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Evaluation of the healing activity of Cucurbita spp. leaf and seed extracts on experimental thermal burns

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Abstract

Burns are the most common skin injuries associated with many complications and high incidences of wound infections. Many compounds of plant products have been assessed to accelerate wound healing or reduce contamination. The possible healing effect of two *Cucurbita* spp. in skin burns was investigated. *Cucurbita maxima* and *Cucurbita pepo* leaves and seeds were extracted using ethanol and analyzed by gas chromatography–mass spectrometry. Second-degree burn wounds were made on laboratory rats, and the burn wounds were applied topically with extracts for 14 days and silver sulfadiazine (SS) for comparison. Serum vascular endothelial growth factor (VEGF) was analyzed post-treatment. Hydroxyproline content and histopathological changes were evaluated in skin biopsy. Phytochemical analysis displayed that *C. maxima* leaf extracts contain linolenic acid (21.94%), phytol (6.45%), and ascorbic acid (9.09%), and they were characterized by glucopyranoside (8.49%) and limonene (5.39%) compounds. The *C. pepo* leaves were characterized by palmitic acid (48.04%) and alkaloid (tetratriacontane: 13.11%). The seeds extracts of both *Cucurbita* spp. revealed a high ratio of linoleic acid, palmitic acid, and ascorbic acid. The leaf extracts accelerated wound contraction by 100%, with normal skin appearance, due to their more structural tissue layers, regular collagen fibers, ability to reduce inflammation, and high hydroxyproline content compared to the seed extracts and SS group. The bioactivity of unsaturated fatty acids, ascorbic acid, monoterpene, glycosides, and alkaloids provided the therapeutic property of these extracts. Serum VEGF was significantly reduced in the groups treated with extracts compared with those treated by the SS group. The leaf extracts of both *Cucurbita* species exhibited a healing efficiency on burn wounds, which were more effective than the seed extracts. Thus, they can be used as a promising plant derivative in burn treatments.

Keywords

animal model; collagen; healing property; plant compounds, skin injury; vascular endothelial growth factor

1. Introduction

Burn injuries have one of the highest mortality rates in the world. Survivors suffer from chronic disabilities due to scar formation and psychological and physical problems [1]. Burns mostly result from thermal, chemical, electrical, and radiation exposure [2]. They are considered as one of the major public health problems in Basrah Province, and the common skin injuries are flame (51%) and scalds (41.7%) [3]. Approximately 50% of samples collected from people in Baghdad Province who suffered from burning are classified as second degree; flame and scalds were also the most relevant causes [4]. Various burn treatments have been utilized to reduce microbial contaminations and facilitate advanced skin healings [5].

Wound repairs include, physiological process that could be categorized into phases: inflammation, hemostasis, proliferation (granulation), and remodeling (maturation) of the extracellular matrix [6]. The inflammation phase comprises the coagulation process and the response of the immune system [7]. The homeostasis phase consists of clotting and fibrin formation and the activation of complementary immune system [8]. The proliferation phase involves the formation of granular tissues that contain fibroblast and endothelial cells for vessel angiogenesis [9]. While, the maturation phase involves collagen and the deposition of extracellular matrix proteins [10]. Angiogenesis is a vital stage for wound repairing as it provides oxygen and nutrition to the wound area. Vascular endothelial growth factor is the crucial proangiogenic mediator stage because it stimulates the proliferation, migration, differentiation, and survival processes of endothelial cells [11]. Hydroxyproline represents a biochemical marker of collagen in tissue [12], and it enhances the collagen deposition, proliferation, and migration of fibroblasts [13]. Finally, it stimulates collagen synthesis through promoting angiogenesis and improving the repair process [14]. The rate of hydroxyproline is an indicator of fiber stabilization and collagen synthesis, and consequently, it refers to the rate of wound healing [12]. The histological sections of burn wounds are critical for estimating the healing process. The histological score for wound healing shows different grades of healing phases that assess the important criteria in histological evaluation [15].

The normal process of wound repair can be impaired by many factors, such as oxidative stress and pathological status of the body, with wound infection being the main cause of mortality in burn injuries [16]. Therefore, many investigations focused on determining the appropriate treatments or compounds to decrease healing time and reduce microbial infections, for example, using antimicrobial agents [17]. However, in many cases these agents cause skin irritation or allergic reaction, which increases the recovery time [5]. Moreover, their actions are to prevent infection rather than heal, and sometimes they exhibit a negative effect during the repair process [18]. Other agents cause a cytotoxic effect; for example, silver derivative ointment is toxic to keratinocytes and fibroblasts [19]. Antimicrobial agents can occasionally lead to resistance [20]. Some studies referred to various substances useful in burn wound treatment, but wound healing is challenging. Therefore, researchers have attempted to extract compounds with the ability to accelerate the healing process. For example, plant derivative products can enhance healing in burn injuries [21]. The antioxidant contents of some medical plants showed therapeutic effect on wound repairing by protecting from oxidative damage and enhancing the healing process [22]. For instance, the fatty acid (oleic acid) extracted from pumpkin seeds showed an efficient activity in moderating the inflammatory response in the wound areas through synthesis of phospholipid membrane and enhancing epithelial tissue repairing [23,24].

Many studies referred to the possibility of using medical plants to treat burn injuries, while others developed compounds to accelerate the healing process and decrease microbial infections with less adverse effect compared with the available burn ointments [25,26].

Cucurbita species belong to the Cucurbitaceae family, they are one of the most widely used vegetable plants as food and traditional medicine due to their health benefits [27]. Among various medicinal plants, the *Cucurbita* species are a vital medicinal plant cultured in most regions of Iraq; the fruits are used in daily nutrition. The plant is an annual creeping herbaceous; its stem and leaves are covered with bristles; the leaves are lobed; the flowers are yellow axillary; and the fruits are large, elongated green or yellowish in color with many seeds. In Iraqi traditional medicine, the plant seeds are eaten to kill nematodes and treat prostate enlargement [28].

Pumpkin contains many functional compounds, including phytosterol, tocopherol, and monounsaturated

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fatty acids [29]. The oil extract of *Cucurbita maxima* seeds showed various therapeutic activities, including hypocholesterolemia and hypoglycemia activities, lowering blood pressure, reducing the growth of gastric cancer and breast cancers [30,31], diuretic properties [32], and antibacterial effect [33]. The pharmaceutical effects of *C. maxima* extracts include antidiabetic [34], antioxidant [35], and anticancer activities [36]. Moreover, the oil extracts of *Cucurbita pepo* showed antioxidant activity because of their high phenolic compounds [37]. Meanwhile, the steroid fraction of the seed extract displayed anti-inflammatory action [38]. The ethanoic fruit extract exhibited inhibitory effect against cancer HeLa cell line [39], while the methanol extracts of the leaves showed antibacterial effect [40]. Given their therapeutic potential on skin injuries, and limited studies in their role, the present study was conducted to estimate the healing activity of leaves and seeds ethanol extracts from *C. maxima* and *C. pepo*, which were planted and distributed in Iraq, on burn wounds on an experimental animal model and compared these effects with those of traditional medical ointment silver sulfadiazine.

