ANTIBACTERIAL SCREENING AND PHYTOCHEMICAL STUDY OF NINE MEDICINAL PLANTS FROM ERITREA

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Summary

Ethnobotanical survey was conducted in two administrative regions, Zoba Ma'akel and Zoba Semenawi Keih Bahri, Eritrea. Nine medicinal plants that are used to treat diseases associated with bacterial infection were collected and identified. Extracts were obtained using methanol, dichloromethane and hexane. Twenty seven crude extracts were screened for antibacterial activities against two Gram-positive and three Gram-negative bacteria using disk diffusion assay. Preliminary phytochemical screenings were also done for all plants. The results from this study demonstrated that the plants have antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Minimum inhibitory concentration value against the sensitive bacteria were also determined and found to be in the range of 8 - 250 µg/Disk. The result suggests that the extracts of these plants have a potential to treat bacterial infection. The preliminary phytochemical test revealed that the plants contain class of compounds which have a potential antibacterial activity. The use of the plants in Eritrean traditional medicine for disease associated with bacterial infection could be justified by their antibacterial activity.

Key-words: Antibacterial; Crude extracts; Eritrean traditional medicinal plants

Introduction

Infectious diseases are the most primitive types of diseases which challenge the survival of human beings (1). In treating such infections, which are mainly caused by microorganisms such as bacteria (2), human beings have identified the use of different herbs since ancient times (3). This practice, which evolved through a long process of trial and error, still passes from generation to generation (4, 5). According to the World Health Organization report, more than 80% of the people in Africa depend on traditional medicine for their health care needs (6). Moreover, over 50% of all modern clinical drugs are of natural product in origin (7). For example, many of the drugs currently used to treat bacterial and other infections were first isolated from natural sources including ethnomedicinal plants (8). Plant metabolites are proved to be the most important group of compounds with wide range of antimicrobial activity (9).

In Eritrea the use of herbs to treat different types of disease is a wide spread practice (10). However, little has been done to study the antibacterial activity of Eritrean flora. The present investigation is part of the study of Eritrean medicinal plants by Medicinal Plants and Drug Discovery Research Center of the University of Asmara. In the study, nine plants from four families were studied.

Ethnobotanical survey was conducted to identify plants used to treat disease associated with bacteria. The results of the ethnobotanical survey and documented use of the plants in Eritrean traditional medicine (11) provides the base for selecting the plants and parts of the plant used in this study. The leaves and aerial parts of selected plants with supposed antibacterial properties were collected. The crude extracts of the plants were tested for antibacterial activity and preliminary phytochemical studies were also conducted for all methanol extracts. The objective of the study was to validate the traditional use of the plants in Eritrean traditional medicine.

Materials and methods

Ethnobotanical survey and plant collection

Ethnobotanical survey was conducted from November to August 2006 in Zoba Ma'akel and Zoba Semenawi Keih Bahri areas included in the survey were: Asmara, Beleza, Shegrini, Betgirgish, Arberebu'a, Adi guadad, Mai-hinzi, and Ghinda. These areas are inhibited by Tigrinya, Tigre, and Saho ethnic groups. The ethnobotanical data was collected using digital record, free-listing, semi-structured and open-ended interviews, with traditional healers, and community elders.

The plant materials listed in Table 1 were collected from different places in Eritrea based on the information obtained from the ethnobotanical survey. Identification of the plants was done according to Flora of Ethiopia and Eritrea (12, 13, 14, 15). One species of *Sansevieria* could not be keyed out by the Flora of Ethiopia and Eritrea but it is identified temporally nearest to *sansevieria canaliculata* (16). Voucher specimens of the plants were deposited at the herbarium of the University of Asmara. Samples for laboratory investigation were air-dried in shade at room temperature (25-28 ^{o}C), for at least two weeks. They were then dried at 40 ^{o}C in an oven for seven days to completely remove residual moisture, before milling into fine powder. The powders were stored at room temperature for future use.

