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Determination of Biological Activities of the Root Bark of *Senna singueana*

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Authors' contributions

This work was carried out in collaboration between both authors. Author ZYD was supervised the whole work as well as organized the manuscript as a whole and KTH did the experimental part and analysis. All authors contributed to manuscript finalization. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: *Senna singueana* is a medicinal plant which is used for the treatment of different kinds of diseases and the plant was selected for the study because of its numerous uses. The main objective of this study was to determine the antioxidant and antibacterial activity of the extracts of the root barks of *S. singueana*.
Methodology: The antibacterial activities of the extracts (determination of Minimal Inhibitory "MIC" and Minimal Bactericidal Concentration "MBC") were determined by using agar well diffusion method. In addition to this the total flavonoid and total phenolic contents were determined by using aluminum chloride colorimetric complex assay and Folin-Ciocalteu method respectively.
Results: Our results revealed that the total flavonoid content of the extracts is ranged from 30.39 mgQE/100 g to 240.83 mgQE/100 g. The extracts also showed good antioxidant activity and total phenolic content as well as weak to moderate antibacterial activity against some bacteria.
Conclusions: The extracts of the root bark of *Senna sinueana* showed good total flavonoid content, DPPH radical scavenging activity and antibacterial activity. In addition to this, the extracts also showed the presence of some important compounds by phytochemical analysis.

Keywords: Senna singueana; antioxidant activity; antimicrobial activity; total phenolic content; total flavonoid content.

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ABBREVIATIONS

TFC : Determination of Total Flavonoid Content
TPC : Total Phenolic Content
GAE : Gallic Acid Equivalent
QE : Quercetin Equivalent
DPPH : 2,2-diphenyl-1-picrylhydrazyl
FRAP : Ferric Reducing Antioxidant Power
MIC : Minimum Inhibitory Concentration
BMC :Minimum Bactericidal Concentration
DMSO : Dimethyl Sulfoxide

1. INTRODUCTION

Senna singueana is a plant belonging to family fabaceae (leguminosae) and subfamily caesalpinoideae. It is a shrub or small tree which is 1-15 m high [1,2]. It is a hermaphroditic species flowering from April to June and its fruits are ready for collection around September. It is widespread in Namibia, in Savannah vegetation belt on all soil types. It is also widely distributed in other African countries including Niger, Northern Nigeria, Mali, Sudan, Eastern and Southern Africa, Ethiopia and Eritrea [3]. The plant has different medicinal values for instance; its root bark is used against convulsions, gonorrhoea, bilharzia, heartburn, stomach-ache, constipation, wounds and snake bites [4,5]. The ash from the burnt roots mixed with porridge provides a remedy for stomach pains. In some parts of Ethiopia, the leaves as well as the bark of the plant are traditionally used for the treatment of a form of skin cancer locally called 'Minshiro Nekersa' [3,4,5]. Herein we reported phytochemical analysis, total flavonoid content, antioxidant activity and antibacterial activities of the extracts of the root bark of *S. singueana*.

2. METHODS AND MATERIALS

2.1 Plant Materials

The fresh root barks of *S. singueana* were collected from Bahir Dar University, Bahir Dar, which is 565 Km away North-West direction from Addis Ababa, Ethiopia in December 2017. The plant was identified by a botanist Dr. Ali Seidu at the Department of Biology, Bahir Dar University, Ethiopia and has Voucher number KTH 01.

2.2 Chemicals and Reagents

The analytical grade chemicals and reagents used for this study were ethyl acetate, methanol, petroleum ether, 10% ferric chloride, Wagner's reagent (Iodine in potassium iodide), aluminum chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), nitric acid (HNO_3), sodium carbonate, iodine, NaH_2PO_4, Na_2HPO_4, phosphoric acid, sodium molybdate, sodium tungstate, trichloroacetic acid, potassium hexacyanoferrate (II), iron chloride, bromine, Ascorbic acid, Gallic acid, DPPH, Quercetin, ascorbic acid, ammonia solution, acetone, chloroform, Tetracycline and Mueller Hinton agar.

2.3 Extraction and Isolation

The air-dried and ground 600 g plant sample was successively extracted with petroleum ether, ethyl acetate and methanol using maceration technique for 48 hours in each solvent. The extract was filtered and residual solvent from each extract was removed using Rotary evaporator under reduced pressure.

