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Comparative study of *Hyptis suaveolens* leaves antioxidant activity before and after essential oil extraction

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Abstract
Context: *Hyptis suaveolens* (L.) Poit; (Lamiaceae) is an aromatic plant whose leaves contain essential oils (less than 1%) and also non-volatile bio-active substances (More than 8%). However, after essential oil extraction, the leaf residues are generally poorly exploited. Which poses an efficient and optimal management problem for this natural resource. The valuation of these residues as substitute antioxidants is considerable.
Methodology: After hydroethanolic (70/30; v/v) maceration of 100g of powder front and after extracting essential oil taken separately, a successively fractionate extract was obtained from solvents of increasing polarities. Next, the total polyphenols, flavonoids and tannins are determined. Finally, the antioxidant activities of extracts and fractions are evaluated by trapping the DPPH radical and then that of the ABTS radical.
Results: The contents of total polyphenols and tannins decreased from 39.167±2.222 to 20.833±1.111 mg EAG/g and 8.733±0.156 to 6.500±0.267 respectively during essential oil extracting process. Still, the proportioning of total flavonoids gave more significant concentrations afterwards with 20.583±0.389 mg EQ/g against 15.417±0.222 mg EQ/g before. The antioxidant activity increased after extracting essential oil with TEAC value of 256.47 µm ol/L TE to 290.91 µm ol/L TE for ABTS method and IC50 value of 0.28mg / mL to 0.27 mg/mL for DPPH. Comparatively to ascorbic acid (IC50 = 0.05 mg/mL), *Hyptis suaveolens* leaves have moderate antioxidant activity.
Conclusion: *Hyptis suaveolens* leaves have moderate antioxidant activity before and after essential oil extraction. The plant hydrodistillation residue could be used as a substitute antioxidant.
Prospect: The determination of the molecular structures could increase the interest for these residues.

Keywords: *Hyptis suaveolens*, after essential oil extraction, antioxidant activity

1. Introduction
Hyptis suaveolens is a plant used in over twenty-two countries for these medicinal properties [3]. All parts of the plant are used in traditional medicine in the treatment of many ailments such as respiratory ailments, gastrointestinal infections, antirheumatic, antisuporifics, colic, colds, indigestion, fever, abdominal pain, burns, wounds, cramps and multiple skin complications [4-7]. However, the leaves are the most used, followed by the seeds and the whole plant [3].
The development of medicinal practices in recent decades especially that of aromatherapy has generated an increasingly strong demand for essential oils. In doing so, we are witnessing a resurgence of problems of adequate management of residues because essential oil represents only about 5% of the vegetable raw material. This is why a study on the exploitability of these residues is current. Thus, certain authors such as Moure *et al*., Have proposed various ways of upgrading these by-products as sources of phenolic compounds, fibers and proteins [8]. Other authors believe that these by-products are potential sources of mineral elements and bioactive compounds with antioxidant properties [9, 10, 11]. As a result, interest in natural antioxidants is growing and growing every year [12]. These products constitute a promising economic prospect in the formulation of functional foods because antioxidants are of great benefit to human health [13]. The exploitation of these residues also offers a real opportunity in the perspective of the sustainable and efficient management of raw materials [14]. It is in this context that our laboratory has been developing a program for the recovery of these residues for several years.

Thus, in recent studies, we have shown on the one hand that the hydroethanolic extracts of the leaves of *Hyptis suaveolens* after extraction of the essential oil have a more pronounced antimicrobial activity than the hydroethanolic extracts of the leaves before extraction of the essential oil [15]. On the other hand, it has been shown that the accentuation of the antimicrobial activity after extraction of the essential oil would be due to the facilitation of the extraction of secondary metabolites and especially of certain phenolic compounds such as flavonoids after the departure. Essential oils [16]. These results could suggest an increase in the antioxidant activity of the leaves of the plant in view of the increase in the extractable quantities of polyphenols and total flavonoids known for their antioxidant activities.

The present study aims to contribute to the valorisation of hydrodistillation residues from *Hyptis suaveolens* leaves as a substitute antioxidant.

