PHYTOCHEMICAL AND BIOLOGICAL STUDIES OF *Hellenocarum amplifolium* GROWN WILDLY IN SULAYMANIYAH, IRAQ

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ABSTRACT

This study investigated the phytochemical composition and biological activities of *Hellenocarum amplifolium* from four regions in Sulaymaniyah Province, Iraqi Kurdistan: Hawraman, Qandil, Penjwin, and Sharbazir. The plant specimens were harvested during the pre-flowering phase, specifically between April and May 2021. Chemical analysis revealed that the Hawraman samples contained the highest levels of phenolics, flavonoids, and bioactive compounds (e.g., gallic acid, quercetin, kaempferol), while Qandil samples were richest in essential oils (e.g., linalool, limonene, α-pinene). Hawraman extracts exhibited the strongest antioxidant activity and provided significant protection against ochratoxin A-induced cytotoxicity. The extract also demonstrated notable antibacterial effects against *Escherichia coli* at 5000 µg/mL, surpassing some reference antibiotics. These findings highlight *H. amplifolium* as a promising source of antioxidative and antimicrobial compounds. The study aimed to profile the plant's phytochemicals, evaluate its antioxidant and antibacterial activities, and assess its protective effects against OTA-induced cytotoxicity.

Keywords: Phenols, Volatile oils, Antioxidant, Nephroprotection, Ochratoxin A.

دراسة كيميائية وبيولوجية لل *Hellenocarum amplifolium* النامي بريا في السليمانية، العراق

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المستخلص

بحثت هذه الدراسة التركيب الكيميائي النباتي والفعالية البايولوجية لنبات *Hellenocarum amplifolium* الذي جُمع من أربع مواقع جغرافية ضمن محافظة السليمانية في إقليم كردستان العراق، وهي: هورامان، قنديل، بنجوين، وشاربازير، وذلك خلال الفترة السابقة لمرحلة الإزهار (أبريل–مايو 2021). أظهرت التحاليل الكيميائية أن عينات هورامان احتوت على أعلى تركيز من المركبات الفينولية الكلية والفلافونويدات الكلية، بما في ذلك مركبات فعالة كيميائيًا مثل :حمض الغاليك (Gallic Acid)، حمض التانيك (Tannic Acid)، كويرسيتين (Quercetin)، حمض الفيريوليك (Ferulic Acid)، كامبفيرول (Kaempferol)، وإيمودين (Emodin). بينما كانت عينات قنديل الاغنى بالزيوت الطيارة مثل :لينالول (Linalool)، سيمين (Symin)، ليمونين (Limonene)، تربينين (Terpinene)، كامفور (Camphor)، ألفا–بينين (α–Pinene)، وكامفين (Camphene). أظهرت مستخلصات هورامان اقوى نشاط مضاد للأكسدة، كما قدمت حماية ملحوظة ضد السُمية الخلوية الناتجة عن أوكراتوكسين A. كما أظهرت هذه المستخلصات تأثيرا مضادا للبكتريا، حيث أدى تركيز 5000 ميكروغرام/مل إلى تثبيط نمو *Escherichia coli* بشكل ملحوظ متفوقا على بعض المضادات الحيوية التقليدية المستخدمة. تشير هذه النتائج إلى أن نبات *Hellenocarum amplifolium* مصدر واعد للمركبات المضادة للأكسدة والمضادة للميكروبات. وهدفت الدراسة إلى تحديد التركيب الكيميائي للنبات، تقييم نشاطه المضاد للأكسدة والبكتيريا، ودراسة تأثيراته الوقائية ضد السمية الخلوية الناتجة عن OTA .

الكلمات المفتاحية: نبات بارازا، الفينولات، الزيوت الطيارة، مضادات الأكسدة، أوكراتوكسين A.

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INTRODUCTION

The Apiaceae family formerly classified as *Umbelliferae* is considered one of the most significant families among angiosperms, comprising approximately 3,800 species distributed across 466 genera (23). This botanical family is renowned for its extensive repertoire of bioactive phytochemicals, including but not limited to terpenoids, saponins, flavonoids, coumarins, polyacetylenes, and steroidal compounds, many of which have demonstrated promising pharmacological potential. Moreover, numerous species within Apiaceae are prominent sources of essential oils, from which over 760 structurally diverse constituents with notable medicinal relevance have been isolated and characterized (25). One notable member of this family is *Hellenocarum amplifolium*, a geographically restricted species whose native range extends from eastern parts of southern Europe through western Türkiye and Iran to the northeastern territories of Iraq (31). Locally referred to as "Baraza" in the Kurdistan region of Iraq, the plant is traditionally consumed in its entirety as a vegetable in both fresh salads and cooked dishes. It also holds considerable importance in folk medicine, where it is employed to relieve toothache, combat halitosis, and manage urinary tract disorders, including the removal of kidney stones and cleansing of the renal system. Despite its widespread ethnomedicinal use, there remains a pressing need for scientific validation to substantiate these traditional claims (25). Among the key threats to food safety and public health in such regions is Ochratoxin A (OTA), a potent mycotoxin synthesized by fungal genera *Aspergillus* and *Penicillium* (15). These fungi are ubiquitous in stored food commodities and pose risks to several physiological systems, notably the hepatic, renal, immune, reproductive, and developmental systems (13). OTA contamination is particularly problematic in staple crops, especially under poor storage conditions conducive to fungal proliferation. In a recent investigation conducted in Sulaymaniyah Province, Al-Nashi (2) identified *Penicillium*, *Aspergillus*, and *Rhizopus* as the dominant fungal genera contaminating wheat flour samples, especially during the humid months of November and December. Ochratoxins are classified into three major types of OTA (Ochratoxins A), OTB (Ochratoxin B), and OTC (Ochratoxin C) (16). Human exposure to OTA occurs primarily via ingestion of plant-derived foodstuffs contaminated with the toxin and secondarily through the consumption of animal products derived from livestock fed OTA-contaminated feed (24, 17). The International Agency for Research on Cancer (IARC) has designated OTA as a Group 2B carcinogen, indicating it is *carcinogenic to humans* based on sufficient evidence of carcinogenicity in experimental animals (1). Accordingly, the present study was designed to analyze the phytochemical constituents of *Hellenocarum amplifolium* collected from different regions within Sulaymaniyah Province and to assess its antioxidant and antimicrobial properties using standardized biological assays.

