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ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF *ERYNGIUM AQUATICUM* ESSENTIAL OIL

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Abstract

Eryngium aquaticum L. (Apiaceae), a culinary herb enjoyed in global cuisines, is also valued for its medicinal properties. The essential oil (EO) extracted from *E. aquaticum* leaves by hydro-distillation was subjected to antibacterial and antibiofilm activity using a microtiter plate-based *in vitro* assay against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Compounds identified by GC-MS analysis of EO were screened against the transcriptional regulatory proteins SarA of *S. aureus* and LasR of *P. aeruginosa* by molecular docking analysis. The minimum inhibitory concentration (MIC) was recorded as 250 µg/mL against both of these two pathogens. The EO of *E. aquaticum* also showed concentration-dependent antibiofilm activity against these pathogens, with a maximum inhibition of 50.9 and 48.03% against *P. aeruginosa* and *S. aureus*, respectively, at the highest concentration (500 µg/mL) tested. The GC-MS analysis identified 17 compounds and all of them showed moderate to weak binding affinity for the active sites of SarA and LasR, with pentanedioic acid (2,4-di-*t*-butylphenyl) mono-ester showing the best docking score against SarA (-5.7 kcal/mol) and LasR (-8.0 kcal/mol). This study suggests that *E. aquaticum* can be a good source of EO with antibacterial and antibiofilm activity against *P. aeruginosa* and *S. aureus*.

Keywords: *Eryngium foetidum*, Essential oil, Antibacterial, Antibiofilm, Molecular docking

Introduction

The emergence of bacterial drug resistance, though widely reported after penicillin's introduction, has ancient roots, predating antibiotic discovery by millennia (Paun et al., 2021). While some resistance mechanisms are inherent to bacteria, others remain genetically silent and activate only in response to challenging environments. The emergence of drug resistance has outpaced the introduction of new antibiotics, meaning that counteracting this issue requires alternative attempts than just the introduction of newer antibiotics (Mühlen & Dersch, 2016). Investigations into bacterial drug resistance have identified the formation of biofilms as one of the key mechanisms by which bacteria develop protection in unfavorable environments. Microbes are much harder to kill inside a biofilm than in their planktonic form, and disrupting the biofilm can restore the effectiveness of antibiotics (Stewart & Costerton, 2001). Although some surfactants have been developed for use in combination with antibiotics, their application is largely limited to topical preparations because their non-specific mechanisms can disrupt human cells as well as bacterial ones (Percival et al., 2019). Thus, current research trends focus on finding suitable antibiofilm agents that can be used to help repurpose antibiotics that are ineffective against resistant bacteria.

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There is long history of using essential oils (EOs) to treat bacterial infections. Numerous studies also proved the antibacterial activity of different plant EOs against various human pathogens (Hou et al., 2022). As a result, EOs have found their way to be used in traditional medicine, cosmetic industry and in modern medicine as antibacterial agents (Angane et al., 2022). Some individual components of essential oils possess strong antibacterial activity and are commonly used alone or in combination for various purposes, including cosmetics, therapeutics, and food preservation. EOs have also been found to impart a synergistic effect or reverse the antibiotic resistance of MRSA, VRE, *Acinetobacter baumannii*, and *Enterococcus faecalis* when given in combination with antibiotics (El Omari et al., 2022; Rosato et al., 2020). Investigation of individual components of EO revealed that they vary in their antibacterial activity or the strength of the activity (Knobloch et al., 1989). As a result, the antibacterial activity of a given EO depends on the composition and individual components present in it (Moleyar & Narasimham, 1992). Use of EO by the people of a given geographic location depends on the availability of source plants that produce EO with good antibacterial activity (Angane et al., 2022).

Eryngium aquaticum L. (family: Apiaceae) also known as 'Bon dhonia' in Bangladesh, is used both as a culinary item for its aromatic properties or as medicine for several ailments. The plant is native to the West Indies but has been naturalized and is commonly cultivated throughout South Asia, the Pacific Islands, and the warmer regions of southern Europe. The plant is used to treat body ache, joint pain, malaria, fever, diarrhea, dysentery, ulcer, skin diseases and many other ailments by different ethnic groups around the world (Devi et al., 2021; Torres et al., 2017). The methanol and ethanol extract of the leaves of *E. aquaticum* has been reported for antibacterial activity against various Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Tabarak et al., 2016). The plant extract has been reported for several pharmacological activities including analgesic, anti-inflammatory, anticonvulsant, antidiabetic, anthelmintic, and antimalarial activity (Hemachandra et al., 2021; Paul et al., 2011). The composition of the EO of *E. aquaticum* from different geographic locations has been reported with a varying number of constituents and their amount (Paul et al., 2011). To the best of our knowledge, no biological activity including antimicrobial activity has been conducted so far with the EO of *E. aquaticum* except its antioxidant capacity (Thomas et al., 2017). As a part of our investigation of the search for bioactive natural products from traditional medicinal plants (Ali et al., 2018; Karmakar et al., 2021; Mitra et al., 2023; Zihad et al., 2022), we now explore the antibacterial and antibiofilm activity of the EO of *E. aquaticum*.

Materials and Methods

Plant collection

Fresh leaves of *E. aquaticum* were collected from the district of Barguna of Bangladesh during the month of July in 2023 and a voucher specimen was submitted to the Bangladesh National Herbarium with the accession number of DACB 91374 for future reference.