2. Materials and methods

2.1. Plant materials and collection

C. maxima (pumpkin) and *C. pepo* (squash) leaves and fruits with seeds were obtained from one of the farms in Basrah. The plants were classified and cultured in the College of Science, University of Basrah, Iraq, in August 2018. Fresh leaves and seeds were used, the plants materials were cleaned with water, dried at room temperature, grinded with an electric grinder, and stored at −7 °C.

2.2. Chemical and reagents

Absolute ethanol, hydrochloric acid, formaldehyde, xylene, and chloroform were bought from Scharlab (Spain). Ketamine hydrochloride, xylazine, chloramine T agent, and pure hydroxyproline were gotten from Sigma−Aldrich (USA). Silver sulfadiazine (SS) cream was also used (Flamazine, 1% w/w, Jorden).

2.3. Preparation of extracts

Extraction was performed in accordance with the method of Ladd et al. [41]. Twenty grams of dried grinded plant material was placed in thimbles of Soxhlet apparatus with 250 ml of absolute ethanol and then extracted in 45−50 °C for 24 h. The process was repeated several times to obtain a sufficient amount of each extract. The extracts were processed in a rotary evaporator and stored at −7 °C.

2.4. Gas chromatography−mass spectrometry (GC-MS) analysis

GC-MS analysis of plant extracts was performed using a Shimadzu GC−2010 system coupled with Shimadzu GC−2010 Ultra network mass selective detector and equipped with a DB-1 MS capillary fused silica column (30 m, 0.25 mm ID, and 0.25 mm film thickness) in the college of Agriculture, University of Basrah. The extracts were esterification according to Kitson et al. [42]. Ethyl ester (1 µl) in methanol for leaves extracts and methyl ester (1 µl) in hexane for seeds extracts were injected and analyzed. The operating conditions were as following: for leaves extracts: column oven temperature 40 C°, injection temp. 250C°, injection mode split, split ratio 30. For seeds extracts: column oven temp. 40C°, injection temp. 280C°, injection mode direct. The relative percentages of the separated compounds were calculated via total ion chromatography by using computerized literature data and MS data obtained from the NIST 08 library [43].

2.5. Experimental animals

Male Wistar albino rats (200−250 g, 3 months old, n = 72) were obtained from the college of Veterinary medicine, University of Basrah. The animals were housed individually in standard cages under constant conditions (temperature 25± and 12:12 h light: dark cycles) and with free access to a standard diet and water. The animal Care and Experimental Ethic Standard Protocol were followed according to University Animal Ethics Committee.

2.6. Thermal burn experimental model

All experimental rats were anesthetized via intramuscular injection of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg) with a 2:1 ratio. The dorsal surface of the rats (near the neck region) was shaved and treated with an antiseptic (70% ethanol). Second-degree burn wounds were made by applying a hot plate (diameter 1.5 cm × 0.5 cm), which was placed on a burner for 5 min, and then on the skin for 10 s with equal pressure. Only a single burn wound was made for each animal [44].

2.7. Application of extracts and obtaining biological samples

After inducing thermal burn wounds on the experimental animals, the rats were randomly divided into six groups (12 rats each): group A: treated with *C. maxima* leaf extract; group B: treated with *C. pepo* leaf extract; group C: treated with *C. maxima* seed extract; group D: treated with *C. pepo* seed extract; group E: treated with silver sulfadiazine cream as reference group (SS); and group F: untreated burn wounds. The burn areas were treated with a topical application of 0.52 µl/mm^2 of experimental extract once daily [45] starting from the day of wound induction up to 14 days. Rats (n = 6) from each group were anesthetized and scarified after 7 and 14 days of the experimental period. Blood samples were obtained from heart punctures, and the serum was stored at −70 °C. The skin biopsy samples (tissue from wound area) were collected for hydroxyproline determination and histopathological study.

2.8. Wound contraction rate

The wound contraction rate was evaluated using graph sheets at each experimental period of 7 and 14 days by using the following formula [46]: % wound contraction on day X = [(area on day zero - area on day X)/area on day zero] × 100.

2.9. Hematological and biochemical study

The hematological parameters The hematological parameters from all groups included red blood cells (RBC 10^{12} µl), white blood cells (WBC 10^6 µl), hemoglobin (Hb g/dl), packed cell volume (PCV %) and platelets (10^9 µl), which were automatically analyzed (SYSMEX XT 2000i blood analyzer). The serum vascular endothelial growth factor (VEGF) was measured by using an ELISA kit (Rat VEGF, Thermo Fisher Scientific, USA).

2.10. Determination of hydroxylproline content in skin biopsy

The hydroxyproline level in skin tissue was determined as described previously according to Bergman and Loxley [47]. After desiccation, the tissue was hydrolyzed with hydrochloric acid (6 N) at 105 °C for 3 h and then oxidized by adding chloramine T agent. Solution absorption was recorded using a spectrophotometer at 557 nm. The value of tested solutions was estimated by using a standard curve from pure hydroxyproline.

2.11. Histological study

Skin specimens from each experimental groups after 14 days of treatments and skin biopsy from burn onset rats (positive control) and from the healthy rats (negative control) were fixed in 10% neutral formalin solution at room temperature for 24 h. Section preparation and tissue processing were conducted as described previously according to Luna [48]. Sections with 7 µm thickness were stained with a hematoxylin–Van Gieson stain and then examined and photographed using a Leica digital light microscopic camera (Leica, Allendale, NJ). The morphometric analysis of histological sections was performed using image J program (1.53e) to estimate the thickness of the epidermis, diameter of hair follicle and sebaceous glands, the density of collagen fibers, using five replicate for each group and for each morphometric parameter.

2.12. Statistical analysis

The data was analyzed by a general linear model using the statistical program SPSS version 22. The morphometric data was analyzed by one-way ANOVA. The comparison between means was according to the least significant differences ($p \leq 0.05$). The data as expressed as mean ± S.D (standard deviation).

3. Results

3.1. Plant extract composition

The results of GC-MS analysis of both ethanol leaf and seed extracts are shown in Tables 1 and 2. *C. maxima* leaves contained linolenic acid (9, 12, 15-octadecatrienoic acid 21.94%), ascorbic acid (9.09%), glycosides (glucopyranoside 8.49%), phytol (6.54%), limonene (monoterpenoid 5.39%) and eucalyptol (1.96%), whereas *C. pepo* leaves comprised palmitic acid (hexadecanoic acid 48.04%), alkaloid (tetratriacontane 13.11%), phytol (2.83%), linolenic acid (5.01%), and ascorbic acid (3.48%). *C. maxima* seeds contained linoleic acid (9, 12-octadecadienoic acid 45.34% & 23.89%), palmitic acid (15.12%), and ascorbic acid (4.73%), whereas *C. pepo* seeds comprised linoleic acid (58.25% & 6.42%), palmitic acid (16.65%), stearic acid (octadecanoic acid 8.96% &1.09%), and ascorbic acid (1.35%).

