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Effects of the Oral Administration of Methanolic Extracts of Some Jordanian Medicinal Plants on Wound Healing in Diabetic Rats

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Abstract

***Background**: Wounds are one of the major health problems in diabetic patients. The current used drugs have several side effects, urging the need for new natural sources for therapeutics.*

***Materials and methods**: This study aimed to evaluate the wound healing potential of the methanolic extract of G. arabica and M. sylvestris leaves, and R. coriaria fruits. The methanolic plant extracts were orally administered to the rats to determine their effect on wound-healing process.*

***Results:** These extracts significantly (P < 0.01) increased the wound contraction in non-diabetic and diabetic rats. In addition, the plant extracts increased the fibroblasts proliferation and migration resulting in strong highly healing activities. The present finding showed that the plant extract has no cytotoxic effects. The proliferation assay exhibited the lowest cell mortality after treatment with plant extract. The present in vitro study showed that treatment with plants extracts increased cell proliferation and migration after 48h as compared to controls.*

***Conclusion:** These finding may indicate that the methanolic leaf extract of the above plants can be used as new therapeutics for wound healing activates in diabetic rats.*

***Keywords:** Diabetes; Wounds, Wound healing; Medicinal plants; Cytotoxicity.*

Introduction

Plants are considered as a great treasure of resources for human. They are available in the various forms that are well recommended in traditional medicine all over the world [1,2]. Plants offer many advantages of being source of the medicinal remedies, since they are easily available in the local surroundings, having no side effects, cost effective and easy to use [3,4]. In Jordan, there are about 363 species of medicinal plants that are used in traditional medicine, and have many pharmacological effects [5].

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Diabetes mellitus is a severe metabolic disorder and one of the world's most common chronic diseases. It affects about 381 million adult populations globally [6], moreover, it is estimated that about 175 million cases are still undiagnosed [5].

The disease is becoming increasingly prevalent. For example, in the united states, 11 million people were diagnosed in 2001, accounting for about 4% of the whole population, and the number is expected to rise to 29 million by 2050 [8]. The International Diabetes Federation (IDF) estimates that these numbers will continue to grow up in the future [9]. Type 2 diabetes is the most common, accounting for 85-90 % of diabetic patients. However, type 1 diabetes accounts for about 10-15 % only [10].

The present study undertaken to provide a scientific approach for the effectiveness of selected plants species in the treatment of the wounds in diabetic patients. The basic criterion for the selection of these plants was that the use of these plants in traditional medicine. Most importantly, a large number of these plants being used by the tribal communities for the treatment of wounds, cuts and skin diseases. However, it seems that there are no scientific reports to support their use in wound healing. Therefore, three plants species were selected to be evaluated for their potential wound-healing activity *in vitro* and *in vivo*. Those plants are *Rhus coriaria, Globularia arabica* and *Malva sylvestris* (Figure 1).

Figure 1: Representative photos for the plant species investigated in this study: Rhus coriaria (A), Globularia arabica (B), and Malva sylvestris (C).

R. coriaria (Figure 1A) belongs to the family Anacardiaceae, commonly known as "Summac" in the Mediterranean countries. It is a very popular spice and a major souring agent [11]. It has been shown to possess many pharmacological activities including antimicrobial, hepatoprotective, hypoglycemic, antioxidant, DNA protective and antibacterial effects [12]. In Jordan, the people use it in folk medicine as anti-inflammatory agent [13], and as a traditional spice due to its souring taste [14] *R. coriaria* is also considered as a rich source of hydrolysable tannins, Gallo tannins [15]. Moreover, it has been reported previously that *R. coriaria* has a high potential activity in wound healing [16].

G. arabica (Figure 1B), which belongs to the family Plantaginaceae, is the only species of *Globularia* that grows in Jordan [17,18]. It is commonly used by Jordanian people in traditional medicine due to its known antimicrobial and anti-tumorous activities [5,14] The leaves of *G. arabica* are ground to a powder or chewed and then sprinkled or smeared onto a wound to heal it [19]. According to Rodríguez-Pérez et al [20], *Globularia* spp methanolic extract improves burn wound healing and inflammation in rats, and possesses antibacterial and antioxidant activities.

M. sylvestris (Figure 1C) belongs to the family Malvaceae and commonly known as Mallow. It is native to Europe, North Africa, and Asia [21]. Its therapeutic properties has been described as anti-inflammatory, anticancer, positive effectiveness on gingivitis, abscesses, tooth pain, urological disease, insect bites, ulcerous wounds and against specific disorders in several systems of the body [22]. Young leaves are eaten raw in salads; leaves and shoots are consumed in soups and as boiled vegetables [21]. The anti-inflammatory properties of *M. sylvestris* can be attributed to the presence of substances such as mucilage, flavonoids, and tannins [23]. Some previous studies have reported that the flowers of *M. sylvestris* are used as a remedy for cut wound, dermal infected wounds, eczema, and inflammatory disease such as gastritis and bronchitis [24]. Moreover, the chloroform flower extract of *M. sylvestris* has been shown to possess wound healing activities in alloxan-induced diabetic rats [25].

