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EVALUATION OF ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *CICHORIUM INTYBUS* (L.) GROWING WILD IN EAST PART OF KOSOVO

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ABSTRACT

Now is known that organic extracts of medicinal plants can be used as antibacterial agents. Effectiveness of extract on bacterial growth depends sort of medicinal plant. In this research we used a medicinal plant *Cichorium intybus* (L.) which was grown wild in Kosovo and was used for a long time against bacterial infections. Antibacterial efficiency of *Cichorium intybus* (L.) were examined using methanol, ethyl acetate, acetone, diethyl ether, water and chloroform extracts and tested against three Gram positive bacteria Staphylococcus aureus (food isolate), Staphylococcus *aurous* (clinical isolate), *Listeria monocytogenes* (clinical isolate) and a Gram negative bacteria *Escherichia coli* (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. More pronounced activity was shown by water and ethyl acetate extracts of the *Cichorium intybus* (L.). The ethyl acetate extract showed antibacterial activity against *Staphylococcus aureus*. Aqueous extract of the plant with concentration 5 mg/ml showed a stronger antibacterial activity against bacteria *Eshcheria coli*. Also aqueous extracts of the *Cichorium intybus* (L.) showed a stronger antibacterial activity as penicillin G against bacteria *Staphylococcus aureus*. The antibacterial activity of the *Cichorium intybus* (L.) was due to the presence of various secondary metabolites such as phenols and flavonoids . Hence, this plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals.

Key words: *Cichorium intybus* (L.), antibacterial activity, agar disk diffusion method, organic extracts.

INTRODUCTION

The propitious geographic position of Kosovo, along with her heterogeneous relief with many productive river valleys, plane lowlands, mountain slopes, as well as the diversity of soil types, are responsible for the heterogeneousness of the Kosovo flora (Mehmeti *et al*., 2007).

Higher and aromatics plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts (Hulin *et al*., 1998). Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases (Perumal *et al*., 2000).

Cichorium intybus (L.) belongs to family Asteraceae and it is a small aromatic biennial or perennial herb. It grows as a wild plant on roadsides in its native Europe, and in North America and Australia, where it has become naturalized (Mehmood *et al*., 2012). The tuberous root of this plant contains number of phytochemicals like lactones, coumarins, flavonoids and vitamins (Varotto *et al*., 2000). The plant root is used as antithepatotoxic, antiallergenic, anti-inflammatory, appetizer, digestive, stomachic, liver tonic, cholagogue, febrifuge, alexeteric and also as tonic (Siddhan *et al*., 2007).

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm its activity (Nair *et al*., 2005). The effect of the plant extracted from microorganisms have been studied by a number of researchers in different parts of the world (Mehmood *et al*., 2012; Ghaderi *et al*., 2012; Renu *et al*., 2013; Petrovic *et al*., 2004; Liu *et al*., 2013; Gilani *et al*., 1994; Shad *et al*., 2013; Brković *et al*., 2006; Masood *et al*., 2014). All parts of this plant possess great medicinal importance due to the presence of a number of medicinally important compounds such as alkaloids, inulin, sesquiterpene lactones, coumarins, vitamins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins and tannins (Zahid *et al*., 2015).

Our research group was interested to analyze the chemical profile of different medicinal plants, which are growing in the region of Kosovo and Albania (Haziri *et al*., 2013,). The aim of this study was to investigate the antibacterial activity of different solvent extracts of *Cichorium intybus* (L.).

MATERIALS AND METHODS

Plant material: The aerial part of *Cichorium intybus* (L.), growing wild in east part of Kosovo, was collected in May of 2014. Voucher specimens (FF/2014001) were deposited in the herbarium of the Department of Veterinary, University of Prishtina. The plants were dried at room temperature.

Preparation of plant organic extracts: Aerial part of *Cichorium intybus* (L.) (100 g) were extracted with 250 ml of each organic solvent (methanol, ethyl acetate, acetone, diethyl ether, water and chloroform) separately for 24 hours at room temperature and the solvents were evaporated by vacuum rotary evaporator (EYELA N-1000, Japan). The extraction process yielded methanol (10.39 g), ethyl acetate (4.0 g), acetone (6.64 g) diethyl ether (6.24 g), water (12.68 g) and chloroform (5.75 g) extracts. Solvents (analytical grade) for extraction were obtained from commercial sources (Sigma–Aldrich, Merck).

