PHYTOCHEMICAL EVALUATION AND DETERMINATION OF THE CONTENT OF SOME NATURAL ANTIOXIDANTS (POLYPHENOLS AND FLAVONOIDS) FROM *FICUS CAPENSIS*, *NEWBOULDIA LAEVIS* AND *CARPOLOBIA LUTEA*

Abstract

Scientific interest in the search for natural antioxidants (secondary metabolites) from medicinal plants has increased considerably in recent years, due to the involvement of free radicals in the genesis of many diseases. The aim of the present study is to determine qualitatively and quantitatively the secondary metabolites of aqueous and hydroethanol extracts from the leaves of the medicinal plants under investigation. Qualitative determination is based on staining and/or precipitation reactions, while quantitative determination is based on the assay of secondary metabolites. The results of their qualitative determinations revealed an abundance of polyphenols and saponins, a significant presence of flavonoids and anthocyanins, and a moderate presence of alkaloids, terpenes and catechic tannins in the aqueous extract. In the hydroethanol extract, on the other hand, there is an abundance of flavonoids, a significant presence of polyphenols, saponins and anthocyanins, and a moderate presence of alkaloids, terpenes and catechic tannins. Gallic tannins and quinones are absent in both extracts. While their quantitative assay revealed an abundance of flavonoids (314.51±5.14 mgEAG/g extract) and total polyphenols (66.19±2.44 mgEQ/g extract). Thus, the high flavonoid and polyphenol content of the various extracts (aqueous and hydroethanolic) of the leaves of the plants studied would justify their use in traditional medicine in the care of sickle-cell patients.

Key words: Medicinal plants, polyphenols, flavonoids, sickle cell disease.

1. INTRODUCTION

For centuries, natural products have been an important source of new active compounds used to treat a wide range of diseases [1]. A number of studies have been carried out on the biological properties of extracts from certain medicinal plants, leading to the discovery of numerous active ingredients used in modern medicine [2]. Examples include quinine, theophylline, morphine and vitamin A. It is estimated that over half of all bioactive molecules in clinical use come from medicinal plants [3]. In fact, the various plant organs take part in variable physico-chemical and metabolic reactions. These metabolic variabilities are physiological adaptation responses to

environmental conditions and stresses [4]. In addition, the aerial parts of plants are the seat of photosynthesis and are exposed to solar stresses, including ultraviolet rays. All these stresses can result in the formation of free radicals, leading to the synthesis of distress substances that can cause leaf wilting, leading to plant dieback and/or death. Similarly, free radicals can also affect the genetic integrity and stability of plants [4]. However, despite constant stress, plants can survive, grow and multiply by synthesizing anti-free radical substances. Plants are therefore natural sources of antioxidant substances [5]. However, the plant kingdom is not the only one stressed by abiotic and biotic factors: the human organism produces free radicals on a daily basis, which are unstable, highly reactive compounds containing a single electron. Because of their stability, free radicals attack certain biological molecules, creating other free radicals and triggering chain reactions that damage numerous cellular components such as proteins, lipids and DNA. These free radicals are thought to be at the root of certain chronic diseases (cardiovascular, cancerous and neurodegenerative), as well as ageing. Thus, the ultimate cause of most pathologies is free radicals [6]. The role of free radical scavengers is to reduce the harmfulness of free radicals, stopping the process by neutralizing these highly reactive species. For some years now, these scavengers have been synthetic antioxidant molecules. However, the use of synthetic antioxidant molecules is currently being called into question because of the potential toxicological risks they may pose. This is why the active search for new sources of natural antioxidants is of the utmost importance [7]. In this case, the focus is increasingly on medicinal plants as sources of bioactive substances.

The use of medicinal plants for their therapeutic, cosmetic, chemical, dietary, pharmaceutical, agri-food and industrial properties is an ancient practice [8]. In fact, according to Béné*et al* [9], medicinal plants are part of the history of every continent. Also, according to the World Health Organization (WHO) in 2013, around 80% of people in developing countries rely on traditional medicine, particularly herbal medicine, for their health care needs. Africa's floristic heritage is rich in medicinal plants with proven efficacy. Indeed, it has been shown that the continent is home to almost 5,000 medicinal species [10-13]. What's more, research continues to show that medicinal plants contain a variety of biologically active chemical principles with different pharmacological activities: antioxidant, anti-inflammatory, analgesic, etc. [12-14].

