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RESEARCH ARTICLE

Antibacterial and Phytochemical Screening of Root Extracts of *Senna singuaena*

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ABSTRACT

For centuries plants have been used throughout the world as drugs and remedies for various diseases including infections. *Senna singueana* (Del.) Lock (Fabaceae) has many traditional uses against infections and related disorders. The aim of the present study was to evaluate the antibacterial potential and phytochemical properties of root extracts of *S. singueana.* Root part of the plant was extracted by maceration using methanol, acetone and chloroform; and extracts were screened for their antibacterial potential against seven standard bacteria species: *Staphylococcus aureus* (ATCC215223), *Streptococcus pneumonia* (ATCC49619), *Streptococcus pyogenes* (ATCC19615), *Escherchia coli* (ATCC259292), *Klebsiella pneumonia* (ATCC70060), *Pseudomonas aeruginosa* (ATCC27853), and *Salmonella typhi* (ATCC1912/R). Antibacterial screening was done using the well diffusion method. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) determinations and phytochemical screening was also done on the extracts. Results showed that the different extracts displayed significant ($p<0.05$) antibacterial activities; and the methanol extract was more active. Alkaloids, carbohydrates, glycosides, phenols, steroids, tannins, and triterpenes were detected in the root extract of the plant. Lowest MIC of 400 µg/ml was shown by the methanol extract against *S. pneumonia* and *S. pyogenes;* and lowest MBC of 500 µg/ml was exhibited by the methanol extract against *S. pneumonia, S. pyogenes,* and *S. typhi*. The chloroform extract also had MBC of 500 µg/ml against *S. pneumonia*, and *S. typhi*. The tested extracts seem to demonstrate bactericidal mechanism of action. In conclusion, root extracts of *S. singueana* demonstrated antibacterial activities against both gram positive and gram negative bacteria and this in turn may, at least partly, rationalize the traditional use of the plant against various infections.

Key words: Antibacterial Activity, Phytochemical Screening, *Senna singueana*

INTRODUCTION

For centuries plants have been used throughout the world as drugs and remedies for various diseases [1, 2]; and medicinal plants served as rich source of antimicrobial agents since antiquity. Unfortunately, in recent years, antimicrobial resistance has become a major public health concern globally [3]; and the emergence of multidrug resistant pathogenic strains and adverse effects of synthetic antibiotics have led to rapid search for new antimicrobials [4]. *Senna singueana* (Del.) Lock (Fabaceae) has been traditionally used throughout Africa to manage numerous disorders including infections [5]. Few scientific reports support its potential use against infections. Anthraquinone and tetrahydroanthracene derivatives with antimicrobial activities have been isolated from the root bark of this species [6]. Furthermore, methanol root extract of the plant displayed antioxidant activity and has been suggested to offer protection against hepatic and oxidative injuries [7] indicating that it may serve as potential herbal medicine. The objective of the present study was to carry out antibacterial activity and phytochemical screening of extracts from root of

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S. singueana so as to provide some scientific verification to its traditional claims.

MATERIAL AND METHODS

MATERIALS

Solvents and Chemicals:

Solvents: Acetone, Methanol (HiMedia laboratories, India), Chloroform (BDH Chemicals Ltd, England); Distilled Water (Labora International PLC), Chemicals and Reagents: Mayer's Reagent, Molisch's Reagent, Ferric Chloride, Concentrated Sulphuric Acid (Sigma-Aldrich Chemicals), Concentrated Hydrochloric Acid (BDH Chemicals Ltd, England), 10% ammonia (Techno Pharmachem, India), Libemann–Buchard Reagent (Blulux Laboratories, India)

Plant Material:

Root of *S. singueana* was collected from Central Zone of Tigray, Northern Ethiopia. The plant was authenticated by Mrs Shoa and a specimen (voucher number of TG002/2006) was deposited in the National Herbarium at the Department of Biological Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

Test Organisms:

The test microorganisms used in this study: *Escherchia coli* (ATCC259292), *Salmonella typhi* (ATCC1912/R), *Staphylococcus aureus* (ATCC215223), *Klebsiella pneumonia* (ATCC70060), *Streptococcus pyogenes* (ATCC19615), *Pseudomonas aeruginosa* (ATCC27853) and *Streptococcus pneumonia* (ATCC49619) were obtained from University of Gondar, Department of Microbiology which were maintained on nutrient agar slope/slant at -20^{o}C (deep freeze). The strains were checked for purity on the basis of standard microbiological culture, biochemical tests and then used for their sensitivity to test samples.

