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Article

Phytochemistry, antioxidant and biocide activity of *Parthenium hysterophorus* L. vs *Artemia salina* L.

Jorge Alberto Alejandre-Rosas[1]
Miguel Ángel Gallegos-Robles[1,§]
Uriel González-Salas[1]
Antonio González-Jacob[1]
José Luis García-Hernández[1]
Bernardo Murillo-Amador[2]

1 Facultad de Agricultura y Zootecnia-Universidad Juárez del Estado de Durango. Carretera Gómez Palacio-Tlahualilo km 32, Ejido Venecia, Gómez Palacio, Durango, México. CP. 35111.

2 Centro de Investigaciones Biológicas del Noroeste, SC. Avenida Instituto Politécnico Nacional núm. 195. Colonia Playa Palo de Santa Rita Sur, La Paz, Baja California Sur, México. CP. 23096.

Autor para correspondencia: garoma64@hotmail.com.

Abstract

The importance of medicinal plants is highlighted, mentioning that 80% of the world's population uses traditional medicine (WHO). Mexico is the second richest country in knowledge of traditional medicine, after China. *Parthenium hysterophorus* L., known as the bitter broom, is mentioned, and although its use in Mexico is not widely documented, it has been recorded in Papantla, Veracruz and Güémez, Tamaulipas. The study focuses on the phytochemistry of *P. hysterophorus*, identifying secondary metabolites, such as alkaloids, flavonoids, saponins, and tannins. Antioxidant activity was evaluated using the DPPH method, showing high percentages of inhibition in aqueous and ethanolic extracts. In addition, the biocidal activity in *Artemia salina* was analyzed, revealing moderate toxicity for the aqueous and ethanolic extract. The plant material was collected in the central area of the state of Veracruz in July 2022. The results suggest a potential therapeutic and pharmacological use of the plant, and a use in crop protection; nevertheless, more research is needed to better understand its phytochemistry and medical applications.

Keywords:

biological activity, free radicals, medicinal plants, natural products.

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Introduction

Medicinal plants are a vital resource for human health, they are used by approximately 80% of the world's population (WHO) (De la Cruz-Jiménez *et al*., 2022). In Mexico, a country with a rich tradition in traditional wild plant medicine, where various cultures have used plants such as Asteraceae, Fabaceae, Rubiaceae, and Malvaceae to treat diseases. Nonetheless, there is still much to be studied about the ethnobotanical richness of the country, as is the case of *P. hysterophorus*, widely distributed outside its area of origin (Cruz-Pérez *et al*., 2021).

This species is native to northwestern Mexico and the United States, endemic to the Americas, and is distributed throughout the Asian continent and part of European countries (Lalita and Kumar, 2018), it is known by its common name as: bitter broom, arrocillo, carrot grass, star weed, congress grass, wild feverfew, ragweed, bitter weed, white top, and the 'Scourge of India' (Espinosa-Rivero *et al*., 2015).

This plant adapts easily to different environments thanks to its biomass allocation and phenotypic plasticity, which gives it a competitive advantage (Rathee *et al*., 2021). *P. hysterophorus* is used medicinally to treat peptic ulcers and inflammations (Espinosa-Rivero *et al*., 2015) and as an antimicrobial due to compounds, such as parthenin, a sesquiterpene lactone and other active metabolites, such as caffeic acid, 3,7-dimethyl ether, quercetagetin, ferulic acid, vanillic acid, p-coumaric, P-hydroxy-benzoin and vanillic acid, 6-hydroxy kaempferol, 3-0-arabinoglycoside, and some other unidentified alcohols (Alfaro-Jiménez *et al*., 2022).

Despite being considered an aggressive weed in agriculture, it also has potential pharmacological properties. Jaiswal *et al*. (2022) mention that the flavonoids found in *P. hysterophorus*, such as kaempferol and quercetin, show antioxidant, anti-inflammatory, and antimicrobial activity, as well as inhibitory potential for cancer cell growth; Rai and Lall (2021) presented results on its analgesic and antitumor activity, as well as for its potential in the treatment of neurodegenerative diseases, pointing out secondary metabolites that may be useful in the treatment of inflammatory diseases, such as rheumatoid arthritis, and have therapeutic applications in the field of cancer and other diseases.