Antibacterial activity: The antibacterial activity of the extracts methanol, ethyl acetate, acetone, diethyl ether, water and chloroform of *Cichorium intybus* (L.) were determined applying the Kirby-Bayer (Barry, 1991) method or disk method (d=5.5 mm, maximum capacity 10 mg). Organic extracts samples were tested *in vitro* against bacterial strains; *Staphylococcus aureus* (food isolate with code 3221), *Staphylococus aureus* (clinical isolate with code 3319), *Listeria monocytogenes* (clinical isolate with code 2653) and *Escherichia coli* (clinical isolate with code 2813). Discs were previously wetted with Dimethylformamide (DMF)solution of the organic extracts with three different concentrations, 1, 3 and 5 mg/ml and then placed in a Petri dish (d=15 cm). The disks were incubated at 37^0C for 48 h; the control was also maintained with DMF penicillin in a similar manner.

RESULTS AND DISCUSSION

Table 1 presents the yields of extracts derived from plant *Cichorium intybus* (L.)

Table 1. Yields of extracts derived from plant *Cichorium intybus* (L.)

Extract	Yield (%)
Methanol	10.39
Ethyl acetate	4.0
Acetone	6.64
Diethyl ether	6.24
Water	12.68
Chloroform	5.75

In this study, the antibacterial activity of different extracts of this plant was evaluated on: *Staphylococcus aurous* (clinical isolate), *Escherichia coli* (clinical isolate) *Staphylococcus aurous* (food isolate) , *Listeria 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 *Cichorium intybus* (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	-	-
	3	-	-	6	-
	5	6	-	6	-
Ethyl acetate	1	-	6	6	-
	3	-	-	6	-
	5	6	6	6	6
Acetone	1	-	-	-	-
	3	6	-	6	-
	5	6	-	-	-
Diethyl ether	1	-	-	-	-
	3	6	-	-	-
	5	-	6	-	6
Water	1	-	-	-	6
	3	-	-	-	6
	5	8	-	-	8
Chloroform	1	6	-	6	6
	3	-	-	-	-
	5	6	-	-	-
Penicillin	1	4	2	4	2
	3	6	6	8	6
	5	8	8	10	10

(-) no inhibition zone

Extracts of methanol (5 mg/ml), ethyl acetate (5 mg/ml), acetone (3 and 5 mg/ml), diethyl ether (3 mg/ml), chloroform (1 and 5 mg/ml) and water with concentration 5 mg/ml shows antibacterial activities against *Escherichia coli* (Table 2). The extracts of methanol and ethyl acetate with concentration of 5 mg/ml (6 mm) resulted in a lower activity than the penicillin G of the same concentration. The extracts of acetone and diethyl ether with concentration of 3 mg/ml have the same inhibition zone as the standard (6 mm). The extract of acetone with concentration of 5 mg/ml resulted in lower activity than the penicillin G with the same concentration. Chloroform extract with concentration 1 mg/ml (6 mm) created an inhibition zone higher than the penicillin G with the same concentration (4 mm). The

extract of water with concentration of 5 mg/ml has the same inhibition zone as the penicillin G (8 mm).

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

The extracts of methanol (1 mg/ml), ethyl acetate (1 and 5 mg/ml) and diethyl ether with concentration 5 mg/ml shows antibacterial activity against *Listeria monocytogenes* (Table 2). The extracts of methanol and ethyl acetate with concentration of 1 mg/ml (6 mm) resulted in a higher activity than the penicillin G (2 mm). A better activity was the ethyl acetate extract (5 mg/ml), which resulted in the same activity as the penicillin G (inhibition zone 6 mm). The diethyl ether extract with concentration of 5 mg/l (6 mm) resulted in lower activity than the standard penicillin G with the same concentration 5 mg/l (8 mm). The other extracts such as methanol (3 and 5mg/ml), ethyl acetate (3 mg/ml), acetone (1, 3 and 5 mg/ml), diethyl ether (1 and 3 mg/ml), chloroform (1, 3 and 5 mg/ml) and water with 1, 3 and 5 mg/ml did not create any inhibition zone (Table 2 and Figure 2).

