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Research Article

Advancing the metabolite profiling, anti-inflammatory, and analgesic effects of *Laurus nobilis* leaves

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Abstract

Laurus nobilis leaves are renowned for antirheumatic, antimicrobial, and antioxidant activity. This work aimed at metabolite profiling of proanthocyanidins, flavonoids, and lignans in methanol–aqueous leaf extract by means of liquid chromatography–high-resolution mass spectrometry. Further, the study evaluated the anti-inflammatory and antinociceptive effects of the extract, administered alone or in combination with diclofenac, in the carrageenan-induced mouse paw inflammation model. At 250 mg/kg for 10 days, the extract showed a moderate anti-inflammatory effect, but this was less than that of diclofenac. The combined treatment reduced edema, but to a smaller extent than diclofenac and the extract alone. In the hot plate test, the extract had an antinociceptive effect, exceeding that of diclofenac, while the combination was less effective than either treatment alone. In conclusion, B- and A,B-type procyanidins alongside acylated kaempferol glycosides hold significance for the observed anti-inflammatory and analgesic activity. While diclofenac remains more potent in reducing inflammation, the extract demonstrated superior antinociceptive activity. The lack of enhanced efficacy in the combined treatment suggests possible pharmacodynamic interactions, warranting further investigation into the mechanisms underlying these effects.

Keywords

Anti-inflammatory and analgesic effects, flavonoids, *Laurus nobilis*, lignans, proanthocyanidins

Introduction

Medicinal plants have long been used for the treatment of diseases based on their therapeutic effects rooted in biologically active chemical compounds. Today, these natural compounds continue to underpin modern drug development, providing the basis for many medicines used in pharmacies (Marrelli 2021). Among these plants, *Laurus nobilis* L. (bay laurel) (Lauraceae) appears to be a representative species (Khaled Khodja et al. 2023). The plant is a Mediterranean evergreen aromatic species, widely cultivated for culinary, medicinal, and industrial purposes for centuries (Anzano et al. 2022; Dobroslavić et al. 2022; Paparella et al. 2022; Khaled Khodja et al. 2023).

Laurel leaves are commonly used as a spice and flavoring agent in the food industry, while traditional medicine has employed laurel preparations for the treatment of digestive disorders, infections, rheumatism, and inflammatory

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conditions (Dias et al. 2014; Caputo et al. 2017). Based on these traditional uses and the increasing interest in plant-derived bioactive compounds, *L. nobilis* has become an important subject of phytochemical and pharmacological research (Dobroslavić et al. 2022; Paparella et al. 2022).

Phytochemical investigations have demonstrated that *L. nobilis* contains a diverse range of secondary metabolites, including essential oils, phenolic compounds, flavonoids, tannins, proanthocyanidins, and lignans (Alejo Armijo et al. 2017; Chahal et al. 2017; Dobroslavić et al. 2022; Paparella et al. 2022). The volatile fraction of laurel leaves is dominated by monoterpenes and oxygenated terpenoids, such as 1,8-cineole, sabinene, α-pinene, and linalool, which contribute to the typical aroma of the plant and exhibit antimicrobial and antioxidant activities (Caputo et al. 2017; Chahal et al. 2017).

In addition to volatile constituents, increasing attention has been focused on the nonvolatile polyphenolic fraction of *L. nobilis* extracts (Alejo Armijo et al. 2017; Dobroslavić et al. 2021, 2022). Thus, polyphenols represent one of the most important groups of phytochemicals in laurel leaves and are largely responsible for their antioxidant and pharmacological properties. Chromatographic analyses, such as high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS), have revealed the presence of numerous phenolic acids, including gallic acid, protocatechuic acid, caffeic acid, ferulic acid, and *p*-coumaric acid, in laurel extracts (Alejo Armijo et al. 2017; Karadağ et al. 2025). Flavonoids constitute another major class of polyphenolic compounds present in *L. nobilis* leaves (Khaled Khodja et al. 2023). Compounds such as rutin, naringin, hesperidin, and quercetin derivatives have been identified and contribute significantly to the antioxidant capacity of the plant (Alejo Armijo et al. 2017; Karadağ et al. 2025). Both phenolic acids and flavonoids exhibit anti-inflammatory activity through modulation of inflammatory enzymes and signaling pathways, which further enhances the pharmacological value of laurel extracts (Dobroslavić et al. 2021, 2022).

