

PHYTOCHEMICAL PROFILING AND IN VITRO ANTIOXIDANT EVALUATION OF PHENOLIC COMPOUNDS IN *EUPHORBIA ABYSSINICA* (CAMEL SLIPPER PLANT) LEAF EXTRACT

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Abstract

Euphorbia abyssinica (Camel Slipper plant) is an important medicinal herb in East African traditional medicine, reputed for treating inflammatory, infectious, and dermatological conditions. While its biological activities are attributed to various secondary metabolites, a detailed phytochemical profile of its phenolic fraction remains underexplored. This study aimed to systematically extract, characterize, and evaluate the antioxidant potential of phenolic compounds from *E. abyssinica* leaves. An optimized ultrasonic-assisted extraction using a hydroethanolic solvent (70:30 ethanol/water) at pH 3 was employed. The phenolic composition was analyzed via Reverse-Phase High-Performance Liquid Chromatography with Photodiode Array detection (RP-HPLC-PDA). Total phenolic content (TPC) was quantified using the Folin-Ciocalteu assay, and in vitro antioxidant activity was assessed via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, with results expressed as Gallic Acid Equivalents (GAE) and efficient concentration (EC). HPLC analysis identified and quantified five major phenolics: chlorogenic acid (45.9 µg/mL), kaempferol (9.18 µg/mL), caffeic acid (7.25 µg/mL), vanillic acid (3.81 µg/mL), and syringic acid (3.77 µg/mL). The extract exhibited a high TPC of 79.33 mg GAE/100 mL. It demonstrated potent DPPH radical scavenging activity, with inhibition rates up to 77%, and an EC range of 10.65–11.55 mg GAE/100 mL. The results confirm that *E. abyssinica* leaves are a rich source of bioactive phenolics, with chlorogenic acid as the dominant constituent. The strong correlation between TPC and antioxidant activity provides a scientific basis for the plant's ethnomedicinal uses in managing oxidative stress-related pathologies and supports its potential for development into standardized nutraceutical or cosmetic products.

Keywords: *Euphorbia Abyssinica*, Phenolic Compounds, HPLC, Folin-Ciocalteu Reagent, Total Phenolic Content, DPPH Assay, Antioxidant Activity, Efficient Concentration, Chlorogenic Acid.

INTRODUCTION

Euphorbia abyssinica, commonly known as the Camel Slipper plant, stands as a prominent member of the Euphorbiaceae family, endemic to the diverse ecosystems of East Africa.[1]] For centuries, it has occupied a significant niche within indigenous pharmacopoeias across Ethiopia, Somalia, and Kenya, where its therapeutic applications

have been passed down through generations of traditional healers.[2] This sustained ethnobotanical relevance is intrinsically linked to the plant's rich reservoir of secondary metabolites, which are believed to underpin its broad-spectrum biological activities.[3] Notably, phytochemical investigations have identified a complex array of bioactive constituents within *E. abyssinica*, including abietane-type diterpenoids (e.g., abyssinin), triterpenoids (e.g., lupeol and betulinic acid), flavonoids, and alkaloids.[4-7] These compounds collectively contribute to the reported anti-inflammatory, analgesic, antimicrobial, and bronchodilatory properties ascribed to the plant's various extracts.[8,9]

The purported medicinal uses of *E. abyssinica* are extensive, addressing conditions ranging from dermatological ailments such as wounds, burns, and skin infections to systemic inflammatory disorders like arthritis and rheumatism. Its application in managing respiratory conditions, including asthma and bronchitis, further underscores its therapeutic versatility. [11-14] Beyond traditional medicine, the plant's extracts have garnered interest in the cosmetic industry, attributed to their purported skin-soothing and anti-aging effects, likely a consequence of their antioxidant and anti-inflammatory capacities.[15] Despite this longstanding empirical use, a comprehensive scientific elucidation of the specific phytochemicals responsible for these effects, particularly the phenolic fraction, remains an area of active research. Phenolic compounds, encompassing subclasses such as phenolic acids and flavonoids, are of paramount interest due to their well-documented roles as potent antioxidants and modulators of key cellular pathways involved in inflammation and oxidative stress.[16]

Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the biological system's ability to detoxify them, is a fundamental pathological contributor to a multitude of chronic diseases, including neurodegenerative disorders, cardiovascular diseases, diabetes, and cancer.[17] Endogenous antioxidant defenses can be overwhelmed, leading to cellular damage. Consequently, exogenous antioxidants, particularly those derived from natural sources, are sought as potential therapeutic or preventive agents. The antioxidant efficacy of plant extracts is largely attributed to their phenolic content, which can neutralize free radicals through various mechanisms, including hydrogen atom transfer and single-electron transfer.[18] Therefore, the systematic quantification of total phenolic content and the evaluation of antioxidant activity are critical steps in validating the ethnomedicinal claims of plants like *E. abyssinica* and in assessing their potential for development into standardized phytopharmaceuticals or nutraceuticals.[19]

Modern analytical techniques provide robust methodologies for such investigations. High-Performance Liquid Chromatography (HPLC) coupled with photodiode array (PDA) detection allows for the precise separation, identification, and quantification of individual phenolic compounds within a complex plant matrix.[20] Complementary to this, spectrophotometric assays offer efficient means for broader assessments. The Folin-Ciocalteu method is a gold standard for estimating total phenolic content by exploiting the redox reaction between phenolics and the phosphomolybdate-phosphotungstate reagent.

Furthermore, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay serves as a widely accepted model for evaluating free radical neutralizing capacity, providing a direct measure of a sample's antioxidant potential. Determining the efficient concentration (EC50), often expressed in Gallic Acid Equivalents (GAE), allows for the standardized comparison of antioxidant strength across different samples.[1]

Given the established ethnopharmacological importance of *Euphorbia abyssinica* and the central role of phenolics in mediating antioxidant and associated health benefits, a detailed phytochemical analysis of its phenolic profile is warranted. This study therefore aims to: (1) optimize an extraction protocol for phenolic compounds from the leaves of *E. abyssinica*; (2) identify and quantify major individual phenolic constituents using RP-HPLC-PDA; (3) determine the total phenolic content via the Folin-Ciocalteu assay; and (4) evaluate the in vitro antioxidant activity through the DPPH radical scavenging assay, calculating the corresponding efficient concentration.

The findings from this research will contribute to the scientific validation of this traditional remedy, provide a clearer understanding of its phenolic chemistry, and establish a foundation for its potential application in developing evidence-based natural health products targeting oxidative stress-related pathologies.

MATERIALS AND METHODS

1. Plant Material and Preparation

Fresh leaves of *Euphorbia abyssinica* (Camel Slipper plant) were collected, authenticated, and thoroughly cleaned. The plant material was shade-dried at room temperature until a constant weight was achieved. The dried leaves were subsequently ground into a fine powder using an electric grinder. The powdered material was stored in airtight containers, protected from light and moisture, until extraction.

2. Chemicals and Reagents

All chemicals and solvents used were of analytical or HPLC grade. Ethanol, hydrochloric acid (HCl), acetic acid, acetonitrile, and methanol were purchased from Sigma-Aldrich. The Folin-Ciocalteu reagent and sodium carbonate were obtained from Fluka. Gallic acid (used as a standard), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and reference phenolic compounds (caffeic acid, vanillic acid, syringic acid, kaempferol, and chlorogenic acid) were sourced from Sigma-Aldrich. Distilled and deionized water was used throughout the experiments.

3. Extraction of Phenolic Compounds

Phenolic compounds were extracted using an optimized ultrasonic-assisted extraction (UAE) method. Briefly, 5 g of the dried leaf powder was mixed with 50 mL of a hydroethanolic solvent (70% ethanol: 30% water, v/v). The pH of the mixture was adjusted to 3.0 using 1M HCl.

The suspension was then subjected to ultrasonic treatment in an ultrasonic bath (frequency: 40 kHz, power: 132 W) for 2 hours at a constant temperature of 37°C. The resulting extract was filtered through Whatman No. 1 filter paper. The filtrate was centrifuged at 4000 rpm for 15 minutes to remove any residual particulate matter. The supernatant was concentrated under a gentle stream of nitrogen gas to near dryness and then reconstituted with the extraction solvent to a final volume of 5 mL. The concentrated extract was stored in amber-colored glass bottles at 4°C until further analysis.

