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Quantification of Phenolic Compounds, Evaluation of Antioxidant and Antimycobacterial Activities of Extracts of *Clerodendrum splendens*, *Sansevieria trifasciata* and *Sacoglottis gabonensis*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

An *In vitro* study was conducted to investigate the extracts of three plants: *Clerodendrum splendens*, *Sansevieria trifasciata* and *Sacoglottis gabonensis*, which are traditionally utilized in Côte d'Ivoire for the treatment of Buruli ulcer. The objectives of this study were to quantify phenolic compounds, assess antioxidant activity against the DPPH● radical and evaluate the effects of aqueous and hydroethanolic extracts from the leaves, roots and barks on a strain of *Mycobacterium ulcerans*. Quantitative analysis revealed significant concentrations of total polyphenols, flavonoids, condensed and hydrolyzable tannins. The highest total phenolic content was found in the decoction and hydroethanolic macerate of *Sacoglottis gabonensis* (DSG: 367.15±4.88; MhSG: 317.51 ± 3.87 mgEAG/g). Total flavonoids were more abundant in hydroethanolic and aqueous macerates of *Clerodendrum splendens* (MhCS: 73.64±3.01; MCS: 44.31±0.50 mgEQ/g), as well as in decoctions of both *Sacoglottis gabonensis* and *Clerodendrum splendens* (DSG: 46.17±1.13 and DCS: 41.40±0.90 mgEQ/g). Condensed tannins were primarily found in the decoction and hydroethanolic macerate of *Sacoglottis gabonensis* (DSG: 30.29 ± 1.00 and MhSG: 21.36 ± 0.68 mgEC/g) and in the macerate of *Clerodendrum splendens* (MCS: 23.12±0.34 mgEC/g). Hydrolyzable tannins were more concentrated in the hydroethanolic and aqueous macerates and decoction of *Clerodendrum splendens* (MhCS: 2.01±0.13; MCS: 1.5±0.03 and DCS: 1.68±0.14%). The antioxidant activity, assessed against the stable DPPH, showed that the extracts of *Sacoglottis gabonensis* (CR_{50}: decoction 0.0085; aqueous radical and hydroethanolic macerates: 0.0209 and 0.0062) and *Clerodendrum splendens* (CR_{50}: decoction 0.005) exhibited activities comparable to that of vitamin C (CR_{50}: 0.002), which served as a reference. All extracts demonstrated significant activity against the *Mycobacterium ulcerans* strain CD1539 at concentrations ranging from 0.1 mg/mL to 0.5 mg/mL, with enhanced activity noted for the plant decoctions.

Keywords: Clerodendrum splendens; Sansevieria trifasciata; Sacoglottis gabonensis; phenolic compounds; antioxidant activities; antimycobacterial activity; buruli ulcer.

1. INTRODUCTION

Buruli ulcer (BU) is an infectious disease caused by *Mycobacterium ulcerans,* ranking as the third most common mycobacterial disease after tuberculosis and leprosy [1]. It poses a significant health challenge in approximately 33 countries, predominantly in tropical regions, with a notably high prevalence in West Africa [2]. Cases have also been documented in Japan, Mexico, South America, Papua New Guinea and Australia [3]. In Côte d'Ivoire, BU is hyper-endemic with a cumulative total of 22,000 cases, including nearly 10,000 new cases reported in 2015 [4]. The regions of Lacs (capital Yamoussoukro) and Gbêkê (capital Bouaké) are identified as areas with high prevalence [4, 5]. The mode of transmission remains uncertain, but it is suspected to occur through insect bites or by direct contact with contaminated environments. The infection typically begins with a painless skin lesion (nodule, plaque or edema), often located on the arms, legs, or face, which can progress to an ulcer if left untreated [6]. Current treatment protocols involve a dual therapy regimen combining Rifampicin and Clarithromycin for a duration of 8 weeks. This treatment approach is effective in healing early lesions, stabilizing the disease, or reducing lesions to facilitate less invasive surgical interventions [7]. If untreated, this infection can result in permanent deformities and disabilities. In West Africa, many patients in rural areas are increasingly resorting to self-medication and traditional herbal remedies. Research has been conducted to evaluate the efficacy and safety of these plants, leading to the identification of several medicinal species traditionally used to treat BU [8-12]. Some studies have analyzed the chemical composition of these plants and their antibacterial activities against *M. ulcerans* in West Africa [13], particularly in Benin [10, 14] in Ghana [15] and in Côté d'Ivoire [16,17]. In Côte d'Ivoire, *Sacoglottis gabonensis* is well recognized for its use in treating Buruli Ulcer, attributed to its potent antimycobacterial properties [18-20]. However, other plants such as the leaves of Clerodendrum splendens and the roots of *Sansevieria trifasciata* are also utilized, albeit without prior studies confirming their effectiveness against Buruli ulcer. These plants are frequently employed in mixtures for treating this infection.

Clerodendrum splendens (Lamiaceae) also known as *Clerodendron splendide* or “Charmille flamboyante”, has various vernacular names depending on the region, including Flaming de-cristo vermelha in Brazil, Makinda ngolo in Congo, Bharangi in India, Anboka yuki in the Central African Republic, Siangouê-lah (Gouro) and Troubatrou (Malinké) in Côte d’Ivoire, and Phuang kaeo daeng or Phuang naak in Thailand [21]. This shrubby vine, characterized by its striking red flowers, can grow up to 4 meters in height. Its evergreen, glossy green leaves are oblong and opposite, measuring 5-18 cm long and 4-10 cm wide. The tubular flowers, typically carmine red (though occasionally white), open into a corolla and feature four stamens and a prominent style. Due to its vibrant and attractive flowers (Fig. 1), it is often used as an ornamental plant in residential areas [22]. *C. splendens* is commonly employed in traditional medicine for its antimalarial, anti-inflammatory and antibacterial properties [23]. It also utilized to treat coughs, buboes, venereal infections, skin diseases, rheumatism, asthma, uterine fibroids, and various skin diseases as a cicatrizing [24-26].

