

RESEARCH ARTICLE

Preliminary Phytochemical Investigation and Effect of *Cucurbita maxima* Seeds Oil on Burn Wound Healing in Iraq

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ABSTRACT

Cucurbita maxima seeds oil were extracted by hot and cold method, identified their phytochemical components as it contains alkaloids, flavonoids, saponins and tannins and analyzed to explain its ability to heal burn wound. Male albino 32 Sprague-Dawley rats were divided into four rats in each cage which included a positive control group (Mebo ointment): two rats for first-degree burn and two for the second one, negative control group: two rats for first-degree burn and two for the second one, cold method: 100% concentration with two rats for first-degree burn and two for the second one and hot method: 100, 75, 50, 25 and 10% concentration with two rats for first-degree burn and two for the second one for each concentration. The results showed that the extracts of *C. maxima* seeds oil at first and second-degree burn by the cold method were not efficient so it could neglect where it compared to the hot procedure at the same time the concentrations 100, 75, 50, 25, and 10% were significant as LSD5% were more than 0.01 when compared with the negative standard, while when compared with the positive standard 100 and 75% were significant while 25, 10% were insignificant and 50% had the same activity as the positive standard. Also, there is a significant difference between concentration at fifth week; 100 and 75% has the largest significant differences which is 0.01 among the other concentrations. At second-degree burn we notice that all the concentrations 100, 75, 50, 25, and 10% were significant as LSD5% were more than 0.01 when compared with the negative control, while when compared with the positive control 100 and 75% were significant while 25, 10% were insignificant and 50% had the same activity as the positive standard. Also there is a significant difference between concentrations at fifth week, 75% has the largest significant difference among the other concentrations. As a final result the concentration 100 and 75% were the best concentration in the treatment of burn wound as the 100% contained a large amount of extract and 75% contained a sufficient quantity of extract in addition to the ointment base vaseline which aid in keeping the skin wet enough to avoid shrinkage of it and help in the rehabilitation of skin epithelial cells. This study of *C. maxima* seeds oil is the first study in the healing of burn wound on rats in Iraq

Keywords: Burn, *Cucurbita maxima*, Phytochemical investigation, Wound.

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INTRODUCTION

The medicinal plants used in many countries in traditional medicines and assessed as important sources for the invention of new drugs.^{1,2} A large diversity of topical preparations, including poultices and ointments, are reported to be valuable in burn wound management one of them with burn healing effect is *Cucurbita* species which is known as a pumpkin.³ The pumpkin plant, *C. maxima* from the family Cucurbitaceae, is a large perennial or annual climbing herb.^{4,5} Pumpkin seeds contain many valuable active constituents and have been traditionally used for clinical, therapeutic, and herbal applications. Pumpkin seeds used as safe diuretic

and deworming agents, while the seeds oil used as a nervine tonic.⁶ The seed's decoction is beneficial to cure both renal and liver disorders. Seeds contain fixed oil composed of arachidic, stearic, palmitic, myristic, behenic, unsaturated fatty acids, phospholipids, carpaine, benzylisothiocyanate, benzyl glucosinolate, glucopaeolin, hentriacontane, β -sisterol, caricin (singrin) and myosine⁷ see Figure 1.

Chemical constituents of *C. maxima* seeds

Phytochemical screening exhibit the major natural chemical compounds identified in *C. maxima* seeds, including flavonoids, tannins, phenols, saponins, terpenoids, glycosides, alkaloids, and cardiac glycosides, steroids and coumarins.⁸

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Figure 1: *C. maxima* fruit, leaves, seeds.

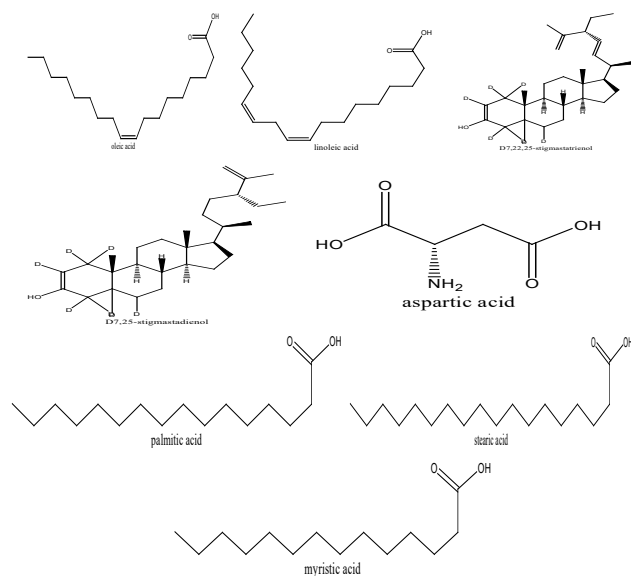


Figure 2: Main components identified in *C. maxima* seeds.

