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# Radical scavenging capacity and total polyphenols content of the hydro-ethanolic extract and fractions from *Eugenia uniflora* L

**Gouollaly Tsiba, Timoléon Andzi-Barhé, Hubert Makomo, Blondy Mboungou, Sarrha Boumba, Marie Claire Makambila and Pascal Robin Ongoka**

**Abstract**
The fight against these radicals, calls for the use of polyphenolic compounds whose content often remains reserved for plants. This is the purpose of this present work, which proposes to measure the contents in polyphenols and evaluate radical scavenging capacities of the extract and the fractions obtnained after the separation on a polyamide column of *Eugenia uniflora.* For that purpose, we noted that the chromatographic profiles on TLC, remain dominated by fluorescence compounds (Orangy yellow, lemon green and blue) materializing the presence of polyphenols (flavonoids and phenols acids) in the leaves. The quantitative analysis of flavonoidic compounds of the hydro-ethanolic extract and the four fractions vary between 11.3 and 29.8 mgECa/gMs. The hydro-ethanolic extract and the fraction F1 have the smallest total flavonoids content, respectively 11.3 and 15.6 mgECa/g MS while those of the fractions F2, F3 and F4 are the highest ones, respectively 27.6, 28.4 and 29.8 mgECa/gMs. The contents in total polyphenolic compounds are the highest compared with total flavonoidic compounds and show very high concentrations for the fractions F4 (259.1 mgEAG/gMs) followed by F3 (226.2 mgEAG/gMs) and F2 (202.4 mgEAG/gMS). The hydro-ethanolic extract and the fraction F1 have the lowest content. This large production of polyphénolic compounds is confirmed by the radical scavenging ability very pronounced observed on the DPPH radical for the fractions F2 (3.1 µg/mL), F3 (2.7 µg/mL) and F4 (2.7 µg/mL) comparable with that of quercetin (2,7 µg/ml) and the gallic acid (1.6 µg/ml) two reference compounds used in the present study. The results observed correlate positively with total phenol content, strongly plead in favour of the use of this extract and fraction as potential food additives in replacement of synthetic compounds.

**Keywords:** radical scavenging, hydro-ethanolic, fractions, *Eugenia uniflora*

## 1. Introduction
Free radicals and their activated derivatives, that we can group together under the term reactive oxygen species (ROS), are continually produced in cells, in particular during oxidative metabolism. It has recently been shown that they can have a role in various diseases onset [1].
However, they have been considered for a long time as potentially toxic because they have the power of damaging different vital cellular constituents, such as lipids, proteins and the DNA, and the power of leading to the death of cells out of oxidative stress [1] and the latter, would be responsable for several pathologies. Thus, the fight against these radicals requires the use of polyphenolic compounds whose richness often remains reserved for plants.
It's from this perspective that 80 % of the African population is continuing to have recourse to traditional medicine for primary health treatment [2]. This medicine uses among other the rich and varied flora in the surrounding environment for medicinal formulas preparation. *Eugenia uniflora*, is part of those plant species whose use in African and South American pharmacopeia is no longer the subject of any doubt. A lot of research work carried out on the secondary metabolites mention the presence of phenolic compounds belonging to the flavonoids and phenols acids family, also the terpenpoids and alcaloids family [3-8]. The identified alcaloids have been correlated with its antidiabetic activity. So, Bakr and *at al.* [6] have been able to isolate five compounds from the methanolic extract including three flavonols and two phenols acids: Myricetin 3- O-(4", 6"-digalloyl glucopyranoside, Myricetin 3-O-glucopyranoside, quercetin, gallic and ellagic acid.
Previous studies focused on biological activities of extracts made it possible to highlight the following pharmacological properties: antidepressant, antihypertensive, antimicrobial, anti-diarrheal, anti-inflammatory, antipyretic, antioxidant and toxicological [3-12].

In Congo, the plant is used for the treatment of diabetes, diarrheal diseases and is part of a formula for high blood pressure.

To the previous work may be added the present study that sets itself the goal of measuring the qualitative and quantitative potential of total polyphenols and evaluating the radical scavenging power of the extract and the fractions obtained after the separation on polyamide.

## 2. Materials and Methods

### 2.1 Vegetable matter

The leaves were harvested in an area of Brazzaville and dried at room temperature, for about a week. The dry vegetable matter is ground with an IKA-WERKE Gmbh-CO-KG, D-79219, Staufen-type device, with a sieve of granulometry 0.25mm.

