	Medium	Medium
12	High	Yes	Low	Yes	82.243	Anomalous	No	No	2.274	Moderate	High	Negative	No	Yes

Caco-2 (Human Colon Adenocarcinoma Cell Line), Pgp-sub (P-glycoprotein), HIA (Human Intestinal Absorption), BBB (Blood Brain Barrier), PPB (Plasma Protein Binding), VDss (Volume of Distribution at Steady State), CYP2D6-inh (Cytochrome P450 2D6 Inhibitor), CYP3A4-sub (Cytochrome P450 3A4 Substrate), cl-plasma (Plasma Clearance), t0.5 (Elimination Half-life), hERG (Human Ether-à-go-go-Related Gene Inhibition), AMES (Ames Test for Mutagenicity), Carcinogenicity, H-HT (Human Hepatotoxicity).

Table 7. Binding Affinity of Bioactive Compounds from Fenugreek Seed Complexed with Gyrase-B.

Ligand	Binding Affinity (kcal/mol)	Interacting Amino Acid Residue
Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]	-7.9	LEU531, GLY533, PHE381, VAL349, LEU534
Thiocyanic acid, ethyl ester	-3.7	TRP387, HIS388, TYR385, ALA202
Thiophene, tetrahydro-2-methyl	-4.4	ALA202, LEU390, LEU391
Methyl 4-O-methyl-D-arabinopyranoside	-6.2	THR206, HIS207, ALA199
Cyclodocosane, ethyl	-9.6	LEU294

2-Piperidinone, N-[4-bromo-n-butyl]	-6.4	HIS388, HIS207, HIS386, ALA202, PHE210
Cyclododecanone, 2-(6-chloro-1-oxohexyl)	-6.6	ARG120, PRO86, VAL89, LEU93, TYR115, VAL116
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	-6.3	ARG120, VAL89, LEU93, VAL116, VAL349, TYR355, LEU353, LEU384, TYR385, TRP387, ALA527, VAL523, PHE518
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	-6.8	ARG120, TYR355, PHE209, PHE205, ILE377, LEU534, PHE381, VAL349, LEU352, TYR385, TRP387, VAL523, PHE518, ALA527
Decanamide, N-(2-hydroxyethyl)	-6.7	HIS207, ASN382, TYR385, HIS386, HIS388, LEU391, ALA202, LEU390, ALA199, TRP387
Oxacyclotetradecane-2,11-dione, 13-methyl	-7.4	ALA527, VAL349
Trans-traumatic acid	-6.3	ASN375, GLY227, VAL523, VAL349, TYR385, LEU352
Ciprofloxacin	-9.2	LEU352, GLY526, TYR385, VAL523, VAL349, ALA527, TYR355
4DUH Co-Crystallized (RLI)	-9.4	ARG44, ASN43, HIS39, LEU152, PRO153, CYS47, CYS36, PRO156

4. Conclusion

This study highlights the promising antibacterial potential of Fenugreek seed-derived phytochemicals, particularly against DNA gyrase-B. The compound cyclodocosane, ethyl emerged as a lead candidate with superior binding affinity compared to ciprofloxacin, supporting its potential as a novel antibacterial agent. *In silico* ADMET profiling further suggests that selected compounds possess desirable pharmacokinetic and safety profiles. These findings position fenugreek as a valuable source of natural compounds for antimicrobial drug discovery. Further experimental investigations are warranted to validate these computational predictions and advance the development of fenugreek-based antibacterial therapies.

Acknowledgement

We wish to acknowledge the academic staff of the Department of Biotechnology, Nigerian Defence Academy, Kaduna for their impactful insights that enriched the outcome of this work.

