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Phenolic composition and antioxidant capacity of leaves infusions of *Annona muricata* L. (Annnonaceae) from two regions of Mexico

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Abstract:

Annona muricata L. (soursop) is a fruit of great economic importance and one of the plants most used in alternative medicine for its phytochemical composition. This work evaluates the phenolic composition and antioxidant capacity of infusions from *Annona muricata* leaves of three different ecotypes from two regions of Mexico. The infusions were characterized for total soluble phenolics, flavonoids, condensed tannins, total anthocyanins, and the antioxidant capacity was evaluated using different assays. The infusions of the analyzed ecotypes showed differences in polyphenol content and antioxidant activity. A positive relation between phenols and antioxidant capacity was observed for ABTS, FRAP, and ORAC. Epigallocatechin gallate (1.22-6.49 mg/g), rutin (3.47-8.73 mg/g), ellagic acid (5.25-14.13 mg/g), epicatechin (0.83-7.57 mg/g), and chlorogenic phenolic acids (4.42 mg/g) were quantified by HPLC. The infusion of leaves of the different soursop ecotypes showed differences in phenolic composition, antioxidant capacity and phenolic acids.

Keywords: *Annona muricata* L.; Antioxidant capacity; Ecotypes; FTIR; Growing regions; HPLC; Infusions; Tropical fruits

1. Introduction

Soursop (*Annona muricata* L.) belongs to the Annonaceae family (Fig. 1) (Villarreal-Fuentes et al., 2020). Besides, it is one of the plants widely used in alternative medicine due to the benefits it offers for human health, with the leaves being the most commonly utilized part (Makuasa et al., 2020). Traditionally, it is prepared as an infusion (Coria-Téllez et al., 2019).

Soursop leaf infusions are used for their bioactive properties in the treatment of viral, parasitic, inflammatory, and even cancer-related diseases (Rustanti et al., 2020; Mohammadhosseini et al., 2023). These properties are primarily attributed to alkaloids, phenols, and acetogenins (Coria-Téllez et al., 2018). Studies have been reported in the literature that determine these compounds in aqueous extracts (Coria-Téllez et al., 2019; Iyanda-Joel et al., 2019) using mainly spectrophotometric (Makuasa et al., 2020), spectroscopic (Rustanti et al., 2020) and chromatographic methods (Balderrama-Carmona et al., 2020). However, there are few studies available that link the growing region to differences in bioactive compound content. Syed Najmuddin et al. (2017) analyzed the differences in phenol and flavonoid content, as well as the antioxidant capacity of aqueous extracts from soursop leaves collected from seven different

Figure 1. *Annona muricata* L leaves. a) Leaves from the Guerrero region and b) Leaves from the Nayarit region.

locations in Malaysia.

In Mexico, four varieties of soursop (*A. muricata*) were registered in 2022 under the names GUANAY-1, GUANAY-2, GUANAY-3, and FRANFRON-22. These varieties are listed in the National Catalog of Plant Varieties maintained by the Ministry of Agriculture and Rural Development (,SADER, 2023; ,SNICS, 2023). In Venezuela, Leal (2015) pointed out that there were at least two types of soursop. Sacramento et al. (2003) identified three selections of soursop from the region of Bahia, Brazil. Pinto et al. (1994) reported three types of Colombian soursop; however, previous studies did not analyze the differences in the leaves of *A. muricata* and focused solely on the fruit of the plant.

In Mexico, soursop is regarded as a priority fruit resource of significant economic importance, specifically the *Annona* species, with a national production of 30,790 t (,SADER, 2023; Villarreal-Fuentes et al., 2020), distributing in the Pacific - from Sinaloa to Chiapas - and in the Gulf of Mexico - from Veracruz to Yucatan - where there are their main cultivation areas (Escobedo-López et al., 2019). However, there are currently no reports indicating the impact of cultivation regions and the selection of soursop on the content of bioactive compounds and antioxidant capacity in its leaves of Mexican origin. Thus, this research aims to evaluate the phenolic composition and antioxidant capacity of infusions and methanol extracts of three selections of soursop leaves from two regions of Mexico for the differentiation of functional phytochemicals.

2. Experimental

2.1 Plant material

Annona muricata L. leaves from three ecotypes of the species (Greñuda, Intermedia, and Lisa) were collected from two Mexican states (Fig. 2), Nayarit (Tepic, 21° 30′ 0″ N 104° 54′ 0″ W, accession numbers UAN01020719, UAN02020719, and UAN03020719) and Chiapas (Cantón el Carmen, 14° 46′ 24″ N 92° 13′ 24″ W, accession number XAL M006166, MEXU633636, and HEM12591), in the months of July-August 2020. The selection of the plant material was made considering their healthy phytosanitary characteristics. The leaves were freeze-dried (Labconco, LYPH Lock 4.5, USA), milled (electric mill, NutriBullet®, Los Angeles, USA), and stored in closed plastic bags (Ziploc®) at room temperature in the absence of light until analysis.

