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Antibacterial and Inhibition of Cholinesterase Activities of Volatile Organic Compounds from *Schinus terebinthifolia* Fruits

Atividade Antibacteriana e Inibitória da colinesterase de Compostos Orgânicos Voláteis dos frutos de Schinus terebinthifolia

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In this study, the chemical profile and antimicrobial activity, along with acetyl- and butyryl-cholinesterase (AChE and BuChE) inhibition properties, of volatile organic compounds (VOCs) derived from hexane extract of *Schinus terebinthifolia* Raddi fruits are investigated. The ripe fruits of this plant were collected in Salvador, Bahia, Brazil, dried, and extracted via maceration with hexane. The hexane extract and the first silica gel fraction, eluted by column chromatography (CC), were subjected to gas chromatography-mass spectrometry (GC-MS) analysis. Detailed examination of the VOCs composition of hexane extract and derivatized compounds revealed the presence of fatty acids, predominantly palmitic acid (23.25%) and linoleic acid (16.05%), along with monoterpenes and steroids. In the antibacterial assay against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, the VOCs demonstrated significant activity against *P. aeruginosa* and *S. aureus*, with a minimum inhibitory concentration (MIC) of 50 μg mL^{-1} for both pathogens. Notably, the biological assay presented an atypical result against Gram-negative bacteria, which generally resist some antimicrobial agents. Furthermore, the cholinesterase inhibition assay indicated moderate inhibitory activity for the hexane and methanol extracts.

Keywords: *Schinus terebinthifolia*; volatile organic compounds; fatty acids; antimicrobial activity; *Pseudomonas aeruginosa*.

1. Introduction

Schinus terbinthifolia Raddi is a tree native to South America, belonging to the Anacardiaceae family, and is widely distributed throughout Brazil. This species holds significant commercial, cultural, and medicinal value. The fruits of *S. terebinthifolia* have been widely used as a spice in traditional Brazilian dishes and in various cuisines worldwide. The ripe fruits are commonly known as "pink pepper" or "aroeira" and are currently marketed as a complement to black pepper. Pink peppers from *S. terebinthifolia* and *S. molle* are similar to black peppercorns but milder, slightly sweet, and fruity.[1] Since the first edition of the Brazilian Pharmacopeia in 1929, it has been recognized for its pharmaceutical properties.[2] This plant has been reported mainly as an anti-inflammatory and antibacterial in folk medicine.[3] Currently, it is part of the list of phytotherapeutics in the Brazilian "National List of Essential Medicines," used in treating cervicitis, vaginitis, and cervical vaginitis.[4,5]

Concerning the chemical composition of the fruits of this species, there is significant scientific interest due to its chemical complexity associated with its antimicrobial activity and bioactive potential attributed to the presence of phenolic acids, terpenoids, and flavonoids.[6] In general gallic acid,[7–9] masticadienoic and masticadienolic acids,[10,11] myricetin,[12,13] quercetin,[14] and galloyl glucosides[15] are the most reported constituents for *S. terebinthifolia*.

Numerous studies have characterized the essential oil (EO) from leaves and fruits of this species, and mono and sesquiterpenes are the main constituents. In fruits, α-pinene is frequently reported as the main compound,[16] alongside minors such as α-phellandrene, α-carene, and limonene.[17] While these EO profiles vary significantly depending on seasonality, extraction methods, and geographical origin,[15] in the present study, the focus is on the volatile organic compounds (VOCs) of the hexane extract fraction, which shares overlapping terpenoid constituents with EO but may also include other lipophilic metabolites. Through the analysis of this understudied fraction, we aim to identify its unique chemical profile and bioactivity properties, thereby complementing the previous EO-centering research. The EO of *S. terebinthifolia* has demonstrated a broad spectrum of biological activities. The oils exhibit antimicrobial effects against *Escherichia coli, Staphylococcus*

