Tropical Journal of Pharmaceutical Research December 2021; 20 (12): 2559-2565
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i12.15

Original Research Article

Isolation and identification of the chemical ingredients of Prosopis farcta leaf extract using ^{13}C and ^{1}H-NMR spectroscopy

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Sent for review: 10 December 2020 *Revised accepted: 27 October 2021*

Abstract

Purpose: *To determine the chemical constituents of the aqueous and alcohol extracts of Prosopis farcta leaves.*

Methods: *Air-dried leaves of P. farcta (500 g) were cleaned and extracted with distilled water five times for 6 weeks to obtain the aqueous extract (fraction I), and later with 75 % ethanol five times for 6 weeks to obtain the alcohol extract (fraction II). The constituents presented in the aqueous and alcohol extracts were separated by thin-layer chromatography using silica gel GF 254 and different solving systems. The total phenolic content of the extract was expressed in terms of Gallic acid in mg per gram of extract. Finally, the aqueous extract was subjected to column chromatography and the structures of different constituents were confirmed using ^{13}C, ^{1}H-NMR, and infrared (IR) spectroscopy.*

Results: *Chemical tests, thin-layer chromatography, and IR results showed that P. farcta extract contains gum, mucilage, tannins, proteins, amino acids, and nitrogenous compounds. The calibration curve ($y = 0.5516 + 0.0017$, $r^2 = 0.9983$) was constructed using serial dilutions of Gallic in distilled water; the total phenolic content was found to be 219 mg of Gallic acid per gram of extract. ^{13}C and ^{1}H-NMR showed that P. farcta leaf extract contains β-sitosterol and hesperidin.*

Conclusion: *P. farcta leaf extract contains two alkaloids and other nutritional components that may be beneficial for herbal medicine and nutrition purposes.*

Keywords: *Prosopis farcta, NMR, IR spectroscopy, β-Sitosterol, hesperidin*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Prosopis farcta (Shock) is a member of the Mimosacea family. It is a loose, straggling, thorny shrub that is 30–300 cm when branched [l], making it very conspicuous in cultivated lands. The genus *Prosopis* is commonly distributed in the lower forest zone, steppe region, and on the alluvial plain in the Arabian Peninsula region in Iraq, Jordan, Syria, Bahrin, and Tunisia [2-4].

Many plants of the genus *Prosopis* are known to have medicinal properties and are used by humans [5,6], including *P. juliflora* seeds and *P. farcta* pods and roots, which have anti-dermatophytic, antifungal, and antimicrobial activities [7-10]. *P. farcta* beans contain some sulfate materials, which can be used as skin remedies [11]. Some plants are used in folk medicine as astringents in rheumatism and as remedies against scorpion stings and snake bites [12,13], where their pods and seeds contain flavonoids and alkaloid Juliprosopine [5,6,14]. Omidi Arash, et al. mentioned that animals fed with *P. farcta* beans their demonstrate reductions in low-density lipoprotein (LDL) and increases in high-density lipoprotein (HDL) [15]; on the other hand, the roots of *P. farcta* are used in folk medicinal plants to treat diabetes mellitus [16-18].

Some studies have mentioned that *P. farcta* pods and roots contain volatile oils that can be used to treat swelling, and they also have anti-dysenteric and antispasmodic properties [17-19]. It is known that *P. farcta* pods, beans, and roots are used in folk medicine in the treatment of renal colic with no side effects [9]. Preliminary studies on *P. Africana Taub* have described its use in Africa as a remedy for toothache and other illnesses [10]. There are two alkaloids related to *Carpane* and *Cossino*, namely prosopine and prospinine, which have been isolated [20].

Mesquite gum was obtained from *P. juliflora* (family Mimosacea) in the southern United States and Mexico. The tears are smooth, light, and range from yellowish-brown to dark brown in color, and are more or less opaque. Mesquite forms a somewhat adhesive mucilage and can be used as an emulsifying agent [2]. Further, *P. rubriflora* and *P. ruscifolia* are considered nutritional sources for humans and animals [17,20]. Ansari *et al* found that their fruits contain protein, organic matter, and crude fat, and are considered a source of nutrients [14,20].

Nuclear magnetic resonance (NMR) is an analytical technique that is used in the qualitative and quantitative analysis of materials found in complex mixtures. NRM is considered an important analytical method given its high reproducibility, and given that it is a rapid analysis technique for the identification and authentication of active ingredients in plant extract [23].

The chemical ingredients present in *P. farcta* leaves have not yet been identified. Therefore, the aim of the present study is to predict the chemical constituents of *P. farcta* leaf extract, which may help inform future studies that aim to identify its clinical usefulness.