2.2 Sample preparation

Tea bags designed for herbal teas were used for infusion preparation, with each bag containing 3.0 g of lyophilized soursop leaves. The infusions were prepared using 240 mL of boiling water and allowed to repose for 5 min (Coz-Bolaños et al., 2018). As a control and for comparative purposes, the polyphenolic compounds from soursop leaves

Figure 2. Collection sites of *Annona muricata* ecotypes. *This figure was created with DIVA-GIS 7.5 software using the coordinates of the sampling areas.

were extracted using water/methanol (ME) solutions in ratios of 70:30 and 40:60 (v:v) for total soluble phenols and flavonoids, respectively. Absolute methanol was utilized for the extraction of tannins. The mixtures were stirred for 10 min and centrifuged at 5000 rpm for 10 min and the supernatant was filtered using Whatman filter paper (Deshpande et al., 1987; Singleton et al., 1999; Dewanto et al., 2002).

2.3 Determination of phenolic compounds

Total soluble phenols (TSP) were determined using Folin-Ciocalteu reagent (Hycel) and Na_2CO_3 (7.0% w/v) (Meyer) solution according to Singleton et al. (1999). Absorbance was read at 750 nm using a Multiskan GO (Thermo Fisher Scientific, 51119200, USA). A gallic acid (Fermont, Mexico) standard curve was elaborated using known concentrations of this phenolic standard, ranging from 0.020 to 0.200 mg/mL.The results were expressed as milligrams of gallic acid equivalents per milliliter (mg GAE/mL).

2.4 Determination of total flavonoids

The total flavonoid (TF) contents were determined using $NaNO_2$ (5.0% w/v) (J. T. Baker) solution, $AlCl_3$ (10% w/v) (J. T. Baker) and NaOH (1.0 M, J. T. Baker) following the procedure described by Dewanto et al. (2002). Absorbance was measured at 510 nm using a Multiskan GO. Catechin (Sigma-Aldrich®, USA) was used as a standard. The results were expressed as milligrams of catechin equivalents per milliliter (mg CE/mL).

2.5 Determination of condensed tannins

The methodology reported by Deshpande et al. (1987) was used for the determination of condensed tannins (CT). Briefly, a 0.5% vanillin (Sigma-Aldrich®, USA) solution prepared in acidified methanol with HCl (4.0%, J. T. Baker) was used. Absorbance was read at 500 nm using a Multiskan GO. To estimate the concentration of tannins, a calibration curve was obtained using known concentrations of catechin (Sigma-Aldrich®, USA). Condensed tannin contents were expressed as milligrams of catechin equivalents per milliliter (mg CE/mL).

2.6 Determination of total anthocyanins

The anthocyanin contents (AC) were studied according to the Abdel-Aal et al. (1999) method. The infusion was measured and adjusted to pH 1 with HCl (4.0 N, Meyer). The absorbance of the sample was read by a Multiskan GO at 535 nm. Total anthocyanin content per sample (mg/mL) was calculated as cyanidin 3-glucoside (Eq. (1)):

$$C = (A/\varepsilon) \times (vol/1000) \times MW \times (1/(sample\ \ wt)) \times 10^6 \quad (1)$$

Where C represents the concentration of total anthocyanin (mg mL^{-1}), A denotes the absorbance reading, ε is the molar absorptivity (for cyanidin 3-glucoside, $\varepsilon = 25{,}965$ cm^{-1}), vol indicates the total volume of infusion, and MW refers to the molecular weight of cyanidin 3-glucoside, which is 449 g/mol.

2.7 Antioxidant activity assays

The method of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) to evaluate the antioxidant activity (AA) was carried out according to Re et al. (1999). The $ABTS^+$ radical was prepared at a concentration of 7 mM using the ABTS reagent (Sigma-Aldrich®, USA) by dissolving it in a potassium persulfate (Fermont, Mexico) solution with a concentration of 2.45 mM, and the mixture was kept in the dark at 4 °C for 12 h before use. Subsequently, the $ABTS^+$ solution was diluted in phosphate buffer at pH 7.4 to obtain an absorbance of 0.7 ± 0.020 at 734 nm using a spectrophotometer (Hach DR3900, USA). The antioxidant capacity was calculated by comparing the absorbance to a Trolox standard curve. The result was expressed as the equivalent Trolox per milliliter (mmol TE/mL).

A solution of 1,1′-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich®, USA) was prepared at a concentration of 400 µM in metanol (Reasol) to determine the antioxidant capacity according to Dewanto et al. (2002) and the absorbance was read at 515 nm by a Multiskan GO. The antioxidant activity was determined using a Trolox (Sigma-Aldrich®, USA) calibration curve and expressed as Trolox equivalents per milliliter (mmol TE/mL).

The ferric reducing antioxidant power (FRAP) method reported by Benzie et al. (1996) also was used to determine antioxidant capacity. 2,4,6-tripyridyl-s-triazine (TPTZ) (10 mM) was prepared using a solution consisting of acetic acid (300 mM), sodium acetate buffer (300 mM) and HCl (40 mM). Also, an iron (III) chloride solution (20 mM) was prepared using distilled water. For the formation of the FRAP reagent, the buffer solutions of sodium acetate, TPTZ and iron chloride were mixed in a ratio of 10:1:1 (v/v/v), respectively and the absorbance was read at 595 nm using a Multiskan GO. The antioxidant activity was finally determined using a Trolox calibration curve and expressed as Trolox equivalents per milliliter (mmol TE/mL).