Generally present in lower concentrations than other polyphenols, proanthocyanidins represent another group of phenolic compounds contributing to the antioxidant potential of *L. nobilis* (Alejo Armijo et al. 2017; Dobroslavić et al. 2021). Additionally, lignans and neolignans are important phytochemical constituents of the Lauraceae family, considered its potential chemotaxonomic markers (Li et al. 2018). Several lignans, such as syringaresinol, sesamin, and related derivatives, have been isolated from Lauraceae species and have shown antioxidant, anti-inflammatory, and cytotoxic activities (Li et al. 2018).

The biological activity of *L. nobilis* extracts is closely associated with the presence of all these phenolic constituents, inhibiting inflammatory mediators and reducing oxidative damage (Dobroslavić et al. 2021; Alejo Armijo et al. 2017). Thus, they have a potential role in the prevention of inflammation-related diseases.

Despite the numerous studies on the phytochemical composition, mainly on the essential oil, and biological activity of *L. nobilis*, comprehensive analyses focusing on proanthocyanidins, flavonoids, and lignans combined with the anti-inflammatory effects of the respective extracts remain limited.

Therefore, the aim of the present study is to investigate the phenolic composition of *L. nobilis* leaf extract, with particular emphasis on proanthocyanidins, flavonoids, and lignans, and to evaluate their potential contribution to the anti-inflammatory and analgesic activities of the plant.

Materials and methods

Plant materials

Laurus nobilis leaves were collected from a herbal garden (Orizovo, Stara Zagora region) in Bulgaria at 150 m a.s.l. (42.21°N, 25.16°E) during the full flowering stage in July 2024. The plant was identified by one of the authors (D.Z.) according to www.worldfloraonline.org. A voucher specimen was deposited at the Herbarium Facultatis Pharmaceuticae Sophiensis, Medical University–Sofia, Bulgaria (No. 11 950). Plant material was dried at room temperature.

Sample extraction

Air-dried powdered leaves (52 g) were extracted with 80% methanol (MeOH) (1:20 w/v) by sonication (80 kHz, ultrasound bath Biobase UC-20C) for 15 min (× 2) at room temperature. The extracts were concentrated *in vacuo* and lyophilized (lyophilizer Biobase BK-FD10P) to yield 2.9 g crude extract. The lyophilized extract was subjected to further ultra-high-performance liquid chromatography–high-resolution mass spectrometry (UHPLC–HRMS) and pharmacological analyses.

Chemicals

Acetonitrile (for LC–MS), formic acid (for LC–MS), and methanol (for HPLC) were supplied by Chromasolv (Sofia, Bulgaria). The authentic standards used for compound identification, isovitexin, homoorientin, orientin, rutin, isoquercitrin, hyperoside, luteolin 7-*O*-glucoside, kaempferol 3-*O*-rutinoside, isorhamnetin 3-*O*-rutinoside, kaempferol 3-*O*-glucoside, quercitrin, isorhamnetin 3-*O*-glucoside, apigenin 7-*O*-glucoside, and quercetin, were purchased from Extrasynthese (Genay, France).

Ultra-high-performance liquid chromatography–high-resolution mass spectrometry

The UHPLC–HRMS analyses were carried out as previously described [10.3390/plants14030415] on a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) in negative ion mode with the *m/z*

range from 150 to 1500. The separation was performed on a C18 column, and the temperature was 40 °C. The mobile phase contained 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution program was as follows: 0–1 min from 0 to 5% B; 20 min–30% B; 25 min–50% B; 30 min–70% B; and 33 min–95% B. The flow rate was 0.3 mL/min, and the injection volume was 1 µL. Data acquisition was performed, and data were processed using Xcalibur 4.2 software (Thermo Scientific).

Experimental animals

Adult male H-line mice weighing 30–40 g were used in the study. The animals were housed under standard laboratory conditions (ambient temperature 20 ± 2 °C, humidity 72 ± 4%, and a 12/12 h light/dark cycle) with free access to water and standard pelleted food. The mice were acclimatized for 7 days before the experiments. All procedures were approved by the Institutional Animal Care Committee (No. 448/10.10.2025) and conducted in accordance with EU Directive 86/609/EEC and Good Laboratory Practice (GLP) guidelines.

Experimental design and grouping

Mice (n = 6 per group) were randomly divided into five groups:

- **Group I (control)**—negative control; untreated animals.
- **Group II (carrageenan control)**—0.1 mL of 1% λ-carrageenan, subplantar injection.
- **Group III**—0.1 mL of 1% λ-carrageenan + diclofenac 5 mg/kg, per os, single dose.
- **Group IV**—0.1 mL of 1% λ-carrageenan + *Laurus nobilis* extract 250 mg/kg, per os for ten days.
- **Group V**—0.1 mL of 1% λ-carrageenan + *Laurus nobilis* extract 250 mg/kg (per os for ten days) + diclofenac 5 mg/kg, per os single dose.

