

Phytochemical Screening and Assessment of Antibacterial, Antioxidant and Antihelminthic Activities of *Sarcocephalus latifolius* Leaves

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ABSTRACT

The main objective of our study was to determine the chemical composition and to assess the antioxidant, antibacterial and antihelminthic activities of the leaves extracts of *S. latifolius*. The antibacterial activity of *S. latifolius* leaves extracts was assessed using micro-dilution method. In end, the antioxidant capacity of different plant extracts has been evaluated by determining their ability to reduce iron (III) to iron (II) and DPPH radical. The results obtained revealed that the dry matter of the powder of the *S. latifolius* leaves is rich in fibers, crude proteins, lipids and carbohydrates. Its water content was moderate (14.3 %); the leaves of *S. latifolius* are rich in mineral elements like calcium (Ca), magnesium (Mg), potassium (K) and iron (Fe); the plant has the main groups of secondary metabolites such as total polyphenols, flavonoids, tannins, alkaloids, bound and free quinones, steroids and tri-terpenes, while it is poor in anthocyanins, leucoanthocyanins and saponins. The bacterial strains used were less sensitive to *S. latifolius* leaf extracts (MIC greater than or equal to 500 µg/mL). The extracts of this plant showed significant antioxidant and antihelminthic activity, with a strong correlation between the methods used to evaluate the antioxidant potential of DPPH and FRAP. Due to their interesting functional properties, this plant could be used in the formulation of a nutraceutical with strong antioxidant power to improve the antioxidant status of consumers, but also in the management of helminthiasis.

Keywords: Bioactivity, Evidence-based Medicine, Medicinal plant, *Sarcocephalus latifolius*, Traditional medicine.

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I. INTRODUCTION

In time, the plants have always been used as medicines. Herbal medicines are considered to be less toxic and milder than pharmaceutical drugs. It is estimated that about 28% of the medicines used in the world are actually of plant origin [1]. The claimed natural origin of these medicines gives them the reputation of being safer and better than so-called

chemical medicines. Africa has important plant biodiversity [2]. Its flora is particularly rich in medicinal plants that local populations know and use. It has been reported that there are about 300,000 medicinal species and that only 10% have been studied from a chemical and pharmacological point of view [3]. Indeed, the World Health Organization (WHO) estimates that more than 80% of the African population continues to rely on medicinal species to provide primary health care [4], [5]. The Democratic Republic of Congo is a reservoir of

biodiversity, both flora, and fauna [6], [7]. Its flora abounds in medicinal plants of biopharmaceutical interest and is capable of providing new bioactive molecules [8]. Thus, the study of plant chemistry is still of brilliant topicality in spite of its antiquity. Among the compounds of plant, origin are coumarins, alkaloids, phenolic acids, tannins, terpenes, flavonoids, etc. [9]. [5] which are endowed with interesting pharmacological properties including antioxidant, antibacterial, and antihelmintic activity. To this end, the search for natural substances with antibacterial, antioxidant and antihelmintic activity is an important scientific challenge in the treatment of several common diseases. In order to scientifically value the phytotherapeutic richness of the DRC, our choice was made on *S. latifolius* used in traditional Congolese medicine in the hypothesis that it would contain secondary metabolites likely to confer antihelmintic, antibacterial and antioxidant properties. The objective of this study was to chemically and bio-actively analyze the leaves of *S. latifolius*.

II. MATERIAL AND METHODS

A. Material

Plant: The leaves of *S. latifolius* were used in this study. These leaves were harvested in the Northern Province-Ubangi more precisely in the territory and sector of Yakoma. The Systematic analysis of this plant was carried out at the Laboratory of Systematic Botany and Plant Ecology, Department of Biology, Faculty of Sciences, University of Kinshasa. These samples were dried out in room temperature ($\pm 27^\circ\text{C}$) at the Molecular Bio-Prospection Laboratory, Department of Biology for two weeks and then ground to obtain fine powder for further analyses.

Worm specimens: In this study, two worm specimens (*Behamia rosea* Michaelsen and *Behamia itoliensis*) were collected from the ponds of Monastère Prieure Notre Dame de l'Assomption and identified at the Natural Resources Management Laboratory, Faculty of Agricultural Sciences, University of Kinshasa.

