

## EVALUATION OF THE ANTIBACTERIAL AND WOUND HEALING ACTIVITY OF *QUERCUS PERSICA*

EBRAHIMI AKRAM<sup>1\*</sup>, KHAYAMI MASOUD<sup>2</sup>, NEJATI VAHID<sup>2</sup>

1- Department of Biology, Payame Noor University, Izeah, Khozestan, Iran.

2- Department of Biology, Faculty of Sciences, Urmia University, Urmia, Iran.

\*Corresponding author: E-mail: [akfa9999@yahoo.com](mailto:akfa9999@yahoo.com)

---

**Abstract:** *Quercus persica* is one of the four species oak growing in the zagrossian region of Iran. This plant contains different components of therapeutic value. In this investigation, antibacterial and wound healing effects of methanolic extract of fruits of *Quercus persica* has been studied. Milled oak fruit that their hull was separated, extracted with methanol in Soxhlet's apparatus. The effect of extract in three concentration (25, 50, 75 mg/ml) were tested using agar diffusion method on *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* RTCC 1898 and *Escherichia coli* O157:H7 and in form topical administration on excision wound in rats. Results showed that all of concentrations were effective on inhibition of bacteria, but this effect with 50 and 75 mg/ml concentration of extract was significant for bacteria. Also in comparison with tested antibiotics, the effect of 75 mg/ml concentration of extract was similar or higher than them. Also in the extract-treated wounds indicated that epithelialize faster, and the rate of wound contraction was significantly increased in comparison to control wounds. This results suggest that *Quercus persica* possesses compounds with antibacterial and wound healing properties.

**Key words:** *Quercus persica*, Antibacterial, Extract, Wound healing.

---

### INTRODUCTION

In recent years, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes. In addition to this problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, immune suppression and allergic reactions. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infection diseases from various sources such as medicinal plants. (Berahou *et al.*, 2007). Historically, plants have provided a good source of anti-infective agents and many of them remain highly effective against microbial infections. Besides, they are cost effective and have fewer side effects. (Khosravi and Behzadi, 2006).

Other part of this study is contain investigation on wound healing effect of plant. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Initial stages of wound healing involve an acute inflammatory phase followed by synthesis of collagen and other extracellular matrix which are later remodeled to form scar. (Shetty *et al.*, 2007).

Wound management involves dressing, pain killers, the use of anti-inflammatory agents, and drugs that promote healing. Current method used to treat difficult wounds includes debridement, irrigation, antibiotics, tissue grafts, and proteolytic enzymes. (Nayak, 2006).

Search in the references and local information suggest that *Quercus* specieses can use to treat wounds due to different properties of tannins that available in them.

*Quercus persica* is a predominant species of oak plants in the zagroos forests of iran. Their leaves is simple, ovate form with dentate margins. The acorn is the fruit of oak trees and contain in a cupule. (Sabeti, 2003). The species of oak, the *Quercus* genus, are classified into the Fagaceae family. Acorns contain considerable amounts of tannin and other anti-nutritional substances. Given in large amounts they may be toxic. (Saffarzadeh *et al.*, 1999). Acorns are used traditionally for food and for making bread, and for medicinal purposes, and some uses suggest it could be used at industrial levels. (Hosseini, 2005).

Tannins are one of the major components of *Q.persica* and Importance of oak trees is most due

to presence of tannins in their different partes. Thus the purpose of this study was to investigate the antimicrobial and wound healing activity of this plant.

Despite previous reports on antimicrobial and wound healing properties of other specieses of the *Quercus* (Antimicrobial activity of *Q.acerifolia* and *Q.macrolepis* (Shahidi *et al.*, 2004) and *Q.brantii* (Khosravi and Behzadi, 2006) and wound healing properties of *Q.infectoria* (Umachigi *et al.*, 2008 ; Haidari *et al.*, 2005; Malekinejade, 1996) our study is the first on the antibacterial and wound healing effects of fruit of this species.