2. Materials and Methods

2.1. Plant material

The leaves of *Hyptis suaveolens* were collected in July 2017 in Yamoussoukro (6047'18.762" North and 5015'25.9992" West) in the center of Côte d'Ivoire and identified by Mr. Amani N'Guessan, botanist at the National Polytechnic Institute Félix HOUPHOUËT-BOIGNY (INP-HB) from Yamoussoukro. A specimen of *Hyptis suaveolens* is listed in the CSRS herbarium under the number: Coll n °C: 18027/bdcsrs: 65599. The leaves were divided into two (2) lots. The 1st batch dried directly in the shade at room temperature in the laboratory (26 to 30 °C) for 7 days and the 2nd batch also dried under the same conditions after extraction of essential oil by hydrodistillation. The various dry leaves of the 1st and 2nd batches were then ground using an electric grinder of the IKA M20 brand. The various ground materials obtained were sieved using a sieve of 0.5 mm mesh. The various powders obtained were stored at 4 °C. until their subsequent use.

2.1.1. Preparation of total hydroalcoholic extracts

The total hydroalcoholic extracts were prepared according to the method described by Yapi Yayé *et al*. [17]. A mass of 100 g of ground material from each sample was macerated in 1 L of an ethanol/water mixture (70/30: v/v) under a magnetic stirrer for 24 hours. After decantation, the mixture was successively filtered through cotton wool and Watman No. 2 paper. The operation was repeated three (3) times on the same ground material. The filtrate obtained was concentrated at reduced pressure at a temperature of 40 °C. using a rotary evaporator of the BUCHI 461 type and then lyophilized to give the total hydroalcoholic extracts before (EHA1) and after (EHA2) extraction of the essential oil.

2.1.2. Fractionation of the various total hydroalcoholic extracts

The various total hydroalcoholic extracts (Before and after) obtained were fractionated successively from solvents of increasing polarities (hexane, dichloromethane, ethyl acetate, ethanol and water) according to the method reported by Bouamama *et al*. [18]. 10 g of each hydroalcoholic extracts (EHA1 and EHA2) were dissolved in 100 mL of water and partitioned successively with hexane (3x 100mL), dichloromethane (3x 100 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. The different organic phases obtained were separately dried over anhydrous sodium sulfate. After filtration and removal of the solvents under reduced pressure, the fractions with hexane (FHEX: 1.632 g and 1.232 g), with dichloromethane (FDCM: 1.823 g and 1.997 g), with ethyl acetate (FAE: 2.079 g) and 2.573 g), with ethanol (FEth: 0.961 g and 1.236 g) and water (FAq: 3.404 g and 2.953 g) respectively before and after extraction of the essential oil were obtained.

2.2 Polyphenol assays

The determination of the total polyphenols was carried out according to the method described by Wood *et al*. [19]. To a volume of 30 µL of extract, 2.5 mL of Folin-Ciocalteu reagent diluted to 1/10. The resulting mixture was kept for 2 min in the dark at room temperature (27±03 °C) then 2 mL of sodium carbonate (75 gL-1) was added thereto. The resulting solution was then incubated at 50 °C for 15 min. The absorbance reading was carried out with a UV-visible spectrophotometer at a wavelength of 760 nm against a blank consisting of 5 mL of Folin-Ciocalteu reagent diluted to 1/10 and 4 mL of sodium carbonate (75 gL-1). Gallic acid was used as a reference standard for quantifying the total polyphenol contents expressed in mg of gallic acid equivalent per gram of extract (mg EAG / g of extract).

2.3 Flavonoids assays

The content of total flavonoids was determined according to the method described by Marinova *et al*. [20]. Volumes of 0.75 mL of 5% (w/v) sodium nitrite and 0.75 mL of 10% (w/v) Aluminum chloride were added to 2.5 mL of extract. ratio 1/500 (m/V). After 5 min 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 and then stirred vigorously. Absorbance was measured at a wavelength of 510 nm. Quercetin was used as a reference standard for quantifying the total flavonoid contents expressed in milligrams of quercetin equivalent per gram of extract (mg EQ.g-1 of extract).

