

Research Article**Assessing the combined effects of plants extract leaf *Olea europaea* and *fasciculifolius asteragalus* with Saline on healing of skin on second-degree burn wounds in rats****Arsalan Azizi¹, Hossein Kavyani²(corresponding) and Shahrbanoo Asgarian**¹Assistant professor, Department of pathology, school of medicine,
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Email: Hosseinkavyani7@yahoo.com**ABSTRACT**

The use of herbal medicines in the treatment of skin lesions such as bed sores or wounds, with different studies and the effectiveness of them is common. The aim of this study was to determine the combined effects of the plant extract leaf *Olea europaea* and *fasciculifolius asteragalus* with saline burn on second-degree healing in rats is created in the skin.

Method: In order to evaluate and compare the effects of plant extracts [leaf *Olea europaea* and *fasciculifolius asteragalus* with saline] and the role of each burn healing in mice, in this study, 20 adult male Wistar rats weighing approximately 250 to 300 g was prepared. After anesthetized mice and control, degree burn wounds 2 in the area have created, then extract obtained in proportion to the value of 30 g of each mixed and made into a poultice. This poultice obtained within 15 days with daily replacement was on the wound. Then compare the result of wound healing based on speed, quality, scarring and infection in burn area were reported in each group. At the end of the day treatment blood samples tested for enzymatic function TNF α and TGF β measured. Data were analyzed using statistical spss analysis.

Findings: Kruskal-Wallis statistical method was used in this study, the findings suggest that the effects of plant extracts to accelerate recovery time in the intervention group and the shape and size of the wound is created, as well as the presence of inflammatory factors in blood serum, groups representing the it is the Significant level test in this case approximately [$p < 0.005$].

Conclusion: This study showed that the extracts of medicinal plants used in this study have a significant impact on wound healing and can even disinfect the wound and the formation of new tissue collagen at the wound site are effective.

Keywords: compounds plant, mouse, burns.

INTRODUCTION:

Burn is one of the worst medical conditions that can damage all physical and mental dimensions and can affect all ages, a person is highly susceptible to infection due to skin degeneration as the first defensive barrier in the body, infections in the burn can be caused by a

bacterium, fungus or virus, and factors such as: age, depth and extent of burn can affect these infections [1]. Despite all the advances in the control and treatment of burn wounds and the presence of special care for these patients, the

main cause of death in patients with burns is infection [2].

Today, new therapies are used to heal wounds including: 1- biologic dressing of pair of curtains umbilical in burn wound [3]. 2- medical engineering and skin substitutes including autologous keratinocyte plates. 3- new treatments with cellular colony stimulation factors and platelet growth factor PDGF. 4- treatment with various stem cells of the bone marrow, fat, umbilical cord and epithelium. 5- gene therapy and transfer of platelet growth factor PDGF. 6- wound healing with flap graft. 7. application of honey in wound healing and treatment of skin and mucosal diseases. The greatest problem with the use of new treatments is the high cost of treatment, the limited use of wound healing, and the high recovery time [4]. One of the methods of treating wound healing is the use of herbal medicines. Medicinal plants have therapeutic and regenerative properties due to the presence of alkaloids, essential oils, flavonoids, tannins, terpenes, saponins and phenolic compounds, they have therapeutic and restorative value plants cause fresh blood flow to the wound and have an antimicrobial effect in the wounds, which is very important in terms of treatment [5]. Herbal remedies can completely disinfect the wound environment and remove the pericarpic tissues and spread the wounds to each other, plants also have a good microbial-free place, as a lining at the site of an open-wound opening of fractures and accelerate treatment in the wounds [6].

In order to study *gumastaragalu* plant and leaf *Olea europaea* composition with Saline and the role of each in wound healing by the burn wound method, studying the process and healing and healing procedures for each of these materials, and on the other hand, in order to compare the effectiveness of these two with each other as well as comparing them with the control group, the present study done. *Fasciculifolius Astaragalu* the Fabaceae family or Leguminous subfamily is populated Which is a blabber. According to the results, the extracted extract of the *astaragalu* plant has a restorative effect on the pathological ulcers

