



## Study the effects of oiled and flavonoid extracts of *Opuntia Ficus-Indica* on healing of avulsion skin wounds in a rabbit's models

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### Abstract

The largest organ of the body system of the body is the skin. So that, it is play a very important central roles in the protection of body against infections, chemicals and mechanical forces. The health of the skin plays a pivotal role in maintaining it, physiological homeostasis of body, fluid imbalance, and thermal dys-regulation. This study was applied in 18 rabbits with avulsion wound in the skin of dorsal part of body back region, the injuries of skin layers involve remove the full-thickness of skin layers, then studying the effectiveness of *opuntia ficus-indica* extracts on wound healing in two forms (oiled and flavonoid extract).

**Results:** after seven days of our study appears the excellent ability of flavonoid group on avulsion wound healing and antimicrobial to compare with oiled group and control group.

**Conclusion:** flavonoid extract of *opuntia ficus-indica* may useful in the treatment of skin injury of extremities and in any part of body.

**Aims of study:** to assessment the efficacy of oiled and flavonoid extract of *opuntia ficus-indica* on avulsion wound healing and study the ability of oiled and flavonoid extract as antimicrobial.

**Keywords:** *opuntia ficus-indic*, avulsion wound, wound healing and skin rabbits injuries

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### INTRODUCTION

The skin wound healing is a very complex process and dynamic procedure for changing the devitalized, dead tissues of skin layers and cellular structures of skin (Sorg et al, 2017). The function of the protective barrier of the skin is the main reason, which is the presence of layers in the skin for wound healing to be restored, in order to protect him from damage and infection. These need to understand the functions and overlaps of many cells and cell mediators from multiple skin sites. However, prolonged wound healing phases or stages increased the body responses to injury restrict normal wound healing and may be associated with formation of scar tissue, so the healthy condition of skin plays an important role in maintaining the body's physiological balance. In this way, it plays central roles in the protection of the body from infections and external mechanical forces by thermal dysregulation and fluid imbalance (Landen et al, 2016).

Cactus (*Opuntia ficus-indica*) is an evergreen plant that abounds in desert areas. It has flat and oval leaves, the trunks of which are stacked one on top of the other at individual angles that originate directly from the root.

The surface of the leaf is already covered with sharp spines at the knots (Butera et al, 2002) (**Fig. 1**).

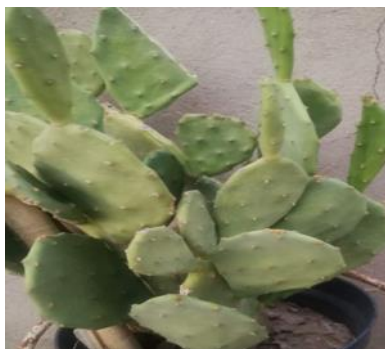
*Opuntia ficus-indica* has been used in wide fields, Involved healthcare, nutrition and also in some forms as cosmetic agents (tea, jam, juice and oils extracted from prickly pear seeds) and is used as a herbal remedy for various health problems in different countries, and the fruits of Aloe Vera act as an anti-ulcer or anti-diarrhea medicine and also an oral anti-hemorrhoid medicine and a treatment for whooping cough. Cactus groups are distinguished by their interesting contents of antioxidants, pectin polysaccharides and fiber (Alimi et al, 2010; Valente et al, 2010).

Cactus contains substantial amounts of vitamins (E, C, A), amino acids, fibers and antioxidant compounds such as betaxanthin, betacyanin, phenols and flavonoids (Khémiri et al, 2019). The scientific recent studies report that cactus have a highlight presence of natural particles that the high potential expert in health and human medicine may possess (Kuti, 2004; Landen

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**Fig. 1.** Cactus (*Opuntia ficus-indica*)

et al, 2016). It is known that *Opuntia ficus-indica* contains a high proportion of polyphenols show antioxidant and anti-inflammatory properties, and is an extraction of biologically active compounds from solid plant material using solvent impermeable base common in plant medicine, this point steps in the manufactured of phytochemical products (Sorg et al, 2017; Valente et al, 2007). In a way that arouses curiosity or interest the isolated products from the cactus flavonoids, neobetainin, alkaloids and indicaxanthin show have been various sugars, which extracts are abundant and have anti-diabetes and anti-skin effects (Harbon and Baxter, 1993; Galati et al, 2003; Tesoriere et al, 2004)

## MATERIALS AND METHODS

### Animals

The present experimental study was applied under the society of cruelty of animals to prevent it in Iraq, in accordance the ethics of college of veterinary medicine for study on the laboratory animals. The study population consisted of eighteen adult rabbits (10-12) month's old age weighting (1.8-2.3) kg divided randomly in to three groups:

- 1- Vaseline group (Control).
- 2- Oiled group.
- 3- Flavonoid group.

