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Multidimensional Evaluation of *Hedychium ellipticum* Essential Oil through Phytochemical Analysis, Target Prediction, and Pharmacokinetic Screening

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Abstract

The *Hedychium* species encompasses approximately 80 distinct varieties, showcasing their adaptability as plants primarily valued for their striking, diverse, and fragrant flowers used in decorative applications. The unique characteristics of *Hedychium* essential oils suggest their potential use in developing plant-based preservatives aimed at extending stored food products shelf life. This study examined phytoconstituents, antimicrobial, and antioxidant properties of the essential oil derived from *Hedychium ellipticum* found in Uttarakhand. The oils were acquired through the hydrodistillation method before their characterization via GC-MS. The chemical examination of the oil identified 20 chemical compounds. The primary components detected include Terpinolene, Iso-isopulegol, Caryophyllene, Piperitol, Humulene, Farnesene, Rosifoliol, terpinene-4-ol, and trans-ocimene present in essential oil. The essential oil shows moderate to strong broad-spectrum antimicrobial activities. To elucidate the possible molecular basis of the observed antimicrobial activity, molecular docking studies were performed using the major compounds, iso-isopulegol and terpinolene, against key bacterial target proteins involved in DNA replication, cell wall biosynthesis, lipid metabolism, and folate pathways. Docking analysis revealed stable ligand-protein interactions, having binding affinities between -5.3 to -6.7 kcal/mol. Notably, iso-isopulegol showed strong binding with *Klebsiella pneumoniae* LpxH (-6.5 kcal/mol) and penicillin-binding protein 2X, while terpinolene exhibited the highest affinity toward the LpxH/JH-LPH-33 complex (-6.7 kcal/mol). These findings indicate a multi-target interaction profile of the major monoterpenoid constituents, supporting a polypharmacological mode of antibacterial action. Overall, the combined experimental and in silico results highlight *H. ellipticum* essential oil serves as a viable natural source of bioactive chemicals with prospective applications in pharmaceutical products.

Keywords: *Hedychium ellipticum*, Hydrodistillation, Essential Oil, GC-MS, Antimicrobial Activity, Molecular Docking

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INTRODUCTION

The genus *Hedychium*, belonging to the family Zingiberaceae, comprises a group of flowering plants widely recognized for their ornamental and aromatic value.[1] In the middle of the eighteenth century, Rumphius called some *Hedychium* species "Ginger Lily" or "Garland Flowers", but in botanical terms, he called them *Gandasulinum*. They were later appropriately identified as *Hedychium* species by Koenig.[2] *Hedychium* species produce lovely, frequently fragrant flowers. Many of these flowers are grown and used as popular hair ornaments for women in Eastern Asia because of their sweet scent. In addition to being used in traditional medicine, essential oils derived from the subterranean tuberous rhizomes or, on occasion, the whole plant of *Hedychium ellipticum* are utilized to create exquisite and high-quality perfumes.[3] Hedychium (Zingiberaceae) is a genus of perennial herbs characterized by tuberous rootstocks, distributed across Asia and Madagascar.[4] Numerous species are utilized in traditional medicine to address bronchitis, asthma, gastrointestinal ailments, blood cleansing, and antiemetic needs, especially among the hill tribes of Uttarakhand.[5] One of the hotspots for the *Hedychium* genus is northeast India, which is home to many endemic species. The highest number of species found in any Indian state is actually 32, found in the northeastern state of Meghalaya. There are ten species in Sikkim, seven in Uttarakhand, and two in Himachal Pradesh.[6] It is prevalent in moist, shaded locales, steep inclines, and grassy places at altitudes of 1000-3000 meters in the Himalayas. This species has also been reported in ethnobotanical reports of India and it is consumed by the locals as a tonic, blood purifier, and treatment for asthma, bronchitis, eye, and stomach ailments. Flowering and fruiting occur from July to October.[7] Phytochemical studies on this genus have been conducted in our ongoing investigation into physiologically active metabolites from aromatic and medicinal plants. The essential oils of *H. ellipticum* that were steam-distilled demonstrated strong antimicrobial activity in biological investigations.[8] Natural antioxidants derived from plants have drawn more notable focus in the past few years due to their ability to defend the body against free radical damage, slow the progression of numerous chronic illnesses, and prevent lipid-oxidative rancidity in food.[9] Customers are growing uneasy about the idea of having their food treated with different chemicals to extend its shelf life as a result of growing health consciousness. The welfare of its consumers is not given enough thought by the food industry. All too frequently, cost-effectiveness and profitability take precedence over consumer health and safety. For instance, although butylated hydroxytoluene (BHT), a common antioxidant, has long been known to promote cancer, it is still used in the meat packing industry.[10]

The natural antioxidants found in herbs and spices are typically categorized as volatile compounds, vitamins, and phenols, which include flavonoids and phenolic acids.[11] The function of reactive oxygen species (ROS) in oxygen-dependent organisms is predicated on their participation in many processes that are recognised to exacerbate cell damage, aging, and lipid peroxidation induction. These processes lead to food deterioration and have an impact on food's colour, flavour, texture, and, most importantly, nutritional value.[12] There are numerous ways for living things to produce free radicals as well as ROS. Antioxidants, along with vitamin C, vitamin E, and carotenoids, are abundant in many medicinal plants.[13] For instance, it has been shown that some ginger species found in tropical nations have potent antioxidant properties that enable them to substitute α-tocopherol.[14] A blend of volatile and aromatic compounds makes up an essential oil. These aromatic compounds, which are distinguished by the presence of specific aroma compounds called terpenoids, have a propensity to foster an environment that is hostile to pathogens. They are not naturally aromatic, but they do have carbon, hydrogen, and/or oxygen. The structure of terpene molecules is made up of isoprene units, which are C_5H_8 and this is a common feature.[15] By turning hydrogen and lipid peroxides into reactive free radicals, like in the Fenton-type reaction, the iron in the ferrous state helps speed up the oxidation of lipids. Though at a tenfold slower rate than Fe2þ, Fe3þ ions can also generate radicals from peroxides. The Fe^{2+} ions are thought to be the most potent pro-oxidants among the several types of metal ions. The chelating agent ferrozine does not form a complex with bound Fe2þ, but it

does with unbound Fe2þ. Since colour decrease may be measured, it is possible to estimate the co-existing chelator's metal-chelating activity.[16] Essential oils work incredibly well as antifungal and antimicrobial agents. Surprisingly, though, a lot of terpenes are already utilized as dietary supplements (like vitamin A) and flavourings like lemon and coriander Flavors.[17] Not as preservatives, many of the chemical ingredients or essential oils are already present in a large number of industrially produced foods. One of the strongest known antioxidants, phenols, is also present in various essential oils. Therefore, essential oils rich in phenol, like wintergreen or clove oils, are not only good flavourings, antifungals, and antimicrobials, moreover, they possess antioxidant properties that can extend food preservation and offer unforeseen health advantages.[18] This research presents the essential oil compositions, as well as the antioxidant and antimicrobial activity, of *H. ellipticum*.