Carrageenan-induced paw edema model

Acute inflammation was assessed using the carrageenan-induced paw edema model, one of the most extensively validated *in vivo* methods for screening anti-inflammatory compounds. This experimental approach was first introduced by Winter et al. (1962) and remains widely employed because of its simplicity, reproducibility, and sensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) and natural products. The model closely reflects the vascular and cellular events associated with acute inflammatory reactions, making it suitable for the pharmacological evaluation of novel therapeutic agents (Winter et al. 1962; Di Rosa et al. 1971).

Inflammation was induced by subplantar injection of 50 µL of 0.5% (w/v) λ-carrageenan dissolved in sterile saline solution (0.9% NaCl) into the right hind paw of each mouse. Carrageenan, a sulfated polysaccharide obtained from red algae, provokes a localized inflammatory response characterized by edema formation, increased vascular permeability, leukocyte migration, and the release of multiple inflammatory mediators (Morris 2003; Necas and Bartosikova 2013).

The carrageenan response is classically described as biphasic. The first phase, occurring during the initial hour after injection, is mainly mediated by histamine, serotonin, and bradykinin, which promote vasodilation and plasma exudation. The second phase, usually most evident between the 2nd and 6th hour, is associated with neutrophil infiltration and increased production of prostaglandins, nitric oxide, tumor necrosis factor-α, interleukin-1β, and other cytokines (Di Rosa et al. 1971; Nantel et al. 1999; Posadas et al. 2004). Because cyclooxygenase-derived mediators are prominent during the later phase, the model is especially responsive to NSAIDs such as diclofenac.

Paw swelling was quantified using a plethysmometer (Ugo Basile Co., Italy), which provides an objective measurement of paw volume displacement in milliliters. Measurements were taken immediately before carrageenan injection and again at 1 h, 3 h, and 24 h after induction of inflammation. These time points were selected to capture the early mediator-driven response, the peak inflammatory phase, and the resolution stage of edema. Similar schedules have been reported in previous pharmacological studies evaluating plant-derived anti-inflammatory agents (Morris 2003; Sharma et al. 2007).

The extent of inflammation was expressed as a percentage increase in paw edema relative to baseline according to the following formula:

$$\%\text{Paw edema increase} = \frac{a - b}{b} \times 100$$

where a is the paw volume measured at the selected time point after carrageenan injection and b is the initial paw volume recorded before induction of edema.

Hot plate test

Analgesic activity was evaluated using the hot plate assay, a standard method for measuring responses to thermal nociceptive stimuli. This model is widely accepted for assessing centrally acting analgesic agents because it involves supraspinally integrated responses to acute heat-induced discomfort (Eddy and Leimbach 1953; Le Bars et al. 2001). Unlike peripheral pain models, the hot plate test is particularly useful for detecting substances that modulate pain processing within the central nervous system.

At the 4th hour after carrageenan administration, mice were individually placed on a heated metal plate maintained at 55 ± 0.5 °C. The latency to the first nociceptive reaction, such as paw licking, paw withdrawal, stamping, or jumping, was recorded in seconds. This response latency reflects the threshold of thermal pain perception and serves as an indicator of hyperalgesia or analgesia (Mulder and Pritchett 2004; Yam et al. 2018).

Carrageenan-induced inflammation is frequently accompanied by increased pain sensitivity due to peripheral sensitization and enhanced excitability of nociceptive pathways. Therefore, prolongation of response latency after treatment suggests attenuation of inflammatory pain and antinociceptive activity. The hot plate test has been extensively used for evaluating herbal extracts, opioids, and non-opioid analgesics in preclinical research (Bannon and Malmberg 2007; Deuis et al. 2017).

To prevent tissue injury, a cut-off time of 30 s was established. Animals failing to respond before this limit were removed immediately from the apparatus; however, none of the animals in the present study reached the cut-off value.

The analgesic effect was expressed as percentage inhibition compared with the control group using the following equation:

$$\%\text{Inhibition} = \frac{a-b}{b} \times 100$$

where a is the mean reaction latency of the treated group and b is the mean reaction latency of the control group. Higher values indicate greater analgesic efficacy.

Statistical analysis

Statistical analysis was performed using MedCalc software. Comparisons among groups were made using the non-parametric Kruskal–Wallis test, followed by pairwise comparisons with the Mann–Whitney *U* test. Differences were considered statistically significant at $p \leq 0.05$.