Seeds are a useful source of vitamins mainly vitamin B along with vitamins (C, D, E, K) beside zinc, magnesium, manganese, phosphorous, phytosterol such as $\Delta 7,22,25$ -Stigmastatrienol, Spinasterol ($\Delta 7, 22$ -stigmastadienol), $\Delta 7,2$ -Stigmastadienol, $\Delta 7$ -stigmasterol, $\Delta 7$ -Avenasterol, β -Sitosterol ($\Delta 5$). The seeds oil is also composed from myristic, oleic, stearic linoleic and palmitic acid 30.3% as in Figure 2. Seeds are rich in iron, minerals, zinc, Mg, proteins and calories. Seeds also contain omega 6 and omega 3 fats and high in fiber.⁹

Pharmacological Activities of *C. maxima* Seeds

Anticancer activity, anti-obesity,¹⁰ diuretic activity,¹¹ cardiotoxic activity,¹² human over active urinary bladder,¹³ humoral immune response,¹⁴ protease inhibitory of seed coat extract,¹⁵ anti-inflammatory in seed extract-fore gut induced injury,¹⁶ CNS stimulant activity,¹⁷ anti-parkinsonism activity,¹⁸ anti-giardial activity,¹⁹ anti-helminthic activity,²⁰ anti-diabetic/anti hyperlipidemia,²¹ cytotoxicity, and antibacterial activity.²²

Burn wound Healing Activities of *C. maxima* Seeds

Burn injury can be defined as skin damage caused by extreme heat, electricity radiation or corrosive chemicals.^{23,24}

Depending on the burn depth, they are divided into first-degree, that is superficial, a second-degree partial thickness, and third-degree full thickness. The process of healing of burn wound is composed of five main phases: (1) inflammation, in which the chemoattractant mediator attract immune cell to wound area, (2) Re-epithelialization that begins with the proliferation and epithelial cells migration at edge of the wound, might belong to the lack of neighbor cells, (3) Granulation with extracellular matrix produced by fibroblasts,²⁵⁻²⁷ (4) Neovascularization that is induced by a pro-angiogenic factor (produced chiefly by macrophage), and (5) The last phase is wound contraction.²⁸⁻³⁰ The epidermal growth factors, transforming growth factor $\beta 1$ and $\beta 2$, interleukin-1, a family of fibroblast growth factors, vascular endothelial growth factors, platelet-derived growth factors, tumor necrosis factor α , insulin-like growth factor-1 and colony-stimulating factor-1 are cytokines that are contained in the wound healing process.^{31,32}

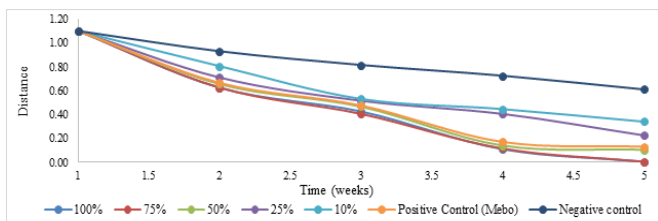
EXPERIMENTAL WORK

The seeds of *C. maxima* were collected, identified at the department of Pharmacognosy and medicinal plants, Kerbala university, College of pharmacy, Iraq, washed and dried separatory at shade ventilation place, then grind by a mechanical grinder to coarse powder and weighed. The experimental part included cold and hot method, first 350 gms of powdered *C. maxima* seeds was weighed and extracted by soxhlet apparatus in n-hexane solvent for about ten hours and then filtered. The volume was reduced by vacuum rotatory evaporator to get a crude extract and 350 gm of powdered *C. maxima* seeds was weighed and macerated in n-hexane solvent, leave for about ten days and then filtered, reduced volume by vacuum rotatory evaporator to get a crude extract. Preliminary phytochemical identification of flavonoids, saponins, tannins and alkaloids presence are detected in the powdered and crude extract of *C. maxima* seeds.³³⁻³⁶

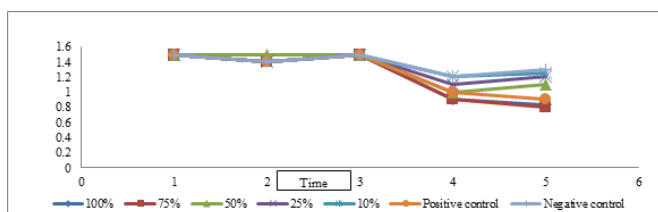
Topical Assessment of Burn wound Healing Activity