References

- [1] World Health Organization. Global antimicrobial resistance and use surveillance system (GLASS) report 2022. World Health Organization; 2022 Dec 8.
- [2] Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therapeutics*. 2015 Apr;40(4):277.
- [3] Koche DE, Shirsat RU, Kawale MA. An overview of major classes of phytochemicals: their types and role in disease prevention. *Hislopia Journal*. 2016;9(1/2):1-1.
- [4] Kim DK, Ediriweera MK, Davaatseren M, Hyun HB, Cho SK. Antioxidant activity of banana flesh and antiproliferative effect on breast and pancreatic cancer cells. *Food Science & Nutrition*. 2022 Mar;10(3):740-50.
- [5] Manandhar S, Luitel S, Dahal RK. *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. *Journal of Tropical Medicine*. 2019;2019(1): 1895340.
- [6] Wani SA, Kumar P. Fenugreek: A review on its nutraceutical properties and utilization in various food products. *Journal of the Saudi Society of Agricultural Sciences*. 2018 Apr 1;17(2):97-106.
- [7] Bano D, Tabassum H, Ahmad A, Mabood A, Ahmad IZ. The medicinal significance of the bioactive compounds of *Trigonella foenum-graecum*: a review. *International Journal of Research in Ayurveda & Pharmacy*. 2016;7(4):84-91.
- [8] 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;20(12):1453-7.

- [9] Sindhusa VB, Rajasekar A. Preparation and evaluation of antimicrobial property and anti-inflammatory activity of fenugreek gel against oral microbes: an *in vitro* study. *Cureus*. 2023 Oct 25;15(10). Molecular Microbiology. 2018 Apr;108(2):115-27.
- [10] Singariya P, Mourya KK, Kumar P. Evaluations of Some Organic Compounds in Ethyl Acetate extract of 'Marwar Dhaman' by Gas Chromatography-Mass Spectrometry. *The Journal of Plant Science Research*. 2017;33(1):17. [17] Alotaibi BS, Hakami MA, Jawaid T, Alshammari N, Binsuwaidan R, Adnan M. Identification of potential *Escherichia coli* DNA gyrase B inhibitors targeting antibacterial therapy: an integrated docking and molecular dynamics simulation study. *Journal of Biomolecular Structure and Dynamics*. 2024 Nov 21;42(17):8885-96.
- [11] Bansal Y, Mujib A, Mamgain J, Kumar S, Dewir YH, Magyar-Tábori K. Synthesis and Accumulation of Phytocompounds in Field-, Tissue-Culture Grown (Stress) Root Tissues and Simultaneous Defense Response Activity in *Glycyrrhiza glabra* L. *Sustainability*. 2024 Feb 15;16(4):1613. [18] Carty M, Menzel R. Inhibition of DNA gyrase activity in an *in vitro* transcription-translation system stimulates *gyr A* expression in a DNA concentration dependent manner: Evidence for the involvement of factors which may be titrated. *Journal of Molecular Biology*. 1990 Jul 20;214(2):397-406.
- [12] Christyapita D, Gnaneswari MD, Sharma S, Michael RD, Subramani PA. *In silico* insights into the molecular mechanisms of *Eclipta alba* leaf fractions enhancing nonspecific immune mechanisms and resistance to *Aeromonas hydrophila* in *Oreochromis mossambicus* (Peters). *Aquaculture Reports*. 2025 Jul 15;42:102774. [19] Amaro RE, Baudry J, Chodera J, Demir Ö, McCammon JA, Miao Y, Smith JC. Ensemble docking in drug discovery. *Biophysical Journal*. 2018 May 22;114(10):2271-8.
- [13] Almatroodi SA, Almatroodi A, Alsahli MA, Rahmani AH. Fenugreek (*Trigonella foenum-graecum*) and its active compounds: a review of its effects on human health through modulating biological activities. *Pharmacognosy Journal*. 2021;13(3): 813-21. [20] Pinzi L, Rastelli G. Molecular docking: shifting paradigms in drug discovery. *International Journal of Molecular Sciences*. 2019 Sep 4;20(18):4331.
- [14] Bush NG, Evans-Roberts K, Maxwell A. DNA topoisomerases. *EcoSal Plus*. 2015 Dec 31;6(2):10-128. [21] Rao H, Ahmad S, Aati HY, Basit A, Ahmad I, Ghalloo BA, Shehzad MN, Nazar R, Zeeshan M, Nasim MJ. Phytochemical screening, biological evaluation, and molecular docking studies of aerial parts of *Trigonella hamosa* (branched Fenugreek). *Arabian Journal of Chemistry*. 2023 Jul 1;16(7):104795.
- [15] Collin F, Karkare S, Maxwell A. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. *Applied Microbiology and Biotechnology*. 2011 Nov;92(3): 479-97. [22] El Gadali K, Rafya M, Maatallah M, Mehdi A, Ouahrouch A, Benkhalti F, Sanghvi YS, Taourirte M, Lazrek HB. Synthesis, structural characterization and antibacterial activity evaluation of novel quinolone-1, 2, 3-triazole-benzimidazole hybrids. *Journal of Molecular Structure*. 2023 Jun 15;1282:135179.
- [16] Samadpour AN, Merrikh H. DNA gyrase activity regulates DnaA- dependent replication initiation in *Bacillus subtilis*.

