Original Article

Antioxidant And Wound Healing Properties Of Prosopis Farcta And Adiantum Capillus Plant Extracts: An In Vitro Study

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DOI: 10.47750/pnr.2023.14.03.480

Abstract

Background: Plant extracts have been used as therapeutic agents and have become important for human health worldwide for a long time. The present study aimed to investigate and compare the effect of Adiantum capillus, and Prosopis farcta plant extracts on an in-vitro wound healing.

Methods: Ethanol extracts of both plants have been prepared and used in the present investigation to study the immunomodulatory role of human peripheral blood mononuclear cells (PBMC). Total phenolic content (TPC) of both extracts was measured by using the aluminum chloride colorimetric method. In vitro antioxidant actives were determined by the DPPH, ABTS, and FRAP free radical scavenging activities were also measured.

Results: Both extracts of Prosopis farcta and Adiantum capillus showed abilities to scavenge DPPH and ABTS free radicals. The Results were showed that the A. capillus extracts had higher antioxidant potential and polyphenol contents than P. farcta extracts.

Conclusion: The whole plant extract of Adiantum capillus-veneris is capable for free radical scavenging molecules and it can be used as a potential source of natural antioxidants for wound healing treatments.

Keywords: Adiantum capillus, Prosopis farcta, antioxidant activity and wound healing.

1. Introduction

The wound is defined as a disruption of the anatomy, physiology, and structure of the skin due to mechanical, physical, chemical, and microbial agents resulting in the breakdown of tissue surfaces or basement membranes(1). Natural products have been confirmed to enhance the wound healing process and their primary or secondary constituents have been shown to contribute potential repair mechanisms including; activation of epithelial cells, growth factors, cytokines, extracellular matrix (ECM), reactive oxygen species (ROS), and several inflammatory mediators(2). Plant-derived products nowadays are one of the basic resources in most pharmacological treatments, which play an important role in the prevention and treatment of various human diseases(3). Recently, it has been an excessive challenge for the researchers to develop new products from plant and microorganisms as biological sources of products with potential benefits(4). Natural antioxidants have been of great interest owing to their ability to scavenge free radicals. Free radicals have been associated with the development of a series of diseases, including cancer, neurodegeneration, and inflammation(5). Many pharmacological and immunological studies were done on wound healing but many problems have remained in hospitals and clinics with prevention or treatment of open wounds like burns or skin injuries after radiation therapy, diabetic ulcers, and bedsores(6). Wound healing is a complex cellular and immunological process resulting in the restoration of anatomical and functional injured tissue. It includes several cellular and biochemical overlapping phases including hemostasis, inflammation, proliferation, and tissue remodeling(6). Medicinal Plants are preferred for wound healing treatment

in many countries due to their availability and low toxicity(7). The genus Prosopis belongs to the family Leguminosae and has about 50 species found in southwestern Asia, west of the Middle East, Turkey, Iran, northern Africa, and the USA. Prosopis farcta contains many therapeutics, such as glucoside, naringenin, caffeic, resveratrol, quercetin, myricetin, daidzein, luteolin, kaempferol, ferulic, and salicylic acids. It is used as a therapy for many illnesses such as wound healing, and prostate disease, and for its antibacterial, antioxidant, antidiabetic, antihyperlipidemic, cardioprotective, anticancer, hepatoprotective, menstruation, fertility, and pain relief properties. Also, it is shown that P. farcta has an antidiabetic potential for local consumers in Turkey(8). While Adiantum capillus belongs to (Family: Adiantaceae) which is one of the most common species due to it is medically importance for clinical and nutrition purposes. It is world-widely distributed in moist and shady places. In general, Adiantaceae occurs in the mountainous region. In Europe, it was found on the Atlantic coast as far as Ireland (9, 10). Adiantum capillus-veneris is a plant with wide range of chemical constituents which exerted many pharmacological effects. It contained flavonoids, phenylpropanoids, carbohydrates, carotenoids, triterpenoids, aoleananes, alicyclic, and many other chemicals. It has antimicrobial, antioxidant, anti-inflammatory, anticholesterolemic, analgesic, hypoglycemic, neuroprotective, antilithiatic, antiproliferative, antidermatitic, and many other effects (10).

