Phytochemical Screening and Anti-microbial Activity of Polygala sadebeckiana Gurke extracts on bacterial isolate from wound samples of patients with “Shimetere”

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Abstract

Background

As Guraghe community believed that modern medication use especially through injection route seriously aggravate the disease, the root part of *Polygala sadebeckiana Gürke* is commonly used as traditional medicine in the management of *"Shimetere"*, which is a common skin and soft tissue infection in the community. The aim of this study was to evaluate anti-microbial activity of Polygala sadebeckiana Gürke extract on bacterial isolate from wound samples of patients with "*Shimetere*".

Methods

Agar well diffusion method was used to evaluate antibacterial activity and *agar dilution method* was utilized to determine minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MIC). The crude extract was tested against isolated bacteria at the concentration of 25, 50, 75 and 100 mg/mL in triplet (3x). The positive controls were azithromycin (15µg) cloxacillin disk (5µg) and the negative control was dimethylsulfoxide (5%). The group means comparisons were made using one-way ANOVA at a significance level of $p < 0.05$ and the results were presented as mean ± standard deviation. The presence of secondary metabolites from crude extract was checked by standard testing procedures.

Results

S. aurous and *S. pyrogen* were the two identified bacteria from 9 (60%) and 3(20%) of wound samples, respectively. All identified bacterial strains were susceptible for the reference antibiotics. Tannins and saponins were the most abundant secondary metabolites found in the crude extracts. The average inhibition zone of the plant extract with 100, 75, 50 and 25 mg/mL concentration were 27, 20.33, 15.25, 11.96 mm ($p < 0.000$) for *S.aurous* and 30.02, 24.50, 19.07, 15.77 mm ($p < 0.000$) *S.pyrogen* bacteria. MIC and MBC of the crude extract were 1.67 and 10 mg/ml for *S. aureus* and 0.98 and 4 mg/ml for *S.pyrogen*.

Conclusion

Polygala sadebeckiana Gürke contained significant tannins and saponin as a secondary metabolites and had antibacterial activities against isolated bacteria (*S. aurous* and *S. pyrogen*) from "*Shimetere*".

INTRODUCTION

Human skin is thought as the first-line defense by acting as a physical barrier that protects the attack of microbial. However, when the defensive barrier is damaged, infections of soft tissue and skin may develop. *Staphylococcus aurous (S. aurous)* is the main cause of these infections [1].

Bacterial soft-tissue infections and skin are among the major communal health concern and hard to treat owing to the high occurrence of resistant bacterial strain such as methicillin-resistant *S. aurous(MRSA)* to the oldest generation antimicrobials in addition with unavailability and the higher cost of the newer generation drugs [1, 2].

"Shimetere" (its direct Amharic meaning "simu yemaytera", English meaning "never call its name"), which is considered as a common skin infection in Gurage community. According to many clinicians working at the health care facilities of the Gurage Zone, *"Shimetere" has similar* clinical presentations as cellulites. The symptoms of redness, pain, swelling and heat are the main characteristics of cellulitis [3].

Recommendation of definitive antimicrobial therapy of skin infections primarily depends on local practice, microorganism resistance rates and patient factors like hypersensitive to penicillin groups [3].

However, modern medicine is the not the choice of patients with "Shimetere" in Guraghe community owing to the fear of medication use via injection route might seriously aggravate the disease. For this reason, medicinal plants are becoming the focus of intense study in recent years to decide whether their traditional uses are supported by actual pharmacological effects or are merely based on falsehood [4].

Antimicrobial resistance bacteria like MRSA are associated with many clinical problems worldwide, considered as the other concern for modern medicine use [5]. Misusing of various antimicrobial drugs in the health care facility is considered the main reasons behind the emergence of antimicrobial resistance [6]. Antimicrobial over use in the health care facility has also significant role on the transmission of resistant microorganism strains [7].

Increased resistance of drug is the reason of serious microbial infections, various adverse effects, long term hospital stay and raised mortality rate. Over-prescribing of antimicrobial is connected with high risk of untoward drug effects, more frequent re-admission and risen medicalization of self- restricting conditions [8].

Moreover antibiotic resistance challenge has been attributed absence of new development of drug by the pharmaceutical manufacturing due to decreased economic encouragements and difficult regulatory requirements. Continued overuse of antibiotic in hospitals, in developing countries, contributed to a choice pressure that has persistent resistant microbial strains, forcing a shift to broad spectrum and more costly antibiotics [9].

Now days, the ever-increasing harm from drug-resistant bacteria calls for a world wide effort to search for novel solutions that can also be based on the natural products from plants that are selected on the basis of documented ethno-medicinal use. A focused phytochemical screening, backed by medicinal plant data, often guide s to the development of new compounds that can play a major role in the world efforts against microbials [10].

