Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
https://doi.org/10.4025/actascibiolsci.v47i1.74960

Chemical composition and antimicrobial activity of three *Pimenta racemosa* var. *ozua* (Myrtaceae) extracts from the Dominican Republic

Tania Isabel Muñoz-Martínez[1], Lolymar de los Ángeles Romero[2], Luis Orlando Maroto-Martín[2] and Yameiri Mena[3]

[1]Laboratorio de Cultivo In Vitro, Área de Ciencias Básicas y Ambientales, Instituto Tecnológico de Santo Domingo, Av. de Los Próceres 49, Santo Domingo 10602, Distrito Nacional, República Dominicana. [2]Área de Ciencias Básicas y Ambientales, Instituto Tecnológico de Santo Domingo, Distrito Nacional, República Dominicana. [3]Laboratorio de Investigación, Área de Microbiología, Instituto Especializado de Estudios Superiores Loyola, San Cristóbal, República Dominicana. *Author for correspondence. E-mail: taniai.munozmartinez@gmail.com

ABSTRACT. *Ozua* is an endemic flowering plant from the island of Hispaniola belonging to the Myrtaceae family. Many Caribbean species within this family remain phytochemically unexplored, including *Pimenta racemosa* var. *ozua* Urb. & Ekman. While some studies have investigated the anti-inflammatory, antinociceptive, and anti-ulcerogenic properties of *ozua*, the antimicrobial potential remains unknown. In this investigation, the essential oil (EO), ethanolic extract (EE), and petroleum ether extract (PEE) of *Pimenta racemosa* var. *ozua* were analyzed to determine their chemical composition and antimicrobial activity against ten microorganisms of clinical interest. The extracts were obtained via steam distillation and Soxhlet extraction. The main secondary metabolites were quantified by spectrophotometric assays, and phytochemical profiles were established by gas chromatography-mass spectrometry (GC-MS). *In vitro* antimicrobial assays were performed using agar-well diffusion, and multi-well dilution assays to determine the minimum inhibitory concentrations (MICs). The profiles obtained showed an abundance of terpenoids, and terpenes in the EO, while tocopherols and phytosteroids predominated in the Soxhlet extracts. EO exhibited significant antibacterial activity, EE showed antimicrobial activity, and PEE demonstrated limited activity against *Pseudomonas aeruginosa*.

Keywords: Myrtaceae; methyleugenol; natural products; essential oils; bioactivity.

Received on April 25, 2025
Accepted on June 24, 2025

Introduction

The *Pimenta* genus, a significant group within the Myrtaceae family, comprises 15 species to the Caribbean and holds considerable economic and ethnopharmacological value. Various *Pimenta* species are extensively used in traditional medicine for the treatment of ailments such as colds, viral infections, bronchitis, dental and muscle pain, rheumatism, and arthritis (Al-Gendy et al., 2017; Ismail et al., 2020; Youssef et al., 2021). Among these, *Pimenta racemosa var. ozua* Urb. & Ekman, an endemic variety to Hispaniola Island, has great bioactive potential yet remains largely understudied.

Most chemical analyses to date have focused on *P. racemosa* var. *racemosa*, profiling major compounds in essential oil such as 1,8-cineole, eugenol, α-terpineol, and limonene (Abaul et al., 1995; Ayedoun et al., 1996; Bello et al., 1995; Contreras-Moreno et al., 2014; Tucker et al., 1991). However, there is a lack of comprehensive phytochemical data specific to var. *ozua*, with prior studies reporting its anti-inflammatory and antinociceptive potential through methanolic and aqueous leaf extracts (Fernández et al., 2001; García et al., 2004). Additionally, the few available reports present discrepancies among studies and often lack proper botanical validation, including herbarium voucher specimens. The absence of verifiable plant material complicates result reproducibility and hinders inter-study comparisons, particularly concerning seasonal or geographic variation in chemical composition.

To address these gaps, this study aims to investigate the chemical composition and antimicrobial properties of essential oil (EO), ethanolic extract (EE), and petroleum ether extract (PEE) derived from the dried leaves of *P. racemosa var. ozua*. Using gas chromatography-mass spectrometry (GC-MS) and *in vitro* antimicrobial assays, we seek to evaluate their efficacy against clinically significant bacterial and fungal strains. We hypothesize that the phytochemical composition of the essential oil (EO), ethanolic extract (EE),

and petroleum ether extract (PEE) contain bioactive secondary metabolites with significant antimicrobial activity, varying depending on the extraction solvent used.

Materials and methods

Botanical material

Pimenta racemosa var. *ozua* leaves were collected in September 2022 from a mixed subtropical humid pine forest in Santiago Rodríguez, Dominican Republic, (19°21'25.7"N 71°15'50.7"W) during the fruiting stage. This phase is associated with increased biosynthesis and accumulation of secondary metabolites; therefore, it would allow bioactive profiling. Voucher specimen (Piña 296 | JBSD 134342) was deposited in the JBSD Herbarium of the Rafael M. Moscoso National Botanical Garden.

Extraction methods

Leaves were dried at 60°C for 72 hours, and gravimetric moisture content (MC) was measured. One hundred twenty g of the pulverized dried leaves were subjected to hydrodistillation for 4 hours using a Clevenger modified apparatus to obtain the essential oil (EO). Three g of the pulverized dried leaves were subjected to a Soxhlet-type apparatus for four h, with ethanol 90% (v v^{-1}), and pure petroleum ether as respective solvents to obtain the ethanolic extract (EE) and the petroleum ether extract (PEE). Both extracts were concentrated using a Scilogex RE100 rotary evaporator at 45–50°C until the solvents were eliminated. The extracts obtained were stored in amber vials at 4°C until analysis. The extractions were carried out in quintuplicate. For each method, the total yield was defined as grams of volume extracted per gram of the pulverized leaf material into the extraction apparatus (w w^{-1}) (Elyemni et al., 2019).

