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ANTIMICROBIAL ACTIVITY & PHYTOCHEMICAL SCREENING OF EXTRACTS OF THREE DIFFERENT ERITREAN MEDICINAL PLANTS

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ABSTRACT

Plants and plant-based products are the bases of many of the modern pharmaceuticals we utilize today for the treatment of a variety of ailments. The objective of this study was to determine the bioactive chemical constituents and to evaluate extracts of three Eritrean medicinal plant's leaves, stem and root for *in vitro* antimicrobial activities by using well diffusion method. Three medicinal plants that are used to treat diseases associated with bacterial and fungal infections were collected and identified. Extracts were obtained using water, alcohol and acetone. The *in vitro* antimicrobial activity of crude alcoholic, acetone and water extracts of the plants against bacterial and fungal microbes were investigated using well diffusion method. The extracts exhibited antimicrobial activities with zones of inhibition ranging from 13 to 24, 15 to 23 and 6 to 26 mm for alcohol, acetone and water extracts respectively. Minimum inhibitory concentration of eight selected plant extracts that showed good activity was also determined using the broth serial dilution technique. More over the minimum bactericidal concentration was performed by sub culturing from the serial dilutions prepared for the MIC. Phytochemical screening revealed the presence of saponin, steroids, tannin, glycosides, alkaloids and flavonoids in the extracts. The ability of the crude extracts of these plants to inhibit the growth of bacteria and fungi is an indication of their broad spectrum antimicrobial potential which may be employed in the management of microbial infections and they could be a potential source of antimicrobial agents.

Keywords: ***Caralluma speciosa*, *Corolia africana* and *Myrica aboria*, phytochemical screening, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).**

INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Medicinal plants represent a rich source from which antimicrobial agents may be obtained. Plants are used medicinally in different parts of the world and are a rich source of many potent and powerful drugs [1] .Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world. Moreover, over 50% of all modern clinical drugs are of natural product in origin [2]. For example, many of the drugs currently used to treat bacterial and other infections were first isolated from natural sources including ethno medicinal plants [3]. Plant metabolites are proved to be the most important group of compounds with wide range of antimicrobial activity [4].

In Eritrea the use of herbs to treat different types of disease is an ordinary practice . However, little has been done to study the antibacterial activity of Eritrean flora. In the present study, three Eritrean traditional medicinal plants with a reputation for healing various infectious ailments were investigated. Interviews with traditional healers and community elders and documented use of the plants in Eritrean traditional medicine provides the base for selecting the plants and parts of the plant used in this study [5, 6]. The leaves, seeds, barks and root 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 types of extracts. The objective of the study was to validate the traditional use of these plants in Eritrean traditional medicine and try to preserve those plants which are found to have antimicrobial activity.

Materials and methods

Plant collection

The plant materials listed in Table 2 were collected based on the information obtained from traditional healers and community elders through open-ended interviews, in areas of Asmara (tselot), Shegrini, Betgirgish and Nefasit**.** These areas are populated by Tigrinya, Tigre, and Saho ethnic groups. Identification of the plants was done according to Flora of Ethiopia and Eritrea. Voucher specimens of the plants were deposited at the herbarium of the Asmara College of Health Sciences, School of Pharmacy. Samples for laboratory investigation were air-dried in shade at room temperature (25-28 ° C), for at least two weeks. They were then dried at 40°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. At the end 5 mg of the powder was dissolved in 10 ml of the respective solvent to prepare a final concentration of 500µg/ml of the extract solution.

Table 2: Ethno botanical profile on the traditional usage of the three selected traditional medicinal plants in Eritrea

Botanical name /Family name	Local name	Parts used	Site of collection	Indications	Voucher Number
Caralluma speciosa	Ango Harmaz	Leaf , whole plant	Tselot, Betgirgish,	Wound healing, Ear infections	CS-12
Corolia africana	Awhi	Leaf, seed and bark	Shegrini	Wound healing,	CA-23
Myrica aboria	N`hba	Leaf , whole plant	Nefasit	Wound healing, Tonsillitis	MA-17

Test strains

For the antibacterial screening *Escherichia coli* (ATTC 8739), *Pseudomonas aeruginosa* (ATTC 10145), *Staphylococcus aureus* (ATCC 6538) and *Candida albicans* (SC 5314) 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°c. The turbidity of the culture was adjusted with sterile saline solution to match 0.5 McFarland turbidity standards [7].

