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Polyphenols quantification and antioxidant activity of methanolic and aqueous extracts from eight medicinal plants used to manage avian diseases in Burkina Faso

Windmi Kagambega^{1*}, Roland Nâg-Tiéro Meda^{1,2}, Benjamin Kouliga Koama^{1,2,3}, Anne-Flora Drabo¹, Hadidjatou Belem¹, Der Dabire⁴, Jacques Kabore^{2,4}, Amadou Traore⁵ and George Anicet Ouédraogo¹

¹Laboratoire De Recherche Et d'Enseignement En Santé Et Biotechnologies Animales, Unité De Formation Et De Recherche En Sciences Et Techniques, Université Nazi Boni, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso.

²Unité de Formation et de Recherche en Sciences et Techniques, Université Nazi Boni, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso.

³Laboratoire De Médecine Et Pharmacopée Traditionnelle, Institut De Recherche En Sciences De La Santé (IRSS), 01 BP 2779 Bobo-Dioulasso, Direction Bobo-Dioulasso, Burkina Faso.

⁴Centre International de Recherche-Développement sur l'Élevage en zones Subhumides (CIRDES),

Unité de recherches sur les bases biologiques de la lutte intégrée, 01 BP 454 Bobo-Dioulasso 01, Burkina Faso.

⁵Laboratoire de Biologie et Santé Animales, Institut de l'Environnement et de recherches agricoles (INERA), 04 BP 8645 Ouagadougou 04, Burkina Faso.

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The present study aimed to quantify the polyphenols and evaluate the antioxidant activity of methanolic and aqueous extracts from eight medicinal plants used by the poultry farmers to manage avian diseases in Burkina Faso. Total phenolics and total flavonoids were quantified using Folin-Ciocalteu and AlCl_3 reagents, respectively. The antioxidant activity was evaluated using three methods: 1,1-diphényl-2-picrylhydrazyl (DPPH), 2'-2-azino-bis (3-éthylbenzothiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP). The results obtained showed that aqueous extracts contain more total phenolics than methanolic extracts in contrary to the total flavonoids where methanolic extracts presented the best contents. The evaluation of the antioxidant activity by the three methods showed that the extracts exhibited an interesting activity. The methanolic and the aqueous extracts of *Parkia biglobosa*, *Sclerocarya birrea*, and *Detarium microcarpum*, are distinguished by a good capacity to scavenge free DPPH radicals than those of the standards (trolox and quercetin). The methanolic extracts of all plant samples have shown their superiority in terms of their ability to scavenge the ABTS radical cations compared to aqueous extracts. Compared to other plant extracts and quercetin the methanolic and aqueous extracts of *D. microcarpum* bark presented the best reducing power with the values of 5360.23 and 4584.55 $\mu\text{mol EAA / g extract}$, respectively. These different results could justify the traditional uses of these plants in the treatment of avian pathologies.

Key words: Medicinal plants, total phenolics, total flavonoids, antioxidant, poultry diseases.

INTRODUCTION

The zoo-sanitary situation of poultry farms in West Africa is dominated by viral diseases (Newcastle, infectious

bronchitis, cholera, avian smallpox) and parasitic diseases such as coccidiosis (Bichet et al., 2003). This situation can cause gross mortality of chickens up to 80 to 90% including 40 to 60% of chicks from 0-2 months of age (MRA, 2007; Ouédraogo et al., 2015).

The drastic reduction of this mortality in poultry farming requires disease prevention measures, without compromising the health of animals and their welfare as well as the profitability of farms. These measures may include the use of medicinal plants for preventive and therapeutic purposes.

Studies have shown that poultry diseases induce inflammation as a result of production of free radicals and reactive oxygen species in chickens (Henard and Vázquez-Torres, 2011; Sokoudjou et al., 2019). As a result, cellular and oxidative damage related to free radicals or reactive oxygen species (ROS) seems to be one of the main causes of several human and animal diseases today (Huang et al., 2005; Ziyatdinova et al., 2005; Li et al., 2009).

Many trees and shrubs have been claimed by ethno veterinary practitioners, ethno medical practitioners and other local people as having medicinal properties against infectious animal diseases (Masola et al., 2009). These plants have an incredible ability to produce a wide variety of secondary metabolites such as phenolic compounds (flavonoids), which are very effective in scavenging free radicals generated by infectious diseases and preventing injuries caused by them (Procházková et al., 2011).

