



Original Research

High-performance thin-layer chromatography fingerprinting, total phenolic and total flavonoid contents and anti-platelet-aggregation activities of *Prosopis farcta* extracts

Farzad Kobarfard^{1,2}, Seyed Abdulmajid Ayatollahi^{1,3*}, Nafiseh Khosravi-Dehaghi^{4,5}, Mehrdad Faizi⁶, Salimeh Amidi², Miquel Martorell^{7,8}, Muhammad Iqbal Choudhary⁹, Hafiz Ansar Rasul Suleria¹⁰, Javad Sharifi-Rad^{1*}

¹Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

²Department of Medicinal Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

³Department of Pharmacognosy and Biotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

⁴EvidenceBased Phytotherapy & Complementary Medicine Research Center, Alborz University of Medical Sciences, Karaj 19839-63113, Iran

⁵Department of Pharmacognosy, School of Pharmacy, Alborz University of Medical Sciences, Karaj 19839-63113, Iran

⁶Department of Pharmacology and Toxicology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

⁷Department of Nutrition and Dietetics, Faculty of Pharmacy, University of Concepcion, Chile

⁸Unidad de Desarrollo Tecnológico (UDT), Universidad de Concepción, Chile

⁹H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

¹⁰Department of Agriculture and Food Systems, The University of Melbourne, Melbourne 3010, Australia

*Correspondence to: majid_ayatollahi@yahoo.com; javad.sharifrad@gmail.com

Received April 8, 2020; Accepted May 3, 2020; Published June 25, 2020

Doi: <http://dx.doi.org/10.14715/cmb/2020.66.4.2>

Copyright: © 2020 by the C.M.B. Association. All rights reserved.

Abstract: Cardiovascular diseases are a leading cause of worldwide death and excessive platelet is closely related with their pathogenesis. Different plants and natural compounds have demonstrated anti-platelet effects. The aim of this study was to report the high-performance thin-layer chromatography (HPTLC) fingerprinting and anti-platelet-aggregation activities of different leaf extracts (*n*-hexane, chloroform, ethyl acetate, methanol and aqueous) of *Prosopis farcta* (Syrian mesquite) plant. The results showed a 100% inhibition of aggregation activity after plasmatic adenosine diphosphate (ADP) aggregation activation of ethyl acetate, ethanolic, methanolic and aqueous extracts, at 60 mg/mL concentration. The IC₅₀ ADP value of these extracts ranged between 4.07 and 11.39 mg/mL. Moreover, these extracts reported the highest amounts of phenolic and flavonoid contents. In conclusion, phytochemicals present in *P. farcta* leaves have anti-platelet-aggregation activities. Future studies are needed to identify the compounds with anti-platelet potential present in *P. farcta*.

Key words: Syrian mesquite; Antiplatelet; Cardiovascular disease; Phytochemical; HPTLC.

Introduction

Cardiovascular diseases (CVD) are a leading cause of worldwide death. Thrombosis provoked by increased platelet activity and triggering platelet aggregation is one of the CVD causes (1). Platelets halt blood loss after tissue plasma but excessive platelet aggregation also plays a crucial role in various CVD such as hypertension, arterial disease, heart failure, stroke and atherosclerosis (2, 3). Food intake is directly associated with CVD and epidemiological studies suggest that regular consumption of flavonoids is inversely associated with CVD risk (4, 5). Flavonoids have an important role to inhibit blood clot formation and in the reduction of risk factors of CVD (6, 7). This protective effect of flavonoids against CVD complications may be related to different mechanisms including anti-platelet properties.

Prosopis farcta (Banks & Sol.) J.F.Macbr. (Fabaceae or Leguminosae family), commonly known as the Syrian mesquite, is native northern Africa, and Asia (8) and is distributed from India to Iran (9, 10). In traditional medicine, *P. farcta* has been used for the treatment

of some diseases (11, 12) such as constipation and to reduce fever (13), treatment of inflammation, diabetes (14) and cardiac or chest pain (15). Moreover, *in-vitro* biological activities have been attributed to this genus such as antioxidant, antimicrobial and anticancer (11, 16, 17). Antioxidant and anticancer activities of *P. farcta* has been correlated with its high content in phenols and flavonoids (18). Harzallah-Skhiri and BenJannet (19) reported that *P. farcta* has a rich phenolic and flavonoid content such as rutin, myricetin and caffeic acid derivatives. *In-vivo* studies of *P. farcta* have reported antimicrobial effects (20), effects against cardiovascular disorder (10, 21-24) and neuroprotective effects (20).

Several food plants extract exhibit anti-platelet activity (6), however, no anti-platelet activities have been documented for *P. farcta* extracts. The present study aimed to determine anti-platelet-aggregation activities of this plant and report high-performance thin-layer chromatography fingerprinting. To the best of our knowledge, this is the first report of high-performance thin-layer chromatography (HPTLC) fingerprinting and anti-platelet-aggregation activities of different leaf ex-

tracts of *P. farcta*.

Materials and Methods

Preparation of *Prosopis farcta* extracts

Fresh leaves of *P. farcta* plants were collected from Tehran, Iran in July 2018 (Figure 1). A botanist from the Department of the Faculty of Pharmacy, Alborz University of Medical Sciences of Iran, identified taxonomically the plants. Leaves were washed three times with distilled water and then dried at 25 ± 5 °C for one week. The dried leaves were powdered by a mechanical grinder and then 400 g of powdered leaves was dissolved in 800 mL of 85% ethanol using a shaking water bath (90 rpm) at room temperature for 24 h. Then, filtering was performed (Whatman No. 1 filter paper) and this was concentrated with a rotary evaporator (Laborota 4000, Heidolph, Germany) at 40 °C for 30 min. This act performed three times to achieve maximum extraction with ethanol. The solid residue (i.e., the extract) was stored at 4 °C until further analysis.

