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Research Article

Treatment of burns associated with diabetes with tannins extracted from *Haloxylon salicornium* and *Zygophilium coccinium*

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Abstract: The use of plant extracts is a successful method of treating diseases. Desert plants are rich in many anti-oxidant compounds, so they have been used since ancient times in folk medicine. The most important of these compounds are tannins that contain in their composition an oxidative hydroxyl group, which resists the free radical compounds, which are used in this study. From two plants of *Haloxylon salicornium* and *Zygophilium coccinium* (after proving their antioxidant activity by DPPH assay, with different concentrations for both plants), tannins were extracted and were used to treat burns in laboratory rabbits with diabetes and divided into different. The incidence of infection was confirmed by a histological, clinical, and microbiological study of the effect of extracts on the treatment period for the stability of their effectiveness for treatment. The results showed the effectiveness of these compounds on microorganisms and accelerated the treatment period despite diabetes and getting fast and effective results. Medicinal plants.

Keywords: Burn, Diabetes mellitus, Tannins, *Haloxylon, Zygophilium*.

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Introduction

Diabetes is a collection of metabolic diseases characterized by hyperglycemia brought on by abnormalities in insulin secretion, action, or both. Long-term harm, dysfunction, and failure brought on by the chronic hyperglycemia of diabetes are especially susceptible to affecting the eyes, kidneys, nerves, heart, and blood vessels (Vandorsten et al. 2013). On the skin, diabetes symptoms can also manifest. When the skin receives higher blood glucose, chronic cutaneous infections are triggered. Bacterial and fungal infections cause pruritus and other symptoms of skin illness, which are exacerbated by hyperglycemia (HG). Long-term HG results in an overproduction of reactive oxygen species (ROS), which in turn leads to oxidative stress in DM and is mostly accompanied by a weakened antioxidant defense system (Matough et al. 2012). The body's antioxidant defenses can no longer stop the rising ROS formation as a result. Oxidative stress is associated with elevated blood levels of glycolipid, glycoprotein, and hypertension which are all linked to DM. The antioxidant defense system works with enzymes like glutathione peroxidase, catalase, and superoxide dismutase to counteract the harmful effects of oxidative stress (Kaneto & Matsuoka 2012). The complex wound-healing cascade is impaired in DM for a variety of reasons, including inflammation, proliferation, angiogenesis, apoptosis, decreased chemotaxis, and matrix formation, decreased bacterial resistance, and degradation of the antioxidant defense system. Each one of these impairs wound healing. Additionally, peripheral vascular disease in the legs is frequently present in

persons with uncontrolled diabetes because of atherosclerosis, which can eventually lead to foot amputation (Forbes & Cooper 2013).

Several drugs are used to treat burns and wounds caused by diabetes, but some of them are unsuccessful, causing tissue damage and occasionally severing organs. Therefore, medicinal plants with antioxidant active components have been used in addition to their therapeutic benefits to combat free radicals created in damaged tissue. This chemical turns microorganisms in the affected area harmless (Lu & Fuchs 2014). Any plant that contains substances in one or more of its organs that can be utilized for therapeutic purposes is referred to as a medicinal plant. Due to the compounds found in various plant tissues that have unique physiological effects on humans, plants have traditionally played a significant role in traditional medicine. Numerous phytochemicals can be extracted from plants and used as pure compounds or as extracts that are effective in treating a variety of illnesses and have a substantial potential role in clinical pathology (Chandrasekharnath et al. 2013).

The experimental plants in the current study were chosen due to their high antioxidant content and their use in folk medicine for the treatment of burns. *Haloxylon salicornicum* is an Amaranthaceae family of desert plants. This plant has a long history of usage in traditional medicine as a diuretic, ulcer preventive, hypoglycemic, and anti-microbial. Qualitative phytochemical analysis revealed that the plant's aerial portions included alkaloids, cardiac glycosides, anthraquinones, flavonoids, saponins, coumarins, sterols, tannins, volatile oils, and volatile bases Ashraf et al. (2012). A small perennial herb, *Zygophilium coccineum* grows in sandy, salty conditions close to the ocean. It features delicately tan flowers and succulent leaves. The growth and distribution of *Zygophyllum* are influenced by the chemical composition of the soil in their habitats (Hammoda et al. 2013). Previous investigations have identified *Z. coccineum* as a therapeutic herb (El-Shora et al. 2016). The leaves, fruits, and stems are used in traditional medicine to treat arthritis, rheumatism, asthma, and hypertension. The herb is additionally used as a diuretic, antihistaminic, local anesthetic, and anti-diabetic medication. Tannins may chelate free radicals, encourage wound closure, boost angiogenesis, and stimulate fibroblast proliferation. The majority of studies have demonstrated that tannins contain antibacterial and astringent properties that speed up wound healing by encouraging wound contraction, promoting angiogenesis, and activating fibroblasts (Yiannak-opoulou 2014). Therefore, this study aimed to prepare alcoholic extracts of *H. salicornicum* and *Z. coccineum* and their effect to treat burns associated with diabetes to understand their efficacy in treatment compared to the routine drugs used for this purpose.

Materials and methods

Animals: Forty-two albino white healthy male rabbits weighting 750-900g and 2-3 months in age, were obtained from the animal house in the Veterinary Medicine College of AlMuthanna University. They were kept in standard, separate cages, given full access to tap water, and fed continuously with standard pellets. The stainless-steel caging (40x60x80cm) had slotted floors and was used to cage animals individually.

The cage racks and pans were cleaned once a week and three times a week, respectively. All cages were kept in temperature-controlled spaces (20-22°C) with minimal lighting. The animals were separated for two weeks before to the experiment to allow for acclimatization. All animals were kept fasting for 21 hours before starting the experiments.

Study design: Animals were randomly distributed into seven groups with six rabbits each. All animals of the six groups were infected with diabetes, and after the onset of the infection and symptoms, on the 14 days, a second-degree burn occurred on their skin in the back area.

