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Research Article

Antioxidant Potential and Phytochemical Screening of Extracts from *Senegalia nigrescens*-A Traditional Swazi Medicinal Plant

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Abstract: *Senegalia nigrescens* has been used in traditional Swazi medicine. *S. nigrescens* is used in the treatment of wounds, toothache, diabetes, dysentery, snake bites, convulsions and skin diseases. In this study, we aimed to evaluate the antioxidant potential, determine the half-minimal inhibition concentration (IC_{50}) values and analyze phytochemical constituents of various solvent extracts obtained from the leaves and stem-bark of *S. nigrescens*. The maceration technique together with the hot solvent extraction approach was used for the obtainment of various solvent extracts. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging potential, IC_{50} values and phytochemical analysis were performed as per established procedures. The radical scavenging potential of extracts from leaves and stem-bark of *S. nigrescens* and positive control (ascorbic acid) were found to be in the ranges of 7.55 ± 0.004 ~ 62.19 ± 0.004%, 9.04 ± 0.003 ~ 63.24 ± 0.006% and 50.98 ± 0.002 ~ 71.0 ± 0.007%, respectively at a concentration range of 200 ~ 3,000 µg/mL. The methanol extracts from the leaves, stem-bark and positive control exhibited IC_{50} values of 921.69, 735.74 and < 200 µg/mL, respectively. Several classes of phytochemicals were identified in these extracts which include alkaloids, steroids, terpenoids, flavonoids, phenolics and tannins. We concluded that *S. nigrescens* showed a weak to moderate DPPH radical scavenging potential and possessed various classes of phytochemicals.

***Keywords*:** *Senegalia nigrescens*, fabaceae, antioxidant activity, IC_{50} values, DPPH assay, phytochemical screening

1. Introduction

Senegalia nigrescens belongs to the Fabaceae family.[1] *S. nigrescens* is native to sub-Saharan Africa and commonly known by vernacular names *viz*. knob-thorn and umkhaya. *S. nigrescens* is a spiky deciduous tree and it has a conical crown that gradually becomes rounded as it grows. The tree grows up to 10-15 meters tall with a spreading canopy and dark grey bark that becomes fissured on maturation.[1] During spring and early summer, the tiny flowers from this tree bloom as spherical clusters at the ends of branches. The leaves of this tree have 4-8 pairs of tiny, oval-shaped leaflets that are pinnately compound.[2] The woods obtained from this tree are long-lasting and therefore, they have been used in making furniture, construction materials and other wood-based products.[3] The bark and pods of *S. nigrescens* have high contents of tannins and therefore, they have been used to tan leather in leather industries.[4] The gum obtained from this tree is used as thickener and stabilizer in food industries. The honey obtained from the nectar of *S. nigrescens* has a distinctive flavor and therapeutic qualities.[5]

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In Eswatini, *S. nigrescens* has traditionally been used to heal wounds and recuperate from dysentery. Studies revealed that *S. nigrescens* has also been used in the treatment of diabetes,[6] toothache, snake bites, dysentery,[7-8] convulsions and skin problems[9] and to lessen pain and swelling.[10] Phytochemicals such as flavonoids, terpenoids, alkaloids, steroids, phenolics and tannins have been reported from the leaves and bark of Acacia genus which include *S. nigrescens*.[10-16] These phytochemicals are responsible for various pharmacological and biological properties of *S. nigrescens* including antimicrobial, antibacterial, anti-inflammatory, anticancer, anti-quorum sensing cytotoxic and antiproliferative activities.[10] However, our literature search showed that the potential of *S. nigrescens* as a source of antioxidant has not previously been explored, especially the species collected in the Kingdom of Eswatini. Therefore, in this study, we aimed to evaluate DPPH radical scavenging activity, determine IC_{50} values and conduct qualitative phytochemical analysis of hexane, chloroform, ethyl acetate, acetone and methanol extracts, obtained separately from leaves and stem-bark of *S. nigrescens* collected in the Kingdom of Eswatini.

2. Materials and methods

2.1 *Collection and identification of plant materials*

Leaves and stem-bark of *S. nigrescens* were collected at Lubhuku and Mpaka in the Lubombo region of Eswatini and they were identified by Dr. M. N. Dludlu, Department of Biology, University of Eswatini (UNESWA). The specimen for leaves (SSLS2023) and stem-bark (SSSB202) were deposited at the S2.8 Research Laboratory at UNESWA.

