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Acmella oleracea (L.) R.K. Jansen essential oils: Chemical composition, antioxidant, and cytotoxic activities

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ABSTRACT

Acmella oleracea is an important food and medicinal plant in the Amazon; it is used to treat toothaches, hepatitis, malaria, fever is also described as an anticancer plant. This work reports for the first time the comparison of chemical profiles, antioxidant, and cytotoxic capacities of inflorescence (INF) and leaf (LEA) *A. oleracea* essential oils (EOs) obtained by hydrodistillation (HD) and steam distillation (SD). The essential oils were obtained by hydrodistillation and steam distillation and analyzed by GC and GC-MS. The antioxidant potential was evaluated by the co-oxidation method of the β-carotene/linoleic acid, and the cytotoxic activity against healthy human embryonic kidney (HEK-293), gastric ascites (AGP-01), melanoma (SK-MEL-19), lung carcinoma (A549) malignant cell lines by the MTT method. The essential oils obtained from INF by HD and SD showed the highest yields (HD = 0.68; SD = 0.5%). The monoterpene hydrocarbons (0–49.97%) and sesquiterpene hydrocarbons (25.79–50.63%) were predominant in the analyzed oils. The main constituents (>10%) identified in the EO samples were caryophyllene oxide (0.3–28.11%), *E*-caryophyllene (6.71–27.31%), myrcene (0–25.03%), germacrene D (0.06–19.56%), guaiol (0–14.23%), 1-pentadecene (0–13.88%), and β-pinene (0–10.04%). The chemometric analysis displayed the formation of three distinct groups of chemical compositions as follows: group I, LEA EOs (HD and SD); group II, INF EO (HD); and group III, INF EO (SD). The INF (14.3%) and LEA (9.0%) EOs showed antioxidant action with no significant difference ($p < 0.05$). The INF EO (HD) showed higher cytotoxicity against the malignant lineages of gastric ascites (IC50 5.31 μg/mL), melanoma (IC50 6.43 μg/mL), while the LEA EO (SD) was more cytotoxic against the lung carcinoma cell line (13.39 μg/mL), and higher toxicity was also observed against the healthy embryonic kidney cell line (IC50 5.14 μg/mL). *Acmella oleracea* is chemically influenced by the volatile extraction methods, and this influences on the antioxidant or cytotoxic capacity of the species.

1. Introduction

The Malignant neoplastic diseases are one of the main causes affecting human health, leading to deaths and sequelae. Data from the World Health Organization (WHO), indicate that about 9.6 million people in the world died as a result of oncological diseases in the 2018

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(WHO, 2020). In Brazil, malignant skin neoplasms, lungs, and stomach are the most recurrent and accounted for more than 44,000 deaths in 2020. Estimates for the year 2023 indicate that around 63,000 people will be affected by one of these types of cancer (Instituito Nacional de Câncer, 2020, 2022).

In the human body, the decrease in cellular antioxidants and the increase in reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide (O^{2-}), and hydroxyl ($OH^{\bullet}$), can trigger destructive processes in biomolecules (Bhat et al., 2015), as in enzymes, proteins, and especially DNA, causing the breakage of single and double strands, leading to toxic and potentially mutagenic effects that manifest themselves in the form of pathologies such as cancer (Troadec et al., 2017; Yan et al., 2013).

Currently, there are several procedures to treat cancer, such as surgery, radiotherapy, chemotherapy, and immunotherapy, as well as the use of synthetic chemotherapy; however, these forms of fighting the disease have limitations and adverse side effects such as pain, fatigue, anemia, weight loss, and hair loss, in addition to the fact that drugs are toxic even at therapeutic doses. In this way, the search for new drugs to combat oncological diseases has become more incisive, mainly derived from substances identified in plants (Bonam et al., 2018; Urruticoechea et al., 2010).

Plants are a source of bioactive molecules against cancer; about 70% of antineoplastic drugs were developed or derived from natural products and plants (Twilley et al., 2020). Among the food and medicinal plant species found in the Amazon region, *Acmella oleracea* (L.) R.K. Jansen, commonly known as Jambu, is a small species with herbaceous characteristics, has showy inflorescences of a golden yellow color when mature, and stands out in Amazonian cuisine, being used in various food forms (Nakajima, 2020; Rondanelli et al., 2020).

Due to their anesthetic and pungent effect when in contact with the oral mucosa, *A. oleracea* is also described for its use in ethnopharmacology for the treatment of various regional diseases. It is used by traditional communities in the Amazon for the treatment of toothaches, hepatitis, malaria, and fever, and is also described as an anticancer plant by native Indonesian populations (Graham et al., 2000; Oliveira et al., 2015; Stein et al., 2021).

A. oleracea presents several bioactive molecules, with spilanthol being the main one responsible for the analgesic and anesthetic effects presented by the plant (Stein et al., 2021). This species also produces essential oils with chemical composition predominance of terpene metabolites such as *E*-caryophyllene (48.64%–33.61%), β-pinene (17.3%), and myrcene (17.4%) (Benelli et al., 2019; Borges et al., 2012; Spinozzi et al., 2021).

Several essential oil extraction methods are known; techniques such as hydrodistillation (HD) and steam distillation (SD) are the most used to obtain essential oils. HD is the most conventional, the principle is based on sample immersion in water in a distillation system with heating. The sample volatiles are boiled, forming a heterogeneous mixture at the end of the process. The SD extraction follows the same HD principle, but the plant sample is suspended and there is no direct contact with the water. The extraction takes place by passing water vapors through the sample that drag off the plant volatiles (Asbahani et al., 2015).

Different extraction methods can generate different essential oil chemical composition, the pharmacological activity are intrinsically linked to the chemical diversity of these oils (Zhu et al., 2020). So, it is necessary to identify more promising methods for obtaining molecules for studies on the pharmacological activities of plants of origin (Taban et al., 2018; Zhu et al., 2020).

Therefore, considering the pharmacological importance and the need to investigate the chemical and biological properties of the Amazon essential oil, the objective of this work was to evaluate the chemical composition, antioxidant potential, and cytotoxic effect of volatile oils from inflorescences and leaves of *Acmella oleracea* obtained by hydrodistillation and steam distillation.

2. Material and methods

2.1. Planting and botanical identification

For the species planting, an adapted methodology was carried out in which seeds of *A. oleracea* were planted under sandy-clay soil, arranged in lines with a spacing of about 30 cm between seedlings (Raposo et al., 2018). The cultivation was held for 40 days, and with the maturation of its inflorescences, plants of *A. oleracea* were harvested.