This study aims to investigate and compare the effect of Adiantum capillus, and Prosopis farcta plant extracts on an in-vitro wound healing through standard in-vitro assays on angiogenesis, fibroblast proliferation assay, and in-vitro assay for protection against damage by free reactive radicals to fibroblasts.

2. Materials and Methods

2.1. Plant Extraction Technique

To prepare the ethanol extract of both plants, 100 g fine powder was soaked in 1000 mL of 95% ethanol for 3 days. The mixture was then filtered by using filter paper and extracted under compact pressure in a rotating evaporator (Buchi, Switzerland). while the aqueous extract of both plants was prepared by soaking 100 g of fine powder in 2000 mL distilled water and then for 4 h the mixture was Shaken in a water bath. Latterly, the filtered mixture was stored in a freezer to make a small ice block then the plant extract was carried by using a freeze-drying machine (Labconco, Kansas, USA). All extracts were kept at −20 ◦C until the tests were performed.

2.2. Ferric Reducing Antioxidant Power (FRAP) assay

The ferric-reducing activity of both Adiantum capillus and Prosopis farcta extracts was estimated by using a method described by Benzie and Strain(11). The reaction mixture consisted of 10 mmol L-1 2,4,6-tripyridyl-s-triazine (TPTZ), 300 mmol L-1 acetate buffer, in 40 mmol L-1 of HCl and 20 mmol L-1 of FeCl3.6H2O. The working reagent of FRAP was processed newly by combining 2.5 mL TPTZ solution, 25 mL acetate buffer, and 2.5 mL FeCl3.6H2O. Then a prepared mixture was incubated in a water bath at 37 ◦C for 5 min and then a blank reading was taken by spectrophotometry at 593 nm. After that, 90 µL distilled water and 30 µL of each extract were added to 900 µL of the working FRAP reagent. Absorbance was deliberated at 0 min instantly upon adding the working FRAP reagent after vortexing with a Genie-2 vortex (Scientific Industries Inc., Bohemia, NY, USA). Then after 4 min, an absorbance reading was taken. All results were expressed by millimoles of ferric-reducing activity of the extract per one gram of the dried weight based on three experiments and BHT was used as the positive control.

2.3. Total Flavonoid Content (TFC) assay

The TFC of each plant extract was measured by using the aluminum chloride colorimetric method (12, 13). Concisely, in a separate test tube, 0.5 mL of extract solutions (1 mg mL-1) was added and mixed with 0.1 mL of 1 mol L-1 potassium acetate 1.5 mL of 95% ethanol, 0.1 mL aluminum chloride, and 2.8 mL distilled water. Then mixtures were incubated at room temperature for 30 min. By using a spectrophotometer, the absorbance readings were taken at 415 nm. Total flavonoid content was expressed as milligrams of quercetin equal to grams of dried plant material.

2.4. Free Radical Scavenging Activity

2.4.1. DPPH Assay

The method of Zahra et al (3), was used for the DPPH assay with quite changes. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay The scavenging activity of stable DPPH free radical was measured depending on literature methods (14, 15) with slight modifications. Ascorbic acid as a reference standard and plant extracts (1 mg mL−1) were prepared as stock solutions, and a set of dilutions with five different concentrations were tested. 5 µL of samples/standards were loaded, followed by 195 µL of DPPH reagent. Then the mixture was mixed forcibly and incubated in the dark for 2h at room temperature, and by using a spectrophotometer the absorbance was measured at 515 nm. The percentage of DPPH free radical scavenging activity was calculated by the following equation as:

DPPH (%) = [(absorbance of blank − absorbance of sample)/absorbance of blank] × 100(16). The results were represented as (IC50 value) the concentration of extract that was required to reduce 50% of the hydroxyl radical produced.

2.4.2. ABTS Assay

The total antioxidant capacity assay was accomplished by using an improved method described by ZA Amin et al(17) Briefly, 2,2-azinobis (3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) radical cation is produced by reacting 2.45 mmol L−1potassium persulfate and 7 mmol L−1 ABTS via incubation in the dark at room temperature for 12–16 hrs. The ABTS solution was diluted with methanol to an absorbance of 0.700 ± 0.020 at 734 nm and equilibrated at 30 ◦C, while plant extracts were diluted with distilled water. To prepare 1 mL of diluted ABTS, 10 µL of each plant extract solution was added and mixed thoroughly. The reactive mixture was permitted to stand at room temperature for 15 min and the absorbance was measured at 734 nm immediately.

