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Phenolic Compound Content and Antioxidant Activity of Extracts from the Leaves of *Anogeissus leiocarpus* (Combretaceae), a Plant Used in the North of Côte d'Ivoire for the Traditional Treatment of Gastrointestinal Disorders in Broiler Chickens

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Anogeissus leiocarpus (Combretaceae) (DC.) Guill. & Perr. is a plant found in West Africa, from Senegal to Cameroon and extending to Ethiopia and East Africa. In the north of Côte d'Ivoire, this plant is commonly used by livestock farmers to treat various pathologies, including digestive and gastrointestinal disorders. The aim of this study was to determine the phenolic compound content and antioxidant properties of 70% hydroethanol and aqueous extracts of A. leiocarpus' leaves.
Phenolic compound contents were determined by spectrophotometric methods. Antioxidant activities were assessed using ABTS, DPPH and FRAP tests.
The 70% hydroethanolic and aqueous extracts of *A. leiocarpus* presented roughly equivalent concentrations of total polyphenols (608.41 and 558.25 mg EAG/g respectively). However, the hydroethanolic extract was richer in flavonoids (539.60 mg EQ/g) than the aqueous extract (388.95 mg EQ/g). In addition, the average inhibition rate of the ABTS radical in the presence of the 70% hydroethanol extract was 11.56 µM Trolox equivalent/g, compared with 8.32 µM Trolox equivalent/g with the aqueous extract. The average chelation percentages were 31.93% (70% hydroethanol extract) and 20.92% (aqueous extract). As for the reduction of DPPH radical, the ICs_{50} for the 70% hydroethanol and aqueous extracts were 4.4 mg/mL and 6.0 mg/mL respectively.
This study confirms that Anogeissus leiocarpus is a plant containing phenolic compounds. The presence of these phenolic compounds in this plant, together with its antioxidant activities with low values (IC_{50}), could justify its widespread use by livestock farmers in northern Côte d'Ivoire, particularly those rearing broiler chickens.

Keywords: Anogeissus leiocarpus; antimicrobial activity; Salmonella typhimurium; flavonoid content.

1. INTRODUCTION

The traditional African pharmacopoeia constitutes a veritable phytopharmacy that could be used in several areas of health care, for both humans and animals and even for plant [1,2,3]. In the specific case of animals, various studies have shown the use of plant-based remedies for their therapeutic management.

Anogeissus leiocarpus (DC.) GUILL. & PERR. (Combretaceae) is a plant found in West Africa, from Senegal to Cameroon and extending to Ethiopia and East Africa [4]. Growing in dry forests and gallery forests [4,5], its distribution extends from the Sahara to the outer layer of tropical rainforests. The decoction of *Anogeisus leiocarpus* leaves is used to treat small ruminants suffering from gastrointestinal parasitism, according to an ethnoveterinary study conducted in Burkina Faso [6]. In the north of Côte d'Ivoire, this plant is commonly used by livestock farmers to treat various pathologies including digestive and gastrointestinal disorders [7]. Julienne et al*.* [8] confirmed in their studies that *A. leicocarpus* is used in veterinary medicine, particularly in the treatment of parasitic diseases caused by *Haemonchus contortus.* The methanolic extract of *A. leiocarpus* stem bark has also demonstrated anti-trypanosomiasis activity against four strains of Trypanosoma (Shuaibu et al., 2008a) as well as leishmanicidal activity (Shuaibu et al., 2008b). *A. leicocarpus* also has strong antihelminthic activity [8].