Liquid-Liquid Extraction Procedure

The crude extract (100 g) was solved in 100 mL of ethanol and then was added 100 mL of distilled water and 500 mL of *n*-hexane. After separation of the *n*-hexane fraction, the remaining solution was extracted with 500 mL chloroform to give a chloroform fraction, and then with 500 mL of ethyl acetate and 500 mL of methanol. All five of the fractions, including the aqueous fraction, were concentrated using rotary evaporation (25). The fractions so obtained were *n*-hexane, chloroform, ethyl acetate, methanol and aqueous. Scheme used in fractionation of crude ethanol extract is summarized in Figure 2.

Plant fingerprinting with high-performance thin-layer chromatography (HPTLC)

HPTLC was conducted on Silica gel 60 F 254 HPTLC Plate 10*10 (Merck, Germany) as the stationary phase. A total of 20 mg each of the different extracts were spotted. For terpenoid compounds identification the mobile phase was toluene:ethyl acetate:acetone:methanol (50:25:25:5) and anisaldehyde-sulfuric acid reagent was used for derivatization. For flavonoid compounds identification the mobile phase was ethyl acetate:water:methanol:*n*-heptane (16:1:3:2) and derivatization reagent was natural product reagent. For antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) the mobile phase was ethyl acetate:water:methanol:*n*-heptane (16:1:3:2) and derivatization reagent was DPPH. Chromatography bands were observed under UV, fluorescence and visible lights.

Total phenolic compounds

The total phenolic contents (TPC) of the different extracts were determined by spectrophotometric method (UV-1800, Shimadzu, Japan) using Folin-Ciocalteu reagent following Nickavar, Alinaghi (26) protocol. Linear calibration curve (absorbance versus concentration) for 25, 50, 75, 100, 150, and 200 µg/mL rutin solution in ethanol was developed (Figure 3). The results were expressed as rutin equivalents (µg rutin equivalents/mg of the extract).



Figure 1. *Prosopis farcta* plant used for extract analyses in this study.

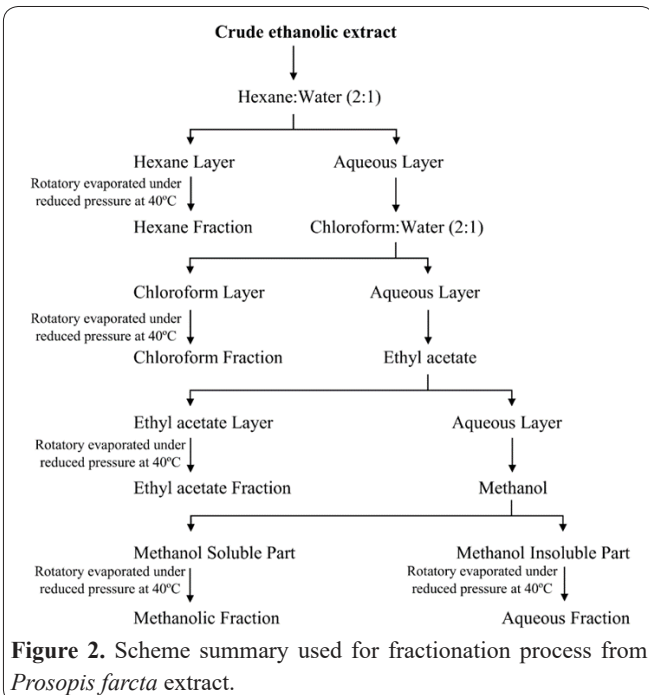


Figure 2. Scheme summary used for fractionation process from *Prosopis farcta* extract.

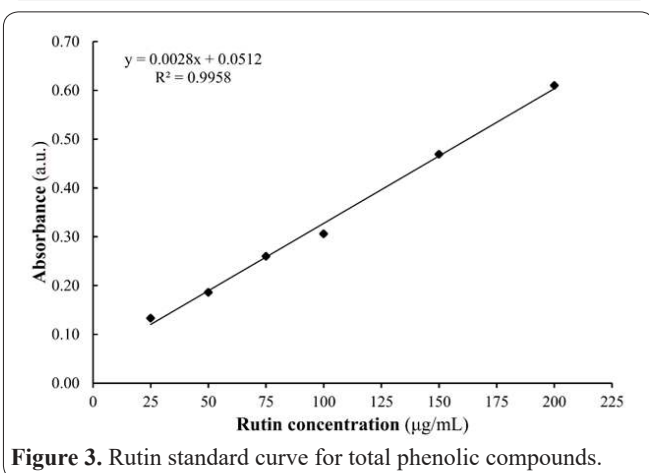


Figure 3. Rutin standard curve for total phenolic compounds.

