Trop J Nat Prod Res, November 2025; 9(11): 5602 – 5607

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

# Tropical Journal of Natural Product Research

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

*Original Research Article*

# Phytochemical Screening, Total Phenolic, Flavonoid, Flavonol, and Tannin Contents and Antioxidant Activity of Extracts from Wild *Herniaria hirsuta* Growing in Morocco

Achraf Hamik[1], Majda Benabbes[1], Abdelmounaim Laabar[2], Mouna Ameggouz[3]*, Ayoub EL Hamri[4], Soufiane Drioua[3], Nour-Eddine Loud[5], Fatima Zahra Benkhouili[1], Rania Jerada[3], Hanae EL Monfalouty[6], Jaouad EL Harti[1]

[1]*Laboratory of Therapeutic Chemistry, Faculty of Medicine and Pharmacy, Rabat Institutes, Mohammed V University, Rabat, BP 6203, Morocco*
[2]*Laboratory of Pharmacology and Toxicology, Biopharmaceutical and Toxicological Analysis Research Team, Faculty of Medicine and Pharmacy, University Mohammed V of Rabat, Morocco*
[3]*Laboratory of analytical Chemistry, Faculty of Medicine and Pharmacy, Rabat Institutes, Mohammed V University, Rabat, BP 6203, Morocco*
[4]*Departement of Chemistry, Faculty of Sciences, Mohammed V University, Rabat, Morocco*
[5]*Laboratory of Pharmacognosy, Mohammed V University, Faculty of Medicine and Pharmacy, Rabat BP 6203, Morocco*
[6]*Laboratory of Plant Chemistry, Organic and Bio organic Synthesis, Faculty of Medicine and Pharmacy, Rabat Institutes, Mohammed V University, Rabat, BP 6203, Morocco*

.

ARTICLE INFO

*Article history:*
Received 14 April 2025
Revised 10 November 2025
Accepted 17 November 2025
Published online 01 December 2025



ABSTRACT

*Herniaria hirsuta* is a medicinal plant widely distributed in North Africa, Europe, and Asia. The plant is used in Moroccan traditional medicine for the treatment of a number of ailments. This study aimed to investigate the phytochemical profile and antioxidant activity of extracts of *Herniaria hirsuta* aerial parts. Plant extracts were obtained through aqueous decoction (EXD), hexane extraction (FHE), and ethanol extraction (FET) using a Soxhlet apparatus. Phytochemical analyses including preliminary phytochemical screening, total phenolic, flavonoid, flavonol, and tannin contents were done according to standard procedures. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging, and ferric reducing antioxidant power (FRAP) assays. EXD had the highest percentage yield (30.26 ± 1.89%), followed by FET (26.66 ± 1.65%) and FHE (14.5 ± 1.27%). EXD also had the highest polyphenol content (5.18 ± 0.54 mg GAE/g), followed by FET (4.03 ± 0.67 mg GAE/g) and FHE (3.24 ± 1.81 mg GAE/g). FHE exhibited the highest levels of flavonoids (14.35 ± 0.98 mg QE/g) and flavonols (8.35 ± 0.24 mg QE/g), while EXD showed lower flavonoid and flavonol contents (7.230.34 mg QE/g and 5.34±0.13 mg QE/g, respectively). Tannin content was highest in FHE (2.95±0.46 mg CE/g) and lowest in FET (0.28±0.14 mg CE/g). Among the extracts, EXD demonstrated superior antioxidant activity with $IC_{50}$ values of 703.33±0.98 µg/mL, 29.09±0.87 µg/mL, and 814.34±1.14 µg/mL in the DPPH, ABTS, and FRAP assays, respectively. These findings highlight the potential of *Herniaria hirsuta* in preventing and treating diseases associated with oxidative stress.

***Keywords:*** *Herniaria hirsuta*, Phytochemical analysis, Antioxidant activity, Polyphenols, Tannins.

## Introduction

Oxidative stress, defined as an imbalance between the production of reactive oxygen species (free radicals) and antioxidant defenses, plays an important role in the development and progression of various diseases, including neurodegenerative disorders, cardiovascular disease, cancer, and diabetes.[1] Natural compounds from plant extracts are effective in suppressing or inhibiting cellular damage caused by free radicals.[2–4] Antioxidant properties of plants have helped to delay, reduce, and eliminate oxidative damage to target molecules.[5,6]

*Corresponding author. Email: mouna_ameggouz@um5.ac.ma, mouna.ameggouz@yahoo.com
Tel: +212620054405

Citation: Hamik A, Benabbes M, Laabar A, Ameggouz M, El Hamri A, Drioua S, Loud N-E, Benkhouili FZ, Jerada R, El Monfalouty H, El Harti J. Phytochemical Screening, Total Phenolic, Flavonoid, Flavonol, and Tannin Contents and Antioxidant Activity of Extracts from Wild *Herniaria hirsuta* growing in Morocco. Trop J Nat Prod Res. 2025; 9(11): 5602 – 5607
https://doi.org/10.26538/tjnpr/v9i11.47

