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Article

Phytochemical profile, antimicrobial and antioxidant activity of extracts of *Gnaphalium oxyphyllum* and *Euphorbia maculata* native to Sonora, Mexico

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Abstract:

The use of synthetic chemical compounds to preserve foods or treat diseases of bacterial origin is limited because they can cause health damage. Therefore, the food and livestock industries seek natural strategies to preserve foods and preserve the health of animals intended for human consumption. In this sense, some extracts of plant from Sonora, Mexico could be an alternative due to the great diversity of plants and the fact that some of them are traditionally used to treat diseases. On the other hand, there are few studies that support the biological activity of ethanolic extracts of *Gnaphalium oxyphyllum* (E1) and *Euphorbia maculata* (E2). In this study, phytochemical content was determined by spectrophotometry, antimicrobial activity was determined by agar diffusion and antioxidant activity was evaluated by ABTS, DPPH and FRAP. The results showed that the E1 and E2 extracts had total phenols, total flavonoids, flavones and flavonols, total flavanones and dihydroflavonols, as well as total tannins, total chlorogenic acid and total polysaccharides. In addition, both extracts showed higher antimicrobial activity against *Listeria monocytogenes* ATCC 19115, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Salmonella enterica* serovar Typhimurium ATCC 14028 when 1 mg ml^{-1} was used ($P<0.05$). In addition, they presented antioxidant activity by the methods of ABTS, DPPH and FRAP. Therefore, the antimicrobial and antioxidant potential of these plants represents a natural alternative to control some Gram-positive and Gram-negative bacteria in the livestock industry, as well as for food preservation.

Key words: *Gnaphalium oxyphyllum*, *Euphorbia maculata*, Antimicrobial activity, Antioxidant, Natural alternative, Food industry.

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Introduction

Consumer interest in avoiding foods with synthetic chemical compounds has increased due to their potential harm to health. In the scientific community, there is a growing interest in the search for natural strategies for food preservation; as well as in livestock production to prevent recurrent diseases of domestic animals[(1)]. Some of the natural alternatives that have been considered in the food industry and in veterinary medicine include the use of probiotics, bacteriocins, antioxidants and chemical compounds derived from plants[(1,2)]. Considering the above, plant extracts have advantages, since, in some of them, their antioxidant and antimicrobial potential has been shown[(3)]. In this context, Mexico is one of the countries with great plant biodiversity worldwide, ranks fourth with approximately 31,000 different species of plants. Of these, it is estimated that more than 3,350 are used in the preparation of traditional medicine treatments[(4)], and in some of

these plants, it has been seen that they have the same active ingredient that is used in the preparation of commercial drugs[5]. However, the studies carried out with plants native to Mexico are incipient since phytochemical compounds and their biological activity lack scientific evidence of their activity. In addition, there are few scientific studies that have characterized the antimicrobial activity of plants native to Sonora, Mexico[6-9] and those that evaluate their antioxidant activity are very few. Particularly, *Gnaphalium oxyphyllum* is a plant known as "Gordolobo" in Sonora and is endemic to northwestern Mexico. It is traditionally used in the treatment of some conditions, such as flu, asthma, cough, fever, bronchitis, swelling, stomach diseases, wounds, low back pain, in the prevention of malaria and urinary tract problems derived from prostatitis and neuritis. As well as for angina pain, antipyretic and to lower blood pressure[10,11]. In addition, its ability to inhibit the growth of some pathogenic bacteria and fungi has been demonstrated[11,12]. However, the antimicrobial and antioxidant activity of the ethanolic extracts of this plant has not been evaluated[11]. On the other hand, *Euphorbia maculata* is a plant native to northwestern Mexico, locally known as "Golondrina". It is traditionally used to treat stomach upsets and eye problems, in addition, in Chinese medicine it is used in blood disorders such as hematuria, hemoptysis, epistaxis and hemafecia, for the treatment of anthrax and some wounds. However, the antimicrobial, antifungal and antioxidant activity has been poorly documented, and there are no studies that evaluate its antimicrobial potential in ethanolic extracts[13,14]. The evidence indicates that these plants are of high biological value, but they have been little studied and have not been harvested in Sonora, Mexico, so the biological activity of the plants can be compromised, because their phytochemical profile can vary depending on factors such as altitude, cultivation site, agronomic and environmental conditions in which they grow[15]. Therefore, and considering that plants can also be used as a food supplement[16], it is interesting to evaluate the nutritional value, antimicrobial, antioxidant activity and phytochemical profile of these plants grown in Sonora, Mexico.

