

Chemical Composition, Allelopathic Potential, and Antimicrobial and Anticholinesterase Activity of *Citrus sinensis* (L.) Peel Oil

Vítor Hugo Neves da Silva

Universidade Federal do Amazonas

Ívina Thayná Miranda Trindade

Universidade Federal do Amazonas

Mayanna Emília Tenório Vicente

Universidade Federal do Amazonas

Geovania Santos de Souza

Universidade Federal do Amazonas

Paulo José Sousa Maia

Federal University of Rio de Janeiro

Maiara de Souza Nunes Ávila

Universidade Federal do Amazonas

Ingrid Reis da Silva

Centro de Biotecnologia da Amazônia

Bruna Ribeiro de Lima

São Paulo State University

Dominique Fernandes de Moura do Carmo (✉ dominiquefmc@ufam.edu.br)

Universidade Federal do Amazonas

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Abstract

Citrus sinensis is renowned for its notable biological effects. This research aimed to characterize the chemical composition of oil derived from the peel of *Citrus sinensis* (L.), referred to as CsPO, and to assess its antimicrobial efficacy against strains of *Enterococcus faecalis* (ATCC29212), *Escherichia coli* (ATCC25922), *Candida albicans* (ATCC10231), and *Mycobacterium smegmatis* (ATCC14468). Additionally, the investigation sought to analyze its impact on the growth of butter bean (*Phaseolus lunatus*) radicles, evaluate its inhibitory effects on acetylcholinesterase enzyme (AChE) both *in vitro* and *in silico* through molecular docking simulations, and determine its potential phytotoxicity. The analysis of the CsPO composition using gas chromatography coupled with mass spectrometry (GC-MS) identified thirty-three (33) monoterpene and sesquiterpene compounds, e.g., β -citronelene (7.33%), β -pinene (9.63%), car-3-en-2-one (8.43%), eugenol (4.24%), limonene-10-ol (8.26%), α -humulene (10.52%), α -neo-clovene (4.83%), and β -acoradiene (12.47%). CsPO demonstrated varying levels of antimicrobial activity, ranging from low (+) to moderate (++). In the allelopathy assay, one of the treatments exhibited alterations in radicular growth and germination rate of butter bean seeds. The *in vitro* anticholinesterase assay revealed an inhibitory concentration (IC₅₀) of 72 μ g/mL. These findings were corroborated by the *in silico* anticholinesterase assay, highlighting the potential of β -citronelene, limonene, β -acoradiene, and α -humulene to inhibit acetylcholinesterase. The *in silico* assay yielded binding energy values between -6.2 and -9.6 kcal/mol, closely resembling those of the galantamine inhibitor (-9.8 kcal/mol, RMSD 0.3678 Å). This study underscores the significant inhibitory activity of CsPO against AChE, suggesting its potential use in treating conditions like Alzheimer's disease and other relevant ailments. Furthermore, this study aims to enhance the scientific understanding of this essential oil, thus contributing to its potential applications in agriculture and medicine.

INTRODUCTION

The *Citrus* genus, a member of the Rutaceae family, thrives abundantly in tropical regions. Its species are renowned for their medicinal properties, primarily attributed to the presence of potent antioxidant compounds like flavonoids, found in extracts derived from their fruit and leaves [1]. Various categories of chemical compounds have been distinguished within *C. sinensis*, spanning its fruit, bark, leaves, juice, and root. These encompass an array of groups including flavonoids, steroids, hydroxyamides, alkanes, fatty acids, coumarins, peptides, carbohydrates, carbamates, alkylamines, carotenoids, volatile compounds, and essential nutritional elements such as potassium, magnesium, calcium, and sodium [2].

Essential oils derived from *C. sinensis* play an important role in both the pharmaceutical and pesticide industries [3]. These oils exhibit significant chemical diversity, containing substances such as hydrogenated and oxygenated monoterpenes, hydrocarbon sesquiterpenes, and oxygenated sesquiterpenes. This diverse composition imparts a wide range of biological activities to these oils.

