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Evaluation of phytochemical compounds in the leaves and fruits of *Alchornea cordifolia* in three localities in Côte d'Ivoire

Zioh Nonhondé Horline Degrâce [1,*], Zouzou Souné Carole [1], Traoré Fakana Drissa [1], Kouamé Kan Benjamin [1] and Bolou Sahi Rosly [2]

[1] *Agro-valorization Laboratory, Jean Lorougnon Guédé University, UFR Agroforesterie, BP 150 Daloa, Côte d'Ivoire.*
[2] *Laboratory of Nutrition and Food Technology, Institut National Polytechnique Félix Houphouët-Boigny (INP-HB), BP 1093 Yamoussoukro, Côte d'Ivoire.*

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Abstract

Alchornea cordifolia is a plant of the *Euphorbiaceae* family used for its therapeutic properties in Africa. The objective is to enhance the value of the leaves and fruits of *Alchornea cordifolia* by studying phytochemical parameters. The plant material consists of fruits and leaves of *Alchornea cordifolia* for phytochemical screening by the Evans method, followed by the determination of total polyphenols, total flavonoids, total tannins, condensed tannins and anthocyanins by the spectrophotometric method. The results showed the presence of flavonoids, polyphenols, tannins, saponins, alkaloids. The highest total polyphenol content was found in mature leaves (3.34 mg EAG/g). At the total flavonoid level, the most concentrated mature leaves contained 0.85 mg EQ/g. For condensed and total tannins, the highest levels were 0.99 mg EC/g and 1.94 mg EAT/g respectively in fruit. Finally, in terms of anthocyanin content, mature leaves had the highest content (5.53 C3GE/g). The presence of secondary metabolisms in the Alchornea cordifolia plant justifies its use from a therapeutic point of view.

Keywords: *Alchornea cordifolia*; Therapeutic properties; Leaves and Fruits; Phytochemical parameters

1. Introduction

Wild plants, also known as spontaneous plants, are an important part of everyday life in rural households and play an important role in the subsistence of a large proportion of the world's population [1]. In tropical countries, a great deal of attention is paid to the role of wild plants in the diet and health of rural populations [2,3]. Wild plants also make significant contributions to food security and health by providing a wide range of essential nutrients for humans. As a result, they are one of the mainstays of diets and healthcare treatments in developing countries and an important source of income for rural populations [2,3].

Alchornea cordifolia (Schumach. & Thonn.) Müell. Arg. is a climbing plant or shrub of the *Euphorbiaceae* family, which is generally distributed in forest scrub, in secondary thickets, in forest galleries in savannahs, in fallow land, on relatively wet soils and forms part of the coastal vegetation made up of isolated shrubs in Côte d'Ivoire [4,5]. *Alchornea cordifolia* leaves contain bioactive compounds such as tannins, alkaloids and saponins, which have been shown to have antimicrobial properties, including inhibition of the growth of *Salmonella typhi*, *Escherichia coli* and *Shigella*. [6]. Consequently, the choice of the nutritional and technological valorisation of the leaves and fruits of *Alchornea cordifolia* Schum in order to show its nutritional particularity lies in the fact that little work has been done on this in Côte d'Ivoire. The aim of this study is to assess the phytochemical compounds in the leaves and fruits of *Alchornea cordifolia* harvested in three regions in order to enhance the value of natural resources and understand their therapeutic potential

* Corresponding author: Zioh Nonhondé Horline Degrâce

2. Material and methods

2.1. Biological material

The biological material used in this study consisted of leaves (young, mature and dried) and fruits of *Alchornea cordifolia* collected in Daloa, Seguela and Yamoussoukro (Figure 1).