4. High-Performance Liquid Chromatography (HPLC) Analysis

The qualitative and quantitative analysis of individual phenolic compounds was performed using an HPLC system (Agilent 1260 Infinity) equipped with a quaternary pump, an autosampler, a thermostated column compartment, and a Photodiode Array (PDA) detector. Separation was achieved on a reverse-phase C-18 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of solvent A (2% acetic acid in water) and solvent B (acetonitrile:water, 70:30, v/v). A gradient elution program was applied at a constant flow rate of 1.0 mL/min as follows: 0 min, 15% B; 9 min, 19% B; 15 min, 29% B; 19 min, 37% B; 22 min, 21% B; and 35 min, 15% B. The column temperature was maintained at 30°C, and the injection volume was 20 µL. Phenolic compounds were detected and quantified at three specific wavelengths (280 nm, 315 nm, and 350 nm) based on their characteristic absorbance maxima. Identification was conducted by comparing the retention times and UV spectra with those of authentic standards. Quantification was performed using external calibration curves constructed for each reference compound.

5. Determination of Total Phenolic Content (TPC)

The total phenolic content of the extract was determined spectrophotometrically using the Folin-Ciocalteu (FC) assay. A stock solution of gallic acid (400 mg/100 mL) was prepared to generate a standard calibration curve. A series of gallic acid solutions with concentrations of 20, 40, 60, 80, and 100 mg/100 mL were prepared. For the assay, 0.1 mL of the standard solution or the plant extract was mixed with 5 mL of distilled water, followed by the addition of 0.5 mL of the FC reagent. After 5 minutes, 2 mL of a 20% (w/v) sodium carbonate solution was added.

The final volume was adjusted to 10 mL with distilled water, and the mixture was incubated in the dark at room temperature for 2 hours. The absorbance of the developed blue color was measured at 755 nm against a reagent blank using a UV-Vis spectrophotometer (Shimadzu UV-1800). The TPC was expressed as milligrams of Gallic Acid Equivalents (GAE) per 100 mL of extract, calculated from the linear regression equation of the standard curve ($Y = 0.0096X$, $R^2 = 0.9987$).

6. Determination of Antioxidant Activity by DPPH Radical Scavenging Assay

The free radical scavenging activity of the extract was evaluated using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method. A stock solution of DPPH (0.48 mmol/L) was freshly prepared in methanol. The maximum absorption wavelength for the DPPH

solution was confirmed to be 517 nm via a spectral scan between 350–650 nm. For the assay, 0.5 mL of the plant extract was mixed with 5 mL of the DPPH solution.

The mixture was vortexed and left to stand in the dark at room temperature for 55 minutes. The absorbance of the resulting solution was then measured at 517 nm against a methanol blank. A control reaction was prepared by mixing methanol with the DPPH solution. The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\text{Scavenging Activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the DPPH solution without extract, and A_{sample} is the absorbance of the DPPH solution with the extract.

7. Determination of Efficient Concentration (EC)

The antioxidant efficiency of the extract was further expressed as the Efficient Concentration (EC), defined as the concentration of the extract (in mg GAE/100 mL) required to achieve 50% scavenging of the DPPH radicals.

This was determined by interpolating from the dose-response curve of the extract's scavenging activity. The EC value was calculated by comparing the scavenging activity of the extract with that of a gallic acid standard, for which a concentration of 7.5 mg/100 mL was found to produce 50% inhibition (IC_{50}).

8. Statistical Analysis

All extractions and analyses were performed in triplicate ($n=3$). Data are presented as mean \pm standard deviation (SD). Statistical analysis, including the calculation of linear regression for calibration curves, was performed using Microsoft Excel.

RESULTS

1. Phytochemical Profiling by High-Performance Liquid Chromatography (HPLC)

The hydroethanolic extract of *Euphorbia abyssinica* leaves was subjected to reverse-phase HPLC analysis for the identification and quantification of individual phenolic compounds.

A representative chromatogram, illustrating the separation profile, is presented in **Figure 1**. Five major phenolic compounds were successfully identified based on the comparison of their retention times and ultraviolet (UV) spectra with those of authentic standards.