2.4 Phytochemical Analysis

The phytochemical analysis of methanol, ethyl acetate and petroleum ether extracts of the root barks of *S. singueana* were studied by slight modifications based on standard procedures described on different literatures [6,7].

2.5 Determination of Total Phenolic Content (TPC)

The total phenolic content of the crude extract was determined by using the Folin-Ciocalteu method [8]. A standard Gallic acid curve was constructed by preparing the dilutions in methanol from the stock solution of Gallic acid and it was constructed by preparing the dilutions of (20, 40, 60, 80 and 100 µg/mL) in methanol from the standard solution of the Gallic acid. 1 mL of each of these dilutions were mixed with 5 mL of Folin-Ciocalteu reagent and allowed to stand for 6 minutes. Then 4 mL of 10% sodium carbonate solution was added to the reaction mixture and the absorbance was recorded after it was mixed and it was allowed to stand for 30 minute at 765 nm spectrometrically. The total phenolic content of the extracts of *S. singueana* was calculated as Gallic acid equivalents (mgGAE/g).

2.6 Determination of Total Flavonoid Content (TFC)

Aluminum chloride colorimetric complex assay was used to determine the total flavonoid content

of the extracts. Quercetin was used as standard and flavonoid content was determined as Quercetin equivalent. A calibration curve for Quercetin was drawn for this purpose as described before [9]. From the standard Quercetin solution, the dilutions of (20, 40, 60, 80 and 100 µg/mL) concentrations were prepared in methanol. 0.25 mL of each of the Quercetin dilution was mixed with 1.25 mL of distilled water and then with 0.075 mL of 5% Sodium nitrite and allowed to stand for 5 minutes of mixing. Then 0.15 mL of 10% Aluminum chloride solution was added and allowed to stand for 6 minutes at room temperature after which 0.5 mL solution of 1M sodium hydroxide was added sequentially. 100 ppm of the extracts was prepared and the same procedure was repeated with the extracts of petroleum ether, ethyl acetate, and methanol and total flavonoid content was calculated as Quercetin equivalents (mgQE/g). All procedures were performed in triplicate. The absorbance of this reaction mixture was recorded at 510 nm on UV spectrophotometer Cary 60 Agilent technologies.

2.7 Measurement of Free Radical Scavenging Activity

2.7.1 DPPH radical scavenging assay

The free radical scavenging activities of the extracts was determined by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. A fresh 0.002% solution of DPPH was prepared in methanol. 20, 40, 60, 80, and 100 ppm of ascorbic acid which is used as standard and plant extracts were prepared by using the standard method. 1.5 mL of the ascorbic acid and extracts were mixed with 3 mL solution of DPPH and allowed to stand in darkness for 15 minutes. Control sample was prepared by taking 3 mL of DPPH and 1.5 mL methanol and its absorbance was recorded at 517 nm. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control. The absorbance was again recorded at 517 nm. The percentage inhibition of DPPH by extracts was calculated by using following formula:

$$\% \text{ Inhibition} = \frac{A-B}{A} X100;$$

Where;

A is the absorbance of pure DPPH in oxidized form while B is the absorbance of the sample taken after 15 minutes of reaction with DPPH [10].

2.7.2 Ferric reducing antioxidant power (FRAP) assay

The reducing power of the crude extracts was determined according to the standard method with slight modification [11]. 2.5 mL of different concentrations of plant extracts was mixed with phosphate buffer solution (PH = 6.6, 0.2M) and potassium hexacyanoferrate ($[K_3Fe(CN)_6]$) (1%). The mixture was incubated at 50°C for 20 min in a water bath. Then trichloroacetic acid (10%) was added to the mixture to terminate the reaction. The upper layer of the solution was mixed with 5 mL of distilled water and 0.5 mL of $FeCl_3$ solution (0.1%). The reaction mixture was incubated for 10 minutes at room temperature and the absorbance developed blue color was measured at 700 nm by using UV-spectrophotometer against a blank solution.

$$\text{Percentage (\%) reduction power} = \frac{(\text{Asample}-\text{Ablank})}{\text{Asample}} \times 100,$$

Where;

A is the sample absorbance of the sample, A is the blank absorbance of the sample.