Sansevieria trifasciata (Asparagaceae), commonly referred to as snake plant or mother-in-law's tongue [27,28], is known by various names across Africa. In Côte d'Ivoire, it is called Tèman in Baoulé, Kpa-kpè in Bété, Sikè-wè in Dioula. In Senegal, it is referred to as Ndolé, bua in Bambara and Kakalé in Maninka. In Sierra Leone, it is known as Woko-le in Bulom, Nomoliyo in Kissi and Nomoni in Gola [29]. Native to tropical regions of West Africa, this herb serves as an ornamental plant, a source of fiber, and a medicinal resource. It thrives in sunny or lightly shaded areas, preferring moist, fertile soil. This herbaceous, succulent, perennial plant can reach heights of up to 90 centimeters. Its leaves form a basal rosette, are flat, thick, leathery, and sword-shaped, with grayish-white transverse bands, measuring between 30 cm to 1 m in height and 5 to 6 cm in width. The flowers, which are greenish or grayish white, grow in clusters measuring 40–75 cm long [30]. Its traditional medicinal uses are well-documented for treating various conditions, including asthma, earaches, ulcers, jaundice, pharyngitis, abdominal pain, colic, diarrhea, eczema, gonorrhea, hemorrhoids, hypertension, menorrhagia, sexual weakness, itchy skin, and urinary diseases [31-33].

Sacoglottis gabonensis (Humiriaceae) is a tree that can exceed 25 meters in height and features rough bark [34]. The bark is of young trees is relatively smooth, while that of older trees displays brown to dark brown horizontal lenticels. This species is highly branched and has a rounded, open crown. The leaves measure alternate and simple, stipulate (approximately 1 mm long), and shed quickly. The petioles are 6 to 10 mm in length, and the leaf blade is ovate to elliptical or oblong, measuring 6 to 15 cm long and 2.5 to 6 cm wide, with a cuneate base, an acuminate apex, a crenate margin, and 6 to 12 pairs of lateral veins [20]. *S. gabonensis* is widely utilized for treating ailments. In Nigeria, bark infusions are employed to address gonorrhea, abdominal pain, diarrhea, ovarian disorders, vaginal infections, and fever [35]. In Gabon, it is used for treating venereal diseases [36]. In Côte d’Ivoire, this plant is traditionally used to treat leprosy and skin disorders. Ethnopharmacological studies have also highlighted its application in the treatment of Buruli ulcer [37,38].

Fig. 1. *Clerodendrum splendens* leaves

Fig. 2. *Sansevieria trifasciata* leaves

Fig. 3. *Sacoglottis gabonensis* Trunk [39]

Fig. 4. *Sacoglottis gabonensis* leaves [39]

2. MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Plant material

The plant material comprises leaves of *Clerodendrum splendens*, roots of *Sanseviera trifasciata* and bark of *Sacoglottis gabonensis*, all collected in January 2024. *Clerodendrum splendens*'s leaves were collected in the commune of Abobo (5°23′21.145″ N, 4°00′59.236″ W) (autonomous district of Abidjan). *Sanseviera trifasciata*'s roots were collected in Azaguié (5°38′00″ N, 4°05′00″ W) (Agnéby-Tiassa region). The bark of *Sacoglottis gabonensis* was sourced from Alépé (5°29′46″ N, 3°39′49″ W) in the Mé region. The plant materials were identified at the National Floristic Center (CNF) in Abidjan in accordance with the available herbarium specimens (N°s uci 017405; 13134 and 131).

2.1.2 Biological material

The CD1539 strain of *M. ulcerans* was isolated and characterized by the laboratory of the National Reference Center Buruli (CNR-BU) at the Tuberculous and Atypical Mycobacteria Unit of the Pasteur Institute of Côte d'Ivoire.

2.2 Methods

2.2.1 Preparation of extracts

The plant material was thoroughly cleaned and air-dried at room temperature (25°C) in a ventilated area for 2 weeks. Subsequently, it was ground into a fine powder an electric grinder (BLENDER HGB66E, reference HGB660EI). A total of ten grams (10 g) of powdered plant material was macerated in (3×100 mL) of distilled water or 80% ethanol for 24 h with continuous stirring. The resulting aqueous (MSG, MST and MCS) and hydro-ethanolic (MhSG, MhST and MhCS) macerates of *S. gabonensis* bark, *S. trifasciata* roots and *C. splendens* leaves were then filtered and concentrated under reduced pressure at 40°C using a rotary evaporator. Additionally, five grams (5 g) of the plant powder were dissolved in (3×100 mL) of distilled water and boiled for 15 minutes. The resulting decoctions (DSG, DST and DCS) were also filtered and concentrated under reduced pressure at 40°C using a rotary evaporator.

2.2.2 Quantitative analysis

2.2.2.1 Determination of total phenols (TP)

Total phenols were quantified in the extracts (MSG, MST, MCS, MhSG, MhST, MhCS, DSG, DST and DCS) by the spectrophotometric method described in [40]. To 1 ml of a 1/10 extract diluted, 1.5 ml of a Na_2CO_3 solution (17%, m/v) and 0.5 ml of Folin-Ciocalteu reagent (0.5 N) were added. Following a 30-minute incubation at 37°C, the absorbance was measured at 720 nm against a negative control (without plant extract). A calibration curve was established using various concentrations of gallic acid, ranging from 0.048 to 25 µg/ml, under the same experimental conditions. The total phenol content was calculated according to formula (1) and expressed as milligrams (mg) of gallic acid equivalent per gram (g) of dry matter (mg EAG/g DM).

$$TP = (V \times C \times d)/m \quad (1)$$

V: volume of crude extract (ml); **C**: average concentration (mg/ml);
d: dilution factor; **m**: mass of pulverized dry matter (g).