2.2 *Drying and grinding of plant materials*

After drying the leaves and stem-bark separately at room temperature for three to four weeks at S2.8 Research Laboratory, UNESWA, they were ground into 792.640 g of leaf powder and 1.082 kg of stem-bark powder using a laboratory-scale grinding machine (KM-1500 Cutting Mill, MRC Laboratory Equipment).

2.3 *Solvents, reagents and chemicals*

Hexane, chloroform, ethyl acetate and sodium carbonate (all from Promark Chemicals); methanol, mercuric chloride, sodium chloride, disodium hydrogen carbonate, potassium chloride, potassium dihydrogen phosphate, potassium iodide, glacial acetic acid, sulphuric acid and hydrochloric acid (all from Minema); acetone and 2,2-diphenyl-1-picrylhydrazil (both from Sigma-Aldrich), ascorbic acid (from Rochelle chemicals), sodium dihydrogen phosphate (from MCB-Lab and Med Suppliers) were used in this research and they were all analytical reagent (AR) grades.

2.4 *Extraction and obtainment of various solvent extracts*

Various solvent extracts were obtained as per methods established by Selepe et al.[17-19] A mass of 100.290 g of leaf powder was soaked and macerated for 48 hours in 800 mL of hexane taken in a 2 L RB flask. Hexane crude extract obtained using this technique was kept in a sample vial. The marc obtained in this maceration process was then subjected to hot solvent extraction using hexane for approximately 12 hrs. The hexane crude extract prepared in this hot solvent extraction was combined with hexane crude extract obtained from the maceration technique. A total mass of 1.845 g of hexane crude extract from leaves was obtained. By following the same extraction method, 2.095, 3.126, 4.636 and 13.875 g of chloroform, ethyl acetate, acetone and methanol leaf crude extracts were received respectively from 100.103, 100.572, 100.492 and 100.836 g of leaf powder. Similarly, 0.854, 2.459, 3.345, 9.015 and 13.670 g of hexane, chloroform, ethyl acetate, acetone and methanol stem-bark crude extracts were obtained respectively from 150.179, 150.467, 150.818, 150.673 and 150.725 g of stem-bark powder.

2.5 *2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and IC_{50} values*

DPPH radical scavenging assay was employed to evaluate various extracts of *S. nigrescens* using procedures reported by Selepe et al.[17-19] Briefly, a stock solution for each extract and positive control (ascorbic acid) were prepared

separately at a concentration of 3,000 µg/mL by dissolving 30 mg of each extract or positive control in 10 mL of 50% methanol (v/v). Serial further dilutions such as 2,000, 1,500, 1,000, 800, 500 and 200 µg/mL were prepared separately from each one of these stock solutions and they served as test solutions and positive controls, respectively. Solutions without extracts were also prepared, which served as negative controls.[17-19] A solution of 3.94 mg of DPPH in 100 mL methanol (0.1 mM DPPH) served as an oxidant.[17-19] A mixture of sodium chloride (8.0 g), potassium chloride (0.2 g), sodium dihydrogen phosphate (1.44 g) and potassium dihydrogen phosphate (0.245 g) in 1,000 mL distilled water served as phosphate-buffered saline (PBS, pH = 7.4). The test solution contained 0.1 mL extract solution (or positive control), 1.0 mL of 0.1 mM DPPH solution and 0.45 mL of PBS buffer. The optical density (OD) of this test solution was measured at 517 nm by using a UV-spectrophotometer (Infinite M 200, Tecan US, Inc.) after 30 minutes incubation. The average value of three experiments was used to calculate the percentage inhibition of radical scavenging potential of extract (or positive control) with help of the equation given below.[17-19]

$$\text{DPPH Scavenged (\%)} = [(\text{OD of Control} - \text{OD of Test})/\text{OD of Control}] \times 100$$

OD of Control = Absorbance of negative control and OD of Test = Absorbance of extract solution (or positive control). Various concentrations *viz*. 200, 500, 800, 1,000, 1,500, 2,000 and 3,000 µg/mL of each extract or positive control were plotted against percentage inhibition of DPPH radical to obtain IC_{50} values.

