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RESEARCH ARTICLE

Valorization of the Biological Activity of *Euphorbia hirta*

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ABSTRACT

Aim: The main objective of our study it to valorization of the biological activity of euphorbia hirtathe. **Material & Methods:** Phytochemical analysis of Euphorbia hirta revealed a variety of compounds, including saponins, polyphenols, flavonoids, tannins, terpenoids, coumarins, and cardiac glycosides. Quantitative measurements indicated that the methanolic extract from the roots is particularly rich in phenolic compounds, with a concentration of 128.45 ± 5.1 µg GAE/mg of extract. Conversely, the methanol extract from the stems had the highest flavonoid content, at 36.11 ± 0.45 µg QE/mg of extract. **Result:** The antioxidant capacity of the polar extracts was evaluated using the DPPHº method, showing that the root methanol extract exhibited significant antioxidant activity, with an IC50 value of 07.04 ± 0.14 µg/ml. **Conlusion:** Additionally, a correlation was noted between the levels of polyphenols and the concentration of phenolic compounds present.

Keywords: Antioxidant potential, 2, 2-Diphenyl-1-picrylhydrazyl, *Euphorbia hirta*, IC50, phytochemical composition

INTRODUCTION

Euphorbia hirta, commonly known as asthma weed or rubber weed, is a flowering plant that belongs to the *Euphorbiaceae* family, which is characterized by a wide range of species with diverse properties. While this plant is native to tropical regions, it has adapted to various climates and is now found in many parts of the world, thriving in disturbed areas and along roadsides.[1]

Typically, *E. hirta* grows as a herbaceous annual with a sprawling growth habit, often reaching heights of approximately 30–60 cm. Its stems are typically green and can exhibit a reddish hue, while its leaves are simple, ovate, and arranged alternately. The plant produces small, inconspicuous flowers that are clustered in axillary cyathia, contributing to its reproductive success and rapid spread.[2]

In traditional medicine, *E. hirta* has been employed for a variety of respiratory ailments, including asthma, bronchitis, and coughs. It is also noted for its potential anti-inflammatory, analgesic, and antimicrobial properties, which have attracted scientific interest. However, caution is necessary when handling the plant, as its milky latex can cause skin irritation and allergic reactions in some individuals. In addition, due to its vigorous growth, *E. hirta* is often viewed as a weed, competing with cultivated plants.[3]

The increasing scrutiny of synthetic antioxidants as food additives is driven by concerns about their potential toxicological risks, such as long-term health effects. Moreover, the overuse of chemical antibacterial agents in clinical settings has led to the rise of antibiotic-resistant strains of bacteria, creating a pressing need for new therapeutic options to combat infections and prevent food spoilage due to oxidation.[4]

Overall, this study aims to contribute valuable knowledge regarding the phytochemical profile and biological activities of *E. hirta*, potentially

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paving the way for the development of new natural products for health and wellness.

MATERIALS AND METHODS

Plant Material

The whole plant of *E. hirta* was collected from Taounate region, Morocco in March 2014. Taxonomic identification was performed by Dr. A. Ennabili (National Institute of Medicinal and Aromatic Plants, Morocco). Whole plants were dried at room temperature and powdered, stored for further analyses.

Extraction

100 g of each powdered sample (leaves, stems and roots) was extracted successively with hexane (1L), ethyl acetate (1L), and methanol (1L) for 24 h with stirring. The obtained extracts were evaporated and stored for further analyses.

Phytochemical Screening

The ethyl acetate and methanolic extracts were screened for phytochemical constituents.

Test for Phenolic Compounds (Ferric Chloride Test)

A small amount of each extract was dissolved in 5 mL of distilled water and a few drops of 1% ferric chloride have been added. The appearance of a dark green color indicates the presence of phenolic compounds.[5]

Table 1: Phytochemical Analysis of *Euphorbia hirta* leave, stem, and root extracts

Extracts	Methanolic extract			Ethyl acetate extract		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Phenolic compounds	+	+	+	+	+	+
Flavonoïds	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Terpenoïds	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+
Saponins	+	+	+	-	-	-
Alcaloïds	-	-	-	-	-	-

Test for Flavonoïds (Alkaline Reagent Test)

A few drops of sodium hydroxide were added to extracts dissolved in distilled water, the appearance of an intense yellow color indicates the presence of flavonoïds. Disappearance of the color after the addition of dilute hydrochloric acid confirms the presence of flavonoids.[5]

Test for Tannins

Each extract dissolved in distilled water, a solution of 1% ferric chloride was added. The appearance of a green color indicates the presence of tannins.[6]

Test for Terpenoïds

A dilute solution (2 mL) in chloroform of each extract, 2 mL of H_2SO_4conc was added. The appearance of a red color at the interface indicates the presence of terpenoïds.[7]

Test for Coumarins

An amount of each extract was dissolved in distilled water, 3 mL of 10% NaOH was added, and the appearance of a yellow color indicates the presence of coumarins.[8]

