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A Comparative Study on the Antifungal Activity of Aqueous Extracts from Different Parts of Wild Lettuce (*Lactuca serriola*) Against Selected Phytopathogenic Fungi

Fuzia ELFituri Eltariki [1,*], Fow-micro@yahoo.com , Sara Alsadik Alsrety [2], Soso29zomoro5690@gmail.com
[1,2] Misurata University, Faculty of science, Department of Biology-Botany, Libya.
[2] Bio-Research &Consultancy Office, research and consultation center, Misurata University, Libya.
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* Corresponding author

Index Terms

***Lactuca serriola*, antifungal activity, plant extracts, *Aspergillus niger*, *Fusarium oxysporum*.**

Abstract

Wild lettuce (*Lactuca serriola* L.) is a medicinal plant traditionally used to treat various ailments. This study evaluated the antifungal activity of cold and hot aqueous extracts from different plant parts (roots, stems, and leaves) against three phytopathogenic fungi: *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum*. Plant materials were collected from Misurata, Libya, air dried, and ground into powder. Extracts were prepared using cold maceration and hot water extraction methods and incorporated into Sabouraud Dextrose by adding 2 mL of extract to Agar at 15 mL of medium (final concentration: 11.8% v/v). Fungal growth inhibition was assessed by measuring radial growth after 7 days of incubation at 25±2°C. Compared to the control (9.00 cm radial growth), the cold root extract reduced A. flavus growth to 1.60 cm (80.12% inhibition), while the hot root extract only reduced it to 7.00 cm (22.22% inhibition). A similar trend was observed for *F. oxysporum* and *A. niger*, where cold extracts also produced much higher inhibition than hot extracts, particularly in root treatments, respectively. Fourier Transform Infrared Spectroscopy (FTIR) analysis revealed the presence of hydroxyl (-OH), carbonyl (C=O), and alkene (C=C) functional groups, indicating bioactive compounds. These findings suggest that *L. serriola* aqueous extracts, particularly from roots, possess significant antifungal properties may have potential as natural antifungal agents and ecofriendly alternatives to synthetic fungicides in sustainable agriculture.

دراسة مقارنة للفعالية المضادّة للفطريات للمستخلصات المائية المستخلصة من أجزاء مختلفة من الخسّ البري (*Lactuca serriola*) ضد فطريات ممرِضة نباتية مختارة

فوزية الفيتوري التريكي[1]، *، سارة الصديق السريتي[2]
[1] جامعة مصراتة، كلية العلوم، قسم الأحياء – علم النبات، ليبيا، البلد، Fow-micro@yahoo.com
[2] مكتب البحوث والاستشارات الحيوية ، مركز البحوث والاستشارات، جامعة مصراتة، ليبيا، Soso29zomoro5690@gmail.com
* المؤلف المراسل

الكلمات المفتاحية

المستخلصات النباتية، النشاط المضاد للفطريات، المكافحة الحيوية

الملخص

يُعدّ الخسّ البري (*Lactuca serriola* L.) نباتًا طبيًا استُخدم تقليديًا لعلاج مجموعة متنوعة من الأمراض. هدفت هذه الدراسة إلى تقييم النشاط المضاد للفطريات للمستخلصات المائية الباردة والساخنة المُحضَّرة من أجزاء مختلفة من النبات (الجذور، السيقان، والأوراق) ضد ثلاثة أنواع من الفطريات الممرضة للنبات *Aspergillus niger* و *Aspergillus flavus* و *Fusarium oxysporum*. جُمعت المواد النباتية من مدينة مصراتة، ليبيا، ثم جُفّفت هوائيًا وطُحنت إلى مسحوق. أُعدّت المستخلصات باستخدام طريقتي النقع البارد والاستخلاص بالماء الساخن، ثم أُدمجت في وسط سابورو ديكستروز آغار بإضافة 2 مل من المستخلص إلى 15 مل من الوسط (بتركيز نهائي 11.8% حجم/حجم).

