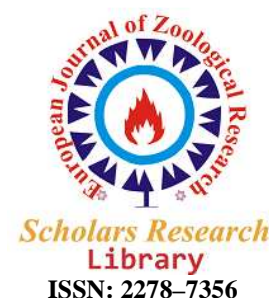




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Investigate the antibacterial effect of black pine essence (*Pinus nigra*) against the bacteria *E.coli*, *Staphylococcus aureus* and *Enterococcus faecalis* in three province of Ardabil, Gilan and Tehran

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ABSTRACT

This study has been done to evaluate the antibacterial effect and chemical constituents of black pine essence (*Pinus nigra*). Leaf sampling was conducted factorial where the first factor included areas, the second factor bacteria and the third factor concentrations in a randomized complete block design with three replications in October, 2012. Leaf samples were collected from three provinces of Ardabil, Gilan and Tehran and after drying; their essence were extracted by water distillation using Clevenger apparatus. Essence compounds were identified by gas chromatograph connected to a mass spectrometer, it showed significant differences between the provinces in terms of oil yields. To investigate the antibacterial properties of the plant, three *E.coli*, *Staphylococcus aureus* and *Enterococcus faecalis* was used and then they were cultivated on Mueller Hinton agar the culture medium. The extracted essence was prepared at four concentrations of 40%, 60%, 80% and 100%, using Dimethyl sulfoxide and was added to the culture medium by well method. Halos were formed after 48 hours around the wells that indicated the lack of bacterial growth. The halos were clearly observed in the *E.coli* and *Enterococcus faecalis* bacteria. But they were not observed around the wells of *Staphylococcus aureus*. The maximum inhibitory effect of black pine essence was on the *Enterococcus faecalis* bacteria and it had little effect on *E.coli* but did not show any effect on *Staphylococcus aureus*. Results showed that black pine essence had the highest anti-bacterial effect on the *E.coli* and *Enterococcus faecalis* in concentration of 100% and the lowest was in the concentration 40%.

Key words: Black pine, *Pinus nigra*, essence, the culture medium, the bacteria, concentration

INTRODUCTION

Our country is one of the richest plant sources in terms of value and diversity in the world, it has covered best species with the highest effective substance in terms of geographical and climatic conditions in most parts of the country and it is leading in providing medicinal herbs [1]. Black Pine (*Pinus nigra*) is a plant belonging to the family Pinaceae, which grows in the northern hemisphere but the history of their dispersion is in the temperate zones. They have narrow and needle leaves and because the leaves are slow to fall appears evergreen [2]. This herb has properties, such as coughs soothing and diuretic. They are also used to relieve chronic catarrh of the respiratory mucous and urinary tract [3]. Black Pine Essence has antifungal and antibacterial properties [4]. Given the therapeutic properties of herbs and also the growing resistance of most of bacteria to drugs, it seems necessary to study the effect of antibiotic and use the natural ingredients to remove bacterial infections.

MATERIALS AND METHODS

Collection of the leaf samples of *Pinus nigra* was performed from three provinces the Ardabil (Fandoglo area), Tehran (Jahan Nama forest park) and Gilan (Asalem area) in the second half of October. The herbs were dried after collecting in shadow state in the greenhouse is equipped with ventilation. After complete drying, the samples were crushed and then their essence was taken by water distillation using Clevenger apparatus. It took four hours of the essence for each sample. After each period, the essence that contained some water was dewatered by Sodium sulfate and for the analysis of the essence compounds; gas chromatograph connected to a mass spectrometer (GC. MS) was used. After analyzing the essences of black pine were identified by the device in three areas of Ardabil 147 combinations, Gilan 137 combinations and in Tehran 155 combinations. Alpha-pinene was the main combination; it was observed in the Gilan 31.89%, Ardabil 30.55% and in Tehran 7.86%.

To evaluate the antibacterial activity of the essence, the agar diffusion method was used as well. For this purpose, first each bacteria is standard in the growth phase were cultured and then bacterial suspension was prepared equal to the standard 0.5 McFarland (concentration of approximately 1.5×10^8 CFU. ml). Bacterial suspension was cultured using a sterile and autoclaved swab in the sterile conditions in the vicinity of the flame on Mueller Hinton agar medium. Then 2 to 4 wells were drilled using a borer cork with a diameter of 5 mm and regular intervals and proper distance from the plate rim. 20 ml of the essence was poured into each well by using a sampler with concentrations of 40%, 60%, 80% and 100%. After completing plates containing the bacteria culture and the essence were put into the incubator at 37 ° C in the same conditions for 48 h with different concentrations. After this period, the diameter of halos that had been created around the wells was measured and recorded using a ruler. Halos diameter or lack of growth around the wells showed the susceptibility or resistance of bacteria to the essence.

