Full Length Research Article

Comparative Analysis of the Hexane Extracts of *Euphorbia hirta* and *Euphorbia hyssopifolia* with Regard to Total Phenolics, Total Flavonoids, Total tannin, GC-MS, and Biological Activities

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ABSTRACT

Background: This research examined the chemical constituents, concentration of phenolic compounds, and biological efficacy of the entire *Euphorbia hirta* and *Euphorbia hyssopifolia* specimens sourced from Morocco.

Methods: GC-MS was employed to analyze the chemical constituents. Three distinct methodologies, the Folin-Ciocalteu approach, the Aluminum Chloride technique, and the vanillin assay under acidic conditions were utilized to quantify the overall concentrations of polyphenols, flavonoids, and tannins in hexane extracts derived from *E. hirta* and *E. hyssopifolia*. The DPPH assay served as a means to evaluate the antioxidant potential of the samples. Furthermore, an investigation was carried out employing the disc diffusion technique to assess the antibacterial efficacy against six pathogenic bacterial strains.

Results: The results relating to the total phenolic content, the total flavonoid content and the total tannin content indicate that *E. hirta* has the highest concentration of polyphenols, and flavonoids, with values of 0.343 mg GAE/g and 11.188 µg QE/ g, respectively. In addition, *E. hyssopifolia* had a higher concentration of tannin, and antioxidant activity with an 0.238 mg CE/g, and IC_{50} value of 0.403 mg/mL, respectively. The results on antimicrobial activity revealed significant efficacy against almost all the strains tested.

Conclusion: As demonstrated by this work, the biochemical composition analysis of the hexane extracts reveals their significant antibacterial and antioxidant properties.

INTRODUCTION

Throughout history, vegetation has served as a source of sustenance, and a storehouse of medical uses; the oldest known method of treating illness was herbalism [1]. The genus Euphorbia, belonging to the *Euphorbiaceae* family, encompasses an extensive array of approximately 2100 species, predominantly found in geographical regions such as Southern Africa, the Middle East, the southern United States, and the Mediterranean region [2-4]. A hallmark characteristic of this genus is the excretion of a milky, noxious latex by specialized laticiferous vessels. This latex comprises a complex amalgamation of diverse compounds, including sugars, proteins, alkaloids, starch, oils, tannins, gums, and resins. It serves as a defensive adaptation to discourage herbivorous insects [5].

Euphorbia hyssopifolia is a herbaceous species typically located in the tropical and subtropical climates of both Africa and the Americas [6]. This plant frequently flourishes along roadways and in agricultural fields, achieving heights of up to 25.89 cm, characterized by opposite phyllotaxy, cyathia featuring glands, and triangular capsules that encase dark seeds marked by transverse striations [7,8]. The therapeutic attributes of *E. hyssopifolia* encompass diuretic and purgative properties, in addition to exhibiting anti-inflammatory effects on the respiratory tract, facilitating bronchial dilation in asthmatic conditions, and providing alleviation from common colds [6], as well as addressing indigestion and alleviating back pain when ingested as a tea or tonic [9].

Euphorbia hirta , referred to as the "asthmatic plant" in the English language, constitutes a globally prevalent medicinal flora [10] characterized by distinct morphological features, notably a slender stem, a purplish or reddish hue, and the potential to attain a height of approximately 40 cm, accompanied by elliptical-oblong foliage. The plant yields tricellular capsules that harbor three angular brown seeds, each enveloped in a yellowish and hairy covering [11-14]. The extract derived from *E. hirta* is conventionally consumed as an aqueous decoction for numerous therapeutic applications. This plant, recognized by its common English designation, has historically been employed to address respiratory ailments, including asthma [15]. Furthermore, current investigations frequently underscore its prospective efficacy against dengue fever [16,17]. Nonetheless, studies have demonstrated that *E. hirta* is a host to a wide array of biologically active constituents [18]. *E. hirta* is esteemed for its significant antifungal, antimalarial, antipyretic, anthelmintic, sedative, antispasmodic, antiasthmatic, and antibacterial attributes [14].

