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Total Phenolic and Flavonoid Contents and Flavonoid Composition of Flowers and Leaves from the Mexican Medicinal Plant *Gymnosperma glutinosum* (Spreng.) Less

Rolando Morado-Castillo[1*], Ramiro Quintanilla-Licea[1], Ricardo Gomez-Flores[2] and Wolfgang Blaschek[3]

[1]Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Laboratorio de Fitoquímica, San Nicolás de los Garza N.L., México.
[2]Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Laboratorio de Inmunobiología y Virología, San Nicolás de los Garza N.L., México.
[3]Pharmazeutisches Institut, Christian-Albrechts-Universität Zu Kiel, Kiel, Germany.

Authors' contributions

This work was carried out in collaboration between all authors. Author RMC wrote the protocol, performed the experimental analysis and wrote the first draft of the manuscript. Authors RQL and RGF designed the study. Author WB advised and managed the research. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: The aim of the present study was to evaluate the total phenolics and flavonoids content of leaves and flowers of *Gymnosperma glutinosum* (Spreng.) Less., and identify their main chemical constituents.
Materials and Methods: *G. glutinosum* leaves and flowers were separately extracted with sequential 85, 80, 75, and 70% Methanol. Total phenolic content was determined using the Folin-Ciocalteu assay with gallic acid as standard. Total flavonoid content was evaluated using the aluminum chloride colorimetric method with quercetin as a standard. TLC and HPLC analysis of

**Corresponding author: E-mail: rolando.moradocs@uanl.edu.mx;*

theextracts were performed, and compounds were identified by retention time and UV spectra in comparison with polyphenolic standards.
Results: Total phenolic content of leaf extracts was 116.7 mg/g GAE (Gallic Acid Equivalents) whereas that of flower extracts was 159.8 mg/g GAE. Total flavonoid content of leaf extracts was 6.7 mg/g QE (Quercetin Equivalents) and that of flower extracts 15.9 mg/g QE. According to TLC and HPLC analysis, chlorogenic acid, astragalin, quercitrin, quercetin, rutin, kaempferol, and vitexin were the main components.
Conclusions: The identified polyphenols, except vitexin, have not yet been reported for *G. glutinosum*. These compounds might be involved in health benefits attributed to *G. glutinosum*. The higher content of flavonoids and other polyphenols in the flower extract suggest that *G. glutinosum* could be more efficient as an herbal remedy when the specimens are flowering, a feature not yet indicated in the Mexican traditional medicine.

Keywords: Gymnosperma glutinosum; polyphenols; flavonoids; Mexican traditional medicine.

1. INTRODUCTION

Within the natural products group, flavonoids and other phenolic compounds are known for multiple biological effects including antioxidant, free radical scavenging activities, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory, anticarcinogenic, antimicrobial, hypolipidemic, antimutagenic, antidiabetic, and many other activities [1-5]. They are also associated with the prevention and treatment of cardiovascular and cerebrovascular diseases [6]. This broad spectrum of bioactivities suggests that polyphenols play an important role in the medicinal properties associated with some herbal remedies and identifying and evaluating their chemical content might be a valuable approach to scientifically validate their ethnobotanical use.

In Mexican traditional medicine, medicinal plants are the most abundant, accessible, and recognized resource, as a result of their ecological diversity and centuries of empirical knowledge [7,8]. *Gymnosperma glutinosum* (Spreng.) Less. (Asteraceae), is a medicinal plant known in Mexico as *Tatalencho, Jarilla, Mota, Hierba Pegajosa, Escobilla, Pegajosa* [9] and its leaves traditionally are used as an aqueous infusion for the treatment of diarrhea, ulcers, joint pain, fever, inflammation, and headaches [10,11]. Phytochemical studies regarding polyphenolic compounds have found 27 highly oxygenated flavonoids from alcoholic extracts of air-dried leaves and stems [12-17], as well as the flavonoid pinocembrin, isolated from chloroform and hexane extracts of aerial parts [18]. Furthermore, the compound D-glycero-D-galactoheptitol has been isolated from methanolic extracts [19].

