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Antibacterial Activity of Different Extracts of *Daucus Carota* (L.) Growing Wild in Kosovo.

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ABSTRACT

In this study the antibacterial efficiency of different organic extracts from *D. carota* (L.) growing wild in Kosovo were examined. Methanol, ethyl acetate, acetone, diethyl ether, water and chloroform extracts were tested against three gram positive bacteria *Staphylococcus aureus* (food isolate), *Staphylococus aureus* (clinical isolate), *Listeria monocytogenes* (clinical isolate) one gram negative bacteria *Escherichia coli* (clinical isolate). The antibacterial activity was determined by using agar disc diffusion method. The inhibition zone of extracts was compared to that of penicillin G as standard. Methanol extract of the plant with concentration 1, 3 and 5 mg/mL showed a stronger antibacterial activity towards bacteria *E. coli*, *L. monocytogenes* and *S. aureus* (clinical isolate). Ethyl acetate extract of the plant with concentration 1, 3 and 5 mg/mL showed a stronger antibacterial activity towards bacteria *S. aureus* (food isolate) with inhibition zone 8 mm. The extract of diethyl ether with concentration of 5 mg/mL has the same inhibition zone as the standard (inhibition zone 10 mm). Thus, this extract shows higher activity than all of the other extracts. Also extracts of acetone, water and chloroform in some concentration showed a good antibacterial activity towards some bacteria with inhibition zone of 6 mm.

Keywords: *Daucus carota* (L.), antibacterial activity, agar disk diffusion method, organic extracts.

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INTRODUCTION

The heterogeneous relief of Kosovo with the diversity of soil types is responsible for the heterogeneousness of the Kosovo flora [1]. In the last years, interest in medicinal plants is more and more increasing, particularly using as antibacterial agents. The reason for that is the growth of antibiotic resistance of bacteria against synthetic drugs [2]. For a long time, plants from the Apiaceae family have been used due to their essential oils. Now it is known that essential oils from Apiaceae family plants show antiseptic, expectorant, diuretic, carminative, vasodilator, or spasmolytic actions [3].

D. carota (L.) is an aromatic plant form Apiaceae family used in traditional medicine, due to recognized antibacterial and antifungal activity of their essential oils (carrot oil). Although this plant has been subject to several investigations [4-11].

Or research group was interested to analyze the chemical profile of different medicinal plants, which are growing wild in the region of Kosovo and Albania [12-19]. The aim of this study was to investigate the antibacterial activity of different solvent extracts from *Daucus carota* (L).

MATERIALS AND METHODS

Plant material

The *D. carota* (L.) plant was collected in May 2016 (Figure 1) in Kosovo. Voucher specimens (FF/2015005) were deposited in the herbarium of the Department of Biology, University of Prishtina. The aerial part of this medicinal plant was air dried at room temperature within three weeks.

Figure 1: *D. carota* (L.) growing wild in Kosovo (photo taken from Arben Haziri)

Preparation of plant organic extracts

The aerial part of *D. carota* (L.) was air-dried and then milled with a mixer. A piece of finely powdered material (200 g) was extracted with 70% methanol (CH_3OH, 4 L) during a 24 h period (three times). After removing the CH_3OH under reduced pressure, the aqueous phase is extracted with four consecutive increasing polarity solvents, diethyl ether ($C_4H_{10}O$), chloroform ($CHCl_3$), ethyl acetate ($CH_3COOCH_2CH_3$) and acetone (CH_3COCH_3). Extraction is done until colorless extracts are taken. The remaining was water extract. Five extracts ($C_4H_{10}O$, $CHCl_3$, $CH_3COOCH_2CH_3$, CH_3COCH_3 and H_2O) were evaporated by vacuum rotary evaporator (EYELA N-1000, Japan) to obtain crude product. The crud product then dissolved in DMSO to prepare the solution with the concentration 1, 3 and 5 mg/mL. These solutions are used in subsequent experiments for testing their antibacterial activity. Solvents (analytical grade) for extraction were obtained from Sigma–Aldrich and Merck.

