# Study the antibacterial effect of N_butanol extract of Urtica dioica.

**Ahmed Abdulaziz Ahmed*, Baydaa Hameed Abdulah **, Yaser Mustafa Kamal *****
***Ministry of health***
**** Musansiriyah university/College of pharmacy***
*****Musansiriyah university/College of pharmacy***

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Corresponding Author email:
Yassir.almullahummadi@uomustansiriyah.edu.iq
orcid: https://orcid.org/0000-0002-7543-

## Abstract:

Since ancient times Urtica dioica has been used as an herbal medicine in Western Asia. The current study demonstrates the anti-bacterial effect of N_butanol extraction of Urtica dioica. The antibacterial effect was tested against some of gram-positive bacteria and gram-negative bacteria isolated from hospitalized patients having urinary tract infection. Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa was used. Urticasea is a family of plant known for their medicinal activity from folk medicine. Urtica dioica is a member of urticasea and it is known for it is antibacterial effect.

**Methods**

The leaves of Urtica dioica were collected in Iraq from jisr dayla district in Baghdad province in april 2019.The N_butanol extraction was compared with the drug ciprofloxacin. Disc diffusion method was used to determine the sensitivity of the samples.

**Results**

The N_ butanol extract shows weak inhibitory effect on Escherichia coli while other strain does not affect by extract comparing with ciprofloxacin.

Conclusion

The N_butanol extract of urtica dioica have weak antibacterial effect when compared with ciproflxacine

**Key words:** Urtica dioica, Ciprofloxacine

## دراسة التاثير المضاد للبكتريا لمستخلص البيوتانول لنبات القريص

**احمد عبد العزيز احمد*,ياسر مصطفى كمال**, بيداء حميد عبدالله*****
***وزارة الصحة***
****كلية الصيدلة /الجامعة المستنصرية***
*****كلية الصيدلة /الجامعة المستنصرية***

### الخلاصة:

منذ العصور القديمة تم استخدام القريص كدواء عشبي في غرب آسيا. توضح الدراسة الحالية التأثير المضاد للبكتيريا لمستخلص البيوتانول لنبات القريص. تم اختبار التأثير المضاد للبكتيريا ضد بعض البكتيريا موجبة الجرام والبكتيريا سالبة الجرام المعزولة من المرضى في المستشفى الذين يعانون من التهاب المسالك البولية. تم استخدام المكورات العنقودية و اشريكية قولونية و كلبسيلا الرئوية و الزائفة الزنجارية .يورتيكاسيا هي عائلة نباتية معروفة بنشاطها الطبي من الطب الشعبي. القريص هو عنصر في عائلة وهو معروف بتأثيره المضاد للبكتيريا.

**طريقة العمل**

جمعت اوراق نبات القريص في العراق من منطقة جسر ديالى بمحافظة بغداد في نيسان 2019 وتمت مقارنة التاثير المضاد للبكتريا بين مستخلص البيوتانول لنبات القريص وعقار سيبروفلوكساسين. تم استخدام طريقة نشر القرص لتحديد مدى تاثر العينات.

**النتائج**

يظهر مستخلص ن بيوتانول تأثير مثبط ضعيف على Escherichia coli بينما لا تتأثر السلالات الأخرى بالمستخلص مقارنة مع سبروفلوكساسين

**الاستنتاج**

مستخلص ن البيوتانول من القريص له تأثير مضاد للبكتريا ضعيف بالمقارنة مع سيبروفلكساسين

**الكلمات المفتاحية:** القريص, السبروفلوكساسين

## Introduction

Nowadays researchers and developers of new drugs are looking for drugs extracted from natural sources. Natural medical products are now widely used, and almost 30% of these products were used in clinical prescriptions. The actions of these products were select due to their medicinal importance.

In the last years, infection have been increased to a high rate and there is a resistance to different type of antibiotics and it was considered major therapeutic problem [(1)]. Because of the requirement to new type of antibiotics and novel strategy in the treatment of sober Gram-negative and Gram-positive infections [2][3]. The increase in the resistance in the most common pathogens, the fast appearance of the new infections, and the possible to use of multidrug-resistant agents in bioweapons [4]. These natural products were used in stomach pain in folk medicine in Turkey [5] and Iran [6][7]. As well as for joint pain, as well as colds and coughs [8]. Also used also used in the hemorrhage of uterus, psychogenic eczema, cutaneous eruption, epistaxis, infantile melena, nervous eczema [9][10][11]. Plants are a very important and safe source of compounds through which they can be developed to chemotheraputic agent. first step to achieve this goal was done in *vitro* antibacterial activity assay [12]. The main objective of this study was to evaluate the anti-bacterial effect of crude extract of *Urtica dioica* and compared them to ciprofloxacine to inhibits different kinds of bacterial strains.