### 2.2 Preparation of extracts

To make the measurement, the extracts of polyphenols, flavonoids and total phenols acids were obtained on mixing 100 g of vegetable matter with 2 × 500 ml of a hydro-ethanolic solution (50% (v/v)). The mixture is shaked up during 72 hours, and then filtered. The filtrate obtained dried concentrated with a rotary evaporator is kept in a cool place (+4 °C) waiting to be analyzed.

### 2.3 Separation of the hydro-ethanolic extract on a polyamide column

1 g of the hydro-ethanolic extract has been chromatographied on a open Polyamide 6 (Fluka) column with a diameter of 2 cm and 50 cm long. The elution is made from a mixture of water and ethanol in accordance with a gradient of decreasing polarity, in the following proportions: 100% $H_2O$, $CH_3CH_2OH/H_2O$ (30/70 v/v), $CH_3CH_2OH/H_2O$ (70/30; v/v), and 100% $CH_3CH_2OH$. The follow-up of different fraction collected is carried out by an analysis on thin layer chromatography (TLC) with the silica gel on a aluminium rack with a eluant made of ethyl acetate / formic acid /water (9.5/0.25/0.25; v/v/v). The plates are first visualized using the UV ($\lambda$ = 254 and 366 nm) then revealed by the reagent of NEU [13] followed by another visualisation using the UV-365 nm. The extract of the four fractions obtained after the separation have been Co-chromatographied with six (06) reference compounds (Rutin, Quercetin, myritecin, chlorogenic acid, gallic acid, cafeic acid,)

### 2.4 measurement of polyphenols [14]

The reagent of Folin-Ciocalteu was used for the evaluation of total phenols of aqueous, hydroethanolic and ethanolic extracts. Folin-Ciocalteu is a mixture of phosphotungstene acid (H3PW12O40) and phosphomolybdène (H3PMo12O40) of yellow color. The method is based on the oxidation of the phenolic compounds by this reagent. This oxidation draws the formation of a new complex molybdenumtungsten of blue color that absorbs to 725 nm. The evaluation of TP is done by comparison of the optic density (D.O) observed to the one obtained from a stallion of known acid Gallic concentration. The total phenol compounds are measured as follow: 0.1ml of the extract hydroethanolic is introduced in an Eppendorff tube of 2 ml, the extract is diluted with 0.9 ml of distilled water. 0.9ml of the reagent of Folin-Ciocalteu (1N) is immediately added after addition of 0.2 ml of Na2CO3 (20%) solution. The obtained mixture is hatched to the ambient temperature during 40 minutes safe from light. The absorbance is measured with the spectrophotometer at 725 nm against a solution of ethanol used like white (control). A right of standardization achieved previously with the Gallic acid in the same conditions that the samples to analyze, permitted to calculate the total phenols contain. The results are expressed in mg equivalent Gallic acid by gram of dry matter (mg E GA/gMs).

### 2.5 Measurement of total flavonoids (FVT) [14]

The colorless solutions of sodium nitrite (NaNO2, 5%) and of aluminum chloride (AlCl3, 10 %) have been used for the evaluation of total flavonoids in aqueous, hydroethanolic and ethanolic extracts. The method is based on the oxidation of the flavonoids by these reagents; oxidation that draws the formation of a brownish complex that absorbed at 510 nm. The comparison of the optic density (D.O) observed to the one obtained from a stallion of known concentration Catechin permits to value the total content in flavonoids by colorimetric effect. In a ball of 10 ml are introduced 250 µl of extract and 1 ml of distilled water successively. To the initial time (0 minute) are added 75 µl of a NaNO2 (5%) solution. After 5 min 75µl of AlCl3 (10%) are added; 6 minutes later, 500µl of NaOH (1N) and 2.5 ml of distilled water are added successively to the mixture. A curve of standardization is elaborated with solutions standards of catechin prepared at different concentrations.

### 2.6 Assessment of the scavenging activity of the hydro-ethanolic extract and fractions (F1, F2, F3 and F4) : qualitative and quantitative analysis [15]

The qualitative analysis of the scavenging activity has been evaluated on pulverizing the solution of 1.1-diphényl-2-picrylhydrazyle (DPPH) at 2 mg/mL on TLC plate of silica gel. The migration solvent was ethyl acetate / formic acid / water (8/1/1). The appearance of pale yellow stains on a purple background shows the scavenging activity.

