Phytochemical composition, Antimicrobial, GC-MS analyses and computational modeling of Fenugreek (*Trigonella foenum-graecum* L) Seeds

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Abstract

Saudi Arabia has several fragrant, decorative, and medicinal plants with strong bioactivity. The current work examines the metabolite profiling of Fenugreek (*Trigonella foenum-gracum* L) ethanol extract for antibacterial, antifungal, antioxidant, and anticancer properties. Additionally, a computer-supported study will determine the pharmacokinetic characteristics and toxicity of the recognized mixes. The moisture, fiber, ash, protein, fat, and carbs in fenugreek seed were 4%, 6.50%, 3.20%, 28.55%, 4%, and 62.48%, respectively. Fenugreek seed flour had physiochemical properties like other edible oils. *Aspergillus flavus*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* were examined for fenugreek seed flour inhibitory activity. Seed oil was found to be highly antibacterial against all tested microbes. Antimicrobial activity was strongest against *E. coli*, with a 20-mm inhibition zone. The highest antibacterial activity was 100% inhibition against *Aspergillus flavus*. The computational modeling reveals that fenugreek compounds bind the TyrRS from *S. aureus*, the human peroxiredoxin 5, and aspartic proteinase from *C. albicans* with high binding scores that reach –9.4 kcal/mol and established promising molecular interactions with some key residues, that satisfactorily explain the in vitro results. According to the study, fenugreek seed is an important antibacterial and antifungal agent for food preservatives and medicine.

Key words: antibacterial, antifungal, computational modeling, fenugreek, phytochemical analysis

Introduction

Fenugreek is scientifically known as *Trigonella foenum-graecum* in Latin. The herb has more than one use. Its culinary, medicinal, and therapeutic uses date back thousands of years to the people of Western Asia and the Mediterranean. Unsaturated fatty acids, including oleic acid, linoleic acid, and linoleic acid, are among the many components found in fenugreek seed oil, which is also rich in antioxidants. The many healthful components of the oil are magnesium, iron, manganese, fiber, and many more. The oil's antibacterial properties effectively kill some kinds of bacteria and fungi [Sulieman et al. 2017a; Sulieman et al 2023].

The fenugreek plant seeds are an excellent food source of soluble fiber, phytonutrients, minerals, and vitamins. Vitamins A, C, and B6, which include thiamine, pyridoxine, folic acid, and niacin, are abundant in this food. Moreover, Fenugreek possesses effects that are hypocholesterolemic, antibacterial, antiparasitic, antifertility, anticancer, and antidiabetic (Wani et al., 2018). Aasim *et al*. (2018) and Rashid *et al*. (2020) note

that Fenugreek's phytochemicals have made it useful in the kitchen, nutraceutical, and medicinal contexts. According to Ruwali *et al*. [6], its nutraceutical applications stem from its therapeutic and health-promoting properties. Concurrently, its flavor is the driving force behind its culinary applications. To increase the concentration of chemicals with biological activity. Many methods have been employed to extract Fenugreek; one of the primary uses of Fenugreek in the food business is its flavour.

Additionally, it protects the liver, fights cancer, aids in lactation, and reduces blood sugar levels. Polyphenols, galactomannans, alkaloids, and saponins are some of the phytochemicals that cause these effects. In addition, research has shown that new technology increases the yield and biological activity of fenugreek extracts (Gavahian et al., 2023).

African bread recipes often call for the addition of fenugreek seeds. 10% fenugreek fibre is mixed with wheat flour for fiber-rich chapati. Diabetic patients benefit from its inclusion since it reduces starch digestion (Sakhare and Prabhasankar 2022). Fortified cookies were made with fenugreek seed flour to increase their mineral and nutritional content (Negu et al*.,* 2020).

The study aimed to determine which bioactive ingredients are present in fenugreek seeds. Additionally, fenugreek oil was studied for many phytochemicals, including its potential as an antioxidant agent and its antibacterial and antifungal properties against harmful microorganisms. After phytochemicals have been identified, their binding activities and molecular interactions with the TyrRS from *S. aureus* (1JIJ), the *C. albicans* aspartic proteinase from (2QZW), and human peroxiredoxin 5 (PRDX5, 1H2D) were explored using computational assays.

Materials and Methods

The raw material

Fenugreek seed samples were acquired from the local Hail market in 2023. The samples were collected from the retailer's store to prevent contamination from dust and dirt and to preserve the spices' flavor and color, which can be affected by direct sunlight exposure.

Proximate analysis

Proximate analysis examines and evaluates a substance or material's essential components or constituents.

The moisture, protein, fiber, ash, and fat contents of the dried fenugreek seed samples were determined using the AOAC (2010) procedures. The carbohydrate content was computed by subtracting the combined percentages of moisture, protein, fiber, ash, and fat from 100. The trials were conducted in triplicates, and subsequently, the means were computed.

Extraction of fenugreek seed oil and estimation of its physicochemical properties

Fenugreek seed oil was extracted using n-hexane, following the AOCS (1971) method, utilizing the Soxhlet apparatus. The experiment was repeated multiple times until enough data was collected for further analysis.

The physicochemical examination of fenugreek seed oil was conducted by the Standard Methods for Examination of Fats, Oils, and Derivatives (1979). The analysis involved the acid value, saponification number (expressed in milligrams of potassium hydroxide per gram of oil), ester value, free fatty acids (measured as oleic acid per 100 grams of oil), and the refractive index at a temperature of 37°C. The essential oils were extracted from fenugreek seeds using hydro-distillation utilizing a Clevenger-type apparatus for 3

hours. The essential oil was dried using anhydrous Na_2SO_4 and then stored in a sealed vial in a dark place at a temperature of 4°C. The essential oil yield was determined using the gravimetric approach by calculating the dry weight (Benhammou et al., 2017).

Preparation of Microbial specimens

The efficacy of fenugreek oil was evaluated against a single strain of gram-positive bacterium, *S. aureus*, and two strains of gram-negative bacteria, *E. coli* and *Sal. typhimurium*, and one strain of mold, *A. flavus*. The bacteria used in the experiment were acquired from the Food Microbiology Laboratory at the University of Gezira**.**

Cultivation of the test organisms

The surface viable counting technique determined the average concentration of viable organisms per milliliter (mL) in the stock suspensions. For each trial, a new stock suspension was prepared, ensuring that the experimental settings remained consistent. This allowed us to obtain suspensions with remarkably similar viable counts. The *A. flavus* fungal culture was cultivated on Saboraud dextrose agar and incubated at 25ºC for four days. The mycelium was collected and rinsed with sterile isotonic saline solution, then resuspended in 100 mL of sterile isotonic saline solution. The resulting suspension was refrigerated for future use.

Testing of plant extract for antimicrobial activities

The antibacterial and antifungal properties of the plant extract were assessed using the agar well diffusion method, as demonstrated by Daoud *et al*. (2019) and Abdel-Rahim & Abdel Moneim (2010). A volume of 1 ml of a recently cultivated bacterial or fungal culture was transferred using a pipette and placed at the center of a sterile Petri dish. Subsequently, the inoculum was combined with molten cooled Muller Hinton agar (MHA) for bacteria strains or Potato dextrose agar (PDA) for fungi in a Petri plate, ensuring thorough mixing. After the agar plates containing inoculums solidified, wells were created using a sterile cork borer with a diameter of 6 mm.