Total flavonoids content

The total flavonoid contents (TFC) of the different extracts were determined by colorimetric method (UV-1800, Shimadzu, Japan) using $AlCl_3$ reagent following Nickavar, Kamalinejad (27) protocol. Linear calibration curve (absorbance versus concentration) for 25, 50, 75, 100, and 150 µg/mL rutin solution in ethanol was developed (Figure 4). The results were expressed as rutin

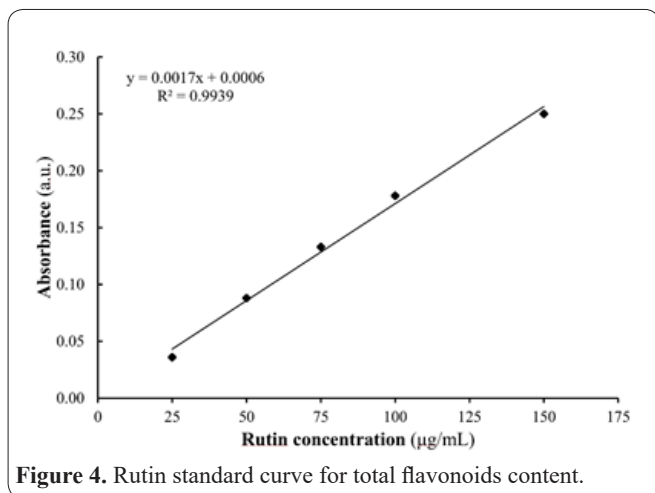


Figure 4. Rutin standard curve for total flavonoids content.

equivalents (mg rutin equivalents/mg of the extract).

Blood collection for platelet aggregation test

Blood to obtain plasma was collected from healthy volunteers in fasting conditions in Falcon tubes with sodium citrate. The participants not take any medication two weeks prior to blood collection. Platelet rich plasma (PRP) was obtained after blood centrifugation (100g for 10 min). After, the residual blood was centrifuged (1500 g for 15 min) to obtain platelet poor plasma. PRP was diluted with PPP to obtain a platelet count adjusted to $250 \pm 25 \times 10^9/L$ (counted under microscope).

Platelet aggregation test

The platelet aggregation test were performed following the Lorigooini, Ayatollahi (28) protocol. The sample extracts were dissolved in 1 mL of dimethyl sulfoxide (DMSO) at 60 mg/mL concentration and 1% of polyvinyl alcohol (PVA) (Sigma-Aldrich, Chemie GmbH, Germany), and the entire stock was filtered using Syringe Filter PVDF 0.22 µm. After one hour of the addition of PVA no particulate matter formed in the solution. Figure 5 shows that a blank mixture of DMSO and PVA in plasma did not affect the platelet aggregation process. Therefore, sample plant extract were prepared in DMSO/PVA 1%. One µL of this DMSO solution was added to 200 µL of plasma, the final extracts concentrations ranged to 0.3125 to 1.25 mg/mL.

Platelet aggregation activities of sample fractions were monitored with turbidimetric method using an optical aggregometer (APACT-4004, LABiTec, Germany). Aliquots of 200 µL of PRP were incubated in aggregometer chamber at 37°C after platelet aggregation activation with adenosine diphosphate (ADP) (Bio/Data, Corp (Germany) or arachidonic acid (AA) (Bio/Data, Corp, Germany) (29). Five min prior the platelet aggregation activation, 1 µL of sample prepared with DMSO and PVA was added to PRP. The platelet aggregation inhibitory activity of extract samples was compared with vehicle (PRP activated without sample extract) (30). The anti-aggregation value of each extract was expressed as either % inhibition or IC_{50} values. The IC_{50} values [inhibitory concentration (mg/mL)] were calculated from logarithmic regression curve (I% against sample concentration).

Statistical analysis

Statistical Package for Social Sciences (IBM SPSS

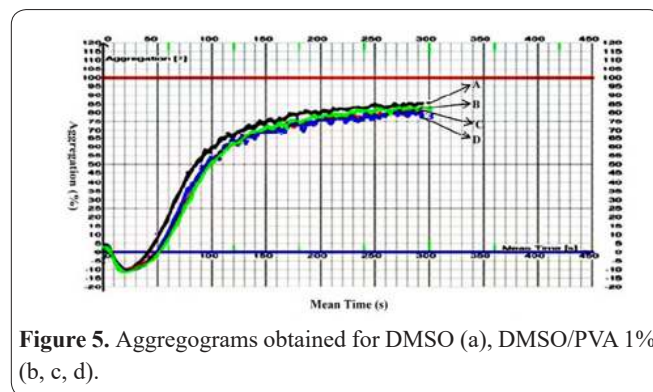


Figure 5. Aggregograms obtained for DMSO (a), DMSO/PVA 1% (b, c, d).

Statistics v.24 for Windows) was used to carry out statistics. Results were shown as mean \pm standard error of mean and $p < 0.05$ was considered statistically significant. All the experiments were carried out in triplicate. One-way analysis of variance (ANOVA) followed by Ducan's post test was used to assess the significant differences between the responses caused by the extracts.

Results

Plant fingerprinting with HPTLC showed the presence of terpenoid compounds in different extracts (Table 1). The highest presence of terpenoids was observed in the *n*-hexane extract while the lowest presence was observed in aqueous extract.

