Antimicrobial Activity evaluation and phytochemical screening of *Silene macrosolen* and *Solanum incanum*: A common medicinal plants in Eritrea.

Bilen Ateshim

Orotta college of medicine and health sciences

Frenai Tekle

Orotta college of medicine and health sciences

Meron Tesfay

Orotta college of medicine and health sciences

Muzit Tekleab

Orotta college of medicine and health sciences

Rahel Tekie

Orotta college of medicine and health sciences

Saimon Weldemariam

Orotta college of medicine and health sciences

Oliver Okoth Achila

Orotta college of medicine and health sciences

Samuel Tekle Mengistu (✉ teklesam7@gmail.com)

Orotta college of medicine and health sciences

Mohammed Elfatih Hamida

Orotta college of medicine and health sciences

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Abstract

Medicinal plants play great roles in the treatment of various infectious diseases. *S.macrosolen* and *S.incanum* are both important medicinal plants used traditionally for treatment of infectious diseases in many places around Eritrea. The periodically emerging new and old infectious microorganisms greatly magnify the global burden of infectious diseases. The majorities of emerging infectious events are caused by bacteria which can be associated with evolution of drug resistant strains and overwhelming of the natural host defenses. Therefore, the search of new or alternative mechanisms to effectively treat and prevent infectious diseases, particularly bacterial diseases, have to be encouraged to effectively reduce these global burden. The objective of the study is to evaluate the in vitro antibacterial activities of the aqueous and solvent crude extract of leaf and stem of *S.macrosolen*, and leaf and root of *S.incanum* against standard strains of selected bacterial species, which can in turn provide a clue for the identification of active constituent responsible for the antibacterial activity. The antibacterial activity of the aqueous (cold and hot water) and solvent extracts (ethanol, methanol, and chloroform) were evaluated on different selected bacterial strains (*E.coli*, *S.aureus*, and *Paeruginosa*) using agar well diffusion method on Mueller-Hinton agar at different concentration with the presence of positive control (Chloramphenicol and ciprofloxacin) and negative control (sterile distilled water and 5%DMSO) controls. The highest inhibition zone was observed for methanol extracted *S.macrosolen* stem and chloroform extracted *S.incanum* root against *S.aureus* at 400mg/ml with 23mm and 24.5mm

respectively. Methanol and cold aqueous extracted *S.macrosolen* stem also showed the highest inhibition of 26mm, 23mm diameter, against *Paeruginosa*, and *E.coli* respectively. The reason for the high inhibition zone could be due to the presence of secondary metabolites such as saponins, tannins, flavonoids, phenols and glycosides. The least result was seen in hot aqueous extract for each plant with no inhibition for all the bacteria. MIC and MBC was determined using tube dilution and plating method for those plant extracts which showed highest and consistent inhibition zone at different concentrations. The MIC and MBC of cold aqueous extract of *S.macrosolen* stem was found at 25mg/ml, and 50mg/ml respectively, against both *E.coli* and *Paeruginosa*, while the MIC of chloroform extracted *S.incanum* root was found at 50mg/ml, however, the MBC was not determined in the concentration tested against *S.aureus*. The paper published after getting the results of the investigation would be anticipated to contribute for the resolution of the burden of the drug resistant bacteria species.

1. Introduction

Human kind has been exposed to infection by microorganisms since before the dawn of recorded history [1]. In treating such infections, mainly bacterial, human beings have identified the use of different herbs since ancient times [2]. The knowledge on plant use is the result of many years of man's interaction and selection on the most desirable, the most vigorous and the most successful plants present in the immediate environment at a given time [3–5]. The continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action has been greatly increased due to the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use [6]. Plants containing medicinal properties have been known and used in some form or other, even by primitive people. Owing to the realization of the toxicity associated with the use of antibiotics and synthetic drugs, which are too costly to be practical for the majority diseases caused by microorganisms [7], developed countries are increasingly becoming aware of the fact that drugs from natural sources are safer and affordable. Therefore, an upsurge in the use of products based on plants is exposed, especially in the field of health care products [7]. Developing countries are rich in medicinal and aromatic plants (MAPs) but, due to difficulty in accessing efficient extraction technologies, value

addition to this rich bio resource is difficult. In most cases, and particularly in very poor countries, the technologies used are inappropriate and not economical. The crucial problem is related to the quality of the product: primitive extraction technologies do not guarantee a stable and high-quality product and, in some cases, inappropriate technologies and procedures result in producing contaminated product which has low market value. In order to assist developing countries to achieve the objective of using rich MAP resource for producing value-added products, dissemination of knowledge of existing extraction technologies and of the latest developments in these technologies is essential [8]. Plant based traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care [9]. Studies on natural products are therefore aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses, and discovery of potential chemotherapeutic agents [10]. With the increasing demand for herbal medicinal products, nutraceuticals, and natural products for health care all over the world, medicinal plant extract manufacturers and essential oil producers have started using the most appropriate extraction technologies in order to produce extracts and essential oils of defined quality with the least variations from batch to batch. Such approach has to be adopted by MAP-rich developing countries in order to meet the increasing requirement of good quality extracts and essential oils for better revenue generation within the country, as well as for capturing this market in developed countries. The basic parameters influencing the quality of an extract are the plant parts used as starting material, the solvent used for extraction, the manufacturing process (extraction technology) used with the type of equipment employed, and the crude-drug: extract ratio. The use of appropriate extraction technology, plant material, manufacturing equipment, extraction method and solvent and the adherence to good manufacturing practices certainly help to produce a good quality extract. From laboratory scale to pilot scale, all the conditions and parameters can be modeled using process simulation for successful industrial-scale production. With the advances in extraction technologies and better knowledge for maintaining quality parameters, it has become absolutely necessary to disseminate such information to emerging and developing countries with a rich MAP biodiversity for the best industrial utilization of MAP resources [8]. In Eritrea the use of herbs to treat different types of diseases is a wide spread practice. However, little has been done to study the antimicrobial activity of Eritrean vegetation.

S. macrosolen is a glabrous, pale or somewhat glaucous perennial, branching from the stock; flowering-stems erect, simple or forking, apparently a little viscid above, 2–3 ft high, often somewhat woody at the base, growing 60 - 90cm tall. The plant is gathered from the wild for local medicinal use. Its location ranges from Northeast Africa - Sudan to Ethiopia and Eritrea, south to Tanzania. It mainly grows in Well-watered grassland, rocky places, and volcanic soils; at elevations of 1,800 - 3,300 meters. The root, known as 'Radix ogkert' or 'Sarsari', is used in the treatment of tapeworms, crushed and pounded root in half index finger size is drunk by tea glass and if the risk comes, powder of *Linum usitatissimum* infusion in water is taken as reducing pain. The dried root of *Silene macrosolen* is smoked to make snakes away and treat "Evil eye" [11]. In Eritrea it is traditionally used for the treatment of segri, gerefta (viral infection), and gonfi. The stem of *Silene macrosolen* is also used for fumigation of house [12].