Materials and Methods

Collection and identification of the plant specimens

In July 2020, leaves of *G. arabica*, *M. sylvestris*, and fruits of *R. coriaria* were harvested from various locations in Jordan. Prof. Sawsan Oran, plant taxonomist, Department of Biological Sciences, The University of Jordan, Amman, Jordan, authenticated the plants. The University of Jordan's Herbarium received voucher specimens.

Preperation of the methanolic extract

G. arabica, *M. sylvestris* leaves and *R. coriaria* fruits were cleaned, dried, and ground in a blender before being immersed in 96 percent methanol. A hundred grams of dried leaves were immersed in 1000 ml methanol (1:10 w/v ratio) for three days at room temperature with constant shaking [26]. The solution was evaporated at 45°C under reduced pressure using filtration and a rotary evaporator (Buchi R200C Rotovapor Complete System W/ condenser C, V500 pump, and V800 controller, Mutah University). The obtained extracts were kept at -20°C in an air tight container. The methanolic extract was prepared using successive extractions. The extracts were weighed to calculate the yield percentage using the following equation: Yield % = (wt. of dry extract/ wt. of dry leaves before extraction) x 100%.

Animals

Male Wistar rats (150-200 g) were used to test the wound healing and anti-inflammatory activity of methanolic extracts of G. arabica, M. sylvestris, and R. coriaria leaf and fruits. The animals were kept in separate standard plastic cages in the animal house at Mutah University's Department of Biological Sciences. The temperature was kept at 23 1°C by alternating between 12 hours of light and 12 hours of darkness. The animals were given unlimited access to pelleted food and water. Jordan University's ethical committee granted the study ethical approval under reference number 47-2021. Before conducting the experiments, the animals were given time to adjust to their new surroundings. During the experiments, the animals were separated using separating cages. Various methods were used to collect blood samples (figure 3). The serum was also obtained for biochemical analysis. The doses of plants extracts administrated *in R. coriaria* fruit extract were chosen based on a previously reported LD_{50} in rats more than (1975 mg/kg body wt.) [27], in *G. arabica* was (up to 1000 mg/kg wt.) [28], However, *M. slyvesries* LD_{50} was (up to 2000 mg/kg wt.) [29].

Induction of diabetes

Diabetes was induced injection of streptozotcin (60 mg/kg body weight). Diabetes was confirmed after 3 days by measuring blood glucose concentration from the tail vein using the On call plus glucometer-strips, Hanover germmany, Mutah University) [30] Diabetics were defined as animals with blood sugar levels greater than 200 mg/dL [31].

Toxicity Study of plant extract

The study used male Wistar rats (150-200 g) that had been fasted overnight. The animals were split into five groups of two each. Groups 1–4 were given 2 mL of plant extract orally, as shown in the table (1).

Table 1: Doses for the toxicity study.

Step	Doses (mg/kg bdw)				
	G 1	G 2	G 3	G 4	G 5
1	100	200	400	800	Saline
2	1000	1500	2000		
3	3000	4000	5000		

The same route was used for the control (group 5) to receive normal saline (2 mL/group). This method has three stages", with the results of each indicating whether testing should be stopped or continued to the next stage. Within 24 hours, general toxicity symptoms and mortality were observed in each group. If there is no death is documented at these stages, the extract substance LD_{50} is assumed larger than 5000 mg/kg body weight, representing a great degree of safety. If death recorded at a certain dose in any of the stages, an assenting test should be made to verify that the extract was the cause of such death. This test simply involves administering the dose of extract that caused death (or the lowest dose that caused death in a condition where more than one death was recorded) to four animals". The patient should then be observed for 1 hour after administration and every 2 hours for the next 24 hours. If at least two of the four animals die, this should serve as verification and authentication of the test results. This method has several advantages, including the use of fewer animals, the investigation of a varied sort of dosages, and the fact that it is inexpensive [32].

According to the following formula LD50 was calculated

LD50: $LD_{50} = (M0 + M1) / 2$

Where M0 = The highest dose of plant extract that gave no death

M1 = The lowest dose of plant extract that gave death.

Experiment design

Diethy ether was inhaled to anesthetize rats. The dorsal aspect was shaved with an electrical clipper (Geemy professional hair clipper), and one fragment was gently extracted from the skin with a 10 mm diameter circular biopsy needle (Disposable Biopsy Punch, ROBBINS Instrument, Mutah University) figure (2) [27].

