



Antioxidant and Antimicrobial Properties of the Essential Oil and Extracts of *Vitex pseudo-negundo* grown in Iran

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Abstract

Essential oil was extracted from *Vitex pseudo-negundo* flowers via hydrodistillation. The oil was then analyzed using Gas Chromatography (GC) and Gas Chromatography/mass spectrometry (GC-MS). Sixty-five components, making up 90.1% of the oil, were identified. The primary constituents included α -pinene (14.6%), limonene (10.7%), iso-dihydrocarveol (8%), α -terpinyl acetate (14.6%), β -caryophyllene (8.7%), bicyclogermacrene (4.9%), and epi-laurenene (8%). This study evaluated the antioxidant and antimicrobial activities of *Vitex pseudo-negundo* extract and essential oil using DPPH and reducing power assays. The IC₅₀ values for antioxidant activity were 98.4 mg/L for the extract and 1006.6 mg/L for the essential oil, indicating superior performance by the extract. The extract contained 70 mg GAE/g of total phenolic compounds and 34 mg CE/g of flavonoids. In contrast, the essential oil had 46.3 mg GAE/g and 8.1 mg CE/g. antimicrobial testing revealed that both the extract and essential oil showed strong antimicrobial activity, attributed to key compounds like α -pinene, limonene, α -terpineol, and linalool.

What is "already known":

- Essential oil may exhibit antibacterial activity, responsible for its bactericidal effect.
- Essential oil was extracted from *Vitex pseudo-negundo* flowers via hydrodistillation.

What this article adds:

- Essential oil of *Vitex pseudo-negundo* yields 0.1% (v/w) with α -pinene (14.6%) as the dominant compound.
- Methanolic extracts exhibit superior DPPH scavenging (IC₅₀: lower than essential oil) compared to synthetic antioxidants BHA/BHT.
- Total phenolic content higher in extracts (70 mg GAE/g) than essential oil (46.3 mg GAE/g).
- Antimicrobial activity stronger against Gram-positive bacteria, with MIC as low as 1 mg/ml for *Staphylococcus epidermidis*.
- Comparisons reveal geographical variations in oil composition, suggesting environmental influences on bioactivity.
- Potential as natural food preservative due to combined antioxidant and antimicrobial effects.

1. INTRODUCTION

Free radicals pose a threat to biological molecules in the body, contributing to numerous diseases, particularly in humans [1]. These reactive species often arise from food oxidation, especially in fats, leading to health hazards for consumers, diminished nutritional value, and undesirable flavors and aromas [2]. Antioxidants counteract these effects by neutralizing free radicals and halting their formation, thus averting associated illnesses. Although synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are widely employed in industries, growing concerns about their potential carcinogenic and nutritional drawbacks have prompted increased interest in natural alternatives among scientists and the public [3].

The genus *Vitex pseudo-negundo* of the family Verbenaceae consists of 250 species of small tropical trees and shrubs. Many such species have been applied as traditional medicinal plants throughout South Europe and Asia. One of these species is *Vitex agnus-castus*. L, which is commonly referred to as the chaste tree and is sparsely found in different parts of the Mediterranean, Central Asia, and Southern Europe [4,5], and known as “Nergundi”. *Vitex negundo*. L is used in traditional medicine as a remedy for arthritis and vermifuge. [6]. Essential oils found in the leaves are used as bathing oils and for sloughing wounds and ulcers. A literature survey shows that an essential oil may exhibit antibacterial activity, responsible for its bactericidal effect [7]. *V. pseudo-negundo*, which is another species of the *Vitex pseudo-negundo* genus grown naturally around seasonal rivers in Iran, has antioxidant properties [8]. It is frequently used in traditional medicine and reportedly shows various biological activities. It is used to treat premenstrual problems, hyperprolactinemia, anxiety, early birth, and stomachache and has diuretic, digestive, and antifungal

characteristics [9,10]. Moreover, the plant contains glucosides [11], phenolic compounds [12], triterpenoids [13], flavonoids [13,14], and iridoids [15].

This study therefore aims to investigate the antioxidant properties of essential oils extracted from *V. pseudo-negundo* and to evaluate their potential application as natural and safe food preservatives.

