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Antioxidant Activity and Phenolic Compounds Content of Extracts from *Ceratotheca sesamoides* Endl and *Striga hermonthica* (Delille) Benth Used as Antheminthic in Burkina Faso

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Abstract

The revaluation of medicinal plants in the veterinary pharmacopoeia is essential for the development of livestock in Burkina Faso. In order to justify the use of *C. sesamoides* and *S. hermonthica* in the treatment of gastrointestinal parasitosis of small ruminants, a quantification of phenolic compounds as well as antioxidant activity and acute toxicité evaluation of both plants were performed. Acute toxicity was evaluated by administering a single dose of 2000 mg/kg body weight of aqueous extract of both plants to mice. The highest total polyphenol content for *C. sesamoides* was obtained in the ethyl acetate fraction with 47.236 ± 2.57 mgEAG/100mg while that of *S. hermonthica* was 74.871 ± 2.57 mgEAG/100mg obtained with the n-butanol Fraction. The dichloromethane extract of *S. hermonthica* obtained the highest total flavonoid content with 7.31 ± 0.48 mgEQ/100 while the highest total flavonoid content of *C. sesamoides* was obtained with ethyl acetate fraction with 5.4273 ± 0.35 mgEQ/100mg. The aqueous extract of *C. sesamoides* obtained the highest content of condensed tannins with 3.028 ± 0.13 mgEAT/100mg. Both plant's extracts did not show any signs of toxicity in *NMRI* mice after administration of the dose of 2000 mg/kg. The antioxidant ac-

tivity by DPPH, FRAP and ABTS methods was good compared to those of Quercetin and Trolox taken as standard.

Keywords

C. sesamoides, *S. hermonthica*, Polyphenols Content, Flavonoids Content, Condensed Tannins, Antioxidant Activity

1. Introduction

Medicinal plants have been used for many decades to alleviate and/or cure human and animal diseases through traditional medicine around the world. Indeed 80% of the African population relies on traditional medicine for their health care needs [1]. In Burkina Faso, about 70% of the population uses traditional medicine for the care of many diseases [2]. According the study of [3], the traditional recipes used for treating animal diseases are 95% plant-based in most regions of Burkina Faso. Among the plant species used for animal care are *C. sesamoides* and *S. hermonthica*, two annual herbaceous plants used by rural herders against gastrointestinal parasites of small ruminants.

C. sesamoides is a wild species of the Pedaliaceae family that can reach 120 cm with a prostrate or erect pubescent stem [4]. It is a slimy species used as a food plant in many African countries [5]. *S. hermonthica* is an annual herb belonging to the Scrophulariaceae family. The stem is erect and scabrous and the leaves can be simple or branched, linear or elliptical with an axillary or terminal spiral cyme inflorescence comprising 40 to 50 flowers [6]. *S. hermonthica* is a parasitic species of cereals such as sorghum and millet whose losses can reach more than 10 million tons of production in Sub-Saharan Africa [7].

C. sesamoides is used in the pharmacopoeia for the treatment of many human and animal diseases among which there are antiparasitic treatments such as against gastrointestinal nematodes [8] [9]. An ethnobotanical survey in southwestern Niger showed that maceration of the whole stem and leaf of *S. hermonthica* is used orally as sheep's anthelminthic [10].

C. sesamoides and *S. hermonthica* contain many secondary metabolites such as total polyphenols and flavonoids as well as condensed tannins that are thought to be responsible for the medicinal properties of several plant species and their antitoxydant capacities [4] [11] [12].

The general objective of our present study is to confirm the medicinal properties in general and anthelmintic properties in particular of *C. sesamoides* and *S. hermonthica* through the quantification of phenolic compounds and the evaluation of antioxidant activity.

2. Material and Methods

2.1. Chemical Reagents

Folin ciolcateu reagent, quercetin, sodium carbonate, aluminum trichloride, iron

III chloride, potassium hexacyanoferrate [K3Fe(CN)6], gallic acid, quercetin, ethanol, vanillin, Na_2HPO_4, NaH_2PO_4, 2,2-diphenylpicrylhydrazyl (DPPH), ABTS [(2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonique)], trichloroacetic, acetic and ascorbic acid, hexane, dichloromethane, ethyl acetate, n butanol. All chemicals and solvents used were of analytical grad.