EXPERIMENTAL

Materials

Whole plants were collected from Mosul in the northern part of Iraq. The plants were subsequently identified by the National Herbarium of Iraq at Abu-Gharib. The leaves of *P. farcta* were separated and dried. Organic solvents (chloroform, n-hexane, ethanol, and methanol) and chemicals (acetic acid, silica gel GF-254, sodium carbonate, gallic acid, and iodine vapor) were of analytical grade and purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Lead acetate, ruthenium red solution, ninhydrin reagent, Dragendoff's and Mayer's reagent, and Folin–Ciocalteu reagent were also purchased from Sigma Aldrich Co. Shimadzu Fourier transform infrared (FTIR; 1700 Japan) and Bruker NMR 400 MHz were used to record IR and NMR spectra.

Preparation of aqueous and alcohol extracts

The air-dried leaves of *P. farcta* (500 g) were cleaned and extracted with 500 mL of distilled water a total of five times with occasional stirring at room temperature for 6 weeks. The combined extracts were then concentrated under vacuum to yield a viscous aqueous extract (15 g; fraction I). Then, the residue was extracted with 500 mL of 75% ethanol for a total of five times at room temperature for 6 weeks, and the combined extracts were concentrated under vacuum to yield a viscous alcoholic extract (10 g; fraction II) [21].

General procedure for thin-layer chromatography

Aqueous and alcohol extract were spotted on three silica gel GF 254 plates using micro pipettes and were kept in the solvent system. Three different solvent systems were used to separate the chemical constituents: chloroform, ethanol, acetic acid, and water (6 mL:3 mL:0.5 mL:1 mL); n-butanol, acetic acid, and water (5 mL:3 mL:2 mL); and 6% acetic acid. The developed TLC plates were visualized using an ultraviolet (UV) chamber at 254 nm and 366 nm. Two-dimensional chromatography was performed using silica gel GF for the separation of amino acids, proteins, and other nitrogenous compounds. First, these compounds were precipitated with lead acetate from the extract.

Then, the dried precipitate was dissolved and spotted on the TLC plate and was kept in the solvent system consisting of chloroform, ethanol, and water (5 mL:3 mL:2 mL); this process continued until the solvent system moved to 90% of the plate. Later, the TLC plate was dried at room temperature and maintained for developing in second direction. The same procedure was repeated using freshly spotted TLC plates and a solvent system consisting of acetone, water, and n-butanol (4 mL:2 mL). Three separate TLC plates were prepared and developed. The developed TLC plates were sprayed using Dragendoff's and Mayer's reagent, separately, for the identification of alkaloid compounds. The second plate was sprayed with Ruthenium red solution for the identification of gum and mucilage residues [12]. The third plate was sprayed with 0.25% ninhydrin reagent and heated on a hot plate for the identification of amino acids and proteins.

Procedure for column chromatography

The total combined extract (fraction II) was partitioned between n-hexane (3:1) and H_2O to yield the n-hexane-derived residue (8 g) and the aqueous-derived fraction (2 g).

The hexane fraction was subjected to a silica gel column (Φ=5, L=50 cm, 200 g). The column was eluted using a hexane–chloroform binary mixture (gradient elution) to yield four subfractions (H1–H4). Subfraction H3 (1.2 g) was subjected to repeated silica gel column chromatography (Φ=3, L=25 cm) using the solvent system hexane–ethyl acetate of different compositions to yield two compounds (9 mg).

The aqueous extract (2 g) was fractionated on Diaion HP-20 (Φ=5, L=50 cm, 15 g), employing gradient elution of methanol in water. Several fractions were combined into two subfractions based on the TLC results, (fraction A and fraction B; % MeOH in H2O; 0.9 and 0.7 g, respectively). Fraction A (30% MeOH/H_2O; 0.9 g) was further fractionated by silica gel (Φ=3 × 40 cm, 15 g), employing gradient elution from chloroform to ethyl acetate; this yielded four fractions (A_1–A_4). Fraction A_4 was fractionated on ODS (Φ =3, L= 25 cm), employing 30 % MeOH/H_2O as an eluent; this yielded five fractions ($A_{4.1}$–$A_{4.5}$). Fraction $A_{4.3}$ was further purified by repeated ODS (Φ=3, L=25 cm) using 20%–30% MeOH/H_2O as eluent solvent systems, which yielded compound 2 (6 mg) [22].

