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Original Article

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Abstract

Artemisia Judaica (Shih Balady, family Asteraceae) is one of the common species of the genus Artemisia that grows in Sinai, Egypt. It is widely used in traditional medicine by Bedouins. In recent years, a lot of research has been done on this plant extract after it has proven effective as an antimicrobial, anticancer, and antioxidant. The present study aims to give (1) preparation of alcoholic extract using ethanol to conduct some laboratory tests to detect the active compound found in the plant with a simplified description of the properties of the Artemisia Judaica plant and the places of growth in Egypt. (2) Evaluation of antioxidant activities by DPPH• assay for the aerial parts of A. Judaica, which was collected from the desert of (Wadi Hagoul) in Egypt. The total phenolic, and flavonoid contents were measured in the plant of interest. (3) Characterization of volatile components using GC/MS analysis.

1. Introduction

For thousands of years, plants have been used to treat various human diseases and hence they are considered important sources of many bioactive compounds. The use of natural products and supplements of medicinal herbs has increased over the past three decades with more than 80%of people worldwide depending on them for some part of primary healthcare (Ekor, 2013).

Artemisia Judaica is a perennial herb that is growing abundantly in North Africa and Middle Eastern countries and Egypt. A variety of aerial parts of the plant (leaves, stems, flowers) were brought from Wadi Hagoul, located on the road linking Cairo and Suez, during its growing season in late winter where the growth and spread of the plant in that region were very dense compared to the amount of its spread in the desert areas or the areas extending on the coast. This type of phytochemicals has been used traditionally in Egyptian medicine for the treatment of gastrointestinal diseases. In addition; many Artemisia species have been used in Iranian traditional medicine as an anti-infectious, anti-bacterial, gastric tonic, digestive and stomachic. Major medicinal effects of Artemisia that have been reported include improved vision, cardiovascular health, capillary strength, connective tissue structure, and enhanced immune system functions, as well as decreased risk of atherosclerosis. (Nezhadali, 2010).

The plant is characterized by being a small annual or perennial herb, with a height of about 70 cm. It branches out from the base with straight ribbed green branches with a rough texture. It is evergreen. The color of its internal parts at the break is greenish-yellow. The plant has a beautiful aromatic smell and bitter taste. flowers yellowish-green tubular, leaves are fragrant and have a soft texture. The upper leaves are smaller than the lower leaves and are prolific. (Jiao et al. 2023).

The stem of the type that contains the anthelminthic substance "santonin" is characterized by its red color in the early growth period, while the stem of the type that does not contain this substance is green in color, and when the plant's growth is complete, the color of the stem in both types turns brown. The Artemisia plant is characterized by its tolerance to a wide range of different temperatures, as it grows in desert and semi-desert areas. The sandy, salty soil is ideal for growing Artimsia, where it grows well.

Artemisia Judaica is grown as a winter crop in October, either by seed or root cuttings. The methods of its cultivation vary according to the regions in which it is grown. In Pakistan, the country where it is available in the largest quantity, it is grown on the slopes of the mountains in the manner of terraces or terraces on which the seeds are sown in hollows at distances of 30 cm. The crop is not irrigated but is left to grow on rainwater. In the regions of the Arab world, wormwood is rarely

grown as a crop except in experimental stations, agricultural research centers, or colleges of pharmacy. The wild plant is collected from its natural areas of spread in desert lands. (Hayat et al. 2009).

Clinically active ingredients: The floral tops and unopened ripe flowers contain artemisia oil and santonin. In flowers, the percentage of oil ranges from 0.3 to 2.5%, and it is separated from it by steam distillation, and it has an almost camphor odor. Santonin is considered the main component of the plant, and it is a crystalline substance that has little solubility in water. It turns yellow when exposed to light, and if it continues to be exposed to light, it turns into a brown resinous substance. The amount of "santonin" varies according to the type of artemisia, the place of its cultivation, and the time of collection (Zhao et al. 2023). Owing to its contents of many bioactive compounds such as; flavonoids, lactones, phenolic, essential oil, and sesqui-terpenoids (Salih et al. 2023). Artemisia Judaica extract was used in many traditional medicines as an anthelmintic, antispasmodic, anti-rheumatic, and antibacterial agent. In recent years, the anti-bacterial and anti-cancer activity of medicinal herbs are highly investigated. (Tit et al. 2023).