With this in mind, this study examined the antioxidant activity of various extracts from the leaves of (*Ficus capensis*; *Newbouldia laevis* and *Carpolobia lutea*). These medicinal plants

have always been used in the Department of Daloa (west-central Côte d'Ivoire) by many traditional practitioners to treat people suffering from sickle-cell anemia.

2. MATERIALS AND METHODS

2.1 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.

2.2 PREPARATION METHODS FOR THE VARIOUS EXTRACTS

2.2.1 Preparing the aqueous extract

The aqueous extract of the basic plant product was prepared according to the method of Zirihi*et al* [15]. 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.

2.2.2 Preparation of hydroethanol extract

The hydroethanol extract of the basic plant product was prepared according to the method of Zirihi*et al* [15]. 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 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.

2.3 Yield calculation

The various yields were calculated using the following formula:

$$\text{Yield (\%)} = \left(\frac{\text{quatité de matiere seche extraite}}{\text{quatité de matiere utilisé}}\right) X100$$

2.4 Qualitative study or phytochemical screening of different extracts

2.4.1 Alkaloid research

Burchard and Dragendorff reagents were used, and 6 mL of each extract was evaporated to dryness. The residue was taken up with 6 mL of alcohol (ethanol) at 60°C. The addition of two drops of Dragendorff's reagent to the alcohol (ethanol) solution produced an orange coloration. Then, the addition of two drops of Burchard's reagent to the previous solution produced a precipitate that indicated the presence of alkaloids [16].

2.4.2 Polyphenol research

Polyphenols were identified by the ferric chloride reaction. A drop of aqueous ferric chloride solution was added to 2 mL of plant extract. A bluish-black or green coloration of varying intensity then indicated the presence of phenolic compounds [17].

2.4.3 Flavonoid research

Flavonoids were determined by the cyanidine reaction. To this end, 2 mL of each extract was evaporated and the residue taken up in 5 mL of twice-diluted hydrochloric alcohol (ethanol). Next, 2 to 3 magnesium chips were added, followed by heat release and a pink-orange or purplish coloration. Next, 3 drops of isoamyl alcohol (ethanol) were added, which either intensified the coloration or did not. This last observation confirmed the presence or absence of flavonoids [18].

2.4.4 Saponin analysis

For their research, 10 mL of the total aqueous extract was placed in a test tube. The tube was shaken for ten seconds and left to stand for ten minutes. A persistent foam height of around 3 cm indicates the presence of saponins [19].

2.4.5 Tannin research

To test for catechic tannins, 5 mL of each extract was evaporated to dryness. Next, 15 mL of Stiasny's reagent was added to the residue. The mixture was then kept in a water bath at 80°C for 30 minutes. The presence of catechic tannins was revealed by the observation of a coarse flake precipitate. As for the gallic tannins, the previous solution was filtered and the filtrate saturated with sodium acetate. The subsequent addition of three drops of $FeCl_3$ produced an intense blue-black coloration, indicating the presence of gallic tannins [16].

2.4.6 Testing for quinone substances

These substances were detected by evaporating 2 mL of each extract to dryness. The residue was triturated in 5 mL hydrochloric acid diluted 1:5. The triturate was placed in a test tube and heated in a water bath at 80°C for 30 minutes. After cooling, it was extracted with 10 mL chloroform and 0.5 mL ammonia was added to the chloroform solution. Red or violet coloration revealed the presence of quinones [16].

2.4.7 Terpene research

This was carried out using Liebermann's reagent. A 5 mL volume of each extract was evaporated to dryness. The residue obtained was then dissolved hot in 1 mL acetic anhydride, then 0.5 mL concentrated sulfuric acid was added to the triturate. The appearance of a purple or violet ring at the interface, turning blue and then green, revealed the presence of terpenes [17].

