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Original Article

Preliminary phytochemical screening and antioxidant activity of *Annona deceptrix* (Westra) H. Rainer an endemic and endangered species of Ecuador

Triagem fitoquímica preliminar e atividade antioxidante de *Annona deceptrix* (Westra) H. Rainer, uma espécie endêmica e ameaçada de extinção no Equador

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Abstract

Annona deceptrix (Westra) H. Rainer belongs to the Annonaceae family which is known to have bioactivities such as antioxidant, antimicrobial, anticancer, anti-inflamatory, pesticide, among others. *A. deceptrix* ethanolic seed and leaf extracts obtained by three extraction methods (Soxhlet, ultrasound, and maceration) were tested for phytochemical and antioxidant activities. Phytochemical screening of plant extracts revealed the presence of catechins, triterpenes, tannins, alkaloids, flavonoids, amino acids, cardiac glycosides, anthocyanidins, reducing sugars, and saponins. Quantitative determination of total phenolic, flavonoid contents, and antioxidant activities of extracts was carried out using colorimetric methods. The highest total phenolic content was 58.14 and 54.08 mg GAE/g DW for Soxhlet extracts from leaves and seeds, respectively. The highest total flavonoid content was 5.03 and 4.42 mg QE/ g DW for macerated and ultrasound-assisted extracts from leaves, respectively. Antioxidant activity by the DPPH method was 196.07 and 146.53 µmol TE/g DW for Soxhlet extracts from seeds and leaves, respectively, and by the ABTS method was 582.68 and 580.40 µmol TE/g DW for Soxhlet and macerated extracts from leaves, respectively. Further research is needed to optimize the use of such bioactive compounds produced by *Annona deceptrix* and apply their biological activities in the pharmaceutical, food, cosmetic, or agrochemical industries.

Keywords: secondary metabolites, Soxhlet, total phenolic content, total flavonoid content, ABTS.

Resumo

Annona deceptrix (Westra) H. Rainer pertence à família Annonaceae, que é conhecida por possuir bioatividades, como antioxidante, antimicrobiana, anticancerígena, anti-inflamatória, pesticida, entre outras. Extratos etanólicos de sementes e folhas de *A. deceptrix* obtidos por três métodos de extração (Soxhlet, ultrassom e maceração) foram testados quanto às atividades fitoquímicas e antioxidantes. A triagem fitoquímica de extratos vegetais revelou a presença de catequinas, triterpenos, taninos, alcaloides, flavonoides, aminoácidos, glicosídeos cardíacos, antocianidinas, açúcares redutores e saponinas. A determinação quantitativa de conteúdo fenólico total, flavonoides e atividades antioxidantes dos extratos foi realizada por meio de métodos colorimétricos. O maior teor de fenólicos totais foi de 58,14 e 54,08 mg GAE/g DW para extratos Soxhlet de folhas e sementes, respectivamente. O maior teor de flavonoides totais foi de 5,03 e 4,42 mg QE/g PS para extratos de folhas macerados e assistidos por ultrassom, respectivamente. A atividade antioxidante pelo método DPPH foi de 196,07 e 146,53 µmol TE/g DW para extratos Soxhlet de sementes e folhas, respectivamente, e pelo método ABTS foi de 582,68 e 580,40 µmol TE/g DW para Soxhlet e extratos macerados de folhas, respectivamente. Mais pesquisas são necessárias para otimizar o uso de tais compostos bioativos produzidos a partir de *Annona deceptrix* e aplicar suas atividades biológicas nas indústrias farmacêutica, alimentícia, cosmética e agroquímica.

Palavras-chave: metabólitos secundários, Soxhlet, conteúdo fenólico total, conteúdo total de flavonoides, ABTS.

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1. Introduction

Plants produce more than 200,000 distinct natural small molecules (Anarat-Cappillino and Sattely, 2014), referred to as secondary metabolites (Bhatla and Lal, 2023). These phytochemicals play an important role in plant adaptation to their environments and are specific to particular botanical groups (Bourgaud et al., 2001).

