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Research Article

Chemical Characterization, Antioxidant, Antimicrobial Potential, and Molecular Docking Studies on the Essential Oil of *Festuca glauca*

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Abstract

Pharmacognostic recognition is growing for *Festuca glauca*, a perennial ornamental grass with a rich phytochemical profile. This species has shown potential in antibacterial and other therapeutic applications. The purpose of this work was to analyze the essential oil (EO) of *Festuca glauca (F. glauca)* for its chemical composition, antibacterial and antioxidant capabilities, and the interaction of its primary phytoconstituents with microbial targets using molecular docking. The EO was extracted using hydrodistillation and evaluated using Gas Chromatography-Mass Spectrometry (GC-MS). Antimicrobial activity was determined using diffusion, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and dilution techniques. Antioxidant activity was assessed utilizing the (2, 2-diphenyl-1-picrylhydrazyl) DPPH and (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) ABTS assays. Piperitone's molecular docking was accomplished with Auto Dock, which is available in PyRx. The EO yield was 2% (w/w), with 31 components, piperitone (73.17%) being the main product. The EO showed significant antibacterial activity against *Klebsiella pneumoniae (K. pneumonia)* (27 mm inhibition zone) and *Pseudomonas aeruginosa (P. aeruginosa*) (28 mm), with MIC values ranging from 156.25-312.5 µg/mL. The antifungal activity was moderate, with *Trichoderma* wild displaying the maximum susceptibility (MIC = 625 µg/mL). In antioxidant tests, the EO showed IC_{50} values of 18 µg/mL (DPPH) and 30 µg/mL (ABTS). Piperitone has high binding affinities to several microbial protein targets (−3.8 to −6.7 kcal/mol), which supports its biological activities. We concluded that the EO of *F. glauca* has excellent antibacterial and antioxidant properties, most probably owing to its high piperitone content, indicating its potential as a natural medicinal agent. More *in vivo* research, safety assessments, and formulation development are required to enable clinical and commercial use.

Keywords: *Festuca glauca,* antioxidants, antimicrobial, molecular docking, piperitone

1. Introduction

Essential oils (EOs) are concentrated, naturally occurring combinations of volatile chemicals taken from various parts of plants (Bogdan, Bungau et al. 2021). They were initially identified for their therapeutic effects and utilized as medicines before finding applications in the perfume and cosmetics industries, as well as cleaning goods, food, and beverages. EOs are known to offer a variety of health benefits, including antibacterial, antifungal, antioxidant, antiparasitic, antiseptic, and antiviral activities, as well as insecticidal qualities (Badr, Badawy et al. 2021, Li, Zhao et al. 2022, Zouirech, Alyousef et al. 2022).

EOs have shown significant antiparasitic efficacy in animal and experimental contexts, attributable to their antibacterial and antiviral characteristics. *Ellettaria cardamomum (E. cardamomum)* oil effectively suppressed *Leishmania* major promastigotes *in vitro* and drastically reduced the lesions in infected animals, without causing systemic harm (Majeed, Alshammari et al. 2023). The oil of *Mentha suaveolens* shows notable

antileishmanial effects, attributable to piperitenone oxide (Bailén, Illescas et al. 2023). EOs, including *Cymbopogon zizanoides (C. zizaniodes)* root oils, possess antiparasitic, antibacterial, and trypanocidal effects (Oliveira, Vieira et al. 2022).

The widespread use of antibiotic therapy has increased antimicrobial resistance among both Gram-negative and Gram-positive microorganisms, rendering certain diseases untreatable and contributing to increasing morbidity and mortality rates globally (Kakoullis, Papachristodoulou et al. 2021, Liu, Tong et al. 2021, Temperoni, Caiazzo et al. 2021). Furthermore, the increased resistance of infections to routinely used antibiotics complicates treatment techniques and prevents appropriate patient care (Kasew, Desalegn et al. 2022, Salam, Al-Amin et al. 2023). Consequently, there is increasing interest in finding and creating natural substitutes for antibacterial agents.

In the United States, infections caused by multidrug-resistant bacteria constitute the third leading cause of death, resulting in around 162,000 adult fatalities each year (Nadimpalli, Chan et al. 2021). Antimicrobial resistance (AMR) is a major issue globally, although it is more severe in less developed countries. Many believe that antibiotic-resistant bacteria primarily reside in South Asia. There is a growing concern on a national and international scale because antibiotic resistance is predicted to be 70% higher in the Asia region (Kang and Song 2013). The rising antibiotic resistance in Pakistan, a South Asian country, represents a major concern both worldwide and regionally. In recent years, Pakistan has witnessed an increase in the prevalence of drug-resistant pathogens and viruses. Quinolone resistance has emerged in the Enterobacteriaceae family in Pakistan over the last decade. In 2016, an epidemic of Extensively Drug-Resistant (XDR) Salmonella showed complete resistance to fluoroquinolones (Yasmin, Akhtar et al. 2013, Abrar, Hussain et al. 2018, Qamar, Yousafzai et al. 2018).

The Poaceae family is among the largest and most varied plant groups and contains many species that are known to have pharmacological and medicinal value (Hassan, Aboel-Ainin et al. 2021, Farouk, Fahim et al. 2023, Kocięcka, Liberacki et al. 2023). Numerous species of this family, including *Pennisetum purpureum* and *Cymbopogon citratus*, have shown a wide range of biological activity, including antioxidant, antifungal, and antibacterial qualities (Budiyanto, Puspitarini et al. 2024; Kiełtyka-Dadasiewicz, Esteban et al. 2024; Singh, Singh et al. 2021; Vinayagam, Santhoshkumar et al. 2021). Their abundance of bioactive secondary metabolites, especially in their EOs, is largely responsible for these actions. The chemical and biological potential of *F. glauca,* a perennial ornamental grass, is still little understood despite the substantial research on numerous Poaceae species.

There are currently no systematic investigations into the chemical profile and bioactive potential of *F. glauca* EO. This study is therefore meant to analyze, for the first time, the chemical composition of *F. glauca* EO using GC-MS, as well as to evaluate its antioxidant and antibacterial activity using recognized *in vitro* assays. Furthermore, molecular docking was used to investigate the interaction of piperitone, the main EO ingredient, with microbial protein targets, offering insights into its potential mechanism of action. The study seeks to establish *F. glauca* as a viable natural source of bioactive chemicals with pharmacological and therapeutic applications.

