Study of Antibacterial Activity of Ethylacetate Total Extract and the Alkaloid Fraction from Flowering Aerial Parts of *Glaucium vitellinum* Boiss. et Buhse against Clinical Isolates of *Staphylococcus Spp.*

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Abstract

**Background:** The purpose of this study was evaluation of the antimicrobial effect of ethyl acetate extract and alkaloid fraction of *Glaucium vitellinum* (*G. vitellinum*) against clinical *Staphylococcus ssp.* isolates from patients of Sina hospital of Tehran.

**Materials and Methods:** The plant of *G. vitellinum* was collected from Khonsar, Isfahan province, during May 2014. It’s flowering and aerial parts were washed, dried, powdered and extracted with methanol and ethyl acetate by using percolator apparatus, separately. In continuation, the alkaloid fraction was separated from metanolic total extract. 100 clinical isolates of *Staphylococcus ssp.* were collected randomly from different clinical samples of patients who referred to Sina hospital of Tehran during 2013-2014. Also, their resistant to common antibiotics were evaluated by disk diffusion method based on the CLSI 2014 protocol. Continuously, the antibacterial effect of ethyl acetate total extract and the alkaloid fraction against clinical isolates of *Staphylococcus ssp.* were evaluated by determining the minimal inhibitory concentration (MIC) by microdilution method based on the CLSI 2014. Standard *Staphylococcus aureus* (PTCC1431) and *Staphylococcus epidermidis* (PTCC 1435) were evaluated, simultaneously.

**Results:** Based on the results, 93% of isolates were coagulase positive and 7% were coagulase negative *Staphylococci ssp.* (CoNS). All coagulase positive cocci were identified as *Staphylococcus aureus* and among 7% CoNS, 3% were identified as *S. epidermidis*, so, in the follow they are named only CoNS.

The MIC of alkaloid fraction of *G.vitellinum* was: 17.87 mg/ml and 23.21 mg/ml against coagulase positive *S.aureus* and CoNS isolates, respectively. Also, MIC of ethyl acetate total extract of *G.vitellinum* was: 73.25 mg/ml and 98.21mg/ml on coagulase positive *S. aureus* and CoNS isolates, respectively.

61.29% of clinical *S. aureus* isolates were sensitive to ethyl acetate total extract and 100% were sensitive to alkaloid fraction while 100% were penicillin resistant while only 60% of them were Trimethoprim/sulfamethoxazole (SXT) sensitive.

Similarly, among CoNS isolates, 42.85% and 100% were sensitive to ethyl acetate total extract and alkaloid fraction, respectively. While 100% were penicillin resistant and only 42% were ciprofloxacin and doxycycline sensitivity.

**Conclusion:** based on the existence of good antibacterial effect for alkaloid portion of *G. vitellinum* against clinical isolates of *Staphylococcus ssp.* doing other in vitro and in vivo complementation tests are recommended for the further studies.
Introduction

In the recent decades emerge of antibiotic resistant pathogens has been a worldwide problem. Also the undesirable some side effects of the antibiotics, urge researchers to search for new sources to combat these problems. Staphylococcus spp. are gram positive, non-motile, oxidase-negative, urease positive and fermentative cocci. Staphylococcus aureus can cause scaling skin syndrome (SSS), food intoxication, toxic shock, impetigo and welding endocarditis. The other species Staphylococcus epidermidis can cause endocarditis, infection of the central nervous system, catheter-related bacteremia and wound infection.

On the other hand, Iran is a good place of growing medicinal herbs or plants and there are a lot of documents about their usage as an alternative treatment. The potential of traditional herbal medicines cannot be underestimated before any examination. Antibacterial properties have been reported to be found in the wide range of medicinal plants.

Glaucium vitellinum (G. vitellinum) is a hairy, biannual plant which is a member of Papaverasea family. G. vitellinum, is a local plant of Khonsar, a city of Isfahan province of Iran, and has many confirmed properties such as pain killer, anti-inflammatory, diaphoretic, sedative and insomnia treatment.

Based on some studies, some of the alkaloids derivates of Glaucium spp showed anti-microbial effect.

Based on our previous study, the alkaloid fraction of G. vitellinum was effective against standard strains of Staphylococcus aureus (PTCC 1431), Klebsiella pneumonia (PTCC1053), Salmonella typhi (PTCC1639), Escherichia coli (PTCC1399) and Pseudomonas aeruginosa (PTCC1430).