Phenolic compounds are one of the classes of bioactive compounds formed by the secondary metabolism of plants. They are responsible for the pigmentation, flavor, and astringency of fruits, and act as protective agents against parasites, insects, and UV light (Albuquerque et al., 2021). In addition, these molecules provide human health benefits due to their antibacterial, antiviral, antioxidant, anti-inflammatory, antimutagenic, and anticancer properties (Roy et al., 2022). Phenolic compounds are considered antioxidant molecules due to the donation of a hydrogen atom and/or an electron to free radicals, causing the breakage of the oxidation reaction chain (Albuquerque et al., 2021).

Currently, due to adverse reactions to the use of synthetic antioxidants, natural antioxidant compounds found in plants are gaining attention (especially phenolic compounds) (Olszowy, 2019). New antioxidant compounds could be identified through studies of the antioxidant activity in different plant species, which could help reveal their value (Chaves et al., 2020), e.g., threatened and/or endemic species.

Ecuador has a large number of fruit trees, some native to the Ecuadorian biomes and others introduced (De la Torre, 2008). Many of these trees are little known or completely unknown, constituting a large number of underexploited native and exotic products of potential interest for agroindustry and as natural sources of bioactive compounds with health benefits. However, information on the phytochemical composition and nutritional value of plant genetic resources is limited or often nonexistent (Guevara et al., 2019).

Annona deceptrix (Westra) H. Rainer (Annonaceae) is an endemic tree to Ecuador, listed as "vulnerable" in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species due to anthropogenic activities such as deforestation and agricultural expansion (Erkens, 2021). This tree grows in the tropical moist lowland forest on the Ecuadorian coast (León Yánez et al., 2019) and is used by locals for food (De la Torre, 2008), and wood harvesting (Erkens, 2021).

Previous studies in species of the genus *Annona* (Annonaceae) show the presence of secondary metabolites such as acetogenins, flavonoids, alkaloids, steroids, and peptides (Leite et al., 2020). Many species from the *Annona* genus have proven biological activities, such as antitumor, antioxidant, antimicrobial, and antifungal (Leite et al., 2020). Therefore, the objective of this study is to obtain a preliminary phytochemical screening and to determine the total phenolic and flavonoid contents, and antioxidant activities (by DPPH and ABTS) of seeds and leaves of *A. deceptrix*. To the best of our knowledge, this is the first report of the phytochemical screening and antioxidant activity of *A. deceptrix*.

2. Material and Methods

2.1. Plant extract preparation

Plant material of *Annona deceptrix* (Figure 1A) was collected from a germplasm bank located in Santa Ana,

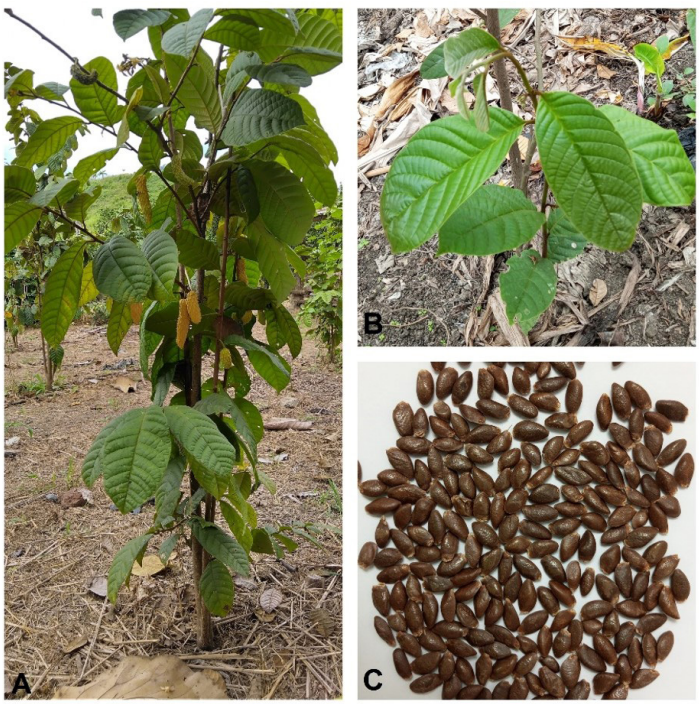

Figure 1. *Annona deceptrix* (Westra) H. Rainer. (A) tree; (B) leaves; (C) seeds. (Pictures: Miryan Pinoargote-Chang).