2. MATERIALS AND METHODS**2.1. Plant Material**

V. pseudo-negundo flowers were collected during the flowering stage from Gonabad, South Khorasan province, southeast Iran.

The flowers were dried at ambient temperature without exposure to sunlight and stored in a cool and dry place

2.2. Isolation of the Essential Oil

Hydrodistillation was used to obtain the essential oil of *V. pseudo-negundo* dried flowers using a Clevenger-type apparatus (3 h), followed by decanting and drying over anhydrous sodium sulfate.

2.3. Preparation of Extract

Thirty (30) g of powdered flowers were mixed with 300 ml of 80% methanol for 24 hours by shaking at ambient temperature. The resulting mixture was filtered using Whatman paper resulting mixture and the filtered extract was protected in refrigerator to keep the phenolic compounds in a suitable container. The resulting mixture was filtered with Whatman paper and the filtered extract was protected in refrigerator to keep the phenolic compounds 4-5 c. In the next step, the extracted flower powder was weighed and mixed again with 300 ml of 80% methanol by shaking at ambient temperature for 24 hours. The mixture obtained from the second step was filtered using Whatman paper as in the first step. Extracts obtained from the first and

second stages after concentration were frozen using a freeze dryer prior to carrying out the experiments [16].

2.4. Gas Chromatography-Mass Spectrometry

A Thermoquest-Finnigan trace gas chromatograph equipped with a DB-5 fused silica column (60 m long, 0.25 mm i.d., 0.25 µm film thickness) interfaced with a Thermo-Finnigan quadrupole mass detector and a computer equipped with Wiley 7 and Nist 1.7 spectra libraries were used to perform GC-MS analysis (column temperature: 60-250°C at a heating rate of 5°C, injector temperature: 250°C, injection volume: 0.1 µl, split ratio: 1:50, carrier gas: He at 1.1 ml/min., ionization potential: 70 eV, ionization current: 150 µA, ion source temperature: 250°C, and mass range: 35-465 mui). The components of the oil were characterized through their GC retention indices using n-alkenes series as the internal reference on a DB-5 column and their mass spectral and fragmentation patterns were compared with literature values 16 and matched with Wiley 7 and Nist 1.7 Mass Spectral Database for GC-MS [17,18].

2.5. DPPH Assay

A previously reported method with minor modifications was used to evaluate the DPPH free radical scavenging activity of the extracts [19,20]. 4 ml from methanolic extract and essential oil added to 1000 µl of DPPH (120 ppm) and was stirred for 30 s. The resulting solution was kept in the dark for 115 min, prior to absorption measurement at 517 nm using a Shimadzu spectrophotometer. The absorption rate of the control sample, which contained 4 ml of methanol and 1 ml of DPPH (120 ppm) was also found after 115 min. The Equation 1 was used to calculate the antioxidant activity.

% Inhibition =

$$(Ac\ 517\ \text{nm} - As\ 517\ \text{nm}) / Ac\ 517\ \text{nm} \times 100 \quad \text{Eq.1}$$

Ac is absorbance of control and As is absorbance of sample. Positive controls were BHA and BHT.

2.6. Reducing Power Assay

Salarbashi *et al.*'s method was used to determine the reducing power of EO/extract [21]. The absorbance of the

solution was measured at 700 nm. BHA and BHT were used as control samples.

2.7. Total Phenolic Determination

For samples of essential oils and extracts, Folin test was performed at a wavelength of 760 nm using Folin – Ciocalteu reagent. Reports were calculated as mg of gallic acid equivalents (GAE) per g of dried extract [16,22].

TFC of the extracts/EO was evaluated using AlCl₃, as previously described with minor modifications [22,23]. In this method, 0.5 ml of the extract or essential oil was mixed with 3 ml of 5% NaNO₂ and 3 ml of 10% AlCl₃. After 6 minutes, the absorbance of the solution was read at 510 nm. Reports were calculated as mg quercetin equivalents (QE) per g (QE/g) of dried extract.

2.8. Antibacterial and aAtifungal Activity

All bacteria and yeast were purchased from Persian Type Culture Collection, Iran.

