Research Article

Phytochemical Study and Effect of Aqueous Extracts of Leaves and Bark of Trunks *Anogeissus leiocarpus* (DC) Guill. And Perr. (Combretaceae) on Free Radicals

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ABSTRACT

Many diseases such as malaria, high blood pressure, infertility, diabetes, cancer, etc., have a genesis linked to oxidative stress caused mainly by the excess of free radicals in the body. The objective of this work is to evaluate the biochemical activities of aqueous extracts of leaves and trunk bark of Anogeissus leiocarpus (DC) Guill. And Perr. (Combretaceae), a medicinal plant of the Ivorian flora. For this purpose, dosages of phytoconstituents, the antioxidant activity as well as the phytochemical screening of these organs are carried out. Antioxidant activity is evaluated by DPPH and ABTS tests. Quantitative studies of phytoconstituants revealed that the leaves contained a high content of total phenolic compounds (12986.46 ± 0.31 µg / g) in relation to trunk bark (10718.89 ± 0.21 µg / g). The mean total flavonoid content was 1555.82 µg / g for the leaves and 525.54 µg / g for the trunk bark. The lowest IC50 (19.8 µg / mL) is also obtained with the leaves. In addition, phytochemical screening, carried out by thin layer chromatography, detected several groups of chemical compounds including tannins, flavonoids, polyphenols, coumarins and xanthones with known antioxidant effects. This work justifies the traditional use of Anogeissus leiocarpus in the treatment of various pathologies and more particularly those involving oxidative stress.

Keywords: Oxidative stress, aqueous extracts, Anogeissus leiocarpus, medicinal plant, Ivorian flora.

INTRODUCTION

In many African countries, the advent of modern medicine and its progress have led people to turn away, somewhat, from traditional medicine. But in recent decades, the use of medicinal plants is gaining renewed interest [1]. According to WHO estimates, more than 80% of the population in Africa still uses traditional medicine to meet their health care needs [2]. The modern pharmaceutical industry itself still relies heavily on the diversity of plant secondary metabolites to find new molecules with novel biological properties [3]. This source seems inexhaustible since each species of the 400,000 known, can contain several thousands of different constituents whose various uses aim to overcome suffering and improve the health of men [4]. In this context, some researchers have performed biological and biochemical tests with many of these plants [4, 5, 6]. However, despite this abundance of medicinal plants, many deaths, related to various pathologies, are observed each year. Given this observation, we believe that the floristic diversity of the Ivory Coast [4] should provoke other contributions in order to make available to our populations more accessible remedies, able to mitigate the oxidative stress. In fact, oxidative stress seems to be the main initial cause of several diseases: malaria, cancer, cataract, diabetes, amyotrophic lateral sclerosis, acute respiratory distress syndrome, pulmonary edema, accelerated aging of the skin, Parkinson's disease, HIV / AIDS, etc. [8, 9]. It is one of the factors in the genesis of multifactorial diseases such as Alzheimer's disease, rheumatism and cardiovascular diseases [10, 11]. Among these certain diseases such as malaria, cardiovascular diseases, etc. remain among the most deadly in the world. In addition, several hundred molecules have been identified in plants. Oak et al. have shown that polyphenols in red wine and green tea have beneficial effects on the reduction of cardiovascular diseases and cancer [12]. However, in Côte d'Ivoire, few studies have focused on the search for antioxidant activities, despite the diversity of its flora. Activities against free radicals in 20 species of non-wood plant products were studied [13]. N'gaman et al. showed that the organs (leaves, stems and roots) of Gmelina arborea Roxb. (Verbenaceae) are rich in phenolic compounds [14]. The antioxidant power of many medicinal plants in Côte d'Ivoire remains to be verified. Faced with this problem, many hopes are placed in the secrets of plants and the emergence of an alternative medicine based on these plants is more than ever on the agenda. It is to contribute to further develop this area of the Ivorian pharmacopoeia that we have chosen to evaluate the biochemical activity of the leaves and bark of *Anogeissus leiocarpus* (DC) Guill. and Perr. (Combretaceae) and perform a phytochemical screening.