Bacterial Strains: In the present study, two bacterial strains were used namely *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 25923. These strains were provided by the Laboratory of Microbiology, Faculty of Pharmaceutical Sciences, University of Kinshasa.

B. Methods

1) Evaluation of the chemical composition

1.1) Determination of humidity

Dry matter was determined by AOAC Method 925.10 (1990). Briefly an empty cup was cleaned, dried, and weighed (PO). To this dish 2 g of sample was added and the whole (P1) was placed in an oven at 105°C for 24 hours. After this drying time, the dish was taken out of the oven and cooled in a desiccator (P2O5) before being weighed again (P2).

1.2) Determination of total ash

Crude ash content was determined by AOAC Method 920.87 [9]. It consists of mineralizing 2 g of the powder contained in the porcelain crucible in a pasteurizing furnace. The vacuum mineralization crucible was cleaned, dried and weighed (PO). The crucible containing the powder was

weighed again (P1) and then placed in the oven at 105°C for 4 hours. After drying, the crucible was removed from the oven, cooled in a desiccator (P2O5) and weighed again (P2). Once weighed, the crucible was placed in the oven at 500°C for about 6 hours, cooled in the desiccator and weighed again. The ash content was the mass of product remaining in the crucible after mineralization in relation to the total dry mass of the product. The result expressed represents the average of three trials.

1.3) Determination of crude proteins

The crude protein content was determined by the Kjeldahl method. 1 g of the powder was weighed into the Kjeldahl tube and 3 g of mixed catalyst was added, followed by 20 ml of concentrated H_2SO_4 . Afterwards, the sample was mineralized until a clear solution was obtained. Then the entire solution was placed in a 100 ml volumetric flask and cooled in the open air. A few minutes later, the solution was returned to the volumetric flask and homogenized. Then 50 ml of 33% NaOH was added to the solution, placed in the nitrogen distiller, and collected 40.5 ml of the distillate in an Erlen Meyer containing 10 ml of 0.1N H_2SO_4 + a few drops of mixed indicators. Finally, we titrated with a 0.1 N solution up to the equivalence point. Crude protein content was converted to nitrogen using Crisand and Sands (1978).

1.4) Determination of total lipids

The fat content was determined by the Soxhlet method. 2 g was weighed for 3 trials and 20 mL of concentrated HCl was added and then disintegrated on the hot plate to obtain a blackish leg. Afterwards, 100 mL of hot water was poured into the leg, then filtered until the blackish leg stuck on the spade was recovered and left to air dry. Then the filter paper was inserted into the cartridge. The empty glass capsule was weighed and 50 mL of petroleum ether was added and placed at the same time as the cartridge to the fat extractor. After this, the cartridge was heated for 40 minutes and rinsed for 15 minutes, then reweighed after cooling in the desiccator and the fat extractor was fixed.

1.5) Determination of total fibre and carbohydrate content

The total ash and carbohydrate content was determined by the method used by Makengo (2018). While the energy value was deducted by applying the following formula:

$$\text{Energy (in Kcal)} = (\% \text{ protein} \times 4) + (\% \text{ fat} \times 9) + (\% \text{ carbohydrates} \times 4)$$

1.6) Chemical screening

The determination of secondary metabolites was performed according to the standard method used by Nkasa *et al.* [10].

1.7) Determination of mineral elements

The trace element content was determined according to the method used by Hanane *et al.* [11]. These mineral elements were measured in the residue of the calcined product at 500°C for 5 hours, with a test sample of 15 g for the leaves. The determination of the trace elements studied (Calcium, Potassium, Magnesium, Iron) was carried out by the aqua regia method used by Hanane *et al.* [11]. 2 to 3 mL of aqua regia were added to 0.15 g of the mineral matter, heated and left to evaporate dry. Then 25 to 30 mL hydrochloric acid (2M) was added until completely dissolved and the results

were read using UV-visible spectrophotometry (LGS 53) at a wavelength of 525 nanometres.

