

Evaluation of antimicrobial activity and phytochemical screening of *Silene macrosolen* and *Solanum incanum*: A common medicinal plant in Eritrea

Abstract

Background: The periodically emerging new and old infectious microorganisms greatly magnify the global burden of infectious diseases. The majority of emerging infectious events are caused by bacteria, which can be associated with the evolution of drug-resistant strains and the overwhelming of the natural host defenses. Medicinal plants play an important role in the treatment of various infectious diseases. The objective of this study is to evaluate the in vitro antimicrobial activities of crude extracts of aqueous and solvents from two Eritrean traditional medicinal plants (*Silene macrosolen* and *Solanum incanum*).

Methodology: Roots and leaves of *Solanum incanum* and stems and roots of *Silene macrosolen* were collected and extracted using standard methods. The extracted ingredients were then subjected to standard bacterial strains (*Escherichia coli* ATCC-25923, *Staphylococcus aureus* ATCC-25922, and *Pseudomonas aeruginosa* ATCC-27853) to determine their antibacterial activity by measuring their zone of inhibition. Phytochemical analysis of the crude extract to see the presence of phytochemical compounds in the extract of selected plants.

Results: The highest inhibition zone was observed for methanol extracted *S. macrosolen* stem and chloroform extracted *S. incanum* root against *S. aureus* in 400 mg / ml with 23mm and 24.5mm respectively. Methanol and cold aqueous extracted stem of *S. macrosolen* also showed the highest inhibition of 26mm and 23mm diameter, against *P. aeruginosa* and *E. coli* respectively. The MIC and MBC of the cold aqueous extract of *S. macrosolen* stem were found at 25 mg / ml and 50mg/ml respectively, against both *E. coli* and *P. aeruginosa*, while the MIC of the chloroform-extracted root of *S. incanum* was found at 50mg/ml, however, the MBC could not be found in the concentration tested against *S. aureus*.

Conclusion: Based on the finding of this study *S. aureus* was found to be more susceptible to the plant extracts than *E. coli* and *P. aeruginosa*, and the methanolic and cold aqueous extracts of the *S. macrosolen* stem revealed the highest antibacterial activity.

Keywords: Antibacterial activity, medicinal plants, extraction, phytochemical, AST

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Introduction

Humankind has been exposed to microorganism infection since before the dawn of recorded history.¹ In treating such infections, mainly bacterial, humans have identified the use of different herbs since ancient times.² Knowledge of plant use is the result of many years of interaction and selection of 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 reemerging infectious diseases. Another major concern is the development of resistance to antibiotics in current clinical use.⁶ Plants containing medicinal properties have been known and used in some form or another, even by primitive people. Due 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 of diseases caused by microorganisms, developed countries are increasingly becoming aware that drugs from natural sources are safer and cheaper. Therefore, an increase in the use of plant-based products 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, adding value to this rich bioresource 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 a contaminated product, which has a low market value. To help developing countries to achieve the objective of using rich MAP resources to produce value-added products, dissemination of knowledge of existing extraction technologies and of the latest developments in these technologies is essential.⁸

In Eritrea, the use of herbs to treat different types of disease is a widespread practice. However, little has been done to study the antimicrobial activity of Eritrean vegetation. In this study, aqueous (hot and cold), ethanol, methanol, and chloroform were used as solvents. The leaves and root parts of *S. incanum* (locally known as 'Engule'), as well as stem and root of *S. macrosolen* (locally known as 'Saero Saero'), were tested in-vitro for their antibacterial activity. The bacteria used were standard strains of *E. coli*, *S. aureus*, and

P. aeruginosa. Visualizing the invitro antimicrobial effect of these locally available traditional medicinal plants opens the gate to further research aimed at the development of new drugs using a modern approach to overcome the antibiotic resistance of microbes. On the other hand, the lack of well-distributed health facilities and the widespread use of traditional medicinal plants (herbs) as a remedy in different countries like India, Ethiopia, and Eritrea have caused great concern in this study.

Methodology

The study was an invitro-experimental study conducted in the Asmara College of Health Sciences. When the experiment, there were both control and experimental groups to maintain the reliability of the results. The extraction procedures were performed in the ACHS Clinical Chemistry laboratory of ACHS; Media preparation and AST (Antimicrobial Susceptibility Testing) procedures were carried out mainly in the Microbiology department of NHL and partly in, and MIC and MBC were carried out in NDQC.