The ethnobotanical censuses carried out in the north of Puebla, Tlanchinal, Hidalgo, Monterrey, Nuevo León, Xalpatlahuac, Guerrero and Zacatecas do not document the use of the plant (Estrada-Castillón *et al*., 2012). Nevertheless, Lara-Reimers *et al*. (2019), in Papantla, Veracruz and Güémez, Tamaulipas, respectively, documented the presence and use of parts of the plant (leaves and stems) for the treatment of gastritis.

A study by Kaur *et al*. (2021) presented data on this weed, which is considered to be harmful; they highlighted that it is a therapeutic medicinal plant and examined the various ethnobotanical uses in traditional medicine, including its application in the treatment of conditions, such as asthma, malaria, skin infections, and gastrointestinal diseases.

In relation to toxicological aspects, they mention the possible adverse effects associated with its consumption or exposure, such as contact dermatitis and allergic reactions; however, it also has a potential herbicidal use, as reported in the extracts of *P. hysterophorus*, *Cleome rutidosperma*, and *Borreria alata*, inhibiting the growth of weeds without causing significant damage to the selected crops.

This suggests a possible potential use of these extracts as natural herbicides in agriculture and weed management (Motmainna *et al*., 2021). For this reason, it is necessary to know and identify the secondary metabolites present in *P. hysterophorus*, to establish the degree of toxicity, using a biological model, to check whether ethanolic and aqueous extracts show antioxidant potential and their innocuity in different concentrations. The present study aims to identify the phytochemistry, *in vitro* antioxidant activity and biocidal activity of *P. hysterophorus*.

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Materials and methods

Study area

The research was conducted at the Faculty of Chemical Sciences, Orizaba Campus, Veracruzana University. Samples of the aerial part of *P. hysterophorus* were collected in an agricultural area in Ixtaczoquitlán, Veracruz, Mexico. (18° 88.95' north latitude and 97° 05.41' west longitude). One of the botanical specimens obtained was registered as 12520 and was deposited in the CORU Herbarium of the Faculty of Biological and Agricultural Sciences of the Veracruzana University.

Plant material

Whole plants of *P. hysterophorus* (stems, leaves, and flowers) were collected and washed with sterile distilled water, removing the damaged plants. The material of the aerial part of the plant was cut into 5 mm pieces and dehydrated in an oven (Thermo Scientific, Hera Therm incubator serial number 4160722) at 40 °C. Subsequently, it was placed in transparent, wide-mouthed glass bottles with a capacity of 4 L to separately macerate the solvents in sterile distilled water and 70% ethanol (2:1 w/v).

The bottles were hermetically sealed and kept at room temperature (25°) and in darkness for 72 h. After this time, the macerate was filtered to separate the solid remains using Miracloth fabric filters (Merck, millipore, USA). Both extracts were concentrated using a Labconco Buchi rotary evaporator at a constant temperature of 40 °C until the evaporation of the respective solvents; after this, the samples were freeze-dried and stored in a moisture-free atmosphere until analysis.

Identification of secondary metabolites

To determine the phytochemical qualitative profile of *P. hysterophorus*, the methodologies considered were those proposed by Domínguez (1973); Martínez *et al*. (2008); Shamsa *et al*. (2008); Pandey and Tripathi (2014); Pandey (2014); Robles-García *et al*. (2016) (Table 1).

Table 1. Preliminary methods for phytochemical determination using qualitative techniques.

Secondary metabolite	Trial	Result	References
Alkaloids	Drangendorff Sonnenschain	Orange precipitate	Shamsa *et al.* (2008); Sreevidya and Mehrota (2003)
Reducing sugars	Fehling Benedict	Orange to red precipitate	Pandey and Tripathi (2014)
Coumarins	Erlich NH_4OH	Orange coloration Observe under UV light-365 nm, blue or green fluorescence	Martínez *et al.* (2008); Domínguez (1973)
Flavonoids	Shinoda 10% NaOH reaction	There is a red coloration of aurones or chalcones Change of coloration from yellow to red indicates the presence of xanthones and flavones; from brown to orange, the presence of flavonols; from purple to reddish, the presence of chalcones; and blue, the presence of anthocyanins.	Martínez *et al.* (2008); Robles-García *et al.* (2016)
Cardiotonic glycosides	Legal Baljet Grignard	Red coloration, unstable	Yadav *et al.* (2014)
Cyanogenic glycosides		Orange to dark red coloration	
		Pink to red coloration	