تم تقييم تثبيط نمو الفطريات من خلال قياس النمو الشعاعي بعد 7 أيام من التحضين عند درجة حرارة 25±2 درجة مئوية. وبالمقارنة مع العينة الضابطة (نمو شعاعي 9.00 سم)، خفّض مستخلص الجذور البارد نمو A. flavus إلى 1.60 سم (بنسبة تثبيط 80.12%)، في حين خفّضه المستخلص الساخن للجذور إلى 7.00 سم فقط (بنسبة تثبيط 22.22%). ولوحِظ نمط مماثل بالنسبة لكلٍّ من F. oxysporum وA. niger، حيث أظهرت المستخلصات الباردة مستويات تثبيط أعلى بكثير مقارنة بالمستخلصات الساخنة، خاصة في معاملات الجذور.

كما أظهر تحليل مطيافية الأشعة تحت الحمراء بتحويل فورييه (FTIR) وجود مجموعات وظيفية مثل الهيدروكسيل (-OH)، والكربونيل (C=O)، والألكين (C=C)، مما يشير إلى وجود مركبات نشطة حيويًا. وتشير هذه النتائج إلى أن المستخلصات المائية لنبات L. serriola، وخاصة المستخلصة من الجذور، تمتلك خصائص ملحوظة مضادة للفطريات، وقد يكون لها إمكانات واعدة كعوامل طبيعية مضادة للفطريات وبدائل صديقة للبيئة للمبيدات الفطرية الاصطناعية في الزراعة المستدامة.

1. INTRODUCTION

Lactuca serriola L. (wild lettuce or prickly lettuce), belonging to the family Asteraceae, is a widespread weed found throughout the Mediterranean basin and other temperate regions [1]. The plant is characterized

by the production of milky latex containing bioactive compounds such as lactucarium, lactucin, and lactucopicrin [2]. Traditionally, L. serriola has been used in folk medicine to treat various ailments including headaches, insomnia, fever, hypertension, and respiratory disorders [3]. Phytochemical studies have revealed that L. serriola contains diverse secondary metabolites including terpenoids, phenolic compounds, saponins, alkaloids, and flavonoids [4][5]. These compounds exhibit multiple biological activities including sedative, analgesic, anti-inflammatory, antioxidant, and antimicrobial properties [6] [7].

Phytopathogenic fungi represent a major constraint to agricultural productivity worldwide, causing substantial crop losses and economic damage. Fungal species belonging to the genera Aspergillus and Fusarium are among the most prevalent plant pathogens. A. niger and A. flavus cause various diseases in crops and also pose health risks to humans and animals through the production of mycotoxins [8]. F. oxysporum is a widespread soil-borne pathogen responsible for vascular wilt diseases in numerous economically important crops, leading to reduced yield and quality [9].

Conventional management of these fungal diseases still relies heavily on synthetic chemical fungicides. However, prolonged and indiscriminate use of such compounds has resulted in environmental pollution, the development of fungicide resistant pathogen strains and adverse effects on non-target organisms and human health [10]. Consequently, there is increasing interest in developing ecofriendly alternatives derived from natural sources, particularly plant based biopesticides.

In this context, several studies have explored the potential of plant extracts from the genus Lactuca as antifungal agents against phytopathogenic fungi. Namik et al [11]. reported that ethanolic extracts of the aerial parts of L. serriola and L. tatarica exhibited inhibitory effects against A. solani, F. acuminatum and A. flavus. However, most previous studies have focused on organic solvent extracts and aerial plant parts, and comprehensive evaluations of cold and hot aqueous extracts prepared from different plant organs (roots, stems and leaves) of L. serriola against major crop pathogens remain limited.

In light of these considerations, it was hypothesized that aqueous extracts of wild lettuce, particularly cold extracts obtained from root tissues, may exert significant antifungal activity against important phytopathogenic fungi. Therefore, the present study aims to:

(1) prepare cold and hot aqueous extracts from different parts (roots, stems and leaves) of naturally dried L. serriola.

(2) evaluate and compare their antifungal efficacy against three important phytopathogenic fungi A. niger, A. flavus and F. oxysporum using an in vitro poisoned food assay. This research aims to contribute to the development of sustainable, environmentally friendly biocontrol strategies for crop protection.

