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Determination of polyphenolic compounds and evaluation of antioxidant activity of *Guiera senegalensis* and *Alchornea cordifolia* leaf extracts used in various traditional medicine recipes in Côte d'Ivoire

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Abstract
Background: The aim of this study is to evaluate the antioxidant activity of *Guiera senegalensis* and *Alchornea cordifolia*, two plants used in various traditional medicine recipes in Côte d'Ivoire. The methodology consisted of infusion extractions and maceration in a 70% hydroethanol solution. Total phenols were determined using the Folin-ciocalteu reagent, total flavonoids by aluminum trichloride (AlCl3) and tannins by vanilla sulfuric acid (1%). Anti-radical potential was revealed by spectrophotometer.
Results: Total phenols were more concentrated in the 70% *A. cordifolia* macerate (62.24 ± 0.32 mg EAG/g). Flavonoids were more concentrated in the 10% infusion for both plants (26.12 ± 0.03 mg EQ/g, while tannins were highest in the 5% *A. cordifolia* infusion (58.69 ± 0.05). Both plant extracts showed good DPPH radical inhibitory power.
Conclusion: Both plants have a high concentration of polyphenolic compounds and good DPPH radical inhibitory power.

Keywords: *Guiera. senegalensis*, antioxidant activity, *Alchornea cordifolia*, traditional medicine, medicinal plants

Introduction
For many Africans, medicinal plants remain the most reassuring means of treating various serious pathologies for which there is no curative solution in modern medicine. Although this is a geocultural consideration, it is justified by numerous experimental studies. Several researchers have shown that medicinal plants are rich in various phytochemical compounds with proven biological activities [1, 2]. In Côte d'Ivoire, various works relating to the knowledge of medicinal plants have been carried out by several authors including Aké-Assi (1984), Tra Bi (1997) [3]. Certain plants are often used alone or in combination. The composition of recipes is guided by the type of pathology being treated. This is why, in the management of various chronic pathologies, recipes are often a blend of plant organs [4]. Not all plants play the same role in the same recipe [4, 5]. In fact, the World Health Organization (WHO) now recommends the use of drug combinations in the treatment of many diseases [6]. Some act directly on the repair of damaged tissue, while others help normalize biochemical reactions to prevent complications. Several complications at the root of chronic diseases are linked to oxidative stress [7]. It has been reported that over a hundred diseases are associated with free radicals [8]. Oxidative stress and free radicals are the primary causes of cancer, autoimmune diseases, cataracts, Alzheimer's disease, male infertility, rheumatism, atheroma and asthma [7]. According to the same author, diabetes, renal failure and Parkinson's disease are diseases that cause secondary oxidative stress [7]. Oxidative stress is the leading cause of death in adults [9]. In premature infants, in neonatology, complications such as retinopathy of prematurity, bronchopulmonary dysplasia, periventricular leuko malacia, intra- and periventricular hemorrhages, and necrotizing enterocolitis are identified as oxidative diseases of the newborn and are promoted by oxidative stress [10]. Oxidative stress, therefore, is a disease of concern and deserves special attention. It is to contribute to the fight against oxidative stress-related diseases that this study was initiated.

Its aim is to assess the antioxidant activity of *Guiera senegalensis* and *Alchornea cordifolia* used in various traditional medicine recipes in Côte d'Ivoire.

Material and Methods

Plant material

The plant material consists of the leaves of *Guiera senegalensis* and *Alchornea cordifolia* (Figure 1). The plants were harvested in August 2023 in Korhogo in the Poro region, then identified using the logitiel "Leaf ID: Al plant identifier" and confirmed by the botanical team at Peleforo GON COULIBALY University, Korhogo, Côte d'Ivoire.

Fig 1: Leaves and flowers of *Guiera senegalensis*

Fig 2: Leaves of *Alchornea cordifolia*

Methods

Preparation of extracts by infusion

Total extracts were prepared by infusion [11]. A mass of 5 g and 10 g of previously ground plant powder were weighed into a square of white cloth and then immersed in 100 mL of boiling distilled water for 5 min. The infused solution is filtered through absorbent cotton and then onto n°3 Wathman paper. The filtrate is evaporated in an oven at 50°C for three days. The resulting 5% and 10% infused extracts were stored in hermetically sealed sterile vials.

Hydroethanol maceration

Total extracts were prepared in hydroalcoholic solution. The filtrate was incubated at 50°C for 72 hours. The 70% ethanol macerate was stored in a hermetically sealed sterile bottle [12].