MATERIALS AND METHODS

Collection of plant material

H. ellipticum was located in Khirsu village, a hill station in the Pauri Garhwal district of Uttarakhand, India. Khirsu is located at an elevation of 1760 meters, 19 kilometers North of Pauri and 160 kilometers west of Dehradun city, India. The location of Khirsu has been selected as a research area for this study shown in the map (Figure 1). The study sites were surveyed in the autumn and monsoon seasons for sample collection and seasonal data acquisition. The plant samples (Figure 2) were collected using a non-destructive method with a short-handled cutting tool, specifically a hoe (Khurpa). A jute bag was employed for the collection, and only the necessary plant samples were carefully obtained by gently scraping the targeted plant species population. The growth of plant species is contingent upon various climatic factors such as temperature, precipitation, and moisture. The sample has been assigned a

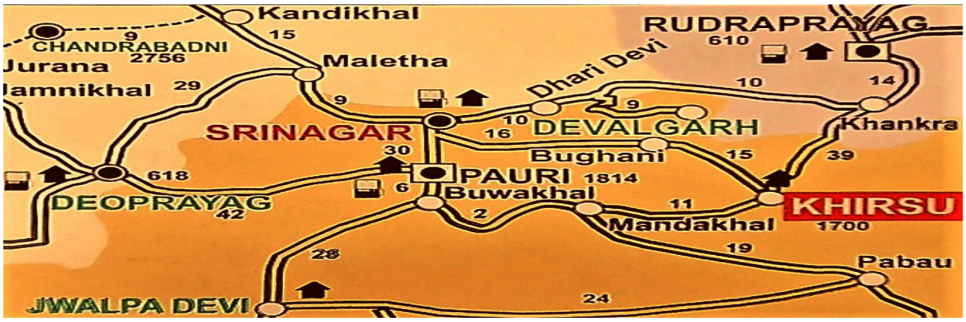

Figure 1. Map showing the site selected for the *Hedychium ellipticum* plant sample collection for the research study

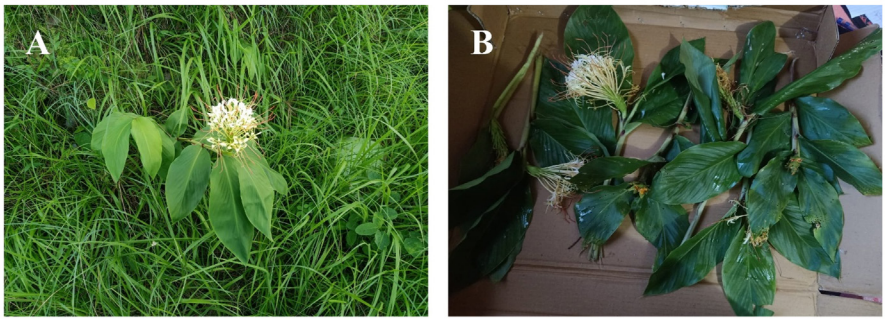

Figure 2. Plant samples of *H. ellipticum* collected from Khirsu village, Pauri Garhwal (A) Natural habitat; (B) collected plant sample

specific name and preserved in the refrigerator at the Department of Botany and Microbiology, Gurukula Kangri (Deemed to be University), Haridwar. Identification of the collected plant, *H. ellipticum* (Accession Number - 1482), was done from the Botanical Survey of India (BSI), Dehradun, Uttarakhand.

Extraction of essential oil

H. ellipticum is noted for its aromatic properties, and the essential oil it produces may have prospective uses in therapeutic aromatherapy, pharmaceuticals, and herbal healthcare (Figure 3). Essential oils derived from the plant *H. ellipticum* were obtained through the steam distillation process. This process is a widely used technique for extracting essential oils from plant material. The extracted essential oil was collected and stored in sterile, airtight containers, such as Eppendorf tubes, to maintain its purity and prevent deterioration. The extracted oil was stored in a cool, dark environment to prevent exposure to light and heat, which may modify its chemical composition.[19]

Determination of chemical profiling through GC-MS

Preparation of an essential oil sample

Essential oils are volatile and hydrophobic in nature; thus, they cannot be directly injected into the GC-MS system without prior dilution. Typically, essential oils are diluted in a suitable solvent (e.g., hexane, methanol, or diethyl ether) to make the sample compatible with GC-MS.

GC-MS Instrumentation

Sample Injector → Column (Gas Chromatography) → Ion Source (Mass Spectrometry) → Mass Analyzer → Detector → Data System

Characterisation and identification of the main components found in the essential oil *H. ellipticum*

GC-MS analyses were conducted with the Model: GCMS-TQ8040 equipped with SH-Rxi-5Sil MS (5% biphenyl 95% dimethyl Polysiloxane) (30 m, 0.25 mmID, 0.25 µm d_f) column and Gas Chromatography Mass Spectrometer with AOC-20i Auto injector mass spectrometer (MS). About 0.1

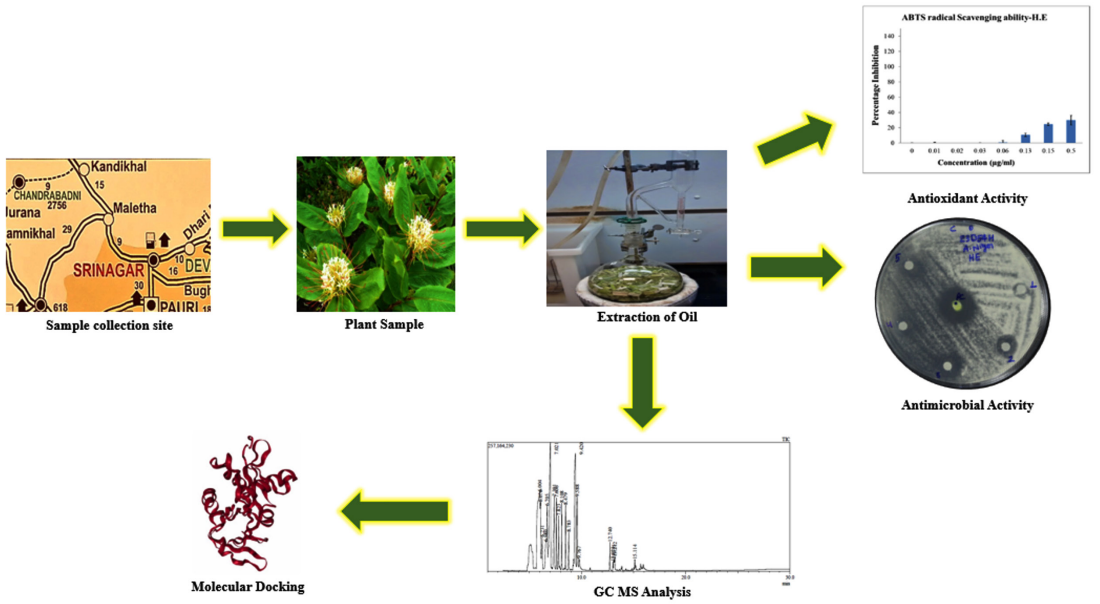

Figure 3. Flowchart illustrating the experimental design for extraction, phytochemical characterization, biological activity assessment, and molecular docking analysis

µL of the essential oil sample, carried by Helium at a flow rate of 1 mL/min, was injected into GC-MS analyser. Each component was identified by comparing its mass spectrum and linear retention index (LRI) with those in the NIST and Wiley libraries.[20]

Antibacterial activity of essential oil

To test the essential oil's antibacterial activity, six different bacterial strains were used and the activity was performed as per the method described by Bauer.[21] The Petri plates with MHA medium were prepared aseptically and inoculated with bacterial cultures. The strains tested were *Klebsiella pneumoniae* (MTTC 432), *Streptococcus pneumoniae* (MTTC 655), *Salmonella typhi* (MTTC 733), *Escherichia coli* (MTCC 118), *Staph. aureus* (MTCC-7443), and *Bacillus cereus* (MTCC 7417), all sourced from the Microbial Type Culture Collection (MTCC), Chandigarh, India. All strains used in this study were kept at 4 °C throughout the study. For each test, 100 µl of bacterial suspension (standardized to 0.5 McFarland turbidity) was spreaded onto the MHA plates. The Ciprofloxacin discs (10 µg) were used as positive controls, and for the vehicle control, a plate with only the solvent. Then, incubated the plates at 37 °C for 24 hours in an incubator (Basil Scientific Corp., New Delhi). After incubation, the zones of inhibition surrounding each disc were measured and documented the findings.[22]

