Regular Article

Evaluation of antibacterial activity of essential oil from Algerian *Pistacia lentiscus* resin

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Abstract

The purpose of this research was to evaluate the in vitro antibacterial activity of the essential oils from the resin of *Pistacia lentiscus* against *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). The agar disc diffusion method was used for microbial growth inhibition at various dilutions of the oils. Results showed that the tested essential oils possess antibacterial activity against *S. aureus* but inactive on *E. coli*. These results may have significant implications for the future development of resin oils of *P. lentiscus* as an antimicrobial agent for the treatment of the infections caused by *S. aureus*.

Key words:

Resin, *Pistacia lentiscus*, essential oil, antibacterial activity, *Staphylococcus aureus*, *Escherichia coli*

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Introduction

The major threat in the treatment of infectious diseases is that the pathogens sometimes become drug resistant (Owlia et al., 2009). *Staphylococcus aureus* and *Escherichia coli* are two pathogens that are associated with serious community-acquired disease (Enright et al., 2000; Oteo et al., 2008). The Gram-positive Bacterium *S. aureus* is the most dangerous of staphylococcal bacterial infections that causes a variety of diseases in animals and humans (Parvizi et al., 2012). This is common in infections, pneumonia and other threatening conditions and even food poisoning (Embrey et al., 2004). Several studies have documented increasing resistance rates in *S. aureus* and *E. coli* to antibiotics (Waters et al., 2011; de Kraker et al., 2011; Gagliotti et al., 2011; Tadesse et al., 2011; Chambers and Deleo, 2009; Karou et al., 2010; Hossain et al., 2008). Synthetic drugs are mainly used but there are increased

chances of side effects and other poisoning conditions on human health (Rodrigues et al., 2013). In this context, it become necessary to find out alternative methods to develop antibacterial agents especially from plants and plant based products (Bachir et al., 2017).

The genus *Pistacia* comprises about 70 genera and over 600 species and belongs to the *Anacardiaceae* family (Hormaza and Wünsch, 2011). Among 15 species of pistachios, only 4 species grow in Algeria, including *Pistacia lentiscus*, *Pistacia terebinthus*, *Pistacia atlantica* and *Pistacia vera* (Belhadj, 1999). *Pistacia lentiscus* L. commonly known as mastic tree or lentisk is a dioecious evergreen shrub that grows up to 3 to 4 m in height and widely distributed throughout the Mediterranean area (Mezni et al., 2012; Koç et al., 2014a). *P. lentiscus* grows north of Algeria and produces a valuable natural resin, which is generally known as

Chios mastic gum. The resin is collected by wounding the trunk and thick branches (Koç et al., 2014a). Mastic gum has been used by healers for several treatments, such as for hypertension, coughs, sore throats, eczema, gastrointestinal disturbances, kidney stones, hepatobiliary disorders, gynaecological diseases, fractures, wounds and jaundice (Al-Habbal et al., 1984; Koç et al., 2014b; Imtiyaz et al., 2013). In the present, the mastic has been commonly used as a flavor or supplement in foods, sweets, beverages, chewing gum, toothpaste and lotions (Koç et al., 2014a).

Mastic gum has been reported as a natural compound with widespread biological properties including antimicrobial and antifungal (Tassou and Nychas, 1995; Magiatis et al., 1999; Koutsoudaki et al., 2005), anticancer (Balan et al., 2007; He et al., 2006; Li et al., 2007; Loutrari et al., 2011), antioxidant, antiinflammatory (Mahmoudi et al., 2013; Triantafyllou et al., 2011), antiparasitic (Eldin and Badawy, 2013), antiatheromatic (Paraschos et al., 2008), hypolipidemic (Paraschos et al., 2008), and hepatoprotective/cardioprotective (Triantafyllou et al., 2007) effects.

The previous chemical investigations reported that the essential oil of mastic contains several secondary metabolites including medicinally important ones (Boelens and Jimenez, 1991; Papageorgiou et al., 1991; Castola et al., 2000; Koutsoudaki et al., 2005; Miyamoto et al., 2014).

Aim of the present work was to investigate the antimicrobial activity of mastic gum essential oil of western Algerian *Pistacia lentiscus* against *E. coli* and *S. aureus*.

Materials and methods

Plant material and essential oil extraction

P. lentiscus resin used in experiments was crude normal, collected during April-June 2010, which corresponds to the period of oleoresin formation, from the forest of Moulay Ismail (Photo 1), about 40 km far from Mascara city, northwest of Algeria. To collect the resin, fine incisions are made in the bark, and drops of sap appear and dry in large, odorous yellow droplets (Photo 2), then picking off.

The ethanol extracts were obtained by weighing out a fraction 2.6 g of *P. lentiscus* resin and soaking in 260 ml of ethanol 96°. The combined hydroalcoholic extract filtered through filter paper and concentrated to dryness under reduced pressure using a rotary evaporator, then stored in the dark at 4°C with no air contact. The extract was further used for screening purposes (Bachir and Benali, 2009).

