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Full Length Research Paper

Evaluation of antibacterial activity and wound healing of *Pistacia atlantica* and *Pistacia khinjuk*

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Pistacia atlantica **and** ***Pistacia khinjuk*** **are two major** ***Pistacia*** **species that grow in the Zagrossian region and classified into the** ***Anacardiaceae*** **family. In this work we investigated the drug properties of these plants. Experiments were carried out by extract of plants** ***in vitro*** **and** ***in vivo*** **(what kind of extract? both of them). In the** ***in vitro*** **experiments, antibacterial activity of four kinds of extract of** ***P. atlantica*** **and** ***P. khinjuk*** **were evaluated using the disk diffusion method on three strains of bacteria (*****Escherichia coli*****,** ***Staphylococcus aureus*** **and** ***Staphylococcus epidermidis*****). All the tests were compared with the inhibitory effect of proper antibiotics commonly used against these bacteria (Gentamicin, Tobramycin and Kanamycin). The** ***in vivo*** **experiment was undertaken to assess the potential of methanolic extract of** ***P. khinjuk*** **in wound healing in wistar rats by using excision wound model. The results showed that extract of two plants had a beneficial drug property. These results suggested that** ***P. khinjuk*** **and P*****. atlantica*** **possess compounds with wound healing and antibacterial activity properties.**

Key words: Antibacterial activity, Wound healing, *Pistacia atlantica*, *Pistacia khinjuk*

INTRODUCTION

The use of plant compounds to treat infections is an old practice in a large part of the world, especially in developing countries where there is dependence on traditional medicine for a variety of diseases. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics. (Abu-Shanbe et al., 2006).The genus *Pistacia L*. is dioecious and deciduous and belongs to the family *Anacardiaceae*. Among 15 known species of *Pistachios*, only 3 species grow in Iran, including, *Pistacia vera* L.1753, *Pistacia khinjuk* Stocks 1952 and *Pistacia atlantica* Desf 1800.They are the most important species of *Pistachio* and for this reason, Iran is known as the origin of *Pistachios*.

The word 'Pistachio' is derived from a Persian name: "pisteh" (Abrishami, 1995). Iran's Pistachio forests are mostly accompanied with wild almond and other species of trees. However, they sometimes occur as pure population or a mixed of two species or sub–species in the same forest. Rechinger (1969) and Khatamsaz (1978) recognized 3 species for Iranian Pistachios. Pistacio trees are reported to possess antibacterial activity (Rasooly et al., 1998; Moraghebi et al., 1999; Mehmet et al., 2004; Koutsoudaki et al., 2005). The fruits in Pistachio, rich in oil , are used by the local inhabitants in many ways as an anti-diarrheal and also as a constituent of cattle feed (Yousfi et al., 2002). However, no investigation of *P. khinjuk* fruit oil of Iran has been carried out. The leaves of *P. atlantica* are used as a stomachic, while its fruits and oleoresin are used in medicine. The sterols are very important due to antioxidant activity and health benefits (Benhassaini et al., 2007). Pistacia plants are known for their medicinal properties since antiquity.

They have played important roles in folk medicine and are used in eczema treatment ,throat infections, renal stones, asthma and stomach ache, and as a astringent , anti-inflammatory ,antipyretic, antibacterial, antiviral, pectoral and stimulant (Kordali et al., 2003). These plants have also been reported to contain phenolic compounds and triterpenoids (Yalpani et al., 1983; Marner et al., 1991). The polyunsaturated fatty acid content of *P.*

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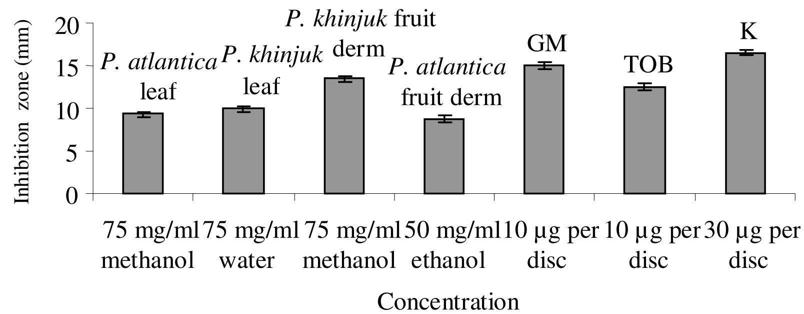

Figure 1. Comparison of antibacterial activity between effective concentration of *P.atlantica , P.khinjuk* and antibiotics.(**GM,** Gentamicin; **TOB,** :tobramycin ,K : kanamycin) on *Staphylococcus aureus*.