Antibacterial and antifungal activities of extracts and isolated compounds from *G. glutinosum*have been previously reported [20,21]. Gomez-Flores et al. [22] found that hexane extracts of *G. glutinosum* leaves possess a strong antimicrobial activity against *Mycobacterium tuberculosis*. Examination of anti-tumoral activity of *G. glutinosum* using an *in vitro* and *in vivo* L5178Y-R murine lymphoma test system resulted in significant cytotoxicity of low-polarity compounds isolated from hexane extracts [19,23,24]. Significant cytotoxicity of methanolic extracts of *G. glutinosum*also indicates the presence of more polar bioactive compounds [19], which might include flavonoids and other polyphenols. In a recent study, the antiprotozoal activity of *G. glutinosum* extracts against *Entamoeba histolytica* was tested [25].

The aim of the present study was to investigate the total phenolic and flavonoid content of leaves and flowers separately and identify potentially bioactive polyphenols by analytical methods. These results could have an impact on the reassessment of the traditional preparation of *G. glutinosum* remedies and its overall acceptance as a medicinal plant.

2. MATERIALS AND METHODS

2.1 Plant Material

Aerial parts of *G. glutinosum* were collected during flowering in July 2013 from an area within the coordinates 24° 39' y -100° 1' in Aramberri, Nuevo Leon, Mexico. One specimen was identified by Dr. Marcela Gonzalez Alvarez and deposited at the Herbarium of Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León (Voucher No. 26671). Plant material was dried at room temperature (Approx. 23ºC). Afterward, flowers and leaves were separated manually.

2.2 Determination of Total Phenolic Content

Total phenolic content was spectrophotometrically determined using the Folin-Ciocalteu reagent [26]. A reflux extraction of 100 mg of plant material was prepared with 50 mL of distilled water in a round-bottomed flask at 100℃ in a water bath for 30 min for leaves and flowers separately. After cooling to room temperature, flask contents were filtered, and the extractwas collected; 2 mL of each extract or gallic acid (as a standard phenolic compound in 2 mL of water) was mixed with 1.0 mL of Folin-Ciocalteu reagent,10 mL of distilled water and 12 mL of 29% aqueous Na_2CO_3. The mixtures were allowed to stand for 30 min, and absorbance was measured at 760 nm in a Cary 50 UV Spectrophotometer. A standard curve was prepared using 0, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250 µg/mL solutions of gallic acid. Values are expressedas acid equivalents (GAE in mg/g of dry mass).

2.3 Determination of Total Flavonoid Content

Quantification of total flavonoid content by colorimetric methods can be imprecise because many flavonoids are glycosylated, resulting in problems in the formation of the colored complex. Acid hydrolysis prior to colorimetric analysis is a common method to avoid this uncertainty in measurement [27].

For analysis, a maceration of 1 g of plant material in 30 mL of MeOH 85% was performed. After 24 h at room temperature extracts were filtered, the solvent was evaporated, and 100 mg of the extractwas mixed with 1.0 mL of 0.5% methenamine, 20 mL acetone and 2.0 mL of 25% HCl. After refluxing for 30 min, the mixture was filtered, deposited in a separation funnel together with 20 mL of water and washed three times with 15 mL of EtOAc. Ethyl acetate phases were collected and washed twice with 50 mL of water (water phases were discarded) and 10 g of anhydrous Na_2SO_4were added. Subsequently, EtOAc phase was filtered and deposited in a volumetric flask.

From the EtOAC phase of each extract (leaves and flowers), 10 mL with approximately 1.0 mg/mL were mixed with 1.0 mL of 10% (v/v) $AlCl_3$ in water and 14 mL of a solution of 5% (v/v) of 98% acetic acid and MeOH. The mixtures were allowed to stand for 30 min, and absorbance values were measured at 425 nm in a Cary 50 UV Spectrophotometer. A standard curve was prepared using 0, 3.91, 7.81, 15.63, 31.25, 62.5, 125 µg/mL solutions of quercetin dehydrate (PhytoLab GmbH). Values are expressedas quercetin equivalents (QE in mg/g of dry mass).

