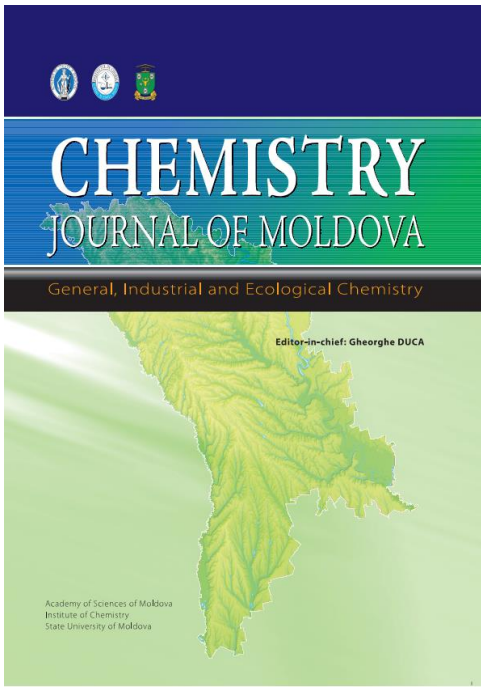

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PHYTOCHEMICAL ANALYSIS AND BIOACTIVITIES OF DIFFERENT ORGANS OF *BUNIUM FERULACEUM* SM.

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Abstract. This study provides an integrated chemical and biological evaluation of hydro-methanolic extracts from the leaves, flowering heads, and tubers of *Bunium ferulaceum* Sm. Phytochemical profiling revealed organ-dependent variations, with leaves rich in phenolics (53.13 mg GAE/g), flavonoids (21.45 mg QE/g), and tannins (10.17 mg TAE/g), while tubers showed the highest triterpene content (8.15 µg UAE/mg). These compositional differences in functional phytochemical classes were statistically correlated with biological responses: triterpenes showed a strong association with anti-inflammatory effects (r= –0.97), while polyphenols, particularly phenolics and flavonoids, were correlated with antibacterial activity (r= –0.91 and –0.90, respectively). The chemical data highlight the coexistence and complementary mechanisms of polyphenols and triterpenoids, supporting a structure-activity relationship that underlies the pharmacological potential of *B. ferulaceum*. Overall, the study emphasizes the chemical rationale behind its bioactivity, providing a solid basis for future isolation and mechanistic studies.

Keywords: *Bunium ferulaceum* Sm, phytochemical analysis, anti-inflammatory activity, antibacterial activity.

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Introduction

Inflammation represents a complex biological response to physical, chemical, or microbial stressors, involving a cascade of biochemical mediators and signalling pathways. While steroidal and nonsteroidal anti-inflammatory drugs remain clinically effective, their prolonged use is limited by severe side effects, including gastrointestinal toxicity, hypertension, and cardiovascular risks [1,2]. In parallel, the emergence of antibiotic-resistant microorganisms due to antibiotic overuse has created an urgent demand for alternative agents with improved safety profiles [3]. Natural products, particularly those derived from medicinal plants, constitute a promising reservoir of bioactive compounds with structural diversity and multifunctional mechanisms. Among these, polyphenols, flavonoids, tannins, and triterpenoids stand out for their capacity to modulate oxidative and inflammatory processes, often acting through redox modulation, enzyme inhibition, and membrane stabilization [4-7].

From a chemical standpoint, *Bunium ferulaceum* Sm. (syn. *Bunium incrassatum* (Boiss.) Amo.), a species of the Apiaceae family endemic to North Africa, is particularly rich in structurally diverse secondary metabolites. Phytochemical reports have identified numerous secondary metabolites belonging to different chemical classes, including coumarins (*e.g.*, scopoletin, scoparone), sterols (*β*-sitosterol), triterpenoids (ursolic and oleanolic acids), and phenolic derivatives (chlorogenic acid, rutin, quercetin) in different plant parts [8-13]. These compounds contain reactive hydroxyl, carboxyl, and methoxy groups responsible for antioxidant, enzyme-inhibitory, and membrane-stabilizing activities [7,14]. For example, the number and position of hydroxyl substituents in flavonoids determine their capacity to chelate metal ions and scavenge free radicals [7,15], while pentacyclic triterpenes contribute to anti-inflammatory and antimicrobial effects through interaction with lipid membranes and modulation of enzymes such as cyclooxygenase and lipoxygenase [5,14,16]. The coexistence of these metabolites within