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

All living cells, particularly those that are close to locations where photosynthesis releases active oxygen are at risk of oxidative activities.[7] Morocco is a Mediterranean country with diverse meteorological conditions (humid and sub-humid climates).[8] With about 4200 species, the Moroccan flora is one of the most diverse and prolific in the entire globe. For millennia, the Moroccan people, particularly those in rural areas, have employed a variety of species for medicinal purposes. Most of these species have been listed in the Moroccan pharmacopeia.[9,10] Medicinal plants contain numerous natural antioxidant compounds, such as polyphenols, flavonoids, tannins, stilbenes, and coumarins.[11] These compounds prevent free radical-mediated oxidative damage by acting at different sites in the pathophysiological pathway.[1,3] The genus Herniaria (Caryophyllaceae) includes several species (*H. glabra*, *H. hirsuta*, and *H. fontanesii*) that are widely distributed in North Africa, Europe, and Asia.[12,13] This genus is used in traditional Moroccan medicine as a diuretic and for the treatment of kidney diseases.[14,15] In Algeria, the plant is used to treat bladder catarrh, bladder stones, and as a preventive treatment against the formation of kidney stones and urinary sand.[14,15] In Europe, Herniariae Herba, which contains *H. glabra* and *H. hirsuta*, is used as a urological drug.[12,15] Phytochemical studies on these species revealed the presence of saponins, flavonoids, and coumarins. While *H. fontanesii* was reported to contain herniariasaponins A–D which are bidesmosidic triterpenoid saponins,[16,17] two monodesmosidic derivatives of medicagenic acid,

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

herniariasaponins E and F, were isolated from the aerial parts of *H. hirsute*.[14] Herniaria has been known for different indications such as edema, bladder catarrh, urine retention, albuminuria, uremia, renal colic, diabetes, most kidneys and bladder diseases, bronchial catarrh, pulmonary phthisis, jaundice, leucorrhea, syphilis, skin diseases due to toxins in the blood, and weak eyesight associated with albuminuria or uremic disorders.[18]

Previous studies have shown that *Herniaria hirsuta* is used to treat various ulcers, and lax wounds.[19] Additionally, it is known for its aseptic, lightly spasmolytic, and potent anti-inflammatory effects in the urinary tract, kidney, and gall bladder.[19] The main objective of this study was to investigate the phytochemicals, total phenolic, flavonoid, flavonol, and tannin contents of Moroccan *Herniaria hirsuta* extracts obtained by decoction and Soxhlet extraction, and to determine the antioxidant capacity of these extracts.

## Materials and Methods

*Plant collection and identification*

Aerial parts of *Herniaria hirsuta* were collected in April 2021 from M'rirt province of khenifra béni Mellal in the Khenifra region of Morocco (Figure 1). The plant material was identified and authenticated at the Scientific Institute of Rabat, where it was cataloged in the institute's herbarium, and assigned the voucher specimen number RAB123456. After collection, the plant material was meticulously cleaned and air-dried at room temperature. The dried plant material were finely powdered and stored in a cool, dark, dry environment to protect them from exposure to light and humidity until further use.

**Figure 1:** Map of M'rirt province of khenifra béni Mellal, Moroco[36]

*Preparation of extracts*

To formulate the extract, 30 g of finely powdered aerial parts of *Herniaria hirsuta* was subjected to hot aqueous extraction for 30 minutes (referred to as EXD). Subsequently, an equivalent amount of the same powder was packed into a cotton cellulose cartridge and extracted with 500 mL of two distinct solvents via the Soxhlet extraction technique. Initially, hexane was employed (referred to as FHE), followed by ethanol for the residual plant material (designated as FET), yielding two distinct fractions. The resulting three extracts (EXD, FHE, and FET) were subsequently filtered through Whatman filter paper, followed by solvent evaporation under vacuum conditions using a rotary evaporator at a bath temperature ranging between 35 to 40ºC, alongside reduced pressure and a controlled rotational speed. Finally, the extracts were stored at 4ºC until required for further analysis.

*Preliminary phytochemical screening*

The extracts EXD, FHE, and FET were subjected to preliminary phytochemical screening employing established protocols for the qualitative assessment of phytochemical constituents within the aerial components of *Herniaria hirsuta*,.[1,20,21]

*Quantitative phytochemical analysis*

*Determination of total phenolic content*

The total phenolic content (TPC) of EXD, FHE, and FET extracts was evaluated using the Folin-Ciocalteu colorimetric technique, following a method previously described with modifications.[22] Briefly, a 200 µL portion of each extract solution was combined with 1000 µL of Folin-Ciocalteu reagent (10%) and 800 µL of Sodium Carbonate ($Na_2CO_3$) solution (7.5%). Subsequently, the mixture was incubated at room temperature for 30 minutes in the dark. Following incubation, the absorbance of each sample was measured at 765 nm using a UV-VIS 6300PC spectrophotometer against a blank. Gallic acid was used to prepare the standard curve. TPC of the extracts were expressed in milligrams of gallic acid equivalents (GAE) per gram of extract.