Minimum inhibitory concentration of essential oil

0.5 MacFarland Standard dilution of selected bacteria were used for the study. Into each microcentrifuge tube, added 100 µl of diluted log-phase bacterial cultures. Then 5 µl of treatment dilutions at different concentrations into the designated tubes. The tubes set incubated for 24 hours. After that, transferred all the samples to a 96-well plate and measured turbidity at 630 nm using an ELISA Plate Reader (iMark Bio-Rad). For the positive control, ciprofloxacin (10 µg) was used as described by Rawat et al.[2]

Antifungal activity of essential oil

The antifungal activity of essential oil was evaluated by using the method from Bauer et al.[21] First, Petri dish of Sabouraud Dextrose Agar (SDA) was prepared and added the fungal strains *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 871). After adjusting the fungal culture to 0.5 McFarland Unit (about 1.5 x 10^8 CFU/mL), spread 100 µl onto each plate. Then, discs loaded with 10 µl of various concentrations (ranging from 0-500 mg/ml) onto the plates. For controls, one disc per plate had only the solvent, and another had Amphotericin B (25 µg) as a positive control. Plates were incubated at 37 °C for 24 hours using a Basil Scientific Corp. India incubator. After incubation, zone of inhibitions were measured with the help of Vernier Calliper as described by Kappen et al.[23]

Antioxidant activity of essential oil

DPPH scavenging assay

In a 96-well plate, 0.1 mL of 0.1 mM DPPH solution was combined with 5 µl of the test drug from a separate stock. Blank duplicates were made with 0.2 ml of DMSO/Methanol and 5 µl of the chemical at different concentrations, and the reaction was done three times. The plate was left in the dark for 30 minutes. After that, the loss of color was measured at 495 nm using a microplate reader (iMark, Bio-Rad). A reaction with 20 µl of deionised water was used as the control. The scavenging activity was reported as "% inhibition" compared to the control. The IC_{50} value was calculated using GraphPad Prism Software.[7]

DPPH Scavenging activity = ((Abs Control-Abs Sample) / Abs Control) × 100

ABTS radical scavenging ability

ABTS radicals were made by combining APS (2.45 mM) with an ABTS solution (7 mM), the mixture was then diluted 100 times to create the ABTS free radical reagent. Next, 10 µl of different concentrations of the standard (Ascorbic Acid -SD Fine- F13A/0413/1106/62 and samples were added to 200 µl of ABTS free radical reagent in 96-well microplates. The reaction were allowed to react at room temperature for 10 minutes in the dark. The change in color, which shows the decolorization, was measured at 750 nm using a microplate reader (iMark, Bio-Rad). The results were compared to the negative control. The IC_{50} was calculated using GraphPad Prism.[24]

Table 1. Compounds detected in the essential oil of *Hedychium ellipticum*

No.	Area%	R.Time	Compound Name	Biological activity	Ref.
1	21.77	6.004	Terpinolene	Antioxidant Activity, Antimicrobial Activity, and Anti-inflammatory Properties	30
2	17.99	7.021	Iso-isopulegol	Antidiabetic Activity, Anti-inflammatory Effects. Antioxidant Activity, and Antimicrobial Activity	31
3	15.31	9.42	Iso-isopulegol	Anti-inflammatory Effects, Antimicrobial Activity, and Antioxidant Activity	31
4	6.98	7.391	Terpinolene	Antioxidant Activity, Antimicrobial Activity, Anti-inflammatory Properties	30
5	5.4	6.705	Ocim-(4E,6Z)-ene <allo-> (also known as allo-ocimene)	Antimicrobial Activity, Anti-inflammatory Properties, Antioxidant Effects, Insecticidal and Repellent Effects	32
6	4.81	9.588	Dihydrocarveol <1,6->	Antioxidant Activity, anti-inflammatory properties	33
7	3.7	6.076	Decalol <beta->	Antioxidant Activity, analgesic properties, and anti-inflammatory activity	34
8	3.12	8.108	Dihydrocarveol <1,6->	Antioxidant Activity, anti-inflammatory properties	33
9	3.04	7.606	Dihydrocarveol <1,6->	Antioxidant Activity, anti-inflammatory properties	33
10	2.89	8.479	Dihydrocarveol <1,6->	Antioxidant Activity, anti-inflammatory properties	33
11	2.75	7.823	Ocim-(4E,6Z)-ene <allo->	Antioxidant Activity, anti-inflammatory activity, Mosquito Repellent Activity	35
12	2.7	6.58	Terpinene <alpha->	Antioxidant Activity, anti-inflammatory properties, analgesic activity	36
13	2.49	6.231	Linalyl phenylacetate	Antioxidant Activity, Anti-inflammatory Effects, Antimicrobial and Antifungal Activity	37
14	2.38	8.783	Menth-2-en-1-ol <cis-, para->	Antimicrobial Activity, Antioxidant Activity, Antidiabetic Activity	32
15	1.47	12.74	Caryophyllene	Anti-inflammatory Activity, Analgesic (Pain Relief) Properties, Antioxidant Properties	38
16	0.91	9.767	Piperitol <trans->	Anti-inflammatory Activity, Antioxidant Activity, Antimicrobial and Antifungal Properties	39
17	0.81	13.212	Humulene <alpha->	Anti-inflammatory Activity, Antimicrobial and Antifungal Properties, Antioxidant Properties	40
18	0.63	13.115	Farnesene	Insect Repellency and Insecticidal Activity, Antioxidant Properties, Antimicrobial Activity	41
19	0.45	15.114	Rosifoliol	Antioxidant Activity, Anti-inflammatory Effects, Antimicrobial and Antifungal Activity	42
20	0.4	13.023	Farnesene	Insect Repellency and Insecticidal Activity, Antioxidant Properties, Antimicrobial Activity	41

Molecular docking of the major compound against pathogenic bacteria

The interaction between the major chemical component of essential oil and the protein of vulnerable pathogenic bacteria will be predicted using the molecular docking technique. The protein ligand of susceptible pathogenic bacteria will be downloaded from the PDB library or protein database, and their molecular docking with active molecule or major molecule of essential oil of plant was analysed through AutoDock Vina.[25-27] or other compatible software.

The protein selected for molecular docking of major chemical constituent of essential oil i.e., Iso-isopulegol and Terpinolene were 6RKS: *E. coli* DNA Gyrase - DNA binding and cleavage domain in State 1 without TOPRIM insertion, 3SWD: *E. coli* MurA in complex with UDP-N-acetylmuramic acid and covalent adduct of PEP with Cys115, 8JTP: Crystal structure of apo Enoyl-Acyl Carrier Protein Reductase (FabI) from *Klebsiella pneumoniae*, 6PJ3: Crystal structure of the *K. pneumoniae* LpxH/JH-LPH-33 complex, 1QMF: Penicillin-binding protein 2x (pbp-2x) acyl-enzyme complex, 2z2l: Penicillin-Binding Protein 2X (PBP2X) from *S. pneumoniae*, 5CFZ: Crystal structure of *E. coli* FabI in apo form, 6WAA: *K. pneumoniae* Topoisomerase IV (ParE-ParC) in complex with DNA and compound 34 (7-[(1S,5R)-1-amino-3-azabicyclo[3.1.0]hexan-3-yl]-4-(aminomethyl)-1-cyclopropyl-3,6-difluoro-8 methylquinolin-2(1H)-one).