Solanum incanum is densely stellate-tomentose, shrub 3-5 feet. High. Leaves sinuate, ovate or ovate – elliptic, obtuse at the apex, unequal at the base, up to 7 inches (in) long 6 in broad, dark-green above, paler beneath, densely stellate – hairy on both surfaces. Although likely to be native to the three countries, *Solanum incanum* is invasive in parts of Kenya, Uganda and Tanzania [13]. Mostly as a weed of disturbed and overgrazed areas and road sides, but also found in various types of woodland, and along the margins of riverine and evergreen forest [14]. *S. incanum* is effective for control of cattle ticks when used as water extracted (World Agro- forestry Center, 2014). It is one of the important traditional medicinal plants which almost depend on its analgesic properties. The fruit of *S. incanum* are used in Kenya for treatment of skin mycotic infections. The leaves are used in composite and lower the risk of high

blood pressure, stroke and heart disease. Throughout tropical Africa a sore throat, angina, stomach-pain, colic, headache, painful menstruation and liver pain are treated with *S.incanum*.

In this Study aqueous (hot and cold), ethanol, methanol, and chloroform leaf and root extracts of *S.incanum* as well as stem and root extracts of *S.macrosolen* were tested in-vitro for their antibacterial activity. The bacteria used were standard strains of *E.coli*, *S.aureus*, and *P.aeruginosa*.

2. Methods And Materials

2.1. Study Design

The study was an invitro-experimental study done in Asmara College of health sciences (ACHS) on certain Eritrean local plants to assess their antibacterial activity. Upon running the experiment, there were both control and experimental groups to maintain the reliability of the results. The extraction procedures were performed in Clinical Chemistry laboratory of ACHS; Media preparation and AST procedures were conducted majorly in Microbiology department of National health laboratory (NHL) and partly in National drug quality control (NDQC); and MIC and MBC were carried out in NDQC.

2.2. Collection of Plant Materials and Extraction

Information regarding to the ethno botanical uses of *S.incanum* and *S.macrosolen* were collected after interviewing traditional users and local people in South-west sub zone of zoba Maekel around Villago and Bet-mekae, and Northern Red sea region around Mai-Habar respectively .The plant *S.incanum* (Root and Leaf) was collected from the fields of Villago and Bet-mekae whereas *S.macrosolen* (Stem and Root) was collected from Northern Red sea region around MaiHabar. The collection was made in February to march 2018 and was identified at the Department of Plant Biology herbarium, EIT, Mai-nefhi, Eritrea by three senior botanists. The collected roots, stem and leaves were washed with water thoroughly to free from debris. The roots of *S.incanum* and *S.macrosolen* were sliced and shade dried for three weeks and the leaf of *S.incanum* and the stem of *S.macrosolen* were shade dried for two weeks. Then they were grounded finely by using dry grinder and passed through a sieve and stored for further use in a tightly closed container.

Different extraction solvents namely aqueous (cold and hot), ethanol, chloroform as well as methanol were used for the preparation of plants extracts to be used against bacteria. 100 grams of each powdered plant material was mixed with a respective 2000 ml of extraction solvent. The mixture was then kept in an agitator for 3 days with occasional shaking for the cold extract, whereas the hot aqueous extract was kept in a water bath at 80oC for 4 hours with continuous stirring. The extracts were then filtered using Whatmann filter paper and were concentrated using Rotary evaporator so as to obtain the crude extract and then they were kept in sterile bottles under refrigerated conditions until use.

The crude extracted which was concentrated using rotary evaporator, was reconstituted by distilled water for aqueous extract, 5% DMSO for methanol and ethanol, and 1 00% DMSO for chloroform extract. This was done by weighting One gram of each dried powdered crude extract of leaves, stems and roots in analytical balance and mixing it with respective reconstituents to make a total solution of 2.5ml thereby preparing a stock solution of 400mg/ml, which is a standard concentration. Different concentrations were then prepared from the stock solution i.e. 200mg/ml and 100mg/ml. The crude extract solutions from *S.incanum* and *S.macrosolen* was stored inside sterilized bottle and kept in the refrigerator at 4°C until used for the antibacterial 16 test. The extracts were tested for sterility by plating it on Muller Hinton agar and incubating for 24hrs at 37°C.

2.3. Phytochemical Analyses

Phytochemical screening was done in order to detect the presence of plant constituents such as alkaloids, flavonoids, saponins, and tannins, in the plant extract. A portion of the extract was used to test for the following plant constituents: flavonoid, saponins, and tannins, phenols, glycosides and proteins using the methods described by kokate (2001) and Harbone (1998).

Tests	Procedures
Flavonoids	To 1mL of the extract, a few drops of dilute sodium hydroxide were added. An intense yellow color was observed, which become colorless on the addition of few drops of dilute HCl acid, which indicates the presence of flavonoids. The presence of flavonoids was also confirmed by another test i.e. few drops of 10% ferric chloride solution were added to 1mL of plant extract. A green or blue color indicated the presence of phenolic nucleus.
Saponins	In a test tube containing about 5ml of plant extract of, a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3minutes. A honey comb like froth formation confirms the presence of saponins.
Tannins	Five ml of the plant extract and a few drops of 1% lead acetate were mixed. A yellow precipitate was formed, which indicates the presence of tannins.
Phenols	(a) Two ml of distilled water followed by drops of 10% aqueous FeCl_3 solution were added to 1ml of the extract. Formation of blue or green indicates the presence of phenols. (b) One ml of the plant extract was diluted to 5ml solution with distilled water and to this few drops of 1% aqueous solution of lead acetate was added. A yellow precipitate was formed which indicates the presence of phenols.
Glycosides	A small amount the extract was dissolved in 1ml of water and aqueous sodium hydroxide solution was added. Formation of yellow color indicates the presence of glycosides.

2.4. Antimicrobial Activities of the Extracts

2.4.1. Acquisition of bacterial strains

The study was emphasized on three Standard strains of both gram positive bacteria (*S.aureus*- 25922) and gram negative bacteria (*E.coli*- 25923, and *Paeruginosa*- 27853) with their specific American Type culture collection (ATCC) numbers. They were collected from the Department of Microbiology in NHL and Fred hollows. The bacterial species were cultured in the selective media for confirmation of their presence. Then they were maintained in nutrient agar slopes and stored in the refrigerator at a temperature of 4°C.

