# Investigation of Antimicrobial Activities of *Lactuca Sativa* (L.) Extracts against Clinical Pathogens

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**Abstract**

The present study describes the antimicrobial activity of 3 extracts of *Lactuca sativa* Linn. (Lettuce), collected from the local markets in Baghdad. Successive petroleum ether, methanol and ethyl acetate+water extracts of *Lactuca sativa* leaves were tested (in vitro) for their antimicrobial activities by agar-well diffusion assay. All extracts displayed varied levels of antibacterial or antifungal activity. The petroleum ether extract exhibited antibacterial effect against all of the tested bacteria and the diameter of zones varied between 14-20 mm. The petroleum ether extract of *Lactuca sativa* leaves presented a good activity against *Escherichia coli*, *Staphylococcus aureus* and *Yersinia enterocolitica*, 20 mm, 19 mm and 19 mm, respectively. The ethyl acetate+water extract presented a good activity against *Geotrichum candidum* and *Botrytis cinerea*, 20 mm and 12 mm, respectively. The results obtained in this study appear to confirm the antibacterial and antifungal potential of *Lactuca sativa* leaves, as well as its usefulness in the treatment of diseases that may be as a result of infection.

***Keywords***: antimicrobial activity, *Lactuca sativa* L. , disc diffusion method

**الكشف عن التاثير المضاد للبكتيريا والمضاد للفطريات لمستخلصات اوراق نبات الخس**

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**الخلاصة**

تصف الدراسة الحالية تاثير ثلاثة مستخلصات من نبات الخس والذي تم جمعة من الاسواق المحلية لمدينة بغداد , حيث تم اختبار تاثير مستخلصات الايثر النفطي والميثانول و خلات الاثيل+ الماء لاوراق نبات الخس كمضادات للبكتيريا ومضادات للفطريات عن طريق استخدام مادة الاجار كوسط زرعي وفحص مدى انتشارها في الوسط الزرعي . واظهرت النتائج ان جميع انواع المستخلصات لنبات الخس لها تاثير متفاوت كمضادات للبكتيريا والفطريات , وكان لمستخلص الاثير النفطي تاثير مضاد

لكل انواع البكتيريا المستخدمة والفطريات 14-20 ملم وفعالية جيدة ضد الإيشريشية القولونية، المكورات العنقودية الذهبية ويرسينيا القولون، 20 ملم، 19 ملم و 19 ملم، على التوالي . كذلك اظهرت النتائج ان لخلات الاثيل + الماء نشاط فعال ضد التيربية ضد العفن الرمادي وابوتراتيس 20 ملم و 12 ملم على التوالي. تظهر النتائج التي تم الحصول عليها في هذه الدراسة لتأكيد القدرة المضادة للبكتيريا و للفطريات لمستخلصات اوراق نبات الخس وكذلك فائدة اوراق نبات الخس السريرية في علاج الامراض الناجمة عن الاصابات الكبتيرية والفطرية .

**الكلمات المفتاحية**: نشاط مضادات المايكروبات ، نبات الخس ، طريقة نشر القرص .

## Introduction

There is an ever continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to the alarming increase that has been witnessed in the incidence of both new and reemerging infectious diseases. A further big concern is the development of resistance to the antibiotics in current clinical use. *Lactuca sativa Linn.* (Lettuce) is a well-known plant worldwide due to its use in the preparation of salad, soup and vegetable curries [1]. Also, this plant exhibit excellent medicinal properties.

The latex sap of *Lactuca sativa* possess antifungal properties [2]. Stem of *Lactuca sativa* has shown depressant effects [3]. The seed oil is reported to have sedative, hypnotic, analgesic and anticonvulsant properties [4]. *Latuca sativa* is also proved to have hypoglycaemic effect [5]. Also it is a rich source of carotene and vitamin C. It is also a fair source of vitamin E [1, 6, and 7]. In the light of presence of these valuable phytoconstituents, it was thought that the plant may possess antioxidant activity.

Traditional uses of lettuce seed in Iran were applied to relieve inflammation, gastrodynia and osteodynia. It was demonstrated that methanolic extract of the seed, contains triterpenoids, saponins and simple phenols that possesses antinociceptive and anti-inflammatory effects [8]. Xu *et al* demonstrated that lettuce seeds have a new flavonol glycoside with a rare structure type, *Lactuca sativo* side A, together with 3 known compounds, japonica A, isoquercitrin and caffeic acid [9] . Since, lettuce seed's extract contains saponin, and it was demonstrated that this compound has negative effects on reproductive system of male rats and one of the considerable uses of brewed lettuce seed in traditional medicine, is to reduce semen, sperm and sexuality [8, 10].