The plates were incubated at 37°C for 24 to 48 hours. The fenugreek seed extract was applied on sterile discs using three different dosages (1 mg, 2 mg, and 3 mg per disc). Every concentration was evaluated three times against the target species. The growth inhibition diameter zones were recorded together with the average and the mean values after incubation. Ampicillin and amphotericin B were used as reference molecules.

Phytochemical profiles

Following Raaman (2006) and Banso and Adeyemo (2006) procedures, phenols, tannins, alkaloids, flavonoids, terpenoids, sterols, cardiac glycosides, and saponins were found in fenugreek seeds.

Phenols

Ten ml ethanol extract was added to 3 drops of 5% FeCl3. A bluish-black color implies phenolic chemicals.

Alkaloids

There were two ways to detect alkaloids:

In Dragendorff's test, 500 µl of Dragendorff's reagent was added to 5 ml of ethanol extract along the test tube side after adding 2 ml MeOH and 2 ml 1% HCl. Orange or orange reddish-brown precipitate indicated a positive result. The extract was tested with two drops of Mayer's reagent in 1 ml. White or creamy precipitate indicates alkaloids.

Flavonoids

Adding 2 ml NaOH 2% to 5 ml extract made it intensely yellow. The hue disappears when diluted HCl is added, indicating flavonoids.

Salkowski-tested terpenes

Mixing 5 ml extract with 2 ml chloroform. Then 3 ml conc. H_2SO_4 was added. A reddish-brown colour implies terpenoids.

Steroids

Five ml extract with two ml H_2SO_4 received two ml glacial acetic anhydride. Colour changes from violet to blue or green suggest steroids.

Keller-Kiliani glycosides

Three drops of 5% $FeCl_3$ and one millilitre of glacial acetic acid were added to 2.5 millilitres of extract. On the side of the test tube was put 0.5 ml of concentrated H_2SO_4. Cards containing cardiac glycosides will be coloured green or blue.

Saponins

We added 10 ml distilled water to 3 g seed powder. Five minutes were spent shaking the solution. Stable foam indicates saponins.

Tannins

Three drops of 5% $FeCl_3$ solution were added to 2 ml of diluted extract. The green, black, or blue colour indicated tannins.

G.C.–M.S. analysis

The GC-MS study used a Perkin Elmer Clarus 600 GC System with an Rtx 5MS capillary column (30 m 0.25 mm i.e. 0.25 m film thickness; max. temp. 350 ◦C) and Clarus 600C MS. The carrier gas was ultra-high-purity helium (99.9999%) flowing at 1.0 mL/min. Ion source, transfer line, and injector temperatures were 280, 270, and 270 ◦C. This gas ionized at 70 eV. The EM voltage was estimated using autotune. Every data came from full-scan mass spectra between 40 and 550 amu.

Analysis conditions

The split ratio of 1 L injected sample was 10:1. The oven was programmed to maintain 280 °C for 25 minutes at 80 °C each minute from 60 °C. Conditions for G.C.–M.S. leaf oil analysis: As noted, G MS detected FAME molecules. The helium flow was 0.7 mL/min. The ion source, transfer, and injector were heated to 250, 250, and 220 °C. After starting at 50 °C (kept for 1 minute), the oven was heated to 250 °C at 40 °C per minute. All data was collected by obtaining full-scan mass spectra from 35 to 500 amu. Spectra were compared to mass spectral libraries to identify compounds (Idriss et al., 2022). We used manufacturing conditions to determine the system's calibration and minimal detection limits (Al-Huqail et al., 2018).

In-silico Study

The antimicrobial and antioxidant activities of the fenugreek seed oil were also assessed using silico modeling and interaction assay. For this purpose, the TyrRS from *S. aureus* (1JIJ), the aspartic proteinase from *C. albicans* (2QZW), and human peroxiredoxin 5 (PRDX5, 1H2D) have been retrieved from RCSB data bank. Then, the active sites of these receptors were targeted to study the antibacterial, antifungal, and antioxidant effects, respectively. ChemDraw was used to draw the chemical structures of the fenugreek seed oil and identify phytochemicals if the compound did not exist on the PubChem website. The docking approach was carried out based on the CHARMm force field as previously published (Ben Saad et al., 2023; Rahmouni et al., 2024; Mhadhbi et al., 2023; Alreshidi et al., 2023) following the preparation of both ligands and receptors by removing water molecules and supplementing both polar hydrogens and Kollman charges... The assessment of binding scores bond categories were realized as previously reported Ben Saad et al., 2023; Mhadhbi et al., 2023; Alreshidi et al., 2023) 1JIJ, 2QZW, and 1H2D have been selected as they are commonly associated with the pathogenesis of infectious diseases, particularly from *S. aureus* and *C. albicans* and pro-antioxidant pathways (Ben Saad et al., 2023; Rahmouni et al., 2024; Mhadhbi et al., 2023; Idriss et al., 2022). Bioavailability and pharmacokinetic properties of the fenugreek-identified phytochemicals have also been studied as previously described (Alreshidi et al., 2023; Rahmouni et al., 2022). The computational assessment of these parameters was based on the ADME/Tox measurements (for absorption, distribution, metabolism, excretion, and toxicity) (Othman et al., 2021; Bédoui et al., 2024)..

Statistical analyses

The statistical analysis was conducted using the GraphPad software program developed by SPSS Inc., based in Chicago, IL. The data are presented as the mean plus the standard error of the mean (SEM). The statistical analysis involves conducting a one-way analysis of variance (ANOVA) and subsequently applying the Newman-Keuls post hoc test. The statistical differences will be deemed significant if the p-value is more than 0.05.

Results and discussion

Proximate chemical composition of fenugreek seeds

Figure 1 shows fenugreek seeds' proximate chemical makeup. The contents of moisture, ash, fat content, crude fibers, and carbohydrates of fenugreek seeds ranged between (4.10- 4.30%, 3.00-3.40%, 27.11-28.43%, 3.82-4.12%, and 48.26-51.11%, respectively.

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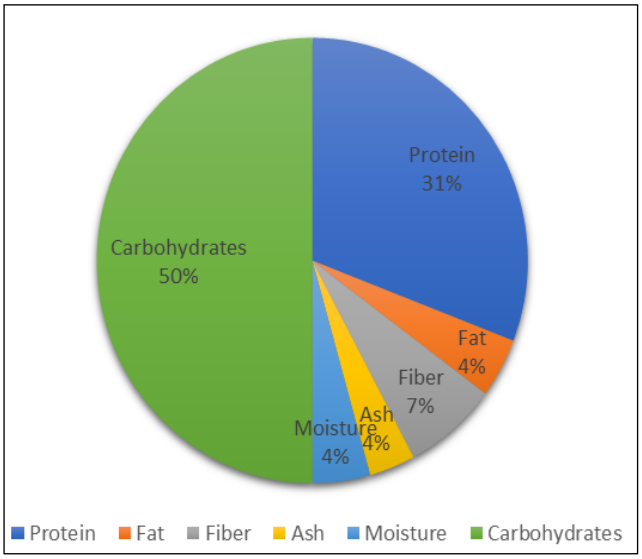

Figure 1. Proximate chemical composition of fenugreek seed oil

Physicochemical properties of fenugreek seed oil

Table (1) shows the physico-chemical properties of fenugreek seed oil. These properties were found to be Acid value: 4.75 mg KOH/g, saponification: 195 mg KOH/g, ester: 190.25, free fatty acids: 2.38 mg/100g oil, and the refractive index of fenugreek seed oil was 1.4640 at 37°C. When consuming fenugreek seed oil, its strong smell is lessened.

