

Antibacterial activity of ethanolic extract of leaves 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 such as 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 leaves has an affective impact by inhibiting the growth of these microorganisms in Vitro. The highest rate of inhibition of the ethanol extract for the leaves against Bacillus pumillus is reached to (18) mm at the concentration (75) mg/ml, but the least inhibition is (9) at the concentration (25)mg/ml compared to the inhibition of the both antibiotics Gentamicin (CN) (18)mm and Ceftriaxone (CRO) (Zero)mm as control, Whereas the (MIC) for Staph.aureus is reached to (13) mm at the concentration (25)mg/ml compared to the both antibiotics (CRO) and (CN) respectively (19 and 6)mm. The results also showed that the (MIC) to inhibit the E.coli is (8)mm at concentration (50)mg/ml compared to the both antibiotics (CRO) and (CN) respectively (Zero and Zero)mm whereas the (MIC) to inhibit Micrococcus bacteria is (11)mm at the concentration (25)mg/ml compared to the two antibiotics (CRO) and (CN) respectively (22 and 27)mm. The results shown that the ethanol extract for the leaves 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. leaves contained many active compounds alkaloids , flavonoids , Phenoles , tannins and the terpenes.

Key words: Cnicus benedictus L. ,Inhibitory activity , preparatory primary investigation , antibiotics.

الفعالية التثبيطية للمستخلص الايثانولي لاوراق نبات الشوك المبارك Cnicus benedictus L.

الخلاصة:

هدفت الدراسة التحري عن الفعالية التثبيطية للمستخلص الايثانولي لاوراق نبات الشوك المبارك في نمو Escherichia coli, Bacillus pumillus, Staphylococcus aureus, Micrococcus المسببة للالتهابات. لقد بينت نتائج الدراسة ان المستخلص الايثانولي لاوراق ذات تأثير فعال في تثبيط نمو تلك الجراثيم خارج جسم الكائن الحي، اذ بلغ اعلى معدل تثبيط للمستخلص الايثانولي لاوراق ضد بكتريا (Bacillus pumillu) 18 ملم عند التركيز (75) ملغم/مل واقل تثبيط (9) ملم عند التركيز (25) ملغم/مل بالمقارنة مع تثبيط المضاد الحيوي (Gentamicin (CN) 18) ملم و (Ceftriaxone (CRO) (صفر) ملم كسيطرة ، بينما بلغ MIC بالنسبة لبكتريا Stap. aureus ((13) ملم عند التركيز (25) ملغم/مل بالمقارنة مع المضاديين الحيويين (CRO) و (CN) على الترتيب (6 و 19) ملم. كذلك اظهرت النتائج ان MIC لتثبيط بكتريا (E.coli 8) ملم عند التركيز (50) ملغم/مل بالمقارنة مع المضاديين الحيويين (CRO) و (CN) على الترتيب (صفر و صفر) ملم بينما بلغ MIC لتثبيط بكتريا Micrococcus ((11) ملم عند التركيز (25) ملغم/مل بالمقارنة مع المضاديين الحيويين (CRO) و (CN) على الترتيب (22 و 27) ملم. اظهرت النتائج امتلاك المستخلص الايثانولي لاوراق النبات فعالية تثبيطية مهمة ضد انواع البكتريا المرضية المستخدمة في الدراسة، فضلا عن ذلك اظهرت نتائج الكشف الكيميائي التمهيدي أن المستخلص الايثانولي لاوراق نبات الشوك

المبارك يحتوي على العديد من المكونات الفعالة ، كما يحتوي على الفلافونيدات بأنواعها ومنها المضادة للاكسدة والفينول والتانينات Tannic acid والتربينات.

الكلمات المفتاحية: نبات الشوك المبارك، الفعالية التثبيطية، الكشف الاولي التمهيدي ، مضادات حيائية.

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 inflorescence (Capitulum head) 3 – 4 cm. in diameter , 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

Materials and methods:

Collection of plant Sampling:

Collection of plant County (Mount Sinjar and Sulaymaniyah) during flowering stage (the fifth month - the seventh month) class and Plant classification 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

conditions of liver . The powder of leaves 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 acetelene , 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.

half of the solvent is evaporated under reduced pressure at (35-45) °C by rotary evaporator (8) .

2- Phytochemical tests procedure :

The extract are 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 leaves.

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.

3-Lead-acetate test : To 1 ml of the test solution , a few drops of aqueous basic lead acetate solution were added. Yellow colour precipitate 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.