2.4 Thin Layer Chromatography Analysis

For TLC analysis, plant material (750 mg) was extracted with 5.0 mL of MeOH, under reflux in a hot water bath for 10 min, cooled and filtered. All extracts were analyzed by TLC on 20 X 20 cm plates (Silica Gel 60 F_{254}, Merck) and compared with methanolic extracts, obtained under the same conditions, of *Solidago virgaurea* L. and *Arnica montana* L. (typical members of Asteraceae). A reference solution of 1.0 mg chlorogenic acid, 2.5 mg quercitrin and 2.5 mg of rutin in 10 mL of MeOH was used. The mobile phase consisted of ethyl acetate: methylethyl ketone: H_2O: formic acid (30:18:6:6). Plates were revealed with UV at 365 nm after spraying with a solution of Natural Products Reagent in MeOH (10 g/L) and Macrogol 400 (50 g/L). Compounds were identified by R_f values as reported elsewhere.

2.5 Extractions for HPLC Analysis

Plant material was ground to a fine powder. Extracts of flowers and leaves were obtained separately by maceration of 50 g of plant material in 500 mL of MeOH/H_2O (different concentrations) at room temperature. The procedure involved four different consecutive extractions (MeOH/H_2O 85%, 80%, 75% and 70%) for 40 h at room temperature for each one. Next, extracts were transferred to a chromatographic column with a matrix of Amberlite FPX66 (filled with 100 g of resin in 2 L of distilled H_2O). Unwanted substances were eluted with 1.2 L of distilled H_2O. Enriched polyphenol extracts were eluted with 600 mL of EtOH 75% at two drops per second. The solventwas removed under vacuum on a rotatory evaporator; then 50 mL of H_2O were added to the enriched polyphenol extracts, and insoluble material was discarded. Water soluble fractions of each enriched polyphenol extract were frozen at -20ºC for 48 h and lyophilized.

2.6 High-performance Liquid Chromatography

Samples (water soluble fraction of the enriched polyphenol extracts) were analyzed in a Waters HPLC instrument (Waters e2695 Separation

Module, Alliance) with a PDA Detector (Waters 2998). Column: C18 Aqua 5mm, 250 X 4.6 mm (Phenomex Inc.). Flow rate: 1 mL/min. Gradient elution: H_2O-Acetonitrile; starting with 100% H_2O and remaining isocratic for 10 min, then using gradient elution to reach 50% of H_2O and 50% of acetonitrile at 40 min and 100% acetonitrile at 50 min. From minute 51 to 60 elution stays isocratic with 100% H_2O. Standards of 46 different polyphenols were used for reference. The most representative for the present work were Chlorogenic acid (Carl Roth GmbH), Kaempferol (PhytoLab GmbH), Quercetin (PhytoLab GmbH), Quercitrin (Extrasynthese SAS), Rutin (PhytoLab GmbH), Vitexin (PhytoLab GmbH) and Astragalin (PhytoLab GmbH).Compounds were identified by retention time and UV spectra in comparison with standards under the same conditions.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic and Flavonoid Content

Concentration measurement is based on creating a calibration curve with standard solutions at different concentrations. The total phenolic content of the extracts was calculated with a standard curve using gallic acid ($y = 0.0018x + 0.013$, $R^2 = 0.9999$), whereas the total flavonoid content was calculated using a calibration curve with quercetin as standard ($y = 0.0239x + 0.0842$, $R^2 = 0.9877$). Analysis showed that flowers contain greater amounts of phenolics and flavonoids than leaves (Table 1). In Mexican traditional medicine, there exists no indication for the use of flowers of *G. glutinosum* [10,11]. Based on the results, it would be reasonable to improve medicinal effects by using flowering plants (in summer and autumn seasons), as polyphenols, particularly flavonoids, are well known bioactive natural products [28].