A voucher specimen was incorporated into the Herbarium "Dra Marlene Freitas da Silva", from the University do Estado do Pará (UEPA) under the number MSF009916, and the species was registered in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN) under registration number A984D4E.

2.2. Drying material and processing

The *A. oleracea* samples were dried at room temperature for seven days. After drying, inflorescences (INF) and leaves (LEA) were separated, crushed with a mixer processor, and immediately subjected to extraction processes (Santos et al., 2022).

2.3. Extraction of essential oils by hydrodistillation (HD) and steam distillation (SD)

The dried samples were submitted separately to hydrodistillation in duplicate in modified Clevenger apparatus for 3 h, the condensing system temperature was 10–15 °C. The steam distillation was carried out in the same conditions, but a boiler was used as a heating source. The essential oils obtained in both extraction methods were centrifuged for 5 min at 3000 rpm and dehydrated in anhydrous sodium sulfate (Na_2SO_4) under the same conditions in a centrifuge (Maia and Andrade, 2009).

2.4. Yield calculation of essential oils

The yields of essential oils were expressed in percent and calculated from humidity-free biomass (HFB) through the relationship between oil volume, mass plant sample, and humidity according to equation 1 below:

$$\%\ \mathbf{EO}\ (\mathbf{HFB}) = \left(\frac{\mathbf{volume\ of\ oil\ obtained}\ (\mathbf{mL})}{\mathbf{material\ mass}\ (\mathbf{g}) - \left(\frac{\mathbf{material\ mass}\ (\mathbf{g}).\ \mathbf{humidity}\ (\%)}{\mathbf{100}} \right)} \right) \mathbf{x\ 100}$$

Residual moisture of the samples was determined using a moisture tester (Gehaka®), model IV 2000, by infrared drying in parallel to extraction.

2.5. Chemical composition analysis

The obtained essential oils were diluted in *n*-hexane in a ratio of 2 μL of oil to 500 μL of solvent and analyzed simultaneously in these two systems: gas chromatography with a flame ionization detector (GC-FID, Shimadzu Corporation, Tokyo, Japan) and gas chromatography with a mass spectrometer (GC-MS, Shimadzu Corporation, Tokyo, Japan). The GC-MS was equipped with an AOC-20i autoinjector and a Rxi-5MS silica capillary column measuring 30m × 0.25 mm and 0.25 μm thickness (Restek Corporation, Bellefonte, PA, USA).

Essential oils were analyzed under the following conditions: injector temperature: 250 °C; oven temperature setting: 60–250 °C (3 °C/min); helium was used as a carrier gas with a linear velocity of 36.5 cm/s (1.0 mL/min) in splitless mode containing 1.0 μL of essential oil solution. Electronic ionization was at 70 eV with ionization sources and transfer line temperatures of 220 °C and 250 °C, respectively. Mass spectra were

obtained by automatic scanning every 0.5 s, with mass fragments in the range of 40–450 m/z. Retention indices were calculated for all volatile components using a homologous series of C8–C40 *n*-alkanes (Sigma-Aldrich, Milwaukee, WI, USA) according to the linear method of van Den Dool and Kratz (van Den Dool and DecKratz, 1963).

Quantitative data regarding volatile constituents was obtained using a Series 2010 GC operated under conditions like those of the GC-MS system. Relative amounts of individual components were calculated by peak area normalization using the flame ionization detector (GC-FIC). The GCMSolution software containing the Adams and FFNSC-2 libraries was used to identify the compounds obtained in GC-MS (Adams, 2007; Mondello, 2011).

2.6. Antioxidant capacity by β-carotene/Linoleic acid

β-carotene/linoleic acid solution was prepared using 1 mg of β-carotene diluted in 5 mL of chloroform. In 1 mL of this mixture, 25 μL of linoleic acid, 200 μL of concentrated Tween, and the addition of water saturated with oxygen produced an absorbance between 0.6 and 0.7 under a wavelength of 470 nm in a spectrophotometer. The essential oil solution in ethanol was prepared at a concentration of 1 mg/mL. A 200 μL aliquot of this solution was submitted to a reaction medium containing 2300 μL of β-carotene/linoleic acid solution in a water bath at 50 °C for 120 min. Absorbance readings of the reaction media were taken at times 0 and 120 min. The antioxidant capacity of the trolox standard was analyzed under the same conditions, and all experiments were performed in triplicate (Barros et al., 2022).

2.7. In vitro cytotoxic activity by the MTT test

The in vitro cytotoxicity analysis of essential oils was performed using the MTT test, which is a calorimetric analysis based on a redox reaction in which there is a change in the yellow color of 3-(4,5-dimethyl-2-thiazole)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) to a violet-colored formazan product due to cleavage of the MTT salt by mitochondrial enzymes and microsomes found only in metabolically active cells (Mosmann, 1983).

The cytotoxic activity of the oils was tested against human tumor cell lines ascites (AGP-01), melanoma (SK-MEL-19), lung carcinoma (A549), and a healthy embryonic kidney cell line (HEK-293). Cells were maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a 5% CO_2 atmosphere.

Oil samples were dissolved in dimethylsulfoxide (DMSO) to 10 mg/mL in a single-dose assay to a final concentration of 25 μg/mL. Cells were plated in a 96-well plate (100 μL/well) at a concentration of 5 x 10^3 cells/well. After the single dose assay and detection of preliminary cytotoxic activity, the oils were incubated again with the cells, but at different concentrations (25 μg/mL–0.3 μg/mL) for a period of 72 h. The negative control received the same amount of DMSO (0.001% at the highest concentration), and doxorubicin was used as a positive control (Ghosh et al., 2005; Jaganathan et al., 2011). Cytotoxic activity was identified using a multiwell scanning spectrophotometer set to a wavelength of 570 nm.

2.8. Statistical analysis

The chemical variability of the *Acmella oleracea* volatiles was evaluated by multivariate statistical analysis. Hierarchical cluster analysis (HCA), considering Euclidean distance and Ward linkage, was used to verify the similarity of the samples of volatiles obtained by the different methods used. Principal component analysis (PCA) was applied to verify the interrelationship in the components of the samples (>5.0%) using the OriginPro software trial version from OriginLab Corporation (Northampton, MA, USA). Statistical significance in the antioxidant capacity tests was assessed using the Tukey test ($p < 0.05$) calculated using the GraphPad Prism software, version 5.0. The IC50 values were evaluated in the same software, and the selectivity index (SI) data were defined based on the relationship between the cytotoxicity of oils against healthy cells and their action against malignant cells, adopting selectivity for SI (>2) and general toxicity for SI (<2) (Badisa et al., 2009).