2. LITERATURE OF REVIEWS

2.1 Botanical fungicides and limitations of synthetic fungicides

Synthetic fungicides have played an essential role in the management of plant diseases; however, their extensive and prolonged use has led to several serious drawbacks. Numerous studies have reported that continuous application of chemical fungicides can disturb natural biological systems, promote the emergence of fungicide resistant fungal strains and cause environmental contamination and potential risks to human and animal health, as well as adverse effects on non-target organisms. In response to these concerns, increasing attention has been directed toward the development of biopesticides as safer and more sustainable alternatives. Plant derived fungicides, in particular, are often biodegradable, less persistent in the environment and may exhibit lower toxicity compared with their synthetic counterparts [10;12]. Examples of commercially or experimentally used botanical fungicides include cinnamaldehyde, jojoba oil, laminarin and Melissa extracts, which have shown promising antifungal activity against a range of phytopathogenic fungi [12].

2.2 Phytochemical profile of Lactuca species

Species of the genus Lactuca are known to contain a rich diversity of secondary metabolites that underpin their traditional medicinal uses. Comprehensive phytochemical reviews have documented the presence of terpenoids, phenolic compounds, saponins, lignans, coumarins, phytosterols and sesquiterpene lactones in Lactuca species. These constituents are directly associated with a wide spectrum of biological activities, including sedative, analgesic, anti-inflammatory, antioxidant and antimicrobial effects [13]. In L. serriola, the milky latex (lactucarium) and root tissues, in particular, have been reported to contain fatty acid esters, triterpenes and sesquiterpene lactones that may contribute to both plant defense and potential pharmacological actions [14;15]. The presence of phenolic compounds and flavonoids with strong antioxidant capacity has also been demonstrated in L. serriola using chromatographic and FTIR analyses.

2.3 Antifungal activity of L. serriola and related species

Plant derived products are widely recognized as important sources of antimicrobial agents, including antifungal compounds. Cowan showed that several classes of phytochemicals, such as phenolics, terpenoids and alkaloids, exhibit broad spectrum activity against bacteria and fungi through multiple mechanisms, including membrane disruption, enzyme inhibition and interference with nucleic acids. Sesquiterpene lactones, which are characteristic metabolites of many Asteraceae species, have also been implicated in plant defense and antimicrobial activity. In line with this, recent studies have investigated the antifungal potential of Lactuca

species. A study from Azerbaijan reported that ethanolic extracts of the aerial parts of L. serriola and L. tatarica inhibited the growth of the phytopathogenic fungi A. solani, F. acuminatum and A. flavus. The crude ethanolic extracts showed stronger inhibitory effects than the untreated controls and, in some cases, than the unextracted plant material, suggesting that solvent extraction can concentrate active constituents. Nevertheless, these investigations have primarily focused on organic solvent extracts and aerial organs, and relatively few studies have evaluated aqueous extracts or compared different plant parts of L. serriola against important phytopathogens such as A. niger, A. flavus and F. oxysporum [16].

2.4 Research gap and rationale of the present study

Although the available literature provides a strong theoretical basis for the antifungal potential of L. serriola through its rich content of phenolic compounds, flavonoids and sesquiterpene lactones there is still a clear gap in our understanding of how extraction method (cold vs hot aqueous extraction) and plant part (roots, stems and leaves) influence its antifungal efficacy against major crop pathogens. Most previous studies have emphasized ethanolic or other organic solvent extracts and aerial tissues, while comprehensive comparative evaluations of cold and hot aqueous extracts from different organs of L. serriola remain scarce. Addressing this gap is essential for assessing the practical potential of L. serriola as a source of water based, environmentally friendly antifungal agents that can be more easily adopted in sustainable crop protection strategies. Chadwick et al. reported that these compounds possess notable antiinflammatory and antimicrobial properties and play an important role in protecting plants against pathogenic microorganisms. Given that L. serriola contains sesquiterpene lactones, these metabolites may partly account for its observed antifungal activity in the present study [17].

3. MATERIALS AND METHODS

3.1 Equipment

Petri dishes, syringes, a 9 mm cork borer, inoculating loops, glass slides, funnels, forceps, a Bunsen burner, screw capped glass tubes, graduated cylinders, volumetric flasks, porcelain evaporating dishes, plastic bags, glass beakers, an incubator, a light microscope, and an autoclave.