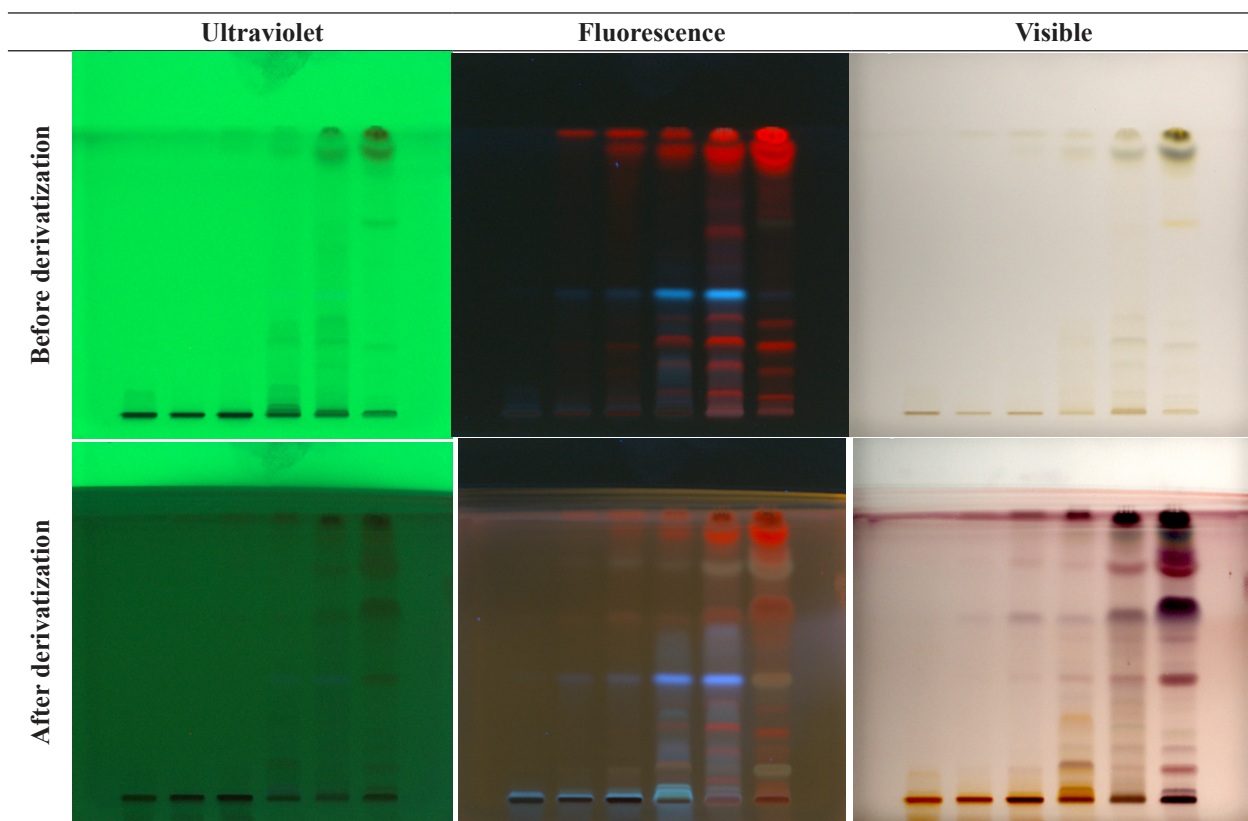
In Table 2 is showed the presence of flavonoid compounds in different *P. farcta* leaf extracts by HPTLC fingerprinting. Ethyl acetate extract showed the highest presence of flavonoid compounds.

Antioxidant screening test using HPTLC method is showed in Table 3. Ethyl acetate extract showed the highest antioxidant activity based on this method.

Total phenolic and flavonoid contents of the studied *P. farcta* leaf extracts are shown in Table 4. The highest amounts of phenolic and flavonoids compounds were present in the ethanolic extract. Ethanolic extract had the highest phenolic content (491.78 ± 2.51) followed by ethyl acetate (331.07 ± 1.63), methanolic (300.71 ± 3.84), aqueous (229.28 ± 1.76), chloroform (170.35 ± 3.24), and hexane (29.28 ± 2.31 µg rutin/mg extract) extracts. Ethanolic extract had the highest flavonoid content (210.88 ± 3.14) followed by ethyl acetate (187.35 ± 2.43), methanolic (109.41 ± 1.48), chloroform (77.05 ± 1.21), aqueous (31.47 ± 0.82), and hexane (15.29 ± 0.92 µg rutin/mg extract) extracts.