Table 1. The physicochemical properties of fenugreek seed oil.

Acid value Mg KOH/g	Saponification value Mg KOH/g	Ester value	Free fatty acids mg/100goil	Refractive index
4.75	195	190.25	2.38	1.4640

2.3 Antibacterial properties of fenugreek oils

The findings regarding the antimicrobial efficacy of the ethanol extract of Fenugreek are presented in Table (2). The investigated microorganisms include *S. aureus*, two gram-negative bacteria *E. coli* strains, and *Sal. typhimurium*, was effectively inhibited by the plant extract at various concentrations (1, 2, and 3 mg/disc); the last concentration (3 mg/disc) exhibited a statistically significant difference ($p \leq 0.05$) when compared to the reference drug ampicillin ten µg/disc. The ethanol extract of Fenugreek exhibited an inhibition zone of 15.00 ± 1.5 mm against *E. coli* and 16.11 ± 0.10 mm against *Sal. typhimurium* at a concentration of 3 mg/disc. The seeds of fenugreek exhibit more pronounced antibacterial properties when tested against *E. coli*. At 1, 2, and 3 mg/disc concentrations, the inhibition zones measured 12.56±0.12, 13.44±0.25, and 16.11±0.10 mm, respectively.

The results presented in Table (2) demonstrate that the Fenugreek extract exhibited significant inhibitory activity against the growth of the examined fungus, *Aspergillus flavus*. Specifically, the mean inhibition zone measured 14.11±0.12 mm at 1 mg/disc (lower concentration) and 16.87±0.32 mm at a concentration of 3 mg/disc (higher concentration). The data in the Table indicates that Fenugreek exhibits a more pronounced inhibitory effect against fungi than bacteria. Furthermore, as the concentration of the plant extract increases, so does the inhibitory effect.

Table 2. The inhibition zone of Fenugreek hydroethanolic extract expressed as means of three replicates (mean±SD)

Microorganisms Tested	Fenugreek extract			Standard molecules*
	1 mg/disc	2 mg/disc	3 mg/disc	
S. aureus	9.12±1.15^{c*}	12.84±0.52^{b}	15.02±0.50^{c}	25.25 ±1.12^{a}
E. coli	12.56±0.12^{b}	13.44±0.25^{b}	16.11±0.10^{b}	11.22±0.33^{c}
Sal. Typhyi	8.72±0.30^{c}	13.00±0.30^{b}	15.24±0.10^{c}	7.42±0.27^{d}
A. flavus	14.11±0.12^{a}	15.30±0.32^{a}	16.87±0.32^{a}	21.65±1.22^{b}

Different letters reflected different significant levels in respect to mean±SD
*: Ampicillin for bacterial strains and amphotericin B for *A. flavus*.

Phytochemical analysis

Table (3) shows the detected main classes within Fenugreek seeds. It was clear that Fenugreek is rich in alkaloids, tannins, saponins, glycosides, flavonoids, and steroids, but it lacks terpenoids.

Table 3. The phytochemical screening test for Fenugreek seeds

Main class	Status
Alkaloids	+
Tannins	+
Saponins	+
Glycosides	+
Flavonoids	+
Steroids	+
Terpenoids	-

- Means the main class was not detected

+ Means the main class was detected

GC-MS analysis

Table (4) and Figure (2) show the names of the detected compounds from the ethanol extract of fenugreek seeds corresponding with their retention time, molecular formula, and %concentrations. Twenty-five compounds were detected of which Ethyl methane Sulfonate (12.41%) was the main component among the ethanol extract of fenugreek seeds, followed by n-Hexadecanoic acid (9.12%), 4-Butyl-2(4-nitrophenyl)-1,3-thiazole (8.21%), 9-Octadecatrienoic acid (6.23%), 9,12-Octadecadienoic acid (Z, Z)- (6.12%), α-Tocopherol (5.46%), 2-Furanmethanol (5.32%), Cholest-5-ene 3-bromo- (4.51%), Dimethyl trisulfide (4.23%), Methylaminobenzoic acid (4.21%), 1,4-Benzene dicarboxylic acid dimethyl ester (3.86%), Pentanal (3.45%), N-methylhomopiperazine (3.22%), and Trigonelline (3.21%).