Total of 32 male albino Sprague-Dawley rats (4 in each group) of weight range 250–400 g and age 6 to 7 months were used. The rats were housed in standard vivarium conditions (relative humidity of 60%, temperature about $25 \pm 1^\circ\text{C}$ and 14 hours dark and 10 hours light cycles), and they were fed with water ad libitum and standard laboratory food. The dorsal part of the animals was shaved under anesthesia by ketamine HCL injection 40 mg/kg. The first and second-degree burn wounds were occurred by using an electrical heater with a circular probe (110°C heat for 10 second, and radius of cm^{-1}). Experimental rats were divided into four groups:

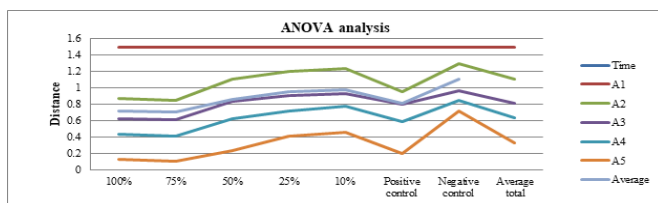
- Positive control group (Mebo ointment): two rats for first-degree burn and two for the second one
- Negative control group: two rats for first-degree burn and two for the second one.
- Cold method: 100% concentration with two rats for first-degree burn and two for the second one.



Scheme 1: Comparison between concentrations of method No.1 with positive and negative control by distance of first-degree burn and the average of replicates.



Scheme 2: Comparison between concentrations of method No.1 with positive and negative control by distance of second-degree burn and the average of replicates.



Scheme 3: Comparison between the average of each replicates with the total average for each concentration in second-degree burn.

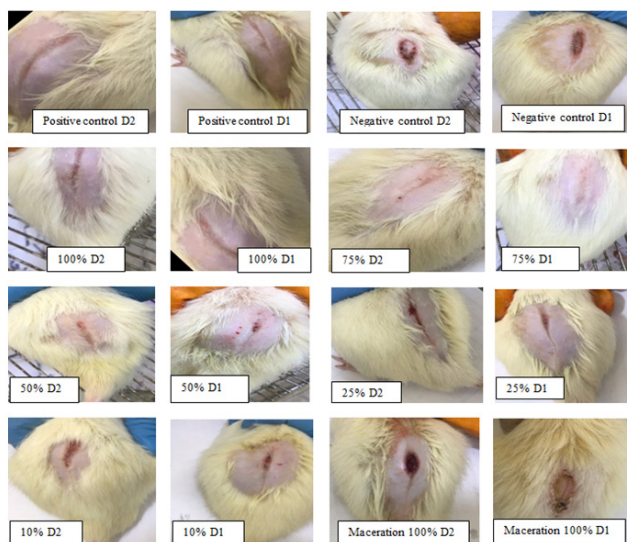


Figure 3: Burned wound of rats after five weeks for all concentrations of hot and cold method in addition to the positive and negative control.

- Hot method: 100, 75, 50, 25 and 10% concentration with two rats for first-degree burn and two for the second one for each concentration.

Starting 24 hours after burn induction, ointments from the extract and base followed by topical application of vaseline to

cover all wound area 3 times daily for five weeks. Closure of wounds was monitored by continuous photography, using a paper ruler as scale. As the percentage of wound size decrease, rat healing increase.

Statistical Analyses

All data analyses were carried out by the SPSS using ANOVA analysis and compared by LSD 5% To determine the significant and insignificant study.

RESULTS

The results show that the percent yield of crude extract from extraction method no. 1 was higher than that obtained from extraction method no. 2, as shown in Table 1.

Chemical Identification of the Crude Extracts

The chemical tests that done to detect the presence of flavonoids, saponins, tannins, and Alkaloids in the crude extracts of *C. maxima* as in Table 2.

Topical Assessment of Burn Wound Healing Activity

The topical application of seeds oil extracts give different percentage of healing in the recommended period as in Tables 3-8 and Scheme 1-3, and shown in Figure 3.

DISCUSSION

The selection of the best method of extraction based on the percentage yield of crude extract. The results showed that the percentage yield of crude extract from extraction method no.1 was higher than that obtained from extraction methods no.2, so it more dependent.