## MATERIAL AND METHODS

### Plant materials

The fruit of *Quercus persica* were collected from the south-zagroos (south west of iran) in October 2007.The taxonomic identification of the plant material was confirmed by a plant taxonomist in the botany laboratory and herbarium of sciences faculty of urmia, iran,where a voucher specimen was deposited(No.1412).

### Preparation of extract

The collected plant material was dried in the shadow, separated their hulls and powdered using an electric blender.The dried and ground fruit(10g) was extracted with 200cc methanol in a soxhlet extractor for 24 h. The methanol extract was concentrated to dryness under reduced pressure amount of pure extract was 2/06 g. The obtained extracts were stored in a refrigerator at +4 °C until use.

### Preparation of discs containing extracts

Different concentrations of 25,50 and 75 mg/ml were prepared from the methanol extract. The concentration were incorporated into sterile blank paper discs (Padtan Teb Inc.,Tehran, Iran) and were dried at 37 °C.

### Tested bacterial strains

The antimicrobial activity of different concentrations of extract was individually tested against three pathogenic bacteria including *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* RTCC 1898 and *Escherichia coli* O157:H7.

### Disc-diffusion assay

The agar disc diffusion test was employed according to the method of Bauer and Kirby under strict adherence to NCCLS criteria for the determination of antibacterial activities of the extract.Briefly, in disc diffusion method, Muller Hinton agar (MHA) medium was inoculated with standard bacteria and discs with different

concentrations of the extract were placed in appropriate positions in the plate. also negative controls prepared using the corresponding solvent. The plate as incubated at 37 °C for 24h. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested bacteria. A similar qualitative assay was use to determine the susceptibility patterns of selected in-use antibiotics: Gentamicin (GM=10 µg/disc),Kanamicin (K=30µg/disc) and Tobramicin(TOB=10µg/disc) and then results of both were compared.(Khosravi and Behzadi, 2006).

### Preparation of the extract for wound healing study

Fruit powder was extracted with methanol in a soxhlet apparatus.Extract was concentrated by evaporation. After the extract was dissolved in eucerin as a vehicle during the study (5%).

### Experimental Animals

Healthy female rats weighing between 150 g and 200 g were used for the study.They were individually housed and maintained on a 12 h light/dark cycle, temperature 22± 2°C, with normal food and water.The surgical interventions were carried out under sterile conditions using ketamine anesthesia.After infliction of wounds as described in the succeeding paragraph,5 animals each were randomly assigned to treatment (extract) or controls.

### Excision wound creation

The rats were anesthetized with ketamine hydrochloride and shaved on both sides of the back with an electric clipper, and the area of the wound to be created way out lined on the back of the animals with using a circular stainless stencil the full thickness of 2 cm length and 0.2 cm depth of the excision wound was created along the marking using toothed forceps, a surgical blade and pointed scissors. (Nayak, 2006).

In the topical study, ucerin (Base ointment) was applied to the ucerin group(n=5), control group were not treated(n=5), wherease the experimental group(n=5) was treated by daily application of the 5% methanolic extract ointment of *Q.persica* fruit.Changes in wound area were calculated, giving an indication of the rate of wound contraction.

### Wound Area Measurements

The measurements of the wound areas were taken on the 1 st, 3th,6 th, 10 th,14 th, 18 th,22th and 26th days for groups using transparency paper and a permanent marker.The wounds areas of all groups were recorded and measured on graph paper.

### Wound healing percent

The percentage wound healing was determined using the following formula:  
 Wound healing percent = (wound area in day 1 - wound area in day x / wound area in day 1) × 100.

**Statistical analysis**

All the tests were repeated 3 times for precise results and all data were expressed as means ± standard errors. One-way analysis of variance (ANOVA), Tukey MRT test and Excel programme were used for data interpretation. Values of p < 0.05 were accepted as being statistically significant.

