

PHYTOCHEMICAL ANALYSIS AND ICP-OES NUTRIMENTAL CONTENT IN EXTRACTS OF CASTOR-OIL PLANT (*R. COMMUNIS*), SWEET ACACIA (*V. FARNESIANA*) AND PINK GRASS (*R. REPENS*)

ANÁLISE FITOQUÍMICA E CONTEÚDO NUTRICIONAL POR ICP-OES EM EXTRATOS DE MAMONA (*R. COMMUNIS*), ACÁCIA-DOCE (*V. FARNESIANA*) E CAPIM-ROSA (*R. REPENS*)

ANÁLISIS FITOQUÍMICO Y CONTENIDO NUTRIMENTAL POR ICP-OES EN EXTRACTOS DE RICINO (*R. COMMUNIS*), ACACIA DULCE (*V. FARNESIANA*) Y PASTO ROSADO (*R. REPENS*)



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ABSTRACT

In the present investigation, several specialized secondary metabolites were extracted and identified to combat entomological pests and the nutritional aspects of three plant species were analyzed. Three wild plants were evaluated: castor-oil plant (*Ricinus communis*), sweet acacia (*Vachellia farnesiana*) and pink grass (*Rhynchelytrum repens*). The pure extracts were evaluated with the following specialized secondary metabolites analysis: flavonoids, sterols, triterpenes, phenols, quinones, saponins, catechins, tannins, triterpenes and anthocyanins. Macronutrient and micronutrient analyses of the extracts of the three plants extracts of castor-oil plant (*Ricinus communis*), sweet acacia (*Vachellia farnesiana*) and pink grass (*Rhynchelytrum repens*) were evaluated with the following methods. The Dumas method was used to analyze the percentage of nitrogen (w/w); the microwave ICP-OES (Inductively Coupled Plasma/Optical Emission Spectrophotometer) method was used to analyze the percentages of macronutrients in percentage (w/w) of phosphorus, potassium, calcium, magnesium, sulfur and sodium (mg/kg) and the percentage of chlorine with silver nitrate/argentometry. Micronutrient analyses of plant extracts were evaluated by microwave digestion/ICP-OES digestion in mg/kg of iron, zinc, manganese, copper, boron, nickel and molybdenum.

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Keywords: Specialized Secondary Metabolites. Plant-Metabolite-Insect. Nutriment Quality

RESUMO

Na presente investigação, vários metabólitos secundários especializados foram extraídos e identificados para combater pragas entomológicas, e os aspectos nutricionais de três espécies de plantas foram analisados. Três plantas silvestres foram avaliadas: higuierilla (*Ricinus communis*), huizache (*Vachellia farnesiana*) e capim-rosa (*Rhynchelytrum repens*). Os extratos puros foram avaliados com a seguinte análise de metabólitos secundários especializados: flavonóides, esteróis, triterpenos, fenóis, quinonas, saponinas, catequinas, taninos, triterpenos e antocianinas. As análises de macronutrientes e micronutrientes dos extratos das três plantas higuierilla (*Ricinus communis*), huizache (*Vachellia farnesiana*) e capim-rosa (*Rhynchelytrum repens*) foram avaliadas com os seguintes métodos. O método de Dumas foi utilizado para analisar a porcentagem de nitrogênio (p/p); o método ICP-OES (Espectrofotômetro de Emissão Óptica/Plasma Acoplado Indutivamente) por micro-ondas foi utilizado para analisar as porcentagens de macronutrientes em porcentagem (p/p) de fósforo, potássio, cálcio, magnésio, enxofre e sódio (mg/kg) e a porcentagem de cloro com nitrato de prata/argentometria. As análises de micronutrientes dos extratos vegetais foram avaliadas por digestão por micro-ondas/digestão ICP-OES em mg/kg de ferro, zinco, manganês, cobre, boro, níquel e molibdênio.

Palavras-chave: Metabólitos Secundários Especializados. Metabólitos Vegetais-Insetos. Qualidade dos Nutrientes

RESUMEN

En la presente investigación se extrajeron e identificaron varios metabolitos secundarios especializados para combatir plagas entomológicas y se analizaron los aspectos nutricionales de tres especies vegetales. Se evaluaron tres plantas silvestres: la higuierilla (*Ricinus communis*), el huizache (*Vachellia farnesiana*) y la hierba rosa (*Rhynchelytrum repens*). Los extractos puros se evaluaron mediante el análisis de los siguientes metabolitos secundarios especializados: flavonoides, esteroides, triterpenos, fenoles, quinonas, saponinas, catequinas, taninos, triterpenos y antocianinas. Los análisis de macronutrientes y micronutrientes de los extractos de las tres plantas —higuierilla (*Ricinus communis*), huizache (*Vachellia farnesiana*) y hierba rosa (*Rhynchelytrum repens*)— se evaluaron con los siguientes métodos. Se utilizó el método de Dumas para analizar el porcentaje de nitrógeno (p/p); el método ICP-OES (plasma acoplado inductivamente/espectrofotómetro de emisión óptica) por microondas se utilizó para analizar los porcentajes de macronutrientes en porcentaje (p/p) de fósforo, potasio, calcio, magnesio, azufre y sodio (mg/kg) y el porcentaje de cloro con nitrato de plata/argentometría. Los análisis de micronutrientes de los extractos vegetales se evaluaron mediante digestión por microondas/ICP-OES en mg/kg de hierro, zinc, manganeso, cobre, boro, níquel y molibdeno.