The treatments were (G1) treated with *H. salicornicum* tannins (H.T) ointment, (G2) treated

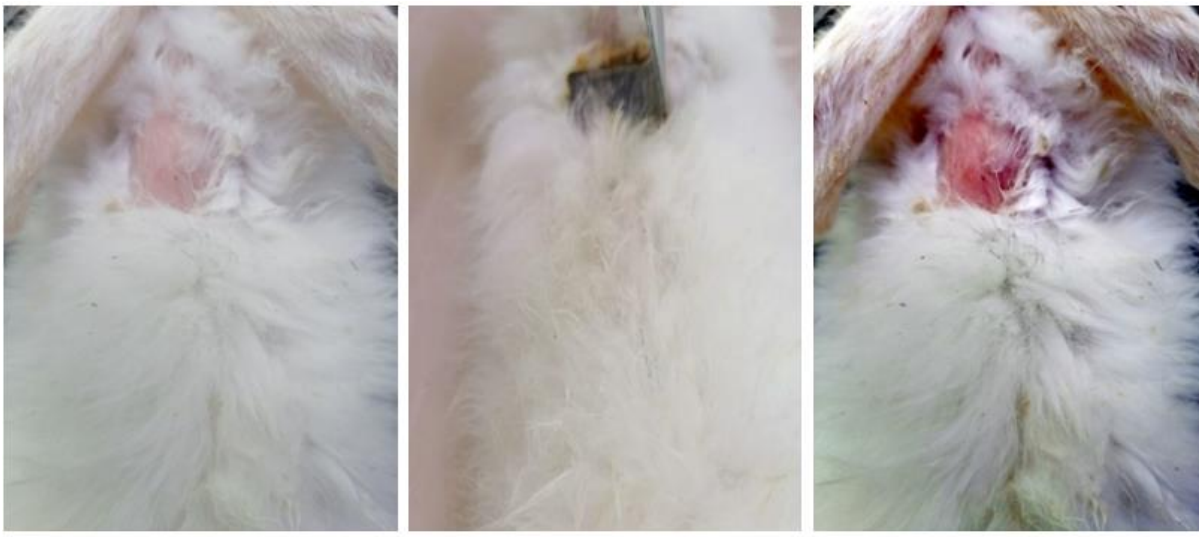

Fig.1. Clinical evolution observed in the experimental model of deep second-degree thermal burns in male albino rabbit. (a) Animal's skin after shaving, (b) Thermal lesion obtained with heated aluminum piece (c) Injured skin.

with *Z. coccineum* tannins (Z.T) ointment, (G3) treated with *H. salicornicum* crude (H.C) ointment, (G4) treated with *Z. coccineum* crude ointment, (G5) treated with chemotherapy (hamazine 1%) ointment, (G6) infected animals without treatment (negative control group), and (G7) healthy animals (positive control group).

Diabetes mellitus induction of rabbits: Diabetes was induced in rabbits by a single injection of alloxan monohydrate (150mg/kg. BW) dissolved in normal saline. The injection was performed over a period of 1 min (intraperitoneally) and then, the rabbit became diabetic. A solution of alloxan was used immediately after preparation and additionally, an oral solution of 20% glucose in tap water was provided ad libitum for 2 days after alloxan induction then was given to rabbit to counter the shock of blood sugar. After 72 hours of injection, animals with a blood glucose level of more than 200mg/dl were selected for the study (Manohar et al. 2012). Rabbits were weighed weekly throughout the study. There was a negative relationship between the highest blood glucose levels and body weight gain.

Measurement of blood glucose concentration: Glucose concentration was measured before administration and subsequently at 24, 48, and 72 hours after administration from the vein of the ears. Baseline nonfasting blood glucose levels were normal for all animals (115±13mg/dl). After the administration of alloxan, there was a characteristic response in blood glucose level concentrations of glucose in the blood of rabbits were determined with an on-call-plus glucometer using the strip method a drop of blood at one end of the strip on the glucometer after 10s. Blood samples were collected using syringes with a volume of 2ml, blood samples were taken at regular intervals from the heart after disinfection with 70% alcohol. The blood sample was 2CC and the serum was separated by centrifuging the sample for 15 minutes at 2500rpm. ELISA was used to determine the insulin levels in serum (Itelima et al. 2014).

Surgical technique of burn: After two weeks of infecting the animals with diabetes and after verifying the disease and the appearance of symptoms such as loss of appetite, lack of weight, frequent urine, excessive drinking of water, high blood sugar, and low level of insulin in the blood, the surgical operation was performed under sterile conditions. The surgical procedures were made under general anesthesia using a mixture of Ketamine hydrochloride (10mg/kg) and xylazine (3mg/kg)

administered intramuscularly (i/m) (Hillyer & Quesenberry 1977). Thermal injuries were made with a solid aluminum piece 2cm in length and width and a mass of 50g, the temperature was previously heated in boiling water until it reached 100°C as measured with a thermometer as shown in Figure 1 for 15 seconds. The aluminum piece was kept in touch with the animal's dorsal proximal region of skin. Analgesia with sodium dipyrone (40mg/kg) was provided intramuscularly immediately following the procedure and was maintained for three days straight through oral administration of sodium dipyrone (200mg/kg) in the animals' drinking water (Tavares Pereira et al. 2012).

Histological analysis: A histological examination was carried out by animals randomly selected and underwent an anesthesia combination of 10% ketamine (90mg/kg) and 3% xylazine (10mg/kg), intramuscularly (Hillyer & Quesenberry 1977). By administering large intraperitoneal dosages of sodium pentobarbital (100mg/kg), euthanasia was accomplished (De Luca et al. 1996) for the removal of the liver and pancreas tissue samples to confirm that injury and damage occurred before burning. To demonstrate the presence of a second-degree burn after burning, a piece of one of the animals' afflicted skins underwent histological testing. For histological studies, tissue samples were promptly fixed by immersion in buffered 10% formalin, processed in paraffin blocks, sectioned at 5µ, and stained with hematoxylin and eosin (H&E). In a histological investigation, the development of skin healing following thermal stress was assessed using a binocular optical microscope (Zeiss-Axiostar model) and a comparative descriptive analysis of the experimental groups.

Independent pathologists with experience in examining burn wound specimens conducted the histological analysis in the following: (1) Granular tissue, characterized by the presence of fibroblasts, myofibroblasts, and neovascularization, (2) inflammatory response, indicated by the presence of polymorphonuclear leukocytes (PNM), and (3) fibrosis, indicated by the density of collagen fibers determined by the intensity of blue color seen under optical microscopy due to staining with hematoxylin and eosin (H&E) (Kumar et al. 2006).

Clinical evaluation: Based on the following criteria, the clinical course of burn-related skin lesions was assessed over the course of 24 days: blistering, swelling, redness, crust, bleeding, secretion, granulation tissue, and scar tissue. A caliper was used to measure the wound retraction 3, 7, 10, and 14 days following the burn induction. Wound contraction was measured as a percentage decrease in the size of the initial wound.

% size wound contraction on day X=[(area on day 0 – open area on day X)/area on day 0] × 100. (16)

Alcoholic extract of *H. salicornicum* and *Z. coccineum:* Fresh *H. salicornicum* and *Z. coccinium* were collected as the aerial parts of the plant in March from southern Sammawa, Iraq. Powder of the aerial parts of the plants (50g) was macerated with 80% ethanol (250ml) with a stirrer by a magnetic bar for 24 hours at room temperature and filtered using filter paper. The clear filtrate was concentrated using a rotary evaporator at 40°C (Prakash, P. & Gupta et al. 2005).

