مجلة كلية التربية الاساسية
كلية التربية الاساسية – الجامعة المستنصرية
Journal of the College of Basic Education Vol.30 (NO. 125) 2024, pp. 17-27

Determination of Antioxidant and Antimicrobial Activity of Prickly lettuce (Lactuca serriola L.) Leaves Extract

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Abstract
The qualitative detection of active compounds in hot aqueous extract of Prickly lettuce (Lactuca serriola L.) leaves showed that it is rich with glycosides, alkaloids, terpenes, saponins, tannins, and flavonoids. The antioxidant activity (Inhibition %) was 9.35, 16.43, 29.17, 53.82, and 76.53% at 12.5, 25, 50, 100, and 200 mg/mL of extract, respectively; the LSD value was 7.053 that refer to found significant differences at ($P \leq 0.05$) in the concentrations used. The inhibition zones of Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Pseudomonas aeruginosa at ($P \leq 0.05$) were 12, 8, 9, and 6 mm, respectively, with average 8.75 mm and LSD value 2.703 at 12.5 mg/mL; 14, 10, 11 and 8 mm, respectively, with average 10.75 mm and LSD value 2.965 at 25 mg/mL; 18, 12, 15, and 11 mm, respectively, with average 14 mm and LSD value 3.071 at 50 mg/mL; 20, 13, 17, and 14 mm, respectively, with average 16 mm and LSD value 3.186 at 100 mg/mL, while, they were 22, 14, 19, and 16 mm, respectively, with average 17.75 mm and LSD value 3.622 at 200 mg/mL, the LSD values were 4.375, 3.502, 4.673, and 4.029 at ($P \leq 0.05$) for inhibition zone of S. aureus, B. cereus, E. coli, and P. aeruginosa, respectively, at 12.5, 25, 50, 100, and 200 mg of Prickly lettuce leaves extract.

Keywords: Active compounds, Antimicrobial activity, Antioxidant activity, Plant extracts, Prickly lettuce.

Introduction

Plants are considered an important source for providing wide biological active substances that could be used in large applications such as industry, agriculture, and other scientific, due to it safe to use, without side effects, and economically available (Alsoufi & Aziz, 2022), which encouraged researchers to study they for using as an antioxidants and antimicrobial agent against wide type of microorganisms (Alsoufi & Aziz, 2023). Prickly Lettuce (Lactuca serriola L.) is an annual plant that is considered a weed of orchards and field crops (Riar 2009), it grows from 30 to 200cm throughout the temperate, the leaves emit milky latex when cut, and it has a hairless reddish stem (Dandrea et al., 2008). The plant was mentioned in many ancient civilizations, such as the Egyptian and Greek civilizations, through its use in several fields. The tender leaves represent one of the parts suitable for consumption in a raw or cooked form, despite the bitter taste it possesses, which is more than doubled in the older leaves in the lower part of the plant and after the process. Flowering, but eating large quantities of it can lead to indigestion (Lebeda et al., 2008; Everitt et al., 2007). The plant is used in many medical fields, as the milky juice produced by the plant represents one of the important resources for use in alternative medicine due to it containing a substance, lactucarium, which is used as an antispasmodic, an aid in digestion, a diuretic, a hypnotic, an anesthetic, a sedative, and a treatment for eye ulcers. This substance has weak opium properties, but it does not cause indigestion in small quantities. It also does not cause addiction; this substance is used internally to treat insomnia, anxiety, and disorders. Nervousness in children, dry and whooping cough, and rheumatic pain, the plant's flowers can be used in their fresh or dried form, but by caution, as an overdose can lead to death by affecting the work of the heart (Kuang et al., 2008; Fragiska, 2005). So this study was aimed at the detection of chemical compounds and estimation of antimicrobial and antioxidant activity in this plant.

Materials and Methods

Samples Collecting

Prickly lettuce (Lactuca serriola L.) leaves were collected in September 2023 from Baghdad city; leaves were cleaned from dust to prepare them for tests.

