

Antibacterial Activity of Ethanolic Extract of Roots of the Blessed Thistle (*Cnicus Benedictus* L.)

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Abstract

The aim of this study is to investigate the activity of the ethanolic extract of *Cnicus benedictus* L. on pathogenic bacteria *Escherichia coli* , *Bacillus pumillus* , *Staphylococcus aureus* and *Micrococcus* . In the clinical significance of these characteristics being causing the infections . The results of this study are showed that the ethanol extract of roots has an affective impact by inhibiting the growth of these bacteria because your study only on bacteria in Vitro. The highest rate of inhibition of the ethanol extract for the roots against *Bacillus pumillus* is reached to 12 mm at the concentration 75 mg/ml, but the least inhibition is 10 at the concentration 25mg/ml compared to the inhibition of the both antibiotics Gentamicin CN 21mm and Ceftriaxone CRO Zeromm as control, Whereas the MIC for *Staph.aureus* is reached to 12 mm at the concentration 25mg/ml compared to the both antibiotics CRO and CN 8 and 5mm respectively. The results also showed that the MIC to inhibit the *E.coli* is 10mm at concentration 25mg/ml compared to the both antibiotics CRO and CN respectively Zero and Zero mm whereas the MIC to inhibit *Micrococcus* bacteria is 11mm at the concentration 25mg/ml compared to the two antibiotics CRO and CN respectively 23 mm 23 mm for each above antibiotic. The results shown that the ethanol extract for the roots has a significant inhibitory effect against some species of pathological bacteria using in the study. In addition , the results of the primary chemical investigation are indicated that the ethanol extract of the *Cnicus benedictus* L. roots contained many active compounds alkaloids , flavonoids , Phenoles , tannins and the terpenes.

Keywords: component : root , Antibacterial , antibiotics.

Introduction

The progress in methodology lead to high specific identification for materials that are useful [1] . The blessed thistle (*Cnicus benedictus* L.) is one of the most important medicinal plant and it is belong to family Asteraceae which considered of big family in plat kingdom. Most or sub memebers of this family are bisexual , female flowers is radial and corella has two form ; tubular or ligulate , fruit Achene [2] . It is 60 cm in hight , leaves 30 cm. with hairs and small spines at the margin .Flowers are yellowish placed on head inflorcence (Capitulum head), this family has about 1120 genus and 125000 species distributed in all the world , In Iraq there is 342 wild species and 58 cultivated [2] .

The blessed thistle has long rich history in traditional medicinal uses , Greeks called it " Knekos" and this plant belongs to Romans. In middle centuries this plant was called *Carduus benedictus* or blessed thistle because of it is care effects, others mentioned that the scientific name (*benedictus*) it came in honor of saint " Benedict " [3] . This plant has very bitter taste and it has anti-septic ,antimicrobial activity and antipyretic. It is also used to stop bleeding and stimulates the milk yield . It is also works well against all the toxins and helps in the healing of inflammatory conditions of liver . The powder of roots protects against the disease if it taken and it has active effect after bite of dogs , scorpions and snakes [4,5]. Kemper [6] had described the uses of this plant as tea , it is useful for idle digestive tract and colon problems , also it increase the energy and uses for high and low acidity in the stomach , as well as it is treats the reduced effectiveness of pancreas and disorder of liver , especially in treatment of jaundice and blood disease . Also had been mentioned that it is wild plant grows in Mediterranean sea and extends to thousands of meters . In Europe , this plants grows in the fields and gardens and it is found also in southern Europe and Asia [7] .

This plant has many active materials (primary and secondary metabolism) which is essentials oil , Cnicin bitter taste , lignin , poly acetheline , flavonoids , triterpins , phytosterols , tannis , citral , paraffin , mucous , mineral rare , iodine [5]. This study was carried out search effect of aqueous and ethanolic extracts for the plant *Cnicus benedictus* L. against different pathogen bacteria such as *B.pumilus* , *E.coli*, *Staph.aureus* and *Micrococcus*. The potency of allelopathy for the extract in number of germs in culture was studied using diffusion method.