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Secondary metabolite	Trial	Result	References
Quinones	Sulfuric acid Börntraguer	Red coloration, anthraquinone Purple red coloration, benzoquinones From greenish yellow, it changes to red, anthrones	Domínguez (1973)
Saponins	Foaming Lieberman Bouchard Rosenthaler	Foam with a height of 8-10 mm stable for 30 min Blue or green coloration at the interphase Pink, red, magenta, or violet coloration	
Sesquiterpenes	Reaction with ferric hydroxylates	Red, violet, or pink coloration	Robles-García *et al.* (2016)
Tannins	Gelatin $FeCl_3$	White precipitate Blue to black coloration (gallic acid) Green coloration (catechol derivatives) Formation of a blue coloration (phenolic compounds)	

Determination of antioxidant activity

The method of the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was used; to this end, a 0.1 mM stock solution was prepared and stored at -4 °C in the dark. The solution was adjusted in absorbance from 1.1 ±0.02 to 517 nm. Once the solution was adjusted, 19.6 ml of the DPHH solution was taken and made up to a volume of 50 ml with methanol in an volumetric flask and placed in darkness; three solutions were prepared in volumetric flasks with the aqueous and ethanolic extracts undiluted at different concentrations, 100, 500, and 1 000 µg ml^{-1} (1 ml sample and 3 ml reagent) in triplicate.

It was left to react for 30 min, then stirred in vortex, and read on the spectrophotometer at 517 nm. Pure methanol was used as a blank (Rivas-Morales *et al*., 2016). The results were converted to percentage of inhibition and expressed as antioxidant capacity in µmol equivalents (AAE to ascorbic acid). Trial data were done in triplicate (Rojano *et al*., 2008).

Antioxidant activity

Antioxidant activity was estimated using the solution of 1,1-diphenyl-2-picrylhydrazil DPPH as a reference (Brand-Williams *et al*., 1995) and the equation suggested by Fukumoto and Mazza (2000). The data obtained are expressed in percentage values of inhibition and as an antioxidant percentage. The tests were performed in triplicate.

$$\%\ \text{Inhibition}=\frac{\text{A-A1}}{\text{A}}*100$$

Where: A= control absorbance; A1= sample absorbance.

Determination of biocidal activity

Five glass fish tanks were used with 1.5 L of artificial seawater (38 g L^{-1}) at room temperature (26 °C) and oxygenated by pumps. Each tank was added with 0.5 to 0.8 g of *Artemia salina* cysts (Brand Azul, Eclosión azul). Hatching took place between 24 and 48 h after seeding. With a Pasteur pipette, 10 mature phototropic nauplii of *A. salina* were taken and placed in glass vials (2 ml 12 × 32 mm) and different concentrations of the aqueous and ethanolic extracts resuspended in sterile distilled seawater were added (0 µg ml^{-1}, 50 µg ml^{-1}, 100 µg ml^{-1}, 200 µg ml^{-1}, 300 µg ml^{-1}, 400 µg ml^{-1}, 500 µg ml^{-1}, 600 µg ml^{-1}, 700 µg ml^{-1}, 800 µg ml^{-1}, 900 µg ml^{-1} and 1 000 µg ml^{-1}) in triplicate for both extracts, leaving them 24 h at room temperature protected from extreme temperatures together with the control group that did not have the extract (Meyer *et al*., 1982).

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The toxicity of the extracts was determined after 24 h under observation with an optical microscope. The lack of movement of the nauplii for 10 min was considered as proof of toxicity. The degree of toxicity of the extract was defined according to the range in which the LC_{50} values were found according to the categories: extremely toxic (1-10 µg ml^{-1}), highly toxic (10-100 µg ml^{-1}), moderately toxic (100-500 µg ml^{-1}), slightly toxic (500-1 000 µg ml^{-1}), practically non-toxic (1 000-1 500 µg ml^{-1}), relatively harmless (≥ 1 500 µg ml^{-1}) (CYTED, 2014). The concentration range determined the Lethal Concentration (LC_{50}) by the Probit method of analysis (Finney, 1971).

Statistical analysis

The design was completely randomized with three replications and three observations per replication. An analysis of variance was performed using the statistical program of Minitab 20.1 (2022). Fisher's test was used to determine the differences between averages of each of the variables evaluated. The determination of biological and antioxidant activity with three replications was considered statistically significant for $p \leq 0.05$. When necessary, data were adjusted for normalization.