Based on the fact that there is no scientific research reporting on the antifungal activity of this plant, we decided to take this opportunity to screen for both its potential both antibacterial and antifungal activity. The aim of this study was to evaluate the *Lactuca sativa* extract in vitro

antibacterial and antifungal activity of three different extracts from leaves, collected from the local market in Baghdad.

## Materials and Methods

### Plant extracts

The extraction process was completed at three steps. First, 30 g of sample in powder was extracted with 250 ml of petroleum ether by using Soxhlet equipment for 8 hrs Following evaporation of the petroleum ether, 5 g of plant material whose lipids had previously been removed, was again extracted with 50 ml of 70% methanol at 40 °C in a shaker (Gerhardt Germany) at room temperature for 2 hrs and then filtered. Next, 200 ml of water + ethyl acetate (1:3) was added to the remaining solid material and after shaking for 1 hr filtered. Following filtration with Whatman filter paper (No 1), all extracts were concentrated and evaporated to dryness *in vacuum* at 55 °C using a rotary evaporator [11]. The yields from the different extracts were weighed, recorded and dissolved in dimethyl sulphoxide
(DMSO) to a final concentration of 100 mg/ml. The extracts were then stored at 4 °C and further used for an antimicrobial activity test.

### Test Microorganisms

The extracts inhibitory effects on a total of 22 microbial species including 12 bacteria, 8 fungus and 2 yeasts, were used as test organisms in this study. These microbial strains were obtained from the Department of Microbiology , College of medicine .They included gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* NRRL B-3711, *Bacillus subtilis* NRRL B-209, *Micrococcus luteus* NRRL-B 1018, *Enterococcus faecium* NRRL B-3502, *E.faecalis* ATCC 29212; and gram-negative bacteria: *Proteus vulgaris* NRRL B-123, *Pseudomonas gingeri* 3146, *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* NRRL-B 3567, *Salmonella typhimurium* ATCC 14028, *Yersinia enterocolitica*. The following eight fungal strains were also tested, *Candida albicans* Y- 12983, *C. glabrata*, *Aspergillus flavus* NRRL 1957, *A. niger* ATCC 10949, *A. fumigatus* NRRL 163, *A. parasiticus* NRRL 465, *Botrytis cinerea* (AHU 9424), *Geotrichum candidum*, *Fusarium graminarum*, *F. solani* (wild types).'

**Determination of antimicrobial activity**

The antimicrobial activities of the petroleum ether, methanol and ethyl acetate + water extracts from the plant sample were evaluated by means of agar-well diffusion assay [12, 13] with some modifications. Fifteen milliliters of the molten agar (45 °C) were poured into sterile petri dishes (Ø 90 mm). Cell suspensions containing 108 CFU/ml cells for bacteria, 107 CFU/ ml cells for yeasts, and 105 spore/ml of fungi were prepared and evenly spread onto the surface of the agar plates of Mueller-Hinton agar (Oxoid, UK) for bacteria, or Sabouraud dextrose agar (Oxoid, UK) medium for yeasts and fungi using sterile swab sticks. Once the plates had been aseptically dried (Ø 10 mm) wells were bored using a sterile corn borer. Extracts (100 µl) were placed into the wells and the plates were incubated at 37°C for 24 h for bacterial strains, 48 h for yeasts and 72 h for fungi at room temperature. Vancomycine and tetracycline (30mg/ml) for bacteria and amphotericine (10 mg/ml) for yeasts and fungi were used as positive controls. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. The tests were performed in triplicate.

**Results and Discussion**

The plant material was subjected to an extraction process, with petroleum ether, methanol and ethyl acetate+water. The yields were 8% for the petroleum ether extract, 3.8% for methanol extract and 2.2% for the ethyl acetate+water extract.

As shown in (Table 1 ) , the extracts from the *Lactuca sativa* plant displayed antibacterial and antifungal activity against all/or some of the tested gram positive and gram negative bacterial and fungal strains, yeasts and molds, with the diameters of zone inhibition ranging between 11 and 20 mm. The most active extract was that obtained from petroleum ether and this extract inhibited the growth of all the bacterial strains tested, specifically *E. coli* (20 mm) *S. aureus* (19 mm) and *Y. enterocolitica* (19 mm). Furthermore, among the fungi studied, *G. candidum* and *B. cinerea* were susceptible to all extracts while *A. flavus, A. parasiticus, A. fumigatus, F. solani* and *C. glabrata* were resistant against all extracts. Thornes, working in 1954, sought an agent to treat vaginal candidiasis in his pregnant patients. Coumarins have been found to inhibit Lactuca *sativa in vitro.* Hydroxycinnamic acids, related to coumarins, have been seen to be inhibitory on gram positive bacteria. Phytoalexins, hydroxylated derivatives of coumarin, are usually uniform within

a plant family but diverse within the plant kingdom. They are antimicrobial compounds synthesized by a plant in response to infection or stress. They are produced in carrots, for example, in response to fungal infection and can be presumed to have antifungal activity. All in all, data about the specific antibiotic properties of coumarins are scarce, although many reports give reason to believe that some utility may reside in these phytochemicals [14, 15]. The petroleum ether extract of investigated species had an *in vitro* potential antimicrobial activity against all bacteria. The data indicated that yeast *C. albicans* extracts of *Lactuca sativa* leaves exhibited the strongest inhibition effect. The presence of coumarin ompounds in *Lactuca sativa leaves* may be responsible for the inhibition activity.