C. Evaluation of Biological Activities

1) Evaluation of antibacterial activity

The antibacterial activity was evaluated by the micro-dilution method described by Bongo *et al.* [12] in liquid medium. The extract to be tested (20 mg) was previously dissolved in 250 µl of DMSO (Dimethyl sulfoxide) and the final volume was adjusted to 5 ml with Mueller Hinton culture medium (final DMSO concentration equal to 5%). The bacterial suspension is prepared by placing in 2 ml of physiological water for each strain, two colonies isolated from the strains to be tested (*Staphylococcus aureus* and *Escherichia coli*) by incubating for 24 hours in order to obtain 0.5 McFarland (108 cells/mL) at 37 °C. The bacterial suspension is then diluted to 106 cells/mL (1/100 dilution). The micro-dilution test is performed in 96-well round bottom sterile polystyrene microplates. Briefly, 100 µl of culture medium is placed in the wells (2-9) and then in the 11th and 12th = control columns). Using a micropipette, 200 µl of each extract to be tested (1000 µg/ml) is placed in wells 1 (the wells of the first column). 100 µl of each extract stock solution is then taken for serial 2-in-2 dilutions up to the ninth column. The last 100 microliters (column 9) are discarded. 5 µl of inoculum (108 CFU/ml) is taken aseptically with a micropipette and added to all wells of the microplate except the wells of the 11th column for bacterial growth control (inoculum and culture medium). The wells of the 12th column will be used as a sterility control of the culture medium. The microplates are incubated in an oven at 37 °C for 24 hours. After this period, 3 µl of Resazurin 1% dye (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) is added to each well and the microplates are then incubated for 4 hours. The minimum inhibitory concentration (first wells showing no bacterial growth) is determined after 24 hours.

2) Evaluation of antihelminthic activity in vitro

In vitro antihelminthic activity was evaluated according to the method used by Dash *et al.* [13] The extracts of the plants to be tested were previously dissolved in physiological water (0.9%) (5mg/mL) in order to have a stock solution. The stock solution was diluted serially to optimize concentrations ranging from 10 mg/ml to 0.6125 mg/ml. A standard solution (Albentel) which served as a positive control was prepared under the same conditions as the extract. Distilled water was used as the negative control. The worms, previously washed, were divided into three batches containing three specimens per petri dish. In the first batch: a series of boxes containing the extract and earthworms, with three worms per box for each concentration; in the second batch: the worms were dewormed (standard) using the same procedure as in the first batch and the last batch, with distilled water (negative control). The boxes were placed at room temperature for 48 hours while proceeding by observation. The rate of paralysis of each concentration for each given dose as a function of contact time was also evaluated. The mortality rate (TM) was also determined for each of the concentrations of the extracts according to the following formula:

$$TM (\%) = (\text{No. of dead worms} / \text{total no. of worms in petri dish}) \times 100$$

3) Evaluation of antioxidant activity

3.1) Total reducing power

The antioxidant capacity of different plant extracts has been evaluated by determining their ability to reduce iron (III) to iron (II) [14]. Briefly, in a test tube, 1 mL of each extract is mixed with 2.5 ml of a phosphate buffer solution (0.2 M, pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution. This is incubated for 30 minutes at 50°C in a water bath. Then 2.5 ml 10% trichloroacetic acid is added and the mixture is centrifuged at 3000 rpm for 10 minutes in a centrifuge. Then 2.5 ml of the supernatant is taken and mixed with 2.5 ml of distilled water and 0.5 ml of a 0.1% aqueous solution of $FeCl_3$. The absorbance is read at 700 nm. The total reducing power is expressed as ascorbic acid equivalent (mg ascorbic acid/g powder).

4) DPPH radical method

4.1) Sample Preparation

Dissolve 10 mg dry extract of each sample in 1mL methanol for polar extracts. Make dilutions to have the following concentration levels: 8mg/ml, 6mg/mL, 4mg/mL and 2mg/mL.

4.2) DPPH-test

4.2.1) Principle

This method is based on the degradation of the DPPH- (2,2 DiPhenyl-1-PicrylHydrazyl) radical. The DPPH- radical is a purple colored radical, the addition of antioxidant reduces this radical and causes discoloration of the mixture; this discoloration of the radical measured by spectrophotometer at 517 nm is proportional to the concentration of antioxidants [15].

4.2.2) Preparation of the DPPH radical

- Dissolve 3.2 mg of DPPH in 100 mL of 80% methanol.
- Store the solution in a dark place for at least one hour.
- The absorbance of this solution should be adjusted to 0.7 ± 0.05 with 80% methanol.

