Antibacterial, antioxidant, and GC-MS profiling of five Yucatán plant leaf extracts against *Listeria monocytogenes*, *Salmonella enteritidis*, and *Escherichia coli*

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ABSTRACT

Objective: To determine the antibacterial and antioxidant activity and to identify the volatile compounds in extracts from *Bixa orellana*, *Cnidoscolus aconitifolius*, *Mentha spicata*, *Piper auritum*, and *Lippia origanoides* from Yucatan.

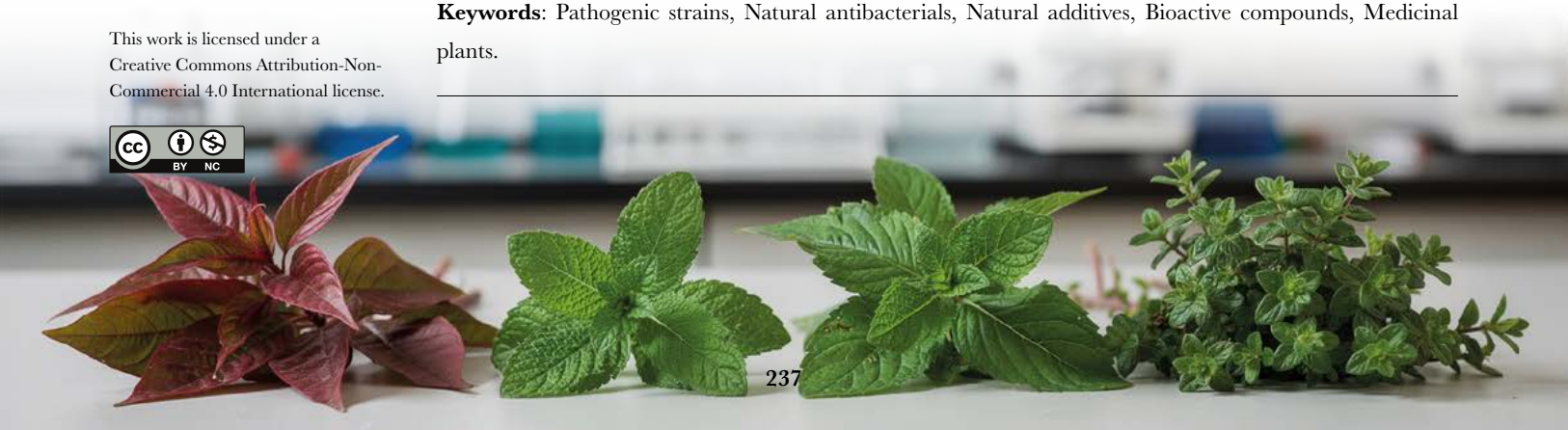
Design/methodology/approach: Antibacterial activity was evaluated using the disk diffusion method; antioxidant activity was assessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, and volatile compounds were identified by gas chromatography-mass spectrometry.

Results: In antibacterial assays, *L. origanoides* and *M. spicata* significantly inhibited *L. monocytogenes*; *B. orellana* was effective against *E. coli*, and *L. origanoides* extract showed significant inhibition of *S. enteritidis* at 40 mg/mL. In antioxidant activity, *B. orellana*, *L. origanoides*, and *M. spicata* showed the highest antioxidant capacity percentages (92.1-100%, $p<0.05$) at 1 mg/mL. Chromatographic analysis tentatively identified the presence of compounds such as carvacrol, phytol, ishwarene, palmitic acid, and linolenic acid.

Limitations on study/implications: Although further MIC/MBC determinations and food-matrix validation are required, the high biological activity observed in *B. orellana*, *L. origanoides*, and *M. spicata*, suggests their potential as natural preservative candidates.

Findings/conclusions: The extracts with the highest antioxidant and antibacterial effects at 1 and 40 mg/mL, respectively, are *B. orellana*, *L. origanoides*, and *M. spicata*. GC-MS analyses tentatively identified the presence of bioactive compounds such as phytol, β-Cyclocitral, linolenic, and palmitic acid, carvacrol, thymol, guaiol acetate, epiglobulol and ishwarene. Results suggest that these three plant extracts represent promising natural alternatives for application in agro-food systems, specifically as potential antioxidant and selective antimicrobial agents..

