



ISSN Print: 2664-6552  
 ISSN Online: 2664-6560  
 Impact Factor: RJIF 5.5  
 IJCRD 2023; 5(2): 17-23  
<https://www.chemicaljournal.in/>  
 Received: 11-05-2023  
 Accepted: 18-06-2023

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 the References

## Phytochemical criblage and evaluation of the antiradical activity of the leaves, roots and roots of *Euphorbia hirta* L. (Euphorbiaceae): A Medicinal plant from Ivory Coast

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DOI: <https://doi.org/10.33545/26646552.2023.v5.i2a.50>

### Abstract

The present study is based on phytochemical screening and evaluation of the free radical scavenging activity of hydroethanolic extracts of *Euphorbia hirta* leaves, stems and roots, with a view to confirming or invalidating the traditional use of this plant in Côte d'Ivoire. Phytochemical sorting of secondary metabolites using color tube reactions and Thin Layer Chromatography (TLC) revealed polyphenols (Flavonoids, coumarins, tannins), sterols and terpenes in extracts of *Euphorbia hirta* leaves, stems and roots. Alkaloids were found only in the roots, and phenolic acids in the leaves and stems.

The presence of these secondary metabolites is thought to underlie the antioxidant activities observed by both qualitative (tube and TLC) and quantitative (Spectrophotometric) analysis methods towards the DPPH free radical. However, quantitative analysis by spectrophotometry showed a better antioxidant activity in leaves with an  $IC_{50} = 0.06381$  mg/mL compared with that observed in stems and roots with  $IC_{50}$  of 0.08312 mg/mL and 0.26137 mg/mL respectively.

**Keywords:** *Euphorbia hirta*, phytochemical screening, free radical scavenging activity

### Introduction

One of the most serious problems in the biological and medical sciences is oxidative stress. This is a situation where the cell can no longer resist the excessive production of toxic free radicals. This leads to a number of diseases ranging from the simplest to the most dangerous, such as cancer, diabetes, cardiovascular and degenerative diseases (Kamkara *et al.*, 2010; Cheurfalem, *et al.* 2016) <sup>[12, 8]</sup>. Synthetic antioxidants do exist, but many of them have been shown to have adverse effects on the body (Brieger *et al.*, 2012) <sup>[6]</sup>. With this in mind, this study focused on *Euphorbia hirta*, with a view to carrying out phytochemical screening and assessing the antioxidant activity of its leaves, stems and roots. Indeed, *E. hirta*, a member of the Euphorbiaceae family, is a plant commonly used in the traditional treatment of female disorders, respiratory ailments, infestations in children, dysentery, jaundice, pimples, gonorrhea, digestive problems, diarrhea, fever, diabetes, hypertension and tumors (Kumar *et al.*, 2010; Ayensu, 1980) <sup>[14, 1]</sup>. At the end of this study, the aim will be to confirm or refute the use of *E. hirta* leaves, stems and roots as a good antioxidant capable of preventing or treating pathologies linked to oxidative stress.

### Materials And Methods

#### Material

##### Plant material

The plant material consists of *Euphorbia hirta* leaves, stems and roots. The plant was selected on the basis of an ethnobotanical survey conducted among women who trade in traditional medicines (Herbalists) in the large market of the town of Korhogo in northern Côte d'Ivoire. The plant was harvested in March 2022 in Korhogo, in the village of Dramanikaha.

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The various plant organs were dried for 3 weeks in a room at room temperature, sheltered from the sun. Finally, these dried organs were crushed in a mortar and sieved to obtain fine powders which were used to prepare the various extracts to be tested.

### Technical equipment

The technical equipment consists of the usual laboratory glassware, an electronic balance (Denver Instrument SI-234), a water bath (Neo-Tech SA), a hot plate (Rommelsbcher), a spectrophotometer (Jenway) and a fume hood (Erlab and Trionyx).