2.6 *Phytochemical screening*

Using methods previously reported by Pillai et al,[20] all extracts were screened for the presence of phytochemicals *viz*. alkaloids, steroids, phenols, flavonoids, saponins, glycosides, coumarins, carbohydrates, proteins, tannins, terpenoids and phlobatannins. The tests conducted and experimental procedures used for the detection of various classes of phytochemicals are listed below.

2.6.1 *Detection of alkaloids*

A filtered solution of 0.1 g of each extract in 6 mL of 2% sulphuric acid was obtained.

Dragendorff's test: A filtered solution of 0.07 g of extract in 4.0 mL of 2% sulphuric acid was prepared. Four drops of Dragendorff's reagent were added to 2.0 mL filtrate. A formation of orange-red precipitate indicated the presence of alkaloids.

Mayer's test: Four drops of Mayer's reagent were added to 2.0 mL filtrate. A formation of white creamy precipitate indicated the presence of alkaloids.

2.6.2 *Detection of steroids*

A filtered solution of 0.1 g of extract in 5 mL of chloroform was prepared.

Salkowski's test: A filtered solution of 0.05 g of extract in 2.5 mL of chloroform was prepared. Four drops of concentrated sulphuric acid were added to this filtrate. The mixture was allowed to stand at room temperature. A formation of a yellow or red precipitate indicated the presence of steroids.

2.6.3 *Detection of terpenoids*

A filtered solution of 500 mg of each extract in 5.0 mL of distilled water was prepared. A volume of 2.0 mL of chloroform and 2.0 mL of concentrated sulphuric acid were added to 2.0 mL of filtrate. The mixture was then heated. A formation of greyish color indicated the presence of terpenoids.

2.6.4 *Detection of phenolics*

Ferric chloride test: A filtered solution of 250 mg of extract in 2.5 mL of distilled water was prepared. Four drops of 10% aqueous ferric chloride were added to this filtrate. A formation of bright yellow solution which changes to dark green indicated the presence of phenolic compounds.

2.6.5 *Detection of tannins*

A filtered solution of 0.1 g extract in 5.0 mL of 99% ethanol was prepared. Four drops of 1% ferric chloride solution were added to 2.0 mL filtrate. A formation of blue-black, green or blue-green color indicated the presence of tannins.

2.6.6 *Detection of flavonoids*

A filtered solution of 0.05 g of extract in 5.0 mL of distilled water was prepared.

Alkaline test: A volume of 5.0 mL of 10% sodium hydroxide was added to 2.0 mL of filtrate. An intense yellow solution was formed, which decolorized on the addition of four drops of dilute sulphuric acid that indicated the presence of flavonoids.

Lead acetate test: A volume of 2 mL of 10% lead acetate was added to 2.0 mL of filtrate. A formation of yellow precipitate indicated the presence of flavonoids.

2.6.7 *Detection of coumarins*

A filtered solution of 0.1 g of extract in 5 mL of distilled water was prepared. A volume of 1.0 mL of 10% sodium hydroxide and 1.0 mL of chloroform was added to 2.0 mL of this filtrate. A formation of yellow color indicated the presence of coumarins.

2.6.8 *Detection of saponins*

A mass of 0.1 g of extract was dissolved in 5 mL of distilled water in a test tube. The reaction mixture was shaken well and allowed to stand. Formation of foam to a height of ~ 1.0 cm and that persisted for ~ 15 minutes indicated the presence of saponins.

2.6.9 *Detection of glycosides*

Keller-Killiani's test: A filtered solution of 500 mg of each extract in 5.0 mL of distilled water was prepared. A volume of 2 mL of glacial acetic acid containing a few drops of ferric chloride was added to the filtrate. A volume of 1.0 mL of concentrated sulphuric acid was further added to the mixture along the walls of the test tube. A formation of a brown ring at the interface or violet color below the ring indicated the presence of glycosides.

2.6.10 *Detection of carbohydrates*

A filtered solution of 0.1 g of each extract in 5.0 mL of distilled water was prepared.

