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Antibacterial and antifungal activities of crude extracts from willow-leaf lettuce (*Lactuca saligna* L.)

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Abstract: The antimicrobial activity of various extracts from *Lactuca saligna* L. (willow-leaf lettuce) was investigated in this work. Leaves and roots were collected from the Saatly district in Azerbaijan, and both aqueous and ethanolic extracts were prepared. The antimicrobial effects were evaluated using the disk-diffusion method against a selection of bacterial and fungal strains, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus anthracoides*, and *Candida albicans*. Results showed that the aqueous root extract exhibited the most potent antibacterial activity, particularly against *E. coli* and *K. pneumoniae*, while the ethanolic root and leaf extracts demonstrated moderate activity against *B. anthracoides* and *C. albicans*. The study provides evidence supporting the traditional medicinal use of *L. saligna* for treating various infections. Furthermore, the findings suggest that *L. saligna* could be a promising source of natural antimicrobial agents, highlighting the need for further research to isolate and identify the active compounds responsible for its antimicrobial properties. This work contributes to the exploration of plant-based alternatives in the search for novel antimicrobial substances, particularly against drug-resistant pathogens.

Keywords: *antibacterial agents, antimicrobial activity, disk-diffusion method, microbial inhibition, plant extracts, traditional medicine*

INTRODUCTION

Herbal medicine remains a cornerstorne of traditional practices worldwide. Exploring plants with a long history of folkloric use is crucial for encouraging safe applications and identifying promising sources of new pharmaceuticals. Over the past 20 years, interest in natural substances as antibacterial agents has surged. To uncover the origins of their therapeutic benefits, many extracts from traditionally used plants have been examined. Some natural products have since been approved as new antibacterial drugs, yet the need remains to find innovative molecules effective against highly resistant infections [Parekh, Chanda, 2007].

Due to their extensive chemical diversity, natural products–whether pure compounds or standardized plant extracts–offer significant potential for pharmacological applications. The effectiveness of these products depends on factors such as extraction methods, target microorganisms, and the compound solubility. Many antibacterial agents have been derived from accessible sources, including horticultural and agricultural products (e.g., tea leaves, citrus, grapevine, berries, and hops) and medicinal plants (e.g., rosemary, pine, sage) [Klančnik et al., 2010].

While antimicrobial agents have historically reduced public health risks from bacterial infections, misuse has led to increasing resistance, necessitating new approaches for drug development. Bioactive components from traditional medicines represent a promising avenue, as thorough screening could reveal potent molecules with relevant antimicrobial properties. Plant extracts often benefit from synergistic interactions among active compounds, leading to enhanced bioavailability, reduced toxicity, and the potential to inhibit bacterial resistance mechanisms [Ghosh et al., 2008; Vaou et al., 2021]. Common plant compounds like coumarins, terpenes, and flavonoids exhibit antibacterial effects, with flavonoids particularly noted for their low toxicity [Adamczak et al., 2019].

The Asteraceae family has long been used in traditional medicine worldwide for applications such as fever treatments, pain relief, and wound healing [Achika et al., 2014]. The genus *Lactuca* L. within Asteraceae has shown anti-inflammatory, antimicrobial, analgesic, and sedative properties, among others [Abdel Bar et al., 2023]. *L. saligna* L., commonly known as willow-leaf lettuce,

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is a perennial plant, vertical rooted. Its stems are 30-70 cm high with dark green leaves. The capitulum is a narrow-cylindrical, 8-11 mm long, 1.5-2 mm wide. The ligules are yellow, turning violet-blue when dried. Seeds are brownish-yellow, narrow and elongated, 2.5-3 mm long, around 1 mm wide. Flowering occurs in June, with fruiting from July to August.

L. saligna is distributed across England, Germany, Russia, and the Mediterranean region [Flora..., 1975; Flora..., 1976]. According to Zohary, its center of origin is Southwest Asia [Zohary, 1991]. Although *L. saligna* is primarily distributed across Europe, central and western Asia, it has also established populations in Australia and North America. In Egypt, *L. saligna* is a rare wild plant, commonly referred to as "Hawa" [Khalil et al., 1991].