2.4.2. Preparation and standardization of Bacterial Inoculums

Standardization of bacterial inoculums was done by picking five colonies of each organism into a normal saline to form the bacterial suspension and thus should be used within 15 min. The microbial inoculum was standardized at 0.5 McFarland. In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range i.e. 1.5×10^8 CFU/ml. Original McFarland standards were made by mixing specified amounts of barium chloride and sulphuric acid together. Mixing the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. A 0.5 McFarland standard is prepared by mixing 0.05 ml of 1.175% barium chloride dehydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$), with 9.95 ml of 1% sulfuric acid (H_2SO_4). The standard could be compared visually to a suspension of bacteria in sterile saline or nutrient broth [15].

2.4.3. Preparation of Nutrient Media

Mueller Hinton agar, Nutrient agar and broth, Mac Conkey, and Mannitol salt agar were used according to Kumar et al 2011. Different amounts of the different Medias were mixed with distilled water according to the instruction written on the media bottles and then sterilized in autoclave at 121°C and 151 Barr pressure for 15 minutes. The sterilized media were allowed to cool to a temperature of about 50°C and dispensed into Petri dishes inside the safety cabinet to make a media of 4mm thickness. The solidified plates were kept in the refrigerator of about 2-8°C. Then the plates become ready to be used for the antibacterial studies.

2.4.4. Test for Antibacterial Activity of the Extracts in agar well diffusion assay

Sterile Muller Hinton agar plates were prepared and a well of about 6.0mm diameter with sterile cork borer was aseptically punched on each agar plate to make 5 ditches or wells on the plates. A sterile cotton swab was used to spread the inoculums evenly on the surface of the agar where the excess was drained off during spreading the bacterial inoculums. The plates were left on the bench for 1 hour so that the inoculums will diffuse into the agar, then 100µl volume of varying concentrations of the extracts (100mg/ml, 200mg/ml, and 400mg/ml) was dropped in each of the appropriately labeled wells. A negative control i.e. 5% and 100% DMSO as well as distilled water was set up for each plate by adding the same volume as that of the extract and a positive control of ciprofloxacin (for Gram positive) and chloramphenicol (for Gram negative) were used. After incubation of 24hr, the zone of clearance around each ditch was measured using vernier caliper. The zone of clearance measurement was taken from the edge of the zone to the point where the extract is applied. Hence the diameter of the zone of inhibition which represents antibacterial activity was measured in millimeter.

2.4.5. Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

The MIC was determined using the tube dilution broth method. This was done when the plant extract showed strong antibacterial activity in the agar well diffusion method consistently at the three different concentrations. The tubes were filled with 0.5 ml of nutrient broth. The extract was prepared by taking 1 g of the plant extract and mixing it with 2.5ml of 5%DMSO for complete dissolution of the extract to prepare a concentration of 400mg/ml [16]. Then 0.5 ml of the plant extract suspension was dispensed into the first tube reducing the concentration by half before serial dilutions were done by transferring 0.5 ml of the nutrient broth containing the extract from the first tube to the second tube, the procedure was repeated until the last tube (eighth tube). 0.5 ml of the bacterial suspension (0.5 McFarland) was then dispensed into each tube. One tube (without extract or drug) was used as a negative control, whereas one tube with antibiotics, Chloramphenicol (for Gram negative) and Ciprofloxacin (for Gram positive) were used as positive control.

The tubes were incubated aerobically for 24 hours at 37°C. The MIC values were determined as the lowest concentrations of the extract capable of inhibiting bacterial growth by looking at the turbidity of the tubes, it was then confirmed by plating the tubes. The MBC was determined by plating the tubes which did not show turbidity on nutrient broth. The lowest concentration of the plant extract that did not yield any colony on the solid media after sub culturing and incubating for 24 hours was taken as the MBC [17].

3. Statistical Analysis

All analysis was undertaken in duplicates and each experiment was repeated two times. Quantitative values were presented as means $\pm$ Standard Deviation (SD). Mixed Design ANOVA was used to evaluate the statistical

differences between concentrations of plant extracts followed by Tukey HSD *post-hoc* test. Statistical analysis was performed using statistical package for social sciences (SPSS), version 26.0 software and Microsoft Excel 2007. Differences at $P < 0.05$ were considered significant.

4. Results

4.1. Phytochemical Analysis

Phytochemical screening of *Solanum incanum* was performed and all solvent extracts were found to be positive for saponins, whereas all extracts were negative for flavonoids except methanol extract of *Solanum incanum* root and chloroform extract of *Solanum incanum* leaf, likewise tannins, phenols, and glycosides were positive in most of the extracts and negative in some of the extracts as shown in Table 2. The phytochemical screening of *Silene macrosolen* was also performed, glycosides was positive for all the extracts except for hot aqueous extract of *Silene macrosolen* root, others like flavonoids, phenols, tannins and saponins show various results with different extracts, as can be seen in the table below. Phytochemical analysis was not performed for chloroform extract of *Silene macrosolen* root. See Table 3.

Table 2
The phytochemical analysis of *S. incanum*

Phytochemical	Test Method	Ethanol		Methanol		Chloroform		Hot Aqueous		Cold Aqueous	
		Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root
Flavonoids	Alkaline reagent test	–	+	–	+	–		–		–	–
Saponins	Foaming test	–	+	+	+	–		+	+	+	+
Tannins	lead acetate test	+	–	+	+	+		–	–	+	+
Phenols	Ferric chloride test	+	+	+	–	+		–	–	+	+
Glycosides	Glycosides test	–	+	+	+	+		+	–	+	+

Table 3
The phytochemical analysis of *S.macrosolen*

Phytochemical	Test Method	Ethanol		Methanol		Chloroform		Hot Aqueous		Cold Aqueous	
		Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
Flavonoids	Alkaline reagent test	-	-	+	-	-	+	-	-	-	-
Saponins	Foaming test	+	+	+	+	+	+	+	+	+	+
Tannins	lead acetate test	+	+	+	+	+	-	+	+	+	-
Phenols	Ferric chloride test	+	+	+	+	+	+	-	-	-	+
Glycosides	Glycosides test	+	+	-	+	-	-	-	+	+	-

