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Review Article

STUDY OF THE FREE RADICAL SCAVENGING ACTIVITY OF EUPHORBIA HIRTA

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ABSTRACT:

The analysis of the phytochemical composition of Euphorbia hirta identified a range of compounds, including saponins, polyphenols, flavonoids, tannins, terpenoids, coumarins, and cardiac glycosides. Quantitative assessments showed that the methanolic extract from the roots is particularly high in phenolic compounds, measuring 128.45 ± 5.1 µg GAE / mg of extract. In contrast, the methanol extract from the stems had the highest concentration of flavonoids, at 36.11 ± 0.45 µg QE / mg of extract. The antioxidant capacity of the polar extracts was assessed using the DPPHº method, revealing that the root methanol extract exhibited significant antioxidant activity, with an IC50 value of 07.04 ± 0.14 µg / ml. Furthermore, a correlation was observed between the levels of polyphenols and the presence of phenolic compounds.

Keywords: Euphorbia hirta, phytochemical composition, antioxidant potential, DPPHº, IC50.

INTRODUCTION

Euphorbia hirta, commonly referred to as asthma weed or rubber weed, is a flowering plant belonging to the spurge family (Euphorbiaceae). Although it is native to tropical regions, this species can be found in various parts of the world. Typically, Euphorbia hirta grows as a herbaceous annual with a sprawling growth habit, reaching heights of approximately 30-60 cm. This plant is frequently utilized in traditional medicine, particularly for respiratory ailments such as asthma and coughs, and is noted for its potential anti-inflammatory and antimicrobial properties. Caution is advised when handling the plant due to its milky latex, which can be irritating. Additionally, it is sometimes considered a weed because of its rapid growth and spread. The use of synthetic antioxidants as food additives is increasingly being scrutinized due to the potential toxicological risks associated with these compounds. Furthermore, the overuse of chemical antibacterial agents in medicine has contributed to the emergence of antibiotic-resistant bacterial strains. This highlights the urgent need to develop new therapeutic agents to combat bacterial resistance and food oxidation. Investigating plants offers significant potential for discovering new extracts or compounds with antimicrobial and antioxidant properties. In this context, the objectives of this study were: (i) to analyze the chemical composition of polar extracts from the leaves, stems, and roots of Euphorbia hirta; (ii) to evaluate the antioxidant capacity of various polar extracts, particularly those from the roots, which have not been previously studied; and (iii) to explore possible correlations between phenolic compounds and antioxidant activities.

Material and method

Plant material

The whole plant of Euphorbia 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 plant were dried at room temperature and powdered, stored for further analyses.

Extraction

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

Phytochemical screening

The ethyl acetate and metanolic exracts 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].

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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 flavonoïds [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 Saponines
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, 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 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, 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 glycosids [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%) and1% copper sulphate solution was 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]. 1ml of dilute solution of each extract was mixed with Folin Ciocalteu reagent (2,5ml). 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 hours 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 Arvouret-Grand et al [11].1ml of dilute solution of each extract was mixed with a 2% solution of aluminium trichloride (AlCl3) in methanol (2 mL).The optical density at 415 nm was measured against 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:

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

The data are presented as mean of triplicate and the concentration required for a 50% reduction (IC_{50}) of DPPH° radical was determined graphically

Statistical analysis

Data were expressed as the mean ± standard deviation of at least three measurements. Data were analyzed using ANOVA 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 analysed using IBM SPSS Statistics 21 (statistical software), Pearson correlation coefficient (ρ) was calculated by Excel .

Results and discussion

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

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

	Methanolic extract			Ethyle acetate extract		
	Leaves	**Stems**	**Roots**	**Leaves**	**Stems**	**Roots**
Phenolic Compounds	+	+	+	+	+	+
Flavonoïds	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Terpenoïds	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+
Saponins	+	+	+	-	-	-
Alcaloïds	-	-	-	-	-	-

Totals phenolic content and Totals flavonoid content

The methanolic extract of roots showed the highest Totals 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 1). 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 totals 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).

Table 2 : Totals phenolic content

Extracts	Totals 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 ethyle acetate	47,67±1,39
Stems extract with ethyle acetate	48,12±1,86
Roots extract with ethyle acetate	80,40±2,46

Table 3: Totals flavonoid content

	Totals flavonoid content (µg QE/mg of extractt)
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 ethyle acetate	5,63±0,12
Stems extract with ethyle acetate	15,42±0,12
Roots extract with ethyle acetate	10,52±0,13

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

Table 4: Antioxidant activity

	IC_{50} (µg/mL)
Leaves extract with methanol	$25{,}65 \pm 1{,}07^{a}$
Stems extract with methanol	$22{,}11 \pm 1{,}11^{b}$
Roots extract with methanol	$10{,}01 \pm 0{,}17^{a, b}$
Leaves extract with ethyle acetate	$48{,}28 \pm 4{,}70^{a, b, c}$
Stems extract with ethyle acetate	$33{,}67 \pm 1{,}14^{b, c}$
Roots extract with ethyle acetate	$18{,}85 \pm 0{,}12^{c}$
Ascorbic acid	3,32±0,03

Conclusion

In 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, 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 Euphorbia 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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