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Original article

Chemical composition and antimicrobial activity of essential oil from *Piper marginatum* leaves obtained by hydrodistillation in pH4, pH7 and pH10

Composición química y actividad antimicrobiana del aceite esencial de hojas de *Piper marginatum* obtenido por hidrodestilación con pH4, pH7 y pH10

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Abstract

The present study aimed to evaluate possible variations in the chemical composition and antimicrobial activity of essential oils extracted from *Piper marginatum* leaves obtained by hydrodistillation at pH4, pH7, and pH10. When compared, the chemical profiles of the three oils were different. A reduction of major phenylpropanoids *Z*-asarone, dillapiole, and *E*-asarone was observed for samples pH4-EO and pH10-EO as compared to sample pH7-EO. Sesquiterpenes macrocarpene (10.8%), viridiflorene (9.1%), caryophyllene (7.7%), and *E*-nerolidol (6.5%) were the major compounds identified in the pH10-EO sample. Compound α-santonin (36.1%) was identified as a major constituent of the essential oil obtained at pH4. The antimicrobial activity of the essential oils also showed variations in their minimum inhibitory concentration values (MIC). The oil obtained at pH4 exhibited strong activity with an MIC of 78.1 µg/ml for both *Escherichia coli* and *Candida utilis* microorganisms. We evidenced the influence of water pH on the chemical composition of essential oils obtained by hydrodistillation, as well as its importance as a parameter in studies using the hydrodistillation method.

Keywords: *Escherichia coli*; Phenylpropanoid, Piperaceae; Hydrodistillation.

Resumen

El presente estudio tuvo como objetivo evaluar posibles variaciones en la composición química y la actividad antimicrobiana de los aceites esenciales de hojas de *Piper marginatum* obtenidos por hidrodestilación con pH4, pH7 y pH10. La comparación indicó que los perfiles químicos de los tres aceites eran diferentes. Se observó una reducción de los principales fenilpropanoides, *Z*-Asarona, dilapiol y *E*-asarona, en las muestras pH4-EO y pH10-EO comparadas con la muestra pH7-EO. Los sesquiterpenos macrocarpeno (10,8 %), viridiflorene (9,1 %), cariofileno (7,7 %) y E-nerolidol (6,5 %) fueron los principales compuestos detectados en la muestra pH10-EO. El compuesto α-santonina (36,1 %) fue el componente principal del aceite esencial obtenido con pH 4. La actividad antimicrobiana de los aceites esenciales también mostró variaciones en los valores de la concentración mínima inhibitoria (CMI). El aceite obtenido con pH4 exhibió una fuerte actividad, con una CMI de 78,1 µg/ml tanto para *Escherichia coli* como para *Candida utilis*. El estudio mostró la influencia del pH del agua en la composición química de los aceites esenciales obtenidos por hidrodestilación y la importancia del pH del agua como un parámetro que debe especificarse en los estudios que utilizan el método de hidrodestilación.

Palabras clave: *Escherichia coli;* Fenilpropanoide; Piperaceae; Hidrodestilación.

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Introduction

Piper marginatum is a medicinal plant from the Piperaceae family widely distributed in the Amazon rainforest and popularly known as *capeba*, *malvarisco*, pepper-do-mato, *nhandi*, *nhandú*, or pepper-of-the-Indians (**Andrade *et al.***, 2008). It is commonly used in infusions against gastrointestinal and liver diseases and snake and insect bites (**Hurtado *et al.***, 2016; **Chaves & Santos**, 2002). Chemical studies of *P. marginatum* have revealed that the plant tissues accumulate amides, prenylated benzoic acids, flavonoids, phenylpropanoids, and aristolactams (**Reigada *et al.***, 2007). In previous studies with essential oils from *P. marginatum* leaves, terpenes and phenylpropanoids have been detected and shown to have antioxidant, leishmanicidal, larvicidal, and antifungal activities (**Brú & Guzman**, 2016; **Moraes *et al.***, 2014). Essential oils are usually obtained by hydrodistillation by crushing the plant in water and heating it in a Clevenger apparatus (**Lima *et al.***, 2021; **Melo-Guerrero *et al.***, 2020). The hydrodistillation of essential oils can cause the loss and degradation of certain volatile compounds due to the long extraction times by thermal or hydrolytic effect (**Elyemni *et al.***, 2019). There are many advanced and innovative methods for obtaining essential oils with optimized extraction times and temperatures besides reduced artifacts and the loss of polar molecules, but hydrodistillation remains the simplest and oldest method used to obtain essential oils (**El Asbahani *et al.***, 2015). In our study, we aimed at obtaining essential oils from *P. marginatum* leaves by hydrodistillation and pH control of the decoction water to evaluate possible variations in the chemical composition and antimicrobial activity.