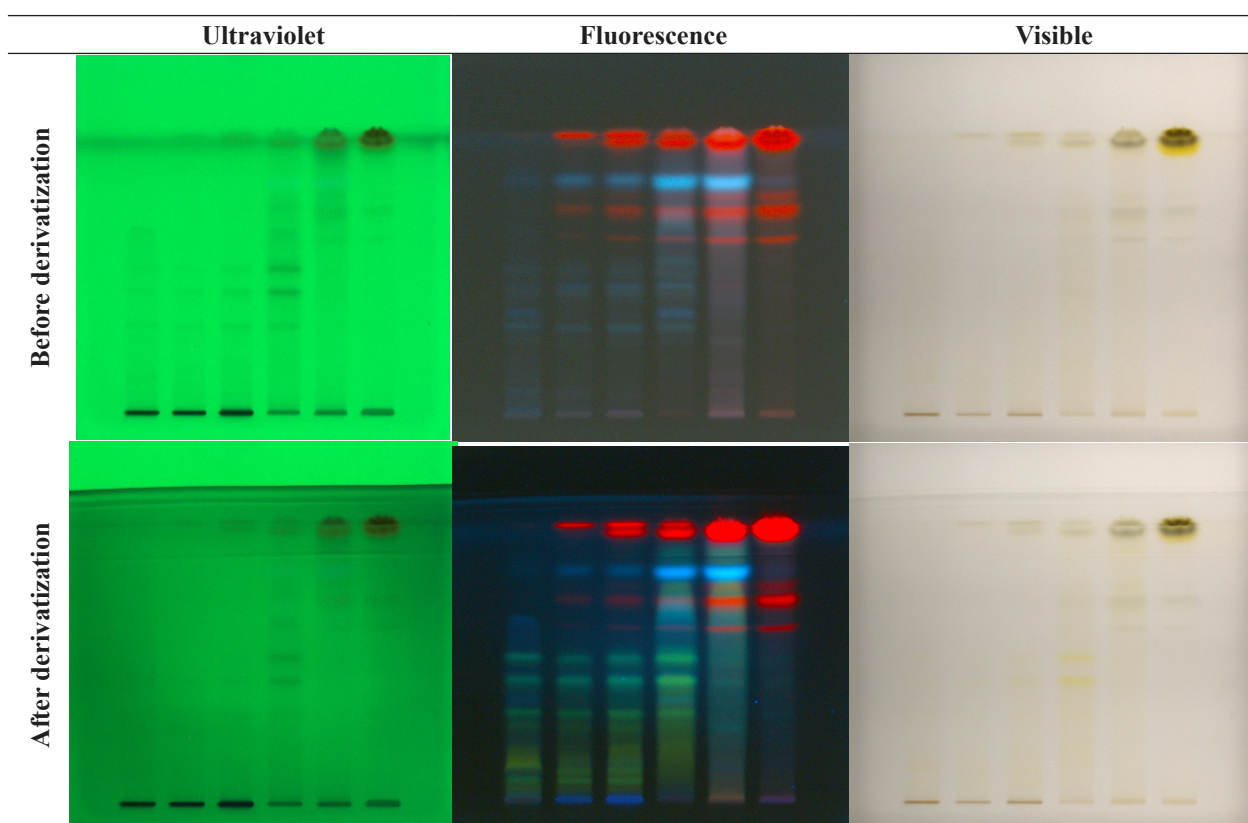
The aggregometry test results are shown in Table 5. The highest inhibition after AA aggregation activation was observed in the ethanolic extract (55.8 ± 1.5 %) followed by aqueous (51.4 ± 0.8 %), ethyl acetate (35.9 ± 0.7 %), hexane (32.6 ± 0.9 %), chloroform (31.9 ± 1.2 %), and methanolic (3.1 ± 0.4 %) extracts at 60 mg/mL concentration. After ADP aggregation activation, at the same concentration, ethyl acetate, ethanolic, methanolic and aqueous extracts showed an inhibition of 100%, chloroform and hexane extracts showed 36.0 ± 7.0 and 17.0 ± 4.0 % inhibition, respectively. IC_{50} for ADP activation was obtained for ethyl acetate (11.39 ± 0.27 mg/mL), ethanolic (5.94 ± 0.31 mg/mL), methanolic (4.07 ± 0.23 mg/mL), and aqueous (5.40 ± 0.12 mg/mL) extracts. The lowest IC_{50} value was obtained for methanolic extract and the highest value for ethyl acetate extract.

Table 1. Terpenoid compounds present in *Prosopis farcta* extracts detected by HPTLC.

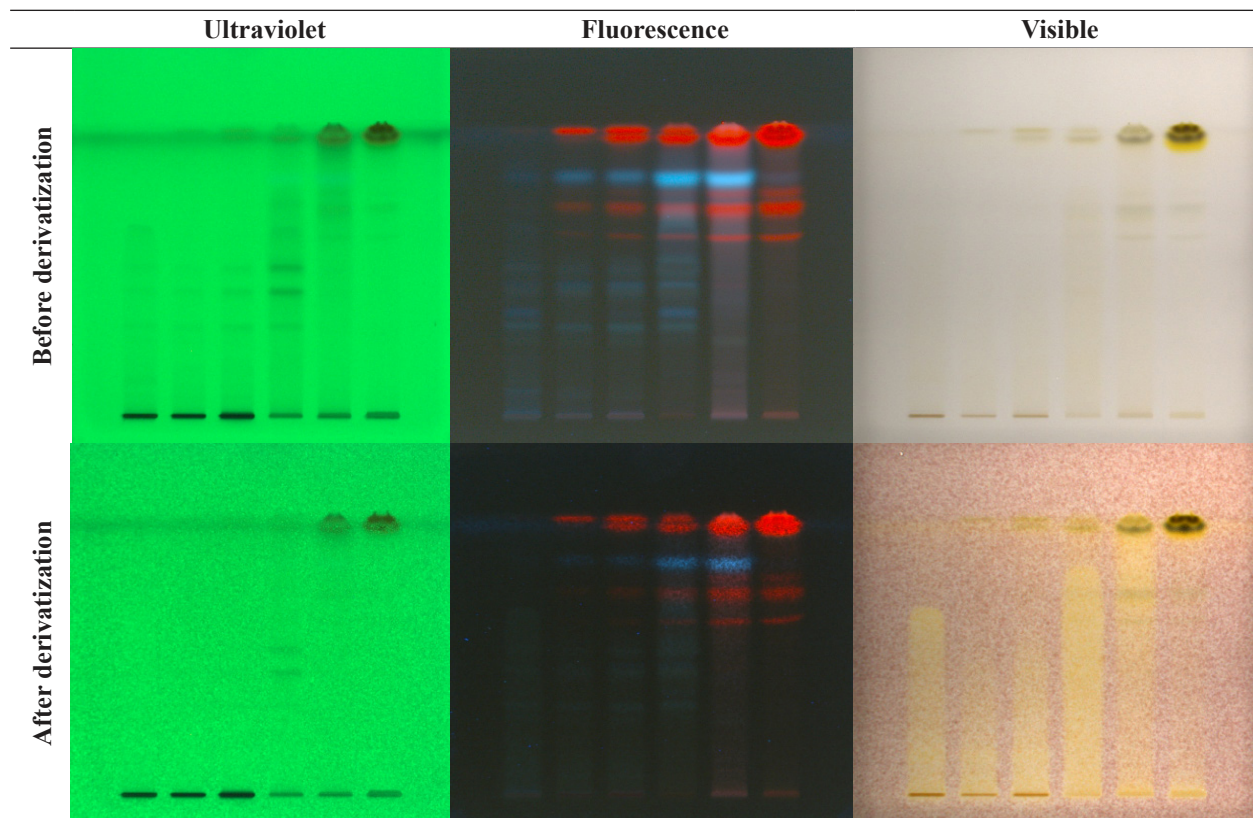


For each HPTLC plate the sample extract order (left to right) was aqueous, methanol, ethanol, ethyl acetate, chloroform, and hexane. Anisaldehyde-sulfuric acid reagent was used for terpenoid identification. Terpenoids appeared as purple-blue lines after spraying the plate with the reagent under the visible light.