## Material and method

**Preparation of the extract:** The leaves of *Urtica dioica* were collected from jisr dayla district in Baghdad in Iraq in april 2019.the leaves of the plant were washed, leave to dry and then grinded to fine powder. 350 grams of the powdered plant material was soaked in 2500ml (1:7) 80 % ethanol, with intermittent shaking.After 3 days, the ethanol extract was filtered off, repeated the process 3 times for 9 days. The filtrate was evaporated till dryness under vacuum using rotary evaporator. A dark greenish residue was obtained. The remaining residue then was suspended in 500 ml distilled water and partitioning respectively with n-hexane, chloroform, ethyl acetate, and n-butanol until reach clear layer for each fraction.The dry n_butanol extract dissolved in10% Dimethylsulphoxide to make a desire concentration 30mg/ml and then testing its antibacterial effect .

### Antibacterial activity test

Antibacterial test was done by Disc Diffusion method according to the method described by the National Committee for Clinical Laboratory Standard (NCCLS) (2002). The bacteria were obtained from AL_KINDI general hospital and it is isolated from hospitalized patients having urinary tract infection. *Pseudomo-nasaeruginosa, Escherichia coli, Staphylococcus aureus, and Klebsiella pneumonia* were used. The tested bacteria

were taken aseptically by an inoculating loop and put it in a test tube that contain 5 mL distilled water. Adequate new inoculums were added till the turbidity equaled 0.5 McFarland ($10^8$cfu/ mL) standards (bioMerieux, Marcy d'Etoile, France). Then taken 1 ML of Bacterial suspension and add to 20 mL of Muller_Hhinton agar. To achieve Disc Diffusion method, a well was made in plates with the aid of a cork-borer (5mm) and add the extract of N_butanol of *Urtica dioica*.The ciprofloxacine was used as a positive control.The plates incubate during night at 37 C. After a day examine the diameter of inhibition zone. The Results were obtained by examination of the inhibition zone [13] [14]. Inhibition zone with diameter of 5mm and over was considered as significant vulnerability of each tested bacteria to the N-butanol extract of *urtica dioica*, while the diameter of inhibition range from 5mm to10 mm was considered as slight and marked as one plus(+), diameter range from 10 mm to15 mm was consider as moderate and mark as two plus (++), and the diameter above 15mm marked as three plus (+++),while when there is no inhibition zone the mark is (-) .

**High performance liquid chromotagraphy (HPLC)**

The N_butanol extract of *Urtica dioica* was analysed by HPLC for determination of major phytoconstituents.

## Result

The HPLC analysis showed that the extract contain both Rutin which is flavinoid and Chlorogenic acid (figure 1,2) which is polyphenolic compound .The aim was to assess activity of the antibacterial effect of *Urtica dioica* N_ butanol extract against different type of bacteria that cause urinary tract infection, compared to the drug ciprofloxacine. The results showed in table (1) and by figures(3, 4, 5, 6) illustrating the inhibition zone of both *Urtica dioica* N_butanol extract and ciprofloxacine and figure 7 showed the bar chart of inhibition zone .*Urtica dioica* N_butanol extract demonstrated an antibacterial activity against *Escherichia coli*, one of the most important species of gram negative bacteria that causes urinary tract infection. *Klebsiella* pneumoniae, *Staphylococcus aureus*, *Pseudomonas aeroginosa* resistant and dont effect by N_butanol extract of *Urtica dioica*.