MATERIALS AND METHODS

Plant Material Collection and Authentication: Fresh, whole-plant specimens of *Hellenocarum amplifolium* were systematically and randomly harvested prior to the onset of the flowering phase, specifically between late April and May 2021. The sampling was conducted across four ecologically distinct sites within Sulaymaniyah Governorate, located in the northeastern part of the Kurdistan Region, Iraq: Hawraman, Sharbazher, Penjwin, and Qandil. Following collection, the botanical identity of the plant material was taxonomically confirmed by experts at the Iraqi National Herbarium, affiliated with the Ministry of Agriculture in Baghdad, Republic of Iraq. This verification ensured accurate species identification and compliance with taxonomic standards.

Table 1. Physical and chemical characteristics of the soil of the locations under study.

Locations	N	P	K	CaCO3	O.M	Sand	Clay	Silt	S. T	E.C	pH	Ca	Mg	Na	Cl^-	HCO_3
	(mg/kg soil)			(g/kg soil)		(g/kg soil)				(dSm^{-1})		(mg/L-1)				
Hawraman	129	12.3	232	67.3	64.3	404	88	508	Silt loam	1.9	7.3	2.1	1.2	2.6	5.3	1.5
Sharbazher	98	14.2	232	253.1	55.3	424	88	488	Loam	0.7	8.1	9.3	5.3	1.6	6.3	2.3
Penjwin	123	12.6	214	310.2	64.5	544	148	308	Sandy loam	0.7	8.2	8.2	2.1	1.3	5.2	2.6
Qandil	123	12.1	112	40.8	61.3	324	148	528	Silt loam	0.4	8.2	2.4	1.0	1.4	3.5	1.4

*** Soil analyses were conducted in the Central Laboratory, College of Agricultural Engineering Sciences, University of Baghdad.**

*** Abbreviations: N, Nitrogen; P, Phosphor; K, Potassium; CaCO3, calcium carbonate equivalent; OM, Organic matter; S.T, Soil texture; EC, soil electrical conductivity; Ca, Calcium; Mg, Magnesium; Na, Sodium; Cl, Chloride ion; HCO3, Bicarbonate Ion**

Table 2. Averages of temperature (^oC), rainfall (mm), and relative humidity (%) of the locations under study from January-April 2021

Location	Variable	Month				Average
		January	February	March	April	
Hawraman	Temperature ^OC	8.1	10.6	13.7	21.7	13.52
	Rain mm	97.8	101.2	81	53.5	83.375
	Relative Humidity %	50.5	49.6	52.1	31.2	45.85
Sharbazher	Temperature ^OC	13.1	14.8	19.5	21.7	17.27
	Rain mm	68.4	71.4	40.4	10.7	47.725
	Relative Humidity %	51.6	49.6	41.3	28.6	38.98
Penjwin	Temperature ^OC	5.2	9.5	17.4	23.4	13.87
	Rain mm	165.5	145.5	101.7	75.8	122.125
	Relative Humidity %	51.6	48.1	48.2	44.6	48.12
Qandil	Temperature ^OC	7.1	11.7	18.6	22.8	15.05
	Rain mm	192.5	117.4	118.4	97.3	131.4
	Relative Humidity %	58.7	52.9	55.5	43.8	52.72

Preparation and Extraction of *Hellenocarum amplifolium:* Freshly collected specimens of *Hellenocarum amplifolium* were initially washed with distilled water to remove any adhered debris and then subjected to shade-drying at an ambient temperature of approximately 28 °C for a period of ten days. Once completely desiccated, the plant material was finely ground using an electric mill to obtain a homogeneous powder. The powdered samples were subsequently stored in airtight, opaque containers at 4 °C to prevent photodegradation and preserve their phytochemical stability. For the extraction procedure, 1.0 gram of the powdered plant material was accurately weighed and transferred into a clean, sterile glass tube. Ten milliliters of 80% ethanol (v/v) were added as the extraction solvent. The mixture was incubated in a temperature-controlled water bath at 38 °C for two hours, with manual shaking every 20 minutes to facilitate the solubilization of bioactive constituents. After incubation, the samples were left undisturbed at room temperature (~25 °C) for an additional 22 hours to allow complete diffusion. The mixture was then filtered using Whatman No.1 filter paper, and the resulting filtrate was collected and stored at −20 °C until subjected to further phytochemical and bioactivity analyses (6).

