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Phytochemical study and antioxidant activities of leaves of *Euphorbia heterophylla* L. (Euphorbiaceae)

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Abstract: Secondary metabolites from the leaves of Euphorbia Heterophylla were extracted by maceration in an ethanol-water mixture (70/30: v/v) with a yield of 16.70%. The resulting hydroalcoholic extract was fractionated successively with hexane, dichloromethane, ethyl acetate, ethanol and water to give the different fractions with respective yields of 13.75; 10.92; 16.78; 11.38 and 45.30%. The phytochemical screening carried out on the ground material, the hydroalcoholic extract and the fractions showed the presence of sterols, terpenes, polyphenols, flavonoids, anthocyanins, tannins and saponins as well as the absence of alkaloids. The ethyl acetate fraction is the richest in total polyphenols and flavonoids with contents of 321.3333±4.6188 mg GAE/g DM and 482.6667±2.3094 mg QE/g DM, respectively. This fraction showed greater antioxidant activity by the DPPH radical scavenging test (IC50 = 134 µg/mL) than that of ascorbic acid (IC50 = 142 µg/mL) at a concentration of 1000 µg/mL. This antioxidant potential was corroborated by the ABTS+• radical-cation scavenging test with a higher TEAC value of 376.2778 ± 6.4037 µmol TE/L of extract.

1. Introduction

Plants are important source of food and active ingredients against diseases and are used in therapy by 80% of the population of developing countries (OMS, 2013). Indeed, they contain various biomolecules that can serve as models to synthesize other more active molecules (Soro *et al.*, 2012), (Alkadi, 2021). It appears necessary to know the active ingredients of medicinal plants in order to study their effectiveness, their action mode and their side effects on human health. Among the diversity of medicinal plants, *Euphorbia Heterophylla* (*E. heterophylla*) is widely used as a purgative, laxative and to expel intestinal worms (Mosango, 2008), (Falodun *et al.*, 2006). It has healing (James and Friday, 2010), antimicrobial (Meenakshi *et al.*, 2010), (Uduak and Kola, 2010), anti-inflammatory (Falodun *et al.*, 2006), antioxidant and vermifuge (Keerthana *et al.*, 2014) properties. Its latex is used in the preparation of arrow poison and fish poison (Falodun *et al.*, 2006). In Côte d'Ivoire, seeds are used to enrich the eggs of laying hens (ISA Warren) with omega-3 polyunsaturated fatty acids (Kouakou *et al.*, 2015). The decoction of the leaves is used for the treatment of indigestion and dysentery (Saraka *et al.*, 2018) and the crushed leaves are used for the treatment of malaria (Dingui *et al.*, 2021).

Preliminary phytochemical screening of the non-volatile fraction of *E. heterophylla* leaves revealed the presence of tannins, alkaloids, saponins, flavonoids, glycosides, steroids, triterpenes, coumarin, anthocyanin, anthracene derivatives and reducing sugars (Falodun *et al.*, 2006), (Nalule *et al.*, 2013). Its crude protein content varies from 16 to 27% (Bindelle *et al.*, 2007). The plant contains 7.2% vegetable oil dominated by 67.2% polyunsaturated fatty acids of which α-linolenic (C18:3 n-3) and linoleic (C18:2 n-6) acids represent 56.5 and 10.7% of total fatty acid, respectively (Kouakou *et al.*, 2019). The leaves of the plant also contain an essential oil dominated by 9,12-octadecanoic, hexadecanoic acids and methyl ester of hexadecanoic acid (Wellington *et al.*, 2019), as well as 1,8-cineole and Camphor (Elshamy *et al.*, 2019). Recently, some polyphenols have been isolated and characterized from the leaves of *E. heterophylla* (Tostes *et al.*, 2019). Despite its therapeutic potential and remarkable nutritional properties, *E. heterophylla* has been the subject of little chemical investigation. In addition, to our best knowledge, the plant acclimatized in Côte d'Ivoire has not been subjected to any chemical study. The present work therefore aims to carry out a phytochemical study of the leaves of *E. Heterophylla* from the Ivorian flora.

2. Materials and methods

2.1. plant material

The leaves of *E. Heterophylla* were collected in July 2018 in Yamoussoukro (6°53'04.7" North and 5°13'54.9" West) in the centre of Côte d'Ivoire, in the rainy season. The plant was identified by Mr. Amani N'GUESSAN, botanist at the National Polytechnic Institute Felix HOUPHOUET-BOIGNY (INP-HB) of Yamoussoukro. The leaves were dried in the shade at room temperature (28 ± 2°C) for 21 days and then crushed. The resulting powders were sieved through a 0.4 mm mesh sieve and conserved at 4°C in a hermetically closed bottle until further use.

