BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

Chemical composition and antioxidant capacity of purified extracts of *Prosopis pallida* (Humb. & Bonpl. ex Willd.) Kunth (Fabaceae) fruits from Northern Peru

[Composición química y capacidad antioxidante de extractos purificados de frutos de *Prosopis pallida* (Humb. & Bonpl. Ex Willd.) Kunth (Fabaceae) del norte de Perú]

Luz A. Suárez-Rebaza[1], Ricardo D. D. G. de Albuquerque[2,3], Ewaldo Zavala[3], Pedro M. Alva-Plasencia[1], Mayer M. Ganoza-Suárez[4], Mayar L. Ganoza-Yupanqui[3] & Rainer W. Bussmann[5]

[1]Departamento de Farmacotecnia, Facultad de Farmacia y Bioquímica, Universidad Nacional de Trujillo, Trujillo, Perú
[2]Laboratório de Tecnologia em Produtos Naturais, Universidade Federal Fluminense, Niterói, Brazil
[3]Departamento de Farmacología, Facultad de Farmacia y Bioquímica, Universidad Nacional de Trujillo, Trujillo, Perú
[4]Escuela de Medicina, Facultad de Medicina, Universidad Nacional de Trujillo, Trujillo, Perú
[5]Department of Ethnobotany, Institute of Botany, Ilia State University, Tbilisi, Georgia

Reviewed by:
Daniela Russo
Basilicate University
Italy

Marcelo L. Wagner
Universidad de Buenos Aires
Argentina

Correspondence:
Mayar L. **GANOZA-YUPANQUI**
mganoza@unitru.edu.pe

Section Biological activity

Received: 6 January 2022
Accepted: 25 May 2022
Accepted corrected: 12 June 2022
Published: 30 September 2023

Citation:
Suárez-Rebaza LA, de Albuquerque RDDG, Zavala E, Alva-Plasencia PM, Ganoza-Suárez MM, Ganoza-Yupanqui ML, Bussmann RW. Chemical composition and antioxidant capacity of purified extracts of *Prosopis pallida* (Humb. & Bonpl. ex Willd.) Kunth (Fabaceae) fruits from Northern Peru
Bol Latinoam Caribe Plant Med Aromat
22 (5): 594 - 606 (2023).
https://doi.org/10.37360/blacpma.23.22.5.43

Abstract: The purpose of this work was to evaluate the antioxidant capacity of *Prosopis pallida* (Fabaceae) fruits "algarrobo", in addition to determine their main chemical components. Fruit purified extracts from the regions of Tumbes, Piura, Lambayeque, and La Libertad (Peru) were evaluated using infusion, decoction, and alcoholic extraction at concentrations of 45, 70 and 96%. To measure the antioxidant capacity of the extracts, the determination of total phenolics content was performed, followed by the antioxidant evaluation using DPPH, FRAP and ABTS. The chemical identification was carried out through UHPLC-UV-MS/MS. The results showed that the decoction of the sample from the region of La Libertad, as well as the ethanol extracts at 45% of the samples from Tumbes and Lambayeque presented greater antioxidant capacity. Moreover, ten major substances of these extracts were identified: nine phenolic derivatives (vicenin II as the main constituent of the extracts) and one diterpene (7-oxodehydroabietic acid).

Keywords: *Prosopis pallida*; Antioxidant capacity; Polyphenols; Vicenin II; Chemical composition

Resumen: El propósito de este trabajo fue evaluar la capacidad antioxidante de frutos de *Prosopis pallida* (Fabaceae) "algarrobo", además de determinar sus principales componentes químicos. Se estudiaron extractos purificados de frutos de las regiones de Tumbes, Piura, Lambayeque y La Libertad (Perú), mediante infusión, decocción y extracción alcohólica en concentraciones de 45, 70 y 96%. Para medir la capacidad antioxidante de los extractos, se realizó la determinación del contenido de fenoles totales, seguida de la evaluación antioxidante mediante DPPH, FRAP y ABTS. La identificación química se realizó mediante UHPLC-UV-MS/MS. Los resultados mostraron que la decocción de la muestra de la región de La Libertad, así como los extractos de etanol al 45% de las muestras de Tumbes y Lambayeque presentaron mayor capacidad antioxidante. Además, se identificaron diez sustancias principales de estos extractos: nueve derivados fenólicos (vicenin II como componente principal de los extractos) y un diterpeno (ácido 7-oxodehidroabiético).

Palabras clave: *Prosopis pallida*; Capacidad antioxidante; Polifenoles; Vicenina II; Composición química

INTRODUCTION

Functional foods are increasingly recommended for the maintenance of a healthy life, as they provide additional pharmacological effects. Among these types of foods, those of vegetable origin, show a high content of specific secondary metabolites, such as polyphenols, mucilages, alkaloids and terpenes (Vattem & Maitin, 2020). Among these, polyphenols which includes flavonoids, phenolic acids, and tannins, are substances that have pronounced antioxidant activity (Pandey & Rizvi, 2009). These compounds can capture free radicals that can be generated in the cells of the human body because of the combination of many environmental factors, including atmospheric pollution. When the increase in the intracellular content of free radicals exceeds the antioxidant defenses of the cell, oxidative stress occurs, through which damage to biological molecules such as lipids, proteins and nucleic acids is induced. Oxidative stress occurs in various pathological states in which cellular functionality is altered, contributing, or feeding back into the development of degenerative diseases such as atherosclerosis, cardiomyopathies, neurological diseases, and cancer (Avello & Suwalsky, 2006). The consumption of foods rich in polyphenols has been suggested to avoid these pathologies and some types of fruits are known to have high contents of polyphenols, as is the case of the genus *Prosopis* (Fabaceae) (Patel *et al*., 2018; Sharifi-Rad *et al*., 2019). Among the species of this genus, *P. pallida*, popularly known in Peru as "algarrobo" (Figure No. 1), is one of the richest vegetable foods known for their high concentration of starch and proteins, being an important part of the diet of many indigenous people. The pulp is sweet and is incorporated as flour e.g., in soups, and being mixed with water to prepare a drink called “atole”, or with milk, as it is considered a rich food for children. Furthermore, the “algarrobo” gum is used as a stabilizing agent, thickener, and additive in different industrial fields (Tamayo *et al*., 2008). However, chemical studies about the secondary metabolites of this species and its antioxidant potential are still scarce. Therefore, the objective of this work was to measure the antioxidant capacity of purified extracts of *P. pallida* fruits by different mechanisms, as well as to evaluate its main chemical components of pharmacological interest.

MATERIALS AND METHODS

Preparation of Extracts

Four samples of *P. pallida* fruits were collected from the regions of Tumbes (04°05’39.3’’S - 80°41’28.9’’ W) (Sample 1), Piura (05°10’43.7’’S - 80°37’09.8’’ W) (Sample 2), Lambayeque (06°28’32.6’’S - 79°42’12.29’’ W) (Sample 3) and La Libertad (07°27’17.8’’S - 79°30’05.2’’W) (Sample 4) (**Figure No. 2**), and were identified in the Herbarium Truxillense (HUT) of the National University of Trujillo (Numbers: HUT 59850, HUT 59858/59859, HUT 59851 and HUT 59852/59853, respectively). The fruits (pods) of *P. pallida* were dried at 40°C in a stove with forced circulation air (Memmert®) oven and the seeds were separated and pulverized until obtaining homogeneous samples. The extracts were prepared at 10% w/v, using three hydroalcoholic extracts (Ethanol 96%, 70% and 45%) and two aqueous extracts (infusion and decoction) (Torres-Guevara & Ganoza-Yupanqui, 2017). The extracts were completely dried in a rotary evaporator (Heidolph®), and then resuspended in distilled water and brought to -80°C in a deep freezer (Artiko®); for better stability, the water was removed using a freeze dryer (Millrock Technology®) and were stored in amber jars at 4°C (Zavala-Urtecho *et al*., 2018). The resulting dry extracts were suspended using an ultrasound bath in 15 mL of distilled water and then mixed with an adsorbent resin (Amberlite® XAD-7HP). The resin was washed with 1 L of distilled water and eluted with 1 L of HPLC grade Methanol. The eluate was concentrated under reduced pressure and then stored at -20°C until use (Cattaneo *et al*., 2014; Quispe *et al.*, 2014).