The present study is focused on the description Simplified of the properties of the Artemisia Judaica plant and places of growth in the Arab Republic of Egypt and shows some of the active compounds and antioxidant effects of ethanolic extract of Artemisia Judaica leaves and stem.

2. Materials and Methods

2.1. Plant extraction

Fresh aerial parts of the Artemisia Judaica (AJ) plant were gathered from Wadi Hajul, in Egypt during late winter 2022. The plant extract was prepared accordingly to the procedure described by Williamson (Williamson et al. 1996), with slight modifications. The plant material (2000 g) was air-dried at room temperature. The herb was ground to obtain a moderately coarse powder using mechanical mortar grinding and pass through a sieve to obtain the fine powder, the resultant dry powder was soaked immediately in ethyl alcohol (96%) at room temperature (100 g/1L) for 9 days (in 3-day intervals), the organic phase filtered through filter paper, then the filtrate was concentrated under vacuum using the rotatory evaporator and percolated several times till exhaustion. The yield (a dark-green viscous residue) was chilled in the refrigerator at 5 °C until use. The tested solution was freshly prepared every day by dissolving 2 g of the viscous residue in 10 ml of 1% solution of tween-80 in distilled water. To identify the chemical constitution of the aerial parts from Artemisia Judaica ethanolic extract gas chromatography-mass spectrometry (GC-MS).

2.2. Characterization of the volatile components

2.2.1. GC-MS spectroscopic analysis

A sample of 1 µL of each oil was diluted to 10.0 µL with GC grade n-hexane and then analyzed by GC-MS (Varian Chrompack CP-3800 GC/MS, Saturn, Netherlands) equipped with a DB-5 GC-column (5% diphenyl, 95% dimethyl polysiloxane, 30 m × 0.25 mm i.e., 0.25-µm film thicknesses). In the MS detector, an electron ionization mode of 70 eV was used. The temperature in the MS source was set at 180 °C. The temperature column was also programmed from 60 °C for 1 min (isothermal) to 246 °C at a constant rate of 3°C/min, with the lower and upper temperatures being held for 3 min. Helium was used as a carrier gas (0.9 mL/min). Quantitative analysis was performed using GC/FID instrument (Hewlett – Packard HP-8590, USA) equipped with optima-5 column (5% diphenyl, 95% dimethyl polysiloxane; 30 m × 0.25 mm, 0.25 µm film thickness) and a split-split less injector (split ratio 1:50). The temperature of the oven was increased from 60 °C to 250 °C at a rate of 3 °C/min, then held constant at 250°C for 5 min. The temperatures of the injector and detector were maintained at 250 °C and 300°C, respectively. The relative peak areas were used to calculate the relative percent concentrations of the detected compounds. A standard solution of C8–C20 n-alkanes mixture was analyzed under the same chromatographic conditions.

2.2.2. Identification of the volatile oil constituents

Chemical constituents of the different essential oils were identified by comparison of their mass spectra with those found in the database library (Wiley 275 library, New York, USA) or with authentic compounds and then confirmed by comparison of their Kovats retention indices with those of authentic compounds and/or literature (ĎÚRANOVÁ et al. 2023).

2.3. Determination of phytochemical contents

2.3.1. Total phenolic assay

The total phenolic content was determined using the Folin–Ciocalteu method as described by (Attard 2013). Briefly, the procedure consisted of mixing 10 µL of sample/standard with 100 µL of Folin-Ciocalteu reagent (Diluted 1: 10) in a 96-well microplate. Then, 80 µL of 1M Na_2CO_3 was added and incubated at room temperature (25 °C) for 20 min in the dark. At the end of incubation time, the resulting blue complex color was measured at 630 nm. Data are represented as means ± SD.

2.3.2. Total flavonoids assay

The total flavonoid contents were determined using the aluminum chloride method as described by (Kiranmai et al. 2011), with minor modifications to be carried out in microplates. Briefly, 15 µL of sample/standard was placed in a 96-well microplate, then, 175 µL of methanol was added followed by 30 µL of 1.25 % $AlCl_3$. Finally, 30 µL of 0.125 M $C_2H_3NaO_2$ was added and incubated for 5 min. At the end of incubation time, the resulting yellow color was measured at 420 nm. Data are represented as means ± SD.

