Trop J Nat Prod Res, May 2024; 8(5):7161-7165

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Analysis of Antioxidant Activities, Bioactive Compound Content, and Cytotoxic Effects of Green Oak and Red Oak Lettuce from Hydroponic and Organic Cultivation Systems

Noppakun Pakdeenarong[1]*, Kwanyuen Leamsamrong[2], Vijitra Luang-In[3]

[1]*Department of Biology, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand*
[2]*Department of Chemistry, Faculty of Science and Technology, Rajabhat MahaSarakham University, Maha Sarakham 44000, Thailand*
[3]*Natural Antioxidant Innovation Research Unit, Department of Biotechnology, Faculty of Technology, Mahasarakham University, Maha Sarakham 44150, Thailand*

ARTICLE INFO

Article history:
Received 28 March 2024
Revised 20 April 2024
Accepted 30 April 2024
Published online 01 June 2024

ABSTRACT

The antioxidant properties of *Lactuca sativa* var. *crispa* L. (green oak and red oak lettuce) grown under hydroponic and organic cultivation systems were compared using water and ethanol extraction. The 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay was implemented to explore the cytotoxicity of hydroponically grown green oak and red oak lettuce ethanolic extracts toward the human liver cancer HepG2 cell line. Total flavonoid content (TFC) in hydroponic green and red oak lettuce aqueous and ethanolic extracts was higher than in organically grown lettuce. The TFC of hydroponic red oak lettuce extracted with ethanol was 2.92±0.10 mg GAE/g DW, higher than organically grown (1.73± 0.09 mg GAE/g DW). The total phenolic content (TPC) of organic lettuce was higher than lettuce grown hydroponically. Antioxidant potential was assessed by the DPPH, ABTS, and FRAP assays. The antioxidant potential of organic lettuce extracts was higher than hydroponically grown lettuce, indicating that the action of the hydroponic system enhanced flavonoid contents, with hydroponic extracts inhibiting cancer cell growth. The green oak lettuce extract had a moderate cytotoxic effect (IC_{50}= 93.81±1.94 µg/mL), significantly stronger than the extract of red oak (IC_{50}= 347.50±8.39 µg/mL) exhibiting a very weak effect. Results showed that the hydroponic cultivation system stimulated the production of flavonoids and enhanced the cytotoxicity of plant extracts toward the HepG2 cell line.

Keywords: Flavonoid, Antioxidant activity, Cytotoxicity, Hydroponic lettuce

Introduction

The World Health Organization advises at least 400 grams of vegetables per day, which has propelled contemporary focus on healthy eating of salads into the spotlight. Lettuce is rich in essential nutrients including vitamins C and A, calcium, iron, carotene, folic acid, lutein, phenols, and fiber.[1] Green and red lettuce are popular choices among health-conscious individuals. Recent studies on lettuce have investigated its antioxidant, phenolic, anthocyanin, and flavonoid contents, revealing potential anticancer properties.[2] A diet abundant in lettuce may contribute to reducing the risk of age-related diseases; an effect attributed to its free radical scavenging activity.[3] The minimal processing requirements and extended shelf life of lettuce have made it a staple in salad bars and fast-food outlets, further enhancing its perception as a healthy food choice.[4] Hydroponic technologies, designed to optimize crop growth and minimize environmental impact, have expanded cultivation areas globally. The problem of concern arises from the consumption of lettuce harvested from hydroponic systems, leading to health implications.[5]

***Corresponding author. E mail: noppakun@msu.ac.th
Tel: +66885724885

Citation: Pakdeenarong N, Leamsamrong K, Luang-In V. Analysis of Antioxidant Activities, Bioactive Compound Content, and Cytotoxic Effects of Green Oak and Red Oak Lettuce from Hydroponic and Organic Cultivation Systems. Trop J Nat Prod Res. 2024; 8(5):7161-7165. https://doi.org/10.26538/tjnpr/v8i5.17

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Comparing the antioxidant potential of organic and hydroponically grown red oak and green oak lettuce, this study investigated whether lettuce grown hydroponically possessed anticancer properties. Whereas, organic farming, recognized for its sustainability and positive impact on soil fertility, contrasts with the technological advancements of hydroponic farming. This work aimed to test the effects of different cultivation techniques on the antioxidant content of salad vegetables, providing insights into the potential health benefits associated with these popular farming practices.