Chemicals and culture medium lactophenol cotton blue stain, 0.45 µm cellulose membrane filters, streptomycin, cotton and 70% (v/v) ethanol. Sabouraud Dextrose Agar (SDA) was used as the culture medium for fungal isolation and antifungal assays. The medium was prepared at an initial pH of 5.6, and streptomycin was added at a concentration of 500 mg/L to prevent bacterial contamination. The tested fungi were identified on the basis of colony morphology and microscopic examination as A. niger (black colonies), A. flavus (green colonies) and F. oxysporum (pink colonies). Fungal cultures were incubated at 25 ± 2 °C and subcultured regularly to maintain their viability. For each fungus, control plates containing SDA without plant extracts were prepared in parallel and included in all measurements and statistical analyses.

3.2 Plant Material Collection

Plant samples of L. serriola (leaves, stems, and roots) were collected during the flowering stage from two locations in Misurata, Libya: Al Gheran (32° 21′ 00″ N 15° 02′ 00″ E; A) and Zawiya Al Mahjoub (32° 23′ 05″ N 14° 59′ 05″ E; B) (Figure 1). Plant material sused in this study are shown in Figure 2. After collection, the plant materials were thoroughly cleaned to remove debris and contaminants, then air dried in the shade at room temperature for 10 days to preserve bioactive compounds. Dried plant materials were ground into fine powder using an electric laboratory (Figure 3) and stored at room temperature in sealed containers protected from direct sunlight until use.

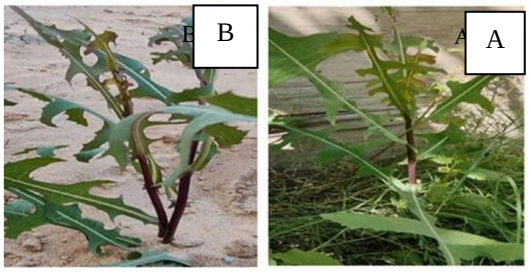


Figure 1. Wild lettuce (L. serriola) plants at the collection sites in Misurata, Libya: (A) Al Gheran site; (B) Zawiya Al Mahjoub site.



Figure 2. Plant samples (leaves, stems, and roots) of L. serriola.

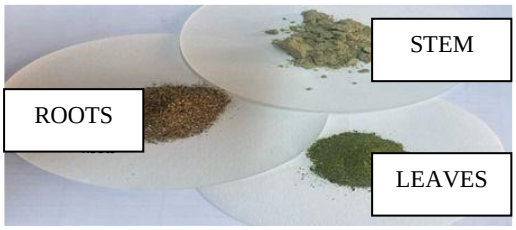


Figure 3. Ground plant samples (leaves, stems, and roots) of L. serriola.

3.3 Preparation of Aqueous Extracts

3.3.1 Cold Aqueous Extract

Ten grams of dried, powdered plant material (leaves, stems or roots) were mixed with 100 mL of distilled water in 250 mL Erlenmeyer flasks. The mixtures were shaken for 1 h on a mechanical shaker and then

left at room temperature for 24 h with intermittent shaking to enhance solvent penetration into the plant tissues. The suspensions were filtered through sterile filter paper (Figure 4), and the filtrates were concentrated under reduced pressure using a rotary evaporator. The concentrated extracts were sterilized by filtration through 0.45 µm cellulose membrane filters and stored at 4 °C until use [11]. For the antifungal assays, 2 mL of each sterilized extract were added to 18 mL of molten Sabouraud Dextrose Agar (SDA) in each Petri plate before solidification, resulting in a final concentration of 10% (v/v). Each treatment was prepared in triplicate.

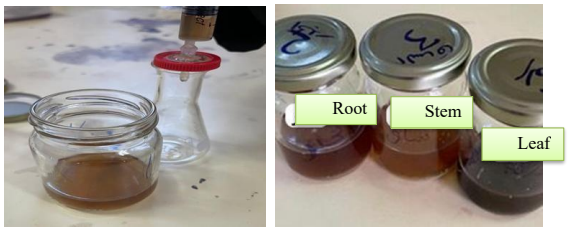


Figure 4. Cold aqueous extracts (root, stem, and leaves) of wild lettuce L. serriola.

