Evaluation of the Antibacterial and Wound Healing Activity of *Quercus persica*

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Abstract: *Quercus persica* is one of the four species oak that growing in the zagrossian region in 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, was extracted with methanol in Soxhlet's apparatus .The effect of extract in three concentration (25,50,75mg/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.

Keywords: *Qurcus 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 [1]. Historically, plants have provided a good source of anti-infective agents and many of them remain highly effective in the fight against microbial infections. Besides, they are cost effective and have fewer side effects [2].

Other problem is wound healing. 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 [3]. 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 [4]. 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.

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Quercus persica is a predominant species of oak plants in the zagroos forests of iran. Their leaves is simple, ovate form with dentate margines.The acorn is the fruit of oak trees and contain in a cupule [5]. 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 [6].. 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 [7].

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* [8] and *Q.brantii* [2] and wound healing properties of *Q.infectoria* [9-11] 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 and botany sourses in

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the botany laboratory and herbarium of sciences faculty of urmia, iran.

Preparation of Extract

The collected plant material was dried in the shadow, separated their hulls and fruits ground to a powder. The dried and ground fruit(10g) was extracted with 200cc methanol in a soxhlet extractor for 24 h. The methanolic extract was concentrated to dryness under reduced pressure amount of pure extract was 2/06 g. The obtained extract were stored in a refrigerator at +4 ºC 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 ºC.

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 ºC 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 [2].

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± 2ºC, 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 [4]. 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}, 3^{rd}, 6^{th}, 10^{th}, 14^{th}, 18^{th}, 22^{nd} and 26^{th} 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

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*

Antibiotices			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	*E. coli*
12.5±0.2c	16.5±0.2a	15±0.5ab	16.5±0.2a	14.25±0.3b	10±0c	*S. aureus*
22.5±3.1a	17.5±0.2ab	16±0.5b	18.5±0.2ab	16±0b	12.5±0.2b	*S. epidermis*

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

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 [12]. 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. (Figure **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 (Figure **2**) is facility.

By amounts of wound areas can calculated the wound healing percent. One-way analysis of variance

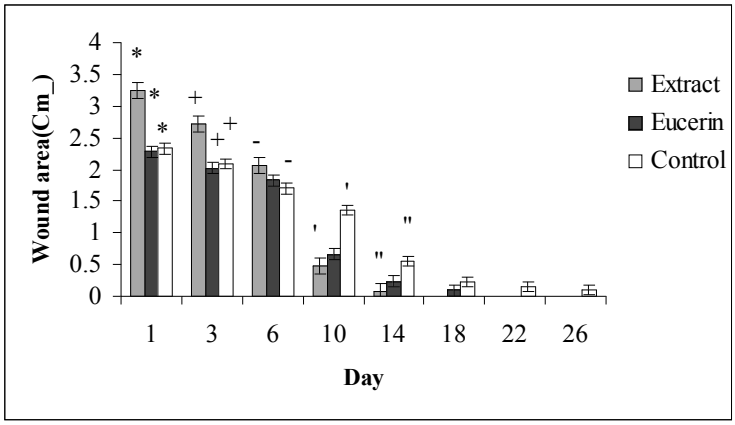

Figure 1: Comparison of mean of wound area variations in different days of control, eucerin and experimental (extract). *,+,','' : significant difference ; at $P = 0.05$.

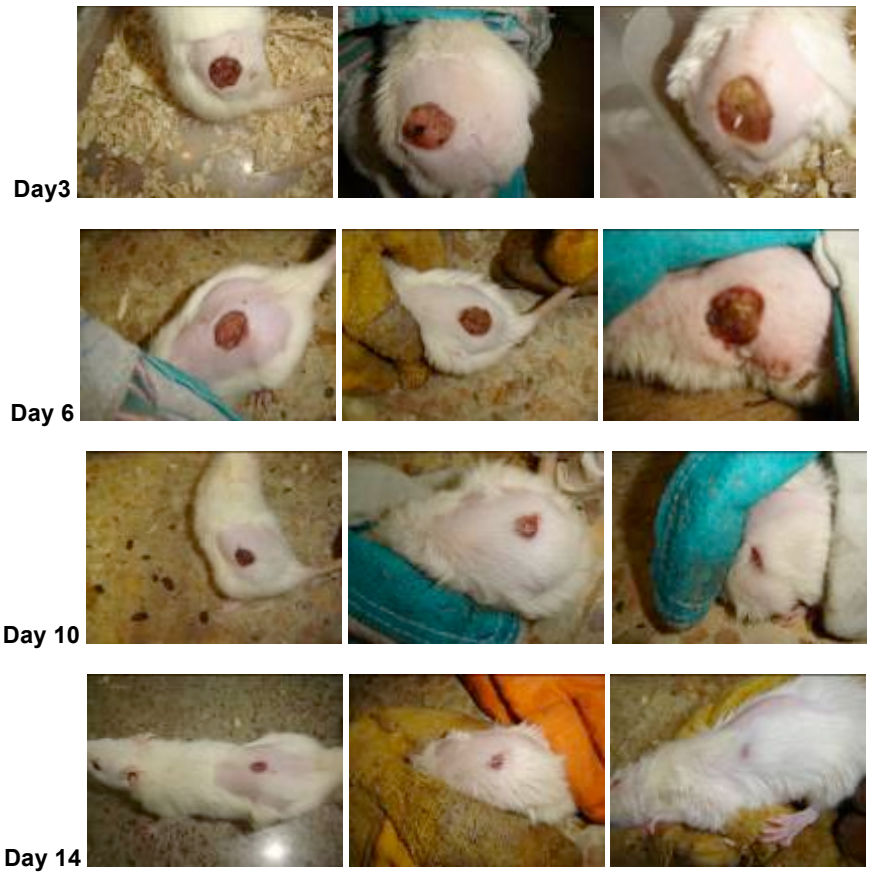

Figure 2: Photographical representation of contraction rate in different days of control, eucerin and experimental wounds.

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**).

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 [13].

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

Table.2: Comparison of Mean of Wound Healing Percent in Different Days of Control, Eucerin and Extract Wound Healing (%)

26	22	18	14	10	6	3	Day Treat
-	-	-	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

that point. According to these studies, tannins can be toxic to filamentous fungi, yeasts, and bacteria [14].

The methanolic extract of *Q.acerifolia* (palmer) seeds and *Q. macrolepis* Kotschy stem gum hadn't inhibition effect on *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* [8], 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, ß-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 enzymereveal 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 [9]Also investigation indicated the efficacy of this plant in wound healing [10, 11].

In other study, applying a herbal medicine contained Acorn and blammint 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 proteolitic 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 [15].

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 is 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 [16].

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 [2] and ethanol and acetone extract of leaves of *Q. persica* on *E.coli* [17] , and their tannins [18] 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 histologocal are needed for careful determine the type and concentration of effective materials, also effect mechanisms of extract on wound healing phases.

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