Results

Based on the retention times, MS and MS/MS accurate masses, fragmentation patterns in MS/MS spectra, relative ion abundance, and comparison of retention times with reference standards and literature data, 60 metabolites were identified or tentatively annotated in *L. nobilis* leaf extract (Suppl. material 1: table S1). Identification confidence levels for metabolite profiling were assigned according to the essentials of Çiçek et al. (2024) to be of maximum scientific relevance (Çiçek et al. 2024). Identification confidence levels were as follows: confirmed structure, including confirmed stereochemistry (A2); confirmed structure except for one or more stereochemical aspects (B); tentative identification matched with a standard compound, a match of at least *t*R, MS, and MS/MS with an actual authentic standard analyzed in parallel, preferably supported by other online data (C); tentative identification based on libraries, model compounds, or related evidence (D), relatively reliable evidence (D1); and relatively poor evidence (D2).

Proanthocyanidins

Numerous proanthocyanidins (PACs), including four dimers, five trimers, seven tetramers, and four pentamers, were tentatively identified in the assayed extract (Suppl. material 1: table S1). The approach for their annotation by MS/MS was described elsewhere (Gevrenova et al. 2025). According to the bonding between (epi)catechin units, 11 compounds were characterized by C-C interflavan linkage and could be referred to as B-type PACs (Tang et al. 2022). A hybrid A,B-type with an additional C-O bond was established in six PACs, while an A-type was deduced in one compound (Suppl. material 1: table S1). The key points in the PAC fragmentation pattern are neutral losses of 126 Da (heterocyclic ring fission, HRF, $C_6H_6O_3$), 152 Da (retro-Diels–Alder fission, RDA, $C_8H_8O_3$), and 288 ($C_{15}H_{12}O_6$)/290 Da ($C_{15}H_{14}O_6$) (quinine methane or interflavan fission, QM) (Symma and Hensel 2022; Gevrenova et al. 2025).

The ESI-MS/MS spectra of the isobaric compounds **3**, **6**, and **19** at *m/z* 577.135 [M-H]–, consistent with $C_{30}H_{25}O_{12}$) were acquired (Suppl. material 1: table S1). The fragmentation pathway was delineated by the following transitions: *m/z* 577.135→425.088 (RDA), *m/z* 577.135→407.077 (RDA), *m/z* 577.135→289.072 (QM), and *m/z* 577.135→287.056 (QM), as has been observed in B-type PAC dimers (Gevrenova et al. 2025). The aforementioned PACs could be related to procyanidin B-2, B-4, B-5, and B-7, previously reported in *L. nobilis* leaves (Sakar and Engelshowe 1985). Moreover, dimeric PAC **20** at *m/z* 575.119 $[M-H]^-$, (consistent with $C_{30}H_{23}O_{12}$) yielded prominent QM fragment ions at *m/z* 289.072 (16%) and 285.040 (31%), indicating a C-O linkage (Rush et al. 2018; Gevrenova et al. 2025). Thus, **20** was assigned as an A-type dimer. It is worth noting that this is the first report of A-type PACs in the leaf extract; until now, they have been established in *L. nobilis* wood (Alejo-Armijo et al. 2019).

Four isobars, **5**, **11**, **15**, and **18**, shared the same $[M-H]^-$ at 865.199, consistent with $C_{45}H_{37}O_{18}$ and the structure of trimer PACs (Suppl. material 1: table S1). Subsequent losses of 288 Da (QM fission) led to the indicative fragment ions at *m/z* 577.135 and 289.072 (**5**), while RDA fragmentation resulted in ions at *m/z* 713.153 $[M-H-152]^-$/695.143 $[M-H-(152+H_2O)]^-$ and 425.089/407.077 (**5**). Thus, the aforementioned trimers were referred to as B-type PACs. Regarding **10** ($[M-H]^-$ at *m/z* 863.183), the abundant QM ions at *m/z* 289.072 (45%) and 285.040 (36%) pointed to an A-type linkage, and it was assigned as a hybrid A,B-type trimer (Rush et al. 2018). This is in line with the previously isolated cinnamtannin B-1 from *L. nobilis* leaves (Dall'Acqua et al. 2009).

PAC tetramers were registered in ESI-MS/MS as singly charged ions at *m/z* 1151.246 [M-H]– (**1** and **12**) and doubly charged ions at *m/z* 575.119 ($[M-2H]^{2-}$ (**9** and **16**), consistent with $C_{60}H_{47}O_{24}$ (Suppl. material 1: table S1). The assignment of hybrid A,B-type was discernible by the prominent QM ions at *m/z* 863.184 (ΔM 288 Da, 18.2%); 573.103 (ΔM 290 Da, 2.9%); and 285.040 (ΔM 288 Da, 15.5%) (**1**). Two B-type linkages were deduced from the RDA ions at *m/z* 999.199 and 711.136/693.124, while the third bond was assigned as A-type. The doubly charged ion at 575.117 (**19**) afforded indicative RDA ions at *m/z* 499.096 $[M-2H-76.023]^{2-}$ and 490.090 $[M-2H-85.030]^{2-}$ accompanied by the HRF ion at 449.088 $[M-2H-126.031]^{2-}$, suggesting B-type bonds, while an A-type was deduced from the QM ions at *m/z* 289.072 and 285.041 (Suppl. material 1: table S1). Three B-type bonds were suggested for the structure of **13** ([M-

H]$^-$ at *m/z* 1153.262), corroborated by the QM ions at *m/z* 289.072 (13.1%) and 287.056 (12.5%).