Sensitivity test was carried out using the well diffusion assay [8]. Agar plates were prepared using sterile MHA. Wells of 6mm in diameter were bored using a sterile well borer. 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 and then 40ul of the extracts were poured in to the respective holes. Test plates were then incubated at 37°c for 18-24 hrs. Holes filled with alcohol, acetone and water were used as negative control. 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 broth dilution method [9]. Serial dilutions of the plant extracts were prepared in a liquid broth which is inoculated with a standardized number of organisms and incubated for a prescribed time. The lowest concentration (highest dilution) of extract

preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). Additionally, minimum bactericidal concentration was also performed on some selected plants by sub culturing three dilutions (the MIC dilution and two higher dilutions). MBC was defined as the lowest concentration of the test samples that showed no visible bacterial colonies on the agar plate.

Preliminary Phytochemical Screening

Identification tests for the various chemicals were carried out to test the presence of various chemical constituents. The crude extracts were subjected to various preliminary phytochemical tests for the presence or absence of different classes of compounds [10]. Thin layer chromatography (TLC) 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.

Identification Tests

The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannin by the following procedure:

Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added [11]. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent [12].The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation

Glycoside

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer [11].

Terpenoids and steroid

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violetcolor was observed for terpenoids and green bluish color for steroids [11].

Flavonoids

Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. Thesolution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids andorange color for flavones [11].

Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins [13].

Reducing Sugar

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

3. Results and discussion

Results for the antibacterial activity of the plants, the organic solvents and the standard antibiotic gentamicin are shown in Tables 1,2,3 & 4. A total of seventeen extracts representing three plant species and two negative controls were screened for their antibacterial activity. Extracts from *Myrica aboria* had a better inhibitory activity against *Staphylococcus aureus* and *Candida albicans* than against the other microorganisms. *Myrica aboria* Leaf acetone was more active than the other extracts against all the tested micro-organisms. Alcohol extracts of *Corolia africana* seed were more active than alcohol extracts of *Corolia africana* bark and leaf against *Staphylococcus aureus*. Both acetone and alcohol extracts of *Corolia africana* seed were found to be highly effective against *Candida albicans.* The activity of *Corolia africana* against E.coli was not satisfactory in which antimicrobial activity was only seen in the seed extracts of the plant, with the acetone and alcohol extracts showing inhibition zone of 14mm and 10mm respectively and the alcohol extracts of both leaf and bark showing no activity. The highest activity of the plant *Corolia africana* was seen in acetone extract of its leaf which showed an activity of 38mm against *E.coli.*

Caralluma speciosa was the weakest plant among the tested plant species against almost all the tested microorganisms, showing no activity against *E.coli* in all its three extracts. Moreover its antimicrobial activity against the other tested microorganisms was not much satisfactory against the other microorganisms. Its highest activity was seen against *Candida albicans* with 18mm in its acetone extract.

The results from this study demonstrated that the plants have antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. *Staphylococcus aureus* is the causative agent of most skin infections and septicemia in addition it is responsible for most secondary bacterial infections. *Pseudomonas aeruginosa* is known to cause burn wound infection and urinary tract

infection [13]. *Candida albicans* is a diploid fungus (a form of yeast) and a causal agent of opportunistic oral and genital infections in humans[14,15]. Systemic fungal infections (fungemias) have emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). Despite a difference in their concentrations, as seen in the tables below, the antimicrobial activity of the most active plant extracts was almost comparable to that of the standard antibiotic gentamicin hence indicating their potential as a source of antibiotics in pharmaceutical industries.

Moreover, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were found to be the major isolates from ear discharges [16]. 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. It should be noted that antimicrobial activity is dependent on the solvent used, difference in extraction techniques, season of plant collection and the medium used in antibacterial screening. So any difference of activity of the plants tested in any other previous studies made, if present, may be attributed to the above factors [17].

Table1. Results showing the inhibition zones of the different extracts of *Myrica aboria* against the four microorganisms tested

Plant Extract	Microorganisms Tested			
	S.aures	***E.coli***	***Candida albicans***	***Pseudomonas aeuroginosa***
***Myrica aboria* water**	26mm	-	24mm	---
***Myrica aboria* leaf alcohol**	17mm	-	25mm	11mm
***Myrica aboria* leaf acetone**	23mm	15mm	21mm	16mm
***Myrica aboria* water**	17mm	12mm	-	11mm
Myrica aboria leaf alcohol	24mm	10mm	19mm	17mm

*Concentration of extracts is 500µg/ml;

Table2. Results showing the inhibition zones of the different extracts of *Corolia africana* against the four microorganisms tested

Plant Extract	Microorganisms Tested			
	S.aures	***E.coli***	***Candida albicans***	***Pseudomonas aeuroginosa***
***Corolia africana* leaf alcohol**	16mm	-	18mm	16mm
***Corolia africana* leaf acetone**	16mm	38mm	-	-
***Corolia africana* leaf water**	-	-	22mm	-
***Corolia africana* seed acetone**	15mm	14mm	24mm	15mm
***Corolia africana* seed alcohol**	24mm	10mm	23mm	14mm
***Corolia africana* seed bark**	-	-	21mm	15mm
***Corolia africana* bark acetone**	18mm	10mm	12mm	16mm
***Corolia africana* bark alcohol**	22mm	-	20mm	17mm
***Corolia africana* bark water**	18mm	17mm	26mm	--

*Concentration of extracts is 500µg/ml;

Table3. Results showing the inhibition zones of the different extracts of *Caralluma speciosa* against the four microorganisms tested.