Keywords: Pathogenic strains, Natural antibacterials, Natural additives, Bioactive compounds, Medicinal plants.



INTRODUCTION

There is a rich biodiversity of plants, some of which additionally serve as food preservatives. Some of these plants can improve color and flavor of foods, and serve in the development of new agricultural products for their bioactivity (Uuh Narvaez & Segura Campos, 2020).

The bioactive compounds (BC) from these plants are advantageous for food preservation and disease prevention (Batiha *et al.*, 2021). Globally, foodborne illnesses affect 1 in 10 people. Around 31 pathogenic bacteria found in different foods cause approximately 420,000 deaths per year worldwide. Species of *Salmonella* sp., *Listeria* sp., and *E. coli* are the most dangerous pathogenic strains globally (Lee & Yoon, 2021). Consequently, plants to inhibit or eliminate these pathogens are constantly being analyzed.

Cnidoscolus aconitifolius is a widely consumed plant in Nigeria and the Mesoamerican region. The BC in *C. aconitifolius* prevent cardiovascular disease when it is consumed in food or teas (Manzanilla Valdez & Segura Campos, 2021; Orji *et al.*, 2016). Additionally, *C. aconitifolius* extract is used as a nephroprotective and hepatoprotective in phytotherapy (Manzanilla Valdez & Segura Campos, 2021). Other plants, like *L. origanoides*, *P. auritum*, *B. orellana*, and *M. spicata*, are also used in many forms (extract, powder, essential oil) in cuisines all over the world and in traditional medicine (Bautista-Hernández *et al.*, 2021).

Extracts can be used to inhibit the growth of Gram (+) and Gram (−) strains, but, the natural habitat of the plants, such as geographical location and climatic conditions, will affect the quality of the extracts (Olvera-Aguirre *et al.*, 2020). This is why it is so important to repeat and document these studies in different geographical regions. Additionally, it is important to make a comparative profiling of these five species collected from Yucatán under a single extraction protocol; paired bioactivity + GC-MS volatiles. To that end, the objective of this study was to determine the antibacterial and antioxidant activity and identify the volatile compounds in extracts from *B. orellana*, *C. aconitifolius*, *M. spicata*, *P. auritum*, and *L. origanoides* from Yucatan.

MATERIALS AND METHODS

Plant matter and storage

The collected species (*Bixa orellana*, *Cnidoscolus aconitifolius*, *Piper auritum*, and *Lippia origanoides*) were from established crop in agricultural research areas at "La Curva" research and production unit, located at 21.075058 N and −89.504310 W, in the municipality of Conkal, Yucatan, Mexico. The fifth plant species, *M. spicata*, was purchased fresh from a known established crop from a local farm in the municipality of Xcanatun, Yucatan, Mexico located at 21° 04′24″N and 89° 37′50″W.

Five hundred grams of fresh leaves were separated from the plants on the same day, washed by submerging them into tap water for one minute and then disinfected with 2 mL of commercial 0.08% ionized silver solution for each L of water for 15 minutes. The leaves were then dried at 40 °C for 72 h. After being completely dried, the leaves were then ground in a Cemotec™ 1090 Sample Mill (Thermo SCIENTIFIC, Galicia, Spain) to obtain a uniform particle and then finally pulverized in a Cyclotec™ 1093 Sample Mill

(Thermo SCIENTIFIC, Galicia, Spain). The powder obtained was then stored in dark airtight bags at −20 °C until extraction.

Extraction Process

Extractions of every plant sample were performed three times with independent biological replicates made from 5 different plants. A solid-liquid extraction (magnetic stirring, 2h) was carried out at a ratio of 1:20 (w/v, 25 °C) in water-ethanol (50%) (1:1 v/v), and roto evaporated (Rotavapor R-215, water bath B-49, vacuum pump V-700, and a vacuum controller V-850. Buchi®, Switzerland) to remove the solvents (ethanol was removed at 77 millibars and 60 rpm; water was removed instantly adjusting pressure to 32 millibars and 180 rpm both at 50 °C). The remainder was frozen at −20 °C for 72 h. At the end of this period, the samples were lyophilized until they had a constant weight. The extracts' yield was calculated as dry matter. The solidified samples were ground and stored at −20 °C in dark airtight bags until analysis. The extraction yield was calculated with Equation 1.