*Determination of total flavonoid content*

The determination of total flavonoid content (TFC) followed the procedure outlined by Ordoñez *et al*. (2006)[23] In summary, 0.5 mL of each extract was combined with an equal volume of 2% Aluminum chloride ($AlCl_3$) solution. The mixture was incubated at room temperature for one hour, after which the absorbance was recorded at 420 nm against a blank. Quercetin was employed for the construction of the calibration curve. The TFC were reported as milligrams of quercetin equivalents (QE) per gram of extract.

*Determination of total flavonol content*

The total flavonol content (TFlC) of the extracts was determined using the method outlined by Yermakov *et al*. (1987).[24] Each extract or standard (quercetin) was subjected to a solution containing 2 mL of $AlCl_3$ (20 mg/mL) and 6 mL of sodium acetate solution (50 mg/mL). Following a 2.5-hour incubation period, absorbance readings were taken at 440 nm. A calibration curve of quercetin was prepared, and the overall quantity of flavonols present was expressed as milligram quercetin equivalent per gram of each respective extract (mg EQ/g extract).

*Determination of total tannin content*

The determination of total tannin content (TTC) in the extracts followed the protocol outlined by Julkunen-Tiitto (1985).[25] Specifically, 50 µL of each extract was combined with 1.5 mL of 4% vanillin solution in methanol and 750 µL of concentrated hydrochloric acid (HCl, 37%). After a 20-minute incubation period, the absorbance was measured at 500 nm using a UV-VIS 6300PC spectrophotometer. Catechin was employed for the construction of the calibration curve.The total condensed tannin content was expressed as milligrams of catechin equivalents (CE) per gram of extract.

*Assessment of antioxidant activity*

*Determination of DPPH free radical scavenging activity*

The capacity of EXD, FHE, and FET extracts derived from *Herniaria hirsuta* to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed following the method outlined by Şahin *et al*. (2004).[26] A series of extract concentrations (ranging from 200 to 2000 µg/mL) were prepared, and 50 µL of each concentration, along with ascorbic acid (used as a positive control at the same concentration range), or methanol (serving as a negative control), was added to 2 mL of a methanol solution of DPPH at a concentration of 0.0023%. After 20 minutes of incubation in the dark at room temperature, the absorbance of the resulting mixture was measured at 517 nm using a spectrophotometer. The percentage inhibition of DPPH radical was calculated using Equation 1.

$$\%\ Inhibition\ of\ DPPH = \frac{Absorbance\ of\ Blank - Absorbance\ of\ Test}{Absorbance\ of\ Blank} \times 100 \quad \text{---------- (1)}$$

*Determination of ABTS free radical scavenging activity*

The capacity of the extracts to neutralize the ABTS radical was assessed following the method established by Pukalskas *et al*. (2002)[27] Initially, a solution of ABTS radical cation ($ABTS^{\cdot+}$) was prepared through the reaction between 7 mM ABTS and 70 mM potassium persulfate in methanol, maintained at room temperature in the dark for 16 hours. Subsequently, the $ABTS^{\cdot+}$ solution was diluted with methanol to achieve an absorbance range between 0.700 and 0.734 at 734 nm. Thereafter, 100 µL of plant extracts at varying concentrations (ranging

from 200 to 2000 µg/mL) were mixed with 2 mL of the diluted $ABTS^{\cdot+}$ solution in methanol and allowed to react for 1 minute. The absorbance was then measured at 734 nm, and the percentage of inhibition was calculated using the same method as described earlier for the DPPH test.

*Ferric-reducing antioxidant power assay*

The ferric-reducing antioxidant power (FRAP) of the investigated extracts was determined following the protocol outlined by Oyaizu (1986).[28] Various concentrations (500 - 5000 µg/mL) of the extracts (200 µL) were combined with 2.5 mL of 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide ($K_3Fe(CN)_6$). The resultant solution was incubated at 50°C for 20 minutes. Following this, the mixture was acidified using 2.5 mL of trichloroacetic acid (10%). Subsequently, 2.5 mL of the solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% iron (iii) chloride ($FeCl_3$), and the absorbance was measured at 700 nm. Catechin was used as the standard (positive control).

*Statistical analysis*

The experiments were conducted three times independently, and the data were presented as mean ± standard deviation (SD). Statistical analysis involved comparison via one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, using GraphPad Prism 8 software. Significant difference was determined at a threshold of $p < 0.05$.

## Results and Discussion

*Phytochemical constituents of the extracts*

The results of the comprehensive analysis of secondary metabolites in the aerial parts of *Herniaria hirsuta*, as depicted in Table 1, reveals a nuanced biochemical landscape that hints at both ecological and pharmacological significance. The results provided offer a comprehensive insight into the phytochemical composition of extracts derived from *Herniaria hirsuta*. Phytochemical screening revealed a diverse array of secondary metabolites present in the aerial parts of *Herniaria hirsuta*. The presence of flavonoids, renowned for their antioxidant properties, suggests that *Herniaria hirsuta* may possess mechanisms for oxidative stress mitigation, which could be advantageous in its natural environment and potentially beneficial to human health if harnessed effectively. Moreover, the detection of tannins underscores the plant's potential role in defense against herbivores and pathogens, as these polyphenolic compounds are known for their astringent properties and ability to deter feeding and inhibit microbial growth.[29] This defensive arsenal could confer an evolutionary advantage to *Herniaria hirsuta*, allowing it to thrive in diverse ecosystems.