Test for Saponins

A diluted solution of each sample (10 mL) in distilled water was stirred vigorously until the formation of foam. A few drops of olive oil have been added, the mixture was stirred vigorously for a few minutes, and the formation of an emulsion was confirmed the presence of saponins.[7]

Test for Alcaloïds

A quantity of the extract was stirred with a few drops of dilute hydrochloric acid and then filtered. The filtrate was used for the following tests:[5]

Mayer Test

A few mL of filtrate, one or two drops of the Mayer reagent are added. The appearance of a white or creamy precipitate indicates the presence of alkaloids.[5]

Wagner Test

A few mL of filtrate, a few drops of Wagner reagent are added. The appearance of a reddish-brown precipitate confirms the presence of alkaloids.[5]

Test for Cardiac Glycosides (Keller-Kiliani Test)

A small quantity of each extract was mixed with 2 mL of glacial acetic acid solution containing one drop of 0.1% ferric chloride. The mixture was then treated with 1 mL of concentrated sulfuric acid. The formation of brown ring at the interface or a greenish ring in the acetic acid layer confirms the presence of cardiac glycosides.[7]

Test for Amino Acids and Proteins

An amount of each extract was dissolved in distilled water and then filtered. The filtrate was used for the following tests:[5]

Biuret Test

A few mL of the filtrate, a drop of copper sulfate solution (2%) to 2 mL of ethanol (95%) was added, followed by the addition of an excess of potassium hydroxide pellets. The appearance of a pink color in the ethanol layer indicates the presence of proteins.[5] A few mL of the filtrate, an equal volume of sodium hydroxide solution (5%), and 1% copper sulfate solution were added. The appearance of a violet coloration indicates the presence of the amino acids.[9]

Total Phenolic Content

Total phenol contents of each extract were determined using the Folin–Ciocalteu method.[10] 1 mL of dilute solution of each extract was mixed with Folin–Ciocalteu reagent (2,5 mL). After 5 min, sodium carbonate solution (75 g/L in water, 2 mL) was added and the reaction mixture was allowed to stand for 2 h at room temperature, then the optical density at 765 nm was measured against water blank. Gallic acid was used as a standard calibration curve (0–300 μg/mL), The results were expressed as μg of gallic acid equivalent (GAE)/mg of extract.

Total Flavonoids Content

Total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand *et al*.[11] 1 mL of dilute solution of each extract was mixed with a 2% solution of aluminum trichloride (AlCl3) in methanol (2 mL). The optical density at 415 nm was measured against a blank sample consisting of a methanol (2 mL) and extract (2 mL) without AlCl3. Quercetin was used as a standard calibration curve (0–50 μg/mL), The results were expressed as μg of quercetin equivalent (QE)/mg of extract.

Antioxidant Tests

Reduction of 2, 2-diphenyl-1-picrylhydrazyl (DPPHº)

The radical scavenging activity, using free-radical DPPH assay, was determined according to the method introduced by Arvouet-Grand *et al*.[12] Briefly 2 mL of each extract (at different concentrations in methanol) was mixed with 2 mL of DPPHº solution (0.004%) in methanol. After an incubation of 30 min, the absorbance was measured at 517 nm using MeOH as blank. 2 mL of DPPHº solution (0.004%) mixed with 2 mL of Methanol were used as control. The absorbance (A) of the control and samples was measured, and The DPPHº scavenging activity was determined using the following equation:

$$\text{DPPH}^{\circ}\text{ scavenging activity (\%)} = ([A_{control} - A_{sample}]/A_{control}) \times 100$$

The data are presented as a mean of triplicate and the concentration required for a 50% reduction (IC_{50}) of DPPHº radical was determined graphically.[13]

Statistical Analysis

Data were expressed as the mean ± standard deviation of at least three measurements. Data were analyzed using the analysis of variance test (Fisher) and determination of the Pearson correlation coefficient (ρ) was used during this work to evaluate and correlate results between them. The data were statistically analyzed using IBM Statistical Packages for the Social Sciences Statistics 21 (statistical software); Pearson correlation coefficient (ρ) was calculated by Excel.[14]

RESULTS AND DISCUSSION

The phytochemical screening of the extracts revealed the presence of phenolic compounds, flavonoids, tannins, terpenoids, coumarins, and cardiac glycosides in all organs of the plant for the two polar solvents. However, saponins are located only in the methanol extract.

Total Phenolic Content and Total Flavonoid Content

The methanolic extract of roots showed the highest total phenolic content (128.45 ± 5.2 µg GAE/mg of extract). This extract is significantly higher than those obtained from the extracts of leaves, stems, and roots obtained in ethyl acetate ($P < 0.05$) [Table 2]. Phenolic compounds are known by their effects to scavenge free radicals,[15] their presence in large quantities justified a better antioxidant activity. The methanolic extract of stems showed the highest total flavonoids content (36.11 ± 0.45 µg QE/mg) of extract, this extract is significantly higher than when compared to the others extract ($P < 0.05$) [Table 3].

