



Research Article

DETERMINATION OF PHENOLIC COMPOUNDS AND ANTIOXIDANT TEST OF AQUEOUS EXTRACTS FROM THE LEAVES AND STEMS OF *STROPHANTHUS SARMENTOSUS* DC (*APOCYNACEAE*), HARVESTED IN MALI

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Abstract

Plants represent an inexhaustible reservoir of bioactive molecules, characterised by great structural diversity and a broad spectrum of biological activities. *Strophanthus sarmentosus* (Apocynaceae) is a plant widely used in traditional African medicine, but one that remains largely unexplored from a phytochemical and pharmacological perspective. This plant contains several groups of secondary metabolites, including flavonoids, tannins, alkaloids and saponosides. The aim of this study was to assess the total phenolic and flavonoid content, as well as the antioxidant activity, of aqueous extracts from the leaves and stems of *S. sarmentosus*. Total polyphenol contents were determined using the Folin-Ciocalteu method, total flavonoid contents by colorimetric assay, and antioxidant activity was assessed using the DPPH free radical scavenging assay. The results showed that total polyphenol content ranged from 364.8 ± 52.3 mg GAE/g in the leaves to 339.6 ± 7.6 mg GAE/g in the stems. Total flavonoid contents ranged from 192.8 ± 14.7 mg CtE/g for the leaves to 72.4 ± 39.0 mg CtE/g for the stems. Antioxidant activity, expressed as the 50 per cent inhibitory concentration (IC₅₀) against the DPPH radical, was 40.03 ± 0.78 µg/mL for the leaves and 42.01 ± 1.07 µg/mL for the stems, values close to that of ascorbic acid, which was used as a positive control (IC₅₀ = 42.90 ± 2.37 µg/mL). These results indicate that the leaves and stems of *S. sarmentosus* are promising sources of phenolic compounds and exhibit significant antioxidant activity, justifying increased interest in this species in the search for new natural agents with therapeutic potential.

Keywords: *Strophanthus sarmentosus*, Total polyphenols, Total flavonoids, Antioxidant activity, DPPH.

INTRODUCTION

Microbial resistance to antibiotics is now a major public health problem and is driving research into new naturally occurring antimicrobial substances. In this context, medicinal plants represent an important source of bioactive molecules likely to be of interest to the pharmaceutical industry. Interest in herbal medicines has also grown in developing countries, where traditional medicine remains widely used. However, their safety should not be taken for granted: it depends, in particular, on the plant species, the part used, the dose and the method of preparation. The therapeutic properties of medicinal plants are mainly attributed to the presence of natural bioactive compounds that accumulate in their various organs (Cissé *et al.*, 2022). These substances include, in particular, phenolic compounds, flavonoids, tannins and alkaloids, some of which exhibit significant antioxidant activity (Tuyg'unovna, 2023). The potential for discovering new molecules remains considerable, as only a small proportion of the approximately 400,000 known plant species has been the subject of in-depth phytochemical and pharmacological studies (Badiaga, 2011). Africa possesses a particularly diverse flora rich in plant species, many of which are used in traditional medicine. However, many species remain poorly studied from a phytochemical and pharmacological perspective (Cissé *et al.*, 2022). This is particularly the case for *Strophanthus sarmentosus*, a plant belonging to the *Apocynaceae* family and

