Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Isolation and Characterization of Flavonoid C-glycosides from *Prosopis glandulosa* Torr. Leaves

Neeraj Kumar Patel, L. M. Sharath Kumar, Jignesh Gajera, Atul N. Jadhav, Ganesh Muguli, U. V. Babu[1]

Phytochemistry Department, The Himalaya Drug Company, [1]R&D Center, The Himalaya Drug Company, Bengaluru, Karnataka, India

Submitted: 29-10-2017 Revised: 06-12-2017 Published: 14-08-2018

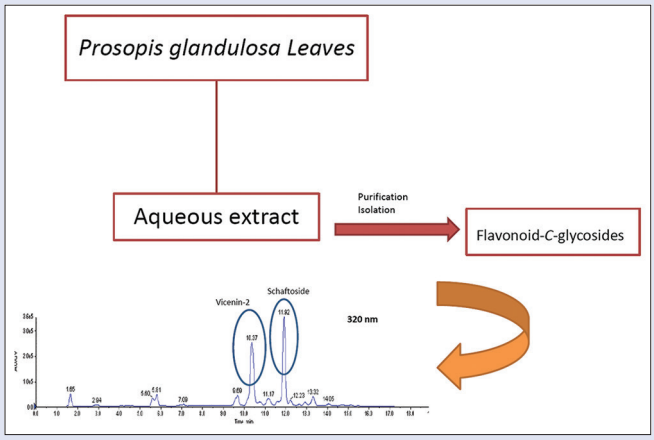
ABSTRACT

Background: *Prosopis glandulosa* Torr. (Fabaceae) is native to Africa, America, and Asia. In India, it is abundantly found in southern parts. The plant is reported to contain polyphenols, flavonoids, mesquitol-catechin dimers, indolizidine alkaloids, and triterpenes, imparting antitumor, antimicrobial, anti-infective, and anti-parasitic activities. **Aim:** The present study deals with the isolation and characterization of flavonoid *C*-glycosides from *P. glandulosa* Torr. leaves**. Materials and Methods:** *P. glandulosa* leaves were subjected to hot aqueous extraction at 90°C–100°C to yield crude aqueous extract (PGA). PGA was fractionated and then subjected to chromatography over Diaion® HP-20 column and semi-preparative high-performance liquid chromatography to provide flavonoid *C*-glycosides. **Results:** We have identified and characterized vicenin-2/isomer and schaftoside (s) from *P. glandulosa* leaves through liquid chromatography-electron spray ionization-mass spectrometry/mass spectrometry (LC-MS/MS) and nuclear magnetic resonance. LC-MS/MS chromatogram displayed peaks at retention time of 9.69 and 10.37 with *m/z* 594 and major fragments at *m/z* 473 $[M-H-120]^-$ (loss of glucose unit) for "vicenin-2/isomer." Interestingly, five major peaks were observed with retention times of 10.75, 11.17, 11.92 (major), 12.92, and 13.32 corresponding to the same mass of *m/z* 564 with major fragments at *m/z* 473 $[M-H-90]^-$ (loss of arabinose unit) and 443 $[M-H-120]^-$ (loss of glucosyl unit), together termed as "schaftosides." **Conclusion:** To the best of our knowledge, this is the first report on flavonoid *C*-glycosides (vicenin-2/isomer and Schaftosides) from *P. glandulosa* leaves which may act as chemomarker(s) for the standardization and quality control of this plant.

Key words: Flavonoid-C-glycosides, liquid chromatography-electron spray ionization-mass spectrometry/mass spectrometry, *Prosopis glandulosa*, schaftoside, vicenin-2

SUMMARY

- *Prosopis glandulosa* Torr. (Fabaceae) is native to Africa, America, and Asia
- Flavonoid C-glycosides such as vicenin-2, schaftoside/Isoschaftoside and quercetin glycosides were reported in *Prosopis* nigra and *Prosopis alba* pods and *Prosopis chilensis* mesocarp
- However, to the best of our knowledge, this is the first report on flavonoid C-glycosides (vicenin-2/isomer and Schaftosides) from *P. glandulosa* leaves.