Total polyphenol assay

The polyphenol assay consisted in dissolving 1 g of extract in 10 mL of methanol. The solution was centrifuged at 3000 rpm/15 min and 0.5 mL of the supernatant was collected. To this solution were successively added 2.5 mL of Folin-ciocalteu reagent and 2 mL of sodium bicarbonate solution. The mixture was vortexed and incubated in the dark for 30 min at room temperature. Absorbance was read at 765 nm against a blank. Total polyphenol content was calculated from the regression equation of the gallic acid calibration curve and expressed as milligram equivalent of gallic acid per gram of extract (mg EAG/g extract) [13].

Total flavonoids assay

In this method, 1 g extract was dissolved in 10 mL methanol. The solution was vortexed and then centrifuged at 3, 000 rpm/15 min. One volume (0.5 mL) of AlCl3 solution (2% w/v) was mixed with 0.5 mL plant extract, to which 0.5 mL distilled water and 0.5 mL sodium acetate solution were added. After 30 min incubation at room temperature, the absorbance of the mixture is read at 430 nm against a blank. Quercetin is used as the standard, and the quantity of flavonoids is estimated in milligram equivalents of quercetin per gram of extract (mg EQ/g extract) [14].

Determination of condensed tannins

The extract solution is prepared from 1 g extract dissolved in 10 mL methanol. This solution is vortexed and centrifuged at 3000 rpm/15 min. To a test tube containing 0.5 mL of the centrifuged solution, 2.5 mL of a 1% vanillin solution prepared in concentrated sulfuric acid is added. The mixture is incubated for 30 min and the absorbance is read at 500 nm against a blank. The quantity of tannins is determined from the calibration line established with tannic acid, and is expressed in milligrams of tannic acid equivalent per gram of extract (mg EAT/g extract) [14].

Evaluation of free radical scavenging activity by spectrophotometry

Antioxidant activity was evaluated by the DPPH free radical scavenging method according to **Blois (1958)** [15]. DPPH (1, 1-diphenyl1-2-picrylhydrazyl) is solubilized in absolute ethanol to obtain a solution with a concentration of 20 µg/mL. Different concentrations of the extract (2000 µg/mL, 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL) are prepared in absolute ethanol. 2.5 mL extract and 1 mL DPPH ethanolic solution are introduced into dry tubes. After shaking, the tubes are placed in the dark for 30 min. The absorbance of the mixture is measured at 517 nm against a blank consisting of 2.5 mL pure ethanol and 1 mL DPPH solution. DPPH inhibition percentages are calculated according to the formula below. Ascorbic acid was used as a reference control. Concentrations required to trap 50% (IC50) of DPPH are determined.

$$\%\,PI = \left(\frac{\text{Abs control} - \text{Abs extract}}{\text{Abs control}}\right) \times 100$$

- % PI: Percentage of Inhibition, - Abs control: absorbance of control tube, - Abs extract: absorbance of the extract.

Statistical analysis

Statistical analysis of results was carried out using Statistica software version 7.1, followed by Graph pad Prism software version 8.0.2 for graphing and plotting. Fisher's minimum significant difference (LSD) test was used to determine significant differences between several means. For all

statistical analyses, differences were considered significant at the 5% significance level.

Results and discussion

Extraction yield

Extraction yields are reported in Table I. The highest yields were obtained with the hydroalcoholic extract in both plants, followed by the 5% infusions. The lowest yield was obtained with the 10% infusion. The highest yield (30.5 ± 0.21%) was obtained with the hydroalcoholic extract of *Alchornea cordifolia*, and the lowest (21.83 ± 0.76%) with 10% infusions of *Guiera senegalensis*. Extracts varied in appearance and color (Table I). There was no statistical difference between extraction yield by maceration and by infusion (5%) at $p \leq 0.05$.