A thorough examination of medicinal and potentially toxic plants can aid in the discovery of more effective and safer medicinal substances. The objective of the present work is to conduct qualitative and quantitative assessments, the determination of the bioactive compounds in *E. hyssopifolia* and *E. hirta* by GC- MS analysis, and to study their antioxidant and antibacterial properties.

METHODS

Preparation of plants material and samples

The specimens of the two plants were gathered in Rabat (33°57 ‘55.1" N 6°53 ‘37.1" W), with the harvest taking place in October 2022. To produce our extracts for this study, we utilized the entire plant.

Preparation of Extracts

Hexane extracts of *E. hirta* and *E. hyssopifolia* were prepared by maceration. Nevertheless, 20 g of powder from each plant was mixed with 200 mL of n-hexane. The mixture was allowed to macerate for 48 hours at room temperature and within darkness. After 48 hours, the mixture was filtered using a paper filter. The obtained extracts were stored at 4 °C.

The following formula was used to get the extraction yield based on the dry plant material’s weight:

EY = (Mass of extracted material / Initial mass of powder) × 100

Phytochemical Screening

To determine which key classes of secondary metabolites were present in our extracts and potentially responsible for the observed actions, phytochemical screening was carried out. Conventional characterization reagents were utilized in order to identify the following chemical groups: polyphenols, alkaloids, anthocyanins, reducing compounds, tannins, flavonoids, and quinones [19].

GC-MS Analysis

Gas chromatography– mass spectrometry was used to examine the extracts of *E. hirta* and *E. hyssopifolia* under the following circumstances: the temperature at the injector port was 250 °C. The oven was initially set at 40 °C, and over the course of 18 minutes, the temperature was progressively raised by 8 °C every minute until it reached 260 °C. We made use of the BR-5 ns FS capillary column. The helium injection volume in the undivided mode was 1.0 $mL.min^{-1}$. The analysis took 105 minutes in total. The mass spectrometry detector (MSD) was configured for electronic impact ionization, with a scan range of 50 to 500 *m/z* and an ionizing energy of 70 eV. The temperature of the ion source tripled to 150 °C after initially rising to 230 °C. The electron multiplier voltage (EM voltage) was maintained at 1100 V above the self-regulatory threshold with a solvent delay of 3 minutes [20].

Quantification of Bioactive Contents

The oxidation of polyphenols leads to the reduction of the Folin-Ciocalteu reagent, which serves as the foundation for these analytical procedures adapted from Zargoosh et al., [21] in order to identify phenolic constituents. To calculate the concentration of flavonoid compounds in their samples, the researchers made adjustments to the procedures outlined in Zirari et al., [22]. The Hagerman [23] technique, on the other hand, employs vanillic acid as a reagent for quantifying tannins.

Antioxidant Activity

The antioxidant activity of extracts from *E. hirta* and *E. hyssopifolia* was measured by looking at DPPH radical scavenging activity, described in our previous study [24].

Antibacterial assays

Bacterial Strains

The antibacterial properties of *E. hirta* and *E. hyssopifolia* against the bacterium *Escherichia coli*, *Staphylococcus aureus* , *Staphylococcus aureus* methicillin- resistant, *Klebsiella pneumoniae* , *Acinetobacter baumannii*, *Staphylococcus epidermidis*, and *Enterobacter cloacae*.

Antibiotics

The antibiotics used were Oxacillin (5µg) Streptomycin (10µg), ampicillin (10µg), and Spiramycin (100 µg).

Disc Diffusion Method

In this study, extracts from *E. hirta* and *E. hyssopifolia* were evaluated for their antibacterial activities using the Kirby-Bauer disk diffusion technique [25]. For this evaluation, Whatman N° 3 paper disks (6 mm in diameter) were sterilized by boiling in sterile glass vials for 30 minutes to eliminate any potential inhibitors of microbial growth. The disks were then inoculated with bacterial suspensions (10^8 CFU) via swabbing. Antibiotic disks were placed on Mueller-Hinton (MH) agar plates, and varying concentrations of the plant extracts were applied to each disk. Subsequently, the inoculated Petri dishes were incubated at 37 °C in the dark.