$$Yield(\%) = \frac{mass\ of\ dried\ extract}{mass\ of\ dried\ plant\ material} \times 100 \quad (1)$$

Antioxidant Activity

The DPPH radical scavenging activity was determined by using the methodology described by Uuh Narváez *et al.* (2021) with slight modifications. First 175 μL of DPPH radical (100 μM in methanol) and 25 μL (1 mg/mL) of the extract were used. These were then mixed and held in the dark for 30 minutes. Absorbance was measured at 517 nm. This was repeated four times. The DPPH radical scavenging activity was calculated as:

$$DPPH\ radical\ scavenging\ activity(\%) = \left[(Ab - Ae) / Ab\right] \times 100 \quad (2)$$

where: *Ab* is the absorbance of the DPPH radical without extract and *Ae* is the absorbance of the DPPH radical with extract.

The neutralization activity of $ABTS^{\bullet+}$ was repeated 4 times and carried out following the method proposed by Uuh Narvaéz *et al.* (2021) with some modifications. The $ABTS^{\bullet+}$ cation was released through the interaction of $ABTS^{\bullet+}$ (7 mM) with potassium persulfate (2.45 mM) for 16 h in the dark. The solution with the released $ABTS^{\bullet+}$ cation was adjusted to an absorbance of 0.7 (±0.02) with ethanol at 734 nm. Subsequently, 10 μL of extract (1 mg/mL) was added to 195 μL of $ABTS^{\bullet+}$ radical solution and the absorbance was measured after 6 minutes at 734 nm. The results were calculated using Equation 2.

Bacterial strains and culture media

The biotechnology and microbiology laboratory at the Yucatán Autonomous University culture collection supplied the bacterial strains: *Listeria monocytogenes* ATCC 51414, *Salmonella enteritidis* ATCC 13076, and *Escherichia coli* ATCC 10536. The strains

were maintained at −20 °C in BHI containing 40% of glycerol (v/v). Disk diffusion was performed on Muller Hinton agar purchased from BD Bioxon (Mexico).

Antibacterial activity

The extracts' antibacterial activity was assessed using the disk diffusion method on Mueller Hinton agar following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) with six repetitions per treatment. This was realized following the methodology of Madrazo-León *et al.* (2022).

Disk diffusion test

Bacterial suspensions, adjusted to a turbidity matching the 0.5 McFarland standard, were inoculated onto Mueller Hinton agar in 100 mm Petri dishes using sterile swabs. Sterile paper disks with a 6 mm diameter were then placed on the surface of the agar, each loaded with 10 μL of each extract at concentrations of 10, 20, and 40 mg/mL. Penicillin and gentamicin (500 μg/mL) were used as positive controls for Gram-positive (*L. monocytogenes*) and Gram-negative (*S. enteritidis* y *E. coli*) bacteria, respectively. Sodium nitrite (10 mg/mL) was included as an additional control, while distilled water served as the negative control and as a solvent for the extracts. The inoculated plates were incubated for 24 hours at 37 °C. After incubation, inhibition diameters zones were measured to assess bacterial susceptibility. Antibacterial activity was classified according to inhibition zone diameter as follows: no inhibition "−" (0 mm), inactive "+" (<10 mm), partially active "++" (10-13 mm), and active "+++" (>14 mm) (Madrazo-León *et al.*, 2022).

Bioactive compounds identification

GC-MS analysis

The secondary metabolites of the extracts were performed with the gas chromatography technique coupled to mass spectrometry described by Gómez-Gutiérrez *et al.* (2023).

Data analysis

For the study, a completely randomized design was used (Equation 3) (Cochran & Cox, 1965). Data were analyzed using SAS statistical software, version 9.0. An analysis of variance (ANOVA) was performed using the PROC ANOVA procedure, and mean comparisons were conducted using Tukey with significance was determined at $p \leq 0.05$.

$$Y_{ij} = \mu + T_i + e_{ij} \quad (3)$$

where: Y_{ij} (observed value of dependent variable) antioxidant and antibacterial activity of PE; μ overall mean; T_i (fix effect of the treatment) T1: *B. orellana*, T2: *L. origanoides*, T3: *M. spicata*, T4: *P. auritum*, T5: *C. aconitifolius*, and e_{ij} random error term.