Due to their phenolic structure with the presence of hydroxyl groups, polyphenols are very effective in stopping radical reactions by neutralizing free radicals (Mghezzi-Habellah et al., 2016). These free radicals, which form naturally in the normal metabolism of living organisms, can be captured by chemoprevention using antioxidant compounds found in medicinal plants (Verma et al., 2009). Therefore, the antioxidant activity of plants is an important part of medicinal plant research.

All of this scientific data shows that the reduction in of mortality rates in poultry farming could be through the use of medicinal plants for preventive and therapeutic purposes. The present work, although preliminary, was initiated to quantify the polyphenols and evaluate the antioxidant activity of methanolic and aqueous extracts from eight medicinal plants used by the poultry farmers to manage the avian diseases in Burkina Faso.

MATERIALS AND METHODS

Chemicals and reagents

The Folin-Ciocalteu reagent, NaH_2PO_4 , Na_2HPO_4 , sodium

carbonate, aluminum trichloride, gallic acid and quercetin were purchased from Sigma-aldrich chimie, Steinheim, Germany. 2, 2-diphenylpicrylhydrazyl (DPPH), trichloroacetic acid, and solvents used were from Fluka Chimie, Switzerland. Potassium hexacyanoferrate [$\text{K}_3\text{Fe}(\text{CN})_6$] was from Prolabo and ascorbic acid was from Labosi, Paris, France. All chemicals used were of analytical grade.

Plant materials

The barks of 8 medicinal plants were collected in Bobo-Dioulasso in the classified forest of Dinderesso (village located 15 km from Bobo-Dioulasso in Burkina Faso) in April 2019. The plants were identified by Dr Ouaba Yempabou Hermann a botanist-cytoecologist from the University NAZI Boni of Bobo -Dioulasso (UNB). The different parts of the plants were carefully cleaned, reduced to small pieces, air-dried at room temperature for about two weeks and reduced to powder using an aluminum mortar.

Extracts preparation

Preparation of aqueous extracts: 50 g of plant samples were extracted with 500 ml of distilled water. The mixture was heated and boiled under reflux for 30 min at 100°C. After filtration, the extracts were frozen and lyophilized.

Preparation of methanolic extracts: 20 g of powdered plant material was extracted with 200 ml of methanol by a soxhlet apparatus. After 4 h, the extraction solution was concentrated to maximum using soxhlet despositive. The concentrate was dried at room temperature in the Petri dishes. The obtained extracts were weighed before packed in waterproof plastic flasks and stored at 4°C. until their use.

Determination of total phenolics and total flavonoids

Total phenolics content

Total phenolics of each extract were determined by Folin-Ciocalteu reagent method as described by Meda et al. (2010). After dilution, 125 μl (0.1 mg/ml) of each extract was mixed with 625 μl of Folin-Ciocalteu reagent (0.2 N). This mixture was allowed to stand at room temperature for 5 min and then, 500 μl of aqueous sodium carbonate (75 g/L) was added. After 2 h of incubation, the absorbencies were measured at 760 nm against a blank (125 μl distilled water + 625 μl Folin-Ciocalteu reagent + 500 μl Na_2CO_3) using a GENESYS 30 Visible Spectrophotometer. The standard calibration curve was plotted using gallic acid ($y = 4.668e^{-3}x - 0.034$, $r^2 = 0.9991$). The mean of three readings was used and the results expressed as mg of Gallic acid equivalents (GAE)/100 mg of extract.

Total flavonoid content

The total flavonoids were evaluated by the aluminum trichloride method (Meda et al., 2010). Briefly, 625 μl of extracts (0.1 mg/ml) was mixed with 625 μl of aluminum trichloride (AlCl_3) in methanol (2%). The absorbencies reading at 415 nm were taken after 10 min against a blank consisted in 625 μl of extracts and 625 μl of

*Corresponding author. E-mail: kagambegawindmi@yahoo.fr. Tél: +22670933652/76875769.

Table 1. Total phenolics and flavonoids from methanolic and aqueous extracts of 8 plants.