aureus, Streptococcus mutans, Salmonella typhimurium,[18–20] and *Candida albicans*.[6] Studies employing Fatty acids (FAs), particularly palmitic acid, link their presence in mixtures as Gram-positive bacteria like *Streptococcus agalactiae* and *Enterococcus faecium* inhibitions.[21] However, while EOs generally show good efficacy against Gram-positive bacteria, their activity against Gram-negative strains is limited due to the latter's restrictive outer membrane enriched with lipopolysaccharides.[22] This is exemplified by *Pseudomonas aeruginosa*, a multidrug-resistant nosocomial pathogen,[23–25] emphasizing a need for novel antimicrobial strategies. Unlike EOs, which prioritize volatile terpenes, the hexane fraction's lipophilic profile may enhance synergistic interactions between FAs and terpenes, potentially overcoming Gram-negative resistance mechanisms. For instance, palmitic acid's membrane-disrupting properties and terpenes like α-phellandrene that impair efflux pumps could offer a dual-action approach against *P. aeruginosa*. By bridging the compositional gap between EOs and hexane-derived VOCs, this work aims to expand the biological potential of *S. terebinthifolia* beyond conventional EO applications.

In addition to their antimicrobial properties, plant-derived VOCs have garnered attention for their potential to inhibit cholinesterase enzymes, which play a critical role in neurodegenerative disorders, such as Alzheimer's.[26] Cholinesterase inhibitors are a primary therapeutic strategy for managing Alzheimer's symptoms by enhancing cholinergic neurotransmission.[27] Recent studies have highlighted the cholinesterase inhibitory potential of terpenoids,[28] which are prominent constituents of *S. terebinthifolia* fruits. Notably, α-pinene and limonene, major components identified in the species' volatile profile, have exhibited significant acetylcholinesterase (AChE) inhibitory activity in previous studies involving various t plants species.[29–31] Furthermore, the traditional use of *S. terebinthifolia* in folk medicine for cognitive enhancement, although poorly documented, provides an ethnobotanical rationale for exploring its neuropharmacological potential.

The present study investigated the chemical composition of the hexane extract (HE) and the VOC-enriched fraction of the hexane extract (VOC-HE) from *S. terebinthifolia* fruits. The antimicrobial activity against three strains of drug-resistant microorganisms and the inhibitory potential against acetyl- and butyryl-cholinesterase (AChE and BuChE) were also evaluated. This dual approach aims to contribute to exploring the biological and chemical relevance of *S. terebinthifolia* fruits, bridging its traditional uses with emerging applications in combating antimicrobial resistance and neurodegenerative disorders.

2. Experimental

2.1. General procedures

The solvents used in the preparation of the extracts, in the partitioning, in the solubilization of the samples, the TLC/CC elution (hexane, ethyl acetate, chloroform, dichloromethane, and methanol) were of analytical grade from Anidrol (Diadema, SP), and Tedia (Rio de Janeiro, RJ). For column chromatography (CC) and TLC was employed silica gel 60 [of particle diameter of 0.063-0.200 μm from Aldrich (St. Louis, MO, USA] and 0.25 mm thickness silica gel 60 F254 plates from Merck (Darmstadt, Germany). The TLC was monitored by exposing the plates to UV light (254 and 366 nm – Spectroline model CM-10, Fluorescence Analysis Cabinet, Melville, NY, USA) exposure to iodine vapors. The column dimensions for CCs were 60 mm (diameter) × 600 mm (length) with approximately 100 g of silica gel 60 (Sigma Aldrich, St. Louis, MO, USA). Gas chromatography-mass spectrometry (GC-MS) analyses were carried out on Shimadzu mod. QP2010SE equipment (Kyoto, Japan) using an Rtx-5MS (5% diphenyl and 95% dimethylpolysiloxane) fused silica capillary column (30 m × 0.25 mm, 0.25 mm of film thickness) and helium as carrier gas. The compounds were identified by comparing the samples' mass spectra with databases (NIST21, NIST107, and WILEY229) and a FAME standard (Aldrich). In the silylation reactions, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCSI) reagents were acquired from Sigma-Aldrich® (St. Louis, MO, USA). For antimicrobial activity, 96-well plates, Muller Hinton broth from Merck® (Darmstadt, Germany), resazurin developer from Sigma Aldrich® (St. Louis, MO, USA), laminar flow hood and oven for bacteriological culture adjusted to 37 °C were used.