Table 2. Flavonoid compounds present in *Prosopis farcta* extracts detected by HPTLC.



For each HPTLC plate the sample extract order (left to right) was aqueous, methanol, ethanol, ethyl acetate, chloroform, and hexane. Natural product reagent was sprayed on the HPTLC plate for flavonoid identification. Sharp blue lines in fluorescent light and light-yellow spots at the visible spectrum reveal the presence of flavonoids.

Table 3. Antioxidant activity identified in *Prosopis farcta* extracts detected by HPTLC.

For each HPTLC plate the sample extract order (left to right) was aqueous, methanol, ethanol, ethyl acetate, chloroform, and hexane. DPPH reagent (1,1-diphenyl-2-picrylhydrazyl) was used for antioxidant screening test. Antioxidant activity appeared as yellow spots on the purple background (visible).

Table 4. Total phenolic and flavonoid contents of the studied *Prosopis farcta* extracts.

Extracts	Phenolic content (μg rutin/mg extract)	Flavonoid content (μg rutin/mg extract)
<i>n</i> -Hexane	29.28 ± 2.31^a	15.29 ± 0.92^a
Chloroform	170.35 ± 3.24^b	77.05 ± 1.21^b
Ethyl acetate	331.07 ± 1.63^c	187.35 ± 2.43^c
Ethanol	491.78 ± 2.51^d	210.88 ± 3.14^d
Methanolic	300.71 ± 3.84^e	109.41 ± 1.48^e
Aqueous	229.28 ± 1.76^f	31.47 ± 0.82^f

Values are expressed as mean \pm standard error of mean of three measurements ($n = 3$). Statistical analysis: ANOVA one way followed by Duncan's post test was used to assess the significant differences ($p < 0.05$). For each determination (phenolic or flavonoid content), samples connected by the same symbol are not statistically different.

Table 5. Aggregometry test results of the studied *Prosopis farcta* extracts.

Extracts	% Inhibition AA (at 60 mg/mL)	% Inhibition ADP (at 60 mg/mL)	IC ₅₀ ADP (mg/mL)
<i>n</i> -Hexane	32.6 ± 0.9^a	17.0 ± 4.0^a	-
Chloroform	31.9 ± 1.2^a	36.0 ± 7.0^b	-
Ethyl acetate	35.9 ± 0.7^b	100.0 ± 0.0^c	11.39 ± 0.27^a
Ethanol	55.8 ± 1.5^c	100.0 ± 0.0^c	5.94 ± 0.31^b
Methanolic	3.1 ± 0.4^d	100.0 ± 0.0^c	4.07 ± 0.23^c
Aqueous	51.4 ± 0.8^e	100.0 ± 0.0^c	5.40 ± 0.12^b

AA: arachidonic acid, ADP: adenosine diphosphate. Values are expressed as mean \pm standard error of mean of three measurements ($n = 3$). Statistical analysis: ANOVA one way followed by Duncan's post test was used to assess the significant differences ($p < 0.05$). For each determination, samples connected by the same symbol are not statistically different.

Discussion

Anti-platelet aggregation activity is widely reported in plant extracts. In this study we reported for the first time the anti-platelet aggregation activities of different extracts of *P. farcta* leaves, the phenolic and flavonoid content and the presence of terpenoids and flavonoids

on them and their antioxidant activity by HPTLC method. The ethyl acetate, ethanolic, methanolic and aqueous extracts, obtained by a liquid-liquid extraction procedure, reported a 100% of inhibition of aggregation activity after plasmatic ADP aggregation activation at 60 mg/mL extract concentration. For these extracts, the IC₅₀ ADP value ranged between 4.07 and 11.39 mg/mL.

Moreover, these extracts reported the highest amounts of phenolic and flavonoid contents. Phytochemicals concentrations are closely related with the anti-platelet aggregation activities reported in extracts (31, 32).

In a previous study we reported the phytochemical screening of *P. farcta* leaf methanolic extract, reporting the presence of saponins, glycosides, alkaloids, tannins and flavonoids (17). In another study, Harzallah-Skhiri and BenJannet (19) reported the presence of tannins, galloyl tannin, vicenin-2, luteolin *C*-glycoside, apigenin *C*-glycoside, *iso*-orientin, myricetin 3-*O*-glucoside, vitexin, caffeic acid derivative, isovitexin, rutin, kaempferol 3-*O*-glucoside, isorahmnetin 3-*O*-glucoside, chrysoeriol 7-*O*-glucoside, kaempferol 3-*O*-rutinoside and isorhamnetin 3-*O*-rutinoside

in *P. farcta* leaves by high performance liquid chromatography (HPLC) method. In our study, we found the highest amounts of phenolic and flavonoid content in ethanolic extracts. Faggio, Sureda (33) reported that the inhibitory activity of phenolic compounds greatly depends on the phenolic class and is mainly due their anti-inflammatory and antioxidant capacities.