2.8 Total Antioxidant Activity by Phosphomolybdate Assay

To carry out phosphomolybdate assay, the procedure which followed by Umamaheswari and Chatterjee [11] was used. The phosphomolybdate reagent was prepared by mixing equal volumes (100 mL) of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammoniummolybdate. Test samples were prepared by dissolving 1 mg of plant methanol extract or any of its sub-fractions in 1 mL of methanol. Then, 0.1 mL of the sample was dissolved in 1 mL of reagent solution in a test tube which was capped with silver foil and incubated in a water bath at 95 °C for 90 min. After cooling the sample to room temperature, the absorbance was observed at 765 nm against a blank. Ascorbic acid was used as a standard. Various concentrations of ascorbic acid (20.40, 60, 80 and 100 mg/L) were prepared and the same protocol was carried out in order to plot a standard curve. The results were expressed as mg of ascorbic acid equivalent (AAE) per mg of

the dried weight of the sample as determined from the equation of the standard calibration curve.

2.9 Antimicrobial Activity Test

2.9.1 Agar diffusion method

The microorganisms for the test were collected from the Biology Department, College of Science, Bahir Dar University. The 5 mm diameter sterile discs (Whatman No 3 paper) were placed on the surface of the inoculated agar in petri dishes, and 20 µL each test solutions ware applied onto the discs. After addition of test solutions on the discs, the extract was allowed to diffuse for 5 minutes and the plates ware then be kept in an incubator at 37°C for 24 hrs. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with ruler and results was expressed as Mean ±std of replicate tests. Standard discs of the antibiotic disc (tetracycline, 30 µg/disc) were serving as the positive antibacterial control. For negative control the same volume (20 µL) DMSO poured on paper disks was used. Antibacterial activity was recorded if the zone of inhibition was greater than 6 mm as described before [11,12]. The disk diffusion assay was used as a preliminary test to select the most efficient extracts.

2.9.2 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts was determined using the agar dilution method as described in EUCAST, (2000) [13]. One mL of each test concentration of each crude extract was thoroughly mixed with 19 mL of Muller Hinton agar growth medium and poured to Petri dishes. The medium was allowed to solidify at room temperature. The inoculated Petri dishes were incubated at 37°C for 48 hr. Parallel to this, petri dishes without extract were used as controls and the results were compared against these controls [11].

2.9.3 Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration (MBC) of the extracts was determined as described in Ninga et al. [14]. Muller Hinton agar growth medium was prepared and autoclaved at 121°C for 15 minutes. The medium was poured in to sterile Petri-dishes and allowed to cool and solidify. The contents of the MIC Petri-dishes that did not show growth or showed growth less than 80% of the controls were sub-cultured on to the prepared Petri-dishes. The Petri-dishes were then incubated at 37°C for 48 hr. Then after, the Petri-dishes without growth represent the minimum bactericidal concentration (MBC). After 48 hr., the results were recorded and taken as MBC.

2.10 Method of Data Analysis

The results were reported as mean ± standard deviation (SD). The data for activities were expressed as the average of three measurements, and all the remaining data were expressed as mean ± standard deviations of triplicates using MS Excel 2010, Origin 8, IBM SPSS statistics 22 (one-way Anova) to show the significant difference of antimicrobial activity between extracts.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

This study showed the presence of different kinds of bioactive compounds in different solvent extracts of the root barks of *S. singueana* by using color change as a confirmatory test. According to this study, methanol extract was found to have a wide range of bioactive compounds including flavonoids, phenols, terpenoids, saponins, alkaloids, tannins, steroids and glycosides because of its high polarity. The ethyl acetate extract was also positive for flavonoids, phenols, terpenoids, alkaloids, steroids and glycosides since ethyl acetate has medium polarity. However, the petroleum ether is being highly non-polar in nature and was able to extract very limited phytochemicals only as steroids and terpenoids. The result of the test for phytochemicals was summarized as follows in Table 1.

3.2 Determination of Total Flavonoid Content (TFC)

The total flavonoid content of the extracts of the root bark of *S. singueana* was determined in terms of Quercetin equivalents per 100 g of the dry weight of the sample (mg QE/100 g) as shown in Table 2. The ethyl acetate extract had the highest TFC followed by methanol and petroleum ether extracts. The concentration of flavonoids in the extracts of the root bark of the *S. singueana* ranged from 30.39 mgQE/100 g

(for petroleum ether extract) to 240.83 mgQE/100 g (for ethyl acetate extract). The change of TFC from one extract to another is due to the polarity change of the solvents used for extraction.