Materials and Methods

Collection of plant Sampling

The plant was collected in Sinjar Mount and Sulaymaniyah during flowering stage and it was classified in herbal Faculty of Sciences / University of Baghdad, Doctor. Khalil Al-Shammarie.

1- Preparation of extracts:

Weight of dry powder (20 gm.) and using put in (Soxhlet) extraction containing ethyl alcohol (200ml. 70%) for 4-5 hours. The half of the solvent was evaporated under reduced pressure at (35-45) °C by rotary evaporator [8] .

2- Phytochemical tests procedure :

The extract were subjected to the following tests to identify their chemical constituents :

A- Procedure :

- *Test for alkaloids: [9,10]*

The following tests was applied for identification of alkaloids in the extract solution of roots.

- 1- Dragendorff's test : to 1 ml of the test solution , 1 ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicates the presence of alkaloids.
- 2- Mayer's test : to 1 ml of test solution , a few drops of the Mayer's reagent was added. Formation of white or pale yellow precipitate indicates the presence of alkaloids.
- 3- Wagner's test: 1 ml of test solution , a few drops of the Wagner 's reagent was added. Formation . A yellow or brown precipitate indicates the presence of alkaloids.

- *Test for Flavonoids:[11]*

- 1-Alkaline test : on filter paper a mixture of test solution with few ml of ammonia was applied and checked under U.V. light, appearance of fluorescence colour indicates the presence of flavonoids.

2-Ferric chloride test : 1 ml of test solution mixed with few drops of the neutral ferric chloride solution , formation of blackish red colour indicates the presence of flavonoids.

- *Test for carbohydrate: [12].*

1- Molisch's test : mix 1 ml of extract with few drops of α -naphthol solution (10% in 95 % ethanol) , and then 1 ml of concentrated sulphuric acid was added on the sides of the test tube purple or reddish violet colour at the inter phase layer reveals the presence of carbohydrates.

2- Anthrone test : 1 ml of extract was mixed with 2 ml of anthrone reagent in a test tube , the mixture was incubated for 5 min. in a boiling water bath for 5 minutes , formation of green to blue colour indicates the presence of carbohydrates .

- *Test for phenolic compounds and Tannins: [9].*

1- Ferric chloride test : To 1 ml of extract , a few drops of 0.1 % ferric chloride solution was added. Dark blue or greenish black colour solution indicates the presence of tannins or phenolic compounds. While brown colour indicates the presence of pseudotannins.

- *Test for terpenoids :[13].*

1-Trim- Hill test: To 1ml of acidic extract solution,1ml of Trim- Hill reagent was added in a test tube, heated on a water bath. The appearance of blue colour indicates the presence of diterpenoids while green colour indicates the presence of monoterpenoids.

2-Liebermann- Burchard's test: to 1ml of test solution, few drops of acetic anhydride was added, heated to boiling ,cooled and then 1ml of conc. Sulphuric acid was added along the sides of the test tube. Formation of a pink colour indicates the presence of triterpenoids.

1- Test for coumarins [14].

2- Sodium hydroxide test: the extract solution was placed in a test tube in the presence of DW. The tube was covered with filter paper soaked in sodium hydroxide solution(diluted and boiled), Yellow fluorescence indicates the presence of coumarins after examination under ultra- violet.

- *Test for lignin : [14].*

1- Labat test: 1ml of the extract solution was mixed with 2ml of 10% gallic acid, formation of olive green colour indicating the positive reaction for lignins.

2- Lignin test: Formation of red colour, when 2% furfuraldehyde was added to the extract solution indicates the presence of lignin.

- 3- The preparation of media:

Used the Nutrient broth from the dissolve 28 grams of it in a liter of distilled water, and by heating with mediated magnetic stirrer, and device Autoclave under a temperature of 121 °C and pressure of 1.5 atmosphere for 30 minutes, and then pour in test tubes.

Also Used the Tryptone Soya Agar of dissolve 40 grams of it in a liter of distilled water, and by heating with stirring mediated magnetic motor, and infertility device Autoclave under a temperature of 121 °C and pressure of 1.5 atmosphere for 30 minutes, and the cold and then pour in the dishes.