METHODS

Extraction:

Root of *S. singueana* was collected, washed with tap water until the sand and mud were removed from the part, dried, size reduced using a hammer, and powdered using grinder. Different extracts were prepared from the powdered plant material by maceration using methanol, chloroform and acetone as solvents. Each time, the extracts were filtered, concentrated under reduced pressure using rotary evaporator, and dried in an oven at a temperature of 35 ^{o}C. The dried extracts were then transferred into vials and stored at room temperature for further use.

Phytochemical Analysis:

The preliminary phytochemical analyses of the methanol, acetone, and chloroform extracts were carried out using the methods described by Idris *et al*., (2009) [8] and Shakeri *et al*., (2012) [9].

Antibacterial Activity Screening:

Agar well diffusion method was employed to do antibacterial screening of extracts. Stock culture was prepared by inoculating each culture from the slants to a flask in sterile broth (brain heart infusion - BHI) and then incubated for 24 hours at 37 °C. The stock culture was serially diluted by ten-fold with sterile BHI broth and 0.1 ml of each dilution was spread over nutrient agar plates and incubated at 37 °C for 24 hours. Antibacterial activity testing of the different extracts was done using well diffusion method following the procedure described by Valgas *et al*., (2007) [10] with slight modification. One loop full (loop diameter 3 mm) of each bacterial suspension was uniformly spread using sterile cotton swab on a sterile Petri dish Muller Hinton (MH) agar and 5 wells (each 6 mm diameter) were made on the MH agar of each Petri dish. Three concentrations (200, 400, and 800 µg/ml) from each sample extract were prepared using dimethylsulphoxide (DMSO). 100 µl of sample extracts and negative control (DMSO) were added to the formed wells. Standard Ciprofloxacin disc (5µg /ml) was used as a positive control. After 24 hours of incubation at 37 ºC, zone of inhibition (millimeter) of each test sample was measured using digital calibrator. Tests were performed in triplicates.

MIC and MBC Determination:

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determinations were done using different dilutions (100, 200, 300, 400, 500, 600, 700, and 800 µg/ml) from each extract. Inoculums were added to test tubes containing the different dilutions and DMSO (control) and then incubated at 37 ^{0}C for 24 hours. MIC was determined as the lowest concentration of an extract that inhibited visual growth in the liquid media. To determine the MBC, 20 µl samples from the tubes with higher than or equal to the MIC were sub cultured on nutrient agar plates and incubated overnight at 37 ^{o}C. A reduction in colony counts by 99.9% from the original inoculum size was considered to represent the MBC.

Statistical Analysis:

Data was analyzed using SPSS version 20. Inhibition zones were expressed as mean ± standard deviation. One way ANOVA followed by Dunnett's multiple comparison was employed to compare results between extracts and between bacteria. Results were considered statistically significant at 95 % confidence level and P-value < 0.05.

RESULTS

Extraction:

The percentage yield (w/w) of different extracts of *S. singueana* root is summarized in (**Table 1**). The methanol extract showed higher percentage yield; and percentage yield decreased as solvent polarity decreased.

Table 1: Percentage yield of different extracts of *S. singueana* root

Type of extract	Percentage yield (w/w)
Methanol	4.30
Acetone	2.25
Chloroform	1.55

Phytochemical Screening:

The phytochemical screening results showed that alkaloids, carbohydrates, glycosides, phenols, steroids, tannins, and triterpenes were detected in at least one extract of *S. singueana* root whereas flavonoids and saponins were not detected (**Table 2**).

Table 2: Preliminary phytochemical screening results of different root extracts of *S. singueana*:

Phytochemical Groups	Results		
	Methanol	Chloroform	Acetone
Alkaloids	+	-	-
Carbohydrates	-	+	-
Flavonoids	-	-	-
Glycosides	+	+	-
Phenols	+	+	+
Saponins	-	-	-
Steroids	+	-	-
Tannins	+	-	+
Triterpenes	-	+	-

(+) denotes phytochemical group was detected and (-) not detected

Antibacterial Activity Screening:

Different extracts from the root of *S. singueana* demonstrated antibacterial activities against standard bacteria of both gram-positive and gram-negative strains. The inhibition zones of the different extracts are summarized in (**Table 3 & 4**).