The total phenolic contents were measured using the Folin–Ciocalteu index method [23]. To prepare the stock solutions, different extracts were accurately and separately weighed and dissolved in a 10 mL volumetric flask consisting of methanol to get 1 mg/mL solutions. To stock solutions of extract (0.1 mL), Folin–Ciocalteu reagent (0.5 ml) and 6 mL of water were added, followed by 1.5 mL of 20% sodium carbonate. Finally, the volume was adjusted to 10 mL with water. The reaction mixture was kept at room temperature for 20 minutes to complete the reaction. Finally, absorbance was measured using a UV-Vis spectrophotometer at 760 nm.

The calibration curve was prepared following the above procedure using standard solutions of Gallic acid (0.5 mg/mL, 0.4 mg/mL, 0.3 mg/mL, 0.2 mg/mL, and 0.1 mg/mL with distilled water). The regression equation was computed from the calibration curve. The total amount of phenolic content in the extract was presented as the equivalent to milligrams of Gallic acid in 1 g of dry extract (mg GAE/g).

IR and NMR spectroscopy

A sufficient amount of dried plant extract was mixed with KBr powder. Discs were prepared and IR spectra were recorded to identify the tannic substances, gummy residues, and alkaloids.

Alcohol extract was suspended in water and then partitioned with n-hexane to yield n-hexane and aqueous-derived fractions [24]. The n-hexane and aqueous-derived fractions were subjected to different fractionation and purification steps using normal and reversed-phase silica gel and elution with different solvents featuring different compositions to yield two compounds. Both compounds were dissolved in $CDCl_3$ and the NMR spectra were recorded.

RESULTS

The aqueous and alcoholic extract were examined by TLC using chloroform, ethanol, acetic acid, and water (6 mL:3 mL:0.5 mL:1 mL) as the mobile phase. The chromatogram revealed the presence of four major spots together with the other two minor spots when visualized under UV light.

The aqueous extract (fraction I) was divided into four parts and analyzed by TLC. The results showed the presence of gum and mucilage components. The gummy substance was isolated by titration with ethyl alcohol; the gummy substance was the precipitated and dried under vacuum. TLC developed with

chloroform, ethanol, acetic acid, and water (6 mL:3 mL:0.5 mL:1 mL) showed the presence of one spot and no reaction with Ruthenium red solution. However, the pink color developed due to a complex formation between the polysaccharide moieties of the gum and the dye. The IR spectroscopy using the KBr disc was obtained and compared with that of gum arabic. The IR absorption bands at 3,700–3,500 cm OH stretching vibration and 1,000–300 cm c–o stretching vibration were the main characteristic bands of the gummy residue; these were identical to the characteristic bands of the gum arabic [13].

Part two was used to identify the presence of tannins. Chromatograms were developed using n-butanol, acetic acid, and water (5 mL:3 mL:2 mL) and 6% acetic acid, separately. The results revealed the presence of two spots in each solvent with Rf values of 0.68 and 0.46 with the n-butanol, acetic acid, and water solvent, and 0.8 and 0.57 with 6% acetic acid solution. These values were comparable with standard substances, tannic acid, and pyrogallol. The sample and standard gain were a bluish-green color with neutral ferric chloride solution and a sharp blue color when viewed under UV light. The spot with Rf 0.8 was found to be identical to that of tannic acid. The IR spectra of both the sample and standard tannic acid revealed the presence of the characteristic absorption bands at 3,500 cm OH stretching, 2,900 CH_2 stretching, and 1,710 cm^{-1} carbonyl group stretching.

The aqueous extract was further used to identify the presence of proteins, amino acid, and nitrogenous compounds. Those compounds were precipitated with lead acetate solution, and two-dimensional chromatography of the precipitate with chloroform, ethanol, and water (5 mL:3 mL:2 mL) revealed the presence of one major spot, which was detected under UV light.

An attempt was made to isolate this spot using a preparative TLC plate with acetone, water, and n-butanol (4 mL:2 mL:2 mL). IR spectroscopy of the isolated compound's residue showed an absorption band at 1,760 cm for C–NH amide stretching, which indicates the presence of a peptide band. Hydrolysis of these substances was carried out using 6N HCl acid for 2 days at 80°C. The hydrolysate extract yielded a purple to blue color with Ninhydrin reagent, which indicates the presence of certain amino acid residues.

In addition, the aqueous extract was used to identify the presence of alkaloids using Mayer's and Dragendoff's reagent tests. Mild turbidity was obtained with Mayer's reagent and orange turbidity with Dragendoff's reagent; these findings indicate the probability of the presence of alkaloids.