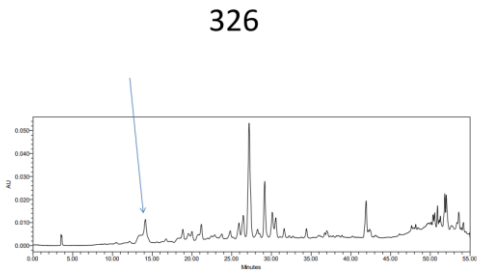


**Figure (1): Chlorogenic acid found in HPLC analysis at 14.8 minutes**

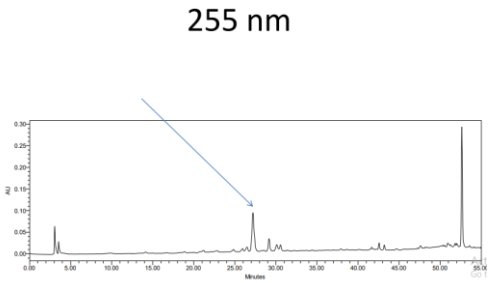


**Figure (2): Rutin found in HPLC analysis at 27 minutes**

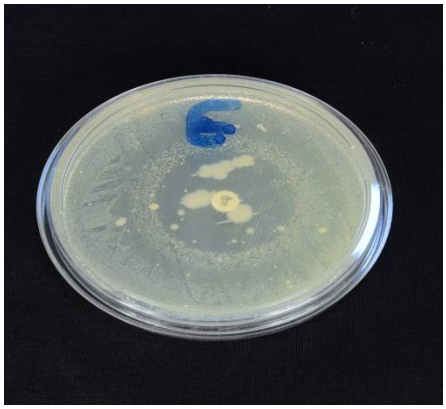

**Figure (3): - Effect of N_butanol *Urtica dioica* extract 30 mg/ml (A) and ciprofloxacine (B), against *E. coli* isolate growth.**

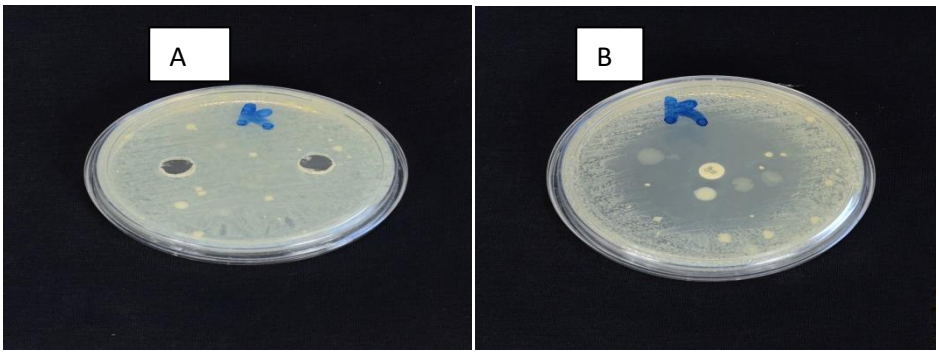


**Figure (4): - Effect of N_butanol *Urtica dioica* extract 30 mg/ml (A) and ciprofloxacine (B), against *Klebsiella* isolate growth**

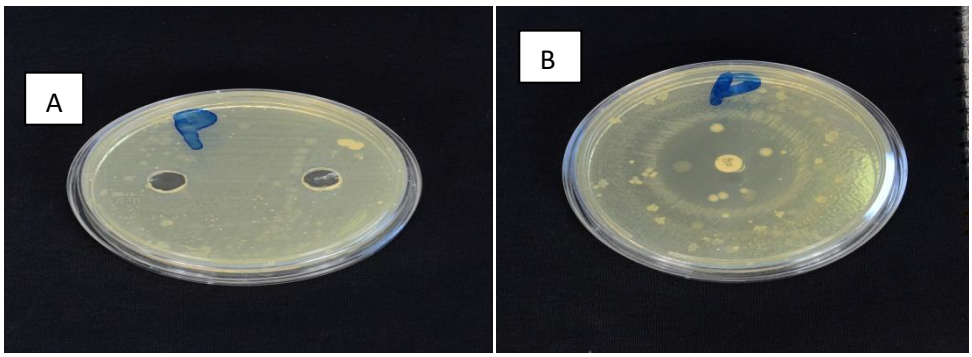


**Figure (5): - Effect of N_butanol *Urtica dioica* extract 30 mg/ml (A) and ciprofloxacine (B), against *Pseudomonas* isolate growth**