Citruses stand as some of the most extensively cultivated crops worldwide, with oranges taking the lead. The production of oranges, encompassing both the fruit and its juice, is primarily centered in four

countries. Chief among these is Brazil, a pivotal player, responsible for one-third of global fruit production and almost half of the world's total juice production [4]. Notably, within Brazil, Rio Preto da Eva emerges as the largest orange producer in the Amazonas State, contributing to a substantial 70% of the region's total output. Notably, between 2019 and 2020, this harvest experienced a remarkable increase of 35% [5]. Additionally, following the onset of the Covid-19 pandemic, the demand for citrus fruits witnessed a significant surge due to their substantial vitamin C content and consequential immunological benefits.

Essential oils can be obtained from various parts of *Citrus* plants through cold pressing of their peels and through distillation methods. In these oils, it is possible to find compounds like terpenes, aliphatic sesquiterpenes, oxygenated derivatives, and aromatic hydrocarbons. Notably, these oils showcase diversity across different *Citrus* species, featuring varying concentrations of substances such as limonene, α -pinene, β -pinene, myrcene, linalool, and terpinene. These discrepancies are inherent to the specific species and are influenced by their growing location. Particularly, when it comes to essential oils extracted from citrus fruits, monoterpenes emerge as the most significant constituents [6].

Currently, clinical trials are being conducted to test reversible cholinesterase inhibitors as a potential treatment for Alzheimer's disease. This approach revolves around the inhibition of the enzyme acetylcholinesterase (AChE). This particular enzyme plays a crucial role in breaking down acetylcholine, a neurotransmitter present in the synaptic space. Through the inhibition of AChE, the breakdown of acetylcholine is slowed down, leading to increased availability of this neurotransmitter for cholinergic transmission [7]. Notably, Conforti and colleagues [8] discovered anticholinesterase activity in *Citrus medica* L. cv. (diamond citron) and linked it to the substantial presence of monoterpenes in the fruit's peel.

Research conducted on 17 variations of monoterpenoids possessing the p-methane skeleton revealed noticeable AChE inhibitory activity in specific compounds. γ -terpinene and terpinen-4-ol displayed such activity at concentrations of 1.2 mM, reaching 22.6% and 21.4%, respectively. Furthermore, other terpenes, including limonene and linalool (Figs. S1A-B Supplementary material), exhibited inhibition on AChE at concentrations of 164 μ g/mL, showing rates of 27% and 37%, respectively.

Chaiyana and colleagues [9] made a similar observation regarding *Citrus hystrix* DC. They attributed the 10% inhibition of AChE by *Citrus hystrix* DC. to the essential oil extracted from the plant's leaves, which contain acyclic and monocyclic monoterpenes (e.g., citronellal and β -phellandrene). The complexity of terpenoid structures that trigger AChE inhibitory effects makes it difficult to predict the potential structure-activity relationship. Nonetheless, certain characteristics, such as the presence of a hydrophobic ligand, seem to correlate with higher inhibition effectiveness, as the AChE active site is known to be susceptible to hydrophobic interactions [8].

Plants exhibiting favorable impacts on cognitive impairment, such as those with anti-AChE, anti-inflammatory, and antioxidant properties, or other pertinent pharmacological attributes, hold promise for Alzheimer's disease treatment [10]. *Citrus*-derived essential oils possess therapeutic applications owing to

their ability to alleviate anxiety and depression, mitigate pain, promote wound healing, and combat fungal and bacterial infections [11].

In addition to the attributes mentioned earlier, citruses have captured scientific attention for their potential in treating various other conditions. For instance, *C. sinensis* has demonstrated potent *in vitro* effects against rotaviruses and other microorganisms [12]. *Citrus aurantium L.*, commonly referred to as bitter orange, finds use in traditional medicine across Brazil and other regions to address concerns such as anxiety, insomnia, and as an anticonvulsant, indicative of its impact on the central nervous system (CNS), among other attributes [13]. Notably, Chaiyana and Okonogi [14] have established a positive link between essential oil usage and enzyme inhibition associated with Alzheimer's disease, a significant finding given the widespread availability of these oils.