Abbreviations used: PGA: Aqueous extract of Prosopis glandulosa leaves; PGM: Methanol fraction of PGA; PGAM: Aqueous-methanolic fraction of PGA; LC-ESI-MS/MS: Liquid chromatography-electron spray ionization-mass spectrometry/mass spectrometry

Correspondence:

Dr. U. V. Babu,
Phytochemistry Department, R and D Center, The Himalaya Drug Company, Makali, Tumkur Road, Bengaluru - 562 123, Karnataka, India.
E-mail: dr.babu@himalayawellness.com

DOI: 10.4103/pm.pm_505_17

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Prosopis glandulosa Torr. (Fabaceae), known as "Honey Mesquite," is native to Africa, America, and Asia. In India, it is abundantly found in southern parts. *Prosopis* spp. has been known by humans to cure various ailments. Leaf and bark aqueous extracts of *Prosopis* spp. have been recommended to cure bronchitis, mouth and throat infections, urinary diseases, and parasite and skin infections.[1-3]

Prosopis spp. reported to contain polyphenols, flavonoids, mesquitol-catechin dimers, indolizidine alkaloids, and triterpenes. Indolizidine alkaloids from *P. glandulosa* exhibited a broad spectrum of antimicrobial and anti-infective activities. Pollens of *P. juliflora* containing hydroxy cinnamic acids and flavone glycosides are a rich source of antioxidants responsible for high free radical scavenging activity.[4-7]

Flavonoid *C*-glycosides such as vicenin-2, schaftoside/isoschaftoside,[8-10] and quercetin glycosides majorly contribute in the phenolic compounds' composition of flour from *Prosopis nigra* and *Prosopis alba* pods and *Prosopis chilensis* mesocarp.[6,7] However, to the best of our knowledge,

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Patel NK, Sharath Kumar LM, Gajera J, Jadhav AN, Muguli G, Babu UV. Isolation and characterization of flavonoid C-glycosides from *Prosopis glandulosa* Torr. leaves. Phcog Mag 2018;14:451-4.

there is no report on flavonoid *C*-glycosides from *P. glandulosa*. Hence, the present study aimed at the isolation and characterization of flavonoid *C*-glycosides in *P. glandulosa* Torr. leaves.

MATERIALS AND METHODS

Plant materials

Leaves from *P. glandulosa* Torr. were collected from Nelamangala region, Bengaluru, Karnataka, India, in October 2016, and were authenticated by Dr. Kannan R (a botanist). A voucher specimen (PG-001) has been deposited at the Pharmacognosy Division, The Himalaya Drug Company, Bengaluru, Karnataka, India.

Solvents and chemicals

Solvents used were locally obtained from approved vendors and were of LR or AR grades. For LC-MS analysis, organic solvents were of LC-MS grade and were purchased from Thermo Fisher (Hanover Park, IL, USA). Reagent grade buffers and standards (>95% by high-performance liquid chromatography [HPLC]) were supplied by Sigma Aldrich (Bengaluru, Karnataka, India). Purified water was prepared using a Milli-Q purification system (Millipore, Billerica, MA, USA). All aqueous solvents were filtered through 0.45 µM syringe filter and degassed in an ultrasonic bath prior to use.

Extraction and isolation of flavonoid C-glycosides

The coarse powdered material (60 mesh) of *P. glandulosa* leaves (25 kg) was subjected to hot aqueous extraction at 90°C–100°C and this process was repeated thrice. Plant extracts were filtered through Whatman Filter paper No. 1, combined and concentrated to dryness on a rotary evaporator at 80°C–90°C to yield 5.129 kg crude aqueous extract of *P. glandulosa* (PGA).