Palabras clave: Metabolitos Secundarios Especializados. Interacción Planta-Metabolito-Insecto. Calidad de los Nutrientes

1 INTRODUCTION

Secondary metabolites play an important role in protecting plants against various environmental stresses and insect pest emergence [1]. It has been estimated that approximately 100,000 secondary metabolites have been described in different plant species and are classified into multiple groups, nitrogen-containing compounds, terpenes, thiols, and phenolic compounds [2]. Focusing on the new model of plant-metabolite-insect interactions may represent a useful approach to find a new generation of organic bioinsecticides. Indeed, plants produce allelochemicals such as phenolic compounds, alkaloids and terpenoids targeting the behavior and physiology of hexapod arthropods. Thus, several compounds belonging to the above chemical classes may represent ideal ingredients for developing sustainable integrated pest and vector management programs. Minimal research has been conducted on nutritional and phytochemical analysis as well as biological evaluation of secondary metabolites of plant origin. Badamenti et al. (2022) carried out work on the isolation and structural elucidation of several bufadienolides from *Drimys panchayati* and evaluated their acaricidal activity against a serious agricultural pest, *Drimys panchayati*, the two-spotted spider mite, *Tetranychus urticae* [3]. Regarding the lethal and sublethal effects of plant-based insecticides and acaricides, Ebadollahi et al. (2022) evaluated the efficacy of some essential oils of *Thymus* species against the stored-produce beetle *Rhyzopertha dominica* [4]. On the other hand, Changbunjong et al. (2022) investigated the fumigant activities and contact toxicity of bitter orange (*Citrus aurantium*) essential oil against the stable fly *Stomoxys calcitrans* [5]. Plata-Rueda et al. (2021) studied the sensitivity and behavioral response of the mealworm *Tenebrio molitor* to oregano (*Origanum vulgare*) essential oil [6]. Walker, (2020) tested three Apiaceae essential oils, namely anise (*Pimpinella anisum*), dill (*Anethum graveolens*) and fennel (*Foeniculum vulgare*) against *Lymantria dispar*, showing them as potential agents for gypsy moth control [7]. Song and Zhou (2020), highlighted the promising toxicity of essential oils extracted from plant species from Chilean Patagonia and Cameroon against agricultural (*Spodoptera littoralis*) and public health (*Culex quinquefasciatus* and *Musca domestica*) insects [8]. On the other hand, color pigmentation patterns can be indicative of cuticular damage in the exoskeleton of insects of the order Odonata [9]. Therefore, in the present investigation, several special secondary metabolites were extracted and identified to combat entomological pests, and the nutritional aspects of three plant species were analyzed. The objectives of this research are to analyze the content of secondary metabolites by phytochemical analysis and the nutritional content of the extracts of higuierilla (*Ricinus communis*), huizache (*Vachellia farnesiana*) and pink grass (*Rhynchelytrum repens*).

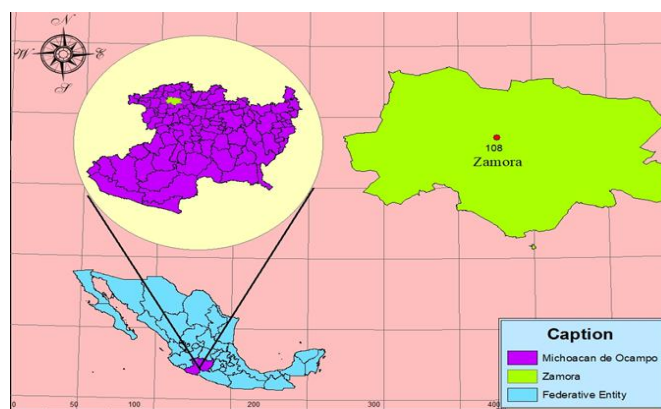
2 MATERIAL AND METHODS

2.1 COLLECTION OF BIOLOGICAL MATERIAL

Three types of wild plants were collected: higuierilla (*Ricinus communis*), huizache (*Vachellia farnesiana*) and pink grass (*Rhynchelytrum repens*) pathogen- and disease-free in Zamora, Michoacan, Mexico (Figure 1-2).

Figure 1

Biological sample collection area used in this project. Geographical coordinates: North Latitude: 20°04'32.99.99" and West Latitude 102°16'19.39"



Source: Authors (2025).

Figure 2

*Biological sample collection. (a) Phenotype aspect of higuierilla (*Ricinus communis*), (b) Phenotype aspect of huizache (*Vachellia farnesiana*) and (c) phenotype aspect of pink grass (*Rhynchelytrum repens*) in Zamora, Michoacan, Mexico*



Source: Authors (2025).

2.2 DRYING OF PLANT MATERIAL

The biological samples of higuierilla (*Ricinus communis*), b) huizache (*Vachellia farnesiana*) and c) pink grass (*Rhynchelytrum repens*) were dried on newspaper for 10 days and then in a drying oven (mod. Novatech) for 72 hours at 34 oC with the objective of dehydration in

order to obtain the extracts for secondary metabolite analysis. The analyses were performed in triplicate.