2.2.2.2 Determination of total flavonoids (TF)

The quantification of TF was conducted using spectrophotometry, following the method described by Avouet-Grand and collaborators

[41]. A quantity of 0.01 g of dry extract was dissolved in 10 mL of distilled water. To 1 mL of this solution, 1 mL of 2% aluminum chloride ($AlCl_3$) in methanol was added. After a 10-minute incubation, the absorbance was measured at 415 nm using a spectrophotometer, with a blank sample (without plant extract) serving as the reference. A calibration curve was established using various concentrations of quercetin, ranging from 0.048 to 25 µg/ml, under the same experimental conditions. The total flavonoid content was calculated according to formula (2) and expressed as milligrams of quercetin equivalents per gram of the dry mass of the plant powder (mg EQ/g DM).

$$TF = (V \times C \times d) / m \quad (2)$$

V: final volume of the extract (mL); **C**: concentration of the extract (mg/mL);
d: dilution factor; **m**: dry matter mass of plant material (g)

2.2.2.3 Determination of condensed tannins (CT)

The quantification of CT was performed using spectrophotometry, following the method described by Heimler and colleagues [42]. To 400 µL of extract or standard, 3 mL of a 4% vanillin solution in methanol and 1.5 mL of concentrated hydrochloric acid were added. The mixture was incubated for 15 minutes, after which the absorbance was measured at 500 nm using a spectrophotometer. Calibration was conducted with various concentrations of catechin (0.39 to 200 µg/mL), under the same experimental conditions. The CT content was calculated according to formula (3) and expressed as milligrams of catechin equivalents per gram of dry matter (mg EC/g DM).

$$TC = (V \times C \times d) / m \quad (3)$$

C: concentration of the extract (mg/mL); **V**: volume of the mixture analyzed (mL);
d: dilution factor; **m**: dry matter mass of plant material (g).

2.2.2.4 Determination of hydrolyzable tannins (HT)

The quantification of HT was carried out using spectrophotometry, as described by Dif et al. [43]. An amount of 0.01 g of extract was dissolved in 10 ml of water. After homogenization, 1 ml of this solution was mixed with 3.5 ml of a solution of $FeCl_3$ (0.01 M in 0.001 M HCl). The absorbance was measured at 660 nm using a UV spectrophotometer. The proportion of HT was calculated according to the expression (4):

$$HT\ (\%) = (Abs \times M \times V) / E\ mole \times m \quad (4)$$

HT: hydrolyzable tannins; **Abs**: absorbance; **E mole**: 2169 gallic acid (constant expressed in moles); **M**: molar mass = 300 g/mol; **V**: volume of extract used; **m**: sample mass.

2.2.3 Evaluation of antioxidant potential by the DPPH test

The method employed is based on the protocol established by Brand-Williams and colleagues [44], with modifications regarding the concentrations used. An amount of 0.003 g of DPPH was dissolved in 100 mL of 70% ethanol to create a solution with a concentration of 0.3 mg/mL. Then 0.01 g of *S. gabonensis*, 0.01 g of *C. splendens* and 0.2 g of *S. trifasciata* were solubilized in 10 mL of 70% ethanol. For each decoction and macerate, a series of concentrations was prepared. The concentrations for *S. gabonensis* were as follows: 0.625×10^{-1}; 0.312×10^{-1}; 0.156×10^{-1}; 0.781×10^{-2}; 0.391×10^{-2}; 0.195×10^{-2}. For *S. trifasciata*, the concentrations were: 10; 5; 2.5; 1.25; 0.625 mg/mL. For *C. splendens*, the concentrations were: 0.625×10^{-1}; 0.312×10^{-1}; 0.156×10^{-1}; 0.781×10^{-2}; 0.391×10^{-2}; 0.195×10^{-2} mg/mL. All preparations were made using the same solvent. In test tubes containing 1 mL of plant extract at the specified concentration, 2 mL of the DPPH solution was added. The tubes were incubated in the dark for 30 min, then the absorbance was measured at 517 nm using a spectrophotometer. A positive control was carried out with a solution of a natural antioxidant (vitamin C) as a reference. The percentage of reduction of DPPH by each tested plant extract was calculated by the following formula:

$$\%\ PR = [(A_b - A_e) / A_b] \times 100 \quad (5)$$

% PR: percentage reduction; $\mathbf{A_b}$: absorbance of white (nm)
$\mathbf{A_e}$: sample absorbance (nm).

The concentration that reduces DPPH by 50% (CR_{50}) is determined graphically using a regression curve between the percentages reduction (PR) and the concentrations.

2.2.4 Study of antimycobacterial activity

2.2.4.1 Preparation of culture medium

The preparation of Löwenstein-Jensen (L-J) culture medium incorporated with plant extracts

was carried out according to the method described by Adebo and colleagues [45]. A quantity of each plant extract was added to an appropriate volume of Löwenstein-Jensen medium to achieve final concentrations of 0.1 mg/mL; 0.3 mg/mL and 0.5 mg/mL. The mixture was homogenized using a blender and then aseptically distributed into sterile sterile screw-top tubes at a volume of 5 ml per tube. The Löwenstein-Jensen tubes were positioned at an incline and allowed to solidify at +85°C for 40 minutes. Sterility tests were carried out on 1% of the tubes from the series. Following validation, the Löwenstein-Jensen medium tubes supplemented with extracts were stored at +4°C until further use.

