EVALUATION OF THE ANTIOXIDANT ACTIVITY OF AQUEOUS AND HYDRO-ETHANOLIC EXTRACTS OF *FICUS CAPENSIS, NEWBOULDIA LAEVIS* AND *CARPOLOBIA LUTEA*.

Abstract

Scientific interest in the search for natural antioxidants (secondary metabolites) in medicinal plants has increased considerably in recent years, as free radicals are at the root of many diseases. The aim of this study was to evaluate the antioxidant activity of aqueous and hydroethanol extracts from the leaves of a basic plant product consisting of equal quantities of *Ficus capensis*, *Newbouldia laevis* and *Carpolobia lutea*. Quantitative determination of antioxidant activity was based on the flavonoid content of the base plant product. It was assessed by DPPH (2, 2-diphenyl-l-picrylhydrazyl) reduction tests and ferric ion reducing power (FRAP). The results of this study showed that the hydro-ethanol extract of the basic plant product is richer in flavonoids than that of the aqueous extract. In addition, the ICs_{50} of the aqueous and hydro-ethanolic extracts of the base plant product with DPPH were 2.13 ± 0.01 µg/mL and 1.19 ± 0.03 µg/mL respectively. In the FRAP test, the reducing powers of the hydroethanolic extract were generally higher than those of the aqueous extract at all concentrations tested. Particularly at the high concentration of 10 µg/mL, the reducing powers of the aqueous and hydroethanol extracts of the base plant product are 435.86 ± 1.29 µg/mL and 453.68 ± 0.71 µg/mL respectively. The hydroethanol extract therefore has better antioxidant activity than the aqueous extract.

Key words: Extracts, flavonoids, antioxidant.

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INTRODUCTION

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Free radicals are thought to be at the root of a number of pathologies, including arthritis, asthma, cancer, diabetes, heart disease and atherosclerosis [1]. According to [2], the use of natural products (fruit, vegetables) rich in antioxidants could play an important role in preventing these diseases. However, the high cost of health care and modern medicines, as well as socio-economic factors, mean that a large proportion of the population use medicinal plants to treat themselves [3]. Today, 80% of the population have used herbal medicine at least once [4]. This

attitude may also be linked to ancestral culture and civilization, which rely wholly or partly on phytotherapy because of the efficacy, accessibility and availability of medicinal plants [5]. In addition, numerous studies have shown that medicinal plants contain various biologically active chemical principles that exert different pharmacological activities: antioxidant, antiimflammatory, analgesic, etc [6 , 7 , 8 , 9 , 10].

It is in this context that this study investigated the antioxidant activity of aqueous and hydro-ethanolic extracts of the leaves of the equal mixture of *Ficus capensis*, *Newbouldia laevis* and *Carpolobia lutea*. These medicinal plants have always been used in the Department of Daloa (central-western Côte d'Ivoire) by several traditional practitioners to treat people suffering from sickle-cell anemia.

MATERIALS AND METHODS

Plant material

The leaves of the medicinal plants used in this study were harvested in December 2019 in Daloa (Haut-Sassandra region, central-western Côte d'Ivoire). They were identified at the Agrovalorisation laboratory of the UFR Agroforestry of the Université Jean Lorougnon Guédé in Daloa. They were then air-dried for two weeks in the laboratory, and finely ground separately in an electric grinder to obtain distinct plant powders. The plant powders obtained from each plant were then mixed in equal quantities to produce a basic plant product, which was stored in a dark, dry place. Finally, the basic plant product obtained was used throughout the work, i.e. for the preparation of the various extracts (aqueous and hydro-ethanolic) and subsequent tests.

METHODS

PREPARATION OF DIFFERENT EXTRACTS

Preparing the aqueous extract

The aqueous extract of the basic plant product was prepared according to the method described in [11]. To do this, one hundred grams of the basic plant product initially obtained was dissolved in one liter of water, then homogenized using a blender to obtain a homogenate. Next, the homogenate obtained was wrung out once on a square of white cloth, then filtered successively with absorbent cotton and finally on Wattman paper to obtain a filtrate. Finally, the filtrate obtained was oven-dried at 50°C for 48 hours to obtain the aqueous extract.