For isolation of C-glycosides, 4.8 kg of PGA was sequentially washed with 12 L each of MeOH (3x) and MeOH:H_2O (4:1) (1x) to provide methanolic (PGM) (220 g) and aqueous-methanolic (PGAM) (210 g) fractions, respectively, of *P. glandulosa* leaves. 200 g of PGAM was loaded on a Diaion HP-20 column (Sigma Aldrich, Bengaluru, Karnataka, India) (57.5 cm × 3.25 cm) stepwisely eluted with 1 L each of 100% H_2O, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, and 100% MeOH. The eluates were evaporated under vacuum to afford H_2O (F1) (29 g), 20% MeOH (F2) (21 g), 40% MeOH (F3) (52 g), 60% MeOH (F4) (56 g), 80% MeOH (F5) (26 g), and 100% MeOH (F6) (15 g) fractions. A portion of 40% MeOH fraction (16 g) was purified through repetitive Shimadzu semi-preparative HPLC LC-8A system (Shimadzu, Kyoto, Japan). Purification was carried out in a gradient mode using Chemsil RP18 column (250 mm × 20 mm i. d., 5 µm, ChemIndia, India) at a flow rate of 7 mL/min; injection volume of 5 mL and sample concentration of 20 mg/mL. The mobile phase composed of HPLC-grade acetonitrile (ACN) (B) – 0.1% aqueous orthophosphoric acid (A) as follows: 0–20 min of 10%–20% of acetonitrile (binary), 20–50 min of 25% acetonitrile (linear), 50–55 min of 25%–10% acetonitrile (binary), and 55–70 min of 10% acetonitrile (linear). The effluent was monitored at 280 and 320 nm and the peak fractions were collected at R_t 25.12–28.10 and 29.54–31.30 for vicenin-2/isomer and schaftosides, respectively.

Liquid chromatography-mass spectrometry/mass spectrometry analysis

Sample preparation

Standard solutions (vicenin-2/schaftosides) (5 mg/5 mL) were prepared in LC-MS-grade MeOH. 500 mg of PGA was taken in a 50 mL volumetric flask and the volume was made up with MeOH as extracting solvent and sonicated for 30 min in a ultrasonic bath at 25°C. The extracted solution was filtered through a 0.45 µm-syringe filter. The filtered solution was directly analyzed for the presence of C-glycosides.

Conditions

Chromatographic conditions

Chromatographic system consisted of a binary gradient pump Shimadzu LC-20 AD and Shimadzu DGU-20A3 degasser. Separation was achieved through phenomenex kinetex phenyl-hexyl column (150 mm × 4.6 mm, 5 µm) and column temperature of 40°C was maintained through Shimadzu CTO-10 AS VP column oven. Peak elution was monitored at 320 nm using Shimadzu SPD-20A UV/VIS detector. The mobile phase composed of a buffer (0.1% formic acid in water) in pump-A and methanol (LC-MS grade) in pump-B delivered at a flow rate of 1 mL/min. The methanol was linearly ramped from 10%–35% in 10 min and then held at 35% for 5 min and decreased to 10% in the next 3 min followed by re-equilibration at 10% for an additional 2 min. The injection volume 10 µL was injected through Shimadzu SUL-HTC autosampler. The total run time for analysis was 20 min.

Mass spectrometric conditions

The API 2000 (Applied Biosystems/MDS SCIEX, Canada) mass spectrometer was coupled with electron spray ionization (ESI) source and a chromatographic system. Batch acquisition and data processing were controlled by Analyst 1.5 version software (SCIEX, Canada). Molecular ion response was checked in both positive and negative ionization modes. Good intense response was obtained in negative ionization mode. The final MS/MS optimized parameters values were as follows: declustering potential – 120 V, focusing potential – 300 V, entrance potential – 10 V, Curtain gas – 30 psi, source temperature – 420°C, source gas – 50 psi and 60 psi, and ion spray voltage – 4500 V. Collision energy of 25 V–45 V was set for each targeted parent ion to generate daughter ions.

Nuclear magnetic resonance analysis

The nuclear magnetic resonance (NMR) spectra of isolated compound/enriched fraction were recorded on a Bruker Avance 400 (Bruker, Rheinstetten, Germany) spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C in DMSO-d_6, respectively.