MATERIALS AND METHODS

Preparation of Raw Extracts

The crude extracts were obtained according to the method described by Zihiri et al. [5]. In 1 liter of distilled water, 125 g of organ powder are macerated twice, for 24 hours, with stirring. The mixture is filtered twice under

vacuum on Whatman® 3 mm filter paper. The aqueous total extract is a powder obtained after lyophilization of the filtrate. These extracts were kept in the refrigerator.

Assay of Total Phenols

The amount of total phenols of the crude plant extracts is determined by the Folin-Ciocalteu colorimetric method. It is based on the principle that in a basic medium, the Folin-Ciocalteu reagent oxidizes the oxidizable groups of the polyphenolic compounds present in the sample. Reduction products (metal oxides W8O23 / Mo8O23) of blue color, have an absorption maximum whose intensity is proportional to the amount of polyphenols present in the sample. The phenols are quantified in the crude extract diluted with distilled water at several concentrations and incubated at 40 ° C., by adding 0.5 ml of Folin-Ciocalteu reagent (0.5N) and 1.5 ml of sodium carbonate (NaCO3) (17%, w / v). The absorbance is determined at 760 nm (maximum wavelength of gallic acid) against a blank (not containing extract) taken as reference. The quantification of the polyphenols is done according to a calibration line (y = ax + b) carried out by a gallic acid standard extract at 100 µg / mL, at different volumes (0, 0.2; , 4, 0.6, 0.8 and 1 mL) under the same conditions as the sample.

Assay of Total Flavonoids

The content of flavonoids is determined according to the method of Djeridane et al. [15]. This method is based on the formation of the flavonoid-aluminum complex with an absorption maximum at 430 nm. Quercetin is used for the calibration curve. 1 mL of the extract (0.1-0.4 g / mL) diluted in distilled water is mixed with 1 mL of the 2% $AlCl_3$ solution diluted in methanol. The sample is incubated for 15 minutes at room temperature. The absorbance is measured at 430 nm with a UV-VIS spectrophotometer. Two readings are done for each extract. A calibration range established with quercetin can be used to determine the total amount of flavonoids, expressed in micrograms of quercetin equivalent (QE) per milligram of extract (µg EQ / mg) [16].

Antioxidant Activity

DPPH TEST (1, 1-diphenyl-2-picrylhydrazyl)

It is a method adapted from Pajero et al., [17]. The ability of an antioxidant to fix free radicals is done by measuring the decrease in purple staining due to the reduction of the DPPH radicals. A color ranging from mauve to yellow is observed. The DPPH solution was prepared by dissolving 5 mg of DPPH in 250 mL of methanol. This DPPH solution (0.02 mg / mL) was stored at 4 ° C, protected from light. In a series of eight tubes each containing 2.5 mL of plant extract sample at different concentrations (0-100 µg / mL), 2.5 mL of the DPPH solution was added. The control tube was prepared by adding 2.5 mL of DPPH and 2.5 mL of ethanol. After 15 min of reaction in the dark, the reading of the OD was carried out at 517 nm with the spectrophotometer.

ABTS TEST (2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid)

This method is adapted from Arnao et al., [18]. It is based on the discoloration of the ABTS • + [2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] blue-green color radical in colorless ABTS in the presence of antiradical compounds. Indeed, solutions of ABTS and potassium persulfate are mixed in the same proportions. The mixture is stirred and stored in the dark and away from heat for 6 hours to 3 days to form the stable ABTS • + cation radical. On the day of the test, the conserved solution is diluted with methanol in order to obtain an absorbance close to 1.1 ± 0.2 at the wavelength 734 nm. Then, 2850 µL of the ABTS • + solution is added to 150 µL of each extract for 2 hours in the dark and protected from heat. Then, the absorbance is measured spectrophotometer at 734 nm. Methanol served as white. The antioxidant activity of the extract is compared to that of a reference antioxidant in terms of equivalence or in terms of inhibition [19]. The reactions that take place may be of the ABTS / trans-3, 3 ', 4', 5, 7-pentahydroxyflavan (catechin) or ABTS / 1, 3, 5-trihydroxybenzene (phloroglucinol) type.