Benedict's test: A volume of 2.0 mL of Benedict's reagent was added to 2.0 mL of filtrate and this mixture was heated in a boiling water bath for 2 minutes. A formation of brick-red precipitate indicated the presence of reducing sugars.

Fehling's test: A few drops of dilute hydrochloric acid were added to 2.0 mL of filtrate and then Fehling's A and B solutions were added to this mixture and heated. A formation of red precipitate indicated the presence of reducing sugars.

2.6.11 *Detection of proteins*

Biuret test: A filtered solution of 0.5 g of extract in 10 mL of distilled water was prepared. A mixture of four drops of 2% copper sulfate solution and 1.0 mL of 95% ethanol were added to 2.0 mL of filtrate and then excess potassium hydroxide pellets were added on the ethanolic layer. A formation of a pink color in the ethanolic layer indicated the presence of proteins.

2.6.12 *Detection of phlobatannins*

A filtered solution of 0.5 g of extract in 5 mL of distilled water was prepared. A volume of 2.0 mL of 1% hydrochloric acid was added to this filtrate and the reaction mixture was boiled. A formation of red precipitate indicated the presence of phlobatannins.

2.7 *Statistical analysis*

STATISTICA software version 10.0 was used for statistical analysis of DPPH radical scavenging activity. Statistical significance was evaluated at a 95% confidence level by applying Turkey's multiple range tests. When $p \leq 0.05$, statistically significant differences were reported.

3. Results and discussion

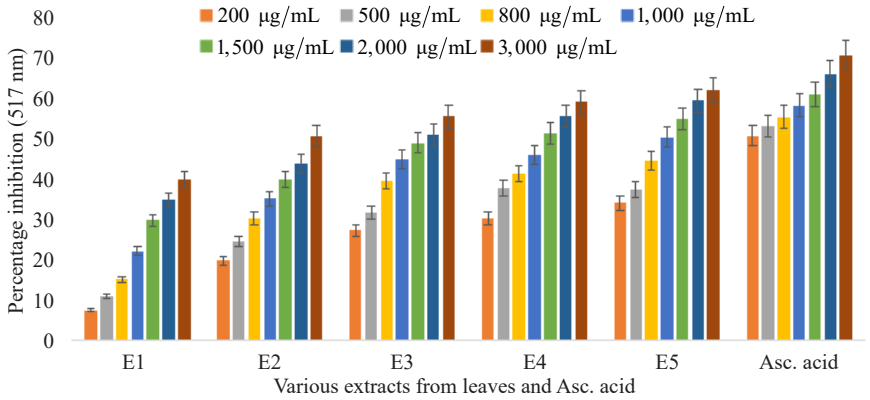
Table 1. DPPH radical scavenging potential of extracts obtained from *S. nigrescens*