B. ferulaceum defines a chemically rich matrix with potential synergistic bioactivity, supported by structure-activity relationship (SAR) models in natural product chemistry [17,18]. Although several studies have previously investigated *B. ferulaceum*, most have focused on individual plant parts (tubers, aerial parts, or seeds) or on the qualitative identification of specific metabolites. Comprehensive quantitative comparisons of major bioactive classes (phenolics, flavonoids, tannins) among different organs and particularly the quantification of triterpenes, which has not been previously reported remain limited.

Quantitative phytochemical analyses using colorimetric assays such as the Folin-Ciocalteu method for total phenolics and the $AlCl_3$ method for flavonoids allow for standardized assessment of these compound classes [17,19]. When integrated with correlation analyses between metabolite concentrations and biological activity, such approaches provide a rational framework for exploring chemical-biological relationships [18].

The present study aims to establish a chemical basis for the bioactivity of *B. ferulaceum* by quantitatively characterizing its major phytochemical groups (phenolics, flavonoids, tannins, and triterpenes) in hydro-methanolic extracts from different organs and correlating these data with anti-inflammatory and antibacterial outcomes. This chemically driven approach distinguishes the work from previous qualitative reports and contributes to understanding the structure activity relationships underlying the pharmacological potential of *B. ferulaceum*.

Experimental

Materials

Methanol (95%), sodium carbonate, aluminium chloride, sodium chloride, sodium phosphate (96%), hydrochloric acid (37%), acetic acid (99%), perchloric acid (70%), bovine serum albumin (≥98%), and diclofenac sodium (≥98%) were purchased from Sigma-Aldrich (Germany). The phosphate buffer (pH 7.2–7.6) and sodium phosphate buffer (pH 7.4) were also obtained from Sigma-Aldrich. The Folin-Ciocalteu phenol reagent (2.1 N) was supplied by Chem-Lab (Belgium). Standard compounds, including gallic acid (≥97%), vanillin (≥99%), tannic acid (≥86%), quercetin, and ursolic acid (≥95%), were acquired from Sigma-Aldrich (Germany). Fresh blood was obtained from a healthy donor.

The leaves and flowering heads (FH) were collected in April and the tubers in September 2022, from Sougueur, Wilaya of Tiaret (West of Algeria) geographically located at N 35° 11′ 01″, E 1° 29′ 45″, A 900 m. They were washed thoroughly with water, dried in shadow for 20 days. The different parts were ground into fine powder, weighed and stored in sealed flasks under dark conditions.

Physical measurements

The Camspec M550 UV-Vis spectrophotometer (United Kingdom), featuring a double beam and quartz cuvettes (l= 1 cm), was used for absorbance measurements. The pH Meter HI-2211 (Hanna Benchtop, Romania) and the incubator BE300 (Memmert, Germany) were also utilized.

Methods

Preparation of plant extracts

Twenty grams of shade air-dried of each grounded material were macerated in 200 mL of 70% (v/v) hydro-methanolic solvent, for 24 h with constant stirring at room temperature and then filtered. The solid residue obtained was subjected to a second extraction using a methanol/water mixture (50:50 v/v) for 24 h [8]. After the second filtration, the filtrates from both processes were combined and concentrated until dryness in rotary vacuum evaporator at 40°C. The obtained residues were conserved in the dark at 4–6°C be used later.

Phytochemical analysis

Total phenolic content (TPC)

The TPC in hydro-methanolic extracts was determined by Folin-Ciocalteu reagent assay [20]. A 100 µL sample of the extract was combined with 100 µL of Folin-Ciocalteu reagent, diluted at a ratio of 1:20. This mixture was added to 2 mL of 2% Na_2CO_3, thoroughly mixed, and incubated for 30 minutes in the dark at room temperature. After incubation, the absorbance was measured at 700 nm. Gallic acid served as the standard for constructing the calibration curve, with TPC expressed as mg of gallic acid equivalent per gram of dry extract (mg GAE/g dry extract).