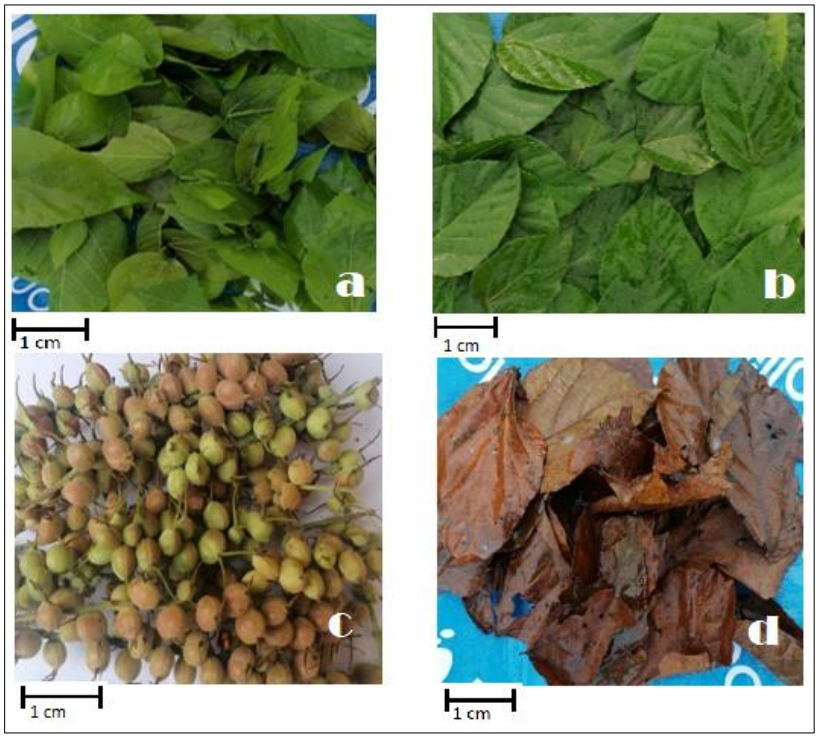

Figure 1 Leaves and fruit of *Alchornea cordifolia* a. young leaves b. mature leaves c. fruit d. dried leaves

2.2. Sampling

Leaf-by-leaf sampling of *Alchornea cordifolia* was carried out from 7 a.m. in the period February to March and September to October. Leaves (young, mature and dried) and fruit were collected and placed in plastic bags on which the stage of maturity and sampling location were indicated. They were then transported to the Agro-valorization Laboratory at the Jean Lorougnon Guédé University in Daloa.

2.3. Production of *Alchornea cordifolia* leaf and fruit powder

The samples were washed with tap water and dried in the shade at room temperature (25-30°C) for a fortnight. 350 g of each leaf (young, mature, dried) and fruit were taken, ground using a grinder and sieved through a 200 micron sieve to give a fine powder. This fine powder was packaged in labelled bags and stored at room temperature for the various analyses.

2.4. Phytochemical screening of *Alchornea cordifolia* leaves

Phytochemical screening was used to identify the various compounds present in *Alchornea cordifolia* powder. Phytochemical compounds were determined using the method developed by Evans [7]. The results were classified according to the reaction obtained (positive or negative).

2.4.1. Identification of tannins

Reaction with 1% ferric chloride

A volume of 1 mL of the plant powder contained in a test tube was added to a volume of 2 mL of distilled water. The resulting mixture was stirred until a homogeneous solution was obtained. The presence of tannins in the plant powder

extracts was determined directly after the addition of one or two drops of 1% (w/v) ferric chloride. The appearance of a blue or blue-black colour indicates the presence of tannins.

2.4.2. Alkaloid research

Mayer test

The Mayer test was used to determine the presence of alkaloids in the leaves and fruit of *Alchornea cordifolia*. To do this, a few drops of Mayer's reagent were added to a 2 mL volume of the plant powder solution. The formation of a white or white-yellow precipitate indicates the presence of alkaloids.

2.4.3. Research into saponins

Saponins from *Alchornea cordifolia* leaves were determined by the presence of persistent foam. A volume of 3 mL of distilled water was added to a volume of 2 mL of crushed dry plant material in a test tube. The persistent formation of foam after manual shaking of the test tube indicates the presence of saponins.

2.4.4. Identification of polyphenols

Polyphenols were identified in the presence of a 2% (w/v) alcoholic or ferric chloride solution. One drop of alcoholic or aqueous 2% (w/v) ferric chloride solution was added to a 2 mL volume of the plant powder. The positive reaction was shown by the appearance of a blue-black or green or dark coloration compared with a phenol solution (control).

2.4.5. Flavonoid research

Sodium hydroxide test

0.1N sodium hydroxide (NaOH) was used to test for the presence of flavonoids. Three drops of the 0.1N sodium hydroxide solution were added to 3 mL of crushed dry plant material in a test tube. The appearance of a yellow-orange colour indicates the presence of flavonoids.