In Azerbaijan, it is found in the eastern Greater Caucasus, in the Kur-Araz lowland and Lankaran, inhabiting sodium-rich rocky areas up to mid-mountain elevations [Flora..., 1961]. During the 2012 expedition in Armenia and Azerbaijan, 35 sites were identified, leading to the discovery of five distinct *L. saligna* populations in Azerbaijan [Kik et al., 2014]. Throughout our latest expedition in the summer of 2023, the species was observed in the Kur-Araz lowland, particularly in the Sabirabad and Saatly districts, where it was found growing along roadsides, railways, and in orchards.

Ethnobotanical studies carried out in southern Italy (Cilento and Vallo di Diano National Park), indicated that *L. saligna* aerial parts were traditionally used in soups [Di Novella et al., 2013]. In Andalusia, Spain, the leaves and stems are also used as a food source [Benítez et al., 2023]. In Jordanian traditional medicine, a root decoction serves as an antihelmintic, analgesic and emollient [Al-Khalil, 1995]. In northwestern Algeria, the leaves are used against diarrhea, genitourinary disease and fever [Megharbi, Kechairi, 2021]. Furthermore, latex from *L. saligna* demonstrated significant sedative effects [Ilgün et al., 2020]. In the Unaizah province of Saudi Arabia, a decoction of the entire plant and seeds is recognized as a carminative, diuretic, tonic and refresher, with cooked leaves and stems commonly incorporated into soups [Youssef, 2013].

Finally, *L. saligna* may confer resistance to several plant diseases, including lettuce dieback, aster yellows, downy mildew, leaf spot, and corky root as well as to various viruses and insects, such as lettuce infectious yellows virus, cucumber mosaic virus, tomato spotted wilt virus, cabbage looper, leafminer, and root aphid [Mou, 2008].

The objective of this study is to assess the antimicrobial activity of different extracts obtained from the roots and leaves of *L. saligna* on selected microorganisms.

MATERIAL AND METHODS

Plant material. The *L. saligna* leaves and roots were collected during summer of 2023 from the Saatly district in Azerbaijan (39°56'19.1"N, 48°22'29.1"E; 10 m below sea level). A voucher specimen was deposited in the Herbarium of the Institute of Botany (BAK) after identification according to Flora of Azerbaijan [1961]. All samples were stored at room temperature, protected from direct sunlight, until further use.

Preparation of extracts. The leaves and roots were crushed into small pieces and dried at room temperature. For the root extract, 4.7 g of finely chopped roots were combined with 42 mL of distilled water in a flask and boiled for 1.5 h. After cooling for 6 h, the homogenate was filtered through a piece of white cloth [Senthil-Rajan et al., 2013]. The sample was concentrated using a rotary evaporator (ROVA-N2L, MRC, Israel) with a water bath (WB-2000, ANM Industries, India), yielding 0.9 g of dried extract equal to 19.15%. Additionally, 8 g of root material was soaked in 77 mL of 96% ethanol at room temperature for 72 h through cold maceration [Senthil-Rajan et al., 2013]. The ethanolic solution was then strained, and the process was repeated twice. After concentration, 0.28 g of dried extract was obtained yielding 3.5%.

For the leaf extracts, 38 g of dried leaves were suspended in 200 mL of distilled water, shaken, and an additional 200 mL of water was added [Alabi et al., 2012]. The mixture was left to stand for 24 h, then decanted and filtered through filter paper. This procedure was repeated twice before concentrating the filtrate, resulting in 3.16 g of dried extract with an 8.31% yield. An ethanol extract was prepared by suspending 68 g of dried leaves in 366 mL of 96% ethanol, following the same procedure as the aqueous extract. This produced 3.8 g of dried extract, corresponding to a 5.59% yield. All crude extracts were stored in a freezer at -7.3°C until use.