**Table 1:** Secondary metabolites contents in the aerial parts of *Herniaria hirsuta*.

| Secondary metabolite | Inference |
|---|---|
| Flavonoids | + |
| Tannins | + |
| Alkaloids | + |
| Coumarins | - |
| Terpenoids | + |
| Sterols | - |
| Saponins | - |

+: present; -: absent.

The presence of alkaloids, another class of secondary metabolites, raises intriguing questions about the pharmacological potential of *Herniaria hirsuta*. Alkaloids are notorious for their diverse biological activities, ranging from toxicity to therapeutic effects,[30] and their presence in this plant suggests a rich reservoir of bioactive compounds that merit further investigation for their potential medicinal applications. Similarly, the presence of terpenoids, with their broad spectrum of biological activities including antimicrobial and anti-inflammatory properties,[31] hints at the pharmacological promise of *Herniaria hirsuta* as a potential source of novel therapeutic agents or lead compounds for drug discovery endeavors.

Conversely, the absence of coumarins, sterols, and saponins in *Herniaria hirsuta* reveals intriguing aspects of its biochemical profile. Coumarins, with their anticoagulant and antimicrobial properties, are notably absent, indicating a divergence from other plant species that possess these compounds. Similarly, the absence of sterols, important constituents of cell membranes and precursors to hormones, suggests potential differences in membrane composition and physiological processes compared to plants that do contain sterols. Additionally, the absence of saponins, known for their foaming properties and potential health benefits, may influence the plant's interactions with other organisms and its applications in traditional medicine or industry.

In essence, the detailed analysis of secondary metabolites in *Herniaria hirsuta* provides valuable insights into its biochemical composition and potential ecological roles, as well as its pharmacological significance and utility in various applications.

These findings align with previous studies, underscoring the consistency of the phytochemical composition across different investigations.

These results were compared to previous studies. For example, the study of Uddin *et al*. (2017)[32] revealed the absence of alkaloids, coumarins, and terpenoids in the methanol extract of *Herniaria hirsuta*. In another study, flavonols, sterols, and triterpenes were present in the aqueous extract of the aerial parts of *Herniaria hirsuta*.[33] In addition, the analysis of the methanol extract of *Herniaria hirsuta* revealed the presence of phytochemicals such as tannins, saponins, steroids, coumarins and betacyanins.[32]

*Total Phenolic, Flavonoid, Flavonols, and Tannin contents*

Table 2 presents the total phenolic, flavonoid, flavonol, and tannin content in different extracts (EXD, FHE, and FET) derived from *Herniaria hirsuta*. These values shed light on the diverse bioactive compounds present in these extracts and their relative concentrations.

Firstly, it is important to note the significant variability in the yield of extraction among the different extracts. The EXD extract exhibits the highest yield at 30.26 ± 1.89%, followed by FET at 26.66 ± 1.65%, and FHE at 14.5 ± 1.27%. This variability in extraction yield may reflect differences in the extraction methods used or the solubility of bioactive compounds in the respective solvents.

**Table 2:** Total phenolic, flavonoid, flavonols, and tannin content of extracts of *Herniaria hirsuta* aerial parts

| Parameter | EXD | FHE | FET |
|---|---|---|---|
| Yield of extraction (%) | 30.26 ± 1.89 | 14.5 ± 1.27 | 26.66 ± 1.65 |
| TPC (mg GAE/ g extract) | 5.18 ± 0.54$^a$ | 3.24 ± 0.67$^b$ | 4.03 ± 0.67$^c$ |
| TFC (mg QE/ g extract) | 7.23 ± 0.34$^a$ | 14.35 ± 0.98$^b$ | 13.23 ± 1.20$^b$ |
| TFlC (mg QE/ g extract) | 5.34 ± 0.13$^a$ | 8.35 ± 0.24$^b$ | 7.34 ± 0.13$^c$ |
| TTC (mg CE/ g extract) | 0.69 ± 0.02$^a$ | 2.95 ± 0.46$^b$ | 0.28 ± 0.14$^c$ |

Values are mean ± standard deviation (SD). Different lower case letters (a to c) in the same row indicate the significant difference ($p < 0.05$). Values were compared by using one way ANOVA followed by multiple comparison test.

EXD: aqueous extract; FET: ethanol extract; FHE: hexane extract; TPC: total phenolic content; TFC: total flavonoid content; TFlC: total flavonol content; TTC: total tannin content; mg GAE/ g extract: mg Gallic Acid Equivalent per gram of extract; mg QE/ g extract: mg Quercetin Equivalent per gram of extract; mg CE/ g extract: mg Catechin Equivalent per gram of extract

For total phenolic content (TPC), the EXD extract had the highest TPC at 5.18 ± 0.54 mg GAE/g extract, followed by FET at 4.03 ± 0.67 mg GAE/g extract, and FHE at 3.24 ± 0.67 mg GAE/g extract. Phenolic

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

compounds are renowned for their antioxidant properties and potential health benefits, making the EXD extract particularly noteworthy in this regard.

