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ANTIBACTERIAL AND PHYTOCHEMICAL SCREENING OF ROOT EXTRACTS OF *EUCLEA RACEMOSA* SUBSP. SCHIMPERI

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ABSTRACT: Emergence of multidrug-resistant strains of pathogens and adverse effects of antibiotics have rapid lead search for new antimicrobials. Medicinal plants have gained more importance as a source of alternative and effective drugs. *Euclea racemosa* sub sp. schimperi (DC.) Dandy (Ebenaceae) has many traditional uses against infections and related disorders. The objective of this study was to evaluate the antibacterial potential and identify major phytochemical groups in root extracts of *E. racemosa*. Root part of the plant was extracted by maceration using methanol, acetone, and chloroform. Extracts were subjected to antibacterial screening against seven bacteria strains: *Staphylococcus aureus* (ATCC215223), *Streptococcus pneumonia* (ATCC49619), *Streptococcus pyogenes* (ATCC19615), *Escherichia coli* (ATCC259292), *Klebsiella pneumonia* (ATCC70060), *Pseudomonas aeruginosa* (ATCC27853), and *Salmonella typhi* (ATCC1912/R). Well, the diffusion method was used to perform the antibacterial screening. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and phytochemical screening was also done on the extracts. Results indicated that the different extracts displayed significant ($P<0.05$) antibacterial activities and the methanol extract were more active. Flavonoids, glycosides, phenols, saponins, steroids, tannins, and triterpenes were detected in the root extracts of *E. racemosa*. Lowest MIC (300 µg/ml) was exhibited by methanol extract against *S. pneumonia*, and chloroform extract against *S. typhi*; and lowest MBC of 400 µg/ml was displayed by methanol extract against *S. pneumonia* and *S. pyogenes*, and chloroform extract against *S. pyogenes* and *S. typhi*. It was also shown that the tested extracts seem to demonstrate bactericidal activities. Thus, it was concluded that root extracts of *E. racemosa* demonstrated antibacterial activity against both grams positive and gram- negative bacteria strains; and this may partly justify the traditional use of the plant against infection and related disorders.

INTRODUCTION: Adverse effects of popular antibiotics and multidrug-resistant strains of pathogens have to lead the rapid search for new antimicrobials.

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Because of the long history of plants in the treatment of different human ailments, most of the herbal drugs are believed to be safer than the synthetic drugs with no side effects; therefore medicinal plants have gained more importance as the possible source of alternative and effective drugs. Plants and natural products remain as an untapped reservoir of potentially useful chemical compounds not only as drugs but also as unique templates that could serve as a starting point for synthetic analogs [1]. Although, Ethiopia has rich plant biodiversity, its traditional medicine has not

been investigated either from their chemical composition or in terms of their pharmacological activities [2, 3, 4]. *Euclea racemosa* sub sp. schimperi (DC.) Dandy (Ebenaceae) is traditionally used in the treatment of the wound, teeth infections, eye disorders, head ache, pain, spasm, and also in smoking milk products. The wood of *E. racemosa* produces, when burned, thick, black smoke that was considered ideal for repelling insects and other pests [5]. In Ethiopia, the root/stem part of *E. racemosa* is locally used as a toothbrush and to repel evil eye [6]. Therefore, the objective of the present work was to screen the antibacterial activity and phytochemical properties of *E. racemosa* root extracts.

METHODOLOGY:

Collection of Plant Materials: Root part of *E. racemosa* was collected from Central Zone of Tigray, Northern Ethiopia. Mrs. Shoa authenticated the plant, and a specimen (voucher number, TG001/2006) was deposited in the National Herbarium at the Department of Biological Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

Extraction: The collected roots of *E. racemosa* were washed with tap water until the sand and mud were removed from the parts, dried, size reduced using a hammer, and powdered using the grinder. Different extracts were prepared by maceration using methanol, chloroform, and acetone as solvents. Each time, the extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and dried in an oven at a temperature of 35 °C. The dried extracts were then transferred into vials and stored at room temperature for further use.

Phyto-chemical Analysis: The preliminary phyto-chemical analyses of the methanol, chloroform, and acetone extracts were carried out using the methods described by Idris *et al*., (2009) [7] and Shakeri *et al*., (2012) [8].