found in the tropical regions of Africa (Arbonnier, 2019; Mensah *et al.*, 2019). This species is used in traditional medicine, but its biological properties and therapeutic potential remain insufficiently documented. The information available in the literature regarding the biological activities and chemical constituents of *S. sarmentosus* is limited (Abiola and Aiyelaagbe, 2023). Phytochemical studies on *S. sarmentosus* have revealed the presence of several groups of secondary metabolites, notably tannins, flavonoids, phenolic compounds and alkaloids, in various parts of the plant (Badiaga *et al.*, 2026; Abiola *et al.*, 2025). The quantification of total polyphenols and flavonoids can be carried out using the Folin Ciocalteu method and the colourimetric method using aluminium salts, respectively (Traoré, 2025). The presence of these compounds justifies the interest in assessing the antioxidant activity of this species. Indeed, oxidative stress, resulting from an imbalance between the production of free radicals and antioxidant defence mechanisms, is implicated in several pathological processes, notably cardiovascular diseases, inflammatory diseases and certain cancers (Fioroni, 2023). Assessing the antioxidant activity of *S. sarmentosus* could therefore contribute to making better use of this plant and to identifying natural resources of pharmacological interest. This activity may be linked to the presence of flavonoids, tannins and other phenolic compounds, which are capable of acting individually or synergistically to neutralise free radicals and limit the effects of oxidative stress (Dembélé *et al.*, 2024; Simone, 2024; Moyo *et al.*, 2021). Among the methods used to assess antioxidant activity *in vitro*, the DPPH radical scavenging assay is widely employed due to its

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simplicity, speed and reproducibility. DPPH, or 2,2-diphenyl-1-picrylhydrazyl, is a stable, violet-coloured free radical. In the presence of an antioxidant capable of donating a hydrogen atom or an electron to it, it is reduced to DPPH-H, which is pale yellow in colour; this results in a decrease in absorbance, typically measured at around 515–520 nm. The intensity of this discolouration allows the extract's ability to scavenge free radicals to be estimated (Touré, 2022; Koumaré, 2024). Thus, the phytochemical study and the assessment of the antioxidant activity of *S. sarmentosus* extracts appear relevant to a better understanding of this species biological potential. They could also contribute to the scientific validation of a plant used in traditional medicine and to the search for new natural sources of bioactive molecules.

MATERIALS AND METHODS

MATERIALS

Plant material

The plant material used in this study consisted of leaves and stems of *S. sarmentosus*, collected in May 2025 in the vicinity of Koutiala, Mali. Taxonomic identification of the specimens was carried out at the National Institute for Research in Traditional Medicine and Pharmacopoeia (INRMPT) in Bamako under herbarium number 1154/DMT. After harvesting, the leaves and stems were separated and then dried in the shade at laboratory temperature (25 °C) for three weeks until completely dry. The dried plant parts were then ground and pulverised to obtain a homogeneous powder, which was stored away from light and moisture until analysis.

METHODS

Preparation of the aqueous extract

50 g of finely ground plant material was added to 500 mL of water; the mixture was heated to reflux whilst stirring for 15 minutes. After cooling, the solution was filtered, concentrated under reduced pressure and evaporated at 70 °C for 24 hours until a dry extract was obtained, which was stored at 4 °C and then freeze-dried.

Determination of total polyphenol content

Principle of the Folin Ciocalteu method: The Folin Ciocalteu method is a rapid colorimetric technique for quantifying total polyphenols in an extract. This method is based on the reaction of polyphenols with the Folin-Ciocalteu reagent (Djamilatou *et al.*, 2021; Cicco *et al.*, 2009; Singleton *et al.*, 1999; Singleton and Rosi, 1965), which is a solution containing tungsten and molybdenum ions. When polyphenols (which act as reducing agents) react with this reagent, they reduce the ions in the reagent (causing them to change from blue to green), resulting in a colour change in the solution. The intensity of the green colour is proportional to the concentration of total polyphenols in the sample. This intensity can be measured by spectrophotometry, usually at a wavelength of 725 nm (Traoré, 2025).

Experimental section: The Folin-Ciocalteu method, described and carried out by Singleton and Rosi in 1965 with a slight modification by Touré *et al.* in 2023, enabled us to determine the polyphenol content in the aqueous extracts of the leaves

and stems of *S. sarmentosus*. To 200 µL of extract, 1.5 mL of water is added, followed by 2.5 mL of Folin's reagent diluted 10 times, and 2 mL of 7.5 per cent sodium carbonate (Na_2CO_3). In an alkaline medium (Na_2CO_3), the phenolic compounds reduce this reagent. A blue-coloured molybdenum and tungsten complex is formed. The solution is incubated for 90 minutes in the dark at 25 °C. Absorbance is measured using a spectrophotometer at 725 nm against a blank (cuvette containing 0.5 mL of water). The calibration curve for gallic acid is prepared using different concentrations (20, 40, 60, 80 and 100 µg/mL). Tests are carried out five times for each concentration, and three values are selected. These values are expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