Table 1
The chemical composition of ethanol leaves extracts of *Cucurbita* spp. by GC-mass analysis.

C. maxima	Compound	Retention Time	Molecular weight	Molecular formula	Peak Area%
1.	D-Limonene	6.025	136	C10 H16	5.39
2.	Eucalyptol	6.074	154	C10 H18 O	1.96
3.	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	6.482	136	C 10H16	4.94
4.	beta.-D-Glucopyranoside, methyl	14.301	194	C7 H14 O6	8.49
5.	Ethyl.alpha.-D-glucopyranoside	14.367	208	C8 H16 O6	5.77
6.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	17.855	652	C38 H68 O8	9.09
7.	Phytol	19.568	296	C20 H40 O	6.54
8.	9,12-Octadecadienoic acid (Z,Z)-	19.842	280	C18 H32 O2	1.87
9.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- **	19.924	278	C18 H30 O2	21.94
10.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	23.438	330	C19 H38 O4	2.24
11.	1-Heptacosanol	26.379	396	C27 H56 O	2.15
12.	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)	26.669	402	C27 H46 O2	2.25
13.	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	29.760	414	C29 H50 O	2.35
14.	Nickel tetracarbonyl	3.022	170	C4NiO4	2.09
15.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene	5.216	136	C10H16	7.85
16.	Benzene, 1-methyl-3-(1-methylethyl)-	5.953	134	C10 H14	4.95
17.	Benzaldehyde, 4-(1-methylethyl)-	9.281	148	C10 H12 O	2.15
18.	Acetic acid, 2-(2,2,6- trimethyl-7-oxa-bicyclo[4.1.0] hept-1-yl)-propenyl ester	15.865	238	C14 H22 O3	1.48
19.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	16.468	296	C20 H40 O	1.55
20.	Dichloroacetic acid, tridec-2-ynyl ester	20.177	306	C15 H24 CL2O2	4.95
C. pepo					
1.	d-Mannitol, 1,4-anhydro-	12.247	164	C 6H12 O5	12.61
2.	alpha.-D-Galactopyranoside, methyl	14.309	194	C7 H14 O6	3.15
3.	3-Eicosyne	16.469	278	C20 H38	1.37
4.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	17.846	652	C38 H68 O8	3.48
5.	Hexadecanoic acid, ethyl ester	18.220	284	C18 H36 O2	0.76
6.	Phytol	19.568	296	C20 H40 O	2.83
7.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-**	19.907	278	C 18H30 O2	5.01
8.	Hexadecanoic acid, 2-hydroxy- 1-(hydroxymethyl)ethyl ester	23.446	330	C19 H38 O4	1.07
9.	gamma.-Tocopherol	27.383	416	C28 H48 O2	1.08
10.	Tetratriacontane	27.708	478	C34 H70	13.11
11.	Hexadecanoic acid, cyclohexyl ester	27.921	338	C22 H42 O2	48.04
12.	Stigmasta-7,25-dien-3-ol, (3.beta.,5.alpha.)-	29.232	412	C29 H48 O	2.61
13.	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	29.766	414	C29 H50 O	2.26
14.	Dichloroacetic acid, tridec-2-ynyl ester	20.177	306	C15 H24 CL2O2	2.06
15.	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	25.731	410	C30 H50	0.56
					100.00

3.2. Percentage of wound contraction

Topical application of ethanol leaves extracts (group A, B) enhanced the healing of burn wounds by significantly ($p \leq 0.05$) increasing the rate of wound contraction in the middle of the experimental period (day 7) (56.678% ± 1.91 and 51.332% ± 1.78 for *C. maxima* and *C. pepo*, respectively). All treated rats achieved a complete healing (100%) after 14 days without any scar formation. Compared with the leaves extracts, the ethanol seeds extracts (group C, D) also enhanced wound contractions after 7 days of treatment (22.921% ± 0.976 and 19.973% ± 0.752 for *C. maxima* and *C. pepo*, respectively) and reached a high contraction rate of 80.37% ± 2.542 and 78.512% ± 2.091 after 14 days of treatment. The silver sulfadiazine (SS) cream used in the study reduced wound damage by 30.397% ± 1.322 of lesion contractions, whereas the normal response to burn injury in the untreated group (F) was 11.977% ± 0.877 of wound contractions 14 days post-burn induction (Fig. 1).

Table 2
The chemical composition of ethanol seeds extracts of *Cucurbita* spp. by GC-mass analysis.

C. maxima	Compound	Retention Time	Molecular weight	Molecular formula	Peak Area%
1.	Methyl tetradecanoate *	19.118	242	C 15H30 O2	0.32
2.	3,7,11,15-Tetramethyl-2-hexadecen-1	20.585	296	C20 H40 O	0.50
3.	Methyl hexadec-9-enoate	21.373	268	C17 H32 O2	0.27
4.	Hexadecanoic acid, methyl ester	21.721	270	C17 H34 O2	15.12
5.	l-(+)-Ascorbic acid 2,6- dihexadecanoate	22.238	265	C382 H68 O8	4.73
6.	Methyl 8-heptadecenoate	22.358	282	C18 H34 O2	0.24
7.	Heptadecanoic acid, methyl ester	22.583	284	C18 H36 O2	0.35
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester **	23.376	294	C19 H34 O2	45.34
9.	9,12-Octadecadienoic acid (Z,Z)-	23.663	280	C18 H32 O2	23.89
10.	Methyl 9-cis,11- trans-octadecadienoate	23.942	294	C19 H34 O2	1.77
11.	Methyl 7,10-hexadecadienoate	24.510	266	C17 H30 O2	2.32
12.	Methyl 11-eicosenoate	24.689	324	C21 H40 O2	0.96
13.	Methyl 18-methylnonadecanoate	24.875	326	C21 H42 O2	2.35
14.	3-Cyclopentylpropionic acid, 2- dimethylaminoethyl ester	25.693	213	C12 H23N O2	0.10
15.	Methyl 20-methyl-heneicosanoate *	26.089	354	C 23H46 O2	0.93
16.	Butyl 9,12,15-octadecatrienoate	27.062	334	C22 H38 O2	0.24
17.	Pentane, 3-methyl-	3.023	86	C6 H14	0.43
18.	Cyclohexanol, 4-[(trimethylsilyl)oxy]cis-	25.758	188	C9 H20 O2 Si	0.14
C. pepo					
1.	Methyl tetradecanoate *	19.120	242	C15 H30 O2	0.49
2.	Pentadecanoic acid, methyl ester	20.452	256	C16 H32 O2	0.15
3.	Hexadecanoic acid, methyl ester	21.759	270	C17 H34 O2	16.65
4.	l-(+)-Ascorbic acid 2,6- dihexadecanoate	22.293	652	C38 H68 O8	1.35
5.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester **	23.485	294	C19 H34 O2	58.25
6.	9,12-Octadecadienoic acid (Z,Z)- **	23.681	280	C18 H32 O2	6.42
7.	Octadecanoic acid, methyl ester	23.615	298	C19 H38 O2	8.96
8.	Octadecanoic acid	23.815	284	C18 H36 O2	1.09
9.	Methyl 9-cis,11-trans- octadecadienoate	23.947	294	C19 H34 O2	0.49
10.	Methyl 9,10-methylene-octadecanoate	24.042	310	C 20H38 O2	0.21
11.	Methyl 7,10-hexadecadienoate	24.406	266	C17 H30 O2	0.21
12.	Methyl 11-eicosenoate	24.696	324	C21 H40 O2	1.22
13.	Methyl 18-methylnonadecanoate	24.872	326	C21 H40 O2	1.94
14.	Methyl 20-methyl-heneicosanoate *	26.083	354	C23 H46 O2	0.65
15.	Stigmasta-7,25-dien-3-ol, (3.beta.,5.alpha.)-	26.725	412	C29 H48 O	0.33
16.	Tetracosanoic acid, methyl ester *	27.184	382	C25 H50 O2	0.76
17.	Pentacosanoic acid, methyl ester *	27.695	396	C26 H52 O2	0.20
18.	Hexacosanoic acid, methyl ester *	28.197	410	C27 H54 O2	0.28
19.	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	27.610	410	C30 H50	0.35
					100.00