and burn wounds in mice, According to botanical studies, this plant has a great influence on the treatment and repair of burn wounds And the production of epithelial cells in similar animals in their own group was done much earlier. The study showed that tissue produced in mice used to treat the plant was quicker to repair, It has a restorative effect and is free from burning, so it brings the wound's edges and boils. Ointment *Astaragalu* soothe all sweeteners, eliminate spoiled meat and improve juicy scars. It is used to treat burns and scars [7]. *Astaragalu* at 4% concentration, *micrococcus luteosum* has an antimicrobial activity in the wounds. [8]. Olive scientific name *Olea europaea* a small family tree from Oleaceae and the order of the moraines, in the temperate regions of the tropics and the Mediterranean and warm regions of the world grows [9]. It is reported in the research, compared the leaves Olive as active antibacterials in burn patients with other antibiotics, the results showed that leaf oil Olive has a strong antibacterial effect [10]. Olive leaf with content of 1 to 2% concentration contains phenolic acid, phenolic Alcols, flavonoids, scarids and phenolic and lipophilic compounds, there are also several biological activities that include: antioxidant, anti-carcinogenic, anti-inflammatory, anti-inflammatory, anti-fatigue, laxative, anti-platelet and cardioprotective [11], also, in other studies, the antiviral effect [anti-rotavirus], rota virus from the virus family RNA, in infections of small epithelial cells that cause fever, vomiting and diarrhea in children, it is noted in the Olive leaf [12].

This study investigates the effect of a combination of plants that have a combined pharmacological property on second-degree burns that can create a microbial-free environment, they can be effective in accelerating wound healing, and the scar tissue can be reduced to a small extent in terms of shape and size. by combining the extracts of plants [*fasciculifoliusastaragalu* and leaf Olive], on the wounds, in particular burn wounds, in addition to quick wound healing, prevent scar formation and also cause severe disinfection of the wound

environment, these plants have been used traditionally and have had a positive effect, the preparation of these plants is easy and with cheap chemical drugs, it can also be used as a prevention of bed sore in patients who have been hospitalized for a long time.

METHOD:

This quantitative and experimental study was conducted in 2015 with the approval and approval of the university of medical sciences in the Yasuj after approval by the deputy research council, the aim of this study was to compare effects of Olive leaf extract and *fasciculifoliusastaragalu* beyond normal saline 2nd degree burn healing in male rats by the researcher and colleagues created. In this research, to investigate and study the herbal compounds Olive leaf extract and *fasciculifoliusastaragalu* and the role of each in healing of burn wounds, 20 adult male Wistar rats [250-300 gr] with free access to water and feed of the mice and the dark-dark period of 12 hours, with a temperature of 24 ± 2 ° C, and adequate ventilation were maintained, before the start of the experiment, we divided the animals or mice into two groups of control and test in 5 subunits: 1-first group, control group, 5 mice. 2- second group, Olive leaf, 5 mice. 3- three group, *fasciculifoliusastaragalu* 5 mice. 4- group four, composition Olive leaf and *fasciculifoliusastaragalu*, 5 mice. before the anesthesia of the mice with ether, the hair was shaved at the back of the wound, the site was disinfected before the ulcer was injected. It should be noted that the mice were kept in good laboratory conditions before, during and after the experiment.

The method of creating a burn wound in an animal:

The format of burn wounds in dimensions 1×1.5 Square centimeters. to create second degree burns, a special metal plate with dimensions 1×1.5 Square centimeters provided by placing the same page with hot heating 90 C for the duration 10 Second we created a burn wound in the back of the animal. the wound was placed in a flattened,

tempered and metallic casing at fixed and constant temperature on the anesthetized mice, and injured with the dimensions mentioned[13].

The method hydroAlcolic extraction:

Plant extract Olive and *fasciculifoliusastaragalu*, the leaves were obtained from the plant, initially, the leaves of the plants were thoroughly crushed after being separated, collected, rinsed clean and dried in a dark place without moisture, and then sown in powder form, then the amount 300gr then prepared powder from each plant in order, Alcol 96% with 30 mil liter distilled water into a graduated cylinder to Alcol 70% was prepared, Alcol, in the specialty for leaf powder *fasciculifoliusastaragalu* and Olive leaf individually added to each human being, then kept plants containing powders for 48 hours in a dark room, so that each human being was covered with a cap, then we passed the hydroalcolic solutions of each plant, after passing through the filter of the receiver of suspended particles separately, from cloth or fine tissue or filter paper, and performed extraction with an Alcolic extracting machine at 40 ° C. solution of the extracted extract was Extraction for 30 minutes at 3500 rpm, and the resulting supernatant was exposed to air on a smooth glass until the solvent was evaporated completely and the crystalline powder of the extract was obtained [14]. The extracts were stored until refrigerated. we mixed the desired herbal compounds equally in a specified amount of 30 gr of each plant extract, in order to obtain the desired composition, we used plant compounds at concentrations of 10, 20, 30, 40 and 50 gr for topical administration of mices, an effective dose [ID50] was almost at a concentration of 30 gr [15].