RESULTS AND DISCUSSION

Extraction yield is a critical parameter in determining the efficiency and practicality of obtaining BC from plant materials. A higher yield is a useful indicator of extraction

efficiency and ensures better use of natural resources. In this study, a hydroalcoholic solution (ethanol 50:50 v/v) was selected to balance polarity and optimize the extraction of both hydrophilic and lipophilic compounds.

Rodrigues *et al.* (2020) evaluated three techniques for *C. aconitifolius*: Subcritical Water Extraction (SWE), Microwave Assisted Extraction (MAE), and Soxhlet, reporting yields of 35-39%, 9-18%, and 31-33% (wet basis), respectively. In the present study, the ethanol (50:50 v/v) extraction yielded 26.3% on a dry basis (Table 1), which is lower than SWE and Soxhlet, but higher than MAE. Yield differences may be attributed to factors such as solvent polarity, extraction time and temperature, technique used, and environmental conditions of the plant's origin (Olvera-Aguirre *et al.*, 2020).

Antioxidant Activity

The estimation of antioxidant activity using the DPPH method has been employed to evaluate the presence of BC in plant leaves. The DPPH radical undergoes a color change from purple to yellow upon interaction with BC. Similarly, the $ABTS^{\bullet+}$ assay assesses the reduction in the intensity of the radical's color due to BC (Zeb, 2021).

Antioxidant activity using the DPPH method (Table 1) revealed that the *B. orellana* extract showed the highest antioxidant activity, inhibiting 100% of free radicals at a concentration of 1 mg/mL. The results, however, did not show statistically significant differences ($p>0.05$) between *L. origanoides*, *M. spicata* and *B. orellana*. These findings indicate that all three have a strong capacity to neutralize free radicals and be considered potential sources of antioxidants.

Pavlović *et al.* (2021) assessed *M. piperita* using the DPPH method with extracts obtained through 24 hours maceration in 70% methanol, 70% ethanol, and hot water, combined with ultrasound one hour before and after maceration. The highest scavenging activity was reported at 250 mg/mL, ranging from 61.27 (water), 92.80 (ethanol), and 93.30% (methanol), respectively. In contrast, the present study evaluated *M. spicata* at a dose of 40 mg/mL which achieved a DPPH inhibition of 97.3% (Table 1). Additionally, *B. orellana* and *L. origanoides* also showed higher antioxidant activity compared to those reported by Pavlović *et al.* (2021), high scavenging activity was observed under the present extraction and assay conditions.

Table 1. Antioxidant activity and yield of leaves and extracts from 5 plant species collected in the Yucatán Peninsula.

Plant	Common name	Family	Extract yield (%)	DPPH (%)	$ABTS^{\bullet+}$ (%)
Cnidoscolus aconitifolius	Chaya, Chaya spinach tree	Euphorbiaceae	26.3 ± 0.9^{a}	40.2 ± 2.7^{c}	80.7 ± 1.3^{bc}
Piper auritum	Sacred pepper, Momo	Piperaceae	13.7 ± 1.0^{c}	43.2 ± 2.5^{c}	71.8 ± 1.9^{c}
Mentha spicata	Yerbabuena, Spearmint	Laminaceae	16.4 ± 1.1^{b}	97.3 ± 2.5^{ab}	92.1 ± 0.2^{a}
Lippia origanoides	Oregano	Verbenaceae	12.0 ± 1.4^{c}	98.1 ± 4.0^{ab}	92.5 ± 0.3^{a}
Bixa orellana	Achiote, Annatto	Bixaceae	21.5 ± 0.9^{ab}	100 ± 1.9^{a}	91.6 ± 0.4^{a}
P value	-	-	<0.001	<0.001	<0.001

% yield: dry matter base; values are expressed as mean ±: standard error; different superscripts indicate significant differences between plants (Tukey HDS, $p\leq0.05$).