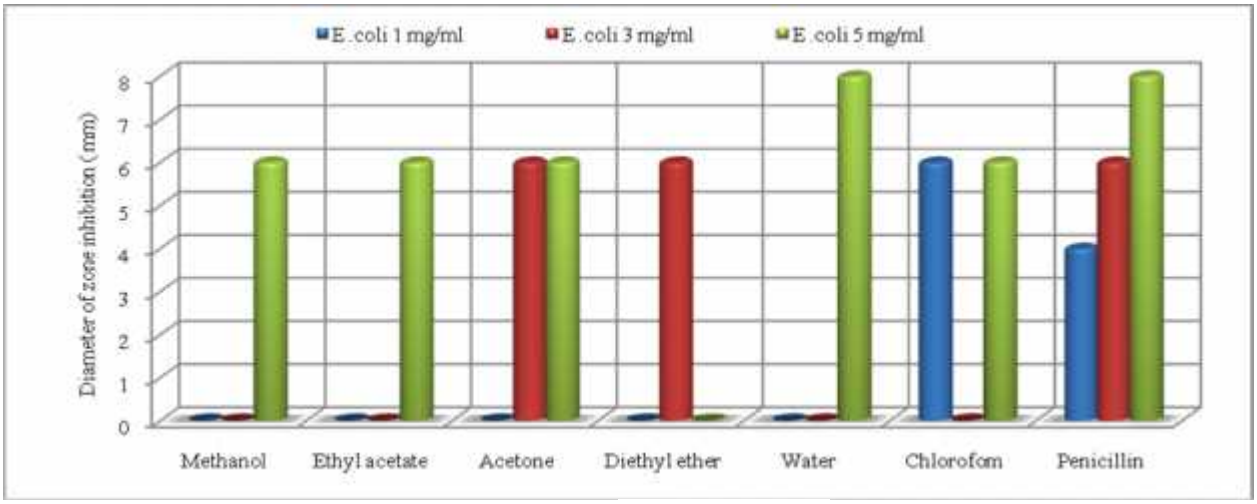

Fig. 1: Antibacterial activity of different extract of *Cichorium intybus* (L.) against bacteria *Escherichia coli* (clinical isolate)

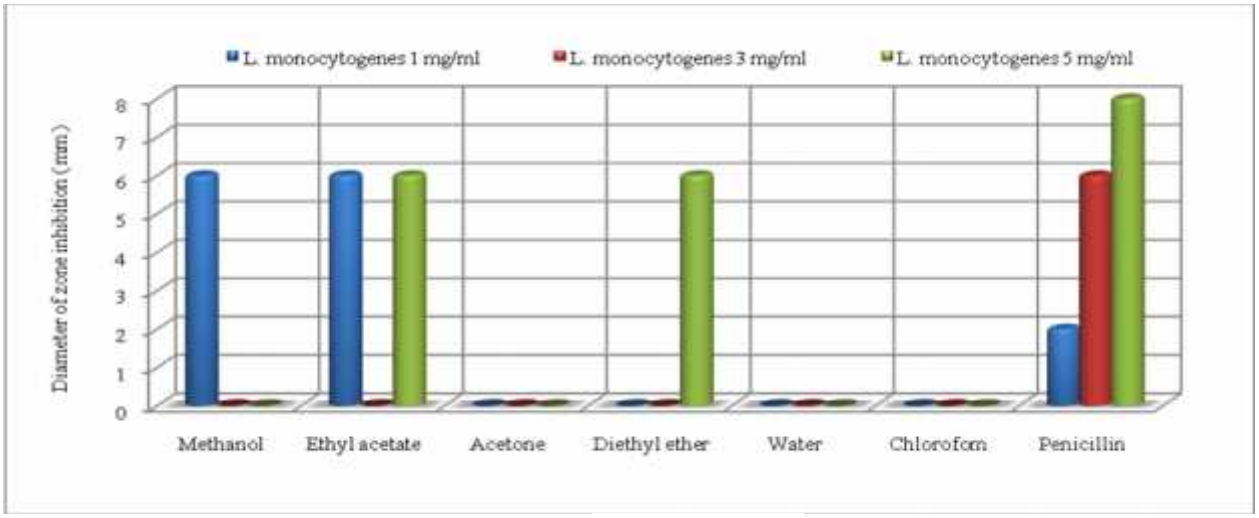

Fig. 2. Antibacterial activity of different extract of *Cichorium intybus* (L.)against bacteria *Listeria monocytogenes* (clinical isolate).