Manabí. The Ecuadorian Ministry of Environment, Water, and Ecological Transition authorized access to biological samples (MAAE-ARSFC-2021-1164) and the use of genetic material (MAAE-DBI-CM-2021-0166). The leaves and seeds (Figure 1B and 1C) of *A. deceptrix* were washed to remove dust and impurity particles, dried at 45 °C for 24 hours in a tray dry (until plant material reached a humidity of 10 ± 3%), and grounded in a blade mill at 25,000 rpm.

Crude extracts of seeds and leaves of *A. deceptrix* were obtained using Soxhlet, ultrasound-assisted, and maceration extraction methods maintaining a 1/10 (w:v) solid-to-solvent ratio. For Soxhlet extractions, 15 g of dry samples was packed with 150 mL of ethanol 96% (v/v). Ultrasound-assisted extraction was carried out in an ultrasound bath operating at 50 W (ISOLab, Germany model 621.05.006). 25 g of dry samples were mixed with 250 mL of ethanol 96% (v/v) in a glass bottle and placed in the ultrasound bath for 60 minutes at 60 ± 4 °C. Maceration extraction was performed in a glass bottle with 25g of dry matter and 250 mL of solvent by soaking in darkness at room temperature (25 ± 2 °C) for 120 hours. All the extracts were filtered with Whatman No. 1 filter paper and stored in amber bottles at 4 °C.

2.2. Chemicals and reagents

Analytical grade chemicals and reagents employed in this study were: Follin-Ciocalteau reagent, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis- (3-ethylbenzothiazoline) -6-sulfonic acid (ABTS), Trolox (6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-2-carboxylic acid), chloride of aluminum, acetic acid, sodium carbonate, gallic acid (Sigma-Aldrich), quercetin, methanol and ethanol absolute (Supelco). Other chemicals were obtained from local suppliers.

2.3. Phytochemical screening

Crude ethanolic extracts of seeds and leaves were screened for secondary metabolites using standard protocols. Standard preliminary phytochemical qualitative analysis carried out were: saponins (Froth test), alkaloids (Dragendorff's test), triterpenes (Liebermann–Burchard test), amino acids (Ninhydrin test), tannins (Ferric chloride test), quinones (Borntrager's test), flavonoids (Shinoda test), cardiac glycosides (Kedde's test), reducing sugars (Fehling's test), catechins, and anthocyanidins (Godlewska et al., 2022; Rondón et al., 2018; Youl et al., 2023). Tests are based on the ability of functional groups to react with specific chemical reagents to give characteristic reactions. Any change of colors or precipitate formation indicated a positive response. Qualitative results are expressed as +++ (strong presence); ++ (moderate presence); + (low presence); and − (absence) of phytochemicals.

2.4. Determination of total phenolic and total flavonoid contents

Total phenolic contents (TPC) of crude ethanolic extracts of seeds and leaves were determined using the Folin–Ciocalteu spectrophotometric method previously described by Martins et al. (2021) with some modifications.

Briefly, 200 µL of each extract dilution (1 mg mL^{-1}) was mixed with 1500 µl of distilled water and 100 µL of Folin–Ciocalteu reagent in a microcentrifuge tube (2 ml). After 5 minutes of incubation at room temperature, 200 µL of a 20% solution of sodium carbonate (Na_2CO_3) was added. Samples were vortexed for 10 seconds, incubated in the dark for 30 minutes at room temperature, and then centrifuged at 500 × g for 2 min. Absorbance was measured at 760 nm in a spectrophotometer (Thermo Scientific GENESYS 180 UV-Vis). TPC was expressed in milligrams of gallic acid equivalent (GAE) per gram of dry weight (DW). All determinations were performed in triplicate (n = 3).