Hydro-distillation

The fresh plant materials were separated from undesirable plants or materials, chopped into small pieces and placed inside a 5 L round-bottomed flask of a Clevenger type apparatus with further addition of distilled water until the plant material (2 kg) is immersed. Hydro-distillation was continued for a period of 6 h with a constant temperature of 90-95 ℃. The condensed EO was separated by decantation and stored at -20 ℃ in glass vial until experiments commenced. The yield of EO was calculated according to the equation (1) where R_{HE} is the yield and *mHE* and *mS* are the masses of EO and plant material, respectively (Hanif et al., 2019).

$$R_{HE}(\%) = \frac{m\mathrm{HE}}{m\mathrm{S}} \times 100 \quad (1)$$

GC-MS analysis of EO

Gas chromatography mass spectrometric (GC-MS) analysis of the *E. aquaticum* EO was conducted using a GC/MS technique with a GC/MS-P2010-Ultra chromatograph from Shimadzu, Japan (serial number 020525101565SA) equipped with a capillary column (Rtx-5ms, 30 m × 0.25 mm × 0.25 μm). The sample was injected in split mode, with helium as the carrier gas at a flow rate of 1.61 mL/min. The temperature program started at 50 °C, increasing at 7°C/min to 180 °C, and then at 10 °C/min to a final temperature of 280 °C, with no hold time. The injection port temperature was set at 300

°C, the ion source temperature at 200 °C, and the interface temperature at 250 °C. The mass analysis was performed with scan mode set within the m/z range of 40-500 and the total run time of the experiment was 28 min. The components were identified by comparing the sample's retention times and mass fragmentation patterns to those in the National Institute of Standards and Technology (NIST) library (Hossain et al., 2017).

In vitro assays

Antibacterial activity test by MIC determination

Preserved cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa* were swabbed on nutrient agar media surface in a Petri dish and incubated overnight with the temperature of the incubator set at 37 ℃. The process was repeated twice to bring the pathogens to normal metabolic conditions. Bacterial culture was collected from the second culture using a transfer loop and mixed with a sterile normal saline solution. The turbidity of the resulting solution was adjusted to that of 0.5 McFarland standard to ensure a bacterial count of 1×10^8 CFU/mL. Serial dilutions were made to ensure a working bacterial count of 5×10^5 CFU/mL when applied in 96-well microtiter plates. The wells of 96-well microtiter plates were added with 100 µL of Mueller Hinton broth with the first well further received 100 µL of stocks of gentamicin or *E. aquaticum* EO prepared with 5% DMSO in distilled water. Serial dilution was such made that the concentration of gentamicin and *E. aquaticum* EO in the first wells were 20 and 500 µg/mL, respectively. The plates were wrapped with parafilm (Sigma Aldrich) and incubated for 18 h at 37℃. After incubation, 5 µL of resazurin solution (6.5 mg/mL) was added to each well and the colour change was recorded after 5 min to determine the minimum inhibitory concentration (MIC). The whole experiment was done in triplicate on three different days (Zihad et al., 2022).

Antibiofilm assay

Antibiofilm assay for *E. aquaticum* EO followed the same procedure as that of the MIC determination except that after the incubation period, the liquid medium from the wells were removed followed by the addition of 100 µL of 1% crystal violet solution and left for 30 min for staining of the cells. The crystal violet solution was discarded and the wells were washed with distilled water to remove any unstained dye. The biofilm forming cells were dissolved with 125 µL of 30% acetic acid solution and were transferred to a new microtiter plate to take the absorbance at 570 nm using a microplate reader (Multiskan GO, Thermo Scientific). The experiment was repeated on three different days and eugenol (Olszewska et al., 2020) was used as the positive control. Reduction in biofilm formation was calculated using equation (2) where OD stands for optical density (Bazargani & Rohloff, 2016).

$$\%\ Inhibition = \frac{OD\ in\ control - OD\ in\ treatment}{OD\ in\ treatment} \times 100 \quad (2)$$

In silico analysis

Molecular docking: ligand preparation

Three dimensional structures of the compounds identified by GC-MS analysis of *E. aquaticum* EO were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in sdf format. In case the 3D structure of any compound is unavailable in PubChem, 2D structures were downloaded and 3D structure was generated by Chem 3D 21.0.0. All the structures were optimized to minimize energy by Chem 3D 21.0.0 and the structures were saved in pdb format. The cocrystal structure ligand of the LasR protein, N-3-oxo-dodecanoyl-L-homoserine lactone (OHN), was used as the control molecule for the LasR protein (Liu et al., 2012; Liu et al., 2006). Meanwhile, ZINC990144, an inhibitor of the SarA protein identified through virtual screening, served as the control molecule for the SarA protein (Arya et al., 2015).

Molecular docking: protein preparation

Crystal structures of the transcriptional regulatory proteins SarA of *S. aureus* (PDB ID:2FRH) and LasR of *P. aeruginosa* (PDB ID: 2UV0) were downloaded from the Protein Data Bank website (https://www.rcsb.org/). Removal of water molecules, ligand and heteroatoms were done with the help of BIOVIA Discovery Studio Visualizer (v.4.5) and the proteins were prepared for molecular docking by AutoDock Tools (v.1.5.7) by the addition of polar hydrogens, surface charges and repairing missing atoms.

Molecular docking: virtual screening

Virtual screening of the ligands was done using the AutoDock Vina based software PyRx (v.0.8) (Dallakyan & Olson, 2015). The dimension and center of the gridbox for SarA (center: x = 37.5, y = -2.7, z = 20.8; size: 26 × 32 × 31) and LasR (center: x = 25.3, y = 38.2, z = 43.2; size: 27 × 24 × 23) proteins were such made to encompass key amino acid residues of the binding site of the ligand as appears in the ligand-bound crystal structure of the protein or as described in the literature (Arya et al., 2015; Liu et al., 2012; Liu et al., 2006). Docking scores were expressed in kcal/mol and binding mode with root mean square deviation (RMSD) of zero were only considered. The docking pose and ligand-protein binding interactions were analyzed using Discovery Studio Visualizer (v.4.5).

