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Original Article

The Antimicrobial Activity of Different Extracts from *Echinophora platyloba* DC.

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Abstract

Background and Aim: *Echinophora platyloba* DC (Apiaceae) is a plant endemic to Kurdistan province of Iran. Aerial part of the plant is regarded as the source of natural antimicrobial agent.
Materials and Methods: The aerial part of *E. platyloba* was extracted with hexane, dichloromethane, acetone EtOH and EtOH: H_2O respectively. The extracts were individually tested against three gram-negative (*Escherichia coli, Shigella flexneri, Acinetobacter baumannii*) and two gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecalis*) via agar dilution methods.
Results: The best inhibitory effect was observed in ethanolic extract, which had a remarkable inhibitory effect on all bacteria especially on *S. aureus*. The results also indicated that dichloromethane extract showed the weakest inhibitory effect on all types of bacteria. Meanwhile, acetone extract exhibited a relatively mild inhibitory effect.
Conclusion: The antimicrobial effects of *E. platyloba* confirmed its potential for folk use. More comprehensive investigations are recommended concerning the significant antibacterial activity of the ethanol extract of *E. platyloba* to determine the exact compounds responsible for observed biological activities.
Keywords: *Echinophora platyloba* DC., Extracts, Antimicrobial, MIC

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Introduction

Medicinal plants have been reliable sources of novel drugs which have a fundamental contribution to human health and well-being (1). *Echinophora* (Apiaceae) consists of four species including *E. cinerea*, *E. platyloba*, *E. sibthorpiana* and *E. orientalis* among which *E. platyloba* DC. and *E. cinerea* are endemic to Iran (2). *E. platyloba* DC. species is known by different local names including Khoshariz (the most common), Tigh Touragh, Tigh Masti, Khoshandar, Tanghez, Kouzang, or Khousharouz. It is a native plan that normally grows wild in Northwest Iran and is used as food seasoning in yoghurt and cheese (3). The *Echinophora* species, which are rich in essential oils, have been used as spices in folk medicine of many countries such as Iran, and local people add the plant to tomato pastes as an

antimicrobial and antifungal preservative (4). Due to the promising increase in bacterial resistances to current antibiotics and other antimicrobial agents, more studies have focused on the characteristics of novel natural antimicrobial agents from plant, animal and microbial sources (5, 6). Plants are great sources of a significant variety of secondary metabolites, including terpenoids, tannins, alkaloids and flavonoids, which have been reported to have in vitro antimicrobial properties (7, 8). The chemical constituents and different biological activities of *E. platyloba*. have been discussed in various articles. The main principles of the essential oils were found as trans-β-ocimene (67.9%), 2-furanone (6.2%), myrcene (6.0%) and linalool (3.1%) (9). The aim of this study was to evaluate the antibacterial effects of hexane, dichloromethane, acetone, ethanol and water–ethanol extracts of the leaves of *E. platyloba* on different bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri*, *Acinetobacter baumannii* and *Enterococcus faecalis* via microdilution method.

Materials and Methods

Plant Material

The aerial parts of the plant were collected from Abidar mountain of Kurdistan province, Sanandaj, Iran, in May 2011. The plant was authenticated by Dr. Masoumi (Registration Number 585 RUH). The plant samples were dried in shadow and ground to fine powder.

Preparation of Extracts

Briefly, 98 g of the plant powder sample was extracted with hexane, dichloromethane and acetone, respectively via Soxhlet method in 60°C for 4 h, and then macerated with EtOH and EtOH: H_2O (50:50). The extracts were then filtered with Whatman filter paper number 1, and were concentrated to dryness *in vacuo* at 45°C by a rotary evaporator (Heidolph laborta 4003, Schwabach, Germany). Subsequently, they were dried yielding a waxy material which was kept in dark at 4°C until tested. Ethanolic and ethanol-water extracts were also obtained by maceration of plant powder (98 g) with 980 cc solvent for 72 h. Extracts were then filtered, and the filtrate was concentrated using a rotatory-evaporator, and finally dried and stored in dark at 4°C.