Total flavonoid contents (TFC) were performed using the aluminum trichloride method with quercetin as a reference according to a method previously described by Ruiz-Reyes et al. (2021) with some modifications.

Briefly, 200 µL of the sample solution was vortexed (10 seconds) with 800 µL of 70% (v/v) aqueous methanol and 1000 µL of 2% (v/v) methanol solution of aluminum trichloride (AlCl3) in a microcentrifuge tube (2 mL). After 15 minutes of dark incubation at room temperature, the absorbance of the supernatant was measured at 430 nm (Thermo Scientific GENESYS 180 UV-Vis Spectrophotometer). TFC was expressed as milligrams of quercetin equivalents (QE) per gram of dry weight. All determinations were performed in triplicate (n = 3).

2.5. Antioxidant activity

Antioxidant activity of *A. deceptrix* seeds and leaves extracts was determined by inhibition of DPPH• (2,2-diphenyl-1-picrylhydrazyl), and Trolox equivalent antioxidant capacity (TEAC) using ABTS•+ (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid), according to the methods previously described by Deghima et al. (2020) with some modifications. Antioxidant activities were expressed as micromole Trolox equivalent per gram of dry weight (µmol TE g^{-1} DW). All determinations were performed in triplicate (n = 3).

2.5.1. DPPH• method

In brief, 50 µL (leaf extract) and 100 µL (seed extract) aliquots of samples were mixed with 950 µL and 900 µL of ethanol absolute in a microcentrifuge tube, respectively, and then were added 1000 µL of a DPPH•–methanol solution. The tubes were vortexed for 10 seconds. After 30 minutes of dark incubation at room temperature, the absorbance at 517 nm was measured (Thermo Scientific GENESYS 180 UV-Vis Spectrophotometer). Methanol was the blank.

2.5.2. ABTS•+ method

In brief, 25 µL (leaf extract) and 50 µL (seed extract) aliquots of samples were mixed with 975 µL and 950 µL of absolute ethanol in a microcentrifuge tube, respectively, and then, 1000 µL of an ABTS•+ –methanol solution was added. The tubes were vortexed for 10 seconds. After 30 minutes of incubation at room temperature in the dark, the absorbance at 734 nm was measured (Thermo Scientific GENESYS 180 UV-Vis Spectrophotometer). Methanol was the blank.

2.6. Statistical analysis

Data were reported as mean ± standard deviation (SD) of three experiments (n = 3). A one-way ANOVA test was applied for the compare of total phenolic content, total flavonoid content, and antioxidant activity of *A. deceptrix* seed and leaf extracts. The normality of errors was evaluated using the Shapiro-Wilk test, and the homogeneity of variances was assessed with Levene's test for the generalized design of blocks and treatments (O'Neill and Mathews, 2002). Tukey's HSD test at 95% confidence level was used to discriminate among means. All analyses were performed with R 4.2.2 software.

3. Results and Discussion

3.1. Phytochemical contents

Phytochemical screening of secondary metabolites present in plants is a widely used technique across a diverse range of plant species (Bomfim et al., 2021; Fernandes et al., 2023; Muniz et al., 2023; Salim et al., 2022; Silva et al., 2021). In the case of phytochemical screening of the ethanolic extracts of *A. deceptrix*, the presence and absence of eleven groups of secondary metabolites were observed, with varying behaviors depending on the plant material and the extraction method employed. Nine groups of phytochemicals could be detected in the seed extracts, while only six groups were determined in the leaf extracts (Table 1).