Material and methods

Preparation of ethanolic extracts

The extracts were obtained from *Gnaphalium oxyphyllum* (E1) and *Euphorbia maculata* (E2), the plants were harvested at the Department of Agriculture and Livestock (DAG, for its acronym in Spanish) of the University of Sonora (DAG-UNISON). The stems and leaves of each plant were dehydrated at 34 °C in a hot air oven (Thelco, Precision Science, model 28, USA). The dehydrated plant material was then pulverized in a mill (Pulvex Mini 100, Mx) to a particle size of 100 microns. Subsequently, 100 g of the pulverized plant material was mixed with 100 ml of 99 % purity ethanol (Sigma-Aldrich, St. Louis MO) in an amber glass bottle and stored for 5 d[17]. Finally, the extracts were filtered with

Whatman No. 41 filter paper and the remaining alcohol in the plant material was evaporated. The yield was calculated by difference in weight of the plant material, and finally, the ethanolic extracts were stored at 4 °C in the dark.

Phytochemical profile of ethanolic extracts

The contents of total phenols and total flavonoids were quantified by the methodologies used by Al-Rifai *et al*[18] and the data were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAEq. g^{-1}) for total phenols, while for total flavonoids, the data were expressed as milligrams of quercetin equivalent per gram of extract (mg QEq. g^{-1}). The content of flavones and flavonols, as well as the content of total flavanones and dihydroflavonols were determined following the methodologies proposed by Popova *et al*[19] and the results were expressed as milligrams of hesperetin equivalent per gram of extract (mg HEq. g^{-1}). The total tannin content was determined by the methodology reported by Price and Butler[20] and the results were expressed in milligrams of catechin equivalent per gram of extract (mg CEq. g^{-1}), while the chlorogenic acid content was quantified following the methodology reported by Griffiths *et al*[21], where the results were expressed as milligrams of chlorogenic acid per gram of extract (mg CA g^{-1}). Finally, the total polysaccharide content was determined by the methodology reported by DuBois *et al*[22] and the data were expressed as milligrams of glucose equivalent per gram of extract (mg GEq. g^{-1}). Calibration curves were used in all determinations and absorbances were read on a spectrophotometer (Spectro Max MD, EU).

Antimicrobial activity of ethanolic extracts

The Gram-positive bacteria *Listeria monocytogenes* ATCC 19115 and *Staphylococcus aureus* ATCC 25923, and the Gram-negative bacteria *Escherichia coli* ATCC 25922 and *Salmonella enterica* serovar Typhimurium ATCC 14028, from the Laboratory of Microbiology of the Department of Chemical-Biological Sciences of the University of Sonora, were used. The bacteria were reactivated in BHI (brain-heart infusion, BD Difco, Sparks, MD) broth culture medium, and two plates with BHI (brain-heart infusion, BD Difco, Sparks, MD) agar were used for each bacterium. Four sterile discs of Whatman No. 41 filter paper of 6 mm in diameter were then placed on each plate and 20 µL of ethanolic extract was added to each disc. Subsequently, the plates were incubated at 37 °C for 24 h and antimicrobial activity was measured in inhibition halos, where halos greater than 3 mm were considered as inhibition[23].

Physicochemical analysis of plants

The analytical methods of the AOAC[24] were used. Total solids were determined by the oven-drying method (990.19); ashes by the gravimetric method (945.46); crude fat by the ether extraction method (920.39); crude protein by the micro-Kjeldahl method (991.20) and moisture by numerical difference. The data were expressed in grams per 100 grams of dry matter (g 100 g^{-1}). Additionally, the pH was measured with an electronic potentiometer (Hanna Instruments pH 211, Cluj, Romania).

Determination of minerals in plants

The amount of calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) from each plant was determined on a model 5000 flame atomic absorption spectrophotometer (PerkinElmer®, CT, USA)[25], while the phosphorus concentration (P) was determined by a colorimetric method of ammonium molybdovanadate in a model 3030 spectrophotometer (PerkinElmer®, CT, USA)[26]. The results were expressed in grams per 100 grams of dry matter (g 100 g^{-1}).