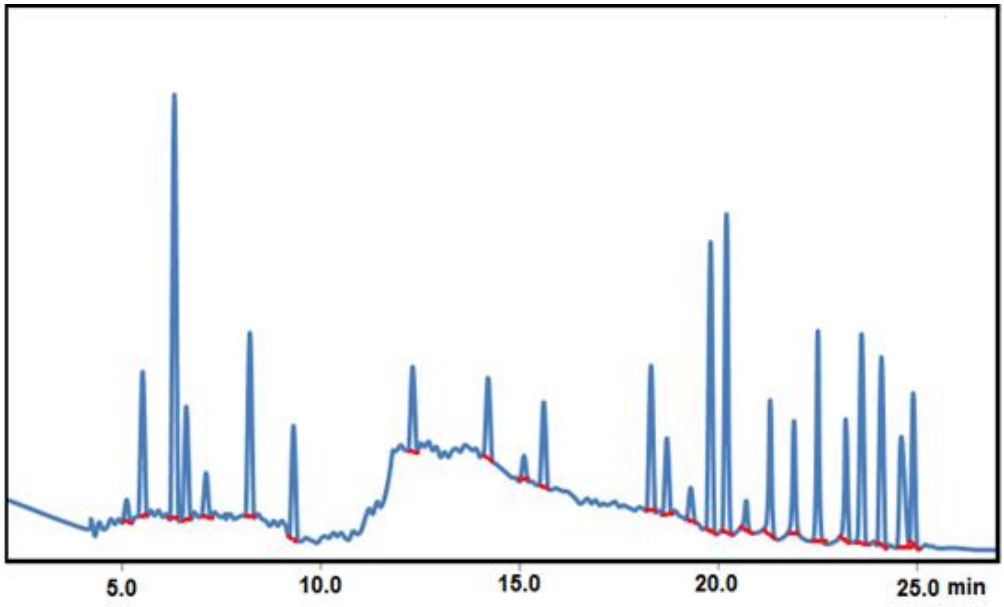

Figure 2. Chromatogram of the ethanol extract of fenugreek seeds

Table 4. GC-MS analysis of ethanol extract of fenugreek seed

No.	R. time	Compound name	Mol. Form	% area
1	5.12	Nitrobutan-3-ol	$C_4H_9NO_3$	0.61
2	5.54	Dimethyl trisulfide	$C_2H_6S_3$	4.23
3	6.04	Ethylmethane Sulfonate	$C_3H_8O_3S$	12.41
4	6.62	N-methylhomopiperazine	$C_6H_{14}N_2$	3.22
5	7.12	Methylsulfonyl methane	$C_2H_6O_4S$	1.24
6	8.24	2-Furanmethanol	$C_5H_6O_2$	5.32
7	9.33	Trigonelline	$C_7H_7NO_2$	3.21
8	12.43	β-Estradiol-3-methyl ether	$C_{19}H_{26}O_2$	2.33
9	14.2	Cyclohexasiloxane dodecamethyl-4,4-methyl-	$C_{12}H_{36}O_6Si_6$	2.31
10	15.13	Decanoic acid	$C_{10}H_{20}O_2$	0.74
11	15.62	4-Hydroxybenzoic acid	$C_7H_6O_3$	2.45
12	18.03	4-Methylaminobenzoic acid	$C_8H_9NO_2$	4.21
13	18.76	2-Methylundecanal	$C_{12}H_{24}O$	2.15
14	19.28	Didodecyl phthalate	$C_{32}H_{54}O_4$	0.89
15	19.88	4-Butyl-2(4-nitrophenyl)-1,3-thiazole	$C_{13}H_{14}N_2O_3S$	8.21
16	20.21	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	9.12
17	20.74	Apigenin 6,8-di C-glucoside	$C_{27}H_{30}O_{15}$	0.83
18	21.35	1,4-Benzenedicarboxylic acid dimethyl ester	$C_{10}H_{10}O_4$	3.86
19	21.94	Pentanal	$C_5H_{10}O$	3.45
20	22.54	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	6.12
21	23.13	Diosgenin	$C_{27}H_{42}O_3$	3.65
22	23.61	9-Octadecatrienoic acid	$C_{18}H_{34}O_2$	6.23
23	24.11	α-Tocopherol	$C_{29}H_{50}O_2$	5.46
24	24.65	Qurecetin 3-arabinoside	$C_{20}H_{18}O_{11}$	3.24
25	24.91	Cholest-5-ene 3-bromo- 3β-	$C_{27}H_{45}Br$	4.51
				100

In silico/ computational modeling of Fenugreek

The fenugreek-identified phytochemicals had different affinities to TyrRS from *Staphylococcus aureus* (1JIJ), aspartic proteinase from *Candia albicans* (2QZW) receptors, and human peroxiredoxin 5 (PRDX5, 1H2D) (Table 5). Recently, it has been reported that binding affinities depend mainly on the 3D chemical structure of the ligands and their structural geometry (Ben Saad et al., 2023; Rahmouni, et al., 2024; Mhadhbi et al., 2023). In the current work, the 25 identified compounds of Fenugreek had negative binding affinities, which support the biological effects. The best binding affinities reached −9.4 kcal/mol for 1JIJ, −8.6 kcal/mol for 2QZW, and −8.3 kcal/mol for 1H2D. These scores concerned mainly compounds no. 21and 24. Compound no. 21 had the highest affinity and established good molecular interactions with the 1JIJ (Table 6).

Table 5. Interactions, bond category, and closest interacting residues for the best-identified compounds with the targeted receptors: 1JIJ, 2QZW, and 1H2D for TyrRS from *Staphylococcus aureus*, aspartic proteinase from *Candia albicans*, and human peroxiredoxin 5 (PRDX5) respectively.

Compound No.	No. H-Bond	Interacting Residues	
		Closest Interacting Residues (Distance, Å)	**No. closest Interacting Residues (Å)**
TyrRS from *Staphylococcus aureus* (pdb id: 1JIJ)			
21 (–9.4)	2	Gly49 (3.53), Gly238 (3.54), Leu223 (5.26), Val224 (5.28), Lys234 (4.90), Lys234 (4.09), Ala239 (4.92), Leu52 (4.47), Pro53 (5.05), Val224 (4.24)	8
24 (–8.6)	7	Arg58 (2.01), Arg58 (2.28), Lys305 (2.13), Asn109 (2.51), Glu302 (2.87), Glu112 (2.95), Glu62 (2.20), Glu62 (4.12), Phe306 (4.33)	7
15 (–7.5)	4	Lys84 (3.94), Arg88 (5.25), Lys84 (2.79), Lys84 (2.71), Asn124 (2.90), Gly38 (2.47), Ala39 (4.55), Pro53 (4.37), His50 (4.44), His50 (4.17), Phe54 (4.93)	8
Aspartic proteinase from *Candia albicans* (pdb id: 2QZW)			
21 (–8.6)	2	Ser12 (2.78), Lys49 (3.54), Val12 (4.79), Pro120 (3.90), Pro120 (4.33)	4
24 (–8.0)	7	Arg192 (2.69), Arg195 (2.31), Arg195 (2.46), Asp32 (2.29), Asp218 (2.56), Thr221 (2.35), Asp37 (2.77), Glu193 (3.24), Tyr84 (5.42), Ala133 (5.47)	9
15 (–6.9)	4	Gly85 (1.88), Thr222 (2.46), Ile223 (2.73), Gly220 (2.86), Asp86 (4.95), Asp86 (3.78), Asp218 (4.44), Ile30 (4.09)	7
Human peroxiredoxin (PRDX5, pdb id: 1HD2)			
24 (–8.3)	6	Asn21 (2.48), Arg86 (2.45), Arg86 (2.27), Gly82 (2.03), Glu16 (4.28), Gly92 (2.69), Leu96 (3.99), Gly92:C,O;Lys93:N (4.34), Arg95 (5.12), Arg95 (4.77), Ala90 (4.84)	9

21 (–7.8)	1	Arg124 (2.78), Pro45 (5.08), Ile119 (4.91), Phe120 (5.26)	4
23 (–6.1)	2	Arg86 (2.33), Arg86 (2.35), Glu16 (3.33), Val69 (5.12), Arg95 (4.33), Lys32 (4.16), Val69 (3.74)	5

The molecular interactions included two carbon H-bonds associated with a network of hydrophobic bonds that contribute to the stability of the complex (Ben Saad et al., 2023; Badraoui,et al., 2023; Akacha et al, 2022) (Figures 3 and 4). These interactions concerned several vital residues. It has been found that it included eight different residues: once for each of Gly49, Gly238, Leu223, Ala239, Leu52, and Pro53, and twice for each of Val224 and Lys234 (Table 6 and Figure 4). Compound no. 24 established the highest number of conventional H-bonds with 1JIJ and 2QZW. Compounds no. 21 and 24 were predicted as the most active for the three targeted receptors and showed deep embedding. Previously, it has been reported that tight embedding (<2.5 Å), as those outlined in our study, is usually associated with potential biological effects, including anti-inflammatory, antiproliferative, antimicrobial, and antioxidant effects ((Ben Saad et al., 2023; Akacha et al, 2022; Alreshidi et al, 2023). Overall, the in-silico modeling approach outlines that both antimicrobial and antioxidant effects of fenugreek compounds are thermodynamically possible. They showed that these biological processes were actual through in vitro tests. Our results support the beneficial, promising impact and health promotion of natural-derived compounds, phytotherapy, and medicinal plants, including *Trigonella foenum-gracum* L (Alreshidi et al, 2023; Othman et al., 2021; Rahmouni et al., 2022).