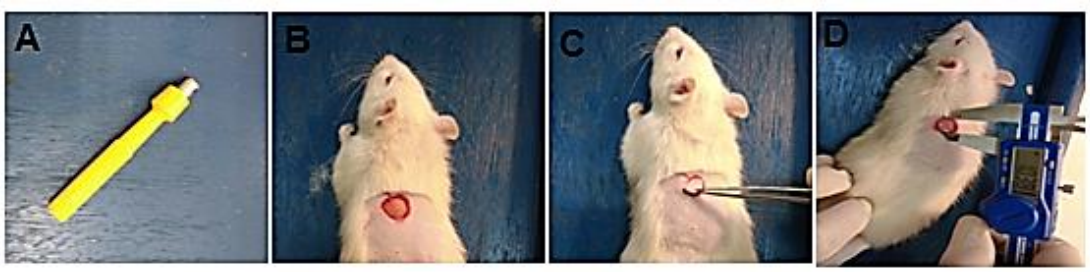

Figure 2: Wound creation in rats. A. 10-mm diameter biopsy punch. B-C. Skin removal. D. Wound Measurement [27].

Contraction, which is primarily responsible for wound closure, was investigated until the wounds were completely covered with epithelium. Using the following formula, wound contraction (WC) was calculated as a percentage change from the initial wound size:

$$\text{Wound Contraction Percentage} = \frac{\text{Original wound size - exact day wound size}}{\text{Original wound size}} \times 100\%$$

The epithelization period was monitored by noting the number of days required for Eschar to fall away, leaving no raw wound behind [33]. Blood samples were collected using different methods figure (3) [27].

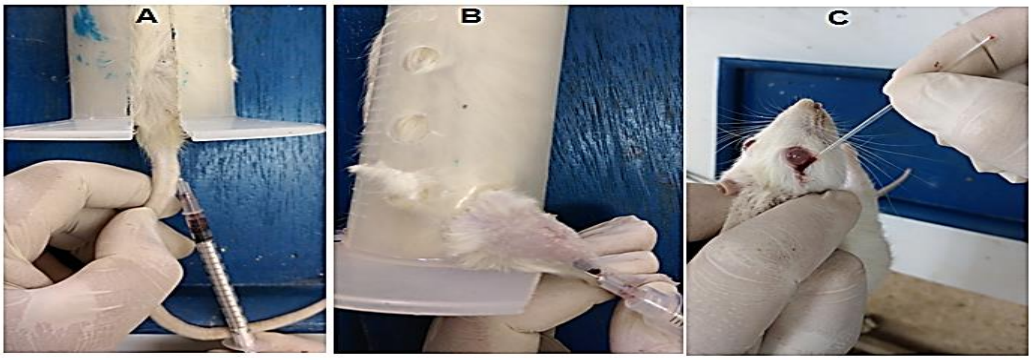

Figure 3: Blood collection methods. A: Tail vein B: Lateral Saphenous Vein C: Retro Orbital Sinus Vein [27].

Ninty rats were used in this experiment. Animals were divided in to two main groups non-diabetic (1-3) and diabetic (4-6) table (2).

Table 2: Treatment groups in orally administrated methanolic plant extracts.

Non-diabetic rats		Diabetic rats	
Group	Treatment	Group	Treatment
1	Normal saline	4	Normal saline
2	Vit.E (100 mg/kg)	5	Vit. E (100 mg/kg)
3	200 mg/kg plant extract	6	200 mg/kg plant extract

Each main group composed of three sub groups of five animals each (n = 5) as follows: Group I: Control group treated with the vechicle (0.5% sodium carboxymethyl cellulose suspension in normal saline). Group II: Standard group treated with Vitamin E (100 mg/kg body weight) after suspended in the vehicle (0.5% sodium carboxymethyl cellulose suspension in normal saline). Group III: Test group treated with plant extract (200 mg/kg body weight) extract in the vehicle. Test drugs were orally administered once a day in an equivalent volume of 5 ml/kg body weight for 15 days. Wound areas were measured used a digital caliper right away, and then repeat the operation on days 0, 3, 6, 9, 12, and 15 after the wound is created. The wound was pictured using digital camera. Contraction which is the mainly contributes to wound closure was studied until day 15. Wound contraction (WC) was calculated as a percentage change of the wound closure.

Fibroblast Proliferation and Migration in Wound Healing Activity of methanolic plant extract *in vitro* study

***In vitro* Cytotoxicity Test**

Plant extracts were tested for cytotoxicity against fibroblast cell lines provided by Prof. Yaser Bustanji's lab at the University of Jordan. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with antibiotics penicillin and streptomycin at a concentration of 100 g/mL. The cells were grown at 37°C in a humidified 5 percent CO2 incubator. Every 3-4 days, the cells were split by discarding the culture media, followed by cell detachment with 1-2 mL trypsin, and the addition of a fresh warm DMEM medium [34]. The plant extract was tested at concentrations ranging from 10 to 100 µg/mL. All cultures were carried out in triplicate. The dose-response curve was used to calculate the IC50.