2. Materials and Methods

2.1 Equipment, Chemicals, and Reagents

Standard microbiology and analytical laboratory equipment were used in the study. Dimethyl sulfoxide (DMSO) (Gaylord Chemical Company, LLC Crown Zellerbach), methanol, Mueller Hinton Agar (MHA)(HIMEDIA Laboratories Private Limited), an Indian origin Bioscience company headquartered in Mumbai, Maharashtra, India, ciprofloxacin, fluconazole, ABTS, DPPH, and other standard microbiological

Table 1: Chemical constituents of *F. glauca* EO

S.NO	Name	Retention Time	Area	Concentration %
1	Biclo[3,1,1]hept-2-ene,2,6,6-trimethyl	9.238	10094	0.08
2	Camphene	9.837	18588	0.14
3	Beta-cymene	10.832	526165	4.07
4	2-carene	12.182	587126	4.55
5	p-Cymol	13.337	43991	0.34
6	Cyclobutane,1,2-bis(1-methylethenyl)trans	13.524	239611	1.86
7	Eucarvone	16.137	10270	0.08
8	(-)-Fenchone	16.330	30927	0.24
9	6-Camphenone	16.693	11847	0.09
10	beta-Linalool	17.158	8773	0.07
11	2-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-trans	17.976	172695	1.34
12	2-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-cis	18.640	120367	0.93
13	Borneol,heptaflourobutyrate (ester)	19.485	42851	0.33
14	Cyclohexene,3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl	19.558	31787	0.25
15	trans-2-Caren-4-ol	19.770	18178	0.14
16	trans-3(10)-Caren-2-ol	20.017	18134	0.14
17	p-Cymen-8-ol	20.140	134691	1.04
18	p-menth-1-en-3-ol,trans-	20.669	86192	0.67
19	Umbellulon	20.919	95870	0.74
20	Verbenone	21.039	16861	0.13
21	Piperitone	21.804	9448507	73.17
22	2(IH)-Naphthalenone,octahydro-4a-methyl-trans	23.816	119561	0.93
23	Caryophyllene	24.849	44835	0.35
24	alpha-Caryophyllene	25.394	116764	0.90
25	delta-Cadinene	26.362	9236	0.07
26	alpha-Limonene diepoxide	26.593	33292	0.26
27	Elemol	26.773	14225	0.11
28	2,4,7,14-Tetramethyl,1-4-vinyl-tricyclo[5,4,3,0(18)]tetradecon-6-ol	27.277	543320	4.21
29	5-Azulenemethanol,1,2,3,4,5,6,7,8-octahydro	27.981	20777	0.16
30	Juniper camphor	28.330	235565	1.82
31	Hexanedioic acid,bus(2-ethylhexyl)ester	32.055	101200	0.78

media were purchased from commercial suppliers.

2.2 Microbial Strains

The Microbiology Laboratory, Department of Pharmacy, KUST, provided four bacterial strains (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa*) and four fungal strains (*Aspergillus parasiticus, Aspergillus green, Trichoderma wild, and T. harzianum)*.

2.3 Sample Collection and Identification

The flowering aerial parts and leaves of *F. glauca* were gathered from the District of Kohat, Khyber Pakhtunkhwa, Pakistan, in September 2023. Samples were carefully washed 2-3 times with deionized water before being air-dried at room temperature and utilized for EO extraction.

2.4 Essential Oil Extraction

EOs, were extracted from the plant *F. glauca using* hydro-distillation using a modified Clevenger apparatus (Okoh, Asekun et al. 2014). The cleaned and chopped plant material was distilled for around 4 hours. The condensed vapors were collected, and the oils formed an upper layer due to their immiscibility and low density. The extracted oil was stored in amber vials, labeled, and kept at 4-6 °C for further examination. Specific gravities were determined using the procedure outlined by ASTM (2006) for liquid fats and oils. This approach comprises figuring out the proportion of the sample's weight per unit volume to that of a unit volume of water at 25 °C (Hati, Dimari et al. 2010).

2.4.1 GC-MS Analysis

An autosampler-equipped Shimadzu QP 2010 Plus GC-MS system (GC-MS 5977B, Agilent Technologies, USA) was used to do chemical profiling of the EOs. Helium was used as the carrier gas in a TRB-FFAP capillary column (30 m × 0.25 mm, 0.25 μm film thickness, polyethylene glycol coating). The injector split ratio was 1:50, injection volume 1 μL, ion source and interface temperatures were set at 240 °C, and pressure was set at 80 kPa. These parameters were set as GC-MS parameters. The oven was set to 40°C for 3 minutes, then ramped up to 90°C at 15°C per minute, 240°C at 2.5°C per minute (held for 4–6 minutes), and lastly 220°C at 10°C per minute (held for 5–6 minutes). The range of mass spectra observed was 40–500 m/z. The manufacturer's software was used for data collection and system control.

2.4.2 Component Identification

Retention times and mass spectra were compared to available data and NIST library standards to determine the components of the oil. Using integrated software, relative abundances were computed from total ion chromatograms.

2.5 Antimicrobial Assays

2.5.1 Agar Well Diffusion (*In vitro* antibacterial characterization)

The Well Diffusion method was used to assess EO's antibacterial activity (Mirtaghi, Nejad et al. 2016). MHA (38g/1L) (Kumar, Prabhakumari et al. 2022) was injected with bacterial cultures. After boring wells, EO serial dilutions (5000–156.25 μg/mL in DMSO) were added. The positive control in this case was ciprofloxacin. Zones of inhibition were evaluated after plates were treated for 24 hours at 37°C.

2.5.2 MIC Determination

In 96-well plates, we adjusted the concentrations of the *Festuca glauca* extract from 3.125 to 100 μg/mL utilizing two series of serial two-fold dilutions. Starting with the initial row, 75 μL of each serially diluted extract was allocated to each well in a vertical arrangement. To maintain consistency, we replicated the technique on a second 96-well plate. The plates were subsequently kept at a temperature of 35–37 °C for an entire day. After the incubation period, the wells were administered a 0.01% solution of 2,3,5-triphenyltetrazolium chloride (TTC). The hue of the tetrazolium salt changed with an extra hour of incubation. The bioactive component suppresses development without any alteration in color. The color is preserved, and growth is completely suppressed at the MIC (Sulieman, Ibrahim et al. 2024). Using the different concentration series, the agar well diffusion method was used to determine