3. Results and discussion

3.1. Essential oils yield by hydrodistillation and steam distillation

The inflorescences and leaves of *A. oleracea* subjected to hydrodistillation and steam stripping showed essential oil yields below 1%, as shown in Fig. 1. The inflorescences extracted by hydrodistillation and steamdistillation showed the highest yields with 0.68 and 0.5%, respectively; leaves extracted by HD and SD showed the lowest oil yields, both with 0.19 and 0.33%, respectively.

It is known that inflorescences have colors and odors that are attractive to pollinating agents such as insects, bats, and birds. The flowers are great producers of mono and sesquiterpenes and other aromatic compounds such as aliphatic alcohols, ketones, and esters contained in essential oils (Zoghbi et al., 2000), which could explain the higher concentration of essential oil in the inflorescences than in the leaves of the studied species.

No studies were identified that report the EO content of *A. oleracea* comparing HD and SD, although it is known that a specimen of *Laurus nobilis* (Lauraceae) also had a higher EO content through HD when compared to SD. This is due to HD performing a greater rupture in the secretory cells with a greater release of essential oil during the extraction process. This effect may be associated with the pattern of heat distribution that is different in the two methods (Taban et al., 2018).

3.2. Chemical compostion of essential oils by hydrodistillation and steam distillation

A total of 62 constituents were identified in *A. oleracea* essential oils, representing an average identification of 88.20% of the total plant's volatiles (Table 1). The class of sesquiterpene hydrocarbons was predominant in the inflorescence samples by SD (25.79%), leaves by HD (50.63%) and SD (46.66%), but the sample of inflorescences by HD showed a greater predominance of monoterpene hydrocarbons with 49.97%.

The main constituents (>10% in one of the extraction methods) were caryophyllene oxide (0.3–28.11%), *E*-caryophyllene (6.71–27.31%), myrcene (1.14–25.03%), germacrene D (0.06–19.56%), guaiol (0–14.23%), 1-pentadecene (3.43–13.88%), and β-pinene (0–10.04%), as shown in Fig. 2.

The inflorescences by HD showed *E*-caryophyllene (27.1%), myrcene (25.03%), germacrene D (10.27%), and β-pinene (10.04%) as main

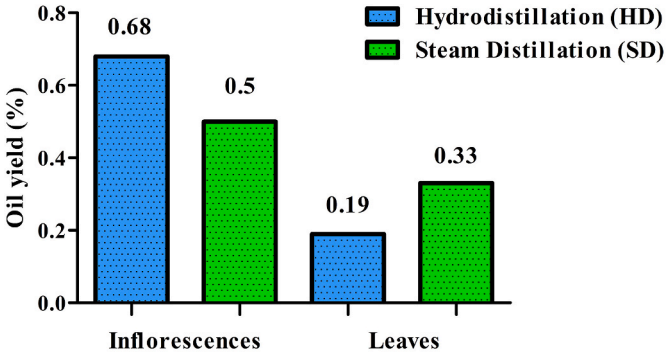

Fig. 1. Essential Oil Yield of the morphological parts of Acmella oleracea under different extraction methods.

Table 1
Chemical composition of essential oils from inflorescences (INF) and leaves (LEA) of *Acmella oleracea* obtained by hydrodistillation (HD) and steam distillation (SD).

N°	RT	RI_C	RI_L	Samples	INF (HD)	INF (SD)	LEA (HD)	LEA (SD)
				Essential oil yield	0.68	0.5	0.19	0.33
				Constituents	(%)			
1	6.549	928	932[a]	α-Pinene	1.13	0.11		
2	7.800	964	969[a]	Sabinene	1.86			
3	**7.921**	**967**	**974**[a]	**β-Pinene**	**10.04**	**4.46**		
4	**8.405**	**982**	**988**[a]	**Myrcene**	**25.03**	**1.14**		
5	8.803	993	1003[a]	*p*-Mentha-1(7),8-diene	0.33			
6	9.683	1017	1024[a]	Limonene		1.83		
7	9.793	1020	1020[a]	β-Phelandrene	9.73			
8	10.010	1026	1026[a]	*Z*-β-Ocimene	1.35			
9	10.406	1037	1044[a]	*E*-β-Ocimene	0.03	0.69		
10	10.858	1049	1054[a]	γ-Terpinene	0.04	0.09		
11	12.050	1081	1086[a]	Terpinolene		0.23		
12	12.504	1093	1095[a]	Linalool	0.19	0.93		
13	13.650	1121	1122[a]	α-Campholenal		0.31		
14	13.781	1124	1128[a]	*allo*-Ocimene	0.43			
15	14.250	1135	1135[a]	*trans*-Pinocarveol		1.92		
16	15.267	1159	1160[a]	*trans*-Pinocamphone		9.29		
17	15.842	1172	1172[a]	*cis*-Pinocamphone		3.25		
18	15.941	1174	1174[a]	Terpinen-4-ol	0.11			
19	16.533	1188	1186[a]	α-Terpineol	0.03	0.25		
20	16.800	1194	1194[a]	Myrtenol		1.78		
21	18.908	1240	1239[a]	Carvone		0.09		
22	20.850	1281	1287[a]	Bornyl acetate		0.6		
23	21.558	1296	1296[a]	*trans*-Pinocarvyl acetate		7.92		
24	22.608	1320	1324[a]	Myrtenyl acetate		0.07		
25	23.214	1334	1335[a]	δ-Elemene	0.62	1.32	0.66	0.65
26	23.675	1344	1349[b]	Terpinyl Acetate		0.06		
27	24.000	1351	1357[a]	Eugenol		0.03		
28	24.917	1372	1374[a]	α-Copaene		0.17		
29	25.342	1381	1387[a]	β-Bourbonene		0.32	0.76	0.52
30	25.638	1388	1389[a]	β-Elemene	0.12	1.21	0.44	0.46
31	**27.129**	**1423**	**1417**[a]	***E*-Caryophyllene**	**27.1**	**6.71**	**27.31**	**22.33**
32	27.318	1428	1430[a]	β-Copaene	0.34	0.29	0.76	0.71
33	27.438	1431	1434[a]	γ-Elemene	0.26	5.9	0.78	0.75
34	27.928	1442	1147[a]	Isogermacrene D	0.1			
35	28.192	1449	1453[a]	Geranyl acetone			0.05	
36	28.361	1453	1452[a]	α-Humulene	1.25	1.51	1.5	1.21
37	28.625	1459	1458[a]	*allo*-Aromadendrene		0.24		
38	29.128	1471	1468[a]	Dodec-(8*Z*)-en-1-ol	1.18			
39	29.533	1480	1478[a]	γ-Muurolene		4.8		
40	**29.666**	**1484**	**1484**[a]	**Germacrene D**	**10.27**	**0.06**	**17.94**	**19.56**
41	**29.921**	**1490**	**1492**[c]	**1-Pentadecene**	**3.43**			**13.88**
42	30.183	1496	1497[b]	Bicyclogermacrene	0.09	2.39		
43	30.241	1497	1495[a]	γ-Amorphene	0.11			
44	30.547	1505	1505[a]	*E*,*E*-α-Farnesene	0.1		0.48	0.47
45	31.717	1533	1529[a]	Kessane	0.1		0.35	2.06
46	32.325	1548	1548[a]	Elemol		2.23		
47	32.641	1556	1559[a]	Germacrene B	0.01	0.56		
48	32.983	1563	1561[a]	*E*-Nerolidol			0.15	0.09
49	33.533	1578	1576[b]	Spathulenol		1.99		
50	**33.724**	**1583**	**1582**[a]	**Caryophyllene oxide**	**0.3**	**2.43**	**28.11**	**25.1**
51	34.208	1594	1594[a]	Salvial-4(14)-en-1-one			0.28	0.23
52	**34.450**	**1600**	**1600**[a]	**Guaiol**		**14.23**		
53	34.667	1606	1614[a]	1,10-di-*epi*-Cubenol		0.3		
54	34.731	1608	1607[a]	β-Oplopenone	0.06			
55	34.817	1609	1608[a]	Humulene epoxide II			1.77	1.37
56	34.800	1609	1600[a]	Rosifoliol		1.85		
57	35.633	1630	1624[b]	*epi*-γ-Eudesmol		0.07		
58	35.742	1633	1629[a]	Eremoligenol		0.07		
59	35.875	1636	1639[a]	*allo*-Aromadendrene epoxide			0.33	0.2
60	36.050	1641	1645[a]	Cubenol			0.19	
61	37.208	1670	1668[a]	14-hydroxy-9-*epi*-*E*-Caryophyllene			0.66	0.55
62	45.182	1892	1888[d]	Spilanthol	0.72			
Monoterpene hydrocarbons					49.97	8.55	0	0
Oxygenated monoterpenes					0.33	17.51	0	0
Sesquiterpene Hydrocarbons					40.37	25.79	50.63	46.66
Oxygenated sesquiterpenes					0.46	23.17	31.84	29.6
Others					5.33	8.68	0.05	13.88
Total Identified					96.46	83.7	82.52	90.14