Microorganism strains

Some pure microorganism strains were obtained from the laboratories of the College of Science, Mustansiriyah University, Iraq, to conduct the necessary tests on them. They included G^+ bacteria (Staphylococcus aureus and Bacillus cereus) and G^- bacteria (Escherichia coli and Pseudomonas aeruginosa). The morphological, cultural, and biochemical characteristics were confirmed according to Mac Faddin (2000).

Hot aqueous extraction

The hot aqueous extract was obtained by mixing 100 g of cut Prickly lettuce leaves into small parts with 300 mL deionized distilled water at 70°C, then mixing it with an electrical blender for 15 min. and filtering by filter paper (Whatman No.1) (Escudero et al., 2000), the filtrate extract was dried by freeze-dried and kept at 4°C until use, then detection phytochemicals: glycosides, alkaloids, terpenes, saponins, tannins and flavonoids (Ullah et al., 2011).

Antioxidant activity

Antioxidant activity (Inhibition %) in hot aqueous extract of Prickly lettuce leaves was determined according to radical scavenging method (Brand-Williams et al., 1995) using 2,2-Diphenyl-1-picryl-hidrazil (DPPH) by mixing 0.1 mL from 12.5, 25, 50, 100, and 200 mg/mL of extract with 3.9 mL DPPH with stirring for 30 min to complete reaction, then filtered through filter paper (Whatman No.1) and absorbency at 517 nm using UV-visible spectrophotometer. Methanol was taken as blank for baseline correction. DPPH solution depends on a control. The antioxidant activity was calculated and expressed as inhibition (%) through the following equation:

$$\text{Antioxidant activity (Inhibition\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Antimicrobial activity

The antimicrobial activity in hot aqueous extract of Prickly lettuce leaves was studied against S. aureus, B. cereus, E. coli, and P. aeruginosa according to well diffusion method for Gupta et al. (2016) by spreader 0.1 mL of the bacterial suspension containing 1.5×10^8 CFU/mL (0.5 McFarland unit) on the surface of sterilized Mueller-Hinton agar medium using a sterile spreader, then made wells (5 mm in diameter and 3 mm in depth) on the agar plate by cork borer and was filled by 0.1 mL (12.5, 25, 50, 100, or 200 mg/mL) of Prickly lettuce extract (sterilized by Millipore 0.22 mμ) and incubated

aerobically at 37°C for 24h, deionized distilled water was using as control. The inhibition zone was measured in millimeters.

Statistical Analysis

SAS (Statistical Analysis System) (2018) program version 9.6th ed., was used to detect the difference factors in the experiments of this study using LSD (Least significant difference) test (Analysis of Variation-ANOVA) to compare between means in results.

Results and Discussion

Phytochemicals of Prickly lettuce leaves

The qualitative detection of active compounds in Prickly lettuce leaves for hot aqueous extraction showed that it is rich in glycosides, alkaloids, terpenes, saponins, tannins, and flavonoids (Table 1).

Table (1): Qualitative detection of active compounds in Prickly lettuce leaves for hot aqueous extraction.

Active compound	Indication
Glycosides	+
Alkaloids	+
Terpenes	+
Saponins	+
Tannins	+
Flavonoids	+
(+): Contain active compound.	

Active compounds are one of the main components in the plant's cells that perform many roles in the life cycle, the importance of glycosides and tannins lies in the ability to provide the plant with the necessary protection against insects and fungi that may be exposed (Kumar et al., 2007). Also, alkaloids are one of the important compounds used in the pharmaceutical industry due to their active effects on the physiological level of the organism (Okwu & Iroabuchi, 2009). Similarly, saponins are used medicinally as an antioxidants, anticancers, anti-inflammatory, and moisturizing mucous membranes, it has also been noted that they have antifungal activity. As well as, terpene compounds that are considered as an anti-agents for virus, bacteria, and fungi growth (Giani et al., 2006).

Antioxidant and Antimicrobial Activity

The antioxidant activity (Inhibition%) was 9.35, 16.43, 29.17, 53.82, and 76.53% for Prickly lettuce leaves hot aqueous extract at 12.5, 25, 50, 100, and 200 mg/mL, respectively, the LSD value was 7.053 that refer to found significant differences at (P≤0.05) in the concentrations used (Figure 1).

