Trop J Nat Prod Res, February 2022; 6(2):194-201

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phytochemical screening and Evaluation of Antioxidant and Antimicrobial Activity of *Solanum linnaeanum* Extracts

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ARTICLE INFO

Article history:
Received 22 January 2022
Revised 01 March 2022
Accepted 04 February 2022
Published online 06 March 2022

ABSTRACT

Solanum linnaeanum belongs to the Solanaceae family and is used in traditional medicine for the treatment of various ailments, including inflammatory diseases. The present study aimed to evaluate the phytochemical profile and assess the *in vitro* antioxidant and antimicrobial activity of the crude hydroethanol extract and the respective chloroform, ethyl acetate, n-butanol and residual aqueous fractions of the leaves, stem bark, and root bark of *S. linnaeanum*. Qualitative and quantitative phytochemical analyses were performed using standard methods, whereas the antioxidant activity was estimated using DPPH, Phosphomolybdenum, and Ferric Reducing Antioxidant Power (FRAP) assays. Antimicrobial activity was determined by the agar disk diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and *Candida albicans*. Qualitative phytochemical analysis revealed the presence of condensed tannins, coumarins, flavonoids, and phenols in all extracts. The ethyl acetate fraction of the leaves showed the highest contents of total phenols (49.527±0.178 mg GAE g^{-1}), total flavonoids (28.743±0.145 mg QE g^{-1}), and condensed tannins (12.133±0.036 mg CyaE g^{-1}). The ethyl acetate fraction of the leaves demonstrated also the highest DPPH radical scavenging power (IC_{50}=347.533±4.219 µg mL^{-1}), highest reduction of the phosphomolybdenum complex (35.091±0.150%) and the highest ferric reducing antioxidant power (322.070±3.375 µMFe^{2+} g^{-1}). *S. linnaeanum* extracts and fractions did not show significant antimicrobial activity against the studied microbial strains. The results contributed to reveal some phytochemical characteristics of this species, and suggest that *S. linnaeanum* is a promising plant that deserves further studies for its exploration as a new source of compounds with antioxidant activity.

Keywords: *Solanum linnaeanum*, Phytochemical, Phenolic, Antioxidant, Antimicrobial.

Introduction

The use of plants for medicinal purposes for the treatment, cure, and prevention of diseases is among the oldest medicinal practices in humanity's history. The production of drugs and the pharmacological treatment of numerous pathologies began with the use of medicinal plants.[1] Traditional medicine remains the most accessible health care system, particularly in rural areas where coverage of national health systems is sparse, deficient, or inexistent. Currently, 80% of the world's population depends on herbal medicines for health care and fight many diseases.[2] The World Health Organization (WHO) also recommended the evaluation of the effectiveness of the plants against human diseases and the development of modern and safe medicines.[3] In Mozambique, medicinal plants are a valuable tool of traditional medicine, being widely used in rural areas as the main source of medicines for primary health care.[4]

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Citation: Chivodze RS, Sulemane AI, Magaia I, Saete VNC, Rugunate SC, Zimila HE, Munyemana F. Phytochemical screening and Evaluation of Antioxidant and Antimicrobial Activity of *Solanum linnaeanum* Extracts. Trop J Nat Prod Res. 2022; 6(2):194-201. doi.org/10.26538/tjnpr/v6i2.5

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The Solanaceae family is one of the largest groups among vascular plants, with approximately 2400 species and 98 genera. Various species of this family have great economic, agricultural, and pharmaceutical importance.[5]

The genus *Solanum* L. is the largest in the Solanaceae family, with about 1500 species. The group comprises a wide range of species of perennial shrubs or creepers.[5,6] In recent years, several works have been published on ethnomedicinal uses, phytochemistry and pharmacological properties of the Solanum genus. [5-9] A variety of chemical constituents have been isolated from Solanum species depending on their geographical distribution and traditional use. The isolated chemical constituents include steroidal saponins, steroidal alkaloids, pregnane glycosides, terpenes, flavonoids, lignans, sterols, phenolic compounds, coumarins and other compounds. Their pharmacological activities include anticancer, antioxidant, antidepressant, antihypertensive, anti-inflammatory, hypolipidemic, hypoglycemic, hepatoprotective and antidiabetic properties.[6,9,10]

One of the most important characteristics of the genus *Solanum* is the high accumulation of alkaloids and flavonoids, which contribute to a broad range of biological effects, including anesthetic, psychostimulant, antimicrobial and antioxidant.[5-7,9]

S. linnaeanum, also known as "Ntuma grande" in the southern region of Mozambique, is among the most ubiquitous members of *Solanum* L. in Southern Africa (South Africa, Zimbabwe, and Mozambique) where it is widely used by local communities for the treatment of various ailments. For instance, the decoction of roots is used by practitioners of traditional medicine in the treatment of epilepsy. Furthermore, the roots, stems, leaves and fruits are used in the treatment of inflammatory, ophthalmological, and stomatological