Culturing of cell lines

The cytotoxicity of the plants extracts was determined microscopically. Different concentrations of the samples (10, 25, 50, and 100 µg/mL) were applied into 24-well microtiter plates containing 5×10^4 cells/ml of the tested cell line. The cells were incubated at 37°C and in a humid atmosphere harboring 5% carbon dioxide. The test was done in duplicate and the cytotoxic effect was detected daily up to 72 hours of culturing changes in cell shape morphology including loss of monolayer, rounding, and shrinking were considered as signs of cytotoxic effect of the tested samples as examined under microscopy. Cultures of cell line without tested extract were used as a negative control [35].

Antiproliferative Assay

The inhibition of cell proliferation was measured using the Giemsa staining method after the tested compounds were applied to cell lines. The media from the wells was aspirated, then washed with 0.5mL PBS and fixed with 0.3mL methanol for 10 minutes at 37°C. Methanol was aspirated, and the plates were left to dry for 2 minutes. Each well received 0.5 mL of Giemsa stain (1:10 in PBS) and was left for 10 minutes. After aspirating the stain, the wells were washed with 0.5 mL deionized water. The bounded stain was extracted using 0.3 mL of 0.1N HCl and the antiproliferative activity was estimated using an enzyme-linked immunosorbent assay (ELISA) microplate reader at 630 nm (ELX 800 Instrument). Cell viability was shown as a percentage of living cells versus control [36]. The percentage of cell death was estimated using the formula:

$$\text{Dead cells (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of treated cells x 100 \%}}{\text{Absorbance of control}}$$

Migration assay

"Fibroblasts were seeded at a density of 35,000 cells per well and incubated until confluent". Using a 200l pipette tip, a scratch was made in each well. Before adding the medium conditioned with different concentrations (10 and 20 g/ml) of each plant extract, the media was removed and the cells were washed in PBS. Optical microscopy was used to examine different areas along the scratches of each well after 0, 12, 24, and 48 hours of induced damage. The distance between the scratch's edges was measured with Image J software and expressed as a percentage of area closure when compared to control untreated cells [37].

Statistical analysis

The data were presented as means standard deviations. The statistical significance of differences between groups was determined using Graph Pad Prism version 7 and one-way analysis of variance (ANOVA) with Tukey's HSD post-hoc test. $P > 0.05$ was considered significant.

Results and discussion

The yielded extracts obtained by methanol extraction of fruit *R. coriaria,* and leaves of *G. arabica* and *M. sylvestris* were about 13.4%, 12.5% and 18.6 % w/w on dry weight basis, respectively.

Toxicity Study

Acute toxicity studies have revealed that all the oral administration doses of the *R. coriaria* and *M. sylvestris* methanolic extract were safe and non-toxic up to (4500 mg/kg). The methanolic extract of *G. arabica* leaf was safe up to (3500 mg/kg) as shown in Table 3.

Table 3: Lethal doses of *R. coriaria, G. arabica* and *M. sylvestris* methanolic extract.

Plant	LD_{50} doses (mg/kg bdw)
R. coriaria	4500
G. arabica	3500
M. sylvestris	4500

Effect of orally administrated methanolic plants extracts on cutaneous wound healing in diabetic rats.

Most of the medicines that are used in the treatment of wounds are external ointments. However, in this experiment the plant extracts were administered orally. The *R. coriaria* methanolic extract (200mg/kg) gave significant ($P < 0.01$) effects on wound healing as compared to the groups that were given vitamin E (Table 4 and Figure 4).

Table 4: Effect of orally administration of *R. coriaria* methanolic extracts on wound contraction in non-diabetic and diabetic rats. Values are mean±S.D (n=5).

Day	Case	Negative control		Vitamin E (100 mg/kg)		*R.coriaria* (200mg/kg)	
		Wound diameter (mm)	Percentage of wound contraction (%)	Wound diameter (mm)	Percentage of wound contraction (%)	Wound diameter (mm)	Percentage of wound contraction (%)
0	Non diabetic	10.63 ±0.31	0	10.39 ± 0.20	0	10.46 ±0.32	0
	Diabetic	10.37 ± 0.23	0	10.28 ±0.11	0	10.34 ±0.09	0
3	Non diabetic	10.37 ±0.29	2.4	9.55 ±0.30	8	9.48 ±0.28	9.3
	Diabetic	10.15 ± 0.21	2	9.61 ±0.18	6.5	9.55 ±0.15	7.6
6	Non diabetic	9.76 ±0.40	8.18	8.78 ±0.33	15.4	8.16 ±0.25	21.9
	Diabetic	9.26 ± 0.20	107	8.81 ±0.32	14.2	8.61 ±0.27	16.7
9	Non diabetic	9.09 ±0.68	14.4	6.93 ±0.64	33.3	6.79 ±0.52	35
	Diabetic	8.24 ± 0.17	20.5	6.96 ±0.43	32.2	6.71 ±0.56	35.1
12	Non diabetic	7.46 ±.0.69	29.8	5.14 ±0.72	50.5	4.84 ±0.63	53.7
	Diabetic	7.33 ±0.21	29.3	5.25 ±0.74	48.9	4.92 ±0.59	52.4
15	Non diabetic	5.96 ±0.76	43.9	1.76 ±0.23*	83	1.89 ±0.30*	81.9
	Diabetic	5.96 ±0.41	42.5	2.14 ±0.36*	79.1	2.54 ±0.44*	75.4
* Significant at $P < 0.01$ over the negative control							

