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Study of Antimicrobial activity of selected Iranian plant extracts on vancomycin resistant *Staphylococcus epidermidis*

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Abstract: *Staphylococcus epidermidis is one the common bacteria causing infections in humans. Its increasing resistance has led to growing use of antibiotic and introduction of new medicines into the market. Strains of Staphylococcus epidermidis are or becoming resistant to many antibiotics, including vancomycin. Since the resistance of the bacteria to vancomycin is increasing, it is important to replace vancomycin with alternative medicines. This study explores antibacterial effects of hydroalcoholic extracts of selected Iranian medicinal plants on clinical vancomycin-resistant strains of Staphylococcus epidermidis. Hydroalcoholic extracts of plant organs with reported experimental antimicrobial effects , Lagenaria vulgaris, Punica grantum, Allium ascalonicum, Persian Marjoram, Urtical duicia, Chicorium intybus, Rubia tinctorum, Trigonella foenum, Descurainia sophia, and Pistaca vera were examined using disk diffusion and dilution antimicrobial susceptibility tests in liquid medium to determine the minimum inhibitory concentration of 15 strains of vancomycin-resistant Staphylococcus epidermidis. The highest and lowest minimum inhibitory concentration were found for the extracts of Allium ascalonicum (MIC_{50}128μg/ml) and Lagenaria vulgaris (MIC_{50} 1000 μg/ml), respectively. Since a growing resistance to vancomycin is observed in Staphylococcus epidermidis, our findings may be important because of the considerable effect of Allium ascalonicum extract on isolated vancomycin-resistant Staphylococcus epidermidis. Further research is required into the clinical applications of extracts.*

Keywords- *Resistant Staphylococcus epidermidis; Medicinal plants*

I. Introduction

Staphylococcus epidermidis is a significant pathogenic bacterium which causes a wide range of infections that sometimes can lead to the death of the infected person [1,2]. The increase in nosocomial infection rate at healthcare and medical institutions largely contributes to the growing economic burden for treating infections. Such infections are among the major causes of death for patients at hospitals [3-5]. *Staphylococcus epidermidis* cells are naturally found in the form of gram-positive, a Coagulase-negative *Staphylococci*(CoNS)arranged in irregular grape-like clusters on human skin and mucous membranes [6,7]. There are many species of CoNS but the most common isolate recovered from clinical specimens is *S. epidermidis* [8].CoNS isolates typically are more resistant to antimicrobial agents than *S. aureus* with the prevalence of resistance to beta-lactams as high as 60–70% [9]. The greatest challenge in treating infections caused by *S. epidermidis* is its increasing resistance to antibiotics, including vancomycin [10-12].

A new approach has developed to using medicinal plants in treating infections. Given the natural availability, limited side effects, and relatively low cost of medicinal plants, researchers are trying to identify antimicrobial activities of these plants [13-17]. The present study explores antibacterial effects of hydroalcoholic extracts from 10 plants whose antimicrobial effects on vancomycin-resistant *S. epidermidis*have been reported by experimental studies.

II. Materials And Methods

2.1. Collection of plants

Ten local medicinal plants with the following Ethno-botanical information were chosen for this study:1- Bottle Gourd seed(*Lagenaria vulgaris*), 2- Pomegranate fruit (*Punica grantum*), 3- Shallot bulb (*Allium ascalonicum*), 4- Parsnip flower (*Persian marjoram*), 5- Nettle leaves (*Urtical duicia*), 6- Chicory root (*Chicorium intybus*), 7- Madder root (*Rubia tinctorum*), 8- Fenugreek seed (*Trigonella foenum*), 9- Cyclops seed (*Descurainia sophia*), and 10- Pistachio nut green shell (*Pistaca vera*).Fresh parts of selected plants were identified, and collected from agriculture & natural resources research center and two regions (Varamin-Pishva35.278117 N, 51.652908 E, Sorkheh Hesar National Park 35.695083 N, 51.589651 E) and analyzed in the laboratory of microbiology department .Fresh plant material was washed under running tap-water, air dried, and then homogenized to fine powder and stored in airtight bottles within dark and dry environment.