2.4 Dosage of total tannins

The tannin content was determined by the vanillin method described by Julkunen-Titto [21]. A volume of 100 µL of each extract was added to 3000 µL of the 4% vanillin/methanol solution, and then mixed vigorously. Then, a volume of 1500 µL of concentrated hydrochloric acid (HCl) was added. The mixture obtained is left to react at room temperature for 20 min. Absorbance is measured at 550 nm against a blank. Different concentrations between 0 and 0.45 µg/ml prepared from a stock solution of tannic acid used as standard, will make it possible to draw the calibration curve. The results are expressed in mg tannic acid equivalent per gram of dry extract (mg EAT/g of dry extract). The contents are calculated from the following formula:

$$C = Ci^* D * 10/m$$

C = mg tannic acid equivalent in 1000 mg of dry extract
Ci = concentration of the sample read (µg/mL),
D = dilution, m = mass of sample (mg).

2.5 Evaluation of antioxidant activity

There are several methods of evaluating the antioxidant activity among which one has the reduction of the cationic radical ABTS. + And the trapping of the DPPH radical. These are methods that have advantages, such as their simplicity and

their widespread use for the rapid presentation of results in the analysis of the antioxidant properties of plants. They also make it possible to provide reliable preliminary information on the presence of the active antioxidant compounds in the extracts.

2.5.1 Trapping of the DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical

The antioxidant capacity achieved on plant extracts was performed based on the degradation of the DPPH radical [22]. The effectiveness of an antioxidant is measured by measuring the decrease in violet colouration, due to the recombination of the DPPH • radicals, which can be measured by UV spectrophotometry. A 6.34 x 10-5 M DPPH solution prepared from 2.5 mg of DPPH dissolved in 100 mL of absolute methanol was protected from light and stored at low temperatures. Vitamin C was used as a positive control. A range of concentrations (500; 300; 100; 50 and 25 µg / mL) of plant extracts or vitamin C (standard antioxidant) was prepared in methanol. Then, 50 µL of each solution was added to 1.95 ml of DPPH solution. After homogenization, the mixture was incubated at laboratory temperature in the dark. After 30 min of incubation, absorbances were measured at 517 nm against a "blank" that contains only methanol. The experiment was repeated three times. The percentage inhibition of the DPPH radical was calculated according to the following equation:

% I = (A0-Ae) * 100/A0

A0 = Absorbance of DPPH without extract at t = 0
Ae = Absorbance of residual DPPH (Extract + DPPH)
The concentration of plant extract responsible for 50% inhibition of the DPPH radical (IC50) was determined from the standard curve of ascorbic acid.

2.5.2 Réduction of the radical-cation of 2,2-azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS + •)

According to Miller *et al*. (1993) [23], the antioxidant activity of an extract lies in its ability to stabilize the cationic radical ABTS + • with a blue-green coloration by transforming it into colorless ABTS + by trapping a proton. The radical-cationic ABTS + • was obtained by mixing 8 mM ABTS (87.7 mg in 20 mL of distilled water) and 3 mM of potassium persulfate (0.0162 g in 20 mL of distilled water) in a ratio of 1: 1 (v/v). Then, the reaction mixture was incubated in the dark at room temperature (27-30 ^{0}C) for 16 hours. Then, the ABTS + • solution obtained was diluted with absolute methanol to obtain a solution of absorbance 0.7±0.02 at 734 nm. In addition, a test portion of 3.9 mL of this dilute solution of ABTS + ° was added to 100 µL of the compound to be tested. After stirring, the mixture was incubated for 6 minutes in the dark. Behind, the residual absorbance of the ABTS +• radical was measured at 734 nm with a UV-visible spectrophotometer and should represent between 20% -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 (µmol / L TE).
The activity of the compounds 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 compound tested. The calibration of the spectrophotometer was carried out by reading the absorbance of different concentrations of trolox (3.75; 5; 6.25; 10; 11.25, 13.75 and 15.10-4 mM) and the level of inhibition (% I) of ABTS + • was determined as follows:

% I = [(Abscontrol - Absextrait)/Abscontrol] x 100

Abscontrol = diluted ABTS absorbance,
Absextrait = diluted ABTS absorbance + sample

3. Results and Discussion

3.1.Quantification of phenolic compound

Hyptis suaveolens leaves are renowned for their antioxidant activity [2]. Likewise, it is reported in the literature that these antioxidant activities are due to the content of phenolic compounds [24]. As a result, the contents of certain phenolic compounds such as polyphenols, flavonoids and tannins were determined in the various hydroalcoholic extracts and fractions resulting from these extracts (Table 1).