In silico ADMET analysis

Canonical SMILES of the compounds identified in GC-MS analysis were retrieved from PubChem database. The canonical SMILES were submitted to online ADMET analysis tool maintained by University of Lausanne and the SIB Swiss Institute of Bioinformatics (http://www.swissadme.ch/).

Results

Result of hydro-distillation and GC-MS analysis

Hydro-distillation of 2 kg fresh plant material of *E. aquaticum* produced 2.1 mL of EO with the yield of 0.105%. GC-MS analysis of the EO resulted in the identification of 17 compounds (**Table 1**). Compound **16** was the most predominant component of the EO with a percent area of 20.47 followed by compound **6** (15.21%) and **14** (14.93%).

Result of antibacterial activity test

E. aquaticum EO showed MIC of 250 µg/mL against both pathogens under investigation while that of gentamicin was 0.78 µg/mL against these pathogens.

Table 1. GC-MS analysis of *E. aquaticum* EO.

Compound number	Name of the compound	Retention time (t_r)	% Area
1	Bicyclo [4.1.0] heptane, 7-pentyl-	12.2	2.72
2	N-(5-azidopentyl)-4-methyl-4-vinylazetidin-2-one	15.2	2.83
3	Cyclododecanol	17.32	7.64
4	Benzaldehyde, 2,4,5-trimethyl	18.43	4.57
5	Tricosanoic acid, isobutyl ester	18.88	4.21
6	8-Hexadecenal, 14-methyl	19.06	15.21
7	Pentanedioic acid, (2,4-di-*t*-butylphenyl) mono-ester	21.08	2.53
8	Pentanoic acid, 5-hydroxy-, 2,4-di-*t*-butylphenyl esters	22.13	3.02
9	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene	22.58	3.36
10	3-Methyl-2-(2-oxopropyl)furan	23.64	3.47
11	*cis*-1-Chloro-9-octadecene	27.51	3.43
12	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	28.77	1.3
13	Methyl 11-methyl-dodecanoate	29.82	3.93
14	13-Octadecenoic acid, methyl ester	31.83	14.93
15	N-propyl 11-octadecenoate	33.92	3.29
16	2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene) pentadecane	35.78	20.47
17	*Z*,*Z*-6,27-Hexatriactontadien-2-one	37.77	3.09

Result of antibiofilm assay

A concentration dependent inhibition of biofilm formation by the test pathogens were observed for *E. aquaticum* EO with the highest inhibition of 48.0% against *S. aureus* and 50.9% against *P. aeruginosa* at the highest concentration of 500 µg/mL employed (**Figure 1**). Inhibition of the biofilm formation for both of the pathogens under investigation followed a concentration dependent manner and no noticeable inhibition was observed below the *E. aquaticum* EO concentration of 15.63 µg/mL.

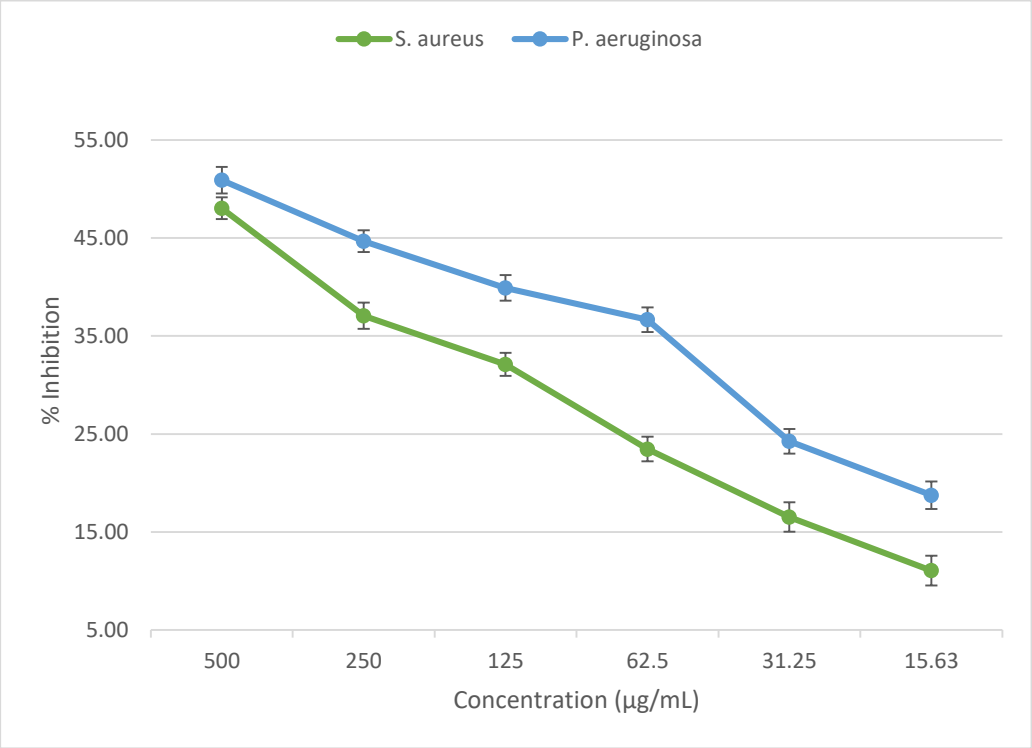

Figure 1. Antibiofilm activity of *Eryngium aquaticum* EO against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Values expressed as mean±SD

Results of molecular docking

The results of molecular docking analysis are presented in **Table 2**. The highest binding affinity for SarA protein was observed for compound **7** (-5.7 kcal/mol) while that for LasR was observed for compounds **7**, **12**, **14** and **15** with the same binding energy of -8.0 kcal/mol. The binding mode of compound **7** with SarA involves two hydrogen bonds with Gln164 (2.91 Å) and Asn158 (2.65 Å) and three hydrophobic interactions with Lys154 (4.50, 4.47 and 3.61 Å) (**Figure 2**). While that of compound **7** with LasR include one hydrogen bond with Ala76 (1.87 Å), one carbon-hydrogen bond with Val76 (3.21 Å), one electrostatic interaction with Asp73 (4.65 Å) and eight hydrophobic interactions with Leu36 (3.93 Å), Tyr64 (3.69 Å), Ile52 (5.42 Å), Leu36 (4.11 Å), Tyr56 (4.95 Å), Tyr64 (4.56 Å), Val76 (4.69 Å) and Ala127 (4.19 Å) (**Figure 3**).