2.2. Plant Material

Whole plants of *C. sesamoides* and *S. hermonthica* were collected in the villages of Katchari and Mamasiol (urban commune of Dori) in September and October 2019. The whole plant samples were then washed with water and dried at room temperature away from sunlight and dust, before being used as whole plant powder for the experiments. The plants were identified at the National Herbarium of Burkina Faso (HNBF) under numbers 8758 and 8759 respectively.

3. Preparation of Extracts

3.1. Aqueous Extraction

Aqueous extracts were made by macerating 100 g of powder of each plant in 900 mL of distilled water for 24 hours under magnetic stirring. The macerate was then filtered twice using absorbent cotton and the filtrate was frozen at −4˚C. The frozen filtrates were then lyophilized using ALPHA 1 - 2 LD Plus freeze dryer and the extracts obtained were placed in a desiccator until their use.

3.2. Hydroacetonic Extraction and Fractionation

Each plants powder (25 g) was extracted with 250 ml of acetone-water mixture (80:20) for 24 hours using an electric mixer. After filtration acetone was evaporated at 60˚C using heidolph brand rotavapor. The resulting aqueous extract (50 ml) was fractionated by successive liquid-liquid partitioning with an equal volume of n-hexane, dichloromethane, ethyl acetate and n-butanol. The organic solutions from the partitioning were then separately pooled and concentrated to dryness to form hexane, dichloromethane, butanol and ethyl acetate fractions. The final aqueous phase was lyophilized. The dichloromethane extract of interest in our present study was stored at 4˚C until use.

Fractionation allows the secondary metabolites to be distributed between the different solvents used, which could allow a concentration of specific molecules in the fraction, avoiding the effect of congestion and thereby optimizing their biological properties. This process would contribute to facilitate the isolation and characterization of interest molecule.

4. Determination of Phenolic Contents

4.1. Total Polyphenols Content

The total polyphenols content was performed by Folin-Ciocalteu method described by [13]. A volume of 125 µL of extract (at 0.1 mg/mL) was mixed with

625 µL of Folin-ciolcalteu reagent (0.2N). After 5 min of incubation in the dark, 500 µL of sodium carbonate (Na_2CO_3, 75 g/L) was added to the mixture. The mixture was then incubated for 2 h in dark before determination of total polyphenol contents at 760 nm against a gallic acid calibration curve ($y = 4668e\text{-}3x - 0.034$, $r^2 = 0.9991$). Each test was repeated 3 times and the results were expressed as mg of Gallic Acid Equivalent per 100 mg of extract (mg GAE/100 mg extract).

4.2. Total Flavonoids Content

The total flavonoids content was determined according to the colometric method [13]. To 625 µL plant of plant solution (at 0.1 mg/mL), 625 µL of aluminum trichloride (AlCl3, 2%) were added. After 10 min of dark incubation, the flavonoids content was evaluated at 415 nm, using a quercetin calibration curve ($Y = 1.259e\text{-}2x$; $r^2 = 0.9990$). The tests were assessed in triplicate 3 times and the results were expressed as mg Quercetin Equivalent per 100 mg of extract (mg QE/100mg extract).

4.3. Condensed Tannins Content

The determination of condensed tannins content was performed using the vanillin acid method [14].

0.5 mL of plant sample diluted in ethanol (1/100) was mixed with 1 mL of sulfuric vanillin solution (70%). After 15 min of incubation of the mixture in the dark at 30˚C, the condensed tannins content was measured at 500 nm against a tannic acid calibration curve ($y = 0.0353x + 0.0016$; $r^2 = 0.9988$).

The tests were repeated thrice and the results were expressed in mg Tannic Acid Equivalent per 100 mg of extract (mgEAT/g extract). Only the condensed tannins content of aqueous end dichloromethane extracts have been done.

5. Antioxidant Activity

5.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Method

This method evaluates the scavenging capacity of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical. 375 µL of extract of each plant (0.1 mg/mL) were mixed with 750 µL of DPPH solution (20 mg/L). After 15 min of incubation in the dark, the absorbance of the mixture was read at 517 nm on a GENESYS 30 spectrophotometer. The DPPH radical scavenging activity of each plant extract was measured using an ascorbic acid calibration curve ($y = -2.224e\text{-}2x + 0.348$, $r^2 = 0.9966$). The test was repeated 3 times and the results were expressed as µmol Ascorbic Acid Equivalent per gram of extract (µmol EAA/g).