Four types of bacterial isolates were used: two negative to gram stain *Bacillus pumilus* and *Escherichia coli* (Gr -ve), and the other is two positive gram stain (Gr + ve) It *Staphylococcus aureus* and *Micrococcus*. Provided from Central Laboratories Department / The State company for Drugs and Industry and Medical Appliances, Samarra. And four concentrations used (5,25,50,75) mg./ml of extracts roots the plant and all concentration were dissolve in (1) ml Buffer No.3, also used antibiotic standard (10mcg) for Ceftriaxone (CRO) and Gentamicin (CN).

Antibacterial activity

The plant was used against positive and negative gram bacterial species and demonstrating its biological effect by the agar Well diffusion method, it was poured 1.5 ml from activated bacteria by nutrient broth agar placed in volumetric flask (250 ml) at 40°C and then move well to ensure that contamination fully spread and poured in petri dish by amount 18 ml and leave solidifies at room temperature . After solidification , we make wells in dishes by cookporar 60µl was putting in each wells in concentration (5,25,50,75)mg./ml , and then dishes were incubated in the incubator under 37 °C for 24 hours for bacteria , inhibition diameters were measured by zone reader device. [15].

Result and Discussion

The ethanolic extract of roots were rich in flavonoids, alkaloids , carbohydrates, phenolic compounds , tannins , coumarin and lignin As shown in Table (1). There are

many researches mentioned that the ethanol extract have an activity of anti- microbial and anti- cancer [16].And an activity of anti-oxidant [17].

Table (1): Phytochemical detection or composition in ethanolic extract.

Phytochemical tests		Extract
		Ethanol
Flavonoids	Zinc-Hcl	+
	Alkaline reagent	+
	Ferric chloride	+
	Lead-acetate	+
Alkaloids	Dragendorff's	+
	Mayer's	+
	Wagner's	+
Carbohydrates	Molisch's	+
	Anthrone	+
Phenolic compounds and tannins	Ferric chloride	+
	Gelatin	Trace
Terpenoids :	Trim-Hill	+
	Liebermann-Burchard's	+
Coumarins	Sodium hydroxide	+
Lignin	Labat	+
	Lignin	+

The plant extracts contain a great deal of the active compounds , and their concentrations are different from a plant to another and from a plant part to another. This makes the tests belonged to using the chemical detectors to investigate to the active compounds in the plant and their ratios a necessary thing prior to studying their medical activities [18].

The results in the table (1) showed that the ethanol extract of the plant roots that contain good quantities of flavonoids . So a large number of the plant contain the flavonoids that are in most plants [19]. The primary chemical detective results showed