Similarly, the total flavonoid content (TFC) analysis reveals intriguing trends. The FHE extract demonstrated the highest TFC at 14.35 ± 0.98 mg QE/g extract, followed closely by FET at 13.23 ± 1.20 mg QE/g extract, and EXD at 7.23 ± 0.34 mg QE/g extract. Flavonoids are known for their antioxidant, anti-inflammatory, and anticancer properties,[34] making the FHE and FET extract particularly promising candidates for further investigation.

Moreover, the analysis of total flavonol content (TFlC) highlights the variability in flavonol concentrations among the extracts. Once again, the FHE extract exhibited the highest TFlC at 8.35 ± 0.24 mg QE/g extract, followed by FET at 7.34 ± 0.13 mg QE/g extract, and EXD at 5.34 ± 0.13 mg QE/g extract. Flavonols are known for their potent antioxidant properties and potential protective effects against chronic diseases,[35] further emphasizing the potential health benefits associated with the FHE and FET extracts.

Lastly, the total tannin content (TTC) analysis unveils interesting insights into the tannin concentrations among the extracts. The FHE extract demonstrated the highest TTC at 2.95 ± 0.46 mg CE/g extract, followed by EXD at 0.69 ± 0.02 mg CE/g extract, and FET at 0.28 ± 0.14 mg CE/g extract. Tannins are known for their astringent properties and potential health benefits, suggesting that the FHE extract may possess notable physiological effects compared to the other extracts.

The results presented in Table 2 underscore the significant variability in the phytochemical composition and bioactive properties of the EXD, FHE, and FET extracts of *Herniaria hirsuta*. These differences may be attributed to the polarity of the solvents used for extraction and environmental factors influencing plant chemistry. These results align with previous research, highlighting the richness of *Herniaria hirsuta* extracts in bioactive compounds.

Several studies have shown the richness of the extracts of the aerial parts of *Herniaria hirsute* in phenolic, flavonoid, and tannin compounds.[33]

Additionally, previous research has reported the presence of saponins, flavonoids, and coumarins in *Herniaria* species, which are important bioactive compounds with potential pharmacological implications.[19] Therefore, the results in Table 2 align with the existing literature on the phytochemical composition and biological activities of *Herniaria hirsuta*.

*Antioxidant activity*

In the present study, the antioxidant activity of the three extracts (EXD, FHE, and FET) of *Herniaria hirsuta* was evaluated using three tests commonly used for the antioxidant characterization of plant extracts: DPPH, ABTS, and FRAP. The antioxidant capacity of each extract tested is presented in Table 3 and Figure 2.

**Table 3:** Antioxidant activity of extracts of *Herniaria hirsuta* aerial parts

| Assay | $IC_{50}$ (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | **EXD** | **FHE** | **FET** | **Quercetin** | **Ascorbic acid** | **Catechin** |
| **DPPH** | 703.33 ± 0.90[a] | 853.60 ± 0.60[b] | 789.89 ± 0.30[c] | 5.49 ± 0.00[d] | - | - |
| **ABTS** | 29.09 ± 0.87[a] | 215.00 ± 0.40[b] | 118.60 ± 0.70[c] | - | 2.52 ± 0.00[d] | - |
| **FRAP** | 814.34 ± 1.10[a] | 965.24 ± 0.40[b] | 870.24 ± 1.00[c] | - | - | 19.24 ± 0.40[d] |

Different letters in the same row (a to c) indicate the significant difference ($p < 0.05$). Values were compared by using one way ANOVA followed by multiple comparison test.

ABTS: ABTS Radical Scavenging Assay; faWSDPPH: DPPH Free Radical-Scavenging Activity; EXD: aqueous extract; FET: ethanol extract; FHE: hexane extract; FRAP: Ferric Reducing Antioxidant Power assay;

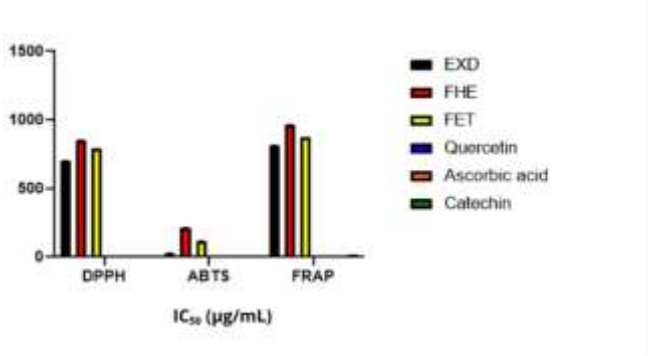


**Figure 2:** Antioxidant capacity of EXD, FHE, and FET extract of Herniaria hirsuta by DPPH, ABTS, FRAP

Table 3 shows the antioxidant capacity of the EXD, FHE, and FET extracts of *Herniaria hirsuta* as evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (ferric reducing antioxidant power) assays. Additionally, the antioxidant activities of quercetin, ascorbic acid, and catechin are provided for comparison purposes.