The oxygen radical absorbance capacity (ORAC) was determined according to Ou et al. (2001) using the 2,2′-azinobis-(2-amidinopropane) hydrochloride (AAPH) (Sigma-Aldrich®) as a peroxyl radical generator, trolox as a standard, and 3′,6′-dihydroxy-spiro [isobenzofuran-1(3H)9(9′)-xathen]-3-one (fluorescein) (Sigma-Aldrich®) as a fluorescent probe. There was an excitation wavelength of 493 nm and an emission wavelength of 515 nm using a fluorometer (TBS-380, Turnerbiosystems). The fluorescence was measured every minute for 2 h. The final ORAC values were calculated using the area under the decay curves and expressed as µmol TE/mL.

2.8 Determination of active compounds by HPLC-DAD

The active compounds determination was made using HPLC-DAD equipment according to Ramamurthy et al. (1992), using a reversed-phase separation on a Zorbax octadecylsilane (ODS)-C18 column (5 µm particle size, 15 cm * 4.6 mm i.d.). A Zorbax ODS-C18 guard column was also used. The mobile phase ran at 1.5 mL min^{-1} and consisted of solvent A: acetic acid/water (2:98 v/v) and solvent B: acetic acid/acetonitrile/water (2:30:68 v/v). During the

analysis, the solvent gradient was programmed from 10 to 100% B in A in 30 min. The UV detector was set to 280 nm, with an injection volume of 20 µL. All solvents used were filtered through 0.45 µm membranes. The different phenolic acids were quantified according to their equivalents by comparison with the retention time and absorption spectra of commercial phenolic compound standards (EGCG, Rutin, ellagic acid, epicatechin and chlorogenic acid) (Sigma-Aldrich®, USA) and reported as mg/g of dry sample.

2.9 FTIR-ATR spectroscopy analysis

A drop of the infusions and ethanolic extracts were placed on the FTIR-ATR (PerkinElmer Spectrum 100 FTIR) for the analysis and the tests were carried out at room temperature (25 ± 2 °C). The spectra were obtained from 16 points with a resolution of 4 cm^{-1} in the range of $4000 - 500$ cm^{-1} (Morales-Chávez et al., 2024).

2.10 Data analysis

All experiments were conducted in triplicate, and the data were presented as the means ± SD. Statistical analysis was performed using Minitab 17 for Windows (Minitab Statistical Software, USA). Significant differences between the samples were tested using two ways analysis of variance (ANOVA) followed by Tukey's HDS comparison test ($p \leq 0.05$), and Pearson's correlation coefficient and significance among antioxidant activity assays and phenolic compounds were calculated ($p \leq 0.05$, 0.01 and 0.001).

3. Results and discussion

3.1 Content of bioactive compounds in *A. muricata* leaves

The infusions from Chiapas (Fig. 3 a) showed the highest TF (0.193 mg CE/mL), AC (0.391 mg C3GE/g), and CT (0.679 mg EC/mL) contents. In contrast, the infusions from Nayarit showed the highest TSP (0.299 mg GAE/mL) content. On the other hand, the ME from Nayarit showed the highest TSP (0.336 mg GAE/mL), TF (0.291 mg CE/mL), and CT (0.629 mg CE/mL). In contrast, the ME from Chiapas demonstrated an AC value of 2.26 mg C3GE/g) which was found to be the highest.

For the ecotypes (Fig. 3 b), the 'Intermedia' ecotype infusions showed higher values of TSP, TF, AC, and CT, with values of 0.313 mg GAE/mL, 0.213 mg, 0.306 mg C3GE/g, and 0.586 mg CE/mL, respectively; however, AC did not present a significant difference in the three ecotypes infusions. Moreover, for the ME, the 'Intermedia' ecotype showed the highest value for TSP (0.369 mg GAE/mL) and CT (0.651 mg CE/mL), and the 'Lisa' ecotype presented the highest contents for TF (0.307 mg CE/mL) and AC (2.72 mg C3GE/g), but the TF content was not significant in the ME of the three ecotypes.

The results of the combination of regions and ecotypes for infusions, as shown in Fig. 3 c, indicate that the Nayarit region combined with the 'Intermedia' ecotype exhibited the highest value of TSP (0.370 mg GAE/mL.

However, the combination of the Chiapas region with the 'Intermedia' ecotype demonstrated elevated levels of TF (0.248 mg EC/mL) and AC (0.400 mg C3GE/mL). Additionally, the same Chiapas region paired with the 'Greñuda' ecotype yielded the highest CT (1.057 mg EC/mL) content.

For the ME (Fig. 3 d), the combination of the Nayarit region and the 'Intermedia' ecotype showed the highest values for TSF (0.419 mg GAE/mL), TF (0.352 mg CE/mL), and CT (0.896 mg EC/mL), while the combination of the Chiapas region and 'Lisa' ecotype presented a high AC (4.64 mg C3GE/g) content. The extraction yields for the infusions were in the range of 79.93-89.17% for TSP, 42.95-107.32% for TF, 8.31-38.83% for AC, and 26.22-298.58% for CT compared to those contained in the ME samples.