Bacterial Strains and Antimicrobial Agents

The extracts were individually tested against three gram-negative (*E. coli* PTCC 1399 (ATCC 25922), *Shigella flexneri* PTCC 1865 (ATCC12022), *Acinetobacter baumannii* PTCC 1855 (ATCC BAA-747)) and two gram-positive bacteria (*S. aureus* PTCC 1784 (ATCC 6538P), *Enterococcus faecalis* PTCC 1778 (ATCC 29212)). Lyophilized cultures of the organisms were obtained from the Persian Type Culture Collection of the Department of Iranian Research Organization for Scientific and Technology (IROST), Karaj, Iran. Antimicrobial powders (gentamycin & oxacilin), suitable for susceptibility tests, were obtained directly from Sigma pharmaceutical companies.

Micro-Well Dilution Assay

Bacteria were cultured on nutrient agar and blood agar at 37°C for 24 h. A 0.5 McFarland standard was used to create inocula densities of 1.5×10^8 cfu/ml in phosphate-buffered saline (PBS) using the direct suspension method for minimum inhibitory concentration (MIC) (10). The final inocula was about 10^6 CFU/ml. Extracts were dissolved in dimethyl sulfoxide (DMSO). Subsequently, the solution was primarily diluted to the highest concentration as a stock solution, and then serial two-fold dilutions were made in a concentration range from 512 to 65536 µg/ml in Mueller-Hinton broth. Minimum inhibitory concentration (MIC) values of extracts against bacterial strains were determined based on a micro-well dilution method in a sterile flat-bottom 96-well polystyrene plates. Further serial dilution techniques were performed to determine the MIC of extracts at concentrations of 512 to 65536 µg/ml after 18 h growth in 37 °C. Negative controls (Mueller-Hinton broth plus bacterial suspension, without antimicrobial substances) and positive controls were also similarly processed (Mueller Hinton broth plus bacterial suspension with appropriate antibiotic). The final volume of each well was 200 µl. The MIC was defined as the first well, without turbidity for growth at 18 h post-inoculation. All wells that showed no visible growth were transferred to Mueller-Hinton agar, and then incubated at 37 °C for 24 h. All experiments were performed in duplicates (Figure 1).

Antibiotic Susceptibility Testing

The susceptibility of *E. coli* ATCC 25922, *S. flexneri*

ATCC 12022, *A. baumannii* ATCC BAA-747, *E. faecalis* ATCC 29212 isolated to gentamycin and *S. aureus* ATCC 6538P to oxacillin was assessed by the standard disk diffusion method according to the clinical and laboratory standards institute (CLSI) guidelines. The MIC for gentamycin and oxacillin was also determined by the broth microdilution method using CLSI criteria. Briefly, a serial dilution of gentamycin and oxacillin was prepared in Mueller Hinton Broth containing 5 $\times 10^6$ CFU/ml bacteria. The culture microtubes were incubated at 37 °C for 18 hours and finally the lowest concentration of antibiotic with no visible bacterial growth was defined as the MIC. Bacteria strain was used as the control. The range of antibiotics concentrations were from 0.125 to 512 µg/ml. The CLSI breakpoints were used for gentamycin (susceptible ≤4 µg/mL; resistant ≥ 8 µg/mL) and oxacillin (susceptible ≤2 µg/ mL; resistant ≥4 µg/mL).