Table 2: Extraction yields

Plantes	Types Extraction	Solvants Extraction	Rendement %	Couleur Extraits	Aspects
Guiera senegalensis	Infusé 5%	Aqueux	30 ± 1[(a)]	Brun	Collant
	Infusé 10%		21, 83 ± 0, 76[(b)]	Brun	Poudre
	Macération 70%	Ethanol	30, 1 ± 0, 3[(a)]	Vert-foncé	Poudre
Alchornea cordifolia	Infusé 5%	Aqueux	29, 17 ± 0, 20[(b)]	Brun	Cristallin
	Infusé 10%		26, 11 ± 0, 10[©]	Brun	Poudre
	Macération 70%	Ethanol	30, 5 ± 0, 21[(a)]	Noir	Poudre

Phenolic compound content

Total polyphenols

Total polyphenol content in plant extracts was determined from the gallic acid calibration line (Y = 1.645 X + 0.0113). The total polyphenol content of G. senegalensis 5 and 10% infusions (62.19 ± 0.05 and 60.16 ± 0.63 mg EAG /g extract respectively) is higher than that of the 70% ethanol macerate (25.84 ± 0.58 mg EAG /g extract) of the same plant. As for A. cordifolia extracts, the 5 and 10% infusions obtained lower total phenol contents (45.72 ± 0.06 and 52.31 ± 0.13 mg EAG /g extract) than the 70% ethanol macerate (62.24 ± 0.32 mg EAG /g extract). The highest levels of total phenols were found in the 5 and 10% G. senegalensis infusions and the 70% A. cordifolia macerate. Statistical analysis showed that there was no significant difference between the total phenol content of the 5% A. cordifolia infusion and the 70% G. senegalensis macerate ($p \leq 0.05$). These two extracts had the highest total phenol content.

Total flavonoid

The total flavonoid content of extracts was determined from the calibration line for quercetin (Y= 6.2 X + 0.0067). The highest flavonoid content of G. senegalensis was obtained with the 10% infused extract (26.12 ± 0.03 mg EQ/g extract), followed by the 5% infused (18.22 ± 0.02 mg EQ/g extract) and the lowest with the 70% ethanolic macerate (13.76 ± 0.16 mg EQ/g extract). A. cordifolia extracts also obtained the highest flavonoid content with the 10% infused (13.39 ± 0.12 mg EQ/g extract). The 70% macerate and 5% infusion of the same plant achieved the same flavonoid content (4.31 ± 0.25 mg EQ/g extract). Statistical analysis showed that there was a significant difference between the flavonoid content of the infused extracts and the 70% macerated at $p \leq 0.05$.

Condensed tannins

The condensed tannin content of extracts was determined from the tannic acid calibration line (Y = 4.3993X + 0.0212). The tannin content of G. senegalensis extracts ranged from 24.77 ± 0.66 to 50.08 ± 0.31 mg EAT/g extract. The highest content was observed in macerated 70% (50.08 ± 0.31 mg EAT/g extract), followed by infused 5% (33.8 ± 1.54 mg EAT/g extract) and 10% (24.77 ± 0.66 mg EAT/g extract). Similar tannin content values were obtained with A. cordifolia extracts. The highest content (58.69 ± 0.05 mg EAT/g extract) was obtained with the 5% infused and the lowest content (10.19 ± 0.63 mg EAT/g extract) with the 70% macerated. Statistical analysis showed that there was a significant difference between the tannin contents of infused and 70% macerated at $p \leq 0.05$.