Table 1: Ethnobotanical data on the traditional usage of the nine selected traditional medicinal plants in Eritrea

Botanical name /Family	Part (tested)	Local name	Site of collection	Indications
Sansevieria erythraeae (Dracaenacea)	Aerial part	Tirmo-I'ka	Debrebizen	Ear infection
Sansevieria sp. cf *canaliculata* (Dracaenacea)	Aerial part	Tirmo-I'ka	Adi guadad	Ear infection
Sansevieria forskaoliana (Dracaenacea)	Aerial part	I'ka-habesha	Sheka wedi besrat	Skin disease
Croton macrostachyus (Euphorbiaceous)	leaf	Tambuk	Ghinda	Ascariasis and Skin disease
Acokanthera schimperi (Apocynaceae)	leaf	Mebtea	Ghinda	Skin irritation
Carissa spinarum (Apocynaceae)	leaf	Agam	Betgergish	wound healing
Salvia schimperi (Lamiaceae)	Aerial part	Aba-hadera	Godaif	Insect repellant and liver disease
Salvia merjamie (Lamiaceae)	Aerial part	Antateh-wollaka	Sembel	Throat inflammation (11)
Salvia nilotica (Lamiaceae)	Aerial part	Antateh-wollaka	Sembel	Sunburn, medicine after vomiting, and painkiller (11)

Test strains

For the antibacterial screening, three Gram-negative bacteria; *Escherichia coli* (ATTC 8739), *Pseudomonas aeruginosa* (ATTC 10145), *Salmonella typhimurium* (ATCC 14028) and two Gram-positive bacteria; *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633) were used.

Antibacterial screening

Bacterial strains were grown on Mueller-Hinton Agar (MHA) plates. Two to three colonies of bacteria were transferred into a tube containing 15 mL nutrient broth and grown overnight at 37 ^{o}C. The turbidity of the culture was adjusted with sterile saline solution to match 0.5 McFarland standards (17).

Sensitivity test was carried out using the disc diffusion assay (18). Agar plates were prepared using sterile MHA. Bacterial strains of standardized cultures were evenly spread onto the surface of the agar plates using sterile swab sticks. Crude extracts were dissolved by the solvent they were extracted. Sterile filter paper (Whatman no. 3) discs (6mm in diameter) were impregnated with 10 µL of each extract solution to have a concentration of 500 µg per disc and placed on the surface of inoculated MH agar plates. Test plates were then incubated at 37 ^{o}C for 18-24 hr. Discs soaked with methanol, dichloromethane and hexane were used as negative control and standard gentamicin disc (10 µg per disc) as positive control. All impregnated disks were dried using dried air before used in the antibacterial test. The diameter of any resulting zones of inhibition was measured in millimeter. The experiment was performed in triplicate.

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) for the extracts that showed activity was also determined using the disc diffusion assay (19). Sterile filter paper discs (6 mm in diameter) were impregnated with concentrations ranging from 4 to 500 µg per disc of plant extract and were placed on the surface of inoculated MH agar media. MIC was defined as the lowest concentration of the test samples that showed no visible growth on the agar (20).

Preliminary Phytochemical Screening

The crude extracts were subjected to various preliminary phytochemical tests for the presence or absence of different classes of compounds (21). Thin layer chromatography was developed using appropriate solvents. The TLC was dried in the open air to remove the solvent. Separated components were detected using suitable reagents. The characteristic colors were observed in UV-light.