The survey, collection and preparation of plant extracts

Information on ethno-botanical uses of *S. incanum* and *S. macrosolen* was collected after interviewing traditional users. The plant *S. incanum* (root and Leaf) was collected from the fields of Villagio and Bet-mekae, Asmara, while *S. macrosolen* (Stem and Root) was collected from the northern Red Sea region around Mai-Habar. The collection was carried out from February to March 2018 and was identified in the Department of Plant Biology herbarium, EIT, Mai-nefhi Asmara, Eritrea. The collected roots, stems, and leaves were thoroughly washed with water to free them from debris. The roots of *S. incanum* and *S. macrosolen* were cut and shade (air) dried for three weeks, and the leaf of *S. incanum* and the stem of *S. macrosolen* were dried in shade (air) (air dried) for two weeks. Then they were ground finely by using a dry grinder and passed through a sieve range of 200 to 450 millimeters. Different extraction solvents, namely aqueous (cold and hot), ethanol, chloroform, and methanol, were used for the preparation of plant 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, while the hot aqueous extract was kept in a water bath at 80° C for 4 hours with continuous stirring. The extracts were then filtered using whatmann filter paper (No.1) and concentrated using a rotary evaporator to obtain the crude extract and then kept in sterile bottles under refrigerated conditions until use.

Standardization of bacterial inoculums and reconstitution and sterilization of extracts

The standardization of the bacterial inoculums was performed by picking (inoculating) five colonies of each bacterium into normal saline to form the bacterial suspension, which was standardized at 0.5 McFarland. The crude extracts that were concentrated using a rotary evaporator were reconstituted with distilled water for aqueous extract, 5% DMSO (dimethyl sulfoxide) for methanol and ethanol, and 100% DMSO for the chloroform extract. This was done by preparing a stock solution of 400mg/ml, which is a standard concentration, and then different concentrations were prepared from the stock solution i.e., 200mg/ml and 100mg/ml. The crude extracts were then stored in the sterilized bottle and kept in the refrigerator at 4° C until used for the antibacterial test. The sterility of the extracts was tested for sterility

by plating them on Muller-Hinton agar and incubating them for 24 hours at 37°C.

Test for antibacterial activity of the extracts in agar-well diffusion assay

Sterile Muller-Hinton agar plates were prepared and a well of about 6.0 mm diameter with sterile cork borer was aseptically punched on each agar plate to make 5 wells on each plate. A sterile cotton swab was used to spread the inoculums evenly on the surface of the agar where the excess was drained off during the spreading of the bacterial inoculums. The plates were left on the bench for 1 hour so that the inoculums would diffuse into the agar, and then 100µl volume of varying concentrations of 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 the extract and positive control of ciprofloxacin (for Gram positive) and chloramphenicol (for Gram negative) were used. After incubation for 24 hours, the clearance zone around each well was measured using a Vercaliper. Therefore, the diameter of the zone of inhibition representing antibacterial activity was measured in millimeters (mm).

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 1g of the plant extract and mixing it with 2.5 ml of 5%DMSO for complete dissolution of the extract to prepare a concentration of 400mg/ml. Then 0.5 ml of the plant extract suspension was dispersed in the first tube reducing the concentration by half before serial dilutions were performed 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) was used as a positive control. The tubes were incubated aerobically for 24 hours at 37° C. MIC values were determined as the lowest concentrations of the extract capable of inhibiting bacterial growth by looking at the turbidity of the tubes. MBC was determined by plating tubes which did not show turbidity in the nutrient broth.

Phytochemical screening of the plants extracts

Phytochemical screening was performed to detect the presence of plant constituents such as flavonoids, saponins, tannins, phenols, and glycosides in the plant extract. A portion of the extracts was used to test for the presence of phytochemicals using the methods described by Kokate and Harbone.^{9,10}

Statistical analysis

All analyses were undertaken in duplicates and each experiment was repeated two times. Quantitative values were presented as means ± Standard Deviation (SD). Mixed design ANOVA was used to evaluate the statistical differences between the concentrations of plant extracts. Statistical analysis was performed using SPSS version 20.0

software (Microsoft 2007). Differences in $P < 0.05$ were considered significant.