3.2 Identification of Polyphenols

By comparative TLC (Fig. 1), it was shown that leaf and flower extracts of *G. glutinosum*present similar composition exceptan unknown band at R_f = 0.89 in the leaves extract. In comparison with other typical members of Asteraceae (*Arnica montana* and *Solidago virgaurea*), *G. glutinosum* extracts showed similarities in the R_f region 0.10–0.60, especially concerning the “light green” colored band at R_f = 0.52. This band was identified as astragalin, according toliterature data [29,30], R_f and color development, after treatment with Natural Product Reagent A. Presence of quercitrin and chlorogenic acid is remarkable and could be proven by comparison with the reference substance. Although by TLCrutin could not clearly be detected, it was found later on by HPLC analysis of the leaf extract. The reason may be that rutin is masked by other unidentified extract components with similar polarity.

Table 1. Total phenolic and flavonoid content of flowers and leaves extracts of *Gymnosperma glutinosum*

Sample	Phenolic content (mg GAE g^{-1} of dry mass±SD)	Flavonoid content (mg QE g^{-1} of dry mass±SD)
Leaves	116.7±0.06	6.7±0.03
Flowers	159.8±0.13	15.9±0.01

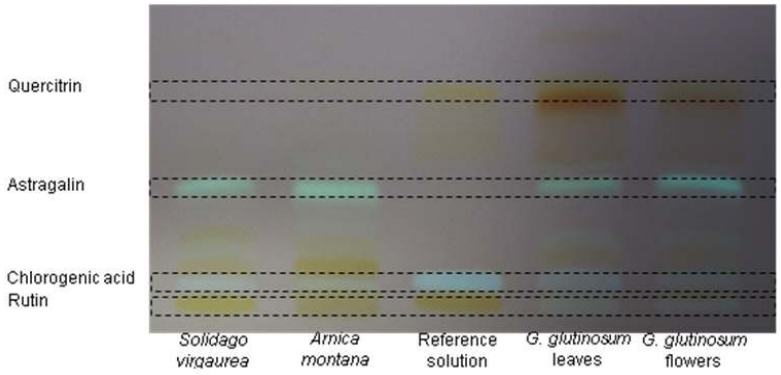

Fig. 1. Comparative TLC of methanolic extracts of *G. glutinosum* with those of *Solidago virgaurea* and *Arnica montana*
Reference solution contains rutin, chlorogenic acid and quercitrin

HPLC profiles of leaf and flower extracts (Fig. 2) showed differences in its composition. In the leaf extract (Fig. 2A), chlorogenic acid (**1**), astragalin (**2**), quercitrin (**3**), quercetin (**4**) and kaempferol (**5**) could be identified. The flower extract resulted in a simpler chromatogram (Fig. 2B) with two main components, namely rutin (**6**) and vitexin (**7**). Chlorogenic acid, quercitrin, quercetin and kaempferol were present only in low amounts. All compounds were identified by retention time and UV spectra, in comparison with standards (Table 2). For *G. glutinosum,* up to 28 flavonoids and other polyphenols [12-17] have been described in some reports. In the present work, the occurrence of 7 polyphenols could be proven which, except vitexin, have not been previously reported.

Table 2. HPLC data of identified polyphenols[a]

Compound	R_t (min)	UV max (nm)
1- Chlorogenic acid	17.8	248, 329
2- Astragalin	30.5	265, 350
3- Quercitrin	31.4	251, 301, 350
4- Quercetin	36.8	254, 376
5- Kaempferol	40.1	266, 364
6- Rutin	28.4	257, 355
7- Vitexin	28.9	214, 269, 339