The extracts of ethyl acetate and diethyl ether with concentration of 5 mg/ml (6 mm) resulted in a lower activity against *Staphylococus aureus* (positive Gram bacteria) isolated in the clinical way, than the standard with the same concentration (inhibition zone of 10 mm). The extract of chloroform with concentration of 1 mg/ml

(6 mm) resulted in a higher activity than the penicillin G (2 mm). Water extract with concentration 1 mg/ml (6 mm) created an inhibition zone higher than the penicillin G with the same concentration (2 mm). The extract of water with concentration of 3 mg/ml has the same inhibition zone as the standard (6 mm). The extract of water with concentration of 5 mg/ml (8 mm) resulted in a lower activity than the penicillin G of the same concentration (10 mm). Aqueous extract of the *Cichorium intybus* (L.) showed a stronger antibacterial activity to bacteria *Staphylococus aureus* isolated in the clinical way. The other extracts such as methanol (1, 3 and 5mg/ml), ethyl acetate (1 and 3 mg/ml), acetone (1, 3 and 5 mg/ml), diethyl ether (1 and 3 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 3).

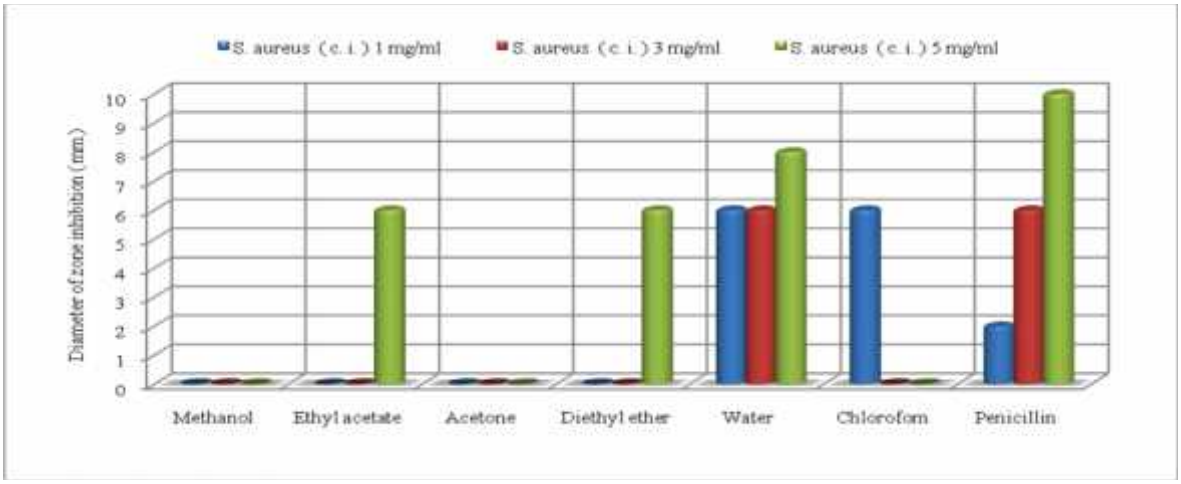

Fig. 3. Antibacterial activity of different extract of *Cichorium intybus* (L.) against bacteria *Staphylococus aureus* (clinical isolate)

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

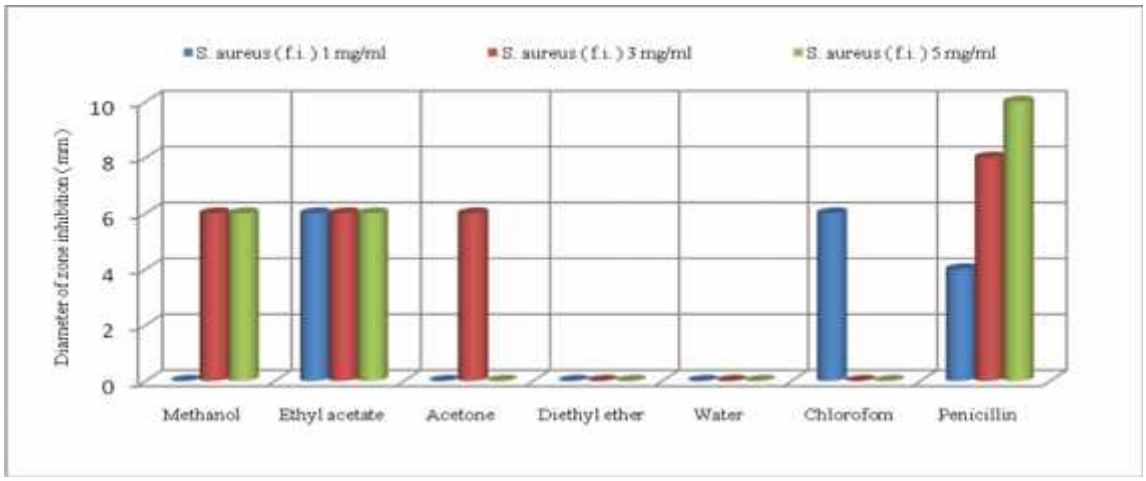

Fig. 4. Antibacterial activity of different extract of *Cichorium intybus* (L.) against bacteria *Staphylococus aureus* (food isolate)