2.2.4.2 Preparation of bacterial inoculum

Under a microbiological safety cabinet, five colonies of the CD1539 strain of *M. ulcerans* were collected using a sterile loop and transferred to a sterile glass tube containing glass beads. After briefly vortexing to separate the bacterial cells, 2 mL of sterile distilled water were added. Following homogenization by shaking, 1 mL of bacterial suspension was transferred to a tube containing 10 mL of sterile distilled water. This stock bacterial suspension was adjusted to a concentration of 10^5 bacteria/mL using opacimetry, in accordance with the BCG standard. The stock suspension was successively diluted to concentrations of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Bacterial suspensions of dilutions 10^{-1}, 10^{-3} and 10^{-5} were selected to inoculate 2 tubes of Löwenstein-Jensen medium for each plant extract, as well as 2 control tubes of Löwenstein-Jensen without extracts. The inoculated tubes were incubated at 33°C in an aerobic atmosphere. Daily monitoring was carried out for one week, followed by biweekly monitoring for 2 months.

2.2.4.3 Determination of antimycobacterial activity of plant extracts

The evaluation of antimycobacterial activity involved of determining the proportion of surviving mycobacteria after exposure to various concentrations of plant extracts. The survival rate is calculated by dividing the mean number of colonies in the tubes containing the extracts by the mean number of colonies in the control tubes (without extracts).

$$Resistance\ rate = \frac{Average\ number\ of\ colonies\ with\ extracts}{Mean\ number\ of\ colonies\ in\ control\ tubes}$$

-If the survival ratio is less than 1, the bacterial strain is considered sensitive to the extract;
-If the survival ratio is greater than 1, the bacterial strain is considered resistant to the extract.

3. RESULTS AND DISCUSSION

3.1 Extraction Yields

Extraction tests conducted using decoction, aqueous and hydroethanolic maceration of *S. gabonensis* bark (SG), *S. trifasciata* roots (ST) and *C. splendens* leaves (CS) yielded varying results, as presented in Table 1.

The decoction produced yield rates of 20.6% for *S. trifasciata* roots, 10.4% for *S. gabonensis* bark and 8.9% for *C. splendens* leaves. The yields from the macerations were comparable across all plant materials, ranging from 7.5% to 9.7%. Notably, *S. trifasciata* roots exhibited the highest yields regardless of the extraction method employed: 20.6% via decoction, 9.3% through aqueous maceration, and 9.7% with 80% ethanol maceration. These findings suggest that the decoction is particularly effective for extracting from the more resilient parts (roots, barks), while maceration is better suited for the more delicate parts (leaves). The hard plant parts typically contain resistant compounds (such as alkaloids, tannins and polysaccharides) that are effectively extracted through the application of heat and boiling water during decoction. The observed difference in yield between the decoctions of *S. trifasciata* roots (20.6%) and *C. splendens* leaves (8.9%) may be attributed to the

Table 1. Yield of crude extracts

	DSG	DST	DCS	MSG	MST	MCS	MhSG	MhST	MhCS
Powder mass (g)	5	5	5	10	10	10	10	10	10
Extract mass (g)	0.52	1.03	0.45	0.88	0.93	0.84	0.75	0.97	0.85
Yield (%)	10.4	20.6	8.9	8.8	9.3	8.4	7.5	9.7	8.5

DSG, DST and DCS: Aqueous decoctions of S. gabonensis, S. trifasciat and C. splendens ; MSG, MST and MCS: aqueous macerates of S. gabonensis, S. trifasciata et C. splendens ; MhSG, MhST and MhCS: Hydroethanolic macerates of S. gabonensis S. trifasciata and C. splendens

greater resistance of the compounds present in the roots, which are more readily released with heat. For *C. splendens* leaves, hydroethanolic maceration yielded 8.5%, which is higher than the 5.7% yield reported by Pandey et al. [46].

3.2 Total Phenol and Flavonoid content, Condensed and Hydrolyzable Tannins

Table 2 presents the varied levels of phytophenols, total flavonoids and both condensed and hydrolyzable tannins from the decoction, aqueous maceration, and hydroethanolic maceration of *S. gabonensis* bark (SG), *S. trifasciata* roots (ST), and *C. splendens* leaves (CS).