Results

Yield percentage of crude extract using different solvents- The percentage was calculated by dividing the weight of the crude extract by the weight of plant powder dissolved at the beginning of the extraction solvent. The aqueous and methanol crude extracts showed the highest percentage yield, however, the chloroform crude extracts were calculated to have the lowest percentage yield (Figure 1).

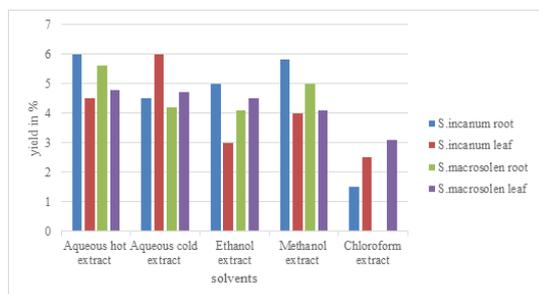


Figure 1 Yield percentage of *S. incanum* and *S. macrosolen* crude extracts using different solvents

Antibacterial activity of plant extracts

The cold aqueous and methanol extracts of the stem of *S. macrosolen* showed the highest inhibition zone ranging from 8mm to 23mm against *S. aureus*, (p -value <0.05). The highest sensitivity of *E. coli* was observed against the methanol extract of the *S. macrosolen* stem 22.5mm and 8mm at concentrations of 400mg/ml and 200mg/ml respectively, (p -value <0.05). The methanol extract showed the highest inhibition zone of 26mm at 400mg/ml against *P. aeruginosa*, (p -value <0.05). The hot aqueous extract of *S. macrosolen* stem did not show any results in all tested bacteria (Table 1). Regarding the antibacterial effect of the *S. macrosolen* root, almost all solvent extracts exhibited antibacterial activity against *S. aureus*, and cold aqueous and Methanol extracts were the highest, with an inhibition zone of 16.5mm and 17mm respectively, (p -value <0.05). *E. coli* was found to be the most sensitive to cold aqueous extract, with inhibition of 11mm, 9mm at 400mg/ml and 200mg/ml, respectively, (p -value <0.05). Cold aqueous and Methanol extract showed the highest activity against *P. aeruginosa*, with a 15mm inhibition zone (p -value <0.05). The hot aqueous extract did not show any result against all bacteria (Table 2). The chloroform extract of the *S. macrosolen* root could not be prepared due to the absolute degradation of the root by chloroform, where filtration was difficult to do.

Table 1 Antibacterial activity of *S. macrosolen* stem against *S. aureus*, *E. coli*, and *P. aeruginosa*. (SD) standard deviations

Bacterial strains	Concentrations (mg/dl)	Cold aqueous	Methanol	Ethanol	Chloroform	p-value	Post hoc (Tukey LSD)
<i>S. aureus</i>	100	8(0)	8(0)	8(0)	8(0)	>0.05	A, B, C, D
	200	15(0)	19(0)	13(0)	0(0)	<0.05	AC>D
	400	23(0)	23(0)	16(0)	10(0)	<0.05	A, B>C>D
	Ciprofloxacin	17(0)	19(0)	18(0)	18±0.0	<0.05	A<B, C, D
<i>E. coli</i>	100	0(0)	0(0)	0(0)	0(0)	<0.05	A, B, C, D
	200	7(0)	8(0)	0(0)	0(0)	<0.05	A, B>C, D
	400	18.5(0.707)	22.5(0.707)	8(0)	0(0)	<0.05	AC>D
	Chloramphenicol	20(0)	21.5(2.12)	21.5(0.707)	24.4(2.83)	<0.05	A, B, C<D
<i>P. aeruginosa</i>	100	0(0)	0(0)	0(0)	0(0)	<0.05	A, B, C, D
	200	8(0)	0(0)	0(0)	0(0)	<0.05	A>B, C, D
	400	16.5(0.707)	26(0)	0(0)	0(0)	<0.05	AC, D

Table 2 Antibacterial activity of *S. macrosolen* root against *S. aureus*, *E. coli*, and *P. aeruginosa*

