Phenolic compound content and antioxidantactivity of extractsfrom the leaves of *Anogeissusleiocarpus*(Combretaceae), a plant used in the north of Côte d'Ivoire for the traditionaltreatment of gastrointestinal disorders in broilerchickens.

Abstract :

Anogeissusleiocarpus(**DC**.)**Guill. &Perr**. (Combretaceae) is a plant found in West Africa, fromSenegal to Cameroon and extending to Ethiopia and East Africa. In the northof the Ivory Coast, this plant iscommonlyused by livestockfarmers to treatvarious pathologies, including digestive and gastrointestinal disorders.

Theaim of thisstudywas to determine the phenolic compound content and antioxidantproperties of 70%hydroethanol and aqueousextracts of *A. leiocarpus'*leaves.Phenolic compound contents weredetermined by spectrophotometricmethods. (**Marinova and al and WOOD and al**). Antioxidantactivitieswereassessedusing ABTS, DPPH and FRAP tests. (**Choong and al ; Parejo and al ; Le and*al***).
The 70% hydroethanolic and aqueousextracts of *A. leiocarpus*presentedroughlyequivalent concentrations of total polyphenols (608.41 and 558.25 mg EAG/g respectively). However, the hydroethanolicextractwasricher in flavonoids (539.60 mg EQ/g) than the aqueousextract (388.95 mg EQ/g). In addition, the average inhibition rate of the ABTS radical in the presence of the 70% hydroethanolextractwas11.56 µM Troloxequivalent/g, comparedwith 8.32 µM Troloxequivalent/g with the aqueousextract. The averagechelation percentages were 31.93% (70% hydroethanolextract) and 20.92% (aqueousextract). As for the reduction of the DPPH radical, the ICs_{50} for the 70% hydroethanol and aqueousextractswere 4.4 mg/mL and 6.0 mg/mLrespectively.
Thisstudyconfirmsthat*Anogeissusleiocarpus*is a plant containingphenolic compounds. The presence of thesephenolic compounds in this plant, togetherwithitsantioxidantactivitieswithlowvalues (IC_{50}), couldjustifyitswidespread use by livestockfarmers in northern Côte d'Ivoire, particularlythoserearingbroilerchickens.

INTRODUCTION

The traditionalAfricanpharmacopoeiaconstitutes a veritablephytopharmacythat can beused in several areas of health care, for bothhumans and animals and even for plant (**Agaie and al, Belemnaba and al, 2007, Souad and*al* 2020, Mario and al 2014**). In the specific case of animals, variousstudies have shown the use of plant-basedremedies for theirtherapeutic management.

Anogeissusleiocarpus(**DC.) GUILL. & PERR**. (Combretaceae) is a plant found in West Africa, fromSenegal to Cameroon and extending to Ethiopia and East Africa (**Arbab, 2014**). Growing in dry forests and galleryforests (**Arbab, 2014; Ouédraogo and al., 2013**), its distribution extendsfrom the Sahara to the outer layer of tropical rainforests. The decoction of *Anogeisusleiocarpus*leavesisused to treatsmall ruminants sufferingfrom gastrointestinal parasitism, according to an ethnoveterinarystudyconducted in Burkina Faso (**Githiori and al., 2005)**. In the north of Côte d'Ivoire, this plant iscommonlyused by livestockfarmers to treatvarious pathologies including digestive and gastrointestinal disorders**(Kone and*al.*, 2019)**. **Julienne and *al*** (**2021**) confirmed in theirstudiesthat*A. leicocarpus*isused in veterinarymedicine, particularly in the treatment of parasiticdiseasescaused by *Haemonchuscontortus***.** The methanolicextract of *A. leiocarpus*stem bark has alsodemonstrated anti-trypanosomiasisactivityagainst four strains of Trypanosoma (**Shuaibu and*al.*, 2008**) as well as leishmanicidalactivity (Shuaibu et al., 2008b). *A. leicocarpus*also has strongantihelminthicactivity (**Juliennea and *al.*, 2021).**
In ourpreviouswork, weevaluated the *in vitro* efficacy*of A. leiocarpus*on multi-resistantstrains of *Salmonella typhimurium*isolatedfrom the droppings of farmedbroilers**(Ouattara and*al.*, 2023)**. Extractsfromthis plant showed good antimicrobialactivityagainstthesemulti-resistant*Salmonella typhimurium*germs. Our studyalsoshowed the presence of several groups of secondarymetabolites, differentlydistributed, with high levels of phenolic compounds in extractsfrom the leaves of this plant **(Ouattara and al., 2023).** Consequently, if farmers are to makebetter use of this plant in broilerrearing, thereisneed to design an improvedphytomedicine to help combat microbialresistance and oxidative stress effectively. In fact, oxidative stress isunavoidable in poultry production and affects the physiological, behavioural and biochemical state of growingchickens, which can cause theirdeath or deterioratemeatquality (appearance, texture, juiciness, tenderness and odour) **(Ali and Li, 2021).** Furthermore, curing an animal treated in a farminvolves not onlyeliminating the germ, but alsoreducing the signs of morbiditygenerallycaused by the massive production of free radicals in the affectedorganism**(Djeridane and*al*, 2006)**. However, severalsyntheticantioxidants are used by broiler breeders as feed additives to combat oxidative stress. Althoughtheseenrichedfoodsupplements are highly effective against free radicals, they are unfortunatelylikely to have side-effects and mayevenbetoxic**(Maman and*al*, 2008, Bramen 1975 ; Bougandoura 2012)**. Plants constitute a naturalreservoir of secondarymetabolites**(Lee and*al*, 2000 ;Cakir and*al*2003 ; Kumaran and*al* 2007)**, and have always been heavilyinvolved in the search for new naturalantioxidantsthat are effective and