Results of ADMET analysis

ADMET analysis of the 17 compounds of *Eryngium aquaticum* EO shows that most of the compounds does not pose any hazard as mutagenic or tumorigenic. Some of the compounds were irritant which is often common with many other EO or EO component. Most of the compounds followed most of the Lipinski's rule of five except that their lipid solubility is slightly higher. This is often common with EOs since most of the essential components are often too non-polar due to their higher content of hydrocarbons and less content of heteroatoms. Different parameters of ADMET of the 17 compounds of *Eryngium aquaticum* EO is presented in **Table 3**.

Table 2. Binding energy of *Eryngium aquaticum* EO components for SarA and LasR proteins.

Compound number	Name of the compound	Binding energy (kcal/mol)	
		SarA	LasR
1	Bicyclo [4.1.0] heptane, 7-pentyl-	-4.4	-7.3
2	*N*-(5-azidopentyl)-4-methyl-4-vinylazetidin-2-one	-3.9	-7.1
3	Cyclododecanol	-4.7	-6.7
4	Benzaldehyde, 2,4,5-trimethyl	-4.6	-7.0
5	Tricosanoic acid, isobutyl ester	-3.8	-6.7
6	8-Hexadecenal, 14-methyl	-4.5	-7.9
7	Pentanedioic acid, (2,4-di-t-butylphenyl) mono-ester	-5.7	-8.0
8	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	-5.2	-7.6
9	1*H*-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene	-5.0	-6.1
10	3-Methyl-2-(2-oxopropyl)furan	-4.1	-6.7
11	*cis*-1-Chloro-9-octadecene	-4.3	-7.9
12	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	-4.7	-8.0
13	Methyl 11-methyl-dodecanoate	-4.4	-7.4
14	13-Octadecenoic acid, methyl ester	-4.6	-8.0
15	*N*-propyl 11-octadecenoate	-4.1	-8.0
16	2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene) pentadecane	-4.5	-7.9
17	*Z*,*Z*-6,27-Hexatriactontadien-2-one	-3.7	-3.6
Control	ZINC990144	-7.9	-
	OHN	-	-8.9

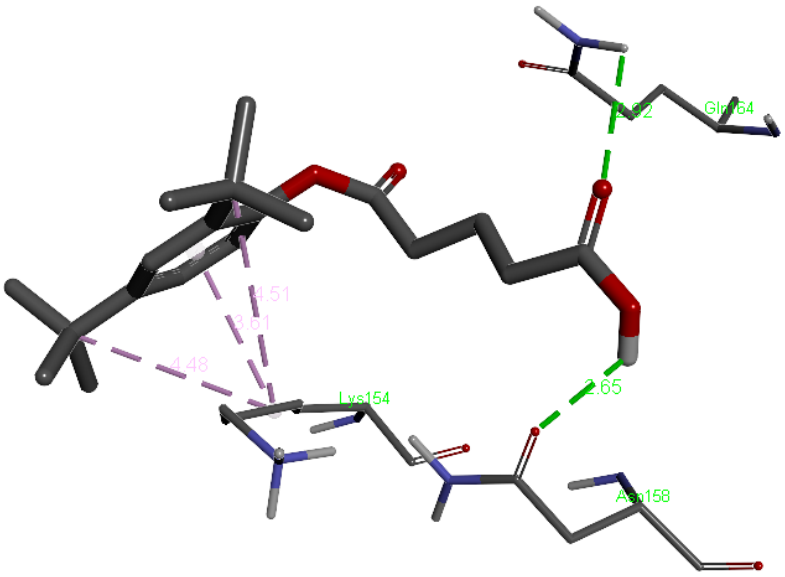

Figure 2. Interactions of compound 7 with SarA of *Staphylococcus aureus*. Bond lengths are in Å; Green: hydrogen bond; Light pink: alkyl and pi-alkyl interactions

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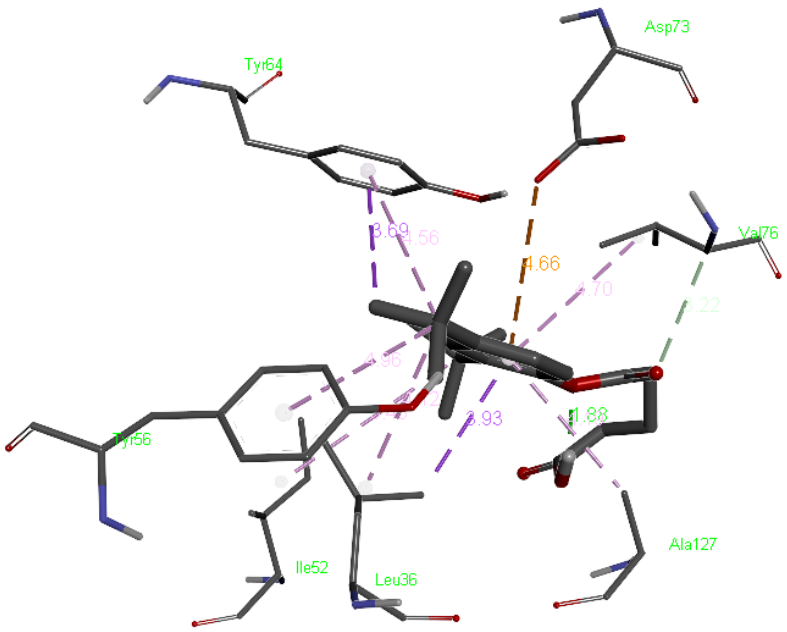

Figure 3. Interactions of compound 7 with LasR of *Pseudomonas aeruginosa*. Bond lengths are in Å; Light green: carbon-hydrogen bond; Orange: pi-anion; Pink: pi-sigma; Light pink: alkyl and pi-alkyl interactions

Table 3. ADMET profiles of *Eryngium aquaticum* EO components.

