



Evaluation of Antimicrobial Activities of Extract From *Johrenia Paucijuga* (D.C.) Bornm

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Abstract

Background: *J. paucijuga* (D.C.) Bornm is known to grow in Azerbaijan. Our investigation has revealed a new distribution area situated in the Gadabay region – Middle Mountain Beltgrasslands of Small Caucasus. The purpose of the study was to evaluate antimicrobial activities of extracts from *J. paucijuga* (D.C.) Bornm.

Materials and Methods: The plant was collected in July 2017 and further investigated for chemical composition. Results of scientific trips have revealed the expansion of the distribution range of some plants and the reduction of other members of the Apiaceae family. Investigations related to the selection of medicinal plants, identification of their biological features, investigation of species ontogenesis, distribution range, chemical composition, evaluation of biological and exploitation reserves are always of importance. Chemical compounds characteristic of this plant - β -sitosterol and β -stigmasterol were detected in our previous research. Extract obtained from *J. paucijuga* (D.C.) Bornm was tested for antimicrobial activity using the disk diffusion method as a part of the cooperation between the Department of Plant Resources of Institute of Botany of Azerbaijan National Sciences Academy and the Department of Medical Microbiology.

Results: Thus, the study of bactericide and fungicide activity of *J. paucijuga* extract has shown that the aqueous extract of this plant (1:10) has antimicrobial and antifungal activity against experimental strains depending on the concentration of the extract of the plant and exposure time. *J. paucijuga* essential oils have shown comparatively higher bactericide activity.

Conclusions: The purpose of the study was to evaluate antimicrobial activities of extracts from *J. paucijuga* (D.C.) Bornm to use it in the prophylaxis of bacterial diseases as preparation antimicrobial hygienic solutions, antibacterial and antifungal ointment. The obtained formula 2 proves that the structural formula of coumarin is the same as the structure formula we are identified (8).

Key words: *Johrenia paucijuga* (D.C.) Bornm, Apiaceae Lindl, antimicrobial, antifungal, β -stigmasterol

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Introduction

Totally 15 species of the genus *Johrenia* (*joreniya*) from the *Apiaceae* family are widely distributed in Iran, Asia Minor, and Transcaucasia. Only *J. paucijuga* (D.C.) Borm is known to grow in Azerbaijan. The distribution of this plant in Azerbaijan (on the lower mountain belt and rocky slopes of Nakhchivan) was described by some authors (1-3). Our investigation has revealed a new distribution area situated in the Gadabay region – Middle Mountain Beltgrasslands of Small Caucasus. The plant was collected in July 2017 and further investigated for chemical composition. Chemical compounds characteristic of this plant - β -sitosterol and β -stigmasterol were detected (4). It is a grassland perennial plant with petiolate, 2-pinnate leaves, yellow leaflets, and oval-shaped fruits.

Results of scientific trips have revealed expansion of the distribution range of some plants and reduction of other members of the *Apiaceae* family. Investigations related to the selection of medicinal plants, identification of their biological features, investigation of species ontogenesis, distribution range, chemical composition, evaluation of biological and exploitation reserves are always of importance.

Extract obtained from *J. paucijuga* (D.C.) Borm was tested for antimicrobial activity using the disk diffusion method as a part of the cooperation between the Department of Plant Resources of Institute of Botany of Azerbaijan National Sciences Academy and the Department of Medical Microbiology.

During antimicrobial and antifungal susceptibility testing representatives of gram-positive bacteria (*Staphylococcus aureus*), gram-negative bacteria (*Escherichia coli*), non-fermenters (*Pseudomonas aeruginosa*, *Klebsiella* spp. and *Antracoides* spp.), fungi (*Candida albicans*) were used as control strains.

Currently, investigation of plants rich with biologically active substances, their natural resources, distribution ranges is of great interest for plant resource scientists. Azerbaijani flora is rich in medicinal plants with antimicrobial, antifungal, antiparasitic, and antiviral effects. Phytochemical investigation of biologically active substances as essential oils, coumarin, flavonoids, sesquiterpene lactones of *Umbelliferae* family representative *Apiaceae* Lindl remains actual (5,6).

Materials and Methods

Plant *J. paucijuga* was collected in Kalaman village of the Gadabay district in July 2019. 50g of finely cut and air-dried aerial part of the plant was extracted by ethanol. After a 3-time extraction, 12 g of greenish resin-like substance was obtained (12%).