The percentage of ABTS free radical scavenging activity was measured as:

ABTS (%) = [(absorbance of blank − absorbance of the sample)/absorbance of sample] × 100 (17). The results were expressed as (IC50 value) the concentration of extract that was required to reduce 50% of the free radical produced.

2.5. PBMC Proliferation Activity

Human peripheral blood mononuclear cells (PBMC)isolation was done by drawing 10 mL of whole blood from a healthy donor and diluting it with the same volume of Histopaque. Then the mixed solution was centrifuged under (relative centrifugal force (RCF) = 157 × g) for 30 min by using a Kubota 2010 centrifuge (Kubota, Tokyo, Japan). The mononuclear layer was transported out and washed safely, then pelleted down with 30 mL phosphate-buffered saline (PBS) and centrifuged three times at RCF = 157 × g for 10 min and resuspended with RPMI media supplemented with NaHCO3 and 2 mM Glutamine (Sigma-Aldrich, Gillingham, UK) besides of 100 g dL−1 fetal bovine serum (FBS). Neubauer hemocytometer (Weber, Teddington, UK) was used for the counting of cells to find out the number of PBMC cells with the same volume of trypan blue. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Merck, Darmstadt, Germany) assay was used to study the effect of the extract on cell viability. 100 µL of cells recessed in RPMI media (100 g dL−1 FBS) was added to the 96-well plate and incubated in an IR jacketed incubator (NuAire Laboratory Equipment Supply, Plymouth, MN, USA) at 37 ◦C for 24 h. After that, 10 µL of the extracts was added and incubated extra for 24h. After the period 10 µL MTT reagent (5 mg mL−1 PBS) was added to each well and further incubated for 4 h. 100 µL DMSO was added and shaken for 20 min to solubilize and extract the formazan crystals. Finally, the microplate was read at 595 nm by using a PowerWave X 340 ELISA (enzyme-linked immunosorbent assay) plate reader (BioTek Instruments, Winooski, VT, USA). All extract samples and controls were tested in triplicate in three independent experiments. The percentage cell viability was calculated using the following formula:

% Cell viability = (absorbance of extract sample − absorbance of control/absorbance of control) × 100 (2).

2.6. In Vitro Wound Healing

The MTT assay was performed to study the cytotoxicity of plant extracts before evaluating the wound-healing activity of Adiantum capillus and Prosopis farcta on human fibroblast cell lines. Cells were grown in DMEM

medium (Dulbecco's Modified Eagle's Medium with 4500 mg glucose/L, 110 mg sodium pyruvate/L, and L-glutamine (from Sigma Aldrich, USA)), supplemented with 10% fetal bovine serum (Sigma Aldrich, USA) under 5% CO2 at 37∘C in a humidified IR Jacketed incubator (from NUAIRE laboratory equipment supply, USA). Cells were counted (0.5 × 105 cells/mL) and transferred into a 96-well plate and incubated for 48 hours before adding both extracts. By using distilled water and 0.25% DMSO as solvents, serial dilutions of extracts were prepared to give final concentrations of 25, 12.5, 6.25, 3.125, and 1.5625 μg/mL and then 10 μL was injected to each well and incubated for 48 hours. 10 μL of MTT (Merck, Germany) solution was added and incubated at 37∘C for four hours. Thus, the solution was taken out by suction, and 100 μL of DMSO was added to each well and then the absorbance was recorded by the ELISA plate reader (PowerWave X340, BIO-TEK Instruments Ltd.) at 595 nm. The percentage of cell growth inhibition was calculated as:

% Cell viability = (abs of extract sample abs of control − abs of control) × 100 (2).

Moreover, an in vitro wound healing assay was done by using the CytoSelect wound healing assay kit (Cell Biolabs, Inc., USA)(18), in which inserts were used to generate a defined wound gap (0.9 mm) properly, then cells were cultured (0.5 × 106 cells/mL) in media containing 10% fetal bovine serum (FBS) and incubated for 48 hrs. until a monolayer was formed around the inserts. The inserts were removed and cells were treated with the experimental samples (200 μg/mL) and incubated for 48 hrs. The proliferation and migration of the cells into the wound field were noticed and then the percentage of wound closure was calculated as:

Total surface area = 0.9 mm ∗ length

Migrated cell surface area = length of cell migration (mm) ∗ 2 ∗ length (19)

Percent closure (%) = migrated cell surface area / total surface area ∗ 100(20).