2.9. Evaluation of MIC and MBC for Essential Oil and Extract

Bacteria from surface of tryptone soya agar plates (HiMedia, India) were collected and adjusted to about 10⁶CFU/ml with tryptone soya broth (HiMedia, India). To perform the experiment, 25 µl of 10⁶CFU / ml of each microorganism and 250 µl of each extract or essential oil were added to wells of 96 well plate. The plates were incubated at 37 ° C for 24 hours. After that, 3-(4,5-dimethylthiazol-2-yl)-2,5-didiphenyltetrazolium bromide (MTT) (100 µl, 5 mg/ml) was added to each well, followed by incubation for another 1 h, to show bacterial growth. Bacterial growth is marked by color change to pink-red. MIC (minimum inhibitory concentration) is defined as the minimum extract concentration, which can inhibit the formation of the pink-red dye. From the wells of MIC and higher concentration of it, samples were taken and spread over the surface of agar plates to investigate the minimum bactericidal concentration (MBC). The lowest concentration of an antibacterial agent, which is required to kill a particular bacterium, is referred to as MBC [21].

3. RESULTS & DISCUSSION

3.1. GC/MS assay

Volatiles component were at least 65 compounds which were recognized in essential oil of the plant and the yield was 0.1% (v / w).

Based on the quantitative analysis, the key components of the essential oil include sesquiterpene hydrocarbons (23.0%), monoterpene hydrocarbons (36.0%), oxygenated monoterpenes (27.2%), oxygenated sesquiterpenes (2.7%), and other compounds (9.9%).

Table 1. Percent composition of the essential oil of flower *Vitex pseudo-negundo*

No	Compound	RI*	percentage
1	α -pinene	940	14.6
2	Camphene	956	0.1
3	Sabinene	976	3.7
4	β -pinene	981	0.4
5	Myrcene	993	3.0
6	α -phellandrene	1004	1.7
7	α -terpinene	1018	0.4
8	Limonene	1030	10.7
9	Z- β -ocimene	1038	t**
10	E- β -ocimene	1052	0.1
11	γ -terpinene	1060	0.5
12	<i>cis</i> -sabinene hydrate	1072	t
13	Terpinolene	1090	0.8
14	<i>trans</i> -sabinene hydrate	1096	t
15	Linalool	1198	0.9
16	Nonanal	1100	0.2
17	isopentyl isovalerate	1105	t
18	methyl butanoate , 3-methyl-3- butenyl (3)	1115	t
19	<i>cis</i> -para-menth-2-en-1-ol	1121	t
20	α -campholenal	1127	t
21	<i>trans</i> -pinocarveol	1140	t
22	<i>trans</i> -para-menth-2-en-1-ol	1141	t
23	Isoborneol	1162	t
24	<i>para</i> -mentha-1,5-dien-8-ol	1171	t
25	terpinen-4-ol	1178	1.2
26	α -terpineol	1188	1.2
27	iso-dihydro carveol	1215	8.0
28	Citronellol	1225	0.1
29	<i>cis</i> -chrysanthenyl acetate	1266	0.2
30	bornyl acetate	1290	0.4
31	methyl geranate	1325	0.4
32	δ -elemene	1339	t
33	α -terpinyl acetate	1350	14.6
34	Isodene	1377	0.4
35	z-caryophyllene	1409	0.1
36	α -gurjunene	1411	0.9
37	β -caryophyllene	1420	8.7
38	β -gurjunene	1434	t
39	α - <i>trans</i> -bergamotene	1435	0.6
40	Aromadendrene	1442	t
41	Z- β -farnesene	1443	0.8
42	<i>cis</i> -muurola-3,5-diene	1450	t
43	α -humulene	1455	1.7
44	allo-aromadendrene	1461	1.7
45	9-epi- β -caryophyllene	1467	t
46	γ -muurolene	1480	0.1
47	β -selinene	1490	0.2
48	Bicyclogermacrene	1501	4.9