2.2. Methods

2.2.1. Extraction of plant material

The hydroalcoholic extract (E_{HA}) was prepared according to the method described by Kassi et *al.* (Kassi *et al.*, 2014). A mass of 100g of crushed sample was macerated in 1 L of an ethanol/water mixture (70/30: v/v) with magnetic stirring for 24 hours. After settling, the mixture was successively filtered on hydrophilic cotton and Wattman N° 2 paper. The operation was repeated three (3) times until the crushed material was exhausted. The filtrate obtained was concentrated under reduced pressure at a temperature of 40°C using a rotary evaporator of BUCHI 461 type and then freeze-dried to give the hydroalcoholic extract (E_{HA}). The extraction was carried out in triplicate.

2.2.2. Fractionation of the hydroalcoholic extract

The obtained hydroalcoholic extract (E_{HA}) was fractionated successively with solvents of increasing polarity (hexane, dichloromethane, ethyl acetate, ethanol and water) according to the method described by Bouamama et *al.* (Bouamama *et al.*, 2006). The hydroalcoholic extract (10 g) was dissolved in water (100 mL) and fractionated successively with hexane (3x100 mL), dichloromethane (3x100 mL) and ethyl acetate (3x 100 mL). The resulting aqueous phase was dried and then extracted with ethanol (3x 100 mL). The resulting solid residue constitutes the aqueous extract. After filtration and removal of the solvents, the hexane (F_{HEX}: 1.375 g), dichloromethane (F_{DCM}: 1.092 g), ethyl acetate (F_{AE}: 1.678 g), ethanol (F_{ETH}: 1.138 g) and aqueous (F_{Aq}: 4.530 g) fractions were obtained. The fractionation was carried out in triplicate.

2.2.3. Screening of secondary metabolite families

The screening method used is that described by Harbone and based on precipitation and/or staining reactions (Harbone, 1998).

2.2.4. Total polyphenols and flavonoids Assays

The total polyphenols were assayed according to the method described by Wood et al. (Wood *et al.*, 2002). To 30 µL of extract were added 2.5 mL of Folin-Ciocalteu's reagent diluted to 1/10th. The mixture obtained was maintained for 2 min in the dark at room temperature (28±2°C) then 2 mL of sodium carbonate solution at 75 $g.L^{-1}$ were added to it. The resulting solution was then incubated at 50°C for 15 minutes. The absorbance was read using a UV-visible spectrophotometer at a wavelength of 760 nm against a blank prepared in the same way except that it contains distilled water instead of the test substance. Gallic acid was used as a standard reference for the quantification of total polyphenol content expressed in mg of gallic acid equivalent per gram of dry extract or matter (DM) (mg GAE/g DM). The tests were carried out in triplicate.

The total flavonoid content was determined according to the method described by Marinova et al. (Marinova *et al.*, 2005). Volumes of 0.75 mL of 5% sodium nitrite solution and 0.75 mL of 10% aluminum chloride solution were added to 2.5 mL of extract in ratio of 1/500 (m/V). After 5 minutes of incubation, the mixture was brought into contact with 5 mL of a 1 M sodium hydroxide solution. The volume obtained was adjusted to 25 mL with distilled water then stirred vigorously. Absorbance was measured at the wavelength of 510 nm. Quercetin was used as a standard reference for the quantification of total flavonoid contents expressed in milligrams of quercetin equivalent per gram of dry extract (mg QE/g DM). The tests were carried out in triplicate.

2.2.5. Antioxidant Assays of the extract and fractions

Oxidation processes are complex because the various antioxidant molecules can be hydrophilic or hydrophobic. There is therefore no universal method for measuring antioxidant activity quantitatively in a very precise manner. Thus, the combination of responses from different and complementary tests provides an indication of the antioxidant capacity of the sample to be tested (Tabart *et al.*, 2009), (Mezni *et al.*, 2022). In this study, we therefore evaluated the antioxidant potential of the hydroalcoholic extract and its fractions by the methods of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS).

2.2.5.1. 2,2-Diphenyl-1-picryl hydrazyl free radical scavenging test

This method is based on the ability of antioxidants to scavenge the DPPH radical. The latter is reduced to hydrazine form (non-radical) by accepting a hydrogen atom. The effect of each extract on DPPH was measured according to the protocol described by Sanchez-Moreno et al. (Sanchez Moreno *et al.*, 1998). Ascorbic acid was used as the standard reference antioxidant. Volumes of 5 mL at different concentrations (50, 100, 250, 500 and 1000 µg/mL) of each extract and fraction were prepared. A volume of 50 µL of the different concentrations of each extract or fraction was added to 1.950 mL of the freshly prepared methanol solution of DPPH (0.025 mg/mL). After 30 minutes of incubation, the absorbances were measured at 517 nm using a spectrophotometer and the percentages inhibition were calculated from **Eqn. 1**.

$$PI\ (\%) = \left(\frac{\mathrm{Ao} - \mathrm{A}}{\mathrm{Ao}}\right) * 100 \qquad \textbf{Eqn. 1}$$

PI: Percentage inhibition; A: Absorbance of diluted DPPH containing the samples to be tested; A0: Absorbance of diluted DPPH (control absorbance)

The 50% inhibition concentration (IC_{50}) was determined by studying the variation in percent inhibition as a function of concentration. A lower IC_{50} value leads to a greater antioxidant activity of the extract or fraction. The tests were carried out in triplicate.