Gallic Acid Calibration Curve and Quantification of Phenolic Compounds

A 1 mg/mL solution of gallic acid (Merck®) in ethanol was used as a standard, six dilutions were prepared in a range between 0.02 and 0.16 mg/mL. Then, 25 µL of each dilution was mixed with 125 µL of the Folin-Ciocalteu (Sigma-Aldrich®) Reagent 10% and stirred for 20 minutes at 45°C. Then 100 µL of 7% Na_2CO_3 was added, it was left stand for 10 minutes in the dark and measured on a microplate spectrophotometer ultraviolet/visible (UV/vis) (Fisher Scientific accuSkan GO®) at 760 nm (Alarcón-Aguilar *et al*., 2018). Then, 10 mg of each purified extract was weighed out and dissolved in 1 mL of its respective solvent. Then 25 µL was taken

and proceeded in the same way as in standard dissolutions. The values were expressed as equivalents of gallic acid per g of lyophilized extract (GAE/g), measurements were made in triplicate (Alarcón-Aguilar *et al*., 2018; Ganoza-Yupanqui *et al*., 2021).

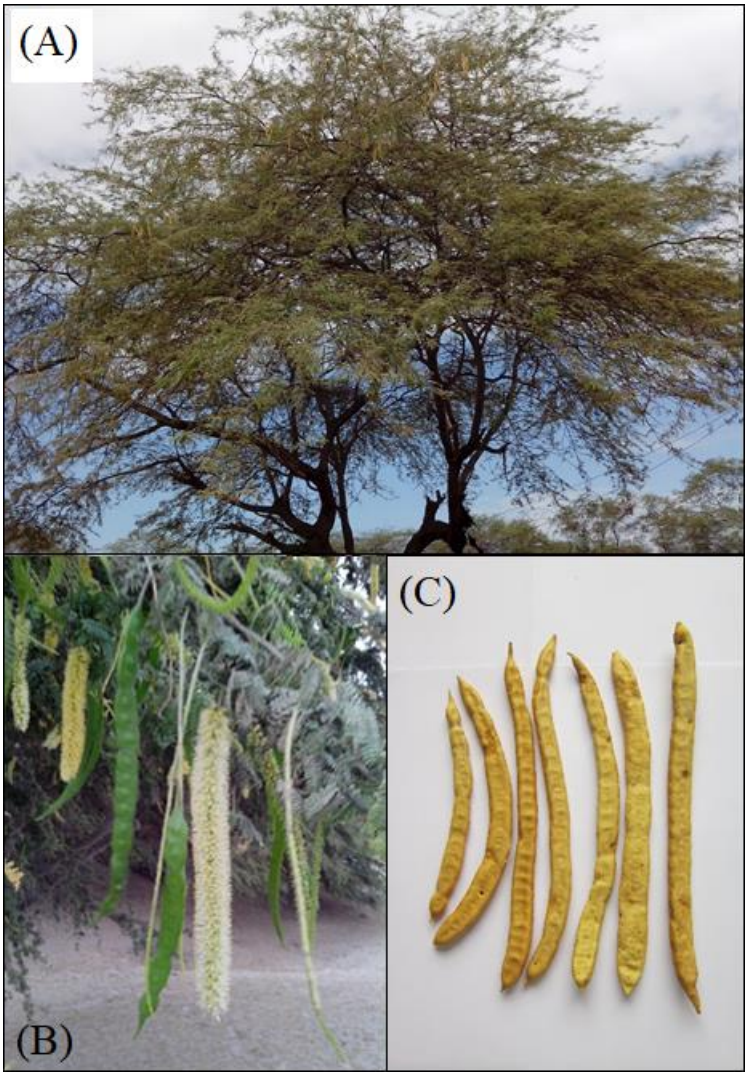

Figure No. 1
***Prosopis pallida* "algarrobo": Tree in its habitat (A), Inflorescence (B), Fruits (C)**

Antioxidant Capacity by Radical 2,2-diphenyl-1-picrylhydrazyl Assay (DPPH)

A 1 mg/mL solution of Trolox (Sigma-Aldrich®) in ethanol was used as a standard, six dilutions in a range between 0.25 and 0.025 mg/mL were prepared. Then, 10 µL of each dilution were mixed with 300 µL of DPPH reagent Sigma-Aldrich® 0.2 mM. The solutions were then stirred for 15 minutes and measured on a UV/vis spectrophotometer (Fisher Scientific accuSkan GO®) at 517 nm using microplate. In the end, the extracts were prepared at 10 mg/mL and measured under the same conditions. The tests were carried out in triplicate and expressed in mg of Trolox equivalents per gram of purified extract (mg TE/g) (Alam *et al*., 2013; Ganoza-Yupanqui *et al*., 2021). The correlation with total phenolic content was made through the determination of Pearson's coefficient.

Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP reagent was prepared using acetate buffer 300 mM (pH 3.6), TPTZ solution (2,4,6-tris (2-

pyridyl)-s-triazine) (Sigma-Aldrich®) 10 mM, HCl 40 mM and a $FeCl_3.6H_2O$ (Sigma-Aldrich®) 20 mM solution. Then, 25 mL of buffer was mixed with 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3.6H_2O$ solution and incubated at 37°C. A 1 mg/mL solution of Trolox (Sigma-Aldrich®) in ethanol was used as a standard, six dilutions in a range between 0.013 and 0.13 mg/mL were prepared. Then, 8 µL of each dilution reacted with 200 µL of the FRAP reagent for 30 minutes at 37 °C and was measured on a UV/vis spectrophotometer (Fisher Scientific accuSkan GO®) at 593 nm using microplate. After, the extracts were prepared at 10 mg/mL and measured under the same conditions. The tests were carried out in triplicate and expressed in mg of Trolox equivalents per gram of purified extract (mg TE/g) (Benzie & Strain, 1996; Benites *et al*., 2019; Ganoza-Yupanqui *et al*., 2021). The correlation with total phenolic content was made through the determination of Pearson's coefficient.

ABTS [2,2'-azino bis (3-ethylbenzothiazolin-6-sulfonic)] radical scavenging assay (ABTS)

A 1 mg/mL solution of Trolox (Sigma-Aldrich®) in ethanol was used as a standard, nine dilutions were prepared in a range between 0.013 and 0.2 mg/mL. Then, it was taken 10 µL of each dilution and 300 µL of ABTS (Sigma-Aldrich®) solution with absorbance 0.7 a 750 nm, approximately, was added. Then, the mix was stirred for five minutes and measured on a UV/vis spectrophotometer (Fisher Scientific accuSkan GO®) at 750 nm using microplate. After, the extracts were prepared at 10 mg/mL and measured under the same conditions. The tests were carried out in triplicate and expressed in mg of Trolox equivalents per gram of purified extract (mg TE/g) (Re *et al*., 1999; Seeram *et al*., 2006; Ganoza-Yupanqui *et al*., 2021). The correlation with total phenolic content was made through the determination of Pearson's coefficient.