To prepare stock solutions, ethanol-derived extracts were dissolved in 1 mL of 100% dimethyl sulfoxide (DMSO) to achieve 150 mg/mL solutions, while aqueous extracts were dissolved in 1 mL of distilled water at the same concentration. These stock solutions, along with their two-fold dilutions (75 mg/mL), were prepared for further use.

Antimicrobial activity. *Microorganisms and growth conditions*. The following microorganisms were selected for evaluating antimicrobial activity:

Staphylococcus aureus (a Gram-positive bacterium), *Escherichia coli* (a Gram-negative bacterium), *Klebsiella pneumoniae* (a Gram-negative bacterium with a prominent polysaccharide capsule), *Pseudomonas aeruginosa* (a Gram-negative opportunistic pathogen), *Bacillus anthracoides* (a spore-forming Gram-positive bacterium) and *Candida albicans* (an opportunistic pathogenic yeast). All microbial strains were provided from the Department of Medicinal Microbiology and Immunology at Azerbaijan Medical University. Bacterial strains were cultured on Mueller Hinton agar, while *C. albicans* was grown on Sabouraud dextrose agar. Cultures were incubated at 37°C. Microbial samples were prepared by suspending 500 x 106 colony-forming units (CFU) in 1 mL of physiological saline solution. Stock suspensions were concentrated and cultivated for 24 h prior to experimentation.

In vitro antimicrobial activity of extracts. The antimicrobial activity of different *L. saligna* extracts was evaluated using the paper disk diffusion method [Bauer et al., 1966]. A cotton swab was used to evenly spread the bacterial and fungal inoculum across the surface of the respective nutrient media in 90 mm Petri dishes. Sterile paper disks (6 mm in diameter) were impregnated with 7 µl of stock solutions and their 1:1 dilutions, then placed on the agar surface. The Petri dishes were incubated for 18-24 h at 37°C. Following incubation, zones of growth inhibition were observed and measured in millimeters. Additionally, commercial antibiotic disks containing ceftriaxone (30 µg), meropenem (10 µg), tetracycline (30 µg), clotrimazole (10 µg), ketoconazole (20 µg), and fluconazole (40 µg) served as positive controls for strain sensitivity assessment. Meropenem and ceftriaxone disks were sourced from Liofilchem S.r.l., Roseto (TE), Italy, while the others were provided by НИЦФ, St. Petersburg, Russia. Distilled water and 100% DMSO (7 µl) are used as negative controls.

RESULTS

In this research, the antimicrobial activity of *L. saligna* against five bacterial strains and one fungal strain was assessed. The effects of ethanolic and aqueous extracts obtained from its roots and leaves are shown in Table 1. The inhibition zones generated by the crude extracts against susceptible microorganisms ranged from 7 to 18 mm. Among the samples, the root aqueous extract exhibited the strongest activity against *E. coli*, with inhibition zones of 18 mm and 14 mm at concentrations of 150 mg/mL and 75 mg/mL, respectively, whereas *P. aeruginosa* proved to be the most resistant strain. This resistance may be due to *P. aeruginosa* inherent defenses, including efflux pumps that expel antimicrobial agent from the cell, enzyme production that deactivates antimicrobial agents, and reduced outer membrane permeability to limit agent entryl [Moore, Flaws, 2011]. Notably, all extracts demonstrated antibacterial activity against *K. pneumoniae*. Additionally, ethanolic extracts of *L. saligna* roots and leaves moderately inhibited the growth of *B. anthracoides*, with inhibition zones of 12 mm and 11 mm, respectively. For *S. aureus*, three out of the four extracts displayed antibacterial effects: both the root ethanolic and aqueous extracts showed a 7 mm inhibition zone at 150 mg/mL, while the aqueous leaf extract produced a 9 mm zone at 75 mg/mL.

The ethanolic extracts from roots and leaves demonstrated antifungal activity against *C. albicans*, whereas the aqueous extracts showed no effect. Negative controls exhibited no inhibition, except for DMSO,

Table 1. Antimicrobial activity of various extracts of *L. saligna*.