Extracts	Concentrations (µg/mL)/inhibition (%)							IC_{50} values (µg/mL)
	200	500	800	1,000	1,500	2,000	3,000	
E1	7.55 ± 0.004^{g}	10.86 ± 0.004^{f}	15.22 ± 0.001^{e}	22.21 ± 0.002^{d}	29.90 ± 0.007^{c}	34.99 ± 0.004^{b}	39.99 ± 0.002^{a}	> 3,000
E2	19.80 ± 0.002^{g}	24.66 ± 0.005^{f}	30.36 ± 0.004^{e}	35.33 ± 0.002^{d}	39.9 ± 0.003^{c}	44.04 ± 0.003^{b}	50.80 ± 0.001^{a}	2,867.89
E3	27.34 ± 0.005^{f}	31.93 ± 0.004^{e}	39.70 ± 0.002^{d}	45.0 ± 0.006^{c}	49.14 ± 0.005^{a}	51.18 ± 0.001^{a}	55.67 ± 0.003^{b}	1,794.78
E4	30.33 ± 0.002^{g}	37.83 ± 0.003^{f}	41.54 ± 0.002^{e}	46.13 ± 0.005^{d}	51.50 ± 0.003^{c}	55.86 ± 0.010^{b}	59.30 ± 0.003^{a}	1,375.76
E5	34.23 ± 0.005^{g}	37.59 ± 0.002^{f}	44.66 ± 0.002^{e}	50.51 ± 0.002^{d}	55.16 ± 0.002^{c}	59.66 ± 0.003^{b}	62.19 ± 0.004^{a}	921.69
E6	9.04 ± 0.003^{f}	13.00 ± 0.007^{d}	15.55 ± 0.007^{d}	17.86 ± 0.006^{c}	20.41 ± 0.005^{b}	24.29 ± 0.008^{a}	35.05 ± 0.005^{e}	> 3,000
E7	11.14 ± 0.004^{g}	15.97 ± 0.005^{f}	19.07 ± 0.003^{e}	23.63 ± 0.007^{d}	31.09 ± 0.004^{c}	44.10 ± 0.005^{b}	52.93 ± 0.004^{a}	2,671.83
E8	13.66 ± 0.005^{f}	17.78 ± 0.005^{e}	23.28 ± 0.005^{d}	36.58 ± 0.004^{c}	45.91 ± 0.005^{b}	51.77 ± 0.010^{a}	53.73 ± 0.003^{a}	1,683.39
E9	17.50 ± 0.000^{g}	22.59 ± 0.006^{f}	28.96 ± 0.005^{e}	42.76 ± 0.006^{d}	50.77 ± 0.004^{c}	57.52 ± 0.004^{b}	61.49 ± 0.003^{a}	1,422.05
E10	23.95 ± 0.044^{f}	31.91 ± 0.002^{e}	50.78 ± 0.001^{d}	53.75 ± 0.005^{c}	57.34 ± 0.002^{a}	59.65 ± 0.006^{a}	63.24 ± 0.006^{b}	735.74
Asc. acid	50.98 ± 0.002^{b}	53.28 ± 0.001^{a}	55.59 ± 0.003^{a}	58.43 ± 0.005^{f}	61.31 ± 0.004^{e}	66.38 ± 0.002^{d}	71.07 ± 0.007^{c}	< 200

E1 = Hexane crude extract from leaves, E2 = chloroform crude extract from leaves, E3 = ethyl acetate crude extract from leaves, E4 = acetone crude extract from leaves, E5 = methanol crude extract from leaves, E6 = hexane crude extract from stem-bark, E7 = chloroform crude extract from stem-bark, E8 = ethyl acetate crude extract from stem-bark, E9 = acetone crude extract from stem-bark and E10 = methanol crude extract from stem-bark. Asc. acid = Ascorbic acid. Values with different superscript letters are statistically different within column

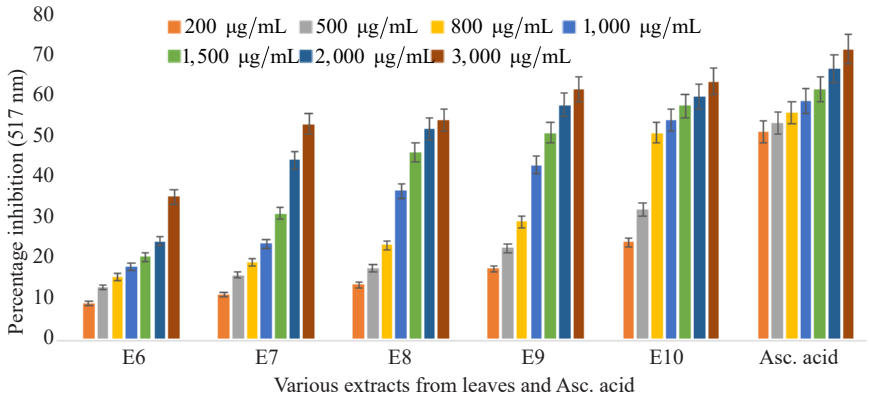
Ten extracts were obtained from *S. nigrescens*. Extracts E1-E5 were respectively hexane, chloroform, ethyl acetate, acetone and methanol extracts obtained from leaves. Similarly, E6-E10 were respectively hexane, chloroform, ethyl acetate, acetone and methanol extracts obtained from stem-bark. DPPH radical scavenging potential of E1-E5 and E6-E10 and positive control (ascorbic acid) at various concentrations (*viz*. 200, 500, 800, 1,000, 1,500, 2,000 and 3,000 µg/mL) are listed in Table 1. At a concentration of 3,000 µg/mL, the positive control exhibited a radical scavenging potential of 71.07 ± 0.007%. At a concentration of 3,000 µg/mL, E1-E5 exhibited radical scavenging potentials of 39.99 ± 0.002, 50.80 ± 0.001, 55.67 ± 0.003, 59.30 ± 0.003 and 62.19 ± 0.004%, respectively. Therefore, E5 and E1 showed the highest and lowest scavenging potential respectively among leaf extracts at 3,000 µg/mL. Extract E5 was a methanol extract obtained from the leaves of *S. nigrescens*. Methanol is a polar solvent and therefore, polar alkaloids, steroids, terpenoids, phemolics, tannins, flavonoids and coumarins might have been dissolved in this methanol extract