Materials and methods

Botanical material

Leaves of *P. marginatum* specimens were collected on *March 2019 from a* fragment of forest located in the city of Recife, state of Pernambuco, Brazil. The species was identified by Dr. Margareth F. de Sales of the Department of Biology of the Federal Rural University of Pernambuco and compared with a voucher specimen previously deposited in the Vasconcelos Sobrinho Herbarium of UFRPE with the number 48210 (**Ramos *et al.***, 2022a).

Obtention of essential oils

For each oil sample obtained, 500 g of fresh leaves of *P. marginatum* were crushed and submitted to hydrodistillation in a modified Clevenger apparatus for 2 h. To obtain the oil in a basic medium, the decoction water was adjusted to pH 10.0 using a 0.1 $molL^{-1}$ sodium hydroxide solution. To obtain the oil in an acidic medium, the decoction water was adjusted to pH 4.0 using a 0.1 $molL^{-1}$ sulfuric acid solution. To obtain the oil in a neutral medium, distilled water was used (pH 7.01). The oils were treated with anhydrous sodium sulfate and stored at ±5 °C for further analysis.

Essential oils analysis

Oil samples were analyzed using a Hewlett–Packard 5890 Series II GC chromatograph equipped with a flame ionization detector and a J & W Scientific DB-5 fused silica capillary column (30 m × 0.25 mm i.d.) with a programmed temperature of 60 to 246 °C at 3 °C/min. The temperatures of the injector and the detector were 260 and 280 °C, respectively. Hydrogen was used as carrier gas at a flow rate of 1.0 mL/min; injection was in split mode (1:30) and the injection volume was 1.0 µL of a solution containing 10 mg/mL of oil in hexane. The amount of each compound was calculated from GC peak areas in the order of DB-5 column elution and expressed as a relative percentage of the total area of the chromatograms.

Chemical identification

Oil samples were analyzed using a Varian GC/MS (GC: Varian 431/GC-MS: Varian 220-MS) system operating in the EI mode at 70 eV equipped with a J & W Scientific DB-5 fused silica capillary column (30 m × 0.25 mm i.d.) and a programmed temperature of

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60 to 246 ºC by 3º C/min. The temperatures of the injector and the detector were 260 and 280º C, respectively. The carrier gas was helium, the flow rate was 1 mL/min, and we used the split mode (1:30) with an injected volume of 1.0 µL of a solution containing 3 mg/mL of oil in hexane. The initial identification of the separated components of the essential oil was done by comparing with previously reported values of retention indices obtained by co-injection of oil samples and C11–C24 linear hydrocarbons and calculated using the Van den Dool & Kratz equation (**Van den Dool & Kratz,** 1963). Subsequently, the MS acquired for each component was matched with those stored in the Wiley/NBS mass spectral library of the GC–MS system and with other published mass spectral data (**Adams,** 2007). Terpenes (β-pinene, *y*-terpinene, *E*-nerolidol, caryophyllene *α*-humulene, and α-pinene,) and phenylpropanoids (*Z*-asarone and *E*-asarone) purchased from Sigma-Aldrich, Brazil, were used to identify the volatile components. Dillapiole was previously obtained from *Piper aduncum* leaves essential oil (91.0 %) (**Ramos *et al*.,** 2022b). All analyses were carried out in triplicate.

Antimicrobial activity

We analyzed this material to evaluate the antimicrobial activity against *Staphylococcus aureus* (02)*, Escherichia coli* (86)*, Bacillus subtilis* (86) bacteria, and *Candida utilis* (1006) and *Candida albicans* (1009) yeasts. We used Saubouraud liquid culture media for yeasts and Mueller Hinton liquid medium for bacteria. The microplates were grown at 37 ºC for 24 h for bacteria and at 30 ºC for 72 h for yeasts. After the incubation period, the microplates were treated by adding 10 µL of a 0.01 % resazurin solution and incubated for 3 h. The MIC was defined as the lowest concentration of the samples that inhibited the growth of the microorganism, with the final concentration fluctuating between 2.5 and 2500 µg/mL (**Ramos & Bezerra,** 2021). Metronidazole was used as a positive control for bacteria and fluconazole for yeasts.