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

diseases. In Mauritius, the fruits of *S. linnaeanum* are traditionally used as an anti-diabetic food, as well as to control blood glucose levels.[8]

Previous phytochemical and pharmacological studies of *S. linnaeanum* occurred particularly on the fruit, where they reported the presence of solanine, solamargine, and solasonine glycoalkaloids and indicated that this species can be considered as a promising medicinal plant that deserves to be further explored for the control of diabetes and related complications such as obesity.[8,11,12]

In Mozambique, most of the medicinal plants are used in traditional medicine without scientific evidence of their bioactivity or biosafety. The scarcity of scientific information in the literature about the phytochemical analysis and bioactivity of different parts of *S. linnaeanum*, inspired the realization of the present study to establish the phytochemical profile and evaluate the antioxidant and antimicrobial activities of the extracts from leaves, stem bark, and root bark of *S. linnaeanum*.

Materials and Methods

Plant material collection and identification

Roots, stems, and leaves of *S. linnaeanum* were collected in February 2020 from Inhaca Island-Kanyaka district, Maputo, Mozambique. The species was identified and authenticated by the botanists from the herbarium of the Inhaca Maritime Biology Station (EBMI), a scientific unit of the Faculty of Sciences of Eduardo Mondlane University, by comparison with an existing specimen with voucher number 1698. Subsequently, the leaves, stem bark, and root bark of *S. linnaeanum* were dried at room temperature for 30 days in the Laboratory of Natural Products of the Department of Chemistry, Eduardo Mondlane University, Mozambique. The dried samples were ground into a fine powder using an electric grinder and stored at room temperature in closed glass containers covered with aluminum foil until extraction.

Preparation of plant extracts and fractions

The crude extracts and fractions of the different *S. linnaeanum* parts (root bark, stem bark and leaf) were prepared following the method described by Zadra,[5] with minor modifications. The crude extract was prepared by maceration under constant agitation at 190 rpm for 24 h using 70% ethanol. The resulting extract was filtered with Whatman No. 1 filter paper and the residue was re-extracted twice following the same procedure. The extracts were combined and concentrated at 40°C under reduced pressure in a rotary evaporator.

Part of the concentrated extract was suspended in distilled water and subjected to a solvent-solvent partition in increasing order of polarity as follows: 3 x 100 mL of chloroform (CF), 3 x 100 mL of ethyl acetate (EA), and 3 x 100 mL of n-butanol (nBu). All fractions including the aqueous residual fraction (Aq) were evaporated to dryness in a rotary evaporator under reduced pressure at 40 °C and stored in a refrigerator at 4°C for phytochemical and biological tests.

Qualitative phytochemical analysis

The preliminary phytochemical analysis for the identification of alkaloids, phenols, flavonoids, tannins, coumarins, anthraquinones, cardiac glucosides, saponins, and steroids was performed according to the methods described by Geetha & Geetha,[13] Tiwari *et al*.[14] and Bargah.[15]

Qualitative analysis with thin layer chromatography (TLC) was carried out through the methods described by Iikasha *et al*.[16] The solvents, spray reagents, and positive controls used in TLC are summarized in Table 1.

Quantitative Phytochemical Analysis

Determination of total phenolic content

The determination of the total phenolic content present in the extracts was carried out using the classic Folin-Ciocalteu colorimetric method as described by Amorim *et al*.[17] Five hundred microliters of 1 mg mL^{-1} methanol solution of each extract was placed separately into the test tubes. Subsequently, 500 µL of 10% Folin-Ciocalteu reagent (v/v) was added, stirred vigorously, and allowed to stand for 10 min at room temperature. Then, 1 mL of 7.5% (w/v) sodium carbonate solution and 8 mL of distilled water was added and the mixture was allowed to stand at room temperature for 2h. The absorbance of the mixture was then measured at 760 nm using a UV/Vis spectrophotometer (Spectroquant® Pharo 300 M). A standard calibration curve of gallic acid at the concentration range of 1-10 µg mL^{-1} was plotted and the results were expressed in milligrams of gallic acid equivalent per gram of dry extract (mg GAE g^{-1}). All assays were done in triplicate and the total phenols content was expressed as mean ± standard deviation.

Determination of total flavonoids content

The total flavonoids were determined using the method of selective complexation of aluminum described by Woisky & Salatino,[18] with minor modifications. Five milliliters of the extract solution (1 mg mL^{-1} in methanol) was placed in a 25 mL volumetric flask and then 0.5 mL of 2% (m/v) aluminum chloride in methanol was added. The absorbance was measured at 425 nm, after 30 min of reaction, using a UV/Vis spectrophotometer (Spectroquant® Pharo 300 M). The content of flavonoids was calculated using the calibration curve of quercetin at the concentration range of 1-10 µg mL^{-1} and the results were expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE g^{-1}). All assays were done in triplicate and the total flavonoids content was expressed as mean ± standard deviation.