3.3.2 Hot Aqueous Extract

Ten grams of plant powder were boiled in 100 mL of distilled water in glass flasks for 20–30 min. After cooling to room temperature, the mixtures were filtered first through sterile gauze and then through filter paper. The filtrates were subsequently concentrated under reduced pressure and sterilized by membrane filtration (0.45 µm), as described for the cold extracts. The sterile hot extracts were stored at 4 °C until use. For each treatment, 2 mL of hot extract were added to 15 mL of molten SDA per Petri plate (final concentration 11.8% v/v), and all treatments were prepared in triplicate.

3.4 Antifungal Activity Assessment

The antifungal activity of plant extracts was evaluated using the poisoned food technique. Two ml of each sterilized extract were added to molten SDA medium (supplemented with 500 mg/L streptomycin to prevent bacterial contamination) before solidification and poured into sterile Petri dishes. Control plates contained only SDA medium without extracts. After medium solidification, fungal discs (9 mm diameter) were cut from the periphery of 3-4 day old actively growing fungal colonies using a sterile cork borer and placed at the center of each test plate. All treatments were performed in triplicate. Plates were incubated at 25±2°C for 7 days. Fungal radial growth was measured by taking two perpendicular diameters of each colony, and the mean diameter was calculated. Growth inhibition percentage (I%) was calculated using the formula:

$$(I\%) = \frac{C-T}{C} \times 100$$

Where C is the mean colony diameter in the control plates and T is the mean colony diameter in the treated plates.

3.5 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectroscopy was performed on aqueous extracts to identify functional groups and chemical bonds present in the plant materials. Samples were analyzed using an FTIR spectrometer in the range of 4000400 cm^{-1}. The obtained spectra were compared with standard databases to identify characteristic functional groups of bioactive compounds.

3.6 Statistical Analysis

All experiments were performed in triplicate, and each value in the tables represents the mean of three replicates. Data were analyzed using GraphPad Prism version 5.01 (GraphPad Software, USA). Results are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare treatments, followed by Tukey's multiple comparison test. Differences were considered statistically significant at $p < 0.05$.

4. RESULTS

4.1 Antifungal Activity of Hot and cold Aqueous Extracts

Hot and cold aqueous extracts of L. serriola exhibited different levels of antifungal activity against the three tested phytopathogenic fungi (Tables 1; Figure 5). In general, cold extracts showed markedly higher inhibition than hot extracts for all fungi and plant parts. For A. flavus, the hot root, stem and leaf extracts reduced radial growth to 7.00, 8.13 and 8.25 cm, corresponding to inhibition percentages of 22.22, 9.72 and 8.33%, respectively, compared with the control (9.00 cm). In contrast, the corresponding cold extracts strongly suppressed *A. flavus* growth to 1.60, 2.43 and 2.45 cm, with inhibition values of 80.12, 69.87 and 69.56%, respectively. A similar trend was observed for *F. oxysporum*: hot root, stem and leaf extracts produced 42.64, 17.84 and 24.03% inhibition (mean growth 3.08, 4.42 and 4.08 cm), whereas the cold extracts achieved 66.85, 48.57 and 37.71% inhibition (mean growth 1.45, 2.25 and 2.73 cm) relative to the control (5.38 and 4.38 cm for hot and cold assays, respectively). For *A. niger*, hot extracts of root, stem and leaf caused 53.00, 36.33 and 17.60% inhibition (4.23, 5.73 and 7.42 cm), while the cold extracts resulted in 52.98, 49.32 and 16.38% inhibition (4.18, 4.50 and 7.43 cm) compared with their controls (9.00 and 8.88 cm). Across both extraction methods, root extracts consistently exhibited the strongest antifungal effects, followed by stem extracts, whereas leaf extracts were generally less effective.