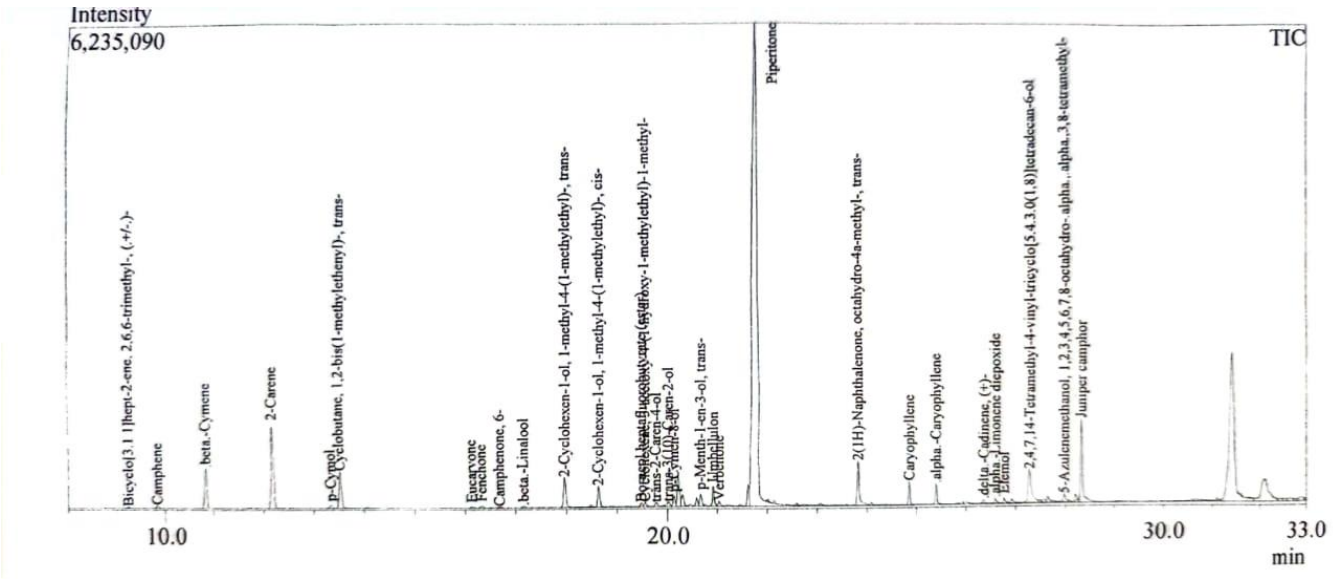

Figure 1: Gas chromatography mass spectrometry spectrum of EO extracted from *F. glauca*

the MIC. As a negative control, DMSO (Shanghai Kean Technology Co., Ltd) was employed.

2.5.3 Minimum Bactericidal Concentration (MBC) by 96-Well Microdilution

We calculated the MBC of the extract from the *Festuca glauca* against the chosen bacterial strains: Each MIC tube that did not show any growth had 100 µL transferred to Mueller-Hinton agar plates. Then, for 24 hours, we incubated the plates at 35-37 °C in an incubator. Then, bacterial colonies were looked for on the plates. When no bacterial colonies are seen at a given concentration, we determined MBC (ALrajhi, Al-Rasheedi et al. 2019, Pasquina-Lemonche, Burns et al. 2020).

2.5.4 Antifungal Activity (Agar Tube Dilution)

Antifungal activity was carried out using the agar tube dilution technique (Ertürk 2006). EO was combined with Sabouraud Dextrose Agar at 45°C and placed into test tubes. Fungal inocula were added to the tubes, which were cultured at 25°C for 5 days. The MIC was calculated as the lowest concentration that did not cause noticeable fungal growth. Fluconazole was utilized as the positive control.

2.6 Antioxidant Assays

2.6.1 DPPH Radical Scavenging Assay

In this study, we used the DPPH (MedChem Express LLC, USA) free radical scavenging method to assess the EO's antioxidant capacity (Braca, De Tommasi et al. 2001). The antioxidant activity of EO was evaluated using a DPPH solution (24 mg/100 mL methanol). After mixing DPPH with EO at serial dilutions (15.62–1000 µg/mL), absorbance at 517 nm was measured 30 minutes later. A UV-Vis spectrophotometer (Shimadzu) was used for this assay. The standard was ascorbic acid. Triplicate experiments were used to calculate the IC_{50}.

2.6.2 ABTS Assay

Utilizing the 2, 2-azinobis [3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) (Muby Chemicals, Ambernath, and Mumbai, India) technique, EO antioxidant capacity was assessed (Re, Pellegrini et al. 1999). After reacting 7 mM ABTS with 2.45 mM potassium persulfate and letting it sit in the dark for 12–16 hours, an ABTS radical cation was produced. At 734 nm, the working solution's absorbance was tuned to 0.70. After mixing EO dilutions (15.62–1000 µg/mL) with ABTS, absorbance was measured one minute later. IC_{50} and percent inhibition were computed. The overall assay is performed by a UV-Vis spectrophotometer (Shimadzu).

2.7 Molecular Docking

Target proteins that were obtained from the PDB (IDs: 6RKS (Loganathan and Krishnan 2025),

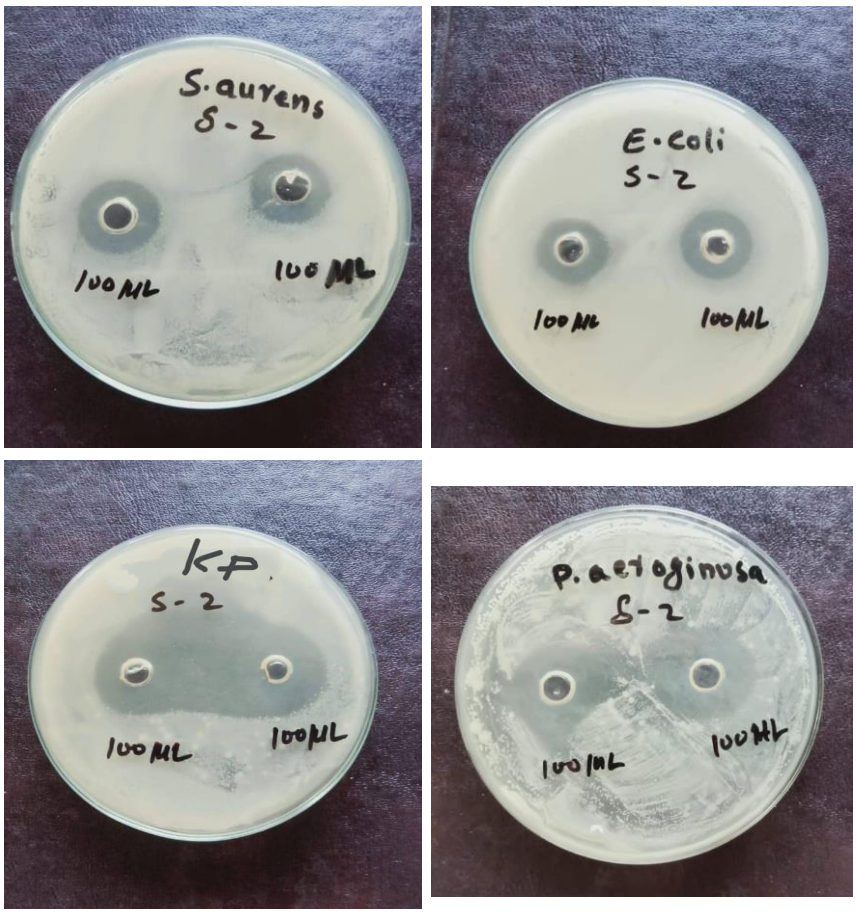

Figure 2: Zone of inhibition of bacteria by *F. glauca* essential oil.