2.5. Quantification of phenolic compounds

2.5.1. Determination of total polyphenols

The total polyphenol content of *Alchornea cordifolia* samples was determined using the method described by Wood and al [8]. The extract solution was obtained after maceration of 1 g of each sample in 50 mL of distilled water for 10 min. A volume of 2.5 mL of Folin-Ciocalteu reagent diluted to one tenth was added to 30 µL of extract. The mixture was kept for 2 minutes in the dark at room temperature (25°C), then 2 mL of sodium carbonate (75 g/L) was added. The mixture was then placed in a water bath at 50°C for 15 minutes and rapidly cooled. Absorbance was measured at 760 nm using a spectrophotometer, with distilled water as the blank. A calibration line was constructed with gallic acid at concentrations ranging from 0 to 0.200 mg/mL. Analyses were carried out in three replicates and the total polyphenol content was expressed in grams of gallic acid equivalent per litre (g GAE/L) of extract. The results obtained by the spectrophotometer can be converted into gram equivalents of gallic acid per gram of sample (mg GAE/g) by multiplying by the dilution factor. The total polyphenol content was estimated using the following formula :

$$\text{Content polyphenols (mg EAG /g)} = \text{C.ext} \times \text{fd}$$

With :
EAG = gallic acid equivalent;
C.ext = concentration of the extract in mg/mL obtained using a spectrophotometer at 510 nm ;
fd = dilution factor

2.5.2. Determination of total flavonoids

The determination of total flavonoids was carried out according to the method described by Marinova and al. [9]. The extract solution was obtained after maceration of 1 g of each *Alchornea cordifolia* sample in 50 mL of distilled water for 10 min. To a 25 mL flask was added 2.5 mL of extract, 0.75 mL of 5% (w/v) sodium nitrite ($NaNO_2$). To the mixture, 0.75 mL of 10% (w/v) aluminium chloride ($AlCl_2$) was added. The mixture was incubated for 6 minutes in the dark. After 6 minutes, 5 mL of sodium hydroxide (1N NaOH) was added and the volume was made up to 25 mL with distilled

water. The preparation was vigorously shaken using a magnetic stirrer (C-MAG HS10 IKA, France) before the total flavonoids were determined at 510 nm using a spectrophotometer. The total flavonoid content was expressed in grams of quercetin equivalent per litre of extract (g EQ/L). Trials were carried out in three replicates on fresh or dried pepper samples. The results were converted to gram quercetin equivalent per gram of sample (mg EQ/g) by multiplying by a dilution factor. The total flavonoid content was assessed using the following formula :

$$\text{Content flavonoids (mg EQ /g)} = \text{C.ext} \times \text{fd}$$

EQ : quercetin equivalent;
C.ext : concentration of extract in mg/mL obtained using a spectrophotometer at 510 nm ;
fd : dilution factor

2.5.3. Anthocyanin determination

The anthocyanin content was determined using the method described by Lee and al [10]. 1 mL of each sample was diluted in 1.5 mL of pH 1.0 buffer and 1.5 mL of pH 4.5 buffer respectively. The absorbance (A) of these two solutions was read at 520 nanometres and 700 nanometres respectively using a spectrophotometer (JASCO V-530, Japan). The concentration of total anthocyanins (CAT) expressed in mg/mL of cyanidin-3-glycoside equivalent is given by the formula :

$$\text{CAT} = \text{A} \times \text{PM} \times 10^3 / \varepsilon \times \text{L}$$

with:
A : (A520 nm - A700 nm) pH 1.0 - (A520 nm - A700 nm) pH 4.5
PM : Molecular weight of cyanidin-3-glucoside (cyd-3-glu) = 449.2 g / mol
F : Dilution factor = 1.5
L : Length of cell = 1cm
ε : Molar extinction coefficient = 26 900 L. mol-1.cm

103: conversion factor from g to mg.

2.5.4. Determination of condensed tannin content

Condensed tannin content was modified by Heimler and al [11]. The method is based on the ability of vanillin to react with the terminal flavonoid group of condensed tannins to form red coloured complexes [12]. In a test tube containing 50 µL of extract solution (5 mg/mL), 3 mL of a 4% methanolic vanillin solution was added. Then 1.5 mL of 37% concentrated hydrochloric acid (HCl) was added. The mixture was incubated in the dark for 15 min at laboratory temperature (25-30°C). The absorbance was then read using a UV/visible spectrophotometer at 500 nm against a MeOH blank. Catechin was used as the standard. The condensed tannin content of the extracts was determined using the catechin calibration curve established from different concentrations (0 ; 1.95; 3.91; 7.81; 15.625; 31.25; 62.5; 125 ; 250 ; 500 and 1000 µg/mL). Levels are expressed in milligrams of catechin equivalent per gram of dry matter (mg CE/g). They were calculated on the basis of the relationship below:

$$Q_{tan}(mg\ EC/g\ de\ MS) = \frac{Conc_{spect} \times FD \times R}{Conc_{ext} \times 100}$$