2.7. Statistical Analysis

All data of the present study was expressed as mean, standard error of the mean (Mean ± SEM), and SPSS (Statistical Package for social science) (Version 24) statistical software was used to analyze the data. and the statistical significance of differences among groups was evaluated by using one-way ANOVA. A value of $P <$ 0.05 was considered significant.

3. Results and Discussion

3.1. Effect of Adiantum capillus and Prosopis farcta extracts on In-Vitro wound closure.

Wound healing is a challenging health problem in medicine especially in the term of management and treatment such as burns and diabetes complications. Table 1 presents the percentage of In-Vitro wound healing of Prosopis farcta extract that reached (91.1%) which was higher than the percentage of wound closure in Adiantum capillus (80%). Our findings agree with the results of an In-vivo study that done in Saudi Arabia(1).

3.2. Ferric Reducing Antioxidant Power (FARP) of Adiantum capillus and Prosopis farcta extracts

In FRAP assay, the ability of Adiantum capillus to reduce Fe3+ to Fe2+ (6.526.7±1.1) that was higher than that of P. farcta (1.610±0.9) as shown in (Table 2). The result of the corresponding study agrees with findings of another results done in Iran (21). There are few reports on the antioxidant

3.3. Total Flavonoid Content (TFC) of Adiantum capillus and Prosopis farcta extracts

The total flavonoid content of Adiantum capillus and Prosopis farcta in the present study is (57.2±0.9), (9.5±0.3) respectively. Our findings is agrees with a result of another study done by Ali Esmail Al-Snafi in Iraq, and Lina Hendawy in Egypt which found that total flavonoid content in Adiantum capillus is (49.62 ± 0.875) in the aqueous

extract, (78.18 ± 1.741) in the methanolic extract, and (50.15 ± 4.79) mg/100g in the ethanolic extract, respectively(10, 22).

While our TFC results is higher than a finding of other studies done by Boukada et al., Santhosh Kumar S. et al, and Abdulqadir A et al. that found total flavonoids contents of hydromethanolic extract of Adiantum capillus-veneris. (4.66 ± 0.01), (7.90 ± 0.90), (23-123 mg QE/g) (5, 23, 24). On the other hand TFC of Prosopis farcta in the present study is lower than a result of many studies that done in Saudi Arabia and India which recorded (135.0),(136.86±0.27) respectively (1, 24, 25).

The flavonoid compounds have a role in free radical scavenging effect in the plant crude extracts. The plant derived phytoconstituents are capable to prevent free radical reaction and prevent our body from oxidative damage and the protection against chronic disease, neurodegenerative and cardio vascular disease(24). Phenolic and flavonoids have been reported to be the most important phytochemicals responsible for the antioxidant capacity(21). The results indicated that the different extracts of A. capillus showed antioxidant activity due to high phenolic compound so it can be used as natural sources of antioxidants.

Plant extracts	**Slide measurements (Squares)**	**Slide measurements (mm)**	**The surface area of the migrated cells**	**Wound closure %**
Adiantum capillus	4	0.72	1.296	80
Prosopis farcta	4.5	0.82	1.476	91.1

Table 1: Effect of Adiantum capillus and Prosopis farcta extract on the percentage (%) of In-vitro wound healing.

Samples	**TFC Absorbance at 415nm**	**FRAP Absorbance at 593nm**
Prosopis farcta	9.5±0.3	1.610±0.9
Adiantum capillus	57.2±0.9	6.526.7±1.1

Table 2: Ferric reducing antioxidant power and total flavonoid content of Adiantum capillus and Prosopis farcta extracts.

3.4. Determination of antioxidant activity

3.4.1. DPPH radical scavenging activity

Both plant extracts were tested for their antioxidant activity by the DPPH method. Each extract (25, 12.5, 6.25, 3.125, 1.57625 mg/µL) was mixed with 3 mL of a methanolic solution containing DPPH radicals (0.1 mM). After 30 min, absorbance was determined at 515 nm. Our study findings show the percentage of DPPH scavenging activity of A. capillus extracts (IC50=148.8±0.05) was higher than in Prosopis farcta (IC50=39.6±0.12) as shown in (Figure 1).