Antimicrobial activity of traditionally used medicinal plants studies is often important in helpful locally accessible and significant plant species especially for the detection of crude drugs as herbal medicine

might be harmless and would reduce resistance created by the pathogens, as they occur in a joined form of more than one molecule in the plant cell [11, 12].

Due to negative attitude towards modern medicine in Guraghe community, *Polygala sadebeckiana Gürke* is commonly utilized as traditional medicine in the treatment of *"Shimetere"* [2, 13, 14].

However there is no experimental study have so far been carried out on *Polygala sadebeckiana Gürke* although the plant is claimed to be used for its antimicrobial activity to treat *"Shimetere"* by Gurage zone traditional healers. Therefore, the aim of this study was to evaluate anti-microbial activity of Polygala sadebeckiana Gürke extract on bacterial isolate from wound samples of patients with *"Shimetere".*

METHODS AND MATERIALS

Description of Plant Collection Areas

Plant was collected by botanists and traditional healers from selected districts of Gurage zone. Gurage zone is located in is southern central Ethiopia. It is situated at 37° 50' 0"–38° 40' 0" E and 7° 40' 0"–8° 30' 0" N. Gurage zone covers an area of 5893.5 km2 with an altitude range of between 1000 and 3600m a.s.l. [13]. The mean yearly temperature is within the ranges of 13–30°C and receive an annual rainfall ranging from 600–1600 mm. Based on the recent classification of vegetation types of Ethiopia, the study area is largely covered by the dry evergreen Afromontane forest and grassland. Land use/cover map of the study area indicated that the cultivated land covered 52% and only 9.9% is covered by natural and human-made forest [14]. This study was conducted from February 2022 - June 2023 GC.

Polygala mooneyi M.G.Gilbert, which is synonym with *Polygala sadebeckiana Gürke.*, specimen was formally collected and identified by M.G.Gilbert, Sebsebe D. and K.Volleson, #8055 from Sidamo (Southern Ethiopia) in 1986 and the specimen was deposited in National Herbarium (ETH), with voucher ID number of ETH000000016 [15].

Study Design

Qualitative phytochemical screening test and in-vitro antibacterial evaluation was done through a randomized experimental design. All experimental tests were done three times (3x) together with the negative and positive controls.

Plant Collection and Preparation

Fresh and healthy roots of Polygala sadebeckiana was collected, cleaned using sterilized distilled water, cut into smaller sizes of about 1–2 cm long, and dried under shade at room temperature for fifteen days. By using wooden-made mortar and pestle, it was ground. Then, it was pounded using an electric grinder into a fine powder. Finally, it was kept in a refrigerator until to be used [16].

Plant Extraction

20–95% of the solvents (polar or/and non-polar) substances are frequently used by the herbal medicine industry to prepare plant crude extracts although a standardized extraction protocol has not been developed yet [16]. Previous studies indicate that methanol is a good solvent to extract the bioactive chemicals in the medicinal plants for antibacterial activities [17].

The powdered root of the plant was macerated in 80% methanol in a conical flask for 2 days with occasional shaking. The filtrate was separated from residue by Whatman No 1 filter paper and the residue was re-macerated by additional methanol for three to five times until the filtrate was free of any visible color. The filtrates was combined and dried in an oven at a temperature of 40^{o}C to remove methanol water. The dried extract was weighed and placed in tightly closed amber colored glass jar and kept in a refrigerator until to be used [18].

Phytochemical analysis

Phytochemical tests was carried out for the methanol extract of the plant using standard procedures to identify the presence of secondary metabolites, including alkaloids, flavonoids, terpenoids, tannins, Polyphenol, glycosides, phytosterols, and saponins [16].

Test for alkaloids

0.25gm of the crude extract was added to five drops of HCl and then it was filtered and finally the filtrate was mixed with Wagner's reagents to form a brown precipitate which indicates the presence of an alkaloid.

Saponins test

0.25gm of the crude extract will boil with 5 ml water for two minutes; the mixture will cool and mix vigorously and left for three minutes. The formation of a foam of 1 centimeter layer demonstrating the existence of saponin.

Test for Polyphenol

0.25gm of the crude extract was added to 4 drops of FeCl3. The formation of a blue-black color demonstrates the existence of phenols and this test is considered as a ferric chloride test.

Test for flavonoids

using an alkaline reagent test, some drops of NaOH solution was added to the crude extracts. The formation of intense yellow color reveals the existence of flavonoids.