Determination of condensed tannins: Cyanidin equivalent

The content of condensed tannins was obtained through the method described by Zemmouri *et al*.[19] Two milliliters of the extract solution (1 mg mL^{-1} in methanol) were mixed in a test tube with 5 mL of acid-butanol solution and traces of ferrous sulfate (77 mg of $FeSO_4.7H_2O$ in 500 mL of butanol - HCl (5:2)). The tubes were covered and placed in a water bath at 95ºC for 50 min. The absorbance was measured at 530 nm using a UV/Vis spectrophotometer (Spectroquant® Pharo 300 M) and the result was expressed in milligrams of cyanidin-3-glucoside equivalent per gram of dry extract (mg CyaE g^{-1}). All assays were performed in triplicate and the condensed tannins content was computed through equation 1.

$$\text{mg CyaE } g^{-1} = [A * V * M * V2]/[l * \text{Ɛ} * v * m] \quad (1)$$

Where: A – is the absorbance of the sample at 530 nm; V - the total volume of the reaction (7 mL); M - the molar mass (484.83 g mol^{-1} of cyanidin-3-glucoside); V2 - the total volume of the extract solution (25 mL); *l* – the width of the optical path of the cuvette (1 cm); Ɛ - the molar extinction coefficient (26.900 $L.mol^{-1}.cm^{-1}$); v - the volume of the extract solution used in the test (2 mL) and m - the mass of the dry weight of the extract (0.025 g).

In vitro antioxidant activity

DPPH radical scavenging assay

DPPH scavenging activity was determined according to the method described by Gawron-Gzella *et al*.[20] A fresh methanol solution of DPPH (1.4 mL) was mixed with 0.2 mL of methanol solution of extract at different concentrations (12.5 - 6000 µg mL^{-1}). Ascorbic acid (5 – 100 µg mL^{-1}) was used as a standard.

The reaction mixture was shaken and left to settle in darkness at room temperature for 30 min. The absorbance was measured at 517 nm using a UV/Vis spectrophotometer (Spectroquant® Pharo 300 M) and the percentages of inhibition (equation 2) were used to determine the IC_{50} value of the samples. All the assays were performed in triplicate and the IC_{50} values of samples were presented as mean ± standard deviation.

$$\text{Inhibition \%} = [(A_{control} - A_{sample}) / (A_{Control})] \times 100 \quad (2)$$

Where: $A_{control}$ - absorbance of DPPH solution without extract; A_{sample} - absorbance of the sample with DPPH.

Phosphomolybdenum reduction assay

The antioxidant activity of the extracts by the phosphomolybdenum method was evaluated using the method described by Prieto et al.[21] Three hundred microliters of 200 µg mL^{-1} methanol solution of the extract were combined with 3 mL of the phosphomolydenum reagent

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate), incubated at 95 °C for 90 min and then cooled to room temperature. The absorbance of the solution was measured at 695 nm using a spectrophotometer (Spectroquant® Pharo 300 M) against a blank consisting of all the reaction mixture and methanol instead of the extract. The percentage of the relative antioxidant activity was computed using equation 3.

$$RAA\% = [(A_{sample} - A_{blank}) / (A_{standard} - A_{blank})] \times 100\% \quad (3)$$

Where: RAA% (relative antioxidant activity) - reducing power of the phosphomolybdenum complex in percentage of ascorbic acid.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing power was estimated through the method described by Firuzi *et al*.[22] Initially, the FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 20 mM ferric chloride hexahydrate, and 2.5 mL of 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine). Next, in a test tube a portion of 2.7 mL of FRAP reagent was diluted with 270 µL of distilled water and added 90 µL of extract (1 mg/mL). After 30 min of incubation at 37°C, the absorbance data were measured at 595 nm using a spectrophotometer (Spectroquant® Pharo 300 M). The antioxidant potential of the extracts was calculated based on a calibration curve of ferrous sulfate ($FeSO_4.7H_2O$) at concentrations ranging from 500 to 2000 µM. All assays were performed in triplicate and results were expressed as micromoles of ferrous sulfate per gram of dry extract ($\mu molFe^{2+}\ g^{-1}$).

Determination of antimicrobial activity

The antimicrobial activity of crude hydroethanol extracts and ethyl acetate fractions was evaluated by the disk diffusion method as described by Klančnik *et al*.[23] and Farjana *et al*.[24] with minor modifications. Four bacterial strains which included Gram-negative *E. coli* ATCC 25922, Gram-positive *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619 and the yeast *C. albicans* were used. The microbial strains were obtained from the Microbiology Laboratory of the Central Hospital of Maputo, and subcultured in the nutritive broth and Sabouraud Dextrose Agar (SDB), and incubated in a bacteriological oven at 37ºC for 24 h and 48 h, for bacteria and fungi, respectively. Afterwards, the cultures were standardized to a turbidity of 0.5 on the Mcfarland scale in 0.9% saline solution, by visual comparison with a standard suspension of barium sulfate equivalent to a suspension of *E. coli* containing 1.5×10^8 *c*ells/*mL*. For testing, 500 µL of the suspension of each microorganism was evenly spread on a Petri dish (90 mm) containing Muller Hinton agar using a sterile swab. Sterile 6 mm diameter paper discs were placed (using sterile forceps) and impregnated with 10 µL of each sample at concentrations between 15.625 and 500 mg mL^{-1}. Dimethylsulfoxide (DMSO) was used as solvent and negative control. Tetracycline 30 µg/disc and Nystatine 100 µg/disc were used as positive controls in the bacterial and fungal sensitivity tests, respectively. The plates were incubated in a bacteriological oven at 37ºC for 24 h. The extract was considered active if the inhibition zone diameter was equal to or greater than 8 mm.