The coumarin compounds and additional volatile components were obtained from the leaves of *Lactuca sativa* by GC/MS analysis, with 49 components identified [3]. We know that many of these determined volatile components are effective against gram positive and gram negative bacteria [16].

However, in this study, the extraction method applied wasn't suitable for isolation of volatile components. The plant *Lactuca sativa,* is found in wide distribution throughout the tropics and subtropics. The leaves of plant are used commonly by the people in food as a vegetable. The human body is able to directly absorb the different compounds and due to the low concentration of these compounds, it has transpired that the plant particularly provides effective protection against infection. This protective property, either through the plant itself or by the compounds contained within, also has the effect of prolonging the shelf life of food in which it is used or prevents it being spoilt by microorganisms. Its current use as a plant additive to other food and consumed by the public may be due to its health-giving properties and its taste. There is a definite need for more research to determine the metabolite synthesis that occurs in this species of plant, as well as the construction of new molecules.

**Table (1):** Antibacterial activity of three extracts from *Lactuca sativa* L. as inhibition zones (mm) (well Ø 10 mm).

| **Tested bacterial strains** | **Petroleum Ether** | **MeOH** | **Ethylacetate + Water** | **Vancomycine (disc Ø 6 mm)** | Tetracycline **(disc Ø 6 mm)** |
|---|---|---|---|---|---|
| ***Bacillus cereus*** | **14±0.1** | **15±0.1** | **12±0.1** | **24±0.6** | **30±0.1** |
| ***Bacillus subtilis*** | **18±0.1** | **19±0.1** | **15±0.1** | **23±0.1** | **34±0.1** |
| ***Enterobacter aerogenes*** | **18±0.5** | **15±0.4** | **12±0.1** | **25±0.1** | **12±0.1** |
| ***Enterococcus faecalis*** | **18±0.2** | **17±0.2** | **18±0.1** | **19±0.1** | **12±0.1** |
| ***Escherichia coli*** | **20±0.4** | **10±0.7** | **-** | **-** | **30±0.1** |
| ***Micrococcus luteus*** | **18±0.1** | **15±0.1** | **14±0.3** | **24±0.1** | **40±0.1** |
| ***Proteus vulgaris*** | **17±0.1** | **16±0.1** | **14±0.1** | **-** | **15±0.1** |
| ***Pseudomonas gingeri*** | **16±0.4** | **-** | **13±0.1** | **23±0.1** | **28±0.1** |
| ***Salmonella typhimurium*** | **18±0.1** | **-** | **15±0.2** | **-** | **14±0.1** |
| ***Staphylococcus aureus*** | **19±0.1** | **18±0.1** | **19±0.1** | **20±0.1** | **29±0.1** |
| ***Streptococcus faecium*** | **17±0.1** | **15±0.1** | **13±0.5** | **18±0.1** | **25±0.1** |
| ***Yersinia enterocolitica*** | **19±1.1** | **16±0.1** | **17±0.1** | **20±0.1** | **29±0.1** |

**Table (2):** Antifungal activity of three extracts from *Lactuca sativa* L. as inhibition zones (mm) (well Ø 10 mm).

| **Tested fungal strains** | **Petroleum ether** | **MeOH** | **Ethylacetate +Water** | **Amphotericine (well Ø 10 mm)** |
|---|---|---|---|---|
| ***Aspergillus flavus*** | - | - | - | - |
| ***A. fumigatus*** | - | - | - | - |
| ***A. niger*** | - | **12±0.1** | **11±0.6** | **12±0.1** |
| ***A. parasiticus*** | - | - | - | - |
| ***Botrytis cinerea*** | **18±0.4** | **14±0.1** | **12±0.1** | - |
| ***Candida albicans*** | **15±0.1** | - | - | - |
| ***C. glabrata*** | - | - | - | - |
| ***Fusarium graminearum*** **(wild type)** - | **12±0.1** | - | **12±0.1** | - |
| ***F. solani*** **(wild type)** | - | - | - | - |
| ***Geotrichum candidum*** **(wild type)** | **14±0.3** | **18±0.8** | **20±0.1** | **14±0.1** |

**–: absence of inhibition**

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