The quantification of phenolic compounds in the extracts of the three studied plants revealed that *S. gabonensis* extracts exhibited the highest total polyphenol content, ranging from 148.85±4.35 to 367.15±4.88 mg EAG/g. This was followed by *C. splendens* extracts, which had values ranging from 108.47±0.12 to 211.80±2.00 mg EAG/g. In contrast, *S. trifasciata* extracts displayed the lowest polyphenol contents, ranging from 12.08±1.64 to 32.21±0.38 mg EAG/g. *S. trifasciata* extracts show the lowest polyphenol contents, ranging from 12.08±1.64 to 32.21±0.38 mg EAG/g. The decoction extracts of all plants demonstrated the highest total polyphenol contents, with values of 367.15±4.88; 149.94±0.67 and 32.21±3.87 mg EAG/g for *S. gabonensis* (DSG), *C. splendens* (DCS) and *S. trifasciata* (DST), respectively. The application of heat during the decoction facilitated the release of phenolic substances [47]. Following decoction, the hydroethanolic maceration extracts showed contents of 317.51±3.87, 211.80±4.35 and 15.43±1.26 mg EAG/g, respectively. The aqueous maceration extracts exhibited the lowest total polyphenol contents across all plant species, with values of 148.85±4.35; 108.47±0.73 and 12.08±1.64 mg EAG/g for *S. gabonensis* (MSG), *C. splendens* (MCS) and *S. trifasciata* (MST), respectively. The phenolic compound contents in *S. trifasciata* root extracts (12.09-32.22 mg EAG/g) are comparable to those reported in studies conducted in Bangladesh (31.99 mg EAG/g) [48] and are higher than those found in polyphenols play a crucial role in counteracting cellular damage caused by free radicals, thereby offering potential health benefits for humans [49]. Extracts from *C. splendens* leaves exhibit higher total flavonoid content compared to *S. gabonensis* bark and *S. trifasciata* roots. The highest flavonoid concentration was found in the hydroethanolic macerate, measuring 73.64±0.24 mg EC/g DM, followed by the aqueous macerate and decoction, which yielded 44.31±0.50 and 41.40±0.90 mg EC/g DM, respectively. For *S. gabonensis* bark and *S. trifasciata* roots, the aqueous decoctions demonstrated greater total flavonoid contents (46.17±1.13 and 29.20±2.14 mg EQ/g DM) compared to the aqueous (33.50±1.35 and 17.41 mg EQ/g DM) and hydroethanolic (29.15±0.78 and 15.26 mg EQ/g DM) macerates. The elevated concentration of flavonoids in the leaves may be attributed to sunlight exposure, which enhances the photosynthesis of phenolic compounds, including flavonoids [49]. In a previous study [50], the flavonoid content in *C. splendens* leaves was reported to be 7.00 mg/g, a significantly lower value than those observed in our study (41.40±0.90 and 73.64±3.01 mg EQ/g). Regarding *S. trifasciata*, several studies have also confirmed the presence of flavonoids in the leaves [51-53], although specific quantitative values were not provided. The results indicate that the decoction of *S. gabonensis* bark contains the highest levels of condensed tannins (30.29±1.00 mg EC/g DM), followed by the aqueous macerate of *C. splendens* leaves (23.12 mg EC/g DM) and the hydroethanolic macerate of *S. gabonensis* (21.36 ± 0.68 mg EAG/g DM). Conversely, *S. trifasciata* roots exhibited the lowest condensed tannin contents, ranging from 0.26±0.22 to 5.37±0.45 mg EAG/g DM. The leaves of *C. splendens* displayed the highest amounts of hydrolyzable tannins, with values of 2.01±0.13% (MhCS), 1.68±0.14% (DCS) and 1.5±0.03% (MCS). Hydrolyzable tannins are predominantly found in the leaves, while condensed tannins are more concentrated in the bark and roots. These differences may be explained by the role of tannins as "chemical defenders" against pathogens and herbivores. Given their exposure to environmental aggressors and the risk of microbial proliferation, leaves can contain up to 25% of their dry weight in tannins [54, 55]. Iroabuchi (2008) reported a condensed tannins content of 3.6 mg/g in *C. splendens* leaves [50], which is comparable to the values obtained in our study (Table 2).

3.3 Antioxidant Profiles Relative to DPPH

The antiradical activity of extracts from *S. gabonensis* bark, *C. splendens* leaves and *S. trifasciata* roots was evaluated by spectrophotometry using the DPPH radical, with vitamin C as the reference antioxidant. Figs 5-7

illustrate the percentage reduction (PR) of DPPH in relation to various concentrations. All extracts demonstrated antioxidant potential, irrespective of the extract concentration. The observed antioxidant activity may be attributed to the synergistic effects of the reducing secondary metabolites present in the studied extracts. Notably, secondary metabolites such as flavonoids and tannins are recognized for their capacity to neutralize free radicals [56-58]. The highest percentage reduction values were observed at concentrations of 0.31×10^{-1} and 0.63×10^{-1} mg/mL (Fig. 5) for the three *S. gabonensis* extracts, yielding reductions of 85.31 and 88.25% (DSG), 88.68 and 89.81% (MhSG), and 84.27 and 87.26% (MSG). These findings indicate that *S. gabonensis* represents a promising source of antioxidant compounds.

C. splendens extracts demonstrated a reduction of over 50% in the DPPH radical, depending on the concentration and extraction method (Fig. 6). At a concentration of 0.0625 mg/mL, all extracts exhibited a reduction exceeding 50% of the DPPH radical, with the hydroethanolic maceration exhibiting the highest percentage reduction of 85.47%.

Table 2. Phytophenol contents of aqueous and hydroethanolic decoctions and macerations

Extracts	Total polyphenols (mgEAG/gE)	Total flavonoids (mgEQ/gE)	Condensed tannins (mgEC/gE)	Condensed tannins (%)
DSG	367.15 ± 4.88	46.17 ±1.13	30.29 ± 1.00	1.04±0.00
MSG	148.85 ± 4.35	33.50±1.35	2.27± 0.91	0.23±0.06
MhSG	317.51 ± 3.87	29.15 ±0.78	21.36 ± 0.68	0.75±0.17
DCS	149.94 ±0.67	41.40 ±0.90	15.45±1.14	1.68 ±0.14
MCS	108.47 ±0.73	44.31 ±0.50	23.12 ±0.34	1.5 ±0.03
MhCS	211.80±2.00	73.64 ±3.01	17.75 ±0.15	2.01 ± 0.13
DST	32.21 ±0.38	29.20 ±2.14	5.37 ±0.45	0.84 ±0.06
MST	12.08±1.64	17.41±2.44	0.26± 0.22	0.63 ±0.01
MhST	15.43 ±1.26	15.26 ±0.62	2.22 ± 0.31	0.47 ±0.08