have very few sideeffects. Withthis in mind, itwouldbe a greatadvantage if a plant-based formulation withantioxidantpropertiescouldbe made available to livestockfarmers in the long term. This is the reasonwhy, weconducted the presentstudy to assess the phenolic compound content and antioxidantproperties of twoextracts of *A. leiocarpus*, a plant from the Ivorianpharmacopoeiathatiscommonlyused by farmers in northern Côte d'Ivoire to treat gastrointestinal disorders in broilerchickens.

MATERIALS AND METHODS

Plant material

It consists of the leaves of *Anogeissusleiocarpus*. Theywereharvested 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.

Preparation ofaqueous and 70% hydoethanolicextracts of *Anogeissusleiocarpus*

The leaves of *Anogeissusleiocarpuswere*washed, cut and dried in the shade for a fortnight. Once dried, the plant materialwasground. 100g of thispowderwas mixed with 1 litre of distilled water. The mixture washomogenised at room temperature in the laboratoryusing a magneticstirrer for 24 hours. The homogenateobtainedwasfilteredtwice on cottonwool and once on Whatmanpaper(3 mm). The volume of the filtrateobtainedwasreducedusinga Med Center Venticelloven at 50°C to give a powderthatconstitutes the total aqueousextract(E.H2O) (**Ouattara and *al.*, 2013)**.

The sameoperationwascarried out using 70% ethanolinstead of distilled water, to obtain the 70% hydroethanolextract(E.HOH) **(Zirihiand *al.*, 2003**). The two plant extractsobtainedwerestored in the refrigerator for furthertesting.

Determination of total polyphenol content

The method of **Wood and *al* (2002)** wasused for the determination of total polyphenols. A volume of 2.5 ml of diluted (1/10) Folin-Ciocalteureagentwasadded to 30 µL of ethanolicextract. The mixture waskept for 2 min in the dark at room temperature, then 2 mL of calcium carbonate solution (75 $g.L^{-1}$)wasadded. The mixture washenplaced in a water bath at 50°C for 15 min and rapidlycooled. Absorbance wasmeasured at 760 nm, usingdistilled water as the blank. A calibration line was made withgallicacid at different concentrations. Analyses werecarried out in triplicate and the concentration of polyphenolswasexpressed in milligrams of gallicacidequivalent per gram of extract (mg GAE/g extract).

Determination of total flavonoid content

The method of **Marinovaand*al* (2005)** wasused to determine total flavonoids. In a 25-mL flask, 0.75 mL of 5% (w/v) sodium nitrite ($NaNO_2$)wasadded to 2.5 mL of extract. 0.75 mL of 10% (w/v) aluminium chloride ($AlCl_3$)wasadded to the mixture and incubated for 6 min in the dark. After incubation, 5 mL of sodium hydroxide (1N NaOH) wasadded and the volume was made up to 25 mL. The mixture wasshakenvigorouslybeforebeingassayedusing a UV-visible spectrophotometer. The readingwastaken at 510 nm. A calibration line wasconstructedwithquercetin at different concentrations. Trials werecarried out in triplicate. Flavonoid content wasexpressed as miligramquercetinequivalent per g extract (mg EQ /g extract).