The results of the presented investigation agree with a result of study done in India, and Iran they found that MeOH extract of A. capillus (IC50=39.01µg/mL), (IC50=41.11±2.03) respectively. Plant extracts showed higher levels of free radical scavenging activity compared to the petroleum ether extract(21, 24).

On the other hand, our findings regarding DPPH of Prosopis farcta extract (IC50=39.6±0.12) is higher than a result of other studies done, they found that IC50 value of the root extract of P. farcta was approximately 20.96 µM, and IC50 value of A. capillus extract was 44.00 ±1.00, 65.85± 3.25 respectively (5, 16, 26). Our results

revealed that both plant extracts are free radical scavengers. However, the Adiantum capillus exhibited more scavenging activity of DPPH than Prosopis farcta extract.

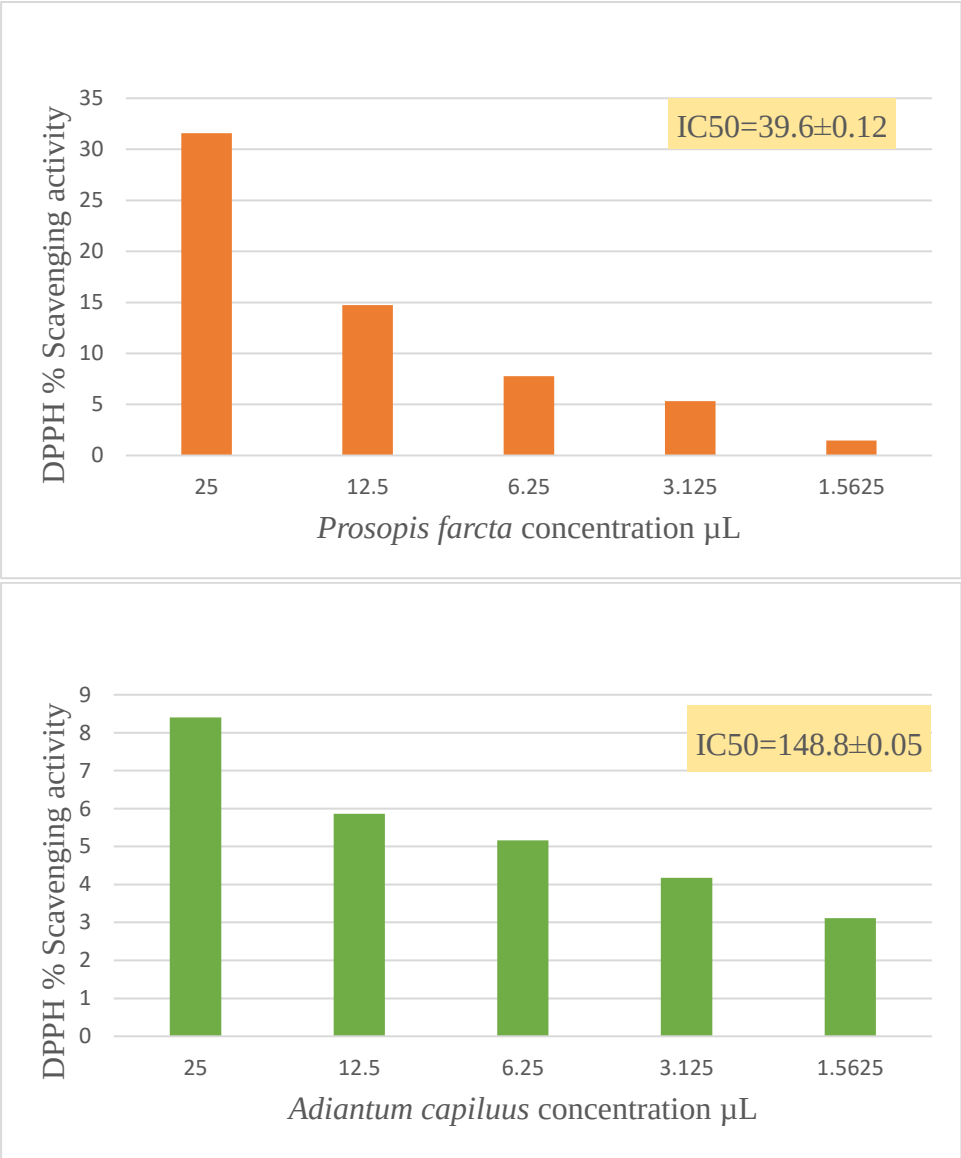

Figure 1. DPPH free radical reducing activity of Adiantum capillus and Prosopis farcta extracts.