The diameter of the inhibitory zone was measured 24 hours post-incubation, with the bacteria

collected for subsequent use [26].

Statistical Analysis

A one-way ANOVA was used to examine the data, and statistical software (SPSS, version 20) was used to assess significant levels and apply Tukey's test (α = 0.05) for multiple comparisons. The mean of three replicates is used to display the results. A *p-value* of less than 0.05 was deemed statistically significant.

RESULTS

Extraction yields

The extracts of *E. hirta* and *E. hyssopifolia* were prepared using hexane. The yield was determined with respect to 20 g of dry plant material and expressed as a percentage. The results obtained are presented in figure 1. The yields of *E. hirta* and *E. hyssopifolia* are respectively 1.78% and 1.38%.

Phytochemical screening

The phytochemical analysis of extracts from *E. hirta* and *E. hyssopifolia* indicated the presence of phenolic compounds, flavonoids, as well as catechic and gallic tannins (Table 1).

GC-MS Analysis

The GC-MS analysis of the extracts from *E. hirta* and *E. hyssopifolia* display the chemical profiles detected through GC-MS analysis. The phytochemical activity and structural information of a few of the chemicals found are shown in Table 2. The peaks observed in the chromatograms were integrated and subsequently matched against the GC-MS NIST II library, which contains a database of spectra for known components. Twenty-seven compounds were elucidated and identified from *E. hirta*, while 26 compounds were identified from *E. hyssopifolia*.

The analysis revealed a variety of compounds, including fatty acids, alcohols, hydrocarbons, sterols, terpenes and esters. Thus, we have for the extract of *E. hirta* 9,19-Cyclolanost-24-en-3-ol (37.755%), 9,19- Cycloergost-24(28)- en-3 (10.185%), D- Cycloolean-14- en-3- one (7.176%), Lup-20(29)-en-3-one (5.459%), with the remaining compounds each contributing less than 5% to the total composition. For the extract from *E. hyssopifolia*, we have Lupeol, trifluoroacetate (24.113%), 1-Dotriacontanol (12.868%), Methyl acetate C (7.036%), 3,7,11,15-Tetramethyl-2-hexa hexadecen-1- ol (6.213%), Clionasterol- H_2O (4.916%), Cholest-22- ene-21- ol, 3,5- dehydro-6-methoxy- (4.044%), with other compounds each accounting for less than 4%.

Quantitative phytochemical analysis

In this study, the amount of total phenol, flavonoid and tannin content of the organic extracts of *E. hirta* and *E. hyssopifolia* is determined as indicated in Figure 1.

The gallic acid equivalent (mg GAE/g MS) was used to express the total phenol content values. The results of the total phenol content showed that *E. hirta* had a significantly higher total phenol content (0.343 ± 0.022 mg GAE/g) than *E hyssopifolia* (0.281 ± 0.029 mg GAE/g). In a similar manner, the total flavonoid content analysis showed that the extract from *E. hirta* had a greater quantity of total flavonoids (11.188 ± 0.325 µg EQ/g) than that of *E. hyssopifolia* (5.938 ± 0.652 µg EQ/g). However, the extract of *E. hirta* had the lowest tannin content (0.163 ± 0.032 mg CE/ g), while the extract of *E. hyssopifolia* had the highest (0.238 ± 0.054 mg CE/ g). The statistical analysis revealed that there are statistically significant differences between the two plants in terms of phenolic compounds, flavonoids and tannins.

Antioxidant activity

The DPPH technique was used to evaluate the extracts' antioxidant capability. This study calculated the IC_{50}, or the concentrations of the extracts of the two plants under investigation required to capture 50% of the DPPH radicals. Figure 1 displays the findings of the antioxidant activity of the *E. hirta* and *E. hyssopifolia* extracts. The antioxidant activity of the *E. hyssopifolia* extract was found to be greater (IC_{50} = 0.403 ± 0.001 mg/mL) than that of the *E. hirta* extract (IC_{50} = 0.556 ± 0.004 mg/mL). The two plant extracts' antioxidant activity has demonstrated a dependence on the dosage given. The DPPH radical scavenging capabilities of the extracts of *E. hirta* and *E. hyssopifolia* were substantially lower than those of the standard, L-ascorbic acid (0.109 ± 0.001 mg/mL) (Figure 1).