2.2. Extraction

Extraction was carried out through maceration process. In this process, 50 gram of dried powder from leaves, seeds, bulbs, or fruits was put into ethanol at 70^{o}C, centrifuged for 72 hours at room temperature, and slowly mixed. Then common filter papers and vacuum distillation apparatus were used to concentrate and dry the extracts [18].

2.3. Microbial strain

Domestic strains of *S. epidermidis*, taken from infected dialysis subjects, infected with nosocomial infections, from Shafa Medical Care Center were identified at the microbiology laboratory using biochemical tests (catalase, coagulase, DNase, and Mannitol salt agar). In the next step, we explored the impact of vancomycin (32 µg/ml) on these strains, and 15 strains were identified as being resistant to this antibiotic at this concentration [19,20]

2.4. Microbial sensitivity test

Kirby-Bauer test (disk-diffusion method),was used to identify strain sensitivity to the 10 plant extracts[21]. A 0.5 McFarland standard sample was prepared for each strain in Mueller-Hinton Broth and cultured on Mueller-Hinton agar. Blank disks were immersed in 100 µg/ml extracts, dried, and placed on a plate with Mueller-Hinton agar at pre-determined intervals. A disk impregnated with distilled water (blank disk) together with a disk containing vancomycin (standard disk) was placed on each plate. The plates were incubated at 37^{o}C for 24 hours and analyzed by measuring the diameter of inhibition zone around the extract-containing disks. For accuracy, the test was performed in duplicates for each extract and the average diameter of inhibition zone was taken into account in case of any variation in the two measurements.

2.5. Determination of minimum inhibitory concentration (MIC)

Sterile 96-well plates were used to determine minimum inhibitory concentration (MIC) through micro broth dilution. In this method, 75 µl of a 0.5 McFarland standard 1:1,000 suspension of the bacterium (equivalent to 1.5 x 10^{8}Cfu/ml) was added to a microwell containing 75 µl of 2-5,000 µg/ml extracts in Muller-Hinton Broth. Control organism suspension and the culture medium were dispensed into one row and herbal extracts with different concentrations were added to one column. The microplates were incubated for 24 hours at 37^{o}C.The minimum concentrations of extracts with no growth-induced turbidity were recorded as MICs [19,20].

2.6. Statistical data analysis

The data were analyzed in SPSS (v=11) using Duncan and Touki tests as well as F test [22,23].

III. Results

Antimicrobial effects of hydroalcoholic extracts of *Lagenaria vulgaris*, *Punica grantum*, *Allium ascalonicum*, *Persian marjoram*, *Urtical duicia*, *Chicorium intybus*, *Rubia tinctorum*, *Trigonella foenum*, *Descurainia sophia*, and *Pistaca vera* on vancomycin-resistant strains of *S. epidermidis* were examined in this study. As seen in Tables 1&2, among the 10 extracts examined, *Allium ascalonicum* has the strongest antibacterial effect on all strains. MIC of *Allium ascalonicum* extract for three strains was 64 µg/ml while the maximum inhibitory concentration was observed only for one strain at 1,000 µg/ml. *Lagenaria vulgar is* extract, with the largest MIC among the extract studied, and affected two strains at 256µg/ml and five strains at a concentration larger than 5,000µg/ml. The smallest and the largest values of MIC_{50}were found for *Allium ascalonicum* extract (128µg/ml) and *Lagenaria vulgaris* extract (1,000 µg/ml), respectively (Table 3). According to F test results, the extracts exhibited different effects on the bacteria ($P\leq0.05$), and Touki test found the largest difference between *Lagenaria vulgaris* and *Allium ascalonicum* ($P\leq0.05$). The smallest $MIC_{90}$was found for *Allium ascalonicum* extract (512µg/ml) while the largest MIC_{90} belonged to *Lagenaria vulgaris*, *Punica grantum*, and *Chicorium intybus* (over 5,000µg/ml). According to Duncan test, the second-most effective extract, following *Allium ascalonicum*, was *Persian marjoram* with MIC_{90} 1,000 µg/ml and MIC_{50}256µg/ml.