Microbial species	Inhibition zone diameter (mm) of plant extracts, 1.05 mg/disk / 525 µg/disk					
	Crude aqueous extract		Crude ethanolic extract		Negative controls	
	Roots	Leaves	Roots	Leaves	DMSO	Distilled water
Klebsiella pneumoniae	8 / 8	– / 8	8 / 7	7 / 8	–	–
Bacillus anthracoides	– / –	– / –	12/12	11/11	–	–
Staphylococcus aureus	7 / –	– / 9	7 /–	– / –	–	–
Escherichia coli	18 / 14	– / –	– / –	– / –	–	–
Pseudomonas aeruginosa	– / –	– / –	– / –	– / –	–	–
Candida albicans	– / –	– / –	10/ 9	9/10	8	–

which produced an 8 mm inhibition zone. The ethanolic root extract produced 10 mm and 9 mm inhibition zones, while the ethanolic leaf extract showed 9 mm and 10 mm inhibition zones at 150 mg/mL and 75 mg/mL, respectively. Thus, a portion of the observed antifungal activity may be attributable to DMSO. Positive controls, detailed in Table 2, included standard antibiotics, with inhibition zones ranging from 7 to 30 mm (Fig. 1). To the best of our knowledge, unlike other *Lactuca* species, the antimicrobial potential of *L. saligna* extracts (aqueous and ethanolic) has not been previously documented. However, according to a recent study, hydromethanolic extracts of the aerial parts of Moroccan *L. saligna* demonstrated a bacteriostatic effect against *S. aureus*, *P. aeruginosa*, and *Salmonella typhimurium*, and a bactericidal effect against *Listeria monocytogenes*, *Enterococcus faecalis*, and *S. aureus* [Bouymajane et al., 2024].

Our previous study demonstrated the inhibitory effects of crude ethanolic extracts from *L. serriola* leaves and roots against *S. aureus*, and the ethanolic root extract also inhibited *C. albicans* [Shukurlu, Muradova,

Table 2. Antimicrobial activity of some standard antibiotics and antifungals used as a positive control.

Test Microorganisms / Antimicrobials	*K. pneumoniae*	*B. anthracoides*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
Ceftriaxone - 30 µg	8	19	7	30	15	n/a
Meropenem - 10 µg	7	26	7	29	21	n/a
Tetracycline - 30 µg	7	27	10	26	13	n/a
Clotrimazole - 10 µg	n/a	n/a	n/a	n/a	n/a	21
Ketoconazole - 20 µg	n/a	n/a	n/a	n/a	n/a	24
Fluconazole - 40 µg	n/a	n/a	n/a	n/a	n/a	29

Note: Numbers show the inhibition zone diameter in mm, n/a – not applicable.