The ESI-MS/MS spectra of PACs **2**, **4**, and **17** with [M-2H]$^{2-}$ at *m/z* 719.152, consistent with $C_{75}H_{59}O_{30}$, were acquired (Suppl. material 1: table S1). An A-type linkage was deduced by the QM ions at *m/z* 576.122 [M-2H-143]$^{2-}$ and 575.120 [M-2H-144]$^{2-}$ alongside the HRF ion at *m/z* 411.072, while B-type was evidenced by QM ions at *m/z* 289.072 and 287.055 (**2**) (Suppl. material 1: table S1). Accordingly, the aforementioned compounds were referred to as A,B-type pentamers. Additionally, B-type pentamer **14** with [M-2H]$^{2-}$ at *m/z* 720.158, consistent with $C_{75}H_{61}O_{30}$) was annotated on the basis of the QM ions at *m/z* 575.119, 289.072, and 287.056, corroborated by RDA ions at *m/z* 644.135 [M-2H-76.023]$^{2-}$and 635.129 [M-2H-85.028]$^{2-}$ (Suppl. material 1: table S1).

Flavonoids

Numerous flavonoids were dereplicated/annotated in *L. nobilis* leaf extract, including five C-glycosylflavones, one C-*O*-glycosylflavone, 19 flavonol *O*-glycosides, one flavanone *O*-glycoside, three flavone *O*-glycosides, and four flavanes. The MS/MS recognition approach was delineated elsewhere (Ak et al. 2021). Based on comparison with MS/MS spectra and retention times of standard references, a series of flavonoids were unambiguously identified as follows: (+) catechin (**23**), homoorientin (**28**), orientin (**29**), rutin (**30**), isoquercitrin (**33**), hyperoside (**34**), luteolin 7-*O*-glucoside (**34**), kaempferol 3-*O*-rutinoside (**39**), isorhamnetin 3-*O*-rutinoside (**40**), kaempferol 3-*O*-glucoside (**41**), quercitrin (**42**), isorhamnetin 3-*O*-glucoside (**43**), apigenin 7-*O*-glucoside (**44**), and quercetin (**49**). Compounds **50–54** could be associated with the same fragmentation patterns, affording the aglycone kaempferol at *m/z* 285.040, corroborated by RDA ions at *m/z* 151.002 ($^{1,3}A^-$) and 107.013 ($^{0,4}A^-$) and coumaric acid (CoA) at *m/z* 163.038 [Co-H]$^-$ alongside *m/z* 145.028 [Co-H-H_2O]$^-$, 119.049 [Co-H-CO_2]$^-$, and 117.033 [Co-H-H_2O-CO]$^-$ (Suppl. material 1: table S1). The coumaroyl, coumaroylhexosyl, and di-coumaroylhexosyl moieties were evidenced by the losses of $C_9H_6O_2$, $C_{15}H_{16}O_6$ (**50**), and $C_{24}H_{22}O_8$ (**51–54**), respectively. Accordingly, **50** was assigned as kaempferol 3-*O*-*p*-coumaroylhexoside, while **51–54** were ascribed to kaempferol 3-*O*-di-*p*-coumaroylhexoside isomers, as has been previously reported (Fiorin et al. 1998; De Marino et al. 2004).

Lignans

Isomeric pairs **56/57**, **58/60**, and **59/61** were associated with the same fragmentation pathway, yielding [M-H-132]$^-$, which is consistent with the loss of $C_5H_8O_4$ (pentose moiety) and aglycons at *m/z* 419.171 ($C_{22}H_{27}O_8$), 389.160 ($C_{21}H_{25}O_7$) and 359.150 ($C_{20}H_{23}O_6$) (Table). The aglycons gave the characteristic transitions [Agl-H-CH_3]$^-$, [Agl-H-CH_3-H_2O]$^-$, [Agl-H-2CH_3-H_2O]$^-$, and [Agl-H-2CH_3-H_2O-CH_2O]$^-$. Accordingly, they were ascribed to lioniresinol, methoxyisolariciresinol, and isolariciresinol, as has been previously reported for 2,7'-cyclolignane-xylosides isolated from *L. nobilis* leaves (Yahara et al. 1992; Li et al. 2018). Compound **55** at *m/z* 521.202 [M-H]$^-$ had the same fragmentation pattern as **59/61** except for the sugar residue ($C_6H_{10}O_5$, hexose). Thus, **55** was assigned as isolariciresinol-hexoside. In the same way, **62** afforded prominent fragment ion at *m/z* 361.166 ($C_{20}H_{25}O_6$) and was related to secoisolariciresinol-pentoside, previously isolated from *L. nobilis* leaves (Yahara et al. 1992).