RT: Retention Time; **RI_C:** Retention Index calculate; **RI_L:** Retention Index of library.
INF: Inflorescences; LEA: Leaves; *n = 2 (standard deviation was less than 2.0).
[a] (Adams, 2007);.

[b] (Mondello, 2011);.
[c] (NIST, 2011);.
[d] (Benelli et al., 2019);.

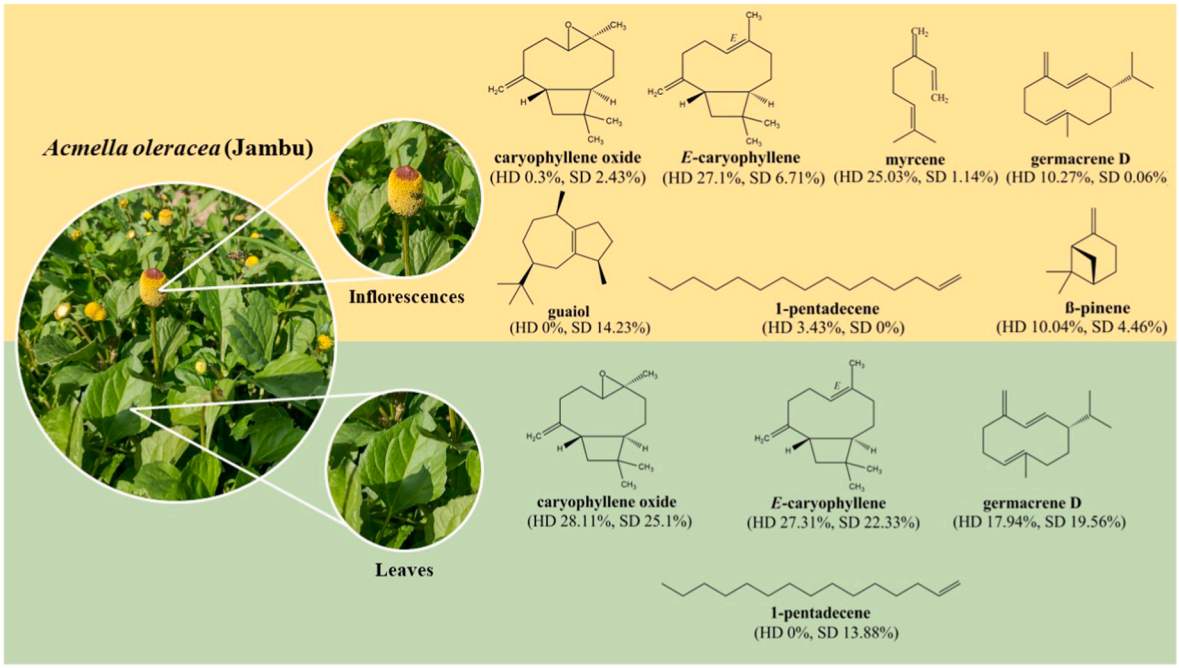

Fig. 2. Structures of the main constituents (>10%) identified in the *Acmella oleracea* essential oils.

compounds. In the extraction by SD, there was a higher occurrence of guaiol (14.23%) and a reduction in the contents of *E*-caryophyllene (6.71%), β-pinene (4.46%), and myrcene (1.14%); however, caryophyllene oxide showed a higher content (2.43%) in SD when compared to HD (0.3%).

The leaf oil by HD showed a predominance of caryophyllene oxide with a content of 28.11%, *E*-caryophyllene also showed a similar content with 27.31%, followed by germacrene D with 17.94%. In the extraction by SD, decreases in the contents of caryophyllene oxide (25.1%) and *E*-caryophyllene (22.33%) were observed, while germacrene D had a higher content with 19.56% in the SD.