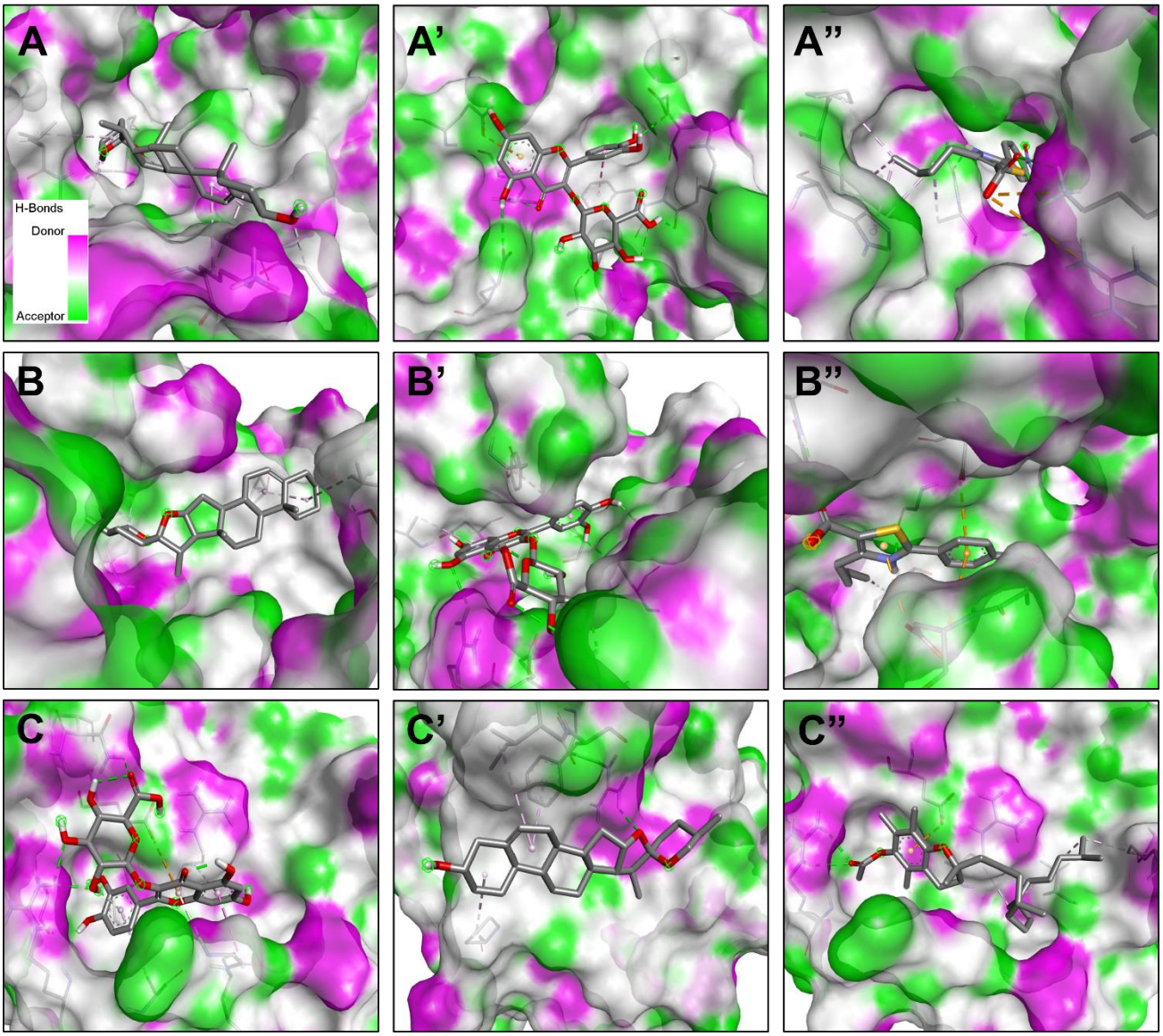

Figure 3: Tridimensional illustrations of the 3 targeted receptors 1JIJ (A, A' and A"), 2QZW (B, B' and B") and 1H2D (C, C' and C") with the 3 predicted best compounds identified in the fenugreek seeds. 1JIJ complexed with compounds no. 21 (A), 24 (A'), and 15 (A"). 2QZW complexed with compounds no. 21 (B), 24 (B'), and 15 (B"). 1HD2 complexed with compounds no. 24 (C), 21 (C'), and 23 (C").