Antibacterial activity

The results of the antibiogram revealed that *Klebsiella* , *Enterobacter* and *MRSA* showed resistance to the four antibiotics tested. In contrast, *S. epidermidis* showed moderate sensitivity to streptomycin, while *E. coli* was moderately sensitive to streptomycin and spiramycin. In addition, *S. aureus* showed sensitivity to streptomycin and spiramycin, while Enterobacter showed moderate sensitivity to streptomycin (Table 3).

The antibacterial activity of *E. hirta* and *E. hyssopifolia* extracts against six pathogenic pathogens was assessed using the disk diffusion test. As seen in figure 2, the inhibitory zone's diameter ranged from 6.17 to 8.33 mm. The largest inhibitory zone was observed in *S. aureus*, the *E. hirta* extract having a diameter of 8.33 mm. Conversely, the smallest diameter was observed in E. coli when exposed to the *E. hirta* extract of 6.17 mm. The statistical evaluation of the diameters of the inhibition zones from the two extracts revealed no significant difference (*p>0.05*).

Figures

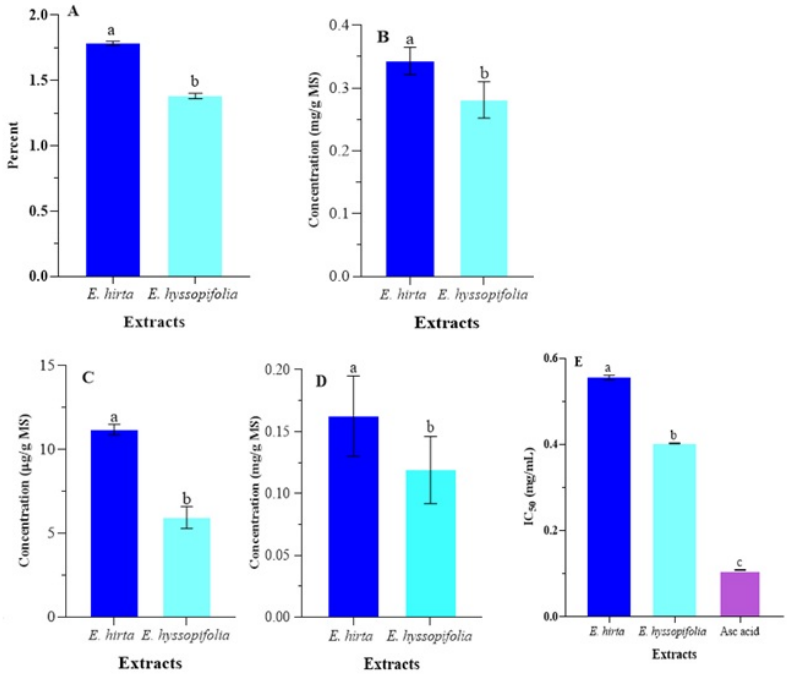

Figure 1: Evaluation of the total content of extraction yields (A), phenolic compounds (B), flavonoids (C), tannins (D), and antioxidant activity (E) of the two plants The letters signify statistically significant differences between the means at the 0.05 significance level.