RESULTS

According to the results of variance analysis, the halos diameter in areas and concentrations of black pine essence and the studied bacteria have shown that there was significant difference between areas and concentration of black pine essence and the bacteria in hinton Agar medium at 1% level (Table 1). The interaction between essence concentration \times area, area \times bacteria and essence concentrations \times bacteria was significant at 1% level (Fig. 4, 5 and 6) and the interaction of area \times essence concentrations \times bacteria was significant at 1% level (Fig. 7). So that the mean Halo diameter in the study area of black pine essence showed that Gilan and Ardabil region were not significantly different and Tehran region had the lowest values (Fig. 1). In culture medium conditions the concentration 100% had the highest halo diameter and 40% had the lowest in essence concentrations of the black pine (Fig.2). The black pine essence had the highest inhibitory effect on the *Enterococcus faecalis* and little effect on the *E.coli* bacteria (Fig. 3).

Table 1-Analysis of variance Halo diameter in culture medium of Muller-hinton agar with areas and concentrations of black pine essence and the studied bacteria

Cs	df	(MS)
Area	2	10.84**
Essence concentrations	3	4.03**
Bacteria	1	2.30**
Essence concentrations \times Area	6	0.11**
Area \times Bacteria	2	1.00**
Essence concentrations \times Bacteria	3	0.30**
Area \times Essence concentrations \times Bacteria	6	0.26**
error	48	0.02
Cv	12.58	

** : Significant at 1% level

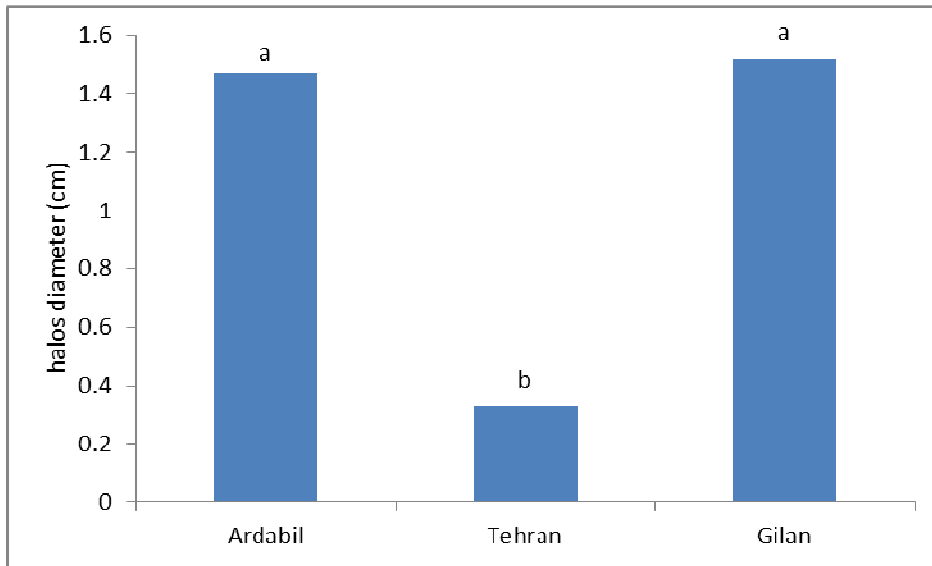


Figure 1 . Comparison the Halo diameter at Hinton Agar medium in the areas of black pine essence

