Original Article

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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Received: 02 March 2015; Accepted: 25 January 2016

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 spp.* 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 spp.* 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 spp.* 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 spp.* (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 spp.* doing other *in vitro* and *in vivo* complementation tests are recommended for the further studies.

Keywords: *Glaucium vitellinum*, Anti-bacterial, Alkaloids, Papaveraceae

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Please cite this article as: Omrani P, Hakemi–Vala M, Baghery-Bejestany F. 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.* Novel Biomed. 2016;4(3):121-5.

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[1].

Staphylococcus spp. are gram positive, non-motile, oxidase-negative, urease positive and fermentative cocci[2,3]. *Staphylococcus aureus* can cause scaling skin syndrome (SSS), food toxication, 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[4,5].

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[6].

Glaucium vitellinum (*G. vitellinum*) is a hairy, biannual plant which is a member of *Papaverasea* family[7]. *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-inflammation, diaphoretic, sedative and insomnia treatment[7-9].

Based on some studies, some of the alkaloids derivates of *Glaucium spp* showed anti-microbial effect[10,11].

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)[12].

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.

Methods

Extraction preparation: The plant was collected from Khonsar (a city of Isfahan province, Iran) during May 2014. It's flowering and aerial parts were washed, dried, powdered and extracted by methanol and ethyl acetate, separately. Then both extracts were gathered, filtered and dried for three hours using percolator apparatus. The alkaloid fraction was separated from methanolic extract, continuously[12].

Bacterial strains: Different clinical samples including blood, urine and body discharges were collected from patients of Sina hospital of Tehran during 2013-14. All samples were cultured on the proper media based on standard microbial protocols for staphylococcus isolation[13]. All used media such as blood agar, Mannitol salt agar, and Mueller Hinton agar were purchased from Merck Co, Germany.

Consequently, Gram staining, urease test, catalase, oxidase, and coagulase tests were used to confirm the *Staphylococcus* identification[13]. Then 100 *Staphylococcus spp.* were collected randomly. Also, species differentiation was done by culturing on Mannitol salt agar, coagulase test and susceptibility to novobiocin disk (Padtan Teb Co, Iran)[13].

Their resistant to common antibiotics (All antibiotic disks were purchased from Mast Co, Uk) were determined in the next step by disk diffusion method based on CLSI 2014 protocol[14].

Consequently, the antibacterial effect of ethyl acetate total extract and the alkaloid fraction was evaluated by determining the minimum inhibitory concentration (MIC) against 100 clinical *staphylococcus spp.* isolates by the microdilution method based on the CLSI 2014. Further Minimum bacteriocidal concentration (MBC) was determined after culture.

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[12]. 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, oxidaze 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 and196 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*.

T test P value	Methicillin	DMSO	Alkaloid Fraction	
<0.0001	0.0±125.0	0.0±0.0	7.349±17.87	*Staphylococcus aureus*
<0.0001			5.103±23.21	*CoNS isolates*
<0.0001			0.0±1.5625	*Standard S. aureus (PTCC1431)*
<0.0001			0.0±0.75	*Standard S. epidermidis (PTCC 1435)*

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

T test P value	Methicillin	DMSO	Total ethyl acetate extract	
0.0001<	0.0±125.0	0.0±0.0	29.95±73.253	*Staphylococcus aureus*
0.17			34.23±98.21	*CoNS isolates*
0.0001<			0.0±78.10	*Standard S. aureus (PTCC1431)*
<0.0001			0.0±62.5	*Standard S. epidermidis (PTCC 1435)*

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

T test P value	Methicillin	DMSO	Total ethyl acetate extract	
0.0001<	0.0±250.0	0.0±0.0	0.0±116.3	*Staphylococcus aureus*
0.0001<			0.0±196.0	*CoNS isolates*
0.0001<			0.0±156.0	*Standard S. aureus (PTCC1431)*
0.0001<			0.0±130.25	*Standard S. epidermidis (PTCC 1435)*

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

T test P value	Methicillin	DMSO	Alkaloid fraction	
0.0001<	0.0±250.0	0.0±0.0	15.0±35.41	*Staphylococcus aureus*
0.0001<			10.21±45.83	*CoNS isolates*
0.0001<			0.0±3.125	*Standard S. aureus (PTCC1431)*
0.0001<			0.0±1.5	*Standard S. epidermidis (PTCC 1435)*

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 *Glaucium* other than vitellinum[10,11.]

Based on the results of Cabo et al. in 1988, the root extract of the *Glaucium flavum*, showed the antibacterial effect against gram-positive bacteria[10].

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[11].

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[13].

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.

However, the species of Glacium 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

genus of the Iranian medicinal plant.

Conclusion

Based on the satisfy 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 Theran. Their personal information was not published and no invasive sampling was done for this research.

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