The alcohol extract was examined using NMR. The isolated compounds were identified by comparing their spectroscopic data with the reported data as β-Sitosterol (1) [25], as well as hesperidin (2) [26]. The isolated compounds are reported for the first time from the leaves of *P. farcta.*

Spectra data

NMR data of β-Sitosterol [1]: white powder; ^{13}C-NMR (Acetone-d6, 100 MHz) δC 37.33 (C-1), 32.54 (C-2), 71.73 (C-3), 43.11 (C-4), 142.39 (C-5), 121.55 (C-6), 32.65 (C-7), 32.82 (C-8), 51.25 (C-9), 36.94 (C-10), 21.40 (C-11), 40.69 (C-12), 43.36 (C-13), 56.97 (C-14), 24.95 (C-15), 28.95 (C-16), 57.69 (C-17), 12.23 (C-18), 19.23 (C-19), 38.26 (C-20), 19.35 (C-21), 34.74 (C-22), 26.84 (C-23), 46.76 (C-24), 29.23 (C-25), 20.08 (C-26), 19.80 (C-27), 23.78 (C-28), and 12.28 C-29); Figures 1 and 2.

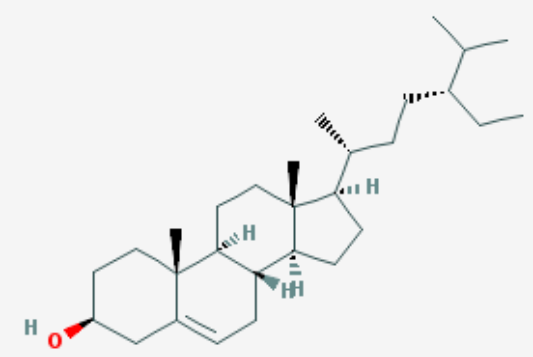

Figure 1: Structure of beta sitosterol

NMR data of Hesperidin [2]: gray powder; ^{13}C-NMR (DMSO-d6, 100 MHz) δC 78.33 (C-2), 42.00 (C-3), 197.00 (C-4), 162.99 (C-5), 96.32 (C-6), 165.09 (C-7), 95.49 (C-8), 162.45 (C-9), 103.26 (C-10), 130.85 (C-1`), 114.10 (C-2`), 146.41 (C-3`), 147.91 (C-4`), 111.97 (C-5`), 117.91 (C-6`), 55.63 (OCH3), 100.56 (C-1``), 72.93 (C-2``), 76.23 (C-3``), 69.55 (C-4``), 75.46 (C-5``), 65.98 (C-6``), 99.38 (C-1```), 70.46 (C-2```), 70.22 (C-3```), 72.01 (C-4```), 68.27 (C-5```), and 17.80 (C-6```); see Figure 3 and Figure 4.

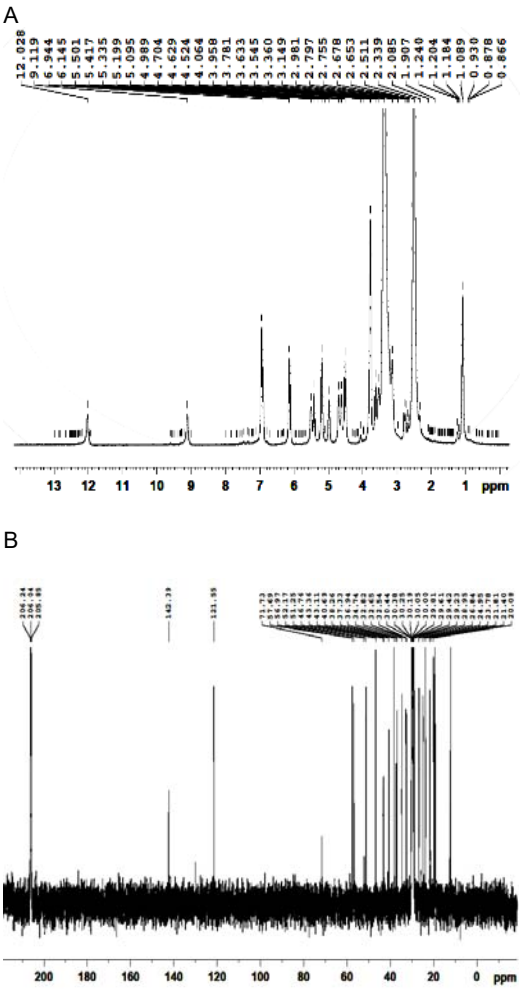

Figure 2: (A & B) ^{1}H and ^{13}C NMR spectra of beta sitosterol.