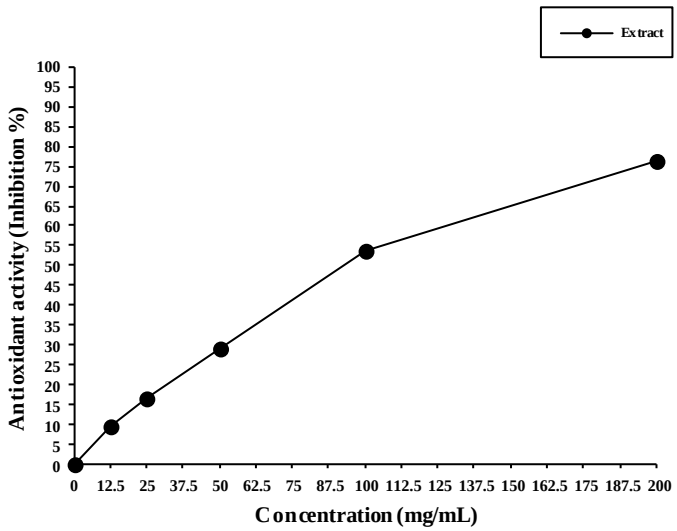

Figure (1): Antioxidant activity (Inhibition %) of Prickly lettuce leaves hot aqueous extract.

The results in (Table 2) showed that the inhibition zone of S. aureus, B. cereus, E. coli, and P. aeruginosa at (P≤0.05) were 12, 8, 9, and 6 mm, respectively, with average 8.75 mm and LSD value 2.703 at 12.5 mg/mL; 14, 10, 11, and 8 mm, respectively, with average 10.75 mm and LSD value 2.965 at 25 mg/mL; 18, 12, 15, and 11 mm, respectively, with average 14 mm and LSD value 3.071 at 50 mg/mL; 20, 13, 17, and 14 mm, respectively, with average 16 mm and LSD value 3.186 at 100 mg/mL, while, they were 22, 14, 19, and 16 mm, respectively, with average 17.75 mm and LSD value 3.622 at 200 mg/mL. The LSD values were 4.375, 3.502, 4.673, and 4.029 at (P≤0.05) for inhibition zone of S. aureus, B. cereus, E. coli, and P. aeruginosa, respectively, at 12.5, 25, 50, 100, and 200 mg/mL of Prickly lettuce extract

Table (2): Effect of antimicrobial activity of Prickly lettuce leaves hot aqueous extract against types of bacteria.

Microorganism's strains	Antimicrobial activity of Prickly lettuce leaves hot aqueous extract (mg/mL)					LSD value
	12.5	25	50	100	200	
	Inhibition zone (mm)					
S. aureus	12	14	18	20	22	4.375 *
B. cereus	8	10	12	13	14	3.502 *
E. coli	9	11	15	17	19	4.673 *
P. aeruginosa	6	8	11	14	16	4.029 *
Average	8.75	10.75	14	16	17.75	4.157 *
LSD value	2.703 *	2.965 *	3.071 *	3.186 *	3.622 *	---

This means having the different letters in the same row differed significantly.
*(P≤0.05): Significant

From the results, hot aqueous extract of Prickly lettuce leaves was more effective for S. aureus and B. cereus than E. coli and P. aeruginosa which is attributed to the structural composition of the cell wall of bacteria, as G^+ lacks a layer of outer membranes, which makes the permeability of substances entering the cell greater compared to G^- which have its inner wall is an internal barrier represented by lipopolysaccharide combined with multiple proteins which can prevent the passage of many harmful substances into the cell (Pliego et al., 2022). Also, the increase in concentration (mg/mL) of Prickly lettuce leaves hot aqueous extract led to higher antioxidant activity (inhibition %).