**RESULTS**

The results of disc diffusion method showed that the three concentration of the extract had effects on *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli*, but the inhibitory effect of 50 and 75 mg/ml concentration of the extract was significant for bacteria. Also, out of the three bacterial species tested, *Staphylococcus epidermidis* was the most susceptible and *E. coli* had a most resistance.

Comparison between extract and antibiotics showed that the effect of 25 mg/ml concentration of the extract on *S. aureus* was same to TOB, the effect of 50 mg/ml concentration lesser than GM and K and higher than TOB and 75 mg/ml concentration had a effect same to K and GM and higher than TOB. In *S. epidermidis* the inhibition zone for 25 and 50 mg/ml concentration was same to GM and lesser than TOB and K and effect of 75

mg/ml concentration was same to K, higher than GM and lesser than TOB. Also the results indicated that on *E. coli*, effect of 25 mg/ml concentration was lesser than three antibiotic and 50 mg/ml concentration was same to GM, K and TOB. The effect of 75 mg/ml concentration was higher than three tested antibiotics. (Table.1).

Number of days required for falling of eschar without any residual raw wound gave the period of epithelization. (Kumara Swamy et al., 2007). This period for extract, eucerin and control groups was 15.2, 21.3 and 28 day respectively. On the other hand, animals treated with the methanol extract of *Q. persica* showed a significant decrease in the epithelization period, as evidenced by the shorter period for the fall of eschar compared to two other group. The drug extract also facilitated the rate of wound contraction significantly. (fig.1) show the comparison of mean of wound area variations in different days of experiment in 3 group. Understanding of this subject with observation of (fig.2) is facility.

By amounts of wound areas can calculated the wound healing percent. One-way analysis of variance of wound healing data showed a significant difference (P=0.01) that with use of Tukey MRT test (P = 0.05.), this differences was indicated. Comparison of wound healing percent in 3 group showed that the difference between the days of 3, 6 and 10 of extract with eucerin was significant. Also the difference between days of 3, 6, 10 and 14 of extract with control and 6, 10 and 14 of eucerin with control was significant (Table.2).

Table.1: Comparison of inhibition zone diameter produced by different concentration of extract with Gentamycin, Tobramycin and Kanamycin in disc diffusion method for *E. coli*, *S. aureus*, *S. epidermis*.

| Antibiotics |            |          | Zone of Inhibition (diameter in mm) in three concentration (mg/ml) |             |           | Bacteria            |
|-------------|------------|----------|--|-------------|-----------|---------------------|
| TOB         | k          | GM       | 75   | 50          | 25        |                     |
| 10.5±0.2c   | 14.5±0.8b  | 14±0b    | 15.5±0.2a  | 13.75±0.1bc | 8.5±0.2d  | <i>E. coli</i>      |
| 12.5±0.2c   | 16.5±0.2a  | 15±0.5ab | 16.5±0.2a  | 14.25±0.3b  | 10±0c     | <i>S. aureus</i>    |
| 22.5±3.1a   | 17.5±0.2ab | 16±0.5b  | 18.5±0.2ab   | 16±0b       | 12.5±0.2b | <i>S. epidermis</i> |

Values are represents as mean ± SE.

a, b, c, .... Means followed by the same letter are not significantly difference; at P = 0.05.

GM: Gentamycin, K: Kanamycin, TOB: Tobramycin

Table.2: Comparison of mean of wound healing percent in different days of control, eucerin and extract.

| Wound healing (%) |             |             |              |              |              |             | day Treat |
|-------------------|-------------|-------------|--------------|--------------|--------------|-------------|-----------|
| 26                | 22          | 18          | 14           | 10           | 6            | 3           |           |
| -                 | -           | -           | 97.23±0.25a  | 85.22±1.01ab | 36.62±0.92de | 16.62±5.1fg | Extract   |
| -                 | -           | 95.96±0.58a | 89.91±0.86a  | 71.05±2.5c   | 19.74±2.3fg  | 11.4±1.1gh  | Eucerin   |
| 94.41±0.26a       | 91.85±0.67a | 90.13±1.71a | 75.97±4.85bc | 41.63±5.28d  | 27.04±4.89ef | 10.3±1.95gh | Control   |