Microbial strains

The bacteria used in the microbiological assays were A Gram positive bacterium; *Staphylococcus aureus* ATCC 25923 and Gram negative bacteria; *Escherichia coli* ATCC 25922. Standard cultures of bacteria from the American Type Culture Collection (ATCC) were obtained from the Hospital University Center (HUC) of Oran City, situated in the North West of Algeria.

Photo 1. The forest of Moulay Ismail, Algeria.

Photo 2. Harvest of the resin.

Assessment of inhibition of bacterial growth

Agar disc diffusion method was used to demonstrate the antibacterial properties of *P. lentiscus* resin extracts.

For disc diffusion, a suspension of each sample tested micro-organism diluted prior to 10^{-1} , 10^{-2} and 10^{-3} - (1 ml of 10^8 cells/ml) was spread on a solid agar medium in Petri dishes (Mueller-Hinton agar). Sterile paper disks in 6 mm diameter were impregnated with the resin oil with the capacity of 13 μ L. These impregnated disks were applied on solid agar medium in petri dishes by pressing slightly. The plates were incubated at 37°C for 24 h, and the diameters of inhibition zones were measured in millimetres (Bachir and Benali, 2012).

Results and discussions

Antimicrobial activity of resin oil determined by disc diffusion

From the preliminary screening studies by disc diffusion method, it was observed that only *S. aureus* was susceptible to resin oil of *P. lentiscus*. The results shown in Fig. 1 and table 1 clearly indicate that resin essential oil is moderate antibacterial towards *S. aureus* and inactive against *E. coli*. Considerable variation in inhibition zone sizes ranging from 08-20 mm was observed in *S. aureus* isolates depending on their sensitivity towards resin oil. The largest zone of inhibition was obtained for with 10^{-1} and 10^{-2} dilutions of *P. lentiscus* on 10^{-1} and 10^{-2} dilutions of *S. aureus* and the lowest for 10^{-2} and 10^{-3} dilutions of resin oil on 10^{-2} and 10^{-3} dilutions of *S. aureus*.

Fig. 1. Results of the antibacterial activity evaluation of the resin essential oil of *P. lentiscus* against *E. coli* and *S. aureus*, using the agar disc diffusion method.

Table 1. Antimicrobial activity evaluation of the essential oil resin of *P. lentiscus* with Agar disc diffusion method.

Bacterial strain		Resin oil dilutions				Control
		10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	
<i>E. coli</i>	10 ⁻¹					
	10 ⁻²	NI	NI	NI	NI	NI
	10 ⁻³					
<i>S. aureus</i>	10 ⁻¹	20 mm	9 mm			
	10 ⁻²	8 mm	20 mm	NI	NI	NI
	10 ⁻³	16 mm	8 mm			

NI: no inhibition

Results of this study demonstrated that the gram-negative bacteria (*E. coli*) was resistant to all tested dilutions of the resin essential oil but the gram-positive bacteria (*S. aureus*) was susceptible when it was tested with the dilutions of 10⁻¹ and 10⁻² of same extract. Usually there is a trend that the essential oils always act on the gram-positive than the gram-negative bacteria (Bidlack et al., 2000). It may be attributed to sensitivity between Gram-positive and Gram-negative bacteria in morphological differences. The Gram-positive bacteria should be more susceptible having only an outer peptidoglycan layer which is not an effective permeable barrier (Santos et al., 2013).

These results confirm previous reports of antibacterial activity for essential oils from resin of *P. lentiscus* have been shown to be more effective against the gram-positive than the gram-negative bacteria (Tassou et Nychas, 1995; Hussain and Tabji, 1997; Ali-Shtayeh et al., 1998; Magiatis et al., 1999; Koutsoudaki et al., 2005; Benhammou et al., 2008; Gkogka et al., 2013). Aksoy et al. (2006), have tested the resin oil of *P. lentiscus* against *Streptococcus mutans* (Gram positive bacteria like *S. aureus*) and they reported its susceptibility to this extract. Many authors conducted studies which support the effectiveness effect of the essential oil of aerial parts of this plant on *S. aureus* (Iauk et al., 1996; Bonsignore et al., 1998; Benhammou et al., 2008; Derwich et al., 2010). On the other hand, and in contradiction to the results obtained by this study, Sakagami et al. (2009) found that these oils were more effective against the two bacteria.

For several years, essential oils of resin of *P. lentiscus* are known for their antimicrobial activity which reported in several studies. These antibacterial properties could be mainly attributed to its major chemical constituents. As reported by Kokolakis et al. (2010), Kordali et al.

(2003), Koutsoudaki et al. (2005) and Magiatis et al. (1999), the essential oil from resin of *P. lentiscus* presented α -pinene, β -pinene and limonene as the major components and are well-known chemicals having antimicrobial potentials (Dorman and Deans, 2000; Magwa et al., 2006).

Conclusion

P. lentiscus, an important medicinal plant is useful in the treatment of wide range of disorders. The present investigation clearly indicates that the essential oil of *P. lentiscus* resin exhibited antibacterial activity against *S. aureus*. On the basis of the present finding, the resin of *P. lentiscus* have antibacterial properties that can be utilized in the production of commercial bactericides and against infections and/or diseases caused by *S. aureus*.

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