April 30, 2021

PharmacologyOnLine

Archives • 2021 • vol.1 • 322-335

PHYTOCHEMICAL STUDY OF THE PLANT SPECIES ZANTHOXYLUM SPRUCEI (RUTACEAE) AND *MELAMPODIUM DIVARICATUM* (ASTERACEAE)

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Abstract

The present work is based on a preliminary Phytochemical Study of the *Zanthoxylum sprucei* and *Melampodium divaricatum* species of the Rutaceae and Asteraceae families, respectively, in which the best conditions for the qualitative extraction of their metabolites are studied. These metabolites were extracted from the plants using the maceration and ultrasonic bath techniques at room temperature, and that of soxhlet at the boiling temperature of the solvent. The solvents used were petroleum ether and ethanol of a different polarity. Chemical tests were developed in order to determine alkaloids, anthocyanins, reducing sugars, phenols, flavonoids, tannins and saponins. The phenolic composition of the extracts was evaluated by determining phenols and flavonoids through the use of spectrophotometric techniques, and the best results for the extracts were obtained by soxhlet and ultrasound. Furthermore, when determining the antioxidant activity of each extract, the extracts of the *Zanthoxylum sprucei* species were found to have a higher yield and a better response to the in vitro activity test than the *Melampodium divaricatum* species.

Key words: *extraction, phytochemical study, alkaloids, flavonoids*

Introduction

The biodiversity of Ecuador ranks sixth in the world (1). With regard to its plants, the Ecuadorian territory covers an area of 256370 km^2 and supports about 20,000 species of vascular plants, of which 5,000 to 8,000 may be medicinal (2). This natural wealth can be explained by the geographical location of Ecuador, the presence of 4 natural regions, and the biological conditions owing to the presence of a range of the Andes mountains (2).

National ethno medical studies have shown that the second largest use of medicinal plants for therapeutic purposes is in the treatment of gastrointestinal diseases (3). Unlike that which occurs with ethno botanical studies, very few works regarding the phytochemical studies carried out in Ecuador have been published. Nevertheless, the work of Rondón (4) concerning the first phytochemical study of 7 medicinal plants from the province of Guayas was recently published. The plants in question were: *Chuquiragajussieui* (Asteraceae), *Pseudognaphalium elegans* (Asteraceae), *Gustaviapubescens* (Lecythidaceae), *Aeghiphila alba Moldenke* (Lamiaceae), *Cleome spinosa* Jacq (Cleomaceae), *Phyllantusacuminatus* Vahl (Phyllantaceae) and *Croton rivinifolius* Kunth (Euphorbiaceae), for which secondary metabolites such as flavonoids and phenols, which are responsible for the antioxidant activity of the ethanol extracts of the plants, were reported. The phytochemical study of the natural in Ecuador, therefore, has an important research potential that would make it possible to transform traditional knowledge into scientific knowledge.

The following work provides a description of studies regarding the composition and antioxidant activity of the leaves of the *Zanthoxylum sprucei* (Rutaceae) and *Melampodium divaricatum* (Asteraceae) species, which grow in Ecuador.

Melampodium divaricatum is popularly known as a false marigold flower, a yellow little star, or a golden flower (5). The infusion obtained from its leaves and inflorescences is commonly used in popular medicine (6). The whole plant has also been popularly used as a curative, diaphoretic (7), diuretic and in the treatment of leucorrhea (8). It is native to South America and is a moderately frequent weed species throughout almost the entire country, where it mainly grows in annual crops, orchards, coffee plantations and on roadsides (5).

The *Zanthoxylum* species, meanwhile, belongs to the Rutaceae family, and about 250 species are known, which grow in the form of trees and shrubs that are found throughout the warm temperate and subtropical regions of the world. Negi (9) carried out a review of the *Zanthoxylum* genus and discovered that they have biological activities of the larvicidal, anti-inflammatory, analgesic, antinociceptive, antioxidant, antibiotic, hepatoprotective, antiplasmodial, cytotoxic, antiproliferative, anthelmintic, antiviral and antifungal types owing to the fact that their chemical constituents principally comprise alkaloids and essential oils.