[a] *Compounds were identified by comparison with standards (Data not shown)*

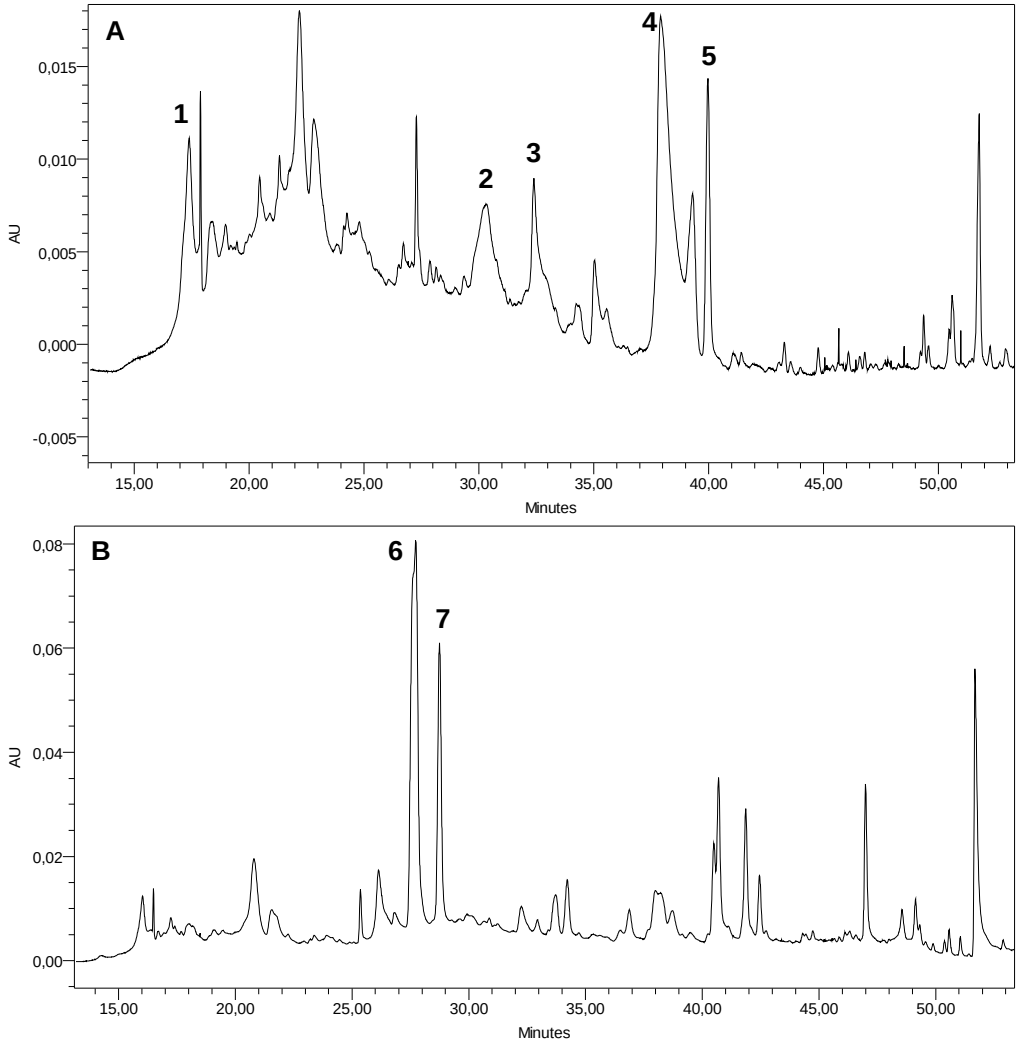

Fig. 2. A. Chromatographic profile, at 270 nm, of the leaves extract of *G. glutinosum*
B. Chromatographic profile, at 270 nm, of the flowers extract of *G. glutinosum*
1=Chlorogenic acid, 2=Astragalin, 3=Quercitrin, 4=Quercetin, 5=Kaempferol, 6=Rutin, 7=Vitexin