4.2. Antimicrobial Activities

4.2.1. Antibacterial activity of plant extracts against *S.aureus*

Two different plants; *S.incanum* (leaf and root) and *S.macrosolen* (stem and root) were screened for their antibacterial effect against *S.aureus*. Most of the plants extract were found to be active at different concentrations against *S.aureus*. Methanol and Cold aqueous extracts of *S.incanum* leaf show the highest inhibition (p -value<0.05) zone ranging from 12mm to 16mm for methanol and 7mm to 15mm for cold aqueous respectively at 200mg/ml, 400mg/ml, likewise chloroform showed inhibition zone of 13mm, 13mm, 11mm respectively at 400mg, 200mg, 100mg, whereas ethanol extract showed inhibition 11mm at 400mg/ml only. Hot aqueous extract did not show any inhibition at any concentration. Chloroform extracted *S. incanum* root showed the highest inhibition zone (p -value<0.05) with 24.5mm, 24.5mm and 23mm respectively at 400mg/ml, 200mg/ml, 100mg/ml, followed by Cold aqueous (p -value<0.05) extracted Solanum incanum root which showed an inhibition zone of 19mm, 17.5mm, 13.5mm respectively at 400mg/ml, 200mg/ml, 100mg/ml, whereas ethanol extracts showed inhibition zone of 18 mm, 17mm, 8mm respectively at 400mg, 200mg, 100mg. methanol extract showed a zone of inhibition of 12mm, 11.5mm, 9mm respectively at 400mg/ml, 200mg/ml, 100mg/ml, However, hot aqueous extract showed the least inhibition zone which did not show any inhibition at all concentrations (Table 4-7).

Table 4
Antibacterial activity of *S.incanum* leaf against *S.aureus*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	18±0.00	0.00±0.00	>0.05	a,b,c
Cold aqueous	0.00±0.00	7.00±0.00	15.00±0.00	18±0.00	0.00±0.00	<0.05	c>b>a
Methanol	0.00±0.00	12.00±0.00	16.00±0.00	19.00±0.00	0.00±0.00	<0.05	c>b<a
Ethanol	0.00±0.00	0.00±0.00	11.00±0.00	18±0.00	0.00±0.00	<0.05	c>b,a
Chloroform	11.00±0.00	13.00±0.00	13.00±0.00	18±0.00	0.00±0.00	<0.05	c,b>a
p-value	<0.05	<0.05	<0.05	>0.05	>0.05		
Where c is 400mg/ml, b is 200mg/ml, a is 100mg/ml M±SD is mean plus or minus standard deviation.							

Table 5
Antibacterial activity of *S.incanum* root against *S.aureus*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	18±0.00	0.00±0.00	>0.05	a,b,c
Cold aqueous	0.00±0.00	7.00±0.00	15.00±0.00	18±0.00	0.00±0.00	<0.05	c>b>a
Methanol	0.00±0.00	12.00±0.00	16.00±0.00	19.00±0.00	0.00±0.00	<0.05	c>b<a
Ethanol	0.00±0.00	0.00±0.00	11.00±0.00	18±0.00	0.00±0.00	<0.05	c>b,a
Chloroform	11.00±0.00	13.00±0.00	13.00±0.00	18±0.00	0.00±0.00	<0.05	c,b>a
p-value	<0.05	<0.05	<0.05	>0.05	>0.05		

Table 6
Antibacterial activity of *S.macrosolen* stem against *S.aureus*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	18±0.00	0.00±0.00	>0.05	a,b,c
Cold aqueous	8.00±0.00	15.00±0.00	23.00±0.00	17±0.00	0.00±0.00	<0.05	c>b>a
Methanol	8.00±0.00	19.00±0.00	23.00±0.00	19.00±0.00	0.00±0.00	<0.05	c>b<a
Ethanol	8.00±0.00	13.00±0.00	16.00±0.00	18±0.00	0.00±0.00	<0.05	c>b,a
Chloroform	0.00±0.00	0.00±0.00	10.00±0.00	18±0.00	0.00±0.00	<0.05	c,b>a
p-value	<0.05	<0.05	<0.05	>0.05	>0.05		

Table 7
Antibacterial activity of *S.marcosolen* root against *S.aureus*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	18±0.00	0.00±0.00	>0.05	a,b,c
Cold aqueous	11.00±0.00	13.50±0.70	16.50±0.70	17±0.00	0.00±0.00	<0.05	c>b>a
Methanol	0.00±0.00	12.00±0.00	17.00±0.00	19.00±0.00	0.00±0.00	<0.05	c>b<a
Ethanol	0.00±0.00	7.00±0.00	12.00±0.00	18±0.00	0.00±0.00	<0.05	c>b,a
Chloroform	0.00±0.00	0.00±0.00	10.00±0.00	18±0.00	0.00±0.00	<0.05	c,b>a
p-value	<0.05	<0.05	<0.05	>0.05	>0.05		

4.2.2. Antibacterial activity of plant extracts against *E.coli*

Cold aqueous extract of *S.incanum* leaf showed the highest inhibition zone (p -value<0.05) of 10mm at a concentration of 400mg/ml however it does not show any inhibition at 200mg/ml and 100mg/ml, whereas ethanol, methanol, chloroform and hot aqueous extracts of *S.incanum* leaf did not show any inhibition zone at the concentrations that were tested. Cold aqueous extract of *S.incanum* root showed the highest inhibition zone (p -value<0.05) with 16mm at 400mg/ml, 200mg, and no inhibition zone at 100mg/ml, followed by chloroform extract (p -value<0.05) with a zone of inhibition of 12.5mm, 11.5mm, 10mm respectively at 400mg/ml, 200mg/ml, 100mg/ml, however ethanol, methanol and hot aqueous extracts of *S.incanum* root did not show any inhibition against *E.coli* at the concentrations that were tested. Methanol extracted *S.marcosolen* stem showed the highest zone of inhibition(p -value<0.05) of 22.5mm, 8mm at a concentration of 400mg/ml, 200mg/ml respectively but it did not show any inhibition zone at 100mg/ml, this was followed by cold aqueous extract (p -value<0.05) with a zone of inhibition of 18.5mm at 400mg/ml, 7mm at 200mg/ml and no inhibition at 100mg/ml. ethanol extract of *S.marcosolen* stem showed inhibition zone of 8mm at 400mg/ml only, whereas chloroform and hot aqueous extracted *S. marcosolen* stem showed no inhibition at all the concentrations that were tested. Cold aqueous extracted *S.marcosolen* root was the one with the greatest activity (p -value<0.05) with inhibition zone of 11mm, 9mm, and 0mm respectively at 400mg/ml, 200mg/ml, and 100mg/ml, followed by methanol extract (p -value<0.05) with 12mm inhibition zone at 400mg/ml, but no inhibition at 200mg/ml and 100mg/ml, while ethanol and hot aqueous extracted *Silene macrosolen* root did not exhibit any inhibition zone at the different concentrations tested. Chloroform extract was not tested against *E.coli*. See Table 8-11.