2.2.5.2. 2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical–cation scavenging test

This test is carried out according to the method described by Miller et al. (Miller *et al.*, 1993). It is based on the ability of compounds to reduce the $ABTS^{+\bullet}$ radical-cation. The solution of the cationic radical $ABTS^{+\bullet}$ was obtained by mixing an 8 mM solution of ABTS (87.7 mg in 20 mL of distilled water) and a 3 M solution of potassium persulfate (0.0162 g in 20 mL distilled water) in a 1:1 (v/v) ratio. The reaction mixture was then incubated in the dark at room temperature (28±2°C) for 16 hours. Then, the $ABTS^{+\bullet}$ solution obtained was diluted in absolute methanol to afford a solution with an absorbance of 0.7±0.02 at 734 nm. In addition, 3.9 mL of the diluted $ABTS^{+\bullet}$ solution was added to 100 µL of the extract or fraction to be tested. After stirring, the resulting mixture was incubated for 6 minutes in the dark. The residual absorbance of the $ABTS^{+\bullet}$ radical was measured at 734 nm using a UV-visible spectrophotometer and should represent between 20 and 80% of the absorbance of the blank. The tests were carried out in triplicate and the results were expressed in µmol Trolox equivalent per liter of extract or fraction (µmol TE/L of extract).

The activity of the extract or fraction is expressed by the Trolox Equivalent Antioxidant Capacity (TEAC) which corresponds to the concentration of Trolox (reference antioxidant) inducing the same antioxidant capacity as a concentration of 1 mM of the extract or fraction tested. The spectrophotometer was calibrated by measuring the absorbance of different concentrations of Trolox solution (3.75; 5; 6.25; 10; 11.25; 13.75 and 15.10^{-4} mM) and the percentage inhibition PI (%) of $ABTS^{+\bullet}$ was determined by **Eqn. 2**.

$$PI\ (\%) = \left(\frac{\mathrm{Ac} - \mathrm{A}}{\mathrm{Ac}}\right) * 100 \qquad \textbf{Eqn. 2}$$

PI: Percentage inhibition
A: Absorbance of diluted ABTS containing the samples to be tested
A0: Absorbance of diluted ABTS (control absorbance)
The antioxidant activity was expressed by **Eqn. 3**.

$$C = \frac{\mathrm{PI\ x\ D}}{4{,}9901} \qquad \textbf{Eqn. 3}$$

C: Trolox Equivalent Antioxidant Capacity (TEAC) in µmol TE/L of extract and D: Dilution factor

3. Results and discussion

3.1. Extraction and fractionation yields

Yields of hydroalcoholic extract (E_{HA}) of *E. heterophylla* leaf powders and the hexane (F_{HEX}), dichloromethane (F_{DCM}), ethyl acetate (F_{AE}), ethanol (F_{ETH}) and aqueous (F_{AQ}) fractions from this extract are given in **Figure 1**. Hydroalcoholic extract was obtained with a yield of 16.70%. This yield is higher than those of the ethanolic and aqueous extracts reported in the literature and which are 11 and 15.8%, respectively (Amit Joshi, 2019). Fraction yields vary from 10.92% for dichloromethane fraction to 45.30% for the aqueous fraction. The results obtained show that the yields of the fractions depend on the solvents used. The best yields were obtained with water and ethyl acetate. These results are similar to those of Soumahoro et al. when extracting secondary metabolites from the leaves of *Hyptis Suaveolens* (Soumahoro *et al.*, 2020).