Tests for phytosterols

the Szarkowski test was done by adding a few drops chloroform to 0.25grams of crude extract and filtered. The filtrate then was mixed with some drops of concentrated H2SO4, shaked and left for a few

minutes. The golden yellow color indicates the presence of phytosterols in the crude extract.

Test for tannins

2 milliliters test solution, and 1% Gelatin solution which contains 10% NaCl was mixed. The formation of white precipitate indicates the existence of tannins.

Tests for glycosides

Keller-Kiliani test was employed. A 0.1gm of plant extract will dissolve in 2 milliliters of glacial acetic acid which contains 1 drop of FeCl3 solution. The mixture poured into a test tube that contains 1 milliliter of concentrated H2SO4. A brown ring at the interphase indicates the existence of glycosides.

Bacterial isolation and characterization

Patients with "*Shimetere*" in the community, diagnosed by local traditional healers, were enrolled in the study. Samples were taken from the infection sites (wound surface) for bacterial isolation and characterization test. Wound surface was rinsed with sterile normal saline, then samples was collected using a sterile cotton swabs; the internal surface of infected area was swabbed slightly; swabs are added instantly into a tube having nutrient broth media then transferred to the biotechnology Laboratory unit of Wolkite University. Culture dependent laboratory methods were used for microbial isolation and characterization. Antibacterial susceptibility test result of isolated bacteria was reported as susceptible, intermediate or resistant by means of the standard set by the Clinical and Laboratory Standards Institute (CLSI) [19].

In-vitro Antibacterial Activity Test

Using *Agar-Well Diffusion Method*, Identified microbial strains was utilized to evaluate antibacterial activities of the crude plant extracts. *Broth-dilution Method* was used to determine MIC and MBC of the crude plant extracts.

Agar-Well Diffusion Method

Following inoculation of identified bacterial strains with sterile swabs at the surface of Mueller Hinton agar plates, it was allowed to waterless at room temperature. Six holes were created at equal distances from each other on the Mueller Hinton agar plate surface. The holes were then filled with the crude plant extracts at different concentration of 100, 75, 50 and 25 mg of extracts with 1mL of solvent (5% DMSO), the negative control of DMSO (5%) and antibiotic disk as a positive control. Cloxacillin (5µg/disk) and azithromycin (15µg/disk) were used as positive control as *S.aurous* and *S.pyrogen* were isolated bacterial strains. The experiment was repeated three times (3x). The plates was then kept at room temperature for about 1 hour to favor diffusion and incubated at the temperature of 37°c for 24 hours. After 24hrs of incubation, the antibacterial activity was determined by measuring the IZ diameter including the hole. The result of bacterial inhibition was evaluated by comparison with growth in negative and positive controls.

Susceptibility pattern of isolated bacteria to the reference drug was determined by measuring the IZ after 24hrs of incubation [20, 21].

Broth-dilution Method

MIC and MBC of crude extract was determined against *S. aureus* and *S. pyrogen by using* broth- dilution method with slight modifications [20, 21]. A 5% DMSO was used to dilute the crude extracts and the concentration of plant extracts ranged from 0.5 to 50 mg/mL. After serial dilution, 0.1 mL of the extract was mixed with 0.1 mL Mueller Hinton agar and poured on different test tubes. 50 µL of standardized inoculum (5 × 105 CFU/ml) were added to each test tube except negative control and incubated at 37°C for 24 hrs. Then, MIC was judged by comparison with growth in positive and negative controls. Finally, MBC was determined by incubation for the MIC test to 150 µL broth in the test tube, and incubated for 48 h at 37°C. The experiment was done three times (3x) for confirmation of the data.

Data analysis

The data were analysed by using Statistical Package for Social Science (SPSS) version 26 and presented as mean ± SD of three replicates. Statistical differences in the mean IZ for individual bacteria with differences in concentrations of the extract were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc at a significance level of $P < 0.05$. MIC and MBC were analyzed using descriptive statistics. The phytochemical tests was recorded as + (plus sign) for positive results for the tested bioactive compounds and – (minus sign) for the negative results for the tested result.

RESULTS

Bacteria isolation and characterization from wound samples

Overall, 15 wound samples of patients with *"Shimetere"* were collected from 5 patients, 3 (60%) were male, and their age ranged from 15 to 40 years. Two types of bacterial strains were isolated from four (80%) of the patients with "Shimetere". In 9 (60%) of the wound samples or 3(60%) of the patients, only *S. aurous* was isolated and in 3(20%) wound samples or 1(20%) of the patients, both *S. aurous* and *S. pyrogen* were identified. No bacteria were identified in the remaining 3 (20%) of the wound samples or 1(20%) of the patients [Fig. 1].

