Original Article

Comparison the Antimicrobial Effects of the Flowering Aerial Parts of *Glaucium vitellinum* Boiss. and Buhse and *Gaillonia aucheri* Jaub. and Spach

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Abstract

Background: With increasing use of antibiotics, the number and type of microbial resistance to antibiotics have been raised and at result the need for synthesis of new antimicrobials or acquire new sources of antimicrobial is indisputable. In this study the antimicrobial effects of the flowering aerial parts of Glaucium vitellinum and Gaillonia aucheri were investigated.

Materials and Methods: The antimicrobial effect of mentioned extracts against *Escherichia coli* PTCC 1399, *Pseudomonas aeruginosa* PTCC 1430, *Salmonella typhimurium* PTCC 1639, *Staphylococcus aureus* PTCC 1431 and *Candida albicans* PTCC 5027 were evaluated by disk diffusion and the microdilution method based on CLSI protocol 2012.

Results: No any zone of inhibition were detected by disk diffusion method against tested microbes for *Glaucium vitellinum.* After deletion of agar interference, the minimal inhibitory concentration (MIC) was determined by broth microdilution method for two plants. None of tested extracts were effective against Gram negative tested bacteria except alkaloid fraction of *G. vitellinum* which was effective against *S. typhi*. In contrast, the extracts and fractions of both plans were effective against tested gram positive bacteria especially *S. aureus*.

Conclusion: The best result of MIC was detected for alkaloid fraction *of G. vitellinum* (0.09 mg/ml) Vs. *G. aucheri* (125 mg/ml) against *S.aureus.*

Keywords: *Gaillonia aucheri*, *Glaucium vitellinum*, antibacterial agents, antifungal agents, MIC

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Introduction

Although the use of antibiotics for humans looks to have good results, but also has created problems in addition to their benefits. With increasing use of antibiotics, the number and type of microbial

resistance to antibiotics have been raised and at the result the need for new antimicrobial agents with new sources is indisputable. In addition to different progressed methods for obtaining new synthetic antibiotics, the use of plant resources for this approach can be considered valuable. Grappling with infectious diseases and the emergence of resistant bacteria in one hand and the adverse side effects of antibiotics in the other hand, explains the necessity and requirement of new antibacterial agents especially with natural origins[1].

In comparison to the most synthetic antimicrobial compounds which have restricted ingredients (usually with the one-dimensional effect) the herbal medicines not only contain a large number of substances with multi-dimensional effects but also their side effects is usually less than common antimicrobials[1]. Existence of documents in traditional medicine of Iran, distribution of a variety of medicinal plants in this country and the need for new drugs, persuade many researchers to investigate on medicinal plants. One of the prototypes is Papaveraceae family consists of 23 genera and 210 species most of which contain isoquinoline alkaloids. Their global prevalence is high, especially in temperate regions of the Northern Hemisphere[2].

There are potential medicinal species in this family, which therapeutic values have been proven and two of important genus are *Glaucium* and *Chelidonium* have been characterized as two important genera in this family[3]. *Glaucium sp.*, have variety alkaloids with important medicinal properties in pharmaceuticals products[2].

Various species of *Glaucium* genus have different uses in traditional medicine, for example; *G. corniculatum* is used as a narcotic and somniferous drug and has been used to relieve diabetes[4]. *G. vitellinum* is appropriate in treating bronchitis, angina, persistent coughs, pertussis and asthma[5, 6].

The other native plant is *Gaillonia aucheri* a member of *Rubiaceae* family with the hard small shrub and thorny branches with a height of 1 meter[7-10]. The leaves are very small with a length of 1.5 cm and dark green[11, 12]. The phytochemical properties and antimicrobial effects of these plants not been studied yet. So the aim of this study was an investigation of antimicrobial effects of the flowering aerial parts of *G. vitellinum* and *G. aucheri* against some gram positive and negative bacteria and *Candida albicans* by two standard methods.

Methods

Plants collection: Aerial parts of *G. vitellinum* and *G. aucheri* were collected in May 2012 from Khansar, Isfahan Province, and Bandar-Abbas, Hormozgan Province, Iran, Respectively. *G. vitellinum* was identified by Gh. Amin while *G. aucheri* was identified by R. Asadpour. Both samples were deposited in the herbarium of the Pharmaceutical Sciences Branch, Islamic Azad University, Tehran under code numbers of 1608 AUPF and 1553-AUPF respectively.

Extraction procedure: Dried powdered plants were separately transferred to the Percolator apparatus which was filled with 80% methanol and left for 3 days. The obtained extracts were concentrated by rotary evaporator. 10 g of the extracts was kept in a dark container for antimicrobial evaluation and the rests were used for alkaloid fractionation[12].