RESULTS AND DISCUSSION

The main constituents identified in the *P. glandulosa* leaves were *C*-glycosyl flavonoids based on the aglycone apigenin. Identification of compounds was carried out by interpretation using LC-ESI-MS/MS. Ultraviolet (UV) spectral data and mass fragmentation pattern were matched with those reported in the literature data. In mass spectrometry, the major diagnostic fragmentations involved the cleavage of two *C*–*C* bonds of the *C*-ring giving two structurally informative fragment ions that provide sufficient information on the number and type of substituents in *A*- and *B*-rings of flavonoids.[11-13] LC-MS/MS chromatogram of *P. glandulosa* leaves revealed the characteristic fragmentation pathway of *C*-glycosylated flavonoids [Figures 1-3]. It has been reported that UV spectra of the *C*-glycosylated flavonoids lie in the range of 324–338 nm and exhibit loss of 120 and 90 amu.[4]

In the LC-MS/MS chromatogram of *P. glandulosa* leaves, peaks at retention time of 9.69 and 10.37 displayed *m/z* 594 with major fragments at *m/z* 473 $[M-H-120]^-$ (loss of glucose unit) which led to an identification as "vicenin-2/isomer." Further, the MS data showed fragment ions at *m/z*: 383 (Aglycone + 113) and *m/z*: 353 (Aglycone + 83) confirming the di *C*-glycosyl flavone fragmentation pattern [Figure 2].[11-13]

Vicenin-2/isomer (>95% pure by HPLC): off white powder (0.234 g), 593 $[M-H]^-$, 473 $[M-H-120]^-$ ^1H-NMR (400 MHz, DMSO-d_6), δ ppm,

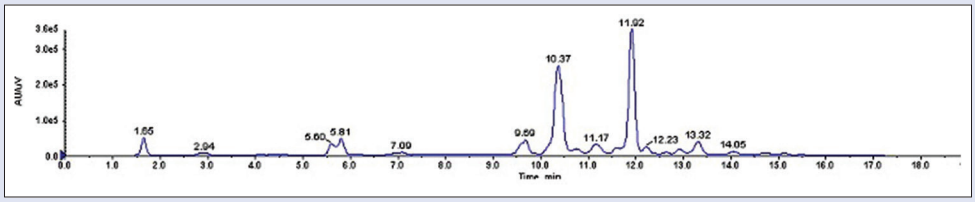


Figure 1: Liquid chromatography-ultraviolet chromatogram of aqueous extract of *P. glandulosa* at 320 nm