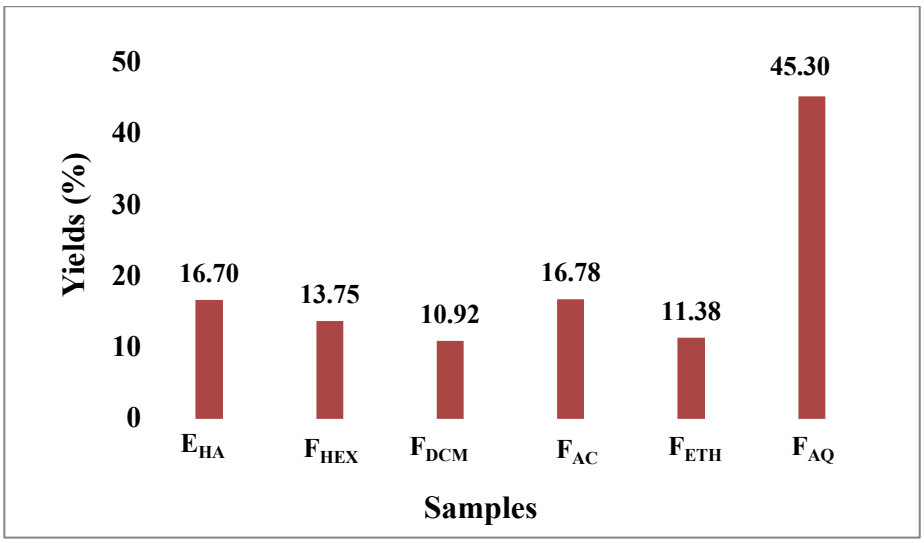

Figure 1. Yields of the hydroalcoholic extract and fractions of *E. Heterophylla* leaves

The leaves of *E. heterophylla* are therefore rich in polar molecules which could be bound to carbohydrates as reported in the literature (Amit Joshi, 2019), (Bonnaillie *et al.*, 2012). Indeed, the nine (9) polyphenols recently isolated from *E. Heterophylla* leaves collected in Brazil were all glycosylated (Tostes *et al.*, 2019). The yield of the hydroalcoholic extract is lower than that obtained for *Alchornea cordifolia* and *Ricinus communis* Linn, two plants from the same family as *E. Heterophylla* (Saraka *et al.*, 2018). Thus, the extraction yields depend on several factors such as nature of plant species, crushed material particles size, temperature, extraction time, agitation degree and method used (Saraka *et al.*, 2018), (Perva Uzunalic *et al.*, 2006).

3.2. Phytochemical screening

The results of phytochemical screening carried out on ground material, hydroalcoholic extract (E_{HA}) as well as on hexane (F_{HEX}), dichloromethane (F_{DCM}), ethyl acetate (F_{AE}), ethanol (F_{ETH}) and aqueous (F_{AQ}) fractions from *E. heterophylla* leaves are given in **Table 1**.

Table 1. Phytochemical constituents of ground material, hydroalcoholic extract and various fractions of *E. heterophylla* leaves

Secondary metabolites Families	Ground material	Extract and fractions					
		E_{HA}	F_{HEX}	F_{DCM}	F_{ACE}	F_{ETH}	F_{AQ}
Sterols and terpenes	+	+	+	+	-	-	-
Polyphenols	+	+	-	+	+	+	+
Flavonoids	+	+	-	+	+	+	+
Anthocyanins	+	+	-	-	+	+	+
Tannins	+	+	-	-	+	+	+
Alkaloids	-	-	-	-	-	-	-
Saponins	+	+	-	-	-	-	+

Presence: +; Absence : -

Phytochemical screening showed that crushed leaves of *E. Heterophylla* contain all the desired secondary metabolites families except alkaloids. The absence of alkaloids in the extract and fractions

has also been reported in the literature (Okeniyi *et al.*, 2012), (Falodun *et al.*, 2008). However, this family is present in the leaves of the plant collected in Uganda [13] (Nalule *et al.*, 2013), Himalayas (Amit Joshi, 2019), Nigeria (Wellington *et al.*, 2019) and Pakistan (Abbassi, 2013), respectively. Our results differ slightly from those of Edoaga et al., who noted the absence saponin and sterols in the plant collected in Nigeria (Edeoga *et al.*, 2005). These results show that we are in the presence of a new chemical race of *E. Heterophylla*.

Hydroalcoholic extract contains all the secondary metabolites families present in the crushed leaves. This result confirms the ability of hydroalcoholic solvent to extract almost all the secondary metabolites present in plant organs (Perva Uzunalic *et al.*, 2006). Secondary metabolites families are variously distributed in the fractions according to the solvent used. The hexane fraction (F_{HEX}) contains only sterols and terpenes while the ethyl acetate, ethanol and aqueous fractions contain polyphenols, flavonoids, anthocyanins and tannins. The absence of all the metabolites families sought in the hexane fraction except sterols and terpenes has also been reported for *E. heterophylla* collected in Pakistan (Abbassi, 2013). However, the alkaloids family is present in the hexane fraction of leaves harvested in Himalayas (Amit Joshi, 2019). Saponins are present only in the aqueous fraction which also contains the greatest number of secondary metabolites, which could justify the use of aqueous decoction in traditional medicine (Saraka *et al.*, 2018). The richness of *E. heterophylla* leaves in secondary metabolites could be the source of its many biological, pharmacological and therapeutic properties reported in the literature (Wellington *et al.*, 2019), (Elshamy *et al.*, 2019).