The TSP, TF, AC, and CT contents reported in this inves-

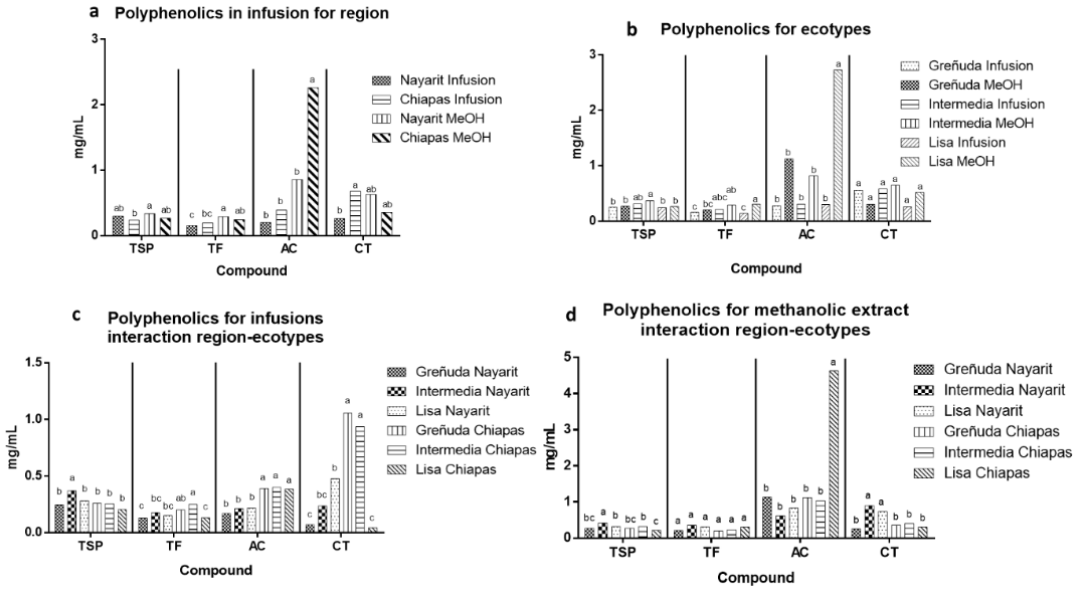

Figure 3. Polyphenolic content in the infusion and methanolic extracts of three *A. muricata* ecotypes leaves. For each value with a common letter are not significantly different (Tukey's test at $p \leq 0.05$).

tigation seem like other studies for *A. muricata* leaf infusions. Hardoko et al. (2018) reported TSP values of 0.253 mg GAE/mL in infusions prepared with dried *A. muricata* leaves. In this study, the TSP content per gram of *A. muricata* prepared as an infusion provided 23.92 mg GAE/g and 71.76 mg GAE/serving. This result is lower than that reported by Green et al. (2015), who determined a TSP content of 50.45 mg GAE/g and an equivalent of 100.91 mg GAE/serving in young soursop leaves. On the other hand, de Moraes et al. (2016) reported a concentration of TSP in leaves of 100.3 mg GAE/g using hot pressurized methanol, solubilizing 33% of the phenolic compounds present in the *A. muricata* leaves, and the extractions yield for this study was superior. While Syed Najmuddin et al. (2017) reported TF values between 0.065 and 0.191 mg CE/mL in leaves obtained from different locations in Malaysia.

Additionally, Hardoko et al. (2018) showed values of 0.014, 0.037, and 0.041 mg CE/mL in *A. muricata* infusions prepared with fresh, dried, and herbal tea leaves, respectively. All these studies report lower values than those obtained in the present research. Moreover, the TF content per gram of ME provided around 7.675 mg CE/g of *A. muricata* leaves. This result is in accordance with Orak et al. (2019), who determined a TF content between 2.62 and 81.32 mg CE/g of *A. muricata* leaves using different solvents for extraction. The AC content in the ME sample for the leaves was higher than that reported for other species. Otherwise, a 240 mL cup of infusion prepared with 3.0 g of leaves should provide 0.918 mg C3GE/serving or 3.82 mg C3GE/L. This concentration is lower than that reported by Lee et al. (2005) for other beverages, such as cranberry juice (13.6 mg C3GE/L) or strawberry juice (63.6 mg C3GE/L).The CT results were within the range reported by Hardoko et al. (2015) and Hardoko et al. (2018) in herbal tea infusions prepared with dried *A. muricata* leaves (0.181 mg CE/mL) and leaves prepared as green tea (0.519 mg CE/mL) and are also similar to the 0.199 mg CE/mL reported in *A. muricata* infusions prepared as black tea. Meanwhile, the CT content in the leaf of ME was found to range from 12.65 to 44.8 mg CE/g. This concentration was significantly higher than the values reported by Aguilar-Hernández et al. (2020) for the pulp, peel, seeds, and columella of *A. muricata*, which were 0.91, 8.21, 0.72, and 4.9 mg of proanthocyanidin equivalents per gram, respectively. The extraction of CT was conducted using an aqueous:methanol solvent system.