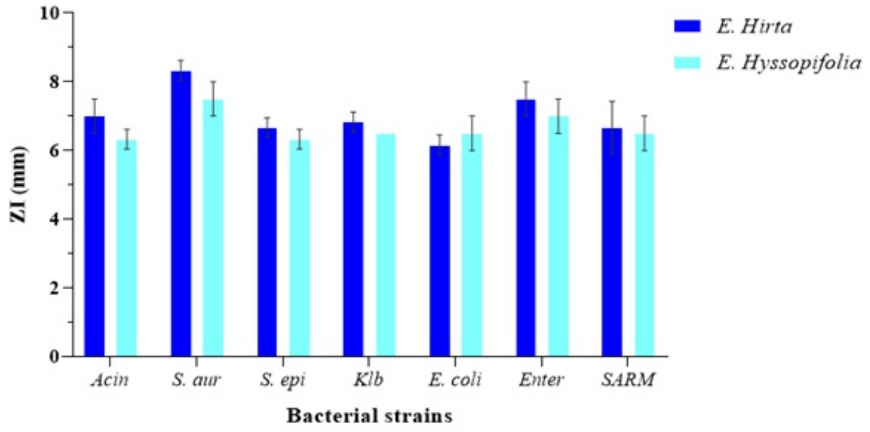

Figure 2: Mean diameter of the pathogen inhibition zones generated by the extracts of E. hirta and E. hyssopifolia.

Tables

Chemical groups	*E. hirta*	*E. hyssopifolia*
Polyphenols	+	+
Catechic tannins	+	+
Gallic tannins	+	+
Leucocyanidins	+	+
Cyanidins	–	–
Anthocyanins	+	+
Quinones	–	–
Alkaloids	–	–

Table 1: Qualitative profile of the phytochemical compounds of E. hirta and E. hyssopifolia. +: Present in trace amount in the extract; –: Absent in the extract.

Compounds \ Plants	*E. hirta*		*E. hyssopifolia*	
	RT (min)	%	RT (min)	%
Oleic diethanolamide	28.987	0.783	-	-
Neophytadiene	48.027	0.484	-	-
Stearic acid	-	-	48.312	0.477
Docosane	48.363	0.711	47.643	1.243
Stearic acid	53.287	1.039	-	-
Nonadecane	54.671	2.965	54.606	1.741
3,7,11,15-Tetramethyl-2-hexa hexadecen-1-ol	54.939	0.657	53.802	6.213
9,12-Octadecadienoic acid	-	-	58.867	1.295
9,12,15-Octadecatrienoic acid	-	-	59.032	1.028
9-Octadecenoic acid (Z)	-	-	59.100	2.012
Neophytadiene	-	-	59.934	0.905
Methyl 8,11,14-heptadecatrie	60.990	2.310	60.911	1.405
Triacontane	-	-	66.630	0.823
Hexacosane	79.192	1.382	-	-
Vitamin E	83.170	0.741	82.904	1.363
1-Heptatriacotanol	-	-	86.263	1.476
D-Friedoolean-14-en-3-one	86.279	7.176	-	-
Methyl cumate E	86.612	2.161	-	-
Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-	-	-	86.664	4.044
Clionasterol -H_2O	86.849	2.303	86.854	4.916
24-Noroleana-3,12-diene	87.178	3.440	87.255	2.033
Lup-20(29)-en-3-one	87.513	5.459	-	-
9,19-Cycloergost-24(28)-en-3	87.63	10.185	88.574	10.307
1-Dotriacontanol	-	-	87.641	12.868
9,19-Cyclolanost-24-en-3-ol	88.052	37.755	-	-
Methyl commate C	88.768	1.709	88.425	7.036
Lupeol, trifluoroacetate	-	-	89.230	24.113
Stigmast-4-en-3-one	89.509	4.245	-	-
Tris(2,4-di-tert-butylphenyl	91.101	2.343	90.618	0.804
Methane, oxybis[dichloro-	103.445	3.053	103.458	3.786
Identified	-	**88.591**	-	**89.888**
Not identified	-	**11.409**	-	**10.112**

Table 2: Secondary metabolites in the extracts of E. hirta and E. hyssopifolia identified using GC-MS. RT: Retention time; %: Peak area.

