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Study of the inhibitory effect of Quercus Coccifera's aqueous extract on Staphylococcus aureus and Pseudomonas aeruginosa In vitro

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Abstract:
The use of therapeutic herbs has become of great importance these days due to the increase in drug resistance. From a long time ago the Venus' navel plant has been used to treat infections. In this study the antibacterial effect of the aqueous extract from the Quercus coccifera (jaft) herb, under laboratory conditions. This study was carried out experimentally. After collecting the Venus navel herb, it was dried in a warm dry environment away from direct sunlight in the shade. The alcoholic extract was prepared using a standard method. Clinical samples of staphylococcus aureus and pseudomonas aeruginosa were acquired from Ilam's health care institutes. The inhibitory effect of the extracts was analysed in the Mueller Hinton using the disk diffusion method for both bacteria. Then MIC and MBC of the extracts was determined using the Macro broth dilution method. At its highest concentration the aqueous extract had an inhibition zone of 27.2 and 23.7 mm on staphylococcus aureus and pseudomonas aeruginosa consecutively. The MIC and MBC for staphylococcus aureus were 10 and 12.5 µg/ml and for pseudomonas aeruginosa they were 10 and 17.5 µg/m consecutively. The results of this study show the strong antimicrobial effect of jaft's aqueous extract on staphylococcus aureus and pseudomonas aeruginosa and if more studies are based on this topic it could be a substitute for common antibiotics.

Key words: aqueous extract, staphylococcus aureus, pseudomonas aeruginosa, MIC, MBC.

Background:
The ever increasing drug resistance amongst bacteria has caused attention to shift to alternative methods of treatment to stop the increasing drug resistance, and also to find drugs with less side effects and toxicity. 50 years have passed since antibiotics have been used to treat infections but inappropriate use of these drugs has caused resistance to them in some species of bacteria **[1].** The drug resistance is one of the reasons that herbal drugs have been receiving attention. Staphylococcus is a gram positive pathogen and is the most important bacteria in skin infections in humans **[2, 3].** Different types of the staphylococcus are common causes of hospital infections across the world **[4, 5].** This bacterium is one of the most important causes of acquired infections **[6],** in society which can lead to bacteraemia, toxic shock syndrome, osteomyelitis and skin infections **[7, 8**]. According to recent reports staphylococcus aureus has developed resistance to some antibiotics **[9].** Pseudomonas aeruginosa is a gram negative pathogen which is mainly associated with infections in the intensive care unit **[10].** This bacterium is innately resistant to a lot of antibiotics **[11, 12].** As science and technology progresses so should the use of herbal drugs. The analysis of antibacterial effects of plants could help in synthesising new antibacterial drugs. Up to now plants such as

aloe Vera, thyme and garlic have been used in treating infectious diseases **[13, 14, 15**] and 75% of people across the world still use herbs to see to their needs **[16].** In western Iran, the fruit of the oak tree is still used to treat gastropathies, acute diarrhea and inflammation **[17].** The fruit of the oak tree has an internal and an external stratum. The internal stratum is also known as the placenta. From the many types of oak available in Iran, the Quercus coccifera strain is found readily in Ilam whose anti fungal properties have been proven to exist in its aqueous extract **[18].** The aim of this study is to analyse the antibacterial effect of the aqueous extract of the inner stratum of the oak fruit on staphylococcus aureus and pseudomonas aeruginosa under in vitro conditions.

Methodology:

Collecting samples

30 clinical samples of the pseudomonas aeruginosa and staphylococcus aureus bacteria were collected from Ilam city's hospitals and were kept in TSB cultivation media and at a temperature of -20 degrees centigrade. Samples were prepared using blood agar culture media in suspensions with different concentrations **[19].** Standard strains of pseudomonas aeruginosa, ATCC1568 and staphylococcus aureus, ATCC417584 were also used.