Ouattara et al. evaluated the *In vitro* efficacy *of A. leiocarpus* on multi-resistant strains of *Salmonella typhimurium* isolated from the droppings of farmed broilers. Extracts from this plant showed good antimicrobial activity against these multi-resistant *Salmonella typhimurium* germs. Also, the presence of several groups of secondary metabolites, differently distributed, with high levels of phenolic compounds in extracts from the leaves of this plant. Oxidative stress is unavoidable in poultry farms that affects the physiological, behavioral, and biochemical state of growing chickens, which can cause their death or deteriorate meat quality (appearance, texture, juiciness, tenderness and odour) [9]. Djeridane et al*.* [10] reported that animal treated in a farm involves not only eliminating the germ, but also reducing the signs of morbidity generally caused by the massive production of free radicals in the affected organism. However, several synthetic antioxidants are used by broiler breeders as feed additives to combat oxidative stress. Although these enriched feed supplements are highly effective against free radicals, they are unfortunately likely to have side-effects and may even be toxic (Maman et al*.*, 2008) [11,12]. Plants constitute a natural reservoir of secondary metabolites (Lee et al*.*, 2000 ; Cakir et al*.,* 2003) [13] and have always

been heavily involved in the search for new natural antioxidants that are effective and have very few side effects. With this in mind, it would be a great advantage if a plant-based formulation with antioxidant properties could be made available to livestock farmers in the long term. This is the reason why, we conducted the present study to assess the phenolic compound content and antioxidant properties of two extracts of *A. leiocarpus*, a plant from the Ivorian pharmacopoeia that is commonly used by farmers in northern Côte d'Ivoire to treat gastrointestinal disorders in broiler chickens.

2. MATERIALS AND METHODS

2.1 Plant Material

It consists of the leaves of *Anogeissus leiocarpus*. They were harvested in the village of Lataha in the Korhogo region (northern Côte d'Ivoire) in March 2022 and authenticated by the Centre National Floristique of the Felix HOUPHOUËT-BOIGNY University in Cocody-Abidjan.

2.2 Preparation of Aqueous and 70% Hydoethanolic Extracts of *Anogeissus leiocarpus*

The leaves of *Anogeissus leiocarpus were* washed, cut and dried in the shade for a fortnight. Once dried, the plant material was ground. Then mixed 100g of this powder with 1 litre of distilled water. The mixture was homogenised at room temperature in the laboratory using a magnetic stirrer for 24 hours. The homogenate obtained was filtered twice on cotton wool and once on Whatman paper (3 mm). The volume of the filtrate obtained was reduced using a Med Center Venticell oven at 50°C to give a powder that constitutes the total aqueous extract (E.H2O) (Ouattara et al., 2013).

The same operation was carried out using 70% ethanol instead of distilled water, to obtain the 70% hydroethanol extract (E.HOH) (Zirihi et al., 2003). The extracts obtained were stored in the refrigerator (º C) for further testing.

2.3 Determination of Total Polyphenol Content

Total polyphenols in extracts determined according to Wood et al. [14]. A volume of 2.5 ml of diluted (1/10) Folin-Ciocalteu reagent was added to 30 µL of ethanolic extract. The mixture was kept for 2 min in the dark at room temperature, then added 2 mL of calcium carbonate solution (75 $g.L^{-1}$). The mixture was then placed in a water bath at 50°C for 15 min and rapidly cooled. Absorbance was measured at 760 nm, using distilled water as the blank. A calibration line was made with gallic acid at different concentrations. Analyses were carried out in triplicate and the concentration of polyphenols was expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

2.4 Determination of Total Flavonoid Content

Total flavonoids carried out according to Marinova et al. [15]. In a 25-mL flask, 0.75 mL of 5% (w/v) sodium nitrite ($NaNO_2$) was added to 2.5 mL of extract. Then added 0.75 ml of aluminium chloride (10% (w/v); $AlCl_3$) to the mixture and incubated for 6 min in the dark. After incubation, 5 mL of sodium hydroxide (1N NaOH) was added and the volume was made up to 25 mL. The mixture was shaken vigorously before being assayed using a UV-visible spectrophotometer. The reading was taken at 510 nm. A calibration line was constructed with quercetin at different concentrations. Assay was carried out in triplicate, flavonoid content was expressed as miligram quercetin equivalent per g extract (mg EQ /g extract).