3.3. Total Phenolic Contents

Total phenolic assays were performed from the gallic acid calibration line (**Figure 2**). The results obtained are shown in **Figure 3**. Total phenolic content of the hydroalcoholic extract is 134.0000±3.4641 mg GAE/g DM. Total phenolic contents vary according to the fractions used. This result is similar to that obtained when determining the total phenolic content of *Anagyris foetida* (Mezni *et al.*, 2022). The highest content (321.3333 mg GAE/g DM) was obtained with the ethyl acetate fraction followed by the aqueous fraction (108 mg GAE/g DM). These two polar solvents are therefore the best solvents for extracting total polyphenols.

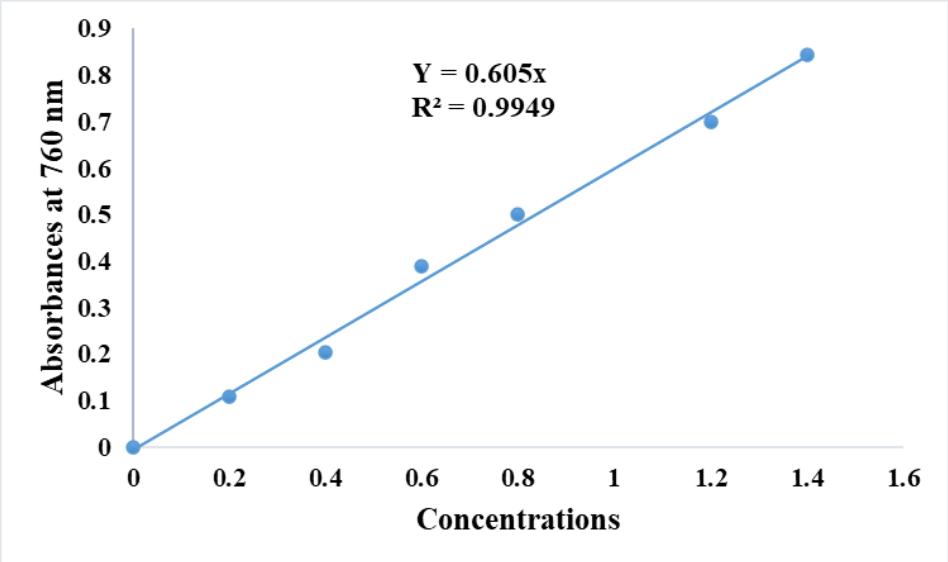

Figure 2. Calibration curve of gallic acid for total phenolic assay

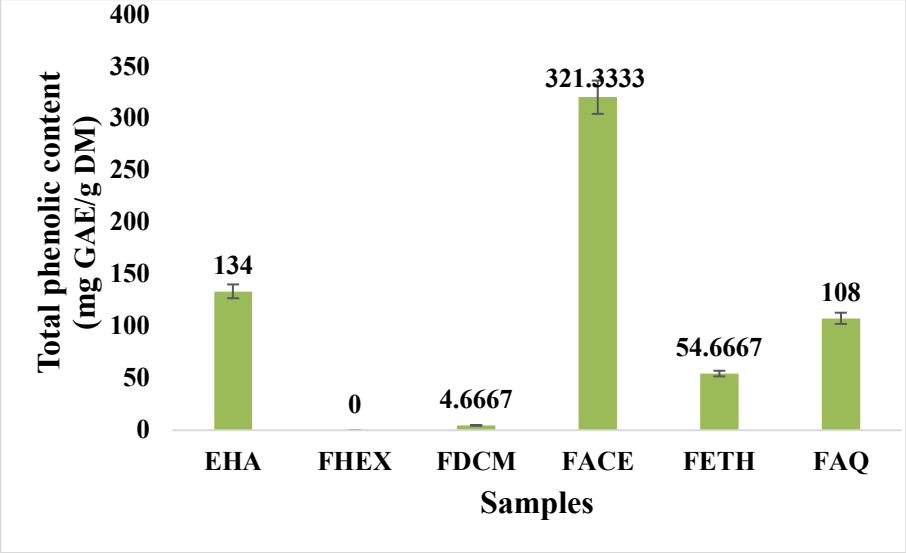

Figure 3. Total phenolic contents of *E. heterophylla* leaves

The abundance of polyphenols in the ethyl acetate fraction has also been reported in the literature (Soumahoro *et al.*, 2020), (Abbassi, 2013), (Kouamé *et al.*, 2021). However, our total polyphenol content in this solvent is much higher than those obtained by Abbasi et al. (190.1± 1.21 mg GAE/g DM) on *E. heterophylla* collected in Pakistan (Abbassi, 2013). This difference could be due to the extraction method, geographical and climatic factors, genetic factors, plant maturation and storage time of the plant material which have a strong influence on the total polyphenol content (Kouamé *et al.*, 2021), (Fiorucci, 2006), (Pedneault *et al.*, 2001).