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition method

The concentrations of each extract were adjusted to 0.1, 0.5, 1.0 and 2.0 mg ml^{-1}, then 1 ml of each extract was mixed with 2 ml of a methanolic solution prepared with the 2,2-diphenyl-1-picrylhidrazyl ($DPPH^{\bullet}$) radical at a concentration of 1 x 10^{-4} M. The mixture was left to react for 16 min in the dark at room temperature. Finally, the absorbance was measured in a spectrophotometer (Spectro Max MD, EU) at a wavelength of 517 nm and the $DPPH^{\bullet}$ solution was used as control[27].

2,2'-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) radical inhibition method

A mixture in 1:1 ratio (v/v) of the 2,2'-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) radical (7 mM) and potassium persulfate (4.95 mM) was prepared and kept in the dark for 16 h at room temperature. The mixture was then diluted with methanol until an absorbance of 1 to 1.5 was obtained. Next, 0.1 mL of each extract was mixed at different concentrations (0.1, 0.5, 1.0 and 2.0 mg mL^{-1}) with 3.9 ml of the $ABTS^{\bullet+}$

solution. Finally, absorbance values were measured on a spectrophotometer (Spectro Max MD, EU) at a wavelength of 734 nm. The $ABTS^{\bullet+}$ solution was used as control[27].

Ferric-reducing antioxidant power (FRAP) method

The FRAP reagent was prepared by mixing 10 parts of sodium acetate buffer solution (300 mM) at a pH of 3.6 with one part of TPTZ (10 mM) (2,4,6-tri (2-pyridyl)-s-triazine) and one part of $FeCl_3$ hexahydrate (20 mM). Then, 0.2 ml of extract was mixed with 3.8 ml of FRAP reagent and the mixture was left to react for 30 min at 37 °C. Finally, absorbance was measured on a spectrophotometer (Spectro Max MD, EU) at a wavelength of 593 nm[27].

Statistical analysis

A completely randomized one-way experimental design was used at 95 % confidence with three repeats per treatment. The mean comparison test was performed by Tukey-Kramer at a significance level of 0.05 and the Pearson correlation coefficient was performed with 95 % confidence. The statistical software used was NCSS version 11.

Results and discussion

The results of the proximate analysis of the plants showed in E2 higher moisture, less total solids and ashes with respect to the E1 plant ($P<0.05$) (Table 1), while no differences were found in the amount of fat and protein of both plants ($P>0.05$). The results in the amount of moisture, total solids and ashes of this study are similar to those found in wild edible plants from Bangladesh and in plants consumed by native tribes of India[28,29]. The variability in the results can be attributed to biological, environmental factors or the age of the plants[30]. In addition, the moisture content of plants could depend on the humidity and temperature of the environment, as well as on the harvest time of the plant, while the ash content refers to the inorganic part of the plant, which includes salts (phosphates, sulfates, chlorides) and some minerals (sodium, potassium, calcium, magnesium, iron and manganese), and their amount may depend on the mineral content of the soil where the plant is established[31]. Likewise, plant lipids are mainly found in the form of triacylglycerols, phospholipids, galactolipids and sphingolipids, and their amount is usually very low in plants[30,32,33], which coincides with what is found in the E1 and E2 plants, and with what was reported in plants from Bangladesh and India[28,29,30]. Although this study found no difference in the amount of lipids between E1 and E2 plants ($P>0.05$),

it has been reported that the variation in lipid content may depend on the species and the environmental conditions in which the plant is found[30,34].

Table 1: Proximate analysis of E1 and E2 plants

Plant	Moisture	Total solids	Ashes	Fat	Protein
E1	61.53 ± 2.24^{a}	38.47 ± 2.23^{a}	5.74 ± 0.63^{a}	2.12 ± 0.12^{a}	11.98 ± 0.85^{a}
E2	68.22 ± 1.22^{b}	31.78 ± 1.13^{b}	4.56 ± 0.73^{b}	2.05 ± 0.15^{a}	11.17 ± 0.73^{a}

E1= *Gnaphalium oxyphyllum*; E2= *Euphorbia maculata*; data expressed in g 100 g^{-1} of dry matter.
^{ab} Different literal indicates difference between the data in the same column (P<0.05).