Results and Discussion

Currently, food processors and consumers have focused on substituting the synthetic preservatives with natural additives (11). Echinophora genus was highly regarded as an antibacterial agent. The E. platyloba clearly demonstrated antibacterial and antifungal properties (12). These activities suggest its potential use as a chemotherapeutic agent and also a food preserving agent. The tested E. platyloba appeared to be effective against a wide spectrum of microorganisms, both gram positive and gram negative microorganism (13). In the present study, the antimicrobial effects of different extracts of the plant was compared with those of gentamicin and oxacillin. An analysis of different extracts of the plant indicated that the highest and lowest antimicrobial effects were observed for the ethanol extract and dichloromethane extract (Table 1 and Figure 2) respectively. There are some scientific evidences confirming the antimicrobial effect of E. Platyloba (14, 15). Entezari et al. indicated that methanolic extract of E. platyloba could inhibit the growth of staphylococcus aureus and pseudomonas aeruginosa. This inhibition could be zero in higher concentrations. Moreover, their findings also proved that E. platyloba could not prevent the growth of Candida albicans, Aspergillus flavus and Aspergillus niger (14). The results of our study concerning the effects on E.coli and S.aureus were consistent with the previous study (14). Although there is no agreement on the acceptable level for the antimicrobial activity of the plant, some scientists suggested a classification for the property of inhibiting microbial plant material offered based on the following criteria: strong inhibition with MIC to 0.5 mg/ml, average inhibition with MIC between 0.6 and 1.5 mg/ml, and a weak inhibition with MIC higher than 1.6 mg/ml (16). Accordingly, this dichloromethane extract had the weakest inhibitory effect on all types of bacteria and acetone extract exhibiting relatively mild inhibitory effects. Meanwhile, the best inhibitory effect was observed in ethanolic extract, which had a strong inhibitory effect on all bacteria especially on S. aureus (512 µg/ml). Hydro-alcoholic extract used in this study had a remarkable effect on bacteria, especially on A. baumannii and S. aureus (4096 µg/ml). Minimum

Table 1: Minimal Inhibitory Concentration (µg/ml) of *E. platyloba* Different extracts and Control Antibiotics.

	E. coli	***S. flexneri***	***A. baumannii***	***E. faecalis***	***S. aureus***
Hexane	16384	4096	512	4096	4096
dichloromethane	32768	32768	32768	32768	32768
Acetone	16384	16384	16384	16384	1024
Ethanol	4096	2048	2048	2048	512
Ethanol-water(50:50)	16384	16384	4096	16384	4096
Gentamycin	1	1	0.5	1	-
Oxacillin	-	-	-	-	16

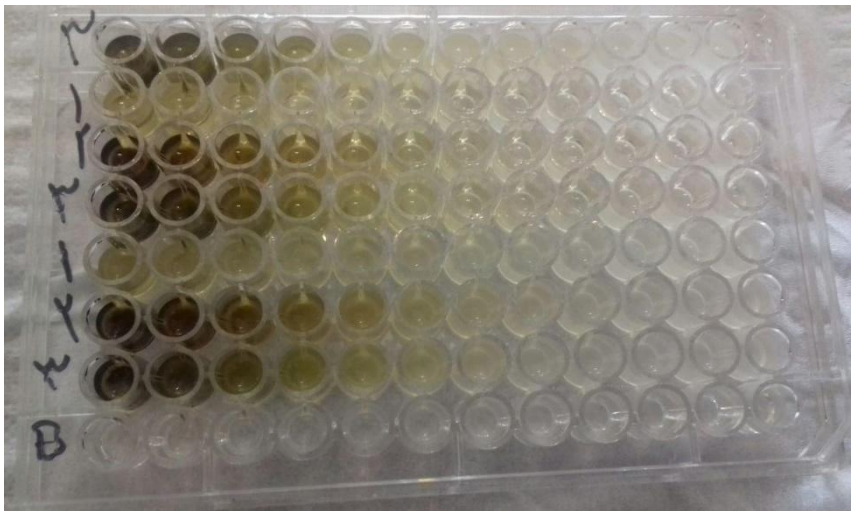

Figure 1. Microdilution Test for the Determination of Minimal Inhibitory Concentration.