7U9K (Becker, Pederick et al. 2023), 8BN6 (Ejidike, Direm et al. 2025), 5O79 (Isah, Sul'ain et al. 2025), 1UKC (Sharma and Choudhary 2024), 5WOA (Yelshanskaya and Sobolevsky 2022), 5ZZD (Godwini, Monicka et al.), and 3AHY (Smith, Romero et al. 2013)) were used in piperitone docking investigations. To prepare proteins, Discovery Studio Visualizer was used. We utilized Auto Dock Vina for docking, which is available through PyRx, which helps in docking using Vina algorithms, and ligand–receptor interaction binding energies (kcal/mol) were examined.

3. Results

3.1. Percentage Yield of *F. glauca* EO

After six hours of hydro distillation, the EO production from *F. glauca* was 2% (w/w) on a dry weight basis.

3.2. Physicochemical Characteristics of the EO

After four days of storage, the colorless, freshly isolated EO from *F. glauca* turned yellow. It was insoluble in water but showed good solubility in ethanol and DMSO. The oil's specific gravity was found to be 0.7.

3.3. Chemical Composition of *F. glauca* EO

GC-MS analysis of the EO discovered 31 phytochemicals based on retention time and concentration (Table 1). Piperitone prevailed with 21.804 minutes retention and 73.17% relative concentration. It was the extract's main component. Monoterpene ketone piperitone is antibacterial, anti-inflammatory, and antioxidant. Tetradecon-6-ol (4.21%), 2-carene (4.55%), beta-cymene (4.07%), and trans-cyclobutane, 1,2-bis(1-methylethenyl) terpenoid or hydrocarbon derivatives were other components of the EOs.

Table 2: The antibacterial activity of *F. glauca* EO

S. No.	Test bacteria	Zones of inhibition in (mm)	Standard (ciprofloxacin) in (mm)
1	*S. Aureus* (ATCC23235)	18mm	22mm
2	*K.pneumoniae* (ATCC13883)	27mm	36mm
3	*E.coli* (ATCC25922)	17mm	21mm
4	*P.aeroginosa*(ATCC15442)	28mm	34mm

These compounds may be antibacterial and antioxidant. Camphene, (-)-fenchone, verbenone, elemol, and caryophyllene were found in low amounts (<1%). These compounds may boost EO biological activity despite their low concentration. Aromatic alcohols include p-cymen-8-ol, trans-2-caren-4-ol, and p-menth-1-en-3-ol. Pharmaceutical and culinary firms use these compounds as antiseptics and flavourings. This study found terpenoids, ketones, alcohols, and esters in the EO, suggesting medical, cosmetic, and agrochemical uses. Piperitone was used as the main bioactive lead molecule for docking and pharmacological studies due to its chemical dominance (GC-MS Figure 1).

3.4. In vitro antibacterial characterization

The *in vitro* antibacterial characterization of *F. glauca* EO was assessed utilizing the agar well diffusion technique to determine MIC and MBC. The EO demonstrated the highest inhibitory zones against *K. pneumoniae* (27 mm), *P. aeruginosa* (28 mm), *S. aureus* (18 mm), and E. coli (17 mm), as indicated in Table 2 and Figure 2. Ciprofloxacin was employed as the standard reference (Chalkley and Koornhof 1985), and DMSO was used as a negative control, demonstrating no inhibitory effect. The *in vitro* antibacterial characterization was robust, with MIC values ranging from 156.25 to 312.5 µg/ml and MBCs universally at 156.25 µg/ml across all tested bacterial strains (Table 3, Figure 3). The activity of *F. glauca* EO appears to be similar to the reference antibiotic. The MBCs of *K. pneumonia, P. aeruginosa, S. aureus, and E. coli* were 156.25µg/ml, while the MICs ranged from 156.25 to 312.5µg/ml.

3.4.2. Antifungal Activity

The antifungal activity of *F. glauca* EO was evaluated using the agar tube dilution method. The EO efficiently inhibited all investigated fungal strains, with MIC values of 2500 µg/ml for *Aspergillus green, Aspergillus parasiticus (A. parasiticus), and Trichoderma harzianum (T. harzianum)*, and 625 µg/ml for Trichoderma wild (see Table 4). Fluconazole was utilized as a positive control.

3.5. Antioxidant Activity

3.5.1. DPPH Radical Scavenging Assay

The EO showed dose-dependent antioxidant activity in the DPPH experiment. The IC_{50} value of *F. glauca* EO was 18 µg/ml, compared to 5 µg/ml of normal ascorbic acid (Table 5). The percent inhibition varied between 95.3 ± 0.45% at 1000 µg/ml and 45.3 ± 0.50% at 15.62 µg/ml.

3.5.2. ABTS Radical Scavenging Assay

In the ABTS assay, the EO demonstrated excellent antioxidant capacity with an IC_{50} value of 30 µg/ml, whereas ascorbic acid had an IC_{50} value of 9 µg/ml. The inhibition percentages varied from 90.20 ± 0.70% to 39.80 ± 0.50% for EO and 96.30 ± 0.50% to 54.70 ± 0.55% for ascorbic acid (Table 6).

3.6. Molecular Docking Studies

Molecular docking was used to determine the binding affinity of piperitone, the main component of the EO, to several bacterial and fungal protein targets. The chemical exhibited positive interactions with all eight target proteins:

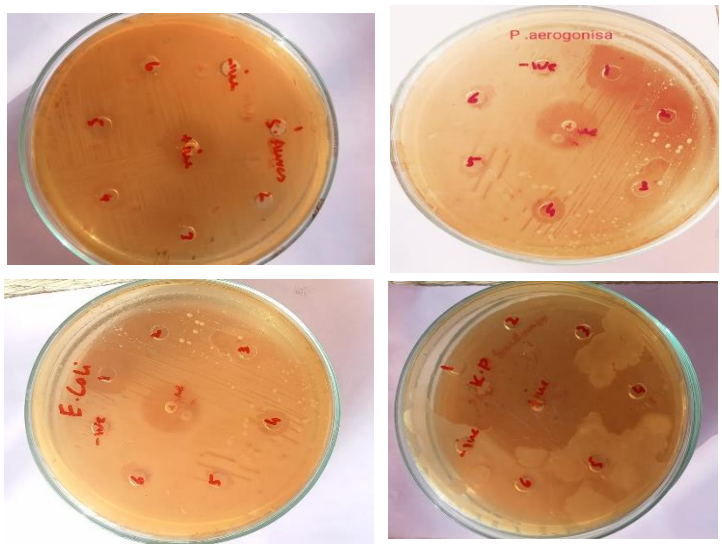

Figure 3: MIC of *F. glauca* essential oil

Table 3: MICs and MBC values for *F. glauca* essential oil

S.No	Test Bacteria	MICs (µg/ml)	MBC(µg/ml)
1	*S. Aureus (ATCC23235)*	156.25	156.25
2	*K.pneumonia (ATCC13883)*	312.5	156.25
3	*E.coli (ATCC25922)*	156.25	156.25
4	*P. aeruginosa (ATCC15442)*	156.25	156.25