(Table 2). Generally, compounds such as phemolics, tannins and flavonoids are regarded as powerful antioxidants since they have hydroxyl groups and these hydroxyl groups have the ability to donate protons and to scavenge free radicals (oxidants). On the other hand, E1 was a hexane extract obtained from the leaves of *S. nigrescens*. This hexane is a non-polar solvent and therefore, non-polar alkaloids, steroids, phenolics, tannins and coumarins might have been dissolved in this hexane extract (Table 2). Additionally, hexane has the ability to extract non-polar fatty matters such as fatty acids and fatty esters. Overall, all these non-polar compounds present in E1 might have relatively weaker antioxidant activity and hence E1 exhibited relatively lower radical scavenging activity. It is noteworthy at this juncture that fatty acids have lengthy non-polar carbon chains at one end and polar carboxylic groups at other end. The polar carboxylic groups have the ability to donate protons and hence fatty acids could scavenge free radicals (oxidants). Similar arguments were also applicable to the methanol extract E10 and hexane extract E6 obtained from the stem-bark of *S. nigrescens*. Extracts, E6-E10 exhibited radical scavenging potentials of 35.05 ± 0.005, 52.93 ± 0.004, 53.73 ± 0.003, 61.49 ± 0.003 and 63.24 ± 0.006%, respectively at 3,000 µg/mL, Therefore, E10 and E6 showed highest and lowest radical scavenging potentials respectively among stem-bark extracts at 3,000 µg/mL. In general, all ten extracts (E1-E10) showed lower scavenging potential relative to positive control. However, at a concentration of 3,000 µg/mL, E4, E5, E9 and E10 showed significant scavenging potential, while E2, E3, E7 and E8 exhibited a moderate scavenging potential. For the purpose of comparison, the bar graphs for scavenging potentials of E1-E5 and E6-E10 together with ascorbic acid are shown in Figures 1 and 2, respectively.



E1-E5 = Refer to the footnote of Table 1. Asc. acid = Ascorbic acid

Figure 1. DPPH radical scavenging potential of leaf extracts of *S. nigrescens*



E6-E10 = Refer to the footnote of Table 1. Asc. acid = Ascorbic acid

Figure 2. DPPH radical scavenging potential of stem-bark extracts of *S. nigrescens*

The IC_{50} values for E1-E10 and ascorbic acid are also listed in Table 1. Ascorbic acid exhibited an IC_{50} value of < 200 µg/mL. The IC_{50} values for E1-E10 were determined to be > 3,000, 2,867.89, 1,794.78, 1,375.76, 921.69, > 3,000, 2,671.83, 1,683.39, 1,422.05 and 735.74 µg/mL, respectively. The result revealed that all ten extracts (E1-E10) had relatively much higher IC_{50} values. However, E5 exhibited relatively highest potency among extracts from leaves with an IC_{50} value of 921.69 µg/mL followed by E4 with IC_{50} value of 1,375.76 µg/mL. Similarly, E10 exhibited relatively highest potency among stem-bark extracts with IC_{50} value of 735.74 µg/mL followed by E9 with an IC_{50} value of 1,422.05 µg/mL. On the other hand, E1 and E6 exhibited the lowest scavenging potential among all extracts with IC_{50} values of > 3,000 µg/mL for each. Overall, all ten extracts (E1-E10) exhibited relatively higher IC_{50} values compared to positive control. In other words, all ten extracts (E1-E10) showed relatively lower scavenging potential compared to positive control. However, E5 and E10 were found to be the most potent among all extracts with IC_{50} values of 921.69 and 735.74 µg/mL, respectively (Table 1).