The aqueous extract of *P. farcta* leaves reported an IC₅₀ ADP value of 5.40 ± 0.12 mg/mL and the presence of phenolic and flavonoid compounds (229.28 ± 1.76 and 31.47 ± 0.82 µg rutin/mg extract, respectively). In rats, parsley aqueous extract of leaves significantly inhibited platelet aggregation activity induced by ADP (IC₅₀ 5.6 mg/mL) (34). Another example is the case of *Allium* species in which the inhibitory platelet aggregation activity has been widely reported (28, 35). Hirsch, Viecili (36) reported several plants with anti-platelet activity, which include saffron (*Crocus sativus* L.), garlic (*Allium sativum* L.), green tea (*Camelia sinensis* (L.) Kuntze), St. John's wort (*Hypericum perforatum* L.), ginger (*Zingiber officinale* Roscoe), *Ginkgo biloba* L., ginseng (*Panax ginseng* C.A.Mey.), and guaviroba (*Campomanesia guaviroba* (DC.) Kiaersk.). In their review they report that phytochemical components such as flavonoids, curcumins, catechins, terpenoids, polyphenols, and saponins present in the plants reported are directly related to anti-platelet activity. Beneficial effects of flavonoids on CVD have been extensively studied and effects on lipid metabolism, capability to reduce cell adhesion and anti-platelet effects have been attributed to them (33). Some mechanisms of platelet aggregation inhibition of flavonoids include the AA pathway inhibition, cytoplasmic Ca²⁺ increase suppression, degranulation blockage, integrin αIIbβ₃-mediated signaling inhibition, platelet granule secretion inhibition and thromboxane formation inhibition (33).

In conclusion, phytochemicals present in *P. farcta* leaves have anti-platelet-aggregation activities. The extracts with highest anti-platelet-aggregation activities also reported the highest phenolic and flavonoid contents. Future studies are needed to identify the individual compounds with anti-platelet potential present in *P. farcta*.

Acknowledgments

We are grateful to Prof. William N. Setzer, Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL, 35899, USA for critically reading the manuscript.

References

- Sobral Filho DC, Monteiro Júnior JGD. High Residual Platelet Activity in Response to Acetylsalicylic Acid in Acute Coronary Syndrome: A New Challenge for Antiplatelet Treatment? *Arq Bras Cardiol.* 2019;113(3):364-6.
- Fuentes E, Gibbins JM, Holbrook LM, Palomo I. NADPH oxidase₂ (NOX₂): A key target of oxidative stress-mediated platelet activation and thrombosis. *Trends in cardiovascular medicine.* 2018;28(7):429-34.
- Siddiqui TI, Kumar KSA, Dikshit DK. Platelets and atherothrombosis: causes, targets and treatments for thrombosis. *Curr Med Chem.* 2013;20(22):2779-97.
- Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. *Am J Clin Nutr.* 2005;81(1 Suppl):317S-25S.
- Mennen LI, Sapinho D, de Bree A, Arnault N, Bertrais S, Galan P, et al. Consumption of foods rich in flavonoids is related to a decreased cardiovascular risk in apparently healthy French women. *J Nutr.* 2004;134(4):923-6.
- Lutz M, Fuentes E, Ávila F, Alarcón M, Palomo I. Roles of Phenolic Compounds in the Reduction of Risk Factors of Cardiovascular Diseases. *Molecules.* 2019;24(2):366.
- Khan H, Jawad M, Kamal MA, Baldi A, Xiao J, Nabavi SM, et al. Evidence and prospective of plant derived flavonoids as antiplatelet agents: Strong candidates to be drugs of future. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association.* 2018;119:355-67.
- Pasiecznik N, Harris PJ, Smith SJ. Identifying tropical *Prosopis* species: a field guide: UK: HDRA Publishing Coventry; 2004. 1-29 p.
- Nahal-Tahmasbi M. Ecological study of American (Pukestanian) Kahoor and effectiveness for compost production in Hormozgan province. *Agric Econ Develop.* 2000;31:305-23.
- Asadollahi K, Abassi N, Afshar N, Alipour M, Asadollahi P. Investigation of the effects of *Prosopis farcta* plant extract on rat's aorta. *J Med Plants Res.* 2010;4(2):142-7.
- Sharifi-Rad J, Kobarfard F, Ata A, Ayatollahi SA, Khosravi-Dehaghi N, Jugran AK, et al. *Prosopis* Plant Chemical Composition and Pharmacological Attributes: Targeting Clinical Studies from Preclinical Evidence. *Biomolecules.* 2019;9(12):777.
- Salari S, Esmailzadeh Bahabadi S, Samzadeh-Kermani A, Yosefzai F. In-vitro Evaluation of Antioxidant and Antibacterial Potential of Green Synthesized Silver Nanoparticles Using *Prosopis farcta* Fruit Extract. *Iran J Pharm Res.* 2019;18(1):430-55.
- Khajoei Nasab F, Esmailpour M. Ethno-medicinal survey on weed plants in agro-ecosystems: a case study in Jahrom, Iran. *Environment, Development and Sustainability.* 2019;21(5):2145-64.
- Henciya S, Seturaman P, James AR, Tsai YH, Nikam R, Wu YC, et al. Biopharmaceutical potentials of *Prosopis* spp. (Mimosaceae, Leguminosa). *Journal of food and drug analysis.* 2017;25(1):187-96.
- Afifi FU. Hypoglycemic Effects of *Prosopis fructa*. *International Journal of Pharmacognosy.* 1993;31(2):161-4.
- Saad A, Ghareeb M, Abdel-Aziz M, Madkour H, Khalaf O, El-Ziaty A, et al. Chemical constituents and biological activities of different solvent extracts of *Prosopis farcta* growing in Egypt. *J Pharmacogn Phytother.* 2017;9:67-76.
- Sharifi-Rad J, Hoseini-Alfatemi S, Sharifi-Rad M, Miri A, Sharifi-Rad M. Phytochemical screening and antibacterial activity of *Prosopis farcta* different parts extracts against methicillin-resistant *Staphylococcus aureus* (MRSA). *Minerva Biotechnologica.* 2014;26(4):287-93.
- Jahromi MAF, Etemadfarid H, Zebarjad Z. Antimicrobial and Antioxidant Characteristics of Volatile Components and Ethanolic Fruit Extract of *Prosopis farcta* (Bank & Soland.). *Trends in*