In the ABTS$^{\bullet+}$ assay, no significant differences were found between *L. origanoides*, *M. spicata*, and *B. orellana* (Table 1), with free radical scavenging ranging from 91.56% and 92.46% at 1 mg/mL. These results confirm the high antioxidant potential of these extracts and their effectiveness as radical neutralizers. Uuh-Narváez *et al.* (2021) reported 100% ABTS$^{\bullet+}$ inhibition in *B. orellana* seed extracts, supporting the notion that both seeds and leaves are valuable antioxidant sources and validating their long-standing use in traditional Mayan gastronomy.

Mentha spicata is widely recognized for its strong antioxidant capacity and ability to scavenge free radicals. Biswas *et al.* (2012) reported up to 76% ABTS$^{\bullet+}$ inhibition in hot water extracts of *M. spicata* leaves using a 1:20 solute-solvent ratio. In line with these findings, the present study confirms that *B. orellana*, *L. origanoides*, and *M. spicata* possess considerable antioxidant potential. Together, the results from both DPPH and ABTS$^{\bullet+}$ assays support their use as promising natural sources of antioxidant compounds. Additional studies will be required to evaluate the most effective dosages in biological systems and the nutritional applications of these extracts.

Antibacterial Activity

The antibacterial activity of the five PE was assessed by measuring the average inhibition zones (mm) against selected bacterial strains (Table 2). Although *B. orellana*, *L. origanoides* and *M. spicata* extracts exhibited some degree of antimicrobial activity, none reached the efficacy levels observed with the positive controls, gentamicin and ampicillin.

In the present study, the highest antimicrobial activity against *L. monocytogenes* was observed with *M. spicata* and *L. origanoides*. Additionally, *L. origanoides* exhibited the strongest effect against *S. enteritidis*, while *B. orellana* performed best against *E. coli*, all at a concentration of 40 mg/mL. These results suggest that these PE partially inhibit *L. monocytogenes*, *S. enteritidis*, and *E. coli* growth.

Table 2. Microbial inhibition zone in mm for *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enteritidis* with hydroalcoholic extracts.

Bacterial strain	C+	C−	SN	*B. orellana*	*C. aconitifolius*	*P. auritum*	*L. origanoides*	*M. spicata*
LM10	17.7 ± 0.7^{Ab}	ND	ND	7.4 ± 0.3^{Cc}	ND	ND	7.57 ± 0.1^{Cc}	6.84 ± 0.2^{Cb}
LM20	17.7 ± 0.7^{Ab}	ND	ND	8.03 ± 0.1^{BCb}	ND	ND	8.12 ± 0.2^{BCb}	7.51 ± 0.1^{Cb}
LM40	17.7 ± 0.7^{Ab}	ND	ND	9.51 ± 0.2^{Ba}	ND	ND	10.2 ± 0.3^{Ba}	10.3 ± 0.6^{Ba}
EC10	21.4 ± 0.6^{Aa}	ND	ND	8.56 ± 1.1^{Cc}	ND	ND	8.26 ± 0.4^{BCb}	7.50 ± 1.2^{Cb}
EC20	21.4 ± 0.6^{Aa}	ND	ND	9.35 ± 1.2^{BCb}	ND	ND	9.09 ± 0.1^{Bb}	8.85 ± 1.1^{BCb}
EC40	21.4 ± 0.6^{Aa}	ND	ND	15.4 ± 1.8^{Ba}	ND	ND	12.6 ± 1.3^{Ba}	12.1 ± 0.1^{Ba}
SE10	19.6 ± 0.5^{Aab}	ND	ND	7.23 ± 0.2^{Cc}	ND	ND	7.48 ± 0.4^{Cb}	6.00 ± 0^{Dc}
SE20	19.6 ± 0.5^{Aab}	ND	ND	8.00 ± 0.2^{Cc}	ND	ND	10.4 ± 1.2^{BCb}	6.00 ± 0^{Dc}
SE40	19.6 ± 0.5^{Aab}	ND	ND	10.1 ± 0.8^{BCb}	ND	ND	12.7 ± 0.4^{Ba}	6.00 ± 0^{Dc}
P value	<0.01	-	-	<0.001	-	-	<0.001	<0.001

LM: *Listeria monocytogenes*; EC: *Escherichia coli*; SE: *Salmonella enteritidis*; 10, 20, 40: indicates mg/mL; C+: 500 μg/mL Penicillin/Gentamicin; C−: distilled water; SN: sodium nitrite (10 mg/mL); ND: not detected; values are expressed as mean ± standard error. Uppercase letters indicate differences between treatments (columns). Lowercase letters indicate differences between doses of the same strain (rows) (Tukey HDS, $p\leq0.05$).