6RKS, 7U9K, 8BN6, 5O79, 1UKC, 5WOA, 5ZZD, and 3AHY, with binding energies ranging from -5.3, -5.1, -4.7, -6.2, -4.2, -3.8, -5.3, and -6.7 kcal/mol, respectively (Table 7, Figure 3. Piperitone makes alkyl bond with LYS249 with bond length (3.92Å) in 7U9K, alkyl bond with PHE23 (5.09Å), HIS21 (5.26Å), LEU338 (4.23Å), TYR91 (4.82Å) in 5O79, alkyl bond with ALA67 (4.12Å), VAL70 (4.73Å), ILE74 (3.85Å) in 6RKS, alkyl bond with ILE540 (4.17Å) in 5WOA. Alkyl bond with ALA345 (3.67Å), CYS341 (4.63Å), LEU346 (4.41Å), ILE342 (5.09Å), LEU138 (5.02Å) in 5ZZD, alkyl bond with TRP417 (5.18Å), TYR298 (4.68Å) and pi-sigma bond with TRP339 (3.94Å), PHR433 (3.68Å) in 3AHY. Alkyl bonds with ILE96 (3.82Å) in 8BN6, and Piperitone forms a pi-sigma bond with TYR81 (3.79Å) and a hydrogen bond with ASN136 (2019Å) in (1UKC). No hydrogen bond interaction was detected with other targets.

4. Discussion

This study focuses on the phytochemical composition, biological potential, and *in silico* interactions of *F. glauca* EO. Hydro distillation yielded 2% (w/w) EO, a reasonable and feasible yield when compared to other *Festuca* species (Sahebkar and Iranshahi 2011), indicating its potential use as a source of bioactive volatiles. Initially colorless, the EO gradually turned yellow, most likely owing to oxidation. Its solubility in DMSO and ethanol, but not in water, demonstrates its lipophilicity. The specific gravity (0.7) is within the normal range for EOs. The GC-MS study detected 31 components, with piperitone (73.17%) being the most abundant—

Table 4: MICs of antifungal activity of EO of *F. glauca*

S. No.	Test fungi	MIC (µg/ml)
1	*Aspergillus green (ATCC16888)*	2500
2	*Aspergillus parasiticus (ATCC15517)*	2500
3	*Trichoderma wild*	625
4	*T.harzinum (ATCC60850)*	2500

Table 5: DPPH scavenging ability of *F.glauca* essential oil

Plant sample	Concentration (µg/ml)	Percent inhibition	IC_{50}
Oil	1000	95.3 ± 0.45	18
	500	87.5 ± 0.85	
	250	79.2 ± 0.50	
	125	76.5 ± 0.50	
	62.50	69.4± 0.50	
	31.25	57.5± 0.50	
	15.62	45.3± 0.50	
Ascorbic acid	1000	98.60 ± 0.50	5
	500	92.40± 0.90	
	250	82.25 ± 0.85	
	125	78.80± 0.65	
	62.50	74.44 ± 1.10	
	31.25	68.91 ± 0.70	
	15.62	60.13 ± 0.90	

known for its antibacterial and antioxidant effects. Bioactive substances, including 2-carene and β-cymene, contribute to the oil's multifunctional properties. In contrast, a previous study shows that *Festuca arundinacea* EO, produced at a significantly lower yield (0.005% w/w), included 71 components. Acids (39.8%) and alcohols (10.4%) were prominent, with lesser quantities of esters, hydrocarbons, aldehydes, and terpenes (Tava, Berardo et al. 1991). Although chemically varied, its poor yield limits practical application. Overall, *F. glauca* EO is more promising for therapeutic and industrial uses than *F. arundinacea* due to its higher yield and concentration of powerful chemicals such as piperitone.

There is an urgent need for novel antimicrobial drugs because the efficiency of existing antibiotics has been reduced due to the emergence of multidrug-resistant organisms (Karaiskos and Giamarellou 2014, Fatima, Purkait et al. 2023, Alara and Alara 2024). Plant-derived chemicals provide promising alternatives, with EOs

Table 6: ABTS scavenging ability of *F.glauca* essential oil

Plant sample	concentration (µg/ml)	Percent inhibition	IC_{50}
Oil	1000	90.20 ± 0.70	30
	500	80.55 ± 0.80	
	250	71.40 ± 0.85	
	125	76.80 ± 0.60	
	62.50	60.35 ± 0.30	
	31.25	49.75 ± 0.90	
	15.62	39.80 ± 0.50	
Ascorbic acid	1000	96.30 ± 0.50	9
	500	82.60 ± 1.10	
	250	77.80 ± 0.95	
	125	73.80 ± 0.85	
	62.50	69.60± 0.90	
	31.25	64.50 ± 0.85	
	15.62	54.70 ± 0.55	

demonstrating high potential (Subramani, Narayanasamy et al. 2017). In this investigation, EO demonstrated significant antibacterial activity against *K. pneumoniae* (27 mm) and *P. aeruginosa* (28 mm), with MIC and MBC values ranging from 156.25 to 312.5 µg/mL, indicating robust bactericidal activities. It also shows antifungal efficacy, with Trichoderma wild showing the highest sensitivity and moderate suppression of *Aspergillus* species. Referring to CLSI guidelines for ciprofloxacin testing, the corresponding cutoff value for resistance in *E.coli, S. aureus,* and *K. pneumoniae* is ≤ 15 mm, and *P.aeruginosa* is ≤ 15 mm. Compared to previous findings, where plant extracts of the Poaceae family were ineffective against pathogens like *E. coli, F. oxysporum, and A. niger*. (Fatima, Akhtar et al. 2022), *F. glauca* EO had broader and more consistent action. While *S. cerevisiae, W. anomalous,* and *S. aureus* were previously identified as the most sensitive bacteria, *F. glauca* EO demonstrated efficiency against both Gram-positive and Gram-negative bacteria and a moderate antifungal spectrum. These findings show its potential as a natural antibacterial agent for both therapeutic and preventative applications.