So, the purpose of this study was evaluation of the antibacterial effect of ethylacetate extract and alkaloid fraction of G. vitellinum against clinical Staphylococcus spp isolates from patients of Sina hospital of Tehran.
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Staphylococcus aureus (PTCC1431) and Staphylococcus epidermidis (PTCC1435) were evaluated, simultaneously. Based on our preliminary study, 125 mg of the dried ethyl acetate extract was weighted and dissolved in 1 ml of 4%DMSO. Also, 100 mg of the alkaloid dried extract was weighted and dissolved in one ml of 4% DMSO. Serial dilution was prepared from each extract and using 100μl of Mueller Hinton broth. Bacterial suspension was made equal to turbidity of 0.5 McFarland standard and immediately before adding to wells diluted 20 times (1:20). Total volume of each well was 200μl (each well was contained 100μl of each extract dilution, 90μl of Mueller Hinton broth and 10μl of diluted bacterial suspension). The microplates were incubated at 37°C for 24 hours. The MIC and MBC were reported based on last turbidity and growth on Mueller-Hinton agar, respectively.

Test accreditation: to accredit the results, all concentrations of total extract and DMSO were cultured in wells to identify their presumptive microbial infection. Also, methicillin and DMSO 4% were used as positive and negative control, respectively.

Statistical analysis: the results were analyzed by STAT software and calculation the multiple T-test.

Results

Frequencies of clinical samples were as follow; 49% blood, 40% body fluids, 7% trachea and 4% wound. Based on the bacterial tests in this experimental study, all Staphylococcus spp. were gram positive cocci, catalase positive, oxidase negative, resistant to bacitracin disk (0.04U) with positive growth on mannitol salt agar. All isolates with positive mannitol fermentation and positive tube coagulase test identified as S. aureus. For all urease positive and coagulase negative isolates, novobiocin disk susceptibility test was used to differentiate S. epidermidis from S. saprophyticus.

So, 93% of isolates were coagulase positive S.aureus and 7% were coagulase negative (CoNS; 3% S.epidermidis, 1% S. saprophyticus and 2% other species). While the antibacterial effect of ethylacetat and the alkaloid fraction against staphylococcus isolates was the main aim of this study, it was mentioned only to S. aureus coagulase positive and CoNS isolates in the following of the paper.

The MIC of alkaloid fraction of G.vitellinum was: 17.87mg/ml and 23.21mg/ml against S. aureus and CoNS isolates, respectively (p value<0.0001). The MIC of standard S.aureus (PTCC1431) and S. epidermidis (PTCC1435) was 1.5625mg/ml and 0.75mg/ml, respectively (table1, p value <0.0001).

Also, the MIC of ethylacetate total extract of G. vitellinum was: 73.25 mg/ml and 98.21 mg/ml against S. aureus and CoNS isolates, respectively (table2). The MIC against S.aureus (PTCC 1431) and S. epidermidis (PTCC 1435) was 78.10 mg/ml and 62.5mg/ml (Table 2, P value <0.0001).

Also, the MBC of ethylacetate total extract of G. vitellinum were: 116.3mg/ml and 196 mg/ml on S. aureus and CoNS isolates, respectively. The MBC were 156 mg/ml and 130.25 mg/ml against standard S. aureus (PTCC 1431) and S. epidermidis (PTCC 1435) (Table 3, P value <0.0001).

The MBC of alkaloid fraction of G. vitellinum were: 35.41mg/ml and 45.83mg/ml against S. aureus, S. epidermidis and CoNS isolates, respectively. The MBC were 3.125mg/ml and 1.5 mg/ml against standard S. aureus (PTCC 1431) and S.epidermidis (PTCC 1435) (Table 4, P value<0.0001).

Table 1: The MIC (mg/ml) of alkaloid extract of G.vitellinum.

<table>
<thead>
<tr>
<th>T test P value</th>
<th>Methicillin</th>
<th>DMSO</th>
<th>Alkaloid Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.0001</td>
<td>7.349±17.87</td>
<td></td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>&lt;0.0001</td>
<td>5.103±23.21</td>
<td>0.0</td>
<td>CoNS isolates</td>
</tr>
<tr>
<td>&lt;0.0001</td>
<td>0.0±1.5625</td>
<td>0.0</td>
<td>Standard S. aureus (PTCC1431)</td>
</tr>
<tr>
<td>&lt;0.0001</td>
<td>0.0±0.75</td>
<td></td>
<td>Standard S. epidermidis (PTCC 1435)</td>
</tr>
</tbody>
</table>
So, 61.29% of clinical S. aureus isolates were sensitive to ethyl acetate total extract and 100% were sensitive to alkaloid fraction while 100% were penicillin resistant in the antimicrobial sensitivity test. Similarly, among CoNS isolates, 42.85% and 100% were sensitive to ethyl acetate total extract and alkaloid fraction, respectively. While 100% were penicillin resistant in the antimicrobial sensitivity test.