Bacteria	Oxacillin		Streptomycin		Ampicillin		Spiramycin	
	Ø (mm)	Pr	Ø (mm)	Pr	Ø (mm)	Pr	Ø (mm)	Pr
S. aureus	0.00 ± 0.00^a	R	15.63 ± 0.56^b	S	0.00 ± 0.00^a	R	20.12 ± 0.12^c	S
S. epidermidis	0.00 ± 0.00^a	R	12.17 ± 0.18^b	MS	0.00 ± 0.00^a	R	15.15 ± 0.16^b	S
Klebsiella	0.00 ± 0.00^a	R	0.00 ± 0.00^a	R	0.00 ± 0.00^a	R	0.00 ± 0.00^a	R
Acinetobacter	6.64 ± 0.19^a	R	6.63 ± 0.28^a	R	6.39 ± 0.11^a	R	6.54 ± 0.33^a	R
E. coli	0.00 ± 0.00^a	R	9.40 ± 0.31^b	MS	0.00 ± 0.00^a	R	12.20 ± 0.23^c	MS
Enterobacter	6.49 ± 0.09^a	R	8.45 ± 0.28^b	MS	6.30 ± 0.07^a	R	6.45 ± 0.11^a	R
MRSA	0.00 ± 0.00^a	R	0.00 ± 0.00^a	R	0.00 ± 0.00^a	R	0.00 ± 0.00^a	R

Table 3: Comparison of the impacts of antibiotics on pathogenic bacteria. Ø: Zone of Inhibition; Pr: Profile; R: Resistant; MS: Moderately sensitive; S: Sensitive. MRSA; Staphylococcus aureus methicillin resistant. Means within the same column share the same letter, they do not differ significantly from one another at the 5% significance level based on Tukey's test.

DISCUSSION

The extraction yields of *E. hirta* and *E. hyssopifolia* were 1.78% and 1.38%, respectively. These results are consistent with previous studies, such as Azaat et al., [27] reported a yield of 1.50% for the n-hexane extract, 2.80% for the ethyl acetate extract, 13.66% for the aqueous extract and 10.63% for the methanolic extract of *E. hyssopifolia*. Koffi et al., [28] obtained a yield of 22.54% for the aqueous extract and 12.21% for the ethanolic extract of *E. hirta*.

Both species included tannins, flavonoids, and phenolic substances, according to phytochemical

screening. There were 27 chemicals found by the GC- MS analysis in *E. hirta* and 26 in *E. hyssopifolia*, including fatty acids, alcohols, hydrocarbons, sterols, terpenes, and esters. These findings are consistent with previous studies on *Euphorbia* species. For instance, Azaat et al., [27] identified similar compounds, such as fatty acids and sterols, in the n-hexane extract of *E. hyssopifolia* . Similarly, Koffi et al., [28] reported the presence of terpenes and sterols in the ethanolic extract of *E. hirta*, aligning with our results. Further study of Rautela et al., [29], they assigned the presence of bioactive compounds, notation D:A- friedooleanan-3- ol, (3.beta)-, Tetracosane and 3- isopropyl-3A in *E. milli* and 2,3- Dihydro- Benzofuran, 2- Hexadecen-1- ol, 3,7,11,15 -Tetramethyl-, [R-[R et 9,12,15-Octadecatrienoic acid giving *E. hirta*.

The relative abundance of these compounds, however, varied, which could be explained by variations in plant maturity, geographic location, or extraction techniques.

Overall, the GC- MS results demonstrate that *E. hirta* and *E. hyssopifolia* are rich sources of bioactive compounds, with compositions comparable to other *Euphorbia* species. However, the unique profiles of each species suggest distinct pharmacological potentials, warranting further investigation.

Due to their critical involvement in global physiological processes, the amount of phenolic compounds in natural products is a crucial criterion for both quantitatively assessing the extract and determining its biological strength [30, 31].

Quantitative analysis showed that *E. hirta* had higher phenolic and flavonoid content, while *E. hyssopifolia* had higher tannin content. These findings align with previous studies, such as Azaat et al., [27], who reported similar trends in phenolic and flavonoid content. A study demonstrated that the n-hexane extract of *E. dracunculoides* had a total phenol content of 8.21 mg/g and a flavonoid content of 4.18 mg/g [32].