Antioxidant Activity

From the values obtained during manipulations performed to evaluate the antioxidant activity, we noticed that the curves have the same shape. The higher the concentration of the extracts is increased, the more anti-radical activity increased until a plateau. Beyond this limit, the activity remains constant [Figure 1]. Furthermore, a lower value of IC_{50} (the concentration of extract which causes 50% inhibition of the activity of DPPHº) indicates a higher antioxidant activity.[16] The antioxidant activity of the methanolic extracts and ethyl acetate obtained with the DPPHº test ranged respectively from 10.01 ± 0.17 to 25.65 ± 1.07 µg/mL and from 18.85 ± 0.12 to 48.28 ± 4.70 µg/mL. The methanolic extract of root showed higher antioxidant activity (10.01 ± 0.17 µg/mL) followed by ethyl acetate extract of roots (18.85 ± 0.12 µg/mL). However, the antioxidant activity of methanolic extract of roots was significantly higher than those obtained from the extracts of leaves and stems ($P < 0.05$). The antioxidant activity of methanolic extracts had been always higher than those of the ethyl acetate extracts for each part of the plant. Numerous studies have shown that antioxidant activity was correlated

Table 2: Total phenolic content

Extracts	Total phenolic content (µg GAE/mg of extract)
Leaves extract with methanol	61.32±2.23
Stems extract with methanol	88.3±1.27
Roots extract with methanol	28.15±5.1
Leaves extract with ethyl acetate	47.67±1.39
Stems extract with ethyl acetate	48.12±1.86
Roots extract with ethyl acetate	80.40±2.46

Table 3: Total flavonoid content

Extracts	Total flavonoid content (µg QE/mg of extract)
Leaves extract with methanol	22.60±0.18
Stems extract with methanol	36.11±0.48
Roots extract with methanol	11.15±0.22
Leaves extract with ethyl acetate	5.63±0.12
Stems extract with ethyl acetate	15.42±0.12
Roots extract with ethyl acetate	10.52±0.13

QE: Quercetin equivalent

Table 4: Antioxidant activity

Extracts	IC_{50} (µg/mL)
Leaves extract with methanol	25.65±1.07
Stems extract with methanol	22.11±1.11
Roots extract with methanol	10.01±0.17
Leaves extract with ethyl acetate	48.28±4.70
Stems extract with ethyl acetate	33.67±1.14
Roots extract with ethyl acetate	18.85±0.12
Ascorbic acid	3.32±0.03

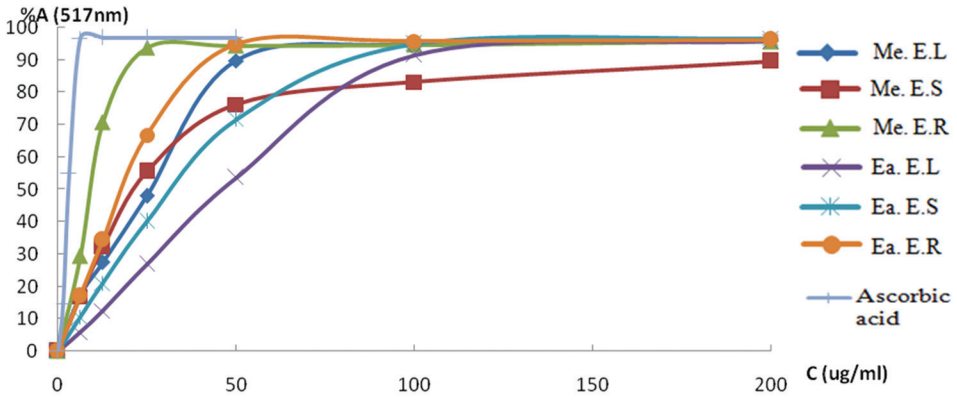

Figure 1: Anti-radical activity of extract of hirta, and ascorbic acid in different concentrations. E: Extract; L: Leaves; S: Steems R: Roots; Me:Methanolic; Ea:Ethyl acetate.

with the presence of phenolic compounds.[15,17] Determination of Pearson correlation coefficient between the values obtained with the DPPHº test and total phenolic content ($P = -0.87$, $P < 0.05$) indicated that antioxidant activity and total phenolic content could be correlated.

CONCLUSION

A high antioxidant activity of the extracts was noted primarily in the methanolic extracts and ethyl acetate of roots. Therefore, the organs can be classified according to their antioxidant capacity by following descending order: Root, stem, and leaf. The good correlation found between antioxidant activity and phytochemical contents indicates that effects observed could be attributed to phenolic compounds. The polar extracts of the roots of *E. hirta* potentially can be considered more active than those of the leaves and stems. They showed a strong antioxidant activity where the possibility of using these extracts in the food industry to remedy the oxidation and in the pharmaceutical industry for the treatment of certain diseases. We can consider these extracts as a new potential source of natural bioactive molecule to be extracted, identify and characterize, and finally exploiting them as dietary antioxidants or as drugs.

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