Publication status: Not informed by the submitting author

Polyphenol and flavonoid contents of Parkinsonia aculeata L., extracts: Phytotoxicity in tomato

Leandris Argentel Martínez, Ofelda Peñuelas-Rubio, Jorge González Aguilera, Jaime Garatuza-Payán, Marcos Vinicius Varjão Romão

https://doi.org/10.1590/SciELOPreprints.4719

Submitted on: 2022-09-07
Posted on: 2022-09-09 (version 1)
(YYYY-MM-DD)

Polyphenol and flavonoid contents of *Parkinsonia aculeata* L., extracts: Phytotoxicity in tomato

Contenido de polifenoles y flavonoides en extractos de *Parkinsonia aculeata* L.: Fitotoxicidad en tomate

Leandris Argentel-Martínez[1*], Ofelda Peñuelas-Rubio[1], Jorge González Aguilera[2]; Jaime Garatuza-Payán[3], Marcos Vinicius Varjão Romão[4]

[1]Departamento de Ingenierías, Tecnológico Nacional de México/Instituto Tecnológico del Valle del Yaqui, Bácum, Mexico.

[2]Editora Pantanal, Nova Xamantina, MT, Brazil.

[3]Departamento de Ciencias del Agua, Instituto Tecnológico de Sonora, Ciudad obregón, Sonora, Mexico.

[4]Instituto Federal de Educação, Ciência e Tecnologia da Bahia, Euclides da Cunha, Bahia, Brazil.

Autor de correspondencia: Leandris Argentel Martínez: oleinismora@gmail.com

ORCID

LAM https://orcid.org/0000-0002-0353-2251

OPR https://orcid.org/0000-0002-7782-3246

JGA https://orcid.org/0000-0002-7308-0967

JGP https://orcid.org/0000-0003-2357-910X

MVVR https://orcid.org/0000-0003-2361-7671

ABSTRACT

Parkinsonia aculeata L. (palo verde) it is native to semidesert region and seasonally dry tropical forests. A more detailed study of the biochemical composition of its organs could offer information for its use for pest and disease biocontrol in species of agricultural and economic interest. This research aimed to determine the content of polyphenols and flavonoids in hydroalcoholic extracts obtained from the stems and leaves of *P. aculeata* and its phytotoxicity in tomato seedlings applied at the early stages of growth. The extracts were applied at 15, 25 and 35 days after emergence (DAE). The highest content of polyphenols and flavonoids was obtained in the leaves, and the polyphenol concentration exceeded that of flavonoids. The hydroalcoholic extracts, of both stems and leaves, presented level 5 phytotoxicity in tomato plants at 15 DAE. However, from 25 DAE, there was no phytotoxicity. At 35 DAE, there was only phytotoxicity when the volume of both organs was 5 mL Plant^{-1}. There was a significant interaction between organ and volumes factors. The study shows that leaf and stem extracts can be used for fusarium wilt biocontrol without causing phytotoxicity in tomato plants from 25 days, using volumes between 1 and 3 mL plant^{-1}.

Keywords: antioxidants, biocontrol, biofungicides, palo verde, rotavapor.

RESUMEN

Parkinsonia aculeata L. (palo verde) es originaria de región semidesértica y bosques tropicales estacionalmente secos, aunque se distribuye en varias regiones del mundo. Entre las principales características de esta especie es el tallo verde, hojas reducidas, legumes moniliformes, mínima cantidad de plagas y enfermedades que la afectan. Un estudio más detallado de la composición bioquímica de sus órganos pudiera ofrecer información para su uso en el biocontrol de plagas y enfermedades en especies agrícolas de interés económico. El presente estudio tuvo como objetivo determinar los contenidos de polifenoles y flavonoides en extractos hidroalcohólicos de tallos y hojas de *P. aculeata* y la fitotoxicidad en plántulas de tomate, aplicados en estadios iniciales del desarrollo. Los extractos fueron aplicados a los 15, 25 y 35 días posteriores a la emergencia (DPE). El mayor contenido de polifenoles y de flavonoides se obtuvo en las hojas y la concentración de polifenoles superó a la de los flavonoides. Los extractos hidroalcohólicos, tanto de tallos como de hojas presentaron fitotoxicidad de nivel 5 en las plantas de tomate, sin embargo, a partir de los 25 DPE no existió fitotoxicidad. A los 35 DPE solo existió fitotoxicidad cuando se aplicó el volumen de 5 mL Planta^{-1} de ambos órganos. Existió interacción significativa entre las fuentes de variación órganos y volumen. El estudio demuestra que se pueden emplear extractos de hojas y tallos para el biocontrol de fusariosis sin causar fitotoxicidad en plantas de tomate a partir de los 25 días, empleando dosis entre 1 y 3 mL planta^{-1}.

