Current Research in Health Sciences, 4(2):93–105, July-December: 2026.
DOI: https://doi.org/10.58613/crhs4213

Current Research in Health Sciences
ISSN (Online): 2584-1084

Journal homepage: https://www.gpub.org/journal-details.php?journal-id=47



Research Article

Phytochemical Profiling and Protective Effects of *Loranthus europaeus* Berry Aqueous Extract Against Acrylamide-Induced Oxidative Stress and Inflammation in Rats

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Article Info

Keywords: *Loranthus europaeus, Aqueous extract, Acrylamide, Antioxidant, Anti-inflammatory, Oxidative stress.*

Received: *25.09.2026;*
Accepted: *12.11.2026;*
Published: *26.11.2026*

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Abstract

Background: Acrylamide is a toxic compound that induces oxidative stress and inflammation. Natural plant extracts rich in polyphenolic compounds may provide protective effects against acrylamide-induced toxicity. **Objective:** Investigate the phytochemical profile and biological activities of the aqueous berry extract of *Loranthus europaeus* (LEPAE) and evaluate its protective effects against acrylamide-induced toxicity in rats. **Methods:** Qualitative and quantitative phytochemical analyses and HPLC profiling were performed to identify and quantify the major phytoconstituents. Antioxidant activities were evaluated using total antioxidant capacity, DPPH radical scavenging, and ferric reducing power assays, while anti-inflammatory activity was assessed using the albumin denaturation inhibition assay. Acute oral toxicity was also evaluated. In the in vivo study, male Wistar rats were divided into three groups (n=10): a negative control group, an acrylamide-treated group (15 mg/kg), and a treatment group receiving LEPAE (250 mg/kg) after acrylamide administration for 60 consecutive days. **Results:** Phytochemical analyses confirmed the presence of phenolic and flavonoid compounds, particularly quercetin, gallic acid, and rutin. The extract demonstrated antioxidant and anti-inflammatory activities in vitro. Acute oral toxicity testing revealed no mortality at doses up to 5,000 mg/kg. Acrylamide administration significantly increased serum malondialdehyde (MDA), interleukin-1β (IL-1β), and interleukin-6 (IL-6) levels, accompanied by a significant reduction in catalase (CAT) levels ($p<0.05$). LEPAE treatment significantly attenuated these alterations, reducing MDA and pro-inflammatory cytokine levels while improving CAT levels. **Conclusion:** The aqueous berry extract of *Loranthus europaeus* demonstrated antioxidant and anti-inflammatory activities and exerted protective effects against acrylamide-induced oxidative stress and inflammation in rats.

1. Introduction

L. europaeus Jacq. is a deciduous hemi-parasitic shrub belonging to the family Loranthaceae, widely distributed across Europe and western Asia [1]. It commonly grows on oak trees, obtaining water and mineral nutrients through specialized structures called haustoria [2]. Its berries contain various secondary metabolites, particularly phenolic acids, flavonoids and terpenoids which have been associated with antioxidant, anti-inflammatory, antimicrobial, and antiproliferative activities [3–5]. The biological properties of plant extracts are influenced

by the extraction solvent, as water selectively recovers several polar and water-soluble constituents [6]. Therefore, studying the aqueous extract of the berries of *L. europaeus* may help determine whether these water-soluble compounds have biological effects that may protect against toxins-induced tissue damage. This is particularly important because oxidative stress and inflammation play a role in the development of tissue damage caused by many environmental and dietary toxins. Acrylamide is an undesirable compound that can arise in foods exposed to elevated temperatures, particularly when asparagine reacts with reducing sugars during thermal processing [7, 8]. In addition to dietary intake, exposure may occur through inhalation of cigarette smoke and contact with certain industrial environments [9, 10].

The toxicological significance of acrylamide extends beyond its occurrence in processed foods. Its ability to disrupt cellular redox balance and trigger inflammatory processes has raised concerns regarding its potential impact on human health, making the investigation of its biological effects and possible protective interventions an important area of research [8, 11]. Following oral administration, acrylamide enters the systemic circulation after gastrointestinal absorption and subsequently reaches various tissues. Its physicochemical characteristics, including low molecular weight and high water solubility, contribute to this process [11, 12]. Hepatic metabolism, mainly mediated by cytochrome P450 2E1 (CYP2E1), converts acrylamide into glycidamide, a reactive epoxide that plays a role in its toxic effects. The formation of this metabolite is linked to ROS generation, impaired cellular redox homeostasis, and enhanced lipid peroxidation [9, 13].

Oxidative injury plays a central role in the biological response to acrylamide exposure. This condition develops when the generation of reactive oxygen species (ROS) surpasses the protective capacity of cellular antioxidant mechanisms, allowing oxidative alterations to affect essential cellular structures and components [14]. Inflammation represents another important aspect of acrylamide-induced toxicity. The inflammatory response may involve the activation of intracellular signaling pathways, particularly nuclear factor kappa B (NF-κB), which regulates the production of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β) and interleukin-6 (IL-6). Increased levels of these mediators may contribute to the progression of tissue injury following acrylamide exposure [13, 15]. Therefore, natural products containing phenolic compounds and flavonoids have been considered potential sources of protection against oxidative and inflammatory damage. Their protective effects are associated with ROS scavenging, inhibition of lipid peroxidation, and enhancement of endogenous antioxidant defenses [16, 17]. *Loranthus europaeus* Jacq. is of particular interest in this regard because its berries contain many biologically active phytochemicals, especially phenolic compounds and flavonoids, which may contribute to its reported pharmacological activities [3].

Despite the reported phytochemical richness and biological activities of *Loranthus europaeus* Jacq., the potential of its aqueous berry extract to counteract acrylamide-induced toxicity remains insufficiently investigated. Accordingly, this study aimed to characterize the extract phytochemical composition, assess its antioxidant and anti-inflammatory properties in vitro, and investigate its protective efficacy against acrylamide-induced oxidative stress and inflammation in vivo.