Table 1. Preliminary phytochemical screening of root bark extracts of *S. singueana*

Phytochemicals	PE	EA	ME
Alkaloids	-	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Sterols	-	+	+
Terpenes	+	-	+
Saponions	-	-	+
Tannins	-	-	+
Glycosides	-	+	+

Key: "+" present "-"not present and ME= methanol extract, EA= ethyl acetate extract, PE= petroleum ether extract

Table 2. Total flavonoid content of the extracts

Extracts	TFC (mgQE/100 g dry weight)
Petroleum ether extract	30.39 ±0.0104
Ethyl acetate extract	240.83±0.026
Methanol extract	147.23 ±0.087

3.3 Determination of Total Phenolic Content (TPC)

The total phenolic content of the extracts of the root bark of the *S. singueana* was examined using the Folin-Ciocalteu's reagent which is expressed in terms of Gallic acid equivalent per 100 g of the dry weight of the sample (mgGA/100 g). The total phenolic content of the extracts of the root bark of *S. singueana* was evaluated and the results are shown in Table 3 and ranged from 20.01 mgGA/100 g (for petroleum ether extract) to 273.46 mgGA/100 g (for methanol extract). This result was supported by the previous reports which stated that the highest solubility of phenols in polar solvents provides the highest concentration of phenolic compounds in the extracts obtained using polar solvents [15,16]. The change of TPC from one extract to another is due to the polarity change of the solvents used for extraction.

3.4 Determination of Antioxidant Activity

The antioxidant activity of the extracts of the root barks of *S. singueana* was evaluated by using ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays.

Table 1. Total phenolic content of the extracts from the root bark of *S. singueana*

Extracts	TPC (mgGA/100 g of dry weight)
Petroleum ether extract	20.01 ±0.067
Ethyl acetate extract	152.859 ±0.135
Methanol extract	273.46 ±0.200

3.4.1 DPPH radical scavenging activity

The antioxidant activity of the extracts from the root bark of *S. singueana* was determined using a methanol solution of DPPH reagent as described before [17]. In the assay, the methanol extract showed a slightly higher activity than ethyl acetate extract, which in turn, was more active than petroleum ether extract as shown in Table 4. This means the phytochemicals soluble in methanol possess a stronger potential to scavenge DPPH free radicals. The DPPH radical is capable of accepting an electron as well as hydrogen. The slightly higher free radical scavenging activity of the methanol extract presumably indicated the presence of a higher content of flavonoids and phenols compared to ethyl acetate and petroleum ether extracts.

3.4.2 Ferric reducing antioxidant power (FRAP) assay

Ferric Reducing power assay of methanol, ethyl acetate and petroleum ether extracts of the root barks of the *S. singueana* was determined and the results are shown in Table 5. The methanol extract was displayed the highest reducing power whereas the ethyl acetate and petroleum ether extracts showed the lowest reducing power. All three extracts showed almost similar increasing trends in reducing power with the increase in extract concentration. In this assay, the presence of reducers (i.e., antioxidants) causes the reduction of the Fe^{3+}, ferricyanide complex to the ferrous form.

3.5 Total Antioxidant Activity by Phosphomolybdate Assay

This assay is based on the reduction of molybdenum (VI) to molybdenum (V) which takes place in the presence of a reducing agent (antioxidant) as shown in Table 6. The

Table 4. Comparison of absorbance and percent of inhibition of the standard and the extracts

Conc. mg/L	Reference		Extracts					
	AA (Standard)		PE extract		EA extract		ME extract	
	absorbance	Inhibition	absorbance	Inhibition	absorbance	Inhibition	absorbance	Inhibition
20	0.5092	49.07	0.925	7.491	0.6190	38.09	0.5901	40.98
40	0.3559	64.4	0.8587	14.12	0.4337	56.63	0.3863	61.37
60	0.2236	77.63	0.7854	21.45	0.3054	69.46	0.2724	72.76
80	0.0951	90.48	0.7502	24.97	0.2446	75.54	0.2023	79.77
100	0.0149	98.5	0.7329	26.71	0.1902	80.98	0.1220	87.79

Note: PE = Petroleum ether, EA = Ethyl acetate, ME = Methanol and AA = Ascorbic acid