Phytochmical Screening

The detection of the different chemical compounds in the extracts was done by thin layer chromatography (TLC) according to the method used by Kouadio et al. [20]. This method allows the detection of several groups of secondary metabolites by specific staining either in the visible or at a given wavelength [14]. Ten (10) mg of extracts were dissolved in 1 mL of absolute methanol to obtain a solution of a concentration of 10 mg / mL. Ten microliters (10 µl), ie 100 µg of this solution were deposited spot on a silica gel plate F254 (stationary phase) using micropillary tube. The plates were placed in tanks previously saturated with eluent or mobile phase $CHCl_3$ -MeOH-H_2O (65: 35: 5 v / v / v), and then dried. These plates were observed before and after revelation either in the visible or under a UV lamp. The frontal ratios (Rf) of the various spots observed are calculated according to the following formula:

$$Rf = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent}}$$

Establishing Terpenoids and Saponosides

These compounds are highlighted with the Godin reagent. After spraying the plate with Godin reagent followed by heating at 100°C. For 10 minutes, the appearance of the various colorations is noted. In the visible, observation of purple spots, red indicates the presence of monoterpenes and saponins in blue [21].

Alkaloids

After spraying Dragendorff reagent and heating the chromatogram at 100°C for 10 min, the alkaloids appear as orange spots in the visible.

Polyphenols

After spraying the chromatogram with the 10% Folin-Ciocalteu reagent and then heating at 100°C for 10 min, the blue spots observed in the visible evidence the presence of polyphenols [22].

Flavonoids and Sesquiterpenic Lactones

After spraying the chromatogram with aluminum chloride ($AlCl_3$) at 5% (w / v) and heating, the presence of flavonoids is indicated by yellow spots visible in the visible or UV at 366 nm [21]. As for sesquiterpenic lactones, they are indicated by fluorescences of various colors at 366 nm[21].

Coumarines

The basic lead acetate (CH_3COO) 2 Pb at 5% (w / v) was used for the spraying of the chromatogram. The 366 nm green and blue UV spots indicate the presence of coumarins.

Tannins

The appearance of spots of various colors (blue, green, black), observable in the visible, after spraying of the chromatogram with a solution of $FeCl_3$ at 10%, shows the presence of tannins.

Anthraquinones and Anthrones

An ethanol solution of 5% KOH was sprayed on the chromatogram. Visible red spots at 366 nm confirm the presence of anthraquinones. Anthrones, on the other hand, are visible at 366 nm in the form of yellow spots [23]. After heating the plate, the terpenes are indicated in purple and the saponins in blue.

Data Processing

The inhibition percentages of DPPH and ABTS are illustrated by histograms. The correlation between the activity of each organ extract and its concentration variation is represented by regression lines. The IC 50 values (Inhibitory Concentration 50%) are determined graphically from the percent inhibition curves as a function of the concentrations. IC50 is the concentration of extract that inhibits 50% of DPPH or ABTS after 30 min of incubation. The lower this value, the greater the antioxidant activity. The results of the antioxidant tests are expressed as mean ± standard deviation (SD).

Statistical Analysis of Results

The choice of the statistical analysis focused on the analysis of the variances (2-way ANOVA) in order to compare the percentages of inhibition of the DPPH and the ABTS according to the type of organ used, the concentration of the 'extract and interaction of these two factors, using the software STATISTICA 7.1 [24]. This technique makes it possible to study a quantitative variable Y according to two factors. This is the effect depending on the type of organ and concentration. The objective is then to test the significance of the average effect of each factor and their interaction in order to show the existence of significant differences between the antioxidant activity of the leaves and that of the trunk bark on the one hand. and to identify, on the other hand, the reasons for these differences. When p <0.05 [25], the difference observed in the variations is said to be significant and the variance is completed by a comparison test of multiple variables (DUNCAN test at the 0.05 threshold).

Student's t-test, using the same software, compared the content of polyphenols and flavonoids in the aqueous extracts of the various organs. This test is an elementary test on quantitative variables to compare averages obtained by populations.

The correlation (R2) between the different dilutions of these tested extracts was verified. It allows to observe the existence of a connection between two variables, but also to note that this relation is linear or not. The equation and the regression curve were also determined. R2 ≥ 0.90 was taken as strongly correlated [26]. Histograms and regression curves were constructed using Excel software.