Pinto *et al.* (2013),Click or tap here to enter text.evaluated the antibacterial activity of the *L. origanoides* methanol extract against antibiotic-resistant strains, including *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans* and *C. parapsilosis*, and found that at doses of 200 mg/mL it inhibited *S. aureus*, *C. albicans* and *C. parapsilosis* with a 12-13 and 11.7 and 7.1 mm inhibition zone, respectively. In the present study, inhibition zones ranged from 12.1 to 15.4 mm using 40 mg/mL, suggesting that the ethanol PE inhibited better *E. coli* strain, than the methanol one used in the Pinto *et al.* (2013) work. That results could be likely due to its BC: r-cymene, carvacrol, thymol, and g-terpinene.

Table 3 summarizes the qualitative classification of inhibition. At 40mg/mL, *B. orellana* was active against *E. coli* and partially active against *S. enteritidis*. *L. origanoides* was partially active against both Gram-positive (*L. monocytogenes*) and Gram-negative (*S. enteritidis* and *E. coli*). *M. spicata* was partially active against *E. coli* at 40 mg/mL.

Overall, *L. origanoides* presented the broadest antimicrobial spectrum, inhibiting both Gram-positive and Gram-negative bacteria. The antibacterial activity observed may be associated with the presence of bioactive compounds capable of disrupting bacterial cell membranes, increasing membrane permeability, causing ion leakage, and ultimately leading to cell death, as previously reported (Olvera-Aguirre *et al.*, 2023). These findings are consistent with previous studies. For example, Arumugam *et al.* (2010) reported inhibition zones of 9 and 21 mm against *E. coli* and *Salmonella typhi*, respectively, using ethanolic extracts of *M. spicata*, whereas aqueous fractions showed no antibacterial activity. This highlights the importance of extraction solvent and phytochemical composition in determining antimicrobial efficacy. Although none of the plant extracts at 40 mg/mL reached the inhibition levels of conventional antibiotics, the observed activity, particularly at higher concentration, inhibit moderately *E. coli* and *S. enteritidis*. That supports the idea of these plants used in traditional cuisine as selective natural antimicrobial agent for applications in food preservation.

Table 3. Degree of inhibition for *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enteritidis* growth produced by five hydroalcoholic extracts from plant species collected in the Yucatan Peninsula.

Bacterial strain	C+	C−	SN	*B. orellana*	*C. aconitifolius*	*P. auritum*	*L. origanoides*	*M. spicata*
LM10	+ + +	−	−	+	−	−	+	+
LM20	+ + +	−	−	+	−	−	+	+
LM40	+ + +	−	−	+	−	−	+ +	+
EC10	+ + +	−	−	+	−	−	+	+
EC20	+ + +	−	−	+	−	−	+	+
EC40	+ + +	−	−	+ + +	−	−	+ +	+ +
SE10	+ + +	−	−	+	−	−	+	+
SE20	+ + +	−	−	+	−	−	+ +	+
SE40	+ + +	−	−	+ +	−	−	+ +	+

LM: *Listeria monocytogenes*; EC: *Escherichia coli*; SE: *Salmonella enteritidis*; 10, 20, 40: indicates mg/mL; C+: 500 μg/mL Penicillin/Gentamicin; C−: distilled water; SN: sodium nitrite (10 mg/mL); (−): No inhibition zone (+): <10 mm, "inactive"; (++): 10-13 mm "partially active"; (+ + +): >14 mm "active".

Identification of volatile compounds

The volatile composition of the five plant extracts (Table 4) provides valuable insight into their biological activities and functional applications. Only compounds representing >5% of the total chromatographic area were considered major constituents. The identified compounds correspond mainly to fatty acids, diterpenes, phenolic monoterpenes, and oxygenated sesquiterpenes associated with antioxidant and antimicrobial activity.