Current understanding of the nutritional quality of hydroponically grown lettuce has recently improved. Moreover, plant phytochemicals can inhibit the growth of cancer cell lines.[6] Therefore, this study examined the phytochemicals in hydroponically grown lettuce and their cytotoxicity toward HepG2 cells, highlighting the broader implications of hydroponic farming on human health risks associated with consuming lettuce harvested from hydroponic systems. This research study marked the first demonstration of the antioxidant capacity of organic and hydroponic red oak lettuce, which exhibited anticancer attributes.

Materials and Methods

Plant culture systems

Green and red oak lettuce varieties grown for 45 days in November to December, 2023 in organic without chemical fertilizers and hydroponic farming systems were obtained from a local farmer in Maha Sarakham Province, Thailand (GPS location; Latitude: 16.1842 Longitude: 103.3003 16° 10′ 38″ N, 103° 18′ 3″ E). Voucher numbers for collected samples are BIO012023 for green oak lettuce and BIO022023 for red oak lettuce and archived at Department of Biology, Mahasarakham University, Thailand.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Plant extraction
The lettuce leaves were finely chopped using scissors. The samples were dehydrated in a high-temperature oven set at 60℃ for a duration of 24 h, and subsequently pulverized into a fine powder using a mortar. The powder was subsequently submitted to Soxhlet extraction using either water or ethanol, while maintaining a temperature of 170℃ for a duration of 6 h. The extraction solution was evaporated using a rotary evaporator (Heidolph Instruments, Schwabach, Germany) under vacuum conditions. After being finished, the concentrated extract was subjected to a hot air oven at a temperature of 50 ℃ for a duration of 12 h in order to complete the drying process.

DPPH radical scavenging test
This assay was conducted as described by Ijoma1 et al. (2023).[7] The radical scavenging capacities of the lettuce extracts against the DPPH radical were conducted. One hundred µL of extract or standard solution was combined with 900 µL of 0.1 mM DPPH radical solution. The absorbance of the triplicate mixtures at 515 nm was recorded after 30 min of dark reaction using UV-Vis spectrophotometer (Thermo Fisher Scientific Inc. Waltham, MA, USA). The % inhibition was computed as follows:

$$\% \text{ Inhibition DPPH radical scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

All the chemicals used were obtained from Sigma-Aldrich, MO, USA.

ABTS radical cation scavenging activity
The ABTS test was used (Long and Halliwell, 2001).[8] One hundred µL of lettuce extracts were combined with 900 µL of ABTS•+ solution (prepared 0. 7 mM ABTS•+, 2.45 mM $K_2S_2O_8$ in distilled water reacted in darkness for 16 h) and allowed to react for 6 min. A_{734nm} readings were measured using UV-Vis spectrophotometer (Thermo Fisher Scientific Inc. Waltham, MA, USA) in triplicate using Trolox as a reference. Radical scavenging was computed using this equation:

$$\% \text{ Inhibition ABTS radical scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

All the chemicals used were obtained from Sigma-Aldrich, MO, USA.

Ferric reducing antioxidant power (FRAP) assay
The ferric reducing ability was performed according to the FRAP method as described by Ijoma1 et al. (2023).[7] FRAP reagent (900 µL) was combined with 100 µL ethanol aliquots of different concentrations of lettuce extracts and reacted for 15 min at 37 °C. The absorbance values of the resulting mixtures in triplicate were measured at 595 nm using UV-Vis spectrophotometer (Thermo Fisher Scientific Inc. Waltham, MA, USA). Trolox was used as a standard solution and the reducing power of the samples increased with the absorbance values. All the chemicals used were obtained from Sigma-Aldrich, MO, USA.

Determination of total phenolic content
Total phenolic content (TPC) was assayed as per Singleton et al. (1999).[9] One hundred µL of the lettuce extracts were combined with 500 µL of 0.2 N Folin-Ciocalteu reagent and 400 µL of 7.5% w/v Na_2CO_3 and reacted for 30 min. A765 nm was observed using UV-Vis spectrophotometer (Thermo Fisher Scientific Inc. Waltham, MA, USA). The results in triplicate were displayed as gallic acid equivalent (GAE) in mg per dry weight. All the chemicals used were obtained from Sigma-Aldrich, MO, USA.