TABLE I. EFFECT OF HOT AND COLD AQUEOUS EXTRACTS OF L. SERRIOLA PARTS ON RADIAL GROWTH AND INHIBITION OF PHYTOPATHOGENIC FUNGI ON SDA MEDIUM AT 25±2°C

Fungus	Plant Part	Mean Growth (cm) in hot extracts	Mean Growth (cm) in cold extracts	Inhibition (%) in hot extracts	Inhibition (%) in cold extract
A. flavus	Root	1.60	7.00	22.22	80.12
	Stem	2.43	8.13	9.72	69.87
	Leaf	2.45	8.25	8.33	69.56
	Control	8.05	9.00	-	-
F. oxysporum	Root	1.45	3.08	42.64	66.85
	Stem	2.25	4.42	17.84	48.57
	Leaf	2.73	4.08	24.03	37.71
	Control	4.38	5.38	-	-
A. niger	Root	4.18	4.23	53.00	52.98
	Stem	4.50	5.73	36.33	49.32
	Leaf	7.43	7.42	17.60	16.38
	Control	8.88	9.00	-	-

Each value represents the mean of three replicates. Control = SDA medium without plant extract.

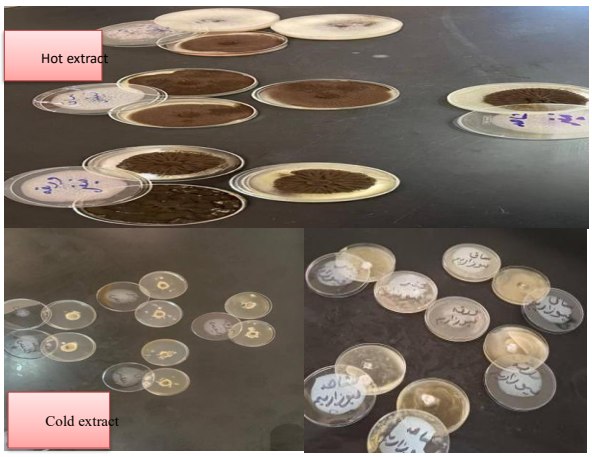


Figure 5. Effect of cold and hot aqueous extracts (root, stem, and leaves) of wild lettuce L. serriola on the radial growth of A. niger, A. flavus, and F. oxysporum at 25 ± 2 °C on SDA medium.

5. FTIR ANALYSIS

FTIR spectroscopy of L. serriola leaf, stem, and root extracts revealed several characteristic functional groups associated with bioactive phytochemicals. Prominent broad absorption bands in the region of 3200–3600 cm^{-1} corresponded to hydroxyl (-OH) stretching vibrations, indicating the presence of phenolic compounds and alcohols. Strong bands around 1650–1750 cm^{-1} were attributed to carbonyl (C=O) stretching of carboxylic acids, esters, and lactones, while absorption bands in the 1500–1650 cm^{-1} region indicated aromatic C=C stretching vibrations typical of phenolic compounds and flavonoids [6]. Additional peaks in the fingerprint region below 1500 cm^{-1} were mainly assigned to C–O stretching vibrations of alcohols, ethers, and esters. The FTIR spectrum of the leaf extract was taken as representative of these functional groups (Figure 6), while The FTIR spectrum of the stem extract showed a similar pattern with minor differences in band intensities (Figure 7), and the root extract spectrum also displayed comparable functional group bands, with some shifts in the fingerprint region that may reflect differences in root specific metabolites (Figure 8). The overlaid FTIR spectrum of L. serriola extracts from the present study and the reference spectrum reported by Awan et al. (2020) showed a high degree of similarity in the major absorption bands (Figure 9), confirming the presence of hydroxyl, carboxylic, and alkene groups previously described for this species. These spectral profiles are consistent with the reported phytochemical composition of Lactuca species, including sesquiterpene lactones, phenolic acids, and flavonoids, and support the potential of L. serriola extracts as a promising source of antifungal compounds.

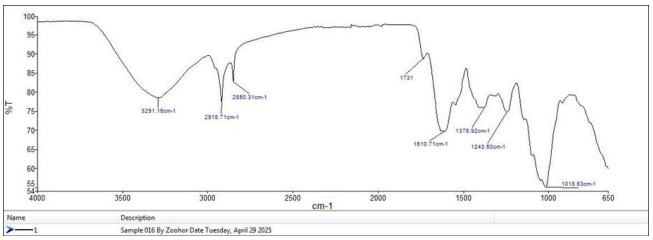


Figure 6. FTIR spectrum of the leaf extract of wild lettuce (L. serriola).