**Discussion**

Based on our literature review, there is no any study on the antibacterial effect of G. vitellinum except our previous study. The only other existed studies are related to Cabo et al. and Semnani et al., who worked on different species of Glauclium other than vitellinum. However Semnani et al. in 2005, showed that extracts of 3 other species of Glaucium (including: G. grandiflorum, G. oxylobum and G. paucibolum) have antimicrobial activity against Gram-negative bacteria. In our previous study, the crude extract and alkaloid sub-fraction of G. vitellinum had significant inhibition activity on the growth of standard S. aureus as a gram positive bacteria and S. typhi as a gram negative bacteria. So existence of good antibacterial effect against gram-positive bacteria has been reported. However, the species of Glauclium in the Cabo et al. and Semnani et al. studies were different, but all the results in addition to our previous and recent studies were strongly confirmed the antimicrobial effect of this species.

### Table 2: MIC (mg/ml) of ethylacetate total extract of G.vitellinum.

<table>
<thead>
<tr>
<th>T test P value</th>
<th>Methicillin</th>
<th>DMSO</th>
<th>Total ethyl acetate extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001&lt;</td>
<td>0.0±125.0</td>
<td>0.0</td>
<td>29.95±73.253</td>
</tr>
<tr>
<td>0.17</td>
<td>0.0±35.0</td>
<td>0.0</td>
<td>34.23±98.21</td>
</tr>
<tr>
<td>0.0001&lt;</td>
<td>0.0±125.0</td>
<td>0.0</td>
<td>0.0±78.10</td>
</tr>
<tr>
<td>&lt;0.0001</td>
<td>0.0±62.5</td>
<td>0.0</td>
<td>0.0±62.5</td>
</tr>
</tbody>
</table>

### Table 3: The MBC (mg/ml) of ethylacetate total extract of G.vitellinum.

<table>
<thead>
<tr>
<th>T test P value</th>
<th>Methicillin</th>
<th>DMSO</th>
<th>Total ethyl acetate extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001&lt;</td>
<td>0.0±125.0</td>
<td>0.0</td>
<td>0.0±116.3</td>
</tr>
<tr>
<td>0.0001&lt;</td>
<td>0.0±35.0</td>
<td>0.0</td>
<td>0.0±196.0</td>
</tr>
<tr>
<td>0.0001&lt;</td>
<td>0.0±125.0</td>
<td>0.0</td>
<td>0.0±156.0</td>
</tr>
<tr>
<td>0.0001&lt;</td>
<td>0.0±35.0</td>
<td>0.0</td>
<td>0.0±130.25</td>
</tr>
</tbody>
</table>

### Table 4: The MBC (mg/ml) of alkaloid fraction of G.vitellinum.

<table>
<thead>
<tr>
<th>T test P value</th>
<th>Methicillin</th>
<th>DMSO</th>
<th>Alkaloid fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001&lt;</td>
<td>0.0±125.0</td>
<td>0.0</td>
<td>15.0±35.41</td>
</tr>
<tr>
<td>0.0001&lt;</td>
<td>0.0±35.0</td>
<td>0.0</td>
<td>10.21±45.83</td>
</tr>
<tr>
<td>0.0001&lt;</td>
<td>0.0±125.0</td>
<td>0.0</td>
<td>0.0±3.125</td>
</tr>
<tr>
<td>0.0001&lt;</td>
<td>0.0±35.0</td>
<td>0.0</td>
<td>0.0±1.5</td>
</tr>
</tbody>
</table>

So, existence of good antibacterial effect of G. vitellinum in our previous study encouraged us to survey this effect against clinical isolates of Staphylococcus spp. collected from Sina hospital of Tehran in the recent study for the first time.
genus of the Iranian medicinal plant.

**Conclusion**

Based on the satisfactory results of alkaloid fraction of *G. vitellinum*, identification of alkaloid ingredients and evaluation their antibacterial effect in the further studies are recommended. Also, doing other *in vitro* tests such as cytotoxicity and mutagenicity and *in vivo* studies of alkaloid fraction in the future is recommended for the substitution of *G. vitellinum* alkaloid fraction to common antibiotics as an alternative treatment.

**Acknowledgment**

The authors like to say their thanks to Dr. Parviz Kavakeb from Sina Hospital of Tehran for his cooperation in sampling and bacterial collection.

**Conflict of Interest**

The authors declare there is no any conflict of interest.

**Ethical Approve**

This project was done on isolated Staphylococcus spp isolates from patients who admitted in Sina hospital of Tehran. Their personal information was not published and no invasive sampling was done for this research.

**References**