RESULTS AND DISCUSSION

Extraction yield and chemical composition of essential oil

The obtained extraction rate for EO was moderate. The EO was preserved in a refrigerator at 4 °C until subsequent utilization. Results of GC-MS analysis of essential oil extracted from *H. ellipticum* plant sample collected from Garhwal, Uttarakhand region, and the identified compounds are displayed in Table 1. The detected compounds are enumerated in accordance with the elution sequence of their retention index.

The output of essential oil from *H. ellipticum* was moderate. In this analysis, 20 chemicals were discovered, accounting for 98.91% of the total recoverable oil. The oil predominantly comprised chemicals including Terpinolene (21.77%), Iso-isopulegol (17.99%), Ocim-(4E,6Z)-ene <allo-> (5.4%), Farnesene (0.63%), Rosifoliol (0.45%), Humulene <alpha-> (0.81%), Caryophyllene (1.47%), Piperitol <trans-> (0.91%), Linalyl phenylacetate (2.49%).

Environmental conditions, the specific part of the plant utilized, the plant's age, the stage of the vegetative cycle, and genetic influences may all affect the chemical makeup of our sample in comparison to similar species from different places. The extraction procedure can influence the yield and chemical composition of essential oils, potentially elucidating the variations in bioactivity.[29]

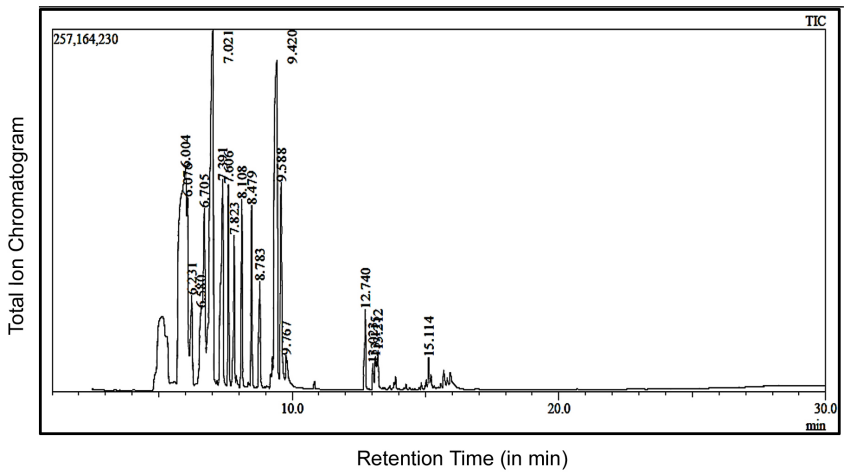

Figure 4. Gas chromatographic profile of *Hedychium ellipticum* (refer to Table 1 for peak identifications)

Table 2. Results for antibacterial activity/MIC against different bacterial strains

Microorganism	Inhibition Zone (in mm)						+ve control (µl)	MIC (µg/ml)
	Essential oil conc. (µg/disc)							
	0	1	5	10	20	100		
Klebsiella pneumoniae	NA*	9.0 ± 0.0	9.5 ± 0.7	9.7 ± 0.0	10.0 ± 0.0	17.5 ± 0.7	26.0 ± 0.0	100
Streptococcus pneumoniae	NA	8.0 ± 1.4	10.5 ± 0.7	11.5 ± 0.7	12.0 ± 0.0	15.0 ± 0.0	35.0 ± 0.0	01
E. coli	NA	6.3 ± 0.0	7.6 ± 0.5	10.0 ± 0.0	11.0 ± 1.0	14.0 ± 1.5	22.0 ± 0.0	0.5
Salmonella typhi	NA	7.6 ± 0.5	8.3 ± 1.15	9.0 ± 1.0	10 ± 1.15	12.0 ± 2.5	29.0 ± 0.3	01
Staphylococcus aureus	NA	NA	9.0 ± 0.0	9.3 ± 0.7	10.0 ± 0.0	12.0 ± 0.0	24.0 ± 0.5	0.05
Bacillus cereus	NA	6.0 ± 0.0	7.6 ± 0.0	8.0 ± 0.0	8.3 ± 0.0	9.6 ± 0.0	25.0 ± 0.0	6.25

*NA-No Activity

The diverse biological actions of the *H. ellipticum* plant were attributed to the predominant chemicals in its essential oils. Terpinolene was the most unique chemical substance of *H. ellipticum*, and there are also Piperitol, Rosifoliol, Farnesene, Caryophyllene, Linalyl phenylacetate, and Iso-isopulegol.

GC-MS analysis of essential oil

The essential oil of the *H. ellipticum* species was analysed by means of the GC-MS technique. The results are shown in Table 1. The essential oil of *H. ellipticum* contained 20 compounds, and some major compounds being Terpinolene (21.77%), Iso-isopulegol (17.99%), Ocim-(4E,6Z)-ene <allo-> (5.4%), Farnesene (0.63%), Rosifoliol (0.45%), Humulene <alpha-> (0.81%), Caryophyllene (1.47%), Piperitol <trans-> (0.91%), Linalyl phenylacetate (2.49%) shown in Figure 4.

The GC-MS study of the essential oil of *H. ellipticum* revealed a diverse array of volatile chemicals, with the primary components identified as Terpinolene (21.77%) reported to have a biological activity like Antioxidant Activity, Antimicrobial Activity, Anti-inflammatory Properties.[30] Iso-isopulegol (17.99%) reported to have biological activity like Antioxidant Activity, Antimicrobial Activity, Anti-inflammatory Effects, Antidiabetic Activity.[31] Ocim-(4E,6Z)-ene <allo-> (5.4%) also reported to have biological activity like Antimicrobial Activity, Anti-inflammatory Properties, Antioxidant Effects, Insecticidal and Repellent Effects.[32] Farnesene (0.63%) reported to have biological activity like Insect repellency and insecticidal activity, antioxidant properties, antimicrobial activity.[40] Rosifoliol (0.45%) reported to have a biological activity like antioxidant Activity, anti-inflammatory effects, antimicrobial and antifungal activity.[41] Humulene <alpha-> (0.81%) reported to have biological activity like anti-inflammatory activity, antimicrobial and antifungal Properties, antioxidant properties.[39] Caryophyllene (1.47%) reported to have biological activity like anti-inflammatory activity, analgesic (Pain relief) properties, antioxidant properties.[37] Piperitol <trans-> (0.91%) reported to have biological activity like anti-inflammatory activity, antioxidant Activity, antimicrobial and antifungal properties.[38] Linalyl phenylacetate (2.49%) reported to have a biological activity like antioxidant activity, anti-inflammatory effects, antimicrobial and antifungal activity.[36]

Antibacterial activity of essential oil

The essential oil of *H. ellipticum* demonstrated a broad antibacterial spectrum, as shown by the disc-diffusion method, effectively suppressing the growth of all six assessed bacterial strains (Table 2). The study results indicate that when the test organism was treated with varying amounts of the sample on an agar plate, the sample demonstrated a maximum zone of inhibition of 17.5 ± 0.7 mm against *K. pneumoniae* and a minimum zone of inhibition of 9.6 ± 0.0 mm against *B. cereus* at a concentration of 100

Table 3. Molecular docking parameters of iso-isopulegol with selected bacterial target proteins