Use of herbal compound created on the wound:

Animals were completely cleaned and disinfected after being fully cleansed in individual cages, we put the herbal extract on the wound burn caused by the mice, in this way, after wound burning, the ulcer was clean and once a day at 9 o'clock, a 1cc of the compound was placed on the surface of the burn wound, the ointment was put on a wound for 15 days with a daily change, in the control group,

we used only the physiology serum on grade 2 burn injuries. The end of the treatment day was prepared from the microbial culture test sites, also, the percentage of wound healing was measured by the ruler in terms of the shape and size of epidermis and dermal tissue and the percentage of recovery was calculated using the following formulas: Percentage of ulcer= $100 \times \frac{X}{Y}$ [X wound length per day / the length of the wound on the first day]

Percentage of recovery = 100- percentage of ulcer
The rate of wound healing in the control and test groups was compared and the outcome of wound healing was reported based on time, quality, scar formation and infection in each group, at the end of the treatment day 15, the mice were anesthetized and blood samples were collected from the heart for performance testing of the factors $TNF\alpha$, $TGF\beta$ [16]. Then, using an easy killing method with cervical spine cuts, the skin tissue samples were prepared from the wound site

and transferred to a 10% formalin solution for tissue retention, after processing and molding, serum sections of 15 microns were prepared by microtome and stained with color of hematoxylin and eosin. the cuts were measured by optical microscope.

FINDINGS:

In this study, a qualitative statistical test was used based on the natural distribution of the smirnormcolomograph test, because the results of this test showed, after the completion of the research interventions, the values of the variables of burn wound size [p=0.01], and factor $TGF\beta$ and $TNF\alpha$ [p=0.02], the level of significance is < 5% smaller and accordingly, these variables do not follow natural tendencies. Regarding the number two groups of study group is more for comparison between a group of variables Kruskal Wallis test results that were used in the following table.

Table 1: comparison of mean and mean burn injuries in male rats treated with Olive leaf extract and *astaragalu* Gum with the serum physiology after the completion of the research interventions

Variable	groups	Number	mean ±Std	mean rating	statistical test	
					statistics	p-value
size of the wounds burns	control [physiological ser]	5	1.3 ± 0.27	18	12.5	0.006
	<i>astaragalu</i>	5	0.1 ± 0.2	8.6		
	Olive leaf	5	0.14 ± 0.11	10		
	compounds Olive leaf and <i>astaragalu</i>	5	0.05 ± 0.03	5.4		

Kruskal Wallis test

Table [1]: shows that the size of the wounds burns after the completion of the research interference between various treatments study is statistically significant [p<0.05] was different, according to table [1], the lowest mean and standard deviation related to the effect of the combined Olive leaf extract and *astaragalu* [0.03 ± 0.05] also the lowest average rating [5.4], and most of the mean and standard deviation related to the control group [1.3 ± 0.27], to determine precisely the difference between the various treatments, using my test Mann Whitney the size of the wounds burns after the completion of the research interference between various treatments studied in the two of them were compared to the results in table [2], has been reported.

Table [2]: compared to the size of the wounds burns after the completion of the research interference between various treatments study

Variable	group	Number [Percent]	mean ±Std	mean rating	statistical test	
					Z	p-value
The size of the burn wound	Olive leaf	[20] ⁵	0.14 ± 0.11	3	-2.67	0.008
	control [physiological ser]	[20] ⁵	1.3 ± 0.27	8		
	<i>astaragalu</i>	[20] ⁵	0.1 ± 0.2	3	-2.67	0.008
	control [physiological ser]	[20] ⁵	1.3 ± 0.27	8		
	compounds Olive leaf and <i>astaragalu</i>	[20] ⁵	0.03 ± 0.05	3	-2.67	0.007
	control [physiological ser]	[20] ⁵	1.3 ± 0.27	8		
	Olive leaf	[20] ⁵	0.14 ± 0.11	6.1	0.63	0.523
	<i>astaragalu</i>	[20] ⁵	0.1 ± 0.2	4.9		

	Olive leaf	[20] ⁵	0.14±0.11	7	-1.5	0.129
	compounds Olive leaf and astaragalu	[20] ⁵	0.03±0.05	4.1		
	astaragalu	[20] ⁵	0.1±0.2	7.1	-0.129	0.196
	compounds Olive leaf and astaragalu	[20] ⁵	0.03±0.05	4.3		

Mann-Whitney tests

According to table [2], the minimum size of the burn wound associated with the plant composition extract Olive leaf and astaragalu with a value [p = 0.007]. It is also observed in this table [2] that the compounds of the extract of plants, both in combination or alone, were significant [p <0.05] compared to the control group.