Table 8
Antibacterial activity of *S.incanum* leaf against *E.coli*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	23.5±2.12	0.00±0.00	>0.05	a,b,c
Cold aqueous	0.00±0.00	0.00±0.00	10.00±0.00	24.5±3.53	0.00±0.00	<0.05	c>b>a
Methanol	0.00±0.00	0.00±0.00	0.00±0.00	23.50±2.12	0.00±0.00	>0.05	c>b<a
Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	24.5±3.53	0.00±0.00	>0.05	c>b,a
Chloroform	0.00±0.00	0.00±0.00	0.00±0.00	24±0.00	0.00±0.00	>0.05	c,b>a
p-value	>0.05	>0.05	<0.05	>0.05	>0.05		

Table 9
Antibacterial activity of *S.incanum* root against *E.coli*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	23.5±2.12	0.00±0.00	>0.05	a,b,c
Cold aqueous	0.00±0.00	16.00±0.00	16.00±0.00	24.5±3.53	0.00±0.00	<0.05	b,c>a
Methanol	0.00±0.00	0.00±0.00	0.00±0.00	23.50±2.12	0.00±0.00	<0.05	a, b, c
Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	24.5±3.53	0.00±0.00	>0.05	a, b, c
Chloroform	10.00±0.00	11.50±0.70	12.50±0.707	25.4±2.38	0.00±0.00	<0.05	b ,c >a
p-value	<0.05	<0.05	<0.05	>0.05	>0.05		

Table 10
Antibacterial activity of *S.macrosolen* stem against *E.coli*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	23.5±2.12	0.00±0.00	>0.05	a,b,c
Cold aqueous	0.00±0.00	7.00±0.00	18.50±0.707	23.5±2.12	0.00±0.00	<0.05	c>b>a
Methanol	0.00±0.00	8.00±0.00	22.50±0.707	23.50±2.12	0.00±0.00	<0.05	c>b>a
Ethanol	0.00±0.00	0.00±0.00	8.00±0.00	24.5±3.53	0.00±0.00	<0.05	c>a,b
Chloroform	0.00±0.00	0.00±0.00	0.00±0.00	25.4±2.83	0.00±0.00	>0.05	a,b,c
p-value	>0.05	<0.05	<0.05	>0.05	>0.05		

Table 11
Antibacterial activity of *S.macrosolen* root against *E.coli*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	23.5±2.12	0.00±0.00	>0.05	a,b,c
Cold aqueous	0.00±0.00	9.00±0.00	11.00±0.707	24.5±3.53	0.00±0.00	<0.05	c>b>a
Methanol	0.00±0.00	0.00±0.00	12.00±0.00	23.50± 2.12	0.00±0.00	<0.05	c>b>a
Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	23.5±3.53	0.00±0.00	>0.05	c>a,b
p-value	>0.05	<0.05	<0.05	>0.05	>0.05		

4.2.3. Antibacterial activity of plant extracts against *P.aeruginosa*

Cold aqueous extracted *S.incanum* leaf was with the highest inhibition zone (p -value<0.05) of 12mm at 400mg/ml, but no inhibition was observed at 200mg/ml and 100mg/ml, this was followed by ethanol extract with 9.5mm at 400mg/ml only, however no inhibition zone was observed for methanol, chloroform and hot aqueous extracted *S. incanum* leaf at all concentrations. Chloroform extract of *S.incanum* root was found to be the most effective (p -value<0.05) with a zone of inhibition of 13.5mm,12.5mm,7mm at concentrations 400mg/ml,200mg/ml,100mg/ml respectively, whereas cold aqueous,ethanol,methanol and hot aqueous did not show any inhibition zone against standard strains of *Paeruginosa* at any concentration tested. Methanol extract of *S.macrosolen* stem showed the highest inhibition zone (p -value<0.05) of 26mm at 400mg/ml only, followed by Cold aqueous extract (p -value<0.05) with 16.5mm, 8mm respectively at 400mg/ml, 200mg/ml, while ethanol, chloroform and hot aqueous extracts of *S.macrosolen* stem did not inhibit the growth of *Paeruginosa* at any concentration. Cold aqueous extracted *S.macrosolen* root was the one with the highest activity (p -value<0.05) with a zone of inhibition of 15mm, 11mm at 400mg/ml, and 200mg/ml respectively, this was followed by methanol extract (p -value<0.05) with 15mm at 400mg/ml only, whereas ethanol, and chloroform did not show any inhibition at all the concentrations. Chloroform extract was not tested against *Paeruginosa*. See Table 12-15.

Table 12
Antibacterial activity of *S.incanum* leaf against *Paeruginosa*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	23.5±2.12	0.00±0.00	>0.05	a,b,c
Cold aqueous	0.00±0.00	0.00±0.00	12.00±0.707	24.5±3.53	0.00±0.00	<0.05	c>a,b
Methanol	0.00±0.00	0.00±0.00	0.00±0.00	25.50± 2.12	0.00±0.00	>0.05	a,b,c
Ethanol	0.00±0.00	0.00±0.00	9.50±0.707	24.5±3.53	0.00±0.00	<0.05	c>a,b
Chloroform	0.00±0.00	0.00±0.00	0.00±0.00	25.4±2.83	0.00±0.00	>0.05	a,b,c
p-value	>0.05	<0.05	<0.05	>0.05	>0.05		

Table 13
Antibacterial activity of *S.incanum* root against *Paeruginosa*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	23.5±2.12	0.00±0.00	>0.05	a,b,c
Cold aqueous	0.00±0.00	0.00±0.00	0.00±0.00	24.5±3.53	0.00±0.00	>0.05	a,b,c
Methanol	0.00±0.00	0.00±0.00	0.00±0.00	23.50± 2.12	0.00±0.00	>0.05	a,b,c
Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	24.5±3.53	0.00±0.00	>0.05	a,b,c
Chloroform	7.00±0.00	11.5±0.707	13.5±0.707	25.4±2.83	0.00±0.00	<0.05	b,c>a
p-value	<0.05	<0.05	<0.05	>0.05	>0.05		

Table 14
Antibacterial activity of *S.macrosolen* stem against *Paeruginosa*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	23.5±2.12	0.00±0.00	>0.05	a,b,c
Cold aqueous	0.00±0.00	8.00±0.00	16.50±0.707	24.5±3.53	0.00±0.00	<0.05	c>b>a
Methanol	0.00±0.00	0.00±0.00	26.00±0.00	23.50± 2.12	0.00±0.00	<0.05	c>a,b
Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	24.5±3.53	0.00±0.00	>0.05	a,b,c
Chloroform	0.00±0.00	0.00±0.00	0.0±0.00	25.4±2.83	0.00±0.00	>0.05	a,b,c
p-value	>0.05	<0.05	<0.05	>0.05	>0.05		