4.2.3) Contacting the sample with the radical

In a test tube, place 20µl of ethanol with 1980 µl of the solution of the DPPH: control solution (3 repetitions). In a test tube, place 20µL of the sample solution for each concentration (at 3 repetitions), add to the 1980 µl solution of the DPPH- radical analysis solution. Allow to incubate in the dark for 30 minutes.

4.2.4) Absorbance reading at 517 nm

Read successively the solutions for each concentration (3 repetitions) with the spectrophotometer at 517nm: the blank (methanol), the control solution and the sample solutions.

Determination of the radical inhibition power

The percentage inhibition of the DPPH radical by the sample was determined using the following formula:

$$\% \text{ inhibition} = [1 - (A_x/A_c)] \times 100$$

where A_x : the absorbance of the DPPH radical in the presence of the extract;

A_c : the absorbance of the DPPH radical (control solution).

III. RESULTS AND DISCUSSION

A. Extraction Yields

Fig. 1 shows the extraction yield of *S. latifolius* leaf extract.

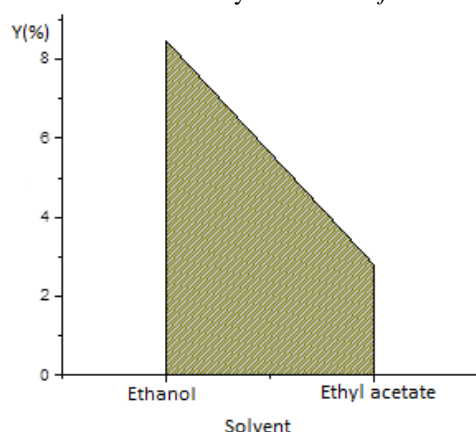


Fig. 1. Extraction yield of *S. latifolius* leaf extracts (Y: yield).

It can be seen from this figure that the yield obtained with ethanolic extract (more polar solvent) is higher than that of ethyl acetate (less polar solvent). This also shows that the quantitatively important metabolites in the leaves of *S. latifolius* are those which pass easily into the more polar solvents, notably polyphenols including anthocyanins, tannins, flavonoids, but also saponins, alkaloids, etc. [5].

B. Phytochemical Composition

Table I shows the results of the chemical composition per 100 grams of dry matter of *S. latifolius* leaves.

TABLE 1: CHEMICAL COMPOSITION OF *S. LATIFOLIUS* LEAF DRY MATTER

Dosed macronutrients	Concentration (in % of dry weight)
Humidity	-
Protein	24,3 ± 2,1
Ashes	11,3 ± 1,5
Fibers	35,3 ± 1,1
Carbohydrates	7,2 ± 1,4
Fat	22 ± 3,6
Energy/kcal	324

As can be seen in this table that *S. latifolius* leaves contain 35.3% fiber, crude protein is 24.3%, lipids 22% and carbohydrates 7.2% respectively. These results are similar to Plassart research (2015) which shows that leaves and fruits contain a significant amount of the basic nutrients such as protein, carbohydrates, lipids and fiber. This high fiber content (35.3%) would be a good source of fiber for a normal diet [16]. The results of the chemical composition of fresh *S. latifolius* leaf material are given in the table below (Table II).

TABLE II: CHEMICAL COMPOSITION OF FRESH *S. LATIFOLIUS* LEAF MATERIAL

Dosed macronutrients	Concentration (% of fresh weight)
Humidity	14,3
Protein	20,8
Ashes	9,7
Fibers	30,2
Carbohydrates	6,2
Fat	18,8
Energy/kcal	521,6

From this table, it can be seen that *S. latifolius* leaves contain 30.2% fiber, 20.8% crude protein, 18.8% fat, 14.3% water, and 6.2% carbohydrates respectively. These results are

in line with Plassart's research [16] which confirms that leaves and fruit contain an appreciable amount of basic nutrients such as protein, carbohydrates, lipids and fiber. Analyses of the nutritional composition of *S. latifolius* by Yesufu and Hussaini [17] also reported that *S. latifolius* contains moderate water content. This proves that the powder from the leaves of the plant can be stored for a long time without being deteriorated. Table III gives the results of the chemical screening of *S. latifolius* leaf extracts.