With :
$Conc_{spect}$: concentration measured by the spectrophotometer.
FD: dilution factor.
R: extract yield.
$Conc_{ext}$: concentration of extract used for analysis

2.5.5. Determination of total tannin content

The determination of total tannins was carried out using the colorimetric method with Folin Ciocalteu reagent as described by Ci and Indira [13] 100µL of extract solution (5mg/mL) was added to a test tube containing 7.5mL of distilled water and 0.5mL of Folin Ciocalteu reagent. Next, 1mL of 35% Na2CO3 was added. The volume was made up to 10mL by adding 900µL of distilled water. The reaction mixture was stirred and left to react for 30 minutes at laboratory temperature (25-30°C). The absorbances were read with a UV/visible spectrophotometer at 700 nm against distilled water used as a blank. A calibration line was drawn from a range of tannic acid concentrations (0 ; 1.95; 3.9; 7.81; 15.62; 31.25; 62.5; 125 ; 250 ; 500 and 1000 µg/mL) used as a standard. Levels are expressed in milligrams of tannic acid equivalent per gram of dry extract (mg EAT/g). They were calculated from the relationship below:

$$Q_{tan}(mg\ EAT/g\ de\ MS) = \frac{Conc_{spect} \times FD \times R}{Conc_{ext} \times 100}$$

With :
$Conc_{spect}$: concentration measured by the spectrophotometer.
FD: dilution factor.
R: extract yield.
$Conc_{ext}$: concentration of extract used for analysis.

2.6. Statistical analysis

Statistical analysis was carried out using Statistica 7.1 software and when a difference was observed a HSD tuckey test was performed to mark this difference at the threshold of ($p < 0.05$) and the excel software is used to make the different graphs.

3. Results

3.1. Phytochemical screening

The phytochemical study of the different types of *Alchornea cordifolia* leaves and fruit revealed the presence of several families of molecules: polyphenols, flavonoids, tannins, alkaloids and saponins (Table 1). Polyphenols are more pronounced in the different types of *Alchornea cordifolia* leaves and fruit in all localities. In the towns, flavonoids were more pronounced in young leaves, mature leaves and fruit than in dried leaves. For tannins, Table I shows abundant presence in young, mature and fruit leaves of *Alchornea cordifolia* in all localities and little presence in dried leaves. As for saponins, their presence was low in the different types of leaves and fruit in all towns. As for alkaloids, their presence is low in the leaves and fruit of all localities.

Table 1 Phytochemical screening of *Alchornea cordifolia* leaves and fruit

Compostions	**Localities**	**Different parts**			
		young leaves	**Mature leaves**	**Dried leaves**	**Fruits**
Polyphenols	Yamoussoukro	++	++	++	++
	Daloa	++	++	++	++
	Seguela	++	++	++	++
Flavonoids	Yamoussoukro	++	++	+	++
	Daloa	++	++	+	++
	Seguela	++	++	+	++
Tannins	Yamoussoukro	++	++	+	++
	Daloa	++	++	+	++
	Seguela	++	++	+	++
Alkaloids	Yamoussoukro	+	+	+	+

	Daloa	+	+	+	+
	Seguela	+	+	+	+
Saponins	Yamoussoukro	+	+	+	+
	Daloa	+	+	+	+
	Seguela	+	+	+	+

++ more present ; + less present

3.2. Total polyphenol content of *Alchornea cordifola* fruit and leaves analysed

The total polyphenol content of *Alchornea cordifola* fruit and leaf samples obtained from this study is shown in Figure 2 below. For each of the samples analysed (fruit, young leaves, mature leaves and dried leaves), Yamoussoukro is the locality whose young leaves, mature leaves, dried leaves and fruit have the highest proportions of polyphenols, respectively 3.12 mg EAG/g, 3.34 mg EAG/g, 1.61 mg EAG/g and 2.32 mg EAG/g. These values are followed by Daloa (2.01 mg EAG/g, 1.96 mg EAG/g, 1.33 mg EAG/g and 2.52 mg EAG/g) and Seguela (2.09 mg EAG/g, 2.03 mg EAG/g, 1.33 mg EAG/g and 1.1 mg EAG/g).