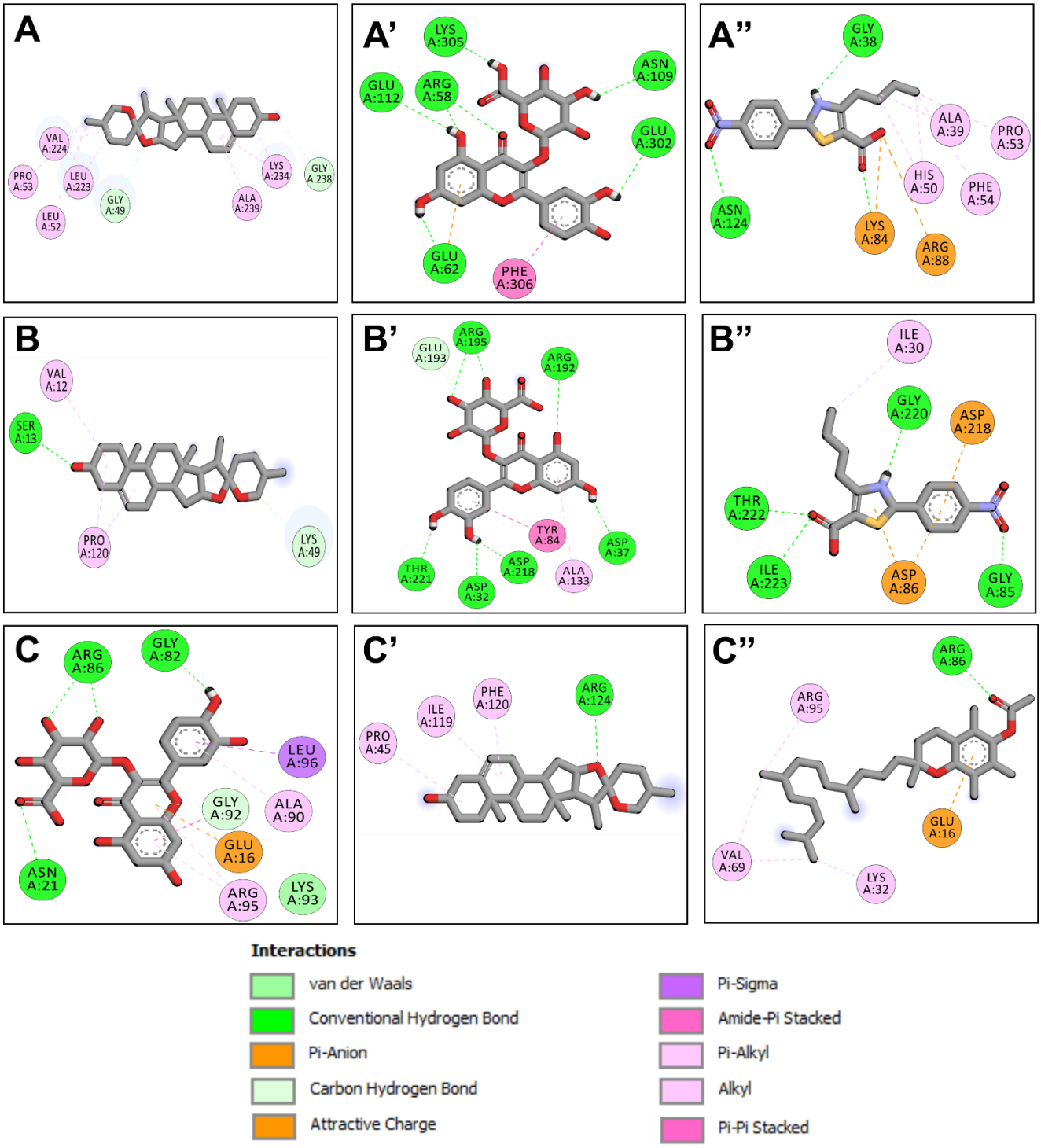

Figure 4: Illustration of the 2D diagrams of interactions of the 3 targeted receptors 1JIJ (A, A' and A"), 2QZW (B, B' and B") and 1H2D (C, C' and C") with the 3 predicted best compounds identified in the fenugreek seeds. 1JIJ complexed with compounds no. 21 (A), 24 (A'), and 15 (A"). 2QZW complexed with compounds no. 21 (B), 24 (B'), and 15 (B"). 1HD2 complexed with compounds no. 24 (C), 21 (C'), and 23 (C").

Table 7 exhibited the pharmacokinetics, drug-likeness, and medicinal chemistry of the fenugreek-identified compounds based on their ADME/Tox (for absorption, distribution, metabolism, excretion, and toxicity) properties. The pharmacokinetic analyses revealed acceptable drug-likeness and medicinal chemical properties for most identified compounds. Interestingly, most of the identified compounds did not inhibit the five assessed cytochrome P450 (CYPs) isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) and possessed good oral bioavailability. Furthermore, most of the fenugreek compounds stand on white and

yellow areas of the mapped boiled egg model (Figure 5), which indicates that these phytochemicals are predicted to be passively absorbed by the G.I. tract and passively permeate the BBB, respectively.

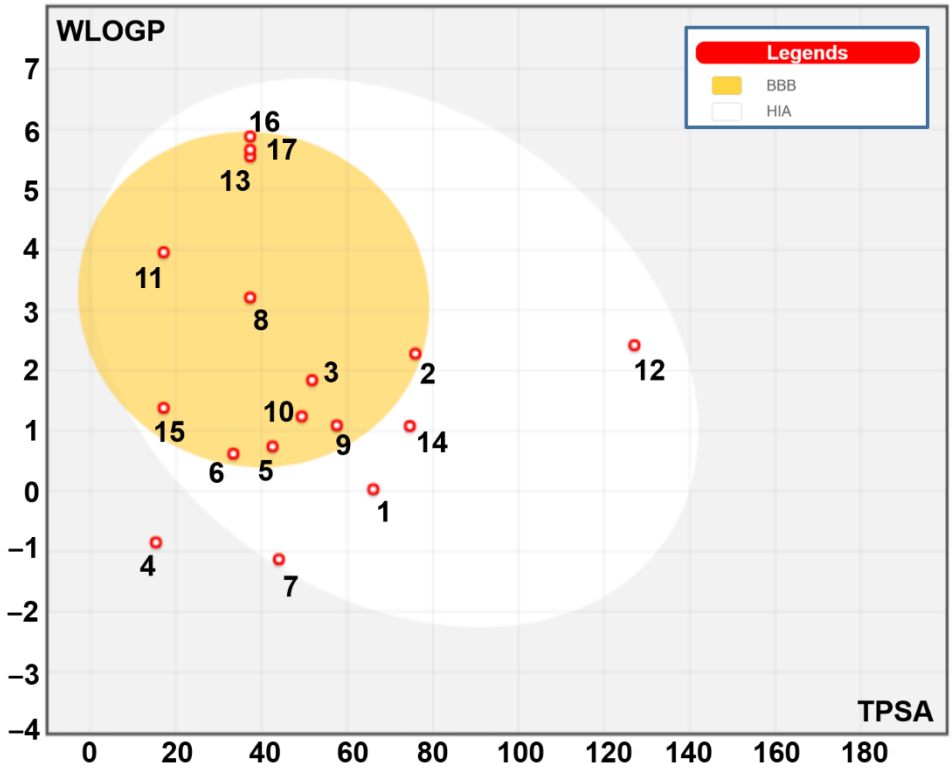

Figure 5. Boiled-egg model of the identified phytochemicals. The yellow and white areas correspond to the BBB (for blood-brain-barrier) permeation and GI (for gastro-intestinal) absorption, respectively.

Table 6. Lipophilicity, pharmacokinetics, druglikeness and medicinal chemistry of the identified compounds based on the ADME/Tox (for absorption, distribution, metabolism, excretion and toxicity) properties.

Entry	**1**	**2**	**3**	**4**	**5**	6	7	10	11	12	13	15	16	18	19	20	22	24
	Lipophilicity & physicochemical properties																	
TPSA	66.05	75.9	51.75	15.27	42.52	33.37	44.01	37.3	57.53	49.33	17.07	127.08	37.3	74.6	17.07	37.3	37.3	227.58
Log Po/w (iLOGP)	1.09	1.91	1.91	1.8	0.6	1.49	-3.11	2.5	0.85	1.17	3.16	2.28	3.85	0.74	1.52	1.52	3.39	1.13
Consensus Log Po/w	-0.24	1.51	1.12	0.32	0.01	0.62	-0.61	3	1.05	1.13	3.87	2.52	5.2	1.13	1.23	1.23	5.07	-0.47
Log S (ESOL) solubility	-0.56	-1.34	-1.09	-0.3	-0.17	-1.09	-1.39	-2.96	-2.07	-2.4	-3.49	-4.27	-5.02	-2.37	-0.86	-0.86	-4.7	-3.27
	Pharmacokinetics																	
GI absorption	High	High	High	Low	High	High	High	High	High	High	High	High	High	High	High	High	High	Low
BBB permeant	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No
P-gp substrate	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes
CYP1A2	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes	No	No	No	Yes	No
CYP2C19	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No
CYP2C9	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes	No	No	No	Yes	No
CYP2D6	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Log Kp (skin permeation)	High	High	High	Low	High	High	High	High	High	High	High	High	High	High	High	High	High	Low
	Druglikeness & Medicinal chemistry																	
Lipinski	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Biovailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.85	0.85	0.85	0.55	0.56	0.85	0.85	0.55	0.55	0.85	0.11
Leadlikeness	1	1	1	1	1	1	1	3	1	1	3	1	2	1	1	1	2	1
Synthetic accessibility	2.48	3.64	2.79	1.52	1.76	1.97	1.04	1.67	1	1	2.28	3.07	2.31	1	1	1	3.68	5.26
TPSA: Topological polar surface area; GI: Gastro-intestinal; BBB: Blood-brain-barrier; P-gp: P-glycoprotein; CYP: Cytochrome P450																		

Discussion

The results showed the presence of all phytochemical classes except terpenoids within fenugreek seeds. This agreed to a great extent with the studies of Kumari *et al*. (2016) and Mahmood and Yahya (2017), who found that *T. foenum-graecum* seeds contain alkaloids, glycosides, tannins, saponins, steroids, terpenoids, and flavonoids. These complex phytochemicals make fenugreek seeds a potential source against several microorganisms. Gram-positive bacteria demonstrated the most extensive zone of inhibition. This phenomenon could be attributed to the lipopolysaccharide layer and periplasmic zone encircling Gram-negative bacteria, which protect the cell membrane against the harmful impacts of the plant extract (Elsharkawy et al., 2019).