Plant Extract	Microorganisms Tested			
	S.aures	***E.coli***	***Candida albicans***	***Pseudomonas aeuroginosa***
Caralluma speciosa alcohol	13mm	--	16mm	12mm
Caralluma speciosa water	16mm	-	16mm	12mm
***Caralluma speciosa* acetone**	16mm	-	18mm	16mm

*Concentration of extracts is 500µg/ml;

Table 4. Results showing the inhibition zones of the positive and negative controls against the four microorganisms tested

Solvent used	Microorganisms Tested			
	S.aures	***E.coli***	***Candida albicans***	***Pseudomonas aeuroginosa***
Gentamicin	19mm	20mm	21mm	17mm
Acetone(Negative control 1)	9mm	7mm	-	11mm
Alcohol(Negative control 2)	13mm	9mm	-	-

*Concentration of gentamicin is 10µg/disc;

An antimicrobial agent with high activity against an organism yields a low MIC value while a low activity against an organism has a high MIC value. The MIC and MBC is normally used to evaluate the efficacy of the agents such as antiseptics, disinfectants and indeed chemotherapeutic agents. Under standard conditions they also support the sensitivity test results [18]. In order for an antibiotic to be effective an MIC or MBC must be able to be achieved at the site of the infection. Here in this study the MIC was performed on some selected plant extracts by the use of broth serial dilution method. The MIC of all the extracts tested was less than 250 µg/ml indicating that the extracts posses good antimicrobial activity. Moreover MBC was also performed on these extracts and satisfactory results were obtained as shown in table 3.

Table 4. Results of the MIC values obtained by the serial dilution method

Plant extracts	Organism tested	MICµg/ml	MBC µg/ml
Myrica aboria alcohol	*S.aures*	31.25	62.5
Myrica aboria leaf acetone	*S.aures*	31.25	62.5
Myrica aboria leaf water	*E.coli*	62.5	125
Corolia africana seed alcohol	*S.aures*	62.5	250
Corolia africana bark alcohol	*E.coli*	62.5	125
Corolia africana leaf alcohol	*E.coli*	62.5	125
Caralluma speciosa seed water	*S.aures*	125	250
***Caralluma speciosa* leaf alcohol**	*E.coli*	125	250

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies [19]. Chemical constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents [13].

The preliminary phytochemical screening of the extracts using different solvent is presented in Tables 5, 6 & 7. The water and alcohol extracts of *Caralluma speciosa* were found to have bioactive chemical constituents like alkaloids and saponin. It was only in acetone extracts that triterpenes was observed. In the water extract, flavonoids and tannins were present which were absent in the organic extracts. This finding gives credibility to the traditional medicinal application of the plants as remedies for internal and external wounds and infections and revealed their potential in the management of wound infections. But this needs further investigation.

Table 5: Phytochemical screening of *Caralluma speciosa* root extracted with different solvents

Phytochemical screened	Acetone	Alcohol	Water
Alkaloids	-	++	+++
Flavonoids	-	-	+
Tannins	-	-	+
Saponins	-	++	++
Terpenoids	-	-	+
Cardiac glycoside	-	-	-
Voltaic oils	++	+	-
Fatty acids	-	+	-
Phenolics	+	-	-
Steroid	+	+++	+++

*N.B "+++" Indicates the presence of the phytochemical in an appreciable amount ''++"present in a moderate amount, "+"present in a trace amount; "-"indicates absence of the phytochemical.

As indicated in table 6 below, the water and alcohol extracts of *Corolia africana* were found to have alkaloids, voltaic oils and steroids. It was only in acetone extracts that triterpenes

was observed. In the water extract, flavonoids was present which was absent in the organic extracts. This finding gives reliability to the traditional medicinal application of the plants as remedies for internal and external wounds and infections and revealed their potential in the treatment of wound infections.

Table 6: Phytochemical screening of *Corolia africana* root extracted with different solvents

Phytochemical screened	Acetone	Alcohol	Water
Alkaloids	-	+++	+
Flavonoids	-	-	++
Tannins	++	-	-
Saponins	-	+	-
Terpenoids	+	-	-
Cardiac glycoside	+	-	-
Voltaic oils	_	++	+++
Fatty acids	-	+	-
Phenolics	+	-	+
Steroid	++	++	+

*N.B "+++" Indicates the presence of the phytochemical in an appreciable amount ''++"present in a moderate amount, "+"present in a trace amount; "-"indicates absence of the phytochemical.