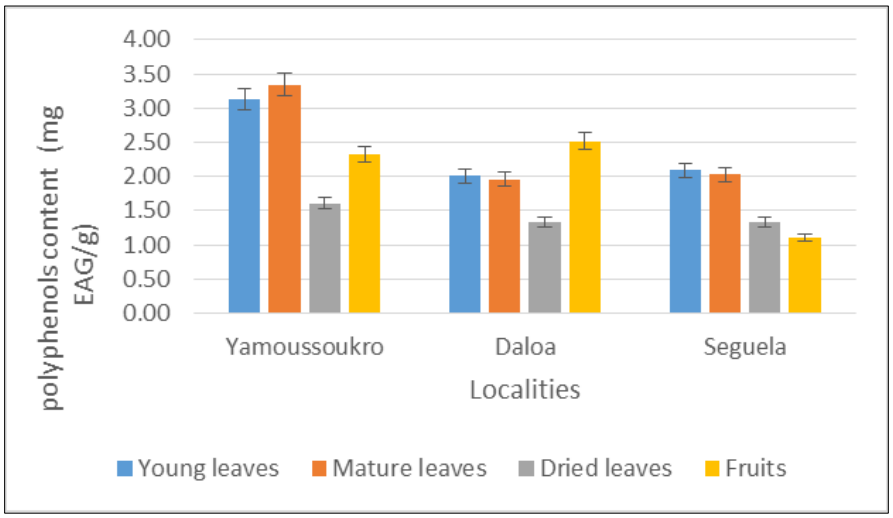

Figure 2 Total polyphenol content of *Alchornea cordifola* fruit and leaf stages by sampling localities

3.3. Flavonoid composition of *Alchornea cordifola* fruit and leaves analysed

Figure 3 below shows the flavonoid composition of the different matrices studied. The proportions obtained vary between 0.43 mg EQ/g and 0.69 mg EQ/g for the Yamoussoukro samples, between 0.65 mg EQ/g and 0.76 mg EQ/g for Daloa and between 0.47 mg EQ/g and 0.85 mg EQ/g for the Seguela locality. Overall, the Daloa samples had higher average proportions, regardless of the type of sample considered.

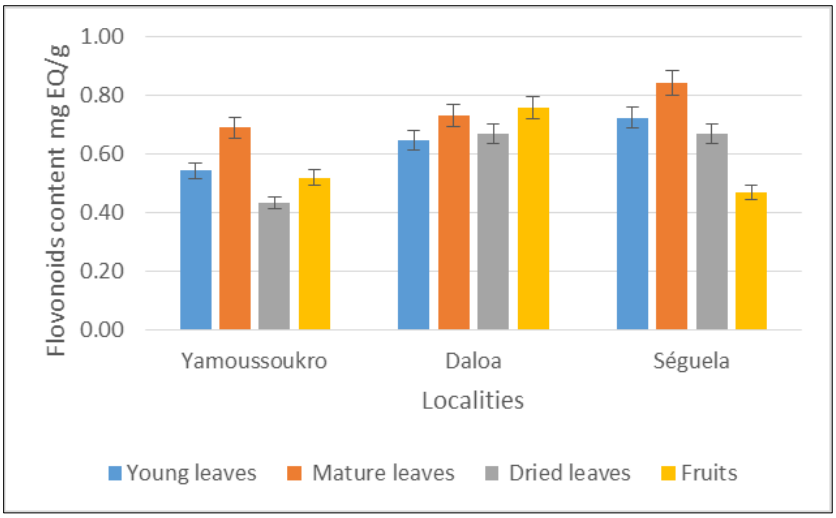

Figure 3 Flavonoid composition of *Alchornea cordifola* fruit and leaf stages according to sampling localities

3.4. Condensed tannin content of *Alchornea cordifola* fruit and leaves analysed

The analysis carried out shows that the leaves and fruit from the town of Daloa contain more condensed tannins (0.09 mg EC/g to 0.99 mg EC/g) than the other localities. As for the elements from Seguela, they contain a low level of condensed tannins, regardless of the part considered (0.09 mg EC/g to 0.15 mg EC/g). However, overall, the fruit and young leaves are the richest parts (Figure 4).