Total flavonoid content (TFC)

As described [9], quercetin was used to set up the calibration curve in methanol (0.005–0.025 mg/mL). For the analysis, both the standard solution and the sample, prepared from a ten-fold-diluted extract in methanol (1 mL), were combined with 1 mL of $AlCl_3$ methanol solution (2%, w/v). Following a 15-minute incubation at room temperature, absorbance was recorded at 430 nm. The TFC was expressed as mg quercetin equivalent per gram of dry extract (mg QE/g dry extract).

Condensed tannin content (CTC)

To determine the tannins present in the extract, 50 µL of extract was added to 1500 µL of a 4% (m/v) vanillin/methanol solution. After being

stirred, 750 µL of concentrated hydrochloric acid was added. The absorbance was measured at 550 nm after 20 minutes of incubation [21]. Tannin content was expressed as mg of tannic acid equivalents per gram of dry extract (mg TAE/g dry extract).

Total triterpene content (TTC)

The TTC was determined using the vanillin–perchloric acid colorimetric method [22]. A 30 µL of the plant extracts, 50 µL of 5% (w) of vanillin prepared in acetic acid, and 100 µL of perchloric acid were mixed. The resulting mixture was incubated at 60°C for 45 min and cooled in an ice-water bath. Then, 450 µL of acetic acid was added. The absorbance was measured at 548 nm. Ursolic acid was used as standard to prepare the calibration curve and the results were expressed as µg ursolic acid equivalent per mg of dry extract (µg UAE/mg dry extract).

Anti-inflammatory activity

The anti-inflammatory potential of *B. ferulaceum* hydro-methanolic extracts was evaluated using two complementary *in vitro* assays: inhibition of heat-induced bovine serum albumin (BSA) denaturation [23] and protection of erythrocyte membranes against hypotonicity-induced hemolysis [24]. Diclofenac sodium served as the reference drug in both tests.

Antibacterial activity

The antibacterial potential of *B. ferulaceum* hydro-methanolic extracts was evaluated against a panel of Gram-positive and Gram-negative conditional-pathogenic bacteria using the disk diffusion method and agar dilution assays. Ciprofloxacin was employed as a reference antibiotic [25,26]. The antibacterial activity was expressed as inhibition zone diameters and minimum inhibitory and bactericidal concentrations (MIC and MBC, respectively).

Statistical analysis

All measurements were carried out in triplicate, and the data are reported as mean ± standard deviations. Analysis of variance (ANOVA) was used for the statistical analysis of data. $p< 0.05$ was considered statistically significant for general analyses. Additionally, Pearson correlation coefficients were calculated to assess the relationships between the concentrations of phytochemicals and their respective anti-inflammatory and antibacterial activities, with significance evaluated at $p< 0.05$ for these correlations.

Results and discussion

Phytochemical analysis

Extraction yield and total phenolic, flavonoid, tannin and triterpene contents

The different parts from *B. ferulaceum* Sm are used for the treatment of various diseases, and its extract contains many bioactive compounds. Phenolic compounds and triterpenes are key bioactive components responsible for its medicinal benefits [8-10]. The extractive yields of hydro-methanolic macerates were 15.14% for leaves, 7.25% for tubers, and 4.75% for FH. Quantitative spectrophotometric analyses of major phytochemical groups are summarized in Table 1. Leaves extract exhibited the highest levels of total phenolics (TPC, 53.13±0.05 mg GAE/g), flavonoids (TFC, 21.45±0.33 mg QE/g), and condensed tannins (CTC, 10.17±0.43 TAE/g), followed by the tuber extract (20.18±0.11 mg GAE/g; 5.66±0.13 mg QE/g; 2.24±0.11 TAE/g). The FH extract showed the lowest concentrations (13.19±0.09 mg GAE/g; 2.62±0.44 mg QE/g; 1.33±0.22 TAE/g). Conversely, total triterpene content (TTC) was highest in the tuber extract (8.15±0.32 µg UAE/mg), followed by leaves and FH (5.25±0.46 and 3.11±0.32 µg UAE/mg, respectively).