2.2. Plant material

Schinus terebinthifolia ripe fruits were collected in the surroundings of Salvador-BA, Brazil (13°0'16''S – 38°30'38''W) in November 2021, identified by Prof. MLS Guedes (Instituto de Biologia), and a sample species (voucher #109728) were deposited in the Herbário Alexandre Leal Costa of Universidade Federal da Bahia. The access to the specimen was registered in the Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado (SisGen) under # A1E7156. The sample had the insects, traces of soil, and other deteriorated plants removed and dried at 40°C in an oven with forced air circulation until less than 10% drying loss. Subsequently, the sample was grounded using a blade mill to obtain a powder with moderately satisfactory granulometry.

2.3. Obtention of the VOCs from hexane extract (VOC-HE)

The hexane extract (HE) was prepared from 813.13 g of *S. terebinthifolia* fruits by maceration for 24 h (1 L 3x). The solutions obtained were filtered and concentrated under reduced pressure using a rotary evaporator to obtain 58.9 g of crude extract. This extract was submitted to a silica gel 60 CC, eluted in a polarity gradient (hex:EtOAc), yielding 16 fractions ($F_{Hex1-16}$) of 150 mL each. The first eluted

fraction $F_{Hex1,}$ named VOC-HE (95:5; hex:EtOAc), was selected for GC-MS analysis and antibacterial activity due to its non-polar elution profile, indicative of volatile and lipophilic compounds known for their bioactive properties.

2.4. Derivatization method

The HE and VOC-HE (1 mg) were derivatized through the silylation reaction. To the samples, 100 µL of TMSCI reagent and 50 µL of pyridine were added separately. The reaction mixture was heated in a water bath at 60 °C for 5 minutes, then cooled to room temperature. This derivatization step was critical to enhance the detection of polar or thermally labile compounds, such as fatty acids, alcohols, or oxygenated terpenoids, which are commonly reported in *S. terebinthifolia* but exhibit low volatility or instability under standard GC-MS conditions. By converting hydroxyl (-OH) and carboxyl (-COOH) groups into trimethylsilyl derivatives, the method improved compound volatility, chromatographic resolution, and detection sensitivity ensuring accurate identification of metabolites relevant to the study's antibacterial and cholinesterase inhibition assays. For instance, silylation prevents the degradation of heat-sensitive compounds like palmitic acid or terpenoid alcohols during vaporization, enabling a comprehensive chemical profile of the VOC-HE.

2.5. Gas chromatography-mass spectrometry (GC-MS) analysis

The HE and VOC-HE were injected into the GC, the injector was set at 290 °C, and the analysis was run with an initial temperature of 50 °C min^{-1}, ranging from 50 °C to 180 °C at a ratio of 15°C min^{-1}, 180 °C to 230 °C at a ratio of 7 °C min^{-1}, and from 230 °C to 250 °C at a ratio of 15 °C min^{-1}, remaining at this temperature for 10 min. Subsequently, the temperature rose to 285 °C at a 4 °C min^{-1} rate, thus remaining for 40 min. The detector temperature at the interface of the GC-MS system was maintained at 290 °C. Ionization was performed using electron ionization (EI) at 70 eV, and the mass spectra were acquired in full scan mode across 35 to 1000 Da.