Table 5. Percentage (%) reduction power of the standard and extracts

		Percentage (%) reduction power			
S.No	Conc. (mg/L)	AA	ME extract	EA extract	PE extract
1	100	92.9366	88.3409	86.0960	60.7677
2	80	88.0819	85.3004	84.1679	55.1033
3	60	83.5336	80.2403	75.4480	43.9348
4	40	77.7235	61.1898	51.4758	22.4183
5	20	60.0330	31.0402	20.0389	2.8368

Note: PE = Petroleum ether, EA = Ethyl acetate, ME = Methanol and AA = Ascorbic acid

product is a green phosphomolybdate (V) complex whose formation is monitored with a spectrophotometer. The assay is often used to estimate the total antioxidant activity of a sample and the results are expressed in terms of Ascorbic Acid Equivalents (AAE). The standard curve equation that is used in calculation was y = 0.0084x - 0.1259, R^2 = 0.9988. The total antioxidant activity of various samples decreases in the following order of methanol showed the highest concentration followed by ethyl acetate then petroleum ether (Table 6). There is a good correlation between the DPPH, FRAP values and the total antioxidant activity measured by phosphomolybdate assay. In this study, methanol fraction showed a higher value than ethyl acetate and petroleum ether extracts which is consistent with that of the previous report [18].

Table 6. Total antioxidant activity by phosphomolybdate assay for extracts

No	Extracts	Concentration in mg/L ± STD
1	Petroleum ether extract	26.178 ± 0.031
2	Ethyl acetate extract	232.96± 0.058
3	Methanol extract	304.15±0.074

3.6 Antibacterial Activities of the Root Bark Extracts of *Senna singueana*

3.6.1 Agar well diffusion method

Different extracts from the root bark of *S. singueana* demonstrated (weak to moderate) antibacterial activities against gram-positive and gram-negative bacteria strains. The methanol extract exhibited maximum zone of inhibition against gram positive bacteria followed by ethyl acetate and petroleum ether extracts. For instance, *Streptococcus pyogenes* was the most susceptible gram positive bacteria followed by *Staphylococcus aureus*. Among the gram negative bacteria, *Salmonella typhi* was the most susceptible followed by *Escherchia coli* and *Klebsiella pneumoniae* for different extracts of *S. singueana* at different concentration. The antibacterial activity of most extract was statistically significant (P ≤ 0.05) compared to the negative control dimethyl sulfoxide (DMSO) and displayed similar potency with that of tetracycline, a standard drug used as positive control in this study. Thus, the present study showed that the different extracts of *S. singueana* root bark possesses significant antibacterial activity and provides possible rationalization to the traditional anti-infection use of the plant. The results showed that for all microorganisms the activity decreased by decreasing the concentration of the extracts as shown in Table 7.

3.6.2 Determination of minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The determination of Minimal Inhibitory Concentration (MIC) is sufficient to indicate the ability of a compound to inhibit microbial replication. With defined ranges of MIC and MBC, the tolerance and resistance shown by microorganisms demands the development of new antimicrobial agents as well as new methodologies to precisely quantify the microbicidal activity of the new pharmaceuticals. Minimum Inhibitory Concentration (MIC) refers to the lowest concentration of an antimicrobial that will inhibit visible growth of a microorganism after 48 hours incubation while minimum bactericidal concentration (MBC) refers to lowest concentration of an antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media (i.e. concentration that will kill the microorganism) [19,20]. MICs and MBCs of different extracts from the root bark of *S. singueana* against both gram-positive and gram-negative strains were determined and the results are shown in Table 8. The lowest MIC was 12.5 mg/mL against *Klebsiella pneumoniae*,

Table 7. Minimum Zone of Inhibition (MZI) of different extracts of *S. singueana* root bark against bacteria