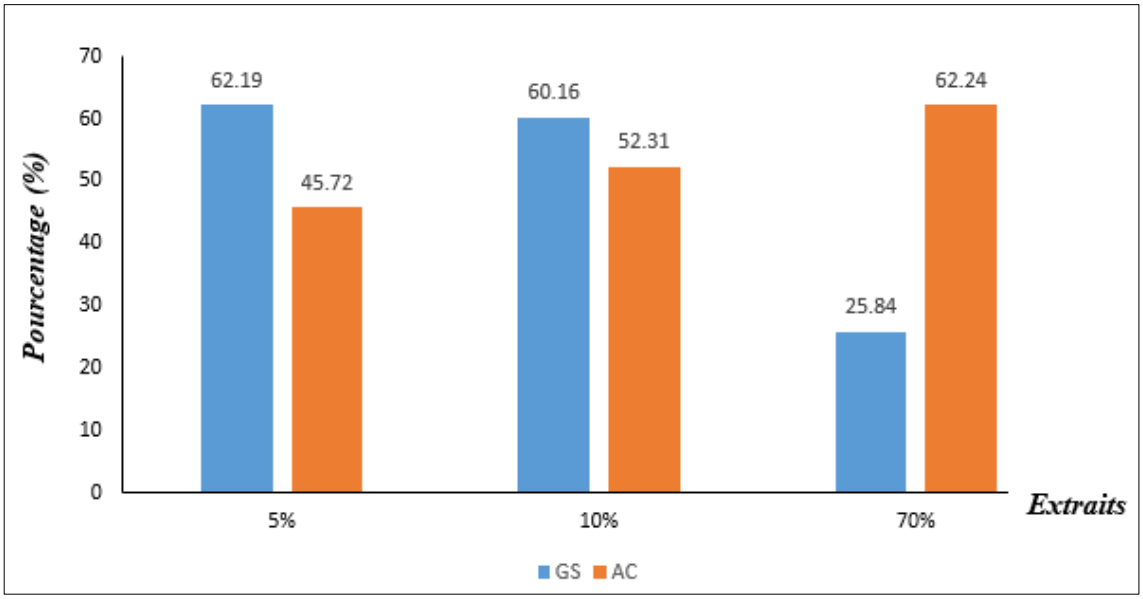

Fig 3: Total phenol content

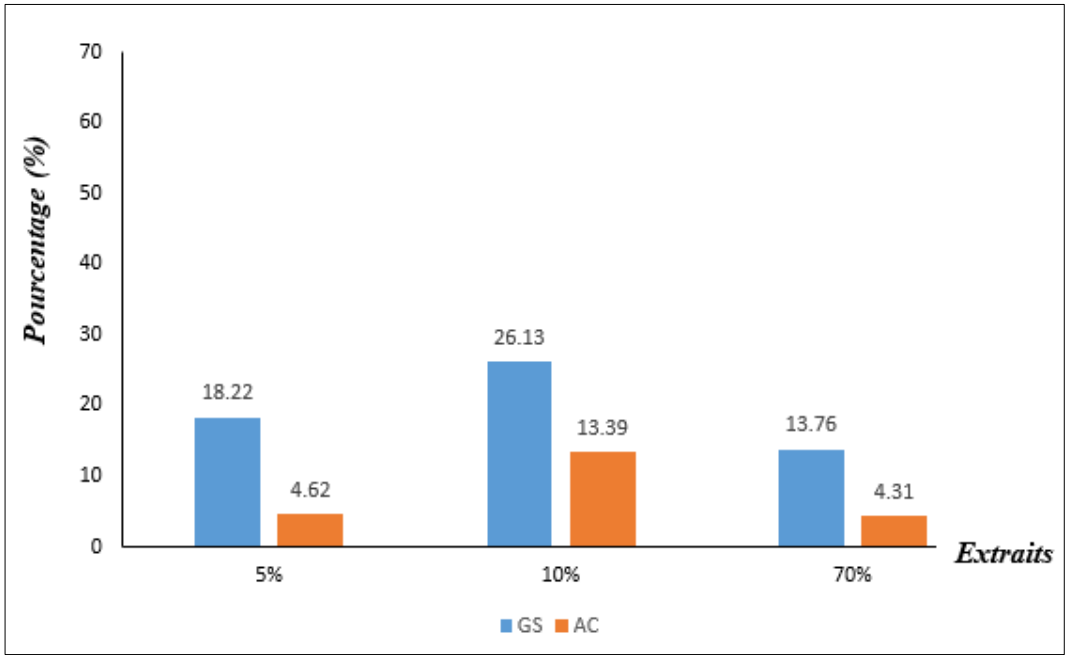

Fig 4: Total flavonoid content

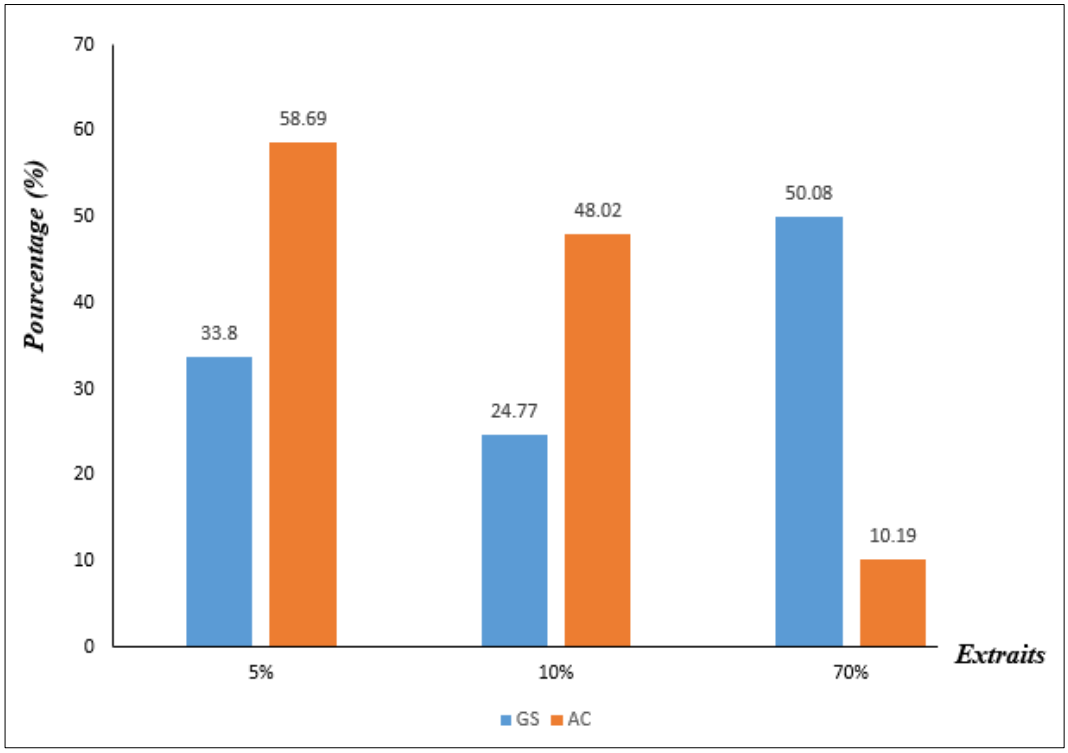

Fig 5: Tannins content

Antioxidant activity

The curves in Figures 6 and 7 show the evolution of different percentages of DPPH (1, 1-diphenyl 1-2-picrylhydrazyl) radical inhibition by infused and macerated extracts of the two plants studied. These curves were used to determine the IC50 (Table II). Vitamin C achieved the highest percentage of DPPH radical inhibition (95%) and the lowest IC50 value (33.33 µg/mL) at the highest concentration (2000 µg/mL). At the same concentration, plant extracts showed variable inhibition percentages ranging from 51.14 to 89.69%. The highest inhibition percentages were obtained with the 5% infusion (89.69% and IC50=55.55 µg/mL) for A. cordifolia, followed by the 70% macerate (86.8% and IC50=52.77 µg/mL) for G. senegalensis. These inhibition percentages and IC50s are close to those of ascorbic acid. At the lowest concentration (62.5 µg/mL) in our experimental study, the 5% infusion of A. cordifolia and the 70% macerate of G. senegalensis inhibited 56.69% and 50.8% of free radicals respectively. The 5% infused and 70% macerated A. cordifolia and G. senegalensis respectively have the best DPPH radical inhibitory powers, making them good candidates for combating oxidative stress. Statistical analysis of the inhibition percentages of the various extracts and vitamin C showed that there was no significant difference between the inhibition percentages of ascorbic acid and 5% infused A. cordifolia. Infused 10% and macerated 70% were statistically different. In the statistical analysis of the inhibition percentages of G. senegalensis extracts, there was no significant difference between the inhibition percentages of 70% macerate and vitamin C. On the other hand, the DPPH

radical inhibition powers of ascorbic acid and those of the 5% infused and 70% macerated extracts of A. cordifolia and G. senegalensis respectively were significantly different. On the other hand, the DPPH radical inhibitory powers of ascorbic acid and those of 5% infused and 70% macerated extracts of A. cordifolia and G. senegalensis respectively are statistically identical at ($p \leq 0.05$).