Besides the aforementioned properties, citruses have drawn scientific interest as regards the treatment of other ailments. For example, *C. sinensis* has shown a powerful effect *in vitro* against rotaviruses and other microorganisms [12]. *Citrus aurantium L.*, commonly known as bitter orange, is used in folk medicine in Brazil and in other countries to treat anxiety and insomnia and as anticonvulsant, indicating a depressing effect on the central nervous system (CNS), among other properties [13]. Chaiyana and Okonogi [14] have found a positive correlation between the use of essential oils and a significant degree of inhibition of enzymes linked to Alzheimer's disease, which is of great importance due to their ready availability.

In research conducted by Ademosun and Oboh [15], essential oils extracted from sweet orange peel and seeds demonstrated the inhibition of cholinergic and monoaminergic enzymes. These oils were also found to contain potential antioxidants, which were observed through the inhibition of lipid peroxidation induced by Fe^{2+} in isolated brain homogenates. Additionally, their study reveals that the essential oil extracted from the bark of the same plant displayed more pronounced inhibition on cholinergic enzymes, while showing a lesser inhibitory effect on MAO and Fe^{2+} -induced lipid peroxidation when compared to the effects exhibited by the essential oil from its seeds.

Alzheimer's disease stands as the primary contributor to dementia in the elderly, impacting approximately 10% of individuals aged 65 and above [16]. Compounds that inhibit acetylcholinesterase activity (referred to as AChE) play a pivotal role in elevating synaptic acetylcholine levels and prolonging its activity, thus aiding in the preservation of cognitive function. Natural AChE inhibitors offer an alternative avenue in the treatment of Alzheimer's disease.

Another noteworthy biological attribute documented in existing literature is the antimicrobial capacity of *Citrus* essential oils. These oils, containing around 400 compounds, are particularly characterized by prevalent components like citrulline and limonene, both of which exhibit broad-spectrum antimicrobial properties [18]. Specifically, limonene has demonstrated efficacy against various microorganisms, including *S. aureus*, *L. monocytogenes*, *S. enterica*, and *S. bayanus*, among others [19].

The term ‘allelopathy’ pertains to plants' ability to disrupt the growth of other plants through the emission of substances into the air or soil. A significant portion of these compounds possesses allelochemical potential [20]. *Citrus* residues offer a valuable reservoir of compounds like D-limonene, flavonoids, carotenoids, dietary fiber, soluble sugars, cellulose, hemicellulose, pectin, polyphenols, ascorbic acid, methane, and oils [21, 22]. Some plants generate allelopathic compounds as part of their secondary metabolism, which can either hinder or foster germination processes.

Based on the aforementioned, the objective of this study is to provide a chemical characterization of the CsPO under consideration. This involved identifying its constituent elements, gauging its biological capabilities, examining its impact on microbial strains through antimicrobial testing, appraising its influence on the germination of *Phaseolus lunatus* seeds (commonly referred to as butter beans) for phytotoxic effects, and evaluating its *in vitro* and *in silico* inhibitory potential on the acetylcholinesterase enzyme.

RESULTS AND DISCUSSION

Yield

As presented in Table 1, the yield of the essential oil obtained from *Citrus sinensis* (L.) peel was 2.05% (v/p).

Table 1. Yield of *Citrus sinensis* (L.) peel oil.

Plant Sample	Month/Year	Temperature	Sample Label	Plant Mass	Yield
<i>Citrus sinensis</i> (L.) peel oil	Nov./2019	27 °C	CsPO*	400 g	2.05%

Santos and colleagues [36] conducted research on the extraction of essential oil from *Citrus sinensis* (L.) peel and obtained a yield of 0.4% (v/p) from segregated whole peels. In a separate study, Fernandes and colleagues [37] obtained an essential oil yield of 2.93% (v/p) for segregated and crushed peels, and 0.53% (v/p) for segregated whole peels in distillations lasting three hours. Due to the variations in extraction methods based on plant parts and intended applications [38], the yield of a specific oil can vary significantly depending on the chosen extraction method.