Disk diffusion results suggest stronger effects of *Allium ascalonicum* on the strains. The disk impregnated with *Allium ascalonicum* extract inhibited growth in all strains. Diameter of inhibition zone was 10 mm for Strain number 11 and 30 mm for two other strains. *Persian marjoram* was the next extract, following Allium *ascalonicum*, in terms of the of inhibition zone diameter. *Persian marjoram* extract created no inhibition zone only in three strains. The smallest inhibition zone (9 mm) was observed for one strain and the largest inhibition zone (20 mm) was found in another single strain. According to the same test, *Lagenaria vulgaris*, *Punica grantum*, and *Trigonella foenum* extracts had the smallest effect in terms of inhibition zone. Inhibition zone around the disks containing *Lagenaria vulgaris*, *Chicorium intybus* extract, and *Trigonella foenum* extracts were observed in 7, 8, and 9 strains, respectively.

Table 1: Minimum concentration of extracts inhibiting the growth of selected isolates of *staphylococcus epidermidis* resistant to vancomycin -microgram per milliliter (µg/ml)-

	Lagenaria vulgaris	*Punica granatum*	*Allium ascalonicum*	*Persian marjoram*	*Urtica dioica*	*Cichorium intybus*	*Rubia tinctorum*	*Trigonella foenum*	*Descurainia sophia*	*Pistacia vera*
1	1000	5000<	64	64	128	1000	128	256	512	1000
2	5000<	512	128	1000	256	512	128	1000	5000<	5000<
3	500<	512	256	512	512	512	256	512	2000	256
4	5000<	256	512	1000	1000	256	256	1000	512	1000
5	1000	5000<	512	1000	5000<	5000<	1000	5000<	5000<	5000<
6	2000	256	128	64	512	64	128	512	1000	1000
7	2000	5000<	512	5000<	1000	2000	1000	1000	5000<	64
8	256	5000<	256	1000	512	5000<	5000<	1000	1000	512
9	256	64	64	256	128	64	64	64	2000	256
10	1000	5000<	256	512	2000	5000<	1000	2000	5000<	1000
11	512	128	64	64	64	64	1000	256	1000	512
12	1000	128	128	64	64	512	128	256	5000<	256
13	2000	512	256	128	128	64	256	1000	2000	1000
14	5000<	2000	1000	5000<	5000<	256	128	64	1000	2000
15	5000<	1000	256	256	256	256	512	512	2000	128

Table 2: Growth inhibition Zone (mm)

	Lagenaria vulgaris	*Punica granatum*	*Allium ascalonicum*	*Persian marjoram*	*Urtica dioica*	*Cichorium intybus*	*Rubia tinctorum*	*Trigonella foenum*	*Descurainia sophia*	*Pistacia vera*
1	-	-	29	-	8	-	-	-	-	15
2	-	-	30	-	8	-	-	-	-	8
3	8	-	27	11	-	9	12	8	-	10
4	8	-	30	9	8	10	10	12	8	9
5	-	8	25	13	-	-	9	-	-	10
6	-	-	11	15	-	12	-	-	8	9
7	-	-	27	11	-	8	-	8	8	11
8	8	8	22	10	-	-	8	-	-	13
9	-	13	20	10	-	-	-	10	10	12
10	-	12	20	20	10	-	-	10	8	10
11	9	-	10	12	8	11	9	12	-	-
12	10	9	28	15	13	9	16	8	9	15
13	13	8	29	13	12	11	16	15	12	-
14	-	-	12	-	-	10	-	12	8	8
15	8	-	22	12	8	12	12	-	-	11

Table 3: MIC_{50} and MIC_{90} values- microgram per milliliter (µg/ml)-

	MIC_{50}	MIC_{90}
Lagenaria vulgaris	1000	5000<
Punica granatum	256	5000<
Allium ascalonicum	128	512
Persian marjoram	256	1000
Urtica dioica	256	2000
Cichorium intybus	256	5000<
Rubia tinctorum	256	1000
Trigonella foenum	512	2000
Descurainia sophia	1000	5000<
Pistacia vera	512	2000