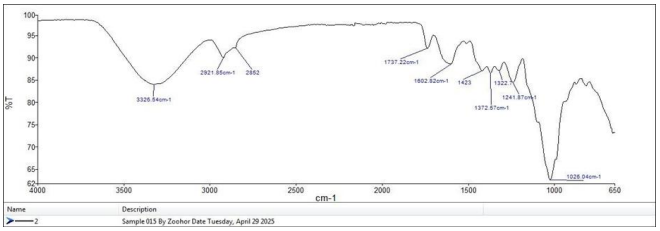


Figure 7. FTIR spectrum of the root extract of wild lettuce (L. serriola).

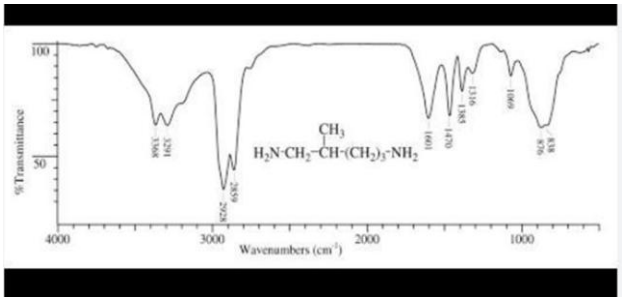


Figure 8. FTIR spectrum of the bioactive compounds of wild lettuce (L. serriola) compared with the reference spectrum reported by Awan et al. (2020).

6. DISCUSSION

The findings of the present study clearly demonstrate that aqueous extracts of *L. serriola* possess notable antifungal activity against the three tested phytopathogenic fungi, *A.*

niger, *A. flavus* and *F. oxysporum*. Cold aqueous extracts consistently produced higher inhibition percentages than hot extracts for all plant parts and fungal species, indicating that the extraction conditions strongly influence the recovery of bioactive constituents. This pattern is particularly evident for *A. flavus*, where the cold root extract achieved 80.12% inhibition, compared with only 22.22% for the hot root extract, despite using the same plant material and concentration.

6.1 Effect of extraction method (cold vs hot)

The superior performance of cold extracts suggests that thermolabile secondary metabolites play a major role in the antifungal activity of L. serriola. Several classes of compounds previously reported in this species including phenolic acids, flavonoids, sesquiterpene lactones and saponins can undergo degradation or structural modification when exposed to prolonged heating. Under cold maceration, these metabolites are more likely to remain intact, thereby preserving their biological activity. In contrast, hot extraction, although efficient in solubilizing certain components, may partially denature or oxidize sensitive molecules, leading to the lower inhibition percentages observed in the hot extract treatments. Similar observations have been reported for other medicinal plants, where cold extraction retained higher phenolic content and stronger antifungal or antioxidant activity than decoction.

The relatively small difference between cold and hot extracts against A. niger in root and stem treatments (52.98 vs. 53.00% and 49.32 vs. 36.33% inhibition, respectively) suggests that some constituents active against this fungus are more heat stable, or present in sufficiently high concentrations to maintain activity even after heating. However, for A. flavus and F. oxysporum, cold extraction clearly enhanced antifungal efficacy, emphasizing the importance of optimizing extraction temperature when developing plant based fungicidal formulations.

6.2 Effect of plant part (roots, stems and leaves)

Across both extraction methods, root extracts consistently exhibited the strongest antifungal effects, followed by stem extracts, whereas leaf extracts were generally less effective. This gradient in activity (roots > stems > leaves) suggests a higher accumulation of defense related metabolites in the root tissues of *L. serriola*. Roots are directly exposed to soil borne pathogens such as *F. oxysporum* and therefore often contain elevated levels of protective compounds, including triterpenes, sesquiterpene lactones and phenolic acids, which have been documented in Lactuca species.

The high inhibition of *F. oxysporum* by the cold root extract (66.85%) supports this interpretation and indicates that root derived constituents are particularly effective against this soil borne fungus. In contrast, the relatively lower activity of leaf extracts, especially against *A. niger* (16.38% inhibition in the cold extract), may reflect lower concentrations or different profiles of antifungal compounds in aerial photosynthetic tissues. These organ specific differences in chemical composition should be considered when selecting plant material for biopesticide production, as using whole-plant or leaf only extracts could dilute the contribution of more active root metabolites.