Chemical Composition

The chemical composition of the CsPO under consideration, along with the area, retention time, calculated Kovats index, and tabulated Kovats index for each compound, are detailed in Table S1 (Supplementary material).

A total of thirty-three compounds, including both monoterpenes and sesquiterpenes, were detected and observed on the chromatogram through GC-MS analysis. Among these, the predominant compounds identified encompass the hydrogen monoterpenes β -citronelene (7.33%) and β -pinene (9.63%), the

oxygenated monoterpenes car-3-en-2-one (8.43%), eugenol (4.24%), and limonene-10-ol (8.26%), as well as the hydrogen sesquiterpenes α -humulene (10.52%), α -neo-Clovene (4.83%), and β -acoradiene (12.47%).

In Ganzhou, China, the essential oil obtained from *Citrus essensis* peel was found to comprise 37 components, constituting 98.05% of the total essential oil [39]. The primary constituents identified were limonene (88.25%), terpinen-4-ol (1.98%), β -myrcene (1.90%), and linalool (1.50%). Similarly, in Guangdong, China, Li and colleagues [40] conducted a characterization of the *Citrus sinensis* peel oil, revealing the presence of 28 components accounting for 85% of the total essential oil. Notably, the key components in this case were d-limonene (49.14%), neral (9.54%), and eucalyptol (3.55%).

Conversely, in Brazil, Dias and colleagues [41] extracted essential oil from *Citrus sinensis* peel collected in Rio Verde (GO). Their analysis identified four components, primarily limonene (98.54%), δ -2-carene (0.27%), δ -2-carene (0.74%), and tricyclene (0.25%), collectively amounting to 99.80% of the oil's content.

In this investigation, the CsPO sourced from Rio Preto (AM), Brazil, exhibited a profile of 33 compounds. Surprisingly, limonene did not dominate the composition, marking a departure from findings in other studies. Instead, the principal constituents in this instance were β -acoradiene (12.47%) and α -humulene (10.52%), two components not previously reported by the mentioned researchers. Moreover, it should be noted that the main components demonstrated a more balanced distribution within this study.

Biological Assays

Determination of Antimicrobial Activity

The antimicrobial activity of CsPO, evaluated by means of the agar-diffusion technique, is depicted in Table 2 and Fig. S2 (supplementary material).

Table 2. CsPO antimicrobial activity against *Enterococcus faecalis*, *Escherichia coli*, *Candida albicans*, and *Mycobacterium smegmatis*.

Strains				
Sample	<i>E. faecalis</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>M. smegmatis</i>
CsPO	++	+	++	++

Low (+): diameter of inhibition halo between 7 and 12 mm; Moderate (++) : diameter of inhibition halo between 13 and 16 mm; High (+++) : diameter of inhibition halo greater than 17 mm; Negative (-): absence of halo.

The findings indicate notable antimicrobial efficacy against *Enterococcus faecalis*, *Escherichia coli*, *Candida albicans*, and *Mycobacterium smegmatis*, with inhibition halos measuring 13 mm (++) , 12 mm (+), 13 mm (++) , and 16 mm (++) , respectively.

According to Bajpai and colleagues [42], the antimicrobial efficacy of essential oils may be attributed to their ability to permeate the bacterial membrane and access the cell interior. This process inhibits the functional attributes of the cell. The presence of porin proteins in the bacterial membrane, the existence of these proteins in gram-negative bacteria, and the intracellular distribution of essential oil constituents are pivotal factors influencing the diffusion and action of essential oils within the cells. These elements contribute to the variation in essential oil activity against different bacterial groups [43, 44]. This alteration in permeability is also reported by Lambert and colleagues [43], supporting the notion that hydrophobic compounds undermine the integrity of bacterial cell membranes.