Statistical analysis

All data were submitted to statistical treatment using software Minitab® 17 and the results were expressed as mean ± standard deviation of three repetitions. The significance of the differences between the means was assessed at a 95% confidence level by the Student t-test and the One-way Analysis of Variance (ANOVA) followed by the Tukey multiple comparison *post-hoc* test.

Table 1: Eluents, spray reagents, and positive controls used in TLC analysis of root bark, stem bark, and the leaf of *S. linnaeanum*

Phytochemicals	Solvent	Positive Control	Spray reagents
Alkaloids	MetOH-NH_4OH (200:3)	Quinine	Dragendorff's reagent
Flavonoids	n-ButOH-CH_3COOH-H_2O (4:1:5)	Quercetin	5% $FeCl_3$ in MetOH
Steroids	Toluene-EtOAc (85:15)	-	10% H_2SO_4 in MetOH
Triterpenoids	n-Hexane-EtOAc (17:3)	-	Lieberman reagent
Saponins	$CHCl_3$-MetOH-H_2O (7:3:1)	-	10% H_2SO_4 in EtOH and 1% vanillin in EtOH
Tannins	1% KOH in MetOH	Tannic acid	1% $FeCl_3$ in 50% MetOH

MetOH – methanol; nButOH – n-butanol; EtOAc – ethylacetate; EtOH – ethanol

Results and Discussion

Phytochemical analysis

The results of qualitative phytochemical screening of the crude hydroethanol extracts and respective fractions from the root bark, stem bark, and leaves of *S. linnaeanum* are summarized in Table 2.

In the present study, the ethyl acetate fraction showed a greater number of secondary metabolites with biological activity, while the aqueous residual fraction showed a smaller number of secondary metabolites.

As shown in Table 2, phenols, flavonoids, condensed tannins and coumarins were present in all extracts and fractions screened. Anthraquinones and saponins were not detected in any fraction whereas alkaloids were only detected in the chloroform and ethyl acetate fractions of leaves.

These results diverge a little from those previously obtained in the fruits of *S. linnaeanum* and other *Solanum* species [7,8,6] since in the present study was noted the absence of saponins and even alkaloids were not identified in most of the fractions of the studied parts of the plant. Steroidal saponins are the typical metabolites in *Solanum* species, from which 134 compounds have been obtained.[6] On the other hand, the genus *Solanum* is known for the prevalence of alkaloids (particularly glycoalkaloids), steroids and phenolic compounds (primarily flavones and flavonols, and their heterosides), which play a crucial role in the plant protection against phytopathogens and are responsible for the pharmacological activities.[12,25] The observed discrepancy should be explained by the influence of various factors such as genetic difference, geographical origin, sample collection period, plant age, among others.[26,27]

The results of quantitative phytochemical analysis of root bark, stem bark, and leaves of *S. linnaeanum* for total phenols, total flavonoids and condensed tannins are summarized in Figures 1a, 1b, and 1c.

The flavonoids content ranged from 0.973 ± 0.042 to 28.743 ± 0.145 mg QE g^{-1} of the dry extract. In general, the extracts and fractions obtained from the root bark exhibited a lower amount of flavonoids compared to the other parts (stem bark and leaf). The flavonoids content decreased in the following order: Leaves > Stem bark > Root bark. These metabolites are primarily responsible for plant protection against ultraviolet light. Thus, leaves showed the highest content of metabolites presumably because are more prone to UV radiation compared to the roots and stem.[28]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

As shown in Figure 1a and Figure 1b the quantitative phytochemical analysis showed that the ethyl acetate fraction had the highest content of total phenols and total flavonoids, while the aqueous residual fraction had the lowest content of total phenols and flavonoids. The phenolic content in the extracts and fractions from all parts of *S. linnaeanum* decreased in the following order: EA > CE > CF > nBu > Aq.

In the methanol extract of *S. linnaeanum* fruits, Mahomoodally & Ramcharum[8] reported a total phenol content of 152.80 ±1.40 mg GAE g^{-1} of dry extract. The total phenolic content quantified in the present study ranged from 1.581 ± 0.116 to 49.527 ± 0.178 mg GAE g^{-1} of dry extract referring to leaves, stem bark, and root bark.