2. Materials and Methods

2.1. Plant Material

The dried fruits of *Loranthus europaeus* Jacq. used in this study were sourced from a herbal supplier in Basrah, Iraq, in August 2025. Botanical authentication was performed by Asst. Prof. Dr. Ula Al-Mousawi, College of Pharmacy, University of Basrah. A reference specimen was deposited under voucher No. 1560. The plant's taxonomic position is summarized in Table 1.

Table 1: Botanical taxonomy of *Loranthus europaeus* Jacq

Taxonomic Level	Classification
Order	Santalales
Family	Loranthaceae
Genus	*Loranthus*
Species	*Loranthus europaeus* Jacq

2.2. Preparation of Aqueous Extract

Aqueous Extract was prepared from the dried berries of *Loranthus europaeus* Jacq. after removing foreign materials, the dried berries were manually ground with a pestle and mortar. For the extraction, 10g of the powdered berries were mixed with 100 mL of distilled water. The mixture was kept at room temperature for 72 hours with intermittent manual agitation, it was then placed on an orbital shaker for 5 hours, followed by ultrasonic treatment in a water bath at 40 kHz for 10 minutes. The mixture was filtered through Whatman No. 1 filter paper. The resulting filtrate was left to evaporate at room temperature under light-protected conditions until dryness. The dried extract was weighed, placed in airtight amber glass bottles, and stored at -20°C until further phytochemical and biological analyses [18, 19]. The extraction yield was calculated using the following equation:

$$\text{Yield } \% = \frac{w_1}{w_2} \times 100 \tag{1}$$

Where,

w_1= weight of dry extract (g), w_2= weight of dry plant material (g)

2.3. Chemicals and Reagents

Acrylamide (CAS No. 79-06-1, purity >99 was used to induce toxicity in the experimental animals. The chemicals and reagents used throughout the study included 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, ascorbic acid, ferric chloride, potassium ferricyanide, trichloroacetic acid, sodium phosphate, ammonium molybdate, sulfuric acid, bovine serum albumin, methanol, trypsin, Tris-HCL buffer, perchloric acid ($HCLO_4$), diclofenac sodium as a reference standard for the proteinase inhibition assay. Chloroform

and 10% neutral buffered formalin were used for animal procedures and tissue fixation, respectively. All chemicals and reagents were of analytical grade unless otherwise specified.

2.4. ELISA Kits

Rat-specific ELISA kits were used to determine the levels of interleukin-1beta (IL-1b), interleukin-6 (IL-6), catalase (CAT), malondialdehyde (MDA). The kits were obtained from SunLong Biotech Co., Ltd. (Hangzhou, China) with catalog numbers EL0040Ra for IL-1β SL0411Ra for IL-6, SL0475Ra for MDA, and SL1084Rafor CAT. All assays were performed according to the manufacturer's instructions. The optical density (OD) was measured at 450 nm using microplate reader, and analyte concentrations were calculated from the corresponding standard curves.

2.5. Preliminary Phytochemical Screening

The aqueous berry extract of *Loranthus europaeus* was subjected to preliminary qualitative phytochemical tests to determine the presence of major groups of secondary metabolites. The screening was carried out using standard colorimetric methods according to previously reported methods [20, 21].

2.5.1. Flavonoids

The presence of flavonoids in aqueous extract was examined by Shinoda and alkaline reagent tests.

Shinoda test

A small quantity of the extract was mixed with magnesium turnings, followed by the addition of concentrated hydrochloric acid. The development of a pink to red color was considered a positive indication for flavonoids.

Alkaline reagent test

The extract was treated with 10% sodium hydroxide solution. A yellow coloration that disappeared following the addition of dilute hydrochloric acid indicated the presence of flavonoids.

2.5.2. Phenols and Tannins

Two qualitative tests were used for the preliminary detection of phenolic compounds, and tannins:

Ferric Chloride Test

A few drops of ferric chloride solution ($FeCl_3$) were added to the extract. The development of a blue-black, or greenish coloration indicated the presence of phenolic compound.

Lead Acetate Test

The extract was treated with 10% lead acetate solution. The formation of a white to pale yellow precipitate indicated the presence phenolic compounds, including tannins.

2.6. Quantitative Estimation of Total Phenolics, Flavonoids and Tannins

The total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) of aqueous berry extract of *Loranthus europaeus* berries were determined by spectrophotometric methods assays as previously reported procedures [22, 23]. The results were quantified using the corresponding reference standards and expressed according to the calibration curve obtained for each assay.

2.6.1. Estimation of Total Phenolic Content (TPC)

Total phenolic content was measured by the Folin-Ciocalteu colorimetric assay. Briefly 1.0 mL, of the aqueous extract was mixed with 1.0 mL of Folin-Ciocalteu reagent. After 3 min, 1.0 mL of 35% saturated sodium carbonate (Na_2CO_3,) solution was added, and distilled water was complete the final volume to 10mL. The reaction mixture was incubated in the dark for 90 min, after which its absorbance was measured at 725 nm using a UV-visible spectrophotometer. Gallic acid was used to prepare the calibration curve, and the TPC was expressed as milligrams of gallic acid equivalents (GAE)/g dry extract.

2.6.2. Estimation of Total Flavonoid Content (TFC)

Total flavonoid content was determined using the aluminum chloride colorimetric method. A volume of 0.5 mL of the extract was mixed with 0.3 mL of 5% sodium nitrite ($NaNO_2$). following 5 min of reaction, 0.3 mL of 10% aluminum chloride ($AlCl_3$) was added. After a further 6 min, 2.0 mL of 1M sodium hydroxide (NaOH) was added, and the mixture was adjusted to 5.0 mL with distilled water. Absorbance was measured at 510 nm. Quercetin was used as the reference standard for constructing the calibration curve, and the results were reported as milligrams of quercetin equivalents (QE)/g dry extract.