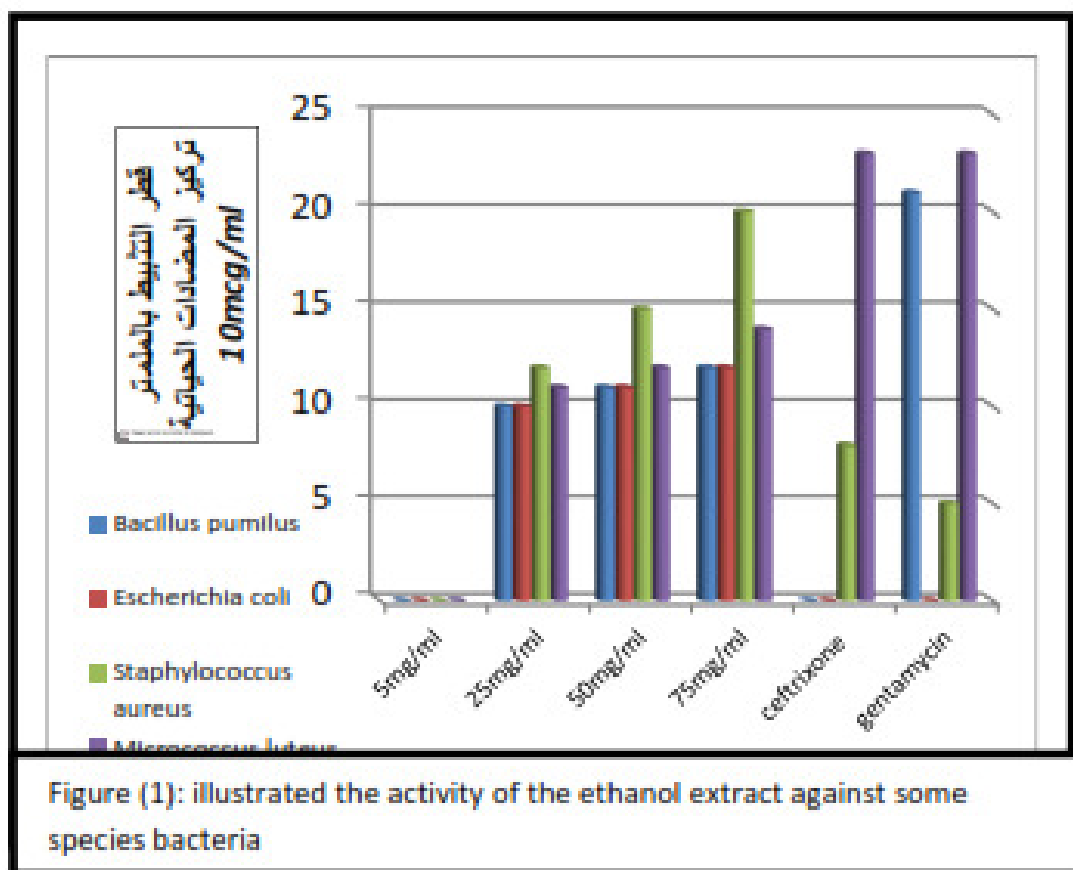
that the ethanol extract of the *C. benedictus* L. contains many active compounds including the alkaloids responsible for the anti-activity to the micro-organism [20]. Flavonoids , anti-oxidant , phenolates , Tannic acid , Tannis and Terpenes are contained in this extract. This result is correspondent to the study of [21]. The nature of the extracts is distinguished by the sticky stature and black green color and aromatic odor . The emergence of the green color is due to the chlorophyll pigment and xanthine , and the distinguished aromatic odor of the plant may be due to containing the *Cnicus benedictus* L. some of the volatile oils [22].

Table (2): the effect of ethanol extract of the *Cnicus benedictus* roots in diameter of the inhibitory area (mm) on some species of the human pathological bacteria and their comparison with the standard anti-biotic.

Microbes (Pathogens)	Leaf Alc. Diameter of the inhibitory area (mm)				Antibiotic disc 10mcg/ml	
	5 mg/ml	25 mg/ml	50 mg/ml	75 mg/ml	Ceftriax one CRO	Gent amy cin CN
<i>Bacillus pumilus</i>	zero	10	11	12	zero	21
<i>Escherichia coli</i>	zero	10	11	12	zero	zero
<i>Staphylococcus aureus</i>	zero	12	15	20	8	5
<i>Micrococcus luteus</i>	zero	11	12	14	23	23