In this context, many researchers are pointed out to this subject such as use of Artemisia (Artemisia monosperma L.) and common sage (Salvia officinalis L.) as an antimicrobial agent against Bacillus subtilis, S. aureus, E. coli, and P. aeruginosa (Aziz et al., 2012); Lavender (Lavandula officinalis L.) as an antimicrobial agent against Acinetobacter baumannii, Enterococcus faecalis, and Enterococcus faecium (Al-Niaame & Aziz, 2013); Pomegranate peel (Punica granatum L.) as an antimicrobial agent against E. coli, Salmonella typhimurium, and Shigella flexneri (Aziz 2015); Bay laurel (Laurus nobilis L.) as an antimicrobial agent against S. aureus, Staphylococcus epidermids, Proteus vulgaris, B. subtilis, E. coli, and P. aeruginosa (Rishan et al., 2016); Lavender (Lavandula officinalis L.) as an antimicrobial agent against E. coli, Salmonella ssp., Aspergillus ssp., Penicillium ssp., Candida albicans, and

Pichia jadinii (Alsoufi & Aziz, 2019); Oregano (Origanum vulgare L.) and Common jasmine (Jasminum officinale L.) as an antioxidant agent (Giannakourou et al., 2019); Rosemary (Salvia rosmarinus L.) as an antimicrobial agent against S. aureus, B. cereus, S. typhimurium, E. coli, P. jadinii, C. albicans, and Penicillium ssp. (Alsoufi & Aziz, 2021); Flaxseed (Linum usitatissimum L.) as an antimicrobial agent against Sh. flexneri, S. typhimurium, and E. coli (Hussien & Aziz, 2021); Welsh onion (Allium fistulosum L.) as an antimicrobial agent against E. coli, Salmonella ssp., P. aeruginosa, and S. aureus (Alsoufi & Aziz, 2022); chickpea (Cicer arietinum) as an antimicrobial agent against E. coli, S. Typhimurium, S. aureus, and B. cereus (Alsoufi & Aziz, 2023); Syzigium aromaticum and Mentha spicata as an antioxidant agent (Oliveira Filho et al., 2023).

Nearly, most plants have potential biological activities as an antimicrobial, antioxidant, anti-inflammatory, anticancer, analgesic, and others (Eftekhari et al., 2021). So, the antimicrobial and antioxidant properties due to having phenolic compounds, terpenoids, flavonoids, carotenoids, α-tocopherols, ascorbic acid, tannins, saponins, and glycosides (Gull et al., 2021). This is consistent with what was found in this study (Table 1).

Conclusion

The study demonstrates the potential use of Prickly lettuce (Lactuca serriola L.) leaves extract as antioxidant agent and as an effective antimicrobial agent against Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Pseudomonas aeruginosa.

References

1. Al-Niaame, A. E & Aziz, R. A. (2013). Study of Lavandula officinalis L. buds of flowers extracts activity against some species of multi drug resistant clinical isolates of bacteria. Iraqi Journal of Biotechnology, 12(2), 82-91.
2. Alsoufi, M. A. & Aziz, R. A. (2019). Use of some plants extracts and puliulan for extending shelf life of apples. Journal of the College of Basic Education, 104(25), 653-672.
3. Alsoufi, M. A. & Aziz, R. A. (2021). Extending the shelf life of food using some biological products. Biochemical and Cellular Archives, 21(2), 4641-4645.
4. Alsoufi, M. A. & Aziz, R. A. (2022). Use of biopreservation technique to increase shelf life for some types of meat. Bioscience Research, 19(2), 1133-1138.