2.6. Antibacterial activity

Two strains of Gram-negative (*Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027) and one Gram-positive (*Staphylococcus aureus* ATCC 6538) were used to test the antimicrobial activity. The broth dilution technique was performed in microplates, and each bacterium was inoculated separately in sterile glass tubes, adding 5 mL of Muller Hinton broth (MHB) and incubated at 37 °C for 24 h; then a test was carried out in another tube with MHB with the same incubation conditions for 6 h to obtain the exponential phase. The turbidity test was conducted, adjusting each broth with bacteria with saline solution to the 0.5 McFarland standard, equivalent to $3x10^8$ CFU mL^{-1} (MacFarland, 1907). Concentrations between 25-1000 µg mL^{-1} samples were tested in triplicate, solubilized in dimethyl sulfoxide (DMSO), and MHB was added. After performing the microdilutions in 96-well plates were incubated at 37°C for 24 h. Chloramphenicol (40 µg mL^{-1}, 200 µL) was applied as a positive control, and DMSO (200 µL) as a negative control. Minimal Inhibitory Concentration (MIC) was determined by adding the developer resazurin (100 µg mL^{-1}), of which 30 µL was added to each well of the microplates. After 2 h, the appearance of the blue color represents the absence of growth, and the pink color represents the presence of bacterial growth.

2.7. AChE and BuChE inhibition test

Cholinesterase inhibitory activities of extracts and chromatographic fractions were performed using a 96-well microplate reader based on the method previously described.[32] The solutions of the samples were prepared employing a 1 mg mL^{-1} solution in MeOH grade HPLC, and as a positive control, eserine (physostigmine) in the same concentration. For the test, 140 µL of phosphate buffer (0.1 mol L^{-1}) pH 7.5 with 0.1% bovine serum albumin, 20 µL of the sample of the standard or MeOH were added to the wells of the microplates, in triplicate, in the case of the blank, and 20 µL of the enzymes (AChE/BuChE) 0.5 U mL^{-1}. After 15 min of incubation at 37 °C, 10 µL of 5-5'-dithiobis-[2-nitrobenzoic] acid (DTNB) 10 mmol L^{-1} and 10 µL of acetylthiocholine iodide (ACTI) 15 mmol L^{-1} was added, and then the reading was performed at 405 nm at time 0 and every 10 min until 60 min. The percentage of inhibition was obtained through the equation:

$$\%I = (AChE - AChI) \times \frac{100}{AChE} \quad (1)$$

Acetylcholinesterase inhibitor (AChI) represents the activity obtained in the presence of the inhibitor and AChE/BuChE in the absence of the inhibitor.

3. Results and Discussion

The HE revealed (Table 1) a predominance of fatty acids (81.12%), particularly linoleic acid (41.04%), alongside terpenoids and sterols (6.99%); these constituents were identified based on the molecular ion and the fragmentations observed from the mass spectrum, compared with the de NIST 08 library data. The analyses were carried out with the silylated derivatives once this procedure permits the molecular ion of compounds of higher molecular masses could be detected. Just spectra of compounds showing ≥ 95% similarity were considered. The identified chemical composition and relative abundance indicated that fatty

acids like linoleic and palmitic acids may contribute to antibacterial activity, while terpenoids (e.g., limonene) and sterols could promote cholinesterase inhibition.

The MS analyses of the VOC-HE from *S. terebinthifolia* fruits permitted to identify 22 constituents (Table 2), comprising 85.08% of the fraction. Palmitic acid (23.25%), linoleic acid (16.05%), margaric acid (13.19%), 1,3,5-cicloheptatriene, 3,7,7-trimethyl (5.03%), stearic acid (3.44%) and limonene (2,37%) were the major constituents of this fraction.