Chemical composition

To determine chlorophyll *a*, chlorophyll *b*, and total carotenoids in the leaf samples (in triplicate), the protocol established by Lichtenhaler and Welbur (1983) was followed. The determination of total phenolic content (TPC), the Folin-Ciocalteu method was used, as described by Singleton et al. (1999). A 10% Folin solution was prepared with 10 mL of the Folin reagent, 90 mL of distilled water, and a 700 mM gallic acid solution to serve as a calibration curve with concentrations between 20 and 100 µg mL^{-1} (2-10%). Each quantification per extract was performed in four replicates. The aluminum chloride colorimetric method was used to determine total flavonoid content (TFC), as described by López-Hidalgo et al. (2021). A calibration curve was created using quercetin (⩾ 95%, Sigma-Aldrich) with concentrations ranging from 2 and 10% (20-100 µg mL^{-1}). The method was used to determine TFC in the EO, as described by Tohidi et al. (2017). Each quantification per extract was performed in triplicate.

Phytochemical profiles

GM-MS were performed on the three *ozua* extracts using a gas chromatograph coupled to a mass spectrometer (Clarus SQ 8Q Perkin Elmer, Waltham, MA) with a TurboMatrix HS-40 sampler from the same manufacturer equipped with a full scan mass spectrometer (MS). Hydrogen was used as a carrier gas, with a constant flow of 1.5 mL min^{-1}. 1 µL of the samples diluted to 10% v v^{-1} in hexane was injected in Split mode, into the Elite 5M5 column (Perkin Elmer) with a thickness of 1.0 µm, inner diameter 0.25 mm, length 60 m and 14-bis (dimethylsiloxy) phenylene dimethylpolysiloxane phase. The oven program had an initial temperature of 90°C and was developed with an increment of 3°C min^{-1} until reaching a final temperature of 320°C. The run was 75 min, and the injector temperature was 250°C, which was constant throughout the process. For GC MS^{-1} detection, an electron ionization system was used, with an ionization energy of 70 eV. The mass scanning range was 50–550 m z^{-1}, while the injector and MS transfer line temperatures were set at 300, and 320°C, respectively. The extract components were identified by comparing their mass spectra and retention times with those reported in the literature (Contreras-Moreno, 2018). The relative proportion of the constituents was expressed as percentages obtained by peak area normalization; all relative response factors being taken as one.

Antimicrobial activity

Standard bacterial lines *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13883),

Enterobacter cloacae (ATCC 13047), *Salmonella enterica* (ATCC 14028) and ceftazidime/cefotaxime-resistant *K. pneumoniae* (ATCC 700603) were acquired from the Clinical Laboratory Reference (Santo Domingo-Dominican Republic), and cephalosporine-resistant *P. aeruginosa* from the Clinical Laboratory of *Instituto Tecnológico de Santo Domingo*. *Fusarium oxyporum* isolate was obtained from Dominican Institute of Agricultural and Forestry Research (IDIAF).

Antimicrobial assay of extracts was performed by agar-well diffusion method in Mueller Hinton Agar (MHA) plates and Sabouraud Dextrose Agar (SDA), as described by Manandhar et al. (2019). Test organisms were inoculated in Nutrient broth and incubated overnight at 37ºC to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5 × 108 CFU mL^{-1}. MHA/SDA inoculated plates were perforated with a sterile six-piece cork-borer (6 mm) and each well was filled with 25 µL of each extract at 20, 40, 60, 80, 90, and 100% (v v^{-1}) concentrations. As positive controls, ciprofloxacin (2 mg mL^{-1}) was used for the bacteria, and fluconazole (2 mg mL^{-1}) for the fungi.

The minimum inhibitory concentrations (MIC) were determined by broth microdilution method in a sterile 96-well microplate, as described by Manandhar et al. (2019). Two-fold serial dilutions of each extract were prepared directly in the wells by adding 30 µL of the extract and 85 µL of Mueller Hinton Broth (MHB). After the addition of a bacterial suspension grown overnight (85 µL) to each well as an inoculum, the plates were incubated at 37°C for 72 hours. Absorbance was measured at 400, 420, and 630 nm every 24 hours to monitor microbial growth (National Committee for Clinical Laboratory Standards [NCCLS], 2004). MIC was defined as the lowest concentration of the extract that completely inhibited visible bacterial growth. Each concentration was done in quintuplicate. Ciprofloxacin (30 µg mL^{-1}) was used as a positive control, while dimethyl sulfoxide (DMSO), ethanol 90%, and petroleum ether were used as negative controls.

Results and discussion

Moisture content and yield

Pimenta racemosa var. *ozua* leaves underwent noticeable odor, texture, and color changes throughout the drying process. The gravimetric moisture content of the fresh *ozua* leaves ranged from 37.9 to 47.6%, with similarities reported by Mujaffar and Bynoe (2020) (45.9%), despite differences in drying times. The EO extracted presented a yield of 1.19% in relation to the oven-dried leaves (Table 1). The results present similarities (1.71%) for the EO obtained from leaves of *P. racemosa* from Egypt (Youssef et al., 2021) and of *P. racemosa* var. *racemosa* (1.6–1.8%) Guadeloupe (Abaul et al., 1995). These similarities may reflect an ecotypic convergence, in response to parallel selective pressures in the environment, or comparable pedoclimatic conditions, such as UV exposure, altitude, climate, and nutrient availability (Abdelmohsen & Elmaidomy, 2025; Vaneková et al., 2020). Similar microclimate and nutritional uptake might have resulted in comparable genetic pathways for essential oil biosynthesis.

The EE presented a yield of 32.7% in relation to the oven-dried leaves and had the highest output, while the PEE presented a yield of 28.7%. This may be attributed to the continuous extraction mechanism inherent to the Soxhlet techniques. The results are consistent with those Sánchez-Zarate et al. (2020) reported for ethanolic extracts of the Mexican *P. dioica* (32.3%).