Determination of total flavonoid content
As explained by Chang et al. (2002)[10], the aluminum chloride assay was conducted to measure total flavonoid concentration (TFC). The mixture of 100 µL lettuce extracts, 500 µL of 5% w/v $NaNO_2$, and 400 µL of 10% w/v $AlCl_3$ was reacted for 15 min. Triplicate absorbance measurements at 415 nm yielded quercetin equivalent (QE) in mg quercetin dry weight. All the chemicals used were obtained from Sigma-Aldrich, MO, USA.

Cancer cell lines and cell cultures
The human hepatocellular carcinoma (HepG2) cell line was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultivated in Dulbecco's Modified Eagle's Medium (DMEM) (Thermo Fisher Scientific, Inc. Waltham, MA, USA), supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Inc. Waltham, MA, USA) and penicillin, 100 µL/mL of streptomycin at 37 ºC under 5% CO_2. DMEM media for cell line culture were renewed every 2 to 3 days until 80% confluency was reached. Cells were washed with 10% phosphate-buffered saline (PBS), pH 7.2 before trypsinization with 0.25% Trypsin-EDTA. DMEM was added to the cell lines and colonies were counted before use.

Cytotoxicity test
The ATCC (Manassas, VA, USA) human liver cancer cell line (HepG2) was cultured in DMEM (Thermo Fisher Scientific Inc., Waltham, MA, USA) with 10% fetal bovine serum, penicillin, and 100 µL/mL streptomycin at 37 ºC under 5% CO_2 and the cytotoxicity of the lettuce extracts against HepG2 cells was tested using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as per Luang-In et al. (2021).[11] The cytotoxicity of plant extracts was measured as cell viability (%) [(A590nm) of treated cells)/(A590nm) of control cells × 100] and IC_{50} value.

Statistical analysis
One-way analysis of variance (ANOVA) was used to identify significant changes between treatment groups, and Duncan's multiple range test (DMRT) was used to compare amongst treatments at $p< 0.05$. The program IBM SPSS statistic version 26 (IBM, Armonk, NY, USA) was used.

Results and Discussion

Determination of total flavonoid and total phenolic content
It was shown that the total flavonoid content of red oak lettuce grown hydroponically was higher than organically grown lettuce, with ethanol proving a suitable solvent. Hydroponic systems yielded higher TFC levels, while organic systems exhibited higher TPC levels. Notably, the hydroponic system demonstrated a tendency to generate higher TFC compared to the organic system. The TFC values of organic green oak lettuce cultivars extracted with water and ethanol (0.74±0.014 and 1.47± 0.02 mg GAE/g DW, respectively) were lower than hydroponically grown lettuce (0.95±0.004 and 1.66±0.04 mg GAE/g DW, respectively) (Figure 1). Conversely, the highest TPC of water-extracted red oak lettuce was observed under the organic cultivation system and in green oak lettuce under the hydroponic system. The TPC of green oak lettuce cultivars from the organic farm extracted with water and ethanol (7.81±0.11 and 8.32±0.09 mg GAE/g DW, respectively) was higher than from the hydroponic farm (7.11±0.95 and 6.30± 0.09 mg GAE/g DW, respectively). Water was identified as an effective solvent for TPC (Figure 2). Hydroponically grown red oak lettuce exhibited the highest TFC, surpassing red oak lettuce from organic systems. The suitability of ethanol as a solvent for TFC was supported by this outcome. This discrepancy in TPC and TFC highlights the nuanced relationship between cultivation methods, solvents, and antioxidant profiles.[12] When tested with DPPH, water-extracted red oak lettuce from organic farms demonstrated the most effective antioxidant activity, closely followed by green oak lettuce under similar conditions. This result aligned with the TPC findings, emphasizing the importance of the cultivation environment. The TPC and antioxidant potential of red lettuce were stronger than green lettuce.[13] Because plant pigment has a certain quantity of phenolics.[14] Fertilizer systems were found to affect the phenolic profiles [15]. Therefore, the hydroponic system may increase total flavonoid content.[16] Significant biological activities are associated with flavonoids and other phenolic constituents due to their antioxidant properties, specifically their capacity to eliminate free radicals.[17] Plant nutrient composition, including secondary metabolites, may be influenced by fundamental distinctions between conventional and organic production methods, particularly in regard to soil fertility management.[17] One prevalent illustration is the consequence of nitrogen scarcity, which is recognized for its ability to stimulate flavonoid