The dichloromethane fraction contains a small amount of polyphenols, whereas the latter are totally absent from the hexane fraction. This result agrees with the phytochemical screening (**Table 1**). However, polyphenols were obtained in the hexane fraction of the plant harvested in Pakistan (Abbassi, 2013). The presence of polyphenols in the leaves of *E. heterophylla* could justify their use in traditional medicine in Nigeria (Ashidi *et al.*, 2010) and Côte d'Ivoire (Saraka *et al.*, 2018).

3.4. Total flavonoids contents

Total flavonoids assays were performed from the gallic acid calibration line (**Figure 4**). The results obtained are shown in **Figure 5**.

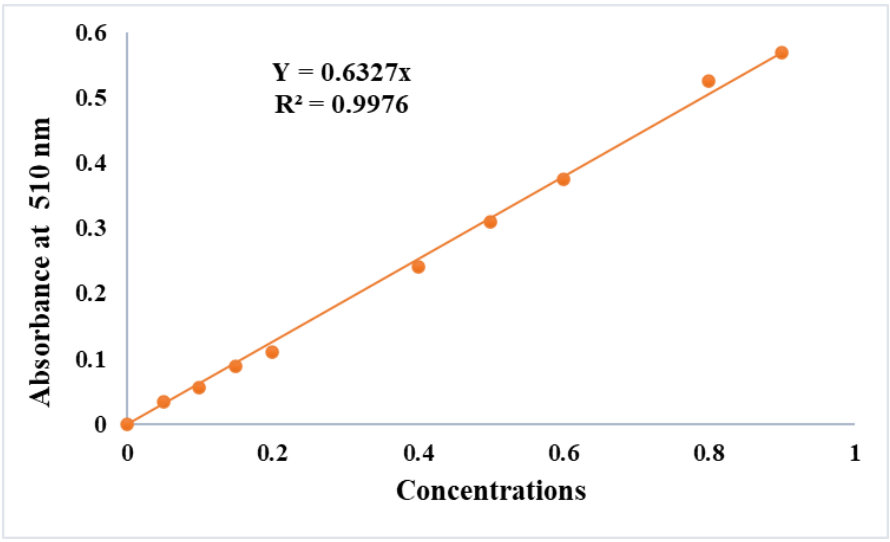

Figure 4. Quercetin calibration curve for total flavonoids assay

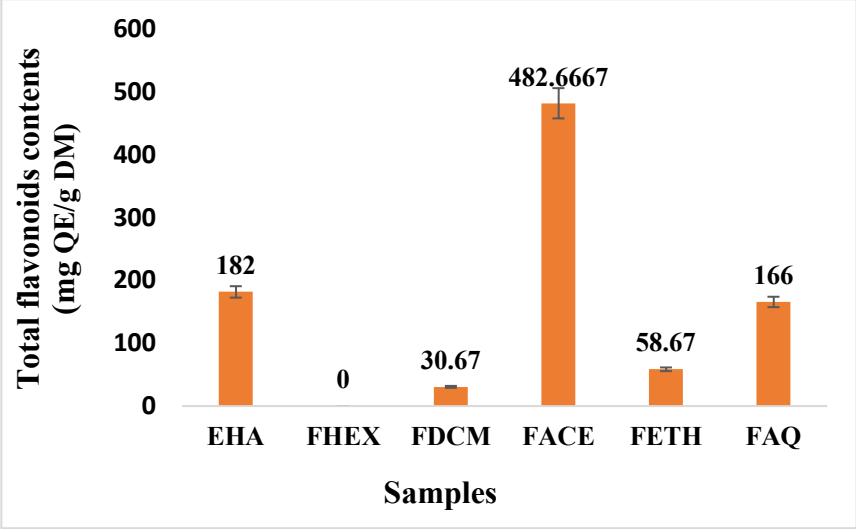

Figure 5. Total flavonoids contents of *E. heterophylla* leaves

Hydroalcoholic extract has a total flavonoid content of 182.0000 ± 9.1652 mg EQ/g DM. As for total polyphenols, the total flavonoids contents vary according to the solvents used. Ethyl acetate fraction was the richest in total flavonoids followed by aqueous fraction with contents of 482.6667±2.3094 and 166±9.1651 mg EQ/g DM, respectively. Total flavonoids content of our hydroalcoholic extract is about 2.6 times higher than that of the aqueous extract of the whole *Euphorbia hirta* plant harvested in Malaysia (Abu Bakar *et al.*, 2020). The abundance of flavonoids in ethyl acetate fraction has also been reported in the literature (Soumahoro *et al.*, 2020), (Kouamé *et al.*, 2021). The hexane fraction did not contain flavonoids as revealed by phytochemical screening of this fraction (**Table 1**).