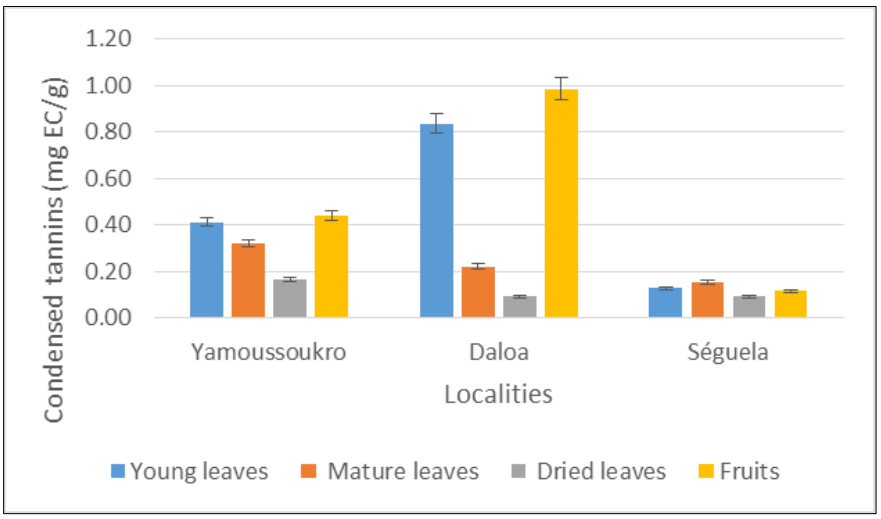

Figure 4 Condensed tannin content of *Alchornea cordifola* fruit and leaf stages by sampling localities

3.5. Total tannin content of Alchornea cordifola fruit and leaves analysed

Figure 5 shows the total tannin content of Alchornea cordifola fruit and leaf stages by sampling location. Young and mature leaves from the three sampling sites contained between 1.15 mg EAT/g and 1.50 mg EAT/g of tannins. The total tannin content of fruit and dried leaves in Yamoussoukro and Seguela did not exceed 1 mg EAT/g. As in Daloa, only the fruit contained 1.94 mg EAT/g of total tannins.

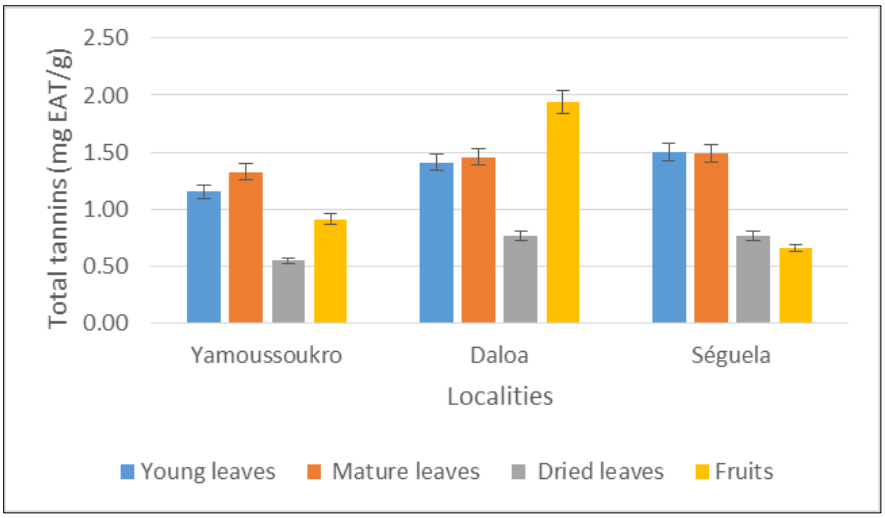

Figure 5 Total tannin content of *Alchornea cordifola* fruit and leaf stages by sampling localities

3.6. Anthocyanin content of *Alchornea cordifola* fruit and leaves analysed

The histogram in Figure 6 shows the anthocyanin content of fruit and leaves. These anthocyanins are more present in mature leaves (4.39 C3GE/g to 5.53 C3GE/g) than in other leaves and fruit. However, samples from the town of Daloa had higher levels than those from other localities.