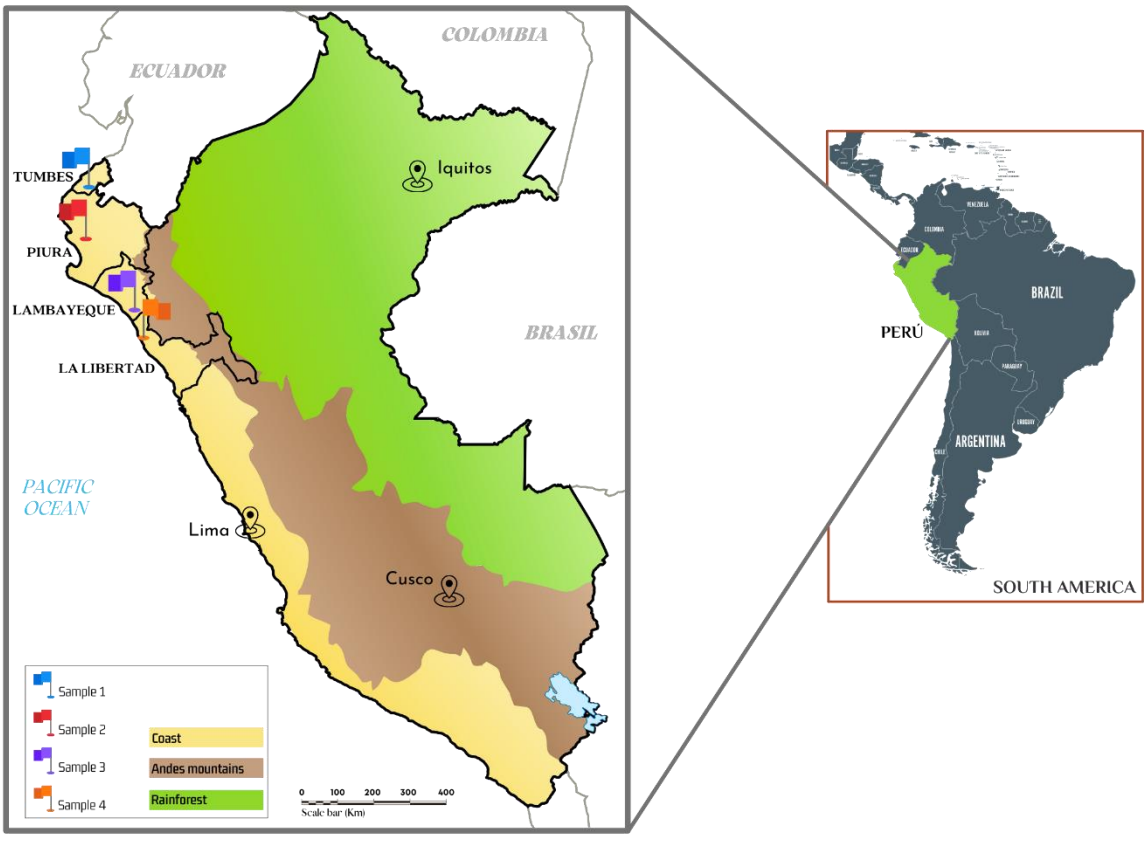

Figure No. 2
Map of *Prosopis pallida* collection sites in Peru

Chemical Identification by UHPLC-UV-MS/MS

A 5 mg/mL solution was prepared using methanol. This solution was passed through a 0.45 µm PTFE syringe filter before passing through the UHPLC-UV-MS/MS system. The analysis was performed on a Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific). Chromatographic separation was achieved on a Kinetex 1.7 µm EVO C18 column (50 × 2.1 mm, 1.7 µm, 100 Å, Phenomenex). Formic acid 0.1% (v/v) in H_2O (A) and MeCN (B) mobile phases were used. The gradient conditions were as follows: 0.0-13 min 5-25% B; 13-15 min 25-30% B; 15-20 min 30-50% B; 20.0-35.0 min 50-70% B; 35.0-40.0 min 70.0-90.0% B; 40.0-45.0 min 5% B. The flow of the mobile phase was 300 µL/min and the injection volume, 3 µL. The column temperature was kept at 30°C. UHPLC equipment coupled to a Q Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with an electrospray ion source (HESI) operated in positive and negative ionization mode. The "spray voltage" was kept at 4000 V for the positive mode and 4200 V for the negative mode. The drying temperature was adjusted to 300°C and the dry gas flow rate was adjusted to 10 L/min. Nitrogen was used as the dry gas, nebulization gas and collision gas. The collision energy was set at 35 eV. HRESIMS and MS/MS spectra were acquired in the range *m/z* 200-2000.

RESULTS AND DISCUSSION

Antioxidant capacity

The determination of phenolic compounds was carried out in the five purified extracts of samples from the four regions (**Table No. 1**). The decoction of Sample 4 presented the highest concentration of phenolic compounds with a value of 90.65 mg GAE/g ($p<0.05$) with respect to the other extracts from the same region (La Libertad) and featured among the best extracts from each region (Ethanol 45% extracts of Sample 1 and 3, as well as infusion of Sample 2). Considering the fact that the decoction of this sample presented the highest value in total phenolics and the extract in 96% ethanol was the one with the lowest concentration in relation to the other samples, it is suggested that the total phenolics of Sample 4 have greater polarity when compared with the phenolic content of the other samples, so that the highest relative percentage of alcohol (96%) reduced the extractive power to obtain these components in Sample 4. When compared with the study by Quispe *et al*. (2014), which reported the phenolic value of freeze-dried syrups from *Prosopis* species cultivated in Piura and Lambayeque, all extracts evaluated in this study had higher values (between 50 and 90 mg GAE/g), while the samples from the study by Quispe showed values of 10.7 (Piura) and 2.43 (Lambayeque) mg GAE/g. However, this comparison must consider the fact that, proportionally, the samples of freeze-dried syrup have a higher amount of sugar (Quispe *et al*., 2014). On the other hand, the previous study with *P. pallida* fruits carried out by Suárez-Rebaza *et al*. (2019) showed lower values of total phenolics and antioxidant potential when compared to the results of our study, as the extracts obtained in the previous publication were not purified through an adsorbent resin column, which aims to concentrate the phenolic content (Suárez-Rebaza *et al*., 2019).