- [23] Raslan MA, Said AA, Alshareef WA, Abo-Salem HM, Raoof GF. Phytochemical study and evaluation of antioxidant and antimicrobial activities through *in vitro* and *in silico* studies of *Citrus volkameriana* Ten. & Pasq. seeds. South African Journal of Botany. 2024 Oct 1;173:19-33.
- [24] Gebrehiwot H, Ensermu U, Dekebo A, Endale M, Nefo Duke T. *In Vitro* Antibacterial and Antioxidant Activities, Pharmacokinetics, and *in silico* Molecular Docking Study of Phytochemicals from the Roots of *Ziziphus spina-christi*. Biochemistry Research International. 2024;2024(1): 7551813.
- [25] Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. The Scientific World Journal. 2017;2017(1):5873648.
- [26] Ralte L, Khiangte L, Thangjam NM, Kumar A, Singh YT. GC–MS and molecular docking analyses of phytochemicals from the underutilized plant, *Parkia timoriana* revealed candidate anti-cancerous and anti-inflammatory agents. Scientific Reports. 2022 Mar 1;12(1):3395.
- [27] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews. 2012 Dec 1;64:4-17.
- [28] Yadav R, Tiwari R, Amin F. The Screening of Analgesic and Anti-inflammatory activities of *Trigonella foenum-graecum* seeds. International Journal of Pharmaceutical Science Invention. 2016;5(7):50-3.
- [29] Mungwari CP, King'ondeu CK, Sigauke P, Obadele BA. Conventional and modern techniques for bioactive compounds recovery from plants. Scientific African. 2025 Mar 1;27:e02509.
- [30] Kumar A, P N, Kumar M, Jose A, Tomer V, Oz E, Proestos C, Zeng M, Elobeid T, K S, Oz F. Major phytochemicals: recent advances in health benefits and extraction method. Molecules. 2023 Jan 16;28(2):887.
- [31] Nandi K, Ghosh R, Mondal S, Sen DJ, Saha D. Source, isolation & impact of glycone and aglycone in human body. World Journal of Pharmaceutical Sciences. 2021 Nov 1:103-13.
- [32] Kytidou K, Artola M, Overkleeft HS, Aerts JM. Plant glycosides and glycosidases: a treasure-trove for therapeutics. Frontiers in plant science. 2020 Apr 7;11:357.
- [33] Bribi N. Pharmacological activity of alkaloids: a review. Asian journal of botany. 2018 Apr;1(1):1-6.
- [34] Koulechova DA, Tripp KW, Horner G, Marqusee S. When the scaffold cannot be ignored: the role of the hydrophobic core in ligand binding and specificity. Journal of Molecular Biology. 2015 Oct 9;427(20):3316-26.