Palabras clave: antioxidantes, biocontrol, biofungicidas, palo verde, rotavapor.

INTRODUCTION

Phytopathologists and producers, with an organic approach, employ various agrotechnical alternatives with the aim of reducing the contaminant load from the excessive application of broad-spectrum fungicides. The use of plant extracts to control pests and diseases is one of those agrotechnical alternatives (Báez *et al*. 2021). Among them, extracts of species such as the governor (*Larrea tridentate* (DC.) Coville) and oregano (*Origanum vulgare* L.) have been used to control phytopathogenic fungi of the genus *Fusarium*, which produces significant losses in the yield of crops of economic interest (Figueroa *et al*. 2019).

Plants sometimes produce a significant amount of primary and secondary metabolites as a regulatory action on a large number of pests and diseases; for this reason, the possibility of being used with a focus on environmental protection through integrated management is being studied (Zoppolo *et al.* 2008). Some of these metabolites are synthesized as defenses (repellents) and others intoxicate and directly eliminate microorganisms and pests. For example, saponins, amygdalins, and norhydroguaiaretic acid are used for the control of fungi and bacteria (Martínez-Olivo *et al.* 2020). Protocatechotic acid has a significant effect on the control of pathogens in general and in particular on preventing basal rot in tomatoes (El-Nagar *et al*. 2020). Among the metabolites that plants synthesize are polyphenols (PP), which are obtained from the shikimic acid biosynthetic pathway (Santos-Sánchez *et al.* 2019). PP are normally antioxidants whose main function is to prevent damage to foliar organs. The damage can be due to biological oxidation induced by abiotic stress or caused by insects and microorganisms (Lee *et al.* 2020), including bacteria and fungi (Rivera-Solís *et al*. 2021).

Many species of the semidesert also show characteristics of tolerance to pests and diseases, subsisting in addition to prevailing adverse conditions such as salinity and drought (Gonzáles *et al*. 2021), asis the case for *P. aculeata*, commonly known as “palo verde or bacaporo” (Adhikari & White 2014). This species has been studied in various regions of the world mainly for clinical purposes (Divya *et al*. 2011; Franco *et al.* 2022), and phytochemical studies have been developed to determine the presence of various metabolites that can be used for the biocontrol of pathogens, such as *Fusarium oxysporum* (Arvizu-Quintana *et al.* 2021). These studies are of great importance in the prevention of environmental contamination after having been proven that they present minimal or no phytotoxicity in the plants of economic interest. An important step is to evaluate whether these extracts obtained from model plants, such as paloverde, affect the physiological, biochemical and/or agronomic performance of the plants. Another important point is to substitute some chemical pesticides with these products. The substitution may reduce the amounts of pollutants in agricultural area. Taking these elements into account, a study was carried out to determine the content of polyphenols and flavonoids in hydroalcoholic extracts obtained from stems and leaves of *P. aculeata* and to evaluate the phytotoxicity in tomato seedlings applied in the initial stages of development.

Materials and methods

Location of the experimental area. The research was developed in the Biotechnology Laboratory of the National Institute of Technology of Mexico, Valle del Yaqui Campus, in the municipality of Bácum, Sonora, Mexico. Leaf and stem samples of *P. aculeata* plants were taken from the semidesert Sonora to obtain hydroalcoholic extracts by percolation according to the process described by Fecker *et al.* (2020). The samples were separated at a rate of 100 g and then remained in 1 L of 76% alcohol during 10 days (Figs. 1a-b, leaves and stems, respectively). After this period, the alcohol was separated using a rotary evaporator at 30 revolutions per minute and at a temperature of 65°C (BUCHI® R215, USA), with an extraction efficiency of 75%. The extracts were kept at 4°C until they were used (Fig. 1c).