RESULTS

Total Polyphenols

The study of the polyphenol content (Fig. 1) showed total phenols in different proportions. This assay revealed that the leaves contained a high content of total phenolic compounds (12.99 ± 0.31 mg / g, ie 12986.46 ± 0.31 µg / g) relative to the trunk bark (10.72 ± 0.21 mg / g, ie 10718.89 ± 0.21 µg / g). Statistical analysis (student test at P <0.05) did not show a significant difference between the levels of the different organs (P = 0.619190> 0.05).

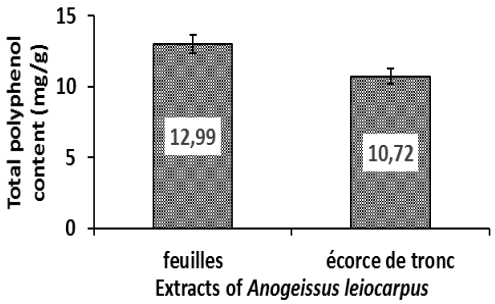

Figure 1: Content of phenolic compounds (average ± SD of three trials) total contents in the organs of Anogeissus leiocarpus

The same lowercase letters translate levels similar to the threshold of α = 5%.

Total Flavonoids

The study of the content of flavonoid compounds (Fig. 2) showed total flavonoids in different proportions. This assay revealed that the leaves contained a significant content of total flavonoid compounds (2 ± 0.02 mg / g, ie 1555.82 ± 0.02 µg / g) relative to the trunk bark (1 ± 0.01 mg / g is 525.54 ± 0.01 µg / g). Statistical analysis (student test at P <0.05) did not show a significant difference in

the flavonoid content of the various organs (P = 0.295642> 0.05).

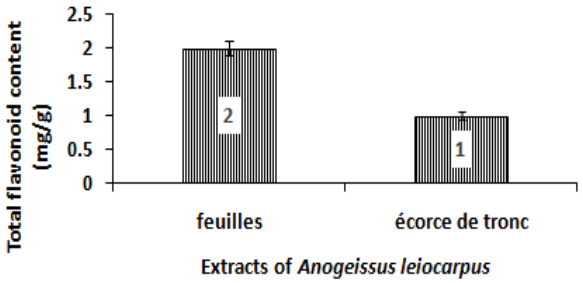

Figure 2: Content of flavonoid compounds (mean ± SD of three trials) total contained in the organs of Anogeissus leiocarpus

The same lowercase letters translate levels similar to the threshold of α = 5%.

3.3 BIOCHEMICAL ACTIVITY

3.3.1 TEST WITH DPPH (2,2-Diphenyl-1-picrylhydrazyl)

The antiradical tests have shown that this plant species has an antioxidant potential. The maximum DPPH inhibition percentage is reached at a concentration of 500 µg / mL (Fig. 3).

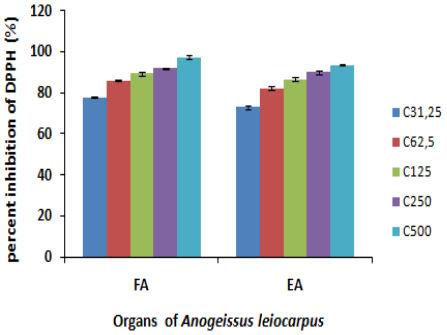

Figure 3: Anti-free radical activity of leaf extracts and trunk bark Anogeissus leiocarpus depending on the concentrations (each value represents the average of three trials ± SD)

The analysis of variance showed a significant difference between the different percent (s) of DPPH inhibition of the extracts. The DUNCAN test located this difference in the type of organ used. In fact, the leaf extract gave an average percentage inhibition of DPPH 88.54% higher than that of the trunk bark (85.15%).

The significant difference between DPPH inhibition percentages is related to the concentration of the extract. Indeed, the higher the concentration, the greater the percentage inhibition is important. This gives 75.33% DPPH inhibition at 31.25 µg / mL; 84.17% at 62.5 µg / mL; 88.08% at 125 µg / mL; 91.08 at 250 µg / ml and 95.54 at 500 µg / ml. The differences observed at the level of the inhibition p.c. are significant at the 5% level.