Alkaloid fractionation procedure: 100ml acetic acid 50% was added to the remained total extract to create an acidic environment. Since alkaloids have basic property, showed salt state in an acidic environment and formed the aqueous phase, then pureed, collected and poured into a decanter. Finally petroleum ether was added for the bleaching out and emitting the dis-alkaloids. This act is repeated until colorless petroleum ether fraction was obtained[13, 14.]

Aqueous phase extract of each plant decants into beaker and was placed inside ice water container. Ammonia was added drop by drop under the hood until the pH of the environment became alkaline.The obtained solution was strewed inside the funnel of the decanter and chloroform was added and mixed until alkaloids solved in chloroform. Then the chloroform phase was collected and concentrated with rotary apparatus and finally dried in the open air[15,16].

Bacterial strains: *Staphylococcus aureus* PTCC 1431, *Escherichia coli* PTCC 1399, *Pseudomonas aeruginosa* PTCC 1430, *Salmonella typhimurium* PTCC 1639 and *Candida albicans* PTCC 5027 were purchased from Iranian Research Organization for Science and Technology.

Disks preparation: Before preparing the soaked

disks, the amount of adsorbed solvent on each disk was measured. Therefore, 10 ml of ethanol was added to 10 paper disks in a covered graduated cylinder and absorbed ethanol was calculated. This experiment was repeated 3 times, and reducing the volume of solvent was determined by the mean of three experiments. Finally, it was cleared that each disk absorbed 0.02ml ethanol.

To prepare the extract with concentration of 0.125 mg/ml, 0.04g of extract, 4ml solvent, 2ml DMSO and 2ml normal saline were added to sterile vials, mixed and dissolve completely. Vials were covered with foil to prevent evaporation of the solvent. Then vials left for 48 hours to resolve the extract in solvent thoroughly.After 48 hours, obtained serial dilutions of the extract were prepared. Then disks were soaked in each concentration of the extract for 4 hours. After that removed the disks and transferred to a sterile Petri dish and kept in incubator to dry.

Disk diffusion method: The preliminary antimicrobial effect of the methanolic total extracts and alkaloid fractions of *G. vitellinum and G. aucheri* against selected microbial strains were performed by disk diffusion method based on in CLSI 2012 protocol[17]. As mentioned before, blank disks were impregnated in the different concentration of the methanolic extracts (125, 250, 500 and 1000 mg/ml). The bacterial suspensions were prepared equal to 0.5 McFarland turbidity and cultured on a Mueller Hinton agar as a microbial lawn by a sterile swab. One disk containing ethanol 96% or and DMSO without extract were and a commercial antibiotic disk were used as a negative and a positive control, simultaneously. All plates were incubated at 37°C for 24 hours. Then the diameter of the zone of inhibition were measured and reported based on mm unit[18,19].

Minimum Inhibitory Concentration (MIC) by microdilution method: MIC for methanolic total extracts and alkaloid fractions in sensitive strains were determined by the microdilution method by using 96 wells microplates based on the CLSI 2012 protocol[18]. The ciprofloxacin, gentamicin, nistatin and ampicillin powders were used as positive and DMSO10% as negative controls, simultaneously. In the microdilution method different concentrations of both plants' extracts and fractions were prepared as follow: 1000, 500, 250, 125, 65.5, 31.25, 15.62, 7.812, 3.906, 1.953, 0.976, 0.488, 0.244, 0.122, 0.061 and 0.0305 mg/ml[19].

Statistical analysis: All data were submitted in an excel sheet and the results were analyzed by SPSS software version 16.0.

Validation method: By the aim of validation of all procedures the following steps were performed:

1) Checking the microbial pollution of extracts and mediums to ensure sterile conditions.

2) Using of sterile medium alone to indicate that there is no any inhibitory effect related to the medium in both disk diffusion and microdilution methods.

3) Using of standard antibiotics as positive controls in both disk diffusion and microdilution methods.

4) Using of a disk containing ethanol 96% or DMSO, without extract as a negative control in the disk diffusion method and DMSO alone in microdilution method.

Results

In this study, antimicrobial effects of the alkaloid fractions and total extracts of *G.vitellinum* and *G.aucheri* plants were determined against four bacteria: *Escherichia coli, Salmonella typhi, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* yeast by disk diffusion and microdilution methods. All detected results were mentioned in the separate paragraphs below.

Disk diffusion: The inhibitory effect of different concentrations (125,250,500 and 1000 mg/ml) of alkaloid fractions and total extracts of *G.vitellinum* and *G. aucheri* against three gram negative bacteria including *E. coli*, *S. typhi* and *P. aeruginosa* and *S. aureus* as a gram positive bacteria and C.albicans as a yeast were determined by disk diffusion method. No any inhibitory effect was detected by this method against tested microbes (table1).