FIGURE 1

Tomato variety used to evaluate phytotoxicity

As an experimental model, tomato seeds of the Río Grande® variety were used, with determined growth, cataloged as susceptible to fusarium wilt (Arellano-Aburto *et al.* 2021). The seeds were sown in 200-well polyfoam trays under semi-controlled conditions in a growth chamber. The conditions inside the chamber were adjusted to 10 hours of light, a temperature of 25°C and a relative humidity of 75%. Peat moss type substrate (PROMIX®) was used for seed germination. At 15 days after emergence (DAE), the seedlings were selected and subjected to the established treatments.

Treatments and experimental design

The treatments consisted of the combination of two sources of variation: A) plant organs, with two levels (stems and leaves); and B) volumes of extracts applied, with four levels (0, 1, 3 and 5 mL plant^{-1}). The level of zero applications of the extract was taken as the control treatment. Each treatment of the eight conformed, had a sample size of 30 plants. The application of the extracts was carried out on the roots and foliar route, in unison, three times after emergence: at 15, 25 and 35 DAE. This last factor was not included as a source of variation, and the results were compared separately in the respective statistical analyses.

The treatments were distributed under semi-controlled conditions, following a completely randomized experimental design with a bifactorial arrangement. In all treatments, the edge effect and neighboring variants were taken into account for phytotoxicity variable measurement. These plants were dispensed with to reduce the possible experimental error.

Evaluated variables

Polyphenol content was determined by the Folin-Ciocalteu method developed by Anesini et al. (2008). For the test, a volume of 125 µL of the gallic acid standard solution was prepared, and 0.5 mL of distilled H_2O and 125 µL of Folin-Ciocalteu reagent were added. These reagents remained in the reaction for 6min, and 1.25 mL of a 7% Na_2CO_3 solution was added. Finally, 1 mL of distilled H_2O was added and left to stand for 90min at a temperature of 17°C and 65% relative humidity.

An absorbance reading was performed on the obtained solution in a UV Vis Genesys 10S spectrophotometer (Thermo Scientific®) at a wavelength (λ) of 760 nm. Then, both stem and leaf extracts were diluted at a 1:5 ratio with 50% methanolic solution, and the total polyphenol content was determined in the same way as gallic acid standards. Then, by interpolating the absorbance of the extracts in the gallic acid curve, it was determined, in triplicate samples, the content of total polyphenols expressed in mg L^{-1} of extract.

Flavonoid content was determined by the method described by Muñoz *et al.* (2007). Samples of 250 μL of the extracts of *P. aculeata* were dissolved in 1000 μL of deionized water. Then, 75 μL of $NaNO_2$ was added and allowed to react for 5 minutes. Subsequently, 75 μL of 10% $AlCl_3$ and 500 μL of 1 M NaOH were added. The mixture was centrifuged at 3500 r.p.m. during 5 minutes. Finally, the absorbance was measured at a wavelength of 510 nm. The final concentrations of total flavonoids were expressed in mg L^{-1} of stem and leaves extract (Herrera *et al*. 2017).

Evaluation of the phytotoxicity of the extracts

The evaluation of the phytotoxicity of hydroalcoholic extracts from leaves and stems of *P. aculeata* was carried out in the initial stages of the Rio Grande tomato variety, following the scale described in Table 1, taking the scale proposed by Esparza-Díaz *et al.* (2010) as a reference.

TABLE 1

Statistical analysis

To compare the concentrations of polyphenols and flavonoids, the theoretical distribution of student probabilities proposed by Gosset (1917) was used, establishing the differences between the organs where they were determined.

For the evaluation of phytotoxicity, a double classification analysis of variance was carried out based on a linear model of fixed effects (Fisher 1937). The number of damaged or dead plants during each treatment (discrete quantitative variable) was taken as a variable response.

When there were differences between the means of the treatments, the test (Tukey's multiple comparison)was used for a significance level of 5% (Tukey 1960). The statistical indicators of coefficient of variation (CV), standard error of the mean of the treatments (ESx) and the coefficients of determination (R^2) were determined, without adjusting for the isolated factors (organs and volume) and for the interaction between these twofactors. STATISTICA professional software, version 14.1 for Windows (Stasoft 2014), was used for statistical processing.