The chemical variability of *Acmella oleracea* essential oil samples was evaluated by multivariate statistical analysis (HCA, hierarchical cluster analysis; PCA, principal component analysis), using constituents with levels greater than 5.0%. The application of hierarchical cluster analysis (HCA) provided the dendrogram shown in Fig. 3, The compositions of the analyzed volatiles were included in three different groups and presented a similarity of 0%.

Group I comprise the oils from the inflorescences extracted by hydrodistillation stripping, while group II concentrates the EO sample from the leaves by hydrodistillation and steam distillation, and group III comprises the volatiles present in the EO of the inflorescences extracted by steam distillation.

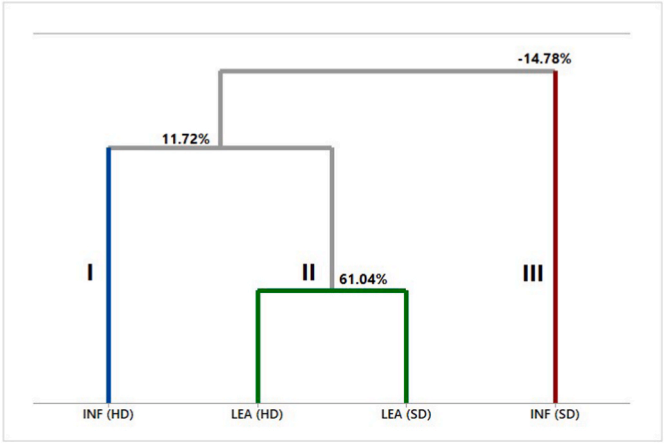

Fig. 3. Chemical constituents identified in *Acmella oleracea* essential oils analyzed by different extraction methods.

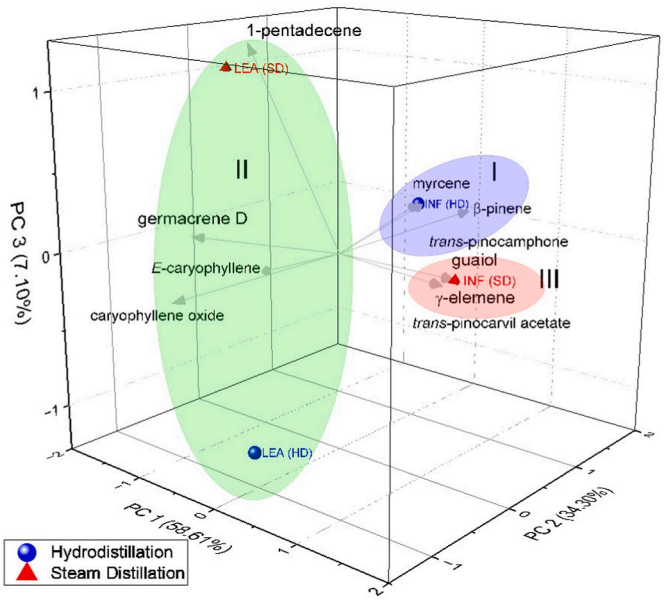

Fig. 4. Analysis of the main components of the constituents present in the essential oils of *Acmella oleracea* obtained by the two extraction methods.

Principal Components Analysis (PCA, Fig. 4) clarified 100.00% of the data variability. PC1 explained 58.61% and showed positive correlations with β-pinene (r = 0.33), *trans*-pinocamphone (r = 1.49), *trans*-pinocarvyl acetate (r = 1.49), γ-elemene (r = 1.46), and guaiol (r = 1.49). The second PC2 component explained 34.30% and showed positive correlations with β-pinene (r = 1.44), myrcene (r = 1.45), β-phellandrene (r = 1.44) and *E*-caryophyllene (r = 0.34). The third component, PC3, explained 7.10% of the data and showed negative correlations with *E*-caryophyllene (r = −0.73) and caryophyllene oxide (r = −0.23). Like the HCA, the analysis of the PCA confirmed the formation of three distinct groups.

The characterization of group I, which includes inflorescences by HD, was made by myrcene (25.03%), β-pinene (10.04%), and β-phellandrene (9.73%). Group II, represented by the EOs of the leaves by HD and SD, was characterized by higher contents of caryophyllene oxide (28.11–25.1%), *E*-caryophyllene (27.31–22.33%), germacrene D (19.56–17.94%), and 1-pentadecene (13.88–0%). While the characterization of group III represented by inflorescences (SD) was given by guaiol (14.23%), *trans*-pinocamphone (9.29%), *trans*-pinocarvyl acetate (7.92%), and γ-elemene (5.9%).

The analysis of the mean contents and standard deviations of the constituents present in the volatiles of *A. oleracea* (Fig. 5) showed that Group I was statistically different (Tukey's test, $p < 0.05$) from Groups II and III by the germacrene D contents (I = 10.2 ± 0.0%; II = 17.9 ± 1.14%; III = 5.9 ± 0.0%) and myrcene (I = 25.0 ± 0.0%; II = 0.0 ± 0.0%; III = 1.14 ± 0.0%).

Group II differed from the other groups by the caryophyllene oxide contents (I = 0.3 ± 0.0%; II = 26.1 ± 2.1%; III = 2.4 ± 0.0%). Furthermore, Group III differed from the other groups by guaiol contents (I = 0.0 ± 0.0%; II = 0.0 ± 0.0%; III = 14.2 ± 0.0%), *E*-caryophyllene (I = 27.1 ± 0.0%; II = 24.8 ± 3.5%; III = 6.7 ± 0.0), and γ-elemene (I = 0.2 ± 0.0%; II = 0.7 ± 0.02%; III = 5.9 ± 0.0).

Applying additional multivariate analyses, in the heat map analysis combined with the hierarchical clustering analysis (Fig. 6), with the classes of compounds, the color pattern varied with the intensity of the color that was gradually increasing, indicating from the smallest to the largest degree. The clustered heatmap (Fig. 6) confirmed the clustering results obtained in PCA and HCA (see Figure 3 and 4).

It is observed that the essential oils from the leaves of *A. oleracea* obtained in HD and SD have the same chemical profile, while the oils obtained from the inflorescences in HD and SD have different chemical profiles. No reports on obtaining *A. oleracea* volatiles by SD were identified in the literature. It is only known that there are a few studies that use HD extraction. In India, two specimens were analyzed by this method, fresh inflorescences of one of the specimens were characterized by limonene (23.6%) and *E*-caryophyllene (20.9 %) (Baruah and Leclercq, 1993), while the fresh aerial parts of the plant presented 2*E*-hexenol (25.7%) and 2-tridecanone (13.1%) (Jirovetz et al., 2005).