Note: *saturated fatty acids, **unsaturated.

3.3. The macroscopic examination of burn wounds contraction

The appearance of burn wounds induced in the laboratory rats were shown in Fig. 2: a1-a4. The burn wounds with all similar appearance. On the 4th day of the experiments, the plant extracts treated groups distinguished with scab layers on the wounds surface. The more contraction on the burned edge compared to the thin scab layer in silver sulfadiazine (reference) group and untreated group (Fig. 2: b1-b6). The brown scab layer observed in the untreated group from day 4−14 of the experiment.

In day 10 (Fig. 2: c1-c4), the burn wound in extract treated groups became more shrinkage and the hair covered the area partially. The reference group showed less shrinkage on the edges and less hair growth.

During the 14th day of treatment (Fig. 2: d1-d6), the complete healing of most burn areas treated with the plant extracts. The skins surface appeared normally without any scar formation and the complete hair growth especially in the groups treated by the leaves,

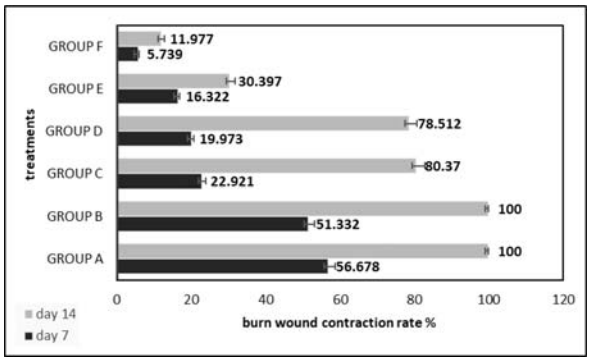

Fig. 1. Effect of ethanol extracts of *Cucurbita* spp. on the percentage of burn wounds contraction after 7 and 14 days of treatments.

followed by the seed treated groups. In the reference group showed a delay in healing of wounds with brown appearance and little hair growth. The untreated animal group, the wounds with irregular edges with large scab formation and small shrinkage.

3.4. Hematological and biochemical parameters

The application of leaf and seed ethanol extracts showed substantial alternations in hematological parameters post 14 days of treatment (Fig. 3). The post-wound treatment in groups A–D showed significant ($p \leq 0.05$) elevation in Hb and PCV levels compared with SS group (E) (Hb: 13.31 g/dl ±0.543, 13.02 g/dl ±0.671, 12.379 g/dl ±0.533, and 12.552 g/dl ±0.513, 10.336 g/dl ±0.421 respectively; PCV: 37.212% ± 0.387, 36.219% ± 0.221, 36.521% ± 0.409, and 36.461% ± 0.021%, 33.87 ± 0.311% respectively). The WBC counts were significantly decreased in the