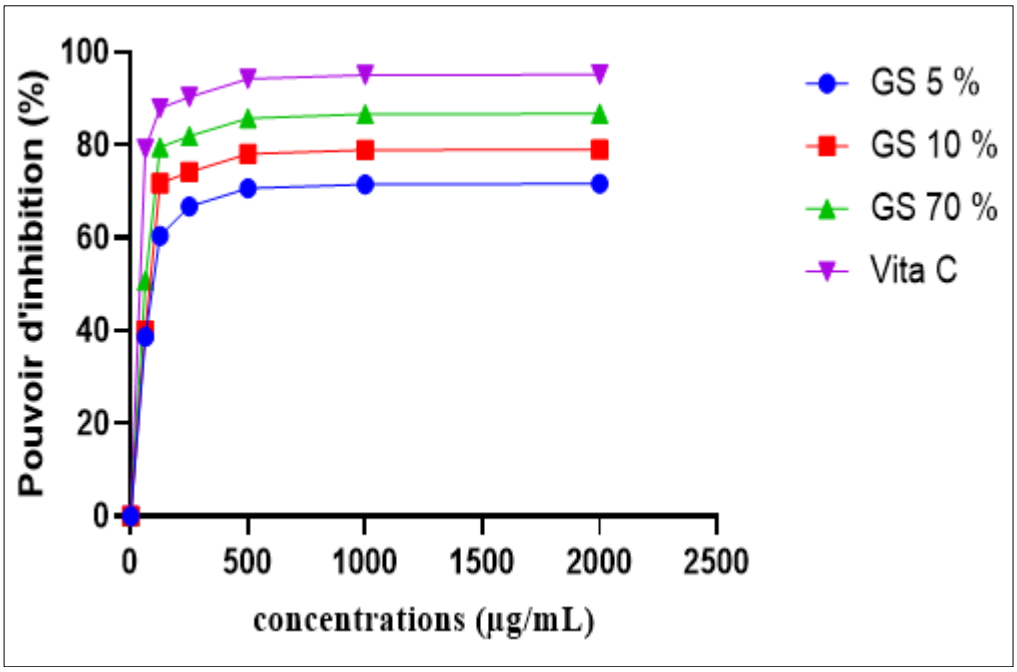

Fig 6: Percentage inhibition curve for *Guiera senegalensis* extracts

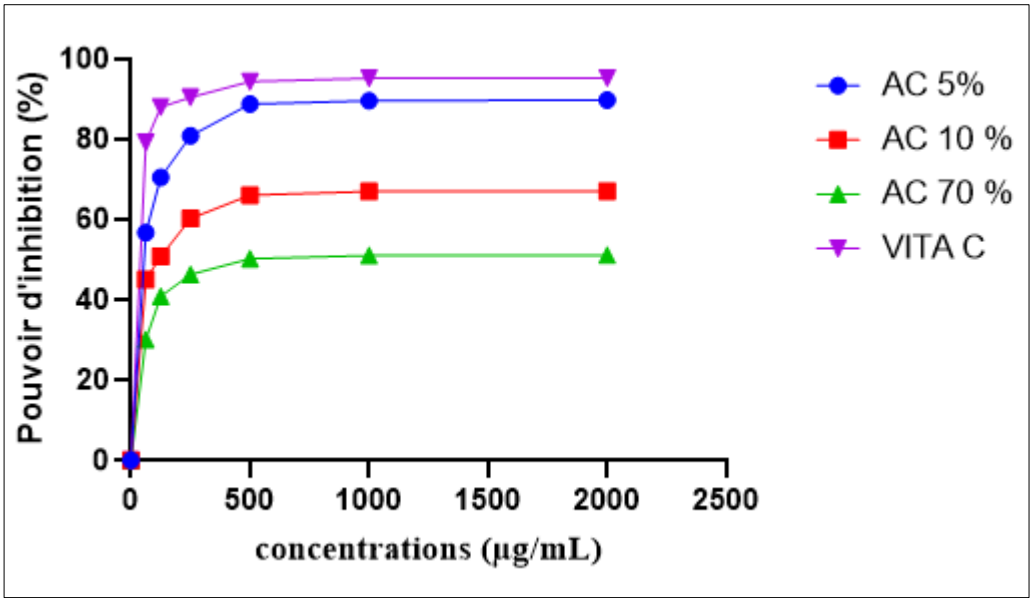

Fig 7: Percentage inhibition curve for *Alchornea cordifolia* extracts

Table 2: 50% inhibitory concentration of extracts

	Guiera senegalensis	***Alchornea cordifolia***
	CI_{50} (µg/mL)	CI_{50} (µg/mL)
Infusé 5 %	111, 11± 4, 81	55, 55 ± 9, 62
Infuse 10 %	88, 88 ± 9, 62	119, 44±12, 72
Macéré 70 %	52, 77 ± 4, 81	450 ± 8, 33
Acide Ascorbique	33, 33 ± 8, 33^{c}	33, 33 ± 8, 33^{c}