Methods

Vegetal material

Fresh leaves from the *Melampodium divaricatum* and *Zanthoxylum sprucei* species were collected in the months of January-February 2019 from the Botanical Garden at the Technical University of Manabí (UTM), Portoviejo, Manabí province in western Ecuador. The botanical identification was carried out by Juan Manuel Moreira and Ana M. Coveña, who are the botanists in charge of the herbarium of the UTM botanical garden. The vouchers of the specimens were deposited with the following codes: *Zanthoxylum sprucei* (Rutaceae) (0122) and *Melampodium divaricatum* (Asteraceae) (0125).

Chemicals and Reagents

The chemicals and reagents employed in this study were: Follin-Ciocalteau reagent, 2,2'-azino-bis-(3-ethylbenzothiazoline) -6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH), sodium nitrite, chloride of aluminum, sodium hydroxide, sodium carbonate, catechin, quercetin, trolox, methanol and ethanol. All the reagents and solvents were supplied by Sigma-Aldrich and are of an analytical grade for study.

Extraction

Each plant material was dried in a Tunnel dryer at a temperature of 40 ℃ for 12 hours and subsequently pulverized. Petroleum ether was used to extract the low polar compounds, while ethanol was used to extract the polar compounds. The extracts of the crude plants were obtained using the ultrasonic bath, maceration and soxhlet extraction methods. About 40 g of finely divided plant material was packed into a thimble and extracted in soxhlet with 400 mL of ethanol. It was subsequently filtered and the solvent was evaporated in a vacuum in order to obtain the corresponding extracts required for analysis. 40 g of the finely divided plant material were placed in a 500 mL flask with 400 mL of ethanol. The mixture was sonicated at 50 W for 1h in an ultrasonic bath. It was subsequently filtered and the solvent was evaporated in a vacuum in order to obtain the corresponding extracts required for analysis. The same amounts of mass and volume that were used for the extraction with the ultrasonic bath were also employed to carry out the maceration. The mixture was left stirring at room temperature for 3 days. It was subsequently filtered and the solvent was evaporated in vacuum in order to obtain the corresponding extracts required for analysis.

Phytochemical screening

The crude extracts were phytochemically evaluated in order to determine the presence of the chemical constituents using standard procedures, which are described below:

Test for alkaloids

Each extract (1 mL) is dissolved in 2 mL of 5% hydrochloric acid and, after mixing and filtering, three aliquots are taken. Wagner, Mayer, Bouchardat, and Dragendorff reagent drops are added to each one. A red-brown precipitate (Wagner), a yellowish-white precipitate (Mayer), a brown precipitate (Bouchardat) and an orange-red precipitate (Dragendorff) indicate the presence of these metabolites (4).

Test for Flavonoids (Shinoda test)

1 mL of absolute ethanol and 3 drops of concentrated hydrochloric acid are added to 10 drops of the extract diluted in isopropyl alcohol. The formation of a red color indicates the presence of aurones and chalcones, while the formation of an orange, red or magenta color indicates the presence of isoflavones, flavonols and flavones, respectively (4).

Test with 10% sodium hydroxide

3 drops of 10% sodium hydroxide are added to 1 mL of extract diluted in isopropyl alcohol. The formation of a yellow-red, brown-orange, purple-red or blue color indicates the presence of xanthones and / or flavones, flavonols, chalcones and anthocyanins, respectively (4).

Test for Saponins. (Without sodium bicarbonate)

Add 1 mL of distilled water to 10 drops of the extract dissolved in isopropyl alcohol (20 mg / mL) in a test tube, shake vigorously to foam, and then allow it to stand for 30 minutes. The saponins content is measured as follows: no froth (absence); froth less than 3 mm high (poor); froth 6 mm in height (moderate) and froth greater than 8 mm in height (abundant) (4).

Test for Saponins. (With sodium bicarbonate)

Add 1 mL of distilled water and 1 drop of saturated sodium bicarbonate solution to 5 drops of the extract dissolved in isopropyl alcohol (20 mg / mL) in a test tube and shake vigorously for 3 minutes. The formation of honey comb-shaped foam indicated the presence of saponins (4).