Table[3]:Intraperitoneal comparison of burn wound size before and after the completion of research interventions in different treatments of the

grups	Descriptive statistics Prior to research interventions			Descriptive statistics after to research interventions			rating mean difference						Wilcoxon statistical test	
	mean	Std. Deviation	median	mean	Std. Deviation	median	Negative Ratings		Posositive Ratings		Equality ranks		Z	P-value
							r	nbe	nu	nra	nbe	nu		
astaragalu	1.5	0	1.5	0.1	0.2	0.15	5	3	0	0	0	-2.02	0.04	
Olive leaf	1.5	0	1.5	0.14	0.11	0.09	5	3	0	0	0	-2.04	0.04	
compounds Olive leaf and astaragalu	1.5	0	1.5	0.03	0.05	0.04	5	3	0	0	0	-2.06	0.03	
control [physiological ser]	1.5	0	1.5	1.3	0.27	1.5	5	1.5	0	0	3	-1.41	0.15	

According to table [3], intra-group comparison of burn wound size before and after the completion of research interventions in the test and control groups under study, there is a significant difference [p <0.05] between the studied groups.

Table[4]:comparison mean and the mean rating TNFα whit Olive leaf extract and astaragalu, compounds Olive leaf and astaragalu whit grup control physiological serafter the completion of research

Variable	group	Number	mean ±Std	mean rating	statistical test	
					statistics	p-value
TNFα	control [physiological ser]	5	2.39 ± 0.61	18	13.33	0.004
	astaragalu	5	0.99 ± 0.17	4.6		
	Olive leaf	5	1.19 ± 0.09	10.4		
	compounds Olive leaf and astaragalu	5	1.19 ± 0.09	9		

Kruskal Wallis test

Table [4], shows that TNFα was significantly different between the studied groups after the completion of research interventions, to determine the exact difference between different treatments, using the Mann-Whitney TNFα test, after the completion of research interventions, between two different treatments, the study was compared in two to two cases, the results of which were reported in table[5].

Table [5]: comparison TNF α after the completion of the research interference between the groups being studied in the two of them

Variable	group	Number[Per cent]	mean \pm Std	mean rating	statistical test	
					Z	p-value
TNF α	Olive leaf	[20] ⁵	0.19 \pm 0.09	3	-2.61	0.09
	control [physiological ser]	[20] ⁵	2.39 \pm 0.61	8		
	astaragalu	[20] ⁵	0.99 \pm 0.17	3	-2.61	0.09
	control [physiological ser]	[20] ⁵	2.39 \pm 0.61	8		
	compounds Olive leaf and astaragalu	[20] ⁵	1.16 \pm 0.09	3	-2.61	0.09
	control [physiological ser]	[20] ⁵	2.39 \pm 0.61	8		
	Olive leaf	[20] ⁵	0.19 \pm 0.09	6.1	-2	0.004
	astaragalu	[20] ⁵	0.99 \pm 0.17	4.9		
	Olive leaf	[20] ⁵	0.19 \pm 0.09	7		
	compounds Olive leaf and astaragalu	[20] ⁵	1.16 \pm 0.09	4.1	-0.52	0.6
	astaragalu	[20] ⁵	0.99 \pm 0.17	7.1		
	compounds Olive leaf and astaragalu	[20] ⁵	1.16 \pm 0.09	4.3	1.56	0.11

Mann-Whitney tests

According to table[5], the TNF α of the test groups were significantly different in comparison with the control group, but there was no significant difference between the two groups in the two groups of inflammatory factors.

Table[6]: comparison TGF β after the completion of the research interference between the groups being studied in the two of them

Variable	groups	Number	mean \pm Std	statistical test	
				statistics	p-value
TGF β	control [physiological ser]	5	2.58 \pm 0.38	45	0.001
	astaragalu	5	1.24 \pm 0.11		
	Olive leaf	5	1.33 \pm 0.07		
	compounds Olive leaf and astaragalu	5	1.42 \pm 0.1		

test variance analysis unilateral

Each of the inhibition factors in the control group was significantly different [P <0.05].