2.6.3. Estimation of Total Tannin Content (TTC)

Total tannin content was assessed using the ferric chloride-potassium ferricyanide method. For the assay 0.5 mL of the aqueous extract was mixed with 1.0 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$), and 1.0 ml of 1% ferric chloride ($FeCl_3$). Distilled water volume was then added to obtain a final volume of 10 mL. the mixture was allowed to react for 5 min, followed by measurement of absorbance at 720 nm. Tannic acid calibration curve was used for quantification, and the results were reported as milligrams of tannic acid equivalents (TAE)/g dry extract.

2.7. HPLC-Based Phytochemical Profiling

HPLC was used to characterize the phytochemical constituents of the aqueous berry extract of *Loranthus europaeus*. The analysis was carried out using a high-performance liquid chromatography system (SYKAM, Germany) equipped with a diode array detector (DAD) and a fraction collector. Separation of the compounds was achieved using a Poroshell EC-C18 column (4.6 mm $\times$ 250 mm, 5μm, particle size). The chromatographic analysis was performed using distilled water containing 0.1% glacial acetic acid as a mobile phase A and acetonitrile containing 0.1% glacial acetic acid as a mobile phase B. A gradient elution program was applied as follows: 60% A and 40% B from 0-3 min, 20% A and 80% B from 4-8 min and 10% A and 90% B from 9-15 min. Flow rate was kept at 1.0 mL/min, with an injection volume of 100 μL, and column temperature of 30 °C. Detection was carried out over the wavelengths range of 280 - 360 nm. The compounds present in the aqueous extract were identified by comparing the retention times of the detected sample peaks with those of corresponding reference standards analyzed under the same chromatographic conditions [24].

2.8. In Vitro Evaluation of Antioxidant Potential

2.8.1. Total Antioxidant Capacity

Total antioxidant capacity of the aqueous extract was determined using the phosphomolybdenum method described previously [25]. For the assay, 1.0 mL of the extract was combined with 1.0 mL of a phosphomolybdenum reagent, which consisted of 0.6 M sulfuric acid (H_2SO_4), 28 mM sodium phosphate, and 4 mM ammonium molybdate. The reaction mixtures were incubated at 95°C for 90 min and then allowed to cool to room temperature. The absorbance was subsequently measured at 695 nm using a UV-visible spectrophotometer. Ascorbic acid was used as the reference standard, and the antioxidant capacity was expressed as milligrams of ascorbic acid equivalents per gram of dry extract (mg AAE)/g dry extract.

2.8.2. DPPH Radical Scavenging Assay

Free radical scavenging activity of the aqueous extract was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay following the method reported by [26]. Different concentration of the extracts was prepared and mixed with 1.0 mL of 0.254mM DPPH solution. The reaction mixtures were kept in the dark at room temperature for 30 min. Absorbance was then measure at 517 nm using a UV-visible spectrophotometer. The ascorbic acid, was used as the reference standard. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{\text{Ac-AS}}{\text{Ac}} \times 100 \tag{2}$$

Where,

Ac represents the absorbance of the control and, As represent the absorbance of the sample.

2.8.3. Ferric Reducing Antioxidant Power Assay

The reducing power ability of the aqueous extract was assessed according to the method previously described [27]. Different concentrations, of extracts were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and, 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixtures were incubated at 50°C for 20 min. followed by the addition of 2.5 ml of 10% trichloroacetic acid (TCA). Then mixture was centrifuged at 3000 rpm, for 10 min. subsequently, (2.5 mL) of the resulting supernatant was mixed with an equal volume of distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3$). The absorbance was measured at 700 nm using a UV visible spectrophotometer. Ascorbic acid was used as the reference standard.

2.9. Inhibition of Albumin Denaturation Assay

The anti-inflammatory potential of the aqueous extract of *Loranthus europaeus* was assessed by measuring its ability to inhibit BSA denaturation. The extract and ibuprofen (reference drug) were tested at concentrations of 0.01–1 mg/mL. Each reaction contained 1.4 mL phosphate buffer (pH 6.4), 100 μL BSA solution, and 1 mL of the test solution. The mixtures were kept at room temperature, heated at 70°C for 5 min, and measured at 660 nm after cooling. The procedure was adapted from a previously published method, with modifications:

$$\text{Inhibition (\%)} = \frac{\text{Ac-AS}}{\text{Ac}} \times 100 \tag{3}$$

Where,

Ac represents the absorbance of the control and, As represents the absorbance of the sample.

2.10. In Vivo Experimental Procedures

2.10.1. Animal Housing

A total of 35 adult male Wistar rats (180–240g) were used in this study. The animals were supplied by the College of Veterinary Medicine, University of Basrah. Of these, 30 rats were included in the main experimental protocol whereas the remaining five animals were designated for the acute oral toxicity assessment of the aqueous extract. Throughout the study, the animals were housed under standardized laboratory conditions, with a temperature of $25 \pm 3^{\circ}$C and a 12-hour light/dark cycle. Food and water were provided ad libitum. Before the experimental procedures commenced, all animals underwent a two-week acclimatization period.

2.10.2. Acute Oral Toxicity Assessment

The acute oral toxicity of the aqueous berry extract was evaluated in five male Wistar rats through sequential oral administration of increasing doses (500, 1000, 2000, 4000, and 5000 mg/kg body weight), following the procedure described by Bruce [28]. Each animal was monitored for 48 hours after dosing, with observations focusing on mortality and clinical signs of toxicity.

2.10.3. Experimental design

Following acclimatization, the 30 rats assigned to the main experiment were randomly allocated into three experimental groups (10 animals per group), as follows: Group I (Untreated Control): Animals received distilled water only. Group II (Positive control): Animals received acrylamide at a dose 15 mg/kg body weight, dissolved in distilled water [29, 30]. Group III (Acrylamide + aqueous extract): Animals received acrylamide at15 mg/kg body weight, followed by aqueous extract of *Loranthus europaeus* at dose of 250 mg/kg body wight. The assigned treatments were delivered orally by gavage over a 60-day period. For the aqueous extract group, 250 mg/kg body weight was selected as the daily dose, representing one-twentieth of the maximum dose tested in the acute toxicity assessment (5000 mg/kg).