Thymol, an isomer of carvacrol, is commonly used to enhance food quality and shelf life with no negative effects on human health (Rathod *et al.*, 2021). It exhibits

Table 4. GC-MS test shows the identification of volatile compounds in ethanolic extracts.

Compound (IUPAC / accepted name)	Common name	% Area (Observed)	Plant species	Reported bioactivity
5-Isopropyl-2-methylphenol	Carvacrol	9.8	*L. origanoides*	Antibacterial, antioxidant
2,6-Di-tert-butyl-4-hydroxyanisole				
Phenol, 2-methyl-5-(1-methylethyl)	—	9.8	*L. origanoides*	Related to carvacrol-like activity
(2E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	Phytol	10.9	*L. origanoides*	Antioxidant, anti-inflammatory
(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	α-Linolenic acid	8.9	*L. origanoides*	Antioxidant (fatty acid)
Hexadecanoic acid	Palmitic acid	8.1	*L. origanoides*	Antioxidant precursor
4,4-Dimethylpent-2-enal	—	7.7	*L. origanoides*	Limited reports
5-Methyl-2-(propan-2-yl)phenol	Thymol	†	*L. origanoides*	Antioxidant, antibacterial
2,6,6-Trimethylcyclohex-1-ene-1-carbaldehyde	β-Cyclocitral	19.0	*M. spicata*	Antioxidant
Ethyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	Ethyl linolenate	14.8	*M. spicata*	Antioxidant derivative
p-Menthane-1,2,3-triol	—	6.8	*M. spicata*	Limited reports
5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	—	5.7	*M. spicata*	Limited reports
Nepetalactone (4aα,7β,7aα)	—	6.4	*M. spicata*	Insect bioactivity
(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	α-Linolenic acid	15.5	*M. spicata*	Antioxidant
Hexadecanoic acid	Palmitic acid	14.4	*M. spicata*	Antioxidant precursor
n-Hexadecanoic acid	Palmitic acid	18.8	*P. auritum*	Antibacterial precursor
(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	α-Linolenic acid	18.6	*P. auritum*	Antioxidant
(2E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	Phytol	14.3	*P. auritum*	Antioxidant
Ethyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	Ethyl linolenate	12.0	*P. auritum*	Antioxidant
Pellitorine	—	†	*P. auritum*	Antibacterial, insecticidal
(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	α-Linolenic acid	40.9	*C. aconitifolius*	Antioxidant
Hexadecanoic acid	Palmitic acid	25.1	*C. aconitifolius*	Antioxidant precursor
(2E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	Phytol	21.1	*C. aconitifolius*	Antioxidant
Ethyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	Ethyl linolenate	10.5	*C. aconitifolius*	Antioxidant
Guaiol acetate	—	29.0	*B. orellana*	Antibacterial, antioxidant
Epiglobulol	—	18.6	*B. orellana*	Antibacterial
(–)-Globulol	—	16.8	*B. orellana*	Antibacterial
Ishwarane	—	†	*B. orellana*	Limited reports
Geranyl-à-terpinene	—	†	*B. orellana*	Limited reports

LO: *Lippia origanoides*; MS: *Mentha spicata*; PA: *Piper auritum*; CA: *Cnidoscolus aconitifolius*; BO: *Bixa orellana*; "−": not founded; †: compound present below 5%.

antioxidant, anti-inflammatory, and antibacterial activities and is mainly found in *Thymus* and *Origanum* species (Escobar *et al.*, 2020; Kachur & Suntres, 2020). Thymol generally shows greater antioxidant potential than carvacrol, attributed to superior hydrogen-donating and singlet and triplet oxygen-quenching capabilities (Rathod *et al.*, 2021).

In this study, carvacrol was among the principal BC identified in *L. origanoides*. This phenolic monoterpene exert antibacterial activity against *E. coli*, *Salmonella*, and *Bacillus cereus* by disrupting and depolarizing the cytoplasmic membrane (Olvera-Aguirre *et al.*, 2023). Carvacrol was one of the strongest compounds in the extract, suggesting its potential application in food preservation.