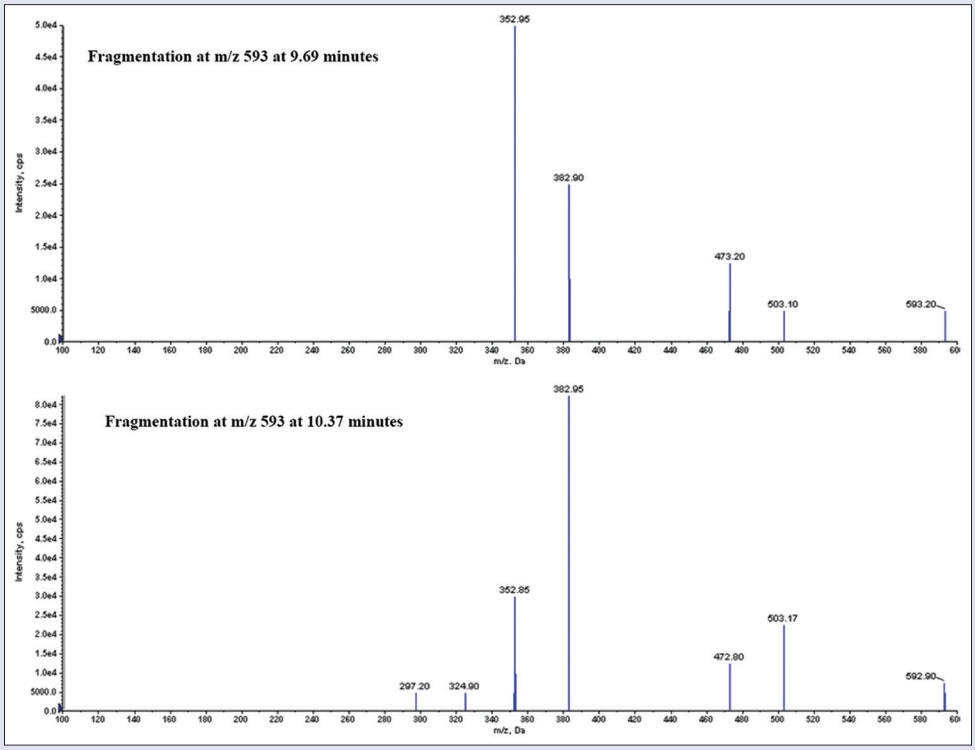


Figure 2: Extracted ion mass spectrometry/mass spectrometry spectra of Vicenin-2/isomer at 320 nm in negative ion mode. The compounds at R_t 9.69 and 10.37 with molecular ion peak [M-H]– at m/z 594 represent Vicenin-2/isomer

10.36 (s, 1H, 7-OH), 9.34 (4'-OH), 8.03 (d, *J* = 8.5 Hz, 2H, H-2′,6′), 6.91 (d, *J* = 8.5 Hz, 2H, H-3′,5′), 6.81 (s, 1H, H-3), 4.80 (d, *J* = 9.8 Hz, 1H, glucose anomeric-H, H-1"), 4.75 (d, *J* = 9.8 Hz, 1H, glucose anomeric-H, H-1'"), 3.50-3.86 (m, sugar-H). The MS and ^{1}H-NMR data were in agreement with the reported data.[10,14]

Surprisingly, five major peaks were also observed with retention times of 10.75, 11.17, and 11.92 (major); 12.92 and 13.32 corresponded to the same mass of *m/z* 564 with major fragments at *m/z* 473 $[M-H-90]^-$ (loss of arabinose unit) and 443 $[M-H-120]^-$ (loss of glucosyl unit) which were identified and together termed as "schaftosides."[11-13] Further, fragment signals observed at *m/z*: 473 $[(M- H) -90]^-$, *m/z*: 443 $[(M- H) -120]^-$, *m/z*: 383 $[(M- H) -120 - 60]^-$, and *m/z*: 353 $[(M- H) -120 - 90]^-$ are characteristics of glucosyl and pentosyl residues. Characteristic fragment ion signals at *m/z*: 383 (Aglycone + 113) and *m/z*: 353 (Aglycone + 83) are typical of di *C*-glycosylflavones as discussed earlier.[11-13] Interestingly, four schaftosides in isomeric pair were tentatively known in the literature as (i) schaftoside (Apigenin-6-*C*-*β*-D-glucopyranosyl-8-*C*-*α*-L-arabinopyranoside), (ii) isoschaftoside (Apigenin-6-*C*-*α*-L-arabinopyranosyl-8-*C*-*β*-D-glucopyranoside), (iii) neoschaftoside (Apigenin-6-*C*-*β*-D-glucopyranosyl-8-*C*-*β*-L-arabinopyranoside, and (iv) neoisoschaftoside (Apigenin-6-*C*-*β*-L-arabinopyranosyl-8-*C*-*β*-D-glucopyranoside) which differ only in configuration and sugar position and the "fifth" schaftoside remains unknown [Figure 3].[8,9,14]