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

synthesis. Fertilizers containing soluble inorganic nitrogen and other nutrients that are more readily accessible to plants are utilized in conventional production systems.[18]

Antioxidant activities

Notably, most organic lettuce extracts exhibited higher radical scavenging activities than lettuce grown under hydroponic cultivation, as determined by free-radical scavenging abilities against the 2,2-diphenylpicrylhydrazyl radical (DPPH•) and FRAP assays. The DPPH assay showed the most significant levels for evaluating antioxidant activity, followed by the ABTS and FRAP assays (Table 1). The ABTS assay results revealed that organic red oak lettuce extracted with ethanol exhibited the highest antioxidant activity, indicating that the choice of solvent significantly impacted antioxidant outcomes. The unique antioxidant activity profile observed in the organic ethanol extraction system, especially with FRAP testing, further emphasized the intricate relationship between cultivation practices, solvent selection, and antioxidant responses.[19] Results suggested an intriguing interplay between cultivation methods and antioxidant properties in oak lettuce extracts.[20] Water-extracted organic lettuce displayed the highest antioxidant effects[21], particularly evident in green oak lettuce. The analysis of antioxidants revealed that the DPPH and FRAP values were significantly higher in organically grown green and red lettuce cultivars compared to those produced hydroponically ($p< 0.05$). However, it is worth noting that green oak lettuce extracts obtained through ethanol extraction method and cultivated hydroponically exhibited a higher ABTS value than those developed organically. ABTS is insoluble in ethanol and DPPH and FRAP are probably more suitable for the extraction of lettuces.[22] Two cultivars from different growing conditions were subjected to DPPH, ABTS radical scavenging, and FRAP assay along with two extraction solvents to determine antioxidant activity. The analysis of hydroponic and organic farms revealed that the hydroponic lettuce exhibited markedly less antioxidant activity compared to lettuce from the organic farm. Consistent with this research, in Colombia, a country in South America, lettuce grown on soil exhibited higher levels of DPPH scavenging compared to lettuce grown in hydroponic systems, for both green and red lettuce varieties.[21] By contrast, lettuce from conventional cultivation recorded higher antioxidant activities in DPPH and FRAP tests than organic lettuce.[23] The antioxidant activities were high in the ABTS assay of hydroponically grown green and red lettuce because the quality of the free radical scavenger is different. Geneva et al. (2021)[24] reported that NPK is essential for lettuce growth and increases antioxidant capacity. They tested the FRAP assay but the DPPH assay showed less activity, while we found that DPPH showed the highest activity. NPK was shown to be essential for antioxidant activity.