Traditionally, *M. spicata* and *C. aconitifolius* are used in Mayan cuisine to prepare pastas and herbal teas. Uuh Narváez & Segura Campos (2020) reported compounds such as epicatechin, p-coumaric acid, caffeic acid, and chlorogenic acid in *C. aconitifolius*. Although different compounds were identified in this study, notably α-Linolenic acid, palmitic acid and phytol, compounds with antioxidant and antimicrobial properties (Murugan *et al.*, 2025).

In *M. spicata*, β-Cyclocitral, Ethyl linolenate, and nepetalactone (4aα,7β,7aα) (monoterpene from the Lamiaceae family), were identified. Nepetalactone act as insect repellent and also serve as attractant for cats and aphids (Gomes *et al.*, 2021). The other two compounds have strong bioactivity (Havaux, 2020).

Piper auritum contains BCs, like fatty acids, phytol, ethyl linolenate, and pellitorine, a pungent alkamide from the Piperaceae family. This compound has antibacterial (*Mycobacterium tuberculosis*, *K. aerogenes*) and insecticidal activity and is valued in food and medicine (Khan *et al.*, 2019). And it was founded in a small quantity that is not sufficient to has an effect on the pathogen strains evaluated.

Linolenic acid, found in *P. auritum* and *C. aconitifolius*, can negatively impact oxidative stability and flavor when heated repeatedly at concentrations >4%, resulting in the accumulation of polar oxidation products and a fishy flavor (Frankel, 2012). Phytol is a common acyclic diterpene found in all five plant species analyzed, and exhibits antibacterial, antioxidant and anti-inflammatory activity (Eksi *et al.*, 2020).

A saturated fatty acid (n-hexadecanoic acid) was detected in all plants except *B. orellana*. This compound function as natural antioxidant and preservative in food (Gómez-Sequeda *et al.*, 2020). Additionally inhibits phospholipase A2 and acts as an anti-inflammatory, antibacterial, and antifungal agent (Aparna *et al.*, 2012).

The last plant analyzed was *B. orellana*, which is known for its antibacterial activity because of the BC in its leaves (alkaloids, triterpenes, steroids, tannins, saponins, flavonoids, phenols, proteins, glycosides and sugars, fats, and oils) with known antibacterial activity. It holds cultural and culinary importance in Mayan traditions (Zarza-García *et al.*, 2021), primarily for its flavoring and coloring properties.

Sesquiterpenes identified in *B. orellana* include guaiol acetate, (−)-globulol, and epiglobulol. Globulol, the main antibacterial in *Eucalyptus globulus*, is effective against *Xanthomonas vesicatoria* and *Bacillus subtilis*. Epiglobulol has been found in *Ficus hispida*, *Myrcia falax*, and *Acmella oleracea* (Fauzi *et al.*, 2021), and shows antibacterial,

antifungal, and antioxidant activity. Ishwarane, a rare sesquiterpene found in this study, has also been identified in *Aristolochia indica* roots (Dos S. Junior *et al*., 2013).

The BCs identified across the five species are primarily antioxidant and moderate antibacterial in nature, supporting their traditional use in food preservation and flavor enhancement. Characterizing and standardizing these plant extracts is essential to determine optimal dosages for applications in food and other industries.

Overall, fatty acids and phytol were common components across most species, whereas phenolic monoterpenes were distinctive in *L. origanoides* and sesquiterpenes in *B. orellana*. These compositional differences may explain variations in biological activity and support the potential application of these extracts as natural additives in agro-food systems. Further studies under practical conditions are recommended.

CONCLUSIONS

The extracts with the highest antioxidant and moderate antibacterial effects at 1 and 40 mg/mL, respectively, are *B. orellana*, *L. origanoides*, and *M. spicata*. GC-MS analyses tentatively identified the presence of bioactive compounds such as phytol, β-Cyclocitral, linolenic, and palmitic acid, carvacrol, thymol, guaiol acetate, epiglobulol and ishwarene. Results suggest that these three plant extracts represent promising natural alternatives for application in agro-food systems, specifically as potential antioxidant and selective antimicrobial agents.

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