Bacterial strains	Concentrations (mg/dl)	Cold aqueous	Methanol	Ethanol	p-value
<i>S. aureus</i>	100	11(0)	0(0)	0(0)	<0.05
	200	13.5(0.701)	12(0)	7(0)	<0.05
	400	16.5(0.701)	17(0)	12(0)	<0.05
	ciprofloxacin	18(0)	20(0)	21.5(2.12)	<0.05
<i>E. coli</i>	100	0(0)	0(0)	0(0)	>0.05
	200	9(0)	0(0)	0(0)	<0.05
	400	11(0)	12(0)	0(0)	<0.05
	chloramphenicol	20(0)	19.5(2.12)	18.5(0.707)	>0.05
<i>P. aeruginosa</i>	100	0(0)	0(0)	0(0)	>0.05
	200	11(0)	0(0)	0(0)	<0.05
	400	15(0)	15(0)	0(0)	<0.05
	chloramphenicol	18(0)	21(0)	22.4(3.53)	<0.05

As shown in Table 3, all the extracts had antibacterial activity against *S. aureus*, while the cold aqueous and methanol extracts showed the highest inhibition zone, with a significant statistical difference. *E. coli* was resistant to all extracts except for cold aqueous extract, which showed 10mm inhibition zone at 400mg/ml. The cold aqueous and ethanol extracts showed antibacterial activity against

P. aeruginosa while for the others they were resistant. The hot aqueous extract did not show any result in all bacteria's; therefore, it is not presented on the table. Moreover; *S. aureus* was sensitive to all solvent extracts of *S. incanum* root and it was most sensitive to chloroform extract which showed a 24.5 mm inhibition zone at 400mg/ml (p -value <0.05). A 16mm inhibition zone was recorded, at

400mg/ml, in cold aqueous extract against *E. coli*, while the others did not show any antibacterial activity. Chloroform, cold aqueous, and ethanol were the extracts which showed antibacterial activity against

P. aeruginosa. On the contrary, the chloroform extract was the most potent, which yielded a result in all three concentrations. Hot aqueous extract did not show any result against all bacteria (Table 4).

Table 3 Antibacterial activity of *S. incanum* leaf against *S. aureus*, *E. coli* and *P. aeruginosa* (SD) standard deviations

Bacterial strains	Concentrations (mg/dl)	Cold aqueous	Methanol	Ethanol	Chloroform	p-value	Post hoc (Tukey LSD)
<i>S. aureus</i>	100	0(0)	0(0)	0(0)	11(0)	<0.05	A, B, C<D
	200	7(0)	12(0)	0(0)	13(0)	<0.05	A<B, D>C
	400	15(0)	16(0)	11(0)	13(0)	<0.05	A, B>C<D
	ciprofloxacin	18(0)	19(0)	18(0)	16(0)	<0.05	A, B, C>D
<i>E. coli</i>	100	0(0)	0(0)	0(0)	0(0)	>0.05	A, B, C, D
	200	0(0)	0(0)	0(0)	0(0)	<0.05	A, B>C, D
	400	10(0)	0(0)	0(0)	0(0)	<0.05	A>B, C, D
	chloramphenicol	23.5(2.12)	24.5(3.53)	23.5(2.12)	24(0)	>0.05	A, B, C, D
<i>P. aeruginosa</i>	100	0(0)	0(0)	0(0)	0(0)	>0.05	A, B, C, D
	200	0(0)	0(0)	0(0)	0(0)	>0.05	A, B, C, D
	400	12(0)	0(0)	7.50±0.707	0(0)	<0.05	A>B, D<C
	chloramphenicol	24.5(3.53)	23.5(2.12)	19.5(3.53)	25.4(2.83)	<0.05	A, B, D>C

Table 4 Antibacterial activity of *S. incanum* root against *S. aureus*, *E. coli* and *P. aeruginosa* (SD) standard deviations