Test for Tannins

1 mL of each extract is dissolved in 1 mL of ethanol and extracted with 3 mL of boiling distilled water for 15 minutes. Once it has been allowed to stand at room temperature, 0.2 mL of 10% sodium chloride solution is added to the mixture and filtered. In addition, 4 drops of 10% ferric chloride solution are added. The observed precipitation is indicative of the presence of tannins (4).

Test for Reducing Sugars

Dissolve 200 µL of the extract in a test tube; add Benedict's reagent until the extract turns bluish, and

then heat in a water bath at a temperature of 60 ° C. It is considered positive if it turns reddish brown (10).

Determination of total phenolic contents

The total content of phenols in the extracts is carried out using the Follin-Ciocalteau colorimetric reaction (11) and employing gallic acid as a reference phenolic compound. The gallic acid calibration curve is prepared by weighing 2 mg of gallic acid and making it up to a volume of 10 mL with distilled water, which is the stock solution at a concentration of 0.2 mg / mL (12). Aliquots of 50 µL are then taken, after which 200 µL of Follin-Ciocalteau reagent and 2 mL of 7% Na_2CO_3 are added and the solution is made up to 5 mL. After 30 minutes in the dark, the absorbance is measured at 765 nm, using the solution prepared without the sample as a blank (12).

Determination of total flavonoids

For the preparation of the Quercetin standard, 2 mg of the Quercetin standard are weighed and dissolved with 70% methanol in a 10 mL volumetric flask, which is considered the stock solution, with a concentration of 0.2 mg / mL. The Aluminum Chloride solution is subsequently prepared by weighing 500 mg of $AlCl_3$ and dissolving it with a solution of 25 mL 5% Acetic Acid in methanol, which leads to a solution with a concentration of 20 mg / mL(13).

In order to perform the calibration curve with the Quercetin standard, aliquots of 5, 10, 15, 20, 25, 30, 35, 40 µL of the standard solution are taken and made up to 1 mL with methanol (70%), after which 1 mL of the $AlCl_3$ solution is added. The same solution is used as a blank without adding the standard compound. After waiting 15 min for the reaction, the absorbance of the solution is measured at a wavelength of 430 nm in the UV-Vis spectrophotometer. The preparation of the extracts is carried out by taking 5 mg of the sample, which is dissolved in 5 mL of 70% aqueous methanol. The determination procedure for total flavonoids is carried out by taking 200 µL aliquots of different extracts of the sample in triplicate and making them up with 1 mL of methanol (70%), after which 1 mL of the $AlCl_3$ solution is added. After waiting 15 min, the absorbance of the solution is measured at a wavelength at 430 nm in the UV-Vis spectrophotometer.

Test with the radical DPPH

The DPPH molecule is known as a stable free radical owing to the delocalization of an unpaired electron over the entire molecule, which is why the molecule does not dimerize, as is the case with most free radicals. Electron delocalization also intensifies the typical deep violet color of the radical, which absorbs in methanol at 517 nm. When the DPPH solution reacts with the antioxidant substrate that can donate a hydrogen atom, the violet color fades. The color change is monitored by employing a spectrophotometer and is used to determine the antioxidant properties (14).

- **DPPH reagent preparation**

0.02 g of DPPH reagent is weighed and made up to 100 mL of methanol in a flask, homogenized and allowed to react for 24 hours in an amber flask in the dark.

- **Wavelength determination**

2.5 mL of the prepared DPPH reagent are taken, after which 15 mL of ethanol are added. A scan is carried out in the spectrophotometer. A maximum absorbance of 0.750 ± 0.05 should appear at a wavelength of 517 nm.

- **Blank preparation**

Add methanol (15 mL) and scan in the spectrophotometer to verify that it does not have absorbance in the working wavelength range in which the maximum of the sample is found.

- **Sample preparation**

3.0 mL of the DPPH reagent are taken and 100, 200, 300, 400, 500 µL of the extract are added. Once the sample and the blank have been prepared, the absorbance is read in a Thermo Scientific, Evolution 60s (UV-Visible) spectrophotometer with Vision Nite software, at 517 nM every 10 minutes.

Essay with the radical ABTS

This is based on the quantification of the discoloration of the $ABTS^{\bullet+}$ radical owing to the

interaction with hydrogen or electron donor species.