The significant difference between DPPH inhibition percentages is related to the interaction between the concentration of the extract and the type of organ used. The same concentration causes different effects for each organ. At 31.25 µg / mL, the trunk bark extract inhibited 72.96% of the DPPH and the leaf extract, 77.71%. At 62.5 µg / mL, the trunk bark extract inhibited 82.16% of the DPPH and the leaf extract 86.19%. The largest p.c. inhibition was observed at 500 µg / mL, with 93.75% for trunk bark and 97.33% for leaves.

There is a good correlation (R2> 0.90) between the activity and the concentration of the extracts. The concentrations used and the antioxidant activity against free radicals follow a logarithmic regression. In other words, as concentration increases, activity tends to increase.

The different IC50 values of the extracts are 19.8 µg / mL for the leaf extract and 20.8 µg / mL for the trunk bark extract (Fig. 4). Antioxidant activity is more important in the leaves than in the trunk bark.

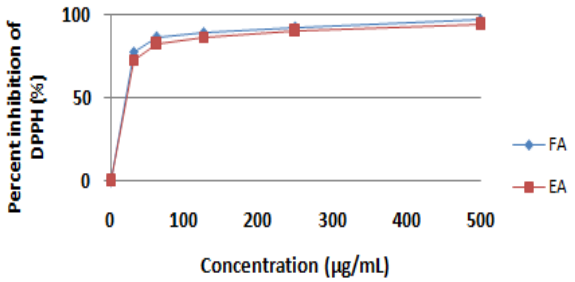

Figure 4: Curves for determining the IC50s of the different extracts

FA: Aqueous extract of leaves; EA: Aqueous extract of the bark of trunk.

TEST WITH ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid)

Tests with ABTS showed percentages of inhibition of ABTS in varying proportions. The maximum percentage inhibition of ABTS is reached at a concentration of 500 µg / mL (Fig. 5).

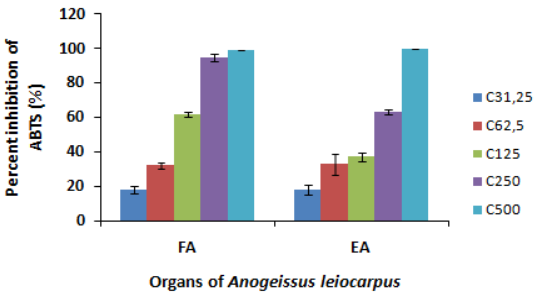

Figure 5: Inhibition activity of ABTS by leaf extracts and bark trunk of Anogeissus leiocarpus depending on the concentrations (each value represents the average of three trials ± SD)

FA: Aqueous extract of leaves; **EA**: Aqueous extract of the trunk bark

The analysis of variance showed a significant difference between the different percentages inhibition of ABTS extracts. The DUNCAN test located this difference in the type of organ used. In fact, the extract of the leaves gave an average percentage inhibition of ABTS of 82.17% higher than that of the trunk bark (65.24%).

The significant difference between the inhibition percentages of ABTS is related to the concentration of the extract. Indeed, the higher the concentration, the greater the percentage inhibition is important. This makes it possible to obtain 23.96% inhibition of ABTS at the concentration of 31.25 µg / mL; 42.97% to 62.5 µg / mL; 65.41% at 125 µg / mL; 84.74 to 250 µg / ml and 91.44% to 500 µg / ml. The differences observed at the level of the inhibition percentages are significant at the 5% level.

The significant difference between the inhibition percentages of ABTS is related to the interaction between the concentration of the extract and the type of organ used. The same concentration causes different effects for each organ. At 125 µg / mL, the trunk bark extract inhibited 37.03% of the ABTS and the leaf extract, 61.73%. At 250 µg / mL, the extract of the trunk bark inhibits 63.29% of the ABTS and the extract of the leaves, 94.99%. The largest inhibition percentages were observed at 500 µg / mL, with 99.28% for trunk bark and 100% for leaves.