Table 15
Antibacterial activity of *S.macrosolen* root against *Paeruginosa*

Solvents	100mg/ml M±SD	200mg/ml M±SD	400mg/ml M±SD	Positive M±SD	Negative M±SD	P-value	Post hoc (LSD)
Hot aqueous	0.0±0.00	0.00±0.00	0.00±0.00	23.5±2.12	0.00±0.00	>0.05	a,b,c
Cold aqueous	0.00±0.00	11.00±0.00	15.00±0.00	24.5±3.53	0.00±0.00	<0.05	c>b>a
Methanol	0.00±0.00	0.00±0.00	15.00±0.00	23.50± 2.12	0.00±0.00	<0.05	c>a,b
Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	24.5±3.53	0.00±0.00	>0.05	a,b,c
p-value	>0.05	<0.05	<0.05	>0.05	>0.05		

1. Minimum Inhibitory concentration and Minimum bactericidal 2. concentration

The plants extracts which show strong antibacterial activity in the agar well diffusion method consistently at the three different concentrations were tested for their MIC and MBC against selected bacterial strains. Cold aqueous extracted *Silene macrosolen* Stem showed MIC at 25mg/ml and MBC at 50 mg/ml against both *E.coli* and

Paeruginosa. Chloroform extract of *Solanum incanum* Root showed MIC at 50mg/ml, however MBC was not determined against *S.aureus*. Negative controls were also run to avoid contamination associated errors. See Table 16.

Table 16
MIC and MBC of plants extracts against selected strains of gram positive and gram negative bacteria.

			Different concentrations in mg/ml	
S.N	Organisms		MIC	MBC
1	<i>S. aureus</i>	Chloroform <i>Solanum incanum</i> Root	50	-
2	<i>E. coli</i>	Cold aqueous <i>Silene macrosolen</i> Stem	25	50
3	<i>P. aeruginosa</i>	Cold aqueous <i>Silene macrosolen</i> Stem	25	50

5. Discussion

In the present study, two plants were subjected to cold extraction to prepare different crude extracts and subsequently percentage yield was calculated. Each crude extract was then dissolved in their respective reconstituents and were applied against standard bacterial strains to find out their antibacterial activity through measuring the zone of clearance. To evaluate the MIC and MBC, broth dilution assay was assessed to discover the lowest concentration where the extract can show static and bactriocidal effect respectively on the bacterial strains. Phytochemical test was also performed to find out the bioactive compounds of the plant extracts.

The antibacterial activities of ethanol, chloroform, methanol and aqueous extracts of *S.incanum* and *S.macrosolen* were examined against standard bacterial strains of

S.aureus, *E.coli* and *Paeruginosa* and their activity were assessed by measuring inhibition zones. The effect of concentration on the antibacterial activity of the plants were assessed and their antibacterial activity increased with increasing the concentrations of their crude extracts. In this study three different concentrations of the extracts were used (400mg/ml, 200mg/ml and 100mg/ml). From these concentrations, the highest effect was seen in 400mg/ml, followed by 200mg/ml and the least effect was seen in 100mg/ml with statistically significant p-value ($p<0.05$). For example, the inhibition zone of 400mg/ml cold aqueous extract of *Silene macrosolen* against *S.aureus* was 23 ± 0.00 mm. However, it is significantly reduced to 15 ± 0.00 mm and 8 ± 0.00 mm in 200mg/ml and 100mg/ml respectively. Similar results have been reported by previous researchers in Eritrea indicating that as the concentration of the plant powder is reduced to half, the sensitivity is also reduced to half [18]. A comparison of antibacterial activity of different solvent extracts were done per each plant material against respective bacteria. Upon the antibacterial activities against *S.aureus*, methanol and cold aqueous extracts of *S.incanum* leaf (16mm and 15mm respectively), *S.macrosolen* root (17.5mm and 16.5mm respectively) and *S.macrosolen stem* (23mm for both) showed the highest inhibition zone ($p<0.05$) at 400mg/ml. However, chloroform extract showed the highest inhibition ($p<0.05$) with 24.5mm at 400mg/ml in *S.incanum* root. Upon the antibacterial activities against *E.coli*, most solvent extract of *S.incanum* leaf did not show any inhibition zone against *E.coli* except the cold aqueous extract with 10mm diameter at 400mg/ml. Cold aqueous extract showed the highest ($p<0.05$) inhibition zone in *S.incanum* root with 16mm. Cold aqueous and methanol extracts showed the highest ($p<0.05$) inhibition in both *S.macrosolen stem* (18.5 mm and 22.5mm respectively) and root (11mm and 12mm respectively). This result is coherent with study by Mamta K, et al. 2011, which concluded that the aqueous root extract of *W.somnifera* hold an excellent potential as an antibacterial agent against *E. coli*. Upon the antibacterial activities against *Paeruginosa*, Cold aqueous extract

showed the highest effect ($p < 0.05$) with 12mm at 400mg/ml in *S. incanum* leaf. In *S. incanum* root, Chloroform extract showed the highest effect ($p < 0.05$) with 13.5mm. In *S. macrosolen* stem, methanol extract showed the highest antibacterial activity ($p < 0.05$) with 26mm at 400mg/ml. Similar to the previous results methanol and cold aqueous extract exhibited highest ($p < 0.05$) antibacterial effect in *S. macrosolen* root with a zone of inhibition of 15mm each at 400mg/ml. From the overall results found, almost all the methanol and cold aqueous extracts of each plant material were found to be the most active against all the experimental bacteria. This is supported by the results of the phytochemical tests, in which the methanol and cold aqueous extracts were positive to almost all the selected phytochemical tests. The yield percentage of these solvent extracts were also found to be the highest. These findings might have an effect in the availability of the active ingredients of these plant extracts, thereby to be the most antibacterial active plant extracts. These results are supported by a study conducted in India, which conclude that aqueous and methanol root extracts of *Withania somnifera* might be exploited as a natural drug for the treatment of several infectious diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications [15]. An overall comparison on the potency of the plant materials was also assessed. *S. aureus* is highly sensitive to chloroform extract of *S. incanum* root, and methanol and cold aqueous extract of *S. macrosolen* stem at 400mg/ml. In addition, *E. coli* and *Paeruginosa* were highly sensitive to methanol extract of *S. macrosolen* stem at 400mg/ml. Therefore, *S. macrosolen* stem was found to be the most potent plant material. Almost all crude extracts showed good antibacterial activities against *S. aureus* which is in agreement with a study done on extracts of *O. limbata* [19]. Generally *S. aureus*, *E. coli* and *Paeruginosa* showed good sensitivity toward cold aqueous and methanol extract but the gram negative bacteria strains *E. coli* and *Paeruginosa* were not sensitive to almost other plants extracts. These could be due to several possible reasons, one being the distinctive feature of gram-negative bacteria is the presence of a double membrane surrounding each bacterial cells. Although all bacteria have an inner cell membrane, gram-negative bacteria have a unique outer membrane. This outer membrane excludes certain drugs and antibiotics from penetrating the cell, partially accounting for why gram-negative bacteria are generally more resistant to antibiotics than other gram-positive bacteria. Concluding as such this may be why the extracts have not shown any effect on the gram negative bacteria being resistant. Overall point of view hot aqueous extraction for each plant on every bacteria has not shown any result. This could be reasoned out by suggesting that the bioactive compound of the plants could have been destroyed due to the high temperature used to extract the plants.