Then, the quantitative analysis of the scavenging activity has been evaluated on mixing 10 mL of the solution of 1,1-diphenyl-2-picrylhydrazyle (DPPH) at 10 mg in 250 ml of ethanol and 100 µL of extract or the fractions at the concentrations of 100-3.12 µg/mL. After that, the activity has been measured at 517 nm in the shelter of the light after 30 minutes of incubation to darkness using a UV-visible spectrophotometer. The pourcentage of inhibition was calculated using the following relation: [(*A*517 white - *A*517 of the sample) / *A*517 white] × 100. *A*517: Absorbance at 517 nm.

## 3. Résults and discussion

### 3.1 Qualitative and quantitative analysis of fractions (F1, F2, F3 and F4)

The chromatographic profiles of the extract and the four (04) fractions obtained after fractionation on a polyamide column (Figure 1) show a succession of spots materializing the presence of compounds with a polyphenolic structures.

So, the following observations can be done after the exhibition of the plate to the lamp UV-366 nm:

- The orangy yellow fluorescences with retention factor (0.3, 0.4, 0.5and 0.7) (Table 1) highlighted in a clear way in the extract and the fractions (F2, F3 and F4), which could be put down to derivates of flavonol ortho-di-hydroxy in the 3' and 4' position [13] (Figure 2). These structures were highlighted and quantized in this species [3-12] and Riham Bakr and *al* [6] were able to quantize these compounds to the contents not exceeding 10 mg/gMs.

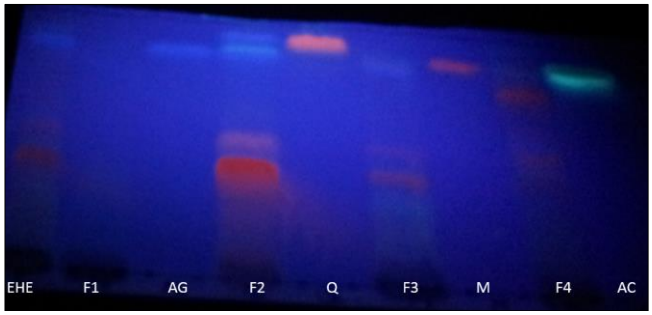



**Fig 1:** chromatographic profiles of extracts and reference compounds. Solvent System : Ethyle Acétate/ formic acid /water (9,5/0,25/0,25) ; Spray Reagent : Neu ; Observation : UV-366 nm. Reference compounds: Q: Quercetin, Ac: Caffeic acid, AG: Gallic acid and M: Myricetin. Hydroethanolic Extract: EHE

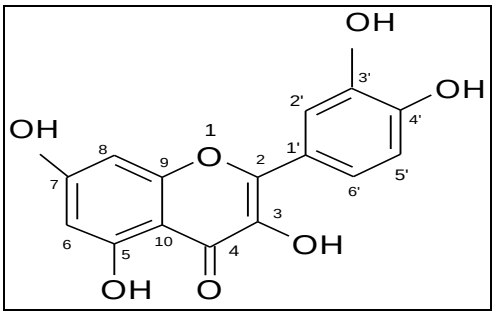
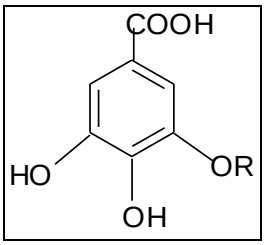



**Fig 2:** 3' 4', 5, 7-trihydroxyisoflavonol : Quercetin

- The blue fluorescence observed specific in the extract and the fractions F2 and F3 to retention factor (0.75 and 0.9) (Table 1), could be put down to derivates of gallic acid (figure 3). The presence of these compounds in the three fractions, show their rather high content in the plant. It is appropriate to not that gallic acid was highlighted and quantized up to 10 mg/gMs in the same species by Riham Bakr and *al* [6].

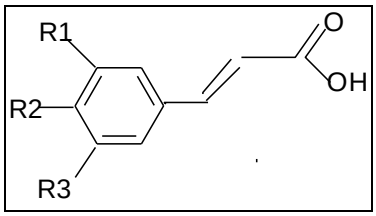



R = H, gallic acid
R = CH3, acide syringique

**Fig 3:** gallic acids Derivates

- No Green blue fluorescence that could meant the presence of dérivates cinnamic acide [13] (Figure 4) has not been highlighted on this thin layer.

F1 fraction shows no fluorescence, it would be rich in very polar polyphenolic compounds and protein [15].