Determination of the *in vitro* antioxidantactivities ofaqueous and 70%hydroethanolextracts.

2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid(ABTS) assay

This methodisbased on the ability of the compounds to reduce the ABTS+° (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid) radical-cation. The test wascarried out using the methoddescribed by **Choongand *al* (2007)**. The ABTS°+ radical-cation wasproduced by reacting 8 mM ABTS (87.7 mg in 20 mLdistilled water) and 3 mM potassium persulphate (0.0162 g in 20 mLdistilled water) in a 1 :1 (v/v) ratio. The mixture wasthenincubated in the dark at room temperature for 12-16 hours. This ABTS°+ solution wasdilutedwithmethanol to obtain a solution with an absorbance of 0.7 ± 0.02 at 734 nm. A test portion of 3.9 mL of thisdiluted ABTS°+ solution wasadded to 100 µL of the test compound. Aftershaking, the mixture wasincubated for 6 min in the dark (T=30±2°C). The residual absorbance of the ABTS+° radical wasthenmeasured at 734 nm using a UV-visible spectrophotometer and wasexpected to bebetween 20% and 80% of the absorbance of the blank. The tests werecarried out in triplicate and the resultswereexpressed in µmol Troloxequivalentper litre of extract (µmol TE/L). A calibration line wasperformedwith 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°+ wasexpressed 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

Antioxidantconcentration or activity(2) :

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

Ironchelation (FRAP)

The chelatingcapacity of the extractswasmeasuredusing the method of **Le and *al* (2007)**. Solutions of extracts and the referenceantioxidant (EDTA 500 µl) wereinitially 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) wasadded to the reaction medium. The mixture wasshakenwell and thenleft to react for 10 min at room temperature, allowing complexation of the residualiron and formation of a chromophore with an absorption maximum at 562 nm. The negative control contains all the reagentsexcept the test sample, whichisreplaced by an equal volume of methanol. Readings are taken at 562 nm against a methanolblank. The sequesteringeffect of the samples on ironisexpressed as a percentage of chelationaccording to the followingequation (3) :

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

2, 2'-diphenyl-1-picrylhydrazyl (DPPH)) radical scavengingassay

The anti-free radical activity of the two plant extractswasmeasuredusing the 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) test according to the method of **Parejoand *al.* (2000)**. A range of concentrations (0-200 µg/mL) of plant extract or gallicacid (referenceantioxidant) wasprepared. A volume of 2.5 mL of this solution was mixed with 2.5 mL of DPPH (100 µM) prepared in methanol. Afterhomogenisation, the mixture wasincubated at room temperature (25°C) in the dark. After 15 min incubation, the absorbance wasread at 517 nm against a "blank" containingonlymethanol. The percentage inhibition of the DPPH radical wascalculatedusing the followingequation :

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

The $IC_{50}$is the concentration of plant extract or quercetinresponsible for 50% inhibition of DPPH radicals. It isdeterminedfrom the graph showing the percentage of DPPH inhibition as a function of extract and Gallic Acid concentrations.

Statisticalanalysis

Data analysis and graphicalrepresentationwerecarried out using Graph Pad Prism 8.0.1 (Microsoft, USA). The mean value isaccompanied by the standard error of the mean (Mean ± SEM). The differencebetweentwo values isconsideredsignificantwhen$P < 0.001$. The resultswerestatisticallyanalysedusing a two-wayanalysis of variance (ANOVA 2)

RESULTS

Phenolic compound content

Determination of phenolic compounds in the twoextractsfrom*A. leiocarpus*leavesshowedthatboth the hydroethanolextract (E.HOH) and the aqueousextract (E.H2O) hadroughlyequivalent concentrations of total polyphenols (608.41 and 558.25 mg EAG/g respectively) (Figure 1). In terms of total flavonoids, the hydroethanolextract of *A. leiocarpus*leaveswasricher (539.60 mg EQ/g) than the aqueousextract (388.95 mg. EQ/g) (Figure 2).