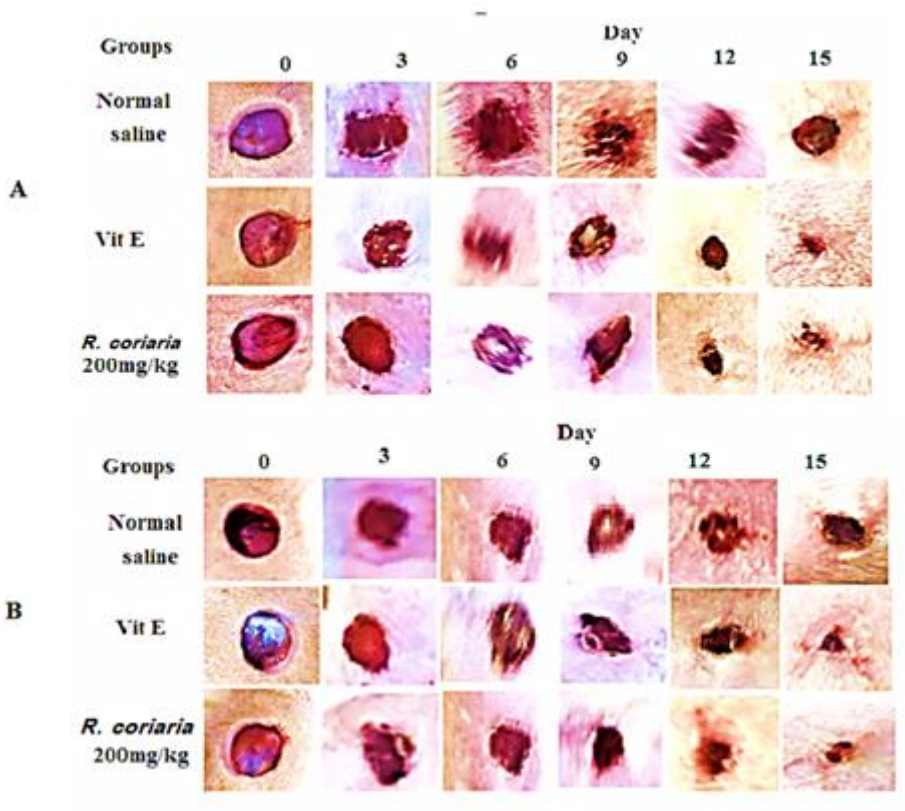

Figure 4: Morphological representations of wound contraction treated orally with *R. coriaria* methanolic extracts in non-diabetic (A) and diabetic (B) rats.

On the other hand, *M. sylvestris* methanolic extract (200mg/kg) gave better healing activity than *G. arabica.* The healing potential of the two plants are shown in (**Tables 5 and 6**), and the photos of the wound healing processes are shown in the (**Figures 5 and 6**), respectively.

Table 5: Effect of orally administration of *M. sylvestris* methanolic extracts on wound contraction in non-diabetic and diabetic rats. Values are mean±S.D (n=5).

Day	Case	Negative control		Vitamin E (100 mg/kg)		*M. sylvestris* (200mg/kg)	
		Wound diameter (mm)	Percentage of wound contraction (%)	Wound diameter (mm)	Percentage of wound contraction (%)	Wound diameter (mm)	Percentage of wound contraction (%)
0	Non diabetic	10.65 ±0.56	0	10.82 ±0.42	0	10.72 ±0.64	0
	Diabetic	10.63 ±0.47	0	11.00 ±0.43	0	11.23 ±0.53	0
3	Non diabetic	10.45 ±0.49	1.8	10.18 ±0.16	5.9	10.05 ±0.53	6.2
	Diabetic	10.35 ±0.41	2.6	10.38 ±0.44	5.6	10.02 ±0.26	10.7
6	Non diabetic	9.57 ±0.41	10	8.73 ±0.53	19.3	8.70 ±0.42	18.8
	Diabetic	9.40 ±0.31	11.5	8.60 ±0.39	21.8	8.18 ±0.12	27.1
9	Non diabetic	8.57 ±0.58	19.5	6.26 ±0.67	42	6.20 ±0.24	42
	Diabetic	8.21 ±0.07	22.7	6.31 ±0.30	42.6	7.42 ±0.27	33.9
12	Non diabetic	7.54 ±0.38	29	3.99 ±0.82	63	5.16 ±0.11	51.8
	Diabetic	7.29 ±0.22	31.4	4.43 ±0.42	59.7	5.30 ±0.22	52.6
15	Non diabetic	5.79 ±0.26	45	1.50 ±0.30*	86	2.65 ±0.60*	75.2
	Diabetic	5.98 ±0.54	43.7	2.07 ±0.60*	81.1	3.18 ±0.66*	71.6
*Significant at P < 0.01 over the negative control.							