2.5 Determination of the *In vitro* Antioxidant Activities of Aqueous and 70% Hydroethanol Extracts

2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay: This method is based on the ability of the compounds to reduce the ABTS+° (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid) radical-cation. The test was carried out using the method » described by Choong et al. [16]. The ABTS°+ radical-cation was produced by reacting 8 mM ABTS (87.7 mg in 20 mL distilled water) and 3 mM potassium persulphate (0.0162 g in 20 mL distilled water) in a 1 :1 (v/v) ratio. The mixture was then incubated in the dark at room temperature for 12-16 hours. This ABTS°+ solution was diluted with methanol to obtain a solution with an absorbance of 0.7 ± 0.02 at 734 nm. A test portion of 3.9 mL of this diluted ABTS°+ solution was added to 100 µL of the test compound. After shaking, the mixture was incubated for 6 min in the dark (T=30±2°C). The

residual absorbance of the ABTS+° radical was then measured at 734 nm using a UV-visible spectrophotometer and was expected to be between 20% and 80% of the absorbance of the blank. The tests were carried out in triplicate and the results were expressed in µmol Trolox equivalent per litre of extract (µmol TE/L). A calibration line was performed with the following concentrations of Trolox : 0.375µM ; 0.5µM ; 0.625µM ; 1µM ; 1.125µM, 1.375µM and 1.5µM and the inhibition rate (% I) of ABTS°+ was expressed as follows (1):

$$\% I = [(Abs\_control - Abs\_extract) / Abs\_control] \times 100 \quad (1)$$

Abs_control = diluated ABTS absorbance,

Abs_extract = diluated ABTS absorbance + sample

Antioxidant concentration or activity:

$$(\mu Méq\ Trolox) = (\%I \times fd)/((4{,}99 \times 10)) \quad (2)$$

2.6 Iron Chelation (FRAP)

The chelating capacity of the extracts was measured using the method of Le et al. [17]. Solutions of extracts and the reference antioxidant (EDTA 500 µl) were initially mixed with 100 µl $FeCl_2$ (0.6 mM in distilled water) and 900 µl methanol. After 5 min, 100 µl Ferrozine (5 mM in methanol) was added to the reaction medium. The mixture was shaken well and then left to react for 10 min at room temperature, allowing complexation of the residual iron and formation of a chromophore with an absorption maximum at 562 nm. The negative control contains all the reagents except the test sample, which is replaced by an equal volume of methanol. Readings are taken at 562 nm against a methanol blank. The sequestering effect of the samples on iron is expressed as a percentage of chelation according to the following equation:

$$\% Chelation = [(Abs\ control - Abs\ test) / Abs\ control] \times 100 \quad (3)$$

2,2'-diphenyl-1-picrylhydrazyl (DPPH)) radical scavenging assay: The anti-free radical activity of the plant extracts was measured using the 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) test" according to Parejo et al. [18]. "A range of concentrations (0-200 µg/mL) of plant extract or gallic acid (reference antioxidant) was prepared. A volume of 2.5 mL of this solution was mixed with 2.5 mL of DPPH (100 µM) prepared in methanol. After homogenisation, the mixture was incubated at room temperature (25°C) in the dark. After 15 min incubation, the absorbance was read at 517 nm against a "blank" containing only methanol". Parejo et al. [18] The percentage inhibition of the DPPH radical was calculated using the following equation :

$$DPPH\ inhibition\ (\%) = (1- (test\ OD / blank\ OD)) \times 100 \quad (4)$$

The IC_{50} is the concentration of plant extract or quercetin responsible for 50% inhibition of DPPH radicals. It is determined from the graph showing the percentage of DPPH inhibition as a function of extract and gallic acid concentrations.

2.7 Statistical Analysis

Data analysis and graphical representation were carried out using Graph Pad Prism 8.0.1 (Microsoft, USA). The mean value is accompanied by the standard error of the mean (Mean ± SEM). The difference between two values is considered significant when $P < 0.001$. The results were statistically analysed using a two-way analysis of variance (ANOVA).