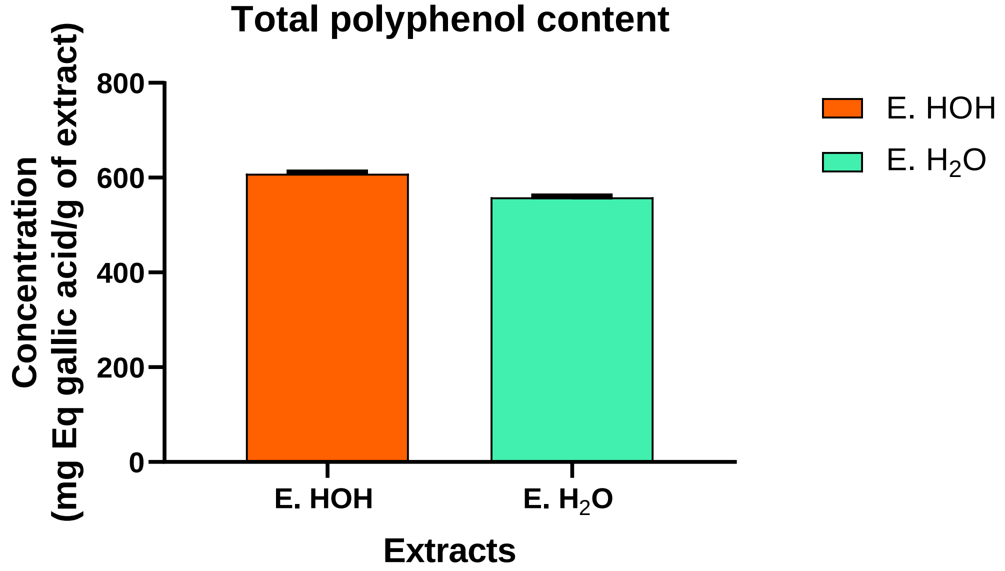

Figure 1 : Total polyphenol content of extractsfrom*A. leiocarpus*

E.HOH = hydroethanolicextract 70% ; E.H2O = Aqueousextract ; ** p (= 0,9404) > 0,005 : différence notsignificant

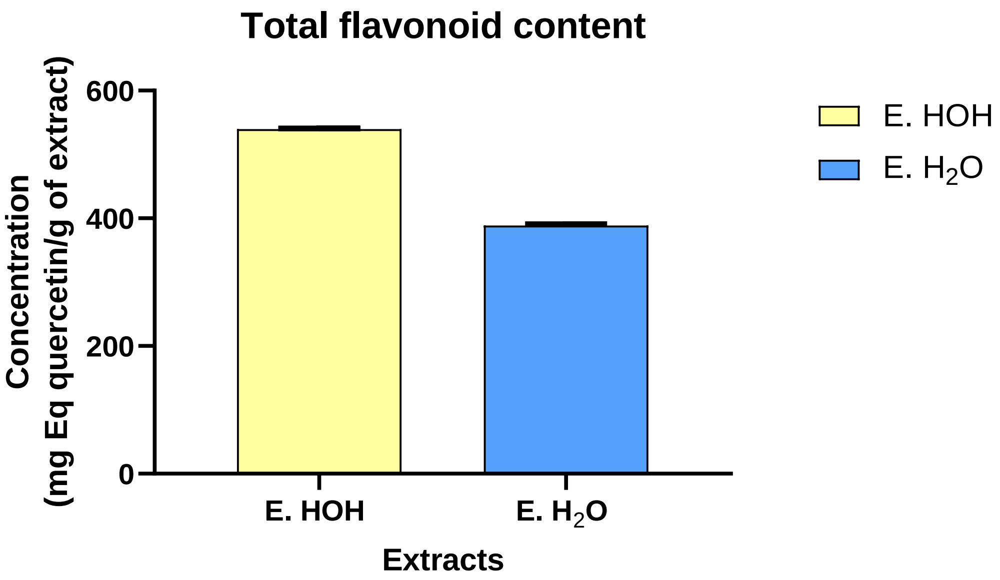

Figure 2 : Total flavonoid content of extractsfrom de *A. leiocarpus*

E.HOH = Hydro éthanolicextract 70% ; E.H2O = Aqueousextract ; ** p (= 0,0027) < 0,005 :significantdifference

ABTS test

For a concentration of 100 mg/mL, the average inhibition rate of the ABTS radical in the presence of the 70% hydroethanolextractwas11.56 µM Trolox eq/g, whereasitwas8.32 µM Trolox eq/g with the aqueousextract. For thesetwoextracts, the inhibition rates obtainedweremuchlowercompared to that of gallicacid, whichrecorded a value of 64.71 µM Trolox eq/g (Table 1).