atlantica ecotype of Algeria which is rich in oil is higher than that of Iranian *P. atlantica* which is rich in monounsaturated fatty acid (Benhassaini et al., 2007). The ratio of unsaturated fatty acid in Iranian *P. atlantica was* 80.13% and in *P. khinjuk* 76.31%. (Saffarzadeh et al., 1999).The oil of *P. atlantica* fruit can be classified as an oleo-linoleic vegetable oil (Yousfi et al., 2002). The present report is the first study on the drug properties of *P. khinjuk*. The aim of this investigation is to determine the drug properties of *P. atlantica* and *P. khinjuk* that grow in Ilam province.

MATERIALS AND METHODS

Plants materials

The leaves and unripe fruits of *P. atlantica* and *P. khinjuk* were collected from mountain of Ivan in Ilam province in May 2007. These plants were identified in herbarium of Department of Biology, Faculty of Sciences, university of Urmia, Urmia, Iran. Leaves and fruits of two the plants were dried in shade and crushed to fine powder.

Preparation of extracts

The dried powders of the plants (10 g) were extracted in soxhlet apparatus with four kind of solvent (200 ml) (methanol, ethanol, ethanol+ water and water). The extracts were evaporated to dryness by oven at 45°C. A semisolid green and brown crude extracts of plants so obtained and weighed.

Bacterial strains

Three bacteria were selected for this study; *Escherichia coli* 0157:H7 (Gram-negative rod), *Staphylococcus aureus* ATCC 25923 (Gram-positive cocci), and *Staphylococcus epidermidis* RTCC 1898 (Gram-positive cocci), obtained from the Urmia University.

Antibacterial activity

The *in vitro* antibacterial activity of different extracts of *P. atlantica* and *P. khinjuk* at 25, 50 and 75 mg/ml was studied by disk diffusion method against three strains of bacteria. Sterilized blank disk (padtan teb) were soaked the disk in aseptic condition until the alcohol was evaporated. The bacterial strains were cultured on Muller Hinton Agar and disks placed for comparison. The Petri dishes were incubated at 37°C for 24 h. After 24 h, inhibition zones appearing around the disks were measured and recorded in mm. The activity of the extracts of two plants were compared with Gentamicin (10 µg per disk), Tobramycin (10 µg per disk) and Kanamycin (30 µg per disk), under conditions studied.

Animal care and wound healing

Healthy wistar albino rats of either sex and approximately the same age, weighing between 150-200 g were used for the study. They were individually housed, maintained in clean polypropylene cages and fed with commercially pelleted rat chow and water. They were housed (animal house in Faculty of Sciences of Urmia University) under controlled conditions of temperature of 23 ± 2°C, humidity of 50 ± 5% and 12 - 12 h of light and dark cycles respectively. The animals were divided into three groups of five animals each for the excision wound model (Tara et al., 2006). Group 1 were not treated and served as a control, Group 2 were treated by daily application of ointment base (Eucerin) and Group 3 were treated by daily application of 5% methanolic extract ointment (extract in Eucerin) of *P. khinjuk*.

Excision wound

A circular piece of full thickness (~500 mm²) was cut off from a predetermined area on the back of the rat. Wounds were traced on 1 mm² graph paper on the day of wounding and subsequently on the alternate days, until healing was complete. Changes in wound area were calculated, giving an indication of the rate of wound contraction. Number of days required for falling of eschar without any residual raw wound gave the period of epithelization (Shetty et al., 2007).The measurements of the wound area were taken on the 1st, 3th, 6th, 10th, 14th, 18th, 22th and 26th days for groups.

RESULTS

Antibacterial activity

In this study, the antibacterial activity of four kind extract of the leaves and derm fruits of *P. atlantica* and *P. khinjuk* having three different concentrations of 25, 50 and 75 mg/ml, are compared with antibiotics and concentrations of 0 used as controls as shown in Figures 1, 2, and 3. It is evident from the Figures that the antibacterial activities increase when increasing the extracts concentration from