The seeds and leaves of *A. deceptrix* have secondary metabolites of the three main categories: nitrogen compounds, terpenoids, and phenolics. Secondary metabolites with the highest prevalence in seed extracts were catechins and in leaf extracts were triterpenes and tannins. Amino acids and cardiac glycosides were moderate to abundant in seeds, and tannins, catechins, flavonoids, and alkaloids in leaves. Anthocyanidins, reducing sugars, triterpenes, alkaloids, and tannins show moderate presence in seeds and alkaloids in leaves. Flavonoids were the phytochemical with the lowest prevalence in seeds and saponins in leaves.

The biosynthesis of secondary metabolites is controlled by different environmental and genetic factors (Zhan et al., 2022). The strong presence of catechins in seeds of *A. deceptrix* could be related to the brown pigmentation of the seed coat, just as the presence of catechins has been demonstrated in the brown seed coats of some legumes (Li et al., 2023; Lim et al., 2021). The strong presence of triterpenes in the leaves of *A. deceptrix* may be because terpenes have a physiological and ecological function related to phytohormones, insecticidal, allelopathic, and pollination by insects (Al-Khayri et al., 2023).

Results presented in Table 1 mostly coincide with the phytochemicals present in ethanolic seeds extract of *A. muricata* (saponins, alkaloids and triterpenoids, flavonoids, anthraquinones, tannins, and cardiac glycosides) and ethanolic leaves extract of *A. muricata* (alkaloids, flavonoids, terpenoids, coumarins and lactones, anthraquinones, tannins, cardiac glycosides, phenols, phytosterols, and saponins) (Gavamukulya et al., 2014; Nguyen et al., 2020b), with the difference that the ethanolic extracts of *A. deceptrix* seeds do not have saponins or quinones and the ethanolic extracts of leaves do not contain cardiotonic glycosides or quinones. Likewise, the results of this study coincide with the results of Ijaiya et al. (2014) who reported that the ethanolic leaf extract of *Annona senegalensis* contains tannins, alkaloids, saponins, flavonoids, and cardiac glycosides.

There are qualitative reports of secondary metabolites in several *Annona* species, some with greater distribution in nature such as flavonoids and tannins, and others more restricted to certain botanical families such as alkaloids. The presence of these bioactive compounds may vary depending on the species and the matrix analyzed, however, leaf extracts of *Annona squamosa*, *A. crassiflora*, *A.muricata* and the results of this study stand out (Table 2).

Table 1. Phytochemical screening of seeds and leaves ethanolic extracts of *Annona deceptrix* (Westra) H. Rainer.

Phytochemical groups	Seeds			Leaves		
	SE	UAE	ME	SE	UAE	ME
Catechins	+++	++	+++	++	++	++
Triterpenes	+	+	+	+++	+++	+++
Tannins	++	-	+	+++	++	++
Alkaloids	+	+	+	++	+	+
Flavonoids	+	-	+	++	++	++
Amino acids	++	++	++	-	-	-
Cardiac glycosides	++	+	++	-	-	-
Anthocyanidins	++	+	+	-	-	-
Reducing sugars	+	+	+	-	-	-
Saponins	-	-	-	-	+	-
Quinones	-	-	-	-	-	-

SE = Soxhlet extraction; UAE = Ultrasound-assisted extraction; ME = Maceration extraction. +++ (Strong presence); ++ (Moderate presence); + (Low presence); – (Absence).

Table 2. Flavonoids, tannins, and alkaloids in *Annonas* seeds and leaves.

Specie	F	T	A	Matrix	Source
Annona deceptrix	+	+	+	Seeds	This study
A. coriacea	+	+		Seeds	Benites et al. (2015)
A. crassiflora			+	Seeds	Ribeiro et al. (2013)
A. mucosa			+	Seeds	Giraldo-Rivera and Guerrero-Álvarez (2018)
A. muricata	+		+	Seeds	Ravaomanarivo et al. (2014)
A. squamosa	+			Seeds	Leite et al. (2021), Ravaomanarivo et al. (2014)
A. sylvatica		+	+	Seeds	Benites et al. (2015), Gonçalves et al. (2015)
A. deceptrix	+	+	+	Leaves	This study
A. coriacea	+			Leaves	Novaes et al. (2018)
A. crassiflora	+	+	+	Leaves	Ferraz et al. (2017), Ribeiro et al. (2013)
A. dioica	+			Leaves	Vega et al. (2007)
A. glabra	+			Leaves	Rocha et al. (2017)
A. mucosa	+	+	+	Leaves	Barboza et al. (2015)
A. muricata	+	+	+	Leaves	Bento et al. (2013)
A. reticulata	+			Leaves	Jamkhande et al. (2016)
A. rugulosa			+	Leaves	Vendramin et al. (2013)
A. squamosa	+	+	+	Leaves	Agrawal et al. (2012), Casamina and Reyes (2022)
A. vepretorum	+			Leaves	Silva et al. (2017)