Essential oils were obtained from plant seeds by hydrodistillation (13). Detection of essential oils was performed on chromatograph (Cana co) using gas-liquid chromatography method (14).

The main aim of the research was the evaluation of the activity of obtained essential oils and extract against gram-negative, gram-positive bacteria and fungi (*C. albicans*). Among investigated microorganisms, Gram-negative *Pseudomonas* is one of the most common causative agents of infections (10-12).

For this purpose, a suspension of 10⁹ microorganisms in 1 ml of saline was prepared. Colonies of a microorganism grown after 24 hour-incubation on nutrient media were picked and used for suspension preparation. The turbidity of obtained suspension was adjusted using the Mc Farland turbidity standard (7-10). Obtained suspensions were poured on the surface of Mueller-Hinton (MHA) and Sabouraud Dextrose agar (SDA). Excess of suspension was absorbed by the pipette.

After inoculation Petri dishes were placed in an incubator for 10 min at 37°C. Sterile discs soaked for 3-5 minutes in investigated substance were applied on the surface of

agar with inoculated microorganism at 2cm distance from each other. Petri dishes with MHA were incubated at 37°C and with SDA – at 28°C.

After the application of disks, the compound diffuses onto agar. Inhibition zones of discs were evaluated after 24-48 hours of incubation. All performed tests were repeated 3-5 times and compared with controls. Sterile zone diameters around discs with impregnated ethanol were taken as controls.

Results and Discussion

The antimicrobial properties of *J. paucijuga* extract were evaluated during the investigation. As control strains during antimicrobial and antifungal susceptibility testing were used representatives of gram-positive bacteria – *S. aureus*, Gram-negative bacteria – *E. coli*, non-fermenters – *P. aeruginosa*, Klebsiella spp. and Antracoides spp. and fungi – *C. albicans*. Results of antimicrobial and antifungal activity of diluted (1:10) extract obtained from *J. paucijuga* are shown in Table 1.

Table 1. Results of the antimicrobial activity of *J. paucijuga* extract evaluated by the disk diffusion method.

Inoculated microorganism	Inhibition zone, mm	Control-ethanol
<i>Antracoides spp.</i>	15 mm	0 mm (–)
<i>E. coli</i>	17 mm	0 mm (–)
<i>P. aeruginosa</i>	16 mm	0 mm (–)
<i>C. albicans</i>	14 mm	0 mm (–)
<i>Klesiella spp.</i>	0 mm	0 mm (–)
<i>S. aureus</i>	18 mm	0 mm (–)

Note: Inhibition zone diameters are represented in mm.

Inhibition zone for *Antracoides spp.* was 15 mm (control “–”); for *E. coli* – 17 mm (control “–”); *P. aeruginosa* – 16 mm (control “–”); *C. albicans*– 14 mm, (control “–”); *Klesiella spp.*– 0 mm (control “–”). The highest inhibition zone was observed for *S. aureus* – 18 mm (control “–”). For evaluation of the antimicrobial activity of the essential oils, the emulsion-contact method was applied using a 2% alcohol solution.

J. paucijuga essential oils have shown higher bactericide activity. 1:3 and 1:4 dilutions in alcohol have inhibited the growth of *S. aureus*. The most effective dilutions for *P. aeruginosa* were 1:4 and 1:6, 1:3 and 1:4 dilutions inhibited *P. aeruginosa* multiplication and growth only after 40 – 60 minutes. 1:3 dilution has considerably inhibited the growth of *E. coli* after 60 minutes and *S. aureus*– after 20 minutes. A weaker solution of essential oils has completely stopped the development of this microorganism after 60 minutes.

According to the results of the investigation, the effect of essential oils depends on the degree of dilution and the duration of exposure. Thus, the study of bactericide and fungicide activity of *J. pucijuga* extract has shown that the aqueous extract of this plant (1:10) has antimicrobial and antifungal activity against experimental strains depending on the concentration of the extract of the plant and exposure time.

Different dilutions of the essential oils of *J. paucijuga* species have been found to have a certain bactericidal effect, depending on the duration of exposure.

Taking into account these features we consider that further research should be conducted in order to develop methods enabling medicinal preparations from *J. pucijuga* (D.C.) Bornm plants.

The purpose of the study was to evaluate antimicrobial and + activities of extracts from *J. pucijuga* (D.C.) Bornm in order to use it in the prophylaxis of bacterial diseases as preparation antimicrobial hygenic solutions, antibacterial and antifungal ointment.

Ethics Committee Approval: Yes

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