Values are represents as mean ± SE.

a, b, c, .... Means followed by the same letter are not significantly difference; at P = 0.05. n =5 in each group

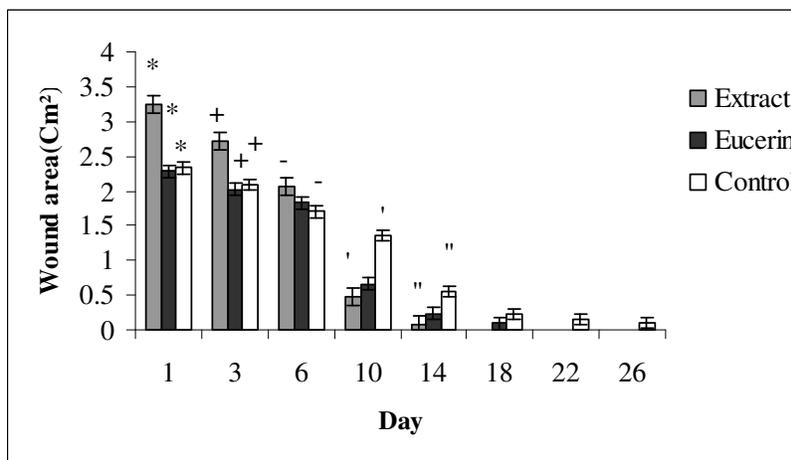


Fig.1: Comparison of mean of wound area variations in different days of control, eucerin and experimental (extract). \*,+,',": significant difference ; at  $P = 0.05$ .

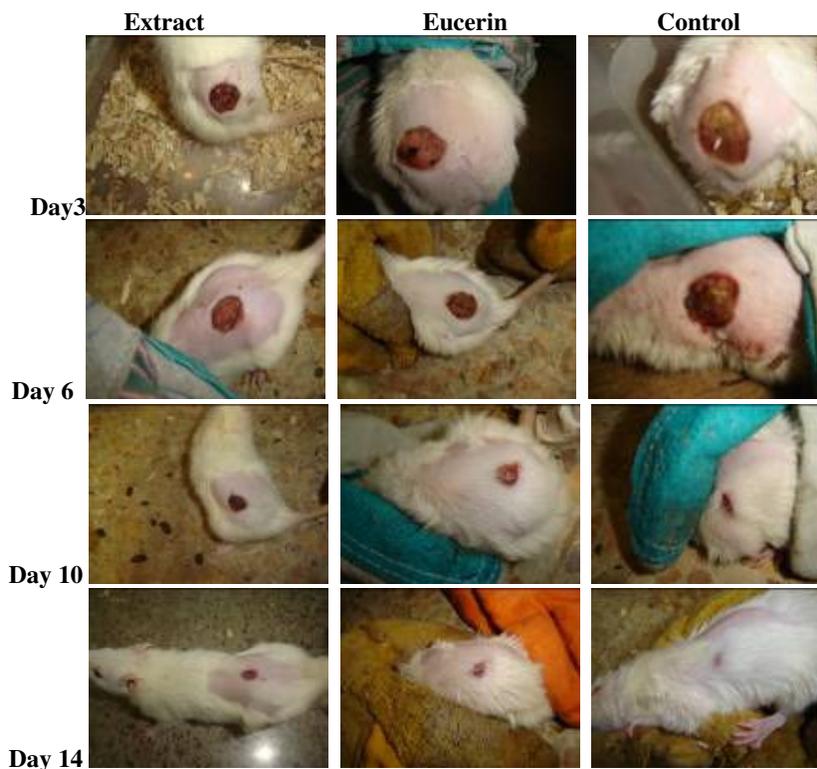


Fig.2: Photographical representation of contraction rate in different days of control, eucerin and experimental wounds.