F = flavonoids; T = tannins; A = alkaloids.

It has been detected four groups of secondary metabolites (cardiac glycosides, anthocyanidins, amino acids, and reducing sugars) present in the seed extracts but were not detected in the leaf extracts of *Annona deceptrix*. Similar results have been reported in other species, e.g., cardiac glycosides have been found in the seeds of several plants of the Apocynaceae family such as *Thevetia peruviana* (Kohls et al., 2012), *Cerbera odollam* and *Cerbera manghas* (Saxena et al., 2023), *Strophanthus kombe* Oliv. (Knittel et al., 2016) and other families including Plantaginaceae, Asparagaceae, and Moraceae. Cardiac glycosides (sugars) are a unique group of secondary metabolites that manipulate the contractile force of cardiac muscles and they are considered one of the most useful drugs in therapeutics. In addition, the flavonoids anthocyanidins are water-soluble natural dyes in plants and fruits with antioxidative and antimicrobial activities (Khoo et al., 2017; Zhao et al., 2023), they are the main pigments in the seed coats of some plants such as *Vigna angularis* (Willd.) Ohwi & H. Ohashi (Zhao et al., 2022). Finally, the presence of amino acids has been reported in seeds of *Annona squamosa* (Mariod et al., 2010).

Catechins, alkaloids, flavonoids, terpenoids, tannins, cardiac glycosides, phenols, and saponins present in *A. deceptrix* seed and leaves extracts would be an indication for its potential use in anti-inflammatory, anti-allergic, antibacterial, and antiviral, heart failure, antioxidant, and anticancer activity among others (Gavamukulya et al., 2014).

3.2. Total phenolic and total flavonoid contents

Total phenolic content (TPC) in ethanolic seeds and leaf extracts of *Annona deceptrix* obtained by three extraction methods show significant differences. On the other hand, the total flavonoid content (TFC) between the six extracts evaluated does not present significant differences (Table 3).

TPC results of this study coincide with those reported by Benites et al. (2015) in methanolic seed extract of *Annona sylvatica* (58.10 ± 1.45 mg GAE/g), as well as the findings published by Fernandes et al. (2016) for other species of aromatic plants such as *Origanum majorana* L. (48.66 ± 3.03 mg GAE/g dw), *Rosmarinus officinalis* L. (46.18 ± 3.02 mg GAE/g dw), and *Melissa officinalis* L. (42.86 ± 3.04 mg GAE/g dw). These plants have been proven to be effective as replacements for synthetic antioxidants (Fernandes et al., 2016).

On the other hand, some reports show a higher total phenolic content in species such as *Annona squamosa* ethanolic leaves extract (316 ±38.16 mg GAE /g) (El-Chaghaby et al., 2014), *A. muricata* ethanolic seeds extract (282.71 ± 8.64 mg GAE/g DW) (Nguyen et al., 2020c), *A. squamosa* ethanolic leaves extract (242.88 ± 6.13 mg GAE/g DW) (Nguyen et al., 2020a), *A. coriacea* methanolic seed extract (147.08 ± 4.20 mg GAE/g), and the lowest total phenolic content reported in *A. muricata* ethanolic leaves extract (2.5 ± 0.2 mg GAE /g dw) (Simo et al., 2018) (609.08±5.82 µg GAE/mg) (Nguyen et al., 2020b).