2.10.4. Sample collection

Following completion of the 60-day treatment period, the animals were anesthetized by chloroform inhalation, and blood samples were obtained by cardiac puncture into sterile tubes without anticoagulated. The blood samples were left at room temperature for 15-20 min and then centrifuged at 3000 rpm for 15 min. Separated serum was collected and stored at -20°C until biochemical analysis.

2.10.5. Statistical Analysis

Statistical analyses were conducted using SPSS Statistics version 23, and GraphPad Prism version 8. The results were expressed as mean $\pm$ standard error. One-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post hoc test, while two-way ANOVA were used for statistical comparisons. P-value < 0.05 was considered statistically significant.

3. Result and Discussion

3.1. Extraction yield

The aqueous extraction of *Loranthus europaeus* berries yielded 0.79g of dry extract from 10g of dried plant material, corresponding to an extraction yield of 7.90% (w/w). The obtained yield may be influenced by the high polarity of water and the solubility of the constituents present in the plant matrix, as solvent polarity is an important factor determining the recovery and profile of phytochemicals during extraction [31]. The applied extraction procedure, combining maceration with orbital shaking and ultrasonication, may have enhanced solvent–plant matrix contacts and facilitated the release of soluble constituents into the extraction medium. In addition, the absence of direct heating during extraction may help minimize thermal degradation of heat-sensitive phytochemicals [32, 33]. Overall, the obtained yield indicates measurable recovery of water-soluble constituents from the dried berries.

3.2. Phytochemical Screening

Qualitative phytochemical screening of the aqueous extract of *L. europaeus* berries revealed the presence of phenolic compounds, flavonoids, and tannins. Strong positive reactions (+++) were observed for phenols and flavonoids, whereas tannins showed a moderate positive reaction (++), as presented in Table 2.

Table 2: Qualitative phytochemical screening of aqueous extracts of *L. europaeus* berries

Phytochemical group	Chemical test	aqueous extract
Phenols and Tannins	Ferric chloride	+++
	Lead acetate	++
Flavonoids	Shinoda /alkaline	+++

Note: +++, strong reaction; ++ moderate reaction

The positive reactions observed in the ferric chloride, Shinoda, and lead acetate tests provide qualitative evidence for the presence of phenolic compounds, flavonoids, and tannin-related constituents in the aqueous extract of *L. europaeus* berries. The strong ferric chloride reaction is consistent with the presence of phenolic constituents, which produce characteristic color development following the addition of ferric chloride. Likewise, the strong Shinoda reaction supports the presence of flavonoids, through their reaction with magnesium under

acidic conditions, with the development of pink-to-red coloration serving as a positive indication of flavonoid compounds. The moderate reaction observed with lead acetate additional supports the presence of tannin through the formation of characteristic yellow to-cream precipitate [34, 35]. These findings are consistent with previous phytochemical investigations of *L. europaeus*, in which phenolic compounds were detected in the fruits. Benabderrahim et al. reported 22 phenolic compounds in methanolic extracts of *L. europaeus* organs, with catechin, epicatechin, chlorogenic acid, hyperoside, and pinoresinol among the predominant compounds, supporting the polyphenolic nature of the species [3]. Overall, the present findings provide preliminary phytochemical evidence that the aqueous berry extract contains varied phenolic and flavonoid constituents, together with tannin-related compounds, which may contribute to its biological activity.

3.3. Quantitative Estimation of Total Phenolic, Flavonoid, and Tannin Contents

The quantitative contents of total phenolic, flavonoid, and tannin compounds in the aqueous extract of *L. europaeus* berries are presented in Table 3. Flavonoids represented the highest quantified bioactive group, followed by phenolic compounds and tannins.

Table 3: Total Phenolic of *Loranthus europaeus* Extracts

Test	Unit	Aqueous extract
TPC	mg GAE\g	5.25 ± 0.85
TFC	mg QE\g	77.5 ± 1.16
TTC	mg TAE\g	4.75 ± 0.68

The aqueous extract of *L. europaeus* berries showed measurable amounts of phenolic, flavonoid, and tannin constituents, with flavonoids representing the predominant quantified group. This finding is consistent with previous phytochemical investigations of *L. europaeus* fruits, which have demonstrated the presence of diverse phenolic and flavonoid constituents [3]. The presence of these compounds is of particular interest because plant phenolics and flavonoids can contribute to antioxidant activity through their ability to donate hydrogen or electrons and the scavenging of reactive species [36]. The measurable tannin content further supports the presence of additional polyphenolic constituents in the aqueous berry extract [37]. Overall, the quantitative profile supports the presence of biologically relevant polyphenolic constituents in *L. europaeus* berries and may partly explain the biological activity observed for the aqueous extract.

3.4. HPLC-DAD Analysis of Phenolic and Flavonoid Compounds in the Aqueous Extract of *L. europaeus* Berries

HPLC-DAD analysis of the aqueous extract of *L. europaeus* berries identified seven phenolic and flavonoid compounds based on comparison of their retention times with authentic standards Figure 1, and Figure 2, respectively. The identified compounds included gallic acid, caffeic acid, ferulic acid, rutin, quercetin, kaempferol, and apigenin. Quercetin exhibited the highest detected level, followed by gallic acid and rutin, while apigenin showed the lowest level among the identified compounds. Overall, the HPLC-DAD profile demonstrated the presence of several phenolic and flavonoid constituents in the aqueous berry extract.