Previous studies of the VOCs of *S. terebinthifolia* fruits have reported the presence of a diversity of terpenes, such as α-pinene (29.16%),[33] δ-3-carene (46,67%),[34] β-myrcene (41%),[19] Δ3-carene,[35] among others. Regarding the composition of the fatty acids, a previous study[21] reported the prevalence of palmitic acid (15.98%), a result aligned with the present investigation. This consistency across studies reinforces palmitic acid's prominence in the species' chemical profile and its potential as a key bioactive agent. Also, in a study with ripe fruits,[5] palmitic acid (5.14%) was detected as the main FA in a hexane fraction of the fruits. However, the relatively high presence of saturated margaric acid (heptadecanoic) was not observed previously. This odd-chain FA commonly occurs as a trace, and the presence of this compound in high levels in fruits is important once saturated plasma phospholipid fatty acid (such as C14:0, C16:0, or C18:0) concentrations were significantly associated with an increased risk of coronary heart disease. However, concentrations of C15:0 and C17:0 FAs showed a significant inverse association with this incidence.[36] Despite their lower relative abundance, the identified terpenes, including α-phellandrene (0.68%), δ-carene (0.45%), and germacrene D (1.24%) detected in the present study, seem to be critical to the observed bioactivity, as the antimicrobial properties of these terpenoids are widely described.

The *in vitro* antibacterial activity of the VOC-HE (Table 3) obtained from the CC of the hexane extract of the *S. therebentifolia* fruits was determined using the dilution assay methodology. The VOC-HE exhibited better activity against Gram-negative bacteria *P. aeruginosa* and Gram-positive bacteria *S. aureus* and lower activity against *E. coli*. The VOC's antibacterial activity of *S. terebinthifolia* has been previously reported against Gram-positive bacteria such as *Bacillus cereus* (MIC of 0.852 mg mL^{-1}), *Staphylococcus aureus* (MIC of 1.704 mg mL^{-1}) and *Listeria monocytogenes* (MIC of 6.820 mg mL^{-1}).[18] Additional study demonstrated more significant activity against S. aureus in an assay performed using the disk diffusion method with an essential oil (EO) presenting predominantly a monoterpene chemical profile.[37] Furthermore, a study reported the antibacterial activity of two samples of S. terebinthifolia collected at different periods, indicating a difference in their bioactivity, with one sample being more active against all tested strains. In this study, the chemical profiles also showed differences: for the most active sample, the main components were identified as germacrene-D (21.0%) and α-phellandrene (18.2%), while the least active sample contained only 8.8% germacrene-D and an undetectable amount of α-phellandrene.[38] Comparison of different strains of Gram-positive and -negative bacteria from two VOC samples (leaves and fruits) that presented α-pinene, α-phellandrene, and D-limonene as major compounds reported more significant activity in both samples for Gram-positive strains [EO leaves: *Bacillus megaterium*

Table 1. Substances identified (% relative area) in hexane extract of *Schinus terebinthifolia* fruits

tR (min)	Compound	M^+	Molecular Formula	Similarity Index (SI)	% Area
		Monoterpene			
28.652	Linalool*	242	$C_{10}H_{18}O$	95	1.54
		Sesquiterpenes			
22.197	Cadinene	204	$C_{15}H_{24}$	97	1.30
25. 421	β-eudesmol*	294	$C_{15}H_{26}O$	95	4.15
		Triterpenes			
60.482	β-sitosterol*	486	$C_{29}H_{50}O$	95	1.08
61.919	Lanosterol	498	$C_{30}H_{50}O$	95	0.98
		Fatty Acids			
33.093	Palmitic acid*	328	$C_{16}H_{32}O_2$	97	13.74
37.054	Linoleic acid*	352	$C_{18}H_{32}O_2$	97	41.04
37.168	Asclepic acid*	354	$C_{18}H_{34}O_2$	96	5.06
39.830	Oleic acid*	354	$C_{18}H_{34}O_2$	96	21.28
40.326	Stearic acid*	356	$C_{18}H_{36}O_2$	97	1.90
		Total identified (% relative area)			92.07
		Unidentified (% relative area)			7.93