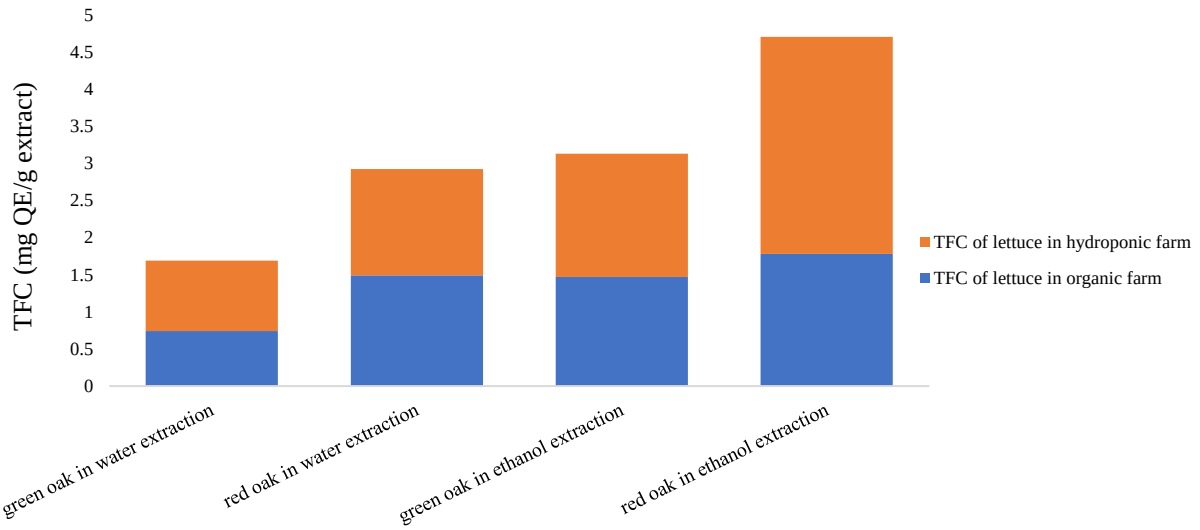

Figure 1: Total flavonoid content (TFC) of green and red oak lettuce cultivated in either an organic or a hydroponic farm and extracted with water and ethanol.

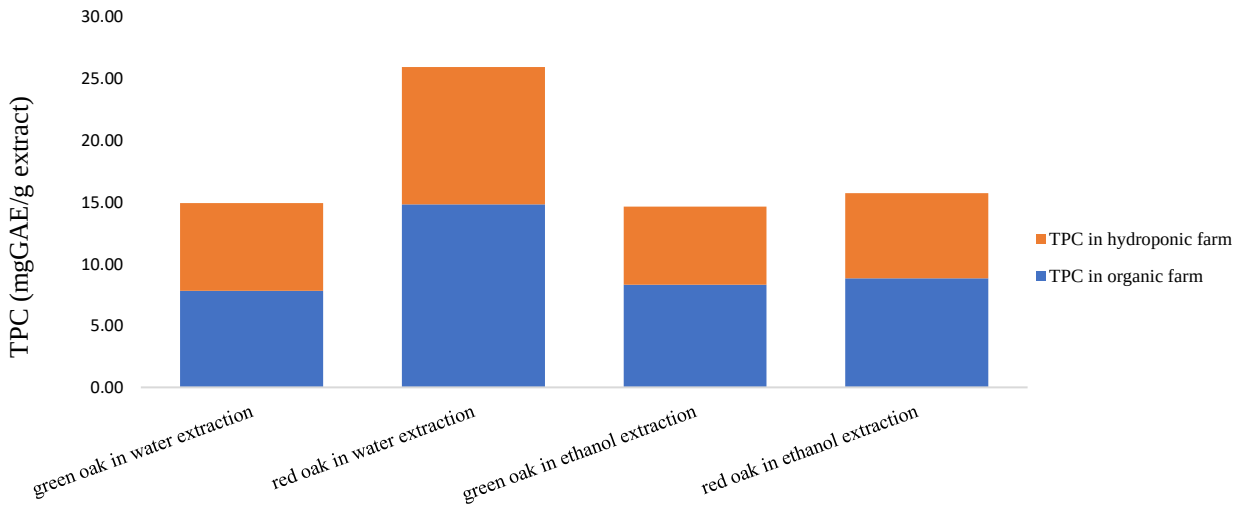

Figure 2: Total phenolic content (TPC) of green and red oak lettuce cultivated in either an organic or a hydroponic farm and extracted with water or ethanol.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Antioxidant activities of green oak lettuce and red oak lettuce extracts from an organic farm and a hydroponic farm