Phytochemical Analyses Determination of Total Phenolic Content (mg GAE/g DW): The total phenolic content (TPC) of *Hellenocarum amplifolium* whole-plant ethanolic extracts was quantitatively assessed utilizing the Folin–Ciocalteu colorimetric method as described by Alsaloom (3) with slight modifications. An aliquot of 100 µL of each extract was transferred into clean glass test tubes, followed by the addition of 4.0 mL of 10% Folin–Ciocalteu reagent. The mixture was gently vortexed and allowed to stand at room temperature (~25 °C) for 5 minutes to permit initial reaction. Subsequently, 2.0 mL of 20% sodium carbonate solution (Na_2CO_3, w/v) was added to neutralize the reaction. The resulting mixture was incubated in the dark at 38 °C for 60 minutes, with periodic shaking to facilitate full color development. For blank controls, 100 µL of distilled water was used in place of the plant extract, while all other conditions remained constant. After incubation, the absorbance of the blue chromophore was measured at a wavelength of 765 nm using a Bio-Tek FLx800 Fluorescence

Microplate Reader (Bio-Tek Instruments, USA). A standard calibration curve was generated using gallic acid solutions prepared at a series of concentrations ranging from 0.1 to 1.0 mg/mL (i.e., 0.1, 0.2, ..., 1.0 mg/mL). The TPC for each sample was calculated based on the linear regression equation derived from this curve and expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g DW) of the sample.

Determination of Total Flavonoid Content (mg QE/g DW): The total flavonoid content (TFC) of the ethanolic extract derived from *Hellenocarum amplifolium* was quantitatively assessed following standardized colorimetric protocols with slight adaptations (5). A quercetin stock solution was initially prepared by dissolving 100 mg of quercetin in 100 mL of distilled water. This solution was serially diluted to obtain a calibration range of 0.05 to 0.50 mg/mL, comprising ten concentrations (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50 mg/mL). For the assay, 0.5 mL of the plant extract was dispensed into a test tube and mixed with 1.5 mL of 80% ethanol. Subsequently, 0.1 mL of 10% aluminum chloride ($AlCl_3$, w/v) and 0.1 mL of 1 M potassium acetate (CH_3COOK) were added sequentially. The final volume was adjusted by the addition of 2.8 mL of distilled water. The mixture was then incubated at room temperature (~25 °C) for 45 minutes in the absence of direct light to allow for the complete development of the chromogenic complex. A reagent blank was prepared under identical conditions, substituting the plant extract with distilled water. The absorbance of the resulting yellow complex was measured at a wavelength of 415 nm using a Bio-Tek FLx800 Fluorescence Microplate Reader (Bio-Tek Instruments, USA). A standard quercetin calibration curve was employed for quantification. The results were expressed as milligrams of quercetin equivalents per gram of dry sample weight (mg QE/g DW).

Determination of Antioxidant Activity Using the DPPH Radical Scavenging Assay: The antioxidant activity of *Hellenocarum amplifolium* ethanolic extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following previously established protocols with minor modifications (3). A stock solution of DPPH was freshly prepared by dissolving 2.4 mg of the radical compound in 100 mL of 95% methanol to yield a stable purple solution. For the assay, 10 mL of the plant extract was added to 3.995 mL of the prepared DPPH solution in a glass tube. The mixture was vortexed for uniform blending and then incubated in the dark at room temperature (~25 °C) for 30 minutes to prevent photo-degradation of the radical species. A blank control was prepared in parallel by mixing 10 mL of 95% methanol with 3.995 mL of the DPPH solution, omitting the extract. After the incubation period, the decrease in absorbance reflecting radical scavenging was measured at a wavelength of 515 nm using a Bio-Tek FLx800 ELISA microplate reader. The percentage inhibition of DPPH free radicals was calculated using the following equation:

% Inhibition = [(Absorbance of Control – Absorbance of Sample) / Absorbance of Control] × 100

where $A_{control}$ represents the absorbance of the blank solution and A_{sample} denotes the absorbance of the extract-treated mixture.