Type of bacteria	Conc. (µg/ml)	MZI of extracts and the standard ±S.D (in mm)				
		ME extract	**EA extract**	**PE extract**	**Tetracycline**	**P-value**
S. aureus	100	20.67±2.52	13.687±3.05	8.67±1.52	23	-
ATCC8737	75	13.50^{ab} ±2.0	12.0^{a}±3.2787	7.0^{b}±1.00		0.05
	50	9.00^{a} ±1.32	9.833^{a} ±2.52	6.3^{b}±0.289		0.045
	25	7.331^{a}±0.041	7.33^{a}±1.26	0.00^{b}		0.008
	12.5	6.33^{a}±0.578	4.83^{a}±0.252	0.00^{b}		0.032
S. pyogenes	100	15.3^{ab}±1.25	10.83^{a}±1.756	8.3^{b}±1.53	18	0.01
ATCC8738	75	13.66^{a}±0.289	9.33^{a}±1.04	6.00^{b}±0.0		0.021
	50	8.50^{a}±1.323	7.50^{a}±0.50	0.00^{b}		0.027
	25	7.67^{a}±0.764	7.00^{a}±0.50	0.00^{b}		0.048
	12.5	6.33^{a}±0.578	6.667^{a}±0.76	0.00^{b}		0.00
E. coli	100	14.67±1.53	11.67±2.51	10.00±2.64	17	-
ATCC8739	75	12.00^{a} ±1.50	8.167^{b} ±1.040	8.33^{b} ±1.52		0.05
	50	8.67±1.61	6.83±1.45	6.33±.2868		-
	25	6.833^{a} ±1.041	2.167^{ab} ±3.75	0.00^{b}		0.025
	12.5	2.00±3.46	-	-		-
K. pneumoniae	100	10.3333±1.53	9.33±1.52	8.667±1.51	14	-
ATCC1129	75	8.00±0.00	8.33±1.25	7.167±0.76		-
	50	7.167^{a} ±0.578	7.00^{ab} ±0.500	2.00^{b}±3.46		0.035
	25	2.00^{a} ±3.46	6.167^{a} ±0.289	-		0.024
	12.5	-	-	-		-
S. typhi	100	15.67±1.52	14.00±2.64	11.00±3.00	19	-
ATCC1130	75	13.67^{a} ±1.607	11.50^{ab}±3.041	7.33^{b}±2.30		0.045
	50	11.00^{a} ±1.802	9.50^{a} ±1.802	0.0^{b}		0.00
	25	9.50^{a} ±2.783	8.1667^{a} ±1.53	0.0^{b}		0.001
	12.5	7.00^{a} ±0.50	6.167^{b} ±0.289	-		0.00

Note: PE = Petroleum ether, EA = Ethyl acetate, ME = Methanol; $^{a, b}$ and ^{ab} has a significant difference at confidence interval ($p<0.05$) and "-" indicates no significant difference, Values are expressed in mean ± STD of 3 individual experiments

Table 8. MICs and MBCs of different extracts of *Senna singueana* root barks

	ME extract		EA extract		PE extract	
Test bacteria's	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Klebsiella pneumoniae	12.5	25	25	50	50	75
Escherichia coli	12.5	25	12.5	25	50	75
Staphylococcus aureus	25	50	25	50	75	100
Streptococcus pyogenes	12.5	25	25	50	50	75
Salmonella typhi	12.5	25	25	50	25	50

Note: PE = Petroleum ether, EA = Ethyl acetate, ME = Methanol

Escherichia coli, *Salmonella typhi, Streptococcus pyogenes* (for methanol extract); and the lowest MBC was 25 mg/mL against *Klebsiella pneumoniae*, *Salmonella typhi, Streptococcus pyogenes, Escherichia coli* (for methanol and ethyl acetate extracts), and *Salmonella typhi* (petroleum ether extract). According to Gebremariam et al. [19], a sample is bactericidal when the ratio MBC/MIC ≤ 4, and bacteriostatic when this ratio is >4. In this study, MBC/MIC ≤ 4 and values have been shown for all the dilutions in which MIC and MBC have been determined and it is indicated that the tested extracts may be acting as bactericidal the growth.

4. CONCLUSION

The results of phytochemical work on extracts of *S. singueana* revealed the presence of alkaloids, phenols, flavonoids, tannins, terpenoids, saponin, and glycosides in different concentration. Based on the results investigated during the study, it can be concluded that all of the extracts (petroleum ether, ethyl acetate and methanol) of the root bark of *S. singueana* possess weak to moderate antioxidant activity which may be due to the presence of phenolic and flavonoid contents in the extracts. The highest contents of phenolic and flavonoid showed significant linear correlation between the values of the concentration of phenolic and flavonoid compounds and antioxidant activity indicated that these compounds contribute to the strong antioxidant activity. Antibacterial effects of root bark of *S. singueana* extracts showed different degrees of inhibition against both Gram-positive and Gram-negative bacteria. The methanol and ethyl acetate extracts showed higher antibacterial activity compared to petroleum ether extract.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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