The alcohol extract of *T. foenum-graecum* seed was tested previously against *Bacillus subtilis* and *Candida parasitosis*. The results showed significant antimicrobial activities, and fenugreek seed can be recommended as a natural source for antibacterial herbal formation (Diab et al., 2023).

Of the 25 detected compounds, 5 were N-containing compounds, four were S-containing, one was Si-containing, 2 were sugar base-containing, and one was Br-containing compounds. Also, it was noticed that there were six acids, one ester, six phenols, and one ether among the detected compounds.

Fenugreek seeds contain functional biomolecules that activate the antioxidative and immunity systems. It contains steroids, essential fatty acids, oils, flavonoids, glycosides, and phenolics. Its extracts are eco-friendlier and more cost-effective than synthetic antibiotics (Chenganmal et al., 2022). Several components detected among fenugreek seeds showed antimicrobial activities, such as 4-butyl-2(4-nitrophenyl), 9,12-octadecadienoic acid, and 9-octadecanoic acids, while n-hexadecanoic acid shows antioxidant, inhibition, and nematocidal activities.

Among the detected compounds from fenugreek seeds, the steroidal saponin (diosgenin) has a pharmaceutical property, the alkaloid trigonelline which is the most distinguished component in Fenugreek, the flavonoids Apigenin-complex, and quercetin-complex has a significant antioxidant property, in addition to the phenolic: 4-hydroxybenzoic acid compound (Kiani et al., 2023). These compounds had a substantial contribution to fenugreek seeds' antimicrobial activity.

Conclusions

According to the findings of this study's chemical analysis, fenugreek seed is abundant in carbohydrates and protein but deficient in fiber, ash, and moisture consumption. The microbiological investigation found that fenugreek oil had high antibacterial efficiency against all the investigated species, with Escherichia coli being the specimen most susceptible to its effects. On the other hand, Fenugreek oil was found to have the most potent inhibitory effect against the *Aspergillus flavus* variety of fungus. The concentrated oil was the sole oil that showed inhibitory effects on the organisms studied. Both binding

affinities and molecular interactions of fenugreek phytochemicals support the experimental in vitro results. This may result in the bioavailability and pharmacokinetic properties associated with antibacterial, antifungal, and antioxidant effects. As a result, fenugreek seed oil is a precious asset to the chemical, medical, and food industries, and it also helps lengthen the shelf life of food products.

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Author Contributions

"Conceptualization, N.M. and A.E.; methodology, A.E. and N.A.; software, R.B.; validation, N.A. and A.E. and N. M.; formal analysis, A.E.; investigation, N.A.; resources, N. M..; data curation, R.B.; writing—original draft preparation, N.M.; writing—review and editing, A.E.; visualization, N.A.; supervision, A.E.; project administration, N.M.; funding acquisition, N.A. All authors have read and agreed to the published version of the manuscript.

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Ethics statement for the use of human and animal subjects

Not applicable

Data Availability

Data will be made available on request

Declaration

Competing interest

The authors declare no conflicts of interest.

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References

Aasim M, Baloch FS, Nadeem MA, Bakhsh A, Sameeullah M, Day S (2018)
Fenugreek (*Trigonella foenum-graecum* L.): An underutilized edible plant of modern world. In Global Perspectives on Underutilized Crops, ed. Ozturk, M., Hakeem, K., Ashraf,

M., and Ahmad, M., 381–408. Cham: Springer. https://doi.org/10.1080/10942912.2020.1825482

Akacha A, Badraoui B, Rebai T, Zourgui L (2022) Effect of Opuntia ficus indica extract on methotrexate-induced testicular injury: A biochemical, docking and histological study. Journal of Biomolecular Structure and Dynamics. 40 (10), 4341-4351. DOI: 10.1080/07391102.2020.1856187

Al-Huqail, AA, Elgaaly GA, Ibrahim M M (2018) Identification of bioactive phytochemical from two *Punica* species using G.C.–M.S. and estimation of antioxidant activity of seed extracts. *Saudi J. Biol. Sci. 25*, 1420–1428. DOI: 10.1016/j.sjbs.2015.11.009

Alreshidi M, Abdulhakeem MA, Badraoui R, Amato G, Caputo L et al (2023) *Pulicaria incisa* (Lam.) DC. as a Potential Source of Antioxidant, Antibacterial, and Anti-Enzymatic Bioactive Molecules: Phytochemical Constituents, In Vitro and In and In Silico Pharmacological Analysis. Molecules. 28 (21), 7439. https://doi.org/10.3390/molecules28217439

AOAC (2010) Official Methods of Analysis of Association of Official Analytical Chemists. 18th Edition, Washington, DC. DOI: 10.4236/ce.2020.118106

AOCS (1971) Official and Tentative Method. 3rd ed. American. Oil Chemist's Society , Chpaign, U.S.A. DOI:10.1007/s11745-001-0424-7

Awad MA, Abdel Moneim ES (2010) Effect of Some essential Oils on Aspergillus flavus Growth and Aflatoxin production.. *Gezira J. of Eng and App Sci.* 2010, 5 (1): 62-70 (DOI:10.4172/2157-7110.C1.044

Badraoui R, Allouche M, El Ouaer D, Siddiqui0 AJ, Ishak S Hedfi A et al (2023) Ecotoxicity of chrysene and phenanthrene on meiobenthic nematodes with a case study of Terschellingia longicaudata: Taxonomics, toxicokinetics, and molecular interactions modelling. *Environmental Pollution*. 316, 120459. DOI: 10.1016/j.envpol.2022.120459

Banso A, Adeyemo S, (2006) Phytochemical screening and antimalarial assessment of *Abutilon mauritianum, Bacopa monifera* and *Datura stramonium. Biokemistri*. 18: 39-44. DOI:10.4314/biokem.v18i1.56390

Bédoui I, Nasr HB, Ksouda K, Ayadi W, Louati N, Chamkha M, Choura C et al. (2024) Phytochemical Composition, Bioavailability and Pharmacokinetics *of Scorzonera undulata* Methanolic Extracts: Antioxidant, Anticancer, and Apoptotic Effects on MCF7 cells. *Pharmacognosy Magazine* 20 (1), 218-229.

Ben Saad H, Frikha D, Bouallegue A, Badraoui R, Mellouli M, Kallel H, Pujo JM., Ben AI (2023) Mitigation of Hepatic Impairment with Polysaccharides from Red Alga Albidum corallinum Supplementation through Promoting the Lipid Profile and Liver Homeostasis in Tebuconazole-Exposed Rats. Pharmaceuticals. 16 (9), 1305. https://doi.org/10.3390/ph16091305

Benhammou N, Larbi B, Bechlaghem N, Ghembaza N, Bekkara F (2017) Assessment of antioxidant potential and phytochemical analysis of *Pituranthos scoparius* crude extract and its fractions. Oriental Pharmacy and Experimental Medicine., 17. DOI 10.1007/s13596-016-0253-7

Chenganmal M, Narayanan C (2022) Phytochemical screening analysis and isolation of bioactive components in Fenugreek using GC-MS study. *Asian J. Plant Science Res*. 12(7):1-7. https://www.imedpub.com/articles/ =48225

Daoud A, Malika D, Bakari S, Hfaiedh N, Mnafgui K, Kadri A et al. (2019) Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of date palm pollen (DPP) from two Tunisian cultivars. Arab. J. Chem. 12 (8): 3075-3086. http://dx.doi.org/10.1016/j.arabjc.2015.07.014 1878-5352 [a] 2015

Diab, A. M.; Al-Khefa B. T.; Khalafallah, M. M.; Salah, A. S.; Farrag, F. A. and Dawood, M. A. O. Dietary methanolic extract of FenugreekFenugreek enhanced the growth, haematobiochemical, immune responses and resistance against *Aeromonas hydrophila* in Nile Tilapia *Oreochromis niloticus. Aqua Res.* 2023, 3055476, 13 pages. http://doi.org/10.115512023/3055476.