Extraction and Quantification of Phenolic Compounds Using RP-HPLC: The extraction of phenolic compounds from *Hellenocarum amplifolium* was performed using 3.0 grams of finely homogenized plant tissue. The material was subjected to solvent extraction using a hydroethanolic mixture (70:30 v/v ethanol: water). The extraction process was facilitated via ultrasonic-assisted extraction (UAE) in a bath sonicator operated at room temperature (~25 °C) for 60 minutes to enhance the release of bound phenolics. Post-extraction, the mixture was filtered through Whatman No.1 filter paper, and 5.0 mL of the filtrate was collected to estimate extraction yield. The remaining filtrate was concentrated under reduced pressure using a rotary evaporator, followed by drying at 38 °C until a constant mass was achieved. The resulting dry extracts were stored in airtight amber glass vials at 4 °C to prevent oxidative degradation prior to chromatographic analysis. Quantitative profiling of individual phenolic constituents was conducted using reversed-phase high-performance liquid chromatography (RP-HPLC). The

instrumentation included a SYKAMN HPLC system equipped with a UV detector, controlled via ChemStation software, and integrated with a Zorbax Eclipse Plus C18-OSD column (dimensions: 25 cm × 4.6 mm, particle size not specified). The column temperature was held constant at 30 °C. Chromatographic separation was achieved using a binary gradient elution system, with methanol (eluent A) and 1% formic acid in water (eluent B) as the mobile phases. The gradient program was configured as follows:

a- 40% eluent B from 0 to 4 minutes.

b- 50% eluent B from 5 to 10 minutes.

The flow rate was maintained at 0.7 mL/min, and the injection volume for both standards and samples were 100 µL, delivered via an automated autosampler. Detection of phenolic compounds was performed at a wavelength of 280 nm, as described in the protocol reported by Radovanović et al. (22).

Extraction and Quantification of Anthraquinone Compounds via HPLC: To isolate anthraquinone derivatives from *Hellenocarum amplifolium*, 100 mg of finely powdered plant material was extracted using 20 mL of 70% methanol under reflux conditions for 60 minutes. The resulting extract was subsequently filtered to remove solid residues and concentrated under reduced pressure using a rotary evaporator. The residue was then reconstituted in methanol to a final volume of 5.0 mL. To prevent degradation or chemical alteration of heat- or light-sensitive constituents, all extracts were subjected to chromatographic analysis immediately following preparation. Each sample was processed in triplicate to ensure analytical repeatability and data reliability. Chromatographic analysis was carried out using a SYKAM HPLC system (Germany) equipped with a photodiode array detector (PDA) and an autosampler. The separation of anthraquinone compounds was performed on a reversed-phase ODS-18 column (25 cm × 4.6 mm, Japan), maintained at a constant temperature of 25 °C. The mobile phase consisted of a mixture of 2% methanol in aqueous acetic acid solution (70:30, v/v), delivered at an isocratic flow rate of 1.0 mL/min. Sample injections were set at a volume of 50 µL, and the detection of anthraquinone peaks was conducted at a wavelength of 254 nm, as per the analytical procedure described by Tan et al. (28).

Determination of Volatile Oil Content (%)

To determine the volatile oil yield, 10 grams of *Hellenocarum amplifolium* powdered plant material were accurately weighed and transferred into a round-bottom distillation flask. To this, 100 mL of distilled water was added, and the mixture was subjected to hydro-distillation using a Clevenger-type apparatus for a continuous period of three hours. This process allowed for the extraction and collection of volatile oil components via steam. Upon completion of the distillation, 20 mL of hexane was added to the aqueous distillate to extract the essential oil fraction. The organic (hexane) layer, containing the dissolved volatile oils, was carefully separated from the aqueous phase, and collected into a clean glass vial. The volatile oil yield was calculated using the following equation:

Volatile Oil Yield (%) = (Volume of Oil (mL) / Weight of Plant Sample (g)) × 100

The extracted oil was then stored at refrigeration temperature (4 °C) in sealed amber vials to prevent oxidation and degradation prior to further compositional analysis using gas chromatography (GC) as described by Wan et al. (29).

Analysis of Volatile Oil Compounds by Gas Chromatography (GC-FID): The compositional analysis of volatile oil constituents extracted from *Hellenocarum amplifolium* was performed at the Department of Environment and Water, affiliated with the Ministry of Science and Technology, Baghdad, Iraq. The analysis was conducted using a Shimadzu GC-2010 gas chromatograph (Shimadzu Corporation, Japan), equipped with a Flame Ionization Detector (FID) and a DM-5Ms capillary column of dimensions 30 m × 0.25 mm internal diameter × 0.25 µm film thickness. The injection port and detector temperatures were maintained at 280°C and 340°C, respectively. The oven temperature program was set to initiate at 100°C, then increased gradually to 300°C at a heating rate of 10°C per minute, ensuring optimal separation of oil constituents. High-purity nitrogen gas (N_2) was used as the carrier gas, operated at a

constant pressure of 100 kPa, providing an inert atmosphere and stable flow conditions throughout the chromatographic run (29).

Antimicrobial Susceptibility Testing of *Hellenocarum amplifolium* Ethanolic Extract: The antimicrobial activity of the ethanolic extract of *Hellenocarum amplifolium* was assessed using the Kirby–Bauer disc diffusion method on Mueller Hinton agar (MHA) (11), following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI), as detailed in the latest M100 document. The assay aimed to evaluate the inhibitory effects of various extract concentrations against *Escherichia coli* (9). Three well-isolated colonies of *E. coli* were suspended in 4 mL of sterile normal saline to prepare the bacterial inoculum, which was then uniformly spread on the surface of MHA plates. Sterile blank paper discs were impregnated with different concentrations of the plant extract (675, 1250, 2500, and 5000 µg/mL) and left to air-dry under aseptic conditions. After drying, the extract-loaded discs were placed onto the inoculated agar surfaces. In parallel, eight reference antibiotic discs were used as positive controls, including:

1- Piperacillin (PRL; 30 µg)
2- Novobiocin (NV; 30 µg)
3- Bacitracin (B; 10 µg)
4- Ciprofloxacin (CIP; 10 µg)
5- Ceftriaxone (CRO; 10 µg)
6- Gentamicin (GNT; 10 µg)
7- Erythromycin (ERY; 10 µg)
8- Amoxicillin (AMOX; 30 µg)

The diameter of the inhibition zones surrounding each disc was measured in millimeters (mm) after appropriate incubation, and the results were compared to the inhibition profiles of the standard antibiotics. All experiments were performed in triplicate to ensure reproducibility and statistical validity.