3. RESULTS

3.1 Phenolic Compound Content

Determination of phenolic compounds in the two extracts from *A. leiocarpus* leaves showed that both the hydroethanol extract (E.HOH) and the aqueous extract (E.H2O) had roughly equivalent concentrations of total polyphenols (608.41 and 558.25 mg EAG/g, respectively) (Fig. 1). In terms of total flavonoids, the hydroethanol extract of *A. leiocarpus* leaves was richer (539.60 mg EQ/g) than the aqueous extract (388.95 mg. EQ/g) (Fig. 2).

3.2 ABTS Test and Chelating Power (FRAP)

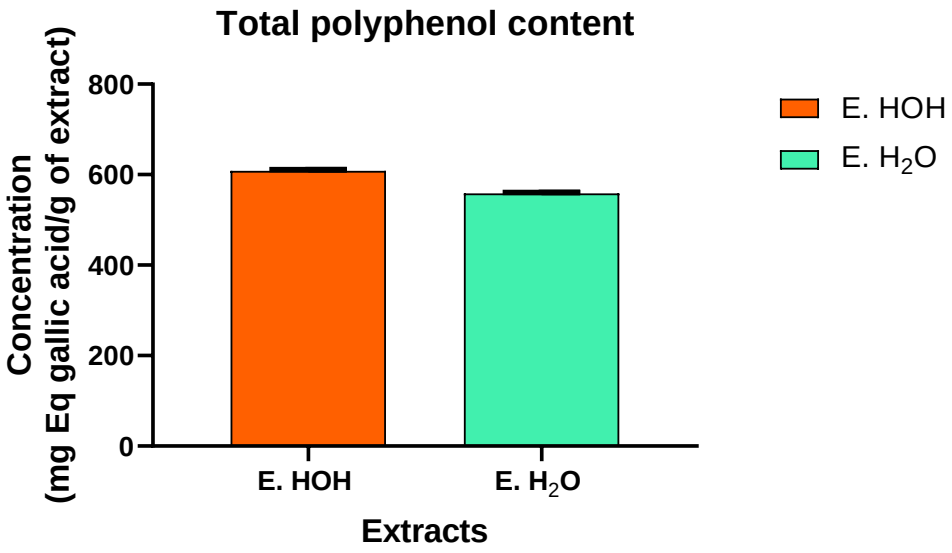
For a concentration of 100 mg/mL, the average inhibition rate of the ABTS radical in the presence of the 70% hydroethanol extract was 11.56 µM Trolox eq/g, whereas it was 8.32 µM Trolox eq/g with the aqueous extract. For these two extracts, the inhibition rates obtained were much lower compared to that of gallic acid, which recorded a value of 64.71 µM Trolox eq/g (Table 1).

The average chelation percentages were 31.93% (70% hydroethanol extract) and 20.92%

(aqueous extract, Table 1). However, these values are significantly lower than those obtained with the reference antioxidant (EDTA), whose average chelation percentage was estimated at 96.74%.