2.3 PHYTOCHEMISTRY ANALYSIS

2.3.1 Flavonoids analyses

Ammonia method. 0.1 g of each plant was weighed in triplicate in an analytical balance (brand). To obtain crude extracts, 10 ml of ethyl alcohol was added and allowed to stand for 30 min. After this period of time, to obtain pure extracts, the plant samples were filtered with Whatman filter paper no. 1, 3 ml of ammonia (NH₃) was added [10].

Alkali action. 0.1 g of each plant was weighed in triplicate in an analytical balance (brand). To obtain crude extracts, 5 ml of distilled water was added and allowed to stand for 30 min. After this period of time, to obtain pure extracts, the plant samples were filtered with Whatman filter paper no. 1, 3 ml of sodium hydroxide (NaOH) was added [11].

Shinoda reaction with the magnesium method. Pure extracts consisting of 5 ml of ethanol and 5 ml of methanol were prepared. 0.5 g of powder of each biological sample was weighed. The crude extract was allowed to stand for 30 min. After 30 min, 3 ml of the pure extract was placed in each test tube to add 0.1 g of magnesium filings and 3 drops of hydrochloric acid (HCl). The test is positive if the extract shows bubbling and turns orange. Shinoda assay this is used for flavonoids with a benzopyrone core in their structure, such as flavones, flavonols benzopyrone nucleus such as flavones, flavonols, flavanones, etc. produce reddish colorations when in aqueous or alcoholic solutions. aqueous or alcoholic solutions.

Sterols and triterpenes with the Salkowski method. Pure aqueous extracts were used. 1 ml of sulfuric acid (H₂SO₄) was added, the test is positive if the extract turns red or yellow for the detection of sterols or methylsterols.

Phenol detection with the ferric chloride method. 1.25 grams of ferric chloride (FeCl₃) was weighed. It was diluted in 8.75 ml of distilled water. It was left to rest for 30 minutes. It was filtered after 30 minutes and 3 drops of ferric chloride solution were added to the pure aqueous extract. The test is positive if the transition of the vire is from dark green to indigo blue is the presence of phenols; if it is full blue it is hydrolyzable tannins and if it turns dark green it is condensed tannins.

Quinones with the methanol and ethanol method. Pure leaf extracts were prepared. 0.5 g of powder of each leaf was rested, 5 ml of distilled water and 5 ml of methanol were added. The sample was filtered and allowed to stand. After 30 min, 1 ml of sulfuric acid (H₂SO₄) was added. If the extract turns red, the test is positive for the presence of quinones.

Saponins with the foam method. Pure methanol extracts were made from a) higuierilla (*R. communis*), b) huizache (*V. farnesiana*) and c) pink grass (*R. repens*). We weighed 0.5 g of powder from each plant, added 5 ml of methanol and left to stand for 30 minutes. After 30 minutes, 1 ml of pure extract was added followed by the addition of 9 ml of distilled water, each sample was placed in a screw cap test tube [12]. The tube was shaken vigorously by hand and allowed to stand for 15 minutes. The results are interpreted as follows:

Table 1

Interpretation of the volume of saponin foam

<i>Foam area</i>	<i>Interpretation</i>
<i>Foam formation greater than 15 mm</i>	<i>High content of saponins: test is positive</i>
<i>Foam formation between 5 to 10 mm</i>	<i>Moderate saponin content: test is positive</i>
<i>Foaming less than 5 mm</i>	<i>Less than 5 mm the test is negative: there is no presence of saponins.</i>

Catechin compounds with the formaldehyde method. Pure alcoholic extracts were prepared. We weighed 0.5 g of leaves and added 5 ml of ethanol and 5 ml of methanol. It was left to stand for 30 min. It was filtered to obtain the pure extract. Then, 5 ml of formaldehyde was added, followed by the addition of 10 drops of hydrochloric acid (HCl). Subsequently, a water bath device was placed. The formation of a precipitate reflects the content of a cationic tannin; the presence of spots or floating compounds reflects the presence of pyrogalllic tannins.

Tannins with the grenetin method. A 1% solution of grenetin was prepared in distilled water. Subsequently, 10 ml of the prepared grenetin solution was added in a test tube and 10 ml of sodium chloride (NaCl) was added over the pure aqueous extract (5 ml). The test is positive with the presence of a precipitate [12].

Triterpenes with the chloroform method. Pure ethanolic leaf extracts were made. 0.5 g of powder of each extract was weighed. Five ml of ethanol was added and left to stand for 30 minutes. After 30 min, the crude extract was filtered to obtain the pure extract. 1 ml of chloroform was added. 1 ml of acetic anhydride was added. The extracts were rested on ice and 2 drops of sulfuric acid (H₂SO₄) were added to each test tube. The test is positive if the pure extract turns pink, green, red, blue or purple [12].

Anthocyanins with the isoamyl alcohol method. Crude extracts were prepared with 5 ml ethanol and 5 ml methanol. It was rested for 30 minutes. The solution was filtered to obtain the pure extract. 5 ml of the pure extract was taken and transferred to test tubes. If the pure extract

turns red it is presence of anthocyanins, but if the alcoholic layer does not turn red it is presence of betacyanin.