### Chemical equipment

Chemical equipment consists of analytical-grade solvents, developers and reagents. These products were purchased from Polychimie (Côte d'Ivoire). For thin-layer chromatography (TLC) tests, we used silica gel 60 F<sub>254</sub> chromatoplates on an aluminum support (Merck, 1980) [18].

### Methods

#### Extractions

The maceration extractive technique was used to obtain organic extracts from the three *Euphorbia hirta* organs studied.

#### Hydroethanol extracts

A 10 g mass of each organ powder was macerated in 100 mL of ethanol/water mixture (70 mL/30 mL) for 24 h. The operation was carried out simultaneously 3 times for each organ. After filtration, the macerates obtained were combined, then placed in an oven at 50 °C for 2 h to remove the ethanol. The extract obtained is kept for 24 h in the refrigerator at 4 °C for precipitation of lipophilic compounds. After decantation, the hydroethanol extracts were obtained. These were used to evaluate phytochemical screening in tubes and antioxidant activities in tubes and by spectrophotometry, and to prepare selective extracts (Ouattara *et al.*, 2016) [19].

#### Selectives extracts

A 15 ml volume of each hydroethanol extract was exhausted by successive fractionations with (3 × 10 mL) hexane (C<sub>6</sub>H<sub>14</sub>), dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) and ethyl acetate (AcOEt). The various selective organic fractions were concentrated in an oven and then stored in a refrigerator at 4 °C. They were used for phytochemical screening and evaluation of antioxidant activity on TLC plates (Ouattara *et al.*, 2016) [19].

#### Phytochemical screening

Secondary metabolites (Tannins, phenolic acids, alkaloids, polyphenols, flavonoids, coumarins, saponins, sterols and polyterpenes) were identified using TLC plate tests and tube tests using color and precipitation reactions.

#### Color reaction tests and tube precipitation

The detection of certain secondary metabolites (polyphenols, flavonoids, coumarins, saponins, sterols and polyterpenes) was carried out using color tests and test tube precipitation. Analytical techniques described in Dohou *et al.*, (2003) [9]; Békro *et al.*, (2007) [4] were used for these tests.

#### Polyphenols detection

A few drops of a 2% (w/v) aqueous iron (III) chloride solution (FeCl<sub>3</sub>) were added to 2 mL of crude hydroethanolic extract. The appearance of a blue-black or green-black coloration indicates the presence of polyphenols (Békro *et al.*, 2007) [4].

#### Flavonoid detection: Shinoda test

5-7 drops of concentrated HCl and 2-5 Mg chips are added to 2 mL of crude hydroethanolic extract. In the presence of flavonoids, a pink-orange coloration is observed after 3 to 5 min. To accelerate the reaction and enhance color, the reaction mass is heated in a water bath for 2-3 min (Békro *et al.*, 2007) [4].

#### Coumarin detection: Potassium hydroxide (KOH) test

10 drops of 10% (w/v) alkaline methanolic KOH solution are added to 3-5 mL of plant extract. The mixture is heated in a water bath. Next, 5-10 mL of distilled H<sub>2</sub>O are added and the reaction mass is vigorously stirred. The resulting solution is neutralized with 10% (v/v) HCl until an acidic solution is obtained. If cloudiness or precipitation is observed, the presence of coumarins is confirmed (Békro *et al.*, 2007) [4].

#### Sterol and terpene detection

An aliquot of hydroethanolic crude extract is dissolved in 1 mL acetic anhydride (CH<sub>3</sub>CO<sub>2</sub>CCH<sub>3</sub>) in a test tube. Next, 0.5 ml of concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) is slowly poured over the walls of the test tube. The appearance of a violet coloration, turning blue and then green, indicates a positive reaction (Békro *et al.*, 2007) [4].