R1, R2, R3 = H, t-cinnamic acid
R1, R3 = H, R2 = OH : p-coumaric acid
R1= H, R2, R3 =OH : caféïc acid
R1=H, R2 =OH, R3 =OMe : férrulic acid
R1=H, R2 =OH, R3 =OMe : isoférrulic acid

**Fig 4:** a few examples phenols acids structures C6-C3

**Table 1:** Recapitulative table of the qualitative analysis of the extract and fractions

| Types of samples | Number of constituents | Spray Reagent | | Probable nature of constituents |
|---|---|---|---|---|
| | | DPPH [14] | NEU [13] UV-365 nm | |
| EHE | 07 | CJC (0.3, 0.4, 0.5, 0.7, 0,8 and 0.9) | FJO (0.3, 0.4, 0.5and 0.7) | Flavonoïds (Flavonol) |
| | | | FB (0.8 and 0.9) | Phénols acids (gallic acids derivates) |
| F1 | 0 | - | - | - |
| F2 | 03 | CJC (0.3, 0.4 and 0.8 | FJO (0.3, 0.4 and 0.5) | Flavonoïds (Flavonol) |
| | | | FB (0.8) | Phénols acids (gallic acids derivates) |
| F3 | 05 | CJC (0.4, 0.5, 0.7, 0,8 and 0.9) | FJO (0.3, 0.4, 0.5and 0.7) | Flavonoïds (Flavonol) |
| | | | FB (0.8 and 0.9) | Phénols acids (gallic acids derivates) |
| F4 | 05 | CJC (0.4, 0.5, 0.7, 0,8 and 0.9) | FJO (, 0.4, 0.5and 0.7) | Flavonoïds (Flavonol) |
| | | | FB (0.8 and 0.9) | Phénols acids (gallic acids derivates) |

LYC: Light Yellow Color; FYO: Fluorescence Yellow orange; BF: Blue Fluorescence);: retention factor; F: Fraction; F1, F2, F3 and F4 correspond to the elution of a mixture of water and ethanol according to a gradient of decreasing polarity, in the following respective proportions: 100% H2O, CH3CH2OH / H2O (30/70 v / v), CH3CH2OH / H2O (70/30, v / v) and 100% CH3CH2OH; EHE. Hydroethanolic Extract.

The quantitative analysis of polyphenols and total flavonoids show that the fractions F2, F3 and F4 are quantitatively richer in polyphenols and total flavonoids than the hydro-ethanolic extract (figure 5). The contents in polyphenols and flavonoids in the extracts are 112.5 mgEAG/gMS and 15.6 mgECa/gMS respectively. The contents in total polyphenols in these fractions are: 202.5; 213.9 and 259.1 mgEAG/gMs and those in flavonoids 27.6; 28.5 and 29.8 mgEca/gMs for the fractions F2, F3 and F4 respectively. It's appropriate to note that these contents have been almost multiplied by two in the fractions F2, F3 and F4. The fraction F1 shows the values in polyphenols and flavonoids very similar to those of the hydro-ethanolic extract that is 95.6 mgEAG/gMs and 11.6 mgECa/gMs. We notice that the type of stationary phase used (polyamide 6-Fluka) makes it possible to enrich these fractions in poly phenolic compounds. The high contents in polyphenols and total flavonoids obtained in the present study, could be justified by the clear observation by Thin Layer Chromatography (TLC) and the presence of these metabolites reported by several authors in the plant [3-12].

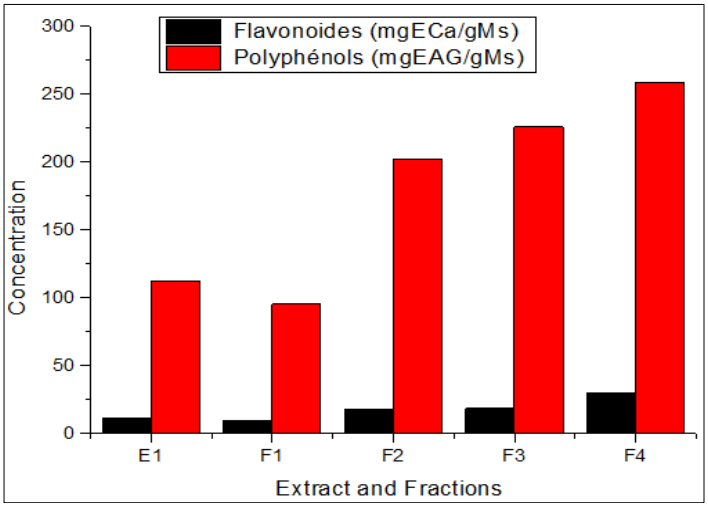



**Fig 5:** measurement of polyphenols and total flavonoids in the hydro-éthanolic extract and fractions.

### 3.2 Assessment of the scavenging activity of the hydro-ethanolic extract and fractions (F1, F2, F3 and F4): qualitative and quantitative analysis

The observation of the scavenging activity on TLC (Figure 6) after the revelation of the plate to DDPH, shows for the extract and the four fractions of spots to retention factor (0.4, 0.5 and 0.7) for the extract and fraction (table 1) and a streak on a purple background observed materializing the presence of scavenging activity. These spots highlighted could be associated with flavonoids and phenols acids specified during the observation on thin layer. This activity is more pronounced for the fractions F2, F3 and F4 whose content in polyphenols has been reported up there. These secondary metabolites are known as powerful scavenging compounds [16-19].