The water extracts of *Myrica aboria* were found to have alkaloids, Flavoring, Tannins, Terterpenes, Phenolics and saponin. It was only in acetone extracts that Cardiac glycoside was observed. In the alcohol extract, Fatty acids, Voltaic oils were present which were absent in the other extracts. This finding gives credence to the traditional medicinal application of the plants as remedies for internal and external wounds and infections and revealed their potential in the treatment of wound infections.

Table 7: Phytochemical screening of *Myrica aboria* root extracted with different solvents

Phytochemical screened	Acetone	Alcohol	Water
Alkaloids	-	-	++
Flavonoids	-	-	+
Tannins	-	-	+
Saponins	-	++	+++
Terpenoids	-	-	+
Cardiac glycoside	+	-	-
Voltaic oils	-	++	-
Fatty acids	-	+	-
Phenolics	-	-	+
Steroid	+++	+++	+++

*N.B "+++" Indicates the presence of the phytochemical in an appreciable amount ''++"present in a moderate amount, "+"present in a trace amount; "-"indicates absence of the phytochemical.

In this study, we used both polar and non-polar solvents for the extraction of active compounds from the three medicinal plants and have demonstrated the presence of antibacterial phytochemicals. Several phytoconstituents like flavonoids, Phenolics, tannins and terpenoids are effective antimicrobial substances against a wide range of microorganisms [20, 21].Hence their presence in the extracts of the tested plants is a good indication of their range of antimicrobial spectrum.

DISCUSSION

The antimicrobial activity has been screened because of their great medicinal relevance during the recent years. Moreover, increase of infections and resistance against antibiotics has become an ever increasing therapeutic problem [22]. The presence of antifungal and antimicrobial substances in the higher plants is well established as they have provided a source of inspiration for novel drug compounds and plant derived medicines have made significant contribution towards human health. The present study was conducted to analyze the phytochemical constituents and antibacterial potential of extracts of different parts of three Eritrean medicinal plants.

Knowledge of the phytochemical constituents of the plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, flavonoids, saponins, essential oils precursors for the synthesis of complex chemical substances [23]. The results of phytochemical screening of extracts of the plants indicate that in order to achieve positive results, the strength of active principle depends on the use of a suitable solvent besides the type of the plant species.

The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobial represents the vast unexploited source for medicine. Plant based antimicrobials have enormous therapeutic potential as they can survive the purpose without any of the side effects that are often associated with synthetic antimicrobials. Continued further research and exploration of plant derived antimicrobials is needed today. Medicinal plants are important source for the development of potential, new chemotherapeutic drugs and the in vitro antibacterial test forms the basis [1, 24].

Many plants have unlimited ability to synthesize secondary metabolites of which many have been isolated. These substances serve as plant defense mechanism against predation by microorganisms, insects and herbivores. Many plants and their extracts are used against microbial infections due to the presence of secondary metabolites such as phenols, essential oils, terpenoids, alkaloids and flavonoids. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Natural products either extracts or pure compounds provide unlimited opportunity for the development of new drugs due to the availability of chemical diversity [25]. To overcome the problem of antibiotic resistance, ethnic medicinal plants have been extensively studied as an alternative treatment for diseases due to their ability to produce a variety of compounds of known therapeutic properties [26, 27] and much consideration has been given to plant extracts and their biologically active compounds [28]. The screening of natural products has been the source of numerous therapeutic agents [29]. The use of higher plants as a source for new potential drugs is still largely unexplored and only a small percentage of them have been subjected to phytochemical investigation and the fractions submitted to pharmacological screening are very low. Such screening of various natural organic compounds and identifying active agents is a need of the hour as due to successful prediction of lead molecules and drug like properties at the inception of drug discovery will pay of later in drug development.

CONCLUSION

Studies on plant extracts could be an alternative for better therapeutic agents from natural sources which are supposed to be more proficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The present study verified the traditional use of *Caralluma speciosa, Myrica aboria* and *Corolia africana* for human ailments and partly explained its use in herbal medicine as rich source of phytochemicals with the presence of tannins, saponins, steroids, flavonoids and terpenoids. Compared to reference antibiotics, the spectrum of antibacterial activity of most of the investigated plant extracts was found to be clearly superior. Thus these plants can be utilized as an alternative source of useful drugs. The demonstration of broad spectrum of *Caralluma speciosa, Myrica aboria* and *Corolia africana* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. The effect of these plants on more pathogenic organisms, and toxicological investigations and further purification, characterization and elucidation of the structure of the bioactive compounds of these plants for industrial drug formulation, however, need to be carried out.

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