2.4 NUTRIENT CONTENT

Dumas method was used to analyze the percentage of nitrogen (w/w). The Dumas combustion method is a comprehensive method for determining the total nitrogen content in a usually organic matrix. The Dumas method is based on the principle of destruction of organic matter through combustion under controlled oxygen supply at very high temperatures [13]. A combustion was generated, where the plant sample was incinerated at 900°C in the presence of oxygen. Then, a reduction was generated, where the nitric oxide produced by the combustion was reduced by tungsten to molecular nitrogen. This was followed by a purification stage, where a series of suitable sorbents (silver wool and tungsten) removed interfering constituents such as hydrogen halides and sulfur oxides from the gas mixture stream. The resulting water vapor was condensed and retained by a desiccant agent (phosphorus pentoxide), subsequent to the reduction process, where fine drying proceeds. Finally, nitrogen detection is performed with a thermal conductivity detector which measures the total nitrogen remaining in a carrier gas flow. The Dumas method is a closed system (the operator is not exposed to it) and does not use acids such as sulfuric acid (H₂SO₄), therefore it does not produce hazardous waste and has no operational risks for personnel (Cruz et al. 2007).

Microwave digestion/ICP-OES. Method was used to analyze the percentages of macronutrients and micronutrients. It was carried out by applying microwave waves at elevated temperatures using acid solutions of 10 ml of nitric acid (HNO₃) that serve the function of digesting the organic samples for subsequent analysis (Hackett et al., 2008). Subsequently, the digester tubes were closed and brought into the microwave oven, the equipment was set to a ramp temperature of 200 °C for 15 minutes. The temperature was maintained at 200 °C for approximately 20 minutes and then brought to room temperature for 10 minutes. Once digested, type 1 water was added to the residue and the residue was volumed to 50 ml in another tube for each sample. Once the previous step was completed, the residue was placed in the corresponding tubes for the ICP-OES equipment and filtered if necessary. The total time of digestion lasted 45 minutes a final volume of 50 ml with Water type 1 was used.

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) conditions. The gas pressure was 100 psi Argon and 100 psi Nitrogen. The flow rate of the gases was: auxiliary gas 0.5 L/min y nebulizer gas 0.5 L/min. The speed of the peristaltic pump: 50 rpm. The reading was carried out for macroelements and microelements.

2.5 STATISTICAL ANALYSIS

For the statistical analysis, the statistical software R studio, version 4.2.2 (2022-10-31) was used. For the specific performance of the principal component analysis, the "FactoMineR" library or package of functions was used; within the same package, the PCA function was used to obtain the "Eigenvalues" or components that summarize the data matrix.

3 RESULTS AND DISCUSSION

3.1 PHYTOCHEMISTRY ANALYSIS

Pohlan et al. (2006), mention that pests are among the most important limiting factors in the productivity of important limiting factors in the productivity of agroforestry systems. These same researchers point out that insect pests are the organisms responsible for 37 to 50% of the are the organisms responsible for 37 to 50% of the losses reported in agroforestry reported in agroforestry worldwide [14]. Brechelt (2004), describes the concept assigned to the term "pest" as follows when an animal, a plant or a microorganism increases its density to abnormal levels and, as a consequence, affects directly or indirectly the human species, either because it harms their health, their comfort, damage to buildings or agricultural, forestry or livestock properties, of which they are or livestock, from which the human being obtains food, fodder, textiles, wood, timber, products, etc. [15].

Qualitative chemical analysis contains relatively simple colorimetric procedures based on chemical transformations with the creation of specific chromophores. The current understanding of plant-insect interaction has had profound consequences in the field of green chemistry research, particularly for its future prospects for the development of new plant-based biorational pesticides [16]. These interactions involve numerous secondary metabolites that may interfere with insect behavior, growth, development or reproduction. The evaluation of the potential bioactivity of natural products against these biological systems is considered of interest, in particular because of the diversity of the hydrocarbon skeletons and the complexity of their functions present in plants [17]. Table 2 shows the results obtained from the extracts of higuerrilla (*R. communis*), huizache (*V. farnesiana*) and pink grass (*R. repens*).

Table 2

Results obtained from the phytochemical analysis of the extracts derived from higuierilla (*R. communis*), huizache (*V. farnesiana*) and pink grass (*R. repens*). **F: Flavonoids; S-T: Sterols and triterpenes; PC: Phenolic compounds; Q: Quinones; S: Saponins; C: Catechins; T: Tannins; TR: Triterpenes; A: Anthocyanins**