5. Alsoufi, M. A. & Aziz, R. A. (2023). Isolation and purification of chickpea (Cicer arietinum) seeds protein, testing their antibacterial activity, and using to extend the shelf life of beef patties. Basrah Journal of Agricultural Sciences, 36(2), 59-67.
6. Aziz, R. A. (2015). Comparison of the inhibitory effect of the alcoholic extract of pomegranate peel and antibiotics against some intestinal bacterial isolates. Iraqi Journal of Science, 50(B2), 1400-1408.
7. Aziz, R. A., Hasan, E. J. & Hussein, Z. G. (2012). Chemical composition and antimicrobial activity of Artemisia monosperma and Salvia officinalis L. plants. Journal of the College of Basic Education, 18(73), 97-106.
8. Brand-Williams, W., Cuvelier, M. E. & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology, 28, 25-30
9. Dandrea, L., Felber, F. & Guadagnuolo, R. (2008). Hybridization rates between lettuce (Lactuca sativa) and its wild relative (L. serriola) under field conditions. Environmental Biosafety Research, 7, 61-71.
10. Eftekhari, A., Khusro, A., Ahmadian, E., Dizaj, S. M., Hasanzadeh, A. & Cucchiarini, M. (2021). Phytochemical and nutra-pharmaceutical attributes of Mentha spp.: A comprehensive review. Arabian Journal of Chemistry, 14(5), 103-106.
11. Escudero, A., Albert, M. J., Pita, J. M. & Perez-Garcia, F. (2000). Inhibitory effects of Artemisia herba-alba on the germination of the gypsophyte Helianthemum squamatum. Plant Ecology, 148, 71-80.
12. Everitt, J. H., Lonard, R. L. & Little, C. R. (2007). Weeds in South Texas and Northern Mexico. Lubbock: Texas Tech University Press.
13. Fragiska, M. (2005). Wild and cultivated vegetables, herbs and spices in Greek antiquity. Environmental Archaeology, 10(1), 73-82.
14. Giani, F., Maria, D., Cardoso, L., Menna, B. & Maurili, J. (2006). Effect of oregano (Origanum vulgare L.) and thyme (Thymus vulgaris L.) essential oils on Trypanosoma cruzi (Protozoa: Kinetoplastida) growth and ultra structure. Parasitology Research, 100(4), 783-790.
15. Giannakourou, M., Strati, I. F., Kriebardis, A. G., Mantanika, V., Poulis, S., Zoumpoulakis, P. & Sinanoglou, V. J. (2019). Shelf life extension and quality improvement of cucumber slices impregnated in infusions of edible herbs. Analytical Letters, 52(17), 2677-2691.

16. Gull, A., Bhat, N., Wani, S. M., Masoodi, F. A., Amin, T. & Ganai, S. A. (2021). Shelf life extension of apricot fruit by application of nanochitosan emulsion coatings containing pomegranate peel extract. Food Chemistry, 349, 129-149.
17. Gupta, D., Dubey, J. & Kumar, M. (2016). Phytochemical analysis and antimicrobial activity of some medicinal plants against selected common human pathogenic microorganisms. Asian Pacific Journal of Tropical Disease, 6(1), 15-20.
18. Hussien, Z. G. & Aziz, R. A. (2021). Chemical composition and antibacterial activity of Linum usitatissimum L. (Flaxseed). Systematic Reviews in Pharmacy, 12(2), 145-147.
19. Kuang, H., van Eck, H. J., Sicard, D., Michelmore, R. & Nevo, E. (2008). Evolution and genetic population structure of Prickly lettuce (Lactuca serriola) and its RGC2 resistance gene cluster. Genetics, 178, 1547-1558.
20. Kumar, G. S., Jayaveera, K. N., Ashok, K. C. K., Sanjay, U. P., Swamy, B. M. V. & Kishore, K. D. V. (2007). Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. Tropical Journal of Pharmaceutical Research, 6(2), 717-723.
21. Lebeda, A., Petrzelova, I. & Maryska, Z. (2008). Structure and variation in the wild-plant pathosystem: Lactuca serriola-Bremia lactucae. European Journal of Plant Pathology, 122(1), 127-146.
22. Mac Faddin J. F. (2000). Biochemical Tests for Identification of Medical Bacteria. (3rd ed.). Lippincott Williams & Wilkins.
23. Okwu, D. E. & Iroabuchi, F. (2009). Phytochemical composition and biological activities of Uvaria chamae and Clerodendoron splendens. E-Journal of Chemistry, 6(2), 553-560.
24. Oliveira Filho, J. G. d., Duarte, L. G. R., Silva, Y. B. B., Milan, E. P., Santos, H. V., Moura, T. C., Bandini, V. P., Vitolano, L. E. S., Nobre, J. J. C., Moreira, C. T., Mitsuyuki, M. C., Junior, S. B. & Ferreira, M. D. (2023). Novel approach for improving papaya fruit storage with carnauba wax nanoemulsion in combination with Syzigium aromaticum and Mentha spicata essential oils. Coatings, 13, 847.
25. Pliego, A. B., Tavakoli, M., Khusro, A., Seidavi, A., Elghandour, M. M. M. Y., Salem, A. Z. M., Márquez-Molina, O. & Rene Rivas-Caceres, R. (2022). Beneficial and adverse effects of medicinal plants as feed