### DISCUSSION

Difference in bacteria resistance may be attributed to lipopolysaccharides in the outer membrane of the Gram-negative bacteria, which make them inherently resistant to external

agents, such as hydrophilic dyes, antibiotics and detergents. (Hayouni *et al.*, 2007).

The present study indicated that the effect of the oak fruit extract on bacteria was concentration-dependent and in high concentration this effect was same or better than tested antibiotics. The antibacterial activity seemed to

depend on the contents of tannin in the plant extracts. Because tannin is one of the major components of *Q.persica* with antimicrobial effects.

Scalbert reviewed the antimicrobial properties of tannins in 1991. He listed 33 studies which had documented the inhibitory activities of tannins up to that point. According to these studies, tannins can be toxic to filamentous fungi, yeasts, and bacteria. (Cowan, 1999).

The methanolic extract of *Q.acerifolia* (palmer) seeds and *Q.macrolepis* Kotschy stem gum had n't inhibition effect on *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* (Shahidi *et al.*, 2004), but our study showed the inhibition effect of methanolic extract of *Q.persica* on three tested bacteria. Comparison between three species shows that *Q.persica* is more active than *Q.acerifolia* and *Q.macrolepis*.

Umachigi and *et al* studied wound healing properties of *Quercus infectoria*. They said that the efficacy of this plant in wound healing may be due to its action on antioxidant enzymes. Phytochemical work reveals that ethanolic extract of galls of *Q.infectoria* contains high amount of tannins, presence of gallic acid, ellagic acid, syringic acid,  $\beta$ -sitosterol and amentoflavone, implied that tannin is one of the active compounds which may be responsible for the antioxidant activity. Also, Studies on the estimation of antioxidant enzymes reveal that the extract significantly increased the levels of superoxide dismutase and catalase, the two powerful antioxidant enzymes of the body that are known to quench superoxide radicals. Besides, Histological examination revealed increased collagen deposition in the drug, treated group as compared to control. (Umachigi *et al.*, 2008). Also investigation (Haidari *et al.*, 2005 ; Malekinejade, 1996) indicated the efficacy of this plant in wound healing .

In other study, applying a herbal medicine contained Acorn and blamint in controlling RAS (Recurrent Aphthous Stomatitis) was successfully more affordable than placebo. In this study suggested that the tannins in combination with proteins, increase their resistance in front of proteolytic enzymes. other factor is flavenoides in plant extract. flavenoides with anti-breaking and haemorrhage of capillaries and increase of healing rate of epithelial wounds with inhibit or activate of enzymes can have main role in wound healing. (Jahanshahi *et al.*, 2004).

Tannin is one of the major components of *Q.persica* with contractive, disinfective, astringent, anti-inflammatory, antibacterial, antioxidant, protein precipitation and ... effects and clear that due to this properties can be favourable in the acceleration of wound healing.

Tannins have been used in dermatology because of their strong astringent property, which positively affects wound healing. When applied topically onto the skin or mucous membranes, tannins cause the precipitation of proteins, which renders the superficial layers impermeable to noxious agents, shrinking colloidal structures. This astringent action deprives bacteria of a favourable growth medium, producing an indirect antibacterial effect. The tannins also promote capillary vasoconstriction, which decreases vascular permeability and causes a local anti-inflammatory effect and impedes the formation of inflammatory exudates, hindering the development of microorganisms. (Lopes *et al.*, 2005).

Despite previous reports on antimicrobial and wound healing properties of other specieses of the *Quercus* , and prove the antibacterial activity of the seed hull methanolic extract. (Khosravi and Behzadi, 2006). and ethanol and acetone extract of leaves of *Q.persica* on *E.coli* (Teimouri *et al.*, 2004) , and their tannins (Kiarostami, 1998) our study was the first on the antibacterial and wound healing effects of fruit of this species.

We could demonstrate antimicrobial and wound healing properties of this plant, but further studies such as biochemical and histological are needed for careful determine the type and concentration of effective materials, also effect mechanisms of extract on wound healing phases.