Preparation of hydroethanol extract

The hydroethanol extract of the basic plant product was prepared according to the method of [11]. This consisted in placing one hundred grams of the basic plant product previously obtained in one liter of 70% ethanol (ethanol-distilled water: 70/30 (V/V)), then mixing it with a blender to obtain a homogenate. The homogenate obtained was then wrung out once on a square of white cloth, then filtered successively with absorbent cotton and on Wattman paper to obtain a filtrate. The filtrate obtained was then oven-dried at 50°C for 48 hours to obtain the hydroethanol extract.

Determination of flavonoids in various extracts

The total flavonoid content of each extract was determined using the aluminum trichloride colorimetric method [12]. To this end, 100 mL of each extract was mixed with 0.4 mL of distilled water, followed by 0.03 mL of a 5% $NaNO_2$ sodium nitrite solution. After 5 minutes, 0.02 mL of a 10% $AlCl_3$ solution was added. 0.2 mL $Na2CO_3$ (1 M) solution was then added to the previous mixture, followed by 0.25 mL distilled water after a further 5-minute rest. The mixture was then vortexed and the absorbance measured at 510 nm. Results were expressed as mg quercetin equivalent per g extract (mg QE/g).

Determination of antioxidant activity (DPPH test)

Determination of free radical scavenging activity by the DPPH assay was carried out using the method described by [13] and slightly modified. To carry it out, an ethanolic solution of $DPPH^{\cdot}$ was prepared by dissolving 4 mg of this product (DPPH) in 100 mL of ethanol. Then, to a volume of 50 µL of each initially prepared extract of given concentration was added 950 µL of the ethanolic solution of DPPH. The extracts and the reference (ascorbic acid) were tested at different concentrations (10; 5; 2.5; 1.25; 0.625; 0.312 µg/mL), then absorbances were measured at 517 nm after 30 minutes incubation in the dark. Three tests were carried out for each concentration of extract tested.

The antioxidant activity associated with the DPPH radical scavenging effect is expressed as percentage inhibition (PI) using the following formula:

$$PI = 100(A_0 - A_1)$$

A_0 : DPPH absorbance

A_1 : sample absorbance

Determination of antioxidant activity (FRAP test)

The reducing power of the extracts was determined by the FRAP method described by [14]. To do this, a 0.4 mL volume of each extract previously prepared at different concentrations was mixed with 1 mL phosphate buffer (0.2 M; pH=6.6) and 1 mL 1% potassium hexacyanoferrate [K3Fe(CN)6]. The mixture was then incubated at 50°C for 30 minutes. After this incubation time, 1 mL of 10% trichloroacetic acid was added, then the tubes were centrifuged at 3,000 rpm for 10 minutes. Again, a 1 mL volume of the supernatant from each tube was mixed with 0.2 mL of 0.1% $FeCl_3$ and allowed to stand in the dark for 30 minutes before measuring absorbances at 700 nm. Antioxidant activity related to the reducing power of extracts is expressed as Reducing Power (RP) using the following formula:

$$PR = 100(Ae-Ab)/Aa$$

Ae: absorbance of extract

Ab: absorbance of white

RESULTS

Determination of flavonoids in various extracts

The results of the quantitative flavonoid assay are shown in Figure 1 and expressed as milligram quercetin equivalent per gram of dry matter (mgEQ/g DM). The results show that flavonoid content is higher in the hydroethanol extract (314.51 ± 5.14 mgEQ/g DM) than in the aqueous extract (135.60 ± 2.47/mgEQ/g DM).

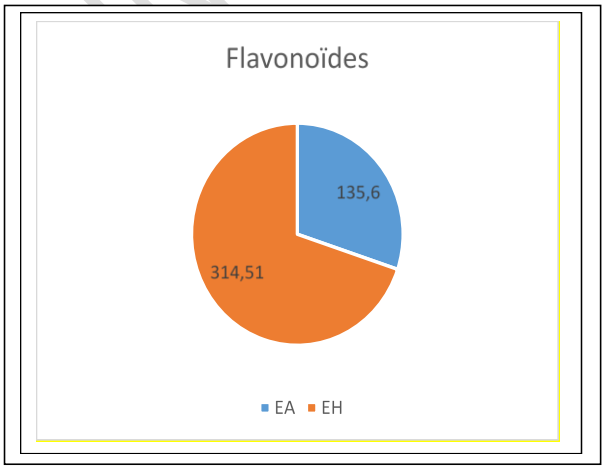

Figure 1: Flavonoid content of different extracts.