Isolation of tannins from *H. salicornicum* and *Z. coccineum*: Powder of the aerial parts of the plants (50g) was treated with 250ml of 10% NaOH. Then the mixture was refluxed at 85°C for 24 hours. The mixture was cooled and filtered for the isolation of suspended and non-dissolved matter. After that 10ml of 10% sulphuric acid was added to the filtrate and the mixture was filtered and washed by dilute sodium bicarbonate to isolate the residue acid and salts (Feeny & Willcocks 1998).

Detection of tannins in plants extracts: It was detected using lead acetate 1% reagent, where 1ml of the reagent was added to 1ml of the extract if a light brown, gelatinous precipitate appears as an indication of the presence of tannins (Santhi & Sengottuvel 2016).

Separation by high-performance liquid chromatography (HPLC): The distribution of the

analyte (sample) between a mobile phase (eluent) and a stationary phase is the foundation of the HPLC separation principle (packing material of the column). Depending on the analyte's chemical components. In analytical chemistry and biochemistry, it is used to separate mixtures of chemicals to identify, measure, or purify the mixture's constituent components (Radovanović et al. 2015). It was used to separate the most important active compounds and the most quantitative ones found in the extracts of the two plants.

Antioxidant activity using DPPH radical scavenging assay: One common test for evaluating the antioxidant activity of plant extracts is the DPPH assay. With higher extract concentration ratios, DPPH radical scavenging activity tends to produce better results. In this test, a DPPH compound is employed as a stable free radical with a dark purple color. When the antioxidant chemicals are returned by giving it an electron or a hydrogen root, the free radical's color changes to yellow (Hu et al. 2011) to become a stable molecule as follows: (DPPH•) + HO-R-OH (DPPH): H + HO-R-O•

The reduction in optical absorbance at 517nm brought on by antioxidants yields information on the DPPH radical's return power. Some chemicals, such as phenolic compounds or flavonoids, may have strong free radical scavenging activities depending on the availability of hydroxyl groups in the plant (Bhakya et al. 2016). 0.1 mM DPPH in methanol solution was prepared, and 0.5mL of this solution was combined with 2.5mL of the methanol extract at various doses (1.25-100mg/mL) and for 30 minutes were spent at 25°C in the dark incubating the reaction solution. With a UV spectrophotometer, the absorbance was measured at 517nm. The positive control was ascorbic acid. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the sample and was calculated using the following formula of % Inhibition of DPPH = [(Ac-As) / Ac] x 100, where Ac is the absorbance of the control (DPPH + methanol) and as the absorbance in the presence of the DPPH + extract (Balan et al. 2016).

1.1 The use of extract concentrates for the treatment of burns: A direct concentration of the extract was applied to the shaved skin of the animal, causing redness and swelling of the skin. The extract was mixed with a neutralizing ointment at a concentration of 50% and applied to intact skin, but it also caused redness. Then a concentration of 25% was used and caused redness of the skin. And after continuous experiments with different concentrations, it was concluded that the concentration of 15% for the crude extract and 12.5% for tannin for both plants did not cause any harm to the skin. Therefore, these concentrations were applied to treat induced skin burns. These ointments are placed twice daily after washing the affected skin until the treatment is complete. The concentrations were made according to the density law, noting that the density of the ointment is approximately 0.85mg/ml, and then it was mixed using a sonicators for 40 seconds to ensure that it was mixed homogeneously.

Basic methods for microbial enumeration: A simple method for the enumeration of bacteria and fungi is based on the quantification of colony-forming units (CFU) per ml or g of sample. For this purpose, serial dilutions were prepared of the sample for determining the number of bacteria per mL in the original solution to take into account the average colonies of the selected dilution the dilution factor and the volume plated.

$$\text{CFU/ml} = \frac{\textbf{A} \text{ colonies (average)}}{\textbf{B} \text{ volume plated (ml)}} \text{ X } \textbf{DF} \text{ (Dilution factor)}$$

$$\text{CFU/ml} = \frac{\textbf{A} \text{ ecolonies (average)}}{\textbf{B} \text{ volume plated (ml)}} \text{X} \frac{1}{\textbf{CF} \text{ (Concentration factor)}}$$

The microbiological evaluation was carried out using “swabs” in the injury area at the moment of surgery and on respective days of biopsies. This sample was transferred to a Petri dish of 20×150mm containing nutrient agar medium in a laminar flow chamber. After 24 h incubation, plates inoculated in triplicate for each sample were evaluated. This

Table 1. The effect of *Zygophilium coccinium* crude (Z-Crude) extracts and *Z. coccinium* tannin (Z-tannin) with different concentrations of DPPH radical scavenging.

% Different concentrations of plants	% RSA (radical scavenging activity) at different concentrations (µg/ml)		
	Scorbic acid	Z-tannin	Z-Crude
100	98.35	82.23	87.19
90	98.21	82.09	86.36
80	97.93	81.82	85.95
70	97.66	80.58	84.44
60	97.25	78.79	83.20
50	96.97	78.37	83.33
40	96.83	77.82	80.30
30	96.56	76.58	78.79
20	96.28	73.83	74.10
10	95.87	69.70	70.80
5	95.32	16.12	66.39
2.5	94.49	7.71	60.47
1.25	94.08	1.38	51.10

routine evaluation was performed to evaluate the degree of contamination of injuries during the healing time at 1, 3, 5 and 7 days after the operation. After that biochemical tests were done for the purpose of diagnosing different bacterial species (Bhakya et al. 2016).

Antibacterial activity of plants extract: The disk diffusion method was used to evaluate the antimicrobial activity of each plant extract. The plant extract residues (50mg) were re-dissolved in 2.5ml of Dimethyl sulfoxide (DMSO) to obtain a concentration of 200mg/ml, then make dilutions by taking 1ml, adding 1ml of distilled water to obtain a concentration of 100mg/ml. Then 1ml of this concentration with 1ml water to obtain a concentration of 50mg/ml and ml of the last with 1ml water for getting a concentration of 25mg/ml. Loaded on sterile filter paper discs (8mm in diameter) after immersing in the four extracts concentrations.

Ten ml of Mueller-Hilton agar medium was poured into sterile Petri dishes (as a basal layer) previously inoculated with bacterial suspension (100ml of medium/1ml of bacteria) to attain 105 cell/ml of medium. Sterile filter paper discs loaded with plant extract were placed on the top of Mueller-Hilton agar plates. The plates were incubated at 37°C for 24 h. The inhibition zones were measured by Vernier caliper, recorded, and considered as an indication of antibacterial activity (Ashraf et al. 2012).