3.2 Antioxidant activity

The results of antioxidant activity (AA) in the infusions showed values of 2.05, 0.834, 1.7 and, 12.32 mM TE/mL for ABTS, DPPH, FRAP, and ORAC assays (Fig. 4a), respectively. Nayarit region presented a higher AA for ABTS, FRAP, and ORAC showing significant difference, while the Chiapas region showed the highest DPPH values, without significant difference. The same behavior was observed for the ME, where Nayarit presented the highest AA for the ABTS, FRAP, and ORAC assays, with 3.35, 1.72 and, 20.37 mM TE/mL, respectively, and Nayarit showed a higher antioxidant activity (2.56 mM TE/mL) using the DPPH assay. The infusion prepared with the 'Intermedia' ecotype showed higher values of AA (Fig. 4b), 2.18, 0.874, 1.93 and, 13.17 mM TE/mL for the ABTS, DPPH, FRAP, and ORAC assays, respectively, although the activity values quantified with the DPPH assay were not significantly different for the three ecotypes. The ME of 'Intermedia' ecotype exhibited higher values of antioxidant activity, measuring 3.49, 2.30, 1.99, and 18.61 mM TE/mL. However, the ORAC assay did not show significant differences among the three ecotypes. The highest values of antioxidant activity in infusions were presented with the combination of 'Intermedia' ecotype from the Nayarit region, with 2.37, 0.882, 2.36, and 15.85 mM TE/mL for the ABTS, DPPH, FRAP, and ORAC assays, respectively; however, the DPPH assay was not significantly different for the AA in all the combinations. The combination of ME with the 'Intermedia' ecotype from the Nayarit

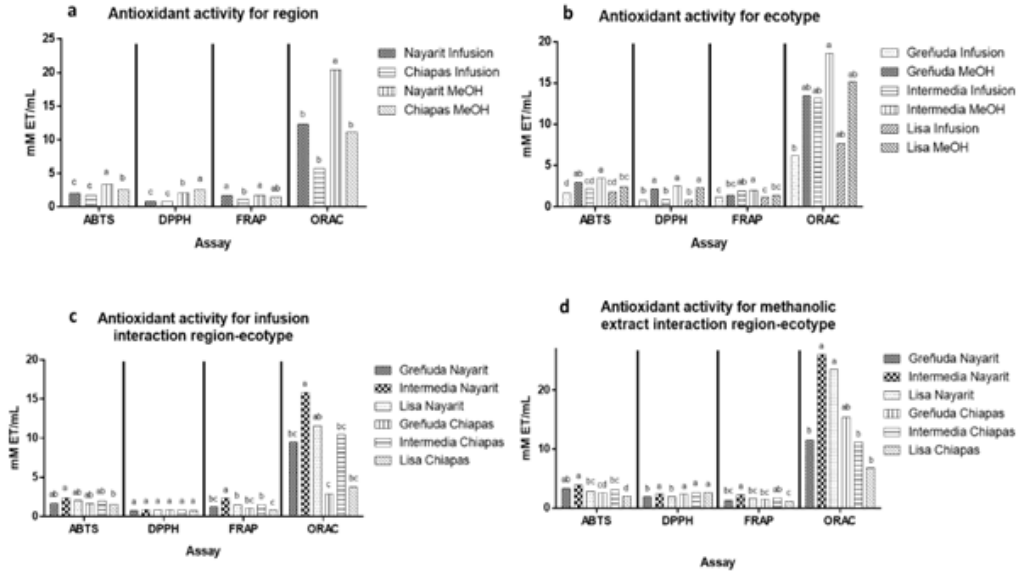

Figure 4. Antioxidant activity in the infusion and methanolic extracts of three *A. muricata* ecotypes leaves. For each value with common letters are not significantly different (Tukey's test at $p \leq 0.05$).

region showed higher AA values of 3.88, 2.44, and 26.04 mM TE/mL for the ABTS, FRAP, and ORAC assays, respectively, and higher DPPH activity was presented for the 'Intermedia' ecotype from the Chiapas region combination with 2.66 mM TE/mL. The extraction yields for AA in the infusions ranged from 51.35% to 76.73%, 23.24% to 45.20%, 71.33% to 105.35%, and 18.70% to 93.82% for the ABTS, DPPH, FRAP, and ORAC assays, respectively, when compared to those found in the ME samples.

Some authors have determined the AA of *A. muricata* leaves using the ABTS assay. For instance, Balderrama-Carmona et al. (2020) reported an AA of 17.93 mM TE/g in an aqueous extract. In this study, the AA of A. muricata infusion was expected to yield 164 mM TE/g and 492 mM TE/serving, respectively. Roduan et al. (2019) demonstrated an AA of 0.539 mM TE/mL and 0.700 mM TE/mL in aqueous and ME, respectively, achieving an extraction yield of 77% for the aqueous extract compared to that obtained from the ME. Orak et al. (2019) reported the AA of *A. muricata* leaves using different solvents, including hexane, dichloromethane, ethyl acetate, and methanol, among which the ME showed the highest activity (0.848 mM TE/g). Another technique to estimate the AA is the DPPH assay. In the present study, the AA measured by DPPH for the plant infusions was 61.6 mM TE/g. Additionally, the infusion and the ME of *A. muricata* presented DPPH scavenging potentials ranging from 22.8% to 25.9% and 57.6% to 70.4%, respectively. Balderrama-Carmona et al. (2020) reported a DPPH value of 2.97 mM TE/g for the aqueous extract of *A. muricata*, which is lower than the value found in this study. Furthermore, George et al. (2015) reported a DPPH scavenging potential of over 90% for the methanolic extract of *A. muricata*, while the aqueous extract demonstrated a scavenging potential of 40%, with an extraction yield of 44.4% in the aqueous fraction compared to that obtained in the ethanolic fraction. Justino et al. (2018) also reported DPPH scavenging potentials for *A. muricata* leaves ranging from 20% to 70% in the aqueous fraction, while the ethanolic fraction exhibited scavenging potentials of 20% to 90%. The AA measured by the FRAP assay in the present study for the infusion of *A. muricata* leaves was between 0.851 and 2.36 mM TE/mL, while that of ME ranged from 1.15 to 2.24 mM TE/mL, which is equivalent to 128.44 mM TE/g. The extraction yield obtained was 47.19%.