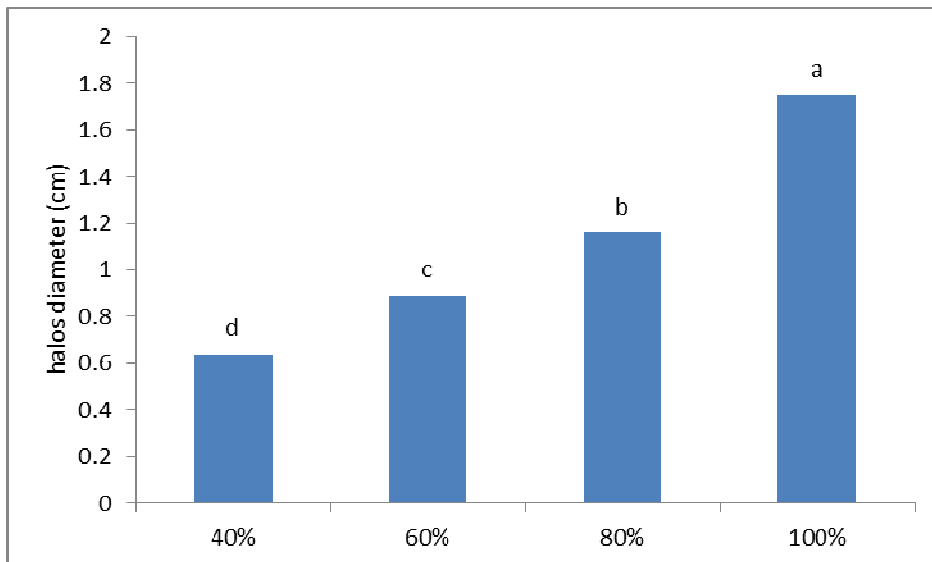


Figure 2 . Comparison the Halo diameter at Hinton Agar culture medium in the concentrations of black pine essence

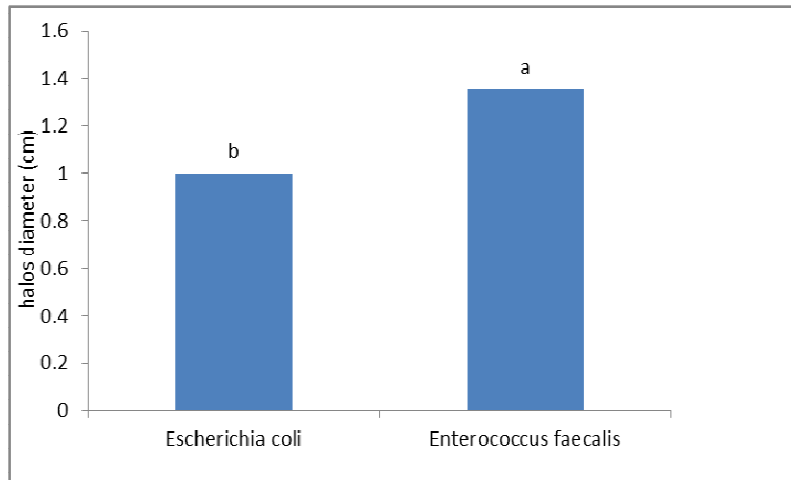


Figure 3 . Comparison the Halo diameter at two types of bacteria Enterococcus faecalis and E.coli