All the rabbits were housed individually indoors beginning 7 days before the first test day in an air conditioned room at (18 to 21 °C) temperature and (45% to 48%) of humidity. They were exposure to 12hrs. of light and 12hrs. of dark as 24-h lighting cycle that included conditions and they were fed a commercial diet for rabbit, with had unrestricted access to water.

### Plants:

Aloe Vera (*Opuntia ficus-indica*) is a permanent evergreen plant rate grows well in climates semi-arid and desert, Aloe Vera leaves In fact are legs shapes, oval and flat shapes, but they are misinterpreted as papers. Aloe Vera leaves were provided locally, plant health was verified and Vouchers were deposited samples in the Basra herbarium (Basrah University, College of Science, Basrah- Iraq). Leaves were ground with a hand grinder and preserved until use time. All

used reagents and solvents were of the highest purity grade available and purchased from (Sigma-Aldrich, 2016).

### 1- Preparation of extracts

#### 1-1- Essential oil extraction

It was extracted amount (30 g) of powder in the powder device Soxhlet using petroleum ether for 24hours. The filtrations extract and evaporated to dry under reduced pressure in the rotary evaporator to give 3.188 g extract (dry extract).

#### 2-1- Flavonoid extraction

Thirty gram of Cactus leaves powder mixture with ethanol were reflux for 6hrs, The extracted filtration through filter paper (whatman No. 541), then add 2% lead acetate water until the composition and structure of deposits flowing, separated sludge filtration paper (Whatman No. 532) and wash with water, methanol and ethyl acetate, respectively.

Salt produced turns into chloride through dissolved in (50 ml acetone and 10 ml 2N HCl) and be nominated through the filter paper (whatman No. 540), the candidate is placed in a Petri dish at room temperature to dry, powder weight brown amorphous Which formed 4,410 gm.

#### 3-1- Preliminary phytochemical analysis

Was subjected to extracts obtained chemical examination of the initial plant to identify the chemical components of cactus leave and methods of analysis used are those described (Harbone and Baxter, 1993; Khandelwal, 2005).

#### 4-1- Thin layer chromatography (TLC)

Was conducted chromatography thin layer of essential oils and flavonoids leaf cactus, was used 10 $\mu$ l of each extract on a thin layer coated generation silica phase fixed (2  $\times$  10 cm) as mobile phase with ratio respectively, these were the words classes for the left in the solvent for 60 min., then dry the by use hair dryer to screen for chemical families (active ingredients) in the extracts.

### Preparation of animals

All the animals were anesthetized with administration of xylazine hydrochloride 5 mg\Kg. bw. and 50 mg\ Kg. bw. ketamine hydrochloride, intramuscularly according to (Satheshkumar, 2005). Make avulsion wound 1cm length in the back (dorsal) of the rabbit by scalpel after given the rabbit's systemic antibiotic (penicillin and streptomycin) for three days as prophylactic dose preoperatively (**Fig. 2**). After them the avulsion wound are imbedded with bacteria and fungi according the groups of the experimental design of our study (**Fig. 2**).

### Antimicrobial Activity

A study of effectiveness of essential oil extract and flavonoid extract against a type of bacteria isolated from the skin known as *staphylococcus aureus* and type of fungus known as *Trichoderma*. In order to study the



**Fig. 2.** Skin avulsion wound, imbedded of pathogens

**Table 1.** The qualitative test of Preliminary for essential oil and flavonoid extract (Absence = - ; Presence=+)

Phytochemical	Essential oil extract	Flavonoid extract
Glycoside	-	+
Phenols	-	+
Flavonoids	-	+
Tannins	-	-
Saponins	-	-
Alkaloids	-	-
Terpenoids	+	-
Sterols	+	-
Carbohydrate	-	-

biological of extract oil and extract flavonoid. The agar well diffusion method was used Muller –Hinton Agar (MHA) for growth of fungus and bacteria to study the ability of extract oil to inhibit Antibacterial efficacy of the extract was measured using the concentration (25,100,200)mg/ml. Prepare the medium by dissolution 30gm of the plant medium in liter of distilled water. Sterilize the medium with an auto clave device at 121°C and pressure 1atm for 30min, and then pour into sterile glass dishes size (5×5cm) (Gowri and Vasantha, 2010). The microliter Essential oil, flavonoid extract were planted with a hole in the center of the plant, the dishes were then placed in the incubator at 37°C for 24hrs and then measured by the diameter of the inhibition zone millimeter.