2.4. Antioxidant activity DPPH• assay

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay was carried out according to the method

of (Boly et al. 2016). Briefly; 100µL of freshly prepared DPPH reagent (0.1% in methanol) was added to 100 µL of the sample in 96 wells plates (n= 6), and the reaction was incubated at room temp for 30 min in dark. At the end of incubation time, the resulting reduction in DPPH color intensity was measured at 540 nm. Data are represented as means ± SD according to the following equation: percentage inhibition= ((Average absorbance of blank-average absorbance of the test)/(Average absorbance of blank))*100.

2.5. Statistical analyses

Data were subjected to statistical analysis using the statistical software program Prism (Graph Pad. Prism. v6). Means and the standard error of the mean (SEM) for each variable were estimated. Differences between the means of different groups were carried out using one-way ANOVA analysis. The results were expressed as mean ± standard error of the mean value (Mean±SEM).

3. Results

3.1. Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was applied for the estimation of the volatile components of A. Judaica extract. The results as shown in Table 1 that are inferred from the chromatogram in Fig. 1, indicated that twenty-four volatile components were characterized. The analyzed sample was intended to investigate its volatile components based on the different recorded retention times, composition percentages, projected molecular structures, molecular weights, and molecular formulas. The characterization of the chemical constituents was projected based on the database library (Wiley 275 library, New York, USA) or with reliable molecules after verification by comparison of their Kovats retention indices with those of consistent molecules and/or literature data. The average peak of a definite molecule was matched with the total abundant area percentage of all molecules in the chromatogram. The objective of the GC/MS analysis is to precisely investigate the effective molecules that establish its perpendicular commercial and traditional utility as herbal medicine. Thus, the results will be a benefit for the deliberation of the biological or therapeutic implications of the extracted plant issues. The sample was screened for 39.76 min, while the total composition percentage of the volatile molecules was determined at 100.01%. The chromatogram in Fig. 1 referred to the relation between the relative abundance of the volatile components recorded at different retention times.

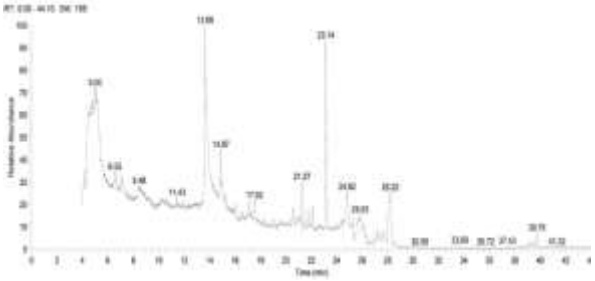

Fig. 1. GC-MS Chromatogram of the volatile components of the extracted Artemisia Judaica.

The most abundant molecules as appeared from the GC/MS analysis corresponding to the total area percentage of the total composition were found as methyl palmitate (23.15 min; 19.03%); 6-pentyl-2H-pyran-2-one (13.64 min; 18.24%); and 3,7-dimethylocta-1,6-diene (28.21 min, 9.69%) (Fig. 2). Methyl palmitate is related to the category of fatty acids and lipids, 6-pentyl-2H-pyran-2-one is related to hydrocarbons and 3,7-dimethylocta-1,6-diene "Dihydromyrcene" is related to the terpenes category.

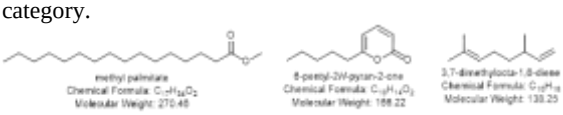

Fig. 2. The structures of the major abundant molecules of A. Judaica extract as obtained by GC/MS.