Compound number	M	T	I	RE	CLogP	S	MW	TPSA	DL	DS
1	n	n	n	n	4.04	-3.53	166	0	-13.1	0.39
2	n	n	n	n	1.71	-1.84	222	46.27	-3.99	0.48
3	n	n	n	n	4.43	-3.37	184	20.23	-5.59	0.37
4	y	y	n	n	2.62	-2.97	148	17.07	-5.57	0.29
5	y	n	y	y	6.17	-4.4	252	17.07	-11.9	0.05
6	y	n	y	y	6.17	-4.4	252	17.7	-11.9	0.05
7	n	n	y	n	4.9	-4.52	320	63.6	-12.1	0.18
8	n	n	y	n	5.25	-4.54	306	46.53	-18.32	0.17
9	n	y	y	n	3.17	-3.34	220	20.23	-4.73	0.15
10	n	n	y	n	1.35	-2.07	138	30.21	-1.98	0.32
11	y	y	n	y	8.22	-5.48	286	0	-31.72	0.05
12	n	n	n	n	6.02	-4.15	270	26.3	-23.2	0.26
13	n	n	n	n	4.89	-3.45	228	26.3	-24.5	0.34
14	n	n	n	n	7.15	-4.68	296	26.3	-30.9	0.21
15	n	n	n	n	8.01	-5.25	324	26.3	-27.3	0.18
16	n	n	y	n	9.96	-6.21	348	0	-7.03	0.09
17	n	n	y	n	14.87	-9.36	516	17.07	-26.1	0.06

M: mutagenic; T: tumorigenic; I: irritant; RE: reproductive effect; S: solubility; TPSA: topological polar surface area; DL: drug likeness; DS: drug score. n indicates no and y indicates yes.

Discussion

Natural products have been the primary source of antibiotics since the discovery of penicillin. Even in the era of medicinal chemistry, antibiotics derived from natural sources, such as cephalosporins, macrolides, aminoglycosides, and tetracyclines, continue to play a crucial role in controlling bacterial infections. However, the constant evolution of resistant bugs suggests newer innovative attempts to counteract bacterial infections. Thus, scientists are more than ever focusing on the discovery of agents that can affect biofilms (Song et al., 2018). Morbidity and mortality by *S. aureus* and *P. aeruginosa* are the most common causes of death worldwide. The ability to form biofilms in various biotic and abiotic surfaces is one of the major reasons behind the failure to treat such bacterial infections. An alternative approach could be the addition of some natural products that do not kill the pathogen but result in phenotypic changes that can reverse the drug resistance. Results have shown that a combination of antibiofilm agents can reduce the MIC of currently available antibiotics (Santativongchai et al., 2022).

Natural products often show weaker antibacterial activity against Gram-negative bacteria than that of Gram-positive bacteria. Interestingly, *E. aquaticum* EO showed almost equal antibacterial and antibiofilm activity against both Gram-positive and Gram-negative bacteria in this study. Since Gram-positive and Gram-negative bacteria have fundamentally different cell wall structures, agents that inhibit cell was synthesis can be more effective against some bacteria than others. However, if the agent affects both types of bacteria equally, it likely works in a different way, by a mechanism that has the same effect on both. From this point of view, it can be concluded that the antibacterial activity *E. aquaticum* EO follows mechanism(s) other than that of cell wall synthesis. Since the composition of a given EO sample is often complex, it is also possible that a range of mechanisms are contributing towards the observed antibacterial activity. Suggested mechanisms through which EOs exert their antibacterial mechanism includes the destruction of bacterial cell wall and cell membrane, disruption of ATP production and protein synthesis (Angane et al., 2022). For example, oregano EO has been found to inhibit respiratory and energy metabolism in methicillin resistant *S. aureus* (MRSA) (Cui et al., 2019). *Litsea cubeba* EO exerts its antibacterial action against MRSA by breaking cell membrane and inhibiting hexose monophosphate pathway (Hu et al., 2019). The antibacterial activity of cinnamon EO against *P. deceptionensis* is attributed to its ability to disrupt the cell membrane. This disruption leads to the leakage of intracellular substances and an increase in oxidative stress within the bacterial cell (Zhao et al., 2023). The high lipophilicity of EO makes them easy to target bacterial cell wall and cell membrane. EO can release lipopolysaccharides from bacterial cell wall and increase the bacterial cell permeability and loss of ATP or other cell components (Hui et al., 2017). EOs are also shown to give antibacterial activity through reduction in intracellular pH, coagulation of cytoplasmic materials, damaging bacterial DNA and interfering quorum sensing process (Angane et al., 2022; Casalino et al., 2023; Shu et al., 2024). Thus, the observed antibacterial activity of *E. aquaticum* EO could include one or more of the above mechanisms that resulted in the inhibition of the pathogens under investigation.