Results and discussion

Results for the antibacterial activity of the plants and standard antibiotic, gentamicen are showen in Table 2. A total of twenty seven extracts representing nine plant species belonging to four families were screened for their antibacterial activity. Extracts from *Sansevieria* species had inhibitory activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Hexane extract of *Sansevieria erythraeae* were more active (12.7±0.5 mm) than its dichloromethane extract (10.0±0.8 mm) against *Staphylococcus aureus.* Extracts of *Sansevieria sp.* cf *canaliculata* both hexane (13.7±0.5 mm) and dichloromethane (10.7±0.5 mm) were more active than respective solvent extracts of *Sansevieria erythraeae* against *Staphylococcus aureus. Sansevieria forskaoliana* hexane (14.0±0.8 mm) and dichloromethane extracts (13.3±0.5 mm) showed the highest activity than all *Sansevieria* plants against *Staphylococcus aureus.* All the methanol extracts of the *Sansevieria* species were not active against any of the tested microorganisms. Hexane extract of *Sansevieria erythraeae* (10.7±0.5 mm), *Sansevieria sp.* cf *canaliculata* (8.2±0.2 mm) and *Sansevieria forskaoliana* (11.3±0.5 mm) were active against *Pseudomonas aeruginosa. Sansevieria forskaoliana* hexane extracts were the only active *Sansevieria* species plant extract against *Bacillus subtilis* (10.7±0.5 mm). Hexane extracts of *Croton macrostachyus* (9.3±0.5 mm), *Acokanthera schimperi* (13.7±0.5 mm), and *Carissa spinarum* (10.3±0.5 mm) were active against *Staphylococcus aureus.* Dichloromethane extract of *Acokanthera schimperi* (13.0±0.8 mm), and *Carissa spinarum* (13.0±0.8 mm) showed higher activity than *Croton macrostachyus* dichloromethane extract (8.3±0.5 mm) against *Staphylococcus aureus.*

Hexane and dichloromethane extracts of *Salvia schimperi* and *Salvia merjamie* were active against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis. Salvia merjamie* hexane and dichloromethane extracts had the highest activity (17.6±0.5 mm) against *Staphylococcus aureus* of all the tested extracts. *Salvia nilotica* dichloromethane extracts were also active against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis.* The highest activity *Salvia nilotica* extracts were observed with the dichloromethane extract (9.8±0.5 mm) against *Staphylococcus aureus.*

The results from this study demonstrated that the plants have antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis. Staphylococcus aureus* is the causative agent of most skin infection and septicemia. *Pseudomonas aeruginosa* is known to cause burn wound infection and urinary tract infection (22). *Bacillus subtilis* occasionally produces disease such as meningitis, endocarditis, endophthalmitis, conjunctivitis, or acute gastro-enteritis in immunocompromised patients (23). Moreover *Staphylococcus aureus* and *Pseudomonas aeruginosa* were found to be the major isolates from ear discharges

(24). These coincide with the traditional use of the plants in Eritrean traditional medicine. Treatment for ear infection, skin disease, throat inflammation, wound healing and liver diseases are some of the uses of the plants in Eritrean traditional medicine. Previous study from Ethiopia showed activity of *Acokanthera schimperi* 80% methanol extract against the same strain of *Staphylococcus aureus* (25); however in our study *Staphylococcus aureus* was not sensitive to methanol extracts of the plant leaves. The difference in activity could result from the solvent used, difference in extraction techniques, season of plant collection and the medium used in antibacterial screening (26).

Table 2: Antibacterial activity of selected plants[a]

Botanical name	Extract	Inhibition zones (mm) against				
		B.s	*E. c*	*P.a*	*S.a*	*S.t*
Sansevieria erythraeae	H	-	-	10.7±0.5	12.7±0.5	-
	D	-	-	-	10.0±0.8	-
	M	-	-	-	-	-
Sansevieria sp.	H	-	-	8.2±0.2	13.7±0.5	-
	D	-	-	-	10.7±0.5	-
	M	-	-	-	-	-
Sansevieria forskaoliana	H	10.7±0.5	-	11.3±0.5	14.0±0.8	-
	D	-	-	-	13.3±0.5	-
	M	-	-	-	-	-
Croton macrostachyus	H	-	-	-	9..3±0.5	-
	D	-	-	-	8..3±0.5	-
	M	-	-	-	-	-
Acokanthera schimperi	H	-	-	-	13.7±0.5	-
	D	-	-	-	13.0±0.8	-
	M	-	-	-	-	-
Carissa spinarum	H	-	-	-	10.3±0.5	-
	D	-	-	-	13.0±0.8	-
	M	-	-	-	-	-
Salvia schimperi	H	10.5±0.5	-	7.0±0.5	10.0±0.4	-
	D	8.3±1.0	-	8.0±0.0	8.8±1.0	-
	M	9.8±0.5	-	-	11.8±1.0	-
Salvia merjamie	H	14.0±0.3	-	11.3±0.2	17.6±0.5	-
	D	13.8±0.4	-	7.0±0.3	17.6±0.5	-
	M	-	-	-	-	-
Salivia nilotica	H	-	-	-	+	-
	D	9.3±0.5	-	8.6±0.0	9.8±0.5	-
	M	8.6±0.4	-	7.3±0.3	-	-
Gentamicin		23.5±0.3	22.5±0.5	18.7±0.4	20.3±0.4	27.7±0.2

