The Quarterly Scientific Journal of Applied Biology

Vol.36, No.4, Sering. 78,Witer 2024, p. 21-24

Journal homepage: https://jab.alzahra.ac.ir

doi.org/10.22051/jab.2023.42990.1548

The Survey on antimicrobial effect of methanolic extract of some species of Apiaceae family in Kurdistan province

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Received: 15/10/2022 Accepted: 17/09/2023

Introduction

Apiaceae with nearly 434 genera and 3780 species is one of the largest plant families in the world. The plants of this family are mostly distributed in the Northern Hemisphere and have low distribution in tropical regions (Bouchra et al 2017). *Zeravschania membranacea* Boiss is native species of Iran. The pleasant aroma of this plant has attracted the attention of the residents of the region in terms of food and medicine. *Zeravschania aucheri* Boiss is one of the plants of Kurdistan that grows in most of the heights of this region. This plant is very aromatic and fragrant, and in traditional medicine, it is considered to be infused or boiled for kidney ailments, removing worms and relieving stomach pain, and its infusion is useful for epilepsy. The essential oil of this species had significant inhibitory activity on *Proteus vulgaris* strain (Pirbalouti et al 2013). *Anthriscus nemorosa* (M. B.) Spreng. is one of the edible plants in Oramanate region. In a study conducted by Hendawy et al. (2019), the essential oil of the dried flowering aerial parts of *Anthriscus cerefolium* (L.) Hoffm and *Anthriscus* ***nemorosa*** was analyzed using GC-MS. The results **showed**

that there are compounds including caryophyllene, δ-cadinene, trans-pinocarveol, spathulenol, germacrene, and caryophyllene oxide in the studied species (Hendawy et al 2019). The purpose of the present study is to qualitative evaluation of the presence of effective substances and to investigate the antimicrobial power of some ecotypes of edible wild species of the Umbelliferae family including *Z. membranacea*, *Z. aucheri* and *A. nemorosa* in the mountainous region of Kosalan in Kurdistan province of Iran.

Material & methods

Sample collection

Plant species were collected from the Kosalan area of Avromanat in the spring of 1400. After identification, herbarium specimens were prepared and kept in the herbarium of Kurdistan University Faculty of Sciences.

Plant extraction

The plant methanolic extract was prepared by rotary evaporator (vacuum distillation).

Qualitative screening of plant chemical compounds

Several phytochemical tests were performed to check the presence of alkaloids, tannins, phenol, flavonoids, sterol, phlobatannin, Saponin and glycoside in the studied species.

Anti-microbial assay

To evaluate the antibacterial activity of plant extracts, three bacterial strains, including two gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and one gram-positive bacteria (*Staphylococcus aureus*), were used. The antibiotic discs, including chloramphenicol, nitrofurantoin, vancomycin, amikacin, ciprofloxacin, meropenem, and imipenem, were used as a comparative positive control.

Based on DDM (disk diffusion method) Pure colonies of each microorganism were suspended in distilled water until a turbidity match McFarland tube number 0.5 (1.5×10^8 CFU/ ml). 100 µl of 0.5 McFarland from each bacterial strain was inoculated into the Müller-Hinton culture medium. Sterile paper discs (6 mm in diameter) were impregnated with 100 µl of each plant extracts at different concentrations (20, 50, 200, and 400 mg/ml). Paper discs were dispensed onto the surface of the inoculated agar plate. The plates were then incubated at 37 °C for 24 hours according to growth requirement of each organism. Each sample was tested in triplicates and antibacterial activity was evaluated by measuring and recorded the zones of inhibition in mm (including the 6 mm disk). Several antibiotic discs was used in order to compare their antimicrobial potency with the plant extracts and as a positive control. DMSO 10% was solvent control.

Measuring the minimum inhibition concentration by the tube dilution method: To determine the MIC, dilution series of 12.5, 25, 50, 100, 200, 400 mg/ml were prepared from the methanolic extract prepared in Mueller Hinton broth medium. According to the definition, the last tube in which there was no turbidity was set equal to MIC. To determine MBC (minimum bacterial concentration), 100 µL of the three tubes before the MIC tube, on Mueller Hinton medium agar

was cultured separately. The lowest concentration of the extract in which bacteria did not grow was reported as MBC.