Table No. 1

Phenolic compounds, expressed in gallic acid, from purified extracts of *P. pallida* fruits

Purified Extract	Phenolic compounds (mg GAE/g ± SD)[a]			
	Sample 1 (Tumbes)	Sample 2 (Piura)	Sample 3 (Lambayeque)	Sample 4 (La Libertad)
Decoction	43.52 ± 0.51	50.39 ± 0.59	69.11 ± 2.96	90.65 ± 1.70[b]
Infusion	63.68 ± 0.50	61.94 ± 1.88[b]	69.58 ± 1.30	81.04 ± 1.58[b]
Ethanol 45%	68.70 ± 1.43[b]	56.84 ± 0.40	85.35 ± 1.87[b]	70.77 ± 0.64
Ethanol 70%	56.85 ± 0.58	61.65 ± 0.52	76.05 ± 0.37	64.29 ± 0.86
Ethanol 96%	63.98 ± 0.61	60.97 ± 0.51	64.67 ± 0.74	44.55 ± 1.01

[a] mg GAE/g ± SD: Average milligrams of gallic acid equivalents per gram of sample ± standard deviation (n=3); [b] extract with higher concentration of phenolic compounds, $p<0.05$ (Tukey HSD post hoc test)

Table No. 2
Antioxidant capacity, using 2,2-diphenyl-1-picrylhydracil assay (DPPH), from purified extracts of *P. pallida* fruits

Purified Extract	DPPH (mg TE/g ± DS)[a]			
	Sample 1 (Tumbes)	Sample 2 (Piura)	Sample 3 (Lambayeque)	Sample 4 (La Libertad)
Decoction	12.67 ± 1.74	31.79 ± 0.62[b]	43.60 ± 1.49[b]	37.03 ± 1.50[b]
Infusion	24.54 ± 1.83	22.77 ± 0.22	27.18 ± 1.06	35.25 ± 0.87
Ethanol 45%	24.40 ± 0.34	18.15 ± 0.28	36.39 ± 1.72	29.49 ± 2.45
Ethanol 70%	18.69 ± 0.10	22.64 ± 3.05	34.97 ± 0.44	29.73 ± 1.12
Ethanol 96%	19.51 ± 2.10	23.12 ± 1.01	27.37 ± 1.39	20.57 ± 1.03

[a] mg TE/g ± SD: Average milligrams of Trolox equivalents per gram of sample ± standard deviation (n=3)
[b] extract with higher concentration of phenolic compounds, $p<0.05$ (Tukey HSD post hoc test)
Pearson's coefficient: 0.7271

The antioxidant capacity of the purified extracts was expressed in Trolox equivalent milligrams for the three methods used. In the DPPH method (**Table No. 2**), the extract with the highest antioxidant capacity was the decoction of Sample 3, with a value of 43.60 mg TE/g. It should be noted that in Samples 2 and 4, the decoctions also presented the highest antioxidant capacity, showing a positive correlation between the concentration of phenolic compounds and antioxidant capacity, with a Pearson's coefficient of 0.7271. In addition, the FRAP method allows to determine the antioxidant capacity of polyphenols with hydrophilic characteristics, so that the reaction is generated with the formation of the complex $[Fe(II)(TPTZ)_2]^{2+}$ (blue) (Shalaby & Shanab, 2013). In this assay, the FRAP method showed the best Pearson's coefficient (0.935) and the greatest significant difference among the extracts was found in the decoction of Sample 4 (**Table No. 3**), with a value of 69.82 mg TE/g ($p<0.05$), compared to the other extract types and to the other regions. While it was determining the antioxidant capacity using the radical cation ABTS, it was found that the Ethanol 45% extracts of Samples 1 and 3 presented the highest antioxidant capacity (95.85 and 82.54 mg TE/g respectively) (Table No. 4), in comparison to the extracts of Sample 4, where the highest antioxidant capacity was determined at the decoction (76.54 mg TE/g), with a significance $p<0.05$. Despite these results, the Pearson's coefficient (0.1126) for the ABTS method in this study was not so high in comparison with the other methods. This is explained by the fact that other substances besides phenolic compounds contribute to the antioxidant mechanism due to the good solubility of both lipophilic and hydrophilic compounds in ABTS assay (Floegel *et al*., 2011; Kuskoski *et al*., 2005).

Table No. 3
Antioxidant capacity, using the Ferric Reducing Antioxidant Power Assay (FRAP), from purified extracts of *P. pallida* fruits

Purified Extract	FRAP (mg TE/g ± DS)[a]			
	Sample 1 (Tumbes)	Sample 2 (Piura)	Sample 3 (Lambayeque)	Sample 4 (La Libertad)
Decoction	28.29 ± 0.10	31.25 ± 1.22	51.97 ± 2.11	69.82 ± 1.62[b]
Infusion	47.70 ± 2.48	37.07 ± 0.92	48.36 ± 2.20	63.62 ± 2.27
Ethanol 45%	55.83 ± 2.19[b]	32.03 ± 1.02	62.35 ± 3.94[b]	39.97 ± 1.40
Ethanol 70%	39.70 ± 1.52	42.29 ± 1.16	52.60 ± 0.42	43.46 ± 0.65
Ethanol 96%	43.34 ± 0.74	43.12 ± 1.01	36.62 ± 0.87	22.90 ± 1.06

[a] mg TE/g ± SD: Average milligrams of Trolox equivalents per gram of sample ± standard deviation (n=3)
[b] extract with higher concentration of phenolic compounds, $p<0.05$ (Tukey HSD post hoc test)
Pearson's coefficient: 0.9350

Table No. 4
Antioxidant capacity, by assay with 2,2'-azinobis (3-ethyl-benzothiazolline-6-sulfonic acid) (ABTS), from purified extracts of *P. pallida* fruits

Purified Extract	ABTS (mg TE/g ± DS)[a]			
	Sample 1 (Tumbes)	Sample 2 (Piura)	Sample 3 (Lambayeque)	Sample 4 (La Libertad)
Decoction	66.44 ± 2.92	72.10 ± 0.59	62.10 ± 2.86	76.54 ± 4.47[b]
Infusion	77.30 ± 2.83	82.67 ± 1.30	52.35 ± 0.89	36.27 ± 3.86
Ethanol 45%	95.85 ± 0.99[b]	80.40 ± 1.31	82.54 ± 1.72[b]	49.26 ± 4.53
Ethanol 70%	82.45 ± 0.49	81.68 ± 2.16	70.68 ± 3.70	49.40 ± 4.84
Ethanol 96%	82.41 ± 0.22	80.12 ± 1.01	47.87 ± 3.31	21.65 ± 1.50

[a] mg TE/g ± SD: Average milligrams of Trolox equivalents per gram of sample ± standard deviation (n=3)
[b] extract with higher concentration of phenolic compounds, $p<0.05$ (Tukey HSD post hoc test)
Pearson's coefficient: 0.1126

Chemical Identification by UHPLC-UV-MS/MS

Ten substances were identified by UHPLC-UV-MS/MS (Table No. 5): eight flavonoids, one phenolic acid and one diterpene. The most intense signal (Figure No. 3) was referent to vicenin II (2) [M-H] at *m/z* 593, a C-glycoside-flavone, showing the fragment *m/z* 395 [M-H-glucose] and other characteristic fragments: *m/z* 473, 383 and 353, according to the literature and software database (Quispe *et al*., 2014; Patel *et al*., 2018; Gonzales-Barron *et al*., 2020). This flavonoid possess several biological activities, including anti-inflammatory, by suppression of the production of tumor necrosis factor-α (TNF-α) and the activation of nuclear factor-κB (NF-κB) induced by LPS (Kang *et al*., 2015), antidiabetic activity by strong inhibition of α-glucosidase and protein tyrosine phosphatase 1B, and suppression of protein-oxidative mechanisms (Islam *et al*., 2014), as well as anticarcinogenic, hepatoprotective, renal-protective, and wound healing activities (Lee & Bae, 2020; Lee *et al*., 2020; Tan *et al*., 2020; Li *et al*., 2021). The other two C-glycoside-flavones identified in *P. pallida* were schaftoside **(3)** and isoschaftoside **(4)**. Both flavonoids presented [M-H] at *m/z* 563, generating the following main fragments: [M-H-90] corresponding to the loss of an arabinose unit *m/z* 473, [M-H-120] corresponding to the loss of a glucose unit *m/z* 443, and [M-H-210] corresponding to the fragmentation of sugar moiety *m/z* 353. The two substances have already been reported for *Prosopis* species (Colombo *et al*., 2006; Colombo *et al*., 2008; Quispe *et al*., 2014; Gonzales-Barron *et al*., 2020). As in the case of Vicenin II, Schaftoside has intense antioxidant, anti-inflammatory, anti-melanogenic, neuroprotective, and hepatoprotective activities (De Melo *et al*., 2005; Du *et al*., 2011; Materska, 2015; Kim *et al*., 2018; Zhou *et al*., 2019; Liu *et al*., 2020) and is also a strong inhibitor of pancreatic lipase (Fernando *et al*., 2019). Isoschaftoside also showed antioxidant, antiparasitic, and antitumor activities, but has in addition significant antimicrobial and antihypertensive properties (Bendini *et al*., 2006; Hooper *et al*., 2010; Du *et al*., 2011; Gomes *et al*., 2014; Zhang *et al*., 2015).