The cationic radical $ABTS^{\bullet+}$ is a chromophore that absorbs at a wavelength of 730 nm and is generated by an oxidation reaction of ABTS (ammonium 2,2'-azino-bis- (3-ethyl benzothiazoline-6-sulfonate) with potassium persulfate. The methodology is carried out as published by Miller (15), with slight modifications.

- **Preparation of 7 mM ABTS solution:**

0.384 g of ABTS are weighed into a 100 mL flask and made up with distilled water.

- **Preparation of 2.45 mM potassium persulfate solution:**

0.0662 g of potassium persulfate are weighed into a 100 mL volumetric flask and made up with distilled water. The 7 mM ABTS solution and 2.45 mM potassium persulfate solution are mixed in equal parts and kept in the dark at room temperature for an estimated time of between 12 and 16 hours, which is sufficient for the formation of the radical. This solution is stable for only two days.

- **Sample preparation**

A stock solution of each sample is prepared by weighing 50 mg of dry extract and dissolving it in 5 mL of methanol (10 mg / mL). A dilution is made by taking 10 µL of the stock solution and making up the volume to 1 mL of methanol. Aliquots of 100, 200, 300, 400 and 500 µL are taken from each separate sample and made up to 1 mL.

- **Technique procedure:**

Take 20 µL of the sample from each extract and place it directly in the spectrophotometer cuvette. 2 mL of the radical solution are added. Wait 7 min and read the absorbance at a wavelength of 730 nm against an ethanol blank. The reagent reference consists of a solution of the $ABTS^{\bullet+}$ radical with the sample solvent.

The percentage of inhibition of the formation of the free radical ABTS is calculated using the following expression:

Inhibition of free radical formation ABTS• and DDPH (%) $= \frac{A_{ABTS} - A_S}{A_{ABTS}} \times 100$

where:

A_{ABTS}: control absorbance (contains all reagents except sample).

A_S: absorbance of the sample.

The IC_{50} is calculated using the regression equation obtained from the concentrations of the extracts and reference substances and the percentage of inhibition of the formation of the radical ABTS ($y = 0{,}1425x + 14{,}684$ $R^2 = 0{,}9923$).

Results and Discussion

Analysis of extraction methods (Yield)

The ethanol extracts of the species studied were obtained using the maceration, soxhlet and ultrasonic bath extraction methods. A multiple range ANOVA test was performed, as shown in Figures 1 and 2. The analysis of these results showed that the null hypothesis could be rejected and, therefore, that at least one method is different from the others.

The maceration and soxhlet methods employed for the *Zanthoxylum sprucei* species were similar to each other, while the ultrasound bath method differed for the two species, since the latter attained higher yields (20%) with respect to the other two (Figure 1). The multiple range tests identified that all the methods were different from each other for the *Melampodium divaricatum* species. It was evident that ultrasound attained the highest yield (20%), as in the case of *Zanthoxylum sprucei* (Figure 2). The yields of the extracts obtained were higher when using the ultrasonic bath-assisted method, i.e., it was the most efficient means to obtain a greater volume of samples with respect to the plants used. Previous reports state that extraction assisted by Ultrasound allows better extraction yields, thus minimizing costs (energy consumption) and process times, which is why it is considered to be environmentally sustainable (16).

Analysis for solvents

Figures 3 and 4 show the multi-range tests carried out for the extraction of both species with different

solvents. The results obtained were different for the two solvents. In the case of the *Zanthoxylum sprucei* species, it was found that ethanol extracts attain a higher yield (15-24%) than ethereal extracts (13-17%). A similar behavior was observed for the *Melampodium divaricatum* species, for which the ethanol extracts were 15-22% and the ethereal extracts were between 13-18%. The rationale for these results can be explained by taking into account the polarity of the solvents. In this respect, Cabrera Suarez, Hirán R., (17) stated that the polarity of the solvent is related to performance owing to the fact that a more polar solvent has a greater power to extract a wide range of metabolites.