Antibiofilm activity of EOs is a recent discovery and it is believed that the antibacterial activity of EOs by part due to its ability to inhibit bacterial biofilm formation (Bai et al., 2023; Stringaro et al., 2018). Since the composition of EO varies with plant species and even with the same plant but with different geographic locations, it is necessary to investigate the antibiofilm activity of individual EO samples to identify its ability to inhibit biofilm formation of different bacterial strains (Angane et al., 2022). With this perspective, we need to identify indigenous sources of plant EO that can be useful as promising antibiofilm agents for possible application in therapeutics. The present investigation is an attempt to investigate the antibiofilm activity of the EO of the plant *E. aquaticum* which has been naturalized in Bangladesh and later found its extensive use as a culinary herb. The EO of *E. aquaticum* showed moderate antibacterial and antibiofilm activity against *S. aureus* and *P. aeruginosa*. The concentration dependent antibiofilm activity showed the highest inhibition at the highest concentration tested and maintained a positive effect up to the concentration of 15.63 µg/mL. These results suggest that the antibiofilm activity of *E. aquaticum* EO might have contributed to its antibacterial activity observed in the microtiter-based MIC test. In case of *E. aquaticum* EO, antibiofilm activity is also a contributing factor as obvious from the *in vitro* study.

Isolation and purification of individual components from an EO sample is often difficult and therefore use of the whole of the EO is a common practice. To predict the underlying mechanism of the antibiofilm activity for individual components of *E. aquaticum* EO, the two most common proteins namely SarA and LasR were chosen for the virtual screening. SarA is one of the most commonly studied protein that regulates the Transcription of 120 genes associated with

biofilm formation in *S. aureus* (Roberts et al., 2006). The wing region of SarA protein consisting of Arg184, Asp188, Glu189 and Arg190 amino acid residues interact with promoter region of target genes (Liu et al., 2006). Thus, the binding of a ligand in this region with strong affinity can help in the inhibition of transcriptional factors that regulate biofilm formation in *S. aureus*. However, due the flexibility of the wing region, it is often difficult to find suitable inhibitors for this protein. Finding a suitable inhibitor using in vitro methods can be challenging when targeting a specific protein. However, molecular docking analysis can aid in identifying a specific inhibitor for a particular protein site at a lower cost. By using virtual screening to search a library of compounds, we can identify molecules with a strong affinity for the key binding site of our target protein. In the virtual screening, most of the compounds of *E. aquaticum* EO showed moderate affinity with the best docking score of -5.7 kcal/mol was observed for compound **7**. Despite not interacting with key amino acids, the molecule was still able to bind to the wing region which involved two hydrogen bonds, measuring 2.62 and 2.92 Å in length. On the other hand, LasR is a vital transcription regulatory protein in *P. aeruginosa*, the absence of which results in the loss of virulence. The pocket of LasR protein that allows the binding of quorum sensing molecules is lined with Tyr56, Trp60, Arg61, Asp73, Leu110, and Ser129 amino acid residues that interacts with the control inhibitor OHN (Bottomley et al., 2007; Liu et al., 2012). Compound **7** formed a number of interactions at the binding site of LasR protein including Tyr56 (4.96 Å) and Asp73 (4.66 Å). Thus, the molecular docking sheds light on the fact that the antibiofilm activity of the *E. aquaticum* EO could be due to the inhibition of transcriptional regulatory proteins of *S. aureus* and *P. aeruginosa* by some of its components including compound **7**. The ADMET analysis revealed that the physicochemical properties of the components of *Eryngium aquaticum* EO followed a pattern similar to other essential oils, with most compounds being notably lipophilic. Despite this drawback of high lipophilicity, the other parameters conformed to Lipinski's Rule of Five. Therefore, the EO from the edible plant *E. aquaticum* can be considered safe for human use. However, to develop a suitable candidate for drug development, optimizing the lipophilicity would be necessary to achieve a better pharmacokinetic profile.

Conclusion

The present investigation reveals the antibacterial and antibiofilm activity of the EO of a commonly used culinary herb *E. aquaticum*. The molecular docking study suggests that inhibition of SarA protein of *S. aureus* and LasR protein of *P. aeruginosa* might have some role in the observed antibiofilm activity of *E. aquaticum* EO. Based on these results, it can be inferred that this herb can impart some degree of protection against foodborne pathogens when used in food. The plant also has no history of toxicity and is extensively used with food in raw form. Thus, the plant is a good source of EO that can be used as a safe antimicrobial agent against pathogens. However, in vitro and in silico activities provide preliminary information regarding the potential biological activity of samples. Therefore, in vivo studies with mechanistic investigations are necessary to understand the underlying mechanisms of the antibiofilm activity of E. *aquaticu*m EO or its components.

Abbreviations

EO: Essential oil; GC-MS: Gas chromatography-mass spectrometry; MIC: Minimum inhibitory concentration; OHN: N-3-oxo-dodecanoyl-L-homoserine lactone; ATP: Adenosine triphosphate; DNA: Deoxy ribonucleic acid.

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Conflict of interest

The authors declare no conflict of interest.

References

Ali, M. T., Blicharska, N., Shilpi, J. A., Seidel, V. (2018). Investigation of the anti-TB potential of selected propolis constituents using a molecular docking approach. *Scientific Reports*, 8(1), 12238. https://doi.org/10.1038/s41598-018-30209-y

Angane, M., Swift, S., Huang, K., Butts, C. A., Quek, S. Y. (2022). Essential oils and their major components: an updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. *Foods*, 11(3). https://doi.org/10.3390/foods11030464

Arya, R., Ravikumar, R., Santhosh, R. S., Princy, S. A. (2015). SarA based novel therapeutic candidate against *Staphylococcus aureus* associated with vascular graft infections. *Frontiers in Microbiology*, 6, 416. https://doi.org/10.3389/fmicb.2015.00416

Bai, J., Li, J., Chen, Z., Bai, X., Yang, Z., Wang, Z., Yang, Y. (2023). Antibacterial activity and mechanism of clove essential oil against foodborne pathogens. *LWT Food Science and Technology*, 173, 114249. https://doi.org/https://doi.org/10.1016/j.lwt.2022.114249