Protective Effect of *Hellenocarum amplifolium* Against Ochratoxin A Induced Nephrotoxicity in Male Rats: A total of twenty-five healthy male albino rats (*Rattus norvegicus*), weighing between 130 and 150 grams, were utilized in this experimental study. The animals were maintained under standardized laboratory conditions, including a temperature-controlled environment (22–25 °C) and a 12-hour light/dark photoperiod. Throughout the experimental period, animals had unrestricted access to food and water. Prior to treatment, rats were acclimatized for two weeks and fed a basal control diet formulated according to the protocol described by Amin et al. (4) noting that dietary protein levels can significantly influence growth and physiological performance in rodents (6). which included: 50% sucrose, 20% casein, 15% corn starch, 5% fiber, 5% corn oil, 3.5% mineral mixture, 1% vitamin mix, 0.3% DL-methionine, and 0.2% choline bitartrate. Following the acclimatization period, the animals were randomly assigned into five experimental groups (n = 5 per group), and treated as follows:

a- Group 1 (Control): Received only the basal diet without any additional treatment.
b- Group 2: Received the control diet with OTA at a dose of (10 µg/mL) administered orally by gavage once daily for four weeks.
c- Group 3: Received the control diet with OTA at a dose of (10 µg/mL) in addition to 0.5 mL of *H. amplifolium* ethanolic extract containing 10 mg/mL, administered daily via oral gavage for 4 weeks.
d- Group 4: Received the control diet with OTA at a dose of (10 µg/mL) in addition to 0.5 mL of *H. amplifolium* ethanolic extract containing 20 mg/mL, administered daily via oral gavage for 4 weeks.
e- Group 5: Received the control diet with OTA at a dose of (10 µg/mL) along with 0.5 mL of α-tocopherol at a dose of 0.02 mg/mL, by oral gavage for 4 weeks.

Throughout the study period, all animals were closely monitored for behavioral changes, signs of systemic toxicity, and mortality rates. Observations were recorded to evaluate the protective potential of *H. amplifolium* extract in mitigating OTA-induced nephrotoxic effects.

Histological Assessment of Renal Tissues

Histopathological evaluation of kidney tissues was performed in accordance with previously established protocols. At the end of the experimental period, rats were humanely sacrificed, and kidney specimens were promptly excised and rinsed in ice-cold normal saline to remove residual blood. The tissues were then fixed in 10% neutral-buffered formalin for a minimum of 24 hours to

preserve morphological integrity. Following fixation, the kidney samples were subjected to standard paraffin embedding procedures. Thin sections of 4–5 µm thickness were prepared using a rotary microtome, mounted on glass slides, and subsequently stained with hematoxylin and eosin (HandE). The stained slides were examined under a light microscope to assess structural alterations, including glomerular integrity, tubular morphology, vascular congestion, inflammatory infiltration, and necrotic lesions. Histological findings were compared across all experimental groups to evaluate the extent of OTA induced renal damage and the potential protective effects conferred by *Hellenocarum amplifolium* extract and α-tocopherol.

Statistical Analysis: All statistical evaluations were performed using XLSTAT software, version 2021.3.1. For the phytochemical parameters, data were analyzed under a Completely Randomized Design (CRD), considering the geographical location as the primary experimental factor. Each treatment was replicated five times to ensure statistical robustness. For the datasets related to biological activities including antioxidant, antimicrobial, and protective assays a one-way analysis of variance (ANOVA) was conducted within the CRD framework to determine the presence of significant treatment effects. Where significant F-values were observed, Duncan's Multiple Range Test (DMRT) was applied to compare the means, with statistical significance accepted at a probability level of $P \leq 0.05$. Results were reported as mean ± standard deviation (SD) unless otherwise stated.