Table 1: Concentrations of phenolics compounds in the various extracts and fractions of *Hyptis suaveolens* leaves before and after extraction of the essential oil.

Extracts and fractions		Total polyphenol content (mg EAG/g dry extract)	Total flavonoid content (mg EQ/g dry extract)	Total tannins content (mg EAT/g dry extract)
Hydroalcoholic extract (EHA)	Before	39.167±2.222	15.417±0.222	8.733±0.156
	After	20.833±1.111	20.583±0.389	6.500±0.267
Hexane fraction (FHEX)	Before	0.483±0.022	1.950±0.033	3.633±0.156
	After	0.333±0.056	2.217±0.056	5.667±0.111
Dichloromethane fraction (FDCM)	Before	4.323±0.004	4.800±0.167	9.767±0.689
	After	7.800±0.000	5.867±0.189	4.567±0.311
Ethyl acetate fraction (FAE)	Before	48.333±0.556	33.333±1.778	5.900±0.133
	After	45.233±0.822	46,000±2,000	4.833±0.156
Ethanol fraction (FETH)	Before	20.833±1.556	21.333±0.889	16.500±0.333
	After	10.633±0.178	29,000±0.667	15.567±0.222
Aqueous Fraction (FAQ)	Before	21.250±1.000	31.333±0.444	8.800±0.067
	After	16.900±0.133	24.667±0.444	6.400±0.200

The results of Table 1 show that the contents of total polyphenols and total tannins of hydroalcoholic extracts (EHA) of *Hyptis suaveolens* leaves decrease during hydrodistillation with variations from 39.167±2.222 to 20.833±1.111 mg EAG/g of extract for polyphenols and from 8.733±0.156 to 6.500±0.267 mg EAT/g for tannins. This decrease is linked to the water-soluble nature of phenolic compounds [25]. These compounds were partially extracted by floral water during hydrodistillation [26]. The total polyphenol contents obtained in this study are higher than those obtained by Sharma *et al*. on the aqueous extract of *Hyptis suaveolens* leaves in India (10.00 and 2.5 mg EAG/g extract) [26] and that obtained by Dossa *et al*. (34.32 mg EAG/g) on the hydroalcoholic extract of *Hyptis suaveolens* leaves in Benin

before extraction of the essential oil [27]. The same is true for the tannin contents of the hydroalcoholic extract before extraction of the essential oil (8.733±0.156 mg EAT / g of dry extract) which are more or less higher than those reported in the literature (5, 50±0.074%) [28]. This difference could be related to the extraction method or to environmental conditions and agricultural practices because these are factors likely to impact the values of the total polyphenol contents [29, 30].

As for the hydroalcoholic extract of *Hyptis suaveolens* leaf residues after extraction of EO, almost no mention is made in the literature in relation to its content of polyphenols or tannins. The high concentration of tannins in the leaves of the plant is said to be the cause of their astringent properties [31].

Unlike total polyphenols and tannins, the total flavonoid content of the hydroalcoholic extract (EHA2) of *Hyptis suaveolens* leaves after extraction of the essential oil (ET) is more abundant than that of the hydroalcoholic extract (EHA1) of before extraction of the EO with respectively 20.58±0.22 mg EQ/g of extract 15.42±0.39 mg EQ/g of extract (Table 1). The increase in the content of flavonoids in the hydroalcoholic extract after extraction of the essential oil would be due to the departure of essential oils, the presence of which would reduce the extraction of flavonoids [32]. Indeed, terpene compounds which are the major components of essential oils, generally form insoluble complexes in extraction solvents with certain phenolic compounds such as flavonoids [33]. The rise in temperature during hydrodistillation would cause the breaking of certain intermolecular bonds, thus promoting the extraction of flavonoids [25, 34]. This increase in the flavonoid content is in agreement with the accentuation of the antimicrobial activities of the hydroalcoholic extract of the leaves of *Hyptis suaveolens* after extraction of the essential oil as reported by our previous work [15]. Indeed, flavonoids are recognized for their antioxidant and antimicrobial properties [35].