Chelating power (FRAP)

The averagechelation percentages were 31.93% (70% hydroethanolextract) and 20.92% (aqueousextract). However, these values are significantlylowerthanthoseobtainedwith the referenceantioxidant (EDTA), whoseaveragechelation percentage wasestimated at 96.74%. Theseresults are shown in Table 1.

Table 1 : Anti oxidantactivities 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

DPPH test

The results of the DPPH radical reductionassay are shown in Figures 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 higherthanthat of the referenceantioxidant (Gallicacid), whichwas 3.6 mg/mL. The IC value$_{50}$isinverselyproportional to the percentage of DPPH inhibition, so a lower IC_{50} corresponds to higherantioxidantactivity. The twoextractsthereforeshowedsignificantantioxidantpowers, but relativelylowerthanthat of gallicacid (the referenceantioxidant).

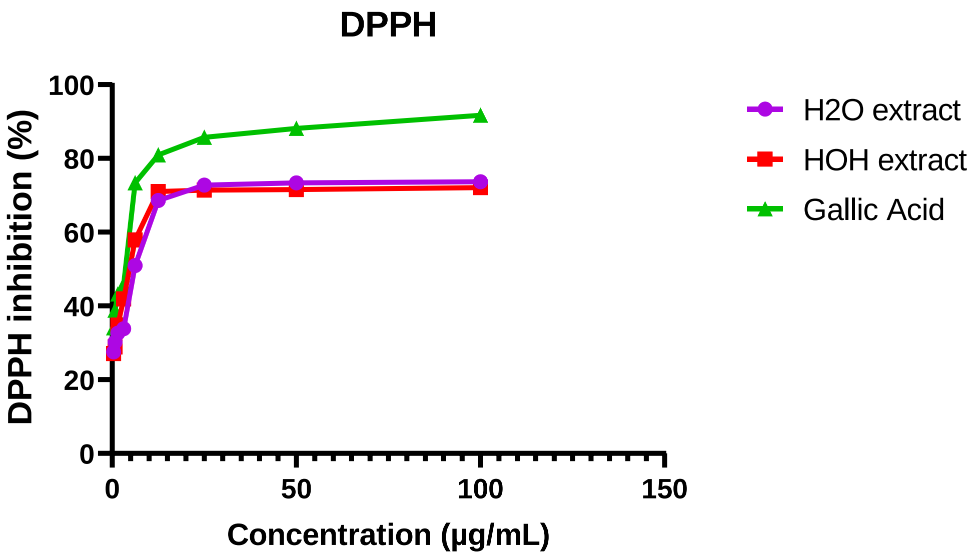

Figure 3: Scavengingability of extracts by DPPH test

E.HOH : Hydroethanolicextract 70% ; E.H2O : Aqueousextract

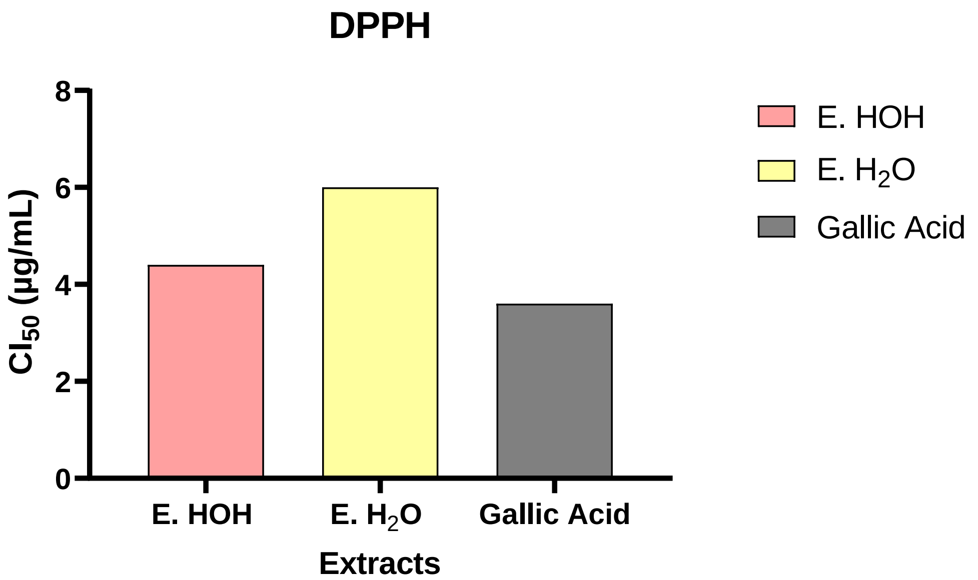

Figure 4: IC50 values of *A. leiocarpus'*extracts by DPPH test

E.HOH : hydro ethanolicextract 70% ; E.H2O : Aqueousextract

DISCUSSION

Determination of the phenolic compounds in the twoextractsshowedthat the hydro-ethanolic and aqueousextracts of *A. leiocarpus*leaveshadroughlyequivalent concentrations of total polyphenols (608.41 and 558.25 mg EAG/g respectively). In terms of total flavonoid content, the hydro-ethanolicextract of *A. leiocarpus*leavescontained a higherlevel (539.60 mg EQ/g) than the aqueousextract (388.95 mg EQ/g).

Our results are in line withthose of **Koné and*al* in 2006**, whoshowedthat*A. leiocarpus*leaves are rich in total polyphenols and flavonoids. The work by theseauthors, whichlooked at a total of six plants, showedthat*A. leiocarpus*contained the highestlevel of total polyphenols (223.13 mg EAG/g, i.e. 4 times more than the leaves of *Veprisheterophylla*, whichcontained the lowestlevel of these compounds. This total polyphenol content isstillmuchlowerthanthatwhichweobtained, whichvariedfrom 558.25 mg GAE/g (aqueousextract) to 608.41 mg GAE/g (hydroethanolextract).