2.5 Quantitative study

2.5.1 Determination of total polyphenols in different extracts

For this study, a 1 mL volume of Folin's reagent (10-fold diluted) was added to 200µL of sample (aqueous or hydroethanol extracts) or standard (prepared in ethanol) with suitable dilutions. After 4 minutes, 500µL of sodium carbonate solution (75 mg/mL) was added to the reaction medium. The mixture was then vortexed for ten (10) seconds and incubated at 40°C for 30 minutes. Then, after 1 hour incubation at room temperature, absorbance was measured at 760 nm. The concentration of total polyphenols was calculated from the regression equation of the calibration range established with gallic acid, and expressed inµgof gallic acid equivalent per milligram of extract (µgGAE/mg extract).

2.5.2Dosage of flavonoids from different extracts

The total flavonoid content of each extract was determined using the aluminum trichloride colorimetric method [20]. 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 of $Na2CO_3$ (1 M) solution was then added to the previous mixture, followed by 0.25 mL of distilled water after a 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).

3. RESULTS

3.1 Performance

Table I shows the yield of the different extracts (aqueous and hydroethanolic) per 100 g of the base plant product. The aqueous extract yielded 19.02%, while the hydroethanol extract yielded 12.56%. The percentage of the aqueous extract is therefore higher than that of the hydro-ethanolic extract.

Table I: Yields of aqueous and hydroethanol extracts

Extraction solvent	Extract mass (g)	Extract yield (%)
Water	19,02	19,02
Ethanol-water (70/30: V/V)	12,56	12,56

3.2 Qualitative study or phytochemical screening of different extracts

The results of phytochemical screening of extracts of the basic plant product are shown in Table II. They reveal that the various extracts prepared contain alkaloids, polyphenols, terpenes, catechic tannins, flavonoids, saponins and anthocyanins in varying proportions. However, they are characterized by the absence of gall tannins and quinones. The aqueous extract, for example, contains an abundance of polyphenols and saponins, a significant presence of flavonoids and anthocyanins, and a moderate presence of alkaloids, terpenes and catechic tannins. The hydroethanol extract showed abundant flavonoids, significant polyphenols, saponins and anthocyanins, and moderate alkaloids, terpenes and catechic tannins.

Table II: Phytochemical screening results

		Extracts	
		Aqueous (EA)	Hydro-ethanolic (EH)
Chemical groups	alkaloids	+	+
	polyphenols	+++	++
	terpenes	+	+
	catechic tannins	+	+
	gallic tannins	-	-
	flavonoids	++	+++
	quinones	-	-
	saponins	+++	++
	anthocyanins	++	++

+++ : Abundance,

++: Significantpresence,

+ : Moderatepresence,

- Absence.

3.3 Quantitative study

3.3.1 Determination of total polyphenols in various extracts

Figure 1 shows the general distribution of polyphenols measured in the two extracts. Specifically, it shows that the value of polyphenols measured in the aqueous extract is 66.19 ± 2.44 mgEAG/gDM (gallic acid equivalent per g dry matter), whereas it is 63.04 ± 2.81 mgEAG/gDM in the hydroethanol extract. This chemical compound is therefore relatively more abundant in the aqueous extract than in the hydroethanol extract.

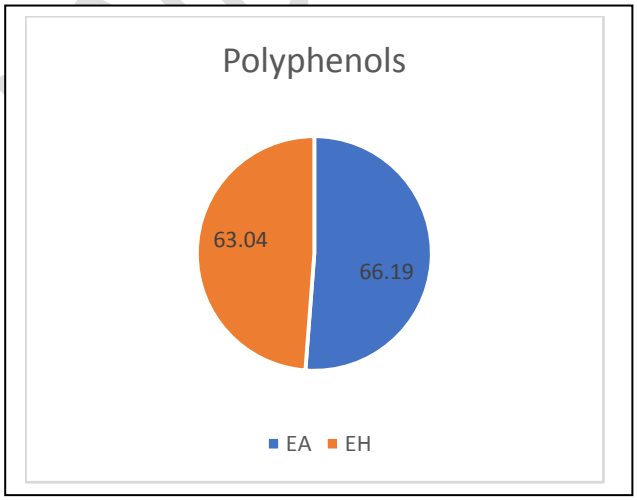

Figure 1: Polyphenol content of different extracts.