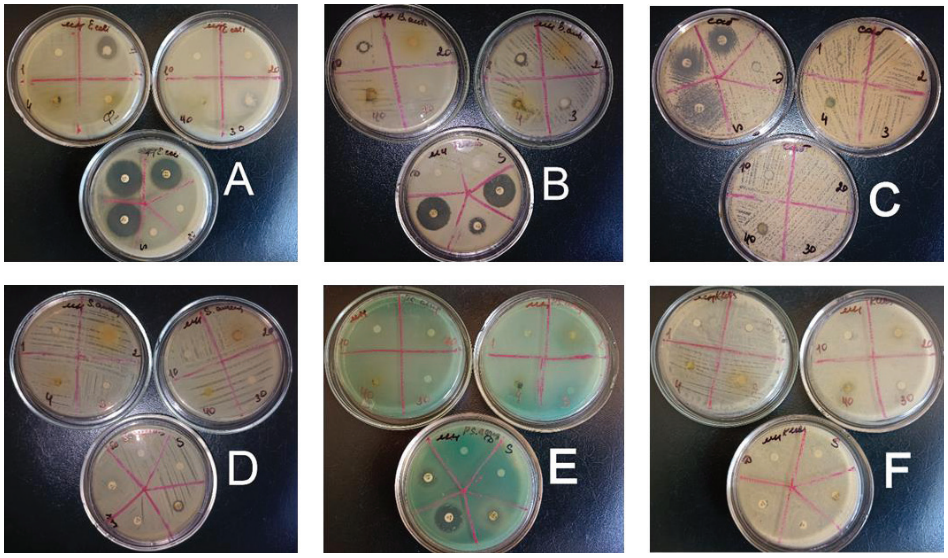

Figure 1. The inhibitory effects of ethanolic and aqueous extracts from *L. saligna* aerial parts and roots against various microorganisms using the paper disk diffusion assay. (A) *Escherichia coli*, (B) *Bacillus anthracoides*, (C) *Candida albicans*, (D) *Staphylococcus aureus*, (E) *Pseudomonas aeruginosa*, (F) *Klebsiella pneumoniae*; 1/10-ethanolic extract of roots (1.05 mg/disk / 525 µg/disk); 2/20 - aqueous extract of leaves (1.05 mg/disk / 525 µg/disk); 3/30 - aqueous extract of roots (1.05 mg/disk / 525 µg/disk); 4/40 - ethanolic extract of leaves (1.05 mg/disk / 525 µg/disk); D-DMSO, S-distilled water; MRP – Meropenem, TET – Tetracycline, CRO – Ceftriaxone, KOT – Clotrimazole, KET – Ketoconazole, ФКЛ - Fluconazole.

2022]. Additionally, ethanolic extracts of roots and leaves from *L. tatarica* inhibited the growth of *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. aureus* and *C. albicans* [Shukurlu et al., 2022]. During the phytochemical analysis of *L. serriola*, we identified hexanal in both roots and leaves, a compound known for its antibacterial properties [Shukurlu et al., 2023]. Lupeol acetate, present in relatively high concentrations in *L. serriola* roots [Shukurlu, 2020], is also known for its antibacterial and antifungal activities [Javed et al., 2021; Muktar et al., 2018]. Moreover, quercetin was detected in the aerial parts of *L. serriola* [Shukurlu, Goger, 2021], and has been shown to exhibit antibacterial effects against certain pathogens [Shu et al., 2011]. Other reserach found that ethanolic seed extract of *L. orientalis* inhibit *K. pneumonia*, *B. subtilis*, *S. aureus*, and *C. albicans*, while chloroform extracts showed activity against *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. aureus*, and *C. albicans* [Zahra et al., 2021]. The methanolic extract of *L. sativa* also demonstrated negative effects against *B. cereus* and *S. aureus* [Afrin et al., 2022]. Similarly, essential oil obtained from the epigeal part of *L. longidentata* showed activity against *B. subtilis* [Federico et al., 2021]. These findings support the traditional uses of *L. saligna* for its antimicrobial properties.

Sesquiterpene lactones of the guaianolide structure were previously identified in *L. saligna*, including lactucin, lactucopicrin, 11β, 13-dihydrolactucopicrin from the aerial parts, and 8-deoxylactucin, jacquinelin, lactuside A, crepidiaside B, salignoside, ixerin F, and macrocliniside A from the roots [Kisiel, Gromek, 1993]. Guaianolide-type sesquiterpene lactones are known for significant antimicrobial potential [Chadwick et al., 2013]. Furthermore, 8-deoxylactucin has shown anti-parasitic activity [Valente et al., 2021].

The antimalarial activity of lactucopicrin and lactucin was previously reported [Bischoff et al., 2004]. Given the antibacterial and antifungal effects of guaianolide-type lactones [Perveen et al., 2019], compounds like lactucin and lactucopicrin may play a key role in the antibacterial activities observed in the investigated extracts.

Isolating and examining the chemical components of *L. saligna* could reveal promising antibacterial agents, potentially effective on their own.