Table 1. Extraction yield of foliar extracts of *Pimenta racemosa* var. *ozua*.

Extraction method	Yield % (w w^{-1})	Density (mg mL^{-1})
Hydrodistillation (EO)	1.19	37.89^{a}
Soxhlet-ethanol 90% (EE)	32.71	659.07^{b}
Soxhlet-petroleum ether (PEE)	28.74	620.40^{c}

F: ANOVA < t: 23.67, p < 0.001. LSD: 0.4591. Different letters indicate statistical difference by LSD.

Chemical composition

The concentration of chlorophylls on the *ozua* leaves (1.68 - 3.87 mg g^{-1}) found in the investigation (Table 2) presents slight variations with the work of Mujaffar and Bynoe (2019) for chlorophyll *a* (4.21 mg g^{-1}) and for chlorophyll *b* (1.6 mg g^{-1}) in West Indian Bay leaves.

TPC and TFC of each *ozua* extract were determined, as shown in Table 3. Based on reported ranges during the fruiting stage (Zhang et al., 2023), the TPC obtained from EO, and EE is considered high (> 70 mg GAE g^{-1}), and

PEE is moderate (20 - 70 mg GAE g^{-1}). While TPC for EO presents higher values than those reported by John and Maharaj (2024) (8.1 mg GAE g^{-1}), for a *P. racemosa* of Trinidad and Tobago, they were significantly lower when compared to those observed in *P. dioica* (270-525 mg GAE g^{-1}) from Mexico (Milenković et al., 2020) and India (Sarathambal et al., 2021). These differences could be the result of ecotype and/or chemotype speciation in response distinct environmental factors known to influence the content and composition of polyphenols in plants (Vaneková et al., 2020). The highest value of TPC was observed in EE, which were inferior when compared to the values obtained by Sánchez-Zarate et al. (2020) for *P. dioica* (516.35 ± 4.96 mg GAE g^{-1}). Lower phenolics in PEE is consistent with previous studies (Abdel-Aleem et al., 2019), albeit of different species, due to the limited affinity of polyphenols for the solvent.

Table 2. Mean content (± standard deviation) of photosynthetic pigments quantified from fresh weight (FW) of the plant leaves in acetone extract for *Pimenta racemosa* var. *ozua*.

Photosynthetic pigments	Concentration (mg g^{-1} FW)
Chlorophyll *a*	3.87 ± 0.01
Chlorophyll *b*	1.68 ± 0.03
Total Chlorophyl *a*/*b*	5.55 ± 0.86
Carotenoids	11.19 ± 0.03

Table 3. Total phenol and flavonoid content of essential oil, ethanolic extract, and petroleum ether extract obtained from dried *Pimenta racemosa* var. *ozua* leaves.

Extract	TPC (mg GAEg^{-1})	TFC (mg QERg^{-1})
EO	77.48 ± 0.06[a]	36.20 ± 0.03[b]
EE	381.47 ± 0.1[a]	56.78± 0.23[a]
PEE	37.87 ± 0.01[b]	14.88 ± 0.01[c]

ANOVA (TPC): F: 10.3, p = 0.0115. Different letters indicate statistical difference by LSD. Kruskal-Wallis (TFC): χ^2: 7.4483, df: 2, p = 0.02413. Dunn's test (Bonferroni): EE vs EO (p: 0.0095). Different letters indicate statistical differences based on Dunn's test.

The TFC values observed in the study differed from previously reported data, with EO showing significantly higher levels compared to *P. racemosa* (2.44 mg QERg^{-1}) (John & Maharaj, 2024). Polyphenolic quantification in several *Pimenta* species have shown minor inconsistencies across studies (Dharmadasa et al., 2015; Lowe et al., 2017; Soysa et al., 2016), even when the same plant part was analyzed under comparable environmental conditions. These discrepancies may be attributed to lower light availability, that dampens flavonoid production (Schweiger & Bernhardt, 2024), or the presence of fungal microorganism, that stimulates phenylpropanoid/flavonoid production as defense system (Jan et al., 2021). The quantification of flavonoids for EE in the present work identified it as the extract with the highest TFC concentration (56.78 mg QERg^{-1}), with values similar to those reported by Santos et al. (2018) for American Myrtaceae (45.2 ± 6.9 mg QERg^{-1}). The low TFC values present in PEE align with previous findings on petroleum ether extracts of Myrtaceae species (Monteiro Cavalcante et al., 2021), likely due to solvent polarity.

Phytochemical profile

The GC-MS analysis revealed notable compositional differences among the extracts, with 32 components identified: 28 in EO, 20 in EE, and 20 in PEE, as shown in Table 4. In the composition of the EO, terpenoids (84.3%), and terpenes (35.52%) predominated, while phytosteroids were most abundant in the EE, representing 62.94%. A qualitative diversity was observed in the chemical profile of the PEE, with phytosteroids (45.92%), terpenoids (20.8%), saturated fatty acids (14.41%), and aliphatic ketones (10.52%) as the main chemical families present.

Analysis of the composition of the EO from dried leaves of *P. racemosa* var. *ozua* identified 11 components previously detected by Tucker et al. (1991). The majority of components, being monoterpene hydrocarbons, are consistent with other studies done with the genus *Pimenta* (Contreras-Moreno, 2018). Limonene, and methyl eugenol have been described as having antioxidant potential, anti-inflammatory effects, and gastroprotective potential (Alencar Silva et al., 2024; Kumar Joshi et al., 2019). The combined effect of the reduction of pro-inflammatory cytokines and the restoration of oxidative stress biomarkers, caused by the compositional diversity of terpenes and terpenoids found in the EO, could explain its topical application for the treatment of rheumatism, swellings, and muscle aches in the Dominican Republic.