In addition, the second order of the volatile molecules with high abundant percentages of compositions was found as oleic acid (24.81 min, 5.04%) "fatty acid"; 2,4-di-tert-butylphenol (14.87 min; 5.60%) "hydrocarbon", methyl (E)-2-((Z)-2-(tert-butyl)pent-2-en-1-ylidene) cyclohexane-1-carboxylate (28.11 min, 4.59%) "hydrocarbon", and (E)-3,7,11,15-tetramethyl-hexadec-2-en-1-ol (21.27 min, 4.71%) "terpene". Other volatile components were characterized with reasonable area percentage relative to the abundance of the total composition percentage and were recognized as methyl (2E,5E)-undeca-2,5,10-trienoate (3.25%), (E)-hexadec-1-en-1-yl acetate (2.03%), tridecanoic acid (2.05%), methyl tetradeca-3,4-dienoate (2.58%), 3-methoxyhexa-1,3,4-triene (1.84%), 6-ethyl-5-hydroxy-7-methoxy-naphthalene-1,4-dione (2.20%), (6E,9E)-18,18-dimethoxyoctadeca-6,9-diene (2.06%), Viridiflorol (1.96%) (Jeleń et al. 2014), 2-(hydroxy-methyl)-5-(2-hydroxypropan-2-yl)cyclohex-2-en-1-ol (3.44%), and 2,6,6-trimethylcyclohex-2-ene-1-carbaldehyde (2.52%).

As shown in **Figure 3**, the interpreted components were classified into three different categories as fatty acids or their lipids, hydrocarbons, and terpenes. The most abundant category was presented by fatty acids and lipids at 40.67%, followed by hydrocarbons at 37.02%, and terpenes at 22.32%. The fatty acids and lipids class include eleven components with the major abundance for methyl palmitate (23.15 min; 19.03%), and oleic acid (24.81 min, 5.04%). Hydrocarbons included eight volatile components with the majority of abundance for 6-pentyl-2*H*-pyran-2-one (13.64 min; 18.24%), 2,4-di-*tert*-butylphenol (14.87 min; 5.60%), and methyl (*E*)-2-((*Z*)-2-(tert-butyl)pent-2-en-1-ylidene) cyclohexane-1-carboxylate (28.11 min, 4.59%). The terpenes category presented five components with the majority of abundance for 3,7-dimethylocta-1,6-diene (28.21 min, 9.69%), and (*E*)-3,7,11,15-tetramethyl-

hexadec-2-en-1-ol (21.27 min, 4.71%). Our findings are in agreement with the literature reports regarding the characterization of the chemical constitutes of the extracted plant with the investigation of fatty acids (**Albasher *et al*. 2020**), hydrocarbons (**Al-Wahaibi *et al*. 2020**), and terpenes (**Nofal *et al*. 2009**).

2. Phytochemical analysis

Table 1. The GC/MS results of the volatile components characterized from A. Judaica extract.