Beginning with the DPPH assay, which measures the ability of antioxidants to scavenge free radicals, the EXD extract exhibited the most potent antioxidant activity with an $IC_{50}$ value of 703.33 ± 0.98 µg/mL, followed by FET at 789.89 ± 0.35 µg/mL, and FHE at 853.60 ± 0.66 µg/mL. These results suggest that the EXD extract possesses remarkable radical scavenging capabilities, potentially attributed to its high phenolic and flavonoid content as observed in previous analyses.

In the ABTS assay, which measures the ability of antioxidants to quench the ABTS radical cation, the EXD extract emerged as the most potent antioxidant with an $IC_{50}$ value of 29.09 ± 0.87 µg/mL, followed by FET at 118.60 ± 0.72 µg/mL, and FHE at 215.00 ± 0.45 µg/mL. Interestingly, potential differences in the mechanisms of antioxidant action among the extracts.

Lastly, the FRAP assay, which evaluates the ability of antioxidants to reduce ferric ions, revealed that the EXD extract exhibited the most potent antioxidant activity with an $IC_{50}$ value of 814.34 ± 1.14 µg/mL, followed by FET at 870.24 ± 1.00 µg/mL, and FHE at 965.24 ± 0.45 µg/mL. These results highlight the remarkable reducing power of the EXD extract, which may be attributed to its high phenolic content as observed in previous analyses.

Comparing the antioxidant activities of the extracts with those of the standard antioxidants; quercetin, ascorbic acid, and catechin, it was evident that while the extracts generally exhibited lower antioxidant activity compared to these standards, they still demonstrated notable free radical scavenging and reducing abilities, underscoring their potential as natural sources of antioxidants. These findings suggest the extracts' potential therapeutic applications in combating oxidative stress-related diseases.

In a previous study on the antioxidant capacity of the aqueous and hydroethanol extracts of the aerial parts of *Herniaria hirsuta*, it was found that the hydroethanol extract exhibited higher antioxidant activity than the aqueous extract.[33]

In summary, the results presented in Table 3 underscore the significant antioxidant potential of the EXD, FHE, and FET extracts of *Herniaria hirsuta*, as evaluated by DPPH, ABTS, and FRAP assays. Further research into the specific antioxidant compounds present in these extracts and their mechanisms of action is warranted to fully elucidate their potential therapeutic applications in combating oxidative stress-related diseases and promoting human health.

Overall, the results presented in this study provide valuable insights into the phytochemical composition and antioxidant potential of *Herniaria hirsuta* extracts, paving the way for further research into their applications in medicine and industry.

## Conclusion

Medicinal plants contain active compounds with therapeutic potential, often attributed to their antioxidative properties. This study on the phytochemical profile of *Herniaria hirsuta* extracts revealed the rich phytochemical constituents of the plant, with the presence of flavonoids, tannins, alkaloids, and terpenoids, alongside variable concentrations of phenolic, flavonoid, and tannin compounds influenced by extraction methods. Aqueous extracts exhibited high phenolic and tannin content, while hexane extracts were richer in flavonoids and flavonols, underscoring the role of solvent polarity in the extraction of phytochemicals. Antioxidant activity assessments using DPPH, ABTS, FRAP assays highlighted differing scavenging abilities compared to standard antioxidants. These findings demonstrate the pharmacological potential of *Herniaria hirsute*, advocating for further research on compound isolation, mechanisms, and optimized extraction methods to fully harness its therapeutic and industrial potentials.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

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

## Acknowledgements

The authors thank Professor Mohammed Sghir Taleb at the Scientific Institute of Rabat for the botanical identification of the plant material.