RESULTS AND DISCUSSION

Total Phenolic and Flavonoid Contents, and Antioxidant Capacity of *Hellenocarum amplifolium:* As summarized in Table 3, the ethanolic extracts of *Hellenocarum amplifolium* collected from the Hawraman region demonstrated significantly higher concentrations of total phenolic compounds (140.08 mg/g) and total flavonoids (24.94 mg/g), along with the highest recorded antioxidant activity (83.66%) among all sampled locations. This pronounced accumulation of bioactive compounds may be attributed to the distinct ecological attributes of the Hawraman area, particularly its elevated altitude (~1733 meters above sea level). High-altitude environments are characterized by lower ambient temperatures and increased exposure to Ultraviolet-B (UV-B) radiation, both of which represent critical abiotic stressors capable of modulating plant secondary metabolism. Such environmental stress conditions are believed to upregulate the enzymatic activity of phenylalanine ammonia-lyase (PAL) a key regulatory enzyme at the entry point of the phenylpropanoid biosynthetic pathway. PAL catalyzes the deamination of phenylalanine to trans-cinnamic acid, a precursor to a wide range of phenolic and flavonoid compounds. The elevation in PAL activity under stress-induced conditions consequently promotes enhanced biosynthesis of antioxidant metabolites, thereby strengthening the plant's intrinsic defensive mechanisms (32). Furthermore, it has been reported that cooler climatic conditions are favorable for the accumulation of phenolic constituents, whereas elevated temperatures may compromise their stability by inducing the breakdown of thermally labile bonds within phenolic structures (21). Overall, the observed spatial variability in the levels of phenolic and flavonoid compounds, as well as antioxidant efficacy, across different geographical origins of *H. amplifolium* may be influenced by a combination of environmental parameters, including light intensity, temperature gradients, precipitation patterns, soil pH, electrical conductivity (EC), and nutrient availability. These factors are known to affect both photosynthetic efficiency and the biosynthetic capacity for secondary metabolites in medicinal and aromatic plants (20).

Table 3. Concentration of total phenol contents (mg/g), total flavonoid contents (mg/g), and antioxidant activity (%) of *H. amplifolium* collected from different locations.

Location	Total Phenols	Total Flavonoids	Antioxidant Activity
Hawraman	140.08 a	24.94 a	83.66 a
Sharbazher	125.01 b	23.58 a	79.80 b
Penjwin	138.30 a	23.25 a	74.50 c
Qandil	122.05 c	23.87 a	80.30 b

***Different letters within each column indicate statistically significant differences between mean values**

Influence of Soil Characteristics in Hawraman on Phenolic Accumulation : The soil composition in the Hawraman region is

notably rich in organic matter, a factor that exerts a considerable impact on the physicochemical properties of the soil, particularly enhancing the bioavailability and uptake of essential macro- and micronutrients by plants. According to findings by Leskovar and Othman (18), increased organic matter content contributes to improved soil porosity, which in turn reduces bulk density and promotes aerobic microbial colonization within the rhizosphere. The resulting enhancement in microbial activity accelerates the decomposition of organic substrates, thereby facilitating the release and mineralization of key nutrients required for plant growth and secondary metabolism. Relative to the other sampled sites, the soil in Hawraman also displayed the highest nitrogen content, which plays a pivotal role in the biosynthesis of phenylalanine an aromatic amino acid that serves as a fundamental precursor in the phenylpropanoid pathway. This pathway is responsible for the production of a wide array of phenolic metabolites, including flavonoids, tannins, and related antioxidant compounds (14). Therefore, the elevated nitrogen availability in Hawraman soils is likely to contribute directly to the observed increase in total phenolic content in *Hellenocarum amplifolium* specimens from this region.

HPLC Analysis of Phenolic Compounds in *Hellenocarum amplifolium:* The qualitative and quantitative profiling of phenolic compounds in *Hellenocarum amplifolium*, as determined by High-Performance Liquid Chromatography (HPLC), is summarized in Table 4. The results revealed significant variations in compound concentrations across the different geographical origins. Specimens collected from the Penjwin region exhibited the highest concentrations of several key phenolic constituents, including Gallic acid (78.91 µg/g), Tannic acid (55.37 µg/g), Quercetin (90.10 µg/g), Ferulic acid (19.77 µg/g), and Kaempferol (17.37 µg/g). In contrast, plant samples obtained from Sharbazher demonstrated the lowest levels of these respective compounds, recording values of 59.13, 36.80, 74.43, 11.40, and 10.90 µg/g, respectively. Interestingly, the concentration trend for Emodin deviated from that of the other phenolics. The highest Emodin content (97.77 µg/g) was detected in extracts from Sharbazher, while the lowest level (74.50 µg/g) was recorded in samples from Penjwin. Among all phenolic compounds analyzed, Emodin emerged as the predominant constituent, followed by Quercetin, suggesting their major contribution to the overall antioxidant and bioactive potential of the species.

Table 4. HPLC analysis of phenolic compound of *H. amplifolium* (µg/g). collected from different locations.

Location	Gallic acid (µg/g)	Tannic acid (µg/g)	Quercetin (µg/g)	Ferulic acid (µg/g)	Kaempferol (µg/g)	Emodin (µg/g)
Hawraman	**62.50 b**	**42.90 b**	**88.93 b**	**13.63 b**	**14.80 b**	**89.80 b**
Sharbazher	**59.13 c**	**36.80 d**	**74.43 d**	**11.40 d**	**10.90 d**	**97.77 a**
Penjwin	**78.91 a**	**55.37 a**	**90.10 a**	**19.77 a**	**17.37 a**	**74.50 d**
Qandil	**60.11 c**	**40.20 c**	**82.93 c**	**12.70 c**	**14.03 c**	**80.60 c**