RESULTS AND DISCUSSION

Polyphenols and flavonoids Content in the hydroalcoholic extract of stems and leaves. Polyphenols concentration and total flavonoids showed highly significant differences between the organs from which the extracts were obtained. The concentration was in both parts, but it was higher in the leaves (Table 2). The result demonstrates the plant ability to store these compounds to be protected from pests and diseases. The storage is more in the leaves than in the stem. Silva *et al.* (2012) evaluated rot control in lettuce (*Lactuca sativa* L.). It was verified that hydroalcoholic extracts of leaves and stems of palo verde at concentrations of 10% were effective in promoting a low severity of the disease (3.7 and 3.3, respectively). These results may explain the low abundance of reports showing the presence of pests and diseases in this species. It was attributed to the synthesis and accumulation of these compounds as main biocontrol agents.

TABLE 2

Various investigations on the use of crude plant extracts have revealed their inhibitory activities on microorganisms. For example, the use of antimicrobial activity of *Pinus* *wallichiana* A.B.Jacks. leaf extracts against *Fusarium oxysporum* f. sp. cubense. (Foc), attributed to the significant presence of flavonoids and polyphenols (Ain *et al.* 2022). These results affirm that the palo verde extract has important potentialities as a biocontrol agent because it comes from a plant. This product easily mitigates into the plant (Stefanovic and Comic 2012), so it would generate little or no phytotoxicity. This characteristic confirms the importance of this study.

Many of these extracts contain terpenoids, alkaloids, tannins, saponins, phenylpropanoids, and flavonoids, which are used to manufacture fungicides and pesticides at high concentrations (Nxumalo et al. 2021).

In Mexico, various plant species extracts have also been obtained. They have a significant concentration of flavonoids and polyphenols used as biocontrol agents of insects. A major control of 48-hour-old larvae with seven concentrations was obtained when the insecticidal activity of mistletoe dust (*Phoradendron densum* Torr. ex Trel.) on *Spodoptera frugiperda* was evaluated by Hernandez *et al.* (2018).

In general, flavonoids play an important role in the protection against biological oxidations induced by biotic and abiotic stresses (Sun *et al.* 2022). The content of polyphenols in plants and fruits varies depending on the genotype, species, environmental conditions, degree of maturity, soil composition, geographic location, and storage conditions (Shen *et al.* 2022). Flavonoids are also a frequent object of research due to their diverse functions, such as nutrient assimilation, protein synthesis, enzymatic activity, photosynthesis, formation of structural components, and defense against adverse environmental factors such as aggression of pathogens and insects (Figueirinha *et al.* 2008; Vélez-Terranova *et al.* 2014).

Phytotoxicity of extracts in tomato seedlings

The application at 15 DAE of treatments T2 to T8 showed highly significant phytotoxicity (p=0.0017) in the Rio Grande tomato variety, causing damage to 77% of the plants of each mentioned treatment (value of 5). For this reason, the hydroalcoholic extract application of *P. aculeata* at 15 DAE is not recommended (Table 3). In the statistical analysis, at this moment of application (15 DAE), although there was a significant interaction between the organ*volume factors (p=0.01796), it was observed that the effect of the applied volume explained 98% of the total variability obtained (R^2(volumes)=0.98). These findings show that any volume used close to emergence can generate phytotoxicity and that this increases significantly as the volume of extract applied increases (Table 3).

TABLE 3

When the treatments were applied at 25 DAE, there were also significant differences between the organs used to obtain the extracts (p=0.00274), as well as between the volumes (p=0.0003), with also a significant interaction (p=0.0179). When the extracts were applied during 25 DAE, a total variability found in phytotoxicity was explained by 64%, and the average phytotoxicity between treatments was 2. A similar result was found at 35 DAE, where the source of volume variation contributed 79% to the total variability found in phytotoxicity, although the average value of phytotoxicity was 1 (Table 3). The results obtained indicate that high volumes of the extract can cause phytotoxicity in plants. Because of this these studies must be carried out to recommend its use. This can be useful to producers to control diseases in seedling without causing damage to the initial morphological and physiological characteristics of plants.

Multiple extracts have been obtained from semi-desert plants for agronomic purposes, with an organic and integrated management approach for pest and disease control (Heikal *et al.* 2021).