Differences among the fungal species in their sensitivity to the extracts may be related to variations in cell wall composition, membrane lipid content and detoxification mechanisms. For example, *A. flavus* was highly sensitive to the cold extracts, particularly from roots and stems, whereas *A. niger* showed comparatively moderate inhibition by leaf extracts. Such species-specific responses are common in studies of plant derived antifungals and highlight the need to tailor extract formulations to target pathogens.

6.3 Comparison with previous studies and FTIR findings

The present results are in line with previous reports on the antifungal potential of Lactuca species. Namik et al. reported that ethanolic extracts of aerial parts of *L. serriola* and *L. tatarica* inhibited several phytopathogenic fungi, including A. flavus. The inhibition percentages obtained in our study with cold aqueous extracts up to 80.12% for A. flavus are comparable to, or in some cases higher than, those reported for ethanolic extracts, despite the use of water as a solvent. This suggests that water soluble constituents of *L. serriola* can provide substantial antifungal effects and may be better suited for environmentally friendly formulations than organic solvent extracts.

FTIR analysis of leaf, stem and root extracts revealed broad O–H stretching bands (3200–3600 cm^{-1}) and C=O and C=C absorption bands in the 1650–1750 and 1500–1650 cm^{-1} regions, respectively, which are characteristic of phenolic compounds, flavonoids and sesquiterpene lactones. These functional groups have been associated with membrane disrupting, enzyme inhibiting and oxidative stress inducing activities in fungi. The strong similarity between the spectra obtained in this study and those reported for *L. serriola* by Awan et al. supports the presence of similar bioactive moieties. Consequently, the FTIR profiles provide a chemical basis for the observed antifungal activity and reinforce the hypothesis that phenolic and lactone type compounds are major contributors to the growth inhibition of A. niger, A. flavus and *F. oxysporum*.

Overall, the combination of bioassay and FTIR data indicates that *L. serriola* represents a promising source of natural antifungal agents. The strong activity of cold root extracts, in particular, suggests that selectively exploiting root material and preserving thermolabile phytochemicals during extraction could yield effective plant-based fungicides suitable for integration into sustainable crop protection strategies.

7. CONCLUSION

The present study demonstrates that aqueous extracts of *L. serriola* possess noteworthy antifungal activity against the phytopathogenic fungi *A. niger, A. flavus* and *F. oxysporum.* Cold aqueous extracts consistently exhibited

stronger inhibition than hot extracts for all plant parts and fungal species, indicating that extraction conditions particularly temperature play a critical role in preserving thermolabile bioactive constituents. Among the tested plant organs, root extracts were the most effective, especially the cold root extract, which produced up to 80.12% inhibition of A. flavus and 66.85% inhibition of F. oxysporum. In contrast, leaf extracts generally showed lower activity, highlighting the importance of plant part selection when designing plant based fungicidal formulations.

FTIR analysis confirmed the presence of functional groups associated with phenolic compounds, flavonoids and sesquiterpene lactones, providing a chemical rationale for the antifungal effects observed in the bioassays. Taken together, these findings suggest that cold aqueous root extracts of L. serriola may have significant potential as natural antifungal agents and could be further developed as ecofriendly alternatives or complements to synthetic fungicides in sustainable crop protection strategies. resulting in higher antifungal efficacy. FTIR analysis confirmed the presence of multiple functional groups associated with antifungal phytochemicals, further supporting the potential use of L. serriola extracts in environmentally friendly disease management strategies.

8. RECOMMENDATIONS

Based on the results of this study, several directions for further research are recommended. First, it is important to isolate, identify and structurally characterize the specific bioactive compounds present in the cold aqueous root extracts of L. serriola that are responsible for the observed antifungal activity.

Second, future work should evaluate different extract concentrations, application rates and exposure times in vitro to determine minimum inhibitory concentrations (MICs) and to optimize the dosage required for effective fungal growth suppression. These studies should be complemented by in vivo or greenhouse trials on economically important crops to assess the efficacy, selectivity and phytotoxicity of L. serriola extracts under more realistic conditions.

Third, it would be valuable to investigate the combined use of L. serriola extracts with other biological control agents or reduced doses of synthetic fungicides to test possible synergistic or additive effects and to support integrated, environmentally friendly disease management strategies.

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