[a]*S.a: Staphylococcus aureus* (ATCC 6538), *P.a*: *Pseudomonas aeruginosa* (ATTC 10145) *B.s*: *Bacillus subtilis* (ATCC 6633) *E.c*: *Escherichia coli* (ATTC 8739), *S.t: Salmonella typhimurium* (ATCC 14028), -: no activity,H: hexane, D: dichloromethane, M: methanol, concentration of extracts 500µg/disk, concentration of Gentamicin 10µg/disk.

The MIC of the active plant extracts (Table 3) range from 8 – 250 µg per disk. Hexane extracts of *Sansevieria forskaoliana, Acokanthera schimperi* and dichloromethane extracts of *Salvia merjamie* had MIC values of 8 µg per disk against *Staphylococcus aureus*. All the plant extracts under this investigation had no inhibitory activity against *Escherichia coli* and *Salmonella typhimurium.*

Table-3: MIC value for Antibacterial activity of selected plants in µg/Disk

Botanical name	Extract	MIC value in µg/Disk		
		B.s	*P.a*	*S.a*
Sansevieria erythraeae	H	-	250	31
	D	-	-	31
Sansevieria sp.	H	-	250	62
	D	-	-	31
Sansevieria forskaoliana	H	62	250	8
	D	-	-	31
Croton macrostachyus	H	-	-	62
	D	-	-	125
Acokanthera schimperi	H	-	-	8
	D	-	-	16
Carissa spinarum	H	-	-	62
	D	-	-	62
Salvia schimperi	H	31	125	125
	D	125	125	125
	M	62	-	62
Salvia merjamie	H	250	16	16
	D	16	31	8
Salvia nilotica	H	-	-	62
	D	125	16	16
	M	125	250	-

The results of the phytochemical analysis show that all the investigated plants contain steroids, triterpenoids, phenols, tannins, phloroglucocides, coumarins, anthranoids, and flavonoids. Alkaloids were detected only from the three *Sansevieria* species plant extracts.

Table 4 Phytochemical screening results of the plants[b]

Class of compounds	Reagent used	S.e	S.sp	S.f	C.m	A.s	C.s	S.s	S.m	S.n
Steroids and Triterpenoids	Sulfuric acid and acetic anhydride ethanol	+	+	+	+	+	+	+	+	+
Phenols, Tannins and Phloroglucocides	Ferrocyanide-Ferric chloride	+	+	+	+	+	+	+	+	+
Coumarins and Anthranoids	5% KOH in ethanol	+	+	+	+	+	+	+	+	+
Flavonoids	10% Antimony chloride solution	+	+	+	+	+	+	+	+	+
Alkaloids	Dragendorff's reagent	+	+	+	-	-	-	-	-	-

[b] S.e : *Sansevieria erythraeae;* S.sp: *Sansevieria* sp.; S.f: *Sansevieria forskaoliana;* C.m: *Croton macrostachyus;* A.s: *Acokanthera schimperi;* C.e: *Carissa spinarum;* S.s: *Salvia schimperi;* S.m: *Salvia merjamie;* S.n: *Salvia nilotica*

Secondary metabolites have proven to be medicinal in nature. They have various protective and therapeutic effects which prevent diseases and maintain a state of well being (27). Phytochemical screening results of different medicinal plants revealed that they contain therapeutically essential secondary metabolites (28) which could account for the antibacterial activity of the plants.

In conclusion, the study suggests that the plants investigated have useful antibacterial activity. The use of the plants in Eritrean traditional medicine for disease associated with bacterial infection could be justified by their antibacterial activity. However there is a need for further investigation of the plants to identify and isolate antibacterial principles.

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