Determination of total flavonoid content

Principle of the colourimetric method using aluminium salts: The total flavonoid content is determined primarily by a spectrophotometric colourimetric method using aluminium trichloride (AlCl_3). AlCl_3 reacts with the hydroxyl groups of the flavonoids to form a stable, yellow-coloured complex. This reaction enables the concentration of flavonoids in a sample to be determined by measuring the intensity of the yellow colour formed, expressed in milligrams of catechin equivalents per gram of extract (Traoré, 2025).

Experimental section: The flavonoid content of the aqueous extracts is determined using a colorimetric assay with AlCl_3 (Dirar *et al.*, 2019; Zhishen *et al.*, 1999) and expressed in milligrams of catechin equivalents per gram of extract (mg CtE/g). 1 mL of diluted extract is placed in a 10 mL flask; 4 mL of water and 0.3 mL of 5% NaNO_2 solution are added. After 5 minutes at 25 °C, 0.3 mL of 10% AlCl_3 solution is added. The mixture is shaken for 6 minutes, then 2 mL of 1 M NaOH solution is added. The reaction mixture is made up to 10 mL with water and then shaken. The absorbance of the mixture is measured on a spectrophotometer at 510 nm against a blank (cuvette containing 0.5 mL of water). The tests are carried out five times on each extract.

Assessment of antioxidant activity (DPPH assay)

The DPPH assay is a rapid spectrophotometric method that measures an extract's ability to scavenge free radicals. Antioxidants are chemical substances, whether natural or synthetic, capable of slowing down or preventing the oxidation of other molecules. Oxidation is a chemical reaction that produces free radicals, unstable molecules that can cause damage to cells and contribute to ageing and various chronic diseases such as cardiovascular disease, cancer and neurodegenerative disorders (Caroline, 2020).

Experimental section: Antioxidant activity was assessed using the DPPH assay (Touré *et al.*, 2023). A volume of 0.2 mL of extracts or the standard (ascorbic acid) at varying concentrations of 25, 75, 100 and 125 µg/mL was added to 7.8 mL of the methanolic DPPH solution (100 µM). The mixture was shaken vigorously and then incubated for 30 minutes in the dark at 25 °C. A decrease in absorbance was measured at 515 nm against the blank (a cuvette containing 0.5 mL of methanol) using a spectrophotometer (Biomate 3S UV-Visible). The inhibition percentages were calculated using the following equation :

$$\% \text{ Inhibition} = \frac{A_{\text{témoin}} - A_{\text{échantillon}}}{A_{\text{témoin}}} \times 100$$

RESULTS AND DISCUSSION

RESULTS

Assay of total polyphenols and total flavonoids:

The results of the determination of total polyphenols and total flavonoids are shown in Table 1.

Table 1. Results for total polyphenols and total flavonoids in aqueous extracts of the leaves and stems of *S. sarmentosus*

	Total polyphenols (mg GAE/g)		Total flavonoids (mg CtE/g)	
Extracts	Leaves	Stems	Leaves	Stems
Aqueous	364.8 ± 52.3 ^A	339.62 ± 7.55 ^B	192.78 ± 14.70 ^B	72.4 ± 39.0 ^A
P-value	0.02756	0.00002	0.00159	0.00619

The concentrations are higher in the leaves, with 364.8 ± 52.3 mg GAE/g of total polyphenols and 192.78 ± 14.70 mg CtE/g of total flavonoids, respectively.

DPPH assay

The results of the DPPH test are listed in Table 2.