The identified compounds may contribute to medicinal effects of *G. glutinosum* considering that most of them have scientifically accepted bioactivities. Chlorogenic acid has been described as antiviral, neuroprotective, and blood pressure reducing substance, in addition to other activities [31,32]. Astragalin is considered to be ubiquitous in the plant kingdom, and in some studies has been effective against allergic diseases [33]. Quercitrin has also been studied as an antileishmanial [34] and an intestinal anti-inflammatory agent [35], but it is better recognized as a pro-drug of its aglycone quercetin, for which anticancer, antibacterial and many other activities were reported [36-38]. Kaempferol exerted antimicrobial, antiviral, cardioprotective, neuroprotective, analgesic, and anticancer activities [39]. Rutin was described as an antidepressant substance, antiplatelet aggregator, and hepatoprotective compound [40,41], whereas vitexin has been studied for its antibacterial, spasmolytic, and antimetastatic effects [42,43]. All these bioactivities are associated with the antioxidant, anti-inflammatory, and radical scavenger effects characteristic for most flavonoids and many other polyphenols [44,45].

4. CONCLUSION

The identified polyphenols, except vitexin, have not been yet reported for *G. glutinosum*. These polar compounds might be involved in health benefits attributed to *G. glutinosum* in Mexican Traditional Medicine, where it is mainly used as an infusion. The higher content of flavonoids and other polyphenols in the flowers extract suggest that *G. glutinosum* could be more efficient as an herbal remedy, using the leaves together with flowers of the collected plant material, which has not been indicated in the ethnobotanical use of this plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry. 2000;55(6):481-504.
2. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther. 2002;96(2-3):67-202.
3. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Ag. 2005;27(2):343-56.
4. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. 2009;2(5):270-78.
5. Dragan S, Andrica F, Serban MC, Timar R. Polyphenols-rich natural products for the treatment of diabetes. Curr Med Chem. 2014;22(1):14-22.
6. Gan RY, Xu XR, Song FL, Kuang L, Li HB. Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. J Med Plants Res. 2010;4(22):2438-44.
7. Rzedowski J. Diversidad y orígen de la flora fanerogámica de México. Acta Bot Mex. 1992;14:47-56. Spanish.
8. Heinrich M, Gibbons S. Ethnopharmacology in drug discovery: an analysis of its role and potential contribution. J Pharm Pharmacol. 2001; 53:425-32.
9. Hernandez T, Canales M, Avila JG, Duran A, Caballero J, Romo de Vivar A, Lira R. Ethnobotany and antibacterial activity of some plants used in traditional medicine of Zapotitlan de las Salinas, Puebla (Mexico). J Ethnopharmacol. 2003;88(2-3):181-88.
10. Martinez M. Catálogo de nombresvulgares y científicos de plantasmexicanas. México: Fondo de Cultura Económica. 1979;857. Spanish.
11. Casas A, Valiente-Banuet A, Viveros JL, Caballero J, Cortes L, Davila P, Lira R, Rodriguez I. Plant resources of the Tehuacán-Cuicatlán Valley, Mexico. Econ Bot. 2001;55(1):129-66.
12. Dominguez XA, Torre B. Two pentamethoxylated flavonoids from *Gymnosperma glutinosum*. Phyto-chemistry. 1974;13:1624-25.