49	β -bisabolene	1506	0.5
50	γ -cadinene	1513	0.3
51	δ -cadinene	1522	1.5
52	<i>trans</i> - γ -bisabolene	1531	0.3
53	α -cadinene	1540	0.3
54	E-nerolidol	1563	0.1
55	Palustrol	1568	0.2
56	Spathulenol	1578	0.3
57	Viridiflorol	1593	0.5
58	epi- α -cadinol	1638	0.7
59	β -eudesmol	1649	0.4
60	α -eudesmol	1651	0.2
61	α -bisabolol	1675	0.2
62	2E,6E-farnesol	1745	0.1
63	Pentadecanol	1775	0.1
64	Z-nerolidyl isobutyrate	1785	<i>t</i>
65	epi-laurenene	1902	8.0
66	Total identified		97.0
67	Major Grouped Compounds		
68	Monoterpene hydrocarbons		36.0%
69	Oxygenated monoterpenes		27.2%
70	Sesquiterpene hydrocarbons		23.0%
71	Oxygenated Sesquiterpenes		2.7%
72	Miscellaneous		8.1%

The primary constituents are α -Pinene (14.6%), limonene (10.7%), iso-dihydrocarveol (8.0%), α -terpinyl acetate (14.6%), β -caryophyllene (8.7%), bicyclogermacrene (4.9%), and epi-laurenene (8.0%). The hydrodistillation technique has previously been employed to analyze the essential oil of *Vitex pseudo-negundo*. The results aligned with the findings of HadjMohammadi et al. However, the concentrations of the chemical compounds they tested (such as limonene (12.2%), α -pinene (35.9%), and bicyclogermacrene (14.5%)) were higher than those observed in this study [27]. Additionally, the primary compounds found in the stems, leaves, and flowers of this plant were limonene, caryophyllene oxide, and myrcene. These were reported at 27.5%, 28.8%, and 17.06%, respectively, by Tayebee et al [28]. GC-MS analysis identified 57 components in the essential oil of *Vitex negundo* Linn. var from Zhangjiazhai Mount, Yimeng Mountain Area, North China, representing 90.313% of the oil. The main compound was β -caryophyllene (26.301%), with notable others including 1,8-cineole, sabinene, *trans*- β -Farnesene, α -terpinyl acetate, caryophyllene oxide, and terpenene-4-ol, contributing 11.857%, 7.813%, 6.154%, 4.663%, 3.609%, and 3.543%, respectively [29]. The oil was obtained by hydrodistillation of pulverized *Vitex*

agnus-castus leaves, yielding 0.8% v/w. Analysis revealed that the main constituents were β -pinene (20.0%), viridiflorol (9.8%), α -pinene (9.1%), and cis-cimene (8.4%). Previous studies have shown that several components in the essential oils of these leaves possess antimicrobial properties. These include 47.14% sesquiterpenes, with α -Copaene comprising 25.26%, and other volatile compounds consisting of five monoterpenes (45.50%) and two fatty acids (7.36%) [7].

3.2. DPPH Radical Scavenging and Reducing Power Assay for Extract and Essential Oil

One of the antioxidant tests performed on the extract and essential oil of *Vitex pseudo-negundo*. was the DPPH test. The DPPH results are shown in Table 2. The DPPH test results indicated that the methanolic/water extract of *Vitex pseudo-negundo* has greater antioxidant activity than its essential oil, primarily due to the phenolic and flavonoid compounds in the flower extract. Notably, the extract's hydroxyl groups play a key role in preventing free radical formation [30]. In this study, BHA and BHT were used as positive controls. As shown in Table 2, the antioxidant power in the DPPH test is expressed in terms of 50% inhibition of free radicals (IC₅₀). The antioxidant power of these two compounds was 52.8 and 59.9 mg/L, respectively.

Table 2. Antioxidant activity (IC50) of different extracts and essential oil of *Vitex pseudo-negundo* compared to synthetic antioxidants (butylated hydroxytoluene; BHT, butylated hydroxyanisole; BHA).