Costa and colleagues [45] demonstrated that the antimicrobial efficacy of essential oils derived from *Citrus* fruit peel is closely tied to their chemical composition, which can be shaped by the extraction technique and the plant's growth environment. In the context of this research, CsPO displayed a moderate level of antimicrobial activity against *E. faecalis*. This suggests that the activity could stem from major components like limonen-10-ol and β -pinene, or possibly from a synergic effect among the various constituents.

Ambrósio and colleagues [46] propose that gram-negative bacteria tend to display higher tolerance to essential oil treatments due to their comparatively impermeable outer membranes. This outer membrane structure obstructs the penetration of hydrophobic essential oils. This phenomenon was observed in the case of *E. coli* (a gram-negative bacterium), which exhibited greater resilience and consequently a smaller inhibition halo (+) when exposed to 30 μ L of the essential oil in the agar-diffusion technique. In the study conducted by Atolani and colleagues [47], the inhibition halo sizes for *E. coli* ranged from 10 to 18 mm, and for the fungus *C. albicans*, they varied between 10 and 16 mm. Conversely, with the gram-positive bacterium *E. faecalis*, CsPO demonstrated significant inhibition. This finding suggests that *E. faecalis* poses a more fragile barrier against external agents compared to other bacterial strains.

The research conducted by Abers and coauthors [48] investigated the antimicrobial potential of the essential oil from *C. sinensis* peel against the bacterium *M. smegmatis*. They experimented with varying volumes of oil, from 20 to 40 μ L, and achieved inhibition halos ranging from 19 to 22 mm, thus confirming its efficacy. The inhibition halos of 13-16 mm observed at 30 μ L underscore the efficiency and applicability of the antimicrobial attribute of this essential oil.

Assessment of Allelopathic Effect

Aromatic fruit essential oils play several roles in plant interactions, apart from being crucial allelochemical sources [49] that influence the germination of different species [50]. It is important to recognize that allelochemicals, contingent on their concentration and the species under examination, can either promote or hinder seed development by modifying numerous metabolic processes linked to germination [51, 52].

CsPO was subjected to an allelopathic investigation by employing various oil concentrations and assessing their impact on seed germination. Table 3 highlights that the rate index, mean germination

time, and rate coefficient remained consistent, indicating that CsPO did not influence these parameters. However, a notable contrast in root growth and germination rate of butter bean seeds was evident in Treatment 4 (T4), in which 500 µL of CsPO was employed. CsPO fostered significant root growth, surpassing the outcomes observed in the treatment utilizing soybean oil. This suggests a favorable allelopathic effect of CsPO, stimulating seed development.

Table 3. Summary of butter bean seed germination means under the effect of CsPO at different concentrations.

Treatment	Germination	AGT ^a	GR ^b	GRI ^c	RL ^d
T0	56 A ^f	2.15 A	0.46 A B ^g	16.74 A	22.96 A B
T1	43 A	2.72 A	0.37 A	10.77 A	15.46 A
T2	59.5 A	2.12 A	0.48 A B	19.32 A	23.22 A B
T3	55.5 A	2.00 A	0.50 A B	16.95 A	26.63 A B
T4	55 A	1.75 A	0.59 B	17.75 A	36.09 B
T5	42 A	2.60 A	0.41 A B	10.73 A	21.55 A B
RC ^e	32.04	22.23	19.14	40.17	36.00
Average	51.83	2.22	0.47	15.38	24.32

^aAGT: average germination time; ^bGR: germination rate; ^cGRI: germination rate index; ^dRL: Root length; ^eRC: rate coefficient; ^fA: highest average (highest activity); ^gB: lowest average (lowest activity).

The rate coefficient values exhibited considerable variability, suggesting the possibility of test-related issues. Seed storage methods stand out among the analysis criteria, as seeds are often treated with plant-derived substances (powders, oils, extracts) before storage. These substances can both eliminate and deter insects, thereby hindering their growth and development. These treatments offer the advantage of uniform seed coverage. To prevent excessive moisture absorption and interference with germination, these treatments should be of short duration [53].