The quantitative data are summarized in **Table 1**. Chlorogenic acid was the most abundant phenolic compound, present at a concentration of **45.9 μ g/mL**. This was followed by kaempferol (**9.18 μ g/mL**), caffeic acid (**7.25 μ g/mL**), vanillic acid (**3.81 μ g/mL**), and syringic acid (**3.77 μ g/mL**). The results demonstrate a rich and diverse phenolic composition in the leaf extract of *E. abyssinica*, with chlorogenic acid representing the dominant constituent.

Table 1: Identification and quantification of phenolic compounds in *Euphorbia abyssinica* leaf extract by HPLC

Phenolic Compound	Retention Time (min)	Concentration ($\mu\text{g/mL}$)
Caffeic Acid	10.3	7.25
Vanillic Acid	23.4	3.81
Syringic Acid	28.6	3.77
Kaempferol	30.1	9.18
Chlorogenic Acid	30.8	45.9

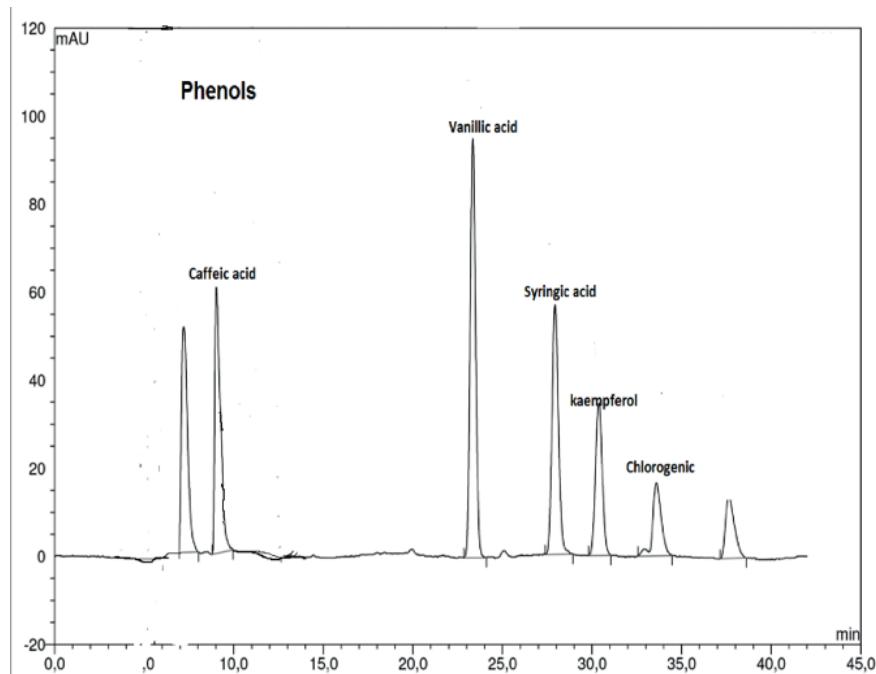


Figure 1. HPLC chromatogram of *Euphorbia abyssinica* leaf extract

Detection was performed using a PDA detector at 280 nm, 315 nm, and 350 nm. Peak identifications correspond to: 1. Caffeic acid, 2. Vanillic acid, 3. Syringic acid, 4. Kaempferol, 5. Chlorogenic acid.

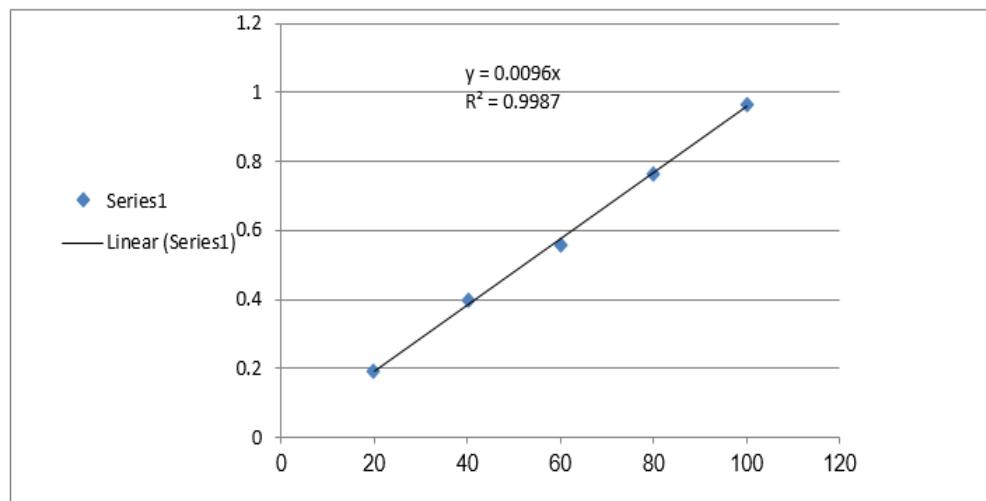
2. Determination of Total Phenolic Content (TPC)