Table 6: Effect of orally administration of *G. arabica* methanolic extracts on wound contraction in non-diabetic and diabetic rats. Values are mean±S.D (n=5).

Day	Case	Negative control		Vitamin E (100 mg/kg)		*G. Arabica* (200mg/kg)	
		Wound diameter (mm)	Percentage of wound contraction (%)	Wound diameter (mm)	Percentage of wound contraction (%)	Wound diameter (mm)	Percentage of wound contraction (%)
0	Non diabetic	10.57 ±0.44	0	10.95 ±0.55	0	10.75 ±0.69	0
	Diabetic	10.96 ±0.7	0	10.66 ±0.41	0	10.81 ±0.42	0
3	Non diabetic	10.25 ±0.08	3	9.95 ±0.49	9.1	9.85 ±0.30	8.3
	Diabetic	10.83 ±0.73	1.18	9.93 ±0.52	6.8	9.88 ±0.46	8.6
6	Non diabetic	9.42 ±0.35	10.8	8.28 ±0.80	24.3	8.56 ±0.38	20.3
	Diabetic	9.98 ±0.52	8.9	8.68 ±0.55	18.6	7.88 ±0.51	27.1
9	Non diabetic	8.10 ±0.22	23.3	6.85 ±1.04	37.4	7.14 ±0.37	33.5
	Diabetic	8.54 ±0.42	22	6.60 ±0.43	38	6.62 ±0.47	38.7
12	Non diabetic	7.06 ±0.55	33.2	3.82 ±0.62	65.1	4.79 ±0.58	55.4
	Diabetic	7.55 ±0.4	31.1	4.94 ±0.8	63.6	4.82 ±0.33	55.4
15	Non diabetic	5.69 ±0.33	46	1.75 ±0.5*	84	2.53 ±0.97*	76.4
	Diabetic	6.04 ±0.7	44.8	1.93 ±0.52*	81.9	3.32 ±0.34*	69.2
* Significant at P < 0.01 over the negative control.							

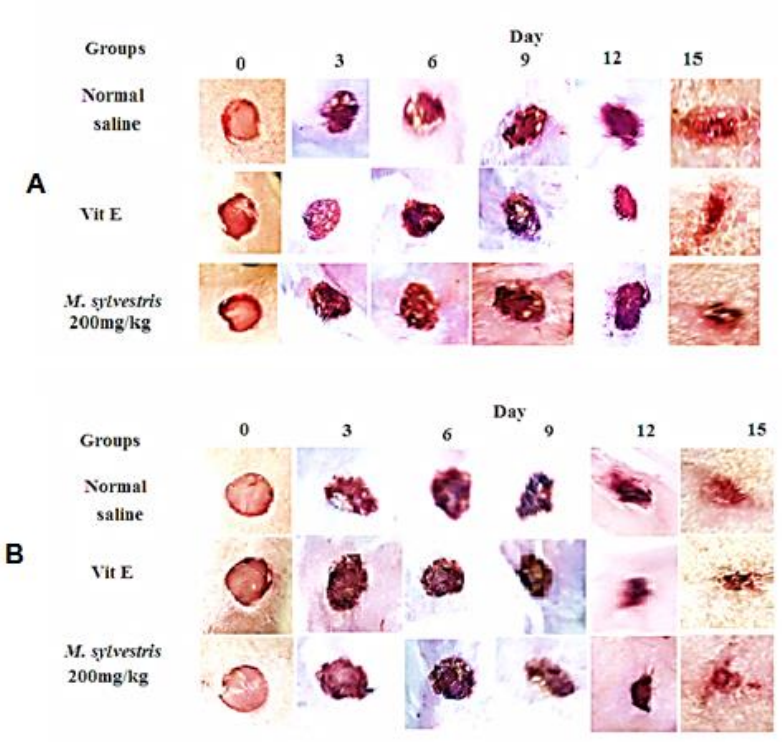

Figure 5: Morphological representations of wound contraction treated orally with *M. sylvestris* methanolic extracts in non-diabetic (A) and diabetic (B) rats.