As shown in Figure 1c, the content of condensed tannins is higher in the ethyl acetate fraction of leaves compared to other extracts and fractions of different parts of the plant. Oliveira[29] reported that the concentration of tannins is typically higher in the parts of vegetables exposed to the sun. In contrast, Haslam[30] reported that substantial accumulation of tannins is often associated with a particular pathological condition, such as insect attack on certain parts of the plant. That can explain the heterogeneous variation observed in condensed tannins content in the three parts of the plant analyzed in the present study: the highest content of condensed tannins was detected in ethyl acetate fraction of the leaves, in chloroform fraction of root bark, and n-butanol fraction of stem bark.

Table 2: Results of qualitative phytochemical screening of root bark, stem bark, and leaves of *S. linnaeanum*

Extract	Alk.	Cond. Tan.	Phen.	Flav.	Ster.	Trit.	Anthr.	Coum.	Card. Gluc.	Sap.
Root bark										
CE	-	+	+	+	+	-	-	+	+	-
CF	-	+	+	+	+	+	-	+	+	-
EA	-	+	+	+	+	+	-	+	+	-
nBu	-	+	+	+	-	-	-	+	+	-
Aq	-	+	+	+	-	-	-	+	-	-
Stem bark										
CE	-	+	+	+	+	+	-	+	-	-
CF	-	+	+	+	+	+	-	+	-	-
EA	-	+	+	+	+	+	-	+	+	-
nBu	-	+	+	+	-	-	-	+	+	-
Aq	-	+	+	+	-	-	-	+	-	-
Leaves										
CE	-	+	+	+	+	+	-	+	-	-
CF	+	+	+	+	-	+	-	+	+	-
EA	+	+	+	+	-	-	-	+	+	-
nBu	-	+	+	+	-	-	-	+	+	-
Aq	-	+	+	+	-	-	-	+	-	-

CE – Crude hydroethanol extract; CF – Chloroform fraction; EA – Ethyl acetate fraction; nBu – n - Butanol fraction; Aq. – Residual aqueous fraction; (+) - present; (-) – absent; Alk. – alkaloids; Cond. Tan. – Condensed tannins; Phen. – Phenols; Flav. – Flavonoids; Ster. – Steroids; Trit. – Triterpenoids; Anthr. – Anthraquinones; Coum. – Coumarins; Card. Gluc. – Cardiac glucosides; Sap. – Saponins.

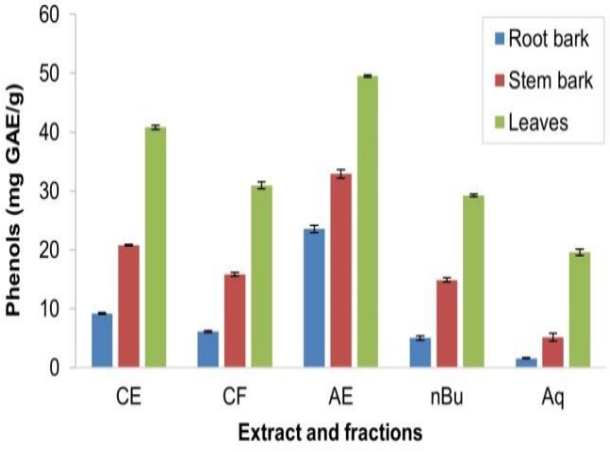

Figure 1a: Content of total phenols expressed in mean ± SD (n = 3) of the extracts of *S. linnaeanum*. CE - Crude hydroethanol extract; CF – Chloroform fraction; EA – Ethyl acetate fraction; nBu – Butanol fraction and Aq – Residual aqueous fraction.

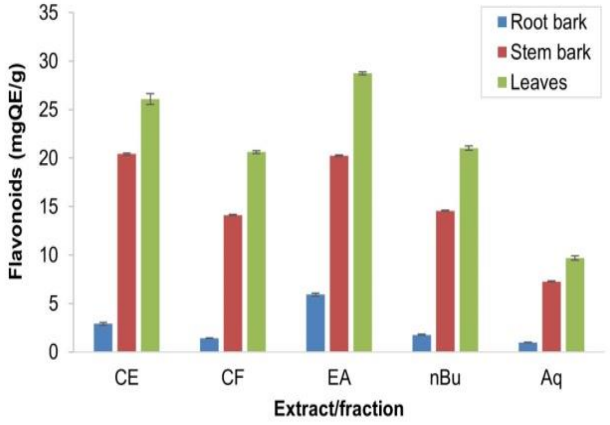

Figure 1b: Content of total flavonoids expressed in mean ± SD (n = 3) of the extracts of *S. linnaeanum*. CE - Crude hydroethanol extract; CF – Chloroform fraction; EA – Ethyl acetate fraction; nBu – Butanol fraction and Aq – Residual aqueous fraction.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