Table 4. Chemical composition of leaf essential oil, ethanolic extract, and petroleum ether extract of *Pimenta racemosa* var. *ozua* leaves.

Chemical compounds	EO	EE	PEE	RT
β-Myrcene	0.08	-	-	4.37
o-Cymene	0.20	0.08	0.18	4.95
D-Limonene	16.11	1.41	2.49	5.01
Caranol	1.71	0.08	12.64	5.07
γ-Terpinene	17.22	-	0.02	5.5
Terpinolene	-	0.09	0.20	5.99
Linalool	3.73	0.04	0.04	6.17
Fenchol	0.03	-	-	6.4
Verbenol	< 0.01	0.04	-	6.77
trans-Isopinocarveol	1.24	-	-	6.84
Isopinocarveol	2.09	0.10	0.37	6.87
Pinocarvone	1.08	-	-	7.25
L-α-Terpineol	3.97	0.09	0.41	7.33
Terpinen-4-ol	8.48	-	0.58	7.52
3-Decanone	3.21	2.13	11.21	7.67
cis-p-mentha-1(7),8-dien-2-ol	< 0.01	10.30	0.55	8.36
D-Verbenone	0.19	-	-	8.39
D-Carvone	0.27	0.19	0.39	8.65
Neral	0.19	-	-	9.05
Thymol	8.15	-	-	9.6
Methyleugenol	27.80	-	-	11.21
Ascaridole	0.05	0.25	0.07	11.22
Caryophyllene	4.53	0.25	0.15	11.49
6-Hydroxyeugenol	-	< 0.01	-	13.2
Longipinocarvone	0.07	-	-	13.84
Humulenol-II	0.26	0.19	0.17	13.96
n-Hexadecanoic acid	0.21	6.36	51.29	18.66
Squalene	< 0.01	0.24	0.99	27.44
Vitamin E	0.16	14.84	6.36	29.81
γ-Tocotrienol	-	0.39		30.01
Stigmasterol	-	0.42	0.07	31.01
γ-Sitosterol	-	62.51	11.80	31.47

RT: Retention time (min); Content %: Equivalent to the amount of substance.

Analysis of the composition of the EE from *ozua* was characterized by being the only one with γ-tocotrienol and verbenol present. PEE presented the most diverse chemical composition among the extracts obtained from the *ozua* leaves, encompassing the second largest concentration of phytosteroids (45.91%), and the greatest of saturated fatty acids (14.41%), and aliphatic ketones (10.52%).

Antimicrobial activity

The antimicrobial activity of EO, EE, and PEE showed variation across the different microbial strains. The EO exhibited the broadest spectrum of activity among the extracts, inhibiting growth in seven of the ten microorganisms tested (Table 5). EO exhibited inhibition zones ranging from 9.57 ± 0.2 mm to 33.13 ± 0.58 mm, with the strongest activity against *E. faecalis* and the weakest against *S. enterica*. This activity is probably due to its high concentration of terpenoids, such as methyleugenol, longipinocarvone, and humulenol-II, which disrupts cellular membrane integrity. Antimicrobial activity has been attributed to polar-substituted aromatic systems, such as terpenoids, which trigger morphological alterations in the membrane. This results in the release of vital intracellular constituents, and the inhibition of target enzymes (Guimarães et al., 2019; Kachur & Suntres, 2020). Furthermore, hydrogel groups present readily form hydrogen bonds with enzyme active sites due to their high reactivity, leading to enzymatic inactivation and causes membrane destabilization or lysis.

The EE demonstrated greater microbial efficacy among the extracts, creating inhibition zones in the drug-resistant bacteria and the fungal strain (Table 6). EE showed inhibition zones between 4.97 ± 0.34 mm and 33.30 ± 0.22 mm, with significant activity against *S. aureus*, *S. enterica*, and *F. oxysporum*. This property is attributed to phytosterols such as stigmasterol, and γ-sitosterol, which can interfere with surface proteins and bacterial membrane composition (Bakrim et al., 2022). Similar findings were reported by Lowe et al. (2017), where the ethanolic extract of *P. racemosa jamaiquina* inhibited *S. aureus*, and *E. faecalis*. Furthermore, Barros Gomes et al. (2020) observed inhibition of *F. oxyporum* by *P. dioica*, aligning with the fungal susceptibility observed in this study.

The PEE displayed minimal antimicrobial activity, producing inhibition zones only in *P. aeruginosa* (Table 7). There is no record of antimicrobial evaluations of *P. racemosa* varieties with ether extracts. In

comparison with other Myrtaceae species, *P. aeruginosa* has shown susceptibility to petroleum ether extracts of *Eucalyptus grandis* (Kwansa-Bentum et al., 2023) and *Eucalyptus camaldulensis* (Ishag et al., 2018). The ineffectiveness of PEE as a general antimicrobial agent may be attributed to the lipophilic nature of its chemical composition. Despite chemical diversity, the lack of structural specificity or potency of the groups presents, and/or the absence of polar compounds may have resulted in a membrane interaction without lethal disruption or selective activity (Rayan et al., 2020).

Table 5. Mean ± standard deviation referring to the inhibition halo form (mm) in ten microorganisms submitted to six concentrations of essential oil obtained from *Pimenta racemosa* var. *ozua* leaves.