The governor species (*Larrea tridentata* (DC.) Coville) has been used as biocontrol of fusarium, with reductions in radial growth of 10% (Martínez-Olivo *et al.* 2020). Neem extract (*Azadirachta indica* A.Juss.) has been used to evaluate antifungal activity against tomato vascular wilt and showed high control efficiency and minimal phytotoxicity in seedlings (Ayvar-Serna *et al.* 2021).

For fungal diseases such as fusariosis, the hydroalcoholic extract of *Acacia farnesiana* was tested for a decade (Rodríguez *et al.* 2012) under in vitro culture conditions, with significant decreases in the mycelial growth of the fungus and minimal phytotoxicity in plants. Rivera-Solis *et al.* (2021) also tested extracts of *Sargassum spp*. as inducers of tolerance to *Fusarium oxysporum* in tomato seedlings without finding significant phytotoxicity. These results demonstrate the practical value of plant extracts in controling diseases that affect agricultural crops and their contribution to caring for the environment by reducing the application of chemical products.

Plant extracts in pest and disease management are currently recognized as environmentally safe, less hazardous and cheaper. In their most natural form, many plant species have insecticidal characteristics (Tavares *et al.* 2021). Its use constitutes an alternative to mitigate contaminant loads due to concentrated chemical products that sometimes generate resistance in organisms.

The production of plant extracts is still important in the discovery of innovative and environmentally safe antimicrobials to overcome problems with resistance to multiple pesticides. The use of extracts to minimize or eliminate the damage caused by pests and diseases can be a contribution to the national and international scientific community. This could have a great economic and ecological significance.

CONCLUSIONS

The hydroalcoholic extracts of *Parkinsonia aculeata* L. present a higher concentration of flavonoids and polyphenols in the leaves than in the stems.

The Río Grande tomato variety presents high phytotoxicity (f=5) at 15 DAE. Because of this, the application of hydroalcoholic extracts of *P. aculeata* at this time is not recommended.

The safe application of the hydroalcoholic extract of *P. aculeata* without symptoms of phytotoxicity appearing, must be carried out from 25 to 35 DAE of the seedlings.

Author Contributions: All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

Adhikari A & White JD (2014) Plant water use characteristics of five dominant shrub species of the Lower Rio Grande Valley, Texas, USA: Implications for shrubland restoration and conservation. Conservation Physiology 2: 005. DOI: 10.1093/conphys/cou005

Ain QU, Asad S, Ahad K, Safdar MN & Jamal A (2022) Antimicrobial Activity of *Pinus wallachiana* Leaf Extracts against *Fusarium oxysporum* f. sp. *cubense* and Analysis of Its Fractions by HPLC. Pathogens 11(3): 347. DOI: 10.3390/pathogens11030347

Anesini C, Ferraro E & Filip R (2008) Total polyphenol content and antioxidant capacity of commercially available tea (*Camellia sinensis*) in Argentina. Journal of agricultural and food chemistry 56: 9225-9. DOI: 10.1021/jf8022782

Arellano-Aburto DA, López-Valenzuela JÁ, Gutierrez-Dorado R, Pineda-Hidalgo KV, Retes-Manjarrez JE & Garzón-Tiznado JÁ (2021) Análisis de resistencia a *Candidatus*

Liberibacter solanacearum en genotipos de tomate. Revista Fitotecnia Mexicana 44(3): 425-425. DOI: 10.35196/rfm.2021.3.425

Arvizu-Quintana EF, Argentel-Martínez L, Peñuelas-Rubio O, Leyva-Ponce A & García-Urías J (2021) Extractos hidroalcohólicos de *Parkinsonia aculeata* L., Sp. Pl. para el biocontrol de *Fusarium oxysporum* Schlecht. Renewable Energy, Biomass & Sustainability 3(2): 46-52. DOI: 10.56845/rebs.v3i2.52

Ayvar-Serna S, Díaz-Nájera JF, Vargas-Hernández M, Enciso-Maldonado GA, Alvarado-Gómez OG & Ortíz-Martínez AI (2021) Actividad antifúngica de pesticidas biológicos, botánicos y químicos sobre el agente causal de la marchitez vascular del jitomate. Revista Fitotecnia Mexicana. 44(4): 617-617. DOI: 10.35196/rfm.2021.4.617