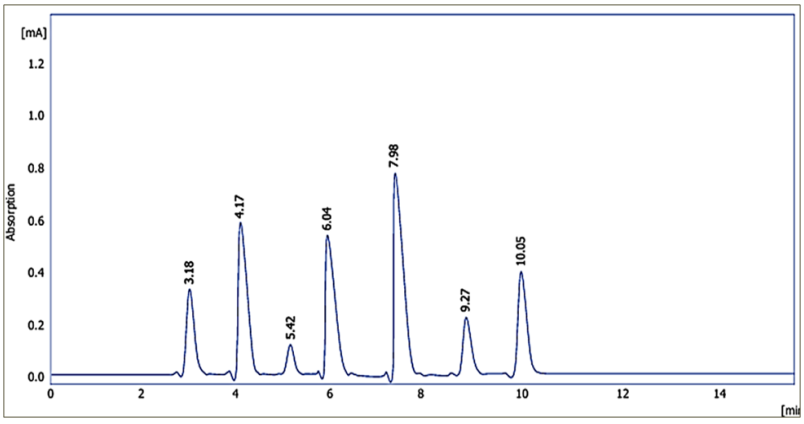

Figure 1: HPLC-DAD chromatogram of the aqueous extract of L. europaeus berries showing separation of phenolic and flavonoid compounds at their respective retention times (min)

HPLC-DAD analysis of the aqueous extract of *L. europaeus* berries revealed seven phenolic and flavonoid compounds, with quercetin showing the highest detected level, followed by gallic acid and rutin. The detection of these constituents agrees with previous phytochemical reports on *L. europaeus* fruits, supporting the presence of diverse phenolic and flavonoid compounds in the berries [3, 38]. The predominance of quercetin, is of particular interest because this flavonoid has well-established antioxidant activity properties related to its ability to scavenge reactive species and donate electron, while gallic acid and rutin also possess antioxidant properties that may contribute to the overall activity of the aqueous extract [39, 40]. Thus, the HPLC profile complements the total phenolic and flavonoid measurements by providing information on individual constituents detected in the aqueous extract.

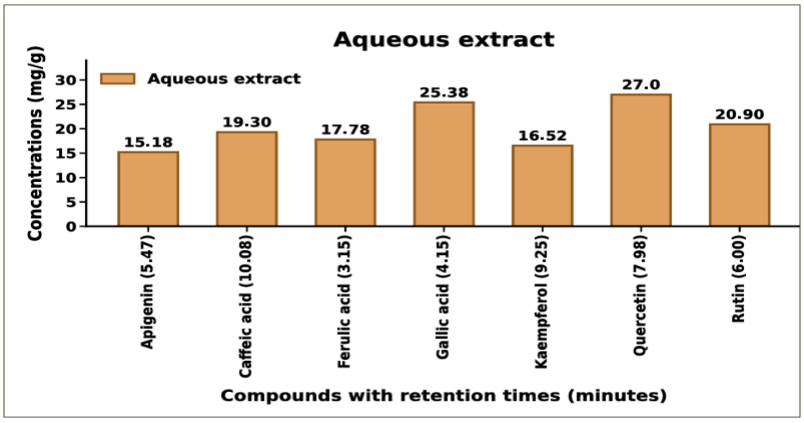

Figure 2: RP-HPLC quantitative analysis of phenolic and flavonoid compounds identified in the aqueous extract of *Loranthus europaeus* berries. Values represent concentrations (mg/g dry extract) and retention times (RT, min), with quercetin showing the highest concentration

3.5. Total Antioxidant Capacity

The total antioxidant capacity of the aqueous extract of *L. europaeus* berries was determined using the phosphomolybdenum assay. The extract showed a total antioxidant capacity of 26.25 ± 0.79 mg AAE/g extract. The measured antioxidant capacity reflects the overall reducing capacity of the extract, based on the reduction of Mo(VI) to Mo(V) in the phosphomolybdenum assay [41]. This activity may be related to the combined contribution of phenolic, flavonoid, and tannin constituents present in the extract.

3.6. Radical Scavenging Activity (DPPH)

The aqueous extract of *L. europaeus* berries showed a concentration-dependent increase in DPPH radical-scavenging activity across the tested concentrations (1–0.01 mg/mL), with inhibition increasing from 18.66% at 0.01 mg/mL to 96.65% at 1 mg/mL. At the three highest concentrations (1, 0.5, and 0.25 mg/mL), the extract showed no significant difference from ascorbic acid ($P > 0.05$) Figure 3. At lower concentrations, inhibition decreased from 79.55% at 0.1 mg/mL to 18.66 at 0.01 mg/mL, while ascorbic acid showed significantly higher inhibition at 0.1, 0.05, and 0.01 mg/mL ($P < 0.0001$). The IC_{50} values were 0.055 mg/mL for the aqueous extract and 0.005 mg/mL for ascorbic acid.

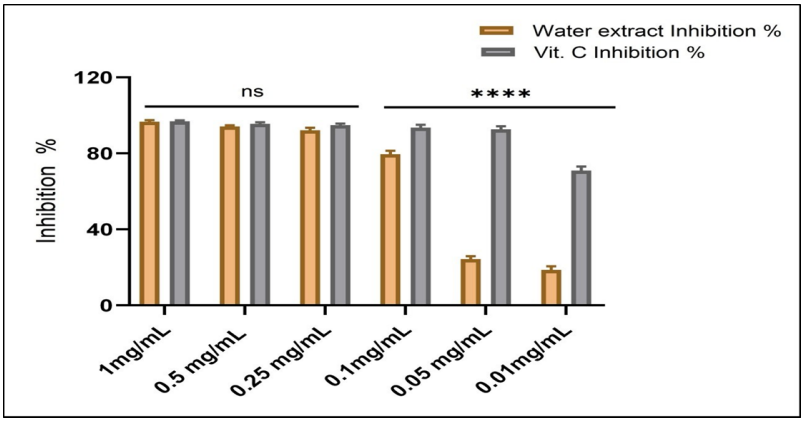

Figure 3: DPPH radical-scavenging activity (inhibition %) of the aqueous extract of *Loranthus europaeus* berries compared with ascorbic acid at 0.01–1 mg/mL. Values are mean ± SD (n = 3). Two-way ANOVA followed by Šídák's multiple comparisons test showed no significant differences between the extract and ascorbic acid at 1, 0.5, and 0.25 mg/mL (ns, $P > 0.05$), whereas the extract was significantly lower at 0.1, 0.05, and 0.01 mg/mL (****, $P < 0.0001$)