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Antibacterial activity

The antibacterial activity of the *D. carota* (L.) extracts obtained by using the different organic solvents such as methanol, ethyl acetate, acetone, diethyl ether, water and chloroform of *D. carota* (L.) are determined applying the Kirby-Bayer [20] method or disk method (d = 5.5 mm, maximum capacity 10 mg). *D. carota* (L.) extracts were tested in an *in vitro* experiment against bacterial strains; *S. aureus* (food isolate with code 3321), *S. aureus* (clinical isolate with code 3319), *L. monocytogenes* (clinical isolate with code 2653) and *E. coli* (clinical isolate with code 2813). For the research we used three different concentration of extracts, 1, 3 and 5 mg/mL in DMSO as solvent and then placed in a Petri dishes (d=15 cm). The disks were incubated at 37 ^{0}C for 48 h; the control was also maintained with penicillin G dissolved in DMSO in a similar manner.

RESULTS AND DISCUSSION

Table 1 presents the yields of extracts derived from plant *D. carota* (L.).

Table 1: Yields of extracts derived from plant *D. carota* (L.)

Extract	Yield (%)
Methanol	13.55
ethyl acetate	5.14
acetone	5.69
diethyl ether	8.67
water	7.78
chloroform	4.09

In this study, the antibacterial activity of different extracts of this plant was evaluated on: *S. aurous* (clinical isolate), *E. coli* (clinical isolate) *S. aurous* (food isolate), *L. monocytogenes* (clinical isolate). The antibacterial activity was determined by using agar disc diffusion method. The zones of inhibition, from extracts were compared to that of penicillin G as standard as shown in table 2.

Table 2: Antibacterial activities of *D. Carota* (L.) organic extracts

Extract	Concentration (mg/ml)	Inhibition zones diameters (mm)			
		***E. coli* (c. i.)**	***L. monocytogenes* (c. i.)**	***S. aureus* (f. i.)**	***S. aureus* (c. i.)**
Methanol	1	6	6	-	6
	3	6	6	2	6
	5	6	6	6	6
Ethyl acetate	1	-	-	8	-
	3	-	6	8	-
	5	-	-	8	-
Acetone	1	6	6	6	6
	3	6	-	6	6
	5	-	-	6	-
Diethyl ether	1	-	-	6	-
	3	-	-	6	-
	5	-	6	10	-
Water	1	6	6	-	6
	3	6	-	-	-
	5	6	6	-	6
Chloroform	1	6	6	-	-
	3	-	6	6	-
	5	-	-	-	-
Penicillin	1	4	2	4	2
	3	6	6	8	6
	5	8	10	10	8

(-) no inhibition zone

Extracts of methanol (1, 3 and 5 mg/ mL), acetone (1 and 3 mg/mL), chloroform (1 mg/mL) and water with concentration 1, 3 and 5 mg/mL shows antibacterial activities against *E. coli* (Table 1 and Figure 2). The

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extracts of methanol and water with concentration of 5 mg/mL resulted in a lower activity than the penicillin G of the same concentration. The extracts of methanol, water and acetone with concentration of 3 mg/mL has the same inhibition zone as the standard (6 mm). Extracts of methanol, acetone, water and chloroform with concentration 1 mg/mL created an inhibition zone higher than the penicillin G with the same concentrations.

The other extracts such as ethyl acetate (1, 3 and 5 mg/mL) acetone (5 mg/mL), diethyl ether (1, 3 and 5 mg/mL) and chloroform with concentration 3 and 5 mg/mL do not create any inhibition zone, in other words they do not show activity (Table 2 and Figure 2).

The extracts of methanol, acetone, water and chloroform with concentration 1mg/mL (inhibition zone 6 mm) resulted in a higher activity against *L. monocytogenes* than the penicillin G (inhibition zone 2 mm). The extracts of methanol, ethyl acetate and chloroform with concentration of 3 mg/mL have the same inhibition zone as the standard (inhibition zone 6 mm).