Detection of microorganisms: A swab was taken from the affected area and then the activated samples were cultured again on the nutrient agar, blood base agar, and MacConkey agar using the streaking method. To distinguish gram-positive and gram-negative bacteria, we first incubate bacterial colonies for 24 hours at 37°C, noting their morphology, including the edges of the colonies, their size, and their color. Next, it was preparing a gram stain and examine the colonies under a microscope with an oily lens. To identify bacterial species, biochemical assays were done (Collee et al. 1996).

Results

DPPH results: With an increased concentration ratio of extracts, the effects of DPPH radical scavenging operation were improved (Table 1, Fig. 2) showing a superiority of the tannin isolated 82.23% at a concentration of 100mg/ml and crude isolated 87.19% at 100mg/ml compared to ascorbic acid

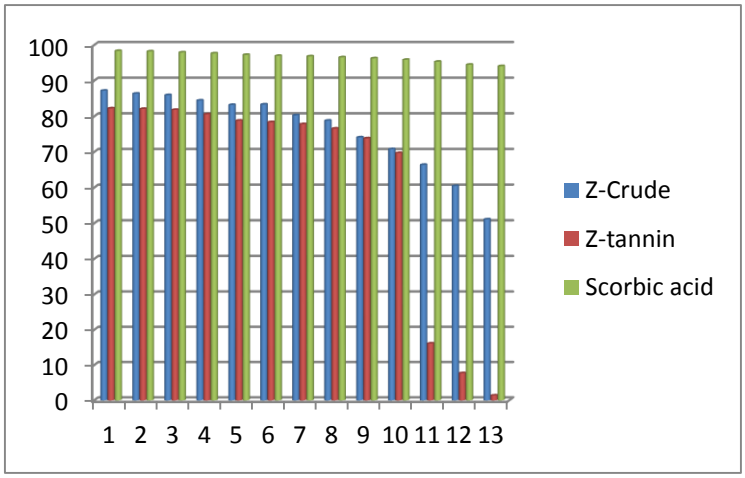

Fig.2. DPPH radical scavenging activity of *Zygophilium coccinium* crude (Z. crude) and *Z. coccinium*_tannin (Z. tannin) isolated with different concentrations when compared to the standard (ascorbic acid).

Table 2. The effect of *Haloxylon salicornium* crude (H-Crude) extracts and *H. salicornium* tannin (H-tannin) with different concentrations of DPPH radical scavenging.

% Different concentrations of plants	% RSA (radical scavenging activity) at different concentrations (µg/ml)		
	Scorbic acid	H- tannin	H-Crude
100	98.35	64.19	81.96
90	98.21	62.67	81.54
80	97.93	60.74	81.27
70	97.66	59.92	80.58
60	97.25	54.41	78.65
50	96.97	44.90	77.96
40	96.83	37.33	76.58
30	96.56	32.78	74.93
20	96.28	22.87	72.59
10	95.87	19.15	69.01
5	95.32	11.85	33.75
2.5	94.49	7.85	30.17
1.25	94.08	1.52	26.45

which recorded 98.35% at 100mg/ml. The lowest inhibition rate was obtained at 1.25 mg/ml. The tannin of *H. salicornium* was 64.19% at 100mg/ml and crude isolated 81.96% at 100mg/ml compared to ascorbic acid which recorded 98.35% 100mg/ml and the lowest inhibition rate was at 1.25mg/ml (Table 2, Fig. 3).

Separation of plant compounds by HPLC: The tannins extract of *H. salicornium* contains two essential compounds of quinic and tannic acids (Table 3, Fig. 4). The tannins extract of *Z. coccinium* contains three active compounds of coumarin, ellagic acid, and tannic acid (Table 4, Fig. 5). The existence of certain unknown compounds that are believed to be derivatives of polyphenols compounds was also shown by the chromatogram's peaks (Figs. 4, 5).

Diabetic infection: The concentration of glucose increased significantly after injection of alloxan until

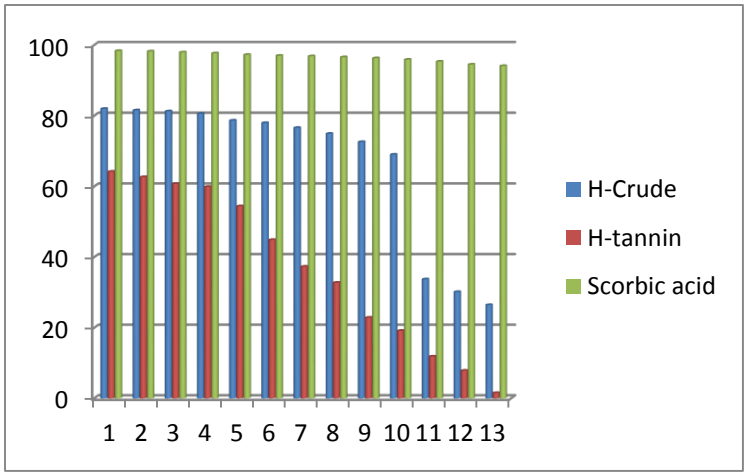

Fig.3. DPPH radical scavenging activity of *Haloxylon salicornium* crude (H. crude) and *H. salicornium* tannin (H. tannin) isolated with different concentrations when compared to the standard (ascorbic acid).

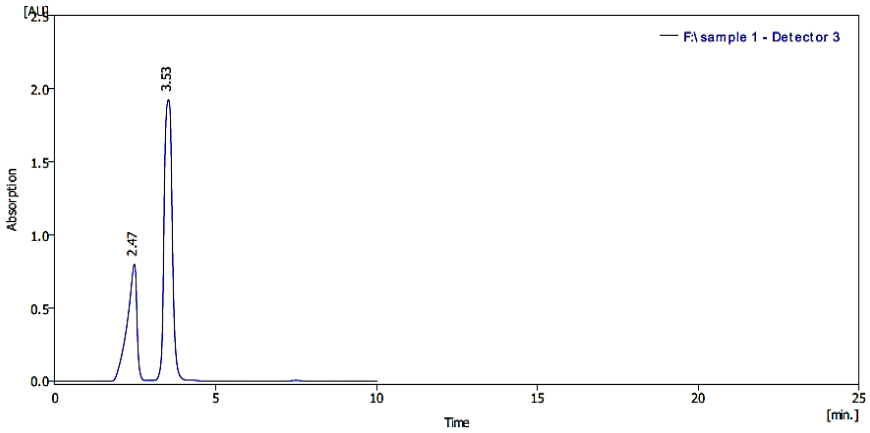

Fig.4. HPLC study of tannins compounds in *Haloxylon salicornium.*

Table 3. Retention time of tannins compounds in *Haloxylon salicornium.*

	Reten. Time (min)	Area (mAU.s)	Heigth (mAU)	Area (%)	Height (%)	WOS (min)	Compound Name
1	2.47	11652.19	712.089	35.7	32.4	0.28	Quinic acid
2	3.527	21032.657	1483.38	64.3	67.6	0.25	Tannic acid
	Total	32684.847	2195.468	100.0	100.0		

glucose reached approximately 250dl/mg in the blood of experimental animals. The proportion of insulin hormone decreased until it reached approximately 0.97ng/ml after the tenth day of the experiment while the insulin level was about 5.5ng/ml in the control group.