The DPPH assay demonstrated that the aqueous extract retained strong radical-scavenging capacity at higher concentrations, with inhibition reaching 96.65% at 1 mg/mL. Similar inhibition was observed at 0.5 and 0.25 mg/mL, with no significant difference from ascorbic acid ($P > 0.05$). This pattern may indicate that sufficient antioxidant constituents were present at these concentrations to react with a large proportion of the available DPPH radicals [42]. A reduction in scavenging was observed as the extract concentration decreased, particularly between 0.1 and 0.05 mg/mL. At these lower concentrations, ascorbic acid produced significantly higher inhibition ($P < 0.0001$), which may reflect the lower amount of active antioxidant constituents available in the crude extract. Similar differences between crude plant extracts and ascorbic acid have been reported [43]. The observed activity may be partly related to the phenolic compounds detected by HPLC-DAD, such as gallic acid, quercetin, rutin, caffeic acid, ferulic acid, apigenin, and kaempferol. These compounds are known to participate in radical scavenging through electron or hydrogen donation [42, 44]. Previous work has also reported DPPH-scavenging activity in *L. europaeus* fruit extracts [5]. The IC_{50} values were 0.055 mg/mL for the aqueous extract and 0.005 mg/mL for ascorbic acid. These values may vary with DPPH concentration and assay conditions [45].

3.7. Ferric Reducing Antioxidant Power Assay

The FRAP assay demonstrated an increase in the reducing power of the aqueous extract of *L. europaeus* berries with increasing concentration, ranging from 0.025 to 1 mg/mL. The highest absorbance was recorded at 1 mg/mL, while the lowest was observed at 0.025 mg/mL. Compared with vitamin C, the aqueous extract showed significantly lower absorbance at all tested concentrations ($p = 0.0013$), as presented in Figure 4.

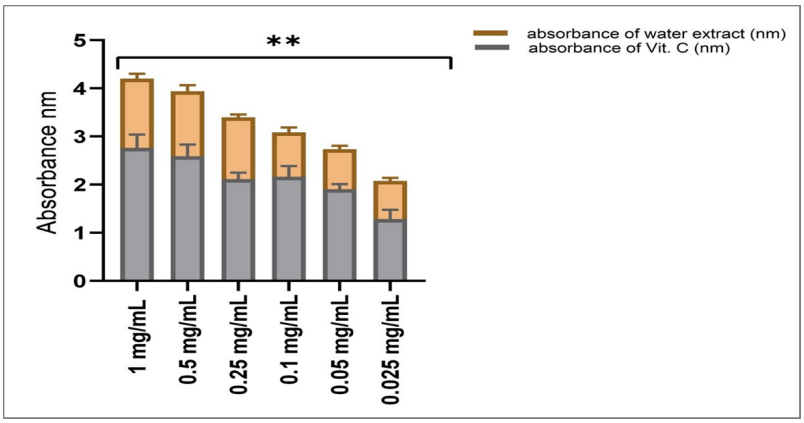

Figure 4: Comparation the ferric reducing power of the aqueous extract of *L. europaeus* berries and ascorbic acid (absorbance = 700 nm). ** shows a significant difference ($p < 0.01$) between the two groups at all tested concentrations

The present results demonstrated a concentration-dependent increase in the reducing response of the aqueous extract, with absorbance rising progressively as the extract concentration increased. This reflects the electron-transfer mechanism underlying the FRAP assay, in which antioxidant compounds reduce ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}), with the degree of absorbance change reflecting the reducing capacity of the tested sample [46]. The observed reducing activity may be related to the phenolic and flavonoid constituents detected in the extract, which can contribute to electron transfer reactions [18].

3.8. Protein Anti-Denaturation activity

The aqueous extract of *L. europaeus* exhibited concentration-dependent inhibition of albumin denaturation, ranging from (45.91% to 65.98%) at 0.01–1 mg/mL, respectively, compared with ibuprofen (51.31% to 80.85%). Ibuprofen showed significantly higher anti-inflammatory activity compared to the aqueous extract across all tested concentrations ($P < 0.001$ to $P = 0.0003$) The IC_{50} values were 0.055 and 0.013 mg/mL for the aqueous extract and ibuprofen, respectively, Figure 5.

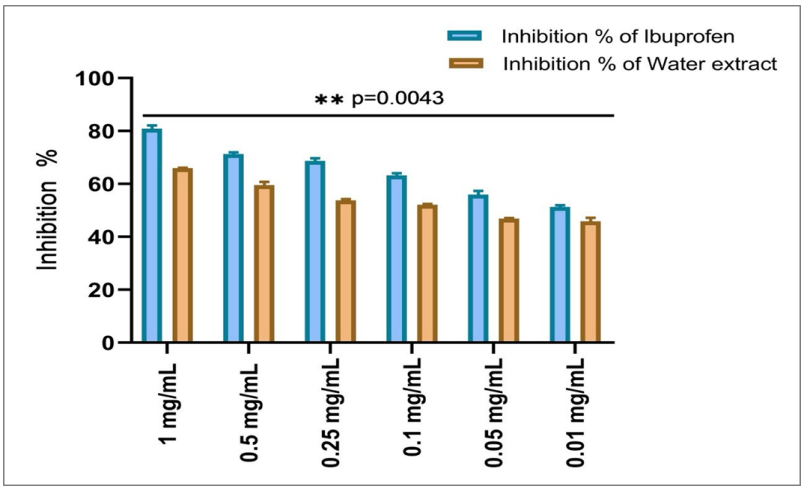

Figure 5: Effect of aqueous extract of *L. europaeus* and ibuprofen on albumin denaturation at different concentrations (0.01–1 mg/mL). Data are presented as mean ± SD (n = 3). Statistical analysis was performed using two-way ANOVA followed by Šídák's multiple comparisons test