Collecting plant and aqueous extract

The oak tree fruit was collected then it was washed and the external stratum was removed and the internal layer was separated. Then after being washed, the collected stratums were dried in a warm dry environment and away from direct sunlight and then they were pestled. 10 grams of the herb powder was mixed with 200 ml of boiling distilled water and was put on the heater stride device for 20 minutes along with mixing. Then the mixture was filtered using a fine fibred sterile cloth and was centrifuged at 3500 rpm for 15 minutes. The mixture was left in open air until the solvent have completely evaporated. The extract powder was kept in a dark glass container at 4 degrees centigrade **[20].**

Disk diffusion method

First a suspension of the bacteria being studied was prepared with a concentration of 0.5 McFarland and using the a sterile valve on top of the plates containing Mueller Hinton Agar culture media, the bacteria were cultivated. After the surface of the plate had dried, paper disks with a diameter of 6mm, impregnated with 10 µl of the extract with different concentrations of 10, 20, 40 and 80 mg/ml were placed on the plates and they were incubated for 24 hours at 37^{C}. After incubation the inhibition zone was measure using a ruler **[21].**

Determining MIC

Different concentrations of aqueous extract were added to similar volumes of bacteria suspension equal to 105 CFU/ml of staphylococcus aureus and pseudomonas aeruginosa in BHI3 liquid culture medium and after 24 hours incubation at 37^{C} and the MIC was determined according to SLCI instructions.

Determining MBC

To determine MBC, 100 µl of three of the concentrations prior to MIC were cultivated separately on Mueller Hinton Agar and the concentration in which no bacteria had grown would be the MBC **[22].**

Determining bacterial sensitivity

To determine the bacterial sensitivity vancomycin (for staphylococcus aureus) and amikacin (for pseudomonas aeruginosa) were used (1µg/ml)

Statistical analysis

The entire halo diameters recorded in relation to the herb extract and positive control were analysed using variance analysis, T-test and repeat measure **[23].**

Results:

Results of the inhibitory effect via the disk diffusion method

In this method, the minimum and maximum inhibitory effects of the aqueous extract on staphylococcus aureus were at 10 and 80 mg/ml consecutively and the inhibition zones for these concentrations were 13.2 and 27.2 mm consecutively. The minimum and maximum inhibitory effects of the extract on pseudomonas aeruginosa were at 10 and 80 mg/ml consecutively and the inhibition zones for these concentrations were 11.1 and 23.7 mm consecutively **Table 1 (see supplementary material)**

MIC and MBC results of the aqueous extract on staphylococcus aureus and pseudomonas aeruginosa

The results obtained for determining the MIC and MBC of the extract on staphylococcus aureus were 10µg/ml and 15µg/ml consecutively. The MIC and MBC of the extract on pseudomonas aeruginosa were 15µg/ml and 25µg/ml consecutively **Table 2 (see supplementary material).**

MIC and MBC results on samples of standard strains of staphylococcus aureus and pseudomonas aeruginosa

MIC and MBC of the extract on the standard sample of staphylococcus aureus were 10µg/ml and 12.5µg/ml consecutively. MIC and MBC for the standard sample of pseudomonas aeruginosa were 10µg/ml and 17.5µg/ml consecutively.

Bacterial sensitivity results

The results showed the average inhibition zone for clinical samples of pseudomonas aeruginosa was 20.1 mm and for standard samples of this bacterium was 23.4 mm. The average inhibition zone for clinical samples of staphylococcus aureus was 23.9mm and for the standard sample it was 27.6mm. The standard samples were more sensitive to the mentioned antibiotics **Table 3 (see supplementary material).**

Discussion:

The analysis carried out in this study shows the antibacterial effect of the placenta of the oak tree fruit and its aqueous extract on staphylococcus aureus and pseudomonas aeruginosa and that it prevents their growth. Also this effect was stronger when compared to standard antibiotics and their difference was statistically meaningful ($p<0.05$). Considering that the size of the inhibition zone means a stronger antibacterial effect, the difference between the extracts effect and the effect of standard antibiotics is discussable. It seems this extract is stronger than other extracts and essences analysed in other studies. In a study on the inhibitory effect of the Helichrysum italicum's extract on staphylococcus and pseudomonas aeruginosa the inhibition zones were 15 and 8 mm consecutively **[24].** In another study effect of some extracts