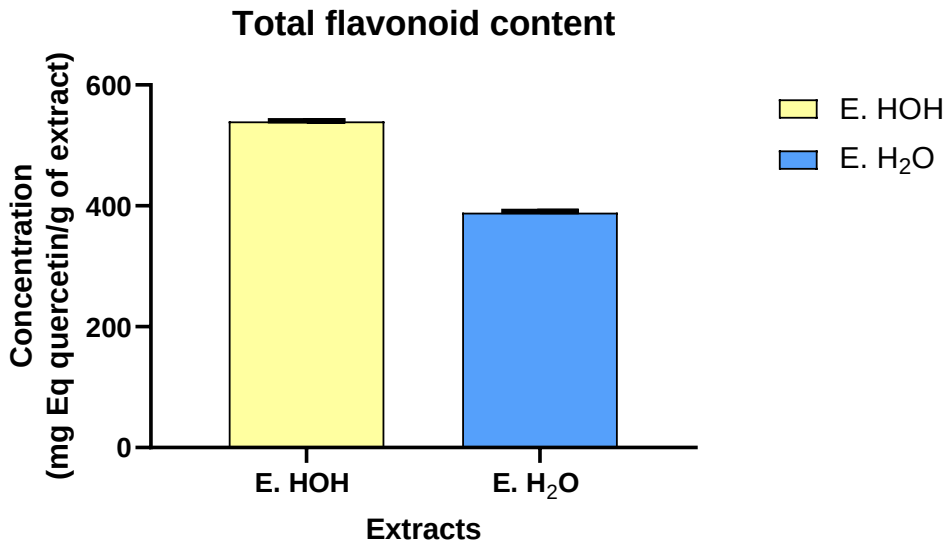
3.3 DPPH Test

The results of the DPPH radical reduction assay are shown in Figs 3 and 4. The concentrations corresponding to 50% inhibition (CI_{50}) for the hydroethanolic (4.4 mg/mL) and aqueous (6.0 mg/mL) extracts of *A. leiocarpus* are higher than that of the reference antioxidant (Gallic acid), which was 3.6 mg/mL. The IC value$_{50}$ is inversely proportional to the percentage of DPPH inhibition, so a lower IC_{50} corresponds to higher antioxidant activity. The two extracts therefore showed significant antioxidant powers, but relatively lower than that of gallic acid (the reference antioxidant).



*E.HOH = hydroethanolic extract 70% ; E.H2O = Aqueous extract ; ** p (= 0,9404) > 0,005 : différence not significant*

Fig. 1. Total polyphenol content of extracts from *A. leiocarpus*



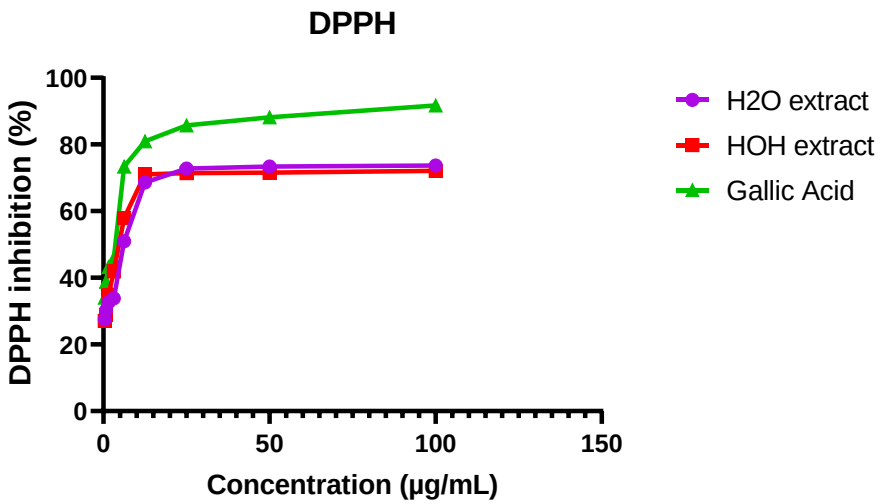
*E.HOH = Hydro éthanolic extract 70% ; E.H2O = Aqueous extract ; ** p (= 0,0027) < 0,005 : significant difference*

Fig. 2. Total flavonoid content of extracts from de *A. leiocarpus*

Table 1. Anti oxidant activities of *A. leiocarpus'* extracts by ABTS and FRAP tests