Our flavonoid contents are higher than those obtained by Sharma *et al*. on the aqueous extract of *Hyptis suaveolens* in India (2.5-1.3 mg EQ/g extract) [26] and obtained by Dossa *et al*. (12.12 mg EQ/g) on the hydroalcoholic extract of *Hyptis suaveolens* leaves in Benin before extraction of the essential oil [27]. This difference could be related to the extraction method used or to environmental conditions and agricultural practices because these are factors likely to impact the values of total polyphenol contents [36].

With the exception of the dichloromethane fraction, the contents of total polyphenols and tannins are greater in all the fractions obtained from these hydroalcoholic extracts before the extraction of the essential oil. This increase in the content of total polyphenols extracted by dichloromethane would probably be linked to the transformation of certain water-soluble glycosides into genins, by loss of their sugars, under the effect of heat. [34], which would make them more soluble in dichloromethane [37]. Ethyl acetate (FAE), whose before and after contents are respectively 48.333±0.556 and 45.233±0.822 mg EAG/g, is the solvent that extracts the maximum of phenolic compounds followed by water (FAq) (21.250±1.000 and 16.900±0.133 mg EAG/g) and ethanol (FEth) (20.83±1.556 and 10.633±0.178 mg EAG/g). These results show that the polyphenols present in the polar or medium polar fractions are mainly in the form of free genins as described in previous work on the fresh leaves of *Hyptis suaveolens* in Nigeria [38]. Studies on the determination of the polyphenol content of the fractions after extraction of the essential oil of *Hyptis suaveolens* have not yet been described in the literature.

Regarding tannins, an increase in the total tannin content soluble in hexane is observed (3.633±.156 to 5.667±0.111 mg EAT/g of dry extract) unlike the other fractions where it decreases (see Table 1). This high level in the hexane fraction could be linked to the departure of essential oils, which would allow the hexane to extract more tannins.

Ethanol is the solvent that extracts the most tannins (16.500±0.333 to 15.567±0.222 mg/g of dry extract) followed by water (8.800±0.067 to 6.400±0.200 mg/g). Then dichloromethane (9.767±0.689 to 4.567±0.311 mg EAT/g), ethyl acetate (5.900±0.133 to 4.833±0.156 mg EAT/g) and hexane (3.633±0.156 to 5.667±0.111 mg EAT/g of dry extract). This is in accordance with the more soluble character of tannins in ethanol and in water (Especially hot) compared to non-polar organic solvents [39, 40]. This variation is explained by the fact that the extraction of tannins depends on their chemical nature, the solvent used and the operating conditions [41].

On the other hand, the total flavonoid contents of the different fractions afterwards are generally higher than those of the fractions before extraction of the essential oil with concentrations ranging respectively from 2.217±0.056 to 46.000±2.000 mg EQ/g of the fraction and 1.950±0.033 to 33.333±1.778 mg EQ/g of the fraction. However, in the case of the aqueous fractions (FAq) there is a decrease in the flavonoid content from 31.333±0.444 to 24.667±0.444 mg EQ / g after extraction of the EO. This decrease in the content of flavonoids in the aqueous fraction after extraction of the essential oil would be due to the extraction of a certain quantity of water-soluble glycosylated flavonoids by the floral water during the hydro distillation [25].

The ethyl acetate (FAE) fractions before and after EO extraction (33.333±1.778 to 46.000±2.000 mg EQ/g) are the richest in flavonoids followed by the aqueous fractions (31.333±0.444 to 24.667±0.444 mg EQ/g) and ethanolic (FEth) (21.333±0.889 to 29.000±0.667 mg EQ/g). The dichloromethane (FDCM) fractions (4.8±0.167 to 5.867±0.189 mg EQ/g) and hexane (FHEX) (1.950±0.033 to 2.217±0.056 mg EQ/g) are the poorest in flavonoids. These results show that the flavonoid content in the fractions depends on the polarity of the solvents used as reported by Marc [42]. The determination of the flavonoid content of the fractions after extraction of the essential oil of *Hyptis suaveolens* has never been studied in the literature.