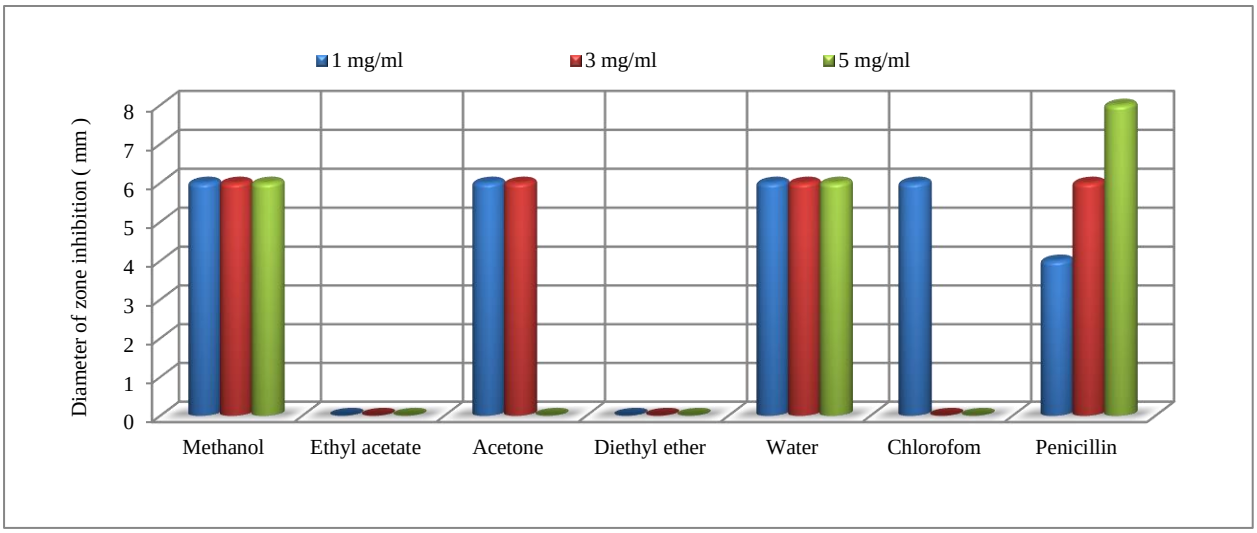

Figure 2: Antibacterial activity of extracts from *D. carota* (L.) against *E. coli* (clinical isolate)

The extracts of methanol, diethyl ether and water with concentration of 5 mg/mL resulted in a lower activity than the penicillin G of the same concentration with inhibition zone of 10 mm. The other extracts such as ethyl acetate (1 and 5 mg/mL), acetone (3 and 5 mg/mL), diethyl ether (1 and 3 mg/mL), chloroform (5 mg/mL) and water with concentration 3 mg/mL did not show any inhibition activity on bacteria *L. monocytogenes* as was shown in Table 2 and Figure 3.

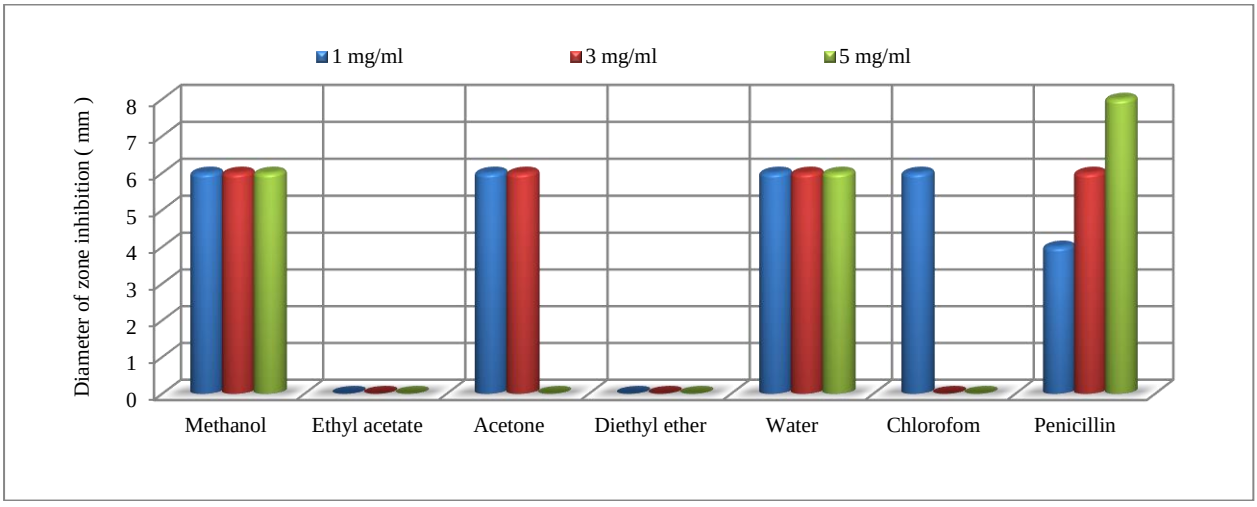

Figure 3: Antibacterial activity of extracts from *D. carota* (L.) against *L. monocytogenes* (clinical isolate)