Anti-inflammatory activity of *Laurus nobilis* extract *in vivo*

Following subplantar administration of 0.1 mL carrageenan into the right hind paw, animals rapidly developed localized swelling characteristic of acute inflammation. The edema response intensified over time and reached its maximum at the 3rd hour after injection. In the carrageenan-treated control group, paw edema rose from 28.55% at 1 h to 47.80% at 3 h, with residual swelling still evident after 24 h (32.30%), confirming successful induction of the inflammatory model.

Treatment with diclofenac (5 mg/kg), *L. nobilis* extract (250 mg/kg for 10 days), or the combined regimen reduced paw swelling relative to the carrageenan control animals. Diclofenac alone produced the most marked anti-inflammatory response during the later stage of inflammation, lowering edema values to 18.85% at 3 h and 9.84% at 24 h. This outcome is in agreement with the established mechanism of diclofenac, which suppresses prostaglandin-mediated inflammatory processes.

The group receiving *L. nobilis* extract alone also demonstrated a reduction in edema formation, although the effect was less pronounced than that observed with diclofenac at the peak phase. At 1 h, edema in this group measured 17.63%, which was lower than both the carrageenan control and diclofenac groups. At 3 h, swelling reached 27.17%, remaining substantially below the untreated inflamed animals, and further decreased to 15.32% after 24 h. These observations indicate that the extract exerted a moderate inhibitory effect on both the initial and delayed phases of carrageenan-induced inflammation.

Co-administration of laurel extract (250 mg/kg for 10 days) with diclofenac (5 mg/kg) also alleviated paw edema, with recorded values of 33.22%, 24.73%, and 18.37% at 1, 3, and 24 h, respectively. Although this treatment was beneficial compared with the carrageenan control group, it did not outperform diclofenac alone and was less effective than the extract monotherapy at the 24 h time point. These findings suggest that the combined treatment did not produce a synergistic response, and its effect may largely reflect the pharmacological action of diclofenac (Fig. 1).

Direct paw volume measurements supported the percentage edema data. In the carrageenan control group, paw volume increased from 0.68 mL before induction to 1.01 mL at 3 h, followed by a slight decline to 0.90 mL after 24 h. In comparison, diclofenac limited the peak volume to 0.73 mL and restored paw size close to baseline at 24 h (0.67 mL). Animals treated with laurel extract showed volumes of 0.88 mL at 3 h and 0.80 mL at 24 h, whereas the

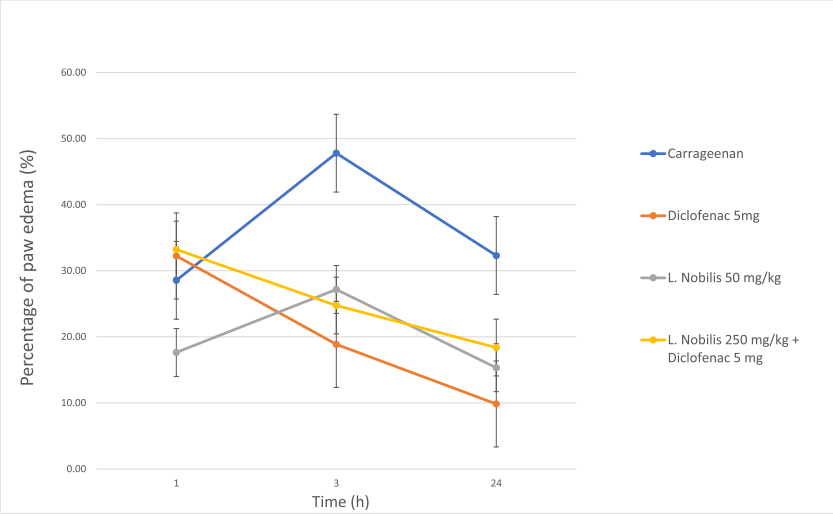


Figure 1. Anti-inflammatory effects of carrageenan, *L. nobilis* extract (250 mg/kg for 10 days), diclofenac (5 mg/kg), and their combination on carrageenan-induced edema.