Schaftosides (>92% pure by LC/MS-UV): amorphous yellow powder (1.830 g), 563 $[M-H]^-$, 443 $[M-H-120]^-$ ^{1}H-NMR (400 MHz, DMSO-d_6), *δ* ppm, 13.64 (s, 1H, 5-OH), 8.00 (d, *J* = 8.4 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 6.79 (s, 1H), 4.73 (d, *J* = 10.8 Hz, 1H, arabinose anomeric-H), 4.70 (d, *J* = 11.1 Hz, 1H, glucose anomeric-H), 4.00-3.00 (m, sugar-H). The MS and ^{1}H-NMR data were in agreement with the reported data.[10,14] These flavonoid *C*-glycosides, namely vicenin-2, schaftoside/isoschaftoside, and quercetin glycosides, majorly contributed in the phenolic compounds' composition of flour from *Prosopis alba* pods and *Prosopis chilensis* mesocarp and depicted antioxidant and protective effects on lipopolysaccharide-induced lipotoxicity in mice.[6,7] Flavonoid *C*-glycosides, namely vicenin-2 and schaftoside/isoschaftoside, are widely distributed in edible fruits and cereals. Vicenin-2 was found to be present in lemon fruit (*Citrus limon*), orange juice (*Citrus sinensis*), wheat (*Triticum aestivum*), and dates (*Phoenix dactylifera*).[15] The edible stems of rhubarb (*Rheum rhabarbarum*), sugarcane juice and bagasse (*Saccharum officinarum*), and wheat grain (*Triticum aestivum*) also reported to have schaftoside/isoschaftoside.[15] *P. glandulosa* is a rich source of indolizidine alkaloids,

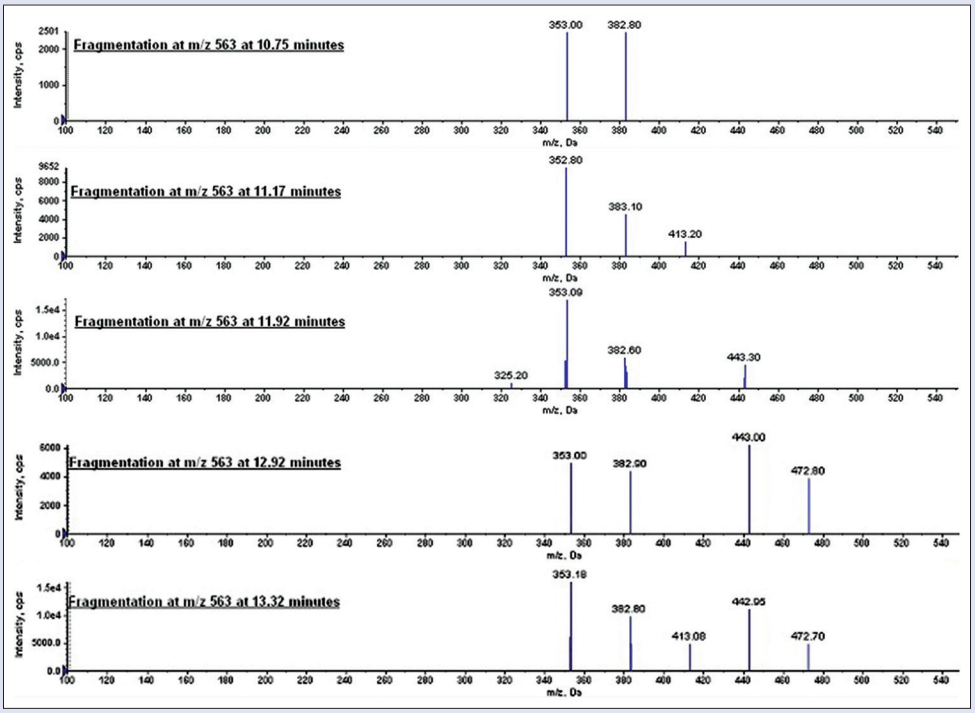


Figure 3: Extracted ion mass spectrometry/mass spectrometry spectra of schaftosides at 320 nm in negative ion mode. The compounds at R_t 10.75, 11.17, 11.92, 12.92, and 13.32 with molecular ion peak [M-H]– at m/z 564 represent schaftosides

triterpenes polyphenols, flavonoids, and mesquitol-catechin dimers that displayed various pharmacological activities.[4-7] However, there was no report on the presence of flavonoid *C*-glycosides in *P. glandulosa* leaves. Literature support that flavonoid *C*-glycosides are biologically active[6,7] and can be used as marker compounds for standardization in addition to be used for identification of *P. glandulosa* leaves.