Histological examinations: The histological changes occurred in the liver of the infected rabbits on day 14 showing hepatocyte fatty degeneration with congested blood vessels and perivascular inflammatory cell accumulation. The pancreas of diabetic group on day 14 showed islets of Langerhans severe damage and inflammatory cell infiltration as a second-degree burn occurred after the burn exhibited greater damage area of desquamation epidermis (Fig. 6).

Clinical examinations: Clinical examinations of the animals showed significant variations among the groups. The animals of G7 showed clinical healing approximately at the twenty-fourth day postoperative while those of the treated groups showed clinical skin healing and return to the normal condition within

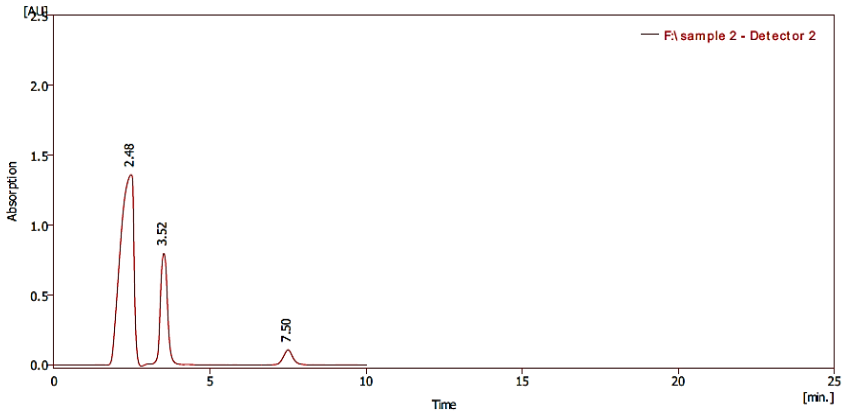

Fig.5. HPLC study of tannins compounds in *Zygophylium coccinium*.

Table 4. Retention time of tannins compounds in *Zygophylium coccinium*.

	Reten. Time (min)	Area (mAU.s)	Heigth (mAU)	Area (%)	Height (%)	WOS (min)	Compound Name
1	2.477	19808.185	812.457	70.8	55.0	0.39	Ella genic acid
2	3.523	7581.702	611.278	27.1	41.4	0.22	Coumarin
3	7.503	607.447	53.522	2.2	3.6	0.2	Tannic acid
	Total	27997.334	1477.257	100.0	100.0		

eight days in G1 and G2. While within twelve days postoperative and in G3, G4 and G5 it was about thirteen or fourteen days. The significant values between the trial groups at the time of recovery were: P=0.036 between G1 and G6; P=0.043 between G2 and G6; P=0.031 between G3 and G6; P=0.041 between G4 and G6; P=0.5 between G5 and G6 (Table 5, Fig. 7).

Microbiology count: There was a noticeable decrease in the number of microorganisms in the part of the burned skin during the treatment period using a concentration of bacteria at 10^{-4} in tannins groups at (P=0.021) compared with the control group and crud groups (P=0.086) (Table 6, Fig. 8).

Antimicrobial activity of the alcoholic extract, tannin, and flavonoid Extracts: The alcoholic extract (methanol 80%) of two plants and tannin extracts have antimicrobial efficacy against *Candida albicans, Staphylococcus aureus*, *Pseudomonas aurogineus*, *Esherishia coli* and *Klepssilia peumonium* that were isolated from burn skin areas of diabetic rabbits using discs dipped in four concentrations of this extracts (200, 100, 50, and 25) on Muller Hilnton agar (Table 7, Fig. 9) in comparison with chemotherapy treatment. The extracts of both plants were the most effective against all types of studied microorganisms and by increasing the concentration, the anti-microbial activity increased with some differences.

Discussion

The use of conventional plant extracts and alternative therapies has recently raised awareness of their accessibility, efficacy, and lack of negative side effects. Since phenols have a polar appearance and are primarily responsible for their antioxidant action, they are proven to be powerful antioxidants (Ahvazi et al. 2012). Studies have linked this extract's tannins' effect on the antioxidants' ability to displace tannins as a possible explanation for this action (Seyoum et al. 2006) and their antioxidant activity increases with concentration.

The results of the current work showed that the extracts have an oxidative activity that has a synergistic effect on the antioxidant effect of DPPH radical. The high contents of total phenolic, total flavonoid, and tannin compounds in *H. salicornicum* and *Z. coccineum* alcoholic extracts can contribute to important antioxidants because of the strong