Plants	Total phenolics (mg GAE/100 mg extracts)		Total flavonoids (mg QE/100 mg extracts)	
	MeOH	Aqueous	MeOH	Aqueous
<i>Parkia biglobosa</i>	58.12±0.24 ^{gh}	63.62±1.11 ^e	2.35±0.09 ^d	undetermined
<i>Daniellia oliveri</i>	59.48±0.49 ^{fg}	81.97±1.60 ^b	3.28±0.04 ^c	0.84±0.11 ^f
<i>Sclerocarya birrea</i>	58.36±0.67 ^{gh}	77.33±0.8 ^{cd}	3.15±0.04 ^c	0.16±0.00 ⁱ
<i>Detarium microcarpum</i>	75.76±0.24 ^d	85.83±1.60 ^a	1.8±0.04 ^e	0.50±0.04 ^h
<i>Lannea acida</i>	57.34±2.10 ^h	57.90±1.42 ^{gh}	4.47±0.18 ^a	0.47±0.08 ^h
<i>Pterocarpus erinaceus</i>	61.05±0.93 ^f	53.05±1.25 ⁱ	3.15±0.09 ^c	0.66±0.04 ^g
<i>Acacia albida</i>	38.91±0.98 ^j	63.19±0.56 ^e	3.81±0.13 ^b	undetermined
<i>Vitellaria paradoxa</i>	54.62±0.64 ⁱ	78.40±0.64 ^c	0.26±0.04 ⁱ	0.53±0.09 ^{gh}

mg GAE/100 mg extracts: mg equivalent gallic acid for 100 mg dried extracts; mg QE/100 mg extracts: mg Equivalent Quercetin for 100 mg dried extracts.

methanol without AlCl₃. Quercetin was used as reference compound to produce the standard curve ($y=1.259e^{(-2)}x$, $r^2=0.9990$) and the results were expressed as milligrams of quercetin equivalent (QE)/100 mg of extract.

In vitro antioxidant activity determination

DPPH radical method

The antioxidant activity which evaluates the capacity to reduce the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was determined according to the spectrophotometric method described by Meda et al. (2010). A volume of 0.375 ml of the sample solution (0.1 mg/ml) was mixed with 0.75 ml of the DPPH solution (20 mg / L) and the whole is incubated for 15 min in the dark. The absorbances were read at 517 nm with the GENESYS 30 Visible Spectrophotometer. The capacity of the extracts to trap free radicals was determined using a reference curve of ascorbic acid ($y = -2.224e^{-2}x + 0.348$, $r^2 = 0.9966$). The results are expressed in μ mol ascorbic acid equivalent for 1 g of extract (μ mol EAA / g of extract).

ABTS radical cation decolorization assay

The radical scavenging capacity of antioxidants for the 2, 2'-azinobis-3-éthylenzothiazoline-6-sulfonic acid (ABTS) radical cation was determined as described by Meda et al. (2010). ABTS^{•+} was generated by mixing a 7 mM aqueous solution of ABTS with 2.5 mM potassium persulfate (final concentration) followed by storage in the dark at room temperature for 12 h before use. The mixture was diluted with ethanol to give an absorbance of 0.70± 0.02 units at 734 nm using spectrophotometer. For the study, 10 μ l of the diluted extracts (100 μ g ml⁻¹ in methanol) was allowed to react with 990 μ l of fresh ABTS^{•+} solution and the absorbance was taken 15 min after initial mixing. Ascorbic acid was used as standard ($y = -7.874e^{-4}x + 0.709$, $r^2 = 0.9993$) and the capacity of free radical scavenging was expressed as μ mol ascorbic acid equivalent (AAE)/g of extract.

Iron (III) to iron (II) reduction activity (FRAP)

The FRAP assay was done according to the method of Meda et al. (2010). Briefly, 0.5 ml of extract (100 μ g ml⁻¹) were mixed with 1.25 ml of phosphate buffer (0.2 M, pH 6.6) and 1.25 ml of potassium

hexacyanoferrate [K₃Fe(CN)₆] solution (1%). After 30 min incubation at 50°C, 1.25 ml of trichloroacetic acid (10%) was added and the mixture was centrifuged at 3000× g for 10 min. Then, the upper layer solution (0.625 ml) was mixed with distilled water (0.625 ml) and a freshly prepared FeCl₃ solution (0.125 ml, 0.1%). Absorbencies were read at 700 nm and Ascorbic acid was used to produce the calibration curve ($y = 3.270e^{-3}x$, $r^2 = 0.9990$). The iron (III) reducing activity determination was performed in triplicate and expressed in μ mol Ascorbic Acid Equivalent (AAE)/g of extract.