DSG, DST and DCS: aqueous decoctions of S. gabonensis, S. trifasciata et C. splendens ; MSG, MST and MCS: aqueous macerates of S. gabonensis, S. trifasciata and C. splendens ; MhSG, MhST and MhCS : hydroethanolic macerates of S. gabonensis S. trifasciata and C. splendens

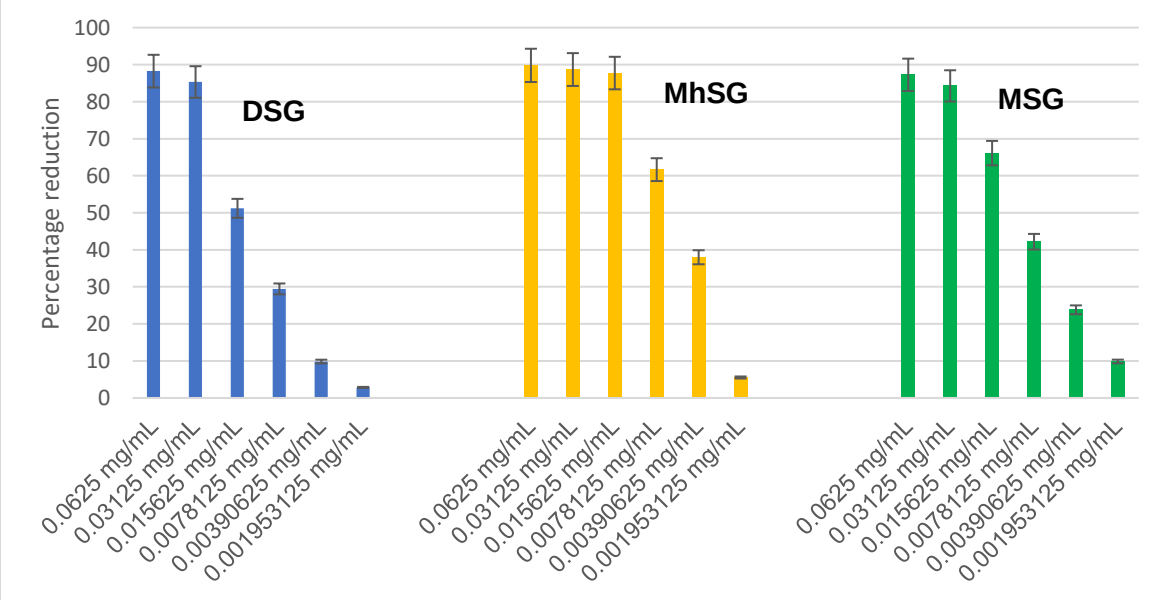

Fig. 5. Percentage reduction of *S. gabonensis* extracts
DSG: aqueous decoction, MhSG: Hydroethanolic macerate, MSG: Aqueous macerate

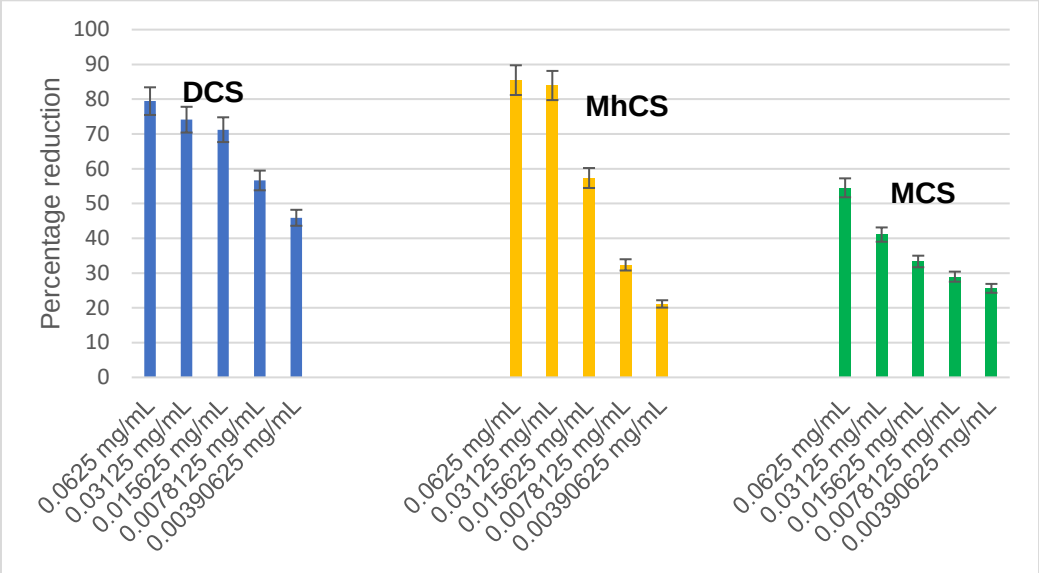

Fig. 6. Percentage reduction of *C. splendens* extracts
DSG: aqueous decoction, MhSG: hydroethanolic macerate, MSG: aqueous macerate