MIC and MBC are very essential while evaluating the antimicrobial activity of plant extracts as a guide towards predicting the efficacy of a promising product. If pharmacokinetic and Pharmacodynamics (PKPD) principles are met by careful selection of a specific promising antimicrobial extracts while given at an appropriate dosage, this will relate to clinical cure, eradication of carrier status of a specific microorganism, as well as prevention of selection of resistance. Interpretation of the MIC gives an understanding of the mode of activity of a given plant extract [20]. MIC and MBC was performed for those who have shown us a promising result in the antimicrobial sensitivity test, where the zone of clearance was recorded to be > 22 mm inhibition for *S. aureus* and > 18 mm inhibition for *E. coli* and *Paeruginosa* with a consistent result at the three different concentrations. The following were the effective ones; *S. macrosolen* stem of cold aqueous extract had shown > 18 mm for *E. coli* and *Paeruginosa*. *S. incanum* leaf of chloroform extract had shown > 22 mm for *S. aureus* based on each concentration. In this study MIC and MBC of *S. macrosolen* stem against *Paeruginosa* and *E. coli* were determined via serial dilution technique. The results of MIC and MBC for both bacteria were recorded to be the same. The end result displayed for MIC and MBC were at 25mg/ml and 50mg/ml. MIC and MBC of *S. incanum* leaf chloroform extract against *S. aureus*, results of this broth dilution assay MIC was seen on the 3rd test tube with 50mg/ml while MBC was not found.

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate urgent steps for screening of plants for secondary metabolites. Recently, much attention has been directed toward plant extracts and biologically active compounds isolated from popular plant species [21].

Phytochemical property of *Solanum incanum* The results of our study indicate that majority of the secondary metabolites like flavonoids, alkaloids, saponins, tannins, phenols and glycosides are contained in *S. incanum* when extracted with different solvents, which is similar to the study conducted by Tewelde and Ghebriel, 2017, where the extract of a *Solanacea* family showed presence for the majority of the bioactive compounds. So this medicinal plant holds promises as source of pharmaceutically important phytochemicals. *S. incanum* possesses numerous biologically active compounds which could serve as potential source of natural drugs in herbal medicine [22]. It was reported that most of the plants of Solanaceae contain alkaloids, tannins, steroids, saponins, as well as phenols [23]. A study held in Kenya demonstrated that different parts of the *S. incanum* plant has almost similar phytochemicals present which are attributed to its antimicrobial activity and hence there is need for more studies to be done on the roots and leaf extracts of *S. incanum* to evaluate their antimicrobial and antifungal properties after studying about the antimicrobial effect of this plant's fruit [24]. The present study was also supported by Tewelde and Ghebriel (2017) which indicates that the extract of *Solanum incanum* showed the presence of saponins that have healing properties as a natural blood cleanser and expectorant. This implies that, it is possible that the presence of certain compounds in the plants like saponins and glycosides might be responsible for its antibacterial activity. Flavonoids were absent in majority of the plant extracts but are present in the methanolic root extract and chloroform leaf extracts. The flavonoid compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, antiinflammatory, anti-carcinogenic, etc. [22]. Our study revealed the presence of tannins in almost all the extracts of *S. incanum* which may be responsible for its antibacterial activity in which similar results were obtained by Tewelde and Ghebriel, 2017. The result of this research also shows the presence of phenols in methanol, ethanol and chloroform extracts but absent in both the hot and cold aqueous extracts. This goes in parallel with the study done by, Tewelde and Ghebriel 2017, which regarded phenols found in fruit extracts of *Solanum incanum* as one of the functional food components in fruits have significant contribution to the health effects of plant- derived products. The results of this study shows that glycosides were absent in almost all the extracts except in cold aqueous extracts, and methanol leaf extract which can be attributed to their higher antibacterial activity. This Statement unifies with the previous study done by, Kemei. E. and Ndukui. J., 2017, which flavonoids, glycosides and terpenoids were weakly present although terpenoids were not performed in this study.

Flavonoids were absent in all the extracts except in ethanol and methanol root extracts of *Silene macrosolen*. This study reveals the presence of saponins in all the plant extracts indicating that it also has healing properties as a natural blood cleanser and expectorant. Phenols were absent in all the extracts except in the ethanol and methanol extract of this plant, this shows that both the stem and root methanol extract of this plant has significant contribution to its antibacterial activity.

6. Conclusion

On the basis of the antibacterial assay of this study *S. aureus* was found to be more susceptible to the employed plant extracts than *E. coli* and *P. aeruginosa*. In the present study the methanolic and cold aqueous plant extracts of *S. macrosolen* stem revealed the strong presence of saponins and glycosides whose presence may contribute to the antimicrobial activities of the plant extracts against the tested organisms. It was also evident that plant methanolic

extracts *S. macrosolen* stem showed greater activity as compared to other extracts. This supports the continuous use of *S. macrosolen* stem in management of wound infection in Eritrean communities, caused by *S. aureus*. Moreover, this study also provides scientific support for the traditionally used medicinal plants to act as a potential source of new drugs in the treatment of bacterial infections.

The future for using plant extracts and plant products is promising, because they are less expensive and less hazardous to the environment. A research that can strengthen the documentation of the indigenous knowledge which contributes for the drug development and for self-reliance in the future is also strongly recommended. Consequently, there is dire need to scientifically validate the claimed medical value of plants commonly used in local communities. So this study leads to further research in the way of isolation and identification of the active compounds from these plants using chromatographic and spectroscopic techniques for proper drug development, so as to standardize it in recommendable dosage form.

7. Limitations

Even though, the present study was in complete success, there were certain limitations that would have added to the significance of the study if they had been performed. Because of the absence of blood agar medium AST could not be performed using the standard strain of *Streptococcus pyogen*. The plant extracts could not be tested for the presence of “Alkaloids” due to shortage of reagents. The AST and phytochemical testing was not performed for chloroform extract of *Silene macrosolen* root because it could not be filtered as the chloroform completely degraded the plant.