Syed Najmuddin et al. (2017) analyzed aqueous extracts of *A. muricata* leaves from different locations in Malaysia and reported a Fe^{3+} reduction potential that ranged from 4.17 to 20.45 µM Fe^{2+}/µg. Roduan et al. (2019) reported activities of 1.25 and 22.82 mg TE/g in aqueous and methanolic extracts, respectively, reaching an extraction yield of 5.47% for the aqueous extract. Another investigation of infusions made from *A. muricata* leaves sourced from a Brazilian region demonstrated a radical reducing potential ranging from 30.8 to 472.8 µg TE/mL (Cercato et al., 2020).

The AA by the ORAC method in the present study for the infusion of A. muricata was 2.88 to 15.85 mM TE/mL, while the ME ranged from 6.79 to 26.04 mM TE/mL, equivalent to 749.2 mM TE/g. The extraction yield obtained was 57.03%. Syed Najmuddin et al. (2017) reported the antioxidant activity of aqueous extracts of soursop leaves from 13 different regions of Malaysia, finding values between 94.66 and 254.7 μM TE/mL. Another investigation utilized solvents with varying polarities to assess the antioxidant capacity of soursop leaves, revealing that the ethyl acetate extract exhibited the highest antioxidant capacity at 3964 µM TE/g (Justino et al., 2018). Nawwar et al. (2012) prepared fractions from a hydroalcoholic extraction of soursop leaves, reporting that the fraction eluted with 60% aqueous methanol demonstrated an antioxidant capacity of 40.5 M TE/g which was significantly higher than the value reported for the hydroalcoholic extract (12.6 M TE/g).

Pearson's correlation coefficients (r) analysis between the TSP and AA in the infusions showed a positive and significant correlation with the ABTS, FRAP, and ORAC assays (Table 1). George et al. (2015) determined the correlations between phenol content and antioxidant activity in infusions of soursop leaves with the DPPH assay, reporting correlations of $r = 0.986$, while for the FRAP assay, the correlation found was $r = 0.995$. The correlations found in this research suggest the contribution of the abundant phenolic compounds of infusions to radical scavenging; however, the antioxidant activity can not only be attributed to phenols since there is evidence of the presence of alkaloids, vitamins, terpenoids, saponins, and essential oils in the leaves of soursop that also possess antioxidant activity (Mannino et al., 2020).

The antioxidant activity of soursop leaves depends mostly on the content of secondary metabolites that can reduce oxidative stress (Cercato et al., 2020). This biological activity was confirmed with the in vitro assays for infusions and methanolic extracts because both extracts were able to scavenge radicals from the ABTS and DPPH assays, which indicates that the compounds present in the extracts can act as donors of a proton of a hydrogen atom or by electron transfer and subsequently reduce the radicals to their stable form (Wołosiak et al., 2022). With the FRAP assay, soursop leaf extracts also showed the potential to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by electron transfer

Table 1. Pearson's correlation coefficients (r) between TSP, TF, AC, CT, and results of antioxidant assays of infusions of *A. muricata* leaves.

	TSP	TF	AC	CT
ABTS	0.801***	0.253	-0.371	0.013
DPPH	0.359	0.420	0.041	0.401
FRAP	0.929***	0.270	-0.475*	-0.075
ORAC	0.733***	0.099	-0.610**	0.202

*Correlation is significant at the following levels: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.00$. TSP: Total soluble phenolics; TF: Total flavonoids; AC: Anthocyanins content and CT: Condensed tannins.

from antioxidant compounds (George et al., 2015). On the other hand, with the ORAC assay, the extracts showed a transfer reaction of hydrogen protons to the peroxyl radicals generated by the decomposition of azo compounds (Syed Najmuddin et al., 2017). Antioxidant activity is the commonly evaluated biological activity in soursop leaves, using mostly *in vitro* assays that are based on the elimination of free radicals. The interest in quantifying these properties largely involves their ability to inhibit the oxidation of molecules by the effect of free radicals or reactive oxygen species (Coria-Téllez et al., 2018; Mannino et al., 2020). *In vitro* assays provide an approximation of antioxidant activity. However, it is plausible that the results may not be applicable to human biological systems because potentially active molecules might not have the opportunity to react fully. This limitation could be due to steric accessibility, as well as the functional groups or substituents of the molecules that may hinder access to the radicals (Roduan et al., 2019).