Test Bacteria, Culturing and Antibacterial Activity Screening: The antibacterial activity screening of extracts was performed against seven bacteria species of both gram-positive and gram-negative strains: *Escherichia coli* (ATCC259292), *Salmonella typhi* (ATCC1912/R), *Staphylococcus aureus* (ATCC215223), *Klebsiella pneumonia* (ATCC70060), *Streptococcus pyogenes* (ATCC 19615), *Pseudomonas aeruginosa* (ATCC27853) and *Streptococcus pneumonia* (ATCC49619). The bacteria were obtained from the University of Gondar, Department of Microbiology and maintained on nutrient agar slope/slant at -20 °C, checked for purity by standard microbiological culture, biochemical tests and then used for their sensitivity to test samples. 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 h 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 h. Antibacterial activity testing of the different extracts was done using well diffusion following the method described by Valgas *et al*., (2007) [9] with slight modification.

One loop full (loop diameter 3 mm) of each bacterial suspension was uniformly spread using a sterile cotton swab on a sterile petri dish Muller Hinton (MH) agar. 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 h of incubation at 37 ºC, the zone of inhibition (millimeter) of each test sample was measured using a 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 800µg/ml) from each extract. Inoculums were added to test tubes containing the different dilutions and DMSO (control) and then incubated at 37 °C for 24 h. 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 °C. A reduction in colony counts

by 99.9% from the original inoculum size was considered to represent the MBC.

Data Entry and Statistical Analysis: Data were analyzed using SPSS version 20. Inhibition zones were expressed as mean ± Standard deviation. One way ANOVA followed by Dunnett's multiple comparisons 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 AND DISCUSSION:

Extraction: The percentage yield (w/w) of different extracts from roots of *E. racemosa* is summarized in **Table 1**. The methanol root extract showed higher percentage yield, and percentage yield decreased as solvent polarity decreased.

TABLE 1: PERCENTAGE YIELD OF DIFFERENT EXTRACTS OF *E. RACEMOSA* ROOTS

Type of extract	Percentage yield (w/w)
Methanol	5.60
Acetone	2.42
Chloroform	1.49

Phytochemical Screening: The phytochemical screening results **Table 2** showed the possible presence of flavonoids, glycosides, phenols, saponins, steroids, tannins, and triterpenes in *E. racemosa* root as those were detected in at least one extract of the plant; whereas alkaloids and carbohydrates were not detected. Some of the phytochemical groups that have been detected in the extracts may contribute to the observed antibacterial activities of the extracts.

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING RESULTS OF DIFFERENT ROOT EXTRACTS OF *E. RACEMOSA*

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 *E. racemosa* demonstrated various degrees of activities against standard bacteria of both gram-positive and gram-negative strains. The inhibition zones of the different extracts are summarized in **Tables 3** and **4.** As can be seen from **Tables 3** and **4**, all concentrations from all tested extracts showed antibacterial activity compared to the negative control (DMSO) which had inhibition zone of 6 mm (size of formed well).

The methanol extracts of *E. racemosa* exhibited a maximum zone of inhibition against gram-positive bacteria followed by chloroform and acetone extracts. *Streptococcus pyogenes* was most susceptible gram-positive bacteria followed by *Streptococcus pneumonia* and *Staphylococcus aureus;* and from the gram-negative bacteria, *Salmonella typhi* was the most susceptible followed by *Pseudomonas aeruginosa, Klebsiella pneumonia,* and *Escherichia coli*. The antibacterial activity of most extracts was statistically significant ($P \leq 0.05$) compared to the negative control (DMSO) and had more or less equivalent potency to that of ciprofloxacin, a standard drug used as positive control in this study.

Thus, the present study shows that the different extracts of *E. racemosa* possess significant antibacterial activity and could serve as a possible justification for the traditional use of the plant against different infectious disorders. Some of the phytochemical groups that were detected in the root extracts of the plant could be responsible for the displayed antibacterial activities.

MIC and MBC Determination: Determination of minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, and minimum bactericidal concentrations (MBCs) are the lowest concentration of an antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media [10]. Accordingly, MICs and MBCs of different extracts from roots of *E. racemosa* against standard bacteria of both gram-positive and gram-negative strains have been done, and the results are shown in **Tables 5** and **6**.

As can be seen from **Tables 5** and **6,** the lowest observed MIC was 300 µg/ml against *S. pneumonia* (methanol extract) and *S. typhi* (chloroform extract); and the lowest MBC was 400 µg/ml against *S. pneumonia*, *S. pyogenes* (methanol extract), and *S. typhi* (chloroform extract). It has been indicated by Djeussi *et al*., (2013) [11] that a sample is bactericidal when the ratio MBC/MIC ≤ 4 and bacteriostatic when this ratio is >4. In the present study, MBC/MIC ≤4 values have been shown for all the dilutions in which MIC and MBC have been determined indicating that the tested extracts may be acting as bactericidal.