Studies carried out with dry inflorescences of *A. oleracea* occurring in Italy reported the presence of *E*-caryophyllene (20.8%) and β-pinene (17.3%) (Benelli et al., 2019), and fresh plant samples had as main constituents *E*-caryophyllene (19.4%) and myrcene (17.4%) (Spinozzi et al., 2021). In Brazil, two other specimens whose essential oils were analyzed showed a predominance of *E*-caryophyllene (30.24%) and thymol (18.30%) in the stems and leaves (Lemos et al., 1991), and a marked presence of *E*-caryophyllene was identified both in the inflorescences (43.85–48.64%) and the leaves (33.61–59.29%) (Borges et al., 2012).

It is possible to observe differences in the contents of the chemical constituents presented in the specimens presented in other works when compared to this present study. This occurs because, in addition to the methods of obtaining the volatiles and the morphological part of the plant used in the extraction, the various environmental factors characteristic of each region influence the obtaining of these metabolites (Gobbo-Neto and Lopes, 2007; Tongnuanchan and Benjakul, 2014), despite all these types of influences, it is noted that some terpenes appear to be specific chemical markers of *A. oleracea* oils, such as *E*-caryophyllene, myrcene, and β-pinene that are recurrent in the specimens in the literature and in the specimen reported here.

3.3. Antioxidant potential by β-carotene and linoleic acid

The evaluation of the antioxidant potential by the method of co-oxidation of the β-carotene/linoleic acid system (Figure 7), allows the analysis of the ability of a substance to prevent and protect the oxidation of β-carotene by free radicals, products of the peroxidation of linoleic acid by water saturated with oxygen (Alves et al., 2010). This method simulates the in vitro attack process of ROS against important biomolecules and the protection capacity of a potential bioactive agent.

The antioxidant test with the β-carotene/linoleic acid system appears to be more suitable for assessing the antioxidant capacity of lipophilic substances such as those contained in essential oils (Kulisic et al., 2004), despite this, no studies were identified that mention the antioxidant capacity of volatile oils from *Acmella oleracea* by the β-carotene method; it is only known that a specimen of *Baccharis trinervis*, also from the Asteraceae family, presented an antioxidant action by this method, with an IC50 of 28.87 μg/mL (Sobrinho et al., 2016).

3.4. Cytotoxic activity by MTT

The essential oils of *Acmella oleracea* (inflorescence HD and leaf SD) showed antiproliferative activity against the three cell lines of human cancer: gastric ascites (AGP-01), melanoma (SK-MEL-19), lung carcinoma (A549), and against a healthy human kidney strain (HEK-293), as shown in Table 2.

Inflorescence oil (HD) characterized by *E*-caryophyllene (27.1%), myrcene (25.03%), germacrene D (10.27%), and β-pinene (10.04%) showed activity against gastric ascites (IC50 5.31 μg/mL), melanoma (6.43 μg/mL), toxicity against healthy embryonic kidney lineage (IC50 5.57 μg/mL) and activity was verified against lung carcinoma (IC50 > 25 g/mL). The selectivity index (SI) demonstrated that the EO of the inflorescences is more selective against the AGP-01 lineage of gastric ascites (SI: 1.04) while the A549 cells of lung carcinoma showed greater resistance against this oil.

The leaf oil by (SD), having main constituents caryophyllene oxide (25.1%), *E*-caryophyllene (22.33%), germacrene D (19.56%), and 1-pentadecene (13.88%), also demonstrated greater cytotoxic activity against the gastric ascites strain (IC50 6.23 μg/mL), followed by the melanoma strain (IC50 7.45 μg/mL), lung carcinoma (13.39 μg/mL) and toxicity was observed against normal (HEK-293) cells (IC50 5.14 μg/mL). Regarding the selectivity index (SI), it is possible to identify that the gastric cancer cell line AGP-01 was also more sensitive to the EO of the leaves (SI: 0.82) and there was greater resistance of the A549 cells of lung carcinoma (SI: 0.38).

Reports of in vitro cytotoxicity of the volatile oils of *A. oleracea* against tumor cells were described only once in the literature, where the oil obtained from the whole plant was mainly characterized by *E*-caryophyllene (19.4%), myrcene (17.4%) and β-pinene (14.7%) and showed cytotoxicity against breast adenocarcinoma (IC50 87.80 μg/mL) and melanoma (IC50 130.9 μg/mL) cell lines (Spinozzi et al., 2021). The IC50 values referring to the toxicity of the oils analyzed in the present work are lower when compared to those in the literature, demonstrating considerably greater cytotoxic activity of the essential oils of the *A. oleracea* species tested here.

The *E*-caryophyllene molecule, the main constituent identified in *A. oleracea*, has several important biological activities, such as synergistic action with other terpenes and cytotoxic activity against epithelial cells with cancerous mutation (Pavithra et al., 2018), this substance is also capable of potentiating the effect of doxorubicin, which shows in vitro cytotoxicity against breast tumor cell lines (Hanušová et al., 2017).

There are other reports of activity of this bioactive against several other malignant cell lines of the colorectal, ovary, bladder, and lung, in