3.4.2. ABTS Free Radical Scavenging Assay

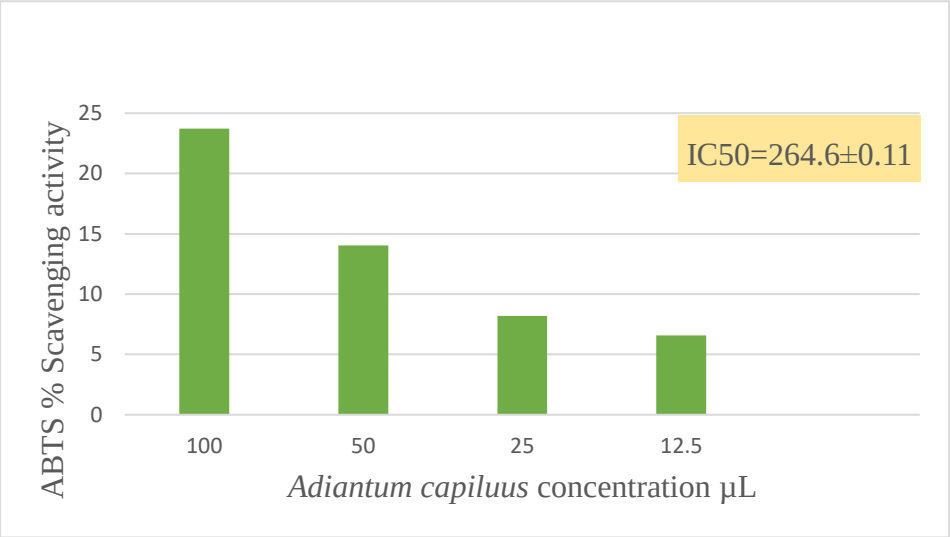

Figure 2 shows the results of the ability of both plant extracts A. capillus, and P. farcta to scavenging ABT radical cations. The highest ABTS radical scavenging rate was found to be A. capillus extract (IC50=264.6±0.11). while, the lowest total scavenging potential was found for P. farcta extract (IC50=142.5±0.52). The scavenging activity of ABTS radical by the plant extract was found to be greater antioxidant potential. Our findings agree with a result of another study that done by Santhosh Kumar S. et al(24).

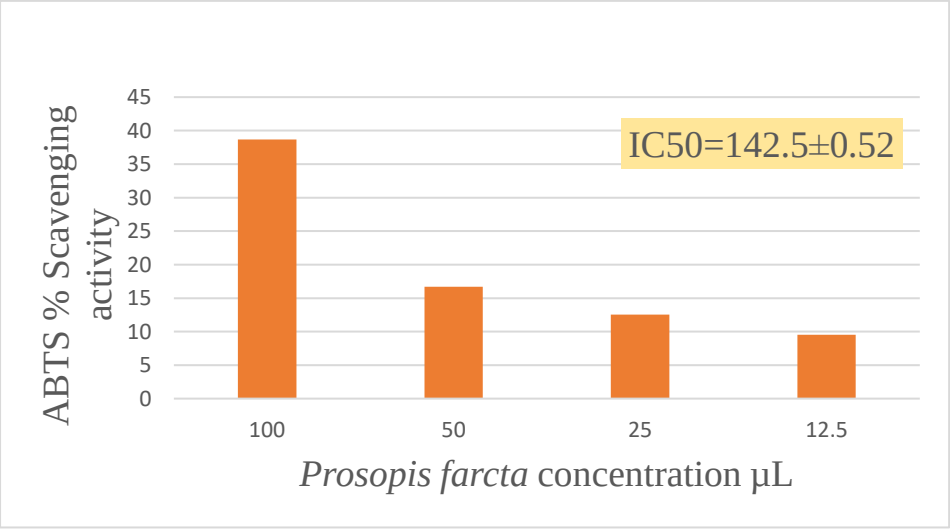

Figure 2. ABTS free radical reducing activity of Adiantum capillus and Prosopis farcta extracts.