The total phenolic content of the extract was determined spectrophotometrically using the Folin-Ciocalteu (FC) assay, with gallic acid as the calibration standard. Initial spectral scanning of the chromogen complex between 700–800 nm established the maximum absorption wavelength (λ_{max}) at 755 nm (Table 2), which was subsequently used for all absorbance measurements.

Table 2: Absorbance scan to determine the optimal wavelength (λ_{max}) for the Folin-Ciocalteu assay

Wavelength (nm)	Absorbance
740	0.724
745	0.726
750	0.730
755	0.736
760	0.729
765	0.728
770	0.727

A highly linear calibration curve was constructed using gallic acid standards ranging from 20 to 100 mg/100 mL. The absorbance values at 755 nm yielded the linear regression equation: $Y = 0.0096X$, where Y is the absorbance and X is the gallic acid concentration in mg/100 mL. The coefficient of determination ($R^2 = 0.9987$) confirmed excellent linearity (**Schema 1**).



Schema 1: Standard calibration curve of gallic acid for the Folin-Ciocalteu assay

Applying this equation to the absorbance values of the triplicate *E. abyssinica* extract samples (**B1**, **B2**, **B3**) resulted in a mean TPC of 79.33 ± 2.35 mg GAE/100 mL (**Table 3**). This finding indicates a substantial concentration of phenolic compounds in the extract, corroborating the qualitative HPLC data.

Table 3: Total Phenolic Content (TPC) of *Euphorbia abyssinica* extract samples

Sample	Absorbance (755 nm)	TPC (mg GAE/100 mL)
B1	0.734	76.45
B2	0.775	80.72
B3	0.776	80.83
Mean \pm SD	0.762 ± 0.023	79.33 ± 2.35

3. Antioxidant Activity: DPPH Radical Scavenging Assay

The in vitro antioxidant potential of the extract was evaluated based on its ability to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Spectral analysis confirmed the λ_{max} of the DPPH solution in methanol to be **517 nm**.

The extract exhibited potent radical scavenging activity. For the tested samples **A1** and **A2**, the percentage inhibition of DPPH radicals was calculated to be **71%** and **77%**, respectively (**Table 4**). These high inhibition percentages demonstrate a significant capacity of the *E. abyssinica* phenolic extract to neutralize free radicals.

Table 4: DPPH radical scavenging activity of *Euphorbia abyssinica* extract

Sample	Scavenging Activity (%)
A1	71
A2	77

4. Determination of Antioxidant Efficiency (EC)

To quantify the antioxidant efficacy relative to a standard, the efficient concentration (EC) was determined. The EC represents the concentration of the extract (in gallic acid equivalents, GAE) required to achieve 50% inhibition (IC_{50}) of DPPH radicals. A gallic acid solution of **7.5 mg/100 mL** was established as the reference point for 50% inhibition.

The calculated EC values for the extract samples were **10.65 mg GAE/100 mL** for A1 and **11.55 mg GAE/100 mL** for A2 (**Table 5**). This indicates that the antioxidant potency of the *E. abyssinica* extract, while substantial, is slightly less than that of pure gallic acid on an equivalent mass basis. The EC values provide a standardized metric for comparing the extract's antioxidant strength with other natural products or synthetic antioxidants.