These findings agree with earlier reports on *B. ferulaceum* methanolic extracts showing high phenolic levels in tubers [27,28], aerial parts [8], flowering heads [29], and seeds [13]. Variations in TPC, TFC, and CTC values among studies may be attributed to geographical origin, environmental conditions, harvest period, and solvent polarity [20].

Table 1

Total triterpene, phenolic, flavonoid, tannin contents and anti-inflammatory activity of plant extracts.

Extract	*Yield* [a] *%*	*Total phenolics* [a] *mg of GAE/g*	*Total flavonoids* [a] *mg of QE/g*	*Total tannins* [a] *mg of TAE/g*	*Total triterpenes* [a] *µg UAE/mg*	IC_{50} [a,b] *(µg /mL)*	IC_{50} [a,c] *(µg /mL)*
Leaves	15.14	53.13±0.05	21.45±0.33	10.17±0.43	5.25±0.46	329.88±0.81	816.71±0.02
Tubers	7.25	20.18±0.11	5.66±0.13	2.24±0.11	8.15±0.32	273.23±0.71	684.33±0.05
FH	4.75	13.19±0.09	2.62±0.44	1.33±0.22	3.11±0.32	344.41±0.02	1221.33±0.11
Diclofenac	-	-	-	-	-	287.22±0.38	714.70±0.64

[a] Each value in the table was obtained by calculating the average of three experiments ± standard deviation. Means with different letters were significantly different at the level of $p< 0.05$; [b] 50% inhibitory concentration, BSA bovine serum albumin; [c] 50% inhibitory concentration, hemolysis. '-' not determined.

The differences observed between plant organs also highlight organ-specific metabolite partitioning, typical of Apiaceae species [13]. To our knowledge, this is the first report quantifying triterpenes in *B. ferulaceum*. In related species, such as *B. bulbocastanum*, triterpenes were undetectable in methanolic extracts [30], suggesting that triterpenoid biosynthesis in *B. ferulaceum* may be species-specific. The moderate TTC values obtained are consistent with the limited polarity of pentacyclic triterpenes, which are better solubilized in less polar solvents.

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Chemically, the coexistence of polyphenols (phenolic acids, flavonoids, and tannins) with triterpenoids provides a valuable bioactive matrix. These two groups of metabolites often exhibit complementary biological functions: polyphenols act primarily through redox modulation and enzyme inhibition, while triterpenes contribute *via* membrane stabilization and modulation of inflammatory mediators [5,31]. Therefore, the phytochemical profile of *B. ferulaceum* supports its pharmacological potential and justifies further chemical-biological correlation studies.

Anti-inflammatory activity

As shown in Table 1 and Figures S1 and S2 (see supplementary information), the hydro-methanolic extracts of *B. ferulaceum* inhibited heat-induced BSA denaturation and erythrocyte membrane haemolysis in a concentration-dependent manner. Among the tested extracts (150–500 µg/mL) for BSA denaturation, the tuber extract showed the strongest inhibition (IC_{50}= 273.23±0.71 µg/mL), surpassing diclofenac sodium (IC_{50}= 287.22±0.38 µg/mL). The leaf extract displayed moderate activity (IC_{50}= 329.88±0.81 µg/mL), whereas the FH extract was least active (IC_{50}= 344.41±0.02 µg/mL). In the haemolytic assay (300–1000 µg/mL), the same pattern was observed: the tuber extract exhibited the highest membrane-stabilizing capacity (IC_{50}= 684.33±0.05 µg/mL). As reflected in Table S1 (see supplementary information), correlation analysis revealed a strong negative correlation between triterpene content and IC_{50} values (r= –0.97 for BSA denaturation; r= –0.93 for haemolysis), confirming that triterpenes are the major contributors to the anti-inflammatory activity. In contrast, phenolics, flavonoids, and tannins exhibited weaker correlations (–0.37<r<0.23), suggesting secondary or supportive roles. A significant positive correlation between the two assays (r= 0.82) indicates that protein stabilization and membrane protection share similar chemical drivers. These findings are consistent with the known pharmacological actions of triterpenoids, including membrane stabilization and inhibition of pro-inflammatory mediators such as cyclooxygenase and lipoxygenase [5]. Polyphenols, particularly flavonoids and tannins, may enhance these effects by scavenging reactive oxygen species and reinforcing membrane integrity [32]. Previous studies on *B. ferulaceum* tuber and aerial parts also reported inhibition of protein denaturation and xylene-induced inflammation [8,10,12,28], further supporting our results. Additionally, quercetin a flavonoid abundant in Apiaceae, and tuber polysaccharides have been implicated in NF-*κ*B and MAPK modulation, providing complementary protection [33].