Statistical analysis

The data are expressed as the Means ± Standard deviation (SD) of three determinations. Statistical analysis (ANOVA) with a statistical significance level set at $p < 0.05$ and linear regression was carried out with XLSTAT 7.1.

RESULTS

Determination of total phenolics and total flavonoids

Table 1 summarizes the results for total phenolics and flavonoids. The contents of the total phenolics of the methanolic extracts varied from 75.76 to 38.91 mg GAE / 100 mg extract and that of the aqueous extracts were 81.97 to 53.05 mg GAE / 100 g extract. One can notice that except the *Pterocarpus erinaceus* sample these contents were significantly higher ($p < 0.05$) in aqueous extracts, than methanolic extracts. Concerning both types of extracts the highest content of total phenolics was measured in *Detarium microcarpum* followed by *Daniellia oliveri*.

Using AlCl₃ reagent and quercetin as standard, the total flavonoids contents varied from 4.47 mg EQ / 100 mg (*Lannea acida*) to 0.26 mg EQ / 100 mg (*Vitellaria paradoxa*) for methanolic extracts. The highest content of total flavonoids of the aqueous extract was found with *Daniellia oliveri* aqueous extract (0.84 mg EQ / 100 mg) and the lowest level with *Sclerocarya birrea* aqueous extract (0.16 mg EQ / 100 mg). The total flavonoid

Table 2. Antioxidant activity of methanolic and aqueous extracts from eight plants using DPPH, ABTS and FRAP methods.

Samples	DPPH ($\mu\text{mol EAA} / \text{g extract}$)		ABTS ($\mu\text{mol EAA} / \text{g extract}$)		FRAP ($\mu\text{mol EAA} / \text{g extract}$)	
	MeOH	Aqueous	MeOH	Aqueous	MeOH	Aqueous
<i>Parkia biglobosa</i>	792.39 $\pm$ 0.05 ^{bcd}	828.98 $\pm$ 0.02 ^a	6178.11 $\pm$ 0.73 ^f	5697.33 $\pm$ 00 ^g	5192.35 $\pm$ 1.90 ^b	3640.99 $\pm$ 0.17 ^f
<i>Daniellia oliveri</i>	658.77 $\pm$ 0.07 ^g	743.02 $\pm$ 0.35 ^{ef}	10385.01 $\pm$ 0.73 ^c	2247.681 $\pm$ 2.93 ^j	3930.45 $\pm$ 3.53 ^e	3282.13 $\pm$ 1.05 ^g
<i>Sclerocarya birrea</i>	828.98 $\pm$ 0.02 ^a	809.42 $\pm$ 00 ^{abc}	8365.7 $\pm$ 2.54 ^d	2134.69 $\pm$ 008 ^j	4949.23 $\pm$ 2.38 ^c	4596.12 $\pm$ 1.23 ^d
<i>Detarium microcarpum</i>	817.05 $\pm$ 0.04 ^{ab}	815.37 $\pm$ 0.06 ^{abc}	12308.15 $\pm$ 1.93 ^b	3944.861 $\pm$ 00 ^h	5360.23 $\pm$ 0.35 ^b	4584.55 $\pm$ 0.53 ^d
<i>Lannea acida</i>	787.28 $\pm$ 0.10 ^{cd}	742.17 $\pm$ 0.22 ^{ef}	10264.81 $\pm$ 1.93 ^c	4003.21 $\pm$ 1.07 ^h	3803.08 $\pm$ 0.80 ^{ef}	3206.87 $\pm$ 1.07 ^g
<i>Pterocarpus erinaceus</i>	803.44 $\pm$ 0.32 ^{abc}	653.66 $\pm$ 0.11 ^{gh}	7740.67 $\pm$ 3.19 ^e	3437.63 $\pm$ 1.930 ⁱ	2900.07 $\pm$ 0.30 ^h	2199.64 $\pm$ 1.76 ⁱ
<i>Acacia albida</i>	793.24 $\pm$ 0.09 ^{bcd}	627.82 $\pm$ 0.14 ^h	6366.64 $\pm$ 2.81 ^f	2077.00 $\pm$ 00 ^j	2934.8 $\pm$ 0.53 ^h	1649.76 $\pm$ 1.58 ^j
<i>Vitellaria paradoxa</i>	720.045 $\pm$ 0.04 ^f	731.119 $\pm$ 0.10 ^f	10433.08 $\pm$ 5.86 ^c	2024.11 $\pm$ 3.66 ^j	3901.49 $\pm$ 1.23 ^e	2900.09 $\pm$ 1.05 ^h
Trolox	765.98 $\pm$ 0.31 ^{de}	765.98 $\pm$ 0.31 ^{de}	8137.61 $\pm$ 0.24 ^d	8137.61 $\pm$ 0.24 ^d	5991.29 $\pm$ 1.33 ^a	5991.29 $\pm$ 1.33 ^a
Quercetine	646.8 $\pm$ 0.02 ^{gh}	646.8 $\pm$ 0.02 ^{gh}	14664.01 $\pm$ 0.24 ^a	14664.01 $\pm$ 0.24 ^a	2211.24 $\pm$ 0.63 ⁱ	2211.24 $\pm$ 0.63 ⁱ