Table 5: Efficient Concentration (EC) of *Euphorbia abyssinica* extract

Sample	EC (mg GAE/100 mL)
A1	10.65
A2	11.55

Commentary: The integrated results from HPLC, TPC, and antioxidant assays present a coherent phytochemical profile of *Euphorbia abyssinica*. The identification of specific phenolics like chlorogenic acid and kaempferol, compounds widely recognized for their antioxidant and anti-inflammatory properties, provides a mechanistic basis for the plant's traditional uses.

The high TPC (**79.33 mg GAE/100 mL**) directly correlates with the strong DPPH scavenging activity (up to **77%**), confirming that the phenolic constituents are major contributors to the extract's antioxidant capacity. The EC values offer a practical measure of this bioactivity, suggesting the extract's potential as a source of natural antioxidants for pharmaceutical or cosmetic applications.

DISCUSSION

The present study provides a comprehensive phytochemical characterization and antioxidant evaluation of a hydroethanolic leaf extract of *Euphorbia abyssinica*, a plant of significant ethnopharmacological importance in East African traditional medicine. The findings robustly demonstrate that the leaves of this species are a rich source of bioactive phenolic compounds, possessing considerable *in vitro* antioxidant potential, thereby offering a scientific basis for some of its traditional therapeutic applications.

The HPLC analysis successfully identified and quantified five principal phenolic constituents: caffeic acid, vanillic acid, syringic acid, kaempferol, and chlorogenic acid. The predominance of **chlorogenic acid (45.9 µg/mL)** is particularly noteworthy. Chlorogenic acid is a well-documented ester of caffeic and quinic acids, renowned for its multifaceted biological activities, including potent antioxidant, anti-inflammatory, antimicrobial, and antidiabetic effects [22, 26]. Its high concentration in *E. abyssinica* suggests it may be a major contributor to the plant's overall bioactivity, potentially underpinning its reported efficacy in managing conditions like hyperglycemia and skin infections [5, 7]. The presence of significant levels of kaempferol, a flavonoid, further enhances the extract's therapeutic profile, as kaempferol is associated with anti-inflammatory, anticancer, and cardioprotective properties through mechanisms involving free radical scavenging and modulation of inflammatory pathways [19, 33]. The co-occurrence of these specific phenolic acids and flavonoids creates a synergistic phytocomplex, likely more effective than isolated compounds, a common advantage of plant-based therapeutics.

The Folin-Ciocalteu assay confirmed a high total phenolic content (TPC) of **79.33 mg GAE/100 mL**. This value aligns with and substantiates the qualitative HPLC data, indicating that the identified compounds constitute a major portion of the extract's phenolic pool. The TPC serves as a key proxy for antioxidant capacity, as phenolics exert their effects primarily through redox reactions. Indeed, the extract exhibited strong free radical scavenging activity in the DPPH assay, with inhibition rates reaching **77%**. This significant antioxidant activity directly correlates with the high TPC, supporting the established principle that phenolic compounds are primary contributors to the radical-neutralizing capacity of plant extracts [15, 27]. The DPPH assay, while a fundamental model, reliably indicates the extract's ability to donate hydrogen atoms or electrons, a crucial mechanism for mitigating oxidative stress implicated in the pathogenesis of chronic inflammatory diseases, aging, and metabolic disorders [29, 32].

To contextualize the antioxidant potency, the efficient concentration (EC) was determined relative to gallic acid. The EC values of **10.65** and **11.55 mg GAE/100 mL** indicate that the extract's activity, while substantial, is slightly lower than that of pure gallic acid on an equivalent phenolic basis. This is an expected result, as gallic acid is a simple, highly active phenolic standard, while plant extracts contain a complex mixture where not all phenolics possess equal radical-scavenging power, and matrix effects may influence the overall activity [30]. Nevertheless, the relatively low EC values confirm a potent

antioxidant effect. More importantly, the integrated approach of HPLC and activity assays moves beyond a simple correlation between TPC and activity; it identifies the specific agents—like chlorogenic acid and kaempferol—responsible for the observed effect, providing deeper mechanistic insight.