3.5. Antioxidant Assays

3.5.1. 2,2-Diphenyl-1-picryl hydrazyl free radical scavenging test

The results of antioxidant assays of hydroalcoholic extract and its fractions obtained during the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging test are recorded in **Table 2**.

Table 2. Percentage inhibition (PI) and 50% inhibition concentration (IC_{50}) of the samples according to the concentrations

Extract and Fractions	Percentage inhibition (PI) (%)					IC_{50} (µg/mL)
	50 µg/mL	100 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL	
E_{HA}	5.909±0.104	14.903±0.714	32.452±0.168	48.318±0.248	**64.387±0.225**	**545**
F_{HEX}	1.691±0.100	2.965±0.100	5.786±0.569	8.554±0.609	12.125±0.840	**……**
F_{DCM}	2.523±0 .483	4.835±0.512	8.768±0.192	12.873±0.197	17.509±0.401	**……**
F_{ACE}	28.495±0.360	38.786±0.350	80.974±0.318	87.232±0.218	**94.627±0.533**	**134**
F_{ETH}	4.672±0.497	7.356±0.350	13.234±0.212	18.875±0.326	24.627±0.446	**……**
F_{AQ}	6.203±0.206	10.846±0.633	28.326±0.350	42.682±0.876	**55.247±0.368**	**770**
AA	17.465±0.655	29.564±0.689	89.906±0.483	95.058±0.182	**96.451±0.713**	**142**

AA: Ascorbic acid

Antioxidant activity of the hydroalcoholic extract, fractions and ascorbic acid (AA) was measured at different concentrations (50, 100, 250, 500 and 1000 µg/mL). The results obtained show that the percentages inhibition of DPPH radical, hydroalcoholic extract and its fractions increase with concentration, the best values being obtained at the concentration of 1000 µg/mL. Similar results have been reported in the literature (Amit Joshi, 2019). Generally, ascorbic acid (AA) has the highest percentage inhibition followed by ethyl acetate fraction (F_{ACE}), hydroalcoholic extract (E_{HA}) and aqueous fraction (F_{AQ}). However, at concentrations of 50 and 100 µg/mL, ethyl acetate fraction exhibits the highest inhibition percentages with values of 28.495 ± 0.360 and 38.786 ± 0.350%, respectively. Ethyl acetate fraction is the most active of the fractions. These results agree with those of Abbasi et al. (Abbassi, 2013). However, ethyl acetate fraction of these authors gave a higher percentage inhibition than ours with a value of 80.09 ± 0.87% at a concentration of 120 µg/mL.

Hexane fraction has the lowest inhibition percentage at any concentration considered. These results agree with those of total phenolic and flavonoids contents which are generally responsible for the antioxidant activity of plant secondary metabolites (Soumahoro *et al.*, 2020), (Kouamé *et al.*, 2021), (Pourmorad *et al.*, 2006). The low DPPH radical scavenging activity of ethanolic fraction could be justified by the presence of compounds which have absorption bands at the same wavelength as the DPPH radical, thus leading to an increase in the optical density and a decrease the percentage inhibition of the radical (Sarr *et al.*, 2015).

To better appreciate the antioxidant activity of hydroalcoholic extract and its fractions, their concentrations corresponding to 50% inhibition (IC_{50}) were determined from the graphs PI (%) = f (concentration). The results in **Table 2** show that ethyl acetate fraction has the best antioxidant activity with an IC_{50} value of 134 µg/mL slightly higher than that of ascorbic acid (142 µg/mL) used as standard reference. These results agree with those of Abassi et al. (Abbassi, 2013) and they reveal the important role of polyphenols and flavonoids in antioxidant activity of plant secondary metabolites. Indeed, several studies have found a good correlation between total phenolic or flavonoids contents and antioxidant activities (Alkadi, 2021), (Lanhers *et al.*, 2005), (Albano and Miguel, 2011). The IC_{50} of the hexane, dichloromethane and ethanol fractions could not be quantified because at the concentrations tested, they do not capture more than 30% of DPPH. Ethyl acetate fraction of the leaves of *E. Heterophylla* from Pakistan, with an IC_{50} value of 36.85 ± 1.8 µg/mL (Abbassi, 2013), is more active than ours. This difference in antioxidant activity could be due to several factors such as the different experimental conditions as well as the climatic and edaphic conditions of the different harvesting localities (Mezni *et al.*, 2022).