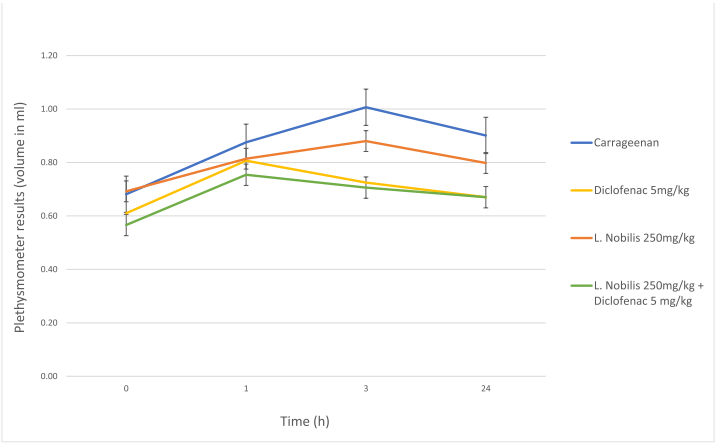


Figure 2. Paw volume in carrageenan, *L. nobilis* extract (250 mg/kg for 10 days), diclofenac (5 mg/kg), and combination groups.

combination group exhibited lower values of 0.71 mL and 0.67 mL at the corresponding time points. Collectively, these results demonstrate that *L. nobilis* possesses appreciable anti-inflammatory activity, although diclofenac remained the most potent treatment in this model (Fig. 2).

Antinociceptive activity of *Laurus nobilis* extract *in vivo*

The analgesic potential of *L. nobilis* extract and diclofenac was assessed using the hot plate assay by measuring the latency to pain response. The carrageenan control group displayed the shortest reaction time (5.7 s), indicating enhanced pain sensitivity. All treated groups showed prolonged response latencies compared with the control group, reflecting an antinociceptive effect.

Administration of diclofenac (5 mg/kg) increased the response latency to 8.60 s, corresponding to 41.68% inhibition of nociceptive behavior. This result is consistent with the well-documented analgesic properties of diclofenac through inhibition of cyclooxygenase activity and reduced formation of inflammatory mediators.

Notably, *L. nobilis* extract at 250 mg/kg for 10 days produced the longest latency time (9.12 s) and the greatest inhibition value (50.25%), exceeding the effect observed with diclofenac alone. This suggests that the extract has substantial analgesic activity and may contain biologically active constituents capable of influencing pain perception pathways. Such activity may be attributed to the presence of flavonoids, terpenes, or phenolic compounds reported in *Laurus nobilis*.

By contrast, the combined administration of laurel extract with diclofenac resulted in a latency of 7.42 s and 22.24% inhibition, which was inferior to both monotherapy groups. Therefore, the combined treatment did not enhance pain relief and appeared less effective than either agent administered separately. This may indicate the absence of additive interaction or possible pharmacodynamic interference between the extract components and diclofenac (Fig. 3).

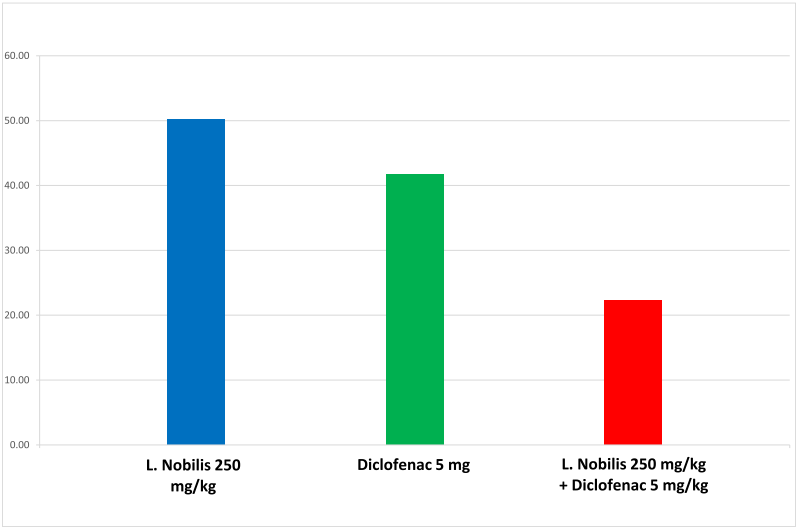


Figure 3. Percentage inhibition compared to the negative control.