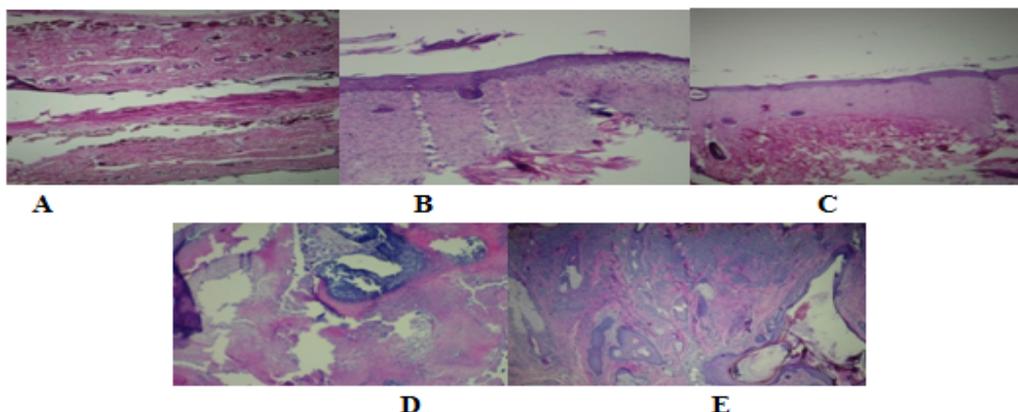


Fig 1: [A, B, C, D and E] Microscopic evolution of burnt skin wounds, 15 days after the application of the physiological serum in the control groups, Zoom in 40 \times 100

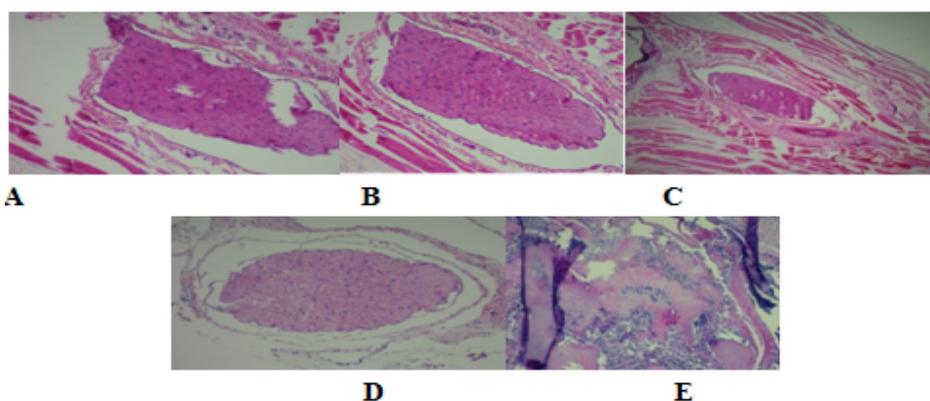


Fig 2: [A, B, C, D and E] Microscopic evolution of burnt skin wounds, 15 days after the application of the plant Olive leaf, Zoom in 40×100 .

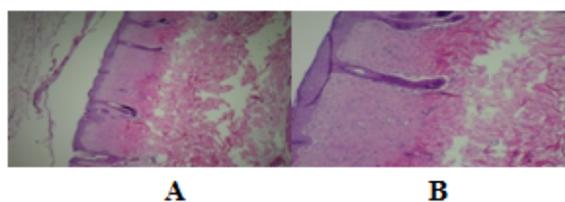


Fig3: [A and B] Microscopic evolution of burnt skin wounds, 15 days after the application of the herbal extracts compounds Olive leaf and Astaragalu, Zoom in 40×100 .

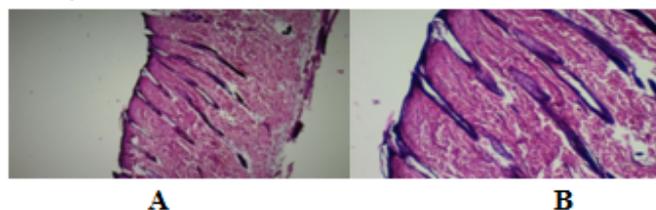


Fig4: [A and B] Microscopic evolution of burnt skin wounds, 15 days after the application of the extract plant Astaragalu, Zoom in 40×100 .