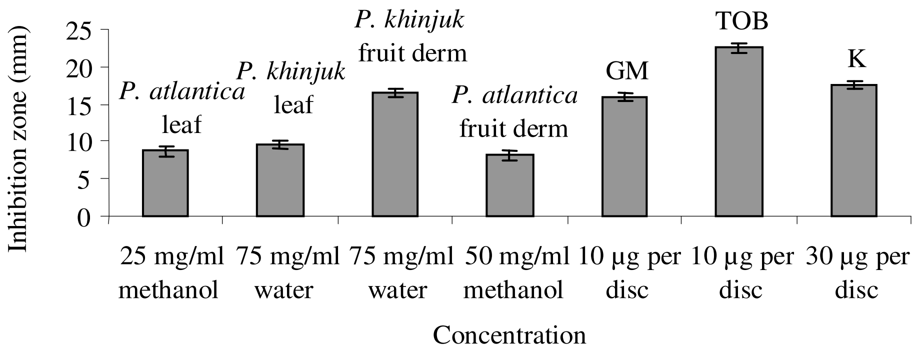

Figure 2. Comparison antibacterial activity between effective concentration of *P. atlantica*, *P. khinjuk* and antibiotics.(GM, Gentamicin; TOB, :tobramycin ,K : kanamycin) on *Staphylococcus epidermidis*.

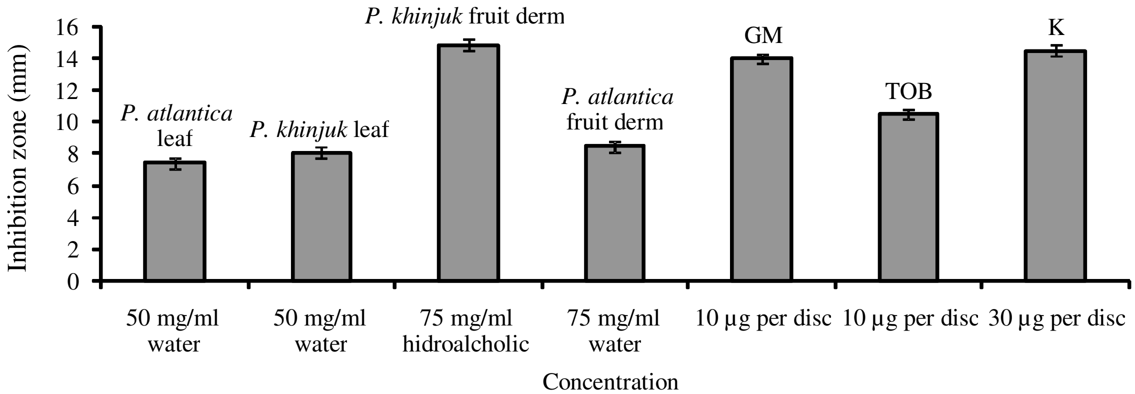

Figure 3. Comparison antibacterial activity between effective concentration of *P. atlantica*, *P. khinjuk* and antibiotics (GM , Gentamicin; TOB, tobramycin ; K, kanamycin) on *Escherichia coli*.

25 to 75 mg/ml. It is also determined that the extracts inhibit the growth of all the bacteria significantly. It was reported that the growth of *E. coli*, one of the most common gram-negative food poisoning bacteria, is significantly inhibited by the hydroalcoholic extract of *P. khinjuk* at a concentration of 75 mg/ml used (Figure 3). As can be seen from the Figures, the extracts with a concentration of 25 to 75 mg/ml has an inhibition zone higher than 7 mm, which is considered as the limit inhibition zone for being a reasonable antibiotic (Magiatis et al., 1999), for all the bacteria studied. The antibacterial activity of the extracts from *P. atlantica* and *P. khinjuk* against the bacteria are found to be less in comparison with those of Gentamicin (10 µg/disk), Tobramycin (10 µg/disk) and kanamycin (30 µg/disk), except for 75 mg/ml hydroalcoholic extract of fruit derm of *P. khinjuk* on *E. coli* that is higher than Tobramycin and same as the Gentamicin and Kanamycin (Figure 3), and 75mg/ml water extract of derm fruit of *P. khinjuk* on *S. epidermidis* that is higher than Gentamicin and same as the Tobramycin and Kanamycin (Figure 2), and 75 mg/ml methanolic extract of fruit derm of *P. khinjuk* on *S. aureus* that is higher than Tobramycin and same as Gentamicin and Kanamycin (Figure 1).