*identified in the silylated form

Table 2. VOC-HE composition of *Schinus terebinthifolia* fruits

tR (min^{-1})	Compound	M^+	Molecular Formula	Similarity Index (SI)	Area (%)
		Monoterpenes			
4.35	1,3,5-cicloheptatriene, 3,7,7-trimethyl	134	$C_{10}H_{14}$	95	5.03
4.53	β-Pinene	136	$C_{10}H_{16}$	96	1.89
5.45	δ-Carene	136	$C_{10}H_{16}$	96	0.45
5.74	*p*-Cymene	134	$C_{10}H_{14}$	96	1.16
5.93	Limonene	136	$C_{10}H_{16}$	96	2.37
15.53	Verbenone	150	$C_{10}H_{14}O$	96	1.66
16.17	α-Phellandrene	136	$C_{10}H_{16}$	94	0.68
		Sesquiterpenes			
17.64	α-Copaene	204	$C_{15}H_{24}$	96	2.01
18.14	β-Elemene	204	$C_{15}H_{24}$	95	0.52
18.71	α-Gurjunene	204	$C_{15}H_{24}$	95	1.37
19.12	Cariophyllene	204	$C_{15}H_{24}$	96	1.11
19.52	α-Bergamotene	204	$C_{15}H_{24}$	96	1.27
20.12	α-Humulene	204	$C_{15}H_{24}$	95	2.05
21.07	Germacrene D	204	$C_{15}H_{24}$	95	1.24
21.55	α-Muurolene	204	$C_{15}H_{24}$	94	0.92
22.28	δ-Cadinene	204	$C_{15}H_{24}$	95	1.83
		Fatty Acids			
33.09	Palmitic acid*	328	$C_{16}H_{32}O_2$	97	23.25
37.07	Linoleic acid	280	$C_{18}H_{32}O_2$	96	16.05
39.36	Margaric acid	270	$C_{17}H_{34}O_2$	96	13.19
39.84	Oleic acid*	354	$C_{17}H_{34}O_2$	97	2.39
40.26	Stearic acid*	356	$C_{18}H_{36}O_2$	95	3.44
		Triterpene			
60.27	β-amyrone	424	$C_{30}H_{48}O$	96	1.20
	Total identified (% relative area)				85.08
	Unidentified (% relative area)				14.92

*Identified in the silylated form

(35.33 mm), *Bacillus mojavensis* (23.67 mm) and *Clavibacter michiganensis* (37.33 mm)]. No activity was observed in any of the samples for Gram-negative strains (*Pseudomonas savastanoi* and *P.syringae* pv. *phaseolicola*).[39] Few reports have been found regarding essential oils that contain significant amounts of fatty acids and have been tested against Gram-negative bacteria.

Previous studies indicated that minor terpenes such as β-pinene, *p*-cymene, and verbenone (RA ≤ 10% in the present study) present antimicrobial activities. β-Pinene destabilizes bacterial efflux pumps in multidrug-resistant strains,[40] while *p*-cymene inhibits quorum sensing in *P. aeruginosa*.[41] Verbenone, exhibits inhibitory growth activity against *Listeria monocytogenes* compromising bacterial viability and resistance mechanism.[42] Even uncommon sesquiterpenes like δ-cadinene suppress biofilm formation, disrupt the cellular structure, and compromise bacterial membrane integrity.[43]

Thus, the combined action of low-abundance terpenes likely amplifies the VOC-HE's bioactivity. For example, α-copaene and α-humulene, though minor constituents are documented synergists in plant essential oils, inhibiting *Staphylococcus aureus* biofilm formation by disrupting extracellular polymeric matrix synthesis.[44] Similarly, caryophyllene modulates bacterial resistance in *P. aeruginosa* by targeting efflux pump proteins.[45] This synergy extends beyond monoterpenes once β-amyrone from *Rosmarinus officinalis* showed modest direct antibacterial activity against *S. aureus*. However, this triterpene in the presence of verbenone and 1,8-cineole suggests a cooperative mechanism that enhances overall bioactivity.[46]

Notably, palmitic acid, the most abundant constituent in the studied fruits, has been previously considered responsible for antimicrobial activity in plant extracts. Its high relative abundance in the VOC-HE suggests a potential

role in the observed antibacterial effects, particularly against Gram-negative bacteria, which are typically less susceptible to fatty acid-mediated disruption due to their complex membrane structure.