Results and discussion

The yield values of essential oils obtained from *P. marginatum* leaves in neutral (pH7-EO), acidic (pH4-EO), and basic (pH10-EO) mediums were 0.13, 0.06, and 0.12 %, respectively. Analysis by gas chromatography revealed qualitative and quantitative variations in the chemical profiles of the three samples (**Figure 1**). We identified a total of 37 compounds: 13 in the pH4-EO samples; 16 in the pH7-EO samples, and 17 compounds in the pH10-EO samples, which corresponded to 72.5 %, 93.0 %, and 69.9 % of the total chemical composition of each oil, respectively (**Table 1**). In the pH7-EO samples, we found monoterpenes, sesquiterpenes, and aromatic compounds, predominantly, phenylpropanoids such as *Z*-asarone (18.2 %), *E*-asarone (15.1%), dillapiole (13.0%) and croweacin (6.0%). The sesquiterpenes macrocarpene (10.8 %), viridiflorene (9.1 %), caryophyllene (7.7 %), and *E*-nerolidol (6.5 %) were the major compounds identified in the pH10-EO samples.

The pH4-EO samples showed the greatest difference between the three oils analyzed; α-santonin sesquiterpene (36.1%) was the major compound and *E*-asarone was the only one present in the three oils. In an aqueous acidic medium, unsaturated terpenes undergo cyclization, hydration, and rearrangement reactions with the formation of carbocation intermediate resulting in mixtures of products similar to those obtained in mediums with neutral pH. Eleven compounds were found only in pH4-EO samples: six of them were identified as oxygenated terpenes and two as non-oxygenated. The six oxygenated terpenes were probably products of the hydration reaction that took place during the process to obtain the oil. In the pH4-EO samples, two carboxylic acids not found in pH7-EO and pH10-EO samples were also identified. The identification of carboxylic acids from essential oils obtained in an acidic medium was expected since the carboxyl groups are protonated while in a basic medium, carboxylates, which have an affinity for the aqueous phase, form.

A quantitative reduction of phenylpropanoids was observed for samples pH4-EO and pH10-EO when compared to sample pH7-EO. The reduction of phenylpropanoids probably occurs due to the oxidative degradation of *Z*-asarone, dillapiole, *E*-asarone, and exalatacin

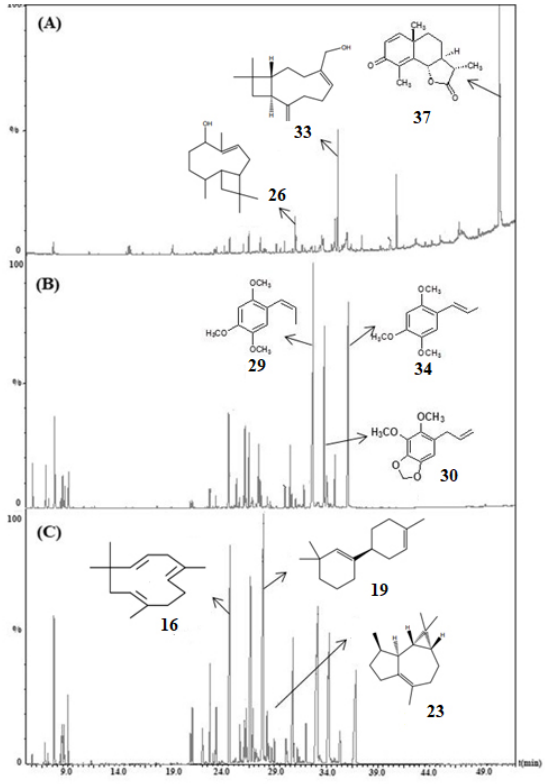

Figure 1. Chemical profiles by GC/MS of essential oils obtained from *P. marginatum* leaves in pH4 (**A**), pH7 (**B**), and pH10 (**C**)

containing allylic and vinylic groups that are susceptible to oxidation in a basic or acidic medium overheating (**Turek & Stintzing,** 2013), and to the formation of phenylpropanoid dimers, which are non-volatile compounds.