Wound healing

The percentage of wound healing was 10.3 ± 1.95, 27.04 ± 4.89, 41.63 ± 5.28, 75.97 ± 4.85, 90.13 ± 1.71, 91.85 ± 0.67, as measured on the 3th, 6th, 10th, 14th, 18th and 22th day respectively in the control group. The healing rate was not altered significantly in any of test on 6th,10th and 14th day as compared to extract group because the percentage of wound healing was 18 ± 3.41, 29.86 ± 0.91, 86.48 ± 1.078, 95.34 ± 2.04, as measured on the 3th ,6th,10th,14th day respectively in the extract group. We have also noted a positive trend in wound contraction rate in extract treated group and a low trend in wound contraction rate in control and Eucerin groups. The percentage of wound healing was 8.88 ± 1.76, 23.36 ± 4.52, 68.77 ± 2.93, 88.05 ± 1.32, 95.12 ± 0.86, 99.7 ± 0.3,

Table 1. Comparison of wound healing (%) in three groups: control, eucerin and extract.

Day	3	6	10	14	18	22
Control	10.3±1.95	27.04±4.89	41.63±5.28	75.97±4.85	90.13±1.71	91.85±0.67
Eucerin	8.88±1.76	23.36±4.52	68.77±2.39	88.05±1.32	95.12±0.86	99.70±0.30
Extract	8.18±3.41	29.86±0.91	86.48±1.078	95.34±2.04	100	-

as measured on the 3rd, 6th, 10th, 14th, 18th and 22nd day respectively in the Eucerin group. (Table1). The results indicated that extract-treated wounds epitheliazed faster and the rate of wound contraction was significantly ($P<0.05$) increased in comparison to control and Eucerin groups. The mean period of epithelialization in the control and Eucerin groups were 28 and 22 days respectively. It was significantly ($P<0.01$) reduced to 16 days in extract of *P. khinjuk* treated group (Table 1).

DISCUSSION

The antibacterial results showed that the crude extracts of two plants inhibited the three bacteria and the activities were considerably dependent upon concentration .The extract with the greatest antimicrobial activity was that of *P. khinjuk* (inhibition zone 13.5 - 16.5 mm), its bioactive components may be α-pinene, terpinolene and other components that we do not know. The antibacterial activity of *P. atlantica* was lower than *P. khinjuk* and those of standard antibiotics under the condition studied. Therefore, we choose the *P. khinjuk* for wound healing activity. Our results agree with the previous antibacterial studies related to these species (Rasooly et al., 1998; Moragheby et al., 1999; Alma et al., 2004; Koutsoudaki et al., 2005). From the botanical point of view, the genus *Pistacia* belonging to the family *Anacardiaceae* comprises 11 European species (Tsokou et al., 2007). Many of these plants yield resin in some degree. Among them, *Pistacia lentiscus* L. var chia is the major source of a resin known as mastic gum, which is highly reputed in traditional medicine field as an antimicrobial agent (Magiatis et al., 1999; Paraschos et al., 2007) and has been an important article of commerce for centuries. Several members of the genus *Pistacia* have been chemically investigated. They are characterized mainly by the occurrence of flavonoids and flavonoid glycosides (Kawashty et al., 2000). Flavonoids are hydroxylated phenolic substances and they have been found in vitro to be effective antimicrobial substances against a wide array of micro-organisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999). These plants have also been reported to contain phenolic compounds and triterpenoids (Yalpani et al., 1983; Marner et al., 1991). Terpenenes or terpenoids are active against bacteria (Cowan, 1999). The chemical analysis of the essential oil of the whole unripe fruits of *P. vera* showed that it was very rich in α-pinene (54.6%) and terpinolene (31.2%) (Tsokou et al., 2007). The percentage of α- pinene from essential oil obtained from oleoresin of *P. atlantica* var.mutica was 94.58%; its antibacterial effects has been approved on various strains of bacteria (Delazar et al., 2003). It should be noted that the two major volatile constituents, α-pinene and terpinolene, are compounds with interesting antibacterial properties (Raman et al., 1995; Carson et al., 1995).

Additionally, terpinolen has been identified as antioxidant agent (Kim et al., 2004) .In our study *P. khinjuk* was shown to possess anti-oxidant property. However confirmation of this suggestion will need well designed clinical evaluation. In excision wound, the extract of *P. khinjuk* showed faster healing compared with control groups and faster wound contraction rate may be due to the presence of flavonoids, which is responsible for the free radical scavenging activity that is believed to be one of the most important components of wound healing (Somashekar, 2007). The most important conclusion drawn from our study is that antibacterial activity of *P. khinjuk* is better than *P. atlantica* at the same concentrations. And according to Behboodi (2003), *P. khinjuk* is the best species to be cultivated in different climates and region. Based on the richness of the local flora and interest of the population in medicinal plants, these zagrossian plants should be subjected to comprehensive scientific evaluation of their chemical, pharmacological, and biological properties.

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