Figure S3 (Supplementary material) illustrates the root growth of butter bean seeds, where genetic factors could have impacted their germination rate. Some scholars have linked the allelopathic attributes of essential oils, alongside other characteristics, to the presence of terpenoids. These compounds can modify cell membrane permeability, diminish enzymatic activity, and disrupt DNA transcription and RNA translation, consequently impeding both seed germination and seedling growth [54].

The substitution of agrochemicals with plant extracts possessing fungicidal properties has undergone extensive investigation in the agricultural domain. Numerous studies have showcased the effectiveness of essential oils and various other plant extracts in suppressing phytopathogenic fungi [55, 56].

***In-Vitro* Assessment of Acetylcholinesterase Inhibition**

The CsPO under investigation displayed significant activity in the acetylcholinesterase inhibition assay, with an IC_{50} value of 72.0 $\mu\text{g/mL}$. This could be attributed to the presence of acyclic and monocyclic monoterpenes in the oil, e.g., linalool and γ -terpinene, respectively. Certain characteristics, such as the presence of a hydrophobic ligand, might contribute to heightened inhibitory effectiveness, given the known susceptibility of the AChE active site to hydrophobic interactions. These characteristics inherent to cyclic and acyclic monoterpenes could influence AChE-inhibiting activity. In the case of bicyclic monoterpenes possessing the carene or pinnae skeleton, the potential for AChE inhibition has been linked to the position of the double bond [8].

Research into the structure-activity relationship between AChE and monoterpenoids has demonstrated that hydrocarbons exhibit more robust inhibition compared to alcohols and ketones. The presence of an oxygenated functional group diminishes the potency of AChE inhibition [7]. More recent investigations on the structure-activity relationship with bisabolane-type sesquiterpenoids, such as that conducted by Fujiwara and colleagues [57], suggest that AChE inhibition potency follows this order: ketones < alcohols < hydrocarbons.

Additionally, certain compounds like coumarins have demonstrated inhibitory effects on AChE. For instance, the compound citroptene (5,7-dimethoxycoumarin), found in citron peel, and auraptene (7-geranyloxycoumarin), present in *C. paradisi Macfad.*, have been proposed as potential erythrocyte AChE inhibitors [8].

***In Silico* Molecular Docking Determination of Acetylcholinesterase Inhibition**

Molecular docking investigations were performed, involving the three-dimensional structure of AChE (1QTI) and the key CsPO components currently under scrutiny. These investigations were targeted at the active site region, previously occupied by galantamine. The active site of this enzyme is situated at the base of its cavity. The so-called 'catalytic triad' – consisting of three amino acid residues, namely, Ser200, His440, and Glu327 – plays a direct role in the hydrolysis of acetylcholine [58]. Moreover, amino acid residues from the anionic subsites (Trp86 and Phe330) and the peripheral subsite (Trp279) are also significant for ligand recognition [59, 60].

Table S2 displays the outcomes of molecular docking investigations involving the primary components of CsPO and AChE. The binding energy values ranged from -6.2 to -9.6 kcal/mol, which closely aligns with the values observed for the acetylcholinesterase inhibitor galantamine (redocking binding energy = -9.8 kcal/mol; RMSD = 0.3678 Å).

The interactions observed for galantamine (Fig. S4 - Supplementary material)) concur with those detailed by Bartolucci and colleagues [32]. These interactions highlight the hydrogen bond formed between the Ser200 residue and the oxygen atom of the O-methyl group, alongside π -alkyl interactions in the anionic subsite residues (Trp84 and Phe330). As outlined by the aforementioned researchers [32], the hydroxyl

group of the galantamine molecule engages with two water molecules, both of which are firmly connected to the catalytic pocket via Ser200. Meanwhile, the cyclohexene ring and two methylene groups of the tetrahydroazepine ring of the galantamine molecule align with the Trp84 indole ring. The double bonds of the cyclohexene ring converge towards the π system of the indole ring, facilitating π - π interactions.