Previous studies with essential oil obtained from the leaves of allopatric species of *P. marginatum* have revealed great variability in the chemical composition with anethole (45.9%), isosafrole (37.3%), *E*-asarone (32.6%), *Z*-asarone (30.4%), anisaldehyde (22.0%). and notosirnol (22.7%) presence, which points to the possibility of having more than seven chemotypes for *P. marginatum* leaves essential oil (**Macêdo *et al.,*** 2020; **Ayres *et al*.,** 2021; **Hurtado *et al*.,** 2016; **Vogler *et al.,*** 2006). However, this is the first report of α-santonin sesquiterpene identification in the Piperaceae species. The proposed biosynthetic route for the α-santonin involves a sequence of cyclization reactions, rearrangements, and oxidation of germacrene from farnesyl diphosphate (**De Kraker *et al.,*** 2001), reactions that can be favored in basic or acidic solutions. Forty compounds were identified in essential oils obtained from *P. marginatum* leaves, stems, and inflorescences including germacrene (**Autran *et al.,*** 2009), a central intermediate in the biosynthesis of several sesquiterpenes (**Xu & Dickschat,** 2020). A new method developed to obtain and simultaneously fractionate essential oils by hydrodistillation allowed the obtention of qualitatively and quantitatively different chemical profiles of essential oils from *P. marginatum* (**Ramos *et al.,*** 2022b). The variation in the chemical composition of the essential oil in a plant species is usually attributed to biotic and abiotic factors such as herbivory, climate, and soil composition, but the parameters of the method used to obtain the oil are usually neglected (**Afshar *et al*.,** 2021; **Vafadar *et al*.,** 2017; **Silva *et al*.,** 2016; **Paolini *et al*.,** 2010). Most studies available in the literature use hydrodistillation to obtain essential oils from plants but do not provide information on the control or specification of the water pH used to obtain the oil. The compounds commonly identified in essential oils obtained from plants

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Table 1. Chemical composition of essential oils obtained from *P. marginatum* leaves in neutral (pH7-EO), acidic (pH4-EO) and basic (pH10-EO) mediums

Compounds	IR[a]	pH4-EO		pH7-EO		pH10-EO	
		IR[b]	%	IR[b]	%	IR[b]	%
1. α-pinene	926	-	-	932	3.4	935	1.8
2. β-pinene	973	-	-	974	3.8	970	1.1
3. β-phellandrene	1025	-	-	1029	6.8	1026	5.0
4. 5-methylhexanoic acid	1033	1029	1.7	-	-	-	-
5. E-ocimene	1044	-	-	1040	2.6	-	-
6. y-terpinene	1054	-	-	-	-	1050	2.4
7. p-methylacetophenone	1179	1185	2.3	-	-	-	-
8. Nonanoic acid	1267	1282	2.4	-	-	-	-
9. β-elemene	1334	-	-	-	-	1333	2.4
10. δ-elemene	1335	-	-	1334	1.0	1336	1.4
11. β-bourbonene	1387	-	-	-	-	1385	3.0
12. Caryophyllene	1417		-	-	-	1415	7.7
13. 4,8-**β**-epoxi-caryophyllene	1423	1408	3.4	-	-	-	-
14. Aromadendrene	1434	-	-	1439	2.1	-	-
15. Metilisoeugenol	1451	-	-	-	-	1447	2.1
16. α-humulene	1452	-	-	-	-	1451	2.0
17. Croweacin	1457	-	-	1453	6.0	-	-
18. Ishwane	1465	1452	2.6	-	-	-	-
19. Macrocarpene	1470	-	-	-	-	1473	10.8
20. α-amorphene	1483	-	-	1478	7.3	-	-
21. Himachalene	1481	-	-	1481	2.1	-	-
22. Methyl isoeugenol	1491	-	-	1495	1.1	-	-
23. Viridiflorene	1496	-	-	-	-	1493	9.1
24. E-y-bisabolene	1529	1536	3.2	-	-	-	-
25. E-nerolidol	1561	-	-	1558	4.7	1459	6.5
26. Caryophyllene alcohol	1570	1560	4.3			-	-
27. Carotol	1594	-	-	1592	1.9	-	-
28. Guaiol	1600	-	-			1596	4.5
29. Z-asarone	1616	-	-	1616	18.2	1615	1.8
30. Dillapiole	1620	1626	1.7	1630	13.0	-	-
31. 4,6-dimethoxy-5-vinyl-1,2-benzodioxide	1653	-	-	1658	3.9	-	-
32. Exalatacin	1655	-	-	-	-	1655	3.2
33. 14-hydroxy-9-epi-(*E*)-caryophyllene	1668	1659	8.5	-	-	-	-
34. E-asarone	1675	1679	3.6	1679	15.1	1672	5.1
35. Z-ligustilide	1734	1726	1.8	-	-	-	-
36. 1-docosene	2189	2190	0.9	-	-	-	-
37. α-santonin	2202	2192	36.1	-	-	-	-
Monoterpenes		-		16.6		10.3	
Sesquiterpenes		58.1		19.1		47.4	
Aromatics		7,6		57.3		12.2	
Others		6,8		-		-	
Total		72.5		93.0		69.9	