Micro-organism	Concentration of Essential Oil (EO) (μL mL^{-1})						Control
	318.97	287.07	255.18	191.38	127.59	63.79	
E. coli	15.27 ± 0.21	14.43 ± 0.17	11.27 ± 0.21	9.73 ± 0.21	8.77 ± 0.21	8.27 ± 0.21	49.5 ± 0.23
E. faecalis	33.13 ± 0.58	32.43 ± 0.09	30.27 ± 0.21	22.33 ± 0.12	15.70 ± 0.24	15.00 ± 0.16	30 ± 0.2
S. aureus	12.07 ± 0.21	11.23 ± 0.19	8.07 ± 0.23	6.53 ± 0.2	5.57 ± 0.21	5.07 ± 0.18	33 ± 0.26
F. oxyporum	0	0	0	0	0	0	8 ± 0.23
K. pneumoniae	14.07 ± 0.17	13.23 ± 0.21	10.07 ± 0.19	8.53 ± 0.08	7.57 ± 0.2	7.07 ± 0.25	27.98±0.8
E. cloacae	14.57 ± 0.29	13.73 ± 0.3	10.57 ± 0.24	9.03 ± 0.31	8.07 ± 0.21	7.57 ± 0.2	22.83±0.87
S. enterica	9.57 ± 0.2	8.73 ± 0.19	5.57 ± 0.16	4.03 ± 0.21	3.07 ± 0.18	2.57 ± 0.21	27.91±0.81
P. aeruginosa	0	0	0	0	0	0	23 ± 0.31
Cephalosporine-resistant *P. aeruginosa*	0	0	0	0	0	0	14.5 ± 0.3
Ceftazidime/cefotaxime-resistant *K. pneumoniae*	10.57 ± 0.88	9.73 ± 0.84	6.57 ± 0.71	5.03 ± 0.75	4.07 ± 0.81	3.57 ± 0.78	15.5 ± 0.11

χ^2: 16.90, df: 5, p = 0.0047. Dunn's test (Bonferroni): Significant differences observed between specific concentration groups (p < 0.05). χ^2: 156.74, df: 9, p < 0.0001. Dunn's test (Bonferroni): Significant differences observed between specific microorganism groups (p < 0.05).

Table 6. Mean ± standard deviation referring to the inhibition halo form (mm) in ten microorganisms submitted to six concentrations of ethanolic extract obtained from *Pimenta racemosa* var. *ozúa* leaves.

Micro-organisms	Concentration of Ethanolic Extract (EE) (mg mL^{-1})						Control
	1064.16	957.74	851.33	638.49	425.66	212.83	
E. coli	9.67 ± 0.21	8.75 ± 0.25	0	0	0	0	49.5 ± 0.23
E. faecalis	0	0	0	0	0	0	30 ± 0.2
S. aureus	33.30 ± 0.22	32.27 ± 0.21	30.33 ± 0.19	22.40 ± 0.14	16.17 ± 0.2	15.23 ± 0.17	33 ± 0.26
F. oxyporum	11.43 ± 0.09	11.33 ± 0.12	11.07 ± 0.09	10.40 ± 0.18	8.77 ± 0.07	0 ± 0.17	8 ± 0.23
K. pneumoniae	8.47 ± 0.54	7.55 ± 0.42	0	0	0	0	27.98±0.8
E. cloacae	8.97 ± 0.31	8.05 ± 0.32	0	0	0	0	22.83±0.87
S. enterica	30.80 ± 0.11	29.77 ± 0.19	27.83 ± 0.24	19.90 ± 0.17	13.67 ± 0.12	12.73 ± 0.22	27.91±0.81
P. aeruginosa	25.33 ± 0.12	23.33 ± 0.32	20.83 ± 0.09	17.83 ± 0.25	17.33 ± 0.22	14.33 ± 0.13	23 ± 0.31
Cephalosporine-resistant *P. aeruginosa*	17.83 ± 0.27	15.83 ± 0.13	13.33 ± 0.2	10.33 ± 0.18	9.83 ± 0.15	6.83 ± 0.26	14.5 ± 0.3
Ceftazidime/cefotaxime-resistant *K. pneumoniae*	4.97 ± 0.34	4.05 ± 0.16	0	0	0	0	15.5 ± 0.11

χ^2 = 23.37, df = 5, p = 0.0003. Dunn's test (Bonferroni): Concentration groups showed significant differences (P < 0.05). χ^2 = 156.24, df = 9, p < 0.0001. Dunn's test (Bonferroni): Significant differences observed between specific microorganism groups (p < 0.05).

Table 7. Mean ± standard deviation referring to the inhibition halo form (mm) in ten microorganisms submitted to six concentrations of petroleum ether extracts obtained from *Pimenta racemosa* var. *ozúa* leaves.

Micro-organism	Concentration of PEE (mg mL^{-1})						Control
	336.75	303.07	269.40	202.05	134.70	67.35	
E. coli	0	0	0	0	0	0	49.5 ± 0.23
E. faecalis	0	0	0	0	0	0	30 ± 0.2
S. aureus	0	0	0	0	0	0	33 ± 0.26
F. oxyporum	0	0	0	0	0	0	8 ± 0.23
K. pneumoniae	0	0	0	0	0	0	27.98±0.8
E. cloacae	0	0	0	0	0	0	22.83±0.87
S. enterica	0	0	0	0	0	0	27.91±0.81
P. aeruginosa	26.33 ± 0.12	23.33 ± 0.13	18.83 ± 0.14	22.13 ± 0.11	20.83 ± 0.12	14.33 ± 0.13	23 ± 0.31
Cephalosporine-resistant *P. aeruginosa*	0	0	0	0	0	0	14.5 ± 0.3
Ceftazidime/cefotaxime-resistant *K. pneumoniae*	0	0	0	0	0	0	15.5 ± 0.11

χ^2: 0.58, df: 5, p = 0.9981. Dunn's test (Bonferroni): No significant differences were observed between specific concentration groups (p < 0.05). χ^2: 363.74, df: 9, p < 0.0001. Dunn's test (Bonferroni): Significant differences observed between specific microorganism groups (p < 0.05).