Table 3: Mean zones of inhibition of different extracts of *S. singueana* root at different concentrations against gram positive bacteria

Test bacteria	Conc (µg/ml)	Mean zone of inhibition ± S.D (mm)				P- values
		Methanol	Acetone	Chloroform	CIP	
Staphylococcus aureus	200	25±1.732	10.33±1.528	18.67±2.082	28	0.016
	400	28.33±2.082	11±2	22.33±2.082	28	0. 000
	800	30.67±1.528	16.33±1.528	23.67±1.528	28	0. 000
Streptococcus pneumonia	200	14+3.6	9.67±0.577	20.67±0.557	18	-
	400	12.33±1.155	10.33±0.557	25±1	18	0. 000
	800	14.33±2.517	10.33±0.577	32±1	18	0.002
Streptococcus pyogenes	200	30.33±0.577	21±1	29+1	26	-
	400	32±1	25.67±1.528	30.67±0.577	26	-
	800	33±0	25.33±0.577	35±1	26	0.012

CIP = Ciprofloxacin (5µg/ml), (-) = statistically not significant

Table 4: Mean zones of inhibition of different extracts of *S. singueana* root at different concentrations against gram negative bacteria

Test bacteria	Conc (µg/ml)	Mean zone of inhibition ± S.D (mm)				P- values
		Methanol	Acetone	Chloroform	CIP	
Escherchia coli	200	14.33±1.55	13±2	7.33±1.528	25	0. 000
	400	17±1	18.67±2.517	11±2	25	-
	800	20±1	20.67±2.012	15.33±1.528	25	-
Klebsiella pneumonia	200	10.67±0.577	9.33±0.577	9.67±0.577	20	-
	400	14±0	12±1	11.67±1.157	20	0. 000
	800	14±0	12.67±0.577	13±1	20	0. 000
Pseudomonas aeruginosa	200	22.33±0.577	25.33±2.08	11.67±1.528	23	-
	400	25±0	28.67±2.08	20.33±0.577	23	0.003
	800	27.67±0.577	34.67±0.577	19.67±0.577	23	0. 000
Salmonella typhi	200	21.67±3.786	14.33±3.786	26.33±2.08	30	0. 000
	400	24.33±3.215	18.67±1.528	26.33±3.05	30	0.001
	800	26.33±0.577	21.67±1.528	30±1	30	0. 000

CIP = Ciprofloxacin (5µg/ml), (-) = statistically not significant

MIC and MBC Determination:

MICs and MBCs of different extracts from *S. singueana* root against standard bacteria of both gram-positive and gram-negative strains were determined and results are shown in (**Table 5 & 6**).

Table 5: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of different extracts of *S. singueana* root at different dilutions against gram positive bacteria

Test Bacteria	Extracts	Concentrations (µg/ml)								MIC (µg/ml)	MBC (µg/ml)
		100	200	300	400	500	600	700	800		
S. aureus	Methanol	+	+	+	+	+	-	-	-	700	700
	Acetone	+	+	+	+	+	+	+	+	N	N
	Chloroform	+	+	+	+	+	+	-	-	700	700
S. pneumonia	Methanol	+	+	+	-	-	-	-	-	400	500
	Acetone	+	+	+	+	+	+	+	+	N	N
	Chloroform	+	+	+	+	-	-	-	-	500	500
S. pyogenes	Methanol	+	+	+	-	-	-	-	-	400	500
	Acetone	+	+	+	+	+	+	+	+	N	N
	Chloroform	+	+	+	+	+	+	-	-	700	700

N = Not determined

Table 6: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of different extracts of *S. singueana* root at different dilutions against gram negative bacteria