***Different letters within each column indicate statistically significant differences between mean values**

Volatile Oil Yield and GC-Based Profiling of Essential Oil Components in *Hellenocarum amplifolium:* The volatile oil content and the compositional analysis of *Hellenocarum amplifolium* essential oil, as determined by gas chromatography (GC), are detailed in Table 5. The data revealed statistically significant variations in volatile oil yield among the different collection sites. The highest oil percentage was recorded in samples obtained from the Qandil region (1.68%), whereas the lowest yield (1.21%) was observed in specimens collected from Hawraman. The GC chromatographic analysis identified seven principal constituents within the essential oil of *H. amplifolium*, namely: Linalool, Cymene, Limonene, Terpinene, Camphor, α-Pinene, and Camphene. Notably, plants collected from Qandil exhibited the highest concentrations of these components, with values of 14.22%, 10.25%, 9.88%, 10.25%, 8.11%, 30.44%, and 4.11%, respectively. Conversely, samples from the Hawraman site displayed the lowest corresponding concentrations, measured at 9.55%, 7.45%, 5.88%, 7.00%, 5.77%, 22.98%, and 2.00%, respectively. Among the identified constituents, α-Pinene emerged as the

predominant compound across all locations, with consistently higher levels compared to the other volatile constituents, underscoring its potential chemotaxonomic and pharmacological relevance in the species.

Table 5. The concentration of volatile oil (%) and its component of *H. amplifolium* collected from different locations.

Location	Volatile oil	Linalool	Cymene	Limonene	Terpinene	Camphor	α.pinene	Camphene
				%				
Hawraman	1.21 d	9.55 d	7.45 d	5.88 d	7.00 d	5.77 d	22.98 d	2.00 d
Sharbazher	1.54 b	12.51 b	8.97 c	6.28 c	7.89 c	6.14 c	24.80 c	2.48 c
Penjwin	1.47 c	10.88 c	9.88 b	8.48 b	9.48 b	7.49 b	27.56 b	3.62 b
Qandil	1.68 a	14.22 a	10.25 a	9.88 a	10.25 a	8.11 a	30.44 a	4.11 a

*Different letters within each column indicate statistically significant differences between mean values

Environmental and Edaphic Factors Contributing to Elevated Volatile Oil Content in Qandil-Sourced *Hellenocarum amplifolium*: Among the studied regions, *Hellenocarum amplifolium* samples collected from the Qandil site exhibited the highest concentrations of volatile oils and their associated chemical constituents. As outlined in Table 2, Qandil recorded the highest values of both precipitation and relative humidity, which are likely to have played a critical role in modulating the accumulation of volatile compounds. These climatic conditions are typically associated with lower ambient temperatures and a reduction in volatilization losses, thereby preserving oil content within plant tissues. Moreover, increased humidity and rainfall levels are recognized for their positive influence on photosynthetic efficiency, a physiological process that underpins the production of both primary and secondary metabolites, including terpenoids and essential oils (26). Enhanced photosynthesis under such conditions may stimulate metabolic pathways involved in isoprenoid biosynthesis, contributing to higher essential oil yields. From a pedological perspective, electrical conductivity (EC) measurements revealed that Qandil soils possessed lower salinity levels relative to the other sampling locations. This observation may be attributed to reduced calcium carbonate ($CaCO_3$) content and elevated organic matter, both of which are known to enhance soil aggregate stability, porosity, and hydraulic conductivity. Improved soil physical structure under these conditions promotes efficient water infiltration and downward percolation, thereby reducing salt accumulation in the root zone through leaching processes.

Antibacterial Activity of *Hellenocarum amplifolium* Extract as Assessed by the Agar Disc Diffusion Method: The concentration-dependent antibacterial effect of *Hellenocarum amplifolium* ethanolic extract against *E. coli*, as observed in this study, is in agreement with those of Sharma et al. (27), who reported a 10 mm zone of inhibition against *E. coli* using a 1% ethanolic *Psidium guajava* leaf extract, outperforming several standard antibiotics (4–7 mm) and second only to ciprofloxacin (15 mm). Recent evidence further supports the strong inhibitory potential of phenolic- and flavonoid-rich plant extracts against uropathogenic *E. coli*. De Fazio et al. (9) conducted a meta-analysis confirming that numerous medicinal plants exert significant antibacterial activity against *E. coli*, reinforcing the potential of botanical extracts as viable alternatives to synthetic antimicrobials. Xu et al. (30) demonstrated that ethanolic plant extracts inhibited the growth of ampicillin-resistant *E. coli*, showing that polyphenolic constituents can remain potent even against resistant pathogens. Similarly, Farah et al. (10) reported bactericidal zones up to 15 mm for selected medicinal plant extracts against multi-drug resistant uropathogenic *E. coli*, closely aligning with our inhibition zone of 13 mm at the highest concentration tested. Collectively, these findings reinforce the hypothesis that bioactive secondary metabolites such as flavonoids, phenolic acids, and terpenoids in *H. amplifolium* are likely responsible for the observed antibacterial effects.

Table 6. Comparing the antibacterial effect of different concentrations of *H. amplifolium* ethanolic extract and some antibiotics against *E. coli*.