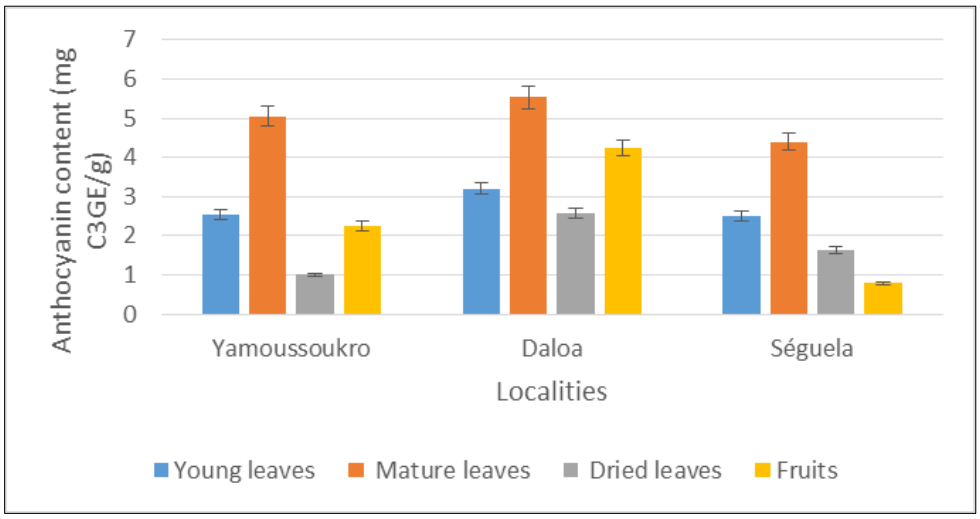

Figure 6 Anthocyanin contents of fruits and different stages of leaves of *Alchornea cordifola* according to sampling localities

4. Discussion

According to Hagerman and al [14], polyphenols are a class of secondary metabolites common in the plant kingdom. In Daloa, Yamoussoukro and Seguela, phytochemical screening revealed the presence of polyphenols, flavonoids, tannins, alkaloids and saponins in the leaves and fruit of *Alchornea cordifolia*. The presence of phytonutrients in the leaves and fruit confirms the traditional use of this plant as a therapeutic herb. These findings are in agreement with those of Tona and al [15] and Kasali [16], who identified the same compounds in *Alchornea cordifolia* leaf extracts.

Among the phytonutrients present in *Alchornea cordifolia* leaves and fruit, total polyphenols, total flavonoids, total tannins, condensed tannins and anthocyanins were also quantified. In general, the results of the study revealed that mature leaves contain more phenolic compounds than young leaves, dried leaves and fruit, with a content of between 1.33 and 3.34 mg EAG/g. These results are much higher than those obtained by Diomandé and al [17] during their work on the aqueous extract of *Albertisia cordifolia* leaves (0.0164±0.16 mg EAG/g) and identical to those of Dieng and al [18]

who obtained 3.12 ± 0.08 mg EAT/g of dry extract for the mature leaves of *Piliostigma thonningii* acclimatised in Senegal. Moreover, the total polyphenol content of young leaves is 61.11 times lower than that obtained by Kouame and al [19] in young unopened leaves of *Piliostigma thonningii* (146.67 ± 4.24 mg EAG/g dry extract) in Côte d'Ivoire. In general, the total polyphenol content of the leaf and fruit categories in our study is higher than that of *Alchornea cordifolia* leaves (0.035 ± 0.001 mg EAG) in the aqueous extract and in the methanolic extract (0.055 ± 0.002 mg EAG/g) obtained by Effo, [20]. The difference in polyphenol content in fruit and leaves at different stages of ripening could be explained by variations in growth that may be linked to climatic and environmental conditions (drought, hot temperatures, intense sun exposure, salinity), which favour the production of secondary metabolites [21]. Polyphenols also play a role in the antioxidant activity of plants, fruit, cereals and other plant-based products [22]. We can therefore deduce that the mature leaves of *Alchornea cordifolia*, which are richer in total polyphenols, are more suitable for therapeutic use [23].