Results and discussion

Secondary metabolites

The ethanolic extract showed a greater amount of secondary metabolites, such as alkaloids and reducing sugars, and a lower presence of flavonoids, sesquiterpene lactones and tannins. The aqueous extract also stood out in alkaloids mainly, followed by reducing sugars, saponins and to a lesser extent by flavonoids.

The difference between the extracts was notable in tannins: positive in the analysis with ferric chloride for the alcoholic extract and negative for the aqueous extract; these data agree with those presented by Jiménez *et al*. (2021); in some of the metabolites, it is important to consider that the time of collection and geographical area influence their presence, plants respond to factors such as number of hours of light, type and fertility of the soil, predators, rainfall regime, among others (Al Ruheili *et al*., 2022) (Table 2).

Table 2. Phytochemical screening present in *P. hysterophorus* extracts.

Metabolites	Extract	
	Aqueous	Ethanolic
Alkaloids	+++	+++
Reducing sugars	++	+++
Coumarins	-	-
Flavonoids	+	++
Cardiotonic glycosides	-	-
Cyanogenic glycosides	-	-
Quinones	-	-
Saponins	++	-
Sesquiterpene lactones	-	++
Tannins	-	++

A composition of secondary metabolites similar to that reported by Rodríguez *et al*.(2000) was observed in ethanolic extracts at 35%, which showed a fungicidal activity against *Stemphylium*, *Fusarium*, *Pyricularia* and *Phytophthora*, where *P. hysterophorus* showed the greatest inhibitory effect on growth of the four fungi evaluated. Fazal *et al*. (2011) found alkaloids in ethanolic extracts with larvicidal and bactericidal activity by their action of blocking neuroreceptors, inhibiting signal

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transductions, intermediates of neuronal signal transduction, and ion channels of vertebrates and insects, and in the ability to intercalate in DNA and stop protein synthesis, induce apoptosis, and inhibit enzymes of sugar metabolism, main source of energy for microorganisms.

Studies such as those by Díaz *et al*. (2011) mention parthenin as a toxic alkaloid with nematicidal and insecticidal activity. The presence of flavonoids highlights its pharmacological uses, including antiparasitic activities (Saucedo *et al*., 2011). On the other hand, Rodríguez *et al*. (2012) identified antifungal activity in aqueous extracts of *P. hysterophorus* against *Pyricularia grisea*, a rice phytopathogen. Another of the metabolites present in *P. hysterophorus* are saponins, which show high antibacterial and antifungal activity by affecting cell integrity and traces of tannins with antimicrobial activity were also found (Patra and Saxena, 2009).

Although Espinosa-Rivero *et al*. (2015) mention that aqueous extracts have low antimicrobial activity against *Helicobacter pylori*, they inhibit its growth and adhesion by blocking the action of urases. These results suggest the possibility of isolating the components of the extract to identify those responsible for antimicrobial activity and use them as active ingredients in drugs. Importantly, metabolites vary in existence or concentration depending on the region and climate, due to the plant's interaction with the environment (Kaur *et al*., 2016).

Antioxidant activity

The antioxidant activity of the aqueous and ethanolic extracts of *P. hysterophorus* showed a high inhibition of DPPH, with 82.19% for the aqueous extract and 73.12% for the ethanolic extract (p# 0.05) (Table 3), similar to that reported by Ahmad *et al*. (2010), 67.07 % in ethanolic extracts. There were no differences in the two extracts evaluated, suggesting that the antioxidant capacity of the aqueous and ethanolic extracts is due to the presence of alkaloids, saponins and, to a lesser extent, flavonoids (Kaur *et al*., 2021; Alfaro-Jiménez *et al*., 2022).

These compounds also have biological effects such as antibacterial, antiviral, anti-inflammatory, antiallergenic, antithrombotic, and vasodilatory activity (Rai and Lall, 2021; Alviter *et al*., 2024).

Table 3. Percentage of inhibition of antioxidants (DPPH), *P. hysterophorus*.

Extract	Absorbance 517 nm	$\lvert x-\bar{x} \rvert^2$	(%)
Aqueous	0.138 a	0.0327 a	82.19 a
Ethanolic 70%	0.209 a	0.121 a	73.12 a
Average	0.173	0.0076	77.65
SD		0.1	

Different letters suggest significant differences for $p \leq 0.05$ according to Fisher.