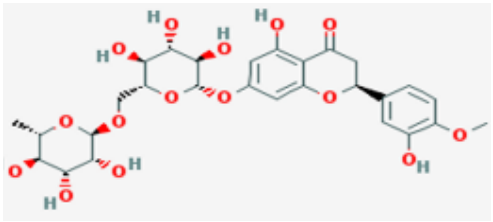

Figure 3: Hesperidin

The total phenolic content of the extract was also determined. The present study revealed that the phenolic content in the dry extract, in terms of its equivalent to milligrams of Gallic acid per dry gram of extract, is 219.24 mg GAE g^{-1}.

DISCUSSION

Different members of the Mimosacea family are used in herbal medicine to treat the signs and symptoms of some unpleasant medical problems. *P. farcta*, *P. juliflora*, and *P. Africana* have been used as antispasmodic and astringent agents in rheumatism; they also exhibit anti-dermatophytic, antifungal, and antimicrobial properties; reduce LDL levels; are used in the treatment of swelling and renal colic; and have anti-dysenteric and antispasmodic properties as well [15-19].

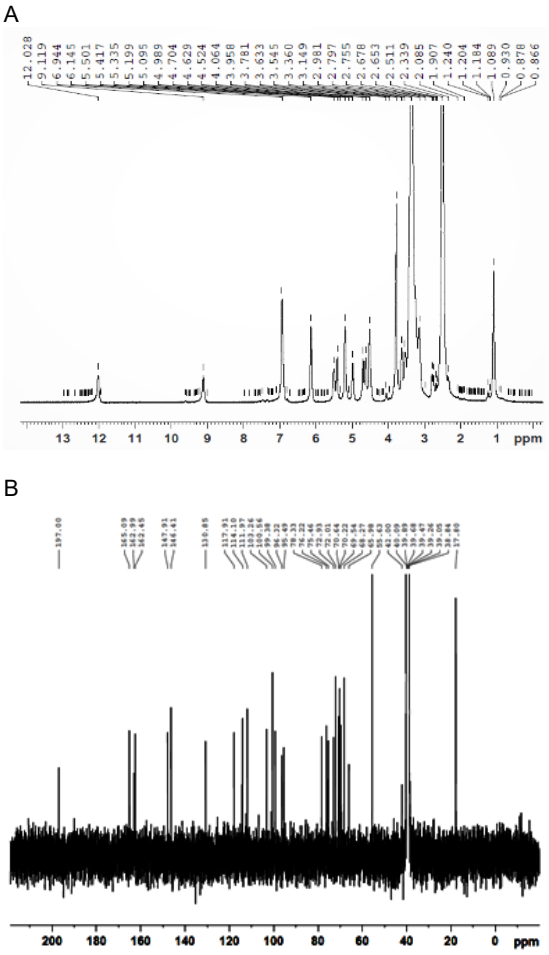

Figure 4: (A) ^{1}H and (B) ^{13}C NMR spectra of hesperidin

Several studies have determined the constituents of the roots, pods, and seeds of these plants [5,6,14], while other studies have determined the constituents in the whole plant. In this study, we tried to determine the constituents in the leaves of *P. farcta*. All of these studies revealed that most of the *Prosopis* species contained two alkaloids [17,20]. As was identified in the present study, the leaf extracts contained two alkaloids: sitosterol and hesperidin. These results provide a clear picture about the active ingredients in these plants, which are used by some to help resolve certain medical problems. Use of these plant extracts

for these purposes may be related to this constituent and its chemical or physical modes of action.

CONCLUSION

Two alkaloids (sitosterol and hesperidin) have been successfully isolated from the leaf extract of *P. farcta*. The structures of these alkaloids have been confirmed by spectroscopic studies. The leaf of *P. farcta* contains two alkaloids (sitosterol and hesperidin), and it also features nutrient materials such as gum, mucilage, proteins, and carbohydrates. However, further clinical studies are required to correlate the various medicinal applications of these plants to the plants' constituents.

DECLARATIONS

Acknowledgement

The authors are grateful to the Deanship of Scientific Research (Grant number 160149) King Faisal University-Kingdom of Saudi Arabia for their support and funding of this research. In addition, we would like to thank Mr. Mohammad Yousef for providing the *P. farcta* leaves used.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Sabah H. Akrawi and Kawkab Saoor designed the study and supervised the project. Sabah H. Akrawi conducted the literature search, experimental work, data analysis, and drafted the manuscript. Hany Ezzat Khalil helped carry out the experimental work. Mahesh Attimarad and Bandar Al-Dhubaib helped draft and review the final manuscript. All authors reviewed and approved the final manuscript for publication.

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