DISCUSSION:

In recent years, much attention has been found out to herbal medicines and their origin, this is mainly due to the demonstration of the side effects of chemical drugs. The problems of the modern drug system, such as high costs and environmental contamination by the pharmaceutical industry, have led to more attention to medicinal plants [17]. The use of herbal medicines in the treatment of skin injuries such as burns, bed sores or ulcers confirmed by different studies and their effectiveness has been customary. Medicinal plants due to parts Alkaloids and phenolic compounds, which are important in terms of therapeutic [18]. In the present study, according to Table [2], the lowest amount of burn wound was

related to plant extract Olive leaf extract and *fasciculifolius* *astaragalu* extract [$p = 0.007$], In Table [2], the compounds of the extract of the plants *astaragalu* as compared to the control group, had a more significant effect on burn wound healing [$p < 0.05$]. According to a research carried out by the Pirbaloty, tissues made after the use of plants *astaragalu* as a drug in mice with burn wounds showed that wound tissue repair was performed faster [19]. in the table [[1 and 2]] Considering the meaning [$p < 0.05$], the effect of Olive leaf on the size of burn wounds showed that the plant had a good effect on burn wounds which is also consistent with the research on the Olive plant, the leaves of the Olive plant have a phenolic effect that contains a very strong antioxidant that

helps in wound healing[20]. The purpose of this study was to investigate the effects of a combination of plants that have the same drug-related properties on burn wounds or litter lesions that can create a microbial-free environment, improve wound healing and scar tissue in terms of shape and size to a small extent. According to Table [3], intra-group comparisons of burn wound size before and after the research interventions in the experimental and control groups under study were significantly different [$p < 0.05$] between the studied groups and well affect the effect of Olive leaf extract after intervention has shown. In a study, they underwent phenolic compounds and Olive leaf antimicrobial activity at the University of Portugal country, in this study, seven phenotypic compounds were obtained by reverse phase DAD / HPLC in vitro from alcoholic extract of Olive leaves, which include: Caffeic Acid, Verbaccocci, Oloropine, L-7-O-Glucoside Luteolin, Rutin, 7-O-Glucoside and Luteolin-4-O-Glucoside, which have antibacterial and antifungal properties, in this research, the Olive leaf on gram-positive bacteria [*Bacillus cereus* and *Staphylococcus aureus*], gram-negative bacteria [*Pseudomonas aeruginosus*, *Escherichia coli* and *Klebsiellapneumoniae*] and fungi included: [*Candida albicans* and *Cryptococcus*], It was effective. The results of this study showed that evaluation of phenolic compounds obtained from Olive leaf phytochemical structure has a significant effect on pathogenic microorganisms in laboratory culture media[21]. In a research titled "Phytochemical analysis", they performed supportive activity of gastric ulcer with extracted Olive leaf extract from Belgrade University. In this study, the mice were randomly divided into 5 groups. In this study, control rats received 50 rats and the rats were treated with Olive leaf extract for treatment. The findings of this study indicate that in the Olive leaf, despite its high antioxidant properties, polyphenolic compounds protect the stomach from mice against ethanol. The findings of this study indicate that in the Olive leaf, despite its high antioxidant properties, polyphenolic compounds protect the stomach from mice against ethanol which Include: Oleuropin, Caffeic Acid,

Luteolin-7-O-Glucoside, O-7-Phenylene Glucoside, Quercetin, Chrysoioid, Luteolin plays an important role in inhibiting the gastric acid pump and glutathione peroxidase in inhibiting free radicals. The results of the research showed, the Olive leaves have a great deal of antioxidant properties in the treatment of stomach ulcers. In this study, phenol content of total flavonoids and tannin in the Olive leaf extracts [OLE] was determined[22].

CONCLUSION:

The results of this study were statistically significant, extract plants used in the present study has a huge impact in terms of rapid recovery wound, size and cellular are able to the environment, and even the wound disinfectant, and are effective in the formation of fresh collagen tissue at the site of the ulcer. In the present study, the combination of herbal extracts Olive leaf and *fasciculifolius* *astaragalu* or each of the extracts of these plants alone had a greater and faster effect on the healing of second-degree burn wounds compared with the control group that used only the wound physiology serum. Therefore, it can be said that the use of extracts of plants in the size of the ulcer, the appearance of the skin without scar, the accelerated recovery time of the burn wounds has a significant role then in using the physiological compounds on the wound, so it can be used as a substitute for medicinal plants instead of using chemical and industrial products.

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