Bacterial strains	Concentrations (mg/dl)	Cold aqueous	Methanol	Ethanol	Chloroform	p-value	Post hoc (Tukey LSD)
<i>S. aureus</i>	100	12.5(0.701)	9.0(0)	8(0)	22(0)	<0.05	A<D>B, C
	200	17.5(0.701)	11.5(0.701)	17(0.0)	22.5(0.701)	<0.05	D>A, C>B
	400	19(0)	12(0)	18(0)	24.5(0.701)	<0.05	D>A, C>B
	ciprofloxacin	18(0)	19(0)	18(0)	20.5(0.701)	<0.05	D>A, C, B
<i>E. coli</i>	100	0(0)	0(0)	0(0)	10(0)	<0.05	D>A, C, B
	200	16(0)	0(0)	0(0)	11.5(0.707)	<0.05	A>D>C, B
	400	16(0)	0(0)	0(0)	12.5(0.707)	<0.05	A>D>C, B
	chloramphenicol	23.5(3.53)	24.5(3.53)	23.5(2.12)	25.4(2.83)	>0.05	A, B, C, D
<i>P. aeruginosa</i>	100	0(0)	0(0)	0(0)	7(0)	>0.05	A, B, C, D
	200	0(0)	0(0)	0(0)	11.5(0.707)	<0.05	D>A, B, C
	400	12.5(0.701)	9(0)	8(0)	22(0)	<0.05	A<D>B, C
	chloramphenicol	17.5(0.701)	11.5(0.701)	17(0)	22.5(0.701)	<0.05	D>A, C>B

Minimum inhibitory concentration and Minimum bactericidal concentration

The plant extracts that 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 (Table 5).

Table 5 Minimum Inhibitory concentration and minimum bactericidal concentration

Organisms	MIC (mg/ml)	MBC (mg/ml)
Chloroform extract of the root of <i>S. incanum</i> root against <i>S. aureus</i>	50	0
Cold aqueous extract of the <i>S. macrosolen</i> stem against <i>E. coli</i>	25	50
Cold aqueous extract of the <i>S. macrosolen</i> stem against <i>P. aeruginosa</i>	25	50

Phytochemical analysis

The result of phytochemical investigation revealed that glycosides were positive for all extracts of *S. macrosolen* except for hot aqueous extract, others such as flavonoids, phenols, tannins and saponins show various results with different extracts, as can be seen in Table 6. The results for *Solanum incanum* also indicate that all solvent extracts were found to be positive for saponins, while all extracts were negative for flavonoids, except methanol and chloroform extracts. Likewise, tannins, phenols, and glycosides were positive in most of the extracts and negative in some of the extracts as shown in Table 6.

Discussion

The use of herbal drugs for the prevention and treatment of various health problems has been in practice since time immemorial. In Eritrea, traditional medicinal plants are widely used in the treatment of human ailments by traditional practitioners (herbalists). Although these practices are able to overcome many of the problems, they also impose undesirable side effects when not used in the proper manner, which implies for scientific research to be held that support this

practice. In the present study, two plants were subjected to cold and hot extraction, and subsequently, antimicrobial activity of the extracts was performed against the standard bacterial strains.

Table 6 Phytochemical analysis (A), *Solanum incanum*; (B), *Silene macrosolen*; (+), presence; (-), absence

Phytochemical	Test Method	Ethanol extract		Methanol extract		Chloroform extract		Hot aqueous extract		Cold aqueous extract	
		A	B	A	B	A	B	A	B	A	B
flavonoids	Alkaline reagent test	-	+	+	+	+	-	-	+	-	-
saponins	Foam test	+	+	+	+	+	-	+	+	+	+
tannins	Lead Acetate test	+	+	+	+	+	+	+	-	+	+
phenols	Ferric Chloride test	+	+	+	+	+	-	-	-	+	+
glycosides	Glycosides test	+	+	+	+	-	+	+	-	+	+