5.2. FRAP Method

The ability of plant extracts to reduce ferric iron to iron salts was evaluated by mixing 250 µL of plant extract (0.1 mg/mL) with 625 µL of phosphate buffer (0.2 M, pH 6.6) and 625 µL of potassium hexacyanoferrate [K3Fe(CN)6] (1%), then

the mixture was incubated at 50˚C in a water bath. After 30 min, 625 µL of trichloroacetic acid (10%) was added to the mixture and the whole was centrifuged at 3000 rpm for 10 min. 0.3125 mL of the supernatant was then taken and mixed with 0.3125 mL of distilled water followed by 0.0625 mL of fresh iron chloride FeCl3 solution (0.1%). The absorbance of the reaction medium was measured at 700 nm. Each test was repeated 3 times and the reduction potential of ferric chloride was expressed as µmol Ascorbic Acid Equivalent per gram of extract (µmol EAA/g).

5.3. Méthode ABTS (2,2'-azinobis-[acide3-éthylenzotiazoline-6-sulfunique])

ABTS●+ cationic radicals were generated by mixing ABTS solution (7 mM) with 2.5 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 12 hours before adjusting its absorbance with ethanol to 0.70 ± 0.02 at 734 nm.

The reduction potential of the ABTS●+ cationic radical by the extracts was determined by mixing 10 µL of extract (at 0.1 mg/mL) with 990 µL of the fresh ABTS solution. After 15 min of incubation in the dark, the absorbance of the mixture was read at 734 nm. The measurements were repeated 3 times and the results were expressed as µmol Ascorbic Acid Equivalent per gram of extract (µmol EAA/g) using the ascorbic acid calibration curve.

6. Acute Toxicity Study

Acute toxicity was performed according to OCDE guideline 423 [15]. Nine (9) NMRI, nulliparous and non-pregnant mice of average weight 26g were divided into 3 lots of 3 mice each distributed as follows: lot 1 which constitutes the control received distilled water at a rate of 0.52 mL, lot 2 received a single dose of 2000 mg/kg body weight of *S. hermontica* aqueous extract and the last lot received the single dose of 2000 mg/kg body weight of *C. sesamoides* aqueous extract. The extracts were administered by gavage using a syringe with a probe. Mice were fasted for 4 h before administration of the extracts. They were refed and hydrated 1h after administration of the extracts. Mice were observed for 1 h, 2 h, 3 h, 4 h, and every 24 h for 14 consecutive days. During the observation period, the signs of toxicity investigated were coat change, sleep, coma, mobility, tremor, change in eye color, convulsions, salivation, diarrhea and mortality.

7. Data Analysis

Data results are expressed as mean ± standard deviation (SD) of three determinations. Statistical analysis with one-way ANOVA followed by Tukey's multiple comparison has been done using Rcmdr packages 27-1 of R 3. 6.2 software with RStudio 5599.7.2 at $p < 0.05$ significance level. Linear regression was carried out with GraphPad Prism 5.0.0.288 with de confidence interval of 95%.

8. Results

8.1. Determination of Phenolic Compounds

Table 1 shows Total Polyphenols (TP) as well as of Total Favonoids (TF) and Condensed tannins (TC) contents. The TF and TP content in the different extracts of both plants was varied. TF content ranged from 2.144 ± 1.66 to 3.814 ± 0.07 mgEQ/100mg for the different extracts of *S. hermonthica* while the TF content of the different extracts of *C. sesamoides* ranged from 2.406 ± 0.23 to 5.4273 ± 0.35 mgEQ/100mg have been registered. However, the ethyl acetate fraction obtained the highest TF content for both plants with 3.814 ± 0.07 mgEQ/100mg 5.4273 ± 0.35 mgEQ/100mg for *s. hermonthica* and *C. sesamoides* respectively.