- Pharmaceutical Sciences. 2018;4(3):177-86.
- 19.Harzallah-Skhiri F, BenJannet H. Flavonoids diversification in organs of two *Prosopis farcta* (banks & sol.) eig.(leguminosea, mimosoideae) populations occurring in the northeast and the southeast of Tunisia. *J Appl Sci Res.* 2005;1(2):130-36.
- 20.Mollashahi M, Tehranipour M, Khayyatzade J, Moosavi B. The neuroprotective effects of *Prosopis farcta* pod aqueous and ethanol extracts on spinal cord α -motoneurons neuronal density after sciatic nerve injury in rats. *Life Sci J.* 2013;10:293-7.
- 21.Saidi MR, Farzaei MH, Miraghaee S, Babaei A, Mohammadi B, Bahrami MT, et al. Antihyperlipidemic Effect of Syrian Mesquite (*Prosopis farcta*) Root in High Cholesterol Diet-Fed Rabbits. *Journal of evidence-based complementary & alternative medicine.* 2016;21(4):Np62-6.
- 22.Keshavarzi S, Bahrami G, Mohammadi B, Hatami R, Miraghaee S. Syrian Mesquite extract improves serum lipids and liver tissue in NFALD modelled rabbits. *J Fam Med.* 2018;16:150-7.
- 23.Asadollahi A, Sarir H, Omid A, Torbati MBM. Hepatoprotective Potential of *Prosopis farcta* Beans Extracts against Acetaminophen-induced Hepatotoxicity in Wister Rats. *Int J Prev Med.* 2014;5(10):1281-5.
- 24.Omid A, Ansari nik H, Ghazaghi M. *Prosopis farcta* beans increase HDL cholesterol and decrease LDL cholesterol in ostriches (*Struthio camelus*). *Tropical animal health and production.* 2013;45(2):431-4.
- 25.Bibi Y, Qayyum A, Nisa S, Waheed A, Chaudhary MF. Isolation Studies From Stem Extract Of *Pistacia Integerrima* Stew. Ex Brand. *Journal of the Chilean Chemical Society.* 2016;61:2916-20.
- 26.Nickavar B, Alinaghi A, Kamalinejad M. Evaluation of the Antioxidant Properties of Five *Mentha* Species. *Iranian Journal Of Pharmaceutical Research.* 2008;7(3):203-9.
- 27.Nickavar B, Kamalinejad M, Izadpanah H. In vitro free radical scavenging activity of five *Salvia* species. *Pakistan journal of pharmaceutical sciences.* 2007;20(4):291-4.
- 28.Lorigooini Z, Ayatollahi SA, Amidi S, Kobarfard F. Evaluation of Anti-Platelet Aggregation Effect of Some *Allium* Species. *Iran J Pharm Res.* 2015;14(4):1225-31.
- 29.Born GV, Cross MJ. The aggregation of blood platelets. *The Journal of physiology.* 1963;168:178-95.
- 30.Amidi S, Kobarfard F, Bayandori Moghaddam A, Tabib K, Soleymani Z. Electrochemical synthesis of novel 1,3-indandione derivatives and evaluation of their antiplatelet aggregation activities. *Iran J Pharm Res.* 2013;12(Suppl):91-103.
- 31.Hirsch GE, Viccili PRN, de Almeida AS, Nascimento S, Porto FG, Otero J, et al. Natural Products with Antiplatelet Action. *Curr Pharm Des.* 2017;23(8):1228-46.
- 32.Zhang P-Y. Cardioprotection by Phytochemicals via Antiplatelet Effects and Metabolism Modulations. *Cell Biochem Biophys.* 2015;73(2):369-79.
- 33.Faggio C, Sureda A, Morabito S, Sanches-Silva A, Mocan A, Nabavi SF, et al. Flavonoids and platelet aggregation: A brief review. *European Journal of Pharmacology.* 2017;807:91-101.
- 34.Gadi D, Bnouham M, Aziz M, Ziyat A, Legssyer A, Legrand C, et al. Parsley extract inhibits in vitro and ex vivo platelet aggregation and prolongs bleeding time in rats. *Journal of ethnopharmacology.* 2009;125(1):170-4.
- 35.Hiyasat B, Sabha D, Grotzinger K, Kempfert J, Rauwald JW, Mohr FW, et al. Antiplatelet activity of *Allium ursinum* and *Allium sativum*. *Pharmacology.* 2009;83(4):197-204.
- 36.Hirsch GE, Viccili PRN, de Almeida AS, Nascimento S, Porto FG, Otero J, et al. Natural Products with Antiplatelet Action. *Current pharmaceutical design.* 2017;23(8):1228-46.