The MIC values further confirmed the trends, with EO showing a range from 19.14 µL mL^{-1} to 143.66 µL mL^{-1}, exhibiting maximum inhibition against *E. faecalis* and *E. cloacae* (Table 8). Microbial susceptibility to EO can be caused by large quantities of D-limonene, terpinen-4-ol, and L-α-terpineol that have been shown to induce morphological changes in *E. coli* and *S. aureus*, causing destruction of the bacterial membrane. The inefficiency against *P. aeruginosa* was also reported by Al-Gendy et al. (2017). EE had MIC values from 31.92 mg mL^{-1} to 159.62 mg mL^{-1}, with the most significant effect on *S. aureus* and *K. pneumoniae*. PEE showed limited antibacterial activity, with a MIC value of 20.20 mg mL^{-1} only against *P. aeruginosa*. None of the extracts inhibited cephalosporin-resistant *P. aeruginosa*. The high concentration of phytosterols and tocopherols in the Soxhlet extracts can explain the antibacterial activity on *P. aeruginosa*, and the variable response to the other microorganisms.

Table 8. Minimum inhibitory concentration (MIC) of the three extracts obtained from *Pimenta racemosa* var. *ozua* leaves against nine species of bacteria.

Micro-organism	*Ozua* leaves extracts			IT
	EO µL mL^{-1}	EE mg mL^{-1}	PEE mg mL^{-1}	
E. coli	38.28	63.85	-	48
E. faecalis	19.14	143.66	-	48
S. aureus	38.28	31.92	-	48
K. pneumoniae	38.28	31.92	-	48
E. cloacae	38.28	95.77	-	72
S. enterica	43.06	63.85	-	48
P. aeruginosa	-	63.85	20.20	48
Cephalosporine-resistant *P. aeruginosa*	-	-	-	72
Ceftazidime/cefotaxime-resistant *K. pneumoniae*	47.84	159.62	-	72

-: Abundant bacterial growth (> 80%); IT: Incubation time in hours.

Conclusion

The results provide the first phytochemical and antimicrobial characterization of *Pimenta racemosa* var. *ozua* in the Dominican Republic, highlighting its unexplored pharmacological potential. The essential oil exhibited strong antibacterial activity, likely due to its high terpenoid content and membrane-disrupting properties. The ethanolic extract, rich in phytosteroids such as γ-sitosterol and vitamin E, was notably effective against drug-resistant bacterial strains and fungal pathogen. Although the petroleum ether extract demonstrated limited activity, restrictive to *Pseudomonas aeruginosa*, its chemically diverse profile warrants further investigation. The study supports the inclusion of this endemic variety in future research on plant-derived antimicrobials and bioactive natural products.

Acknowledgments

The authors are grateful to General Directorate of Customs (DGA), Santo Domingo, Dominican Republic for providing access to the gas chromatography–mass spectrometry (GC–MS) and Willy M. Maurer (Generative Research Coordinator & Researcher) at the Loyola Specialized Institute of Higher Education, San Cristóbal, Dominican Republic (IEESL) for generously providing access to the ELISA microplate reader used in this study.

References

Abaul, J., Bourgeois, P., & Bessiere, J. M. (1995). Chemical composition of the essential oils of chemotypes of *Pimenta racemosa* var. *racemosa* (P. Miller) J. W. Moore (Bois d'Inde) of Guadeloupe (F.W.I.). *Flavour and Fragrance Journal*, *10*(5), 319–321. https://doi.org/10.1002/FFJ.2730100506

Abdel-Aleem, E. R., Attia, E. Z., Farag, F. F., Samy, M. N., & Desoukey, S. Y. (2019). Total phenolic and flavonoid contents and antioxidant, anti-inflammatory, analgesic, antipyretic and antidiabetic activities of *Cordia myxa* L. leaves. *Clinical Phytoscience*, *5*(1). https://doi.org/10.1186/s40816-019-0125-z

Abdelmohsen, U. R., & Elmaidomy, A. H. (2025). Exploring the therapeutic potential of essential oils: A review of composition and influencing factors. *Frontiers in Natural Products: Review Collection*, *4*. https://doi.org/10.3389/fntpr.2025.1490511

Alencar Silva, A., Morais, L. P., Sena Bastos, C. M., Menezes Dantas, D., Batista, P. R., Dias, F. J., & Barbosa, R. (2024). Vasorelaxant effect of phenylpropanoids: Methyl eugenol and eugenol in human umbilical cord vein. *Biomedicine & Pharmacotherapy*, *178*. https://doi.org/10.1016/J.BIOPHA.2024.117227

Al-Gendy, A. A., Moharram, F. A., & Zarka, M. A. (2017). Chemical composition, antioxidant, cytotoxic and antimicrobial activities of *Pimenta racemosa* (Mill.) J.W. Moore flower essential oil. *Journal of Pharmacognosy and Phytochemistry*, 6(2), 312–319. https://www.phytojournal.com/archives/2017/vol6issue2/PartF/6-2-49-620.pdf

Ayedoun, A. M., Adeoti, B. S., Setondji, J., Menut, C., Lamaty, G., & Bessiére, J.-M. (1996). Aromatic plants from tropical West Africa. IV. Chemical composition of leaf oil of *Pimenta racemosa* (Miller) J. W. Moore var. *Racemosa* from Benin. *Journal of Essential Oil Research*, *8*(2), 207–209. https://doi.org/10.1080/10412905.1996.9700597

Bakrim, S., Benkhaira, N., Bourais, I., Benali, T., Lee, L. H., El Omari, N., Sheikh, R. A., Goh, K. W., Ming, L. C., & Bouyahya, A. (2022). Health benefits and pharmacological properties of stigmasterol. *Antioxidants*, 11(10). https://doi.org/10.3390/ANTIOX11101912

Barros Gomes, P. R., Silva Barros Junior, F. R., Batista Reis, J., Oliveira Everton, G., Santos de Oliveira, R. W., Costa Louzeiro, H., Fontenele, M. A., Freitas, A. C., Paula, M. L., & Mouchrek Filho, V. E. (2020). Chemical composition and biological activity of the essential oil of the fruits *Pimenta dioica* against formae speciales of fungus *Fusarium oxysporum*. *Revista Colombiana de Ciencias Químico-Farmacéuticas*, 49(1), 89–100. https://doi.org/10.15446/rcciquifa.v49n1.87010