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The extracts of ethyl acetate, acetone and diethyl ether showed antibacterial activity against *S. aureus* (isolated in food) in all of the concentrations 1, 3 and 5 mg/mL. The extracts of ethyl acetate (3 mg/mL) and diethyl ether with concentration of 5 mg/mL has the same inhibition zone. The extracts of ethyl acetate, acetone and diethyl ether with concentration of 1 mg/mL (inhibition zone 8 mm respectively 6 mm) resulted in a higher activity against *S aureus* (isolated in food) than the penicillin G (inhibition zone 4 mm). The extracts of methanol (inhibition zone 2 mm) acetone, diethyl ether and chloroform (inhibition zone 6) with concentration of 3 mg/mL resulted in a lower activity than the standard of the same concentration with inhibition zone of 8 mm. The extracts of methanol, acetone (inhibition zone 6 mm) and ethyl acetate (inhibition zone 8 mm) with concentration of 5 mg/mL resulted in a lower activity than the standard of the same concentration with inhibition zone of 10 mm. The extracts of water (1, 3 and 5 mg/mL), chloroform (1 and 5 mg/mL) and methanol with concentration 1 mg/mL did not show activity on bacteria *S. aureus* isolated in food (Table 2 and Figure 4).

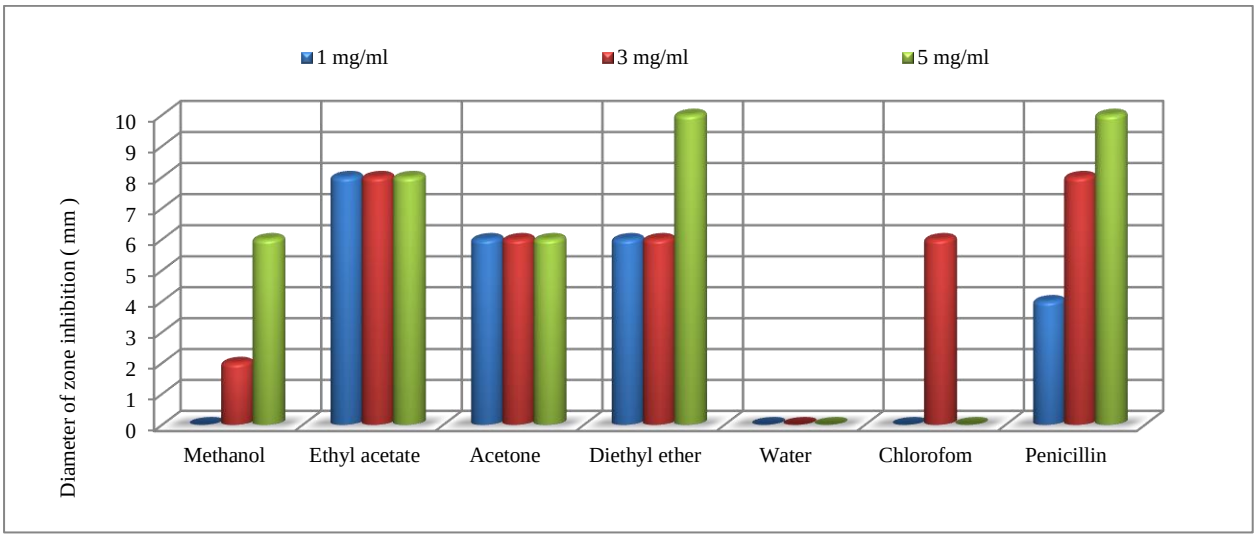

Figure 4: Antibacterial activity of extracts from *D. carota* (L.) against *S. aureus* (food isolate)