There is a good correlation (R2> 0.90) between the activity and the concentration of the extracts. The concentrations used and the antioxidant activity against free radicals follow a logarithmic regression. In other words, as concentration increases, activity tends to increase.

The different IC50 extracts are 98.8 µg / mL for leaf extract and 183 µg / mL for *A. leiocarpus* trunk bark extract (Fig. 6). Antioxidant activity is more important in the leaves than in the trunk bark.

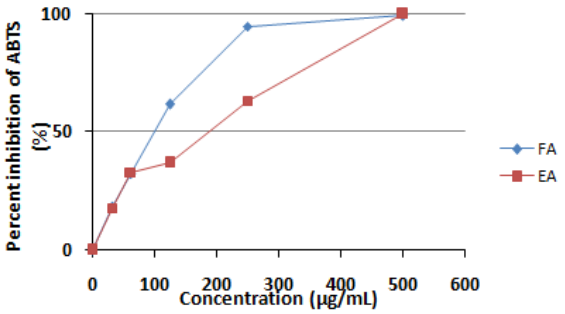

Figure 6: Curves for determining the IC50s of the different extracts

FA: Aqueous extract of leaves; **EA**: Aqueous extract of the bark of trunk.

Phytohemical Investigations

The detected chemical compounds are shown in Table 1. Eleven groups of phytochemicals were identified. The results show the presence of catholic and gallic tannins, alkaloids, polyterpenes, polyphenols, anthocyanins, saponosides, xanthones, naphthoquinones, coumarins and flavonoids in the various organs. However, anthraquinone glycosides and free anthraquinones were not detected in any of the extracts.

Table 1: Compounds identified by TLC in A. leiocarpus stem bark.

Organs		Developing: chloroform / methanol / water (65: 35: 5)							Possible chemical compounds
		Before revelation	After revelation						
			Godin	dragend	Folin	$AlCl_3$	$FeCl_3$	KOH	
Leaves	Rf	0,67	0,88	0,25	0,88 0,52	0,52 0,25	0,25	0,52	Saponosides, catechin and gallic tannins, polyphenols, xanthones, naphthoquinones, polyterpenes, anthocyanins, flavonoids, alkaloids
	Color	Blue	Purple	Orange	Blue Blue	Yellow Yellow	Black	Yellow	
Trunk bark	Rf	0,67	0,87	0,52	0,88	0,52	0,52	0,52	Saponosides, catechin and gallic tannins, polyphenols, xanthones, polyterpenes, anthocyanins, flavonoids, alkaloids
	Color	Blue	Purple	Orange	Blue	Yellow	Black	Yellow	

DISCUSSION

The results of the total phenol compounds assay showed that the leaves contained a higher mean total content of total phenolic compounds (12986.46 ± 0.31 µg / g) relative to the trunk bark (10718.89 ± 0, 21 µg / g). Student's t-test indicated a no significant difference between these two averages (p> 0.05). In general, the unequal distribution of polyphenols in different parts of a plant (leaves, stems, roots, seeds, etc.) has been mentioned by several authors [27, 28]. This unequal distribution could be explained by several factors including biogenetic or environmental factors. Polyphenols are important compounds because they make the so-called phyto-micronutrients. They are the most abundant antioxidants in food since humans consume about 1g / day, nearly ten times more than vitamin C and 100 times more than vitamin E [29]. They

neutralize free radicals and thus help prevent various degenerative pathologies. This result could justify the preference of traditional health practitioners, as to the use of *A. leiocarpus* leaves in the treatment of pathologies such as degenerative rheumatism, urinary schistosomiasis, amenorrhea, sexual impotence, prostate, abdominal pain, tremor-causing diseases, all, diseases that can contribute to aging early and involve oxidative stress [30]. The activity of the plant against these pathologies could be related to polyphenols because of their analgesic, anti-inflammatory, anticarcinogenic, antithrombic, and anti-atherogenic properties [31]. Moreover, the choice of the use of the aqueous extract for the determination of polyphenols is made by reference to the work of Ozen et al., Who found that the aqueous extract of Thymus praecox is richer in polyphenols than the alcoholic extract [32]. This could be explained by the presence of a large number of molecules soluble in water and not soluble in alcohol. However, this is not always verified since other authors report that alcoholic extracts record the highest total polyphenol contents [33, 34].