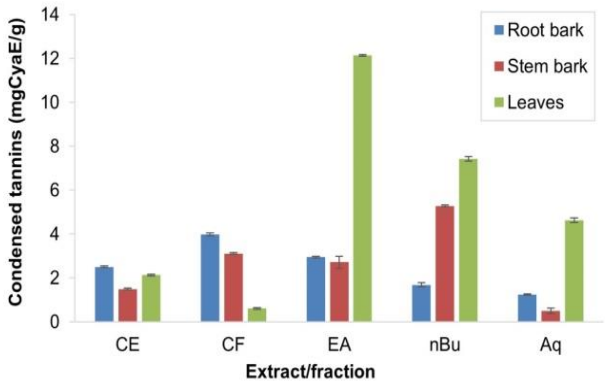

Figure 1c: Content of condensed tannins expressed in mean ± SD (n = 3) of the extracts of *S. linnaeanum*. CE - Crude hydroethanol extract; CF – Chloroform fraction; EA – Ethyl acetate fraction; nBu – Butanol fraction and Aq – Residual aqueous fraction.

Antioxidant activity of S. linnaeanum extracts and fractions

The antioxidant activity can be measured through several methods and there is not a universal method to measure precisely and quantitatively antioxidant activity, due to the different types of free radicals and their different mechanisms of action in living organisms.[31] In the present study, three methods (DPPH, Phosphomolybdenum, and FRAP) were employed to evaluate the antioxidant capacity of extracts and fractions of leaves, root bark, and stem bark of *S. linnaeanum*. The results of the *in vitro* antioxidant activity using the three methods are presented in Table 3, while Table 4 shows the correlation coefficients between antioxidant activity and total phenols, total flavonoids for *S. linnaeanum* root bark, stem bark, and leaves.

The ethyl acetate fractions of leaves, stem bark, and root bark exhibited the lowest IC_{50} values, demonstrating a relatively high capacity to inhibit the DPPH radical. The antioxidant activity by the DPPH method for the three parts of the plant studied decreased in the following order: EA > CE > CF > nBu > Aq. This trend is similar to that of the content of phenols and flavonoids analyzed in this study, suggesting that these metabolites are the underlying reason behind the observed antioxidant activity.

Table 3: Antioxidant activity by DPPH, Phosphomolybdenum and FRAP methods

	Leaves	Stem bark	Root bark
		DPPH	
Extract	**IC_{50} (µg mL^{-1})**	**IC_{50} (µg mL^{-1})**	**IC_{50} (µg mL^{-1})**
CE	492.362 ±2.224[a]	960.630 ±8.772[a]	2005.875 ±6.660[a]
CF	519.151 ±4.226[b]	1030.467 ±7.831[b]	2082.899 ±3.910[b]
EA	347.533 ±4.219[c]	513.788 ±6.713[c]	605.281 ±9.139[c]
nBu	708.311 ±1.799[d]	1073.294 ±8.602[d]	2216.555 ±5.481[d]
Aq	941.652 ±3.168[e]	2083.757 ±2.652[e]	4505.910 ±1.806[e]
AA		38.510 ±0.171	
		Phosphomolybdenum	
Extract	**(%)**	**(%)**	**(%)**
CE	14.780 ±0.260[a]	12.878 ±1.786[a]	10.170 ±0.735[a]
CF	29.617 ±2.271[b]	19.994 ±0.607[b]	16.998 ±0.613[b]
EA	35.091 ±0.150[c]	22.962 ±0.492[c]	20.916 ±0.396[c]
nBu	10.948 ±0.576[d]	8.931 ±0.780[d]	6.799 ±0.607[d]
Aq	7.087 ±0.567[e]	5.676 ±0.908[e]	4.638 ±0.563[e]
		FRAP	
Extract	**(µMFe^{2+} g^{-1})**	**(µMFe^{2+} g^{-1})**	**(µMFe^{2+} g^{-1})**
CE	151.320 ±2.563[a]	77.520 ±1.580[a]	20.370 ±2.240[a]
CF	161.971 ±0.779[b]	101.920 ±3.435[b]	36.920 ±1.751[b]
EA	322.070 ±3.375[c]	183.520 ±3.467[c]	62.370 ±1.447[c]
nBu	86.120 ±3.274[d]	46.170 ±3.786[d]	7.870 ±0.853[d]
Aq	67.370 ±3.005[e]	34.120 ±1.790[e]	4.020 ±1.333[e]

CE – Crude hydroethanol extract; CF – Chloroform fraction; EA – Ethyl acetate fraction; nBu – n-butanol fraction; Aq – Residual aqueous fraction; and AA – Ascorbic acid.
The means followed by different letters in each column are significantly different ($p < 0.05$).

The fact that the ethyl acetate fraction presented better results against the DPPH free radical is due to the high accumulation of polyphenols in this fraction. According to Andrade et al.[32] polyphenols have high solubility in ethyl acetate, which makes this fraction exhibit greater antioxidant activity.