Treatment name	DPPH (mg /L)	RP (mg /L)
<i>Vitex pseudo-negundo</i> extract(80%M)	98.4 ± 0.7	1006.6 ±17.6
<i>Vitex pseudo-negundo</i> Essential oil	227.1± 3.	1408.9 ± 28.3
BHA	52.8 ± 0.7	75.7 ± 0.6
BHT	59.9± 0.8	90.5 ± 0.6

3.3. Reducing Power Assay

The reducing power serves as an important indicator in evaluating antioxidant capabilities [24]. This assay involves reducing agents converting the Fe³⁺/ferricyanide complex to Fe²⁺ [25]. The capacity was determined by the color change from yellow to green-blue, quantified by absorbance at 700 nm; greater absorbance indicates greater

reducing power [26]. The study revealed that higher concentrations of the essential oil, extract, BHT, and BHA led to increased reducing powers. The findings are outlined in Table 2.

3.4. Total Phenolic and Flavonoid Content of Extract and Essential Oil

Table 3. Total contents of phenolic, flavonoid extract and essential oil from *Vitex pseudo-negundo*¹

treatment name ²	TPC ³	TFC ⁴
<i>Vitex pseudo-negundo</i> extract(80%M)	70 ±0.7	34.7 ±0.3
<i>Vitex pseudo-negundo</i> Essential oil	46.3 ±0.3	8.1 ±0.1

¹Values correspond to mean ± standard deviations of three (n=3) measurements. Within each column, different superscript letters indicate significant difference (p <0.001).

²*Vitex pseudo-negundo* (80%M): 80% methanolic extract of *Vitex pseudo-negundo* and essential oil from *Vitex pseudo-negundo*

³TPC = total phenolic content (mg gallic acid equivalent/g extract).

⁴TFC = total flavonoid content (mg quercetin equivalent/g extract).

The antioxidant powers of the essential oil and the extract were both lower than those of BHA and BHT. Given the reducing power of plant extracts and essential oils, the total amounts of phenolic and flavonoid compounds are expected to be important factors. Antioxidant effects in plant extracts and essential oils stem from phenolic constituents [31]. These compounds can transform free radicals into less reactive forms by donating hydrogen or electrons, thereby interrupting radical chain reactions [16,32]. As observed in Table 2, the order of the reducing power is as follows: BHA (75.7mg/L) > BHT (90.5mg/L)

> methanol/water extract (1006.6 mg/L) > essential oil (1408.9 mg/L) As the results show, α-pinene and limonene are the main components of *Vitex pseudo-negundo*, which are reported to be 14.6 and 10.7, respectively, and play an important part in the strength of antioxidant activity. In 2019, Yi Wang et al. investigated the antioxidant power of Myrcene, limonene, geraniol, linalool, nerol, and α-pinene by DPPH and reducing power methods and concluded that α-pinene and limonene had the highest antioxidant power [33]. Other antioxidants in essential oils from *Vitex pseudo-negundo* include α-terpineol and Linalool. In 2014,

Zengin and Baysal studied the antioxidant properties of terpenes by DPPH and FRAP and reported that α -terpineol and Linalool had antioxidant properties [34]. Phenolic compounds (flavonoids and proanthocyanidins) are responsible for the antioxidant ability of medicinal plants. Therefore, many flavonoids act as potential inhibitors for many metabolic enzymes. Antioxidant power which associated with phenolic components is dependent on some factors; especially the number and arrangement of hydroxyl groups, structures, especially and the presence of electron donor and receptor units in the ring structure [16,35]. According to the results displayed in Table 3, the

amounts of phenolic and flavonoid compounds are higher in the extract (70mg of GAE/g and 34mg of CE/g, respectively) compared with the essential oil. However, the corresponding values for the essential oil were 46.3 mg of GAE/g and 8.1mg of CE/g, respectively.

3.5. In vitro antibacterial and antifungal activities of the extract and essential oil

The antimicrobial efficacy of the methanolic extract and essential oil from *Vitex pseudo-negundo* flowers against various foodborne pathogenic bacteria were evaluated using the method described by Salarbashi et al. [21].