Bello, A., Rodriguez, M. L., Castiñeira, N., Urquiola, A., Rosado, A., & Pino, J. A. (1995). Chemical composition of the leaf oil of *Pimenta racemosa* (Mill.) J. Moore from Western Cuba. *Journal of Essential Oil Research*, *7*(4), 423–424. https://doi.org/10.1080/10412905.1995.9698553

Contreras-Moreno, B. Z. (2018). Chemical composition of essential oil of genus *Pimenta* (Myrtaceae): Review. In H. A. El-Shemy (Ed.), *Potential of essential oils,* (pp. 1–25). IntechOpen. https://doi.org/10.5772/intechopen.78004

Contreras-Moreno, B., Rojas, J., Celis, M., Rojas, L., Méndez, L., & Landrum, L. (2014). Componentes volátiles de las hojas de *Pimenta racemosa* var. *racemosa* (Mill.) J.W. Moore (Myrtaceae) de Táchira-Venezuela. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, *13*(3), 305–310. https://www.redalyc.org/pdf/856/85631010010.pdf

Dharmadasa, R. M., Abeysinghe, D. C., Dissanayake, D. M. N., Abeywardhane, K. W., & Fernando, N. S. (2015). Leaf essential oil composition, antioxidant activity, total phenolic content and total flavonoid content of *Pimenta dioica* (L.) Merr (Myrtaceae): A superior quality spice grown in Sri Lanka. *Universal Journal of Agricultural Research*, *3*(2), 49–52. https://doi.org/10.13189/ujar.2015.030203

Elyemni, M., Louaste, B., Nechad, I., Elkamli, T., Bouia, A., Taleb, M., & Eloutassi, N. (2019). Extraction of essential oils of *Rosmarinus officinalis* L. by two different methods: Hydrodistillation and microwave assisted hydrodistillation. *The Scientific World Journal*, *2019*. https://doi.org/10.1155/2019/3659432

Fernández, A., Álvarez, A., García, M. D., & Sáenz, M. T. (2001). Anti-inflammatory effect of *Pimenta racemosa* var. *ozua* and isolation of the triterpene lupeol. *Farmaco*, *56*(4), 335–338. https://doi.org/10.1016/S0014-827X(01)01080-1

García, M. D., Fernández, M. A., Alvarez, A., & Saenz, M. T. (2004). Antinociceptive and anti-inflammatory effect of the aqueous extract from leaves of *Pimenta racemosa* var. *ozua* (Mirtaceae). *Journal of Ethnopharmacology*, *91*(1), 69–73. https://doi.org/10.1016/J.JEP.2003.11.018

Guimarães, A. C., Meireles, L. M., Lemos, M. F., Guimarães, M. C. C., Endringer, D. C., Fronza, M., & Scherer, R. (2019). Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules*, *24*(13). https://doi.org/10.3390/molecules24132471

Ishag, O. A. O., Erwa, I. Y., Diriye, M. A., Lawane, A. A. M., Ahmed, H. M., Ahmed, F. A., Mergani, S. E., Elamin, A., & Omer, A. B. (2018). Antimicrobial potential and phytochemical screening of *Eucalyptus camaldulensis* and *Eucalyptus microtheca* leaves extracts. *South Asian Research Journal of Natural Products*, *1*(3), 120–125.

Ismail, M. M., Samir, R., Saber, F. R., Ahmed, S. R., & Farag, M. A. (2020). Pimenta oil as a potential treatment for *Acinetobacter baumannii* wound infection: In vitro and in vivo bioassays in relation to its chemical composition. *Antibiotics*, 9(10). https://doi.org/10.3390/antibiotics9100679

Jan, R., Asaf, S., Numan, M., Lubna, & Kim, K. M. (2021). Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. *Agronomy*, *11*(5). https://doi.org/10.3390/agronomy11050968

John, C., & Maharaj, R. (2024). Effect of West Indian Bay Leaf (*Pimenta racemosa*) and Turmeric (*Curcuma longa*) essential oils on preserving raw chicken breasts. *Food Technology and Biotechnology*, *62*(2), 150–161. https://doi.org/10.17113/ftb.62.02.24.8155

Kachur, K., & Suntres, Z. (2020). The antibacterial properties of phenolic isomers, carvacrol and thymol. *Critical Reviews in Food Science and Nutrition*, *60*(18), 3042–3053. https://doi.org/10.1080/10408398.2019.1675585

Kumar Joshi, R., Soulimani, R., & Bouayed, J. (2019). Limonene: Natural monoterpene volatile compounds of potential therapeutic interest. *American Journal of Essential Oils and Natural Products*, *7*(4), 1–10. https://dx.doi.org/10.22271/23219114

Kwansa-Bentum, B., Okine, B. A., Dayie, A. D., Tetteh-Quarcoo, P. B., Kotey, F. C. N., Donkor, E. S., & Dayie, N. T. K. D. (2023). *In Vitro* effects of petroleum ether, dichloromethane, methanolic and aqueous leaf extracts of *Eucalyptus grandis* on selected multidrug-resistant bacteria. *PLOS One*, *18*(3), e0283706. https://doi.org/10.1371/journal.pone.0283706

Lichtenhaler, K., & Welburn, A. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, *11*(5), 591–592. https://doi.org/10.1042/BST0110591

López-Hidalgo, C., Meijón, M., Lamelas, L., & Valledor, L. (2021). The rainbow protocol: A sequential method for quantifying pigments, sugars, free amino acids, phenolics, flavonoids and MDA from a small amount of sample. *Plant Cell and Environment*, *44*(6), 1977–1986. https://doi.org/10.1111/pce.14007