The total flavonoid content of the leaf and fruit categories is higher than that of *Alchornea cordifolia* leaves, 0.048 ± 0.001 mg EQ in the aqueous extract and in the methanolic extract 0.054 ± 0.002 mg EQ/g obtained by Effo [20]. The flavonoid content of young leaves (0.54 to 0.72 mg EQ/g dry extract) is much lower than that of Kouame and al [19] in unopened leaves of *Piliostigma thonningii* (68.33 ± 0.94 mg EQ/g dry extract) in Côte d'Ivoire. Leaf contents (0.43 to 0.85 mg EQ/g dry extract) are lower than those of Sombie and al [24] in mature leaves of *Piliostigma thonningii* (2.17 ± 0.13 mg EQ/g dry extract) obtained in Burkina Faso. The results of the flavonoid contents in the fractions show that they depend on the maturity of the leaves and their stages of development (young or mature leaves), but also on the polarity of the solvents used, as reported by Hami and al [25]. The high average concentration of flavonoids in mature leaves could be justified by the fact that plants synthesise flavonoids using solar radiation to protect themselves from oxidation; as exposure to the sun increases, so do flavonoid contents, particularly in the most exposed parts [26]. These flavonoids could therefore be mainly in the form of free genins [27]. In addition to their antioxidant properties, flavonoids are also known for their anti-inflammatory and diuretic properties, according to Becker and al [28]. According to Bruneton [29], they are an important source of antioxidants in the human diet. The condensed tannin content of young leaves is much lower than that of the hydroalcoholic extract of young unopened leaves of *Piliostigma thonningii* (38.33 ± 0.47 mg EAT/g dry extract) according to Kouame and al [19]. The contents of mature leaves are also lower than those of mature leaves of *Piliostigma thonningii* acclimatised from Burkina Faso (12.62 ± 0.02 mg EAT/g dry extract) obtained by Sombie and al.[24]. These results show that the tannins are concentrated in the young mature leaves. According to Ksouri and al [30], tannins have anti-diarrhoeal properties which they confer on plants possessing them. These plants therefore have the ability to inhibit the activities of digestive enzymes by forming insoluble complexes with proteins, which reduces their digestibility and makes them inaccessible to the body [31;32]. Finally, tannins also promote wound healing, which explains their use in post-partum treatment for women [33].

The lowest anthocyanin content was recorded in dried leaves (1 mg C3GE/g) and the highest in mature leaves (5.53 mg C3GE/g). These results are higher than those obtained by Kabran and al [34] who found 0.491 mg/g in *Mallotus oppositifolius* leaves in Côte d'Ivoire and Janat Akhanovna, [35] in *Alchornea cordifolia* leaves (0.03mg/g). The difference in content between different plant species and different leaf stages could be explained by the environmental and physiological conditions of the plant. Leaf age, temperature and light intensity play a crucial role in anthocyanin biosynthesis [35]. Anthocyanins are obvious vital colour pigments, present in all plant tissues throughout the plant kingdom [36]. These pigments are widespread in nature and are responsible for the colours in the various plant organs such as stem, leaf, flower, fruit, root and tuber (orange, red, violet and blue) [37,38]. Anthocyanins are also of dietary importance for human health, with Lo and al [39] reporting encouraging results in the treatment of various syndromes such as cancer and other heart diseases [40].

Determination of total tannins in our *Alchornea cordifolia* leaves (young, mature and dried) and fruit shows that these parts of the plant contain levels of between 0.55 and 1.50 mg EC/g and 0.66 and 1.94 mg EC/g respectively. The total tannin content of *Alchornea cordifolia* leaves is lower than that obtained by Diomandé and al [17] when total tannins were determined in the aqueous extract (0.00469 mg EC/g), ethyl acetate extract (0.7254 mg EC/g) and ethanolic extract (0.99 mg EC/g) of *Albertisia cordifolia* leaves. The differences observed in the results could be due to the diversity of extraction solvents. The concentration of total tannins in plant extracts depends on polarity and affinity with the extraction solvent, as demonstrated by Diomandé and al [17]. In addition, tannins, which give leaves and fruit a very bitter taste, could be the basis of anti-diarrhoeal and antiseptic activities due to their ability to bind and precipitate water-soluble proteins [28]. Hence the justification for using the fruits of *Alchornea cordifolia* due to their high tannin content.

Phytochemical screening also revealed the presence of saponins and alkaloids. Saponins are known for their anti-hyperlipidemic, hypotensive and cardiodepressant properties. They are also known to inhibit intestinal absorption of cholesterol, leading to a reduction in blood cholesterol levels [41]. As for alkaloids, in addition to their antimicrobial action, they also play a role in detoxification and lowering blood pressure, as Awoyinka and al [42] point out.

5. Conclusion

This study has shown that, from a phytochemical point of view, the leaves (young, mature and dried leaves) and fruits of *Alchornea cordifolia* contain total polyphenols, flavonoids, anthocyanins, total and condensed tannins, saponins and alkaloids. Dosage of these bioactive compounds revealed considerable levels, giving the plant good antioxidant power, which would justify its recurrent use in traditional therapy against a number of pathologies. Quantitative analysis also showed that the mature leaves had the highest levels, unlike the other samples. This should justify the preference for mature leaves over other leaves and fruits for therapeutic uses. However, further research is needed to identify the plant's nutritional parameters.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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