Table 2. Results of the DPPH test for aqueous extracts of the leaves and stems of *S. sarmentosus*

Extracts	IC ₅₀
	DPPH (µg/mL)
Aqueous (Leaves)	40.029 ± 0.776
Aqueous (Stems)	42.010 ± 1.072
Vitamin C	42.904 ± 2.37

The highest free radical scavenging activity was observed with the aqueous extract of *S. sarmentosus* leaves (IC₅₀ = 40.029 ± 0.776 µg/mL). These results may help to reinforce the use of *S. sarmentosus* as a promising natural source of antioxidants.

DISCUSSION

The aqueous extract of *S. sarmentosus* leaves has the highest levels of total polyphenols and total flavonoids, at 364.8 ± 52.3 mg GAE/g and 192.78 ± 14.70 mg CtE/g respectively. These results indicate that the leaves are an important source of phenolic compounds. The high content of these secondary metabolites could contribute to the biological properties observed in this species. The antioxidant activity of the extracts was assessed using the DPPH radical scavenging assay. This activity is of particular interest, as oxidative stress is implicated in the onset or progression of several chronic diseases, notably cardiovascular diseases, inflammatory diseases and certain cancers. The genus *Strophanthus* is known for its numerous pharmacological properties, including its antioxidant potential. This could be linked to the presence of flavonoids, tannins and other phenolic compounds, which are capable of neutralising free radicals, possibly through synergistic effects. Aqueous extracts of the leaves and stems exhibited significant antioxidant activity, with IC₅₀ values of 40.029 ± 0.776 µg/mL and 42.010 ± 1.072 µg/mL, respectively. These values are comparable to that obtained with ascorbic acid, used as a standard (42.904 ± 2.37 µg/mL).

The IC₅₀ of the aqueous leaf extract is slightly lower than that of the standard, whilst that of the aqueous stem extract is also comparable and slightly lower. A lower IC₅₀ value indicates a greater capacity to scavenge the DPPH radical under the experimental conditions used. However, our results differ from those reported for the methanolic root extract of *S. hispidus*, which exhibited a much higher IC₅₀ value of 969.1 µg/mL (Mensah *et al.*, 2019). The aqueous extracts of the leaves and stems of *S. sarmentosus* therefore appear to be more active against the DPPH radical than the methanolic extract of the roots of *S. hispidus*. This difference could be explained by several factors, including the species studied, the plant organ used, the extraction solvent, the nature of the extracted compounds and the experimental conditions. However, one should avoid making too direct a comparison between these studies, as extraction protocols and the conditions under which the DPPH test was conducted may vary. Furthermore, phytochemical analysis did not reveal the presence of cyanogenic glycosides in the extracts studied (Badiaga *et al.*, 2026). This absence might suggest a reduced risk of toxicity specifically linked to these compounds. However, it does not allow us to conclude that the plant is entirely safe, as other constituents may contribute to its toxicity. In this context, the cytotoxic activity of the leaf, stem and root extracts of *S. sarmentosus* was assessed using the lethality test on *Artemia salina* larvae (Osamudiamen *et al.*, 2020). The LC₅₀ values obtained ranged from 117 to 270 µg/mL. According to the criteria of Clarkson *et al.* (2004), a LC₅₀ between 100 and 500 µg/mL corresponds to moderate toxicity. The extracts studied therefore exhibit moderate cytotoxicity under the experimental conditions used. The presence of alkaloids, tannins and flavonoids could contribute to this cytotoxic activity, as suggested by Osamudiamen *et al.* (2020). However, the test on *A. salina* constitutes a preliminary toxicity assay. Further studies, based in particular on cytotoxicity tests on cell lines and on *in vivo* toxicological models, would be necessary to confirm this potential and clarify the mechanisms involved.

Conclusion

In conclusion, leaf extracts of *S. sarmentosus* have a higher content of total polyphenols and flavonoids, as well as greater antioxidant activity than stem extracts. This activity may partly explain the traditional use of this plant to treat various conditions, particularly infections and inflammatory diseases. However, further studies are still needed to confirm these properties and determine the mechanisms of action of the bioactive compounds involved.

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