[a] Linear retention indices from the literature (**Adams,** 2007); [b] Retention indices calculated from retention times in relation to those of the n-alkanes series on a 30 m DB-5 capillary column

Table 2. MIC values for essential oils obtained from *P. marginatum* leaves in neutral (pH7-EO), acidic (pH4-EO), and basic (pH10-EO) mediums

Microorganisms	MIC values expressed in μg/mL				
	pH4-EO	pH10-EO	pH7-EO	Metronidazole	Fluconazole
B. subtilis	625	2500	2500	2500	-
E. coli	78.1	2500	2500	78.1	-
S. aureus	625	2500	2500	19.5	-
C. albicans	312.5	1250	2500	-	2500
C. utilis	78.1	625	625	-	2.5

are monoterpenes, sesquiterpenes, phenolics, phenylpropanoics, and heterocyclics, which contain various chemical groups of alcohols, ketones, aldehydes, carboxylic acids, esters, and acetates. These compounds in an aqueous base or acid medium under heating can undergo cyclization, hydrogenation, hydration, and dehydration reactions resulting in variations in the chemical composition of the essential oil (**Turek & Stintzing,** 2013).

Additionally, the antimicrobial potential of essential oils obtained at different pH values also exhibited variation in CMI values against bacteria and yeasts. Our results showed that the pH4-EO oil exhibited greater activity against the gram-negative bacteria *E. coli,* with a MIC of 78.1 μg/ml, while for the yeasts *C. albicans* and *C. utilis* the MIC was 312 μg/ml and 78.1 μg/ml, respectively (**Table 2**). The strong antimicrobial activity observed for the pH4-EO sample may be associated with the presence of the major compound α-santonin, a sesquiterpene that has been used as an effective drug against infectious diseases caused by worms, which was first isolated from the flower bud of *Artemisia santonica* (**Wang *et al*.,** 2019). Activity for the pH4-EO sample can also be attributed to the presence of 5-methylhexanoic and nonanoic acids as oils rich in short-chain fatty acids exhibit strong antimicrobial activity (**Holanda *et al*.,** 2020). The activities of the pH7-EO and pH10-OE samples were considered moderate to weak for all microorganisms tested, with a MIC that varied between 625 μg/ml and 2500 μg/ml for both oils.

Previously, the essential oil from *P. marginatum* leaves containing *E*–nerolidol, *O*-cymene, spathulenol, elemicin, and α-copaene as major compounds showed antimicrobial activity (**dos Santos *et al*.,** 2021).

Conclusion

Our results showed the effect of varying the pH of the water used in the hydrodistillation on the composition and antimicrobial activity of essential oils from *P. marginatum* leaves. The compounds identified in the samples pH4-EO, pH7-EO, and pH10-OE evidenced intermediates and biosynthetic pathways in common, which indicates that the variations in the chemical profiles observed among these three samples were due to the variations in pH values. Our results also revealed the importance of water pH as a parameter that should be considered in future studies to obtain essential oils by the hydrodistillation method.

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Authors' contribution

E.C Oliveira and M.F.F. Silva: Formal analysis and investigation; Ramos, C. S.: Conceptualization, data visualization, writing, editing, and supervision.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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