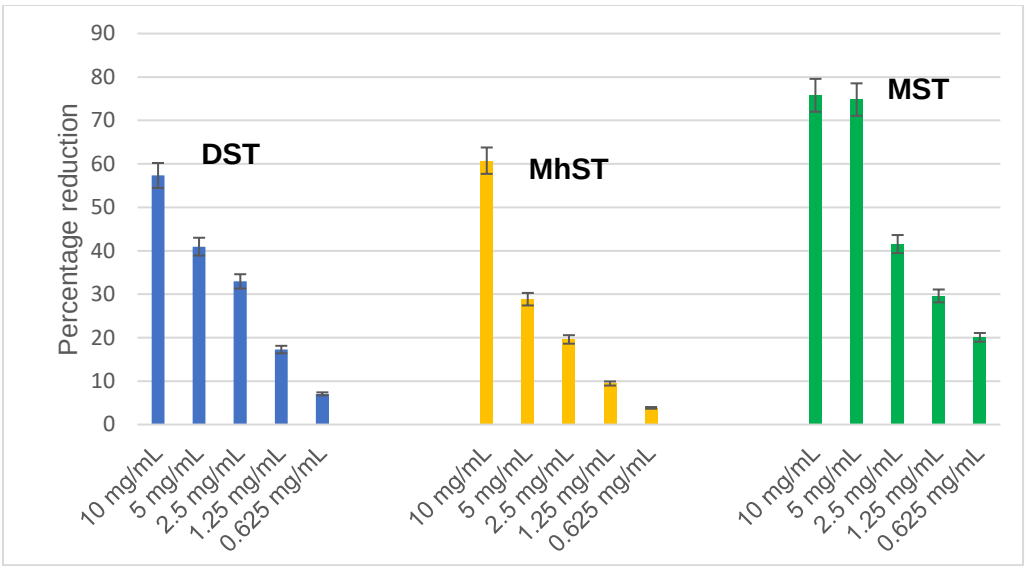

Fig. 7. Percentage reduction of *S. trifasciata* extracts
DST: aqueous decoction, MhST: hydroethanolic macerate, MST: aqueous macerate

This was followed by the decoction (79.43%) and the aqueous maceration (54.50%). At concentrations of 0.312×10^{-1} and 0.156×10^{-1} mg/mL, both the hydroethanolic maceration and decoction also surpassed the 50% reduction threshold for DPPH. However, at a concentration of 0.781×10^{-2} mg/mL, only the decoction remained active. Analysis of the extracts from the roots of *S. trifasciata* at a concentration of 10 mg/mL revealed that all extracts reduced the DPPH radical by more than 50% (Fig. 7). The aqueous macerate exhibited the highest percentage reduction (77.61%), followed by the hydroethanolic macerate (60.74%) and the aqueous decoction (57.34%). At concentrations of 5 mg/mL and 2.5 mg/mL, only the aqueous macerate achieved a reduction exceeding 50%, with similar reduction percentages of 75.78 and 74.80 % at 10 mg/mL.

The percentage of inhibition reflects a plant's ability to reduce free radicals. For a more accurate assessment, it is preferable to determine the effective concentration (CR_{50}),

which is defined as the concentration of extract that results in a 50% reduction in DPPH activity [58]. The lower the CR_{50}, the more effective the extract [59]. The CR_{50} values for the studied extracts were determined graphically using EXCELL software, and the results are presented in Table 3.

Analysis of this table indicates that extracts of *S. gabonensis* exhibit higher antiradical activity compared to those of *S. trifasciata* and *C. splendens*, with lower CR_{50} values (0.085 mg/mL for DSG; 0.0209 mg/mL for MSG and 0.0081 mg/mL for MhSG), which are comparable to that of vitamin C (0.002 mg/mL). This effectiveness is attributed to their richness in phenolic compounds, such as flavonoids and tannins (Table 2). Additionally, the extracts of *C. splendens* leaves demonstrate lower CR_{50} values, particularly the decoction at 0.005 mg/mL, indicating superior antioxidant activity compared to *S. trifasciata* roots. The efficacy of *S. gabonensis* and *C. splendens* in traditional pharmacopoeia can be partly ascribed to the synergistic effects of these well-known antioxidants. The antioxidant activity of *S. trifasciata* roots is lower than that reported by Bangladeshi researchers, who found a CR_{50} of 2.19 mg/g for leaf extracts [48]. Similarly, Filipino researchers reported CR_{50} values of 0.302 mg/mL and 0.094 mg/mL for crude leaf extracts, ethanolic and aqueous, respectively [51]. Regarding *C. splendens* leaves, our CR_{50} values align with those reported by Ghanaian researchers (0.103 mg/mL) [60], but lower than those found by Diabaté and colleagues (0.988 mg/mL) [61].

3.4 Evaluation of Antimycobacterial Activity

Table 4 presents the number of bacterial colonies that survived the action of plant extracts incorporated into the Löwenstein-Jensen medium after 2 months of incubation. The data vary depending on the concentration of the extracts and the bacterial suspension inoculated. The observations are as follow:

-Total inhibition of bacterial growth was achieved for all concentrations of extracts from the decoction of *C. splendens* and *S. trifasciata*.

-Total inhibition of bacterial growth was also observed for the other extracts at higher concentrations of 0.3 mg/mL and 0.5 mg/mL.

-Bacterial survival was significantly lower than that observed in the control tubes.

Table 5 illustrates the bacterial survival rates following exposure to the various plant extracts. The determination of these rates on media containing extracts, compared to control tubes, revealed values below 1, irrespective of the extract or bacterial suspension tested.