Concentration	*E. coli* (mm)
***H. amplifolium* (5000 µg/mL)**	**13 b**
***H. amplifolium* (2500 µg/mL)**	**10 bcd**
***H. amplifolium* (1250 µg/mL)**	**7 de**
***H. amplifolium* (675 µg/mL)**	**5 e**
CIP; 10 µg	**19 a**
CRO; 10 µg	**18 a**
ERY; 10 µg	**11 bc**
GNT; 10 µg	**11 bc**
AMOX; 30 µg	**10 bcd**
PRL; 30 µg	**10 cd**
NV; 30 µg	**8 cde**
B; 10 µg	**Resistant**

***Different letters within each column indicate statistically significant differences between mean values**

CIP: Ciprofloxacin, CRO: Ceftriaxone, ERY: Erythromycin, GNT: Gentamicin, AMOX: Amoxicillin, PRL: Piperacillin, NV: Novobiocin, and B: Bacitracin

Histological Evaluation of Renal Tissues in Response to Ochratoxin A and *Hellenocarum amplifolium* Treatment: The proximal convoluted tubules are highly vulnerable to nephrotoxicity caused by OTA due to their active transport, biotransformation, and xenobiotic clearance functions. OTA induces degeneration of tubular epithelial cells and interstitial fibrosis, often accompanied by functional disturbances (12). In the present study, histopathological examination of OTA-exposed rat kidneys revealed focal chronic inflammatory cell infiltration (Fig. 1-A), vascular congestion (Fig. 1-B and 1-C), and localized interstitial fibrosis (Fig. 1–D), which are in agreement with previously documented OTA induced renal lesions. Conversely, kidney tissues from rats treated with *H. amplifolium* ethanolic extract and α-tocopherol displayed intact renal cortical architecture, with no signs of inflammation, vascular compromise, or fibrosis. This preservation of structure is attributed to the antioxidant potential of phenolic constituents in the plant extract, which may scavenge free radicals and reduce OTA-induced oxidative injury Flavonoids, in particular, have been reported to reduce the steady-state concentration of free radicals in biological systems, thereby providing antioxidant protection (7). Cipriani et al. (8) likewise demonstrated that polyphenolic-rich *Phyllanthus* spp. extracts confer dual antibacterial and nephroprotective effects via oxidative stress reduction and modulation of inflammatory pathways. Furthermore, Mohajeri et al. (19) reported that curcumin functions as a potent antioxidant, neutralizing reactive oxygen species generated by mycotoxin exposure. These findings support the hypothesis that phenolic acids, flavonoids, and terpenoids in *H. amplifolium* contribute to both antibacterial activity and protection against OTA-induced renal damage.

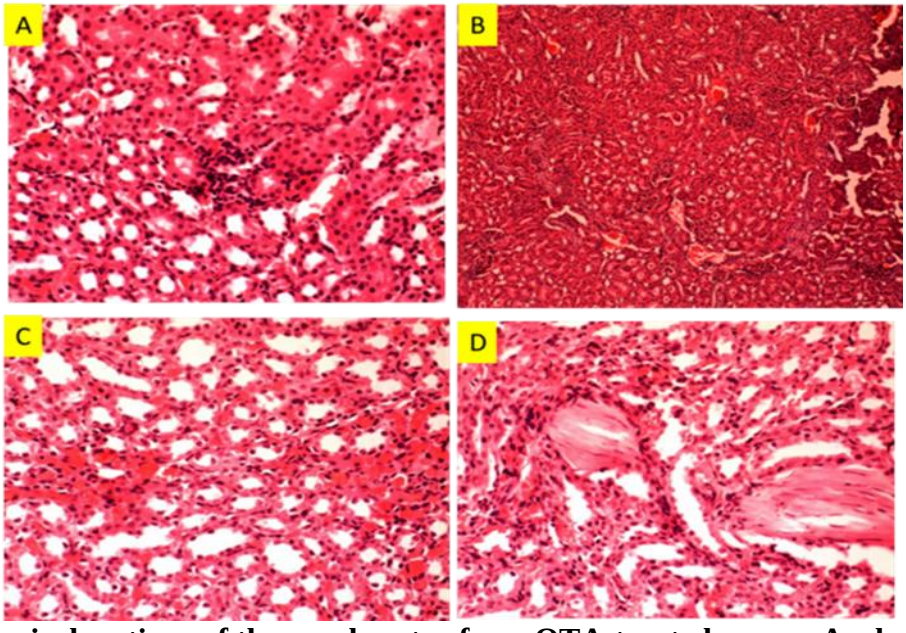

Fig. 1. Histological sections of the renal cortex from OTA-treated group: A- showed chronic inflammation. B and C showed mild and severe congestion. D- show focal fibrosis

CONCLUSIONS

Hellenocarum amplifolium from Sulaymaniyah Province exhibits significant variation in phytochemical composition depending on the collection site. Hawraman populations were richest in phenolics, flavonoids, and bioactive compounds, showing the strongest antioxidant activity and protective effects against ochratoxin A-induced cytotoxicity. The extracts also demonstrated notable antibacterial activity, particularly against *Escherichia coli*. These results suggest that *H. amplifolium* is a valuable natural source of antioxidative and antimicrobial compounds with potential therapeutic and nutraceutical applications.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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