Aerobic organisms breathe in molecular oxygen (O_2) found in the environment, which results in the formation of reactive oxygen species (ROS). When this O_2 is reduced in its passage through the respiratory chain, it forms the super oxide, which in the oxidation-reduction process, can very easily form hydrogen peroxide (H_2O_2), which, in the presence of transition metals, such as iron (Fe^{2+}) and copper (Cu^{+}), produces the Hydroxyl radical (OH) through the Fenton reaction, which, in biological systems, is the most harmful and cause of oxidative damage (Kaur *et al*., 2021).

The high percentage of DPPH inhibition in *P. hysterophorus* extracts highlights their antioxidant capacity, which is attributed to the aforementioned natural compounds. This antioxidant therapy offers an inexpensive alternative to treat diseases related to oxidative stress. Ahmad *et al*. (2011) found similar results in ethanolic extracts of *P. hysterophorus* in Pakistan in terms of their antilarvicidal activity correlated with the presence of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (PO), ascorbate peroxidase (APX) and catalase, which have the function of protecting cells from oxidative damage caused by ROS.

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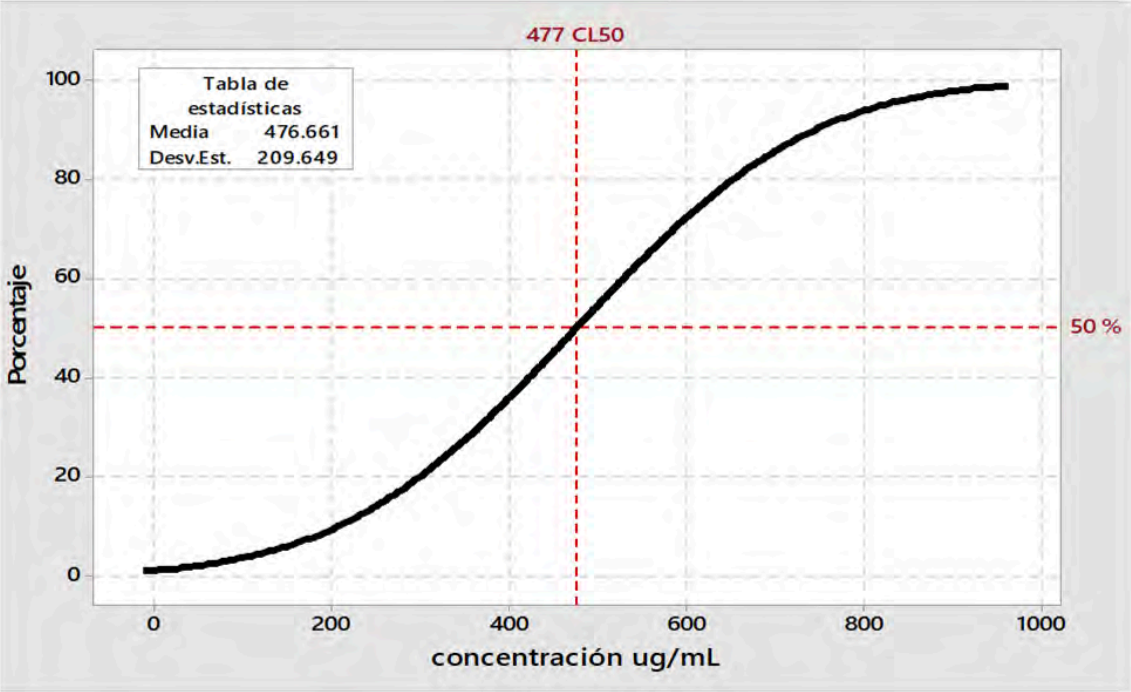
Plants remain the main source of pharmaceuticals and alternative treatments for human diseases (Mofokeng *et al*., 2022). Kaur *et al*. (2021) reported a 55% cell membrane protection against lipid peroxidation in mouse renal cells due to the presence of flavonoids also reported in this study.

Biocidal activity

This study performed the first screening of the toxicity shown by aqueous and ethanolic extracts of *P. hysterophorus*. For this test, the control group kept 100% of the individuals alive during the 24 h duration of the trial, with based on the toxicity characteristics, the LC_{50} indicated that the ethanolic extract at a concentration of 477 µg ml^{-1} (Figure 1).

Figure 1. Survival percentage of *A. salina* in ethanolic extract of *P. hysterophorus* at different concentrations.