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EA: Extrait Aqueux; EH: Extrait Hydro-éthanolique

Determination of antioxidant activity

DPPH test

The results of the two figures (2 and 3) reveal that, in general, DPPH radical inhibition is significant and depends on the extract concentrations (aqueous or hydroethanol) tested. Thus, it can be said that all extract concentrations used are dose-dependent. The hydro-ethanolic extract showed greater activity than the aqueous extract: at a concentration of 5 µg/mL of hydro-ethanolic extract, inhibition was 78.69 ± 1.34%, and 82.07 ± 1.14% at a concentration of 10 µg/mL. The aqueous extract, on the other hand, showed 75.99 ± 1.02% inhibition at 10 µg/mL (the highest concentration). To better appreciate the antioxidant activity of the extracts, their IC_{50} are determined from the straight line equation (y=ax + b). Thus, the IC_{50} of the hydroethanol extract is lower than that of the aqueous extract, with values of 1.19 ± 0.03 µg/ mL and 2.13 ± 0.01 µg/ mL respectively.

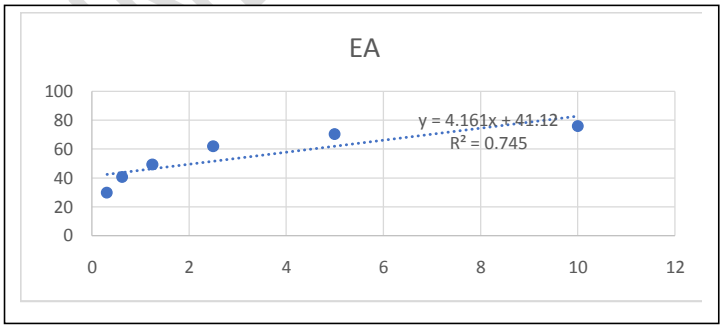

Figure 2: Percentage inhibition of aqueous extract (EA)

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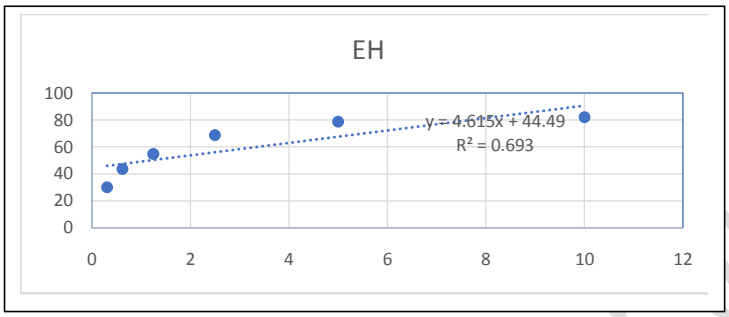

Figure 3: Percentage inhibition of hydroethanol extract (HE)

FRAP test

The results of the FRAP test revealed that the reducing power of the hydro-ethanolic extract was better than that of the aqueous extract. In fact, whatever the extract concentration used (aqueous or hydro-ethanolic), it was found that the aqueous extract had a lower reducing power than the hydro-ethanolic extract. At a concentration of 0.312 µgEAA/mL of aqueous extract and hydroethanol extract, the reducing power is 163.978 ± 0.92% and 175.301 ± 0.98% respectively. Similarly, for concentrations ranging from 0.625; 1.25; 2.5; 5 and 10 µgEAA/mL of aqueous extract and hydro-ethanolic extract, respective reducing powers of 200.476 ± 2.03%; 243.147 ± 0.54% ; 305.712 ± 1.63%; 378.664 ± 0.52% and 435.864 ± 0.23% respectively for the aqueous extract and 226.436 ± 0.94%; 274.356 ± 0.44%; 340.653 ± 0.10%; 390.884 ± 1.12% and 353.679 ± 2.10% for the hydroethanol extract (Figure 4).