Báez M, Torres EI, Gruszycki AE, Alba DA, Valenzuela GM & Gruszycki MR (2021) Actividad antioxidante y antiinflamatoria en extractos hidroalcohólicos de *Kalanchoe daigremontiana* Raym.-Hamet & H. Perrier. Revista Colombiana de Ciencias Químico-Farmacéuticas 50(1): 86-99. DOI: 10.15446/rcciquifa.v50n1.95450

Divya B, Mruthunjaya K & Manjula SN (2011) *Parkinsonia aculeata*: a phytopharmacological review. Asian Journal of Plant Sciences 10(3): 175-181. DOI: 10.3923/ajps.2011.175.181

El-Nagar A, Elzaawely AA, Taha NA & Nehela Y (2020) The antifungal activity of gallic acid and its derivatives against *Alternaria solani*, the causal agent of tomato early blight. Agronomy 10(9): 1402. DOI: 10.3390/agronomy10091402

Esparza-Díaz G, López-Collado J, Villanueva-Jiménez JA, Osorio-Acosta F, Otero-Colina G & Camacho-Díaz E (2010) Concentración de azadiractina, efectividad insecticida y fitotoxicidad de cuatro extractos de *Azadirachta indica* A. Juss. Agrociencia 44(7): 821-833.

Fecker R, Buda V, Alexa E, Avram S, Pavel IZ, Muntean D & Danciu C (2020) Phytochemical and biological screening of *Oenothera biennis* L. hydroalcoholic extract. Biomolecules 10(6): 818. DOI: 10.3390/biom10060818

Figueirinha A, Paranhos A, Pérez A, Santos C & Batista M (2008) *Cymbopogon citratus* leaves: Characterization of flavonoids by HPLC–PDA–ESI/MS/MS and an approach to their potential as a source of bioactive polyphenols. Food Chemistry 110(3): 718-728. DOI: 10.1016/j.foodchem.2008.02.045

Figueroa G, Castro TEA & Castro SHT (2019). Efecto bioplaguicida de extractos vegetales para el control de *Spodoptera frugiperda* en el cultivo de maíz (*Zea mays*). Acta biológica colombiana 24(1): 58-66. DOI: 10.15446/abc.v24n1.69333

Fisher RA (1937) The Design of Experiments. Edinburgh; London: Oliver and Boyd.

Franco ES, Nascimento E, Vasconcelos DA, Silva PA, Novaes TL, Feitosa MG & Maia MB (2022) Polar fraction from *Parkinsonia aculeata* aerial parts extract improves imbalanced metabolic profile and reduces proinflammatory interleukin levels in white adipose tissue in obese rats induced by western diet. Journal of Ethnopharmacology 282: 114557. DOI: 10.1016/j.jep.2021.114557

Gonzáles HHS, Peñuelas-Rubio O, Argentel-Martínez L, Ponce A, Andrade MHH, Hasanuzzaman M, Aguilera JG & Teodoro PE (2021) Salinity effects on water potential and the normalized difference vegetation index in four species of a saline semiarid ecosystem. PeerJ 9: e12297. doi: 10.7717/peerj.12297

Gosset E (1917) Another differences calculus based on standard deviation and confidence interval. Statistical References 26: 66-72.

Heikal G, Saad H & El-Genaidy M (2021) Evaluation efficiency of some insecticides, plant extracts and plant oils on controlling *Myzuspersicae* infesting squash plants under plastic greenhouses. Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control 13(2): 109-116. DOI: 10.21608/EAJBSF.2021.192756

Hernández J, Guzmán U, Cerna C, Aguirre U, Cepeda S & Julio C (2018). Actividad insecticida de polvo de *Phoradendron densum*(Santalaceae) sobre *Spodoptera frugiperda* (Lepidoptera: *Noctuidae*). Memorias Carteles del XLI Congreso Nacional de Control Biológico. Sociedad Mexicana de Control Biológico, A.C., Puerto Vallarta, Jalisco. México. 141-142.

Herrera M, Moreno O, Villares A, Gonzalez J & Belmonte B (2017) Estudio de la abundancia de polifenoles y flavonoides en plantas del Valle del Yaqui, Sonora. Entorno Académico. 19: 12-17.