Test Bacteria	Extracts	Concentrations (µg/ml)								MIC (µg/ml)	MBC (µg/ml)
		100	200	300	400	500	600	700	800		
E. coli	Methanol	+	+	+	+	+	-	-	-	600	700
	Acetone	+	+	+	+	+	+	-	-	700	800
	Chloroform	+	+	+	+	+	+	+	+	N	N
K. pneumonia	Methanol	+	+	+	+	+	+	+	+	N	N
	Acetone	+	+	+	+	+	+	+	+	N	N
	Chloroform	+	+	+	+	+	+	+	+	N	N
P. aeruginosa	Methanol	+	+	+	+	+	-	-	-	600	600
	Acetone	+	+	+	+	+	-	-	-	600	600
	Chloroform	+	+	+	+	+	+	-	-	700	700
S. typhi	Methanol	+	+	+	+	-	-	-	-	500	500
	Acetone	+	+	+	+	+	+	-	-	N	N
	Chloroform	+	+	+	+	-	-	-	-	500	500

N = Not determined

DISCUSSION

Extraction:

Medicinal plants contain different phytoconsituents which are responsible for their biological activities. To identify the phytochemical groups with pronounced activity, extraction was made using solvents of decreasing polarity (methanol, acetone, and chloroform). As can be seen from table 1, methanol extract showed higher percentage yield; and percentage yield decreased as solvent polarity decreased indicating that the constituents were more extractable with polar solvents. This is also in line with traditional practices as most use water for extraction.

Phytochemical Screening:

Most of the phytochemical groups: alkaloids, carbohydrates, glycosides, phenols, steroids, tannins, and triterpenes that were detected in at least one extract of *S. singueana* root are known to display biological activities; and they may contribute to the observed antibacterial activities of the extracts.

Antibacterial Activity Screening:

Different extracts from the root of *S. singueana* demonstrated antibacterial activities against standard bacteria of both gram-positive and gram-negative strains. As can be seen from table 3 & 4, all dilutions of each extract showed antibacterial activity compared to DMSO (negative control) which had inhibition zone of 6 mm (size of formed well). The methanol extract exhibited maximum zone of inhibition against gram positive bacteria followed by chloroform and acetone extracts and *Streptococcus pyogenes* was most susceptible gram positive bacteria followed by *Staphylococcus aureus* and *Streptococcus pneumonia.* Among the gram negative bacteria, *Salmonella typhi* was the most susceptible followed by *Pseudomonas aeruginosa*, *Escherchia coli* and *Klebsiella pneumonia*. The antibacterial activity of most dilutions of each extract was statistically significant $\preceq(P0.05)$ compared to the negative control (DMSO) and displayed similar potency with that of ciprofloxacin, a standard drug used as positive control in this study. Thus, the present study shows that the different extracts of *S. singueana* root possess significant antibacterial activity and provides possible rationalization to the traditional anti-infection use of the plant. Some of phytochemical groups (table 2) detected in the

different extracts may be responsible for the antibacterial activities. Anthraquinone and tetrahydroanthracene derivatives with antimicrobial activity have been isolated from the root bark of *S. singueana* [6] and hence support results of the present study.

MIC and MBC Determination:

The determination of Minimal Inhibitory Concentration (MIC) is sufficient to indicate the ability of a compound to inhibit microbial replication [11]. MIC refers to the lowest concentration of an antimicrobial that will inhibit visible growth of a microorganism after overnight 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) [12, 13]. MICs and MBCs of different extracts from *S. singueana* root against standard bacteria of both gram-positive and gram-negative strains were determined and results are shown in table 5 & 6. The lowest MIC was 400 µg/ml against *S. pneumonia* and *S. pyogenes* (methanol extract); and the lowest MBC was 500 µg/ml against *S. pneumonia* (methanol and chloroform extracts), *S. pyogenes* (methanol extract), and *S. typhi* (methanol and chloroform extracts). Moreover, it has been indicated by Djeussi *et al*., (2013) [14] that a sample is bactericidal when the ratio MBC/MIC $\leq$ 4 and bacteriostatic when this ratio is >4. In the present study, MBC/MIC $\leq$ 4 values have been shown for all the dilutions in which MIC and MBC has been determined indicating that the tested extracts may be acting as bactericidal.

CONCLUSION

Root extracts of *S. singueana* displayed antibacterial activity against both gram positive and gram negative bacteria. Alkaloids, carbohydrates, glycosides, phenols, steroids, tannins, and triterpenes were detected in the extracts and may contribute to the antibacterial action. The observed antibacterial action may, at least partly, rationalize the traditional use of the plant against various infections.

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