The TP content was also varied for the extracts of both plants. A TP content ranging from 11.568 ± 0.30 to 74.871 ± 2.57 mgEAG/100mg was obtained for the extracts of *S. hermonthica* with 74.871 ± 2.57 mgEAG/100mg as the highest value obtained with the n-butanol fraction. the total polyphenol contents of *C. sesamoides* was ranged from 11.425 ± 0.539 to 47.236 ± 2.57 mgEAG/100mg. the highest value of polyphenols in *C. sesamoides* extracts was 47.236 ± 2.57 mgEAG/100mg obtained with the ethyl acetate fraction. Statistical analysis showed high significance ($p < 0.05$) between both plants extracts and fractions for TP and TF contents.

Aqueous extracts and dichloromethane fractions of both plants, of particular interest for our work, were quantified for condensed tannin content. The contents obtained were varied but the aqueous extract of *C. sesamoides* obtained the highest content with 3.028 ± 0.13 mgEAT/100mg.

8.2. Acute Toxicity

The administration of a single dose of 2000 mg/kg of aqueous extract of *C. sesamoides* and *S. hermonthica* did not induce any signs of acute toxicity or mortality in the mice during the 72 hours of observation and after 14 days of follow-up.

Table 1. Total Polyphenols, Total Flavonoids and condensed tannins contents.

Extracts	Condensed Tannins content (mgEAT/100mg)	Total Flavonoids content (mgEQ/100mg)	Total Polyphenols content (mgEAG/100mg)
***S. hermonthica* Hex**	ND	2.144 ± 1.66^{ab}	30.313 ± 0.11^{d}
***S. hermonthica* DCM**	0.2996 ± 0.01	7.307 ± 0.48^{g}	11.568 ± 0.30^{a}
***S. hermonthica* ethyl A**	ND	3.814 ± 0.07^{de}	70.694 ± 0.30^{g}
***S. hermonthica* But**	ND	1.906 ± 0.07^{ab}	74.871 ± 2.57^{h}
***S. hermonthica* (aqueous)**	0.1228 ± 0	1.774 ± 0.199^{a}	16.209 ± 1.012^{b}
***C. sesamoides* Hex**	ND	2.406 ± 0.23^{bc}	12.532 ± 0.15^{a}
***C. sesamoides* DCM**	0.2476 ± 0	2.94 ± 0^{c}	19.92 ± 1.21^{c}
C. sesamoides ethyl A	ND	5.4273 ± 0.35^{f}	47.236 ± 2.57^{f}
***C. sesamoides* But**	ND	4.329 ± 0.16^{e}	35.882 ± 0.15^{e}
***C. sesamoides* (aqueous)**	3.028 ± 0.13	3.7066 ± 0.321^{d}	11.425 ± 0.539^{a}

ND: Not Determined.

8.3. Antioxidant Activity

Table 2 summarizes the free radical scavenging capacity by the DPPH, method reducing potential of ferric ions by the FRAP, as well as the ABTS cationic radical reduction capacity methods. Both plants extracts, showed higher antioxidant capacity with all the methods. The statistical analysis showed high significance ($p < 0.05$) between both plants extracts and fraction for the antioxidant capacities.

9. Discussion

The present study shows a varied content of polyphenols and total flavonoids of the different extracts and fractions of the two plants. The highest TP content was obtained with ethyl acetate and n-butanol fractions for *S. hermonthica* and DCM and ethyl acetate fractions for *C. sesamoides.* The TF content was higher in the DCM fractions for *S. hermonthica* and ethyl acetate for *C. sesamoides*. The content of condensed tannins (CT) which is of particular interest in our work for the control of gastrointestinal nematodes has been varied. The high content of CT was obtained with the aqueous extracts of *C. sesamoides*. However, the CT contents of the DCM fractions of both plants were approximately equal. Our results corroborate those of [16] who obtained a fairly variable TP and TF content in methanolic and aqueous extraction of medicinal plants. In contrast to our study [17] obtained higher phenolic compound contents of forage plants use as anthelminthic from Côte d'Ivoire. Many studies on the antiparasitic and antibacterial properties of extracts of certain plants such as *Anogeisus leiocarpus, Danielia oliveri* as well as *Acacia polyancatha* performed a qualitative determination of condensed tannins marking their presences contrary

Table 2. Antioxidant activity of *C. sesamoides* and *S. hermonthica* extracts.