Discussion and conclusion

An in-depth UHPLC–HRMS analysis of *L. nobilis* methanol–aqueous extract was performed, allowing for the identification/annotation of 60 secondary metabolites. For the first time, a series of PAC trimers and tetramers from both B- and A,B-types were tentatively identified. It is worth noting that coumaroyl and di-coumaroyl deoxyhexosides of kaempferol were annotated in the extract alongside the well-known flavonol glycosides and pentosides of lignans lioniresinol, methoxyisolariciresinol, isolariciresinol, and secoisolariciresinol.

The present study demonstrates that *L. nobilis* leaf extract possesses notable anti-inflammatory and analgesic activities in acute experimental models of inflammation and pain. These effects were confirmed by the reduction of carrageenan-induced paw edema and the prolongation of reaction latency in the hot plate test. Collectively, the findings support the traditional use of *Laurus nobilis* in the management of painful and inflammatory conditions and indicate that this medicinal plant may represent a valuable source of bioactive therapeutic compounds.

Administration of laurel extract at 250 mg/kg significantly reduced edema formation compared with the carrageenan control group, although its activity was lower than that of diclofenac at the peak inflammatory stage. This suggests that the extract may act on both the initial and delayed phases of inflammation. The anti-inflammatory activity of *L. nobilis* could be attributed to its rich phytochemical composition, including flavonoids and phenolic compounds, possessing antioxidant and mediator-modulating properties (Conforti et al. 2006; Ramos et al. 2012; Caputo et al. 2017). As previously described, the presence of flavonoids such as rutin and quercetin defines the investigated anti-inflammatory activity in *in vivo* experimental models (Rotelli et al. 2003). In addition, the occurrence of the identified proanthocyanidins and lignans explains the pronounced effect (Alejo Armijo et al. 2017; Li et al. 2018; Dobroslavić et al. 2021). Thus, through inhibition of oxidative stress and inflammatory signaling pathways, these constituents may collectively contribute to the reduction in paw edema observed in this study.

The combined treatment of laurel extract with diclofenac did not demonstrate superior anti-inflammatory efficacy compared with diclofenac alone. Although the combination significantly lowered edema relative to the carrageenan control group, no additive or synergistic effect was detected. This outcome may indicate that both agents share overlapping mechanisms, particularly interference with prostaglandin-mediated pathways, thereby limiting the benefit of co-administration. It is also possible that pharmacodynamic or pharmacokinetic interactions between plant constituents and diclofenac reduced the expected combined response. Similar findings have been reported in studies where herbal preparations did not potentiate standard anti-inflammatory drugs when tested at fixed doses (Williamson 2001).

The hot plate assay further revealed the marked analgesic potential of *L. nobilis*. In this model, the extract produced a greater increase in latency time than diclofenac, indicating stronger antinociceptive activity under thermal stimulation. Because the hot plate method is particularly sensitive to centrally acting analgesics, this result suggests that *L. nobilis* may modulate nociceptive processing at spinal or supraspinal levels in addition to its peripheral anti-inflammatory effects (Le Bars et al. 2001; Bannon and Malmberg 2007). Bioactive terpenoids and flavonoids present in aromatic plants have been shown to interact with opioid receptors, GABAergic neurotransmission, and transient receptor potential channels, which may partly explain the enhanced pain threshold observed with the extract (Khoddami et al. 2013; Deuis et al. 2017).

In contrast, the combination of *L. nobilis* with diclofenac resulted in lower analgesic activity than either treatment administered alone. This finding indicates that simultaneous administration does not necessarily improve the therapeutic outcome and may even attenuate efficacy. Potential explanations include receptor-level competition, altered absorption, or modulation of intracellular signaling pathways. These results emphasize the importance of

experimentally investigating herb–drug combinations before considering their clinical application.

In conclusion, *Laurus nobilis* leaf extract rich in phenolic compounds, including flavonoids and proanthocyanidins, exhibited significant anti-inflammatory and analgesic activities *in vivo*. Although diclofenac remained more effective in suppressing carrageenan-induced edema, the extract showed superior efficacy in the hot plate model, indicating particularly strong analgesic potential. Combined administration with diclofenac did not enhance activity, suggesting that the extract may be more beneficial as an independent therapeutic candidate rather than as an adjunct treatment at the tested doses. For the first time, numerous proanthocyanidins, including mainly tetramers and pentamers from B- and hybrid A,B-types, as well as lignans and flavonols, were annotated in laurel methanol–aqueous leaf extract. Overall, these findings support the medicinal relevance of *Laurus nobilis* and encourage further studies aimed at developing it as a natural source of anti-inflammatory and analgesic agents.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

Experiments on animals: No. 448/10.10.2025.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
No AI tools were used in the preparation of this manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary material: table S1

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Data type: docx

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