Topical Application of *C. maxima* Seeds Oil on Burn Wound

The main requirements for healing of burn wound involve proliferation and migration of cells such as fibroblasts and vascular endothelial cells and may include increasing the neovascularization in granulation tissue. The increasing number of fibroblasts primarily arises due to the resident fibroblasts' proliferation in response to the growth factors, so it can migrate from surrounding connective tissue into the burn wound position. Also, vitamins C and E are required as they reduce oxidative injury and help promote burn wound healing. Many minerals are associated with healing of burn wounds such as zinc, copper and Iron. Zinc is essential for skin integrity, immune function and growth. Copper is essential to maintain skin strength, blood vessels, and connective and epithelial tissue. Iron is necessary in transporting oxygen through hemoglobin in the bloodstream and myoglobin in the muscle. Other factors that affect healing of burn wound are the antibacterial activity that lead to reduce water evaporation from the surface of burn wound and provide optimum physiologic environment and finely improve scare formation. All the above requirements are present in the extracted oil of *C. maxima* seeds as it contain the necessary vitamins, minerals and also has the antibacterial, anti-inflammatory and antioxidant activity that required for healing of burn wound in addition

Table 1: Weight and percentage yield of each crude extract, obtained from extraction methods no.1, and 2

Extraction methods	Weight of crude extract (gm)	%yield of crude extract (%)
Method no.1	84.40	24.11
Method no.2	49.05	14.01

Table 2: Chemical identifications of the flavonoids, saponins, tannins and alkaloids.

Test name	Result
Flavonoids test	+
Saponins test	+
Tannins	+
Alkaloids test	++

Table 3: Comparison between concentrations of method No.1 with positive and negative control by distance of first-degree burn and the average of replicates.

Conc.	Time														
	1	1	A1	2	2	A2	3	3	A3	4	4	A4	5	5	A5
100%	1.1	1.1	1.1	0.65	0.6	0.63	0.42	0.43	0.43	0.12	0.10	0.11	0.01	0.0	0.01
75%	1.1	1.1	1.1	0.6	0.65	0.63	0.4	0.41	0.41	0.1	0.13	0.12	0.0	0.01	0.01
50%	1.1	1.1	1.1	0.63	0.68	0.66	0.44	0.49	0.47	0.13	0.15	0.14	0.1	0.11	0.11
25%	1.1	1.1	1.1	0.7	0.72	0.71	0.5	0.53	0.52	0.4	0.41	0.41	0.2	0.25	0.23
10%	1.1	1.1	1.1	0.8	0.81	0.81	0.52	0.54	0.53	0.43	0.46	0.45	0.35	0.33	0.34
Positive control	1.1	1.1	1.1	0.64	0.69	0.67	0.45	0.5	0.48	0.15	0.19	0.17	0.13	0.12	0.13
Negative control	1.1	1.1	1.1	0.96	0.9	0.93	0.83	0.8	0.82	0.75	0.7	0.73	0.6	0.62	0.61

Table 4: Comparison between concentrations of method No.1 with positive and negative control by distance of second degree burn and the average of replicates.

Conc.	TIME														
	1	1	A1	2	2	A2	3	3	A3	4	4	A4	5	5	A5
100%	1.5	1.4	1.5	0.91	0.83	0.87	0.61	0.63	0.62	0.42	0.43	0.43	0.11	0.12	0.12
75%	1.5	1.4	1.5	0.90	0.80	0.85	0.60	0.61	0.61	0.41	0.4	0.41	0.10	0.00	0.10
50%	1.5	1.5	1.5	1.0	1.10	1.10	0.80	0.85	0.83	0.63	0.6	0.62	0.25	0.21	0.23
25%	1.5	1.4	1.5	1.1	1.20	1.20	0.90	0.91	0.91	0.70	0.72	0.71	0.40	0.42	0.41
10%	1.5	1.4	1.5	1.2	1.25	1.23	0.92	0.94	0.93	0.75	0.8	0.78	0.43	0.48	0.46
Positive control	1.5	1.4	1.5	1.0	0.90	0.95	0.81	0.79	0.80	0.60	0.58	0.59	0.20	0.19	0.20
Negative control	1.5	1.4	1.5	1.2	1.3	1.3	0.95	0.96	0.96	0.83	0.84	0.84	0.70	0.73	0.72

Table 5: Concentration 100% first-degree burn maceration with n-hexane

Conc.	TIME														
	1	1	A1	2	2	A2	3	3	A3	4	4	A4	5	5	A5
100	1.1	1.2	1.2	0.91	0.93	0.92	0.84	0.82	0.83	0.7	0.65	0.68	0.35	0.38	0.37