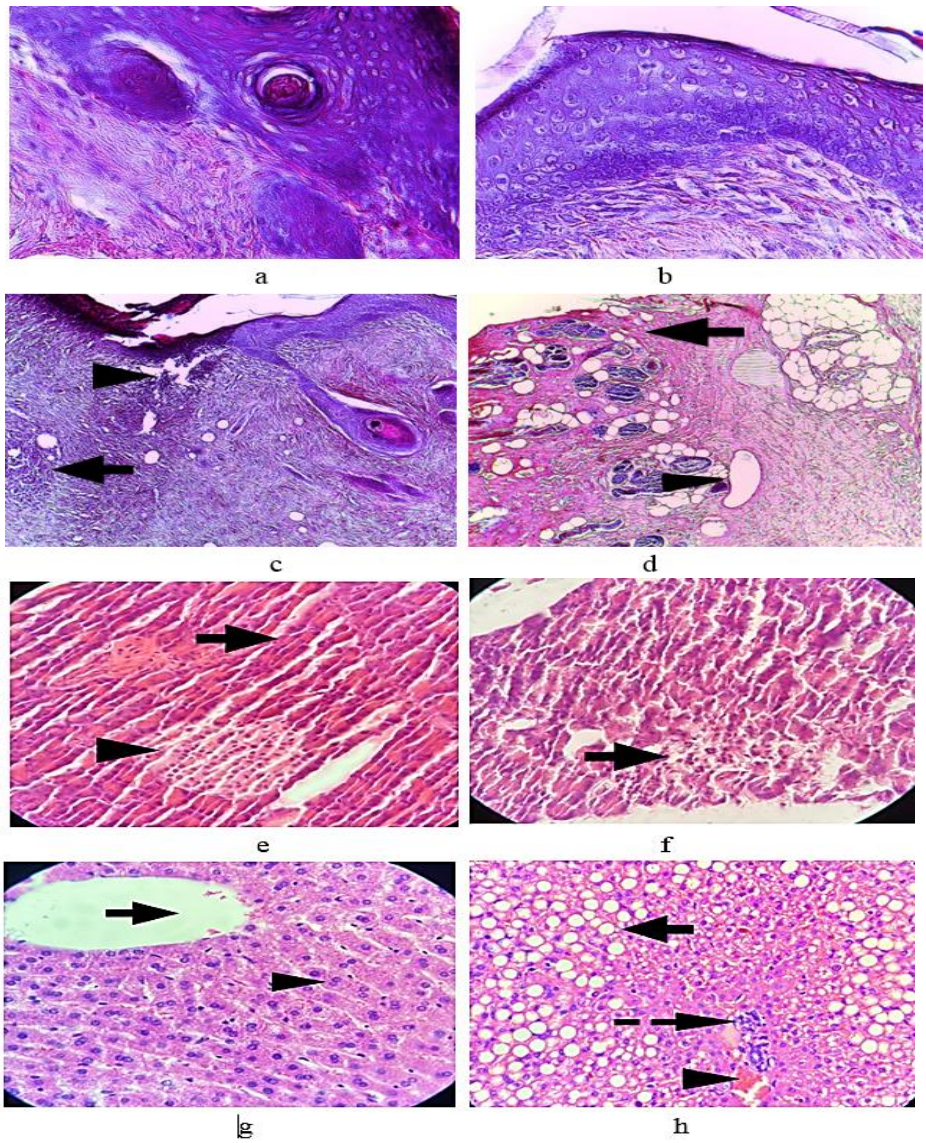

Fig.6. *Photomicroscopy* results: (a) Normal skin *(H&E, 40*X*), (b)* Normal skin *(H&E, 40*X*), (c) photomicroscopy of the skin of a rabbit with the second-degree burn on day 3 after the burn exhibited Coagulation necrosis was identified in the epidermis (arrowhead)*

Group day	G1	G2	G3	G4	G5	G6
1	32000±0.57	24000±0.18	28000±0.52	22000±0.65	27000±0.23	38000±0.39
3	20000±0.96	15000±0.3	20000±0.82	18000±0.68	15000±0.6	95000±0.75
5	12000±0.61	9000±0.27	16000±0.38	17000±0.72	8000±0.52	345.000±0.67
7	4000±0.26	6000±0.81	9000±0.84	11000±0.6	4000±0.82	850.000±0.77

congested blood vessels (arrowhead) and perivascular inflammatory cell accumulation (dotted arrow) (H&E, 400X).

relationship between the concentration of the compounds. Antioxidant phytoconstituents can transfer hydrogen atoms or an electron (Taia 2006). In the current study, the burn wounds in the experimental animals healed faster using the plant extract compared to the control group. Agarwal et al. (2009) and Ranjbar-Heidari et al. (2012) reported that in the aspect of wound healing several plants have a reliable therapeutic effect when topically applied and referred to the high content of active phytochemical ingredients, such as flavonoids, tryptamines, alkaloids, and tannins which proved to

Table 5. W[illegible] abbits each. $P \leq 0.05$, sig[illegible]

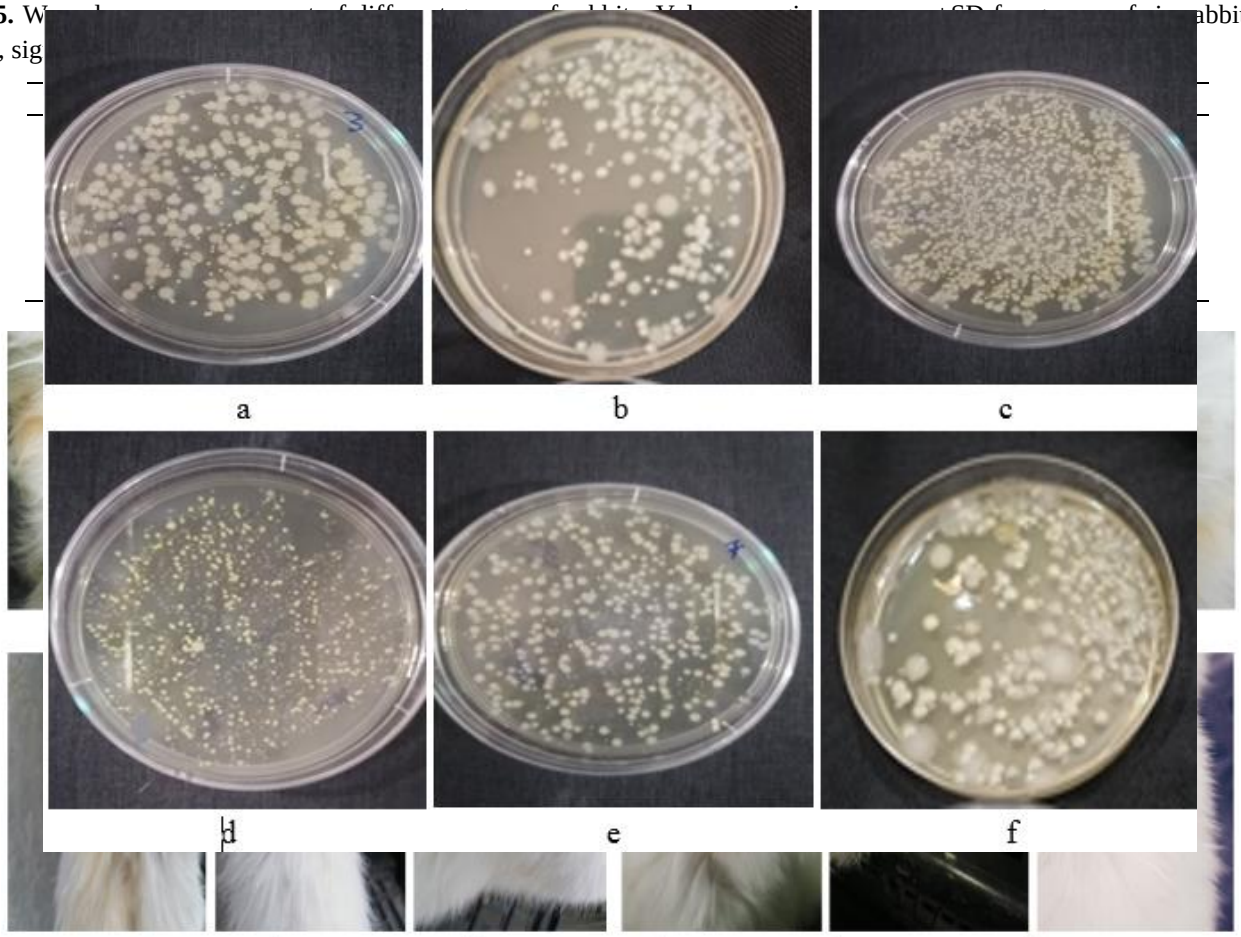

Table 7. The inhibition zone in different groups as mean±SD for groups of six rabbits each.