The aqueous extract of *L. europaeus* berries inhibited heat-induced BSA denaturation in a dose-dependent manner, with the highest inhibition recorded at 1 mg/mL (65.98%). The BSA denaturation assay is commonly used to evaluate anti-inflammatory potential in vitro, since heat-induced denaturation of proteins produces antigenic changes associated with inflammatory damage, and samples that protect protein structure from this denaturation are considered to have anti-inflammatory activity [47]. This inhibitory effect may be related to the phenolic and flavonoid compounds detected by HPLC-DAD in the extract, particularly quercetin and gallic acid, which are known to stabilize proteins through hydrogen-donating and metal-chelating properties [48]. This result agrees with earlier findings on *L. europaeus*, where ethyl acetate and methanol fractions reduced paw edema in rats using an egg-albumin-induced inflammation model [49]. Compared with ibuprofen, the aqueous extract showed significantly lower inhibition at all tested concentrations, with adjusted P values ranging from <0.0001 to 0.0003. The IC_{50} values were 0.055 mg/mL for the aqueous extract and 0.013 mg/mL for ibuprofen, indicating that the aqueous

extract required a higher concentration than ibuprofen to achieve 50% inhibition of protein denaturation under the present experimental conditions.

3.9. Acute Toxicity Finding

Sequential administration of the aqueous berry extract at doses ranging from (500 to 5000 mg/kg body weight) produced no mortality or observable toxic effects during the observation period. Accordingly, the oral LD_{50} was estimated to be greater than 5000 mg/kg. A dose of 250 mg/kg body weight (1/20 of the highest tested dose) was therefore selected for the subsequent in vivo study.

3.10. Serum Oxidative Stress Markers in Acrylamide-Exposed Rats Following Aqueous Extract Administration

Serum analysis revealed significant alterations in both oxidative stress parameters following acrylamide exposure. Compared with the control group, acrylamide-treated rats exhibited higher MDA levels and lower CAT levels. Administration of the aqueous berry extract alongside acrylamide modified these changes, as evidenced by reduced MDA and increased CAT levels relative to the acrylamide group Table 4, Figure 6.

Table 4: Changes in Serum Catalase and Malondialdehyde Levels in Acrylamide-Treated Rats following Aqueous Extract Administration

Parameters	Control	ACR	ACR+ LEP AQE.	LSD	p-value
Catalase pg/ml	15.085 ± 0.728^{a}	7.605 ± 0.637^{c}	9.895 ± 0.877^{b}	0.574494	< 0.0001
MDA ng/ml	18.762 ± 2.189^{c}	59.28 ± 4.09^{a}	34.84 ± 3.58^{b}	2.575981	< 0.0001

Note: values are presented as Mean $\pm$ SD. With different superscript letters (a, b, c) within the same row denoting statistically significantly different at ($p < 0.05$). Abbreviations: ACR: Acrylamide; CAT: Catalase; MDA: Malondialdehyde, AQE: Aqueous Extract.

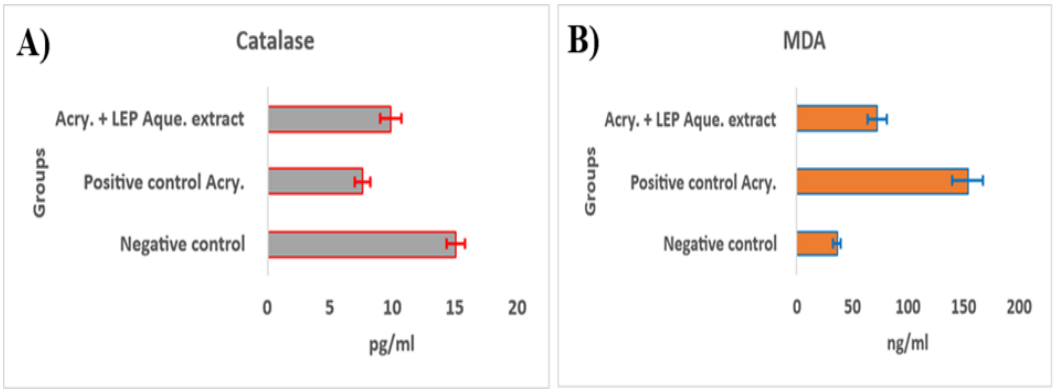

Figure 6: Comparison of serum oxidative stress parameters among the experimental groups following aqueous extract treatment. (A) Catalase (CAT) levels and (B) malondialdehyde (MDA) levels in control, acrylamide-treated, and acrylamide + aqueous extract-treated rats. Data are expressed as mean $\pm$ SD. Statistical analysis was performed using one-way ANOVA followed by Fisher's LSD test, with significance set at $p < 0.05$

The oxidative imbalance observed after acrylamide exposure may be associated with its metabolic conversion by cytochrome P450 2E1 (CYP2E1), leading to the formation of glycidamide, a reactive metabolite involved in cellular toxicity [50]. One consequence of this process is the excessive generation of reactive oxygen species (ROS), which can promote membrane lipid peroxidation. Malondialdehyde (MDA), a product of lipid peroxidation, is therefore commonly used to assess oxidative damage [51]. In parallel, catalase (CAT) represents an essential component of the enzymatic antioxidant system, as it facilitates the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen [52]. In agreement with this oxidative pattern, the acrylamide group in the current study exhibited a pronounced increase in serum MDA accompanied by a reduction in CAT levels relative to the control group. Comparable alterations have been documented in previous experimental studies involving acrylamide exposure [53, 54]. Notably, the addition of the aqueous *L. europaeus* berry extract resulted in a significant shift in both markers toward the control values. Serum MDA decreased, while CAT levels showed a partial increase compared with the acrylamide-only group ($p < 0.0001$). The biochemical response observed in vivo can be considered alongside the antioxidant capacity demonstrated by the extract in the in vitro experiments. Its phytochemical profile, particularly the presence of phenolic compounds, flavonoids, and tannins, may provide a basis for this activity. Furthermore, HPLC-DAD analysis revealed quercetin, gallic acid, and rutin among the identified constituents. These compounds may contribute to the overall antioxidant potential of the extract. Collectively, the results indicate that the aqueous berry extract improved the oxidative status of acrylamide-exposed rats, as reflected by the changes in serum CAT and MDA levels.