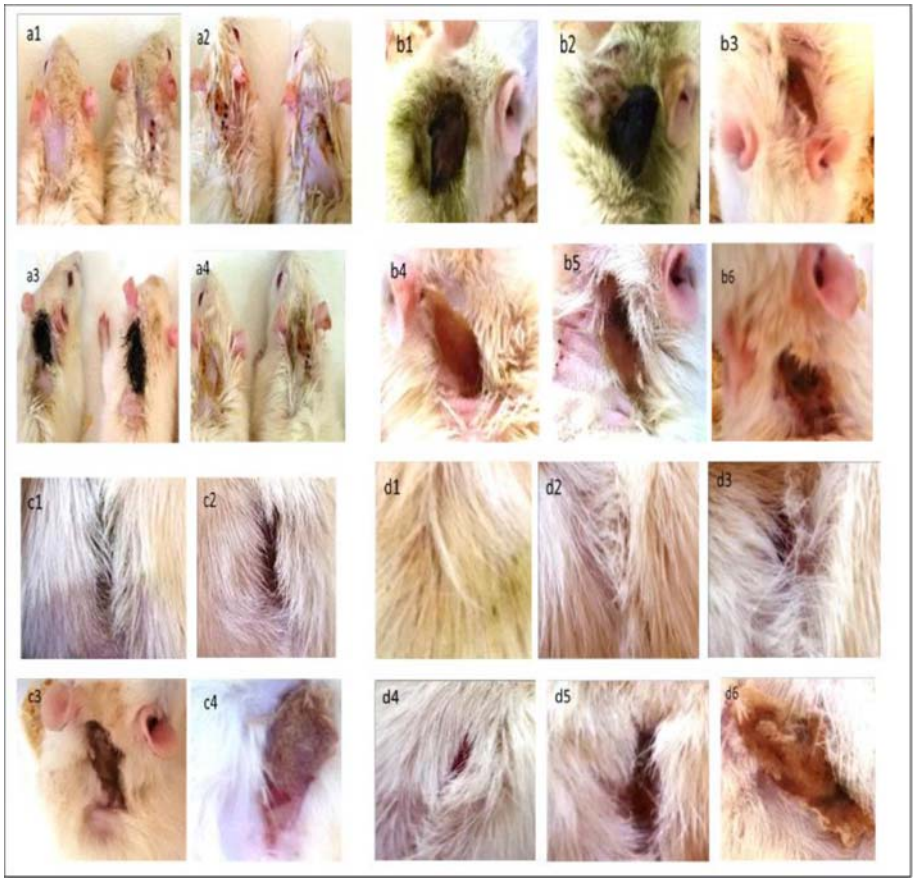

Fig. 2. Macroscopic examination of burn wounds contraction. a: induction of burn wounds in laboratory rats. a1: skin prepared, a2: induced burns, a3: treatment with leaf extracts, a4: treatment with seed extracts. b: the burn wounds of treated rats on the 4th day of treatment. b1,2 wounds treated with leaf extracts, b3,4 wounds treated with seed extracts, b5 wounds treated with ointment (sulfadiazine), b6 untreated burn wound. c: the burn wounds of treated rats on 10th days of treatment. c1 wounds treated with leaf extract, c2 wounds treated with seed extract, c3 wounds treated with ointment, c4 untreated burns. d: the burn wounds of treated rats on 14th day of treatment. d1,2 wounds treated with leaf extract, d3,4 wound treated with seed extract, d5 wounds treated with ointment, d6 untreated burn wounds.

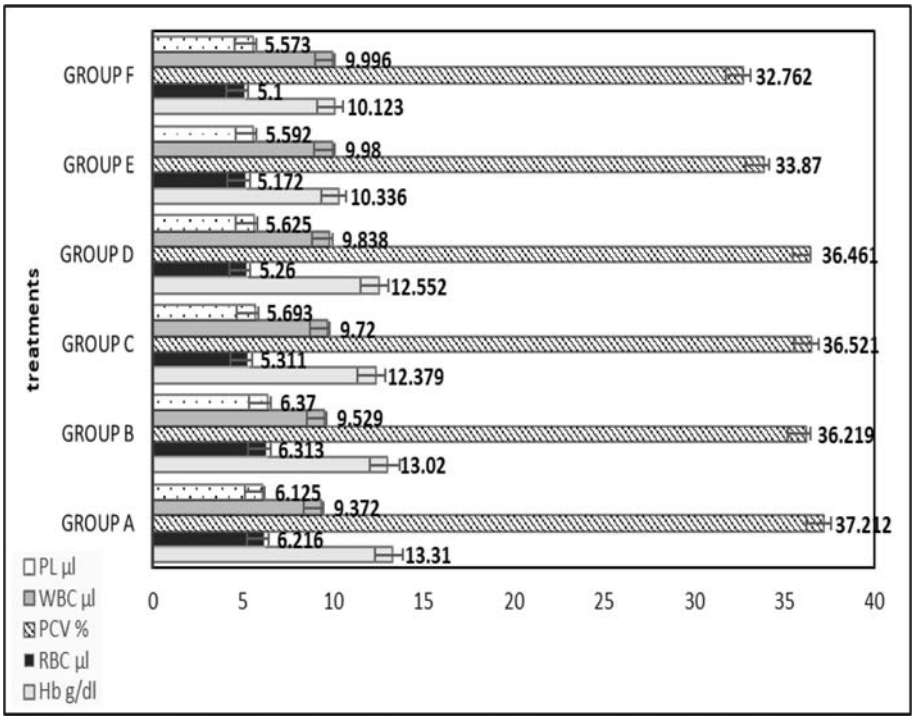

Fig. 3. Effect of ethanol extracts of *Cucurbita* spp. on the hematological parameters after 14 days of treatments.

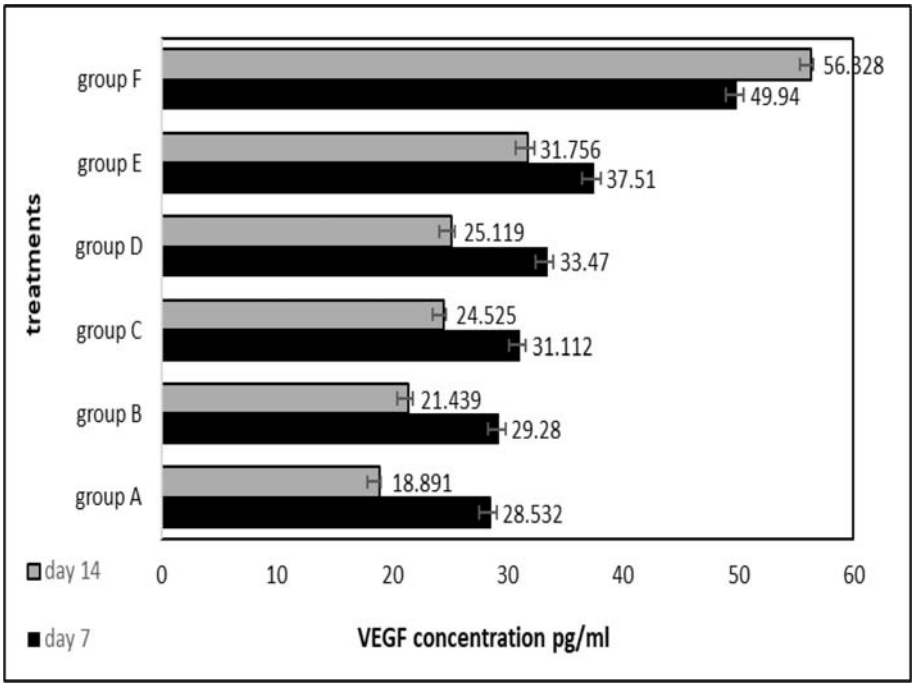

Fig. 4. Effect of ethanol extracts of *Cucurbita* spp. on serum VEGF levels after 7 and 14 days of treatments.

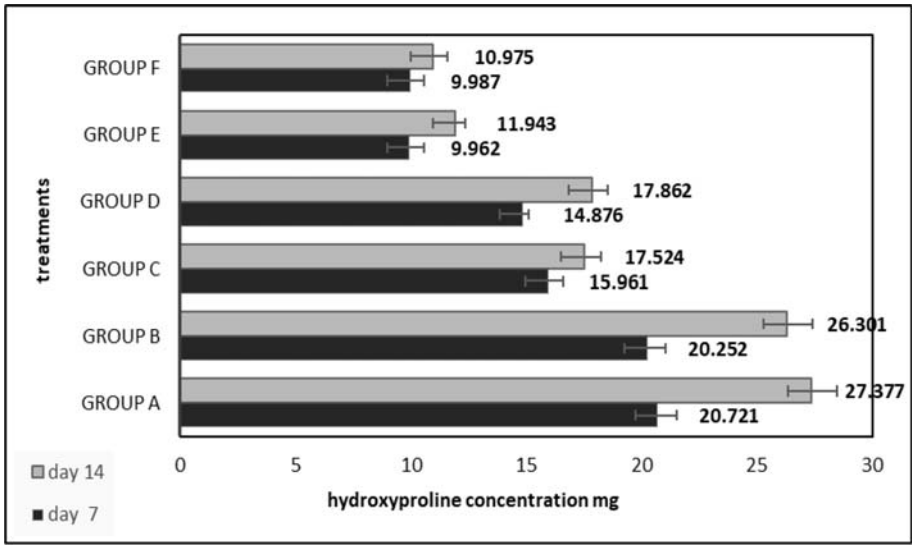

Fig. 5. Effect of ethanol extracts of *Cucurbita* spp. on hydroxyproline content in skin biopsy after 7 and 14 days of treatments.