Ligand + Protein	Cur Pocket Id	Binding affinity	Cavity Vol (Å)	Centre (x, y, z)	Docking size (x, y, z)
iso-Isopulegol + 6RKS	C4	-5.3	1155	176, 168, 86	25, 17, 17
iso-Isopulegol + 3SWD	C1	-5.3	62112	-24, -18, -97	35, 35, 35
iso-Isopulegol + 8JTP	C5	-5.9	5109	-22, -52, -155	26, 35, 30
iso-Isopulegol + 6PJ3	C2	-6.5	223	-9, -43, -12	17, 17, 17
iso-Isopulegol + 1QMF	C1	-6.0	2187	94, 59, 56	29, 17, 30
iso-Isopulegol + 2Z2L	C2	-5.5	6052	25, 14, -1	35, 29, 35

µg/disk. The zone of inhibition refers to the region surrounding a disk on an agar plate where bacterial growth is absent, attributed to the effect of an antimicrobial agent. This method assesses the susceptibility of a specific test organism to a given antimicrobial agent. The MIC of ciprofloxacin against *Streptococcus pneumoniae* was found to be 1 µg/mL. According to CLSI guidelines, an MIC of 1 µg/mL indicates that *S. aureus* is susceptible to ciprofloxacin. The MIC of ciprofloxacin against *Str. pneumoniae* aligns with expected clinical breakpoints, confirming its susceptibility. This suggests that ciprofloxacin is an effective antibiotic for treating infections caused by the strain of *Str. Pneumoniae* (Figure 5).

Antioxidant activity

DPPH radical-scavenging activity

The DPPH radical-scavenging activity of the oil was examined at specified dosage levels. The essential oil exhibited varying efficacy in neutralizing DPPH radicals. Based on the results of the study, it was observed that only sample *H. ellipticum* exhibited low antioxidant property (DPPH) with an IC_{50} value above the dose limit at the maximum used concentration. In comparison to the standard Ascorbic Acid, which had an IC_{50} value of 4.573 ± 0.036 µg/ml (Figure 6).

Antioxidative potential of essential oil determined via ABTS assay

The results indicated that *H. ellipticum* exhibited weak ABTS radical scavenging activity, with an IC_{50} value exceeding the highest concentration tested. In comparison to the standard Ascorbic Acid, which had an IC_{50} value of 2.434 ± 0.043 µg/ml (Figure 7).

Antifungal activity of essential oil

According to the findings of the study, when the test organism (*C. albicans* and *A. niger)* was treated with different concentrations of the sample on an agar plate, the maximum zone of Inhibition was 9.0 mm at 25% dose against the test organism *C. albicans,* as compared to the positive control (Maximum zone of Inhibition 20 mm at a 25 µg dose). A maximum zone of inhibition of 10 mm diameter was observed at 500 and 1000 µg doses of the sample. A clear zone of inhibition was formed around the disc of positive control (Approx 20 mm in diameter) (Figure 8). The zone of inhibition is an area around a disc on an agar plate where no fungal growth is observed due to the presence of an antimicrobial agent. It is used to determine whether a particular test organism is susceptible to the action of a particular antimicrobial agent or not.

Molecular docking of the major compound against pathogenic bacteria

Docking analysis was carried out to examine the binding efficiency and interaction pattern of iso-isopulegol (Table 3), a monoterpenoid alcohol, against a panel of key bacterial target proteins involved in essential cellular processes, including DNA replication, cell wall biosynthesis, fatty acid metabolism, and folate synthesis. The docking results demonstrated that iso-isopulegol exhibits consistent and moderate binding affinities, with binding energies ranging from -5.3 to -6.5 kcal/mol, indicating stable ligand-protein interactions across all selected targets (Figure 9).

The results indicated that, among the selected proteins, the complex of LpxH/JH-LPH-33 from *K. pneumoniae* had the highest binding affinity, with a binding energy of -6.5 kcal/mol

Table 4. Molecular docking of Terpinolene with selected bacterial target proteins

Ligand + Protein	Cur Pocket Id	Binding affinity	Cavity Vol (Å)	Centre (x, y, z)	Docking size (x, y, z)
Terpinolene + 5CFZ	C2	-5.8	321	-21, 33, -9	17, 17, 17
Terpinolene + 3SWD	C2	-5.5	55238	17, 43, -59	35, 35, 35
Terpinolene + 6WAA	C1	-6.0	24820	6, -1, -69	35, 35, 35
Terpinolene + 6PJ3	C1	-6.7	630	-11, -36, -5	17, 17, 17
Terpinolene + 1QMF	C1	-5.7	2187	94, 59, 56	29, 17, 30
Terpinolene + 2Z2L	C1	-5.5	6836	22, 1, -32	34, 34, 23

and a relatively small cavity volume of 223 $Å^3$. This very compact nature of the binding pocket may indicate efficient accommodation of iso-isopulegol and its potential to interfere with the biosynthesis of lipid A, an important route for the integrity of the bacterial outer membrane. Correspondingly, penicillin-binding protein 2X, in its acyl-enzyme complex form (PDB ID: 1QMF), showed a strong interaction with iso-isopulegol, yielding a binding affinity of -6.0 kcal/mol to support its possible role in peptidoglycan cross-link inhibition during bacterial cell wall synthesis.

Iso-isopulegol had equally impressive interactions with the enzymes involved in DNA replication, such as *Escherichia coli* DNA gyrase domains (PDB codes: 6RKS, 6KZV), with binding energies of -5.3 kcal/mol and -5.5 kcal/mol, respectively. These results indicated the probable inhibitory role of iso-isopulegol against the supercoiling and replication of DNA. Furthermore, efficient binding interactions were also obtained against MurA (PDB code: 3SWD), an important enzyme that catalyzes the first committed step of peptidoglycan synthesis, in addition to the enzyme enoyl-acyl carrier protein reductase (FabI; PDB code: 8JTP), an essential enzyme responsible for the synthesis of fatty acids in bacteria. The obtained binding energies in these targets also strengthen the broad-spectrum antimicrobial properties of iso-isopulegol.

Moreover, iso-isopulegol exhibited a strong binding affinity with dihydrofolate reductase (DHFR; PDB ID: 3IX9) and penicillin-binding protein 2X (PDB ID: 2Z2L) from *S. pneumoniae* with binding energy scores of -5.5 kcal/mol, which might disrupt folate metabolic and cell wall biosynthetic pathways. Interestingly, the binding results revealed that iso-isopulegol possessed the capacity to bind effectively within small and large pockets, which is primarily due to its highly flexible nature.

The findings from the studies summarized above suggest that iso-isopulegol acts on a multi-targeted basis (in terms of its antimicrobial effects) and affects multiple key Biological Cellular Processes (BCPs). The polypharmacological properties of iso-isopulegol may help address the problem of antibiotic drug resistance and, therefore, indicate the potential of iso-isopulegol to be developed as a lead compound for novel antibacterial agents. The corresponding docking studies have provided strong support for the experimental multi-target approach (MTA) observation for monoterpenoid compounds. Thus, validation of these results should be performed by means of molecular dynamics simulations and/or further in vitro/vivo validation.