3.3 Determination of active compounds by HPLC-DAD

HPLC analysis showed the presence of different active compounds including phenolic acids and flavonoids, such as rutin, epigalatocatechin gallate (EGCG), and epicatechin. In addition, the compounds detected in infusions were also quantified (Fig. 5 and Table 2). Previous investigations have identified the presence of rutin, ellagic acid, epicatechin, and chlorogenic acid in soursop leaves at concentrations of 5.20, 3.07, 1.83, and 6.25 mg/g, respectively (Souza et al., 2018). Mancini et al. (2018) detected the presence of rutin (4.11 mg/g) and epicatechin (6.23 mg/g). Flavanones such as (+)-catechin, (−)-gallocatechin, and (−)-epicatechin gallate have also been quantified in *A. muricata* (soursop) extracts (George et al., 2015).

The differences in the presence of phenolic compounds between various ecotypes can be attributed to several factors, including the chemical structure of the phenolic compounds, their interactions with other components, and the composition of the solvent (Coz-Bolaños et al., 2018). These factors

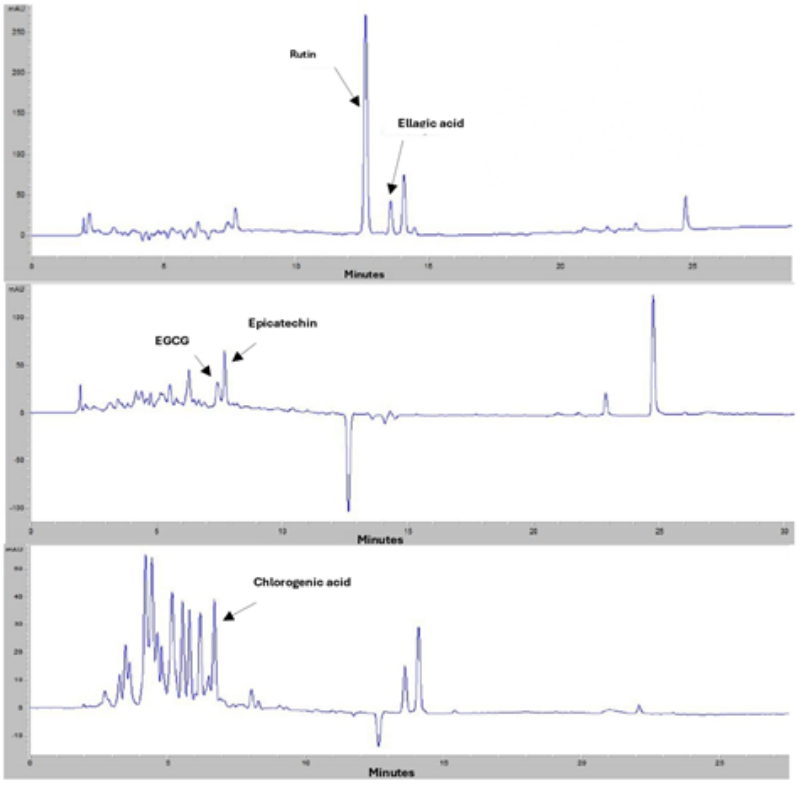

Figure 5. HPLC profile of leaf extracts of soursop ecotypes.

Table 2. Quantification of phenolic compounds by HPLC of leaf infusions of *A. muricata* ecotypes.

Compound	Retention time (min)	Chiapas			Nayarit		
		Greñuda	Intermedia	Lisa	Greñuda	Intermedia	Lisa
EGCG	7.22	1.22±0.08^{d}	4.20±0.40^{b}	1.23±0.08^{d}	3.04±0.24^{c}	6.49±0.28^{a}	2.42±0.18^{c}
Rutin	12.58	7.52±0.21^{b}	7.60±0.49^{b}	5.19±0.17^{c}	8.03±0.13^{ab}	3.47±0.08^{d}	8.73±0.14^{a}
Ellagic acid	13.81	11.66±1.05^{bc}	7.88±0.66^{d}	5.25±0.21^{e}	14.13±0.05^{a}	12.72±0.69^{ab}	10.23±0.03^{c}
Epicatechin	7.60	0.83±0.02^{c}	N.D.	1.20±0.04^{c}	4.56±0.23^{b}	7.57±0.11^{a}	4.29±0.34^{b}
Chlorogenic acid	6.91	N.D.	N.D.	N.D.	N.D.	4.42±0.12^{a}	N.D.

*The results are expressed as the mean ± standard deviation of three replicates. Mean values in the same row with a common letter are not significantly different (Tukey's test at $p \leq 0.05$,) where EGCG is epigallocatechin gallate.

can influence extraction efficiency and the stability of the molecules, as they may lead to oxidation or degradation of these components, ultimately affecting their biological activity (Orak et al., 2019). Furthermore, the HPLC results of *A. muricata* infusions revealed the presence of unidentified components, whose characterization could facilitate a more comprehensive evaluation of the secondary metabolites and their biological effects. In this sense, the beneficial effects of phenolic compounds have been documented, including antihyperglycemic and antiobesity activities (Ortsäter et al., 2012). Additionally, rutin has been reported to inhibit hyperperistalsis and exhibit antihyperglycemic, antilymphoma (Calzada et al., 2020), and anticancer (specifically prostate) activities (Yang et al., 2015). Furthermore, ellagic acid has demonstrated antioxidant, chemopreventive, and antiviral properties against human papillomavirus (Donne et al., 2017). Regarding epicatechin and chlorogenic acid, their antioxidant activities have also been established (Nawwar et al., 2012).