The flavonoids vitexin **(5)** and isovitexin **(6)** are common compounds in the genus *Prosopis* and were also identified in *P. pallida*, (Sharifi-Rad *et al*., 2019). These substances presented [M+H] at *m/z* 433 and generated their characteristic fragments [M+H-$C_4H_8O_4$], corresponding to the fragmentation of sugar moiety (*m/z* 313), and [M+H-$C_4H_8O_4$-CH_2O], corresponding to the following loss of the carbon attached an OH function, derived from the sugar (*m/z* 283) (Yan *et al*., 2013). The biological effects of these two flavonoids have been broadly studied. Vitexin has attracted increased attention due to its wide range of pharmacological activities, including antioxidant, anticarcinogenic, anti-hyperalgesic, antidiabetic, anti-inflammatory, neuroprotective, and endocrinous effects, whereas isovitexin showed antioxidant, anti-inflammatory, antidiabetic, and neuroprotective activities (He *et al*., 2016).

The flavanol naringenin **(9)** was an identified flavonoid aglycone in the extract of *P. pallida*. In the ESI negative mode analysis, it was possible to identify the quasi-molecular ion [M-H] at *m/z* 271. The most characteristic fragments were *m/z* 253,

related to a loss of water; *m/z* 151, corresponding to the A ring and the carbonyl group of C ring; and *m/z* 119, related to the ring B and two carbons of the C ring. As the other flavonoids mentioned above, naringenin has a large broad of biological activities. According to Salehi *et al*. (2019), these activities include anticarcinogenic (melanoma, glioma, liver, prostate and breast cancer), antidiabetic, antihyperlipidemic, anti-inflammatory, antiasthma, antimicrobial, antioxidant, antiplatelet, antioxidant, cardioprotective, expectorant, immunomodulatory, laxative, and hepatoprotective effects; as well as more specific mechanisms, such as anti-Hepatite C, anti-Chikungunya, and anti-Dengue activities, antiaging and neuroprotective effects (including anti-Alzheimer, anticonvulsant, and anti-stroke damage activities); in addition to observed effects on eye protection, promotion of male and female fertility, weight loss and protection against radiations (Salehi *et al*., 2019).

Finally, two methoxy-flavonols with [M-H] at *m/z* 769 **(7)** and 623 **(8)** were observed in the extract analysis. The compound **7,** tamarixetin-3-*O*-rhamnosyl-rhamnosyl-glucoside, showed a methoxy-flavonol aglycone connected with a hexoside, verified by the loss of *m/z* 162 and two hydrogens [M-H-162-H_2], presenting a *m/z* 605, and with a rhamnosylrhamnoside residue, verified by the loss of *m/z* 291 from the previous fragment, that generated a fragment of *m/z* 314, relative to a methyl-flavonol aglycone with phenolic-hydrogen loss. Rhamnosyl was also observed by the presence of fragment *m/z* 357, corresponding to the aglycone connected to the branch portion of the rhamnosyl residue. The loss of the methyl group of aglycone is explained by the fragment *m/z* 299. The compound **8,** tamarixetin-3-*O*-rutinoside, had a methoxy-flavonol connected with a rutinoside, verified by the loss of rutinoside residue [M-H-rutinose] that generated the fragments of *m/z* 314 and 299, corresponding to an aglycone form from methoxy-flavonol. Generally, the presence of methoxy-flavones is characteristic of *Prosopis*, as observed in previous results to this study and corroborated in the literature by the presence of isorhamnetin and tamarixetin aglycones in different species of this genus (Saad *et al*., 2017; Delgado-Nuñez *et al*., 2020). Tamarixetin presents anti-inflammatory activity, so that reduces the secretion of various inflammatory cytokines by dendritic cells after activation with LPS, in addition to enhance the secretion of the anti-inflammatory cytokine interleukin (IL)-10 and specifically increase the population of IL-10-secreting immune cells in LPS-activated splenocytes (Park *et al*., 2018).

The phenolic acid, feruloyl-*O*-dihexoside **(1)** with [M-H] at *m/z* 517, presented as the main fragments *m/z* 337 [M-H-hexoside-H_2O], *m/z* 193 [ferulic acid-H], *m/z* 175 [ferulic acid-H-H_2O], *m/z* 160 [ferulic acid-H-H_2O-CH_3] and *m/z* 89 [C_6HO ring]. Phenolic acid and some of its derivatives are known for their anti-microbial, anti-inflammatory, antioxidant, anticarcinogenic, hepatoprotective, neuroprotective, anti-diabetic, anti-cholesterolemic, and UV-protective activities (de Paiva *et al*., 2013). The encountered diterpene 7-oxodehydroabietic acid **(10)**, belongs to the chemical class of aromatic abietane diterpenoids, which have previously been reported in the genus *Prosopis* (Elmezughi *et al*. 2013). This substance with [M+H] at *m/z* 315 presented fragments related to the loss of a methyl group *m/z* 300 and the loss of the characteristic branch attached to the aromatic ring *m/z* 272, as well as the fragmentation profile observed in abietane diterpenoids *m/z* 272 to *m/z* 243 and *m/z* 205 to *m/z* 177 (Azemard *et al*., 2016). 7-oxodehydroabietic acid is best known for playing a defensive role against herbivorous insects via insect endocrine-disrupting activity (Oh *et al*., 2017).