Phenolic compounds exhibit a broad range of biochemical activities, including antioxidant, antimutagenic, and anticancer properties, along with the capacity to modulate gene expression. Flavonoids, as active components, demonstrate diverse biological effects, contributing to resistance against microbial, ulcerative, arthritic, angiogenic, and cancerous diseases, while also inhibiting mitochondrial adhesion formation [33].

A wealth of bioactive chemicals found in plants have great promise for the creation of novel chemotherapy medications. They are used to treat a wide range of illnesses and fight against different infections, making them essential to both conventional and contemporary medicine. Researchers worldwide are currently looking into the therapeutic uses of compounds that are pharmacologically active and originate from medicinal plants. 80% of people worldwide use herbal medications due to their shown effectiveness, low cost, non-narcotic qualities, and low risk of adverse consequences [34].

Significant progress has been made in identifying and functionally characterizing the bioactive components of phytochemicals, which are categorized as secondary metabolites naturally present in plants [35].

The antioxidant activity of the extracts of both plants can be attributed to the presence of phenolic compounds. Azaat et al., [27] demonstrated the antioxidant activity of the aqueous extract, methanolic, ethyl acetate and n- hexane (0.313, 0.080, 0.193 and 2.078 mg/ mL, respectively). In our study, the results obtained for the extracts were of 0.556 mg/mL and 0.402 mg/mL, respectively of *E. hirta* and *E. hyssopifolia*.

The differences in antioxidant potential among various plants can be attributed to substantial variations in their phenol and flavonoid contents. Furthermore, the type of extraction solvent used can also influence the anti-radical scavenging activity [35].

A recognized method for assessing the antioxidant activity of plant extracts is the removal of free radicals by DPPH. This technique is frequently used to assess the antioxidant capacity of plant extracts since it takes less time to analyze. DPPH is regarded as a highly potent antioxidant because of its capacity to donate hydrogen. In order to prevent these free radicals from playing a harmful role in a number of illnesses, including cancer, their removal is crucial

[36, 37].

Given the significant variation in phytochemical content observed between the two plants, we hypothesized that this might lead to differences in their pharmacological effects. To investigate this, we assessed the antioxidant and antibacterial activities of both plants.

The extracts demonstrated notable antibacterial effects against pathogenic bacteria, with S. aureus showing the highest sensitivity. In comparison with certain *Euphorbia* species, *E. macroclada*, *E. falcata*, *E. Aleppo*, *E. denticulata*, *E. szovitsii*, *E. virgata, E. cheiradenia*, and *E. petiolata* have all been shown to have antibacterial properties against *S. aureus*, *B. megaterium*, *P. vulgaris*, *K. pneumoniae* and *E. coli* [38].

In this research endeavor, we have investigated the extracts derived from *E. hirta* and *E. hyssopifolia* to elucidate their chemical constituents, phytochemical characteristics, antioxidant potential, and antibacterial efficacy. Our study's findings unequivocally show that the phytochemical-rich extracts of *E. hirta* and *E. hyssopifolia* have a great deal of potential for use in medicine. Since our investigation of these plants in Morocco is new, it is challenging to draw firm conclusions about the total amount of phenolic chemicals present. Consequently, it is essential to carry out more research and replicate the analyses. These procedures are essential for verifying the outcomes and pinpointing the precise substances causing the biological effects that have been seen. Consequently, our findings pave the way for the observed biological effects. As a result, our research paves the way for more thorough studies meant to uncover significant biomolecules that may enhance the health of people and animals.

CONFLICT OF INTEREST

No conflicts of interest are disclosed by the authors.

AUTHOR CONTRIBUTIONS

All authors took part in conceptualization of the study, execution of experiments, data analysis, writing manuscript and finally doing revisions.

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AI Use Statement

The authors used an artificial intelligence (AI) tool solely for language editing and improvement of the manuscript. The AI tool was not used for data generation, data analysis, interpretation of results, scientific conclusions, or preparation of figures. The authors carefully reviewed and edited all AI- assisted content and take full responsibility for the accuracy, integrity, and originality of the manuscript and the data presented therein.OM

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