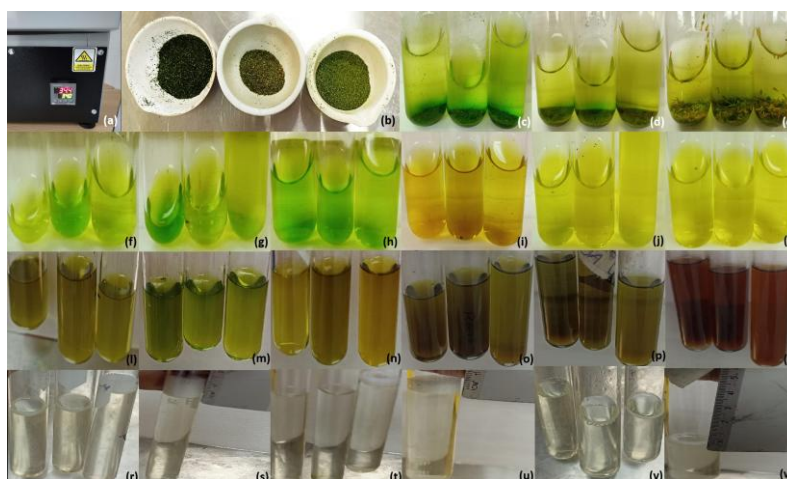
Extract type	Seco ndar y meta bolite	Assay	Results
<i>R. communis</i>	F	Ammonia	+ (chalcones and aurones)
<i>V. farnesiana</i>	F	Ammonia	+ (flavons and flavonols)
<i>R. repens</i>	F	Ammonia	+ (flavons and flavonols)
<i>R. communis</i>	F	<i>Alkali action</i>	+ (flavonols)
<i>V. farnesiana</i>	F	<i>Alkali action</i>	-
<i>R. repens</i>	F	<i>Alkali action</i>	+ (flavonols)
<i>R. communis</i>	F	Shinoda reaction	-
<i>V. farnesiana</i>	F	Shinoda reaction	-
<i>R. repens</i>	F	Shinoda reaction	+
<i>R. communis</i>	S-T	Salkowski reaction	+ (sterols)
<i>V. farnesiana</i>	S-T	Salkowski reaction	+ (sterols)
<i>R. repens</i>	S-T	Salkowski reaction	+ (methylsterols)
<i>R. communis</i>	PC	Ferric chloride	+ (Condensed tannins)
<i>V. farnesiana</i>	PC	Ferric chloride	+ (Condensed tannins)
<i>R. repens</i>	PC	Ferric chloride	+ to phenols
<i>R. communis</i>	Q	Methanol and ethanol	- (there was only a color change)
<i>V. farnesiana</i>	Q	Methanol and ethanol	- (there was only a color change)
<i>R. repens</i>	Q	Methanol and ethanol	- (there was only a color change)
<i>R. communis</i>	S	Foam method	+
<i>V. farnesiana</i>	S	Foam method	+
<i>R. repens</i>	S	Foam method	-

<i>R. communis</i>	C	Formaldehyde	- (there was only a color change)
<i>V. farnesiana</i>	C	Formaldehyde	- (there was only a color change)
<i>R. repens</i>	C	Formaldehyde	- (there was only a color change)
<i>R. communis</i>	T	Grenetin	- (absence of precipitate, but if there was color change)
<i>V. farnesiana</i>	T	Grenetin	- (absence of precipitate, but if there was color change)
<i>R. repens</i>	T	Grenetin	- (absence of precipitate, but if there was color change)
<i>R. communis</i>	TR	Chloroform method	+
<i>V. farnesiana</i>	TR	Chloroform method	+
<i>R. repens</i>	TR	Chloroform method	+
<i>R. communis</i>	A	Isoamyl alcohol method	-
<i>V. farnesiana</i>	A	Isoamyl alcohol method	-
<i>R. repens</i>	A	Isoamyl alcohol method	-

Figure 3 and Figure 4 shows the results of the positive presence of secondary metabolites in the phytochemical analyses.

Figure 3

Extraction process and identification of secondary metabolites in biological samples of higuierilla (*Ricinus communis*), huizache (*Vachellia farnesiana*) and pink grass (*Rhynchelytrum repens*). a) drying oven (mod. Novatech); b) pulverization of plant samples; c) crude extract of *R. communis*; d) crude extract of *V. farnesiana*; e) crude extract of *R. repens*; f) pure extract of *R. communis*; g) pure extract of *V. farnesiana*; h) pure extract of *R. repens*; i) pure extract of *R. communis* positive for flavonoids. *repens*; f) pure extract of *R. communis*; g) pure extract of *V. farnesiana*; h) pure extract of *R. repens*; i) pure extract of *R. communis* positive for flavonoids with NH_3 : chalcones and aurones; j) pure extract of *V. farnesiana* positive for flavonoids with NH_3 : flavones and flavonols; k) pure extract of *R. repens* positive for flavonoids with NH_3 : flavones and flavonols; l) extract of *R. communis* positive for flavonoids with NaOH : flavonols; m) pure extract of *V. farnesiana* negative for flavonoids with NaOH ; n) pure extract of *R. repens* positive for flavonoids with NaOH : flavonols; o) pure extract of *R. communis* negative for Shinoda reaction; p) pure extract of *V. farnesiana* negative for Shinoda reaction; q) pure extract of *R. rapens* positive for Shinoda reaction; r) pure extract of *R. communis* positive for saponins; s) positive evidence of foam in *R. communis*; t) pure extract of *V. farnesiana* positive for saponins; u) positive evidence of foam in *V. farnesiana*; v) pure extract of *R. repens* negative for saponins; and w) negative evidence of foam in *R. repens*



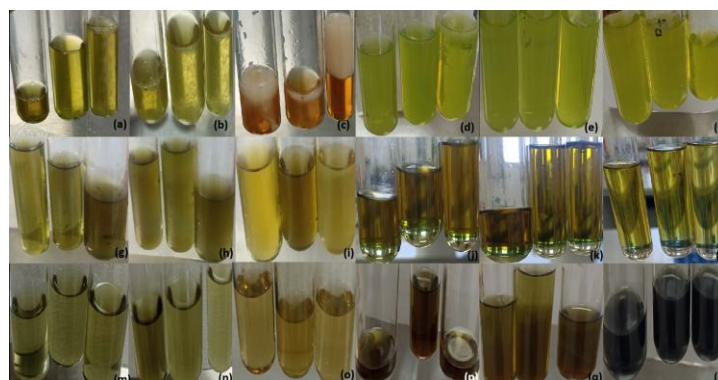
Source: Authors (2025).