#### Detection of saponins: Foam test

A 2 g mass of ground dry plant material is boiled in 100 mL distilled water for 30 min (or 20 min after initial boiling). After cooling and filtration, the solution is made up to 100 mL with distilled water. From this stock solution, 10 tubes (1.3 cm internal diameter) are prepared with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mL; the volume in each tube is readjusted to 10 mL with distilled water. Each tube is shaken vigorously in the horizontal position for 15 sec. after 15 min rest in greenical position, the height (in cm) of the persistent foam is recorded. If it is less than 1 cm in all tubes, the foam index (Im) sought is less than 100. If it is 1 cm in one of the tubes, then the foam index is calculated by the following formula:

$$Im = \frac{1000}{N^{\circ}tube}$$

The presence of saponins in the plant is confirmed with an index greater than or equal to 100 (Dohou *et al.*, 2003) [9].

#### Phytochemical screening on TLC plates

The search for tannins, phenolic acids and alkaloids was carried out using TLC plate tests following the methods described by Mamyrbékova-Békro *et al.*, (2008) [17] and Kabran (2011) [11].

Using capillaries, 2 µL of each selective extract is deposited as a dot 0.5 cm from both edges of the chromatographic plate. The TLC plates are then placed in the migration tank containing the migration solvents (developing agents).

After development, chromatograms were visualized using visible light developers. Colorations appearing as spots are recorded and frontal ratios (Rf) calculated.

### Estimation of antioxidant power

#### DPPH tube screening of hydroethanol extracts

The method used for this test is that proposed by Popovici *et al.* (2009) [20].

In a 0.5 mL volume of extract solution, 1.5 mL of violet DPPH is added, and the positive reaction is reflected by the appearance of a yellow coloration in the medium after 15 min incubation.

#### TLC screening of selectives extracts for DPPH

The TLC screening for antioxidant capacity used is that developed by the method described by Takao *et al.*, (1994) [23].

A 10  $\mu$ L volume of each plant extract solution is deposited on a chromatoplate (silica gel 60 F254, on aluminum support (Merck)), which is then placed in a chromatography tank saturated with migration solvent. After development, chromatograms are dried and then developed with an ethanolic solution of DPPH (0.2 mg/mL). After 30 min of optimal time, extract constituents with potential free radical scavenging activity are revealed as pale-yellow spots on a violet background.

#### Assessment of antioxidant activity by spectrophotometry against DPPH of hydroethanolic crude extracts

The antioxidant potential of the extracts was assessed using the method of Blois (1958) [5].

DPPH was solubilized in absolute EtOH to obtain a solution with a concentration of 0.3 mg/mL. Different concentration ranges (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL) of the extract are prepared in absolute EtOH. 2.5 mL plant extract and 1 mL ethanolic DPPH solution are added to dry, sterile tubes. After shaking, the tubes are placed in a dark place for 30 min. The absorbance of the mixture is then measured at 517 nm against a blank consisting of 2.5 mL pure EtOH and 1 mL DPPH solution. The positive reference control is ascorbic acid (vitamin C). DPPH inhibition percentages are calculated according to the formula:

$$I(\%) = (A_b - A_e) / A_b \times 100$$

I: Inhibition percentage

$A_b$ : Absorbance of blank

$A_e$ : Absorbance of sample

The concentrations required to trap 50% ( $IC_{50}$ ) of DPPH are determined from the graphs showing the percentage inhibition of DPPH as a function of extracts or vitamin C concentrations.

#### Statistical analysis

Analyses of the measurements obtained during the various manipulations were carried out using EXCEL software. It was used to plot the various diagrams and also to determine the  $IC_{50}$  parameter for each extract.

## Results and Discussion

### Results

#### Yields

Maceration is the extraction technique chosen for this study. This technique is reputed to extract a large number of organic compounds, and was used to obtain hydro-ethanolic plant extracts from the various plant organs studied. The extraction yields obtained for these different *E. hirta* organs are summarized in Table 1.