In the study by the sameauthors, the trunkbark of *A. leiocarpus*containedonly 26.53 mg EAG/g of polyphenols and 10.30 mg EQ/g of flavonoids. Theselevels of total polyphenols and flavonoids are alsolowerthanthoseobtained in the presentstudy. *Terminaliamacroptera*root barkshowed the highestantioxidantactivity, followed by *A. leiocarpus*leafbark in tests using ABTS and DPPH, with respective values of 468.8 µM Eq Trolox/g and 350.30 µM Eq Trolox/g (ABTS) and 361.60 mg/mL and 271.87 mg/mL (DPPH) (**Koné and*al*, 2009). Gheldolf and*al*, 2002 ;Holasova and*al*, 2002 and Kumaran and*al*, 2005** have shownthatthereis a linearcorrelationbetween total polyphenol content and antioxidantactivity in these plants.

In anotherstudy by **Barku and*al* (20013)**, the antioxidantactivity of methanolicextracts of 5 medicinal plants wasassessedusing the DPPH test and the ferric ion reducing power (FRAP) test. Thesewere*AmaranthusSpinosus, Anogeissusleiocarpus, Spondiamonbin, Corchorusolitorius and Mallotusoppositifolia.* The resultsshowedthat all these plants have antioxidantactivity. However, *Anogeissusleiocarpus*showed the highestantiradicalactivity (95.86 ± 0.1%) followed by *Cochorusolitorius*(94.19 ± 0.06%), as percentages of DPPH radical inhibition ;while*Amaranthusspinosus*recorded the lowestactivity (40.87± 2.5%) on the one hand and had the highestreducing power followed by *Spondiamonbin* ;*Amaranthusspinosus*having the lowestreducing power, on the other hand. The leaves of *A. leiocarpus*alsohad the highestlevels of total polyphenols (1294.81 ± 30 mg GEA) and flavonoids (540.23 ± 24.5 mg/g), while*Amaranthusspinosus*recorded the lowestlevels of thesesame compounds with the following respective values: 48.01 ± 2.0 mg GEA and 63.16 ± 107 mg/g. Throughthiswork, theseauthorshave shownthat the leaves of *Anogeissusleiocarpus*are rich in total polyphenols and flavonoids on the one hand and constitute an important source of antioxidants on the other.