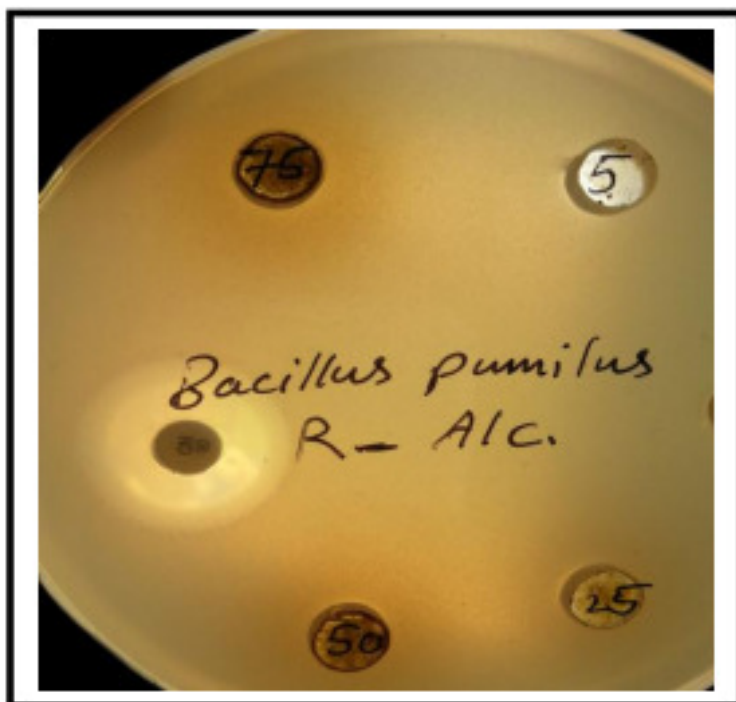
The table (2) and the figure (1) illustrated that the *E.coli* bacteria are influenced by the activity of the extract . The maximum inhibition of the extract occurred at the concentration (75) mg/ml (12) mm compared with the antibiotics (Zero) mm (picture 2), whereas the inhibition of extract against *B. pumilus* arrived at the concentration (75) mg./ml (12) mm in comparison with the antibiotic (CN) that it is inhibition reached at (0)mm and the antibiotic (CRO) that it is inhibition was (Zero) mm (picture 1), the extract also appeared it is effect on *Staph. aureus* , the maximum inhibition is reached (20) mm at the concentration (75) mg./ml in comparison with the antibiotic (CN), It is inhibition is reached to (5) mm and the antibiotic (CRO) (8)mm (picture 3) , whereas the *Micrococcus* are (75) mg./ml , the inhibition reached (14)mm in comparison with the antibiotics (CRO) and (CN), their inhibition are amounted to (23)mm and (23)mm respectively (picture 4).

The active of the crude extracts against some species of bacteria are due to containing these growing some microorganism . This is consistent with inhibition is due to the glycosides , in addition to the influence of the other active groups as alkaloids by which the *C. benedictus* L. is distinguished , especially the alkaloids that solved in the ethyl alcohol and has a capacity of solution superior to the solution in the water .



The importance of this plant is of containing the active tannins in inhibiting the bacteria and viruses by their capacity of inciting the (Phagocyte cells) as well as has an activity in destroying the protein and other compounds into the bacteria cell all because the bacteria used them to attach and the terpenes activated to tear the cellular membranes by the Lipophilic compounds [23]. The alkaloids can make interference with the DNA of the bacteria cells and lead to kill them [24] .

The phenol compounds are distinguished by their properties as anti-bacteria by debarring proton motive oxidative phosphorylation and coagulating the cytoplasm components [25].



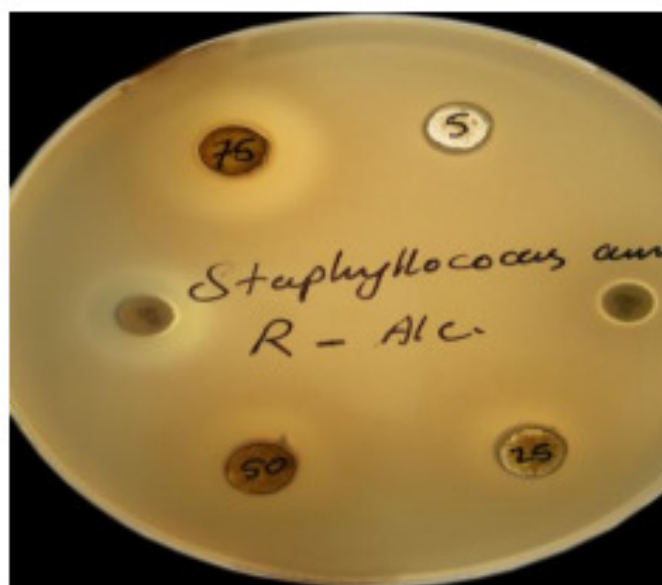
Picture (1) : explained the activity of the ethanol extract of the root Alc. Against *Bacillus pumillus* , and illustrated the concentration by mg./ml and the antibiotic concentration by 10µg/ml.