Data obtained from phytochemical tests

Table 1 shows the qualitative results of the phytochemical tests performed on the ethanol extracts of *Melampodium divaricatum* (Asteraceae) and *Zanthoxylum sprucei* (Rutaceae), obtained with an ultrasonic bath. An abundant presence of phenols, alkaloids, flavonoids and tannins was observed for both species. Moreover, a low presence of anthocyanins was detected for the *Zanthoxylum sprucei* species and no reducing sugars or saponins were detected. In the case of the *Melampodium divaricatum* species, an abundant presence of anthocyanins was detected, although there was a low presence of saponins, and no reducing sugars were detected.

See Table 1

Total phenol and flavonoid content (Follin-Ciocalteau)

As can be seen in Figure 5, the phenolic contents of the extracts have values of 246.94-457.63 mg/g, which is a wide range. The phytochemical results are characterized by the fact that the *Zanthoxylum sprucei* and *Melampodium divaricatum* species have abundant phenolic compounds.

These results have not previously been reported in literature. When comparing them with those of other plant species (Table 2) it will be observed that they are in the same range, which is important, since the evaluation of the plants showed antioxidant activity.

See Table 2

As can be seen in Figure 6, the flavonoid contents for the extracts have values of 19.30-79.06 mg/g.

Upon comparing the values obtained with those of alcoholic extracts of other medicinal plants reported in scientific literature (Table 3), it will be noted that the flavonoid value of the extracts studied is in a permissible range of that of the value of the plants reported in literature, which makes it possible to infer that they may have antioxidant activity.

See Table 3

Antioxidant Capacity DPPH

DPPH is a stable free radical that may be able to accept an electron or hydrogen radical in order to become a stable diamagnetic molecule. The interaction of an antioxidant molecule with DPPH leads to the transfer of an electron or hydrogen atom to it, thus neutralizing its free radical character and converting it to 1-1, diphenyl-2-picrylhydrazine. The reduction in the capacity of the DPPH radical is determined by the decrease in its absorbance at 517 nm, which is induced by antioxidant molecules, and there is a visually noticeable change in color from purple to yellow (18). In this type of assay, the number of DPPH molecules that are reduced appears to be related to the number of hydroxyl groups available (19). Ethanol extracts composed of polar molecules with abundant free hydroxyl groups such as flavonoids, phenols, tannins, among others, may, therefore have a strong scavenging activity. This type of activity was observed for the secondary metabolites in all the extracts analyzed in the present investigation and could be related to the results obtained for the DPPH free radical assay, since these extracts rich in flavonoids, phenols or polyphenols are known as radical scavengers and have antioxidant activity. The in vitro activity with DPPH radical of the two ethanol extracts obtained by the ultrasonic bath extraction method is analyzed in Table 4, as follows. (This table is attached)

See Table 4

When comparing the IC_{50} values obtained for the extracts, they are slightly higher than the standards, and considering that the standards are pure compounds, these values may be adequate if we

compare them with what is reported in literature for certain medicinal plants (Table 5).

See Table 5

Furthermore, the *Zanthoxylum sprucei* plant has lower IC_{50} values than *Melampodium divaricatum*, thus indicating a greater response to antioxidant activity. These results could be related to the nature of the metabolites present in the *Zanthoxylum sprucei* extract, but to corroborate this hypothesis, more in-depth studies are required in order to discover the chemical composition of the extracts.

Antioxidant Capacity $ABTS^{\bullet+}$

The generation of the $ABTS^{\bullet+}$ radical forms the basis of one of the spectrometric methods that have been applied to measure the total antioxidant activity of pure solutions or substances and aqueous mixtures. The technique for the generation of the radical cation $ABTS^{\bullet+}$ involves the direct production of the chromophore $ABTS^{\bullet+}$ green-blue through the reaction between $ABTS^{\bullet+}$ and potassium persulfate ($K_2S_2O_8$). It has three absorption maxima at the wavelengths of 645 nm, 734 nm and 815 nm. The addition of the antioxidants to the pre-formed radical reduces it to ABTS. The degree of discoloration as a percentage of inhibition of the radical cation $ABTS^{\bullet+}$ is, therefore, determined as a function of concentration and time, along with the corresponding value, using the Trolox as standard and under the same conditions (15).