TFC results of this study are low and do not coincide with those reported in other species such as *A. muricata* ethanolic seed extract (86.57 ± 3.20 mg QE/g DW) (Nguyen et al., 2020c), *A. squamosa* ethanolic leaves extract (82.61±0.82 mg QE/g DW) (Nguyen et al., 2020a), but is superior to *A. muricata* ethanolic leaves extract (209.52±1.88 µg QE/mg DW) (Nguyen et al., 2020b).

Table 3. Total phenolic and total flavonoid content of seeds and leaves ethanolic extracts of *Annona deceptrix* (Westra) H. Rainer.

Plant material	Extraction method	Total phenolic content	Total flavonoid content
		(mg GAE/g DW)	(mg QE/g DW)
Seeds	SE	54.08 ± 5.69 ab	1.75 ± 1.34 a
	UAE	31.40 ± 8.61 c	3.00 ± 2.49 a
	ME	34.93 ± 2.70 bc	3.29 ± 2.72 a
Leaves	SE	58.14 ± 9.46 a	4.10 ± 0.35 a
	UAE	47.50 ± 8.75 abc	4.42 ± 0.88 a
	ME	49.95 ± 8.51 abc	5.03 ± 0.41 a

SE = Soxhlet extraction; UAE = Ultrasound-assisted extraction; ME = Maceration extraction; GAE = Gallic acid equivalent; QE = Quercetin equivalent; DW = dry weight. Average ± standard deviation. Common letters along the columns do not differ significantly among ethanolic extracts (HSD Tukey, $p < 0.05$).

The variations found in the content of phenols and flavonoids between the extracts evaluated could be due to genetic and biological variations of the species, geographical origin of the plant, part of the plant used, seasonal changes, soil types, extraction, and drying methods used (Moncayo et al., 2021). In addition, some authors attribute the change in the content of secondary metabolites to the efficiency of conventional and non-conventional extraction methods to critical parameters such as understanding the nature of the plant matrix, the chemistry of bioactive compounds, and scientific experience (Azmir et al., 2013).

The quantity of TPC is the highest with SE, it could be due to gallocatechin and gallic acid content increasing with increasing heating temperature (Ross et al., 2011). On the other hand, the TPC using UAE was lower for each matrix compared to the other extraction methods because the longer the UAE time, the lower the extraction of phenolic compounds observed (Vu et al., 2017). Optimal extraction conditions for extracting out phenolic contents were found to be at sonication for 40 minutes for *Terminalia catappa* leaves (Annegowda et al., 2010), 30 min for *Lactuca sativa* leaves (Hao et al., 2023) and 5 min for banana (*M. cavendish*) peel (Vu et al., 2017). In this study, the time used for UAE was 60 minutes.

Although there are no differences between the flavonoid contents of the extracts evaluated, it is notable that the quantity of flavonoids is greater in the leaves than in the seeds. Furthermore, the extraction method that uses the highest temperature (SE >60 °C, UAE = 60 °C, ME=<60 °C) for the extraction procedure has the lowest number of flavonoids, which increases as the extraction temperature decreases. The decrease in a number of secondary metabolites detected with Soxhlet extraction could be due to the loss or metabolism of thermo-unstable compounds (Moomin et al., 2023). Thermal degradation is the most common mechanism used to explain the drop in polyphenol yield during high-temperature extractions (Antony and Farid, 2022) and this occurs because the content of catechins and epicatechins decreases with increasing heating temperature at the time of extraction (Ross et al., 2011).

With the results obtained, it can be pointed out that the obtaining of different metabolites in the two matrices evaluated with three extraction techniques responds to the extraction of plant secondary metabolites depends on several factors such as the extraction method used, the plant matrix, specific phytochemicals, optimal solvent or solvent mixture (Bitwell et al., 2023; Tzanova et al., 2020).