In vitro antibacterial activity of different organic extracts of *Cichorium intybus* (L.) were tested against three species of Gram positive bacteria (*Staphylococcus aureus* (f. i.), *Staphylococus aureus* (c. i.), *Listeria monocytogenes* (c. i.); and one Gram negative bacteria *Escherichia coli* (c. i.). The antibacterial activity results were compared with those from antibiotic penicillin G and they are shown in Figure 5.

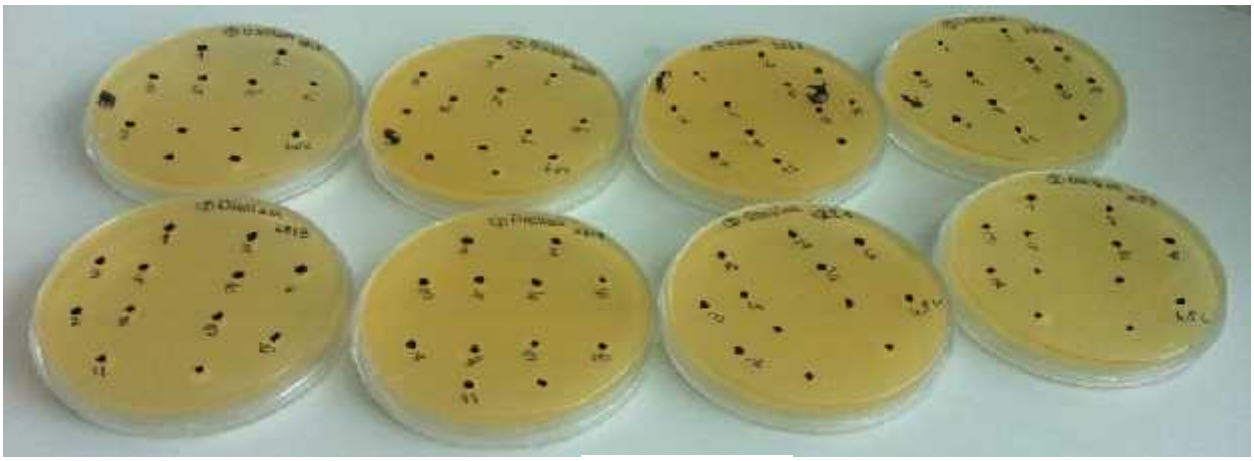

Fig. 5: Antimicrobial activity of different extracts *Cichorium intybus* (L.) against *Staphylococcus aureus* (f. i.), *Staphylococus aureus* (c. i.), *Listeria monocytogenes*(c. i.) and *Escherichia coli* (c. i.).

Conclusion: *Cichorium intybus* (L.) plays an important role in a range of substances of natural origin which are used in our daily life and it`s a plant that is used in traditional medicine for various purposes. This causes more interest in the research of plant *Cichorium intybus* (L.) which has different constituents. The antibacterial activity of organic extracts of the plant was evaluated against a Gram negative bacteria *Escherichia coli* (clinical isolate) and three Gram positive bacteria *Listeria monocytogenes* (clinical isolate), *Staphylococcus aureus* (food isolate) and *Staphylococcus aureus* (clinical isolate) . Plant material was collected in eastern part of Kosovo, and extracted with cold solvents: methanol, ethyl acetate, acetone, diethyl ether, water and chloroform . Extracts were prepared with concentration 1, 3 and 5 mg/ml and activities were determined by measuring of the diameter of zones of inhibition against both Gram positive and Gram negative bacteria using the paper agar disc diffusion method.

Results obtained from water extracts more than surprising, are logical, based on numerous of studies where this extract is analyzed in the content of components. The large amount of metabolites present as flavonoids, phenols, terpenes, alkaloids, etc., in water extracts is responsible for the antibacterial activity.

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