13. Wollenweber E, Dietz VH. Occurrence and distribution of free flavonoids aglycones in plants. Phytochemistry. 1981;20(5):869-932.
14. Yu S, Fang N, Mabry TJ. Flavonoids from *Gymnosperma glutinosum*. Phytochemistry. 1988;171-77.
15. Iinuma M, Mizuno M. Natural occurrence and synthesis of 2'-oxygenated flavones, flavonols, flavanones and chalcones. Phytochemistry. 1989;28:681-94.
16. Wollenweber E, Dörr M, Fritz H, Pependieck S, Yatskievych G, Roitman JN. Exudate flavonoids in Asteraceae from Arizona, California and Mexico. Z Naturforsch C. 1997;52:301-07.
17. Horie T, Ohtsuru Y, Shibata K, Yamashita K, Tsukayama M, Kawamura Y. ^{13}C NMR spectral assignment of the A-ring of polyoxygenated flavones. Phytochemistry. 1998;47(5).865-74.
18. Martinez R, Calderon JS, Toscano RA, Valle-Aguilera L, Mendoza-Candelaria HM. Ent-neoclerodane diterpenes from *Gymnosperma glutinosum*. Phyto-chemistry. 1994;35(6):1505-07.
19. Quintanilla-Licea R, Morado-Castillo R, Gomez-Flores R, Laatsch H, Verde-Star MJ, Hernandez-Martinez H, Tamez-Guerra P, Tamez-Guerra R, Rodriguez-Padilla C. Bioassay-guided isolation and identification of cytotoxic compounds from *Gymnosperma glutinosum* leaves. Molecules. 2012;17:11229-41.
20. Canales M, Hernandez T, Serrano R, Hernandez LB, Duran A, Rios V, Sigrist S, Hernandez HLH, Garcia AM, Angeles-Lopez O, Fernandez-Araiza MA, Avila G. Antimicrobial and general toxicity activities of *Gymnosperma glutinosum*: A comparative study. J Ethnopharmacol. 2007;110:343-47.
21. Serrano R, Hernandez T, Canales M, Garcia-Bores AM, Romo de Vivar A, Cespedes CL, Avila JG. Ent-labdane type diterpene with antifungal activity from *Gymnosperma glutinosum* (Spreng.) Less. (Asteraceae). BLACPMA. 2009;8(5):412-18.
22. Gomez-Flores R, Arzate-Quintana C, Quintanilla-Licea R, Tamez-Guerra P, Tamez-Guerra R, Monreal-Cuevas E, Rodriguez-Padilla C. Antimicrobial activity of *Persea americana* Mill (Lauraceae) (Avocado) and *Gymnosperma glutinosum* (Spreng.) Less (Asteraceae) Leaf extracts and active fractions against *Mycobacterium tuberculosis*. Am-Euras J Sci Res. 2008;3(2):188-94.
23. Gomez-Flores R, Verastegui-Rodriguez L, Quintanilla-Licea R, Tamez-Guerra P, Monreal-Cuevas E, Tamez-Guerra R, Rodriguez-Padilla C. Antitumor properties of *Gymnosperma glutinosum* leaf extracts. Cancer Invest. 2009;27(2):149-55.
24. Gomez-Flores R, Quintanilla-Licea R, Verde-Star MJ, Morado-Castillo R, Vazquez-Diaz D, Tamez-Guerra R, Tamez-Guerra P, Rodriguez-Padilla C. Long-chain alkanes and ent-labdane-type diterpenes from *Gymnosperma glutinosum* with cytotoxic activity against the murine lymphoma L5178Y-R. Phytother Res. 2012;26:1632-36.
25. Quintanilla-Licea R, Mata-Cardenas BD, Vargas-Villareal J, Bazaldua-Rodriguez AF, Angeles-Hernandez IK, Garza-Gonzalez JN, Hernandez-Garcia ME. Antiprotozoal activity against *Entamoeba histolytica* of plants used in northeast Mexican traditional medicine. Bioactive compounds from *Lippia graveolens* and *Ruta chalepensis*. Molecules. 2014;19: 21044-65.
26. Dehghan G, Shafiee A, Ghahremani MH, Ardestani SK, Abdollahi M. Antioxidant potential of various extracts from *Ferula szovitsiana* in relation to their phenolic content. Pharm. Biol. 2007;45(9):691-99.
27. European Pharmacopoeia. 5th ed. Strasbourg: EDQM. 2005;01/2005:1892.
28. Veitch NC, Grayer RJ. Flavonoids and their glycosides, including anthocyanins. Nat Prod Rep. 2011;28:1626-95.
29. Nakabayashi T. Isolation of astragalin and isoquercitrin from Bracken, *Pteridium aquilinum*. Bull Agr Chem Soc. 1955; 19(2):104-09.
30. Wagner H, Bladt S. Plant drug analysis: A thin layer chromatography atlas. 2nd ed. Berlin: Springer-Verlag Berlin Heidelberg. 1996;214-15.
31. Casteluccio C, Paganga G, Melikian N, Bolwell GP, Pridham J, Sampson J, Rice-Evans C. Antioxidant potential of intermediates in phenylpropanoid metabolism in higher plants. FEBS Lett. 1995;368:188-92.
32. Shibata H, Sakamoto Y, Oka M, Kono Y. Natural antioxidant, chlorogenic acid, protects against DNA breakage caused by monochloramine. Biosci Biotechnol Biochem. 1999;63(7):1295-97.