Fig.7. Clinical evolution of injured tissue on: (a)5th day after burn induction in G1 (b) 5th day after burn induction in G2 (c) 5th day after burn induction in G3 (d) 5th day after burn induction in G4 (e) 5th day after burn induction in G5(f) 5th day after burn induction in G6 (g)10th day after burn induction in G1 (h) 10th day after burn induction in G2 (i) 10th day after burn induction in G3 (j) 10th day after burn induction in G4(k) 10th day after burn induction in G5 (l) 10th day after burn induction in G6.

Table 6. The numerate of microorganisms on the injured skin of experiment groups in different days after burn as mean±SD for groups of ten rabbits each.

promote healing of the wounds. Due to a strong influence on the flavonoids and tannins, these substances also have an antibacterial effect on the microorganisms in the area of injury, which was supported by other research, e.g. Bhattacharyya & Jha (2011) found that the phenolic chemicals of plant extracts, which are known to have antibacterial action, are responsible for the breakdown of the membranes and cell walls of microscopic organisms, which can be linked to the antibacterial activity.

Additionally, the presence of active substances such as saponins, tannins, alkaloids, steroids, phenols, and flavonoids may be a contributing factor to the antibacterial activity (Javid et al. 2012) and the interaction between the chemical substances that operate synergistically. In general, the differences in the way positive-Gram and Gram-negative bacteria respond to antibacterial agents are mostly caused by

their cell walls are constructed. While negative bacteria have a cell wall with numerous layers separated by an exterior cell membrane, positive bacteria have a single layer in their cell wall (Javid et al. 2012) and this agrees with Wolfson & Hooper (1985) who found that gram-positive bacteria strains

Fig.8. The numerate of microorganisms in (a) 5th day in animals of G4 (b) 5th day in animals of G3 (c) 3th day in animals of G6 (d) 5th day in animals of G2 (e) 5th day in animals of G1 (f) 7th day in animals of G6.

Treat	*Con.*	*Staph.*	*Pseudo.*	*Klebs*	*E. coli*	*candida*
C.Z	200	26±0.878	23±0.71	33±0.451	32±0.234	28±0.691
	100	19±0.96	21±0.612	21±0.093	27±0.05	22±0.901
	50	13±0.05	17±0.735	11±0.324	12±0.52	22±0.482
	25	6±0.2	8±0.487	7±0.57	9±0.003	15±0.09
T.Z	200	22±0.85	19±0.691	21±0.82	24±0.06	22±0.05
	100	18±0.234	12±0.037	16±0.632	16±0.723	17±0.293
	50	12±0.786	8±0.243	9±0.482	11±0.633	12±0.432
	25	7±0.986	6±0.581	9±0.73	8±0.75	6±0.76
T.H	200	27±0.132	22±0.814	28±0.651	24±0.75	20±0.521
	100	22±0.045	18±0.884	18±0.771	17±0.634	13±0.803
	50	22±0.563	12±0.71	11±0.682	15±0.04	9±0.61
	25	14±0.34	8±0.39	10±0.53	6±0.612	7±0.065
C.H	200	18±0.7	17±0.14	24±0.143	27±0.78	28±0.781
	100	18±0.003	15±0.02	17±0.62	21±0.007	22±0.538
	50	12±0.165	15±0.1	7±0.581	9±0.56	30±0.81
	25	10±0.34	7±0.315	7±0.7	8±0.283	16±0.156

Key: C.Z (crude of *Z. coccinium*), T.Z (tannins of *Z. coccinium*), T.H (tannins of *H. saliconium*), C.H (crude of *H. saliconium*).