## RESULTS AND DISCUSSION

Qualitative analysis for essential oil and flavonoid extract for Cactus leaves. Table (1) appear the indicated the analysis of flavonoid extract of the preliminary phytochemicals, the presence of flavonoids, glycosides and carbohydrates showing as a results of analysis. The same schedule prepares the extract of essential oils that contains compounds from steroid and terpinoid. The preferable sources of chemicals is a vegetarian extracts

**Table 3.** Used concentration and ratio inhibition of oil extract and flavonoid extract against bacteria *Staphylococcus aureus*

Sample	Concentration(mg/ml)	Ratio inhibition(Cm)
Flavonoid extract	100	5
Essential oil	100	5

**Table 4.** Used concentration and the ratio inhibition of essential oil and flavonoid extracts against fungi *Trichoderma*

Sample	Concentration(mg/ml)	Ratio inhibition(Cm)
Essential oil	100	3.6
Flavonoid	100	3.3

that can be used as extract for therapeutic uses (medical treatment), these regarding as alternative phyto-treatment form. The most classification of phytochemical it is basically produced through a plant part as secondary metabolites, despite the function of the phytochemical complex in plants are not clear, however, some chemical plant products appear to be defensive against plant pathogens structural and functional properties. (Balandrin and Kjoke, 2005).

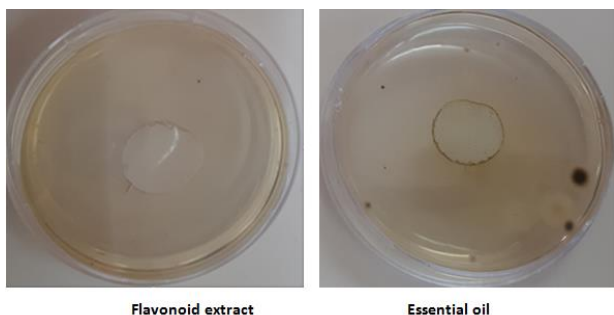
The results presented in **Table 2** shows that the essential extract of Cactus leaves contains five components are given positive test with terpenoids and sterols. The flavonoid extract contains three compounds. These components are given a positive results with Folin-Denis, Lead acetate (basic, 25%), FeCl<sub>3</sub>-K<sub>3</sub>Fe (CN)<sub>6</sub>; (1:1), Benedict reagent and Antimony chloride (10% in CHCl<sub>3</sub>) reagents because they related to the flavonoid family.

The anti-microbial activity of Cactus leaves were tested against pathogenic bacteria (*Staphylococcus aureus*) and Fungi (*Trichoderma*) at dose(0.1) gm the oil extract and flavonoid extract shows moderate to high activity at all concentration against bacteria (*Staphylococcus aureus*) and anti-fungi (*Trichoderma*) activity as in **Tables 3 or 4** and **Figs. 3** and **4**.

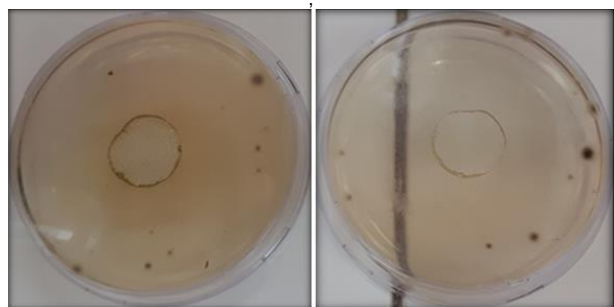
The results show that increase in the zone of inhibition of the bacterial and fungal growth when tested at a concentration of (100mg/ml) of essential oil and flavonoid extract. The basis of treatment for bacterial infections is antibiotics, so that the present study appears clearly the effect of plant extracts naturally as antibacterial and we suggest that can used in the exploited of infection management which produced by pathogen micro-organism in the animals and human procedure manner. A high variability of bacterial genes