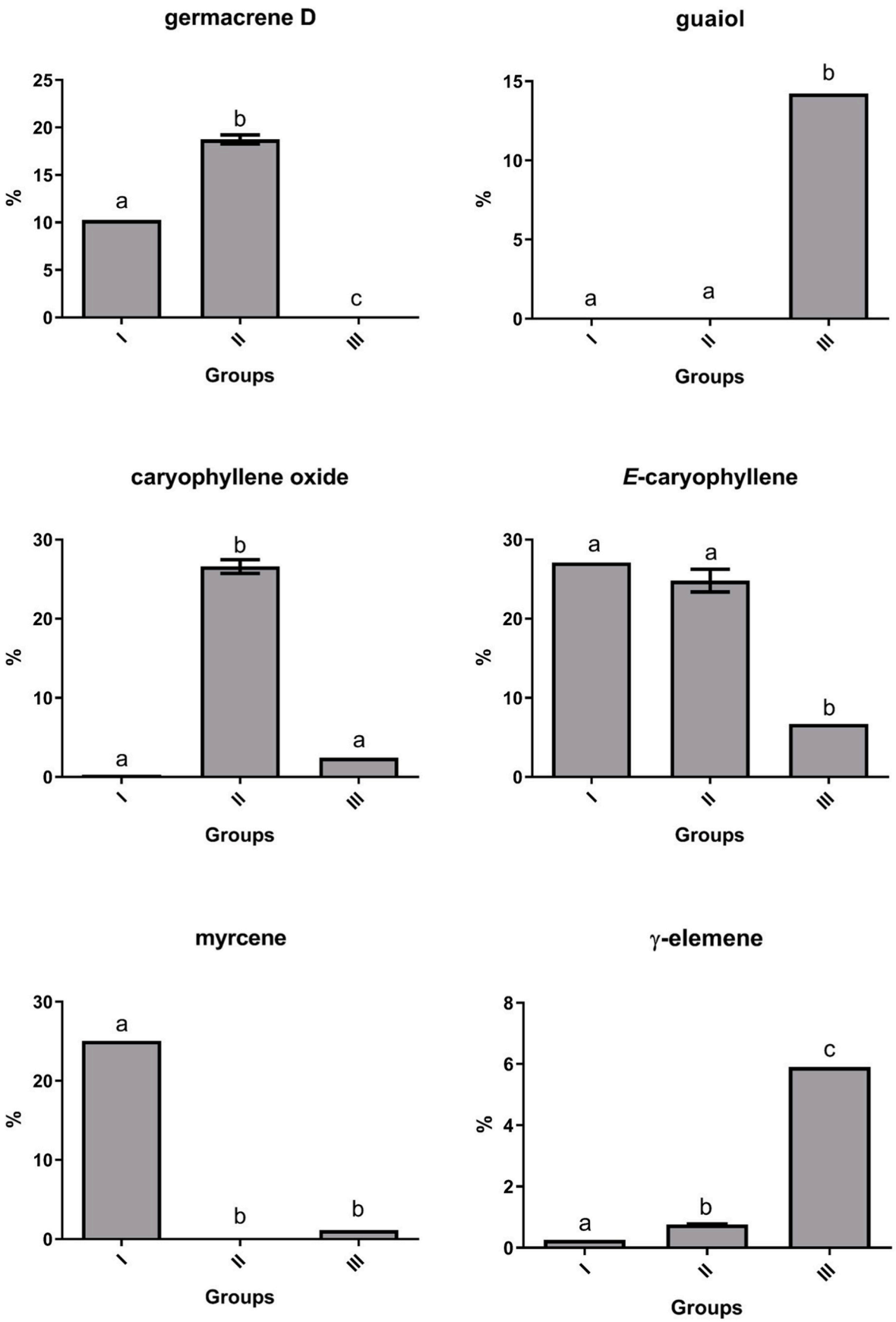

Fig. 5. Constituents of *Acmella oleracea*. Mean ± standard deviation. Values with the same letters in the bars do not differ statistically in Tukey's test ($p > 0.05$).

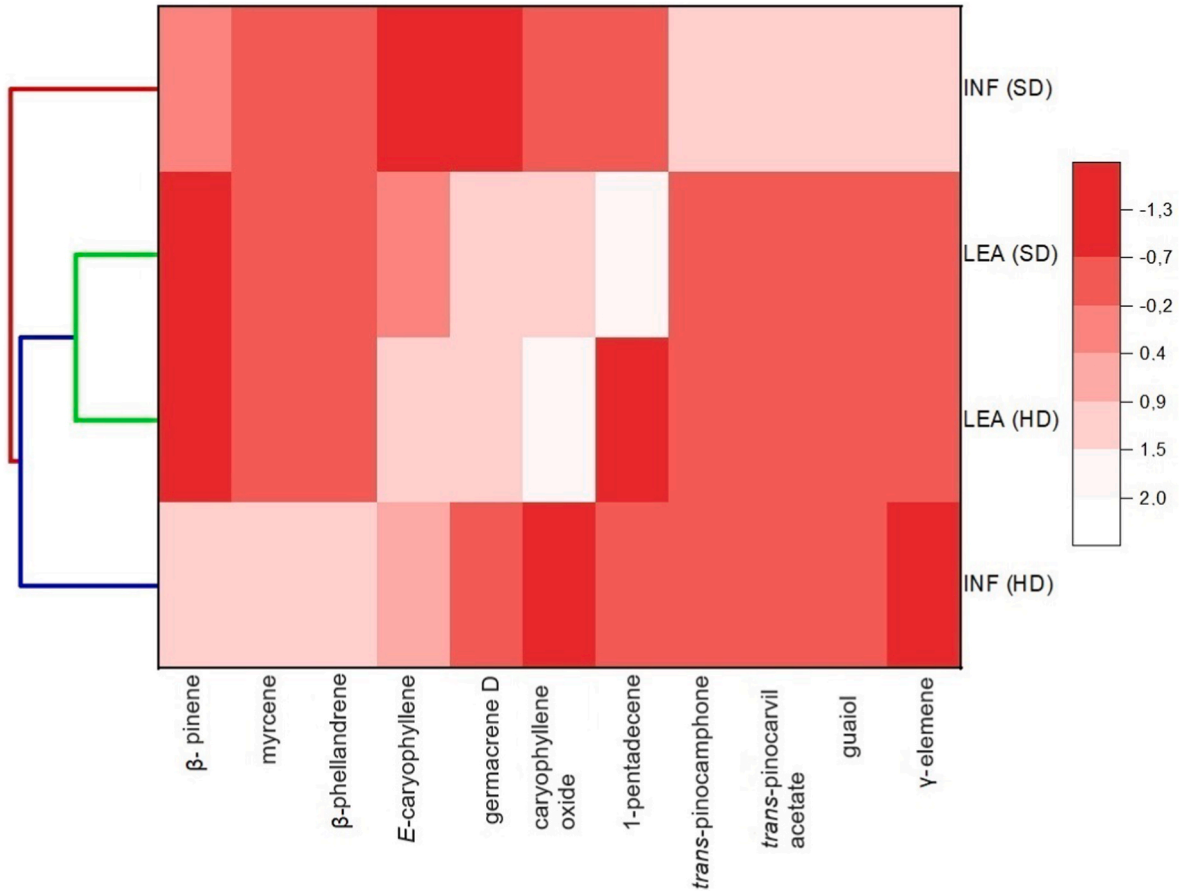

Fig. 6. Class grouping heat map in essential oils from *Acmella oleracea* samples.