Table 4. Antibacterial and Antifungal Activities of the extract and Essential Oil of *Vitex pseudo-negundo*

Name of bacteria	MIC (mg/ml) extract	MBC (mg/ml) extract	MIC (mg/ml) essential oil	MBC (mg/ml) essential oil	MIC (mg/ml)
<i>Bacillus cereus</i> PTCC 1247	2	>128	22/5	22/5	gentamicin 0.005
<i>Staphylococcus aureus</i> PTCC 1337	4	64	<0.08	<0.08	amikacin 0.004
<i>Staphylococcus aureus</i> PTCC 1431	2	>128	45	45	amikacin 0.004
<i>Staphylococcus aureus</i> PTCC 1112	4	>128	0/3	0/3	amikacin 0.004
<i>Staphylococcus epidermidis</i> PTCC 1114	1	>128	0/7	0/7	amikacin 0.004
<i>Staphylococcus epidermidis</i> DSMZ3270	4	>128	45	90	amikacin 0.004
<i>E. coli</i> PTCC 1330	64	>128	>90	>90	gentamicin 0.005
<i>E. coli</i> PTCC 0157	64	>128	>90	>90	gentamicin 0.005
<i>Salmonella typhi</i> PTCC 1609	16	>128	>90	>90	gentamicin 0.005
<i>Listeria monocytogenes</i> PTCC 1165	8	>128	2/81	22/5	gentamicin 0.005
<i>Pseudomonas aeruginosa</i> PTCC 1074	16	16	>90	>90	amikacin 0.04
<i>Candida albicans</i> PTCC 5027	8	>128	2/8	2/8	clotrimazole 0.008

There are several reasons to justify the antimicrobial behavior of the extracts and essential oils depending on the type of compounds and their mechanism of action. One reason is that the phenolic compounds in the extract; especially those of a hydrophobic nature, are able to easily

cross the cell wall of Gram-positive bacteria [36]. Flavonoid compounds have been shown to affect the synthesis of proteins and lipids. In particular, nucleic acids can affect hydrogen bonding between the hydroxyl groups in phenolic compounds and nucleic acids in microbial cells and be

effective in inactivating DNA molecules [37]. Table 4 shows the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) results. Based on the results, phenolic extract and essential oil had some antimicrobial activity on Gram-positive and Gram-negative bacteria studied, with higher activity on Gram-positive one. The antimicrobial effect of the extract and essential oil varied depending on the type of bacteria. In general, Gram-positive bacteria were more sensitive to the phenolic extract in comparison with Gram-negative bacteria. In the case of essential oil, with the exception of *Listeria monocytogenes* PTCC 1165, the results were similar to those of the extract. As shown in Table 4, the MIC of the extract (1 mg/ml) was shown for *Staphylococcus epidermidis* PTCC 1114 Gram-positive bacterium. The most resistant bacteria were *Escherichia coli* PTCC 1330 and *E. coli* PTCC 0157 with MIC (64 mg/ml) and MBC > 128. There were differences between the extract and the essential oil in terms of inhibiting the growth of bacteria. In some cases, depending on the type and species of bacteria, the extract had a greater effect in inhibiting the growth of bacteria. For example, the extracts were more effective on bacteria such as *Bacillus cereus* PTCC 1247, *Staphylococcus aureus* PTCC 1431, *S. epidermidis* DSMZ3270 and *Salmonella typhi* PTCC 1609. *Pseudomonas aeruginosa* PTCC 1074 was the most sensitive bacterium according to the MBC result. As observed in the table, in some bacteria, the essential oil had a greater effect in inhibiting bacterial growth. Another reason for the antimicrobial power of the extract can be the presence of higher amounts of phenolic and flavonoid compounds. In the case of *Candida albicans* PTCC 5027, the antifungal effect of the essential oil was greater than that of the extract, while clotrimazole was used as positive control. Gentamicin was used as positive control for bacteria, except Amikacin was as positive control for all *Staphylococcus* and *P. aeruginosa*. The corresponding MICs are shown in Table 4. It should be noted that phenolic compounds interfere with the bilayer phospholipid membrane, affecting the membrane permeability of microbial cells and causing the release of intracellular