Lee J, Nguyen QN, Park JY, Lee S, Hwang GS, Yamabe N & Kang KS (2020) Protective effect of shikimic acid against cisplatin-induced renal injury: *in vitro* and *in vivo* studies. Plants 9(12): 1681. doi: 10.3390/plants9121681

Martínez-Olivo IE, Reyes-Melo KY & del Rayo Camacho-Corona M (2020) La gobernadora como fuente potencial de agentes antituberculosos. Revista de Ciencias Farmaceúticas y Biomedicina 1: 89-89.

Muñoz O, Copaja S, Speisky H, Peña R & Montenegro G (2007). Contenido de flavonoides y compuestos fenólicos de mieles chilenas e índice antioxidante. Química Nova 30(4): 848-851. DOI: 10.1590/S0100-40422007000400017

Nxumalo KA, Aremu AO & Fawole OA (2021) Potentials of medicinal plant extracts as an alternative to synthetic chemicals in postharvest protection and preservation of horticultural crops: a review. Sustainability 13: 5897. DOI: 10.3390/su13115897

Rodríguez PAT, Arrebato MAR, Baños SB, Triana AC & González DR (2012) Actividad antifúngica de extractos de *Acacia farnesiana* sobre el crecimiento *in vitro* de *Fusarium oxysporum* f. sp. *lycopersici*. Revista Científica UDO Agrícola 12(1): 91-96.

Rivera-Solís LL, Rodríguez-Jasso RM, Flores-López ML, Robledo-Olivo A, Sandoval-Rangel A, Sariñana-Aldaco O & González-Morales S (2021) Extractos de *Sargassum* spp. como inductores de tolerancia a *Fusarium oxysporum* en plántulas de tomate. Ecosistemas y Recursos Agropecuarios 8(1) e2826: doi: 10.19136/era.a8n1.2826

Santos-Sánchez NF, Salas-Coronado R, Hernández-Carlos B &Villanueva-Cañongo C (2019) Shikimic acid pathway in biosynthesis of phenolic compounds. Plant Physiological Aspects of Phenolic Compounds 1: 1-15. DOI: 10.5772/intechopen.83815

Shen N, Wang T, Gan Q, Liu S, Wang L & Jin B (2022) Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. Food Chemistry 132531. DOI: 10.1016/j.foodchem.2022.132531

Silva CL, Souza EB, Felix KCS, Santos AAG, Silva MY & Mariano RLR (2012) Óleos essenciais e extratos vegetais no controle da podridão mole emalface crespa. Horticultura Brasileira 30(4): 632-638. DOI: 10.1590/S0102-05362012000400012

StatSoft (2014) STATISTICA 13.3. StatSoft Incorporation Version 13.3

Stefanovic O & Comic L (2012) Synergistic antibacterial interaction between *Melissa officinalis* extracts and antibiotic. Journal of Applied Pharmaceutical Science 2(1): 1-5.

Sun J, Sun W, Zhang G, Lv B & Li C (2022) High efficient production of plant flavonoids by microbial cell factories: Challenges and opportunities. Metabolic Engineering 70: 143-154. DOI: 10.1016/j.ymben.2022.01.011

Tavares WR, Barreto MDC & Seca AM (2021). Aqueous and ethanolic plant extracts as bioinsecticides-establishing a bridge between raw scientific data and practical reality. Plants. 10(5): 920. DOI: 10.3390/plants10050920

Tukey JW (1960) A survey of sampling from contaminated distributions. In: Olkin I (ed). contribution to probability and statistics: essays in honor to Harold Hotelling. Redwood City: Stanford University Press. Pp. 448-485

Vélez-Terranova M, Campos-Gaona R & Sánchez-Guerrero H (2014). Uso de metabolitos secundarios de las plantas para reducir la metanogénesis ruminal. Tropical and Subtropical Agroecosystems 17(3): 489-499.

Zoppolo R, Faroppa S, Bellenda B & García M (2008) Guía para la producción y consumo saludable. Montevideo, Uruguay: Unidad de Comunicación y Transferencia de Tecnología del INIA. Available at <https://www.paho.org/uru/dmdocuments/alimentos_en_la_huerta.pdf>

Figure 1 - Obtaining extracts from *P. aculeata* L. Sp.Pl. A) macerated leaves; B) macerated stems; and C) extracts obtained after rotary evaporation.