Results & discussion

The results of the qualitative screening of chemical compounds by the method of extracting the whole plant using methanol solvent for the examined species showed that the three studied species lacked sterols. Or if they had, it could not be identified in these tests due to its very low amount. Phenolic compounds were present in all species and no difference was observed between the species. The saponin test was positive in *Z. aucheri* and *Z. membranacea* and no stable foam was formed in *A. nemorosa*. Phlobatanin, glycoside, and tannin tests were also negative except for *Z. aucheri*. The presence of alkaloids was positive only in *Z. membranacea*. The flavonoid test by sodium hydroxide method confirmed the presence of this substance in all species.

The sensitivity and resistance of a number of different Gram-positive and negative bacterial strains were investigated in the presence of four different concentrations of *Z. membranacea*, *A. nemorosa*, and *Z. aucheri* .extracts in three replicates.

The analysis of variance showed that all the studied factors have a significant effect on inhibiting the proliferation of bacteria. These factors include the effect of different plant species, different concentrations of plant extracts, and different strains of bacteria. Of course, the two-by-two interaction of these factors was also significant at the confidence level of 0.05.

The greatest antimicrobial effect was related to the concentration of 200 and 400 mg/ml, and lower concentrations (50 and 20 mg/ml) were completely ineffective. Also, among the different studied species, *Z. membranacea* had the greatest effect on the relative inhibition of *S. aureus* strain proliferation, and *Z. aucheri* was completely ineffective on all strains. Among the studied bacteria, *P. aeruginosa* had the most resistance, so none of the antibiotics used were able to inhibit the proliferation of this bacterial strain. The effect of inhibiting the growth of bacteria by the extract of *Z. membranacea* can be due to the synergistic effect of the presence of alkaloids and saponin, which was confirmed in the qualitative test of their simultaneous presence only in this species.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the methanolic extract of the studied medicinal plants on the studied bacteria are shown different inhibitory and lethal effects in broth culture medium. The highest inhibitory effect in the lowest concentration was related to the extract of *Z. membranacea* on gram-positive bacteria (*S. aureus*). Although *Z. aucheri* (MIC=50, MBC=100) and *A. nemorosa* (MIC=50, MBC=200) were both able to inhibit the growth of *E. coli*, but the lethal effect of *Z. aucheri* at lower concentrations was detected. It should be noted that the effect of growth inhibition (MIC) and lethality (MBC) of all three plant species against *P. aeruginosa* bacteria was weak (MIC = 100, MBC > 100).

According to the results of the antimicrobial test of the studied plant extracts, it was found that among the studied species, *Z. membranacea*, showed a zone of inhibition in three studied strains of bacteria, and among them, the inhibitory effect on *S. aureus* was the highest (17±1.02 mm).

Although the diameter of inhibition zone was smaller compared to the antibiotics that inhibited the proliferation of *S. aureus* (chloramphenicol, vancomycin, and nitrofurantoin). However, using this plant with a pleasant aroma can be a suitable alternative to antibiotics. The *A. nemorosa* also showed an inhibition zone (10±0.02 mm) for *S. aureus* at a concentration of 400 mg/ml. All the extracts, except the *Z. membranacea*, did not affect gram-negative bacteria. In comparison with other tested species of the Umbelliferae family, including Heptaptera anisoptera, which has not reported any antibacterial effect on Gram-positive or negative strains (Nikavar et al 2009), *Z. membranacea* has a better performance. In the study of Pirbalouti et al. (2013) on *Z. membranacea*, moderate to good inhibitory activity (MICs = 32-250 µg/ml) of its essential oil against four bacterial strains, especially Proteus vulgaris strain, has been confirmed.

Conclusion

Based on the results the extract of *Z. membranacea* in optimal concentrations can be preferable to the use of high doses of antibiotics in dealing with *S. aureus*. Because due to the increasing level of bacterial resistance with commonly used antibiotics, using plant-sourced medicine might help to prevent development of more resistant bacteria.

Keywords:

Apiaceae, Antimicrobial effect, Disk diffusion, Minimum Bactericidal Concentration (MBC), Minimum Inhibitory Concentration (MIC)

Acknowledgements

This research was funded by the University of Kurdistan (UOK), Iran, as a research grant (11.99.5722).

Conflict of interest declaration

The authors have no Conflict of interest related to this paper.

Statement on ethics

The research did not involve any animals or humans as subjects. The researchers carefully adhered to moral guidelines, guaranteeing no copying, fraud, artificial data, distortion, repetitive publication, presentation, or overlap.