CONCLUSION

L. saligna L. has a long history of use in traditional medicine, with applications spanning anti-inflammatory, analgesic, and antidiarrheal treatments, among others. However, while the ethnobotanical evidence suggests its broad therapeutic potential, the scientific investigation into its antimicrobial properties is still at an early stage. The results of this study highlight the importance of further research in confirming and elucidating the plant's potential in this regard. The observed activity against selected microorganisms suggests the possibility that *L. saligna* may contain valuable bioactive compounds which could contribute to the development of novel therapeutic agents, particularly in response to the growing challenge of antibiotic resistance. Additional studies are required to identify these compounds and refine extraction methods to enhance their efficacy.

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Şoran südləməsi (*Lactuca saligna* L.) növünün qatı ekstraktlarının antibakterial və antifunqal aktivliyi

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Tədqiqat işində *Lactuca saligna* L. (Şoran südləməsi) növünün müxtəlif ekstraktlarının antimikrob təsiri analiz edilmişdir. Bitkinin yarpaq və kökləri Azərbaycanın Saatlı rayonundan toplanmış, onların həm sulu, həm də etanollu ekstraktları hazırlanmışdır. Antimikrob təsirlər, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus anthracoides* və *Candida albicans* növünün də daxil olduğu müəyyən bakterial və göbələk ştammlarına qarşı disk-diffuziya üsulu ilə qiymətləndirilmişdir. Nəticələrə əsasən, kökün su ilə alınmış ekstraktı, xüsusilə *E. coli* və *K. pneumoniae* ştammına qarşı ən güclü antibakterial təsir göstərmiş, kök və yarpaqlardan etanolla alınmış ekstraktlar isə *B. antracoides* və *C. albicans* ştammlarına qarşı orta dərəcədə aktivlik nümayiş etdirmişdir. Tədqiqat müxtəlif infeksiyaların müalicəsi məqsədilə

L. saligna növünün ənənəvi təbabətdə istifadəsini dəstəkləyici mahiyyətə malikdir. Bundan əlavə, alınan nəticələrə əsasən *L. saligna* təbii antimikrob vasitələrin perspektivliyi baxımından əhəmiyyətlidir və bu səbəbdən onun qeyd edilən xassələrinə cavabdeh olan təsiredici birləşmələri ayırmaq və identifikasiya etmək məqsədilə silsilə tədqiqatlar aparılmalıdır. Mövcud tədqiqat yeni antimikrob birləşmələrin axtarışında, xüsusən də dərman vasitələrinə davamlı patogenlərə qarşı bitki əsaslı alternativlərin araşdırılmasına töhfə verir.
Açar sözlər: *antibakterial vasitələr, antimikrob aktivlik, disk-diffuziya metodu, mikrobların inhibirləşməsi, bitki ekstraktları, xalq təbabəti*

Антибактериальная и противогрибковая активность густых экстрактов Молокана солончакового (*Lactuca saligna* L.)

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В данном исследовании изучалась антимикробная активность различных экстрактов из *Lactuca saligna* L. (Молокана солончакового). Листья и корни растения были собраны в Саатлинском районе Азербайджана, и из них были приготовлены водный и этанольный экстракты. Антимикробное действие оценивали диско-диффузионным методом против несколько бактериальных и грибковых штаммов, включая *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus anthracoides* и *Candida albicans*. Результаты показали, что водный экстракт корней проявил наиболее сильную антибактериальную активность, особенно против *E. coli* и *K. pneumoniae*, в то время этанольные экстракты корней и листьев продемонстрировали умеренную активность против *B. anthracoides* и *C. albicans*. Данное исследование подтверждает использование *L. saligna* для лечения различных инфекций в народной медицине. Кроме того, полученные данные свидетельствуют о том, что *L. saligna* может быть перспективным источником природных антимикробных агентов. Результаты подчеркивают необходимость дальнейших исследований для выделения и идентификации активных соединений, ответственных за антимикробные свойства этого растения. Данное исследование вносит вклад в изучение растительных альтернатив в поиске новых антимикробных веществ, особенно против устойчивых к лекарствам патогенов.
Ключевые слова: *антибактериальные средства, антимикробная активность, диско-диффузионный метод, ингибирование микроб, растительные экстракты, народная медицина*