Molecular docking analysis of terpinolene,

Docking results indicated moderate to strong binding with all of the tested targets, using docking scores between -5.5 and -6.7 kcal/mol (Table 4), demonstrating favourable interactions in general cases (Figure 10). Of these, the highest binding energy was found with the LpxH/JHLPH33 complex of *Klebsiella pneumoniae* (PDB ID: 6PJ3) with -6.7 kcal/mol in a cavity volume of about 630 $Å^3$. LpxH is an essential enzyme in lipid A biosynthesis, and successful Terpinolene binding to this tight pocket indicates the potential to inhibit the formation of the outer membrane of bacteria. Compound terpenolene also exhibited better inhibition towards the topoisomerase IV (ParE–ParC) of *K. pneumoniae* (PDB ID: 6WAA), with a binding affinity of -6.0 kcal/mol. Since topoisomerase IV

is important for DNA replication and chromosome segregation, this interaction would suggest a potential targeting on the bacterial DNA topology and the replicative machinery. Significant binding to also dihydrofolate reductase (DHFR; PDB ID: 3IX9) of the bacteria *S. pneumoniae* was recorded (-6.2 kcal/mol), indicating interference with the metabolism of folates and biosynthesis of nucleotides. Low but confident binding affinities were obtained for binding to FabI (PDB ID: 5CFZ) and MurA (PDB ID: 3SWD), with ΔG bind values of -5.8 and -5.5 kcal/mol, respectively. FabI is the fundamental enzyme of fatty acid biosynthesis, and MurA is responsible for the first committed step in peptidoglycan biosynthesis. The ability of Terpinolene to interact with these enzymes also corroborates its potential in modulating various steps of bacterial cell envelope biogenesis.

Moreover, Terpinolene exhibited constant binding to PBP-2X in both the acyl-enzyme complex (PDB ID: 1QMF; -5.7 kcal/mol) and native form (PDB ID: 2Z2L; -5.5 kcal/mol). PBP-2X

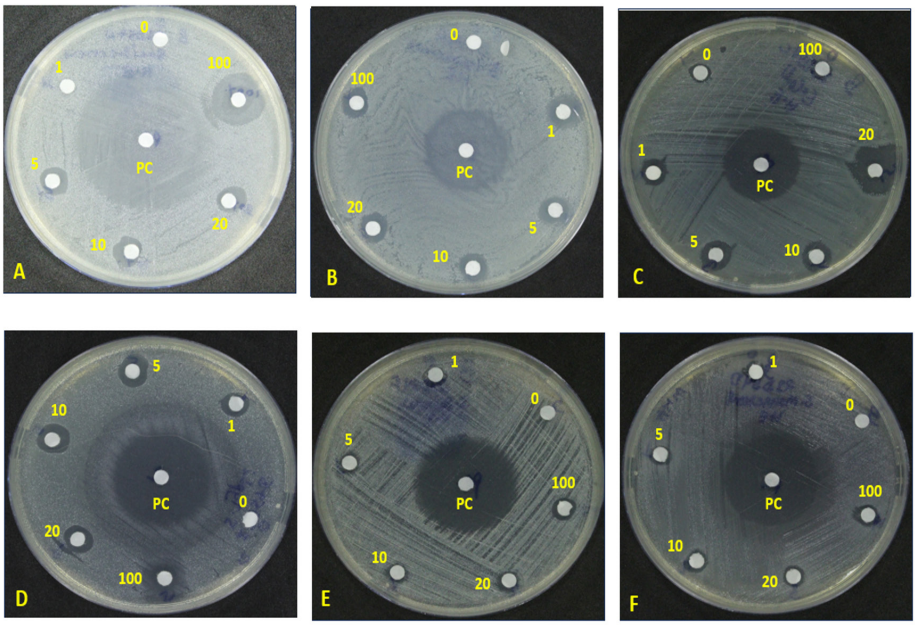

Figure 5. Inhibitory activity of *H. ellipticum* essential oil against bacterial strains, (A) *K. pneumoniae,* (B) *Str. pneumoniae,* (C) *E. coli,* (D) *S. typhi,* (E) *S. aureus,* (F) *B. cereus*

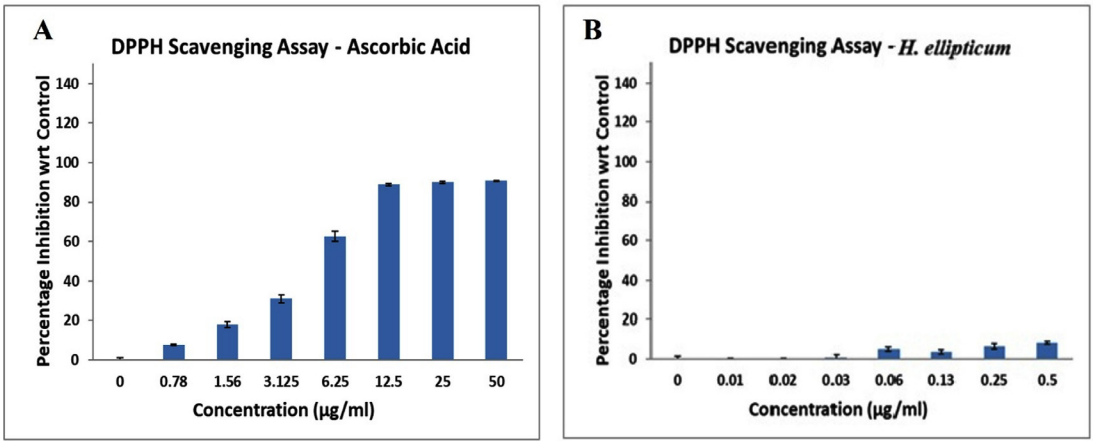

Figure 6. DPPH Scavenging activity; (A) Ascorbic acid (positive control); (B) *H. ellipticum* essential oil

is responsible for peptidoglycan cross-linking and binding of ligands, which may adversely affect bacterial cell wall integrity, especially in Gram-positive organisms.

It is noteworthy that Terpinolene showed binding efficacy with both smaller and larger binding cavities. This is an indication that it is structurally versatile and that its binding process is facilitated by good hydrophobic complementarity. The nature of Terpinolene, as well as its high lipophilicity, suggests that the hydrophobic and van der Waals forces are responsible for the binding. This is typical of monoterpene derivatives.

In general, the docking study reveals that Terpinolene has a complex pattern of multi-target interaction, which binds to a number of crucial protein targets of bacterial origin related to cell wall biosynthesis, lipid metabolism, DNA replication, and folate metabolism. A polypharmacological approach to antibacterial activity is a great advantage in terms of resistance development and explains why Terpinolene has a high potential as a natural antibacterial drug candidate. The binding energies found are moderate and comparable to the binding energies of other small nonpolar terpenes that have previously been reported.

Absorption, distribution, metabolism, and excretion (ADME) properties and drug-likeness prediction of iso-Isopulegol and Terpinolene

The bioavailability radar chart shows iso-isopulegol to be positioned in a region where there

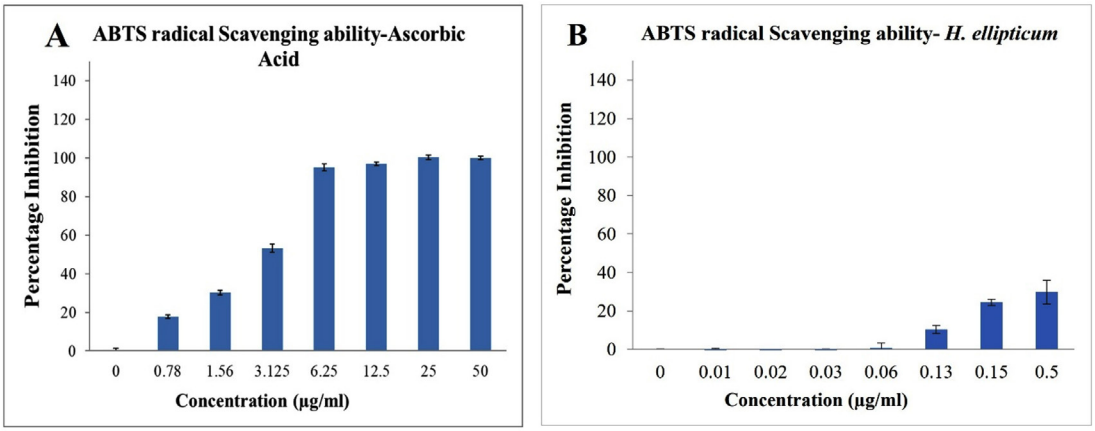

Figure 7. ABTS radical Scavenging activity; (A) Ascorbic acid (positive control); (B) *H. ellipticum* essential oil