This is due to a greater amount of metabolites, in particular the sesquiterpene lactones and tannins present, but they were not found in the aqueous with LC_{50} of 167.6 µg ml^{-1} (Figure 2), they are considered moderately toxic according to the CYTED classification in 2014. Different authors mention the inhibition of key enzymes of energy metabolism (oxidative phosphorylation) and nucleic acid replication (DNA polymerase) due to sesquiterpene lactones (parthenin) together with tannins that could be the key to these levels of toxicity shown by *P. hysterophorus* (Alviter *et al*., 2024).

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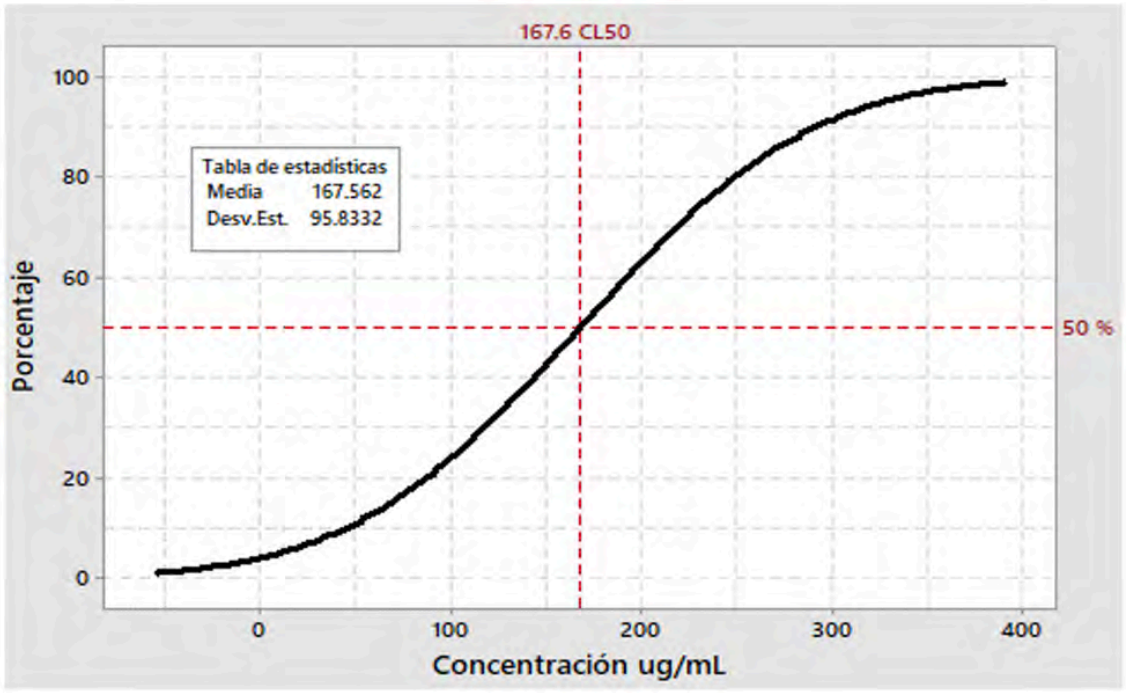
Figure 2. Survival percentage of *A. salina* in the aqueous extract of *P. hysterophorus* at different concentrations.



The use of extracts of *P. hysterophorus* on *A. salina* provides information on how the components of secondary metabolism are an important source of pharmacological products; this widely used trial determines the lethal effect on *A. salina* and thus predicts its ability to produce the death of cancer cells in tissue culture, control insects, or exert a wide range of pharmacological effects (Ahsan *et al*., 2020, Kaur *et al*., 2021).

Conclusions

This study proved the existence of secondary metabolites, such as flavonoids, alkaloids, saponins, and tannins, in the aqueous and ethanolic extracts of *P. hysterophorus*, which have been widely reported with biological activity against microorganisms, plants, animals, and human health. They were determined the percentage of inhibition of antioxidants by means of the DPPH technique with inhibition percentages of aqueous and ethanolic extracts of 82 and 73%, respectively.

The cytotoxic activity was identified in the biological model of *A. salina*, obtaining an LC_{50} of 477 µg ml^{-1} in the ethanolic extract and 167.6 µg ml^{-1} in the ethanolic extract on the nauplii of *A. salina*, considering these concentrations as moderately toxic.

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