including A. Fraasii, A. Holosericea and Achilla taygetea on staphylococcus aureus caused an inhibition zone of 16mm **[25].** In another study it was shown that the inhibition zone created by the Salvia ringens plant on the staphylococcus was less than 10mm **[26].** In Khosravi et al.'s study it was seen that the inner stratum of oak fruit extract had a noticeable antibacterial effect on salmonella and E.coli which correlates with the results of this study **[27, 28].** In another study by Khosravi et al. showed that the extract from lavandula stoechas also had an inhibitory effect staphylococcus aureus which was less than the effect recorded in this study **[29].** In the study carried out by Mirzaei et al. in 2013 showed that the extract from inner stratum of Quercus brantii had no toxic effect on the liver and kidneys of laboratory mice **[30]** and it could be same for the species in this study. In another study by Safari et al. in 2009 on the ethanol and methanol extract of the Quercus brantii species it was proven that this extract affects on various bacteria such as pseudomonas aeruginosa which correlates with this study but the inhibitory effect of the oak's aqueous extract is much more than the ethanol and methanol extract of Quercus brantii and their difference is meaningful (P<.0.05) **[31].** This goes to show that different species of oak have antibacterial and antifungal effects. In another study by Abbasi et al. it was proven that the extract from Scrophularia striata Boiss has a stronger effect compared to the inners stratum's effect on staphylococcus aureus and pseudomonas aeruginosa **[23].** Also in Behdani *et al.*'s study it was shown that Henna extract also has a stronger inhibitory effect compared to the study at hand on staphylococcus aureus and pseudomonas aeruginosa **[32].** The results of this study show that with the increase in the amount of extract in all the samples, the average inhibition zone also increases ($p<0.05$). This may be due to an increase in the bacterial sensitivity of pseudomonas aeruginosa and staphylococcus aureus against higher amounts of extract or an increase in the antibacterial effect of the extract in higher amounts. However it should be mentioned an increase in concentration could lead to an increase in toxicity **[33].** Results showed that the inhibitory effect on bacteria after 48 hours after cultivation wasn't much different than after 24 hours and the difference wasn't statistically meaningful ($P>0.05$). As there is an ever increasing resistance to antibiotics developing in various countries, the results obtained especially those in relation to the effect of high doses of extract on bacteria (staphylococcus aureus and pseudomonas aeruginosa) could be of importance and show that besides the effective results of the aqueous extract the phenolic and alchololic extracts could be useful and should be analysed. The clinical use of this extract requires more extensive studies and if the results prove successful and the results are standardized the herb can be used as a replacement for ineffective antimicrobial drugs.

Conclusion:

The results obtained from the experiments carried out show that the aqueous extract from the inner stratum of oak fruit has a noticeable inhibitory effect on pseudomonas aeruginosa and staphylococcus aureus and if more extensive studies are carried out, it could be a suitable replacement for ineffective antibiotics. In later studies the toxicity of this substance on human and animal cells will be analyzed.

References:

[1] Dadashbeigi M *et al. J of Veterinary Medicine Sanandaj*. 2011 4.
[2] Akram M *et al. Int J Immunopathol Pharmacol*. 2014 **27**: 313 [PMID: 25280022]
[3] Morán A *et al. Biofouling*. 2014 **30**: 1175 [PMID: 25397362]
[4] Hu DL *et al. Tohoku J Exp Med.* 2011 **225:** 161 [PMID: 21971303]
[5] Kilic A & Basustaoglu AC, *Res Microbiol*. 2011 **162**: 1060 [PMID: 21925597]
[6] Gupta S *et al. J Clin Diagn Res*. 2014 **8**: DC09 [PMID: 25386432]
[7] Weichhart T *et al. Infect Immun*. 2003 **71**: 4633 [PMID: 12874343]
[8] Holtfreter S *et al. Int J Med Microbiol*. 2010 **300**: 176 [PMID: 19889576]
[9] Lindsay JA, *Int J Med Microbiol.* 2010 **300**: 98 [PMID: 19811948]
[10] Berezin EN *et al. J Infect Dev Ctries*. 2014 8: 942 [PMID: 25116658]
[11] Livermore DM, *Clin Infect Dis.* 2002 **34**: 634 [PMID: 11823954]
[12] Cornaglia G *et al. Lancet Infect Dis.* 2011 **11:** 381 [PMID: 21530894]
[13] Davis RH *et al. J Am Podiatr Med Assoc*. 1989 **79**: 559 [PMID: 2607423]
[14] Karaman S *et al. J Ethnopharmacol*. 2001 **76**: 183 [PMID: 11390134]
[15] Zhang L *et al. Am J Clin Nutr*. 2006 **84**: 912 [PMID: 17023720]
[16] Liu X *et al. Adv Dent Res*. 2011 **23**: 56 [PMID: 21441482]
[17] Khennouf S *et al. Pharmazie*. 1999 **54:** 75 [PMID: 9987803]
[18] Panahi J *et al. J Pharm Biomed Sci.* 2013 3.
[19] Isenberg HD, *Asm press, Washington DC*. 1992 1.
[20] Panahi J *et al. J of Ilam Uni Med Sci.* 2013 21.
[21] Gandomi-Nasrabadi H *et al. J of Medicinal Plants.* 2012 2.
[22] Celiktas OY *et al. Food Chemistry.* 2007 100.
[23] Abbasi N *et al. J of Medicinal Plants.* 2006 6.
[24] Nostro A *et al. Lett Appl Microbiol*. 2000 **30:** 379 [PMID: 10792667]
[25] Magiatis P *et al. Z Naturforch C.* 2002 **57**: 287 [PMID: 12064728]
[26] Tzakou O *et al. Planta med*. 2001 **67**: 81 [PMID: 11270730]
[27] Khosravi A *et al. J of Arak uni of med scie*. 2003 5.
[28] Suwalak S & Voravuthikunchai SP, *J Electron Microsc.* 2009 **58:** 315 [PMID: 19451663]
[29] Khosravi A *et al. J of Qazvin uni of med scie*. 2004 7.
[30] Mirzaei N *et al. Res J of Biol Scie*. 2013 2.
[31] Khosravi A *et al. Pak J Med Sci.* 2006 22.
[32] Behdani M *et al. J of GMUHS*. 2009 2.
[33] Fredholm BB *et al. Pharmacol Rev*. 2011 **63**: 1 [PMID: 21303899]

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Supplementary material:

Table 1: Inhibitory effects using the disk diffusion method

		Concentration	Inhibition zone (10mm hole size)
Aqueous Extract	**Pseudomonas aeruginosa**	10 mg/ml	11.1mm
		20 mg/ml	14mm
		40 mg/ml	8.81mm
		80 mg/ml	23.7mm
	Staphylococcus aureus	10 mg/ml	13.2mm
		20 mg/ml	19mm
		40 mg/ml	21.3mm
		80 mg/ml	27.2mm
	Pseudomonas aeruginosa ATCC	10 mg/ml	12.4mm
		20 mg/ml	14.9mm
		40 mg/ml	20.3mm
		80 mg/ml	24.8mm
	Staphylococcus aureus ATCC	10 mg/ml	14mm
		20 mg/ml	19.5mm
		40 mg/ml	22mm
		80 mg/ml	27.8mm

Table 2: Results of MIC and MFC results of the aqueous extract

		Samples	Concentration
Aqueous Extract	**MIC**	**Pseudomonas aeruginosa**	15 µg/ml
		Pseudomonas aeruginosa ATCC	10 µg/ml
		Staphylococcus aureus	10 µg/ml
		Staphylococcus aureus ATCC	10 µg/ml
	MBC	**Pseudomonas aeruginosa**	25 µg/ml
		Pseudomonas aeruginosa ATCC	17.5 µg/ml
		Staphylococcus aureus	15 µg/ml
		Staphylococcus aureus ATCC	12.5 µg/ml

Table 3: Results of Bacterial sensitivity

	Samples	Inhibition zone (10mm hole size)
	Pseudomonas aeruginosa	20.1mm
Amikacin	**Pseudomonas aeruginosa ATCC**	23.4mm
	Staphylococcus aureus	23.9mm
Vancomycin	**Staphylococcus aureus ATCC**	27.6mm