Oxidative stress, a harmful process caused by free radicals, is a major factor in the development of many diseases (Sharma and Medicine 2014, Sadiq 2023). Plants' antioxidant capability can be evaluated using their ability to minimize oxidative agents, scavenge free radicals, and reduce cupric ions (Apak, Güçlü et al. 2008, Thatoi, Patra et al. 2014). *F. glauca* EO demonstrated considerable free radical scavenging activity, with IC_{50} values of 18 µg/mL (DPPH) and 30 µg/mL (ABTS). Despite being slightly less efficient than ascorbic acid, these data show significant antioxidant capacity, possibly due to important ingredients like piperitone and β-cymene, which have redox characteristics. *Cymbopogon flexuosus (C. flexuosus*) EO, a member of the same botanical family, also demonstrated high DPPH scavenging action, reaching 78.19% inhibition at 150 µg/mL, similar

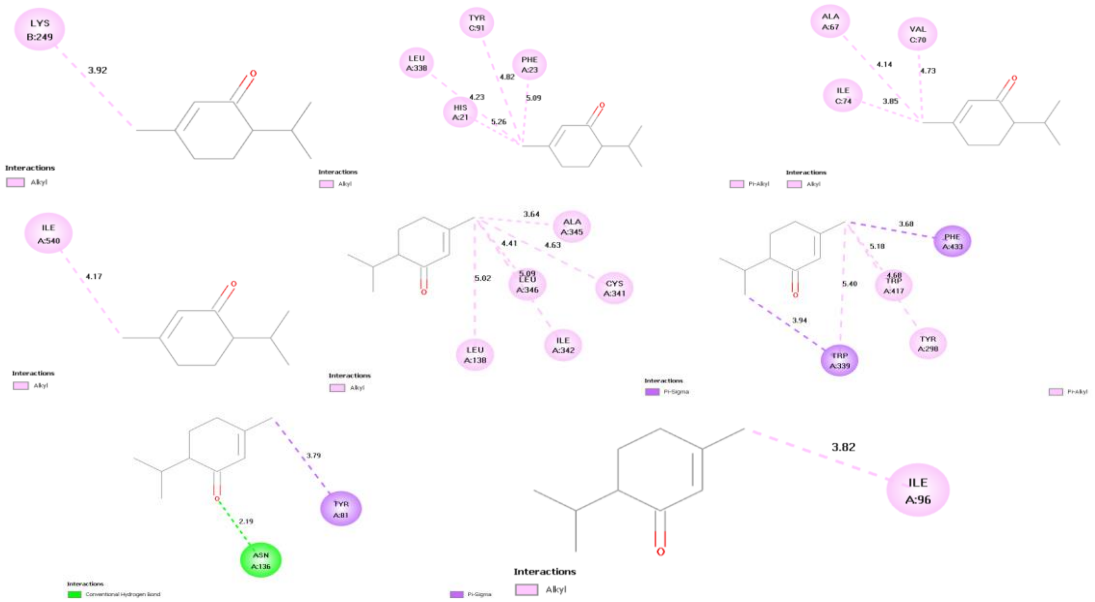

Figure 3. Molecular docking of pepritone with respective target A.5ZZD, B.3AHY, C.7U9K, D.8BN6, E.5O79, F.6RKS, G.1UKC and H. 5W0A respectively

Table 7. Represent compound Piperitone molecular docking with respective target

Compound	Target Proteins	E-value (Kcal/mol)	No of H bonds	Binding residues forming H bonds
Piperitone	6RKS	-5.1	1	ARG:364
	7u9k	-5.3	1	TRP:49
	8bn6	-4.9	0	
	5o79	-4.6	1	ARG:244
	1UKC	-5.2	1	SER:409
	5wOa	-5.9	1	SER:318
	5ZZD	-5.4	1	ARG:291
	3ahy	-5.5	1	ASN:225

to vitamin C (Bhatnagar and Science 2020). Both species' antioxidant action is linked to the ability of EO components to donate hydrogen atoms, thereby transforming DPPH radicals into stable DPPH-H molecules. Furthermore, the presence of major and minor components, as well as potential synergistic effects, improves total antioxidant effectiveness. The similarities in antioxidant reactions between *F. glauca* and *C. flexuosus* highlight the potential of EOs from this plant family as natural antioxidants, with probable uses in controlling oxidative stress-related illnesses. Similarly, Aswathi Moothakoottil Kuttithodi and his colleagues evaluated the EO extracted from *Cinnamomum malabatrum* for its antioxidant properties as well as other pharmacological effects, such as enzyme inhibition and antibacterial activity against a variety of Gram-positive and Gram-negative bacteria (Kuttithodi, Narayanankutty et al. 2023).

Molecular docking showed that piperitone, the EO's active constituent, bound to all eight target proteins, including bacterial and fungal species. With binding energies from -3.8 to -6.7 kcal/mol, these interactions were moderate to strong. The highest affinity fungal protein is 3AHY (-6.7 kcal/mol), followed by 5O79 (-6.2). Multi-microbe antibacterial properties may be present. Most

docking connections were hydrophobic, although LYS249 in 7U9K, PHE23, HIS21, LEU338, TYR91 in 5O79, and ILE540 in 5WOA created alkyl links. In 3AHY, piperitone forms alkyl and π-sigma bonds with residues TRP417 and PHE433. It sticks to protein active sites. The sole hydrogen-bonded ASN136 chemical was 1UKC. Our results agree with previous studies, which show that despite its high binding affinity for mosquito OBP 2L2C, Chemical ID 6987 (Piperitone) did not establish hydrogen bonds with the protein. The data indicate that Glide exhibits a stronger binding affinity for Piperitone with a binding score of 4.19 (SASAKI, WADA et al. 2023). Piperitone binding affinities observed in our study are in line with those of structurally similar substances like piperine, which has shown binding energies between -6.4 and -6.7 kcal/mol against class A and C β-lactamases (Rasuly, Mosawi et al. 2024). Piperitone has a strong binding affinity for fungal targets but weaker polar interactions, therefore primarily forming hydrophobic bonding. Our docking scores for microbial enzymes are well within the range where docking scores range from -5 to -7 kcal/mol, matching EO ingredient binding patterns like carvone, menthone, and thymol (Degfie, Endale et al. 2022).

5. Conclusion

This study identifies piperitone as the main phytochemical ingredient of *F. glauca* essential EO. The EO has high antibacterial, moderate antifungal, and significant antioxidant properties. Molecular docking studies supported these findings, demonstrating piperitone's efficient interactions with microbial target proteins. These findings show that *F. glauca* EO could be a useful natural source of antibacterial and antioxidant compounds. Additional *in vivo* and toxicological investigations are needed to validate its therapeutic applications.

Conflict of Interest

The authors declare that they have no competing interests.

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Consent Forms

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Authors' Contribution

SH and MS performed experimental work, data collection and evaluation, literature search, and manuscript preparation. FA, MS, SA, SKS, FU, and SMH supervised the research work and refined the manuscript for publication. The authors read and approved the final manuscript for publication.

Data Availability

All the relevant data of this manuscript is available from the authors.

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References

Abrar, S., S. Hussain, R. A. Khan, N. Ul Ain, H. Haider and S. Riaz (2018). "Prevalence of extended-spectrum-β-lactamase-producing Enterobacteriaceae: first systematic meta-analysis report from Pakistan." Antimicrobial Resistance & Infection Control **7**: 1-11.

Alara, J. A. and O. R. J. I. D.-D. T. Alara (2024). "An overview of the global alarming increase of multiple drug resistant: a major challenge in clinical diagnosis." **24**(3): 26-42.