**Table 2.** TLC for flavonoid extract and essential oil as preliminary qualitative test

Test sample	Pansaldehyde & Phosphoric acid	Ninhydrin	Folin reagent	Drangdroff	40% H <sub>2</sub> SO <sub>4</sub>	H <sub>2</sub> SO <sub>4</sub> +Chloroform	visible
Essential oil extract	0.7	-	-	-	-	0.7	-
	0.45					0.45	
	0.33					0.33	
	0.23					0.23	
	0.19					0.19	
Flavonoid extract	-	-	0.40	-	0.40	0.40	
			0.35		0.35		
			0.21		0.21		



**Fig. 3.** Skin avulsion wound, imbedded of pathogens



**Fig. 4.** Ability of inhibition of essential oil and flavonoid extract against fungi *Trichoderma*



**Fig. 5.** Appearance of puss after two day

administered to them to be evading quickly antibiotics action by increasing the development of antimicrobial resistance that create a major clinical problems when use antibiotics in the treatment of infectious diseases (Pompeu et al, 2015). The toxicity of certain antibiotics drugs on the human tissue and others problems make the triggered expert increase in the research of new plant origin as antimicrobial drugs and substances. The screening and scientific assessment of plants extracts it is expecting that because its antimicrobial property that may provide a new antibiotic materials considering of high diversity of plants; So that the current study appear clearly the anti-bacterial nature of the cactus extract and we suggestion that the cactus plants can be used in the controlling of bacterial infection diseases in the animals and human systems, this agreement with (Ogbulie et al, 2007).

The clinical observations in our experimental study on the wound differ from group to other one, the main signs characterized by puss formation after 36-48 in all groups after applied the pathogens on wounds **Fig. 5**.



**Fig. 6.** Oiled group after three day



**Fig. 7.** Flavonoid group after three day



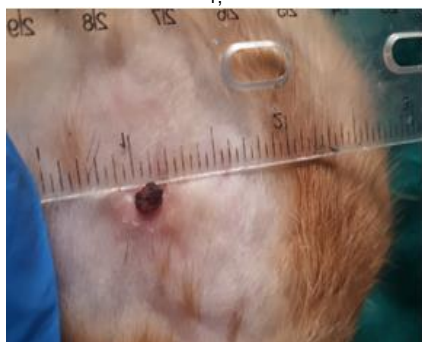
**Fig. 8.** Vaseline group after three day

After three days, applied the flavonoid and oil extract with Vaseline on wound to measurement the activity of it on wound healing by effects on action of pathogens. In flavonoid and oil groups show the narrowing of wound size and arrest of puss formation in day three after applied the flavonoid and oil extract on wound **Figs. 6** and **7** to compare with Vaseline group as control group in the same period **Fig. 8**. This result agrees with (Khémiri et al, 2019) when use the *opuntia ficus-indic* oil has shown a good effect of healing of wound. It also shown the ability of oil extracts on reepithelialization phase by reducing this phase and prevents cutaneous infections.

Continuity applied the extract on the wound until day seven and the recorded changes appeared complete healing with growing new hair and disappearance of the scar tissue in flavonoid group (**Fig. 9**) to compare with control and oiled group, while the oiled group showing the continuity of wound narrowing with scar formation (**Fig. 10**) to compare with control group (**Fig. 11**)



**Fig. 9.** Oiled group after three day



**Fig. 10.** Oiled group after seven days

appeared no changes in the wound size and healing, these results recorded in the same study of (Ammar et al, 2015) when use the extracts of flowers of the *Opuntia ficus-indica* (mucilaginous and methanol) that appear the activity of it in the healing of wound were assessed



**Fig. 11.** Vaseline group after seven days

by using an excision wound in the rats models. The acceleration of contraction of wound and reducing the phases of remodeling was appeared after four weeks from treatment by use the plant extracts, and showing a benefit efficacy on skin wound repair to compare with control group (Trombetta et al, 2006).

## CONCLUSION

after seven days of our study appears the excellent ability of flavonoid group on avulsion wound healing and antimicrobial to compare with oiled group and control group, thus flavonoid extract of *opuntia ficus-indica* may useful in the treatment of skin injury of extremities and in any part of body.

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