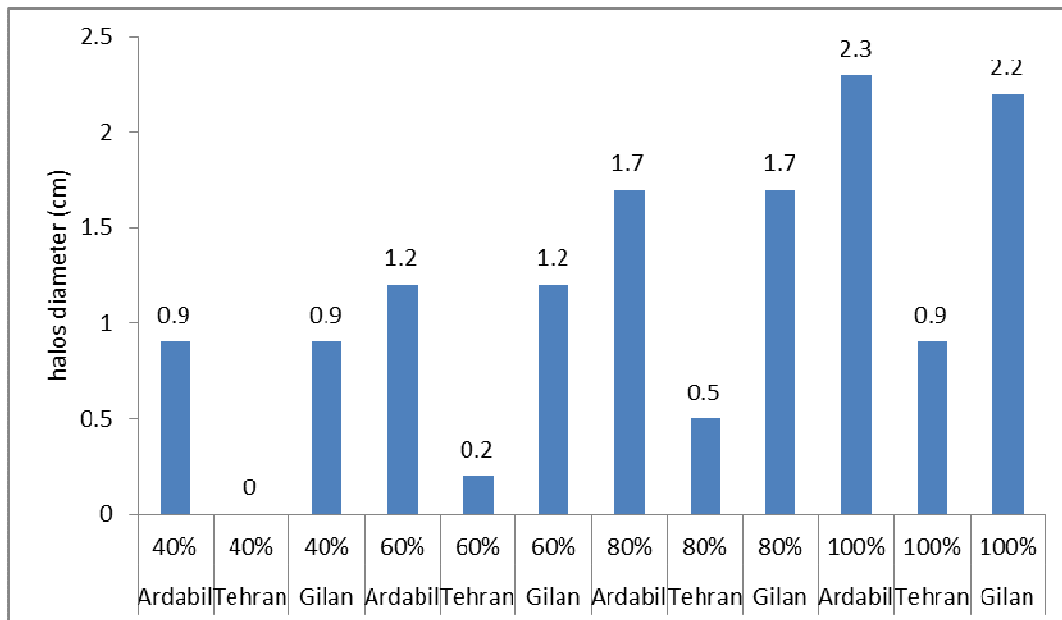


Figure 4 . Interaction essence concentrations × the studied area at hinton Agar medium

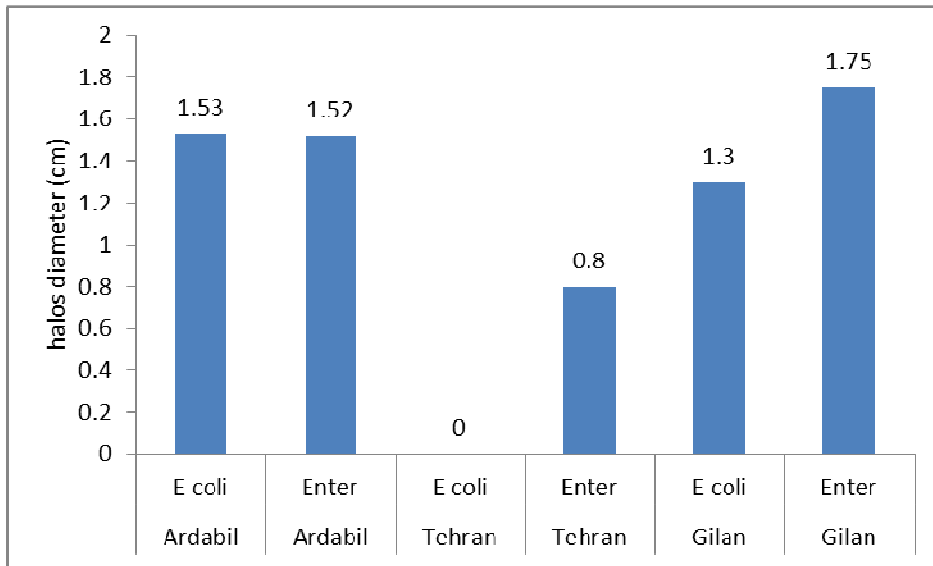


Figure 5 . Interaction area × the studied bacteria at hinton Agar medium

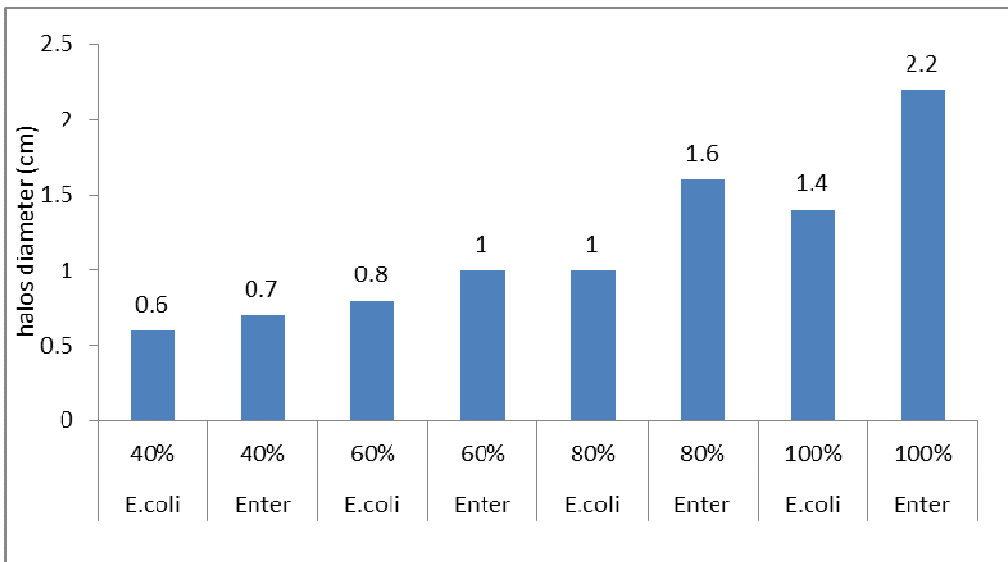


Figure 6 . Interaction essence concentrations × the studied bacteria at hinton Agar medium