3.5.1. 2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical–cation scavenging test

Antioxidant activity of hydroalcoholic extract and its fractions by the $ABTS^{+\bullet}$ radical cation trapping test was carried out from the calibration line of Trolox (**Figure 6**). The results obtained are presented in **Figure 7**. The Trolox Equivalent Antioxidant Capacity (TEAC) of hydroalcoholic extract of *E. heterophylla* leaves is 132.2017 ± 4.6002 µmol TE/L of extract. Ethyl acetate fraction has the highest TEAC (376.2778 ± 6.4037 µmol TE/L of extract) followed by ethanol (186.8174 ± 7.2643 µmol TE/L of extract) and aqueous (161.0543 ± 8.0438 µmol TE/L of extract) fractions. These results show that ethyl acetate fraction had, as for the DPPH test, a greater antioxidant activity for the ABTS test. The hexane and dichloromethane fractions showed low antioxidant activity (more than 11 times lower than the other fractions). Ethyl acetate fraction was also the most active in the $ABTS^{+\bullet}$ radical cation scavenging test performed on the leaves of several plants (Sarr *et al.*, 2015), (Muhammad *et al.*, 2017).

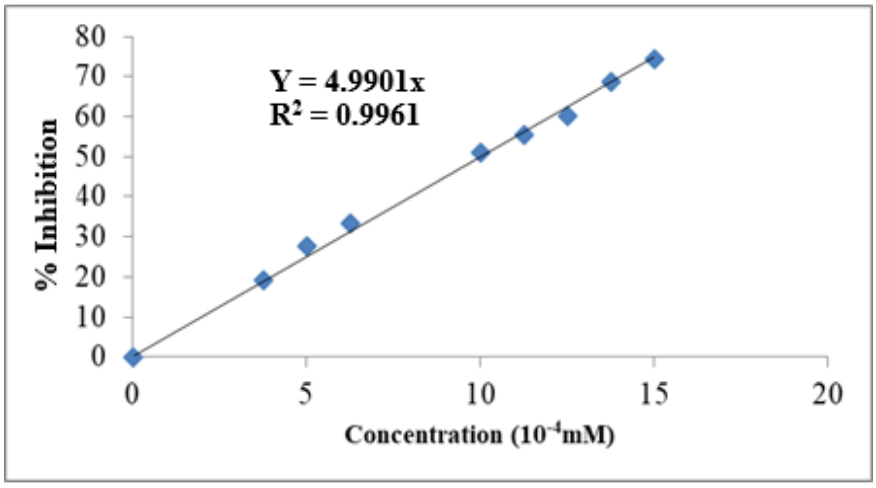

Figure 6. Trolox calibration curve for antioxidant activity assay

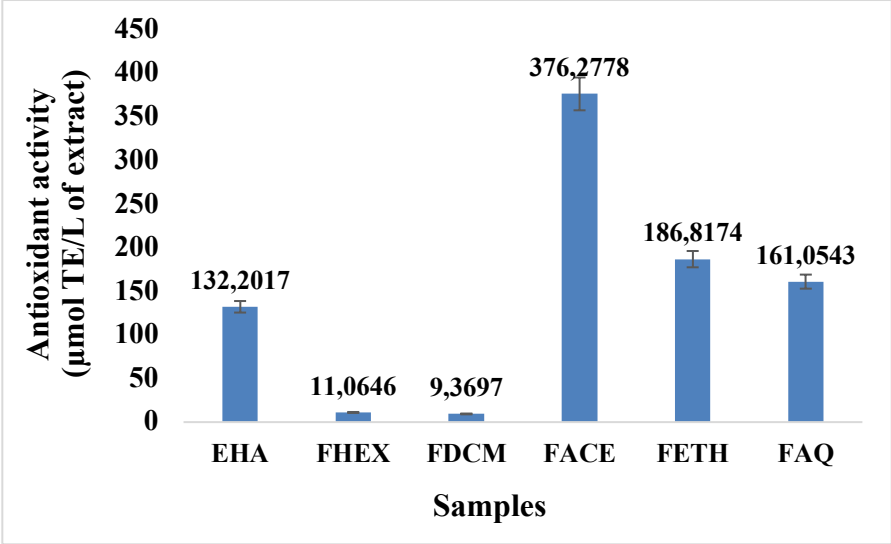

Figure 7. Antioxidant activity of extract and fractions by ABTS test

Conclusion

The leaves of *Euphorbia Heterophylla* contain almost all the secondary metabolites sought in our study except alkaloids. Total phenolic and flavonoids contents are higher in ethyl acetate fraction, which therefore shows greater antioxidant activity compared to that of the standards used. *Euphorbia Heterophylla* leaves could therefore be considered as a potential source of natural antioxidants that are beneficial to human health. Purification and elucidation of the structures of some secondary metabolites are in progress.

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