The effect of concentration on the antibacterial activity of plants was assessed and their antibacterial activity increased with increasing the concentrations of their crude extracts. In this study, three different concentrations of extracts were used (400mg/ml, 200mg/ml and 100mg/ml). From these concentrations, the highest effect was observed in 400mg/ml, followed by 200mg/ml, and the least effect was observed in 100mg/ml with a statistically significant p-value ($p < 0.05$). Previous researchers have reported similar results in Eritrea indicating that as the concentration of the plant powder is reduced to half, the sensitivity is also reduced to half.¹¹ A comparison of antibacterial activity of different solvent extracts was done per each plant material against respective bacteria. Upon antibacterial activities against *S. aureus*, methanol and cold aqueous extracts of *S. incanum* leaf (16mm and 15mm respectively), root of *S. macrosolen* (17.5mm and stem of 16.5mm respectively) and *S. macrosolen* (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 400 mg / ml on *S. incanum* root. Upon the antibacterial activities against *E. coli*, most solvent extracts of *S. incanum* leaf did not show any inhibition zone against *E. coli*, except for the cold aqueous extract with 10mm diameter at 400mg/ml. The cold aqueous extract showed the highest ($p < 0.05$) inhibition zone ($p < 0.05$) in the root of *S. incanum* at 16mm. Cold aqueous extracts and methanol showed the highest ($p < 0.05$) inhibition ($p < 0.05$) in both *S. macrosolen* stem (18.5 and in the 22.5mm respectively) and root (11mm and 12mm, respectively). This result is consistent (consistent) with a study by Mamta K *et al* which concluded that the aqueous root extract of *W. somnifera* has excellent potential as an antibacterial agent against *E. coli*. Upon the antibacterial activities against *P. aeruginosa*, the cold aqueous extract showed the highest effect ($p < 0.05$) with 12mm at 400mg/ml in the leaf of *S. incanum*. In *S. incanum* root, chloroform extract showed the highest effect ($p < 0.05$) with 13.5mm. In the stem of *S. macrosolen*, methanol extract showed the highest antibacterial activity ($p < 0.05$) with 26mm at 400mg/ml. Similarly, to the previous results, methanol and cold aqueous extract exhibited the highest ($p < 0.05$) antibacterial effect ($p < 0.05$) in the root of *S. macrosolen* with a zone of inhibition of 15mm each at 400mg/ml.

The general results indicated that almost all the methanol and aqueous cold 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 for almost all the selected phytochemical tests. The yield percentage of these solvent extracts was also found to be the highest. These findings might have an effect on the availability of the active ingredients of these plant extracts, thereby being the most antibacterial active plant extracts. These results are supported by a study conducted in India, which concludes

that aqueous and methanol root extracts of *Withania somnifera* could be exploited as a natural drug for the treatment of various infectious diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications.¹²

An overall comparison of the potency of the plant materials was also evaluated. *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. Additionally, *E. coli* and *P. aeruginosa* were highly sensitive to the methanol extract of the *S. macrosolen* stem at 400mg/ml. Therefore, the *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 *O. limbata*.¹³ Generally, *S. aureus*, *E. coli*, and *P. aeruginosa* showed a good sensitivity to cold aqueous and methanol extracts, but the gram-negative bacteria strains *E. coli* and *P. aeruginosa* were not sensitive to almost other plant extracts. These could be due to several possible reasons, one of which being that the distinctive feature of Gram-negative bacteria is the presence of a double membrane surrounding each bacterial cell. This outer membrane excludes certain drugs and antibiotics from penetrating the cell, partially explains why gram-negative bacteria are generally more resistant to antibiotics than other Gram-positive bacteria. From an overall point of view, hot aqueous extraction for each plant in every bacterium has not shown any results. This could be explained by suggesting that the bioactive compound of the plants could have been destroyed due to the high temperature used to extract the plants.

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,¹¹ where the extract of a *Solanaceae* family showed presence for the majority of the bioactive compounds.¹¹ So, this medicinal plant holds promises as to the source of pharmaceutically important phytochemicals. A study held in Kenya demonstrated that different parts of the *S. incanum* plant have almost similar phytochemicals present which is attributed to its antimicrobial activity hence there is a 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.¹⁴

Conclusion

Based on the antibacterial assay of this study, *S. aureus* was found to be more susceptible to the plant extracts than *E. coli* and *P. aeruginosa*. In the present study, the methanolic and cold aqueous

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

Limitations

Although the present study was an incomplete success, there were certain limitations that would have added to the significance of the study if they had been performed. Due to the absence of a blood agar medium, AST could not be performed using the standard strain of *Streptococcus pyogenic*. The presence of 'alkaloids' could not be tested due to a shortage of reagents. AST and phytochemical tests were not performed for chloroform extract of *Silene macrosolen* root because it could not be filtered as chloroform completely degraded the plant.

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None

Conflicts of interest

The author declares no conflicts of interest.

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