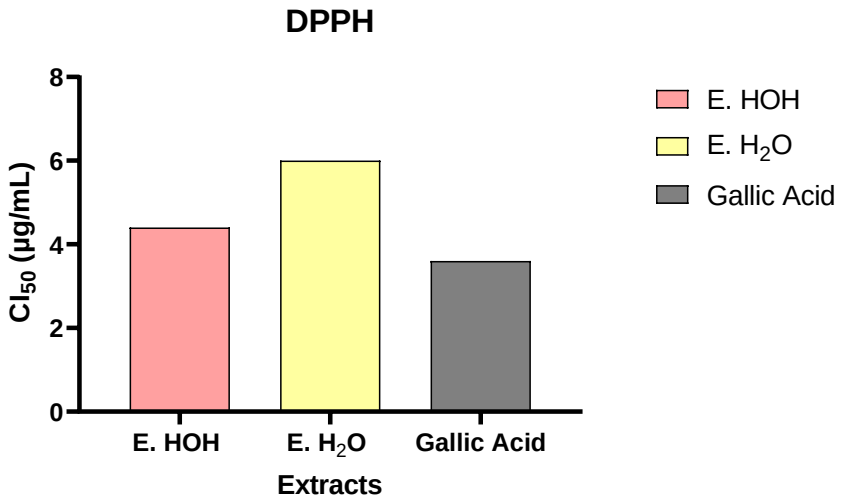
Extract or Reference molecule	Assays	
	ABTS (mM Eq Trolox/g)	FRAP (% of chelation)
E.H2O	8,32 ± 0,199	20,92 ± 0,195
E.HOH	11,56 ± 0,535	31,93 ± 0,078
Gallic Acid	64,71 ± 0,519	
EDTA		96,74 ± 0,275

E.HOH = 70% hydro ethanolic extract; E.H2O = Aqueous extract



E.HOH : Hydroethanolic extract 70% ; E.H2O : Aqueous extract

Fig. 3. Scavenging ability of extracts by DPPH test



E.HOH : hydro ethanolic extract 70% ; E.H2O : Aqueous extract

Fig. 4. IC50 values of *A. leiocarpus'* extracts by DPPH test

4. DISCUSSION

Determination of the phenolic compounds in the two extracts showed that the hydro-ethanolic and aqueous extracts of *A. leiocarpus* leaves had roughly equivalent concentrations of total polyphenols (608.41 and 558.25 mg EAG/g, respectively). In terms of total flavonoid content,

the hydro-ethanolic extract of *A. leiocarpus* leaves contained a higher level (539.60 mg EQ/g) than the aqueous extract (388.95 mg EQ/g).

Our results are in line with those of Koné et al. [19], who showed that *A. leiocarpus* leaves are rich in total polyphenols and flavonoids. The work by these authors, which looked at a total of six plants, showed that *A. leiocarpus* contained the highest level of total polyphenols (223.13 mg EAG/g, i.e. 4 times more than the leaves of *Vepris heterophylla*, which contained the lowest level of these compounds. This total polyphenol content is still much lower than those results obtained in current study.

In the study by the same authors, the trunk bark of *A. leiocarpus* contained only 26.53 mg EAG/g of polyphenols and 10.30 mg EQ/g of flavonoids. These levels of total polyphenols and flavonoids are also lower than those obtained in the present study. The antioxidant activity content of *Terminalia macroptera* root bark (468.8 µM Eq Trolox/g vs 361.60 mg/mL, for ABTS and DPPH tests) showed highest value followed by *A. leiocarpus* leaf bark (350.30 µM Eq Trolox/g vs 271.87 mg/mL, for ABTS and DPPH tests) Koné et al. [19]. Gheldolf et al. [20], Holasova et al. [21] and Kumaran et al. [13] have shown that there is a linear correlation between total polyphenol content and antioxidant activity in these plants.

In another study by Barku et al. [22], the antioxidant activity of methanolic extracts of *Amaranthus Spinosus, Anogeissus leiocarpus, Spondia monbin, Corchorus olitorius and Mallotus oppositifolia* medicinal plants was assessed by the DPPH test and the ferric ion reducing power (FRAP) test. The results showed that all these plants have antioxidant activitycomponent. However, *Anogeissus leiocarpus* showed the highest antiradical activity (95.86 ± 0.1%) followed by *Cochorus olitorius* (94.19 ± 0.06%), as percentages of DPPH radical inhibition ; while *Amaranthus spinosus* recorded the lowest activity (40.87± 2.5%) on the one hand and had the highest reducing power followed by *Spondia monbin ; Amaranthus spinosus* having the lowest reducing power, on the other hand. The leaves of *A. leiocarpus* also had the highest levels of total polyphenols (1294.81 ± 30 mg GEA) and flavonoids (540.23 ± 24.5 mg/g), while *Amaranthus spinosus* recorded the lowest levels of these same compounds with the following respective values: 48.01 ± 2.0 mg GEA and 63.16 ± 107 mg/g. Through this work, these authors have shown that the leaves of *Anogeissus leiocarpus* are rich in total polyphenols and flavonoids on the one hand and constitute an important source of antioxidants on the other.