ALrajhi, M., M. Al-Rasheedi, S. E. M. Eltom, Y. Alhazmi, M. M. Mustafa and A. M. Ali (2019). "Antibacterial activity of date palm cake extracts (Phoenix dactylifera)." Cogent Food & Agriculture **5**(1): 1625479.

Apak, R., K. Güçlü, M. Özyürek and S. E. J. M. a. Çelik (2008). "Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay." **160**: 413-419.

Badr, M. M., M. E. Badawy, N. E. J. J. o. D. D. S. Taktak and Technology (2021). "Characterization, antimicrobial activity, and antioxidant activity of the nanoemulsions of Lavandula spica essential oil and its main monoterpenes." **65**: 102732.

Bailén, M., C. Illescas, M. Quijada, R. A. Martínez-Díaz, E. Ochoa, M. T. Gómez-Muñoz, J. Navarro-Rocha and A. González-Coloma (2023). "Anti-trypanosomatidae activity of essential oils and their main components from selected medicinal plants." Molecules **28**(3): 1467.

Becker, R., J. L. Pederick, E. G. Dawes, J. B. Bruning and A. D. Abell (2023). "Structure-guided design and synthesis of ATP-competitive N-acyl-substituted sulfamide d-alanine-d-alanine ligase inhibitors." Bioorganic & medicinal chemistry **96**: 117509.

Bhatnagar, A. J. J. o. A. and N. Science (2020). "Chemical composition and antioxidant activity of essential oil of Cymbopogon flexuosus." **12**(1): 25.

Bogdan, M. A., S. Bungau, D. M. Tit, D. C. Zaha, A. C. Nechifor, T. Behl, D. Chambre, A. I. Lupitu, L. Copolovici and D. M. J. M. Copolovici (2021). "Chemical profile, antioxidant capacity, and antimicrobial activity of essential oils extracted from three different varieties (Moldoveanca 4, Vis Magic 10, and Alba 7) of Lavandula angustifolia." **26**(14): 4381.

Braca, A., N. De Tommasi, L. Di Bari, C. Pizza, M. Politi and I. J. J. o. n. p. Morelli (2001). "Antioxidant principles from bauhinia t arapotensis." **64**(7): 892-895.

Chalkley, L. and H. Koornhof (1985). "Antimicrobial activity of ciprofloxacin against Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus determined by the killing curve method: antibiotic comparisons and synergistic interactions." Antimicrobial agents and chemotherapy **28**(2): 331-342.

Degfie, T., M. Endale, T. Tafese, A. Dekebo and K. Shenkute (2022). "In vitro antibacterial, antioxidant activities, molecular docking, and ADMET analysis of phytochemicals from roots of Hydnora johannis." Applied Biological Chemistry **65**(1): 76.

Ejidike, I. P., A. Direm, C. Parlak, S. A. Olaleru, C. O. Adetunji, F. M. Mtunzi, A. Ata, M. O. Eze, H. S. Clayton and P. A. Ajibade (2025). "DNA gyrase inhibition by Ni (II)-Schiff base complexes via in silico molecular docking studies: Spectroscopic, DFT calculations and in vitro pharmacological assessment." Results in Chemistry **15**: 102219.

Ertürk, Ö. J. B. (2006). "Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants." **61**(3): 275-278.

Farouk, O. Y., J. R. Fahim, E. Z. Attia and M. S. J. S. A. J. o. B. Kamel (2023). "Phytochemical and biological profiles of the genus Phragmites (Family Poaceae): A review." **163**: 659-672.

Fatima, I., W. Akhtar, N. K. Bangash, S. Kanwal, N. Rauf, T. S. Malik and T. J. P. B.-A. I. J. D. w. A. A. o. P. B. Mahmood (2022). "Volatile profiling, elemental composition and biological activities of aerial parts of seven Poaceae species." **156**(4): 908-925.

Fatima, Z., D. Purkait, S. Rehman, S. Rai and S. Hameed (2023). Multidrug resistance: a threat to antibiotic era. Biological and Environmental Hazards, Risks, and Disasters, Elsevier**:** 197-220.

Godwini, R., J. C. Monicka and S. G. Victoria "Chemical Physics Impact."

Hassan, H. M., M. Aboel-Ainin, S. K. Ali, A. J. J. o. A. C. Darwish and Biotechnology (2021). "Antioxidant and Antimicrobial activities of MEOH Extract of Lemongrass (Cymbopogon citratus)." **12**(2): 25-28.

Hati, S., G. Dimari, G. Egwu and V. Ogugbuaja (2010). "Specific gravity and antibacterial assays of some synthetic industrial essential oils." Science World Journal **5**(1).

Isah, M., M. D. Sul'ain, W.-N.-A. W. A. Wahab, H. Abdullah, S. Jamil, N. Syamira, M. Shabudin, A. N. Shuid and W. R. W. Ishak (2025). "Chemical profiling and mechanistic insights into the antibacterial efficacy of Melaleuca cajuputi leaf extract." BMC Complementary Medicine and Therapies **25**(1): 121.

Kakoullis, L., E. Papachristodoulou, P. Chra and G. J. A. Panos (2021). "Mechanisms of antibiotic resistance in important gram-positive and gram-