Entry	Chemical name	Classification	Retention time (RT, min)	Molecular Weight	Molecular formula	Area %
	Fatty acids and lipids					
1	methyl (2*E*,5*E*)-undeca-2,5,10-trienoate	Ester of fatty acid	6.54	194.27	$C_{12}H_{18}O_2$	3.25
2	methyl (2*E*,6*E*)-2,6-dimethyl-8-oxoocta-2,6-dienoate	Ester of fatty acid	7.05	196.25	$C_{11}H_{16}O_3$	1.57
3	methyl (9*E*,12*E*,15*E*)-octadeca-9,12,15-trienoate	Ester of fatty acid	7.12	292.46	$C_{19}H_{32}O_2$	1.56
4	(*Z*)-hexadec-11-enoic acid	Fatty acid	16.04	254.41	$C_{16}H_{30}O_2$	1.27
5	(*E*)-hexadec-1-en-1-yl acetate	Ester of fatty acid	21.49	282.47	$C_{18}H_{34}O_2$	2.03
6	methyl palmitate	Ester of fatty acid	23.15	270.46	$C_{17}H_{34}O_2$	19.03
7	oleic acid	Fatty acid	24.81	282.47	$C_{18}H_{34}O_2$	5.04
8	tridecanoic acid	Fatty acid	25.29	214.35	$C_{13}H_{26}O_2$	2.05
9	methyl tetradeca-4,6-diynoate	Ester of fatty acid	25.85	234.34	$C_{15}H_{22}O_2$	0.89
10	methyl tetradeca-3,4-dienoate	Ester of fatty acid	26.16	238.37	$C_{15}H_{26}O_2$	2.58
11	cyclohexyl octanoate	Ester of fatty acid	39.76	226.36	$C_{14}H_{26}O_2$	1.40
	Hydrocarbons					
12	**3-methoxyhexa-1,3,4-triene**	Hydrocarbon	6.62	110.16	$C_7H_{10}O$	1.84
13	6-pentyl-2*H*-pyran-2-one	Hydrocarbon	13.64	166.22	$C_{10}H_{14}O_2$	18.24
14	2,4-di-*tert*-butylphenol	Hydrocarbon "phenol"	14.87	206.33	$C_{14}H_{22}O$	5.60
15	(*E*)-2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-2-enal	Hydrocarbon	14.98	206.33	$C_{14}H_{22}O$	1.03
16	2-((benzyloxy)methyl)-5-methylfuran	Hydrocarbon	15.30	202.25	$C_{13}H_{14}O_2$	1.46
17	6-ethyl-5-hydroxy-7-methoxy-naphthalene-1,4-dione	Hydrocarbon	20.55	232.24	$C_{13}H_{12}O_4$	2.20
18	(6*E*,9*E*)-18,18-dimethoxyoctadeca-6,9-diene	Hydrocarbon	21.75	310.52	$C_{20}H_{38}O_2$	2.06
19	methyl (*E*)-2-((*Z*)-2-(tert-butyl)pent-2-en-1-ylidene)cyclohexane-1-carboxylate	Hydrocarbon	28.11	264.41	$C_{17}H_{28}O_2$	4.59
	Terpenes					
20	(1*aR*,4*S*,4*aS*,7*R*,7*aS*,7*bS*)-1,1,4,7-tetramethyl-2,3,4*a*,5,6,7,7*a*,7*b*-octahydro-1*aH*-cyclopropa[e]azulen-4-ol "Viridiflorol"	Sesquiterpene	17.08	222.37	$C_{15}H_{26}O$	1.96
21	2-(hydroxymethyl)-5-(2-hydroxypropan-2-yl)cyclohex-2-en-1-ol	Monoterpene	17.51	186.25	$C_{10}H_{18}O_3$	3.44
22	(*E*)-3,7,11,15-tetramethylhexadec-2-en-1-ol	Diterpene	21.27	296.54	$C_{20}H_{40}O$	4.71
23	2,6,6-trimethylcyclohex-2-ene-1-carbaldehyde	Monoterpene	22.11	152.24	$C_{10}H_{16}O$	2.52
24	3,7-dimethylocta-1,6-diene "Dihydromyrcene"	Monoterpene	28.21	138.25	$C_{10}H_{18}$	9.69
						Σ= 100.01

The phytochemical contents such as phenolic, and flavonoid components were quantitively analyzed to estimate the chemical profile of the extracted aerial parts of A. Judaica. The analyses tended to quantitively estimate the contents of secondary metabolites. Many studies have been reported to investigate the phytochemical contents of the extracted A. Judaica (Acheuk et al. 2017; Allam et al. 2019; Nasr et al. 2020). The result of the phenolic test revealed that the extracted A. Judaica presented a furious source of phenolic contents with 244.42±1.65 µg gallic acid per mg of the extracted sample and 61.10 µg gallic acid equivalent per 1 mg of the extracted sample (absorbance read at 630 nm is 0.997). The analysis of the flavonoid contents revealed the presence of flavonoid contents with 67.33±2.87 µg rutin equivalent per mg of the extracted sample (absorbance read at 420 nm is 0.525). Figure 4 presented the standard curves required for the quantitative calculations of the phenolic, and flavonoid contents using standard reagents such as gallic acid for phenolics, and rutin for flavonoids. The reasonable contents of the phytochemicals will be the major factor that controls the antioxidant mechanism in the reactions between the free radicals in the solution of DPPH, and the reactive species in the plant extract such as phenolics, and flavonoids (Aftab, and Vieira, 2010).

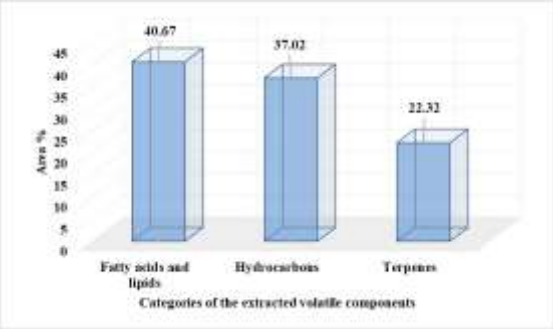

Fig. 3. The characterized volatile components from A. Judaica extract by GC-MS analysis.