**Table 1:** Yields of different extracts of *E. hirta*

		EHF H-Et	EHT H-Et	EHR H-Et
Masses extracted	m <sub>1</sub> (g)	2.5618	1.7225	1.5357
	m <sub>2</sub> (g)	2.816	2.0149	0.8364
	m <sub>3</sub> (g)	2.5737	2.0319	1.7649
Yields	r <sub>1</sub> (%)	25.618	17.225	15.357
	r <sub>2</sub> (%)	28.16	20.149	8.364
	r <sub>3</sub> (%)	25.737	20.319	17.649
	r <sub>moy</sub> (%)	26.51±1,10	19.23±1,34	13.79±3,62

#### Phytochemical screening

Phytochemical screening was carried out to obtain an idea of the presence of secondary metabolites in the three *E. hirta* organs studied.

#### Phytochemical screening in tube

Phytochemical screening for saponins, polyphenols, flavonoids, coumarins and sterol-terpenes was carried out using tube characterization reactions.

Saponins were screened in the decocts of the various *E. hirta* organs. The moss heights used to calculate moss indices were all below 1 cm, so the three organs contained no saponins. The presence of polyphenols was confirmed by the appearance of a blue-black or green-black coloration after the addition of a 2% (w/v) FeCl<sub>3</sub> aqueous solution. Flavonoids were characterized by the Mg test in hydrochloric acid (Shinoda test), and the pink-orange coloration observed indicated a positive reaction. The presence of coumarins was observed by the appearance of cloudiness or precipitation in the various test tubes. Finally, acetic anhydride (CH<sub>3</sub>CO<sub>3</sub>CCH<sub>3</sub>) revealed sterols and terpenes by the appearance of a violet coloration turning blue and then green (Békro *et al.*, 2007) [4]. The various results of phytochemical screening in tubes are shown in Table 2.

**Table 2:** Detection of polyphenols, flavonoids, coumarins and sterol-terpenes

	Saponins	Polyphenols	Flavonoids	Coumarins	Sterols-terpenes
EHF	-	+	+	+	+
EHT	-	+	+	+	+
HER	-	+	+	+	+

(+): présence; (-): absence

#### Phytochemical screening by TLC

This method was used in this study to search only for tannins, phenolic acids and alkaloids in the selective extracts. The colorations and frontal ratios (Rf) of the spots observed are given in Tables 3 and 4.

**Table 3:** Detection of tannins and phenolic acids in the developer CH<sub>2</sub>Cl<sub>2</sub>/A cOEt/ CH<sub>3</sub>COOH (1:4:1) (V/V/V)

Extracts	R <sub>f</sub> (Color): Possible compound
EHFH-Et	0.72 (gray): tannin; 0.42 (green): phenolic acid; 0.25 (green): phenolic acid; 0.22 (gray): tannin; 0.11 (gray): tannin; 0.00 (gray): tannin
EHTH-Et	0.91 (green): phenolic acid; 0.41 (green): phenolic acid; 0.16 (gray): tannin; 0.10 (gray): tannin; 0.00 (gray): tannin
EHRH-Et	0.10 (gray): tannin; 0.00 (gray): tannin

**Table 4:** Detection of alkaloids in the developer CH<sub>2</sub>Cl<sub>2</sub>/AcOEt/C<sub>6</sub>H<sub>14</sub>/ CH<sub>3</sub>COOH (1:2:1:0.1) (V/V/V/V)

Extracts	R <sub>f</sub> (Color): Possible compound
EHF H-Et	No alkaloid identified
EHT H-Et	No alkaloid identifie
EHR H-Et	0.9 (orange): alkaloid; 0.81 (orange): alkaloid

**Summary of phytochemical screening in tubes and by TLC**

The results of qualitative detection tests using thin-layer chromatography (TLC) and tube color reactions on secondary metabolites from *Euphorbia hirta* leaf, stem and root extracts are shown in Table 5.