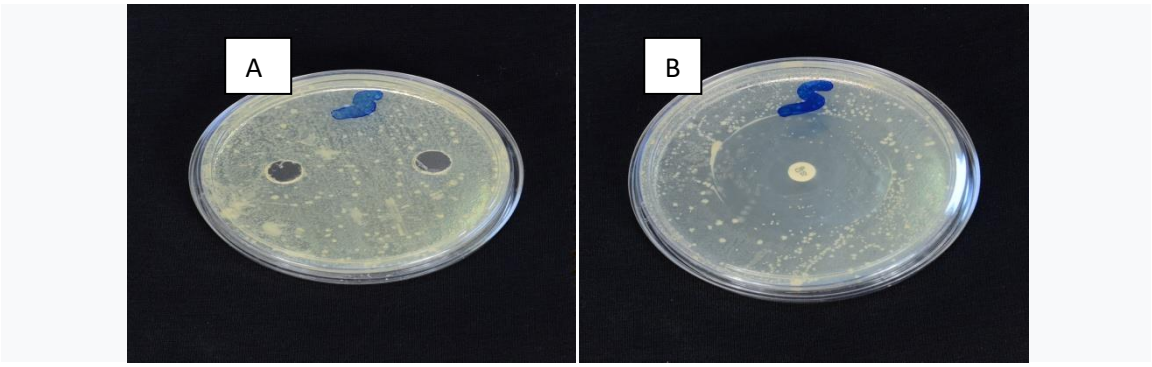


**Figure (6): - Effect of N_butanol *Urtica dioica* extract 30 mg/ml (A) and ciprofloxacine (B), against *Staphylococcus aureus* isolate growth**

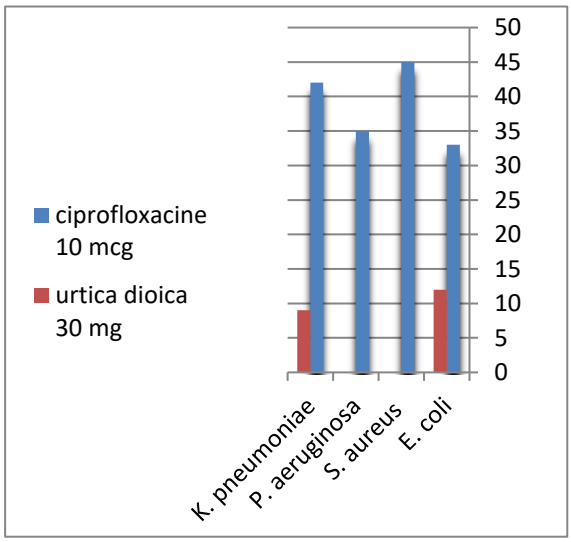


**Figure (7): Bar chart demonstrate the effect of N_butanol extract and ciprofloxacine against some bacterial isolate**

| Microorganisms | n_butanol *urtica dioica* extract (30mg/ml) | ciprofloxacine |
|---|---|---|
| *E-Coli* | ++ | +++ |
| *Klebsiella spp.* | + | +++ |
| *Staphylococcus aureus* | - | ++<br>+ |
| *Pseudomonas aeruginosa* | - | ++<br>+ |

**Table (1) Antibacterial effect of n_butanol *Urtica dioica* extract and ciprofloxacine against some bacterial isolate**

## Discussion

This study focused on antibacterial effect of N_butanol extract of *urtica dioica* .The extract tested against four species of bacteria .The result show there is weak antibacterial effect of of N_butanol extract of *urtica dioica* with *E.coli* while other bacteria did not affected by of N_butanol extract of *urtica dioica*.Gulçin *et al* (2004) reported that antimicrobial activity of the *Urtica dioica* water extract against *E.coli* show weak effect[15].Modarresi *et al* (2012) reported that of N_butanol extract of *urtica dioica*.have weak effect on *E.coli* while no effect on *K.pneumonia and P. aeruginosa and S.aures.*[16]. And because HPLC revealed that of N_butanol extract of *urtica dioica* contain both Rutin which is flavinoid compound and cholinergic which is poly phenolic compound and these results are similar to the result reported from Vaquero *et al* (2007) that reported that Rutin have weak effect on *E.coli* and have no effect on *K.pneumonia and P. aeruginosa and S.aures*[17].Zafar *et al* (2017) reported that Chlorogenic acid have weak effect on *E. coli* and *k. pneumonia* after treatment with methanolic extract of Nasturtium officinale which contain Chlorogenic acid [18].Flavonoid have antibacterial activity because it is ability to bind to the extracellular and other type of soluble proteins to make a complex with cell walls of bacterial, lipophilic

flavonoids have the ability to destroy bacterial cells membranes[19] [20].

Phenolic compounds are hydrophobic, and have a hydroxyl group. The value of this group on the antibacterial activity is known because it great more acidity, the position and how many hydroxyl groups the phenolic compound possess linked to their action on bacteria [21] [22]. Plant products work by two major mechanisms, the first one is linked to its common hydrophobicity, which assist their bond to the bacterial surface and making unstabilization [23] [24]. The second one is due to inactivation of special kind of molecules in the bacteria such as enzymes or receptors by adhesion to precise sites [25] [21] [26].

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