Microdilution method: The MIC of methanolic and alkaloid fractions of *G. vitellinum* and *G. aucheri* against tested bacteria and yeast were determined by microdilution method based on CLSI 2012[8]. As it was shown in (table 2), none of the methanolic extracts and alkaloid fractions of both plants showed any inhibitory effect against tested gram negative bacteria except an alkaloid fraction of *G. vitellinum* against *Salmonella typhi.*

In contrast, the extract of both plants were effective

Table 1: Mean (± SD) of the zone of inhibition (mm) of different concentrations (125, 250, 500 and 1000 mg/ml) of alkaloid fraction and methanolic extracts of *G. vitellinum* and *G.aucheri* plants.

Microbes	Zone of inhibition(mm)						
	G. vitellinum		*G. aucheri*		Antimicrobial disks		
	Alkaloid fraction	Methanolic	Alkaloid fraction	Methanolic	Gentamicin	ciprofloxacin	Nistatin
Escherichia coli	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	25.5± 0.71	-	-
Salmonella typhi	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	19.5±0.71	-	-
Pseudomonas aeruginosa	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	16.5±0.71	-	-
Staphylococcus aureus	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	-	28±1.41	-
Candida albicans	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	-	-	58 ± 1.41

Table 2: Mean (± SD) of MIC alkaloid fractions and total extracts of *G. vitellinum* and *G. aucheri* plants in serial concentration (0.035175-1000 mg/ml).

Microbes	Minimal inhibitory concentration (MIC) *mg/ml*						
	G. vitellinum		*G. aucheri*		Antimicrobial disks		
	Alkaloid fraction	Methanolic	Alkaloid fraction	Methanolic	Gentamicin	Ciproph loxacin	Nistatin
E. coli	1000 ± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0481±0.0183	–	–
S. typhi	11.72± 5.52	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0481±0.0183	–	–
P.aeruginosa	1000 ± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0481±0.0183	–	–
S.aureus	0.09±0.03	0.0± 0.0	125 ±0.0	0.092±0.031	–	±0.0183 0.0481	–
C. albicans	0.09±0.03	0.0± 0.0	0.0± 0.0	2.92±1.38	–	–	0.04±0.01

against *S.aureus*. However, the MIC of alkaloid fraction of *G. vitellinum* was lesser than *G. aucheri* (0.09 vs. 125mg/ml) which means that alkaloid fraction of G. *vitellinum* was stronger than the same fraction of *G. aucheri*.

Discussion

The aim of this study was an evaluation of the anti-microbial effect of methanolic extracts and alkaloid fractions of *G. aucheri* and *G. vitellinum* against three gram negative bacteria including *Escherichia coli* PTCC 1399, *Pseudomonas aeruginosa* PTCC 1430, *Salmonella typhimurium* PTCC 1639, *Staphylococcus aureus* PTCC 1431as a gram positive bacteria and *Candida albicans* PTCC 5027 as a yeast by disk diffusion and microdilution methods. In this study two standard methods were employed for antimicrobial evaluation of mentioned extracts and alkaloid fractions. Based on the simplicity and reproducibility of the disk diffusion method, this method was chosen at first to evaluate the antimicrobial effectiveness of the mentioned extracts of both medicinal plants.

Based on the table 1, no any zone of inhibition was detected against tested microbes by disk diffusion even after 3 times. No any antimicrobial effect of this method for these plants' extracts may be related to their inability to leave the disk and /or to agar diffusion. Also, based on the study of Jiang 2011, in contrast to commercial antibiotics, there is no enough documented information about the solvents, the chemical structure and the active mechanism for all natural materials in comparison to standard antibiotics such as existed in CLSI (Clinical Laboratory Standard Institute) protocols or different pharmacopes (USP or BP)[20]. In addition, some of the extracts are capable to diffuse in agar but agar spreading may be forbidden in other cases. So, choosing the best method in such studies, is depending on to existed information or if no any data available, all existed methods must apply to choose and evaluate the best one in each subject. Therefore, the best method may be different based on the origin of materials, such as the difference in the chosen method in each case is not far from the expected. So, because of no enough data existed for the selected plants of this study after literature review, we proposed two methods for antimicrobial evaluation by priority of disk diffusion because of mentioned advantages of this method and microdilution method in the second position.

Since, no any antimicrobial effect in this study was detected by disk diffusion method at the first step, broth microdilution method was chosen, consequently. By deletion of agar interference the antimicrobial effect of *G. aucheri* and *G. vitellinum* were cleared during broth microdilution assay. Based on the detected results by this method, the extract of both plants were effective against *S. aureus*. However, the MIC of alkaloid fraction of *G. vitellinum* was lesser than *G. aucheri* (0.09 vs. 125mg/ml) which means that alkaloid fraction of G. *vitellinum* was stronger than the same fraction of *G. aucheri* which may relate to better function of the cell wall components.

Conclusion

The antimicrobial effect which was detected in this study related to non-pure total extracts and alkaloid fractions of both tested plants, so, isolation of total extract components and identification their combinations are recommended for future studies. Also, doing further studies to determine the cytotoxicity or mutagenicity of effective fraction is recommended.

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