Extracts	DPPH (µmol EAA/g)	ABTS (µmol EAA/g)	FRAP (µmol EAA/g)
S. hermonthica **Hex**	53.70 ± 1.94^{a}	524.05 ± 16.65^{a}	130.82. ± 3.61^{a}
S. hermonthica **DCM**	258.73± 18.10^{f}	9928.26± 300.25^{b}	1035.83± 9.73^{d}
S. hermonthica **ethyl A**	86.829 ± 7.74^{cd}	752.42 ± 18.14^{a}	328.79 ± 11.82^{bc}
S. hermonthica **But**	82.21 ± 0.25^{bc}	817.33 ± 41.63^{a}	410.98 ± 3.61^{bc}
S. hermonthica **(aqueous)**	108.93 ± 6.40^{e}	9522.05 ± 127.86^{e}	584.66 ± 10.03^{c}
C. sesamoides **Hex**	90.232± 7.37^{ce}	4327.08 ± 249.8^{b}	86.829 ± 7.74^{a}
C. sesamoides **DCM**	258.73± 18.10^{f}	9928.26± 300.25^{e}	1035.83± 9.73^{d}
C. sesamoides **ethyl A**	839.182 ± 6.43^{i}	4665.58^{c}	839.182 ± 6.43^{f}
C. sesamoides **But**	806.87± 1.39^{h}	2286.46± 100.25^{c}	6370.43± 291.46^{e}
C. sesamoides **(aqueous)**	64.344 ± 1.51ab	1098.92 ± 22.83^{f}	249.33 ± 7.74^{ab}
Quercetine	106.85de	14671.58 ± 534.72^{f}	2211.24 ± 36.17^{e}
Trolox	785.99g	8137.61^{d}	785.99^{g}

Reference molecules = Quercetin and Trolox.

to our study which performed a quantitative determination of these metabolites [18] [19]. The different variations in phenolic compound content observed would be attributable to climatic conditions, maturity at harvest, storage and extraction conditions [20]. Also, some parameters of the Condensed Tannins determination method, in this case the vanillin method, whose critical point depends on the nature of the solvent (water, alcohol), the nature and concentration of the acid (Sulfuric, Hydrochloric) as well as the reaction time and the type of standard used, can explain the difference in Condensed Tannins content observed [21].

The toxicity study revealed no evidence of acute toxicity or mortality in NMRI mice. Our results corroborate those of [22] obtained after administration of aqueous and acetonic extracts of *Acacia radiana* and *Acacia nilotica* leaves to NMRI mice. Similarly, [23] obtained an identical result to ours with hydroethanol extracts of *Cogniauxia podolaena* in Congo Brazzaville while aqueous extracts of the same plant were weakly toxic to mice according to the same authors.

The antioxidant activity of both plants extracts and fractions by DPPH, FRAP and ABTS methods revealed high antioxidant activity compared to the standard. DCM fractions and aqueous extracts obtained higher antioxidant activity in general but with a higher inhibition capacity with the DCM fractions. The antioxidant activity of the plants is related to the presence of phenolic compounds such as total polyphenols and flavonoids as well as condensed tannins [24]. Our results are similar to those obtained by [25], who obtained variable phenolic compound content with aqueous and ethanolic extracts of different plant parts with quite high antioxidant activity. Several fractions of the different parts of *Nauclea latifolia* obtained quite high antioxidant activity similar to our results [26]. Also, methanolic extracts of the different parts of *Detarium microcarpum* showed high antioxidant activity by DPPH and FRAP method which was strongly correlated to the total polyphenol content of these extracts which is identical to our results [20]. Indeed, according to [27] phenolic compounds have redox properties that allowed them to act as antioxidant. Antioxidant activity of these two plants shows that they can limit the damage caused by free radicals through enzymatic and chemical defense mechanisms in order to protect animal cells [28].

10. Conclusion

The present study shows the content of total polyphenols and flavonoids as well as that of condensed tannins in different extracts and fractions of *C. sesamoides* and *S. hermonthica* two annual herbs traditionally used in the treatment of gastrointestinal parasitosis of sheep in Burkina Faso. Both plants showed no acute toxicity or mortality in NMRI mice. The presence of these phenolic compounds in these different extracts justifies the antioxidant activity and the use of these both plants in the veterinary pharmacopoeia for the treatment of gastrointestinal parasitosis of small ruminants in the Sahel and North Center region of Burkina Faso. However, more in-depth studies must be conducted to improve and popularize the use of these plants in the fight against gastrointestinal parasitosis of

small ruminants.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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