3.3. Effect of BSG on HepG2 cell migration

To evaluate the ability of both extracts to inhibit HepG2 cell migration, the size of the induced wound was monitored over 33 hr, till closure of the induced wound in control cells. The data illustrated a no marked effect in groups treated with BSG extracted with 1st technique except for cells treated with EC50 of BSG (0.261 µg/ml). Contradictory, the data showed a marked effect of BSG extracted by 2nd technique in inhibiting cell motility in a dose dependent manner (fig.3).

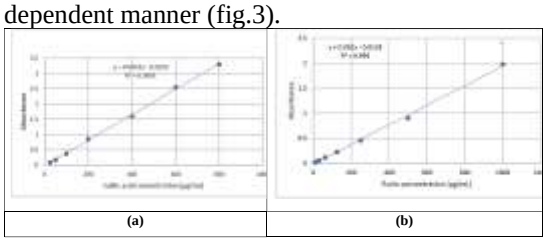

Fig. 4. The standard curves for the calculation of the data of the phytochemical constituents. (a) Gallic acid standard curve for phenolic contents. (b) Rutin standard curve for flavonoid contents.

3.3. Antioxidant activity

The antioxidant activity of the extracted issues of A. Judaica was assessed by applying DPPH• free radical assay. Trolox was used in this test as a standard reference. The results revealed that the extracted plant displayed comparatively moderate antioxidant activity with an IC50 value of 125.1±6.7 µM relative to the result of Trolox (IC50= 28.87±1.24 µM). As shown in Figure 5, the percentages of the scavenging activities of the tested extract and the reference standard were determined at different concentrations of the samples (µg/mL). The increase in the sample concentration will result in an increased % of inhibition of the tested samples.

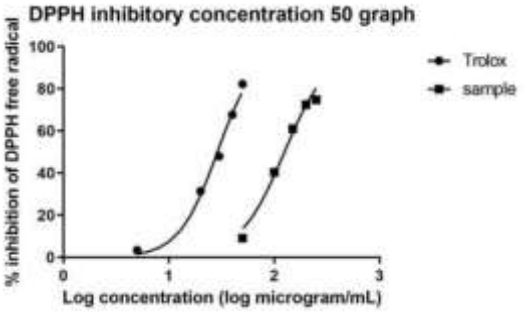

Fig. 5. The relation between % inhibition of DPPH free radical scavenging versus different concentrations of the extracted plant issues (log concentration µg/mL).

The mechanism of the antioxidant activity, in general, is controlled by the ability of the sample constitutes to trap the free radicals of DPPH• in the solution, hence terminating the reaction. Particularly, the sample efficiency for this consequence of trapping the free radicals is completely controlled by the formation of stable free radicals by the components in the extracted plant. Thus, reactive species shuch as phenolics, and flavonoids "form stable free radicals by resonance factor" and can trap the free radicals of DPPH• in the solution. In addition, reactive species such as fatty acids, lipids, oxygenated hydrocarbons, and terpenes that have reactive oxygen species have also great impacts on the mechanism of the free radical reactions. Or findings regarding the antioxidant impacts of the phenolic components are in agreement with the literature reports (Allam et al. 2019). Also, several recent studies have proven the good effects of methyl palmitate (Hamed et al. 2020) and 6-pentyl-2H-pyran-2-one (Zhang et al. 2017) as antioxidant molecules with good efficiency, as they contain oxygen atoms that can inhibit free radicals in solution and terminate the free radical reaction.

Conclusion

The objective of the present work is to characterize the volatile components by GC/MS analysis, phytochemical components, and antioxidant activity of the ethanol extract of the leaves and stem issues of Artemisia Judaica. The results verified that the plant extract is rich in fatty acids, lipids, hydrocarbons, and terpenes. The methyl palmitate (19.03%), and 6-pentyl-2H-pyran-2-one (18.24%) are the most abundant components. The volatile components were classified into three basic categories (fatty acids & lipids, oxygenated

hydrocarbons, and terpenes), in which fatty acids and lipids presented the most abundant components (40.67%). The plant extract also verified good contents of phenolics, and flavonoids in the phytochemical analysis. The plant extract in addition revealed good antioxidant activity (IC50= 125.1±6.7 μM).

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