CONCLUSION

Flavonoid *C*-glycosides such as vicenin-2/isomer, schaftoside/isoschaftoside, and quercetin glycosides are reported in *Prosopis alba* pods-derived products and allied species. However, to the best of our knowledge, so far, flavonoid *C*-glycosides from *P. glandulosa* leaves are not yet reported and this communication may be the first report. In general, this class of compounds are biologically active and can be used as marker compounds for standardization of extract in addition to the identification of this plant.

Acknowledgements

The authors are thankful to Dr. Rangesh Paramesh, CSO, R&D Centre and Management of The Himalaya Drug Company, Bengaluru, Karnataka, India, for his kind support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kirtikar K, Basu B, Blatter E, Kirtikar K, Basu B, Blatter E. Indian Medicinal Plants. 2nd ed. Allahabad, India: Lalit Mohan Basu; 1935.
2. Khejra CD. Khejra, Vanoshdi Chitravali (Jaributti). Hindi Seva Sadan; 2001. p. 269-70.
3. Pasiecznik NM. Prosopis: Pest or providence, weed or wonder tree? Eur Trop For Res Netw Newsl 1999;28:12-4.
4. Cattaneo F, Costamagna MS, Zampini IC, Sayago J, Alberto MR, Chamorro V, *et al*. Flour from *Prosopis alba* cotyledons: A natural source of nutrient and bioactive phytochemicals. Food Chem 2016;208:89-96.
5. Rahman AA, Samoylenko V, Jacob MR, Sahu R, Jain SK, Khan SI, *et al*. Antiparasitic and antimicrobial indolizidines from the leaves of *Prosopis glandulosa* var. glandulosa. Planta Med 2011;77:1639-43.
6. Pérez MJ, Cuello AS, Zampini IC, Ordóñez RM, Alberto MR, Quispe C, *et al*. Polyphenolic compounds and anthocyanin content of *Prosopis nigra* and *Prosopis alba* pods flour and their antioxidant and anti-inflammatory capacity. Food Res Int 2014;64:762-71.
7. Schmeda-Hirschmann G, Quispe C, Soriano Mdel P, Theoduloz C, Jiménez-Aspée F, Pérez MJ, *et al*. Chilean prosopis mesocarp flour: Phenolic profiling and antioxidant activity. Molecules 2015;20:7017-33.
8. Nikolov N, DellaMonic G, Chopin J. Di-C-glycosylflavones from *Crataegus monogyna*. Phytochemistry 1981;20:2780-1.
9. Stevenson PC, Kimmins FM, Grayer RJ, Raveendranath S. Schaftosides from rice phloem as feeding inhibitors and resistance factors to brown planthoppers, *Nilaparvata lugens*. Entomol Exp Appl 1996;80:246-9.
10. Xie C, Veitch NC, Houghton PJ, Simmonds MS. Flavone C-glycosides from *Viola yedoensis* MAKINO. Chem Pharm Bull (Tokyo) 2003;51:1204-7.
11. Benayad Z, Gómez-Cordovés C, Es-Safi NE. Characterization of flavonoid glycosides from fenugreek (*Trigonella foenum-graecum*) crude seeds by HPLC-DAD-ESI/MS analysis. Int J Mol Sci 2014;15:20668-85.
12. Colombo R, Yariwake JH, McCullagh M. Study of C- and O-glycosyl flavones in sugarcane extracts using liquid chromatography-exact mass measurement mass spectrometry. J Braz Chem Soc 2008;19:483-90.
13. Colombo R, Yariwake JH, Queiroz EF, Ndjoko K, Hostettmann K. On-line identification of further flavone C- and O-glycosides from sugarcane (*Saccharum officinarum* L. gramineae) by HPLC-UV-MS. Phytochem Anal 2006;17:337-43.
14. Osterdahl BG. Chemical studies on bryophytes, 22: Flavonoid C-glycosides of *Mnium undulatum*. Acta Chem Scand B 1979;33:400-4.
15. Courts FL, Williamson G. The occurrence, fate and biological activities of C-glycosyl flavonoids in the human diet. Crit Rev Food Sci Nutr 2015;55:1352-67.