Overall, these chemical-biological correlations demonstrate that triterpenes constitute the primary anti-inflammatory agents in *B. ferulaceum*, while polyphenols (flavonoids, tannins, phenolic acids) act synergistically to enhance the overall activity [14,31,34,35].

Antibacterial activity of extracts

The antibacterial potential of *B. ferulaceum* hydro-methanolic extracts was evaluated against 7 conditional-pathogenic strains using disc diffusion and agar dilution assays (Table S2, supplementary information). Both leaf and tuber extracts exhibited notable bactericidal effects, while the FH extract was less active. Gram-positive bacteria were more sensitive than Gram-negative strains, likely due to the absence of an outer lipopolysaccharide layer [26,27]. The leaf extract demonstrated the highest efficacy (MIC= 0.75–11.9 mg/mL), particularly against *S. aureus* and *E. faecalis* (MIC= 0.75 and 0.92 mg/mL), whereas the tuber extract showed

moderate potency (MIC= 1.95–15.65 mg/mL). The FH extract exhibited weak inhibition (MIC > 8.55 mg/mL). Although less potent than ciprofloxacin (MIC= 0.02-1 mg/mL), the leaf extract showed remarkable activity against *P. aeruginosa* (MIC= 11.9 mg/mL; inhibition zone= 18.65 mm), a strain often resistant to multiple antibiotics. While ciprofloxacin acts as a purified synthetic antibiotic targeting a single enzymatic pathway, plant extracts represent complex multicomponent systems acting through multiple mechanisms. Such systems are generally less prone to resistance development, may act synergistically with conventional antibiotics, and provide safer, eco-friendly alternatives for preventive or complementary antimicrobial strategies [15,36]. Correlation analysis revealed strong negative relationships between antibacterial activity and total phenolics (r= –0.91), flavonoids (r= –0.90), and tannins (r= –0.87), confirming that polyphenols are the main contributors to antibacterial potency (Table S1, supplementary information). Triterpenes showed a moderate correlation (r= –0.49), implying a supporting or synergistic role [12].

Chemically, phenolics act through enzyme inhibition, membrane disruption, metal chelation, and protein precipitation [12,26,30]; flavonoids intercalate with DNA and form complexes with microbial proteins, while tannins destabilize cell walls and inhibit extracellular enzymes. Triterpenoids, though less polar, can interact with lipid membranes, enhancing permeability and facilitating the diffusion of phenolic compounds [15,16]. Therefore, the antibacterial efficacy of *B. ferulaceum* extracts likely results from the concerted action of polyphenols and triterpenes, with the former exerting the primary effect and the latter improving membrane interactions and compound uptake.

Conclusions

The present work integrates phytochemical profiling with biological evaluation to elucidate the chemical basis of *B. ferulaceum* bioactivity. Quantitative analyses revealed distinct organ-specific metabolite distributions, with triterpenes dominating tubers and polyphenols (phenolic acids, flavonoids, tannins) prevailing in leaves. Correlation analysis confirmed that anti-inflammatory activity is primarily associated with triterpenes, whereas antibacterial effects are driven by polyphenols. These findings emphasize the central role of chemical composition in mediating biological responses and the complementary functions of these metabolite classes. Overall, this study highlights the chemical diversity and structure-activity relationships within *B. ferulaceum*, establishing a solid foundation for future isolation, characterization, and pharmacological exploration of its bioactive constituents.

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Supplementary information

Supplementary data are available free of charge at http://cjm.ichem.md as PDF file.

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