The quantitative determination of total flavonoids reveals that the leaves are richer in flavonoids (anthocyanins, leucoanthocyanins, flavones, isoflavones) compared to the trunk bark. Indeed, at a concentration of 200 µg / mL, the leaf extract contains an average total flavonoid content of 1555.82 µg / g and the trunk bark contains 525.54 µg / g. This work corroborates that of Cozzi et al., Who reported the presence of several flavonoids derived from quercetin and kaempferol in the organs of *A. leiocarpus* with a low content in the trunk bark [35]. In general, flavonoid levels are higher in the leaves than in the trunk and roots. This can be explained by the fact that plants synthesize flavonoids thanks to solar radiation to protect themselves from oxidation; the more exposure to sunlight increases, and the higher the content of flavonoids, especially in the most exposed parts, usually the leaves. These compounds are very important because of their antioxidant, antimicrobial and anticancer properties [36, 37]. Middleton et al., Showed their antiallergic, anti-inflammatory, anti-thrombotic, anti-tumor and hepatoprotective properties [38]. In addition, previous studies indicate that *A. leiocarpus* contains six molecules of ellagic acid derivatives, four of which have been isolated and characterized. These molecules are 3,3 ',4'-tri-O-methylflavellagic acid, 3,3'-di-O-methylellagic acid, tri-O-methyl-ellagic acid and acid 3, 3'-di-O-methyl-4-β-O-xylopyranosyl-ellagic, a polyhydric alcohol (sorbitol), terpenoids such as α-amyrin, β-amyrin and β-sitosterol [35].

The antiradical activity of the extracts of the various organs was evaluated by the DPPH and ABTS tests. The DPPH test showed a greater ability to capture and neutralize free radicals with the leaf extract than with the trunk bark. The DUNCAN test linked this inhibitory power to the type of organ used, the concentration of the extract and the interaction of these two variables. This could be explained by the unequal distribution of the compounds in the organs. It is probably phenols, flavonoids, tannins, responsible for biochemical activity. The free radicals of ABTS are 99.28% inhibited by the leaf extract and 100% by the trunk bark extract at the concentration of 500 µg / mL. This activity is thought to be related to 3,3 ', 4'-tri-O-methylflavellagic acid, 3,3'-di-O-methylellagic acid, tri-O-methylellagic acid and 3,3'-di-O-methyl-4-β-O-xylopyranosyl-ellagic acid, which are derivatives of ellagic acid and more abundant in the trunk bark, unlike flavonoids [39]. These derivatives are good antioxidants acting as evacuators of free radicals of oxygen and as protectors of DNA against degradation, by alkylating agents. Given this activity and the proportions of the antioxidant substances contained in the plant, it could be recommended in the fight against pathologies related to oxidative stress because these substances are part of the therapeutic treatments against atherosclerosis, chronic polyarthritis, asthma and cancers [40]. Nevertheless, other important pharmacological and toxic studies need to be conducted. On the other hand, the study of correlation showed a positive correlation, with high coefficients (R^2 > 0.90) between the inhibition percentages of DPPH (R^2 = 0.95), of ABTS (R^2 = 0.99) and the concentrations of the extracts. This indicates that these two variables are dependent. This result is similar to that of Prabhjit et al., Who also reported correlation coefficients greater than 0.90, in a study of free anti-radical activities of DPPH of Rubia cordifolia L. (Rubiaceae) [26]. These actions against free radicals are strongly related to the presence of phenolic compounds and flavonoids detected mainly in the leaves because the IC50 obtained with their extracts, are the weakest. However, the smaller the value of the IC50, the more the extract is considered a powerful antioxidant [41]. To our knowledge, antioxidant activities on free radicals, as well as the determination of flavonoids and phenolic compounds of aqueous extracts of leaves and trunk bark of *A. leiocarpus* are reported here for the first time. However, some authors have performed these tests with alcohol extracts [30, 42]. These results, therefore, confirm these previous works and provide other interesting information on the activities related to the plant. From these results, it appears that the antioxidant activity is inversely proportional to the content of flavonoids, and proportional to the content of polyphenols. This would explain that in addition to flavonoids and polyphenols, other molecules would participate in this antioxidant activity. In order to remove any ambiguity, a phytochemical screening was carried out.