The antioxidant capacity $ABTS^{\bullet+}$of ethanol extracts from the *Melampodium divaricatum* and *Zanthoxylum sprucei* plant species at different concentrations is shown in Figure7. The antioxidant capacity was increased as the concentration of all the extracts was increased (100-500 µg/mL). For the concentration of 500 µg/mL, the best response of antioxidant capacity was shown by the *Zanthoxylum sprucei* ethanolic extract (44.23), with a standard deviation of 0.094, while for *Melampodium divaricatum* it was 33.65 with a standard deviation of 0.025.

Upon comparing the results for the $ABTS^{\bullet+}$ antioxidant capacity response obtained by employing the ultrasonic bath extraction method for the ethanolic extracts of the *Melampodium divaricatum* and *Zanthoxylum sprucei* plant species with other total antioxidant activity values reported for other medicinal plants in literature (Table 6), it is possible to state that the plants attained satisfactory results as regards antioxidant activity, since the values oscillate between a range of (27.5-33.65 µg/mL) for *Melampodium divaricatum* and (18.65-44.23 µg/mL) for *Zanthoxylum sprucei*.

See Table 6

Conclusions

The use of phytochemical tests to identify and qualitatively characterize metabolites shows that the plants studied have a broad of chemical compounds, which have a high concentration of flavonoids, anthocyanins, phenols, reducing sugars and tannins in the case of *Zanthoxylum sprucei,* and alkaloids, anthocyanins, phenols, flavonoids and tannins in that of *Melampodium divaricatum*. The phenolic and flavonoid composition of the extracts of the leaves of *Melampodium divaricatum* and *Zanthoxylum sprucei* was determined using spectrophotometric techniques, and it was found that the best results were obtained for the extracts by means of soxhlet and ultrasound. When compared with other studies, this makes it possible to infer that the extracts may have a positive antioxidant activity. It is shown that the extracts of the *Zanthoxylum Sprucei* species respond better to the DPPH test for antioxidant activity in vitro (IC_{50} 73.43 µg / mL) than do those of the *Melampodium divaricatum* species (IC_{50} 121.67 µg / mL), respectively. The same occurs with the ABTS test, for which the total antioxidant activity was 44.23% for the *Zanthoxylum sprucei* species and 33.65% for the *Melampodium divaricatum* species.

Acknowledgments

The authors acknowledge for the support of the Universidad Técnica de Manabí (UTM) in the Convocation Project approved in 2018 with the "Título Tamizaje fitoquímico y determinación de la actividad antioxidante y antibacterial de 8 familias de plantas medicinales que se encuentran en la provincia de Manabí"

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Table 1: Results of the Phytochemical Tests carried out on the ethanol extracts of the *Zanthoxylum sprucei* and *Melampodium divaricatum* species obtained with an ultrasonic bath.

	Phytochemically test						
	Alcaloids	**Antocianines**	**ReductorsSugars**	**Fenols**	**Flavonoids**	**Tanines**	**Saponins**
Ethanol extracts of ***Zanthoxylum sprucei*** obtained using ultrasonic bath	+ +	+	-	+ ++	++	+ +	-
Ethanol extracts of ***Melampodium divaricatum*** obtained using ultrasonic bath	+ +	+ +	-	+ + +	++	+ +	+
+ low presence ++ Relatively abundant presence +++ Abundant presence - No detection							

Table 2: Comparison of values of the total phenol content of alcoholic extracts belonging to the same family of plants published in literature.

Ethanol extracts	**Concentration [mg/g]**	**References**
A. cucullata (Asteraceae)	53.807 ± 0.059	Eruygur N., 2019 (20)
Hieraciumpilosella L. (Asteraceae)	244.16 ± 2.15	Stanojević L., 2009 (21)
Calendulaofficinalis (Asteraceae)	435.102± 0.05	Abdel-Aziem, Sekena H. 2014(22)
Citrus aurantifolia Linn. (Rutaceae)	25.91 ± 6.86	Şeker Karatoprak, G., 2020 (23)
Green prickley ash (Zanthoxylum schinifoliumSieb. et Zucc) (Rutaceae)	46.37±1.08	Lu, M. 2011 (14)
Pericarpium Citri Reticulatae (Rutaceae)	79.5±7.7	Yi, Z. 2008 (24)

Table 3: Comparison of values of the total content of flavonoids of alcoholic extracts belonging to the same family of plants published in literature.