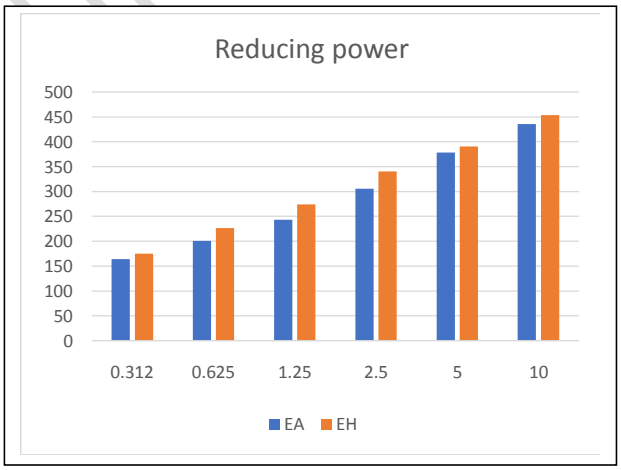

Figure 4:Reducing power of different extracts

EA: Extrait Aqueux; EH: Extrait Hydro-éthanolique

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DISCUSSION

The use of aqueous and hydro-ethanolic extracts has made it possible to extract polar compounds such as flavonoids, which are among the main components of medicinal plants with antioxidant activity, from the basic plant product [15, 1]. These results corroborate the work carried out by [16] on *Piliostigma thonnigii* leaves. However, determination of flavonoid content by the aluminum trichloride method for each extract revealed that the hydroethanol extract had more than double the flavonoid content of the aqueous extract, i.e. 314.51 ± 5.14 mgEqQ/gMS and 135.60 ± 2.47 mgEqQ/gMS respectively. From the flavonoid assay data, it can already be said that flavonoids are predominantly present in both extracts (aqueous and hydro-ethanolic) of the base plant product, with a strong predominance in the hydro-ethanolic extract. This latter observation

is in line with the results of [17] and [18]. Indeed, these authors also found that flavonoid content was higher in the methanolic extract than in the aqueous extract. The results of their study, as well as these, indicate that alcoholic solvents (ethanol and methanol) concentrate more of this phenolic compound than aqueous extract.

Evaluation of the antioxidant activity of the hydro-ethanol and aqueous extracts of the base mixture using a spectrophotometer at 517nm showed that the hydro-ethanol extract exhibited greater antioxidant power in a dose-dependent manner. In fact, according to [19], this reduction capacity is determined by the decrease in absorbance induced by antiradical substances. The antioxidant power results of the various extracts tested at different concentrations in this study showed that the percentage inhibition of the hydroethanol extract was higher than that of the

aqueous extract, whatever the concentration used. These results are in line with those of [19]. Indeed, these authors showed that at all concentrations tested, hydro-ethanolic extracts of *Piliostigma thonningii* leaves and bark significantly inhibited the $DPPH^{\cdot}$ radical in a dose-dependent manner. In this study, the CI values$_{50}$ of each extract determined revealed that the aqueous extract is endowed with a moderate antioxidant power than that of the hydro-ethanolic extract with respective values of 1.19 and 2.13. Antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins have been shown to reduce and decolorize DPPH through their ability to release hydrogen [20]. Polyphenols and in particular flavonoids contained in base product extracts are probably responsible for antioxidant activity. This assertion is in line with work carried out by [21] on extracts of *Irvingia gabonensis*, a species rich in phenolic compounds which are responsible for numerous biological activities including antioxidant and anti-inflammatory activity.

A further assessment of the antioxidant activity of the hydroethanol and aqueous extracts of the base product was also possible using the method of [14] (the FRAP test). This is a simple, rapid and reproducible test. During this test, the reduction of Fe^{3+} /ferricyanide complex to the ferrous form (Fe^{2+}) is triggered by the presence of reducing agents in the plant extracts. According to [22], the ferrous ion (Fe^{2+}) can be assessed by measuring and monitoring the increase in blue color density in the reaction medium at 700 nm. This method revealed that the reducing powers of the base product extracts are dose-dependent. This reducing power of the base product is probably due to the presence of hydroxyl groups in flavonoids (phenolic compounds). This presence of hydroxyl groups could act as electron donors. According to [23], antioxidants can be considered as reducers and inactivators of oxidants. Some previous studies have also suggested that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity [24 , 1].

CONCLUSION

The present study demonstrated the antioxidant activity of aqueous and hydroethanol extracts of the leaves of the mixture *Ficus capensis*, *Newbouldia laevis* and *Carpolobia lutea* (base plant product). According to the results obtained in this study, the basic plant product is a potential

source of flavonoids with naturally-occurring antioxidant properties, justifying its traditional use in the treatment of numerous ailments linked to oxidative stress. However, the hydro-ethanolic extract showed the highest flavonoid content, very high DPPH free radical scavenging activity and high iron reduction capacity. These results could form the basis for research into new compounds with applications in the pharmaceutical industry.

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