An interesting group of secondary metabolites of higher plants are the flavonoid pigments, which are extremely abundant and variable in the degree of oxidation. For this class of compounds a large number of activities have been reported, such as antitumor, antihypertensive, anti-inflammatory, bactericidal, bactericidal, anti-inflammatory, anti-inflammatory, bactericidal or cell stabilizing. Against insects, it has been proved that they have insecticidal activity and growth inhibitory activity, and have been evaluated against a large

number of species, among them *Heliothis virescens*, *Spodoptera exigua*, *Heliothis zea*, *Manduca sexta*, *Amyelosis transitella*, *Ostrinia nubilalis* Hubner, and *Pieris brassicae*. Among the flavonoids that affect development and growth are quercetin, rutin, naringenin, rnaicin, eriodictyol and catechin [18-19].

Figure 4

Extraction process and identification of secondary metabolites in biological samples of higuerrilla (Ricinus communis), huizache (Vachellia farnesiana) and pink grass (Rhynchelytrum repens). a) pure extract of R. communis negative for shinoda reaction; B) pure extract of V. farnesiana negative for shinoda reaction; C) pure extract of R. repens negative for shinoda reaction; D) pure extract of R. repens positive for shinoda reaction; D) pure R. communis extract negative for tannin reaction; e) pure V. farnesiana extract negative for tannin reaction; f) pure R. repens extract negative for tannin reaction; g) pure R. communis extract positive for triterpenes reaction; h) pure V. farnesiana positive for triterpenes reaction; i) pure extract of R. repens positive for triterpenes reaction; J) pure extract of R. communis positive for phenols reaction; k) pure extract of V. farnesiana positive for phenols reaction; l) pure extract of R. repens positive for phenols reaction; M) pure extract of R. communis negative for catechin reaction; n) pure extract of V. farnesiana negative for catechin reaction; o) pure extract of R. repens negative for catechin reaction; P) pure extract of R. communis negative for quinone reaction; q) pure extract of V. farnesiana negative for quinone reaction; and r) pure extract of R. repens negative for quinone reaction



Source: Authors (2025).

Flavonoids are important for host recognition and acceptance by adult insects. When an adult female butterfly or moth lands on a plant, she usually walks on the surface of the leaf or stem. The duration of this walk and the onset of the next behavior may depend on the stimuli received from the compounds on the plant surface. Some species start drumming with their forelegs upon contact with a potential contact with a potential host, others lower their antennae.

At least two factors support the claim that flavonoids play a role in insect-plant interactions. At firstly, that flavonoids can modulate insect feeding and oviposition behavior of insects and, second, that the distribution of flavonoids varies among plant species. However, it is necessary to establish whether the distribution specific flavonoids overlaps with the presence or absence of these flavonoids in host and/or non-host plants. Aldehydes are straight-chain chemical compounds that are saturated (single bond) or unsaturated (double bond) with the carbonyl functional group responsible for the insecticidal activity, such as propanal, 2-methyl-2-butenal and 2-methyl-2-butenal. The biological activity of aldehydes activity lies in acting as a bioinsecticide and with phytotoxicity against insects that insects that attack fruits, vegetables and grains. This functional group can be used as a post-harvest insecticidal control agent, eliminating 100% of the aphid pests that attack grains, with undetectable damage to the functional characteristics of the products tested [20].

Monoterpenes are secondary metabolites biosynthesized in plant essential oils [21]. They consist of an isoprene base structure and, when they contain oxygen, they are structuralize into terpenoids. The insecticidal and acaricidal activity of polyhalogenated monoterpenes obtained from *Plocamium cartilagineum* was demonstrated against insects such as *Spodoptera frugiperda*, a larva that damages corn, sugarcane or on corn, sugar cane or onion; *Heliothis virescens*, larva that damages tobacco, cotton or tomato; and tobacco, cotton or tomato and *Aphis fabae*, an aphid or bean aphid that can also affect beets, among others. Argandoña et al. (2000) isolated two halogenated monoterpenes; mertensene and violacene, obtaining also two derivatives of the first one; dibromomertensene and dihydromertensene, to test their insecticidal activity against insecticidal activity against the tomato larvae *Tuta absoluta* and the cereal aphid *Schizaphis graminum*. When the compounds were tested against tomato larvae, 100% mortality was observed when mertensene was applied and 80% for mertensene and, 80% for violacene and dibromertensene (obtained by the addition of 2 bromides in the double bond of mertensene). In the case of for aphids, violacene showed 92% mortality and the other compounds did not show the other compounds did not show effectiveness at any of the concentrations used. concentrations used [22-27].