Discussion

The aim of this study was to determine the antioxidant potential of *Alchornea cordifolia* and *Guiera senegalensis*, two Ivorian pharmacopoeia plants used in several therapeutic recipes. The work carried out in the course of this study showed that the highest extraction yields were obtained with the 70% hydroalcoholic solution and the 5% infusion for the two plants studied. These results reflect the fact that the hydroalcoholic solution extracts the maximum phytochemical compounds compared with water [16]. Indeed, these authors obtained higher extraction yields with organic solvents than with aqueous solvents. The polarity of the compounds to be extracted and ethanol's ability to solubilize a wide range of phytochemicals may account for this result. The difference in yield between 5% and 10% infusion may be due to the solid/liquid ratio. The higher the solvent volume, the greater the degree of contact between the drug and the extraction solvent. The drug is well soaked, making it easier for the extraction solvent to recover the active ingredient over time. These results are similar to those of Koné *et al*. (2017) [17],

who showed that yields are higher when the volume/mass ratio of the grind is low. Determination of polyphenolic compounds in extracts from these plants showed that total polyphenol content in G. Senegalensis extracts was higher in the infused than in the 70% ethanolic macerate. The same observation was made for total flavonoids. Tannins were more concentrated in the 70% ethanolic macerate than in the infusions. Overall, total phenol and flavonoid content was higher in *Guiera senegalensis* extracts than in *Alchornea cordifolia* extracts. Tannin content was higher in *Alchornea cordifolia* extracts than in *Guiera senegalensis* extracts. This difference in polyphenolic compound content found could be due to several factors including the genetic composition of the plant species studied, the extraction method, the extraction solvent and the possible presence of interfering substances [18]. In the present study, as the extraction solvents (water and hydroalcohol) were of identical polarity, and the leaf powder from each plant had undergone the same extraction methods, the difference in composition would come from the genetic composition of the two plant species. This difference in phenolic compound content, probably linked to the genetic composition of the species, could justify the concomitant use of several species to form an effective recipe for the treatment of several pathologies. This could also explain the management of several biological phenomena during traditional treatments [19]. The phenolic compound content of our extracts is higher than that obtained by Effo, (2018) [20] but lower than that obtained by Mariod *et al*., (2016) [21] in his study on *G. Senegalensis* leaf extracts. This difference can be explained by environmental, geographical, climatic and genetic factors, as well as the degree of ripening of the plant and the storage time of the extracts [22]. These results show that *G. Senegalensis* leaves are richer in total polyphenols and flavonoids than those of *Alchornea cordifolia*. Polyphenols are endowed with biological activities inherent to medicinal plants, thanks to their numerous hydroxyl groups. These hydroxyl groups can interact with free radicals to interrupt the oxidation chain. The high content of total polyphenols, total flavonoids and condensed tannins in *G. senegalensis* and *Alchornra cordifolia* leaves may confer DPPH free radical. In fact, the IC50s of our extracts are variable, with the lowest values obtained with the 70% *Guiera senegalensis* macerate, followed by the 5% *Alchornra cordifolia* infusion. These values are closer to those of the reference molecule (Ascorbic Acid) than those of the other extracts. Both the 70% *Guiera senegalensis* macerate and the 5% *Alchornra cordifolia* infusion show greater inhibitory power than other extracts of the same plant. *G. senegalensis* leaf extracts have a moderate inhibitory effect on the DPPH radical. The inhibitory activity of these extracts remains lower than that of ascorbic acid, taken as a reference molecule. Several research studies have reported results similar to those obtained in our work [23, 24]. Through several complementary methods, these authors have shown that extracts from the leaves of these plants possess good antioxidant activity. The extracts studied are good antioxidants and can be used in the management of pathologies linked to oxidative stress.

Conclusion

This study has shown that these two plants, widely used in traditional medicine, contain various secondary metabolites such as total phenols, total flavonoids and tannins. The content of these compounds varies from plant to plant. The highest levels of polyphenolic compounds were found in extracts infused with both plants. The highest DPPH radical inhibitory power was obtained with the 70% macerated extract of *Guiera senegalensis*, followed by the 5% infused extract of *Alchornea cordifolia*. Both *G. senegalensis* and A. cordifolia leaf extracts are good free radical scavengers. Leaf extracts from the two plants studied have good antioxidant activity. These leaves are good candidates for the management of chronic diseases linked to oxidative stress. This work justifies the use of *G. senegalensis* and *A. cordifolia* in traditional medicine.

Outlook: It will be important to study the toxicity and antimicrobial activity of these plants with a view to developing improved traditional phytomedicines.

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