Likewise, the protein content of E1 and E2 plants was similar to that found in green leafy vegetable plants[35], and it has been reported that the amount of protein in plants may depend on the physiological state, age, environmental conditions and nutrients present in the soil[36]. On the other hand, the content of P, Na and K was higher in plant E1 with respect to plant E2 (P<0.05), while the content of Mg was higher in plant E2 (P<0.05), and no differences were found in the content of Ca between both plants (P>0.05) (Table 2). These results are similar to those found in plants from Iran and India[30,37], and it has been reported that the variability in the mineral content of the plants could be related to the mineral composition of the soil, as well as to the geographical area where the plants are established[38].

Table 2: Mineral content of the E1 and E2 plants

Plant	Ca	P	Mg	Na	K
E1	1.12 ± 0.13^{a}	0.33 ± 0.05^{b}	0.21 ± 0.03^{a}	1.63 ± 0.03^{b}	1.23 ± 0.05^{b}
E2	1.15 ± 0.14^{a}	0.25 ± 0.03^{a}	0.55 ± 0.02^{b}	1.22 ± 0.33^{a}	1.07 ± 0.33^{a}

E1= *Gnaphalium oxyphyllum*; E2= *Euphorbia maculata*; data expressed in g 100 g^{-1} of dry matter.
^{ab} Different literal indicates difference between the data in the same column (P<0.05).

The results of the antimicrobial activity showed that the E1 and E2 extracts inhibited the growth of the four evaluated pathogens (P<0.05) and the greatest inhibition occurred when the pathogens were exposed to 1 mg ml^{-1} of each extract (Table 3). On the other hand, the E1 extract was more efficient in inhibiting *S. aureus* and *L. monocytogenes* with respect to the E2 extract (P<0.05), while both extracts did not show differences in inhibition against *E. coli* and *S. enterica* serovar Typhimurium. Similar results were reported in hexane extract from *Gnaphalium oxyphyllum* flowers, which was able to inhibit the growth of *S. aureus*, *B. cereus*, *E. coli* and *S. enteric* serovar Typhimurium, in addition, the methanolic extract of these flowers inhibited the growth of *S. aureus* and *B. cereus*, while the hexane extract of the leaves of *Gnaphalium oxyphyllum* had antimicrobial activity against *S. aureus*, *B. cereus* and *E. coli*[10]. Another study showed that hexane and chloroform extracts from the aerial part of *Gnaphalium oxyphyllum* inhibited the growth of *S. aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *E. coli* and *Candida albicans*[12]. In addition, it has been reported that the hydroethanolic extract from leaves of *Euphorbia maculata* showed antimicrobial activity against *S. aureus*[39], while methanolic extracts from other plants of the genus

Euphorbia showed antimicrobial activity against *S. aureus*, *Bacillus megaterium*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *E. coli*, *Pseudomonas aeruginosa* and *Candida albicans*, *Candida glabrata*, *Epidermophyton* spp. and *Trichophyton* spp.[40], which is similar to what was found in this study. The antimicrobial activity of the extracts is associated with cell wall damage and decrease in cytoplasmic pH in Gram-positive and Gram-negative pathogenic bacteria, in addition, the antimicrobial activity of plants is attributed to a wide variety of secondary metabolites, such as tannins, alkaloids, phenolic compounds, flavonoids, xanthones and hyperforin[41,42].

In this context, the results showed that the content of total phenols, total flavonoids, flavones and flavonols, total chlorogenic acid and total polysaccharides was higher in the E1 extract with respect to E2 ($P<0.05$) (Table 4), while no difference was found in the content of total flavanones and dihydroflavonols, and total tannins between the extract E1 and E2 ($P>0.05$). On the other hand, the E1 extract had a pH of 4.18 and the E2 extract had a pH of 5.26, while the yield of the extracts of these plants varied from 12.24 to 15.68 %, respectively. Similar results were reported by Rojas *et al*[12], who reported yields of 1.76 % and 5.64 % in hexane and chloroform extracts from the *Gnaphalium oxyphyllum* plant. The differences in the yield of this plant may be due to the polarity of the type of solvent that was used for the extraction of phytochemical compounds. In addition, the variation in the pH of plant extracts may be due to the acidic nature of the compounds present, such as flavonoids, tannins, benzoic acid, oleic acid, stearic acid, lignoceric acid, among others[43]. In this sense, it has been reported that, in *Gnaphalium oxyphyllum* and other species of the genus *Gnaphalium*, the presence of diterpenoids, flavonoids, acetylene compounds and carotenoids was found[10,44], while in *Euphorbia maculata*, the presence of polyphenols and flavonoids has been reported[45,46,47], and in other species of *Euphorbia*, the presence of sesquiterpenes, diterpenes, sterols, flavonoids and other polyphenols has been reported[14].