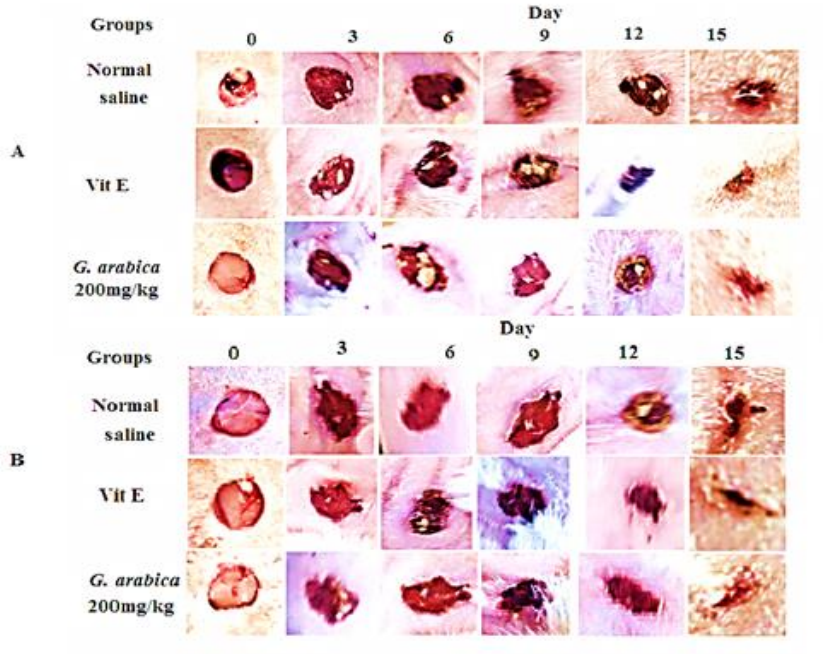

Figure 6: Morphological representations of wound contraction treated orally with *G. arabica* methanolic extracts in non-diabetic (A) and diabetic (B) rats.

Wounds are a clinical problem and they frequently seen as a serious concern in therapeutic practice. The normal wounds are resolved in a few days, however, chronic wounds represent a major problem because of economic and social factors, therefor it must search for new natural products with higher efficacy and lower costs and side effects [38,39]. Moreover, stress or diabetes can cause a delay in the wound healing process or increase the probability of infection in the wound area, which may damage the other organs leading to severe morbidity and long hospitalization [40].

The present study focused on the development of new therapeutic agents using medicinal plants extracts. The results revealed that the untreated group has some degree of wound- healing, which might be due to self-immunity [41], however, the experimental groups which are treated with the methanolic plants extracts showed faster wound healing process. Moreover, the methanolic plant extracts showed no signs of irritation, pain, restlessness, or scratching/biting of the wound site to the rats during the wound treatment period. This wound-healing activity might be due to the influence of the extracts on the various wound healing phases like fibroplasias, collagen synthesis, and wound contraction, resulting in a faster healing process as reported previously [42]. This interpretation is supported by the present results which displayed that the use of plants extracts lead to a higher rate of wound contraction, which causes to faster healing. The best results were obtained from *R. coriaria* methanolic fruit extract which could be recommended as an alternative drug for the treatment of diabetic wounds. It has been previously reported that the aqueous extract of *R. coriaria* can cause a reduction in the wound area, increased collagen deposition and hydroxyproline concentration, and reduced collagenase-2 and Myeloperoxidase enzyme activity leading to faster wound healing [43].

On the other hand, the methanolic extract of *G. arabica* leaves had also showed a good wound healing activity in both diabetic and non-diabetic rats. These results are coincided with that of Es-Safi et al. [44]. who found that the hydromethanolic extract of *G. alypum* possess a considerable antioxidant activity, mainly due to the presence of flavonoid and phenyl ethanoid constituents? Therefore, these antioxidant compounds may contribute to the wound healing activity. Moreover, Fehri and Aiache [45] reported that the *G. alypum* have anti-ulcer properties against stomach mucosal injury by inhibiting the migration of intraepithelial lymphocytes.

In contrast, the methanolic extract of *M. slyvesries* leaves exhibited less wound healing potential compared the other two plant extracts. However, Pirbalouti et al. [46] found that the extract of *M. sylvestris* flowers improved the wound healing process and the histological studies showed increased well organized bands of collagen, more fibroblasts and few inflammatory cells. *M. sylvestris* flower extract was shown to contain anthocyanin, malvin, niacin and folic acid which might be responsible for wound healing, antioxidant and antimicrobial activities.

In vitro fibroblast proliferation and migration in wound healing.

The results of IC_{50} determination and cytotoxicity of plant extracts at concentration of 100µg/ml are shown in Table 7. The lowest IC_{50} was seen in *G. arabica*, while the lowest cytotoxicity was seen in *R. coriaria*.