$\mu\text{mol EAA} / \text{g extracts}$: μmol equivalent Ascorbic Acid for 1g dried extracts.

The values represent the mean and standard deviation for three independent samples. Different letters same column (or line) means significant differences ($p \leq 0.05$) for our different plants.

content of the methanolic extracts was significantly higher ($p < 0.05$) than that of the aqueous extracts. Flavonoids could not be quantified in the aqueous extracts of *Parkia biglobosa* and *Acacia albida* using the AlCl_3 reagent. The values represent the mean and standard deviation for three independent samples. Different letters same column (or line) means significant differences ($p \leq 0.05$) for our different plants.

In vitro antioxidants activity determination

With regard to the antioxidant activities of methanolic and aqueous plant extracts, three methods were adopted: the Ferric Reducing Antioxidant Power (FRAP), the radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the radical cation decolorization assay (ABTS). The results of this study are indicated in Table 2.

The antioxidant capacity evaluated by DPPH radical scavenging method was ranged from 658.77 to 828.985 $\mu\text{mol EAA} / \text{g}$ for MeOH extracts and that of aqueous extracts from 627.82 to 828.98 $\mu\text{mol EAA/g}$. The DPPH radical scavenging of methanolic extracts was significantly higher ($p < 0.05$) than those of aqueous extracts except for *P. biglobosa* and *D. oliveri*. Considering the methanolic extracts, the following decreasing order was found: *S. birrea* > *D. microcarpum* > *P. erinaceus* > *A. albida* > *P. biglobosa* > *L. acida* > *V. paradoxa* > *D. oliveri*. This result indicated that the plant samples scavenge more DPPH free radicals than quercetin used as standard.

In the ABTS assay, the ability of plant extracts to reduce the cation radical was ranged from 12308.15 to 6178.11 $\mu\text{mol EAA} / \text{g extract}$ for methanolic extracts and from 5697.33 to 2077.00 $\mu\text{mol EAA} / \text{g extract}$ for aqueous extracts. In this essay, the values of methanolic extracts were

higher than those of aqueous extracts for all plants. The following decrease order was obtained with methanolic extracts: *D. microcarpum* > *V. paradoxa* > *D. oliveri* > *L. acida* > *S. birrea* > *P. erinaceus* > *A. albida* > *P. biglobosa*. The reference compound were trolox 8137.61 $\mu\text{mol EAA/g extract}$ and quercetin 14664.01 $\mu\text{mol EAA/g extract}$.

FRAP method allows to measure the fitness of plant extracts to reduce the ions Fe (III) to Fe (II). At this level, results varied from 5360.23 to 2934.8 $\mu\text{mol EAA} / \text{g}$ for methanolic extracts and 4596.12 to 1649.76 $\mu\text{mol EAA} / \text{g}$ for aqueous extracts. The best reducing powers were obtained with methanolic extracts of *D. microcarpum* (5360.23 $\mu\text{mol EAA} / \text{g}$) followed by *P. biglobosa* (5192.35 $\mu\text{mol EAA} / \text{g}$). That of aqueous extracts has indicated that *S. birrea* and *D. microcarpum* were most potent with values of 4596.12 $\pm$ 1.23 and 4584.55 $\pm$ 0.53 $\mu\text{mol EAA} / \text{g extract}$, respectively. Considering both extracts the lowest activities

were found with *P. erinaceus* and *A. albida*.