Table 4: Phytochemical profile, pH and yield of E1 and E2 extracts

Phytochemicals	**Extracts**	
	E1	**E2**
Total phenols, mg GAEq. g^{-1}	181.62 ± 0.04^{a}	173.22 ± 0.06^{b}
Total flavonoids, mg QEq. g^{-1}	114.30 ± 0.05^{a}	103.42 ± 0.04^{b}
Flavones and flavonols, mg HEq. g^{-1}	110.15 ± 2.35^{a}	98.33 ± 2.44^{b}
Total flavanones and dihydroflavonols, mg HEq. g^{-1}	23.68 ± 1.89^{a}	21.58 ± 2.16^{a}
Total tannins, mg CEq. g^{-1}	8.21 ± 0.16^{a}	7.92 ± 0.67^{a}
Total chlorogenic acid, mg CA g^{-1}	33.14 ± 1.01^{a}	28.78 ± 1.11^{b}
Total polysaccharides, mg GEq. g^{-1}	257.92 ± 2.19^{a}	236.59 ± 2.16^{b}
pH of the extract	5.26	4.18
Extract yield, %	12.24	15.68

E1= *Gnaphalium oxyphyllum*; E2= *Euphorbia maculata.*

[ab] Different literal indicates difference between the data in the same column ($P<0.05$).

The antioxidant activity of plants is associated with the presence of vitamins, phenolic compounds, carotenoids, among others. Particularly, in this study, it was found that the E1 and E2 extracts showed greater antioxidant activity by the DPPH and FRAP methods when they were evaluated at a concentration of 1 mg ml^{-1} (P<0.05) (Table 5), while in the ABTS method, the greater antioxidant activity of the E1 and E2 extracts was observed when they were evaluated at a concentration of 0.5 mg ml^{-1} (P<0.05). To date, there is no universal method to measure the antioxidant activity of plants because the chemical reagents used by these methods do not react the same with the different types of antioxidants present in plants. For example, $ABTS^{\bullet}$ reacts with lipophilic and hydrophilic antioxidants, which allows it to be applicable in aqueous and lipid systems, while $DPPH^{\bullet}$ can only be dissolved in an organic medium so it reacts well with low polar or non-polar compounds, and both methods are based on the ability of antioxidants to neutralize reference free radicals ($ABTS^{\bullet}$ and $DPPH^{\bullet}$). Therefore, in the E1 and E2 extracts, there could be more phenolic compounds of a hydrophobic nature than of a hydrophilic nature. Likewise, the FRAP method is based on the ability of antioxidants to reduce the ferric ion to the ferrous state and measures the total antioxidant capacity of the sample, which shows the presence of phenolic compounds in the E1 and E2 extracts[48]. These results of antioxidant activity are similar to those reported by Luyen *et al*[49], who observed high antioxidant power in methanolic extracts, ethyl acetate and aqueous extracts of *Euphorbia maculata* using the ORAC method, while other studies have shown the antioxidant activity of plants of the genus *Euphorbia*, where Basma *et al*[50] evaluated the antioxidant activity of leaves, stems, flowers and roots of *Euphorbia hirta* using DPPH and FRAP techniques. In addition, Upadhyay *et al*[51] found antioxidant activity in *Euphorbia hirta* leaves by the DPPH and FRAP methods, while Zhang *et al*[52] reported antioxidant activity in *Euphorbia lathyris* stems, roots, seed and seed cover using the DPPH and FRAP methods.

Table 5: Antioxidant activity of E1 and E2 extracts

Extract	DPPH (mg QEq. g^{-1})		ABTS (mg QEq. g^{-1})		FRAP (mg FeSO4Eq. g^{-1})	
(mg ml^{-1})	E1	E2	E1	E2	E1	E2
0.1	0.028±0.001[a]	0.025±0.003[a]	0.008±0.0001[a]	0.005±0.0002[a]	0.062±0.002[a]	0.054±0.004[a]
0.5	0.127±0.004[b]	0.128±0.006[b]	0.035±0.0002[b]	0.032±0.0002[b]	0.084±0.004[b]	0.072±0.006[b]
1	0.146±0.004[c]	0.140±0.004[c]	0.037±0.0002[b]	0.035±0.0003[b]	0.099±0.003[c]	0.095±0.005[c]
2	-	-	-	-	-	-

E1= *Gnaphalium oxyphyllum*; E2= *Euphorbia maculata;* (-)= not quantifiable.