Phytochemical investigations have revealed eleven (11) large groups of chemical compounds including alkaloids, quinones, flavonoids, terpenes, saponosides, catholic and gallic tannins, anthocyanins, coumarins, phenols and xanthones. These phytoconstituants are, at the origin of the observed biological and biochemical effects, and consequently, determine the therapeutic interests of the plant. Among these compounds, many are known for

their antioxidant effects; according to Alex et al., alkaloids can be involved in the healing of malaria [43]. This would explain the use of *A. leiocarpus* trunk bark against this pathology. The presence of these compounds in this organ differs our results from those of Cozzi et al., Who found only traces of alkaloids in the trunk bark [35]. This heterogeneity in the results may be due to environmental, climatic and organ harvesting conditions, as well as to genetic and experimental conditions. Flavonoids (flavones, flavonols, anthocyanins) are also present in the organs of the plant species. The positive effects of these bioactive molecules on human health are reported by several authors [44, 45]. They are known to have a strong antioxidant activity, by inhibiting on the one hand, the free radical-producing enzymes and on the other hand, by trapping the radicals formed. One of the first stages in the development of neurodegenerative diseases, which often appear with age, is due to a decrease in antioxidant defenses and a mitochondrial increase in the production of radical species [7]. Thus the intake of natural antioxidants by food can only be beneficial for human health. Numerous studies have shown a positive correlation between high flavonoid consumption and decreased risk of cardiovascular and neurodegenerative diseases. Tannins are proton donors to lipid free radicals produced during peroxidation. More stable tannic radicals are then formed, which has the effect of halting the chain reaction of lipid auto-oxidation [46]. Their strong presence in the organs would justify their major involvement in the antioxidant activity observed at the plant level. This concerns both catechism and gallic tannins. These results are similar to those of Cozzi et al., Which indicated high tannin content in the plant [35]. According to these authors, the majority of medicinal uses of *A. leiocarpus* are likely based on its tannin content. No experimental data on their toxicity seems to have been published, which justifies the popular use of drinking newborn children a decoction of bark to relieve their ills. Similarly, coumarins are known to have antioxidant activity similar to that of flavonoids [47]. According to this author, these compounds are capable of preventing the peroxidation of membrane lipids and of capturing hydroxyl radicals, superoxide radicals and peroxides. It reports the anti-inflammatory, antiseptic, antiviral and antioxidant properties of phenols. These would be produced by plants to guard against infections and phytophagous insects. Their detection in the organs confirms the work of Chaabi et al., Who reported in the plant, the presence of P-coumaric acid [48]. As for xanthones, their properties of inhibition towards the lipid peroxidation, as well as their properties of free radical scavengers against superoxide anions have been reported [49].

The results of phytochemical tests justify the fact that compounds, other than polyphenols and flavonoids, are actively involved in antioxidant activity. These are certainly the tannins, coumarins, xanthones whose presence explains the intervention of the plant in the treatment of pathologies induced by oxidative stress and accelerating aging.

The work carried out could provide a demonstration and justification of the traditional use of *A. leiocarpus* in the treatment of oxidative stress diseases.

CONCLUSION

The work carried out confirmed the antioxidant activity of leaves and trunk bark of A. leiocarpus. The antiradical activity showed a higher antioxidant potential in vitro with the leaf extract than that of the trunk bark. This activity is dose dependent since the compounds responsible for the said activity are much concentrated in the aerial parts. Similarly, the determination of polyphenols and total flavonoids has revealed that the aerial part contains a higher content of these compounds. In addition, phytochemical screening has shown the presence of several large groups of secondary metabolites, probably responsible for the therapeutic virtues of the plant.

In view of these results, this plant could constitute a hope in the relief of the oxidative stress related affections, a real threat of public health.

It is therefore possible to extend the ethnopharmacological studies on *A. leiocarpus*, to confirm the results obtained in vitro by in vivo tests and to carry out toxicity tests.

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