**Table 5:** Summary table of phytochemical screening of secondary metabolites

	Polyph.	Flavonoids	Coumarins	Ste-ter.	Saponins	Alkaloids	Tannins	A. phe.
EHF	+	+	+	+	-	-	+	+
EHT	+	+	+	+	-	-	+	+
HER	+	+	+	+	-	+	+	-

(+); présence; (-): absence; Polyph: Polyphenols; Ste-ter.: Sterols-terpene; A. phe. Phenolic acid

**Antioxidants activities****Antioxidant activity in tube**

Après l'ajout de la solution de DPPH dans les extraits éthanoliques des feuilles, tiges et racines de *E. hirta*, une coloration yellow a été observé après quelques minutes d'incubation indiquant une activité antioxydante des trois organes étudiés.

**Antioxidant activity by TLC**

Antioxidant compounds in selective extracts of *E. hirta* leaves, stems and roots appeared as pale yellows spots on a purple background. The results of this analysis are shown in Tables 6 and 7.

**Table 6:** Phyto compounds that trap DPPH in the developer CH<sub>2</sub>Cl<sub>2</sub>/A cOEt/ CH<sub>3</sub>COOH (1:4:1) (V/V/V)

Extracts	R <sub>f</sub> (Color): Possible compound
EHF H-Et	0.92 (yellow): NI; 0.72 (yellow): tannin; 0.65 (yellow): NI; 0.49 (yellow): NI; 0.42 (yellow): phenolic acid; 0.32 (yellow): NI; 0.25 (yellow): phenolic acid; 0.22 (yellow): tannin; 0.11 (yellow): tannin; 0.06 (yellow): NI; 0.00 (yellow): tannin
EHT H-Et	0.91 (yellow): phenolic acid; 0.81 (yellow): NI; 0.69 (yellow): NI; 0.47 (yellow): NI; 0.41 (yellow): phenolic acid; 0.37 (yellow): NI; 0.16 (yellow): tannin; 0.10 (yellow): tannin; 0.06 (yellow): NI; 0.00 (yellow): tannin
EHR H-Et	0.91 (yellow): NI; 0.81 (yellow): NI; 0.69 (yellow): NI; 0.45 (yellow): NI; 0.39 (yellow): NI; 0.31 (yellow): NI; 0.25 (yellow): NI; 0.19 (yellow): NI; 0.10 (yellow): tannin; 0.06 (yellow): NI; 0.00 (yellow): tannin

NI: unidentified compound.

**Table 7:** Phyto compounds that trap DPPH in the developer CH<sub>2</sub>Cl<sub>2</sub>/AcOEt/C<sub>6</sub>H<sub>14</sub>/ CH<sub>3</sub>COOH (1:2:1:0.1) (V/V/V/V)

Extracts	R <sub>f</sub> (Color): Possible compound
EHFH-Et	0.82 (yellow): NI; 0.64 (yellow): NI; 0.46 (yellow): NI; 0.37 (yellow): NI; 0.15 (yellow): NI; 0.06 (yellow): NI
EHTH-Et	0.90 (yellow): NI; 0.57 (yellow): NI; 0.29 (yellow): NI; 0.15 (yellow): NI; 0.09 (yellow): NI; 0.04 (yellow): NI
EHRH-Et	0.9 (Yellow): alkaloid; 0.81 (yellow): alkaloid; 0.59 (yellow): NI; 0.47 (yellow): NI; 0.25 (yellow): NI; 0.17 (yellow): NI; 0.10 (yellow): NI; 0.05 (yellow): NI

NI: unidentified compound.

**Antioxidant activity by spectrophotometry**

In view of its scientific accuracy, spectrophotometric quantification of the antioxidant power of plant matrices was carried out to certify the manifestation of said activity, detected by TLC and tube color reactions.

**Inhibition percentages for various organs and vitamin C**

Figure 1 shows the different percentages of DPPH inhibition by hydroethanol extracts of *E. hirta* leaves, stems and roots, compared with vitamin C.