3.2 PRINCIPAL COMPONENTS ANALYSIS OF NUTRIENT CONTENT

The Inductively Coupled Plasma (ICP) is an ionization source that together with an Optical Emission Spectrophotometer (OES) constitutes the ICP-OES equipment. In this equipment, ICP (inductively coupled plasma) is used to generate excited atoms that emit electromagnetic radiation upon return to their ground state. The wavelength of this radiation is characteristic of

each element (macroelements and microelements), being a very selective technique. The amount of radiation emitted is proportional to the concentration of the analyte. This fact allows, after comparison with standards, to quantify the analyte content with accuracy and precision [28-29]. Induction plasma is an ionized gas (plasma source) containing free electrons and cations that operates under conditions of high temperatures and low pressures, usually operated by a free radiofrequency generator. [30-31].

The elements three asterisks in each column mean that they are the nutritional elements in which each plant stands out for having a greater presence in comparison with the others; those into two asterisks are the nutritional elements that it shares with those next to it and in one asterisk the nutritional elements with the lowest concentration in that plant (Table 3).

Table 3

Macroelement and microelement concentrations (mg/kg)

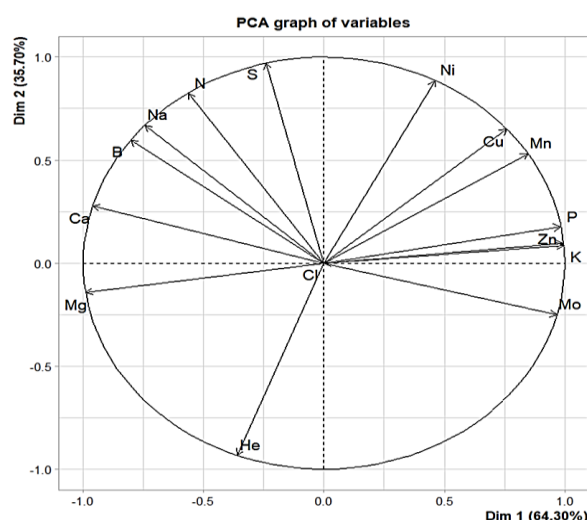
Elements	Pink grass (<i>R. repens</i>)	Huizache (<i>V. farnesiana</i>)	Higuerilla (<i>R. communis</i>)
N	25,600.00*	35,200.00***	41,400.00***
P	3,700.00**	1,700.00****	3,100.00**
K	17,400.00*	10,100.00***	14,600.00**
Ca	6,400.00*	28,500.00***	22,000.00***
Mg	2,300.00**	2,600.00**	2,400.00**
S	2,200.00*	3,000.00****	4,900.00****
Na	90.00*	467,000.00*	563,000.00*
Cl	0.00**	0.00**	0.00**
Fe	81.50****	94.50***	54.00*
Zn	31.40***	8.58****	22.90**
Mn	56.90****	33.40*	59.30***
Cu	9.95****	4.46*	11.70***
B	3.96*	22.50****	24.00****
Ni	1.26*	0.60*	2.17***
Mo	3.80***	0.25*	1.38****

The three plant extracts samples are found in different quadrants (Figure 5). The higuerilla is found in quadrant one, standing out for the dominance of elements such as manganese, copper and nickel. In contrast, iron seems to be the most deficient element for higuerilla. This same plant has similar amounts of phosphorus, potassium and zinc against rosado grass, due to its location in quadrant IV adjacent to it. The pink grass only stands out in one element which is molybdenum, it can be seen that it is the plant with the highest amount of this element, also

for zinc. The opposite quadrant to IV is II, therefore, it can be said that pink grass is the most deficient of the three biological samples in the following elements: nitrogen, calcium and sulfur for macroelements. As for the microelements they are: sodium, boron and nickel (Figure 6). Next, analyzing quadrant III we can find huizache, which stands out for containing a high concentration of elements such as iron, magnesium (2,600 mg/kg), but in this last one it does not seem that the difference is so high in comparison with the other two plants higuerrilla (2,400 mg/kg) and pink grass (2,300 mg/kg). Finally, the element chlorine appears in the middle, since in none of the samples its presence was reported, giving a result of 0 mg/Kg.

Figura 5

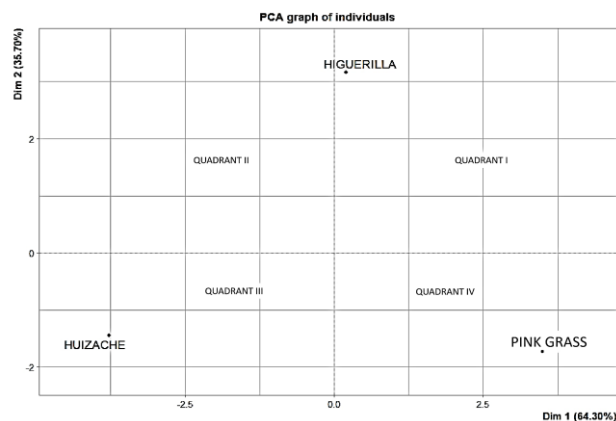
Principal Components Analysis graph of macroelements and microelements variables



Source: Authors (2025).

Figure 6

Principal Components Analysis graph of individuals plants, higuerrilla (R. communis), b) huizache (V. farnesiana) and c) pink grass (R. repens)



Source: Authors (2025).