EA: Extrait Aqueux; EH: Extrait Hydroéthanolique

3.3.2Dosage of flavonoids from different extracts

The results of the quantitative flavonoid assay are shown in Figure 2, expressed as milligram quercetin equivalent per gram of dry matter (mgEQ/gMS). Generally speaking, they show that flavonoids are present in both extracts, but in different proportions. Their assay showed that the flavonoid content in the hydroethanol extract was 314.51 ± 5.14 mgEqQ/gMS, whereas it was 135.60 ± 2.47 mgEqQ/gMS in the aqueous extract. Thus, this organic compound is more abundant in the hydro-ethanolic extract than in the aqueous extract.

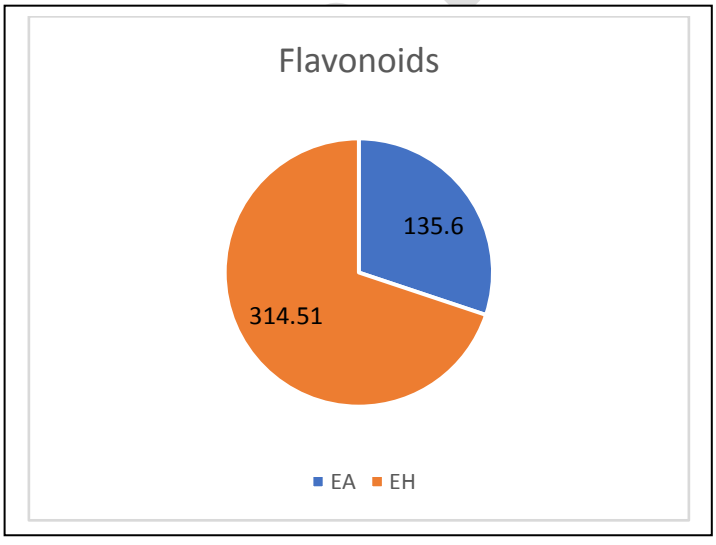

Figure 2: Flavonoid content of different extracts.

EA: Extrait Aqueux; EH: Extrait Hydro-éthanolique

4. DISCUSSION

Extraction of the base plant product with distilled water gave a high yield of 19.02%, compared with 12.56% for extraction with the ethanol-water mixture (70/30 : V/V). These results are broadly comparable to those obtained by Ibrahim *et al* [21], who obtained a yield of 24.25% with distilled water extraction of the whole plant of *C. benghalensis*, compared with 12.56% with methanol. According to Ibrahim *et al* [21], the higher extraction value with distilled water (24.25%) than with methanol (12.56%) suggests that distilled water is a better extraction solvent for the plant studied than methanol. The results obtained in this study follow the same principle. In addition, the work of Okou [11] states that, in general, the use of a given solvent for the extraction of bioactive substances from a medicinal plant can enhance one group of active compounds over another. Thus, according to these same authors, water can be used to isolate bioactive substances such as anthocyanins, tannins, saponins, etc., while ethanol can be used to highlight tannins, polyphenols, polyacetylenes, etc. Furthermore, according to Okou *et al. [*12], the use of distilled water (less polar) in an extraction of medicinal plants also isolates macromolecules like the aforementioned compounds, unlike ethanol (more polar), which cannot highlight these macromolecules apart from the compounds initially mentioned (as they are less soluble in ethanol). Consequently, the work of the latter authors could justify the high yield of distilled water extraction in this study, as in that of Ibrahim *et al* [21].