Lowe, H. I. C., Daley, D. K., Lindo, J., Davis, C., Rainford, L., Hartley, S.-A., & Thoms-Rodriguez, C. (2017). The antibacterial and antifungal analysis of crude extracts from the leaves and bark of *Pimenta* species found in Jamaica. *Journal of Medicinal Plants Research*, *11*(38), 591–595. https://doi.org/10.5897/jmpr2017.6435

Manandhar, S., Luitel, S., & Dahal, R. K. (2019). *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. *Journal of Tropical Medicine*, *2019*. https://doi.org/10.1155/2019/1895340

Milenković, A., Stanojević, J., Stojanović-Radić, Z., Pejčić, M., Cvetković, D., Zvezdanović, J., & Stanojević, L. (2020). Chemical composition, antioxidative and antimicrobial activity of allspice (*Pimenta dioica* (L.) Merr.) essential oil and extract. *Advanced Technologies*, *9*(1), 27–36. https://doi.org/10.5937/savteh2001027m

Monteiro Cavalcante, R. B., Moura, A. J. B., Araújo, M. A. M., & Moreira-Araújo, R. S. R. (2021). Bioaccesibilidad de compuestos fenólicos y capacidad antioxidante en hojas de menta de pimienta orgánica. *Revista Chilena de Nutrición*, *48*(2), 157–162. https://doi.org/10.4067/s0717-75182021000200157

Mujaffar, S., & Bynoe, S. (2020). Microwave drying of West Indian Bay leaf (*Pimenta racemosa*). *West Indian Journal of Engineering*, *42*(2), 87–95. https://journals.sta.uwi.edu/ojs/index.php/wije/article/view/9230

National Committee for Clinical Laboratory Standards. (2004). *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria: Approved standard M31-A2* (2nd ed.).

Rayan, M., Abu-Farich, B., Basha, W., Rayan, A., & Abu-Lafi, S. (2020). Correlation between antibacterial activity and free-radical scavenging: *In-vitro* evaluation of polar/non-polar extracts from 25 plants. *Processes, 8*(1). https://doi.org/10.3390/PR8010117

Sánchez-Zárate, A., Hernández-Gallegos, M. A., Carrera-Lanestosa, A., López-Martínez, S., Chay-Canul, A. J., Esparza-Rivera, J. R., & Velázquez-Martínez, J. R. (2020). Antioxidant and antibacterial activity of aqueous, ethanolic andacetonic extracts of *Pimenta dioica* L. leaves. *International Food Research Journal*, *27*(5), 825–834. http://www.ifrj.upm.edu.my/27%20(05)%202020/DONE%20-%2006%20-%20IFRJ20196.R1.pdf

Santos, P. F. P., Gomes, L. N. L. F., Mazzei, J. L., Fontão, A. P. A., Sampaio, A. L. F., Siani, A. C., & Valente, L. M. M. (2018). Polyphenol and triterpenoid constituents of *Eugenia florida* DC. (Myrtaceae) leaves and their antioxidant and cytotoxic potential. *Quimica Nova*, *41*(10), 1140–1149. https://doi.org/10.21577/0100-4042.20170284

Sarathambal, C., Rajagopal, S., & Viswanathan, R. (2021). Mechanism of antioxidant and antifungal properties of *Pimenta dioica* (L.) leaf essential oil on *Aspergillus flavus*. *Journal of Food Science and Technology*, *58*(7), 2497–2506. https://doi.org/10.1007/s13197-020-04756-0

Schweiger, A., & Bernhardt, H. (2024). Influence of temperature and LED light spectra on flavonoid contents in *Poa pratensis*. *AgriEngineering*, *6*(3), 2167-2178. https://doi.org/10.3390/agriengineering6030127

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, *299*, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Soysa, E. J. S. D., Abeysinghe, D. C., & Dharmadasa, R. M. (2016). Comparison of phytochemicals antioxidant activity and essential oil content of *Pimenta dioica* (L.) Merr. (*Myrtaceae*) with four selected spice crop species. *World Journal of Agricultural Research, 4*(6), 158–161.

Tohidi, B., Rahimmalek, M., & Arzani, A. (2017). Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of *Thymus* species collected from different regions of Iran. *Food Chemistry*, *220*, 153–161. https://doi.org/10.1016/J.FOODCHEM.2016.09.203

Tucker, A., Adams, R. P., & Landrum, L. (1991). Volatile leaf oils of Caribbean Myrtaceae. I. three varieties of *Pimenta racemosa* (Miller) J. Moore of the Dominican Republic and the commercial bay oil. *Journal of Essential Oil Research*. *3*(5), 323-329. https://doi.org/10.1080/10412905.1991.9697952

Vaneková, Z., Vanek, M., Škvarenina, J., & Nagy, M. (2020). The influence of local habitat and microclimate on the levels of secondary metabolites in Slovak Bilberry (*Vaccinium myrtillus* L.) fruits. *Plants, 9*(4), 436–436. https://doi.org/10.3390/PLANTS9040436

Youssef, F. S., Labib, R. M., Gad, H. A., Eid, S., Ashour, M. L., & Eid, H. H. (2021). *Pimenta dioica* and *Pimenta racemosa*: GC-based metabolomics for the assessment of seasonal and organ variation in their volatile components, *in silico* and *in vitro* cytotoxic activity estimation. *Food & Function*, *12*(12), 5247–5259. https://doi.org/10.1039/D1FO00408E

Zhang, H., Wang, M., Yu, G., Pu, J., Tian, K., Tang, X., Du, Y., Wu, H., Hu, J., Luo, X., Lin, L., & Deng, Q. (2023). Comparative analysis of the phenolic contents and antioxidant activities of different parts of two pomegranate (*Punica granatum* L.) Cultivars: 'Tunisia' and 'Qingpi.' *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1265018