Farm type	Lettuce variety	Solvent	IC_{50} (mg/mL) DPPH assay	IC_{50} (mg/mL) ABTS assay	FRAP assay (mg Trolox/g extract)
Organic farm	Green oak lettuce	Water	0.08 ± 0.00^{a}	0.06 ± 0.00^{b}	3.52 ± 0.01^{d}
		Ethanol	0.47 ± 0.07^{g}	0.22 ± 0.09^{f}	4.71 ± 0.05^{b}
	Red oak lettuce	Water	0.12 ± 0.00^{b}	0.04 ± 0.00^{a}	2.983 ± 0.07^{c}
		Ethanol	0.13 ± 0.00^{c}	0.04 ± 0.00^{a}	6.71 ± 0.10^{a}
Hydroponic farm	Green oak lettuce	Water	0.23 ± 0.00^{e}	0.10 ± 0.00^{e}	1.33 ± 0.01^{e}
		Ethanol	0.74 ± 0.11^{h}	0.10 ± 0.00^{e}	4.66 ± 0.09^{b}
	Red oak lettuce	Water	0.17 ± 0.00^{d}	0.08 ± 0.00^{c}	0.89 ± 0.03^{f}
		Ethanol	0.39 ± 0.00^{f}	0.09 ± 0.00^{d}	3.92 ± 0.05^{b}

Values with different superscripts in each column are significantly different at $p < 0.05$

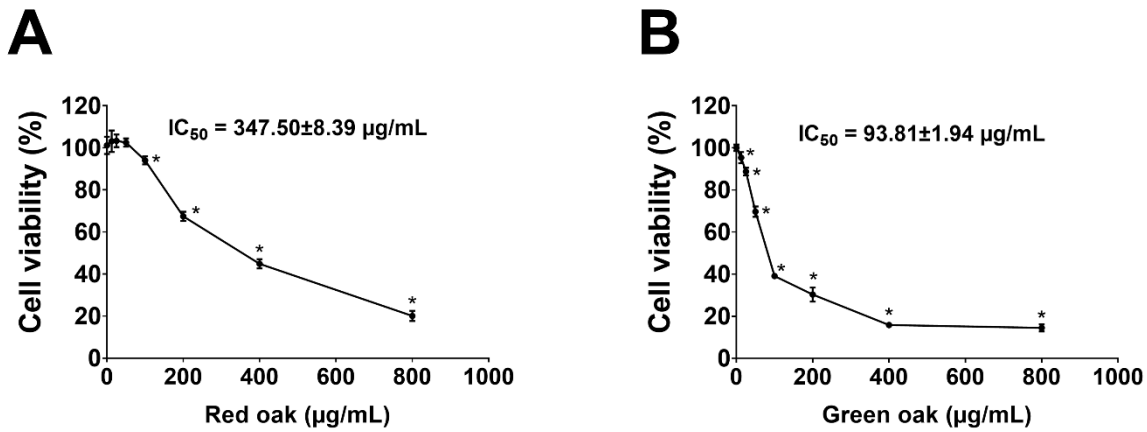

Figure 3: Cell viability of human liver Hep2 cancer cells treated with plant extract for 24 h. (A) Green oak extract. (B) Red oak extract. * Indicates significant differences from the control cells (no extract) ($p < 0.05$).

In addition, red oak lettuce extracted in water and also in alcohol had stronger radical scavenging activity than green oak lettuce[25]. Green oak lettuce showed the highest antioxidant activity for the DPPH assay. Red oak lettuce grown organically scored the highest for water extraction in the ABTS assay. By contrast, red lettuce scored the highest value of FRAP or ABTS.[26] Antioxidants are a large group of chemicals consisting of both polar and nonpolar compounds. Polar and non-polar molecules have different activities and their existence must be considered when selecting the appropriate solution. [27]

Cytotoxicity

The results showed a concentration-dependent decline in the viability of HepG2 cells, which was statistically significant ($p<0.05$). The red oak lettuce extract exhibited minimal cytotoxicity (IC_{50} = 347.50±8.39 µg/mL) against human liver Hep2 cancer cells (Figure 3A). The extract of green oak lettuce exhibited a higher level of cytotoxicity, resulting in reduced cell viability. This effect was observed as doses increased, with an IC_{50} equal to 93.81±1.94 µg/mL, indicating a moderate impact. This is illustrated in Figure 3B.