We observed moderate to strong and positive correlations between the content of total phenols, total flavonoids and the DPPH scavenging activity in the three parts of the plant studied (Table 4), which is in line with the previous reports.[33,34,35]

These results are also in agreement with those presented by Zadra[5] in his study on *Solanum guaraniticum* A.ST.- HIL, where the ethyl acetate fraction exhibited the best antioxidant capacity through the DPPH method. The IC_{50} values, in this case, followed the same pattern as the total polyphenol contents, ie, Ethyl acetate > Crude extract > Butanol extract > Chloroform, suggesting that these metabolites were most responsible for the pronounced antioxidant capacity of the ethyl acetate fraction.

Ethyl acetate fraction from all parts of *S. linnaeanum* analyzed in the present study exhibited greater antioxidant activity (IC_{50} = 347.533 ±4.219; 513.788 ± 6.713 and 605.281 ±9.139 µg mL^{-1}) for leaves, stem bark, and root bark, respectively, than the antioxidant capacity demonstrated by the methanol extract of *S. linnaeanum* fruits analyzed

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

by the DPPH method by Mahomoodally & Ramcharum,[8] with IC_{50} = 2300 µg mL^{-1}.

The reducing power of the phosphomolybdenum complex is another important *in vitro* antioxidant assay used to assess the antioxidant capacity of plant extracts. This method has the advantage of evaluating the antioxidant capacity of both lipophilic and hydrophilic components [21] and allows the assessment of the total capacity of a complex mixture of compounds (extracts and fractions obtained from the plant). The total antioxidant activity decreased in the following order: EA > CF > CE > nBu > Aq. Ethyl acetate fraction of the leaves, stem bark and root bark showed the greatest antioxidant potential with activities of 35.091 ±0.150%, 22.962 ±0.492% and 20.916 ±0.396%, respectively, while the residual aqueous fraction showed the lowest antioxidant potential with activities of 7.087 ±0.567%, 5.676 ±0.908% and 4.638 ±0.563%, respectively.

The correlations between total phenols/flavonoids with antioxidant activity by phosphomolybdenum method are shown in Table 4. The Pearson correlation coefficient (r) was greater for total phenolic content than for total flavonoid content. The antioxidant activity in the phosphomolybdenum assay may be related to a complex mixture of chemical components, with a synergistic effect.[36]

The Ferric Reducing Antioxidant Power (FRAP) of different extracts was estimated from their ability to reduce TPTZ-Fe (III) to TPTZ-Fe (II). The ability to reduce Fe^{3+} is a good indicator of the potential antioxidant activity of a given extract, however, this mechanism is not exclusively for the antioxidant activity.[37] It measures the ability of antioxidant compounds (reducing agents) to act through an electron transfer mechanism, which may not necessarily reflect the antioxidant activity of the sample.[38] Thus, not all reducing agents that can reduce Fe^{3+} are antioxidants and not all antioxidant compounds are capable of reducing Fe^{3+} (as is the case with glutathione-containing thiol groups).[39]

As shown in Table 3, the greatest reducing power of Fe^{3+} in the three parts of *S. linnaeanum* (leaves, stem bark and root bark), was observed in the fraction of ethyl acetate (322.070 ± 3.375; 183.520 ± 3.467 and 62.370 ± 1,447 µMFe^{2+} g^{-1}, respectively). The antioxidant activity by the FRAP method in extracts and fractions of *S. linnaeanum* decreased as EA > CF > CE > nBu > Aq. These results are similar to those found with the phosphomolybdenum method.

The correlation of total phenolic content and flavonoid content with the antioxidant activity by the FRAP method is shown in Table 4. The Pearson correlation coefficient (r) was strong for both phytoconstituents.

Antimicrobial activity of S. linnaeanum hydroethanol extract and ethyl acetate fraction

The results for the antimicrobial activity of crude hydroethanol extracts and ethyl acetate fractions from the *S. linnaeanum* root bark, stem bark, and leaves are given in Table 5. None of the extracts and fractions exhibited antimicrobial activity at concentrations below 31.25 mg mL^{-1}.

The ethyl acetate fraction from stem bark showed the lowest Minimum Inhibitory Concentration (MIC) value (31.25 mg mL^{-1} for bacterium *S. pneumoniae* and 62.5 mg mL^{-1} for fungus *C. albicans*). Ethyl acetate fractions showed better inhibitory effects than crude hydroethanol extracts.

Holetz *et al*.[40] categorized the antimicrobial activity of plant extracts as follows: MIC less than 100 µg mL^{-1} – good; 100 to 500 µg mL^{-1} – moderate; 500 to 1000 µg mL^{-1} – weak; above 1000 µg mL^{-1} – inactive. Therefore, in the present study, the crude hydroethanol extracts and ethyl acetate fractions of *S. linnaeanum* are considered inactive against the selected microorganisms.