## REFERENCES

- Berahou A., Auhmani A., Fdil N., Benharref A., Jana M., Gadhi C.A (2007). Antibacterial activity of *Quercus ilex* bark's extracts. J Ethnopharmacol . 112(3):426-429 .
- Cowan M.M (1999). Plant products as antimicrobial agents. Clin.Microbiol. Rev. 12(4) : 564-582 .
- Haidari R., Siami A., Pakbaz M., Aghazadeh M (2005). Measurement of tannin in four genotype of *Quercus infectoria* olive and application of their gall powder in treatment of wound. J.Aro.Med.Pla.Res.Iran . 21(4): 433-443.
- Hayouni E.A., Abedrabba M., Bouix M., Hamdi M (2007). The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts .Food Chem. 105(3) :1126-1134.
- Hosseini P (2005). Conservation of biodiversity in the central zagros landscape conservation zone (PIMS 2278). Government of the Islamic Republic of Iran. UNDP. GEF . p:35.

- Jahanshahi GH., Moattar F., Soltani MR (2004). Evaluation of a herbal medicine in the treatment of recurrent aphthous ulcer. *Beheshti Univ. Dent. J.* .22(1):19-25.
- Khosravi A.D., Behzadi A (2006). Evaluation of The Antibacterial activity of the seed hull of *Quercus brantii* on some gram negative bacteria. *Pak J Med Sci* . 22(4):429-432.
- Kiarostami kh (1998). Evaluation of antibacterial effects of *Quercus persica* and *Quercus castaneifolia* in tissue culture and perfect plant. *J.Sci.* 11(1):1-8.
- Kumara Swamy H.M., Krishna V., Shankarmurthy K., Abdul Rahiman B., Mankani K.L., Mahadevan K.M and et al ( 2007). Wound healing activity of embelin isolated from the ethanol extract of leaves of *Embelia ribes* burm. *J.Ethnopharmacol.* 109 (3): 529-534.
- Lopes G.C., Sanches A.C.C., Nakamura C.V., Dias Filho B.P., Hernandez L. , de Mello J.C.P (2005). Influence of extracts of *Stryphnodendron polyphyllum* mart and *Stryphnodendron obovatum* benth. on the cicatrisation of cutaneous wounds in rats . *J. Ethnopharmacol* . 99(2): 265-272 .
- Malekinejade H (1996). Identification and collection of *Quercus infectoria* galls and histological study of wound healing by powderes forms. University of urmia.
- Nayak B.Sh (2006). *Cecropia Peltata L(Cecropiaceae)* Has wound-healing potential: A preclinical study in a sprague dawley rat model. *Int j low extrem wounds.* 5(1) :20-26 .
- Sabeti H (2003). Forests, trees and shrubs of iran. 3rd ed. Yazd *University*, p.576.
- Saffarzadeh A., Vincze L., Csapó J (1999). Determination of the chemical composition of acorn (*Quercus brantii*), *Pistacia atlantica* and *Pistacia khinjuk* seeds as non-conventional feedstuffs. *Acta Agr. Kapos* . 3(3):59-69.
- Shahidi Bonjar G.H., Aghighi S., Karimi Nik A (2004). Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of iran. *J.Biol.Sci* . 4(3):405-412.
- Shetty S., Udupa S., Udupa L (2008). Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of *Ocimum sanctum linn* in rats. *eCAM* . 5(1):95-101.
- Teimouri M., Korori S., Moraghebi F., Matinizadeh M (2004). Comparison antibacterial activity of *Quercus persica* and *Quercus ilex*. *Iranian J Pharm Res* . 3(2):76-77.
- Umachigia SP., Jayaveerab KN., Ashok Kumarc CK., Kumard GS., Vrushabendra swamy BM., Kishore Kumarf DV(2008). Studies on wound healing properties of *Quercus Infectoria*. *Trop J Pharm Res* . 7 (1): 913-919.