Elsharkawy, E. R.; Abdallah, E. M.; Shiboob, M. H.; Alghanem, S. Phytochemical, antioxidant and antibacterial potential of *Ducrosia anethifolia* in Northern border region of Saudi Arabia. *J. Pharm. Res. Int.*, 2019, 31: 1–8. **DOI:** 10.9734/jpri/2019/v31i630361

Gavahian, M.; Bannikoppa, A.M.; Majzoobi, M.; Hsieh, C.W.; Lin, J.; Farahnaky, A. Fenugreek bioactive compounds: A review of applications and extraction based on emerging technologies. *Crit Rev Food Sci Nutr*. 2023 Jun 11:1-17. DOI: 10.1080/10408398.2023.2221971

Idriss, H.; Siddig, B.; Maldonado, P. G.; Elkhair, H.; Alakhras, A.; Abdallah, E. M.; Torres, P. H. S.; Elzupir, A. O. Phytochemical Discrimination, Biological Activity and Molecular Docking of Water-Soluble Inhibitors from *Saussurea costus* Herb against Main Protease of SARS-CoV-2. *Molecules* 2022, *27*, 4908. https://doi.org/10.3390/molecules27154908

Kiani, H. S.; Ali, B.; Al-Sadoon, M. K.; Al-Otaibi, H. S. and Ali, A. LC-MS/MS and GC-MS identification of metabolites from the selected herbs and spices, their antioxidant, antidiabetic potential and chemometric analysis. *Processes*. 2023, 11:2721. https://doi.org/10.3390/pr11092721

Kumari, O. S.; Rao, N. B.; Gajula, R. G. Phytochemical analysis and antimicrobial activity of *Trigonella foenum-graecum* (Methi seeds). *J. Medicinal Plants Studies*. 2016, 4(4): 278-281. https://www.plantsjournal.com/archives/2016/vol4issue4/PartD/4-3-38-842.pdf

Mahmood, M. N.; Yahya, I. K. (2017). Nutrient and phytochemical of FenugreekFenugreek (*Trigonella foenum-graecum*) seeds. *International Journal of Science: Basic and Applied Research*. 2017, 36(3): 203-213. https://www.gssrr.org/index.php/JournalOfBasicAndApplied/article/view/8250

Mhadhbi, N.; Dgachi, S.; Belgacem, S.; Ahmed, A.B.; Henry, N.; Loiseau, T. et al.. Design, theoretical study, druggability, pharmacokinetics and properties evolution of a new organo-bromocadmate compound as prospective anticancer agent. Journal of Molecular Structure. 2023, 1274 (2), 134439. DOI:10.3390/cryst13050746

Negu, A.; Zegeye, A. and Astatkie, T. Development and quality evaluation of wheat based cookies enriched with fenugreek and oat flours. Journal of Food Science and Technology. 2020, 57 (10):3573–80. doi: 10.1007/s13197-020-04389-3

Othman, I.M.M.; Gad-Elkareem, M.A.M.; Radwan, H.A.; Badraoui, B.; Aouadi, K. et al. Synthesis, Structure-Activity Relationship and in silico Studies of Novel Pyrazolothiazole and Thiazolopyridine Derivatives as Prospective Antimicrobial and Anticancer Agents. ChemistrySelect. 2021, 6 (31), 7860-7872.https://doi.org/10.1002/slct.202101622

Raaman, N. Phytochemical techniques. ISBN: 8189422308, 9788189422301, 2006. New India Publishing.

Rahmouni, F.; Badraoui, R.; Ben-Nasr, H.; Bardakci, F.; Elkahoui, S. et al. (2022). Pharmacokinetics and therapeutic potential of Teucrium polium against liver damage associated hepatotoxicity and oxidative injury in rats: computational, biochemical and histological studies. Life. 2022, 12 (7). DOI: 10.3390/life12071092

Rahmouni, F.; Hamdaoui, L.; Saoudi, M.; Badraoui, R., Rebai, T. 2024. Antioxidant and antiproliferative effects of Teucrium polium extract: computational and in vivo study in rats. Toxicology Mechanisms & Methods. 2024, 2:1-37. DOI: 10.1080/15376516.2023.2301670

Rashid, F.; Bao, Y.; Ahmed, Z. and Huang, J.-Y. (2020). Effect of high voltage atmospheric cold plasma on extraction of fenugreek galactomannan and its physicochemical properties. *Food Research International* (Ottawa, Ont.). 2020, 138 (Pt A):109776. https://doi.org/10.1016/j.foodres.2020.109776

Ruwali, P.; Pandey, N.; Jindal, K. and Singh, R. V. (2022). Fenugreek (*Trigonella foenum-graecum*): Nutraceutical values, phytochemical, ethnomedicinal and pharmacological overview. *South African Journal of Botany*. 2020, 151:423–31. DOI 10.7717/peerj.16842

Sakhare, S. D.; Prabhasankar, P. Effect of fenugreek fiber on rheological and chapati making quality of whole wheat flour. *Journal of Food Science and Technology*. 2022, 59 (2):532–41. DOI: 10.1007/s13197-021-05037-0

Standard Methods for Analysis of Fats, Oils and Derivatives. International Union of Pure and Applied Chemistry. 6th edn. 1979, 45-70, Pregamon Press, Oxford. DOI:10.1007/s11745-001-0424-7

Sulieman, A. E.; Shaarawy, S. M., Alghamdi, A. A.; Veettil, V. N.; Abdelgadir, M.; Ibrahim, N. A. Evaluation of antimicrobial and synergistic effects of selected medicinal plants of Hail area with antibiotics. *Bioscience Biotechnology Research Communications*. 2017a; (10), 44-50. DOI:10.21786/bbrc/10.1/7

Sulieman, A.E.; Alanaizy, E.; Alanaizy, N.A.; Abdullah, E.M.; Idriss, H.; Salih, Z.A., Ibrahim, N.A.; Ali, N.A.; Ibrahim, S.E.; Abd El Hakeem, B.S. Unveiling Chemical, Antioxidant and Antibacterial Properties of Fagonia indica Grown in the Hail Mountains, Saudi Arabia. *Plants*. 2023, (12), 1354. https://doi.org/10.3390/plants12061354

Wani, A. A.; Kumar P. Fenugreek: A review on its nutraceutical properties and utilization in various food products. *Journal of the Saudi Society of Agricultural Sciences*. 2018; 17 (2): 97-106. https://doi.org/10.1016/j.jssas.2016.01.007