Table No. 5
Identified substances from *Prosopis pallida* by UHPLC-UV-MS/MS

Substances	Rt (min)	M-H	M+H	Main Fragments (m/z)	UV absorption (nm)	References
Feruloyl-*O*-dihexoside **(1)**	6.30	517		337(4), 193(86), 175(100), 160(14), 89(10)	218, 325	NA
Vicenin II **(2)**	7.20	593		473(26), 395(7), 383(61), 353(100)	215, 271, 334	Quispe *et al*., 2014 Patel *et al*., 2018

Schaftoside **(3)**	8.93	563		473(16), 443(17), 425(13), 413(10), 395(7), 383(86), 353(100)	217, 271, 333	Colombo *et al*., 2006 Colombo *et al*., 2008 Quispe *et al*., 2014
Isoschaftoside **(4)**	9.03	563		473(33), 443(47), 425(6), 413(13), 383(74), 353(100)	217, 271, 339	Colombo *et al*., 2006 Colombo *et al*., 2008 Quispe *et al*., 2014
Vitexin **(5)**	10.49		433	415(14), 397(40), 367(19), 337(27), 313(100), 283(51), 217(5)	NA	Yan *et al*., 2013
Isovitexin **(6)**	11.14		433	397(5), 379(15), 361(15), 337(38), 313(69), 283(100), 271(6)	NA	Yan *et al*., 2013
Tamarixetin-3-*O*-rhamnosyl-rhamnosyl-glucoside **(7)**	11.22	769		357(0.8), 315(31), 314(100), 299(25), 271(4), 243(2), 179(2), 151(2)	NA	Saad *et al*., 2017 Delgado-Nuñez *et al*., 2020
Tamarixetin-3-*O*-rutinoside **(8)**	13.17	623		315(67), 314(100), 300(11), 299(22), 271(4), 243(2), 179(1), 151(3)	267, 352	Saad *et al*., 2017 Delgado-Nuñez *et al*., 2020
Naringenin **(9)**	19.82	271		271(48), 227(2), 177(13), 169(10), 151(100), 119(43)	NA	Salehi *et al*., 2019
7-Oxodehydroabietic acid **(10)**	27.20		315	301(2), 300(32), 296(1), 272(0.2), 70(0.5), 53(0.2)	NA	Azemard *et al*., 2016

Rt = Retention Time; NA = not avaliable

CONCLUSION

In the present study it was observed that the decoction of the sample from the region of La Libertad, followed by the extracts of Ethanol 45% of the samples from the regions of Tumbes and Lambayeque presented the highest absolute results of antioxidant capacity using the FRAP and ABTS methods. This demonstrated correlation with total phenolic content. Furthermore, it was possible to identify the main phenolic substances present in the extracts, including Vicenin II as the major constituent, in addition to the presence of diterpene 7-oxidehydroabietic acid. These findings contribute to the chemical and biological knowledge of *P. pallida* and a better understanding of its actions as a functional food/additive, which can direct at the development of new formulations based on its extracts for medicinal use.

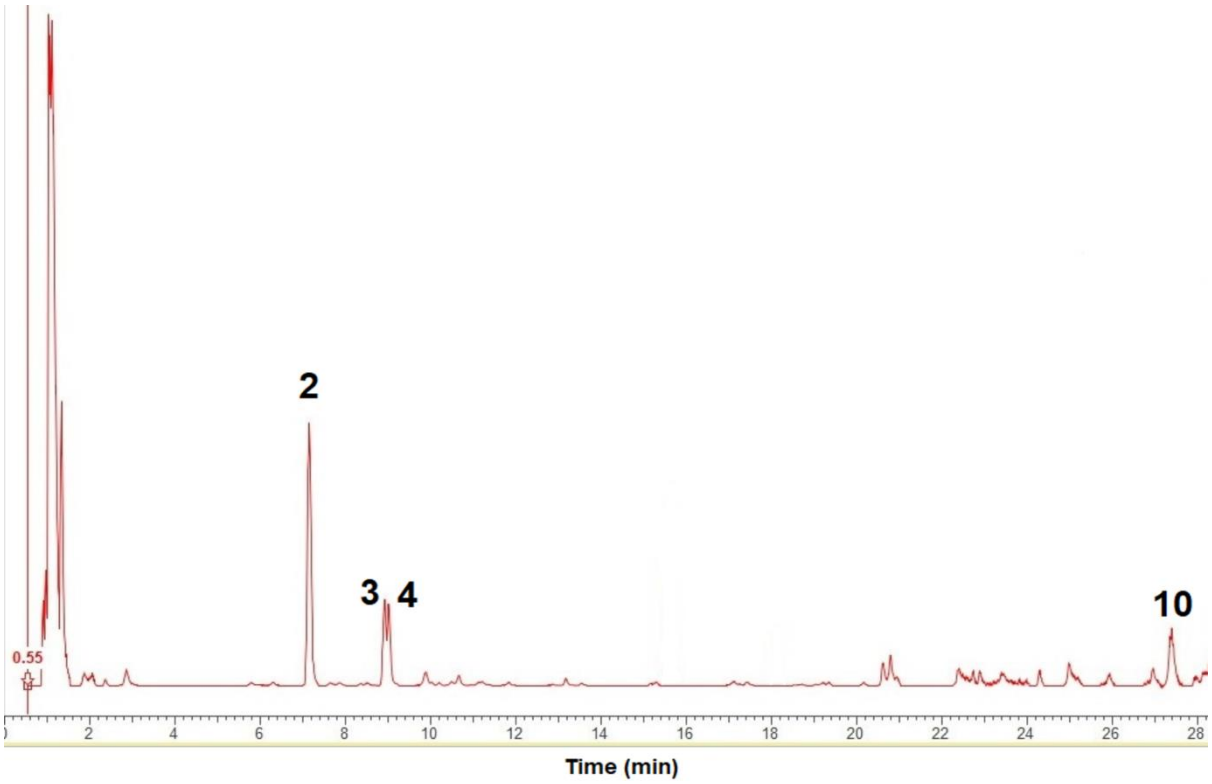

Figure No. 3
General UHPLC-MS/MS chromatogram from *Prosopis pallida* decoction purified extract obtained from the Sample 4 showing their main compounds: vicenin II (2), schaftoside (3), isoschaftoside (4) and 7-oxodehydroabietic acid (10)

REFERENCES

Alam MN, Bristi NJ, Rafiquzzaman M. 2013. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. **Saudi Pharm J** 21: 143 - 152. **https://doi.org/10.1016/j.jsps.2012.05.002**

Alarcón-Aguilar BA, Zavala-Urtecho ED, Suárez-Rebaza LA, Ganoza-Yupanqui ML, Fernández-Sosaya JL. 2018. Polifenoles, capacidad antioxidante y factor de protección solar de *Borago officinalis* L. "borraja" dispensada en las farmacias naturales de EsSalud. **Rev Peru Med Integrativa** 3: 138 - 143. **https://doi.org/10.26722/rpmi.2018.33.94**

Avello M, Suwalsky M. 2006. Radicales libres, antioxidantes naturales y mecanismos de protección. **Atenea** 494: 161 - 172. **https://doi.org/10.4067/S0718-04622006000200010**

Azemard C, Menager M, Viellescazes C. 2016. Analysis of diterpenic compounds by GC-MS/MS: contribution to the identification of main conifer resins. **Anal Bioanal Chem** 408: 6599 - 6612. **https://doi.org/10.1007/s00216-016-9772-9**

Bendini A, Cerretani L, Pizzolante L, Toschi TG, Guzzo F, Ceoldo S, Marconi AM, Andreetta F, Levi M. 2006. Phenol content related to antioxidant and antimicrobial activities of *Passiflora* spp. Extracts. **Eur Food Res Technol** 223: 102 - 109. **https://doi.org/10.1007/s00217-005-0150-7**

Benites J, Ybañez-Julca RO, Ganoza-Yupanqui ML, Mantilla-Rodríguez E, Zavala E, Velasquez S, Gajardo S, Morales B, de Albuquerque RDDG, Rocha L, Martinez JL. 2019. Antioxidant effect and chemical composition of *Ananas comosus* [L.] Merr. peels from Peruvian northern. **Bol Latinoam Caribe Plant Med Aromat** 18: 577 - 585. **https://doi.org/1010.35588/blacpma.19.18.6.40**

Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': the FRAP assay. **Anal Biochem** 239: 70 - 76. **https://doi.org/10.1006/abio.1996.0292**

Cattaneo F, Sayago J, Alberto M, Zampini I, Ordoñez R, Chamorro V, Pazos A, Isla M. 2014. Anti-inflammatory and antioxidant activities, functional properties and mutagenicity studies of protein and protein hydrolysate obtained from *Prosopis alba* seed flour. **Food Chem** 161: 391 - 399. **https://doi.org/10.1016/j.foodchem.2014.04.003**

Colombo R, Yariwake JH, Queiroz EF, Ndjoko K, Hostettmann K. 2006. On-line identification of further flavone C- and O-glycosides from sugarcane (*Saccharum officinarum* L., Gramineae) by HPLC-UV-MS. **Phytochem Anal** 17 (5): 337 - 343. **https://doi.org/10.1002/pca.923**

Colombo R, Yariwake JH, McCullagh J. 2008. Study of C- and O-glycosylflavones in sugarcane extracts using liquid chromatography: exact mass measurement mass spectrometry. **J Braz Chem Soc** 19: 483 - 490. **https://doi.org/10.1590/S0103-50532008000300016**

De Melo GO, Muzitano MF, Legora-Machado A, Almeida TA, De Oliveira DB, Kaiser CR, Koatz VLG, Costa SS. 2005. C-Glycosylflavones from the aerial parts of *Eleusine indica* inhibit LPS-induced mouse lung inflammation. **Planta Med** 71: 362 - 363. **https://doi.org/10.1055/s-2005-864104**

De Paiva LB, Goldbeck R, Santos WD, Squina FM. 2013. Ferulic acid and derivatives: molecules with potential application in the pharmaceutical field. **Braz J Pharm Sci** 49: 395 - 411. **https://doi.org/10.1590/S1984-82502013000300002**

Delgado-Nuñez EJ, Zamilpa A, González-Cortazar M, Olmedo-Juárez A, Cardoso-Taketa A, Sánchez-Mendoza E, Tapia-Maruri D, Salinas-Sánchez DO, Gives PM. 2020. Isorhamnetin: A nematocidal flavonoid from *Prosopis laevigata* leaves against *Haemonchus contortus* eggs and larvae. **Biomolecules** 10: 773. **https://doi.org/10.3390/biom10050773**

Du SS, Zhang HM, Bai CQ, Wang CF, Liu QZ, Liu ZL, Wang YY, Deng ZW. 2011. Nematocidal flavone-C-glycosides against the root-knot nematode (*Meloidogyne incognita*) from *Arisaema erubescens* tubers. **Molecules** 16: 5079 - 5086. **https://doi.org/10.3390/molecules 16065079**

Elmezughi J, Shittu H, Clements C, Edrada-Ebel RA, Seidel V, Gray A. 2013. Bioactive natural compounds from *Prosopis africana* and *Abies nobili*. **J Appl Pharm Sci** 3: 40 - 43. **https://doi.org/10.7324/JAPS.2013.30308**

Fernando WIT, Attanayake AMKC, Pereira HKI, Sivakanesan R, Jayasinghe L, Araya H, Fujimoto Y. 2019. Isolation, identification and characterization of pancreatic lipase inhibitors from *Trigonella foenum*-graecum seeds. **S Afr J Bot** 121: 418 - 421. **https://doi.org/10.1016/j.sajb.2018.10.023**

Floegel A, Kim DO, Chung SJ, Koo SI, Chun OK, 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. **J Food Compos Anal** 24: 1043 - 1048. **https://doi.org/10.1016/j.jfca.2011.01.008**

Ganoza-Yupanqui ML, Muñoz-Acevedo A, Ybañez-Julca RO, Mantilla-Rodríguez E, Zavala E, Gajardo S, Rios M, Benites J, Martinez JL. 2021. Potential antioxidant effect of fruit peels for human use from northern Peru, compared by 5 different methods. **Bol Latinoam Caribe Plant Med Aromat** 20: 611 - 637. **https://doi.org/10.37360/blacpma.21.20.6.44**

Gomes ACC, Sampaio LS, Silva PA, Larnas ME, Sakuragi CM, Junior CBB, Simas NK, Kuster RM. 2014. *In vitro* effect of isoschaftoside isolated from *Syngonium podophyllum* on pig kidney Na+, K+-ATPase. **Quim Nova** 37: 51793613. **https://doi.org/10.5935/0100-4042.20140257**

Gonzales-Barron U, Dijkshoorn R, Maloncy M, Finimundy T, Calhelha RC, Pereira C, Stojkovic D, Sokovic M, Ferreira ICFR, Barros L, Cadavez V. 2020. Nutritive and Bioactive Properties of Mesquite (*Prosopis pallida*) Flour and Its Technological Performance in Breadmaking. **Foods** 9: 597. **https://doi.org/10.3390/foods9050597**

He M, Min JW, Kong WL, He XH, Li JX, Peng BW. 2016. A review on the pharmacological effects of vitexin and isovitexin. **Fitoterapia** 115: 74 - 85. **https://doi.org/10.1016/j.fitote.2016.09.011**

Hooper AM, Tsanuo MK, Chamberlain K, Tittcomb K, Scholes J, Hassanali A, Khan ZB, Pickett JA. 2010. Isoschaftoside, a C-glycosylflavonoid from *Desmodium uncinatum* root exudate, is an allelochemical against the development of *Striga*. **Phytochemistry** 71: 904 - 908. **https://doi.org/10.1016/j.phytochem.2010.02.015**

Islam MN, Ishita IJ, Jung HA, Choi JS. 2014. Vicenin 2 isolated from *Artemisia capillaris* exhibited potent anti-

glycation properties. **Food Chem Toxicol** 69: 55 - 62. **https://doi.org/10.1016/j.fct.2014.03.042**

Kang H, Ku SK, Jung B, Bae JS. 2015. Anti-inflammatory effects of vicenin-2 and scolymoside *in vitro* and *in vivo*. **Inflamm Res** 64: 1005 - 1021. **https://doi.org/10.1007/s00011-015-0886-x**

Kim PS, Shin JH, Jo DS, Shin DW, Choi DH, Kim WJ, Park K, Kim JK, Joo CG, Lee JS, Choi Y, Shin YW, Shin JJ, Jeon HB, Seo JH, Cho DH. 2018. Anti-melanogenic activity of schaftoside in *Rhizoma Arisaematis* by increasing autophagy in B16F1 cells. **Biochem Biophys Res Commun** 503: 309 - 315. **https://doi.org/10.1016/j.bbrc.2018.06.021**

Kuskoski EM, Asuero AG, Troncoso AM, Mancini-Filho J, Fett R. 2005. Aplicación de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. **Ciênc Tecnol Aliment** 25: 726 - 732. **https://doi.org/10.1590/S0101-20612005000400016**

Lee IC, Bae JS. 2020. Hepatoprotective effects of vicenin-2 and scolymoside through the modulation of inflammatory pathways. **J Nat Med** 74: 90 - 97. **https://doi.org/10.1007/s11418-019-01348-x**

Lee BS, Sumin Y, Lee C, Ku SK, Bae JS. 2020. Renal protective effects of vicenin-2 and scolymoside in a mouse model of sepsis. **Braz J Pharm Sci** 56: e18636. **https://doi.org/10.1590/s2175-97902019000418636**