3.4 FTIR-ATR spectroscopy results

Fig. 6 shows the FTIR spectra of leaf infusions from different ecotypes of *A. muricata* in the 4000-650 cm^{-1} region. This technique allows the identification of the characteristic functional groups of the active compounds. The band in the region of 3700 to 3000 cm^{-1} corresponds to the stretching movements and tension vibrations characteristic of the substituted -OH functional groups in benzene rings, showing the presence of polyphenolic compounds (Daud et al., 2016). The peak at 2924 cm^{-1} corresponds to torsional movements of carboxylic acid bonds (Ibrahim et al., 2022). The peaks observed at 2853 cm^{-1} are attributed to the asymmetric stretching of the methyl substituents (-CH_3) of the lactone rings, while the band at 1740 cm^{-1} is attributed to the stretching vibrations (-C=O) of the γ-lactone ring of acerogenins (Hidalgo et al., 2019). The absorptions at 1656, 1513, and 1450 cm^{-1} correspond to the stretching vibration characteristics of the (-C=C-) bonds of flavonoids. The signals, observed in the 1410-1310 cm^{-1} region, are attributed to the bending movements of the bonds of the hydroxyl functional groups (-OH), belonging to phenols such as tyrosol (Grijalva-Verdugo et al., 2018). The bands between 1376 and 1320 cm^{-1} are assigned to the methyl groups (-CH_3) of alkanes and alkenes (Ibrahim et al., 2022). The peaks observed at 1286, 1248, and 1205 cm^{-1} are attributed to the out-of-plane torsional movements of the -OH functional group of carboxylic acids, the stretching vibration of the C-O bond, and the asymmetric stretching of C-O-C bonds, respectively, which belong to the pyran ring structure of tannins (Daud et al., 2016; Grijalva-Verdugo et al., 2018). The signals observed at 1161, 1071, and 1027 cm^{-1} correspond to the ester functional group (O=C-O), attributed to coumarins (Ibrahim et al., 2022). Finally, the 828-719 cm^{-1} bands are related with strong absorption attributed to the stretching vibrations of the C-O bonds in the α and β pyranose compounds found in anthocyanins (Grijalva-Verdugo et al., 2018).

4. Concluding remarks

The infusions of the analyzed ecotypes exhibited differences in polyphenol content and antioxidant activity, similar to the methanolic extract. A positive correlation between phenolic compounds and antioxidant capacity was observed using ABTS, FRAP, and ORAC assays, while a negative relationship between anthocyanins and antioxidant capacity was noted for the FRAP and ORAC assays. The phenolic compounds quantified included epigallocatechin gallate, rutin, ellagic acid, epicatechin, and chlorogenic acid, with chlorogenic acid being identified exclusively in the Nayarit 'Intermedia' ecotype. Additionally, the functional groups of phenolic acids and bioactive compounds were identified. The variability in the bioactive compounds and antioxidant capacities of soursop leaves may be attributed to the genotypic diversity present among the ecotypes of soursop plants, which are propagated by seeds through a halogamous process in an open pollination system. This work offers information for the development of products with pharmacological potential, such as antioxidants and nutraceuticals, with the understanding that there is variation among the collection regions and ecotypes of *A. muricata*.

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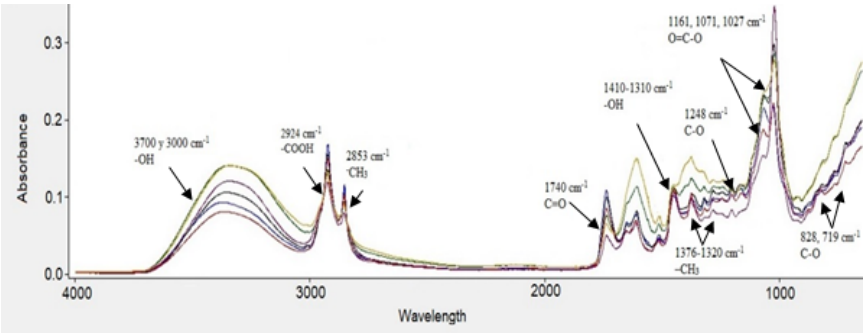

Figure 6. FTIR spectra in the mid-infrared region (4000 − 650 cm^{-1}) of the soursop leaf ecotypes of the Chiapas and Nayarit regions.

Authors contributions
Claudia Grijalva-Verdugo: Methodology and writing original draft; César Leobardo Aguirre-Mancilla, Salvador Horacio Guzmán-Maldonado and Juan Carlos Raya-Pérez: Supervise, conceived and planned experiments; Carlos Alberto Núñez-Colin: Statistical design and data analysis; Jesús Rubén Rodríguez-Núñez: Original idea, and technical project manager.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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

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