Ethanol extracts	Concentration [mg/g]	References
A. cucullata (Asteraceae)	21.372 ± 0.026	Eruygur N., 2019 (20)
Hieraciumpilosella L. (Asteraceae)	82.18 ± 0.53	Stanojević L., 2009 (21)
Solidagograminifolia L. (Asteraceae)	151.41± 2.44	Toiu, A., 2019 (25)
Citrus aurantifolia Linn. (Rutaceae)	7.83 ± 2.66	Şeker Karatoprak, G., 2020 (23)
Green prickley ash (Zanthoxylum schinifolium Sieb. et Zucc) (Rutaceae)	48.62±0.24	Lu, M. 2011 (14)
PericarpiumCitriReticulatae(Rutaceae)	233.4 ± 4.1	Yi, Z. 2008 (24)

Table 4: IC_{50} values obtained when evaluating the sequestering activity of the DPPH radical for the different extracts.

Samples	Concentration (µg/mL)	Captor effect (%)	IC_{50} (µg/mL)
Eugenol	10	25,75	**29,00**±0.14
	20	35,72	
	30	45,79	
	40	54,92	
	50	61,56	
Rutin	5	40,26	**10,26**±0.27
	10	50,90	
	15	55,19	
	20	70,58	
	25	83,72	
Ethanol extracts of Zanthoxylum sprucei obtained with ultrasonic bath	100	51,05	**73,431**±0.09
	200	53,97	
	300	60,25	
	400	63,18	
	500	66,11	
Ethanol extracts of Melampodium divaricatum obtained with ultrasonic bath	100	43,51	**121,677**±0.16
	200	58,16	
	300	67,36	
	400	69,46	
	500	71,55	

Table 5: IC_{50} values DPPH of medicinal plants belonging to the same family referred to in literature

Ethanol extracts	IC_{50}(µg/mL)	References
Citrus aurantifolia Linn. (Rutaceae)	50.83 ± 1.65	Şeker Karatoprak, 2020 (23)
Green prickleyash (Zanthoxylum schinifolium Sieb. et Zucc) (Rutaceae)	94.12± 0.24	Lu, M. 2011(14)
A. cucullata (Asteraceae)	132.55 ± 0.026	Eruygur N., 2019 (20)

Table 6: Total antioxidant activity values $ABTS^{\bullet+}$ of medicinal plants belonging to the same family referred to in literature

Ethanol extracts	Antioxidant capacity(µg/mL)	References
Z. leprieurii (Rutaceae)	55.10 ± 2.14	Yoro, T. 2020 (26)
P. crispa (Asteraceae)	60.03 ± 0.03	Shahat, Abdelaaty A. 2014 (27)
P. cyanocarpa (Asteraceae)	70.03 ± 0.03	Shahat, Abdelaaty A. 2014(27)

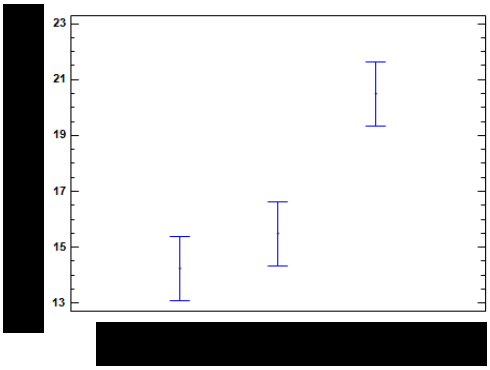

Figure 1: *Zanthoxylum sprucei* multi-rank ANOVA test.

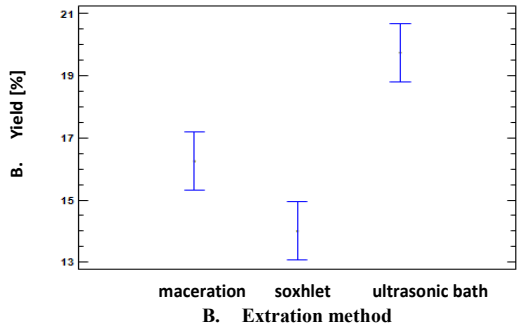

Figure 2: *Melampodium Divaricatum* multi-rank ANOVA test.