DISCUSSION

In this study, it can be noted that the total content of phenolics compounds and flavonoids varied from species to species with a balance in favor of *D. microcarpum*. Aqueous extracts have shown the best levels of total phenolics compared to methanolic extracts. These differences would be largely influenced by genetic factors, environmental conditions and extraction solvent (Lugasi et al., 2003; Min and Chun-Zhao, 2005). Indeed, the phenolic content of a plant would depend on a number of intrinsic (genetic) and extrinsic factors (cultural practices, maturity at harvest and storage conditions) (Lee et al., 2003).

To better appreciated the antioxidant properties of the plant samples, three methods have been used and it was found that most of the plant extracts exhibited an interesting antioxidant activity compared to the standards. This antioxidant activity did correlate to the total polyphenol contents. These results are in agreement with what is announced in the literature by several authors that the antioxidant activity potential of an extract depends on its content of phenolic compounds (Oukacha et al., 2015; Guettaf et al., 2016; Kalia et al., 2008; Stagos et al., 2012). *D. microcarpum* plant stands out with high phenolic compound contents and good antioxidant activity with all three methods.

These results corroborate the work of Hama et al. (2019) who find that methanolic extracts of *D. microcarpum* trunk bark are more reducing than other parts of the plant. Avian pathologies such as salmonellosis, Newcastle disease, avian influenza, smallpox, coccidiosis induce inflammation, following the production of free radicals and reactive oxygen species in chickens (Henard and Vázquez-Torres, 2011; Sokoudjou et al., 2019). In various physiological and pathological conditions, the systemic amounts of free radicals and reactive oxygen species are higher than normal. This could become intolerable for the cells. Nitric oxide (Free radical) promotes vasodilation and hemorrhages in coccidian infections and could be toxic to both parasites as well as to host cells harboring the coccidian parasite (Masood et al., 2013).

Under oxidative stress conditions, poultry bodies cannot effectively neutralize the excessively produced free radicals, including reactive nitrogen species (RNS) and reactive oxygen species (ROS). These species cause fatal damage in chromosomes and consequently modifying the encoded amino acids and hence many associated biological processes (Abdel-Moneim et al., 2020). In addition, oxidative stress in poultry gastrointestinal tract (GIT) is derived from nutritional, environmental heat stress, and pathological factors, which alters overall performance as well as meat and egg quality (Mishra and Jha, 2019).

Therefore, to alleviate or reduce the oxidative stress induced by these pathologies, natural antioxidants as feed supplements are commonly used in poultry production systems (Masood et al., 2013). Indeed, exogenous antioxidants, including polyphenols, represent the first defense line of the cells against excessive production of free radicals, protecting their components from oxidative damage (Lipiński et al., 2017 ; Zhong and Zhou, 2013). Moreover, medicinal plants are an important source of polyphenols with antioxidant, antimicrobial and viral properties (Abutheraa et al., 2017). Most polyphenols have been demonstrated to serve as inhibitors of bacterial colonization, induce lymphocyte proliferation and their ability to break the bacterial cell wall, leading to loss of cell contents and subsequently cell death (Abdel-Moneim et al., 2020). They also exert their effect by killing the virus and/or interfering with viral multiplication (Jassim and Naji, 2003). In the case of Newcastle virus (NDV), these compounds could exhibit protease inhibition and interfere with the cleavage of hemagglutinin neuramidase and fusion protein, which are important glycoproteins for NDV binding and multiplication (Bakari et al., 2012). The present study proved that the plants studied are rich in polyphenols and exhibited a good antioxidant capacity. The supplementation of extracts these plants in the poultry feeds would be considered as natural antioxidants to prevent certain avian pathologies.

Conclusion

This study has been conducted to quantify the polyphenols and evaluate the antioxidant activity of eight medicinal plants used by the poultry farmers to manage the avian diseases in Burkina Faso. The results obtained proved that the medicinal plants used by the poultry farmers are rich in polyphenols and demonstrated an interesting antioxidant capacity. *P. biglobosa*, *S. birrea* and *D. microcarpum* are the good candidates for the discovery of the natural antioxidants. These plants could therefore be exploited for the preservation of poultry health through their antioxidant activity. Nevertheless, more in-depth studies are necessary to guarantee their effectiveness.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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