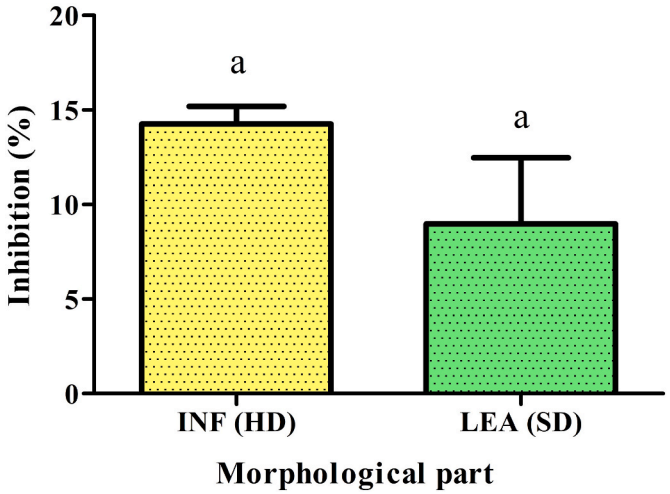

Fig. 7. Comparison of potential antioxidants between essential oil samples from inflorescences by hydrodistillation and leaves by steam distillation.

addition to demonstrating a considerable synergistic effect with the antineoplastic cisplatin, affecting the cell cycle of lung carcinomas through the increase of kinase enzymes and inducing apoptosis through the reduction of proteins expressed by the BCL-2 regulatory gene with cancerous mutation (Ahmed et al., 2022).

Recurrent constituent caryophyllene oxide in the leaves of *A. oleracea* is another anti-cancer agent of great importance, presenting toxicity against tumor lines of hepatocellular carcinoma, using ferroptosis, a new type of cell death induced by the accumulation of reactive species of oxygen in the intracellular medium and by the peroxidation of iron-containing lipids in carcinogenic cells (Xiu et al., 2022).

Caryophyllene oxide molecules also have antimetastatic potential through the passage of matrix metalloproteinases (MPMs) that allow tumor cells to migrate to surrounding tissues through the lymphatic blood systems, depleting nutrients and inducing angiogenesis, thus demonstrating the importance of this bioactive in metastasis (Jo and Kim, 2022).

The two main monoterpenes, myrcene and β-pinene, identified in the essential oils of *A. oleracea* also show mechanisms of action against cancer cells; myrcene has an apoptotic effect on lung carcinoma cells through mitochondria-mediated cell death signaling and increased oxidative stress (Bai and Tang, 2020), and β-pinene induced apoptosis in carcinomas of the oral epithelium, with pathways of action not yet well established in the literature (Machado et al., 2022).

Table 2
Cytotoxic activity of *Acmella oleracea* essential oils.

Oil sample	IC50 (μg/mL)			
	AGP-01 (Gastric Ascites)	SK-MEL-19 (Melanoma)	A549 (Lung Carcinoma)	HEK-293 (Embryonic Kidney)
Inflorescences (HD)	$5.31^{1.04}$ (5.16–5.90)	$6.43^{0.86}$ (6.12–7.25)	>25	5.57 (4.50–5.83)
Leaves (SD)	$6.23^{0.82}$ (6.10–6.85)	$7.45^{0.6}$ (7.13–7.71)	$13.39^{0.38}$ (12.408–13.854)	5.14 (4.98–5.74)
Doxorubicin (μM)	0.25 (0.19–0.33)	0.30 (0.05–0.28)	0.86 (0.69–0.97)	0.90 (0.74–1.05)

>25: cytotoxic activity greater than 25 μg/mL; SI = IC50 in HEK-293 cells/IC50 in a cancer cell (superscript value).

Scientific literature reports of isolated trials with the sesquiterpene hydrocarbon germacrene D and with the 1-pentadecene alkene against tumor lines in vitro or in vivo have not been identified; it is only known that they are two molecules of ecological importance, 1-pentadecene being preliminarily indicated as an active repellent against beetles (*Tribolium castaneum*) and germacrene D a pollination agent for Asteraceae species, in addition to being also indicated as a possible anticancer agent in synergy with other substances contained in volatile oils (Đukić et al., 2021; El-Sherei et al., 2014; Li et al., 2021).

It is possible to observe that the main constituents of the inflorescences and leaves of *Acmella oleracea* have different mechanisms of action against cancer cells. It is noted that in the inflorescences, the combination of *E*-caryophyllene, myrcene, germacrene D and β-pinene is more active against the malignant cells of gastric ascites and melanoma and is less toxic against the healthy embryonic kidney lineage (HEK-293), while in the leaves, the combination of caryophyllene oxide, *E*-caryophyllene, germacrene D and 1-pentadecene is more toxic against healthy cells but less active against the lung cancer cell line.

4. Conclusions

The *Acmella oleracea* volatiles obtained by the two extraction methods present chemical variability, forming different chemical profiles among the extraction methods and the morphological parts. The inflorescences obtained by hydrodistillation present a better essential oil yield, marked by *E*-caryophyllene and myrcene, than steam distillation.

The antioxidant capacity of *A. oleracea* essential oil is minimally influenced by the extraction method or morphological part. Likewise, the cytotoxic activity between the oils is similar. *A. oleracea* is a source of bioactive molecules against cancer cells, generating perspectives for developing antineoplastic herbal medicines using the plant, requiring further studies on synergisms and/or molecular antagonisms in in vivo models.

CRediT authorship contribution statement

Lucas Botelho Jerônimo: Formal analysis, Writing – original draft. **Paulo Vinicius Lima Santos:** Formal analysis. **Laine Celestino Pinto:** Formal analysis. **Jamile Silva da Costa:** Formal analysis. **Eloisa Helena de Aguiar Andrade:** Formal analysis. **William N. Setzer:** Writing – review & editing. **Joyce Kelly do Rosário da Silva:** Writing – review & editing. **José Augusto Carvalho de Araújo:** Formal analysis. **Pablo Luis B. Figueiredo:** Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Abbreviations list

GC/MS	Gas chromatography-mass spectrometry
GC-FID	Gas chromatography–flame ionization detector
$RI_{(C)}$	Calculated Retention Index
$RI_{(L)}$	Literature Retention Index
EO	Essential oil
INF	Inflorescences
LEA	Leaves
HD	Hydrodistillation
SD	Steam distillation
MTT	3-(4,5-dimethyl-2-thiazole)-2,5-diphenyl-2-H-tetrazolium bromide
ROS	Reactive oxygen species
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethylsulfoxide

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