The results in Table 5 indicate that survival rates of the CD1539 strain of *M. ulcerans* were all less than 1, regardless of the concentration of plant extract in the inoculated medium. A lower bacterial survival rate, approaching 0, indicates a more active extract. At a concentration of 0.5 mg/mL of *S. gabonensis* extracts, the survival rate is zero, while for *C. splendens* and *S. trifasciata*, the rates are 0.05 and 0.74, respectively. At a concentration of 0.3 mg/mL, the survival rate was zero for *S. gabonensis* extracts, while it ranged 0.16 to 0.85 for *C. splendens* and *S. trifasciata* extracts. These data suggest that all the extracts from the three plants exhibit significant activity against the *M. ulcerans* strain. This antimycobacterial activity is more pronounced in decoction extracts compared to aqueous and hydroethanolic macerations. The extracts contain phenolic compounds, flavonoids, and both hydrolyzable and condensed tannins, which may inhibit the growth of mycobacteria [62,63]. Furthermore, Koné et al. reported minimal inhibitory concentrations (MIC) ranging from 0.78 mg/mL to 1.56 mg/mL for total aqueous extracts of *Sacoglottis gabonensis* against various mycobacterial strains [19]. Additionally, Soma et al. observed survival rates below 1 for five strains of *M. ulcerans* at a concentration of 20 mg/ml of *Sacoglottis gabonensis* [64].

Table 3. CR_{50} of *C. splendens*, *S. trifasciata*, *S. gabonensis* extracts and vitamin C.

Extracts	DSG	MSG	MhSG	MCS	DCS	MhCS	MST	DST	MhST	Vitamin C
CR_{50} (mg/mL)	0.0085	0.0209	0.0062	0.051	0.005	0.015	1.53	7.79	8.23	0.002

DSG, DST and DCS: Aqueous decoctions of S. gabonensis, S. trifasciata and C. splendens ; MSG, MST and MCS: Aqueous macerates of S. gabonensis, S. trifasciata and C. splendens ; MhSG, MhST and MhCS: Hydroethanolic macerates of S. gabonensis S. trifasciata and C. splendens ; V.C : vitamin C

Table 4. Number of mycobacterial colonies by concentration of extracts and by dilution of inoculum

		Sacoglottis gabonensis			*Clerodendrum splendens*			*Sansevieria trifasciata*			
Bacterialinoculum	**Concentration of extracts (mg/mL)**	**DSG**	**MSG**	**MhSG**	**DCS**	**MCS**	**MhCS**	**DST**	**MST**	**MST**	**Witnesses**
Dilution 10^{-1}	0.1	96	26	9	0	5	18	0	131	135	159
	0.3	0	0	0	0	0	14	0	100	130	
	0.5	0	0	0	0	0	9	0	76	119	
Dilution 10^{-3}	0.1	6	1	0	0	0	1	0	10	10	15
	0.3	0	0	0	0	0	0	0	0	9	
	0.5	0	0	0	0	0	0	0	0	0	
Dilution 10^{-5}	0.1	0	0	0	0	0	0	0	0	0	6
	0.3	0	0	0	0	0	0	0	0	0	
	0.5	0	0	0	0	0	0	0	0	0	

DSG, DST and DCS: aqueous decoctions of S. gabonensis, S. trifasciata and C. splendens ; MSG, MST and MCS: aqueous macerates of S. gabonensis, S. trifasciata and C. splendens ; MhSG, MhST and MhCS: hydroethanolic macerates of S. gabonensis S. trifasciata and C. splendens

Table 5. Bacterial survival rate by extract concentration and inoculum dilution

		Sacoglottis gabonensis			*Clerodendrum splendens*			*Sansevieria trifasciata*			
Bacterialsuspensions	**Concentration of extracts (mg/mL)**	**DSG**	**MSG**	**MhSG**	**DCS**	**MCS**	**MhCS**	**DST**	**MST**	**MhST**	**Witnesses**
10^{-1}	0.1	0.6	0.163	0.05	0	0.03	0.113	0	0.823	0.849	1
	0.3	0	0	0	0	0	0.088	0	0.628	0.817	1
	0.5	0	0	0	0	0	0.056	0	0.447	0.748	1
10^{-3}	0.1	0.4	0.06	0	0	0	0.06	0	0.66	0.66	1
	0.3	0	0	0	0	0	0	0	0	0.6	1
	0.5	0	0	0	0	0	0	0	0	0	1
10^{-5}	0.1	0	0	0	0	0	0	0	0	0	1
	0.3	0	0	0	0	0	0	0	0	0	1
	0.5	0	0	0	0	0	0	0	0	0	1

DSG, DST and DCS: aqueous decoctions of S. gabonensis, S. trifasciata and C. splendens ; MSG, MST and MCS: aqueous macerates of S. gabonensis, S. trifasciata and C. splendens ; MhSG, MhST and MhCS: hydroethanolic macerates of S. gabonensis S. trifasciata and C. splendens

4.CONCLUSION

This study investigated the phenolic composition, antioxidant activity against the DPPH radical and antimycobacterial activity of extracts from the leaves of *Clerodendrum splendens*, the roots of *Sansevieria trifasciata* and the bark of *Sacoglottis gabonensis*, three plants raditionally used in Ivory Coast for the treatment of Buruli ulcer. The extracts were prepared using aqueous decoction as well as aqueous and hydroethanolic macerations. Analytical tests identified revealed the presence of flavonoids, terpenes, sterols, coumarins, anthocyanidins, anthraquinones, alkaloids and saponins. Spectrophotometric analysis quantified phenolic compounds, flavonoids, hydrolyzable and condensed tannins, and assessed the antioxidant activity. The bark of *Sacoglottis gabonensis* and the decoction of *Clerodendrum splendens* exhibited significant antioxidant activity, comparable to that of vitamin C. The findings suggest that the decoction is effective in extracting bioactive compounds from the roots, as well as through the maceration of the leaves. Additionally, the extracts demonstrated notable activity against *Mycobacterium ulcerans*, with bacterial survival rates ranging from 0 to 0.11 for *Clerodendrum splendens*, 0 to 0.60 for *Sacoglottis gabonensis* and 0 to 0.85 for *Sansevieria trifasciata*. The decoctions of all three plants displayed superior antimycobacterial activity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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