This higher cytotoxicity of green oak lettuce extract may be due to higher contents of particular specific compounds responsible for anticancer effects. Dark-colored chlorophyll pigments found in green lettuce varieties may protect against certain malignancies including colon and liver cancers. When comparing the cytotoxicity of the green oak lettuce extract with a recent report [28], this result showed effect of <20% cell viability at 0.8 mg/mL dose against HepG2 was stronger than the cytotoxic attribute of hydroponically and soil-cultivated lettuce of 1 mg/mL dose against HepG2, showing 28.6% cell viability and 33.2% cell viability, respectively. Our study results underscore the complexity of antioxidant properties in oak lettuce extracts, with cultivation methods and solvent choices playing pivotal roles. While water extraction in organic systems tended to enhance TPC and antioxidant effects, the hydroponic system gave higher TFC production. The antioxidant activity varied depending on the specific assay and the solvent used, emphasizing the need for a more comprehensive understanding of these factors when evaluating the overall antioxidant potential of oak lettuce extracts.

Conclusion

The hydroponic system with red oak lettuce efficiently enhanced TFC and the derived lettuce extracts inhibited cancer cell growth by decrease in the viability of HepG2 cells. This finding aligned with the general trend observed in TPC analysis, while using water as a solvent for TPC was more effective than ethanol. A new finding is the hydroponic cultivation system can stimulate the production of flavonoids and enhanced the anticancer attribute of lettuce extracts towards HepG2 cancer cells.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

This research project was financially supported by Mahasarakham University, Thailand.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