[ab] Different literal indicates significant difference between the data of the same column and between the treatments of the same method (P<0.05).

Finally, the ABTS, DPPH and FRAP methods are commonly used to measure the antioxidant activity of phenolic compounds because of the high correlation that can be found between them. Therefore, it has been suggested that it is not necessary to apply more than one method to measure the antioxidant activity; however, it has been reported that this is not always the case, due to the nature of the phytochemicals present in plants[27]. In this study, a high correlation coefficient (R^2) was found between the DPPH,

ABTS and FRAP methods (DPPH *vs* ABTS= 0.99; DPPH *vs* FRAP= 0.93; ABTS *vs* FRAP= 0.88), which confirms the presence of antioxidant phenolic compounds found in E1 and E2 extracts and shows the accuracy of the methods used.

Conclusions and implications

The extracts of *Gnaphalium oxyphyllum* and *Euphorbia maculata* showed the presence of the phytochemicals: total phenols, total flavonoids, flavones and flavonols, total flavanones and dihydroflavonols, total tannins, total chlorogenic acid and total polysaccharides. In addition, both extracts had antimicrobial activity against Gram-positive and negative pathogenic bacteria, as well as antioxidant activity by the DPPH, ABTS and FRAP methods. Therefore, the extracts from plants native to Sonora, Mexico, *Gnaphalium oxyphyllum* and *Euphorbia maculata*, represent a natural alternative in the food and livestock industry to reduce the use of synthetic chemical compounds.

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Table 3: Antimicrobial activity of E1 and E2 extracts against Gram-positive and negative bacteria

	Gram positive				**Gram negative**			
	S. aureus		***L. monocytogenes***		***E. coli***		***S. typhimurium***	
CONC	**E1**	**E2**	**E1**	**E2**	**E1**	**E2**	**E1**	**E2**
0.1	8.43 ± 0.42^{Da}	6.10 ± 0.52^{Ca}	7.50 ± 0.20^{Ca}	4.32 ± 0.31^{Ba}	3.00 ± 0.70^{Aa}	2.30 ± 0.21^{Aa}	2.50 ± 0.15^{Aa}	2.50 ± 0.12^{Aa}
0.5	12.10 ± 0.61^{Eb}	10.13 ± 0.42^{Db}	9.50 ± 0.36^{CDb}	8.11 ± 0.43^{Cb}	5.50 ± 0.70^{Bb}	4.20 ± 0.32^{ABb}	3.50 ± 0.20^{Ab}	3.50 ± 0.14^{Ab}
1	16.00 ± 0.32^{Fc}	14.00 ± 0.36^{Ec}	13.24 ± 0.43^{Dc}	10.34 ± 0.41^{Cc}	8.50 ± 0.70^{Bc}	8.10 ± 0.34^{Bc}	5.52 ± 0.40^{Ac}	6.52 ± 0.22^{Ac}
2	16.22 ± 0.28^{Ec}	14.10 ± 0.51^{Dc}	13.53 ± 0.38^{Dc}	$10.40\pm0.36C^{c}$	8.55 ± 0.70^{Bc}	8.30 ± 0.41^{Bc}	5.54 ± 0.32^{Ac}	6.54 ± 0.32^{Ac}
3	16.31 ± 0.53^{Ec}	14.23 ± 0.39^{Dc}	$13.56\pm0.41D^{c}$	$10.48\pm0.38C^{c}$	8.57 ± 0.70^{Bc}	8.35 ± 0.42^{Bc}	5.56 ± 0.45^{Ac}	6.55 ± 0.31^{Ac}

CONC= concentration of extracts (mg mL^{-1}); E1= *Gnaphalium oxyphyllum*; E2= *Euphorbia maculata*; data expressed in mm of inhibition halo.

Different uppercase literal indicates significant difference between data in the same row and different lowercase literal indicates significant difference between data in the same column ($P<0.05$).