The absence of metabolites with strong antibacterial activity such as saponins, anthraquinones, alkaloids (except in ethyl acetate and chloroform fractions of leaves) and low content of tannins in *S. linnaeanum* extracts may have contributed significantly to the low antimicrobial activity. [41-44]

These results are similar to those reported by Iikasha et al.[16] whereby the hydroethanol extracts of *S. linnaeanum* fruits showed no antibacterial activity (MIC >1000 µg mL^{-1}) against *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *S. boydii* ATCC 9207.

The hydroethanol extract of *Solanum guaraniticum* A. Hil showed a weak activity against *Pseudomonas aeroginosa*, and the others fractions (chloroform, ethyl acetate and n-butanol) were inactive against *Klebsiella pneumonia* and *E. coli*. All extracts of the *S. guaraniticum* were inactive against fungi.[45]

Unlike the antioxidant activity, which showed a high correlation with the contents of phenolic compounds and flavonoids in the present study, there was no evidence of a positive correlation between the antimicrobial activity and the contents of phenolic compounds and flavonoids.

It is not possible to correlate the antimicrobial activity of extracts with the polyphenol content, although these present antimicrobial properties and exhibit inhibitory effects against several microorganisms, due to the presence of interfering substances in the extracts.[46]

In research done through oral sources to practitioners of traditional medicine in local communities, as well as in the scientific literature, no reports were found on the use of the three parts of the plant as antimicrobial drugs. The results of this study justify the non-use of this plant in traditional medicine to combat diseases caused by microorganisms.

Table 4: Correlation coefficient values between antioxidant activity and total phenols and total flavonoids content of *S. linnaeanum*

Part of the plant	Assay	Correlation coefficient			
		Phenolics		Flavonoids	
		r	R^2	r	R^2
Root bark	DPPH	0.838	0.702	0.803	0.645
	Phosphomolydenum	0.811	0.657	0.719	0.517
	FRAP	0.897	0.804	0.823	0.678
Stem bark	DPPH	0.922	0.850	0.914	0.836
	Phosphomolydenum	0.815	0.665	0.799	0.639
	FRAP	0.912	0.831	0.836	0.699
Leaves	DPPH	0.951	0.904	0.945	0.893
	Phosphomolydenum	0.717	0.514	0.653	0.427
	FRAP	0.880	0.775	0.769	0.591

r – Pearson's correlation coefficient; R^2 – determination coefficient.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 5: Minimum Inhibitory Concentration MIC of crude hydroethanol extracts and ethyl acetate fractions of leaves, stem bark, and root bark of *S. linnaeanum*

		MIC in mg mL^{-1} (Inhibition zone in mm ±SD)				
Plant extract		***E. coli***	***S. pneumoniae***	***S. aureus***	***E. faecalis***	***C. albicans***
CE	Root bark	>500	500 (10.5 ± 0.5)	>500	>500	>500
	Steam bark	>500	500 (9.0 ± 0.0)	125 (10.7 ± 0.3)	>500	>500
	Leaves	>500	500 (9.7 ± 0.6)	>500	>500	>500
EA	Root bark	>500	125 (13.5 ± 1.0)	250 (8.7 ± 0.6)	>500	250 (9.7 ± 1.5)
	Steam bark	>500	31.25(15.5 ± 0.9)	250 (8.3 ± 0.6)	>500	62,5 (8.0 ± 0.5)
	Leaves	125 (8.5 ±0.5)	62.5 (15.8 ± 1.0)	125 (8.0 ± 0.0)	125 (8.5±0.9)	125 (8.2 ± 0.3)
Tetracycline 30 µg/disc		(24.0 ± 1.0)	(31.0 ± 0.0)	(20.0 ± 0.0)	(15.3 ± 0.6)	-
Nystatin 100 µg/disc		-	-	-	-	(16.7 ± 0.6)

CE – Crude hydroethanol extract; EA – Ethyl acetate fraction

Conclusion

Extracts from the leaves, stem bark and root bark of *S. linnaeanum* revealed the presence of several secondary metabolites of pharmacological interest. The ethyl acetate fraction showed the best antioxidant activity, which can be attributed to the higher content of polyphenolic compounds detected in this fraction. These results suggest that *S. linnaeanum* leaves, stem bark, and root bark can provide antioxidant properties, offering protection against free radicals, as well as being an important promising source of natural antioxidants. However, *S. linnaeanum* extract was not efficient in inhibiting the growth of the studied microbial strains.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors deeply acknowledge the financial support of the National Research Fund (FNI), through the project to assess the bioactivity and toxicity of medicinal plants used in Matutuine district, Maputo province, Mozambique (UEM – FC ASDI – MEDICINAL PLANTS – Grant Number 21A). We would like to express our sincere gratitude to the Microbiology Laboratory of the Maputo Central Hospital for the availability of the microbial strains used in the assessment of antimicrobial activity, as well as to thank the Ministry of Science, Technology and Higher Education for granting the Master's Scholarship to student Rogério Sebastião Chivodze. We also thank Mr. Francisco Mapanga and Sérgio Mapanga for authenticating the species used in this study.

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