3.3. Antioxidant activity of extracts

In this study, the antioxidant activity of leaves and seeds of *A. deceptrix* is evident. Several studies also report the antioxidant activity of *Annona* species, such as; leaves (Nguyen et al., 2020b) and seeds of *A. muricata* (Nguyen et al., 2020c), leaves of *A. squamosa* (Ibrahim et al., 2020; Nguyen et al., 2020a), bark and leaves of *A. cherimola* (Mohammed et al., 2020).

Antioxidant activities of each of the six ethanolic seeds and leaf extracts of *A. deceptrix* were determined by DPPH and ABTS methods (Table 4). In the DPPH assay, the results show that there are differences between the antioxidant activity of the different extracts evaluated, the highest antioxidant activity was recorded in seeds extract (196.07 µmol TE/g DW) followed by leaves extract (146.53 µmol TE/g DW) both get by SE. On the other hand, the results of ABTS analysis show that there are no statistical differences between the antioxidant capacity of the extracts evaluated. The highest activity was recorded in leaves extract (582.68 µmol TE/g DW) obtained by SE followed by leaves extract (580.40 µmol TE/g DW) obtained by ME.

Among the two assays used for determining antioxidant activity in the present study, ABTS presented the best results followed by DPPH. These findings are in agreement with the reports by Floegel et al. (2011) and Kainama et al. (2020).

These differences found between the two methods for determining the antioxidant activity of *A. deceptrix* extracts are because each extract contains a diverse range of antioxidants that have unique reactions with each radical used. On one hand, some dihydrochalcones and flavanones did not react with the DPPH radical in contrast to the ABTS radical (Platzer et al., 2021). On the other hand, these assays determine the antioxidant effect of substances depending on their polarity, thus DPPH is ideal for lipophilic antioxidants (e.g., terpenes, quercetins, resveratrols, and anthocyanins) but is restrictive for those with little or no lipophilic properties (Lang et al., 2024) and ABTS for hydrophilic (e.g., hydrophilic phenols) and lipophilic antioxidants (Munteanu and Apetrei, 2021).

Table 4. Total antioxidant capacity by DPPH and ABTS methods of seeds and leaves ethanolic extracts of *Annona deceptrix* (Westra) H. Rainer.

Plant material	Extraction method	DPPH•	ABTS•+
		μmol TE/g DW	μmol TE/g DW
Seeds	SE	196.07 ± 17.59 a	446.48 ± 74.79 a
	UAE	87.65 ± 27.03 c	302.58 ± 167.00 a
	M	100.75 ± 11.66 bc	348.65 ± 48.71 a
Leaves	SE	146.53 ± 22.28 ab	582.68 ± 93.97 a
	UAE	119.80 ± 10.37 bc	576.54 ± 114.95 a
	M	126.28 ± 20.40 bc	580.40 ± 104.34 a

SE = Soxhlet extraction; UAE = Ultrasound-assisted extraction; M = Maceration; TE = Trolox equivalent; DW = dry weight. Average ± standard deviation. Common letters along the columns do not differ significantly among ethanolic extracts (HSD Tukey, $p < 0.05$).

4. Conclusion

This study set the baseline of the phytochemical profile and antioxidant activity of leaves and seeds of *Annona deceptrix*. Ethanolic extracts present several phytochemical compounds such as catechins, triterpenes, tannins, alkaloids, flavonoids, amino acids, cardiac glycosides, anthocyanidins, reducing sugars, and saponins. The ethanolic leaf extracts contain the highest quantity of phenols and flavonoids, as well as the highest antioxidant activity according to the ABTS assay. Soxhlet extraction is the most suitable method for obtaining secondary metabolites from leaves and seeds of *Annona deceptrix*. These results allow us to lay the groundwork for future research to test the biological activity of ethanolic extracts of *A. deceptrix* in crop protection as an inhibitor of the growth of microorganisms as well as the effect on insect pests. On the other hand, the determination of the phytochemical content of the leaves and seeds of *A. deceptrix* is the starting point for the production of secondary metabolites using *in vitro* cell and tissue culture techniques and thus conserving the species that has a vulnerable category in its natural ecosystem.

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