The phenol compounds have also a role in inhibiting the growth of bacteria by inhibiting the responsible metabolic reactions by interference non specialized with the proteins , this leads to protein denaturation and then absent the capacity of bacteria to survive [26]. The resins and the substantial oils make the aqueous botanic extracts activated towards the selective microorganisms Especially the bacterial species used in the study [27]. The difference of impact of these auxiliary metabolic products in their influence is due to the difference of types of these active materials and their amounts [28]. There are several elements influencing on the inhibitory activity of the extracts including the habitat , the type of the extract , the ideal method of extract , the way of test using in evaluating the extract and the type of the microorganism Especially the species used in the experiment [29].

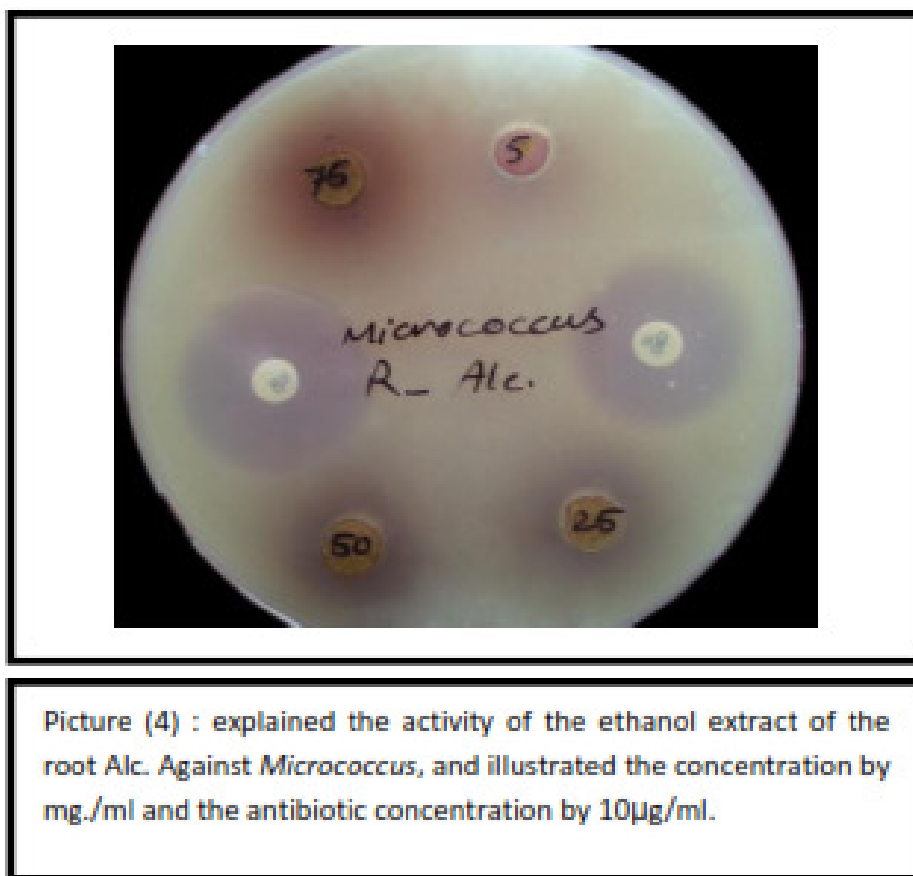
We conclude there is a bio-activity of the roots extracts the roots of the *C.benedictus* L. working against bacteria. The plant has no toxicity to normal cells, because the active compounds, most of which are anti-inflammatory, have no side effects. This gives an opportunity to used it as an alternative from the traditional antibiotics because numerous species of bacteria become assistants to them.



Picture (2) : explained the activity of the ethanol extract of the root Alc. Against *E. coli* , and illustrated the concentration by mg./ml and the antibiotic concentration by 10 μ g/ml.



Picture (3) : explained the activity of the ethanol extract of the root Alc. Against *Staph. aureus* , and illustrated the concentration by mg./ml and the antibiotic concentration by 10 μ g/ml.



Picture (4) : explained the activity of the ethanol extract of the root Alc. Against *Micrococcus*, and illustrated the concentration by mg./ml and the antibiotic concentration by 10µg/ml.

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