leaf extract treatment groups (group A 9.372 × 10^6 µl ±0.121; group B 9.529 × 10^6 µl ±0.106) compared with the seed extract treatment groups (9.72 × 10^6 µl ±0.100, C; 9.838 × 10^6 µl ±0.130, D) and SS standard group (9.98 × 10^6 µl ±0.104). No significant differences were observed in RBC and platelet counts among all experimental groups. The serum concentration of VEGF was significantly lower post 14 days compared to 7 days in the leaf and seed extract treatment groups (A− D) (Fig. 4). Clear significant

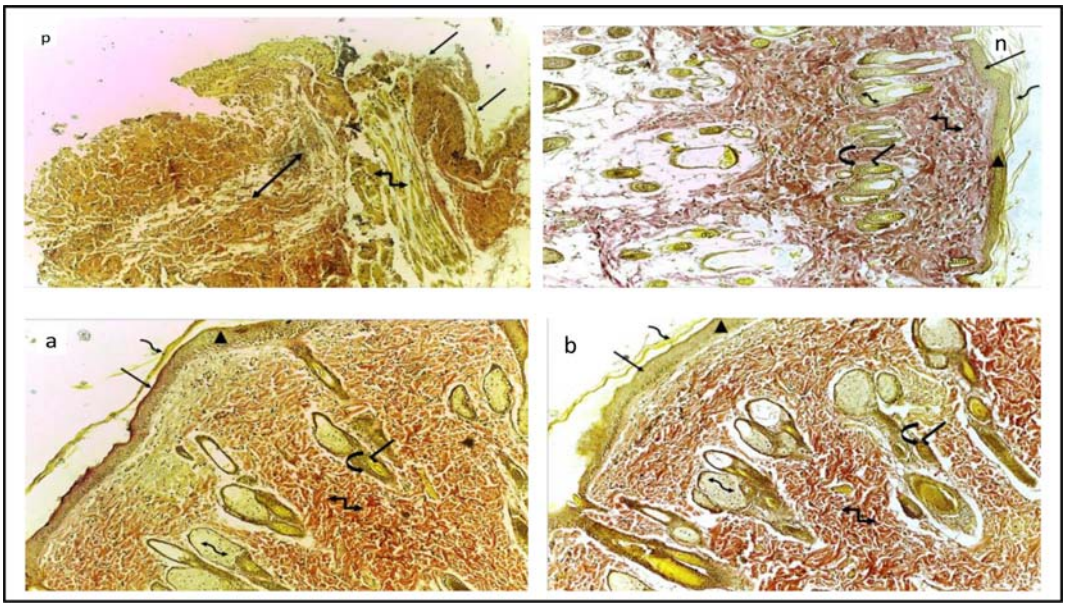

Fig. 6. Histological sections in rats' skin. P: day one of burn, n: normal skin, a: burn wound treated with alcoholic extracts of *C. maxima* leaves (group A), b: burn wound treated with alcoholic extracts of *C. pepo* leaves (group B) after 14 days. Epidermis (→), keratinocytes (▲), keratin deposition (↘). Sebaceous glands (↖↘), hair root sheath (ↄ), collagenous fibers (↖↳). hair follicle (⟂) Haematoxylin−Van Gieson stain. (100X).

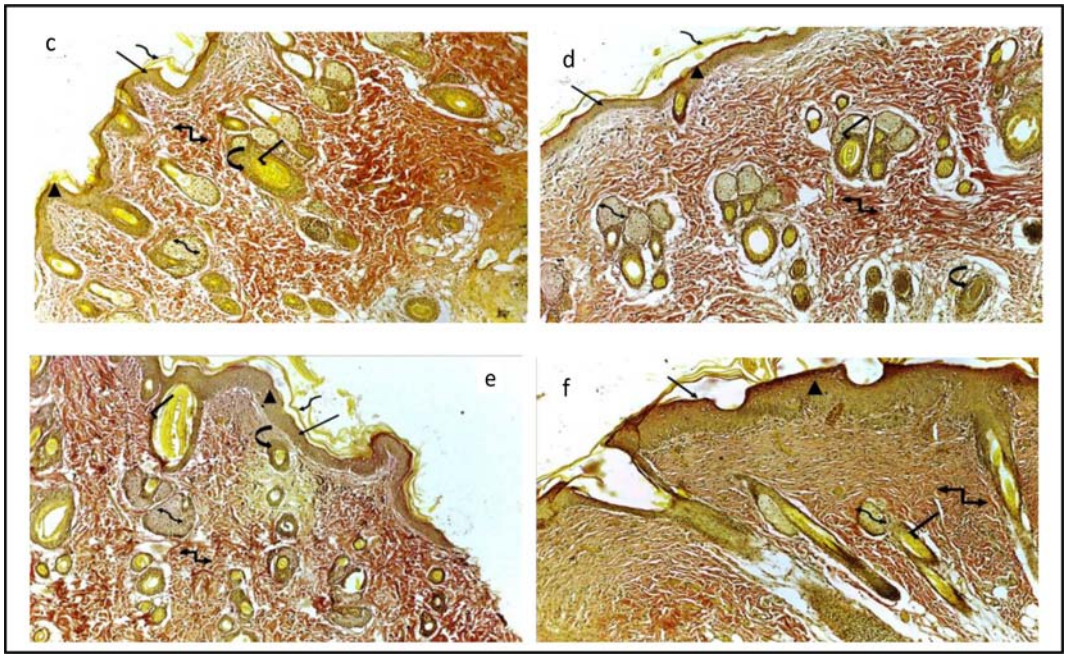

Fig. 7. Histological sections in rats' skin. c: burn wound treated with alcoholic extracts of *C. maxima* seeds (group C), d: burn wound treated with alcoholic extracts of *C. pepo* seeds (group D), e: burn wound treated with silver sulphadiazine (group E), f: burn wound without treatment (group F) after 14 days. Epidermis (→), keratinocytes (▲), keratin deposition (↘), sebaceous glands (↖), hair root sheath (ↄ), collagenous fibers (↰), hair follicle (▬) Haematoxylin−Van Gieson stain. (100X).