33. Kotani M, Matsumoto M, Fujita A, Higa S, Wang W, Suemura M, Kishimoto T, Tanaka T. Persimmon leaf extract and astragaling inhibit the development of dermatitis and IgE elevation in NC/Nga mice. J Allergy Clin Immunol. 2000; 106(1 Pt 1):159-66.
34. Muzitano MF, Cruz EA, de Almeida AP, da Silva SAG, Kaiser CR, Guette C, Rossi-Bergmann B, Costa SS. Quercitrin: An antileishmanial flavonoid glycoside from *Kalanchoe pinnata*. Planta Med. 2006; 72:81-83.
35. Camuesco D, Comalada M, Rodriguez-Cabezas ME, Nieto A, Lorente MD, Concha A, Zarzuelo A, Galvez J. The intestinal anti-inflammatory effect of quercitrin is associated with an inhibition in iNOS expression. Br J Pharmacol. 2004; 143:908-18.
36. Chow JM, Shen SC, Huan SK, Lin HY, Chen YC. Quercetin, but not rutin and quercitrin, prevention of H_2O_2-induced apoptosis via anti-oxidant activity and heme oxygenase 1 gene expression in macrophages. Biochem Pharmacol. 2005; 69:1839-51.
37. Comalada M, Camuesco D, Sierra S, Ballester I, Xaus J, Galvez J, Zarzuelo A. *In vivo* quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway. Eur J Immunol. 2005;35:584-92.
38. Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E. Anti-inflammatory effects of flavonoids: Genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediators Inflamm. 2007;45673.
39. Calderon-Montaño JM, Burgos-Moron E, Perez-Guerrero C, Lopez-Lazaro M. A review on the dietary flavonoid kaempferol. Mini Rev Med Chem. 2011;11(4):298-344.
40. Janbaz KH, Saeed SA, Gilani AH. Protective effect of rutin on paracetamol- and CCl_4-induced hepatotoxicity in rodents. Fitoterapia. 2002;73:557-63.
41. Nöldner M, Schötz K. Rutin is essential for the antidepressant activity of *Hypericum perforatum* extracts in the forced swimming test. Planta Med. 2002;68(7):577-80.
42. Ragone MI, Sella M, Conforti P, Volonte MG, Consolini AE. The spasmolytic effect of *Aloysia citriodora*, Palau (South American cedron) is partially due to its vitexin but not isovitexin on rat duodenums. J Ethnopharmacol. 2007; 113(2):258-66.
43. Choi HJ, Eun JS, Kim BG, Kim SY, Jeon H, Soh Y. Vitexin, an HIF-1α inhibitor, has anti-metastatic potential in PC12 cells. Mol Cells. 2006;22(3):291-99.
44. Tapiero H, Tew KD, Nguyen-Ba G, Mathe G. Polyphenols: Do they play a role in the prevention of human pathologies? Biomed Pharmacother. 2002;56:200-07.
45. Moreno-Jimenez MR, Cervantes-Cardoza V, Gallegos-Infante JA, Gonzalez-Laredo RF, Estrella I, Garcia-Gasca TJ, Herrera-Carrera E, Diaz-Rivas JO, Rocha-Guzman NE. Phenolic composition changes of processed common beans: Their antioxidant and anti-inflammatory effects in intestinal cancer cells. Food Res Int. 2015;76:79-85.

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