TABLE III: RESULTS OF CHEMICAL SCREENING OF *S. LATIFOLIUS* LEAF EXTRACTS

Substances searched	Result
Total Polyphenols	+
Flavonoids	+
Anthocyanes	-
Tanins	+
Leuco-anthocyanins	-
Alkaloids	+
Saponins	-
Bound quinones	+
Steroids	+
Triterpenes	+
Free quinone	+

Legend: +: Compounds present, -: compounds absent.

From Table III, it can be seen that a total of eight secondary metabolites have been identified in the leaves of *S. latifolius*, including polyphenols, flavonoids, tannins, alkaloids, bound and free quinones, steroids and triterpenes. All three are absent, as are anthocyanins, leucoanthocyanins and saponins. These results corroborate those of Ikpefan *et al.* [18] who identified several compounds in the leaves of *S. latifolius*, such as alkaloids, flavonoids, tannins, terpenoids. Ngokam *et al.* [19] isolated from the roots of the plant an indole alkaloid called nauclefolinin as well as five other known triterpenes. Shigemori *et al.* [20] discovered from the trunk bark of *S. latifolius* five indo-monoterpenic alkaloids named strictosamide and nauclefins A, B, C, D and E.

Fig. 2 shows the mineral content of *S. latifolius* leaves.

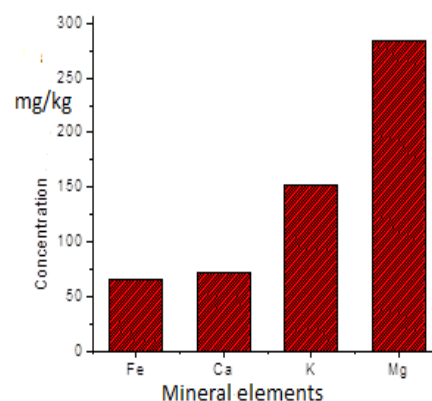


Fig. 2. Mineral elements content of *S. latifolius* leaves.

As can be seen in this figure, magnesium shows a remarkable predominance in the leaves of the plant compared to the other elements detected (Fe, K, Ca). Nevertheless, potassium presents a non-negligible concentration in the leaves of *S. latifolius*. These results confirm Plassart research [16] which reveals that *S. latifolius* is a plant that contains more magnesium, calcium and potassium.

C. Evaluation of Biological Activities

1) Evaluation of antibacterial activity

Table IV shows the results of the antibacterial activity of different extracts of *S. latifolius*.

TABLE IV: RESULTS OF THE ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *S. LATIFOLIUS*

Extracts	CMI ($\mu\text{g/mL}$)	
	<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 8739
Ethanol	1000	1000
Ethyl acetate	500	4000

Legend: ATCC: American Type Cell Collection, MIC minimum inhibitory concentration.

From Table IV it appears that two bacterial strains used have a low sensitivity to *S. latifolius* leaf extracts (MIC greater than or equal to 500 $\mu\text{g/mL}$) [21]. This resistance can be justified by the nature of the bacterial walls but also by the compounds contained in the *S. latifolius* leaves.

2) Evaluation of antioxidant activity

The results of the antioxidant activity assessment are shown in the table below:

TABLE V: PERCENTAGE OF DPPH INHIBITION AND Fe^{3+} REDUCTION IN THE PRESENCE OF *S. LATIFOLIUS* LEAF EXTRACTS

Concentration ($\mu\text{g/mL}$)	Inhibition %		
	VIT C	DPPH	FRAP
10	85,7	68,06	48,5
20	88,2	76,2	51,7
30	88,7	86,3	51,9
40	89,7	86,7	54,4
50	ND	ND	62,1

Legend : ND : not determined, VIT C : vitamin C.

From this table, it can be seen that the percentage of inhibition of the *S. latifolius* leaf extract is a function of the concentration. At 10 $\mu\text{g/mL}$, we have a percentage inhibition of approximately 68% in the DPPH test and 48.5% in the FRAP test. These results show that the extract of our plant showed greater antioxidant activity than the vitamin C used as a positive control. These results are similar to the research of Plassart [16] who reported that the aqueous and ethanolic extracts of *S. latifolius* show a dose inhibition dependent on DPPH radical activity significantly comparable to the activity of ascorbic acid [16]. Since polyphenols are endowed with antioxidant properties [5], these results confirm our previously presented qualitative phytochemical analyses.