Bazargani, M. M., Rohloff, J. (2016). Antibiofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms. *Food Control*, 61, 156-164. https://doi.org/https://doi.org/10.1016/j.foodcont.2015.09.036

Bottomley, M. J., Muraglia, E., Bazzo, R., Carfi, A. (2007). Molecular insights into quorum sensing in the human pathogen *Pseudomonas aeruginosa* from the structure of the virulence regulator LasR bound to its autoinducer. *Journal of Biological Chemistry*, 282(18), 13592-13600. https://doi.org/10.1074/jbc.M700556200

Casalino, G., Dinardo, F. R., D'Amico, F., Bozzo, G., Bove, A., Camarda, A., Lombardi, R., Dimuccio, M. M., Circella, E. (2023). Antimicrobial efficacy of cinnamon essential oil against avian pathogenic *Escherichia coli* from poultry. *Animals*, 13(16), 2639. https://www.mdpi.com/2076-2615/13/16/2639

Cui, H., Zhang, C., Li, C., Lin, L. (2019). Antibacterial mechanism of oregano essential oil. *Industrial Crops and Products*, 139, 111498. https://doi.org/https://doi.org/10.1016/j.indcrop.2019.111498

Dallakyan, S., Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. In J. E. Hempel, C. H. WilliamsC. C. Hong (Eds.), *Chemical Biology: Methods and Protocols* (pp. 243-250). Springer New York. https://doi.org/10.1007/978-1-4939-2269-7_19

Devi, P. B., Deb, P., Singh, H. B. (2021). Ethno-medicinal uses of Eryngo (*Eryngium foetidum* L.) by Meitei community of Manipur, Northeast India. *Indian Journal of Traditional Knowledge*, 20(3), 767-774.

El Omari, N., Charfi, S., Elmenyiy, N., El Hachlafi, N., Balahbib, A., Chamkhi, I., Bouyahya, A. (2022). Essential oils for combating antimicrobial resistance: mechanism insights and clinical uses. In: Kumar, V., Shriram, V., Paul, A., Thakur, M. (eds) *Antimicrobial Resistance.* (pp. 323-355). Springer, Singapore. https://doi.org/10.1007/978-981-16-3120-7_12

Hanif, M. A., Nisar, S., Khan, G. S., Mushtaq, Z., Zubair, M. (2019). Essential oils. In S. Malik (Ed.), *Essential Oil Research: Trends in Biosynthesis, Analytics, Industrial Applications and Biotechnological Production* (pp. 3-17). Springer International Publishing. https://doi.org/10.1007/978-3-030-16546-8_1

Hemachandra, G. H. T. K., Thuvaragan, S., Sanmugarajah, V. (2021). Pharmacological screening of *Eryngium foetidum* Linn–A Review. *Borneo Journal of Pharmacy*, 4(4), 248-259.

Hossain, S. J., Islam, M. R., Pervin, T., Iftekharuzzaman, M., Hamdi, O. A., Mubassara, S., Saifuzzaman, M., Shilpi, J. A. (2017). Antibacterial, anti-diarrhoeal, analgesic, cytotoxic activities, and GC-MS profiling of *Sonneratia apetala* (Buch.-Ham.) seed. *Preventive Nutrition and Food Science*, 22(3), 157-165. 10.3746/pnf.2017.22.3.157

Hou, T., Sana, S. S., Li, H., Xing, Y., Nanda, A., Netala, V. R., Zhang, Z. (2022). Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Bioscience*, 47, 101716. https://doi.org/https://doi.org/10.1016/j.fbio.2022.101716

Hu, W., Li, C., Dai, J., Cui, H., Lin, L. (2019). Antibacterial activity and mechanism of *Litsea cubeba* essential oil against methicillin-resistant *Staphylococcus aureus* (MRSA). *Industrial Crops and Products*, 130, 34-41. https://doi.org/https://doi.org/10.1016/j.indcrop.2018.12.078

Hui, X., Yan, G., Tian, F. L., Li, H., Gao, W. Y. (2017). Antimicrobial mechanism of the major active essential oil compounds and their structure–activity relationship. *Medicinal Chemistry Research*, 26(2), 442-449. https://doi.org/10.1007/s00044-016-1762-0

Karmakar, U. K., Shilpi, J. A., Hossain, M. H., Kundu, P. (2021). Phytochemical screening and antioxidant potential of *Portulaca oleracea* Linn. *Khulna University Studies*, 18(2), 27-39. https://doi.org/10.53808/KUS.2021.18.02.2105-ls

Knobloch, K., Pauli, A., Iberl, B., Weigand, H., Weis, N. (1989). Antibacterial and antifungal properties of essential oil components. *Journal of Essential Oil Research*, 1(3), 119-128. https://doi.org/10.1080/10412905.1989.9697767

Liu, G. Y., Guo, B. Q., Chen, W. N., Cheng, C., Zhang, Q. L., Dai, M. B., Sun, J. R., Sun, P. H., Chen, W. M. (2012). Synthesis, molecular docking, and biofilm formation inhibitory activity of 5-substituted 3,4-dihalo-5H-furan-2-one derivatives on *Pseudomonas aeruginosa*. *Chemical Biology & Drug Design*, 79(5), 628-638. https://doi.org/https://doi.org/10.1111/j.1747-0285.2012.01342.x

Liu, Y., Manna, A. C., Pan, C. H., Kriksunov, I. A., Thiel, D. J., Cheung, A. L., Zhang, G. (2006). Structural and function analyses of the global regulatory protein SarA from *Staphylococcus aureus*. *Proceedings of the National Academy of Sciences*, 103(7), 2392-2397. https://doi.org/10.1073/pnas.0510439103

Mitra, R., Rouf, R., Dutta, A. K., Shilpi, J. A., Uddin, S. J. (2023). Screening of agglutination activities of common vegetables in Bangladesh. *Khulna University Studies*, 20(1), 62-71. https://doi.org/10.53808/KUS.2023.20.01.900-ls