3.11. Serum Inflammatory Markers Following Aqueous Extract Administration in Acrylamide-Treated Rats

Assessment of the inflammatory biomarkers revealed substantial changes following acrylamide exposure. Both IL-1β and IL-6 concentrations were elevated in the acrylamide group compared with the control animals. In contrast, rats receiving the aqueous berry extract in combination with acrylamide showed lower circulating concentrations of both cytokines than those exposed to acrylamide alone Table 5, Figure 7.

Table 5: Changes in Serum IL-1β and IL-6 Levels in Acrylamide-Treated Rats Following Aqueous Extract Administration

Parameters	Control	ACR	ACR+LEP AQE.	LSD	p-value
IL-1b (pg/ml)	36.3 ± 3.46^{c}	153.28 ± 13.87^{a}	71.86 ± 9^{b}	7.427111	< 0.0001
IL-6 (ng/ml)	20.161 ± 1.78^{c}	98.48 ± 9^{a}	51.97 ± 4.23^{b}	4.440881	< 0.0001

Note: Values are presented as Mean $\pm$ SD. With different superscript letters (a, b, c) within the same row denoting statistically significantly different at ($p < 0.05$). Abbreviations: ACR: Acrylamide; AQE: aqueous extract; IL-1b: Interleukin-1 beta; IL-6: interleukin-6.

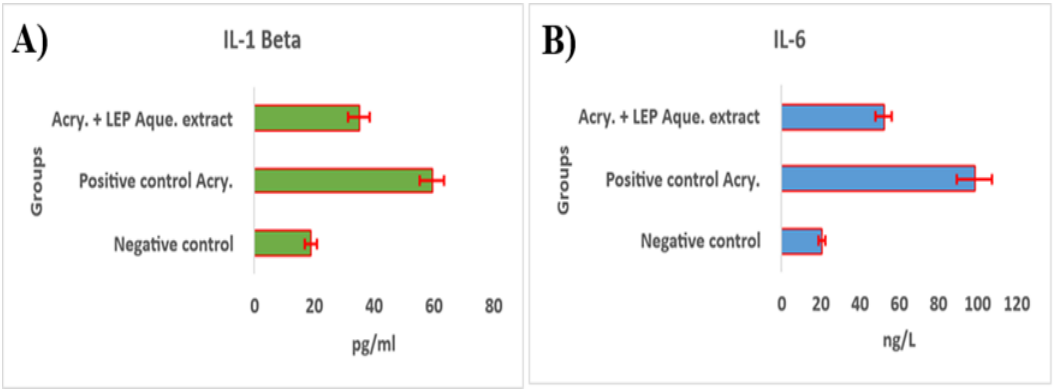

Figure 7: Comparison of serum inflammatory cytokine levels among the experimental groups following aqueous extract treatment. (A) Interleukin-1 beta (IL-1β) levels and (B) interleukin-6 (IL-6) levels in control, acrylamide-treated, and acrylamide + aqueous extract-treated rats. Data are expressed as mean $\pm$ SD. Statistical analysis was performed using one-way ANOVA followed by Fisher's LSD test, with significance set at $p < 0.05$

Oxidative stress and inflammation are closely related processes in acrylamide-induced toxicity, as increased oxidative disturbances may promote inflammatory signaling and cytokine production. Cerrah et al. reported that acrylamide exposure increased MDA, IL-1β, TNF-α, and NF-κB levels in rat liver, while carvacrol attenuated these changes [55]. In the present study, acrylamide administration markedly increased serum IL-1β and IL-6 levels compared with the control group, indicating a clear inflammatory response. This finding is consistent with Ozsan and Kececi, who reported increased IL-1, IL-6, and TNF-α levels in acrylamide-exposed rats, with reduced cytokine levels following treatment with Nigella sativa [56]. Co-administration of the aqueous extract of *L. europaeus* berries significantly reduced both IL-1β and IL-6 levels compared with the acrylamide group ($p < 0.0001$). A similar response was reported by Sayed et al., who found that pomegranate-peel extract reduced acrylamide-induced elevations in serum IL-1β and IL-6 [53]. Together with the improvement observed in MDA and CAT, the reduction in IL-1β and IL-6 suggests that the aqueous extract may attenuate both oxidative and inflammatory disturbances associated with acrylamide exposure.

4. Conclusion

In conclusion, the aqueous berry extract of *Loranthus europaeus* (LEPAE) exhibits notable antioxidant and anti-inflammatory properties, potentially associated with its rich polyphenolic composition, particularly quercetin, gallic acid, and rutin. In vivo findings demonstrated that oral administration of LEPAE at 250 mg/kg attenuated acrylamide-induced oxidative stress and inflammatory responses in rats, as evidenced by reduced serum MDA, IL-1β, and IL-6 levels, along with improved CAT levels. These findings suggest that *Loranthus europaeus* berries represent a promising natural source of protective agents against acrylamide-induced toxicity.

Article Information

Ethical Approval: The experimental protocol received ethical approval from the Ethics Committee of the College of Pharmacy, University of Basrah (Approval No. IEC/2025/128).

Informed Consent: Not applicable.

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: No external funding was received for this study.

Author Contributions: All authors contributed to the conception and design of the study, data collection/analysis, interpretation of results, and preparation and approval of the final manuscript.

AI Use Disclosure: No generative artificial intelligence tools were used in the preparation of this manuscript.

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