Li Y, Zheng Y, Wang H. 2021. Anticancer activity of Vicenin-2 against 7,12 dimethylbenz[a]anthracene-induced buccal pouch carcinoma in hamsters. **J Biochem Mol Toxicol** 35: e22673. **https://doi.org/10.1002/jbt.22673**

Liu M, Zhang G, Wu S, Song M, Wang J, Cai W, Mi S, Liu C. 2020. Schaftoside alleviates HFD-induced hepatic lipid accumulation in mice via upregulating farnesoid X receptor. **J Ethnopharmacol** 255: 112776. **https://doi.org/10.1016/j.jep.2020.112776**

Materska M. 2015. Flavone C-glycosides from *Capsicum annuum* L.: Relationships between antioxidant activity and lipophilicity. **Eur Food Res and Technol** 240: 549 - 557. **https://doi.org/10.1007/s00217-014-2353-2**

Oh HW, Yun CS, Jeon JH, Kim JA, Park DS, Ryu HW, Oh SR, Song HH, Shin Y, Jung CK, Shin SW. 2017. Conifer diterpene resin acids disrupt juvenile hormone-mediated endocrine regulation in the Indian meal moth *Plodia interpunctella*. **J Chem Ecol** 43: 703 - 711. **https://doi.org/10.1007/s10886-017-0861-9**

Pandey KB, Rizvi SI. 2009. Plant polyphenols as dietary antioxidants in human health and disease. **Oxid Med Cell Long** 2: 270 - 278. **https://doi.org/10.4161/oxim.2.5.9498**

Park HJ, Lee SJ, Cho J, Gharbi A, Han HD, Kang TH, Kim Y, Lee Y, Park WS, Jung ID, Park YM. 2018. Tamarixetin exhibits anti-inflammatory activity and prevents bacterial sepsis by increasing IL-10 production. **J Nat Prod** 81: 1435 - 1443. **https://doi.org/10.1021/acs.jnatprod.8b00155**

Patel NK, Sharath-Kumar LM, Gajera J, Jadhav AN, Muguli G, Babu UV. 2018. Isolation and characterization of flavonoid C-glycosides from *Prosopis glandulosa* Torr. leaves. **Pharmacogn Mag** 14: 451 - 454. **https://doi.org/10.4103/pm.pm_505 17**

Quispe C, Petroll K, Theoduloz C, Schmeda-Hirschmann G. 2014. Antioxidant effect and characterization of South American *Prosopis* pods syrup. **Food Res Int** 56: 174 - 181. **https://doi.org/10.1016/j.foodres.2013.12.033**

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radic Biol Med** 26: 1231 - 1237. **https://doi.org/10.1016/S0891-5849(98)00315-3**

Saad AM, Ghareeb MA, Abdel-Aziz MS, Madkour HMF, Khalaf OM, El-Ziaty AK, Abdel-Mogib M. 2017. Chemical constituents and biological activities of different solvent extracts of *Prosopis farcta* growing in Egypt. **J Pharmacogn Phytother** 9: 67 - 76. **https://doi.org/10.5897/JPP2017.0452**

Seeram NP, Henning SM, Niu Y, Lee R, Scheuller HS, Heber D. 2006. Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity. **J Agric Food Chem** 54: 1599 - 1603. **https://doi.org/10.1021/jf052857r**

Salehi B, Fokou PVT, Sharifi-Rad M, Zucca P, Pezzani R, Martins N, Sharifi-Rad J. 2019. The therapeutic potential of Naringenin: A review of clinical trials. **Pharmaceuticals** 12: 11. **https://doi.org/10.3390/ph12010011**

Shalaby EA, Shanab SMM. 2013. Antioxidant compounds, assays of determination and mode of action. **Afr J**

Pharm Pharmacol 7: 528 - 539. **https://doi.org/10.5897/AJPP2013.3474**

Sharifi-Rad J, Kobarfard F, Ata A, Ayatollahi SA, Dehaghi NK, Jugran AK, Tomas A, Capanoglu E, Matthews KR, Djordjevic JP, Kostic A, Kamiloglu S, Sharopov F, Choudhary MI, Martins M. 2019. *Prosopis* plant chemical composition and pharmacological attributes: Targeting clinical studies from preclinical evidence. **Biomolecules** 9: 777. **https://doi.org/10.3390/biom9120777**

Suárez-Rebaza LA, Ganoza-Yupanqui ML, Zavala-Urtecho ED, Alva-Plasencia PM. 2019. Compuestos fenólicos y actividad antioxidante de extractos hidroalcohólicos y acuosos de frutos de *Prosopis pallida* "algarrobo". **Agroind Sci** 9: 87 - 91. **https://doi.org/10.17268/agroind.sci.2019.01.11**

Tamayo LMA, Arteaga-Gonzalez DM, Jaramillo-Garcez Y. 2008. Propiedades farmacológicas del Algarrobo (*Hymenaea courbaril Linneaus*) de interés para la industria de alimentos. **Rev Lasallista Investig** 5: 100 - 111.

Tan WS, Arulselvan P, Ng SF, Taib CNM, Sarian MN, Fakurazi S. 2020. Healing effect of Vicenin-2 (VCN-2) on Human Dermal Fibroblast (HDF) and development VCN-2 hydrocolloid film based on alginate as potential wound dressing. **BioMed Res Int** 2020: 4730858. **https://doi.org/10.1155/2020/4730858**

Torres-Guevara FA, Ganoza-Yupanqui ML 2017. Etnobotánica y sistemas de extracción para compuestos fenólicos, actividad antioxidante y toxicidad de plantas de páramos y bosques nublados del norte peruano. **Rev Peru Med Integrativa** 2: 101 - 109. **https://doi.org/10.26722/rpmi.2017.22.51**

Vattem DA, Maitin V. 2020. **Functional foods, nutraceuticals and natural products: Concepts and applications**. DEStech Publications, Lancaster, USA.

Yan C, Liu H, Lin L. 2013. Simultaneous determination of vitexin and isovitexin in rat plasma after oral administration of *Santalum album* L. leaves extract by liquid chromatography tandem mass spectrometry. **Biomed Chromatogr** 27: 228 - 232. **https://doi.org/10.1002/bmc.2780**

Zavala-Urtecho ED, Ganoza-Yupanqui ML, Ybañez-Julca RO, Mantilla-Rodriguez AE, Gajardo-Solari SKN, Benites-Vilchez JW. 2018. Perfil ultravioleta y actividad regeneradora de liofilizados de cáscara del fruto de variedades *de Mangifera indica*, mango. **Rev Peru Med Integrativa** 3: 40 - 45. **https://doi.org/10.26722/rpmi.2018.31.80**

Zhang Y, Tang HM, Li A, Xu LF, Chen JN, Huang S, He L. 2015. Determination of six C-Glycoside flavones and antitumor activity of water-soluble total flavonoids from *Isodon lophanthoides* var. gerardianus. **Zhongguo Zhong Yao Za Zhi** 40: 1543 - 1547. **https://doi.org/10.4268/cjcmm20150822**

Zhou K, Wu J, Chen J, Zhou Y, Chen X, Wu Q, Xu Y, Tu W, Lou X, Yang G, Jiang S. 2019. Schaftoside ameliorates oxygen glucose deprivation-induced inflammation associated with the TLR4/Myd88/Drp1-related mitochondrial fission in BV2 microglia cells. **J Pharmacol Sci** 139: 15 - 22. **https://doi.org/10.1016/j.jphs.2018.10.012**