Moleyar, V., Narasimham, P. (1992). Antibacterial activity of essential oil components. *International Journal of Food Microbiology*, 16(4), 337-342. https://doi.org/https://doi.org/10.1016/0168-1605(92)90035-2

Mühlen, S., Dersch, P. (2016). Anti-virulence strategies to target bacterial infections. *Current topics in Microbiology and Immunology*, 398, 147-183. https://doi.org/10.1007/82_2015_490

Olszewska, M. A., Gędas, A., Simões, M. (2020). The effects of eugenol, *trans*-cinnamaldehyde, citronellol, and terpineol on *Escherichia coli* biofilm control as assessed by culture-dependent and -independent methods. *Molecules*, 25(11), 2641. https://doi.org/10.3390/molecules25112641

Paul, J. H., Seaforth, C. E., Tikasingh, T. (2011). *Eryngium foetidum* L.: a review. *Fitoterapia*, 82(3), 302-308. https://doi.org/10.1016/j.fitote.2010.11.010

Paun, V. I., Lavin, P., Chifiriuc, M. C., Purcarea. C. (2021). First report on antibiotic resistance and antimicrobial activity of bacterial isolates from 13,000-year old cave ice core. *Scientific Reports*, 11(1), 514. https://doi.org/10.1038/s41598-020-79754-5

Percival, S. L., Mayer, D., Kirsner, R. S., Schultz, G., Weir, D., Roy, S., Alavi, A., Romanelli, M. (2019). Surfactants: Role in biofilm management and cellular behaviour. *International Wound Journal*, 16(3), 753-760. https://doi.org/10.1111/iwj.13093

Roberts, C., Anderson, K. L., Murphy, E., Projan, S. J., Mounts, W., Hurlburt, B., Smeltzer, M., Overbeek, R., Disz, T., Dunman, P. M. (2006). Characterizing the effect of the *Staphylococcus aureus* virulence factor regulator, SarA, on log-phase mRNA half-lives. *Journal of Bacteriology*, 188(7), 2593-2603. https://doi.org/10.1128/jb.188.7.2593-2603.2006

Rosato, A., Sblano, S., Salvagno, L., Carocci, A., Clodoveo, M. L., Corbo, F., Fracchiolla, G. (2020). Anti-biofilm inhibitory synergistic effects of combinations of essential oils and antibiotics. *Antibiotics*, 9(10). https://doi.org/10.3390/antibiotics9100637

Santativongchai, P., Tulayakul, P., Ji, Y., Jeon, B. (2022). Synergistic Potentiation of antimicrobial and antibiofilm activities of penicillin and bacitracin by octyl gallate, a food-grade antioxidant, in *Staphylococcus epidermidis*. *Antibiotics*, 11(12), 1775. https://doi.org/10.3390/antibiotics11121775

Shu, C., Ge, L., Li, Z., Chen, B., Liao, S., Lu, L., Wu, Q., Jiang, X., An, Y., Wang, Z., Qu, M. (2024). Antibacterial activity of cinnamon essential oil and its main component of cinnamaldehyde and the underlying mechanism. *Frontiers in Pharmacology*, 15, 1378434. https://doi.org/10.3389/fphar.2024.1378434

Song, X., Xia, Y. X., He, Z. D., Zhang, H. J. (2018). A review of natural products with anti-biofilm activity. *Current Organic Chemistry*, 22(8), 789-817. https://doi.org/http://dx.doi.org/10.2174/1385272821666170620110041

Stewart, P. S., Costerton, J. W. (2001). Antibiotic resistance of bacteria in biofilms. *Lancet*, 358(9276), 135-138. https://doi.org/10.1016/s0140-6736(01)05321-1

Stringaro, A., Colone, M., Angiolella, L. (2018). Antioxidant, antifungal, antibiofilm, and cytotoxic activities of *Mentha* spp. essential oils. *Medicines*, 5(4), 112. https://doi.org/10.3390/medicines5040112

Tabarak, M., Devendra, K. P., Priyanka, R., Annie, O. (2016). Evaluation of phytochemicals, antioxidant, antibacterial and antidiabetic potential of *Alpinia galanga* and *Eryngium foetidum* plants of Manipur (India). *Pharmacognosy Journal*, 8(5), 459-464. 10.5530/pj.2016.5.8

Thomas, P. S., Essien, E. E., Ntuk, S. J., Choudhary, M. I. (2017). *Eryngium foetidum* L. essential oils: chemical composition and antioxidant capacity. *Medicines*, 4(2), 24. https://doi.org/10.3390/medicines4020024

Torres, N. L., Laurido, C., Pavan, M. F., Zapata, A., Martínez, J. L. (2017). Medicinal plants of Panamá 2: Ethnobotanics of Forestal Reserve La Tronosa, Province of Los Santos. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, 16(4), 361-384. https://blacpma.ms-editions.cl/index.php/blacpma/article/view/191

Zhao, D., Ma, Y., Wang, W., Xiang, Q. (2023). Antibacterial activity and mechanism of cinnamon essential oil nanoemulsion against *Pseudomonas deceptionensis* CM2. *Heliyon*, 9(9), e19582. https://doi.org/10.1016/j.heliyon.2023.e19582

Zihad, S., Hasan, M. T., Sultana, M. S., Nath, S., Nahar, L., Rashid, M. A., Uddin, S. J., Sarker, S. D., Shilpi, J. A. (2022). Isolation and characterization of antibacterial compounds from *Aspergillus fumigatus*: an endophytic fungus from a mangrove plant of the Sundarbans. *Evidence-Based Complementary And Alternative Medicine*, 2022, 9600079. https://doi.org/10.1155/2022/9600079