## References

1. Ameggouz M, Drioua S, El-Guourrami O, Azalmad H, Ouajdi M, Zahidi A, Doukkali A, Satani B, Benzeid H. Phytochemical Analysis and Evaluation of the Antioxidant Activity of *Cedrus atlantica* (Endl.) G. Manetti ex Carrière Stem Extracts. Trop J Nat Prod Res. 2024; 8(3):6741-6750. https://doi.org/10.26538/tjnpr/v8i3.40
2. Ameggouz M, Drioua S, El-Guourrami O, Azalmad H, Metni KB, Koursaoui L, Zahidi A, Doukkali A, Satani B, Benzeid H. Assessment of Acute Toxicity and Analgesic Effect of *Cedrus atlantica* (Endl.) G. Manetti ex Carrière Stem Extracts. Trop J Nat Prod Res. 2024; 8(7):7677-7681. https://doi.org/10.26538/tjnpr/v8i7.7
3. Drioua S, Azalmad H, El-Guourrami O, Ameggouz M, Benkhouili FZ, Assouguem A, Kara M, Ullah R, Ali EA, Ercisli S, Fidan H, Benzeid H, Doukkali A. Phytochemical Screening and Antioxidant Activity of *Vitex agnus-castus* L. Open Chem. 2024; 22(1):20230190. https://doi.org/10.1515/chem-2023-0190
4. Drioua S, El-Guourrami O, Assouguem A, Ameggouz M, Kara M, Ullah R, Bari A, Zahidi A, Skender A, Benzeid A, Doukkali A. Phytochemical Study, Antioxidant Activity, and Dermoprotective Activity of *Chenopodium ambrosioides* (L.). Open Chem. 2024; 22(1):20230194. https://doi.org/10.1515/chem-2023-0194
5. El-guourrami O, Drioua S, Ameggouz M, Salhi N, Sayah K, Zengin G, Zahidi A, Doukkali A, Benzeid H. Antioxidant Activity, Analgesic Activity, and Phytochemical Analysis of *Ammi majus* (L.) Extracts. Int J Second Metabol. 2023; 10(2):23-37. https://doi.org/10.21448/ijsm.1139246
6. Salhi N, El-guourrami O, Rouas L, Moussaid S, Moutawalli A, Benkhouili F, Ameggouz M, Alshahrani M, Awadh A, Bouyahya A, My El Abbes F, Cherrah Y. Evaluation of the Wound Healing Potential of *Cynara humilis* Extracts in the Treatment of Skin Burns. Evid Based Complement Alternat Med. 2023; 2023:5855948. https://doi.org/10.1155/2023/5855948
7. El-guourrami O, Salhi N, Benkhouili FZ, Zengin G, Yilmaz MA, Ameggouz M, Zahidi A, Rouas L, Bouyahya A, Goh KW, Sam TH, Ming LC, Doukkali A, Benzeid H. Phytochemical Composition and Toxicity Assessment of *Ammi majus* L. Asian Pac J Trop Biomed. 2023; 13(4):165. https://doi.org/10.4103/2221-1691.374233
8. Drioua S, El-Guourrami O, Ameggouz M, Benkhouili FZ, Assouguem M, Kara M, Al Kamaly O, Alnakhli AM, Ait Benlabchir A, Benzeid H, Doukkali A. Study of Phytochemical Compound and Antipyretic Activity of *Chenopodium ambrosioides* L. Fractions. Open Chem. 2024; 22(1):20240055. https://doi.org/10.1515/chem-2024-0055
9. Drioua S, Cherkani-Hassani A, El-guourrami O, Ameggouz M, Zahidi A, Bouyahaya A, Shah SI, Al-Worafi YM, Ming LC, Benzaied H, Doukkali A. Toxicological Review of Anticancer Plants Used in Traditional Medicine in Morocco. Prog Microbes Mol Biol. 2023; 6(1):1-45. https://doi.org/10.36877/pmmb.a0000328
10. Drioua S, Ameggouz M, Laabar A, Aasfar A, Faouzi MA, Zahidi A, Ullah R, Alotaibi A, Bouyahya A, Zengin G, Balahbib A, Benzied H, Doukkali A. Chemical Composition and Analgesic and Antidiabetic Activity of *Chenopodium ambrosioides* L. Cell Biochem Funct. 2024; 42(8):e70016. https://doi.org/10.1002/cbf.70016
11. Drioua S, Ameggouz M, Assouguem A, Kara M, Ullah R, Bari A, Lahlali R, Fidan H, El-Guourrami O, Benkhouili FZ, Maamar Y, Benzied H, Doukkali A. Comprehensive Phytochemical and Toxicological Analysis of *Chenopodium ambrosioides* (L.) Fractions. Open Life Sci. 2024; 19(1):20220895. https://doi.org/10.1515/biol-2022-0895
12. Atmani F, Slimani Y, Mimouni M, Aziz M, Hacht B, Ziyyat A. Effect of Aqueous Extract from *Herniaria hirsuta* L. on Experimentally Nephrolithiasic Rats. J Ethnopharmacol. 2004; 95(1):87-93. https://doi.org/10.1016/j.jep.2004.06.028
13. Settaf A, Labhal A, Cherrah Y, Slaoui A, Hassar M. *Herniaria hirsuta* dissout les calculs biliaires cholestéroliques. Espérance médicale. 1999; 6(47):79-82.
14. Mbark AN, Charouf Z, Wray V, Nimtz M, Schöpke T. Monodesmosidic Saponins From *Herniaria hirsuta*. Pharmazie. 2000; 55(9):690-692.
15. Eddouks M, Maghrani M, Lemhadri A, Ouahidi ML, Jouad H. Ethnopharmacological Survey of Medicinal Plants Used for the Treatment of Diabetes Mellitus, Hypertension and Cardiac Diseases in the South-East Region of Morocco (Tafilalet). J Ethnopharmacol. 2002; 82(2-3):97-103. https://doi.org/10.1016/S0378-8741(02)00164-2
16. M'Bark AN, Guillaume D, Kol O, Charrouf Z. Triterpenoid Saponins from *Herniaria fontanesii*. Phytochem. 1996; 43(5):1075-1077.
17. Charrouf Z, Nait-Mbark A, Guillaume D, Leroy Y, Kol O. Herniaria Saponin B, A Novel Triterpenoid Saponin From *Herniaria Fontanesii*. In: Waller GR, Yamasaki K, editors. Saponins Used in Food and Agriculture. Boston, MA: Springer US; 1996 ; 241-245p.
18. Kozachok S, Pecio Ł, Kolodziejczyk-Czepas J, Marchyshyn S, Nowak P, Mołdoch J, Oleszek W. γ-Pyrone Compounds: Flavonoids and Maltol Glucoside Derivatives from *Herniaria glabra* L. Collected in the Ternopil Region of the Ukraine. Phytochem. 2018; 152:213-222. https://doi.org/10.1016/j.phytochem.2018.05.009
19. Peeters L, Van der Auwera A, Beirnaert C, Bijttebier S, Laukens K, Pieters L, Hermans N, Foubert A. Compound Characterization and Metabolic Profile Elucidation After *In Vitro* Gastrointestinal and Hepatic Biotransformation of an *Herniaria hirsuta* Extract Using Unbiased Dynamic Metabolomic Data Analysis. Metabolites. 2020; 10(3):111. https://doi.org/10.3390/metabo10030111
20. Ameggouz M, Drioua S, El-Guourrami O, Azalmad H, Metni KE, Zahidi A, Doukkali A, Satrani B, Benzeid H. *In Vitro* Evaluation of Stem Extracts and Essential Oils from Sawdust of *Cedrus atlantica* (Endl.) G. Manetti ex Carrière for Their