Table 6: Concentration 100% second-degree burn maceration with n-hexane

Conc.	TIME														
	1	1	A1	2	2	A2	3	3	A3	4	4	A4	5	5	A5
100	1.1	1.2	1.2	0.94	0.95	0.95	0.86	0.88	0.87	0.75	0.78	0.77	0.43	0.45	0.44

significant while 25, 10% were insignificant and 50% had the same activity as the positive standard. Also there is a significant difference between concentration at 5th week, 100 and 75% has the largest significant differences which is 0.01 among the other concentrations. At Table 8 second-degree burn we notice that all the concentrations 100, 75, 50, 25, and

to the presence of the phytochemical compounds such as tannins which act as astringent and help in healing. This research study the effect of these requirement on healing of burn wounds in different groups of rats for about five weeks and the results were analyzed by CRD design by SPSS using ANOVA test and examined by the least significant differences (LSD). The results showed that the extracts of *C. maxima* seeds oil by the cold method were not efficient at the definite period so this procedure was neglected where it compared to the hot procedure at the same time. At Table 7 first-degree burn we notice that all the concentrations 100, 75, 50, 2, and 10% were significant as LSD5% were more than 0.01 when compare with the negative standard, while when compared with the positive standard we notice that 100 and 75% were

10% were significant as LSD5% were more than 0.01 when to compare with the negative control, while when compared with the positive control we notice that 100 and 75% were significant while 25, 10% were insignificant and 50% had the same activity as the positive standard. Also there is a significant differences between concentrations at 5th week, 75% has the

Table 7: Comparison between the average of each replicates with the total average for each concentration in a first-degree burn

	Conc.	Time	A1	A2	A3	A4	A5	Average
B1	100%		1.1	0.63	0.43	0.11	0.01	0.46
B2	75%		1.1	0.63	0.41	0.12	0.01	0.45
B3	50%		1.1	0.66	0.47	0.14	0.11	0.50
B4	25%		1.1	0.71	0.52	0.41	0.23	0.59
B5	10%		1.1	0.81	0.53	0.45	0.34	0.65
B6	Positive control		1.1	0.67	0.48	0.17	0.13	0.51
B7	Negative control		1.1	0.93	0.82	0.73	0.61	0.84
	Average total		1.1	0.72	0.52	0.30	0.21	
	LSD 5%		A=0.01		B=0.01		A* B=0.022	

Table 8: Comparison between the average of each replicates with the total average for each concentration in second-degree burn

	Conc.	Time	A1	A2	A3	A4	A5	Average
B1	100%		1.5	0.87	0.62	0.43	0.12	0.71
B2	75%		1.5	0.85	0.61	0.41	0.10	0.70
B3	50%		1.5	1.10	0.83	0.62	0.23	0.86
B4	25%		1.5	1.20	0.91	0.71	0.41	0.95
B5	10%		1.5	1.23	0.93	0.78	0.46	0.98
B6	Positive control		1.5	0.95	0.80	0.59	0.20	0.81
B7	Negative control		1.5	1.30	0.96	0.84	0.72	1.10
	Average total		1.5	1.1	0.81	0.63	0.32	
	LSD 5%		A=0.01		B=0.01		A*B=0.022	

largest significant difference among the other concentrations. As a final result the concentrations 100 and 75% were the best concentration in the treatment of burn wound as the 100% contained large amount of extract and 75% contained a sufficient quantity of extract in addition to the ointment base vaseline which aid in keeping the skin wet enough to avoid shrinkage of it and help in the rehabilitation of skin epithelial cells. It's important to mention that the age of rats affect the immunity and finely the time of burn wound curing because they were exposed to different experiment previously, also the weight effect on the treatment by its relation to the burn degree due to the present layers of adipose tissue. So the null hypothesis of refuse this study was rejected and this study was accepted and this research is concur with (Bardaa *et al.* 2016) study³⁷ and (Bardaa *et al.* 2016) study.³⁸

This research study showed that the seeds oil from *C. maxima* plant extracted by hot method is an important source for burn wound healing in rat when compare with positive control (Mebo ointment) as it has antioxidant and antibacterial agents and anti-inflammatory activity. These findings facilitate the future future clinical applications of *C. maxima* seeds oil and its use traditionally.

CONCLUSION

This research study showed that the seeds oil from *C. maxima* plant extracted by hot method is an important source for burn wound healing in rat when compare with positive control (Mebo ointment) as it has antioxidant and antibacterial agents and anti-inflammatory activity. These findings facilitate the future future clinical applications of *C. maxima* seeds oil and its use traditionally.

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