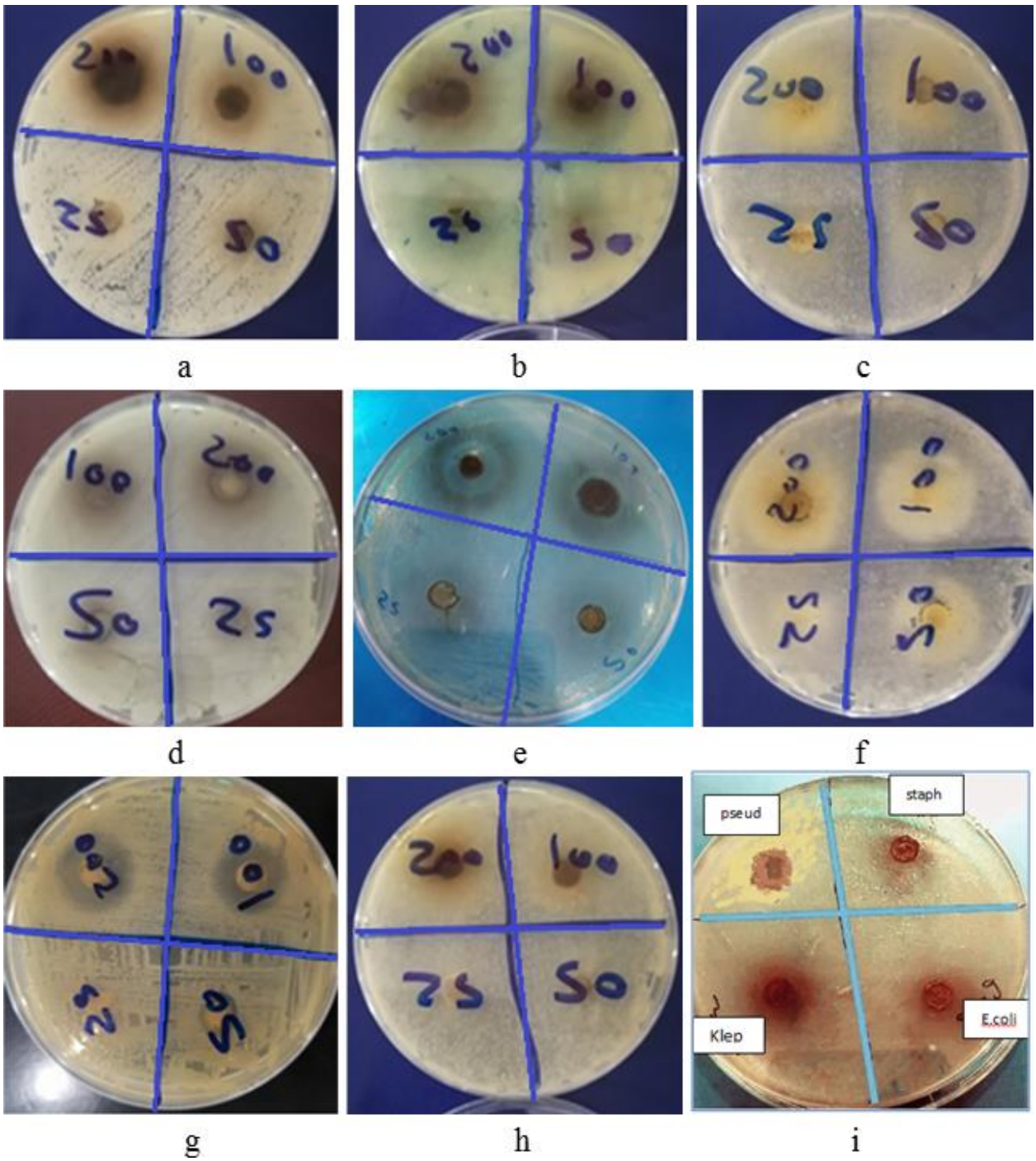

Fig.9. Inhibition zone of (a) *Staphylococcus aureus* with TH, (b) *Klebsiella pneumoniae* with TH (c) *Klebsiella pneumoniae* with CH, (d) *Escherichia coli* with CH, (e) *Staphylococcus aureus* with TZ (f) *Escherichia coli* with TZ, (g) *Klebsiella pneumoniae* with CZ, (h) *Escherichia coli* with CZ, (i) Bacteria types with hamizine 1%.

were generally more sensitive to the extracts. The phenolic chemicals may have an impact on antibacterial action. Additionally, a shift in the strain of bacteria, or the introduction of novel bacterial mutations as a result of storage or frequent transfer of isolate bacteria could be to blame for the lack of inhibitory action. Ali-Shtayeh et al. (1998) also showed that the disruption of bacterial cell membrane structure is one of the antimicrobial pathways of phenolic compounds. Under aqueous conditions the -

OH groups in phenolic compounds are extremely reactive and react with a variety of biomolecules, causing deformation and bacterial growth inhibition. Phenolic compounds also play a role in protein and cell wall-binding, bacterial enzyme inactivation, and DNA intercalation during replication. Ryan (1984) showed that phenolic chemicals can suppress germs due to an iron deficit or hydrogen bonding with vital proteins like microbial enzymes.

Alloxan effectively partially degrades the beta cells of the pancreatic islets, which reduces both the quality and quantity of insulin produced by these cells. The model employs two distinct pathogenic effects, including the selective inhibition of insulin production induced by glucose and the development of reactive oxygen species (ROS), which promotes the selective necrosis of beta cells in the pancreas. Cells become insulin-dependent diabetes mellitus or type 1-like when these two processes work together (Forbes & Cooper 2013). The former is associated with alloxan's specific inhibition of the pancreatic glucose sensor enzyme glucokinase, whilst the latter is more associated with alloxan's ability to engage in redox cycling, which results in the generation of ROS. Significantly, these effects have been linked to alloxan's chemical makeup and structure (Kitabchi et al. 2009). This is consistent with the results of this study of high blood glucose (about 250mg/dl) and low insulin concentration (about 0.95ng/ml).

Gerard (2014) pointed out that the pancreas' work excess eventually causes the beta cells to die. In the muscle, liver, and fat tissues, insulin resistance prevents glucose from reaching the cells. A severe burn could cause systemic inflammation, which would then cause insulin resistance. In addition, the liver continues to produce glucose due to insulin resistance and impaired repair. Laguens et al. (1980) suggested this hypothesis by noting that dilation of cisterns and degranulation of the rER surface. In the hepatocytes of diabetic mice, mitochondrial enlargement and a lack of mitochondrial cristae were observed. The livers of the infected animals showed histological changes that varied from fatty liver cell degeneration to steatohepatitis and periportal fibrosis. This observation was consistent with Forbes & Cooper (2013) who showed that the livers of diabetic mice frequently exhibited abnormalities affecting all of the organ's constituent parts, such as the sinusoids and portal regions, hepatocytes, nuclei, and intracytoplasmic organelles.

Due to the increased production of reactive oxygen species (ROS) and the nonenzymatic glycation of numerous macromolecules, which results in alterations to cellular structure and function and the creation of advanced glycation end products, hyperglycemia causes abnormalities in cellular metabolism (AGEs). By interacting with the particular AGE receptor (RAGE), the synthesis of AGEs intensifies metabolic abnormalities and also promotes the production of reactive oxygen species (Lucchesi et al. 2015). As a result, the basement membrane's structure and biophysical characteristics change, altering the permeability and dilation of blood vessels (Chien et al. 2010).

Burns are frequent traumatic injuries that cause local tissue damage and a systemic mediator-induced reaction. Increased free radical activity and lipid peroxidation are signs of both local and systemic oxidant alterations. It is well-recognized that free radicals and their scavenging mechanisms are crucial for both normal and slow wound healing (Sahib et al. 2009). This agreed with the decreasing levels of antioxidants in the control group. Burn injury, as compared to control, results in a striking decrease in total antioxidant status and a loss in antioxidant scavenging activity (Grunwald & Garner 2008). Targeting this state with antioxidants may be thought of as a promising start for improving the outcome of burns when free radicals are present in amounts that overwhelm natural radical blocking or scavenging processes (Sahib et al. 2009).

Conclusion

Medicinal plants are rich in many effective antioxidant compounds that are used to neutralize free radicals formed in damaged or infected tissues in different parts of the body to reach a state of recovery

and return to a normal neutral state. Tannins are considered the most powerful antioxidants that contain effective hydroxyl groups found in large quantities in desert medicinal plants and different parts according to the type of plant. They were extracted for the first time in this study from *H. salicornium* and *Z. coccinium* collected from the Samawa desert. They were used in folk medicine to treat burns and were used as an ointment to treat burns in diabetic animals, and they proved the high effectiveness in treatment compared to chemotherapy and accelerated the healing period of the wound. Changes from the normal state were recorded in many blood parameters due to the effect of sugar and burning and some of them returned to normal values after the completion of healing, which indicates the therapeutic effectiveness. These extracts and their ability to resist free radicals that destroy tissue.

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