Table 7: Cytotoxicity and IC_{50} of *R. coriaria*, *G. arabica* and *M. sylvestris* methanolic extracts (n=3).

Plant	IC_{50} (µg/mL)	Cytotoxicity (%)
R. coriaria	90 ±5.6	37 ± 4.8
G. arabica	72 ±3.3	47.3 ±5.3
M. sylvestris	82 ±4.2	42 ± 4.8

On the other hand, the ability of plant extracts to stimulate fibroblast proliferation and/or migration was studied by the scratch assay that showed the migration of fibroblasts after scratch and culture with 10 µg/mL and 20 µg/mL of plant extracts. The maximum stimulatory effect was observed after 48 hr of treatment with 20 µg/mL of *R. coriaria* extract, followed by *G. arabica* (Figure 7).

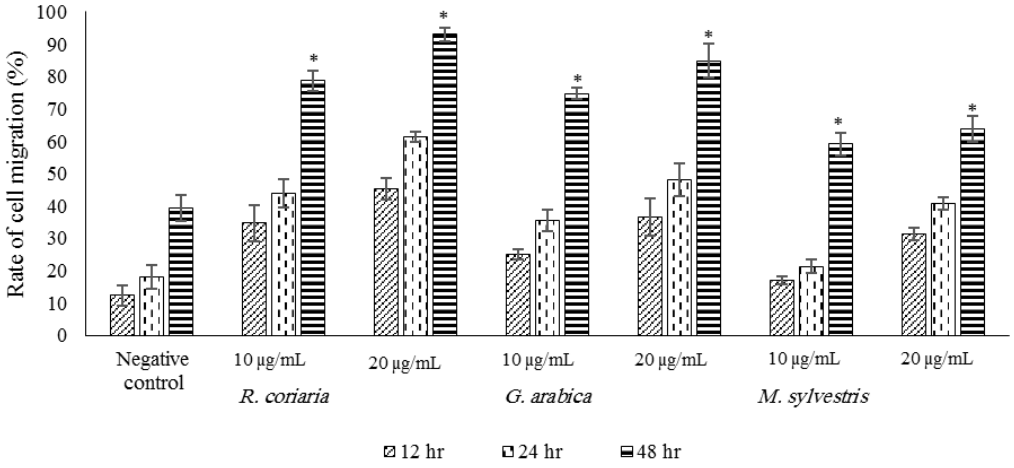

Figure 7: The rate of cell migration of fibroblasts treated with methanolic extracts of *R. coriaria*, *G. arabica* and *M. sylvestris* after 12 hr, 24 hr, and 48 hr compared to the negative control group. *Significant at $P < 0.05$ over the negative control.

Cell migration and proliferation have a major role in the wound healing process. The scratch assay is widely used for *in vitro* study wound healing activity [37]. The fibroblasts are responsible for collagen deposition, which is needed to repair the tissue after injury, because collagen provides strength, integrity and structure required to restore the anatomical integrity and function of the tissues after damaged [47]. The fibroblasts activity during the early proliferative phase is cellular replication and migration. However, at about the third day following injury, the large mass of fibroblasts begins to synthesize and produce a high amount of collagen fibers which determines the activity of the wound healing [48]. The present study showed that the methanolic extracts of *R. coriaria* and *G. arabica* strongly stimulated migration of the fibroblasts. These results support and confirm the present *in vivo* observations, where the methanolic extract of *R. coriaria* and *G. arabica* showed strong excision wound healing activity. These results are in agreement with that of Abdallah et al. [49] and El Hasasna et al. [50,51], who reported that *R. coriaria* methanolic extract increased uterus cervix cell migration capacity and inhibited cervical and breast cancer growth. Therefore, the present study might establish the pharmacological efficacy of the above plant extract on wound healing process.

Conclusion

This research study had focused on the remarkable potential phytotherapy of commonly used plants *G. arabica*, *R. coriaria* and *M. sylvestris* for their pharmacological benefits. The importance of the present work lies in establishing and reporting for the first time the wound healing benefits of these plants in a diabetic rat model. The methanolic extracts of both *G. arabica* and *R. coriaria* showed a strong wound healing activity. Moreover, they are rather safe and showed no signs of systemic toxicity in the rat.

Conflict of interest

The authors declare no conflict of interests.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Ethical Clearance

All experimental protocols were approved under the Department of Biology, Mutah University, Jordan, and all experiments were carried out in accordance with the guidelines established by the FIOCRUZ Committee of Ethics for the Use of Animals (CEUA LW16/14).

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Author's contribution

Ahmad Za'al Alsarayreh: conception and design
Sawsan Attalah Oran: analysis and interpretation
Jumah Mutie Shakhanbeh: data collection and writing
Yaseen Taha Al Qaisi: critical revision of the article
Ibrahim Ismail Alfarrayeh: critical revision of the article
Ayah Algaramseh: Obtaining funding

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