Photoprotective and Antihyperglycemic Activities. Trop J Nat Prod Res. 2025; 9(2):826-832.
Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from *Ephedra intermedia* Indigenous to Balochistan. Sci World J. 2017; 2017:5873648. https://doi.org/10.1155/2017/5873648
21. Poh Hwa T, Cheah YK, Ibrahim J, Radu S. Bioprotective Properties of Three Malaysia *Phyllanthus* Species: An Investigation of the Antioxidant and Antimicrobial Activities. Int Food Res J. 2011; 18(3):887-893.
22. Ordoñez AAL, Gomez JD, Vattuone MA, Isla MI. Antioxidant Activities of *Sechium edule* (Jacq.) Swartz Extracts. Food Chem. 2006; 97(3):452-458. https://doi.org/10.1016/j.foodchem.2005.05.024
23. Yermakov AI, Arasimov VV, Yarosh NP. Methods of Biochemical Analysis of Plants. Agropromizdat, Leningrad. 1987: 122-142.
24. Julkunen-Tiitto R. Phenolic Constituents in the Leaves of Northern Willows: Methods for the Analysis of Certain Phenolics. J Agric Food Chem. 1985; 33(2):213-217.
25. Şahin F, Güllüce M, Daferera D, Sökmen A, Sökmen M, Polissiou M, Agar G, Özer H. Biological Activities of the Essential Oils and Methanol Extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia Region of Turkey. Food Control. 2004; 15(7):549-557. https://doi.org/10.1016/j.foodcont.2003.08.009
26. Pukalskas A, van Beek TA, Venskutonis RP, Linssen JPH, van Veldhuizen A, de Groot Æ. Identification of Radical Scavengers in Sweet Grass (*Hierochloe odorata*). J Agric Food Chem. 2002; 50(10):2914-2919.
27. Oyaizu M. Studies on Products of Browning Reaction--Antioxidative Activities of Products of Browning Reaction Prepared from Glucosamine. Jpn J Nutr Diet. 1986; 44(6):307-315.
28. Huang R and Xu C. An overview of the perception and mitigation of astringency associated with phenolic compounds. Compr Rev Food Sci Food Saf. 2021; 20(1):1036-1074.
29. Thawabteh A, Juma S, Bader M, Karaman D, Scrano L, Bufo SA, Karaman R. The Biological Activity of Natural Alkaloids against Herbivores, Cancerous Cells and Pathogens. Toxins (Basel). 2019; 11(11):656.
30. Mabou FD and Yossa IBN. TERPENES: structural classification and biological activities. IOSR J Pharm Biol Sci. 2021; 16:25-40.
31. Uddin G, Ali J, Feroz S. Antimicrobial, Antioxidant and Phytochemical Analysis of *Herniaria hirsuta*. Univ Swabi J. 2017; 1(1):84-93.
32. Ammor K, Bousta D, Jennan S, Bennani B, Chaqroune A, Mahjoubi F. Phytochemical Screening, Polyphenols Content, Antioxidant Power, and Antibacterial Activity of *Herniaria hirsuta* From Morocco. Sci World J. 2018; 2018:7470384. https://doi.org/10.1155/2018/7470384
33. Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as Potential Anti-Inflammatory Molecules: A Review. Molecules. 2022; 27(9):2901.
34. Chagas M do SS, Behrens MD, Moragas-Tellis CJ, Penedo GXM, Silva AR, Gonçalves-de-Albuquerque CF. Flavonols and Flavones as Potential anti-inflammatory, Antioxidant, and Antibacterial Compounds. Oxid Med Cellul Longev. 2022; 2022(1):9966750.
35. OpenStreetMap contributors, "Mrirt, Morocco," OpenStreetMap, 2025. [Online]. [cited 2025 Nov 2 ]. Available from: https://www.openstreetmap.org.