Phytochemical screening revealed an abundance of polyphenols and saponins in the aqueous extract, flavonoids in the hydroethanol extract, and a significant presence of flavonoids and anthocyanins in the aqueous extract, and polyphenols, saponins and anthocyanins in the hydroethanol extract, and a moderate presence of alkaloids, terpenes and catechic tannins in both the aqueous and hydroethanol extracts. These results corroborate those of Okou [11] as previously cited. Similarly, the work of Okou [11] showed that not only can a solvent isolate specific bioactive compound, but the combination of solvents can reveal a type of bioactive substance that is soluble in both solvents. These latter observations could explain the non-negligible presence of anthocyanins in both the aqueous and hydro-ethanol extracts, and the moderate presence of alkaloids, terpenes and catechic tannins in both the aqueous and hydro-ethanol extracts. Thus, phyochemical screening showed that both extracts contain secondary metabolites and the results of this study are similar to those of Piba*et al [*22] who worked on *Ficus mucoso; Zizyphusmauritiana*and *Tamarindus indica*. According to Pooja *et al* [23], the

richness of these extracts in active chemical compounds could confirm the antioxidant, antihypertensive and anti-inflammatory properties of medicinal plants. Also according to Krief [24]; Yezza and Bouchama [25], all the above-mentioned organic compounds belong to the three major groups of secondary metabolites (phenolic compounds, alkaloids and terpenoids) which possess a wide range of activities (cardiovascular system, immunology, diabetes, stress-related diseases, antimicrobial and antiviral activities, etc.) in human biology.

Quantitative assay of the two extracts showed that the aqueous extract contained 66.19 ± 2.44 mgEAG/gMS of polyphenols, compared with 63.04 ± 2.81 mgEAG/gMS of the same compound in the hydroethanol extract. These results show that polyphenols are relatively more abundant in the aqueous extract than in the hydroethanol extract. However, the value of total polyphenols found in the aqueous extract of the basic plant product in this study is higher than the content obtained by Meziti [26], which is 27.07 ± 0.58 mgEAG/g, and that of Boudiaf [27], which is 23.81 ± 2.67 mgEAG/mg in the aqueous extraction of *Nigella sativa*. Similarly, the polyphenol assay value of the hydroethanol extract of the base product in this study is much higher than that found by Meziti (2009) [26], which is 33.64 ± 0.34 mg EAG/g. On the other hand, these results are below the value obtained by Boudiaf [27] when extracting *Nigella sativa* with chloroform (191.06 ± 23.34 mgEAG/g). Quantitative determination of flavonoids revealed that the aqueous extract contained 135.60 ± 2.47 mgEqQ/gMS, compared with 314.51 ± 5.14 mgEqQ/gMS for the hydroethanol extract of the base plant product. These results therefore show that the hydro-ethanolic extract contains more flavonoids than the aqueous extract. On the basis of the polyphenol and flavonoid assay data, it can be said that flavonoids are present to a greater extent than total polyphenols in both extracts (aqueous and hydro-ethanolic) of the base plant product. Furthermore, the sum of the two chemical compounds (flavonoids and polyphenols) that are phenolic compounds in a given extract shows that the hydro-ethanolic extract contains more of these phenolic compounds (flavonoids and polyphenols) than the aqueous extract. This latter observation is in line with the results of work by Meziti [26] and Boudiaf [27], who also found that the phenolic compound content (flavonoids and polyphenols) was higher in the methanolic extract than in the aqueous extract. This shows that alcoholic solvents (ethanol and methanol) concentrate these phenolic compounds more than aqueous extracts.

5. CONCLUSION

The present study has shown that distilled water is a better solvent for extracting secondary and primary metabolites than ethanol for the base plant product prepared. In addition, phytochemical screening of both extracts revealed the presence of numerous secondary metabolites. This presence is characterized by an abundance of polyphenols and saponins in the aqueous extract, and flavonoids in the hydro-ethanolic extract, a significant presence of flavonoids and anthocyanins in the aqueous extract, polyphenols, saponins and anthocyanins in the hydroethanol extract, and a moderate presence of alkaloids, terpenes and catechic tannins in both the aqueous and hydroethanol extracts. As for the quantitative assay, it revealed a relative abundance of polyphenols in the aqueous extract than in the hydro-ethanolic extract, in contrast to an abundance of flavonoids in the hydro-ethanolic extract than in the aqueous extract. Moreover, flavonoids and polyphenols (phenolic compounds) are more accumulated in the hydro-ethanolic extract than in the aqueous extract.

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