Organ extracts show significant antioxidant potential, irrespective of extract concentration. Inhibition percentages for leaves ranged from 49.394±0.415% to 99.148±0.371%, while those for stems and roots varied respectively from 45.985±1.988% to 96.132±0.612% and from 18.650±1.420% to 88.791±1.180%. These values are generally lower than those of the reference compound (vitamin C), whose values reach 99.705±0.000%.

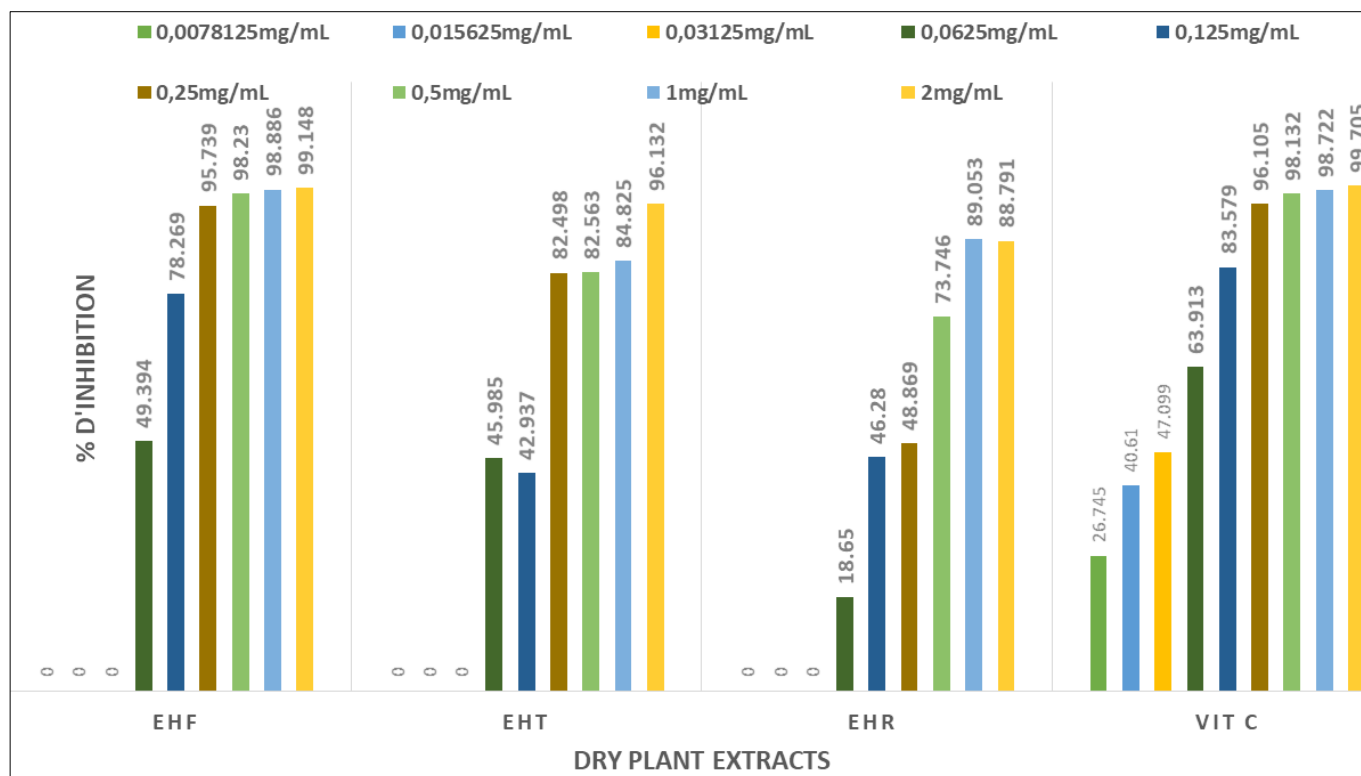


Fig 1: DPPH inhibition by hydroethanol extracts of leaves, stems and roots

#### Determination of IC<sub>50</sub> for vitamin C and extracts from three organs

The IC<sub>50</sub> of the extracts studied and of vitamin C were determined graphically using EXCELL software. Each IC<sub>50</sub> is calculated by solving the equation  $y = ax + b$  of each trend curve with:  $y = 50$ . The mean IC<sub>50</sub> values obtained are shown in Table 8.