compounds. In addition, these compounds lead to changes in membrane function for electron transfer or nutrient uptake and disruption of protein synthesis and enzyme activity [38]. However, in the case of essential oils, different compounds play a role in the antimicrobial properties. This has been also supported by previous studies. For example, in 2019, Guimarães et al. reported that in the control of *Salmonella typhimurium* using eugenol and terpinol, the mechanism of bacterial death is the loss of cell membrane integrity or function [39]. Teneva et al. showed that the antibacterial activity of terpenes may be due to damage to the plasma of the bacterial cell membrane [40]. As pointed out in the results corresponding to the antioxidant part, α -pinene and limonene, which have high antimicrobial activity in addition to antioxidant role, are the most dominant components in *Vitex pseudo-negundo* essential oil. In 2019, Yi Wang et al. investigated the antibacterial activity of a number of terpenes including α -pinene and limonene and concluded that α -pinene and limonene both had antibacterial activity. The combination of α -pinene showed the strongest antibacterial activity [33]. They further reported that terpene compounds affected Gram-positive bacteria more than Gram-negative bacteria, mainly due to the hydrophilic cell wall structure of the latter [33]. In 2014, Zengin and Baysal studied the antibacterial properties of terpenes and reported that α -terpineol and Linalool had antibacterial activity [34]. Therefore, in this study, perhaps α -terpineol and Linalool were among the compounds causing antibacterial activity in essential oil. In addition, in the case of essential oil in the present study, the higher susceptibility of Gram-positive to Gram-negative bacteria can be attributed to the reasons mentioned. To deepen the understanding, comparisons with similar studies on *Vitex* species reveal both consistencies and variations. For instance, a study on *Vitex agnus-castus* essential oil reported major components like β -pinene (20.0%) and α -pinene (9.1%), with notable antimicrobial activity attributed to sesquiterpenes and monoterpenes, aligning with our findings on monoterpene dominance but differing in specific percentages, possibly due to geographical and

extraction differences [7]. Another investigation on *Vitex doniana* leaves and fruits essential oils showed antimicrobial efficacy against Gram-positive bacteria, similar to our results, but with different major compounds like (E)- β -farnesene and citronellol, highlighting species-specific variations [41]. Controversially, while our study found the extract superior in antioxidant activity due to higher phenolics, a report on *Vitex agnus-castus* extracts and oil showed comparable DPPH scavenging, with IC50 values closer between oil and extracts, suggesting solvent choice impacts efficacy [42]. Furthermore, some studies on *Vitex negundo* reported higher β -caryophyllene levels (26.3%), correlating with stronger antifungal activity against *Candida* species, contrasting our essential oil's better performance against *C. albicans* compared to the extract, which may indicate synergistic effects unique to pseudo-negundo[29]. These discrepancies underscore the influence of environmental factors, plant parts, and extraction methods on bioactivity, warranting further standardized comparative research.

4. Conclusion

This study demonstrated that both the essential oil and methanolic extract of *Vitex pseudo-negundo* flowers grown in Iran possess notable antioxidant and antimicrobial properties. GC–MS analysis revealed a complex chemical profile dominated by monoterpene and sesquiterpene compounds, with α -pinene, α -terpinyl acetate, limonene, β -caryophyllene, bicyclogermacrene, and epi-laurenene identified as the major constituents of the essential oil.

Antioxidant evaluations showed that the methanolic extract exhibited significantly stronger free radical scavenging and reducing power activities than the essential oil, which correlated with its higher total phenolic and flavonoid contents. These findings confirm that phenolic compounds play a central role in the antioxidant capacity of *V. pseudo-negundo*.

Antimicrobial assessments revealed that both the extract and essential oil were effective against a broad spectrum of

microorganisms, with greater activity observed against Gram-positive bacteria.

Overall, the results indicate that *Vitex pseudo-negundo* flower extract and essential oil represent promising natural sources of antioxidant and antimicrobial agents. Their combined bioactivities suggest potential applications in food preservation, pharmaceutical formulations, and functional products. Further studies focusing on toxicity, stability in food systems, and synergistic interactions with other natural preservatives are recommended to support industrial utilization.

5. Declarations

5.1. Authors' Contributions

All authors equally contributed to this work.

5.2. Declaration of Interest

The authors of this article declared no conflict of interest.

5.3. Ethical Considerations

All ethical principles were adhered in conducting and writing this article.

5.4. Transparency of Data

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

5.5. Funding

This research was carried out independently with personal funding and without the financial support of any governmental or private institution or organization.

5.6. Using Artificial Intelligent chatbots

No AI chatbot has been used in this study

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