negative pathogens and novel antibiotic solutions." **10**(4): 415.
Kang, C.-I. and J.-H. Song (2013). "Antimicrobial resistance in Asia: current epidemiology and clinical implications." Infection & chemotherapy **45**(1): 22.
Karaiskos, I. and H. J. E. o. o. p. Giamarellou (2014). "Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: current and emerging therapeutic approaches." **15**(10): 1351-1370.
Kasew, D., B. Desalegn, M. Aynalem, S. Tila, D. Diriba, B. Afework, M. Getie, S. Biset and H. W. J. P. o. Baynes (2022). "Antimicrobial resistance trend of bacterial uropathogens at the university of Gondar comprehensive specialized hospital, northwest Ethiopia: A 10 years retrospective study." **17**(4): e0266878.
Kocięcka, J., D. Liberacki and M. J. S. Stróżecki (2023). "The Role of Antitranspirants in Mitigating Drought Stress in Plants of the Grass Family (Poaceae)—A Review." **15**(12): 9165.
Kumar, V. N., C. Prabhakumari and S. John (2022). "Phytochemical screening and antibacterial activity of leaf extracts of Anacardium occidentale L." J. Indian bot. Soc **102**(2): 326-333.
Kuttithodi, A. M., A. Narayanankutty, N. U. Visakh, J. T. Job, B. Pathrose, O. J. Olatunji, A. Alfarhan and V. Ramesh (2023). "Chemical composition of the Cinnamomum malabatrum leaf essential oil and analysis of its antioxidant, enzyme inhibitory and antibacterial activities." Antibiotics **12**(5): 940.
Li, M., X. Zhao and M. J. F. Xu (2022). "Chemical composition, antimicrobial and antioxidant activity of essential oil from Allium tenuissimum L. flowers." **11**(23): 3876.
Liu, Y., Z. Tong, J. Shi, Y. Jia, T. Deng and Z. J. C. b. Wang (2021). "Reversion of antibiotic resistance in multidrug-resistant pathogens using non-antibiotic pharmaceutical benzydamine." **4**(1): 1328.
Loganathan, C. G. and K. Krishnan (2025). "Eco-Friendly Green Synthesis of Novel 1, 2, 3-Triazole Derivatives via Piperazine Scaffold and Their Antimicrobial Potential: In Silico Evaluation Targeting Serine Proteases 6RKS and 1BDD." International Journal of Environmental Sciences: 638-661.
Majeed, Q., A. Alshammari and A. Alanazi (2023). "RESEARCH ARTICLE Antileishmanial effects, cellular mechanisms, and cytotoxicity of Elettaria cardamomum essential oil against Leishmania major infection." Tropical Biomedicine **40**(2): 259-265.
Mirtaghi, S. M., P. T. Nejad, M. Masoumeh Mazandarani, F. Livani and H. J. M. L. J. Bagheri (2016). "Evaluation of Antibacterial Activity of Urtica dioica L. Leaf Ethanolic Extract Using Agar Well Diffusion and Disc Diffusion Methods." **10**(5).
Nadimpalli, M. L., C. W. Chan and S. Doron (2021). "Antibiotic resistance: a call to action to prevent the next epidemic of inequality." Nature medicine **27**(2): 187-188.
Okoh, S. O., O. T. Asekun, O. B. Familoni and A. J. J. A. Afolayan (2014). "Antioxidant and free radical scavenging capacity of seed and shell essential oils extracted from Abrus precatorius (L)." **3**(2): 278-287.
Oliveira, T. A., T. M. Vieira, V. R. Esperandim, C. H. Martins, L. G. Magalhães, M. L. Miranda and A. E. Crotti (2022). "Antibacterial, antiparasitic, and cytotoxic activities of chemical characterized essential oil of Chrysopogon zizanioides roots." Pharmaceuticals **15**(8): 967.
Pasquina-Lemonche, L., J. Burns, R. Turner, S. Kumar, R. Tank, N. Mullin, J. Wilson, B. Chakrabarti, P. Bullough and S. Foster (2020). "The architecture of the Gram-positive bacterial cell wall." Nature **582**(7811): 294-297.
Qamar, F. N., M. T. Yousafzai, M. Khalid, A. M. Kazi, H. Lohana, S. Karim, A. Khan, A. Hotwani, S. Qureshi and F. Kabir (2018). "Outbreak investigation of ceftriaxone-resistant Salmonella enterica serotype Typhi and its risk factors among the general population in Hyderabad, Pakistan: a matched case-control study." The Lancet Infectious Diseases **18**(12): 1368-1376.

Rasuly, M. F., S. H. Mosawi, S. Nazir and Z. Habibzada (2024). "Integrating molecular docking and molecular dynamics simulation approaches for investigation of the affinity and interactions of the piperine with Class D β-Lactamase." Afghanistan Journal of Basic Medical Science **1**(2): 29-41.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. J. F. r. b. Rice-Evans and medicine (1999). "Antioxidant activity applying an improved ABTS radical cation decolorization assay." **26**(9-10): 1231-1237.

Sadiq, I. Z. J. C. m. m. (2023). "Free radicals and oxidative stress: Signaling mechanisms, redox basis for human diseases, and cell cycle regulation." **23**(1): 13-35.

Sahebkar, A. and M. J. J. o. E. O. B. P. Iranshahi (2011). "Volatile constituents of the genus Ferula (Apiaceae): A review." **14**(5): 504-531.

Salam, M. A., M. Y. Al-Amin, M. T. Salam, J. S. Pawar, N. Akhter, A. A. Rabaan and M. A. Alqumber (2023). Antimicrobial resistance: a growing serious threat for global public health. Healthcare, Multidisciplinary Digital Publishing Institute.

SASAKI, K., K. WADA and K. MATUOKA (2023). "Pulegone and Piperitone, Essential Oil Components of Lamiaceae Family, Enhance the Activities of Drug-Metabolizing Enzymes." 千葉科学大学紀要(16): 5-9.

Sharma, N. J. B. and Medicine (2014). "Free radicals, antioxidants and disease." **6**(3): 1.

Sharma, V. and V. K. Choudhary (2024). "Tri-butyltin (IV) phenoxyacetohydroxamate: Synthesis, characterization, biological evaluation and molecular docking studies." Main Group Chemistry **23**(1): 103-111.

Smith, M. A., P. A. Romero, T. Wu, E. M. Brustad and F. H. Arnold (2013). "Chimeragenesis of distantly-related proteins by noncontiguous recombination." Protein science **22**(2): 231-238.

Subramani, R., M. Narayanasamy and K.-D. J. B. Feussner (2017). "Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens." **7**: 1-15.

Sulieman, A. M. E., S. M. Ibrahim, M. Alshammari, F. Abdulaziz, H. Idriss, N. A. H. Alanazi, E. M. Abdallah, A. J. Siddiqui, S. A. Shommo and A. Jamal (2024). "Zingiber officinale Uncovered: Integrating Experimental and Computational Approaches to Antibacterial and Phytochemical Profiling." Pharmaceuticals **17**(11): 1551.

Tava, A., N. Berardo and M. J. P. Odoardi (1991). "Composition of essential oil of tall fescue." **30**(5): 1455-1458.

Temperoni, C., L. Caiazzo and F. J. A. Barchiesi (2021). "High prevalence of antibiotic resistance among opportunistic pathogens isolated from patients with COVID-19 under mechanical ventilation: results of a single-center study." **10**(9): 1080.

Thatoi, H., J. K. Patra and S. J. A. p. p. Das (2014). "Free radical scavenging and antioxidant potential of mangrove plants: a review." **36**: 561-579.

Yasmin, F., N. Akhtar and A. Hameed (2013). "In vitro synergistic effect of ciprofloxacin with aminoglycosides against multidrug resistant-Pseudomonas aeruginosa." Pakistan Journal of Pharmaceutical Sciences **26**(5): 1041-1045.

Yelshanskaya, M. V. and A. I. Sobolevsky (2022). "Ligand-binding sites in vanilloid-subtype TRP channels." Frontiers in Pharmacology **13**: 900623.

Zouirech, O., A. A. Alyousef, A. El Barnossi, A. El Moussaoui, M. Bourhia, A. M. Salamatullah, L. Ouahmane, J. P. Giesy, M. A. Aboul-Soud and B. J. B. R. I. Lyoussi (2022). "Phytochemical analysis and antioxidant, antibacterial, and antifungal effects of essential oil of black caraway (Nigella sativa L.) seeds against drug-resistant clinically pathogenic microorganisms." **2022**(1): 5218950.