IV. Discussion And Conclusion

S. epidermidis is a major pathogenic bacterium known as a cause of infections in urinary system, dermal infection, septicemia, and bone and joint infections. It is also known for its resistance to natural factors such as dryness, antibiotic, and antiseptic treatments, leading to variety of nosocomial infections [6,24]. The problem of treating the infections caused by this antibiotic-resistance bacterium has become so challenging that

today can only be overcome by a few medicines, including one of the recently developed antibiotics, *i.e.* vancomycin which is still used to cure many infections caused by *S. epidermidis* [25,26].

With the development of resistance to vancomycin in enterococci and the transfer of resistant genes to *S. epidermidis*, we now experience the prevalence of vancomycin-resistant or intermediate-resistant strains[12,24]. Since vancomycin is one of the latest medicines developed for this purpose, it is crucially important to regularly search for medicines or anti-staphylococcus compounds. According to a report by CLSI, those strains of *S. epidermidis* whose growth can be inhibited by vancomycin at the concentrations 8 to 16 µg/ml are considered semi-resistant to vancomycin [20]. Strains that are sensitive to smaller concentrations are called sensitive strains while strains with MICs over32µg/ml are considered vancomycin-resistant strains. Antimicrobial resistance in CoNS is similar to that seen in S. aureus, although they generally are more resistant. Reduced vancomycin susceptibility is more frequent in S. haemolyticus and *S. epidermidis* than in *S. aureus* [27,28].

A previous study on the inhibiting effects of 20 herbs on methicillin-resistant *staphylococci* reported an MIC of 200µg/ml while the smallest MIC_{50} was found to be 128µg/l [29]. A similar study explored the impacts of 19 Chinese medicinal herbs on methicillin-resistant staphylococci and reported an MIC ranging between 1.1 and 3.07 µg/l [30]. Yet another study in the same vein measured the antibacterial properties of isoflavonoids isolated from *Erythrinavariegata* against methicillin-resistant staphylococci. Compounds like erycristagallin and orientanol with the MIC3·13–6·25 µg/ml were found to be the most effective one in terms of antimicrobial properties [31].

Another study reported an MIC of 12 µg/ml for alpha-mangostin from *Garciniamangostana L.* in treating vancomycin-resistant *enterococci* and methicillin-resistant *S. epidermidis* [32].

Sakagami et al. found that MIC of Calozeyloxanthone isolated from *Calophyllummooni* against vancomycin-resistant *Enterococci* (VRE) and vancomycin-sensitive *Enterococci* (VSE) with MIC values of 6.25µg/ml and 12.5µg/ml, respectively [33].

A study by Fisgin *et al.* found that Ankaferd Blood Stopper has growth inhibitory effect on clinical isolates Gram negative and positive bacteria, and Methicillin-resistant coagulase-negative *staphylococci*, from a hospital in Turkey. Ankaferd Blood Stopper is a mixture of 5 medicinal plants, *Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum* and *Urtica dioica*, which has been used in managing hemorrhage and bleedings in Turkey [34].

Many of *Allium* family member vegetables are known for their antimicrobial characteristics and also many researches have been done on *Allium* vegetables. N. Benkeblia et al. examined the Antimicrobial activity essential oil extracts of three types of onions and garlic against bacteria and fungi and according to this study garlic showed highest inhibition and green onion the lowest [35].

Given the prevalence of vancomycin-resistant *S. epidermidis* in many countries, the findings of this study, particularly those related to the effects of *Allium ascalonicum* extract on all strains of vancomycin-resistant *S. epidermidis*, can be quite important. The findings in disk diffusion method suggest limited impacts of antimicrobial agents on the strains studied here. However, further studies are required into the clinical application of these herbs. Successful and standardized results may indicate usefulness of these herbs as supplement medicines in treating infections caused by *S. epidermidis*.

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