($p \leq 0.05$) decreasing was observed in leaf extracts in groups A and B 18.891 pg/ml ±0.121 and 21.439 pg/ml ±0.341 respectively compared to seed extracts in Group C and D 24.525 pg/ml ±0.130 and 25.119 pg/ml ±0.371 respectively). In the SS treatment group (E) the VEGF decreased to 31.756 pg/ml ±0.60 post 14 days. In the untreated wound group (F), the VEGF was elevated compared with that in all experimental groups at 7- and 14-days post-treatment, which consist 49.94 pg/ml ±0.541 and 56.328 pg/ml ±0.215, respectively.

3.5. Hydroxyproline content in skin biopsy

Hydroxyproline content related to experimental groups at 7- and 14-days post-wound treatment is presented in Fig. 5. A substantial significant ($p \leq 0.05$) increase in hydroxyproline level were observed in burn wounds treated with the leaf and seed extracts (groups A−D) compared with SS (group E) and untreated burns (group F). High significant ($p \leq 0.05$) levels of protein were found after the 14 day of treatment, especially in the groups treated with ethanol leaf

Table 3
The morphometric parameters in histological sections of the experimental groups.

Experimental groups	Epidermis thickness (micron)	Hair follicle diameters (micron)	Sebaceous gland diameters (micron)	Collagen density %
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A (*C. maxima* leaves extracts)	40.557 ± 1.260b	69.355 ± 3.062b	101.532 ± 1.857a	20.014 ± 1.896a
B (*C. pepo* leaves extracts)	43.415 ± 0.902b	72.153 ± 1.991b	90.580 ± 1.507b	20.093 ± 2.207a
C (*C. maxima* seeds extracts)	31.812 ± 1.498c	85.954 ± 6.079a	80.523 ± 1.367c	17.508 ± 1.885a
D (*C. pepo* seeds extracts)	36.108 ± 5.700b	64.782 ± 3.108b	78.513 ± 6.428c	21.326 ± 1.449a
E (ointment)	40.829 ± 4.144 b	52.525 ± 2.423b	78.631 ± 2.951c	16.180 ± 2.443a
F (untreated)	89.595 ± 11.067a	87.027 ± 16.450a	60.231 ± 4.757d	21.142 ± 1.993a
Normal (health)	33.842 ± 0.482c	44.837 ± 8.729c	53.026 ± 2.965d	17.439 ± 2.631a
Sig ($p \leq 0.05$)	0.000	0.000	0.000	0.038

Small different letters referred to significant at $p \leq 0.05$.

extracts (27.377 mg ± 1.120 and 26.301 mg ± 1.097) compared with the seed extracts (17.524 mg ± 0.714 and 17.862 mg ± 0.675). Groups E and F had a low hydroxyproline content after 14 days (11.943 mg ± 0.437 and 10.975 mg ± 0.6, respectively).

3.6. Histological study

Microscopic examination: The rats' skin sections at day 1 of burn induction (positive control) showed variable histological changes, including complete destruction of the epidermis and no obvious boundaries between the epidermis and the dermis. The skin lost its normal structure, signs of inflammation were present, and the inflammatory cells were extended to the sub cutis layer (Fig. 6p). The normal skin sections of rats (negative control) showed a normal skin layer. The epidermis consists of stratified squamous epithelial cells separated from the dermis by a clear border. The dermis consists of dense bundles of collagen fibers and contains hair follicles and sebaceous glands. The hair follicle is composed of medulla and cortex surrounded by the root's heath. The sebaceous gland is composed of epithelial cells arranged in lobules with central nuclei (Fig. 6n).

The skin sections 14 days post-injury and those treated with leaf extracts (groups A and B) showed a thick epidermis layer with more regular structure that was distinguishable from the dermis layer. Mild keratin deposit was found at the surface layer. The sebaceous gland was large, with a striated duct. Most hair follicles showed a clear medulla matrix, and they were surrounded by root sheaths with keratinocytes. Some inflammatory cells were found. The collagen fibers appeared dense and regular in the section (Fig. 6a and b). Light microscopic observations of burnt skin sections treated with seed extracts (groups C and D) after 14 days showed normal keratinocytes in the regular epidermis layer that was distinguishable from the dermis layer. The large sebaceous glands with adipocytes showed central nuclei. The hair follicles had an internal and external root sheath. Infiltration of mild inflammatory cells were found. Dense regular collagenous fibers were found in some regions of the dermis layer, while the collagenous fibers in other regions were irregular (Fig. 7c and d). Moreover, the skin sections related to burn wounds treated topically with SS cream at 14 days post-injury (group E) showed an irregular epidermis layer with thickness in some regions. No clear epithelialization was observed. Many hair follicles appeared to be atrophied with incomplete root sheath. Some sebaceous glands appeared to be small, and it lost its sac shape with incomplete secretory duct. Irregular and loose collagen fibers were present in the dermis layer (Fig. 7e). The burnt skin sections after 14 days without treatment (group F) revealed inflammatory signs in the dermis layer. The epidermis was thick with an irregular area. The dermis showed irregular dense collagenous fibers. A small number of sebaceous glands was observed, along with some atrophied hair follicles (Fig. 7f).

The morphometric results: Statistical analysis among the experimental groups showed a significant difference ($p \leq 0.05$) in the epidermis thickness and diameters of hair follicle and sebaceous gland, whereas insignificant differences were found in the collagen density among them (Table 3). The epidermis thickness of burn wounds treated with *C. maxima* seed extracts (group C) displayed a value 31.812 micron with insignificant differences from the epidermis in normal skin sections (33.842 micron), whereas it differed significantly ($p \leq 0.05$) compared with the epidermis thickness in sections of groups, A, B, D, and E. The epidermis in the untreated burn wound section (group F) showed high thickness (89.595 micron) with significant differences compared with the other groups. Insignificant differences were observed in the epidermis thickness and diameter of hair follicles among the experimental groups A, B, D, and E. The diameters of hair follicles were large in burn wounds treated with *C. maxima* seed extracts (group C, 85.954 micron) and in the untreated wounds (group F, 87.027 micron), with insignificant differences between them. On the contrary, they differed significantly ($p \leq 0.05$) from those in all other groups. The burn wounds treated with *C. maxima* leaf extracts showed a significant ($p \leq 0.05$) enlargement of sebaceous glands (101.532 micron). The sebaceous glands in burn wounds treated with *C. pepo* leaf extracts (90.580 micron) differed significantly from those in all other groups. The diameters of sebaceous glands displayed insignificant differences between the seed extract-treated groups and ointment-treated group. Insignificant differences were also observed between the untreated burn wounds and normal skin sections.