Table 8: IC<sub>50</sub> values for extracts of leaves, stems, roots and vitamin C

	EHF	EHT	EHR	Vitamine C
IC <sub>50</sub> (mg/mL)	0.06381	0.08312	0.26137	0.03664

#### Discussion

This work is part of the phytochemical screening and evaluation of the antioxidant activity of *E. hirta* leaves, stems and roots.

In order to determine the phytochemical composition and certain biological effects of these organs, hydro-ethanol extractions were first carried out. The yields obtained differed from one organ to another. These range from  $26.51 \pm 1.10\%$  for leaves to  $13.79 \pm 3.62\%$  for roots. Stems had an intermediate yield of  $19.23 \pm 1.34\%$ . These values are relatively noteworthy, which would justify the routine use of the water/alcohol solvent mixture for extraction by various researchers.

Phytochemical screening by TLC and in tubes revealed the presence of polyphenols (flavonoids, coumarins, tannins) and sterols-terpenes in all *E. hirta* organs studied. Alkaloids are only present in the roots. On the other hand, leaves and stems contain phenolic acids, whereas roots do not. Saponins are absent from all three organs studied. Several studies carried out on *E. hirta* have shown similarities with these results. Indeed, work on the chemical composition of the plant has shown the presence of several chemical compounds such as triterpenes, tannins, polyphenols,

alkaloids, coumarins, steroids, flavonoids, tannins etc. ((Tuhin *et al.*, 2017; Lanshers *et al.*, 2005; Kumar, 2010; Bagayogo, 2020) [25, 16, 14, 2].

Evaluation of antioxidant activity by tube, TLC and spectrophotometry has shown that *E. hirta* leaves, stems and roots exhibit antioxidant activity towards the DPPH radical. This observed activity is thought to be due to the synergistic action of the various secondary metabolites detected in the three organs (Bruneton, 1999; Sivapriya & Srinivas, 2007; Kolak *et al.*, 2009) [7, 22, 13]. Indeed, these secondary metabolites contain several pharmacological properties, including antioxidant, antidiarrheal, antimicrobial, antiparasitic, anti-inflammatory, analgesic, antidiabetic, anticancer, antispasmodic, antiviral, antifungal and aphrodisiac properties (Singh *et al.*, 2004; Tona *et al.*, 2004; Kwan *et al.*, 2015; Basma *et al.*, 2011; Tuhin *et al.*, 2017) [21, 24, 15, 3, 25]. All this information could justify the traditional use of *E. hirta* in traditional medicine.

Evaluation of antioxidant activity by quantitative spectrophotometry showed that vitamin C had the best antioxidant power, with IC<sub>50</sub> (Vit. C) = 0.03664 mg/mL. However, the antioxidant activity of the three organs is not negligible compared with that of the reference molecule (vitamin C). By comparing the 50% inhibitory concentration of the hydroethanol extracts of the three organs (IC<sub>50</sub> (Leaves) < IC<sub>50</sub> (Stems) < IC<sub>50</sub> (Roots)), it is noted that the antioxidant power of the leaves is better than that of the stems, which in turn is greater than that of the roots.

#### Conclusion

This study highlighted the antioxidant power of *Euphorbia hirta* leaves, stems and roots. The antioxidant activity observed in these organs is due to the combined action of polyphenols, flavonoids, coumarins, tannins, phenolic acids, alkaloids, sterols and terpenes detected within them. Leaves with the best antioxidant power would be more effective in preventing and treating diseases linked to oxidative stress.

However, it would be wise to verify the innocuousness of these organs on the organism by a study of their toxicity, in order to be reassured of their traditional uses.

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