2-Gelatin test : Mix equal amount of extract solution and gelatin solution (1gm. Gelatin was dissolved in warm 10% aqueous sodium chloride solution at the time of the test) . Formation of white precipitate indicates the presence of tannins.

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:

1-Nutrient broth

Use 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.

2 - Tryptone Soya Agar (OXOID LTD, England)

Use 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 : one negative to gram stain (Gr -ve) It

Escherichia coli and Bacillus pumilus, and the other is positive for the 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).

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	+

mg./ml of extracts leaves the plant and all concentration were dissolve in (1) ml Buffer No.3, also used antibiotic standard (10mcg) for Ceftriaxone (CRO) and Gentamicin (CN).

1- Antibacterial activity :

Antibacterial activity tested against Gram positive bacteria and Gram negative bacteria 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 concentrations of phytochemical compounds of extract . The ethanolic extract for leaves were rich in flavonoids, alkaloids , carbohydrates, phenolic compounds and tannins , coumarin and lignin. 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 (2): the effect of ethanol extract of the Cnicus benedictus leaves 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	Ceftriaxone CRO	Gentamycin CN
Bacillus pumilus	zero	9	10	18	zero	18
Escherichia coli	zero	zero	8	10	zero	zero
Staphylococcus aureus	zero	13	16	18	6	19
Micrococcus luteus	zero	11	13	15	22	27

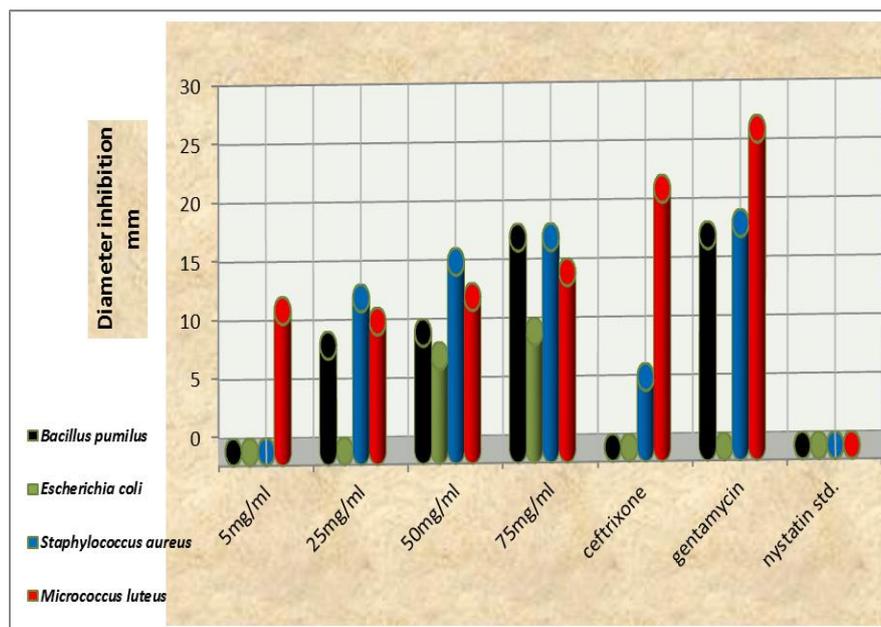


Figure (1): illustrated the activity of the ethanol extract against some species bacteria

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 leaves 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].

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 (10) mm compared with the antibiotics (Zero) mm (picture 2), whereas the inhibition of extract against *B. pumilus* arrived at the concentration (75) mg./ml (18) mm in comparison with the antibiotic (CN) that it is inhibition reached at (18)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 (18) mm at the concentration (75) mg./ml in comparison with the antibiotic (CN), It is inhibition is reached to (19) mm and the antibiotic (CRO) (6)mm (picture 3), whereas the *Micrococcus* are (75) mg./ml, the inhibition reached (15)mm in comparison with the antibiotics (CRO) and (CN), their inhibition are amounted to (22)mm and (27)mm respectively (picture -4).



Picture-1: explained the activity of the ethanol extract of the leaf Alc. Against *Staph. aureus*, and illustrated the concentration by mg./ml and the antibiotic concentration by 10 μ g/ml.



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

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].

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 [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 [29].

We conclude there is a bio-activity of the leaves extracts the leaves of the *C. benedictus* L. working against bacteria. This gives an opportunity to used it as an alternative from the traditional antibiotics because numerous species of bacteria become assistants to them.

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