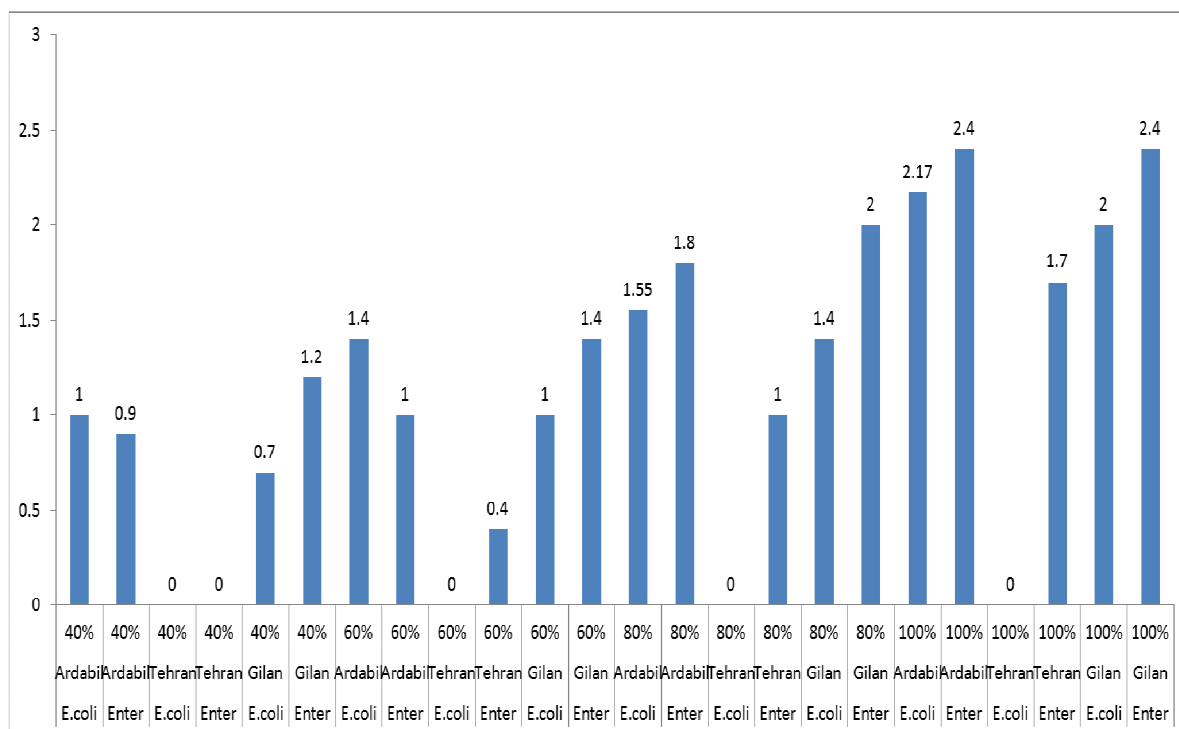


Figure 7 . Interaction essence concentrations × bacteria × studied area at hinton Agar medium

DISCUSSION

In a study which was conducted on the black Pine essence composition by Ekram Sezik, et al [5], the most important compounds at both research was alpha – pinene and camphene, beta - pinene. In the study of Ollandi and Toroglu in 2009 on the antimicrobial activity of black pine extracts in Turkey that had done on the two bacteria, E.coli, and Enterococcus faecalis that was impressive [6]. In this study, the black pine essence was effective on E.coli, and Enterococcus faecalis. Hemmati, et al studied the effect of habitat on the some flavonoids compounds of lime trees (*Tilia platifolia* L.) at two area Gorgan and Kelardasht in 2012, [7]. The results showed that there is a significant difference between the growth areas and studied organs. In this study, plant samples were also collected from three provinces with the cold weather (Ardabil), mild and wet (Gilan) and hot weather (Tehran), results showed a significant difference in the amount of essence compounds.

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