This study confirms that *Anogeissus leiocarpus* is a plant that contains phenolic compounds. It also justifies our previous work, which consisted of searching for secondary metabolites in hydroethanol and aqueous extracts of *Anogeissus leocarpus.* Using the triphytochemical method, this study revealed the presence of phenolic compounds, cardiac glycosides, saponins, sterols, terpenes and alkaloids. However, this study also shows that the content of phenolic compounds, flavonoids and antioxidant activities in *Anogeissus leiocarpus* varies from one extract to another, from one organ to another, from one region to another and from one methodology to another. These remarks corroborate those of other authors who stipulate that the levels of phenolic compounds and their activities may depend on several intrinsic and extrinsic factors. These factors generally include the nature of the organ, the harvesting period, the extraction technique used, the solvent used, the geographical location and the method used to assess antioxidant activity [23,2]

However, the antioxidant activity of extracts from this plant could be attributed to total phenolic compounds and in particular total flavonoids [10]. The presence of phenolic compounds in the leaves of this plant and its interesting antioxidant activity (low IC_{50}) could justify its widespread use by livestock farmers in northern Côte d'Ivoire, particularly those rearing broiler chickens.

Indeed, according to Gerasopoulos et al. [24], dietary supplementation with phenolic compounds exhibits effective antioxidant activity in broilers. Moreover, it has been reported that vitamin E and C supplementation helps chickens combat oxidative stress and boost their immunity [25]. For example, *Equol*, which is also an antioxidant compound obtained from the isoflavonoid *daidzein*, a soy isoflavone, can reduce the oxidative load induced by ROS [26]. *Equol* also protects the intestinal epithelium against oxidative stress by enhancing the expression of antioxidant genes, stimulating the function of antioxidant enzymes and improving antioxidant capacity [27]. Also, the inclusion of

phenolic compounds in poultry farming has been shown to be effective in combating oxidative stress from rearing, processing to consumption of poultry meat. Plants contain powerful antioxidants, [28,29] so their use in diets as additives could improve digestion in broilers. They are natural sources of antioxidants, and studies have shown that these plants are much more potent than synthetic antioxidants [9,12,11,30,31]. In addition, the antimicrobial activities of phenolic compounds have already been demonstrated by several authors. Indeed, the mechanisms of action of certain phenolic compounds involve inhibiting cell wall synthesis, cell membrane function and protein synthesis [32,33,34]. Recently, one of our studies demonstrated the antisalmonial activity of this plant on multi-resistant strains of *Salmonella typhimurium* isolated from the droppings of farmed broilers. The massive use of *Anogeissus leiocarpus* by farmers in the north of Côte d'Ivoire, particularly those rearing broilers, could be justified by its phenolic compound content and antioxidant activities [35-38].

5. CONCLUSION

This study confirms that *Anogeissus leiocarpus* is a plant whose leaves contain variable levels of phenolic compounds. The presence of phenolic compounds in the leaves of this plant, together with its interesting antioxidant activity, could justify its widespread use by livestock farmers in northern Côte d'Ivoire, particularly those rearing broiler chickens. In view of the results obtained, *Anogeissus leiocarpus* could be a potential candidate for the development of improved traditional medicines for the treatment of infectious diseases and oxidative stress in poultry. It would therefore be useful to continue research on this plant with a view to gaining a better understanding of its anti-infectious and antioxidant properties in poultry farming. In the future, it would be interesting for us to study the *in vivo* antioxidant activities of this plant in chicks in order to measure the biomarkers of oxidative stress (catalase, peroxidase, malondialdehyde and nitric oxide).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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