Thus, while fatty acids dominate the chemical profile, the antimicrobial activity observed against *P. aeruginosa* and *S. aureus* likely arises from a dual mechanism: the fatty acids (e.g., palmitic acid) disrupting membrane integrity and the monoterpenes targeting virulence factors (e.g., efflux pumps, biofilms). This synergy aligns with studies showing terpene-fatty acid combinations exhibit broader-spectrum activity than individual components.[47,48]

However, the observed activity against Gram-negative *P. aeruginosa* is noteworthy, as few studies link fatty acid-rich extracts to such effects. For instance, acetone extracts of *S. terebinthifolia* (oleic acid: 38.74%) showed moderate anti-*Pseudomonas* activity (MIC 128 μg mL^{-1}).[49] This finding suggests that abundant fatty acids like linoleic and palmitic acid in our sample may disrupt Gram-negative membranes or efflux pumps, warranting mechanistic studies. These results are interesting when compared with the bacteriostatic and bactericidal effect of the MeOH acidified extracts evaluated against the same microorganisms.[50] The less polar extract showed higher activities.

Concerning AchE inhibition, *Schinus* spp. has already been reported with promising activity in inhibiting AChE. While *S. aroeira* and *S. longifolia* EOs presented AChE inhibition activity of approximately IC_{50} of 20 μg mL^{-1},[51,52] this is the first report for VOCs enriched extract of *S. terebinthifolia* fruits. The moderate BuChE inhibition (enhanced at 60 min) may arise from terpenoids identified in the HE, as similar compounds in other plants inhibit cholinesterases via hydrophobic interactions with the enzyme's active site.[28] Notably, fatty acids like palmitic acid have also been implicated in neuroprotective pathways,[53] suggesting a potential synergistic role between lipidic and terpenoid components in dual bioactivities. This dual antimicrobial-neuropharmacological focus is mechanistically cohesive: membrane-disrupting fatty acids could enhance bacterial susceptibility, while lipophilic terpenoids/sterols may penetrate blood-brain barriers to modulate cholinesterases. Future studies should isolate key compounds to clarify their individual and combined roles in both activities.

4. Conclusions

The volatile fraction of the hexane extract (VOC-HE) from *Schinus terebinthifolia* fruits was rich in fatty acids (palmitic and linoleic acids) and it demonstrated significant antibacterial activity against *Pseudomonas aeruginosa,* a Gram-negative pathogen with high clinical resistance. This rare Gram-negative targeting highlights its potential as a novel antimicrobial agent. The hexane and methanol extracts also showed moderate BuChE inhibition, suggesting neuropharmacological relevance linked to terpenoids/sterols. The dual bioactivity underscores the multifunctional potential of *S. terebinthifolia*'s lipophilic components, bridging traditional uses with applications against infections and neurodegenerative disorders. Further studies should isolate key compounds to clarify their mechanisms and synergies.

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Table 3. Minimum inhibitory concentration (MIC) of hexane fraction with VOCs (VOC-HE) *Schinus terebinthifolia* fruits against *Escherichia coli* (ATCC 8739), *Pseudomonas auruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538)

Microorganisms	MIC (μg mL^{-1})
Escherichia coli	100
Pseudomonas aeruginosa	50
Staphylococcus aureus	50

positive control: chloramphenicol (40 μg mL^{-1})

Table 4. Percentage of inhibition (%I) of AChE and BChE for the hexane and MeOH extracts and eserine (standard) at times 30 min and 60 min

Amostra	% I ± DP*		% I ± DP*	
	AChE		BchE	
	30 min	60 min	30 min	60 min
Hex	41.72 ± 0.02	28.76 ± 0.01	58.57 ± 0.01	51.89 ± 0.01
MeOH	44.64 ± 0.01	36.82 ± 0.03	23.82 ± 0.20	49.94 ± 0.01
Eserine	73.56 ± 0.70	87.67 ± 0.02	72.07 ± 0.10	92.26 ± 0.40

*The results are expressed as mean ± standard deviation (n=3).

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