Among the compounds subjected to testing, α -humulene displayed the most favorable binding energy value (-9.6 kcal/mol). The primary interactions identified involved anionic residues: (1) Try121, Try334, and Phe330, participating in π -alkyl interactions, and (2) Trp84, contributing to π -sigma interactions. These specific residues hold significance as they are linked to the presumed 'anionic' bond with choline in the AChE cavity [60]. The remaining compounds also engaged in π -sigma and π -alkyl interactions with these residues (Figs. S5 and S6 - supplementary material). This implies that these substances establish interactions capable of inhibiting AChE, a conclusion supported by the biological inhibition data.

CONCLUSION

This study investigated the chemical composition and anticholinesterase activity of the essential oil from the peel of *Citrus sinensis* L. collected at Rio Preto da Eva (AM), Brazil, identifying a total of 33 compounds, notably hydrogenated and oxygenated monoterpenes as well as sesquiterpenes. Six major components stood out, including hydrogenated sesquiterpenes β -acoradiene (12.47%) and α -humulene (10.52%), and hydrogenated monoterpenes β -pinene (9.63%), car-3-en-2-one (8.43%), limonen-10-ol (8.26%), and β -citronelene (7.33%). The *in vitro* assays indicate the inhibitory effect of CsPO against the acetylcholinesterase enzyme ($IC_{50} = 72.0 \mu\text{g/mL}$), possibly due to its key compounds. These findings align with *in silico* determinations, emphasizing the inhibitory impact of the same compounds, in particular α -humulene, which demonstrated binding energy values akin to the galantamine inhibitor. The allelopathic effect observed in T4, (500 $\mu\text{g/mL}$) influencing root growth and germination rate, suggests the effect of *Citrus sinensis* secondary metabolisms. Concerning antimicrobial activity, a general conclusion can be drawn: CsPO displays inhibition against the tested bacterial strains and a fungus, with inhibition ranging from low (+) to moderate (++). Based on the *in vitro* and *in silico* findings regarding AChE inhibition potential, it can be deduced that the CsPO compounds possess substantial inhibitory action against AChE. For that reason, further research on this CsPO is warranted for Alzheimer's disease treatment, with specific focus on its prominent terpenoid components, especially β -acoradiene and α -humulene, distinctive to the region where this *C. sinensis* L. species grows. In light of the above, the primary objective of this study is to enhance the understanding of the *Citrus sinensis* species prevalent in the Amazon region. By focusing on one of its derived products, i.e., its essential oil, this research aims to bolster both its economic significance and scientific importance. The antimicrobial, phytotoxic, and anticholinesterase potential of its essential oil suggests that this *Citrus sinensis* species can become a viable natural option for addressing conditions such as Alzheimer's disease and other health-related issues.

Declarations

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Author Contributions:

i) Assessment of Allelopathic Effect: Maiara de Souza Nunes Ávila and Mayanna Emília Tenório Vicente. li) Biological Assays: Mayanna Emília Tenório Vicente, Geovania Santos de Souza, Ívina Thayná Miranda Trindade and Ingrid Reis da Silva.

Chemical Composition analysis: Vítor Hugo Neves da Silva, Geovania Santos de Souza. lii) In-Vitro Assessment of Acetylcholinesterase Inhibition: Ingrid Reis da Silva, Mayanna Emília Tenório Vicente, Ívina Thayná Miranda Trindade and Vítor Hugo Neves da Silva. iv) In Silico Molecular Docking Determination of Acetylcholinesterase Inhibition: Bruna Ribeiro de Lima. v) Supervision and Final draft of article: Paulo José Sousa Maia and Dominique Fernandes de Moura do Carmo. Vi) Dominique Fernandes de Moura do Carmo participated directly or indirectly in all stages of this work.

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