3.2 Evaluation of the antioxidant activity of the leaves of the plant before and after extraction of the essential oil

The presence of phenolic compounds in the leaves of *Hyptis suaveolens* would be sufficient reason to assess their potential antioxidant activity. Indeed, phenolic compounds strongly contribute to the reduction of the oxidative action of reactive oxygen species (ROS). These species are the cause of many cardiovascular diseases. It should be noted that the measurements of the capacity to scavenge free radicals with the DPPH and ABTS methods are easy, fast and very sensitive and therefore they are more frequently used for a preliminary evaluation of the antioxidant potential of different natural substances.

3.2.1 Potential for inhibition of the DPPH radical

The inhibitory concentrations of 50% (IC50) of the DPPH radical of the various extracts and fractions before and after extraction of the essential oil are represented in FIG. 1. Ascorbic acid (AA) was used as a reference antioxidant.

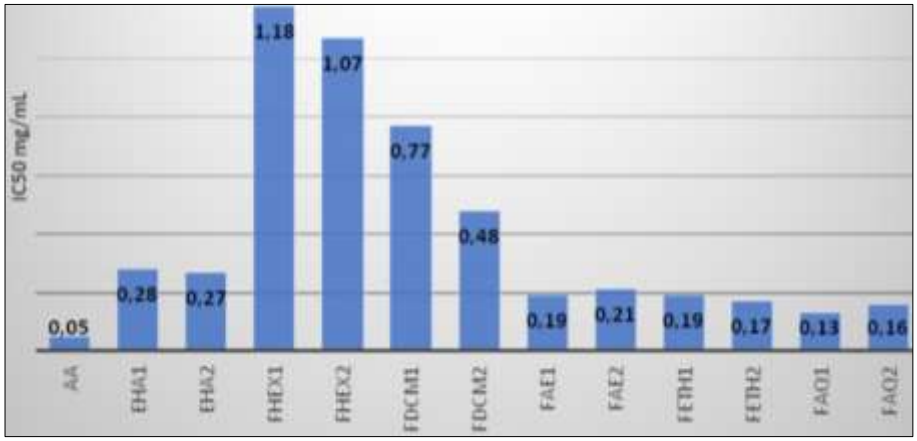

Fig 1: Values of the DPPH inhibition concentrations of 50% of the diffèrent extract and fractions before and after extraction of the essential oil

FIG. 1 shows that the hydroalcoholic extracts of *Hyptis suaveolens* leaves before and after extraction of the EO have IC50s of 0.28 mg/mL and 0.27 mg/mL respectively. These two extracts have very similar IC50s, which means that the hydrodistillation has no influence on the antioxidant activity of the extracts. Thus, the extracts of *Hyptis suaveolens* leaves in particular those of the leaves after extraction of the EO could be used as an additive to other foods as a surrogate antioxidant [43].

Our results obtained on the IC50 values before EO extraction are lower than those reported by Dossa *et al*. [27] with IC50 values (0.28 mg/mL versus 0.58 mg/mL for Dossa *et al*.). This would probably be linked to the difference in the levels of phenolic compounds observed between these two studies.

Our results also show that the polar fractions (ethyl acetate, water and ethanol) have similar antioxidant activities. The antioxidant activities of these polar fractions are greater than those of the non-polar fractions (dichloromethane, hexane). These results are in agreement with the results of a number of authors who have shown that polar solvents extract more antioxidant compounds than solvents of low polarity [44].

However, these polar extracts with IC50 values varying between 0.19 mg/mL and 0.23 mg/mL, remain moderate antioxidant activities in comparison with ascorbic acid whose value of the inhibition concentration of the DPPH radical 50% is 0.05 mg/mL.

The comparison of this capacity of reduction of the DPPH radical with the contents of phenolic compounds of the various extracts and fractions makes it possible to say that the antioxidant capacity of the leaves of *Hyptis suaveolens* would be particularly related to the presence of flavonoids with a correlation coefficient of R = 0.904 against R = 0.816 and R = 0.429 respectively for the total polyphenols and total tannins. This observation is in conformity with that already made by a certain number of authors [45].