The higuerrilla plant is rich in microelements such as manganese, copper and nickel compared to the other two, and in macroelements such as nitrogen. The pink grass has high amounts of microelements such as zinc and molybdenum, and does not stand out with high concentrations of any macroelements, but rather seems to have the lowest concentration of nitrogen, calcium and sulfur. Likewise, it is deficient in microelements such as sodium, boron and nickel. The huizache plant only stands out for being the one with the highest concentrations of calcium and iron. For most of the microelements such as manganese, copper, nickel and molybdenum, it is the plant with the lowest amounts, and for the three plants there is no chlorine and similar amounts of magnesium ranging from 2,300 mg/kg to 2,600 mg/kg. As for the elements phosphorus and potassium, pink grass and higuerrilla have similar values, for the first element from 3,100 to 3,700 mg/kg. On the other hand, for the second element, potassium, the values range from 14,600 to 17,400 mg/kg. The higuerrilla and the pink grass can complement each other well to make a mixture high in microelements such as: manganese, copper, boron, nickel and molybdenum.

Rani et al (2023) analyzed the methanolic leaf extract of *Thalictrum foliolosum* by ICP-MS technique to trace mineral elements and the result revealed the presence of important micronutrients such as manganese (Mn), iron (Fe), zinc (Zn), chromium (Cr), copper (Cu) and potassium (K). These minerals are cofactors for more than 3,000 regulatory enzymes of various metabolic pathways [32]. Wieczorek, et al (2022) mention that ICP-OES is an advanced instrumental method that allows less expensive and less time-consuming trace element analysis with adequate higher precision and repeatability evaluation [33]. Trimmel et al, (2023) analyzed seven certified reference materials for plants: apple tree leaves, peach tree leaves, hay powder, aquatic plant, twigs and leaves of bush, spinach leaves and green tea to determine their mass fractions of 48 elements by ICP-MS/MS, which were lithium (Li), beryllium (Be), sodium (Na), magnesium (Mg), aluminum (Al), calcium (Ca), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), germanium (Ge), arsenic (As), Se, rubidium (Rb), strontium (Sr), iodine (I), Nb, molybdenum (Mo), silver (Ag), cadmium (Cd), Sb, tellurium (Te), barium (Ba), La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Ta, Tl, lead (Pb), Bi, Th and U [34]. On the other hand, Sadee et al. (2023) evaluated the concentration of various metals in vegetables of common local consumption by ICP-MS, these analyzed plants were tarragon, laurel, dill, Syrian mesquite, vine leaves, thyme, arugula, basil, common purslane and parsley. The concentration ranges analyzed were: 76-778 for aluminum (Al), 10-333 for boron (B), 4-119 for barium (Ba), 2812-24645 for calcium (Ca), 0.1-0.32 for cobalt (Co), 201-464 for iron (Fe), 3661-46400 for potassium (K), 0.31-1.53 for lithium (Li), 860-

14330 for magnesium (Mg), 16.20-71.5 for manganese (Mn), 612-4725 for sodium (Na) and 15.8-46 $\mu\text{g g}^{-1}$ for zinc (Zn) [35]. Sultana et al. (2022) compared the results obtained in the determination of the content of essential elements such as manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) in plant samples using different analytical approaches, as well as the most commonly used spectroscopic methods in the field of plant analysis, such as acid digestion in combination with atomic emission (AES) and atomic absorption spectrometry (AAS), being the analysis by inductively coupled plasma optical emission spectrometry (ICP-OES) a sustainable and rapid analytical alternative [36]. Sukru et al. (2022) determined trace metal concentrations of magnesium (Mg), iron (Fe), copper (Cu), lead (Pb), chromium (Cr), arsenic (As), nickel (Ni), zinc (Zn), and Selenium (Se) in dried red plum samples from Turkey. These were determined by ICP-OES technique after solubilization in microwave digestion methods. The concentrations of the elements in dried red plum samples were 44-47 of magnesium (Mg), 15-18 of iron (Fe), 03.1-3.2 of copper (Cu), 4.1-4.4 of lead (Pb), 1.4-1.6 chromium (Cr), 1.1-1.3 arsenic (As), 2.5-2.8 nickel (Ni), 3.4-3.7 zinc (Zn) and 4.2-4.5 selenium (Se) at $\mu\text{g/g}$ concentrations [37].

4 CONCLUSIONS

The specialized secondary metabolites detected in higuierilla (*R. communis*) were: chalcones, auronos, flavonols, sterols, condensed tannins, saponins and triterpenes. The specialized secondary metabolites detected in huizache (*V. farnesiana*) were: flavones, flavonols, sterols, condensed tannins, saponins and triterpenes. The specialized secondary metabolites detected in pink grass (*R. repens*) were: flavones, flavonols, methylsterols, phenols and triterpenes. With respect to the metallome of the extracts analyzed by the ICP-OES technique, whose method determined quickly and with high precision the trace elements, it was quantified that the higuierilla plant is rich in microelements such as manganese, copper and nickel compared to the other two, and in macroelements such as nitrogen. The pink grass has high amounts of microelements such as zinc and molybdenum, and does not stand out with high concentrations of any macroelements, but rather seems to have the lowest concentration of nitrogen, calcium and sulfur. The huizache plant only stands out for being the one with the highest concentrations of calcium and iron.

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