TABLE 3: MEAN ZONES OF INHIBITION OF DIFFERENT EXTRACTS OF *E. RACEMOSA* 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	11±2	24.67±1.528	30	0. 000
	400	25±1	11±2	24.67±1.528	30	0. 000
	800	28.67±1.528	15.67±1.528	29±2	30	0. 000
Streptococcus pneumonia	200	28±0	7.33±1±1.52	10±0	13	0.006
	400	29.33±1.155	8±3	12.33±2.517	13	0.008
	800	29.33±1.155	8±3	12.33±2.517	13	0. 000
Streptococcus pyogenes	200	21.33+1.528	15.67±1.528	26±1	30	0. 000
	400	24.33±1.528	16.67±2.309	27.33±3.055	30	0. 000
	800	30±1	17.67±0.577	29.67±1.155	30	0. 000

CIP = Ciprofloxacin (5µg/ml)

TABLE 4: MEAN ZONES OF INHIBITION OF DIFFERENT EXTRACTS OF *E. RACEMOSA* 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	14.67±1.528	11±2	15.33±3.215	26	0.001
	400	18.33±1.155	13±1	18.67±2.082	26	0.001
	800	25±2	15.33±1.528	22±2.646	26	0.001
Klebsiella pneumonia	200	9.67±0.577	16.67±2.517	22.67±0.577	18	-
	400	10±0	18±2.646	23±0	18	-
	800	12±0	22.33±1.155	25±1	18	0.004
Pseudomonas aeruginosa	200	20.33±0.577	10±1	17.33±1.528	19	0.001
	400	22.67±0.577	10.67±0.577	25±1	19	-
	800	30.33±0.577	11.1±1	26.67±1.528	19	0.001
Salmonella typhi	200	30.33±.577	23±3.6	28.67±2.08	30	0.009
	400	27.33±1.52	21.33±1.52	27.33±2.08	30	0. 000
	800	30.67±.477	25.67±2.52	30.67±1.52	30	0.009

CIP = Ciprofloxacin (5µg/ml), (-) = Statistically Not Significant

TABLE 5: MINIMUM INHIBITORY CONCENTRATIONS (MICS) AND MINIMUM BACTERICIDAL CONCENTRATIONS (MBCS) OF DIFFERENT EXTRACTS OF *E. RACEMOSA* ROOT EXTRACT 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	+	+	+	+	-	-	-	-	500	600
	Acetone	+	+	+	+	+	+	-	-	700	800
	Chloroform	+	+	+	+	-	-	-	-	500	600
S. pneumonia	Methanol	+	+	-	-	-	-	-	-	300	400
	Acetone	+	+	+	-	-	-	-	-	400	500
	Chloroform	+	+	+	+	-	-	-	-	500	500
S. pyogenes	Methanol	+	+	+	-	-	-	-	-	400	400
	Acetone	+	+	+	+	+	+	+	+	N	N
	Chloroform	+	+	+	-	-	-	-	-	400	500

N = Determined

TABLE 6: MINIMUM INHIBITORY CONCENTRATIONS (MICS) AND MINIMUM BACTERICIDAL CONCENTRATIONS (MBCS) OF DIFFERENT EXTRACTS OF *E. RACEMOSA* ROOT EXTRACT 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	600
	Acetone	+	+	+	+	+	+	+	-	800	N
	Chloroform	+	+	+	-	-	-	-	-	400	500
K. pneumonia	Methanol	+	+	+	+	+	+	-	-	700	700
	Acetone	+	+	+	+	+	+	+	+	N	N
	Chloroform	+	+	+	+	+	-	-	-	600	700
P. aeruginosa	Methanol	+	+	+	+	+	-	-	-	600	700
	Acetone	+	+	+	+	+	+	+	+	N	N
	Chloroform	+	+	+	+	-	-	-	-	500	600
S. typhi	Methanol	+	+	+	+	-	-	-	-	500	500
	Acetone	+	+	+	+	-	-	-	-	500	500
	Chloroform	+	+	-	-	-	-	-	-	300	400

N = Determined

CONCLUSION: Root extracts of *E. racemosa* demonstrated antibacterial activity against both gram positive and gram negative bacteria strains, possibly by bactericidal action. Flavonoids, glycosides, phenols, saponins, steroids, tannins, and triterpenes were detected in the extracts and may contribute to their antibacterial action. This anti-bacterial action may serve as partial justification to the traditional use of the plant against various infectious disorders.

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CONFLICT OF INTEREST: Nil

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