The results of the scavenging activity of the hydro-ethanolic extract and the fractions (F2, F3 and F4) after the separation on polyamide column are presented in the figure below (Figure 7). We notice that concentration which inhibits 50 % 50 % of fractions F2 (3,1 µg/mL), F3 (2,7 µg/mL) and F4 (2,8 µg/mL) are very similar to quercetin (2.7 µg/ml) and gallic acid (1.6 µg/ml) two reference compounds used in the present study but higher than in that of the alpha-tocophérol (5.6 µg/mL). This strong inhibition of free radicals of fractions could be justified by their strong concentrations in polyphenolic compounds and by the chromatographic profiles that highlight the flavonic-type compounds and phenolic acids reported in the present study and in the literature [3-13]. In fact, the polyphenolic compounds are known as powerful compounds with a power of reducing free radicals [16-19].

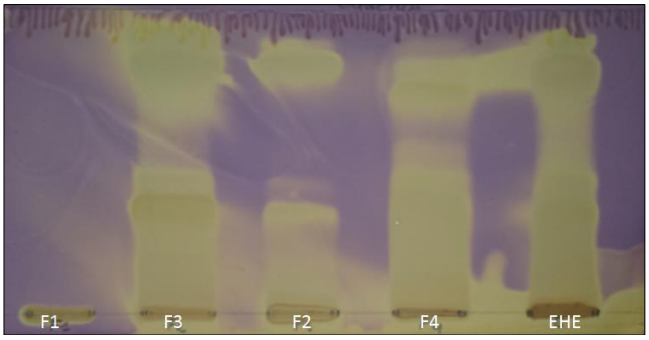



**Fig 6:** scavenging activity in the extract and fractions. F: Fraction; F1, F2, F3 and F4 correspond to the elution of a mixture of water and ethanol according to a gradient of decreasing polarity, in the following respective proportions: 100% H2O, CH3CH2OH / H2O (30/70 v / v), CH3CH2OH / H2O (70/30, v / v) and 100% CH3CH2OH; EHE. Hydroethanolic Extract

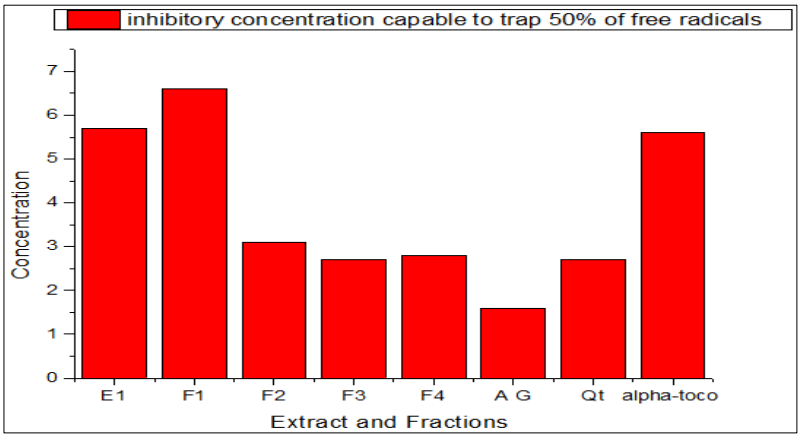



**Fig 7:** scavenging activity in the extract and fractions

## 4. Conclusion

This study has shown that the hydro-ethanolic extract of the leaves of *Eugenia uniflora* and the fractions (F1, F2, F3 and F4) obtained after the separation on a polyamide column show a strong richness in polyphenols and total flavonoids. This content in these metabolites is all the more pronounced for the fractions F2, F3 and F4. In addition, it shows that these same fractions are potentially rich in scavenging compounds. These results reinforce those which have been already obtained and show the interest of the *Eugenia uniflora* leaves for the care and treatment of several diseases including hypertension. They correlate positively with total phenol content strongly plead in favour of the use of this extract and fraction as potential food additives in replacement of synthetic compounds.

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