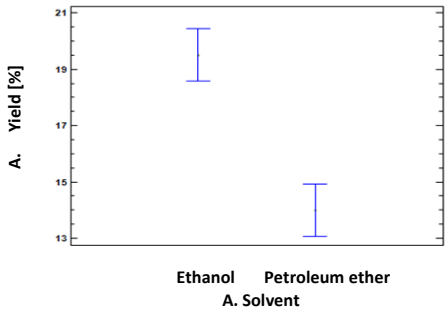

Figure 3: *Zanthoxylum sprucei* multi-rank ANOVA test.

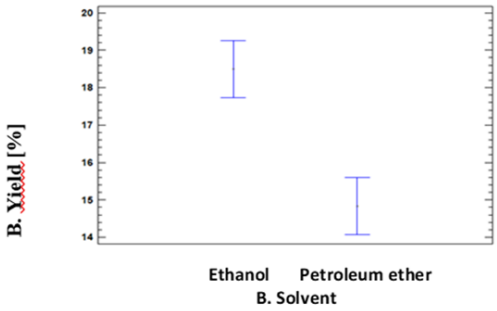

Figure 4: *Melampodium Divaricatum* multi-rank ANOVA test.

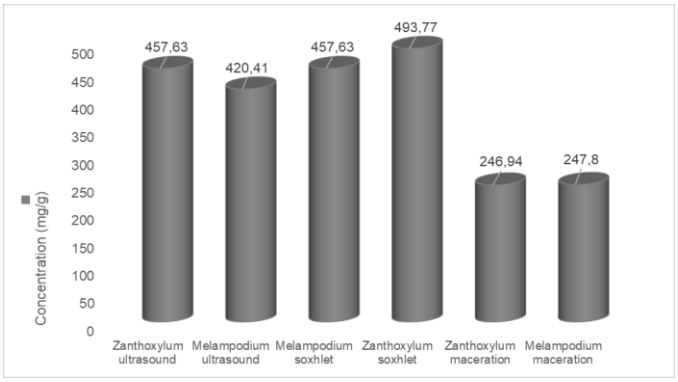

Figure 5: Behavior of phenolic content in the extracts evaluated

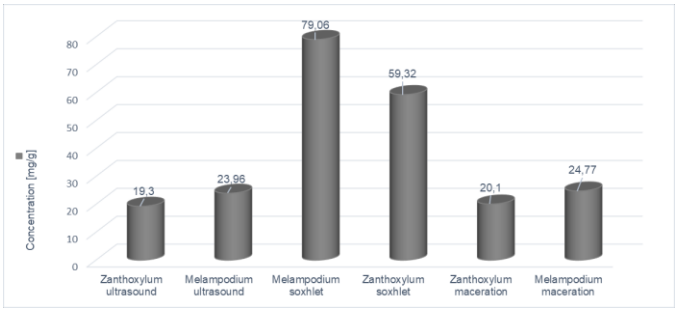

Figure 6: Behavior of the flavonoid content in the extracts evaluated.

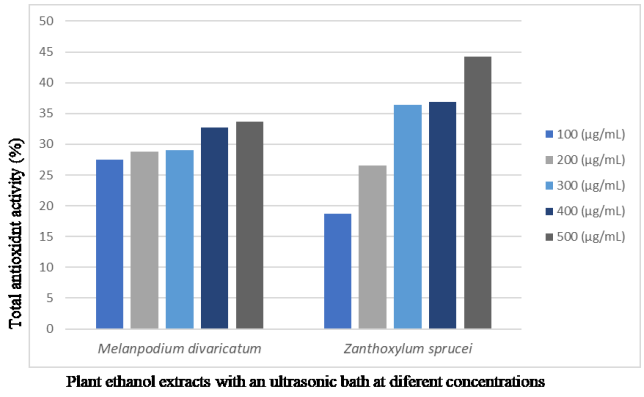

Figure 7: Total ABTS$^{\bullet+}$ antioxidant activity for *Melampodium divaricatum and Zanthoxylum sprucei*