This studyconfirmsthat*Anogeissusleiocarpus*is a plant thatcontainsphenolic compounds. It also justifies ourpreviouswork, whichconsisted of searching for secondarymetabolites in hydroethanol and aqueousextracts of *Anogeissusleocarpus*. Using the triphytochemicalmethod, thisstudyrevealed the presence of phenolic compounds, cardiac glycosides, saponins, sterols, terpenes and alkaloids**(Ouattara and *al*., 2023)**. However, thisstudyalso shows that the content of phenolic compounds, flavonoids and antioxidantactivities in *Anogeissusleiocarpus*varies from one extract to another, from one organ to another, from one region to another and from one methodology to another. Theseremarkscorroboratethose of otherauthorswhostipulatethat the levels of phenolic compounds and theiractivitiesmaydepend on severalintrinsic and extrinsicfactors. Thesefactorsgenerallyinclude the nature of the organ, the harvestingperiod, the extraction technique used, the solvent used, the geographical location and the methodused to assessantioxidantactivity(**Hanenand*al* 2022, and Souad and *al***).

However, the antioxidantactivity of extractsfromthis plant couldbeattributed to total phenolic compounds and in particular total flavonoids**(Djeridane and*al*, 2006)**. The presence of phenolic compounds in the leaves of this plant and itsinterestingantioxidantactivity (low IC_{50})couldjustifyitswidespread use by livestockfarmers in northern Côte d'Ivoire, particularlythoserearingbroilerchickens.

Indeed, according to **Gerasopoulos and *al*. (2015)**, dietarysupplementationwithphenolic compounds exhibits effective antioxidantactivity in broilers. Moreover, it has been reportedthatvitamin E and C supplementationhelpschickens combat oxidative stress and boost theirimmunity**(Minand and *al*.,2018)**. For example, *Equol*, whichisalso an antioxidant compound obtainedfrom the isoflavonoid*daidzein*, a soy isoflavone, can reduce the oxidativeloadinduced by ROS **(Liu and al., 2006)**. *Equol*alsoprotects the intestinal epitheliumagainstoxidative stress by enhancing the expression of antioxidantgenes, stimulating the function of antioxidant enzymes and improvingantioxidantcapacity**(Linand al., 2016)**. Also, the inclusion of phenolic compounds in poultryfarming has been shown to be effective in combatingoxidative stress fromrearing, processing to consumption of poultrymeat. Plants containpowerfulantioxidants,(**Bidie and*al*. 2011, Ercetin et *al*. 2012**)sotheir use in diets as additives couldimprove digestion in broilers. They are natural sources of antioxidants, and studies have shownthatthese plants are much more potentthansyntheticantioxidants**(Ali and Li, 2021, Bougandoura and *al* 2012, Bramen and*al* 1975)**. In addition, the antimicrobialactivities of phenolic compounds have already been demonstrated by severalauthors. Indeed, the mechanisms of action of certain phenolic compounds involveinhibitingcellwallsynthesis, cell membrane function and proteinsynthesis**(Bhuiyan and*al.*2009 ;Pasril and Yuliasanti 2014)**. Recently, one of ourstudiesdemonstrated the antisalmonialactivity of this plant on multi-resistantstrains of *Salmonella typhimurium*isolatedfrom the droppings of farmedbroilers**(Ouattara and al., 2023)**. The massive use of *Anogeissusleiocarpus*by farmers in the north of Côte d'Ivoire, particularlythoserearingbroilers, couldbejustified by itsphenolic compound content and antioxidantactivities.

CONCLUSION

This studyconfirmsthat*Anogeissusleiocarpus*is a plant whoseleavescontain variable levels of phenolic compounds. The presence of phenolic compounds in the leaves of this plant, togetherwithitsinterestingantioxidantactivity (IC_{50}between 4.4 mg/mL and 6.0 mg/mL), couldjustifyitswidespread use by livestockfarmers in northern Côte d'Ivoire, particularlythoserearingbroilerchickens. In view of the resultsobtained, *Anogeissusleiocarpus*couldbe a potential candidate for the development of improvedtraditionalmedicines for the treatment of infectiousdiseases and oxidative stress in poultry. It wouldthereforebeuseful to continue research on this plant with a view to gaining a betterunderstanding of its anti-infectious and antioxidantproperties in poultryfarming. In the future, itwouldbeinteresting for us to study the *in vivo* antioxidantactivities of this plant in chicks in order to measure the biomarkers of oxidative stress (catalase, peroxidase, malondialdehyde and nitric oxide).

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