4. Discussion

Analysis of the leaf extracts showed different compounds in *C. maxima*, including high ratio of alpha-linolenic acid (polyunsaturated fatty acids: PUFA), phytol, monoterpenoids, and vitamin C, compared with the leaf extracts of *C. pepo* that had high content of palmitic acid. *C. maxima* seed extracts

contained a higher ratio of linoleic acid and vitamin C than *C. pepo* seed extracts, which have more saturated fatty acids content, palmitic and stearic acid. Some studies referred to the phytochemical composition of *Cucurbita* spp. Both leaf and seed extracts of *C. maxima* have phenolic glycosides (11E-octadecatrienoic acid) [49], whereas the seeds contain tocopherol, sterol [50], and high linoleic acid content [51]. The leaf extracts of *C. pepo* contain alpha myrin, phytol, and morin [52], while its seeds contain palmitic acid, stearic and linoleic acids [29].

The study results showed that the *Cucurbita* species ethanol extracts enhanced burn wounds contraction rate after 7 days of treatment and the complete healing post 14 days. This effect may be related to the improved biochemical and physiological activities required for tissue restoration due to various phytochemical compounds that may accelerate the healing process. Pumpkin has been widely used for traditional medicine, including burns, boils, and inflammation [53]. Seed extracts of *C. pepo* have antibacterial and anti-inflammatory properties [29,54].

The burn wounds treated with leaf and seed extracts showed less inflammatory responses than the other groups, which may be related to the activity of various compounds in plant extracts acting as anti-inflammatory agents. The therapeutic property of ethanol seed extracts of *C. maxima* showed large spectrum of inhibition against bacteria isolated from burn wound patients, such as *Staphylococcus aureus*, *S. warneri*, and *Pseudomonas aeruginosa* [33]. The leaf extract of *C. pepo* showed inhibitory effect against *S. aureus*, *Enterobacter aerogenes*, *Escherichia coli*, and *Providencia stuartii* [55]. The polysaccharides extracted from *C. maxima* fruits pulp revealed inhibitory effect against *S. aureus* and *E.coli* [56].

The healing potential of *Cucurbita* spp. ethanol extracts, especially the leaf extracts, may be related to the effect of the extracts on hydroxyproline levels in treating wound tissue, which showed increased concentration during the treatment periods of 7 and 14 days, illustrating its activity may relate to the increase hydroxyproline (collagen substrate) influence on the early proliferation phase in treated burn wounds [57]. The activation of dermal fibroblast and keratinocytes enhance collagen synthesis, which is the base of extracellular matrix that maintains the epithelialization of healing phases [21]. Bardaa et al. [58] found that the cold extract of *C. pepo* seed oil has a healing effect on second-degree burns by increasing wound contraction rate from day 3−11 and skin biopsy hydroxyproline content compared with the untreated group and displays high antioxidant activity due to tocopherol ratio. Or the therapeutic efficiency of medicinal plants may be related to the bioactivity of extract components in accelerating wound healing. In this study, the *Cucurbita* leaf and seed extracts showed various phytochemicals, such as linolenic acid, ascorbic acid, limonene, phytol, and linoleic acid. Several studies confirmed the vital effect of these compounds on wound repair. Ascorbic acid (10%) cream showed anti-inflammatory properties and accelerated the wound repair process by enhancing fibroblast proliferation, angiogenesis, and collagen synthesis [59]. The oil mixture containing linolenic acid extracts showed a reduction in thermal lesions by increasing cell proliferation in rats' burn wounds [60]. The pumpkin seed oil containing linoleic acid improved wound repair through fibrin stabilization, fibroblast activation, enhancement of hydroxyproline content, and reduction of macrophage in wounds [58]. The utilization of limonene oil extract efficiently decreased inflammation and enhanced collagen synthesis in wounds induced in laboratory rats [61]. Phytol fraction, which is an active component of *Phlomis russeliana*, showed anti-inflammatory activity that improved the wound healing process in albino mice [62].

In the present study, the leaf and seed extracts were effective in the treatment of second-degree burn wounds compared with silver sulfadiazine, which had less healing activity and low wound contraction rate. These results may be related to the cytotoxic effect and slow healing action of the ointment [63], which is mainly used in treating burn as antimicrobial agent related to the actions of silver ions [64].

The circulating VEGF levels is elevated in the beginning of burn injury until the wounds closure [65]. In this study the animal groups treated with ethanol extracts displayed high levels of serum VEGF in the first week of treatment while reduced in the second week. The explanation of that effect may be attributed to the phytochemical compounds that enhanced the growth factors of skin repair, including VEGF, which in turn stimulated angiogenesis, accelerating healing and collagen deposition in the early period of treatment. This effect was observed by early rate of the wounds contraction. While during the second week, prevent scar formation through appearing the skin sections with a more regular tissue structure. Our suggestions were illustrated by other studies, which showed that during the critical phase of healing, the expression of adhesion molecules and vascular permeability were induced by VEGF, which influenced many processes in the proliferative phase, including re-

epithelialization, angiogenesis, and formation of granulation tissue; moreover, during the remodeling phase, the scar formation was promoted and affected by the VEGF level [66,67].

The creation of second-degree burn wounds on experimental rats showed a complete tissue destruction with significant inflammation. The topical application of ethanol leaves extract resulted in a normal skin appearance. The histological examination of skin biopsy at 14 days post-treatment revealed complete skin tissue healing, regular epidermis, less inflammation in cells, a hair follicle with root sheath formation, and regular collagen fibers compared with the silver sulfadiazine treatment group with incomplete regeneration of the epidermis and the dermis layer, no clear epithelialization, and shrinkage of some hair follicles. These findings indicate the healing properties of *Cucurbita* species leaves extract within a short treatment period. The healing activity of pumpkin seed oils was illustrated in some studies. Bardaa et al. [45] found that the cold extract of *C. pepo* seed oils exhibited a high percentage of wound contraction (96.7%) after 33 days of treatment and increased collagen production. Another study demonstrated that the wound contraction rate was 91.6% and normal structure layers and collagen density were achieved after 11 days of treatment with *C. pepo* cold oil extracts by increasing hydroxyproline content to 25.6 mg/100 gm. tissue, which may be related to the high content of PUFA, tocopherol, and sterol compounds [58].

In untreated burn wound rats (group F), the incomplete formation of epidermis, irregular collagen, atrophied hair follicle, and presence of inflammatory cells may be related to delay in the healing process. This result demonstrated [68] that the prolonged inflammation signs delay tissue repairing, which in turn increases the incidence of wound infection and edema [58].

5. Conclusions

This study was demonstrated for the first time in Basrah province to examine the healing potential of ethanol leaf extracts of both *C. maxima* and *C. pepo* in topical application 0.52 µl/mm^2 on second-degree burn wounds that were induced in laboratory animals. The leaf extracts were more effective than the seed extracts in accelerating tissue repairing and healing properties. This was related to various phytochemical contents. The therapeutic effects include complete wound recovery, improved collagen deposition, and normal skin appearance post-treatment compared with the slow repair rate of silver sulfadiazine. Thus, these results support the consideration of plant extracts as a natural alternative for the treatment of thermal burn wounds. Despite these findings, further studies are required to determine the activity of different solvent extracts in the mechanism of the healing process for future medical application.

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