References

1. Shi M, Gu J, Wu H, Rauf A, Emran TB, Khan Z, Mitra S, Aljohani ASM, Alhumaydhi FA, Al-Awthan YS, Bahattab O, Thiruvengadam M, Suleria HAR. Phytochemicals, Nutrition, Metabolism, Bioavailability, and Health Benefits in Lettuce-A Comprehensive Review. Antioxidants (Basel). 2022; 11(6):1158. Doi: 10.3390/antiox11061158.
2. Zhou W, Liang X, Dai P, Chen Y, Zhang Y, Zhang M, Lu L, Jin C, Lin X. Alteration of Phenolic Composition in Lettuce (Lactuca sativa L.) by Reducing Nitrogen Supply Enhances its Anti-Proliferative Effects on Colorectal Cancer Cells. Int J Mol Sci. 2019; 20(17):4205. Doi: 10.3390/ijms20174205.
3. Temple NJ. Antioxidants and disease: more questions than answers. Nutr. Res. 2000; 20: 449-459.
4. López A, Javier GA, Fenoll J, Hellín P, Flores P. Chemical composition and antioxidant capacity of lettuce: Comparative study of regular-sized (Romaine) and baby-sized (Little Gem and Mini Romaine) types. J. Food Compos. Anal. 2014; 33(1):39-48. Doi: 10.1016/j.jfca.2013.10.001.
5. Velazquez-Gonzalez RS, Garcia-Garcia AL, Ventura-Zapata E, Barceinas-Sanchez JDO, Sosa-Savedra JC. A Review on Hydroponics and the Technologies Associated for Medium- and Small-Scale Operations. Agriculture. 2022; 12(5):646. Doi:10.3390/agriculture12050646.
6. Erharuyi O, Adhikari A, Falodun A, Jabeen A, Imad R, Ammad M, Choudhary MI, Gören N. Cytotoxic, Anti-inflammatory, and Leishmanicidal Activities of Diterpenes Isolated from the Roots of *Caesalpinia pulcherrima*. Planta Med. 2017; 83(1-02):104-110. Doi: 10.1055/s-0042-110407.
7. Ijoma KI, Ajiwe VIE, Odinma SC. The organic extracts from the leaves of *Ficus thonningii* Blume, *Jatropha tanjorensis* J.L Ellis and Saroja and *Justicia carnea* Lindley as potential nutraceutical antioxidants and functional foods. Trends Phytochem. Res. 2023;7:76-85.
8. Long LH, Halliwell B. Antioxidant and prooxidant abilities of foods and beverages. Meth. Enzymol. 2001; 335:181-190.
9. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth. Enzymol. 1999; 299:152-178.
10. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 2002; 10:178-182.
11. Luang-In V, Saengha W, Karirat T, Buranrat B, Matra K, Deeseenthum S, Katisart T. Effect of cold plasma and elicitors on bioactive contents, antioxidant activity and cytotoxicity of Thai rat-tailed radish microgreens. J Sci Food Agric. 2021; 101:1685-1698.
12. Liu X, Ardo S, Bunning M, Parry J, Zhou K, Stushnoff C, Stoniker F, Yu LL, Kendall, P. Total phenolic content and DPPH radical scavenging activity of lettuce (*Lactuca sativa* L.) grown in Colorado. LWT - Food Sci. Technol. 2007; 40:552-557.
13. Ozgen S, Sekerci S. Effect of leaf position on the distribution of phytochemicals and antioxidant capacity among green and red lettuce cultivars. Span. J. Agric. Res. 2011; 3: 801-809.
14. Falodun A, Irabor EE. Phytochemical, proximate, antioxidant and free radical scavenging evaluations of Calliandria surinamensis. Acta Pol Pharm. 2008; 65(5):571-575.
15. Sofo A, Lundegardh B, Martensson A, Manfra M, Pepe G, Sommella E, De Nisco M, Tenore GC, Campiglia P, Copa A. Different agronomic and fertilization systems affect polyphenolic profile, antioxidant capacity and mineral composition of lettuce. Sci. Hortic. 2016; 204:106-115.
16. Viacava GE, Roura SI, Berrueta LA, Iriondo C, Gallo B, Alonso-Salces RM. Characterization of phenolic compounds in green and red oak-leaf lettuce cultivars by UHPLC-DAD-ESI-QToF/MS using MSE scan mode. J. Mass Spectrom. 2017; 52:873-902.
17. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines (Basel). 2018; 5:93. Doi: 10.3390/medicines5030093.
18. Romani A, Pinelli P, Galardi C, Sani G, Cimato A, Heimler D. Polyphenols in greenhouse and open-air-grown lettuce. Food Chem. 2002; 79:337-342.
19. Szulc P, Krauklis D, Ambroży-Deręgowska K, Wróbel B, Zielewicz W, Niedbała G, Kardasz P, Niazian M. Evaluation of the effect of conventional and stabilized nitrogen fertilizers on the nutritional status of several maize cultivars (*Zea mays* L.) in critical growth stages using plant analysis. Agronomy. 2023; 13:480. Doi :10.3390/agronomy13020480.
20. Kimura M, Rodriguez-Amaya DB. Carotenoid composition of hydroponic leafy vegetables. J. Agric. Food Chem. 2003; 51:2603-2607.
21. Zapata-Vahos IC, Rojas-Rodas F, David D. Gutierrez-Monsalve JA, Castro-Restrepo D. Comparison of antioxidant contents of green and red leaf lettuce cultivated in hydroponic systems in greenhouses and conventional soil cultivation. Rev. Fac. Nac. Agron. 2020; 73:9077-9088.
22. Durazzo A, Azzini E, Lazzé MC, Raguzzini A, Pizzala R, Maiani G, Palomba L, Maiani G. Antioxidants in Italian head lettuce (*Lactuca sativa* var. *capitata* L.) grown in organic and conventional systems under greenhouse conditions. J. Food Biochem. 2013; 38:56–61.
23. Arina AN, Azrina A. Comparison of phenolic content and antioxidant activity of fresh and fried local fruits. Int. Food Res. J. 2016; 23:1717.
24. Czech A, Szmigielski M, Sembratowicz I. Nutritional value and antioxidant capacity of organic and conventional vegetables of the genus Allium. Sci Rep. 2022; 12:18713. Doi: 10.1038/s41598-022-23497-y.
25. Geneva M, Kostadinov K, Filipov S, Kirov, E, Stancheva I. Analysis of the antioxidant capacity of lettuce growth at different fertilizer regimes. C.R. Acad. Bulg. Sci. 2021; 74: 145-154.
26. Llorach R, Martínez-Sánchez A, Tomás-Barberán FA, Gil MI, Ferreres F. Characterization of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem. 2008; 108:1028-1038.
27. Kim MJ, Moon Y, Tou JC, Mou B, Waterland NL. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). J. Food Compos. Anal. 2016; 49:19-34.
28. Naseem S, Ismail H. *In vitro* and *in vivo* evaluations of antioxidative, anti-Alzheimer, antidiabetic and anticancer potentials of hydroponically and soil grown *Lactuca sativa*. BMC Complement. Med. Ther. 2022; 22:30. Doi :10.1186/s12906-022-03520-5.