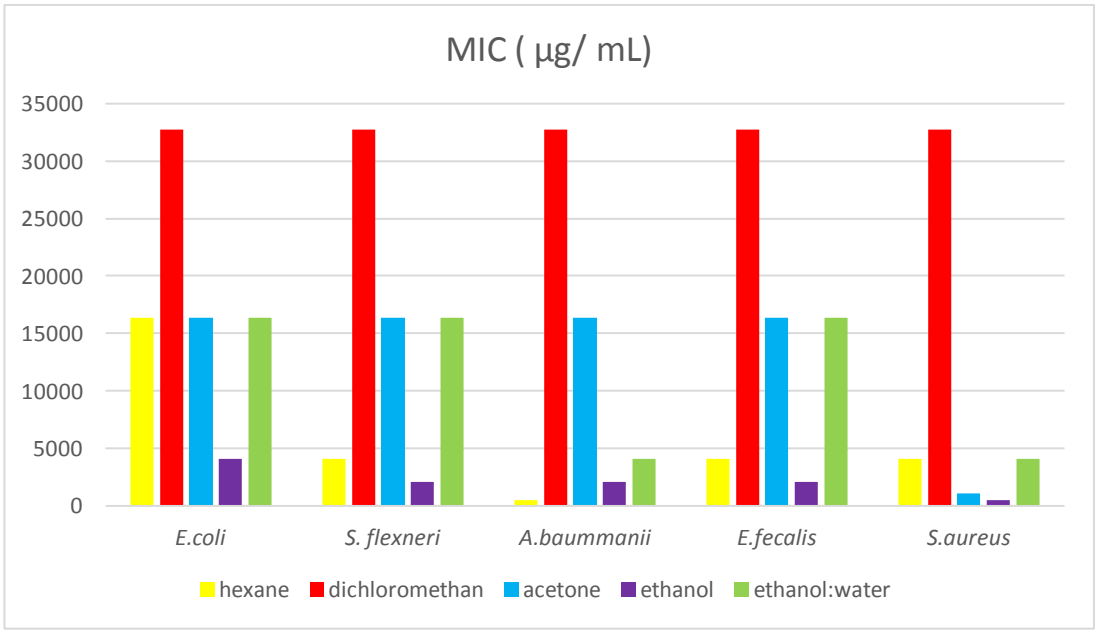

Figure 2. MIC Vale of *E. platyloba* Different Extracts.

inhibitory concentration (MIC) values of E. platyloba D.C on different kinds of bacteria in this study indicated the notable sensitivity of gram-positive bacteria and the relative susceptibility of gram-negative bacteria. Our results clearly indicated that the extracts had a better inhibitory activity against gram-positive bacteria than against gram-negative bacteria. The cell walls of gram-positive and gram-negative bacteria are different. Most studies regarding the antimicrobial mode of action in active constituents of plants have been performed on bacteria, and results have showed that gram-negative bacteria are generally less susceptible than gram-positive bacteria (17). Different compositions in the cell walls of gram-positive and gram-negative bacteria make them sensitive or resistant to the effects of antimicrobial agent. The cell wall of gram-negative bacteria is more complicated, and contains peptidoglycan with lipoproteins in outer membrane tight connections which makes them more resistant to the effects of macromolecules and hydrophobic compounds (18, 19). The lack of the hydrophilic lipopolysaccharide layers in gram-positive bacteria makes them more susceptible to the effects of antimicrobial agents (20). The antimicrobial activity has also been attributed to the presence of some active

constituents in the extract of E. platyloba such as Stigmasterol and Sitosterol (21). In this study, we investigated the antibacterial effects of extracts of the E. platyloba. We indicated that E. platyloba methanol extract had an inhibitory effect on the growth of these bacteria. Moreover, we observed that ethanol extract had the best antibacterial activity.

Conclusion

This study confirmed that with regard to the remarkable growth inhibitory effect of the ethanol extract of *E. platyloba*, polarity could obviously affect the antibacterial activity. Hence, the purification and evaluation of the antibacterial effects of active substances of *E. platyloba D.C* extracts for therapeutic or industrial utilization are recommended. Further studies on therapeutic applications of *E. platyloba* extracts should be undertaken to investigate the safety issues. Although the mechanism of the antimicrobial effect of *E. platyloba* has not been identified, further investigations are required to clarify the exact mechanism of the antimicrobial effect of *E. platyloba*.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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