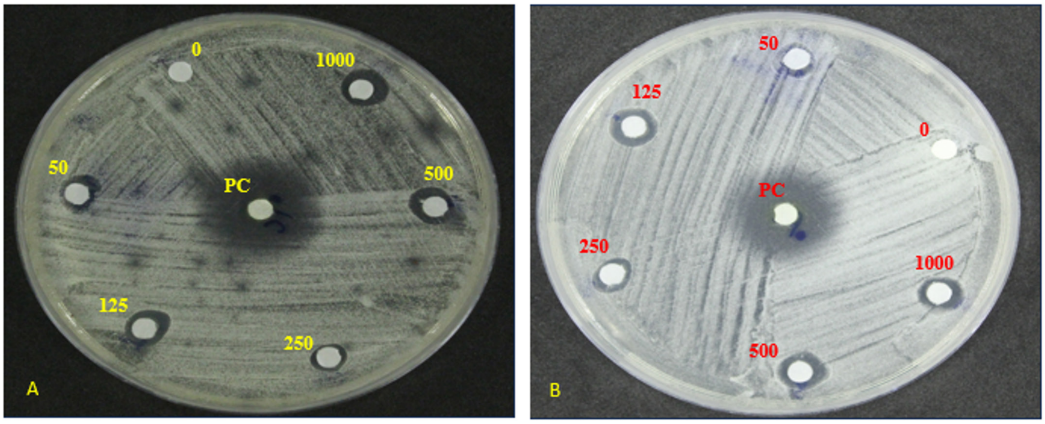

Figure 8. Antifungal activity of *Hedychium ellipticum* essential oils against fungal strains; (A) *Candida albicans;* (B) *Aspergillus niger*

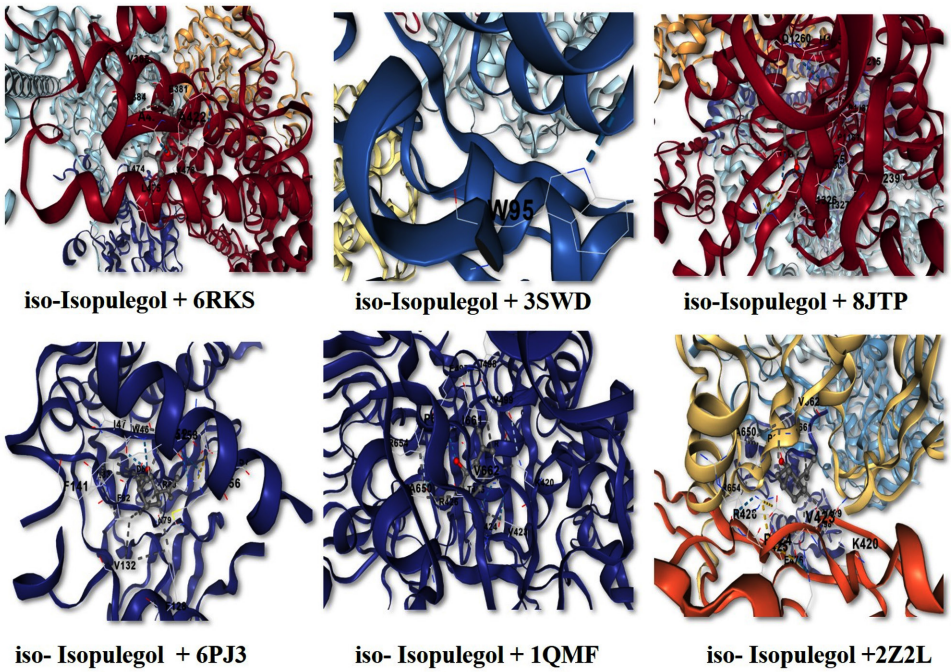

Figure 9. Molecular docking of iso-isopulegol with selected bacterial target proteins

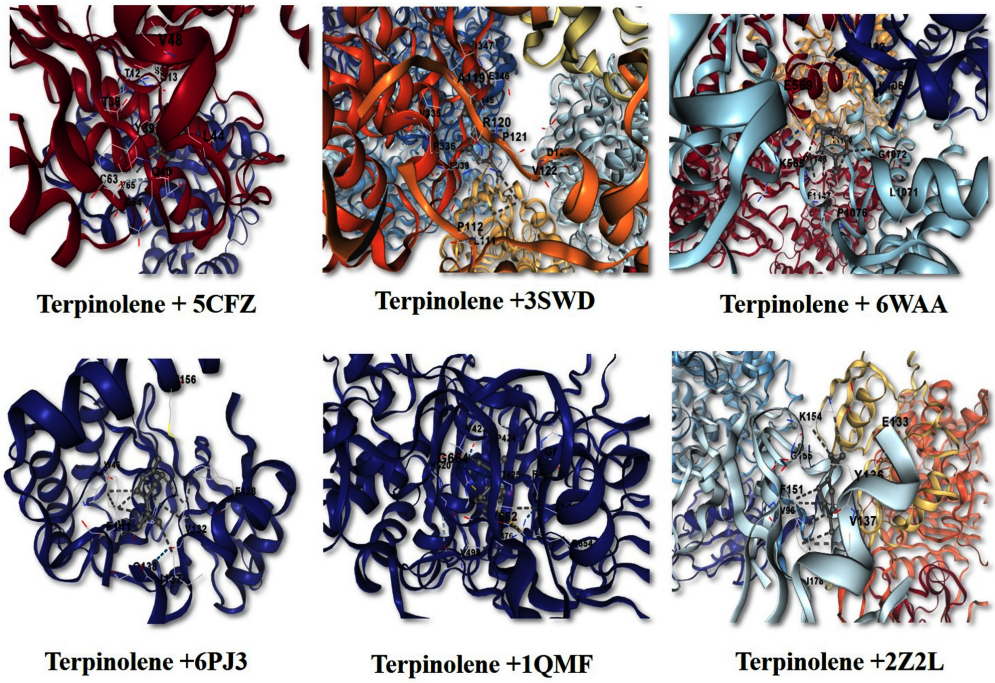

Figure 10. Molecular docking of Terpinolene with selected bacterial target proteins

is optimal physicochemical property availability for bioavailability. The iso-isopulegol structure displays optimal lipophilicity (LIPO), important for efficient cell permeability.[43,44] Another important property is optimal molecular size (SIZE), important for proper cell target engagement, as well as optimal flexibility (FLEX), facilitating cell membrane permeability.[45] Moreover, polarity (POLAR), due to the hydroxyl functional group, is displayed moderately; this differentiates iso-isopulegol from hydrocarbon monoterpenes. Polarity is important in increasing intermolecular interactions. However, its aqueous solubility (INSOLU), due to its lipophilic nature, is expected to be low.[46] Notably, monoterpenoid structures display low solubility. Furthermore, its degree of saturation (INSATU), playing a critical role in its stability and metabolic availability, still stays in the required range.