The extracts of methanol and water (inhibition zone 6 mm) with concentration of 5 mg/mL resulted in a lower activity against *S. aureus* (positive Gram bacteria) isolated in the clinical way, than the standard with the same concentration (inhibition zone of 8 mm). The extracts of methanol and water with concentration of 1 mg/mL (inhibition zone 6 mm) resulted in a higher activity than the penicillin G (inhibition zone 2 mm). The extracts of methanol and acetone with concentration of 3 mg/mL have the same inhibition zone as the standard (inhibition zone 6 mm). The other extracts such as ethyl acetate, diethyl ether, chloroform (1, 3 and 5 mg/mL), acetone (5 mg/mL) and water with concentration 3 mg/ mL do not create any inhibition zone, in other words they do not show activity (Table 2 and Figure 5).

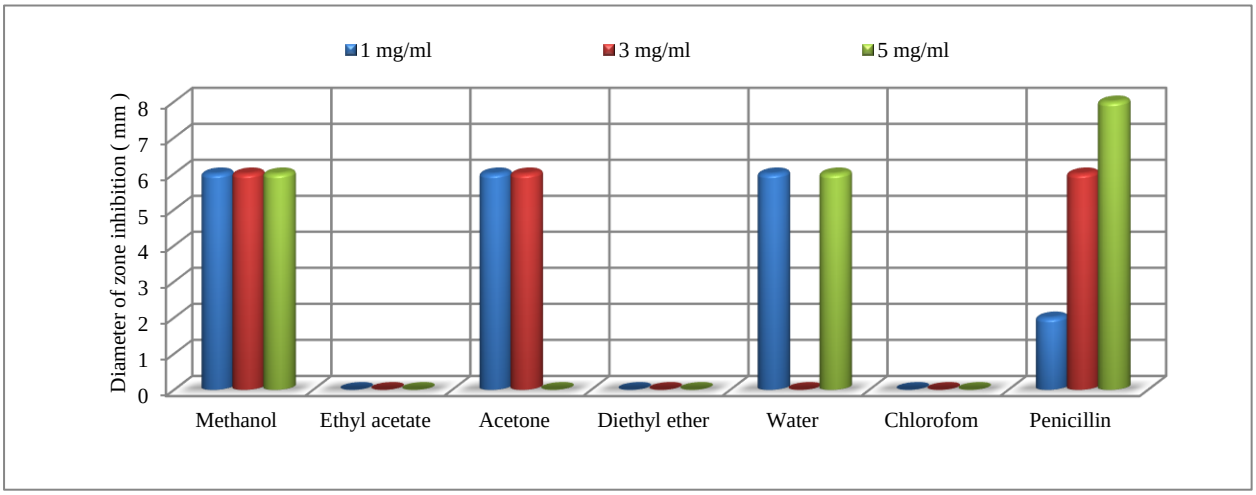

Figure 5: Antibacterial activity of extracts from *D. carota* (L.) against *S. aureus* (food isolate)

CONCLUSIONS

D. carota (L.) is a medicinal plant used in folk medicine for different health illness. The aim of this research was to analyze the antibacterial activities of different extracts from *D. carota* (L.) plant growing wild in Kosovo. The three concentrations of the extracts with ethyl acetate, acetone and diethyl ether showed a very good activity towards bacteria *S. aureus* (food isolate). Thus, these extracts showed higher activity than all other extracts. Diethyl ether extract of the plant with concentration 5 mg/mL showed a stronger antibacterial activity (inhibition zone 10 mm) against bacteria *S. aureus* (food isolate). Water and methanol extracts with concentration 1, 3 and 5 mg/mL showed a stronger antibacterial activity (inhibition zone 6 mm) against bacteria *E. coli*. Methanol extract of the plant with concentration 1, 3 and 5 mg/mL showed a stronger antibacterial activity towards bacteria *E. coli* (clinical isolate), *S. aureus* (clinical isolate) and *L. monocytogenes* (clinical isolate). Results obtained from methanol, acetone diethyl ether and ethyl acetate extracts for antibacterial activity are logical, based on numerous of studies where these extracts were analyzed in the content of flavonoids, phenols, terpenes, alkaloids, etc., and these components are responsible for the biological activity.

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