3.2.2 ABTS radical reduction capacity

The peculiarity of the ABTS method is its ability to scavenge free radicals in both lipophilic and hydrophilic antioxidants. Analyzes were performed using Trolox as a reference (Figure 2). The triplex results were expressed by the Trolox Equivalent Antioxidant Capacity (TEAC) in µmol/L TE.

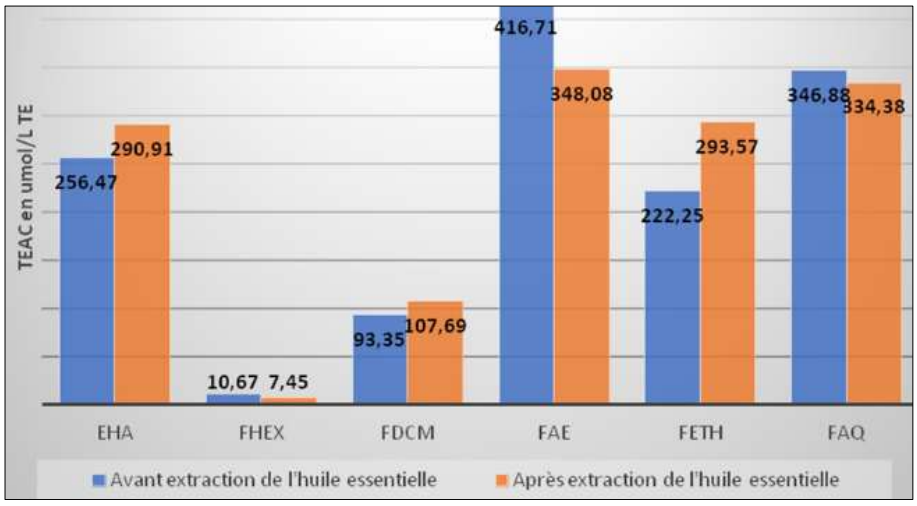

Fig 2: ABTS radical reduction capacity of extracts and fractions of *Hyptis suaveolens* leaves before and after extraction of the essential oil

Unlike the DPPH test, here we note a slight increase in the antioxidant power after extraction of the essential oil with TEAC from 256.47 µmol/L TE to 290.91 µmol/L TE for the hydroalcoholic extracts (figure 2). This is in accordance with the contents of total flavonoids which also increase during hydrodistillation. Thus we could confirm that flavonoids with a correlation coefficient of R = 0.9655 would contribute much more to the trapping of the ABTS radical than total polyphenols and tannins with respectively for R = 0.8701 and R = 0.1208.

Here too, as in the case of DPPH, the polar fractions (ethyl acetate, water and ethanol) have antioxidant activities greater than those of the non-polar fractions (dichloromethane, hexane) [46]. Thus, the strongest antioxidant potentials are observed for the ethyl acetate fractions (416.71 to 346.08 µmol/L TE), followed by the aqueous fractions (346.88 to

334.38 µmol/L TE) and the fractions ethanols (222.25 to 293.57 µmol/L TE) respectively before and after extraction of the essential oil. However, a decrease in this activity is noted for the ethyl acetate fractions and a slight increase for the aqueous fractions after extraction of the EO, unlike their total flavonoid contents which increase for the former and decrease for the latter. This could be explained by the nature of the flavonoids extracted by these two types of solvents [47, 48].

4. Conclusion

Evaluation of the antioxidant activity of *Hyptis suaveolens* leaves showed moderate activity both before and after extraction of the essential oil. If this activity appears similar for the DPPH radicals, there is a clear increase after extraction of the essential oil for the ABTS despite the drop in the contents of polyphenols and total tannins with hydro distillation. So we could say that flavonoids are the main contributors to the anti-free radical activity of the leaves of the plant. Moreover, in view of their moderate antioxidant activity, one could suggest extracts of *Hyptis suaveolens* leaves as additives to other foods as a substitute antioxidant.

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