supplements in poultry nutrition: a review. Animal Biotechnology, 33(2), 369-391.
26. Riar, D. S. (2009). Understanding 2,4-D Resistance In Prickly lettuce (Lactuca serriola L.) and Evaluating Chemical Fallow Systems For The Inland PNW. PhD Thesis, Washington State University, Department of Crop and Soil Sciences.
27. Rishan, A. M., Aziz, R. A. & Al-Temimi, S. S. (2016). Determine the chemical content of Bay (Laurus nobilis L.) leaves extract and its effectivenss against some bacterial species. Iraqi Journal of Market Research and Consumer Protection, 8(1), 281-301.
28. SAS. (2018). Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed., SAS. Inst. Inc. Cary. N.C. USA.
29. Ullah, N., Khurram, M., Amin, M. U., Afridi, H. H., Khan, F. A., Khayam, S. M. U., Ullah, S., Najeeb, U., Hussain, J. & Khan, M. A. (2011). Comparison of phytochemical constituents and antimicrobial activities of Mentha spicata from four northern districts of Khyber pakhtunkhwa. Journal of Applied Pharmaceutical Science, 1(7), 72-76.

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كلية التربية الاساسية – الجامعة المستنصرية
Journal of the College of Basic Education Vol.30 (NO. 125) 2024, pp. 17-27

تقدير الفعالية المضادة للأكسدة والتثبيطية لمستخلص أوراق الخس الشوكي

(Lactuca serriola L.)

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مستخلص البحث:
أظهر الكشف النوعي للمركبات الفعالة في المستخلص المائي الساخن لأوراق نبات الخش الشوكي (Lactuca serriola L.) أنها تحتوي على glycosides وalkaloids وterpenes وsaponins وtannins وflavonoids، وبلغت الفعالية المضادة للاكسدة (التثبيط %) 9.35 و16.43 و29.17 و53.82 و76.53% عند تركيز 12.5 و25 و50 و100 و200 ملغم/مللتر من المستخلص على التوالي، واشارت قيمة LSD التي بلغت 7.053 الى وجود فروقات معنوية عند (P≤0.05)، ولوحظ وجود فروق معنوية (P≤0.05) في اقطار منطقة التثبيط لبكتريا Staphylococcus aureus و Bacillus cereus وEscherichia coli وPseudomonas aeruginosa التي بلغت 12 و8 و9 و6 ملم على التوالي وبمعدل 8.75 ملم وقيمة LSD مقدارها 2.703 عند استعمال تركيز 12.5 ملغم/ مللتر؛ بينما كانت الاقطار 14 و10 و11 و8 ملم على التوالي وبمعدل 10.75 ملم وقيمة LSD مقدارها 2.965 عند استعمال تركيز 25 ملغم/ مللتر؛ وكانت الاقطار 18 و12 و15 و11 ملم على التوالي وبمعدا 14 ملم وقيمة LSD مقدارها 3.071 عند استعمال تركيز 50 ملغم/ مللتر؛ وبلغت الاقطار 20 و13 و17 و14 ملم على التوالي وبمعدل 16 ملم وقيمة LSD مقدارها 3.186 عند استعمال تركيز 100 ملغم/ مللتر، في حين كانت الاقطار 22 و14 و19 و16 ملم على التوالي وقيمة LSD مقدارها 3.622 عند استعمال تركيز 200 ملغم/ مللتر، ولوحظ من خلال النتائج ان قيمة LSD كانت 4.375 و3.502 و4.673 و4.029 عند (P≤0.05) لقطر منطقة تثبيط كل من بكتريا S. aureus وB. cereus وE. coli وP. aeruginosa على التوالي عند استعمال تراكيز 12.5 و25 و50 و100 و200 ملغم/ مللتر من مستخلص اوراق الخس الشوكي.

الكلمات المفتاحية: المركبات الفعالة، الفعالية المضادة للميكروبات، الفعالية المضادة للأكسدة، المستخلصات النباتية، الخس الشوكي.