3.5. Effect of Adiantum capillus and Prosopis farcta extracts on the Percentage of PBMC Cell Viability

Results are mentioned in Figure 3 and show the cell viability, which is a reflection of cell counts. The effects did not show significant fibroblast proliferation ($P < 0.05$) in different concentrations of both extracted plants.

The findings of the current study shows that best concentration of Adiantum capillus extract that recorded higher percentage of PBMC viability is 50µ g/mL while for P. farcta is 100 µ g/mL as shown in (Figure 3). Our finding is comparable with another study findings that done by Nilforoushzadeh, et al. (27).

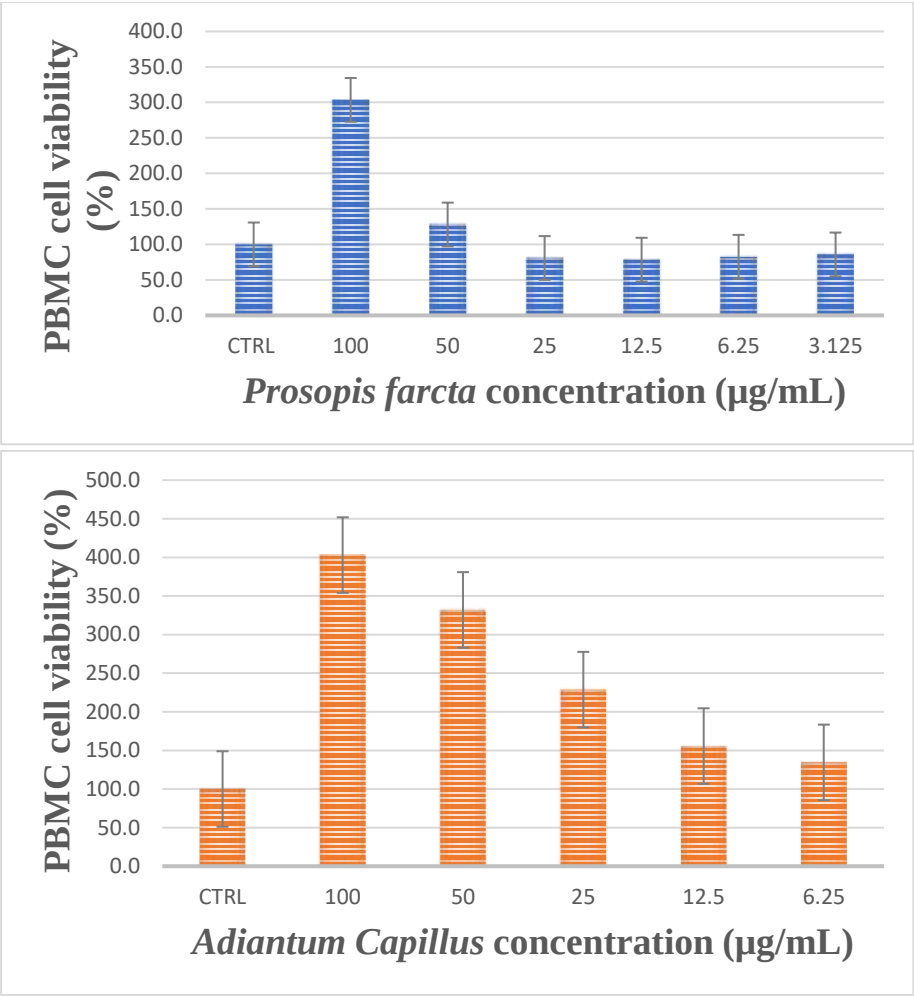

Figure 3: Effect of Prosopis farcta and Adiantum capillus on the percentage of PBMC cell viability.

4. Conclusion

This study concluded that both plant extracts have potential wound healing effect but Adiantum capillus have more wound healing effect than Prosopis farcta extract. But further investigations are needed to obtain complete profile and its various actions. The antioxidant activity that estimated by using the DPPH assay, revealed that the A. capillus-veneris extract had high antioxidant potential, in agreement with the total polyphenolic content. Both plant extracts have potential In-Vitro wound healing effect and further investigation of these plant extracts are needed for more exploration. These results ensure the importance of studied plants and widen new research perspectives as well as the design of new plant-based drugs sourced from indigenous plant material especially for wounds and burns.

Acknowledgment

The authors receive no funds from any private or public institutes.

Conflict of Interest

The authors declare no conflict of interest.

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