3) Evaluation of antihelmintic activity

Table VI gives the time of paralysis of earthworms in the presence of *S. latifolius* extracts.

TABLE VI: TIME OF PARALYSIS OF EARTHWORMS IN THE PRESENCE OF *S. LATIFOLIUS* EXTRACTS

Concentration (mg/mL)	Time of paralysis (minutes)		
	Albentel	Ethanol	Ethyl acetate
10	7	7	6
5	13	12	18
2,5	14	22	28
1,25	23	36	27
0,625	26	42	34

From this table, it can be seen that ethanol and ethyl acetate extracts significantly inhibited earthworm mobility. These results would be due to the presence of the compounds contained in the leaves of the studied plant. However, in the lowest concentration (0.625 mg/mL) of *S. latifolius*, we

observed paralysis of the worms in less than 50 minutes after contact with the plant extracts. A predominance of ethyl acetate extract over ethanolic extract. This difference can be explained by the nature of the compounds that pass into ethyl acetate (less polar solvent). By comparing the extracts of the plant with the standard (Albentel) at low doses (0.625 mg/mL) we notice that there is not a significant difference. Table VII gives the mortality rate of earthworms in the presence of plant extracts.

TABLE VII: MORTALITY RATE OF EARTHWORMS IN THE PRESENCE OF *S. LATIFOLIUS* EXTRACTS

Concentration (mg/ mL)	Mortality rate (%)		
	Ethanol	Ethyl acetate	Albentel
10	100	100	100
5	100	66,6	66,6
2,5	88,9	55,5	66,6
1,25	66,6	55,5	55,5
0,625	55,5	33,3	33,3

It appears from this table that the plant studied has antihelmintic activity. The ethanolic extract has a predominant activity (55.5% mortality rate) compared to the ethyl acetate extract (33.3%). This shows that compounds with a strong deworming activity pass easily into the more polar solvent [22]. By comparing this plant with the positive control (Albentel), we notice that at low concentration (0.625 mg/mL) the acetate extract presents the same result as Albentel (33.3% of the mortality rate) which is a modern product used in the treatment of gastrointestinal worms. These results are justified by the effect that this species contains secondary metabolites such as flavonoids, polyphenols, and tannins endowed with anti-helminthic properties. These results confirm the ethnobotanical data reported by Ademola *et al.* [23] which show the use of *S. latifolius* leaves by the Fulani tribe in Nigeria as regular dewormer against intestinal parasites such as *Haemonchus contortus*.

IV. CONCLUSION AND SUGGESTIONS

In short, the objective of this study was to chemically and bioactively analyze the leaves of *Sarcocephalus latifolius* in order to contribute to the scientific valorization of the phytotherapeutic richness of the DR Congo. In this study, the antibacterial activity of *S. latifolius* leaves extracts was assessed using micro-dilution method and the antioxidant capacity of different plant extracts has been evaluated by determining their ability to reduce iron (III) to iron (II) and DPPH radical. The results obtained revealed that: The dry matter of the powder of the leaves of the plant is rich in fibers, crude proteins, lipids and carbohydrates. While its water content was revealed to be moderate (14.3%); the leaves of *S. latifolius* are rich in trace elements, especially calcium (Ca), magnesium (Mg), potassium (K), and iron (Fe); the plant possesses the major groups of secondary metabolites such as total polyphenols, flavonoids, tannins, alkaloids, bound and free quinones, steroids and tri-terpenes, while it is poor in anthocyanins, leucoanthocyanins and saponins. The bacterial strains used were less sensitive to *S. latifolius* leaf extracts (MIC greater than or equal to 500 $\mu\text{g/mL}$). The extracts of this plant showed significant anti-oxidant and antihelmintic activity, with a strong correlation between the DPPH and

FRAP antioxidant potential assessment methods. Due to their interesting functional properties, this plant could be used in the formulation of a nutraceutical with strong antioxidant power to improve the antioxidant status of consumers, but also in the management of helminthiases.

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