These findings have substantial implications. Firstly, they validate the ethnomedicinal use of *E. abyssinica* for inflammatory and infectious conditions, as oxidative stress is a key component of these pathologies. The antioxidant and anti-inflammatory properties of the identified phenolics can help explain its utility in treating wounds, arthritis, and skin ailments [3, 6]. Secondly, the results highlight the plant's potential as a source of natural antioxidants for the nutraceutical and cosmetic industries. The anti-aging and skin-soothing properties attributed to the plant in traditional cosmetics [6] can be mechanistically linked to the ability of its phenolic constituents to protect skin cells from oxidative damage induced by UV radiation and environmental pollutants [24].

However, this study has limitations. The in vitro nature of the antioxidant assays, while informative, does not directly translate to in vivo efficacy, where bioavailability, metabolism, and tissue distribution become critical factors. Furthermore, the biological activities of *E. abyssinica* are not limited to its phenolic fraction; its documented diterpenoids and triterpenoids likely contribute synergistically to its overall medicinal profile [2, 23]. Future research should focus on in vivo models of oxidative stress and inflammation, isolation of specific active compounds for dose-response studies, and investigation of the safety profile and potential toxicity of standardized extracts to ensure their therapeutic application is both effective and safe [41].

This investigation successfully bridges traditional knowledge and modern science by elucidating the phenolic composition and confirming the significant antioxidant capacity of *Euphorbia abyssinica* leaf extract. The identification of chlorogenic acid as a major component provides a specific chemical marker and a plausible mechanistic lead for its biological effects. These results strengthen the scientific rationale for the traditional use of this plant and underscore its potential as a valuable source of bioactive phenolics for developing standardized phytopharmaceuticals or natural antioxidant products.

CONCLUSION

This study successfully establishes a detailed phytochemical and pharmacological profile for the leaf extract of *Euphorbia abyssinica* (Camel Slipper plant), providing robust scientific validation for its traditional medicinal and cosmetic uses. Through the application of standardized analytical methodologies—including ultrasonic-assisted extraction, high-performance liquid chromatography (HPLC), the Folin-Ciocalteu (FC) assay, and the DPPH radical scavenging assay—the research has conclusively demonstrated that the leaves are a rich reservoir of bioactive phenolic compounds.

The HPLC analysis provided a fingerprint of the phenolic composition, identifying and quantifying five major constituents: caffeic acid, vanillic acid, syringic acid, kaempferol,

and notably, chlorogenic acid as the predominant component. This specific identification of key active molecules, such as chlorogenic acid and kaempferol, moves beyond generic phytochemical screening to offer a plausible mechanistic basis for the plant's reported therapeutic effects, linking its traditional applications to known antioxidant, anti-inflammatory, and antimicrobial properties of these compounds. The high total phenolic content (TPC) of **79.33 mg GAE/100 mL**, confirmed by the FC assay, quantitatively underscores the extract's substantial phenolic pool. This high TPC was directly correlated with significant in vitro antioxidant activity, as evidenced by the DPPH assay, where the extract exhibited potent radical scavenging capacity of up to **77%**. The determination of the efficient concentration (EC) further contextualized this activity, providing a standardized metric (10.65–11.55 mg GAE/100 mL) that facilitates comparison with other natural antioxidants.

Collectively, these findings bridge ethnobotanical knowledge and modern pharmacognosy. They validate the empirical use of *E. abyssinica* for conditions associated with oxidative stress and inflammation, such as wounds, skin disorders, and arthritis. Furthermore, the results strongly support the plant's potential in the cosmetic industry, where its phenolic constituents can be leveraged for their skin-protective and anti-aging properties. The study thus confirms the plant's value as a promising source of natural antioxidants.

However, while this in vitro characterization is a critical first step, it delineates the boundary of current knowledge and highlights necessary future directions. The promising bioactivity must be substantiated through in vivo pharmacological and toxicological studies to evaluate bioavailability, systemic efficacy, and safety profiles. Additionally, research should explore the potential synergistic interactions between the identified phenolics and the plant's other known bioactive classes, such as diterpenoids and triterpenoids. In conclusion, this work provides a rigorous scientific foundation for the valorization of *Euphorbia abyssinica*. It justifies further investment in research aimed at developing standardized, evidence-based phytopharmaceuticals or cosmeceuticals derived from this valuable medicinal herb, ensuring its traditional benefits can be safely and effectively harnessed.

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