The BOILED-Egg plot (Figure 11) predicts that iso-isopulegol is located within the yellow (yolk) region, indicating a high probability of blood-brain barrier (BBB) penetration, while also remaining within the area associated with good human intestinal absorption (HIA).[47] This dual prediction suggests that iso-isopulegol may efficiently cross biological membranes and reach central compartments, which is consistent with reported neurological and antimicrobial activities of monoterpenoid alcohols.[48,49]

Iso-isopulegol was predicted to be a non-substrate for P-glycoprotein, or PGP, which suggests poor active efflux from intestinal epithelial cells or the brain. This property is expected to improve intracellular retention and bioavailability at the tissue level, especially in tissues protected by such efflux transporters as the central nervous system.[50]

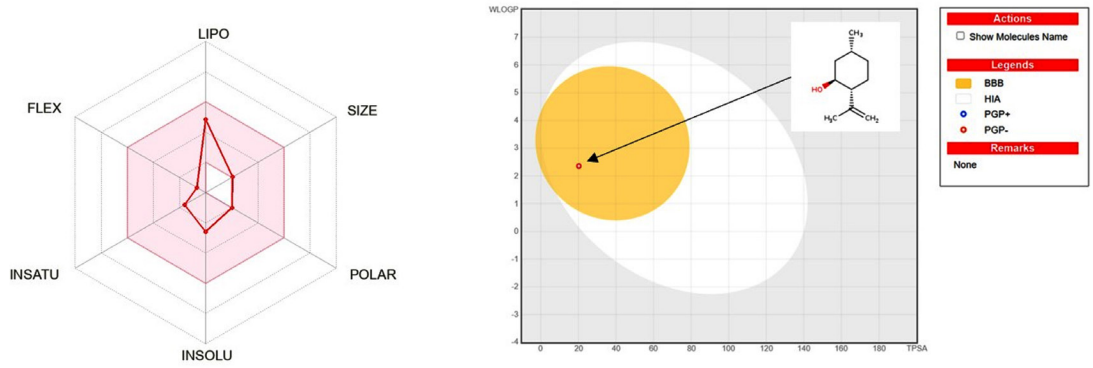

Figure 11. The BOILED-Egg plot showed that iso-isopulegol has a high probability of blood-brain barrier (BBB) penetration

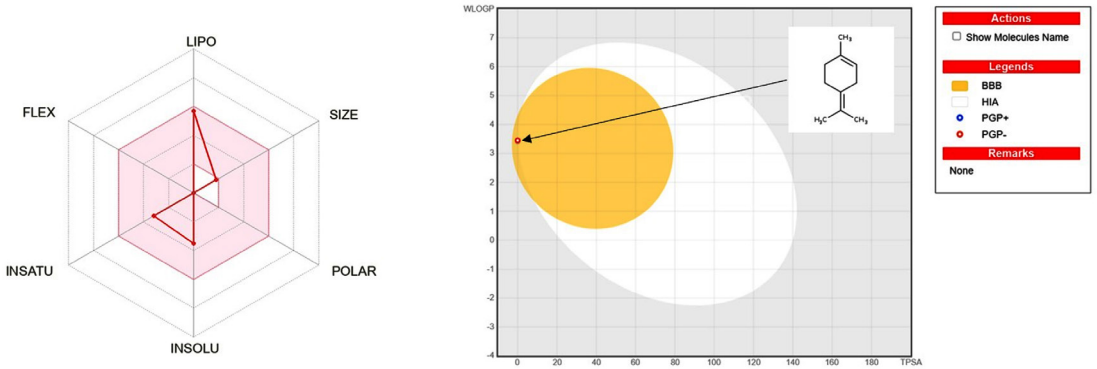

Figure 12. The BOILED-Egg plot showed that Terpinolene has a high probability of blood-brain barrier penetration

The moderate lipophilicity, together with the presence of a hydroxyl group, implies that iso-isopulegol would be metabolically transformed through phase I reactions, mainly by the oxidation mediated by cytochrome P450. The metabolites produced may have increased polarity and thus be excreted easily either renally or biliary. Rapid metabolism is possible, but again it is common in monoterpenoid structures and can be improved through formulation or structural optimization.[51,52]

The ADME (Absorption, Distribution, Metabolism, and Excretion) properties of Terpinolene were evaluated using an in silico Swiss ADME-based pharmacokinetic profiling approach. The results are illustrated through a bioavailability radar and a BOILED-Egg model (Figure 12), providing a comprehensive overview of the molecule's drug-likeness and pharmacokinetic suitability. The bioavailability radar demonstrated that Terpinolene is within the ideal space for most relevant physicochemical parameters, such as lipophilicity (LIPO), size (SIZE), polarity (POLAR), solubility (INSOLU), saturation (INSATU), and molecular flexibility (FLEX).[44] The radar profile analysis shows that the compound is very lipophilic as are monoterpenoid hydrocarbons, and this would favour penetration through membranes. However, low polarity and aqueous solubility were relatively poor systemically, limiting systemic bioavailability as is often seen with volatile terpene molecules.[48,53]

Despite these limitations, the overall radar plot suggests that Terpinolene largely satisfies the criteria for oral bioavailability, particularly in terms of membrane diffusion and molecular size, supporting its potential as a bioactive lead rather than a classical drug molecule.[45]

The BOILED-Egg plot provides insight into gastrointestinal absorption (HIA) and blood-brain barrier (BBB) permeability. Terpinolene is positioned within the yellow (yolk) region, indicating a high probability of blood-brain barrier penetration, while still maintaining favorable human intestinal absorption.[47] This distribution profile suggests that Terpinolene may exert central nervous system (CNS)-related biological effects, which is consistent with reported neuroactive and antimicrobial properties of terpene compounds.[49] The molecule is also predicted to be non-substrate for P-glycoprotein (PGP-), indicating that it is unlikely to be actively effluxed by intestinal or brain efflux transporters. This characteristic enhances its intracellular retention and bioavailability, particularly in CNS tissues.[50] The pronounced lipophilicity of Terpinolene suggests that it is likely to undergo hepatic metabolism, primarily via cytochrome P450-mediated oxidation pathways, a common metabolic route for terpene hydrocarbons. While high lipophilicity may lead to rapid metabolic clearance, it also supports effective tissue penetration.[51,52] The absence of polar functional groups implies that metabolic transformation may be necessary to facilitate renal or biliary excretion.

Collectively, the ADME analysis indicates that Terpinolene possesses favorable absorption and distribution characteristics, particularly with respect to intestinal permeability and BBB penetration. Although its low polarity and solubility may limit conventional oral drug development, these properties are typical of natural terpenes and do not preclude biological efficacy. Instead, they support its role as a bioactive natural lead compound, especially for antimicrobial or CNS-associated applications.[45,49]

The predicted pharmacokinetic behavior of Terpinolene complements molecular docking findings and supports further investigation through formulation optimization, metabolic stability studies, and in vitro/in vivo validation.

CONCLUSION

This study examined the chemical composition, antioxidant activity, and antimicrobial properties of *H. ellipticum*. In conclusion, the essential oil of *H. ellipticum* is predominantly composed of Terpinolene, which is the primary contributor to its biological activity. This study demonstrated that the essential oil of *H. ellipticum* had antimicrobial activity against the investigated microorganisms and possessed antioxidant properties, indicating its potential as a natural therapeutic agent for health and agricultural applications. Consequently, the essential oil of *H. ellipticum* can be utilized in the formulation of antibiotics, bioinsecticides, and food preservatives. Nonetheless, on a broad practical scale, it is

imperative to have a deeper understanding of the impact of sublethal dosages of essential oils on non-target animals, along with possible toxicities to humans. Consequently, additional research on the potential toxicities of the evaluated essential oils is necessary for safety considerations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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