Declarations

Competing interests

The authors declare that they have no competing interests.

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Author details

Bilen Ateshim¹, Frenai Tekle¹, Meron Tesfay¹, Muzit Tekleab¹, Rahel Tekie¹, Saimon Weldemariam¹, Oliver Okoth Achila², Samuel Tekle Mengistu³, Mohammed Elfatih Hamida³

¹Bilen Ateshim. Department of Clinical Laboratory Sciences, Orotta College of Medicine and Health Sciences (OCMHS). Email: bilenateshimcls@yahoo.com

¹Frenai Tekle. Department of Clinical Laboratory Sciences, Orotta College of Medicine and Health Sciences (OCMHS). Email: frenaitekclecls@yahoo.com

¹Meron Tesfay. Department of Clinical Laboratory Sciences, Orotta College of Medicine and Health Sciences (OCMHS). Email: merontesfaycls@yahoo.com

¹Muzit Tekleab. Department of Clinical Laboratory Sciences, Orotta College of Medicine and Health Sciences (OCMHS). Email: muzittekleabcls@yahoo.com

¹Saimon Weldemariam. Department of Clinical Laboratory Sciences, Orotta College of Medicine and Health Sciences (OCMHS). Email: saimonweldemariamcls@yahoo.com

2Oliver Okoth Achila. Unit of Clinical Laboratory Science, Orotta College of Medicine and Health Sciences (OCMHS). Email: oliverachila@gmail.com

³Samuel Tekle Mengistu. Nakfa Hospital, Northern Red Sea Ministry of Health. Email: teklesam7@gmail.com

⁴Mohammed Elfatih Hamida: Unit of Medical Microbiology, Orotta College of Medicine and Health Sciences (OCMHS). Email: mohelfatih77@gmail.com

Corresponding author: Mohammed Elfatih Hamida, email: mohelfatih77@gmail.com, Orotta College of Medicine and Health Sciences (OCMHS), Unit of Medical Microbiology, P.O. Box 1034, Asmara, Eritrea.

[M1]Insert him as author

References

1. Morens D M FGK and FAS. Emerging infections: a perpetual challenge. *Lancet Infect Dis.* 2008;8:710–9.
2. Matasyoh J C, Maiyo Z C NRM and CR. Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum*. *Food Chem.* 2009;113:526–9.
3. Joshi B LS and SA. Antibacterial Property of Different Medicinal Plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum* and *Origanum majorana*. *Kathmandu Univ J Sci Eng Technol.* 2009;5:143–50.
4. Okpako D. African medicine: tradition and beliefs. *Pharm J.* 2006;276:239–40.
5. Magassouba F B, Diallo A, Kouyate M, Mara F and Mara O, et al. Ethno botanical survey and antibacterial activity of some plants used in Guinean traditional medicine. 2007;114:44–7.
6. Rojas R, Bustamante B, Bauer J et al. Rojas R, Bustamante B, Bauer J et al. *J Ethnopharmacol.* 2003;88:199–204.
7. K.S.M. Karthika, Dr. Lankapalli Bullayya, Visakha patnam AP. Antibacterial activity of plant extract *plumbago zeylanica* against clinical bacteria. *PG Coll Res Centre, Dep Microbiol India.* 4AD;6:1603–7.
8. Mercola. Importance of herbal medicine. www.Mercola.com. 2014.
9. Owolabi J, Omogbai EKI OO. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigella Africana* (Bignoniaceae) stem bark'. *Afr J Biotechnol.* 2007;6:882–5.

10. Das BN and Biswas BK. Antibacterial and cytotoxic activities of the leaf extract of *Murrayako enigii*. Int J Life Sci Biotechnol Pharma Res. 2012;1:59–63.
11. Zelalem Getnet, Subramanian Chandrodyam and GM. Studies on traditional medicinal plants in ambagiorgis area of wogera District, Amhara Regional State, Ethiopia. int.j.pure AppBiosci. 2016;4:38–45.
12. Andemariam SW. Legislative regulation of Traditional Medicinal Knowledge in Eritrea VIS-À-VIS ERITREA'S commitments under the convention of biological diversity. Environ Dev J. 2010;6:133–62.
13. Henderson L. Problem plants in Ngorongoro Conservation Area. Final Report to the NCAA. 2002.
14. Goodman GA, Rall TW, Nies AS TP. Las Bases Farmacológicas de la Terapéutica. Editor. Med. Pan Am. Mex. D F. 1991.
15. Pranay Jain and Rishabh Varshney J. Antimicrobial activity of aqueous and methanolic extracts of *Withania somnifera* (Ashwagandha) Pranay Jain* and Rishabh Varshney. J Chem Pharm Res. 2011;3:260–3.
16. Al-Fatimi, M., Wurster, M., Schröder, G., & Lindequist U. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J Ethnopharmacol. 2007;111:657–66.
17. Porter, P. RM Mariita, CKPO Ogol NO and PO. No Title. Res J Med Plant,. 2011;5:54–64.
18. Tewelde Sahle, Ghebriel Okbatinsae. Phytochemical investigation and antimicrobial activity of the fruit extract of *Solanum incanum* grown in Eritrea. Ornam Med Plants. 2017;1:15–25.
19. D. Ahmed, M. Munsha, A. Hannan MB and NB. No Title. J Appl Sci Res. 2009;6:1611.
20. Chan LW, Cheah ELC, Saw CLL, Weng W HP. Antimicrobial and antioxidant activities of *Cortex Magnoliae Officinalis* and some other medicinal plants commonly used in South-East Asia. Chinese Med. 2008;3.
21. Dalal H. Antimicrobial activities of *Solanum incanum*, *Elettaria cardamomum* and *Zingiber officinale*, used traditionally to treat pathogenic microbes. Int J Sci Eng Appl Sci. 2016;2:2395–3470.
22. Mitali Ghosal PM. Phytochemical screening and antioxidant activities of two selected 'BIHI' fruits used as vegetables in Darjeeling Himalaya. Int J Pharm Pharm Sci. 2012;4:567–74.
23. Amadi JE, Salami SO EC. Antifungal properties and phytochemical screening of extracts of African Basil (*Ocimum gratissimum* L.). Agric Biol J N Am. 2010;1:163–6.
24. Kemei. E. Jepkoech and Ndukui. J. Gakunga. Antimicrobial activity and phytochemical screening of *Solanum incanum* fruit extract against clinical samples of *Staphylococcus aureus* collecting from Nakuru Provincial General Hospital Laboratory. Kenya Int Res J Med Biomed Sci. 2017;2:1–8.