All ten extracts (E1-E10) were subjected to phytochemical screening and the observations are summarized in Table 2. Alkaloids and steroids were detected in all ten extracts (E1-E10). Extracts E5 and E10 were methanol extracts obtained from leaves and stem-bark, respectively. As discussed previously that methanol is a polar solvent and therefore, polar alkaloids and steroids might have been present in these extracts. On the other hand, E1 and E6 were hexane extracts obtained from leaves and stem-bark, respectively. Hexane is a non-polar solvent and therefore, non-polar alkaloids and steroids might have been present in these extracts. Similarly, phenolics and tannins were also identified in all ten extracts (E1-E10). Terpenoids were present only in E5, E9 and E10. As discussed previously that E5 and E10 were methanol extracts obtained from leaves and stem-bark, respectively. Since methanol is a polar solvent, polar terpenoids might have been present in these extracts.

Table 2. Phytochemical screening of various extracts from leaves and stem-bark of *S. nigrescens*

Phytoconstituents	Extracts									
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
Alkaloids	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+
Terpenoids	-	-	-	-	+	-	-	-	+	+
Phenolics	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+
Flavonoids	-	+	+	+	+	+	-	+	+	-
Coumarins	+	+	+	+	+	+	+	-	+	-
Saponins	-	-	-	-	-	-	-	-	+	+
Glycoside	-	-	-	-	-	-	-	+	+	-
Carbohydrates	-	-	-	-	-	-	-	-	+	+
Proteins	-	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	+	-	-	-	-	+	+

E1-E10 = Refer to footnote of Table 1. The (+) and (-) signs indicate the presence and absence of phytoconstituent, respectively and these signs remained the same for a particular extract for which more than one phytoconstituent test was conducted

Extract, E9 was an acetone extract obtained from stem-bark. Acetone is also a polar solvent and therefore, polar terpenoids might also have been present in this extract. Phlobatannins were found only in E4, E9 and E10 and coumarins were found in E1-E7 and E9. Flavonoids were available in E2-E6, E8 and E9. Saponins and carbohydrates were found only in E9 and E10. However, proteins were not detected in any of these ten extracts (E1-E10). Previous studies have shown the presence of flavonoids, terpenoids, alkaloids, phenolics and tannins in *S. nigrescens*.[11-16] Therefore, the result from this study was in good agreement with previous studies.[11-16] Additionally, the existence of other phytoconstituents was also reported in this study. The presence of these phytoconstituents in *S. nigrescens* is responsible for its radical

scavenging potential. The present study revealed that the methanolic extracts from both leaves and stem-bark of *S. nigrescens* showed relatively higher radical scavenging potential.

4. Conclusions

Hexane, chloroform, ethyl acetate, acetone and methanol extracts were obtained separately from leaves and stem-bark of *S. nigrescens* collected in the Kingdom of Eswatini. These extracts were evaluated for DPPH radical scavenging potential. The radical scavenging potential of leaves and stem-bark extracts were found to be in the ranges of 7.55 ± 0.004 ~ 62.19 ± 0.004% and 9.04 ± 0.003 ~ 63.24 ± 0.006%, respectively at a concentration range of 200 ~ 3,000 µg/mL. The radical scavenging potential of positive control was found to be in the range of 50.98 ± 0.002 ~ 71.0 ± 0.007% at the same concentration range. The methanol extracts from both leaves and stem-bark were identified as the most potent extracts with IC_{50} values of 921.69 and 735.74 µg/mL, respectively. The positive control showed an IC_{50} value of < 200 µg/mL. Additionally, several classes of phytochemicals were identified in these leaves and stem-bark extracts. Particularly, the presence of antioxidant compounds such as phenolics, flavonoids and tannins in the leaves and stem-bark of *S. nigrescens* was responsible for radical scavenging potential. From this study, we concluded that *S. nigrescens* showed a weak to moderate DPPH radical scavenging potential and possessed various classes of phytochemicals. Since *S. nigrescens* has been used in traditional Swazi medicine, further studies on this plant are required. Particularly, the isolation, characterization and evaluation of active compounds that are responsible for antioxidant and other therapeutic activities are recommended as future perspectives from this plant.

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Conflict of interests

The authors declare that there has been no conflict of interest.

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