Antimicrobial susceptibility pattern of isolated bacteria

Both *S. aurous* and *S. pyrogen* bacteria were susceptible for reference antibiotics (cloxacillin and azithromycin). Cloxacillin (5 µg /disk) showed average diameter in the IZ of 40.9 mm against isolated *S. aureus*. Average diameters in the IZ of azithromycin (15µg /disk) were 41 mm and 46 mm against isolated *S. aureus* and *S. pyrogen* bacteria, respectively [Table 1].

Table 1
Antimicrobial susceptibility pattern of isolated bacteria from each patient using standard antibiotics

Standard drugs used	**Patient serial number**	**IZ of bacterial isolated from each patient samples (diameter in mm)**	
		Staphylococcus Aurous	***Streptococcus Pyrogen***
Azithromycin (15 µg/disk)	01	41.00	46.00
	02	42.00	-
	03	40.50	-
	04	40.50	-
	05	-	-
Average IZ (Diameter (mm) ± standard deviation)		41.00 ± 0.7	46.00 ± 0.00
Cloxacillin (5µg/disk)	01	43	x
	02	39	-
	03	42	-
	04	39.50	-
	05	-	-
Average IZ (Diameter (mm) ± standard deviation)		40.9 ± 1.93	
Note: ¯*Absence of isolated bacteria* ^{x}*Absence of the test*			

Phytochemical Screening of Crude Plant Extracts

The phytochemical screening of methanol extracts of Polygala sadebeckiana Gürke were summarized in Table 2 and Fig. 2. The methanol extracts consisted of all tested secondary metabolites. Tannins and saponins were the most abundant secondary metabolites found in the crude extracts, while all other tested secondary metabolite are slightly available.

Table 2
Results of Preliminary Phytochemical Screening of methanol extract of *Polygala sadebeckiana Gürke*

Test results	Secondary Metabolites						
	Saponin	Tannin	Polyphenol	Alkaloid	Flavonoid	Phytosterol	Glycoside
Moderately Present	+	+	-	-	-	-	-
Slightly present	-	-	+	+	+	+	+
Absent	-	-	-	-	-	-	-

Note: $^{+}$Yes, $^{-}$No

Antibacterial Activities of Methanol Crude Extracts: *Agar-Well Diffusion Method*

The results of the zone of inhibition (mm, diameter) generated by antibacterial activities of methanol crude extracts of *P. sadebeckiana* (Root) were measured and summarized in Table 3 and Fig. 3. The crude extracts concentrations were classified into 100, 75, 50 and 25 mg/mL in three replicates (3x) and exposed to *S. aureus*, and *S. Pyrogen*, which were isolated from patients with *"Shimetere"*.

Table 3
Antibacterial effects of methanol extracts of *Polygala sadebeckiana Gürke* with different concentrations (Mean of IZ of the three replicates (Diameter in (mm) ± Standard Deviation))

	[Concentration (mg/ml)]	Average IZ (Diameter (mm) ± standard deviation)			
		S. Aureus	*p-value*	*S. Pyrogen*	*p-value*
Plant extracts	**100 mg/ml**	27 ± 0.5	*0.000*	30.02 ± 0.42	*0.000*
	75 mg/ml	20.33 ± 0.58		24.50 ± 0.50	
	50 mg/ml	15.25 ± 0.66		19.07 ± 0.40	
	25 mg/ml	11.96 ± 0.48		15.77 ± 0.75	
Azithromycin	**15 µg/disk**	41 ± 0.7		46 ± 0.00	
Cloxacillin	**5 µg /disk**	40.9 ± 1.93		-	

Figure 3 Inhibition zone of extracts with different concentration against *S.aurous* and *S. pyrogen*

MIC and MBC: *Broth Dilution Method*

MIC and MBC test was carried out using a serial dilution of the crude extract by 5% DMSO. The methanol crude extracts were applied to the isolated bacterial strains with concentrations ranging from 0.5µg/mL

to 50 µg/mL and the results are shown in Table 4. The MIC and MBC values of the plant extracts were 1.67 and 10 mg/ml for *S. aureus* and 0.98 and 4 mg/ml for *S.pyrogen*.

Table 4
MIC and MBC of Methanol Extracts of Polygala sadebeckiana Gürke against identified Bacterial Strains (µg/mL)

Bacterial strains	The MIC and MBC of the Crude Extracts (mg/mL)		
	MIC	MBC	MBC/MIC
S. Aureus	1.67 ± 0.76	10.00 ± 1.00	5.99
S. Pyrogen	0.98 ± 1.32	4.00 ± 1.00	4.08

DISCUSSION

Infections of skin include pyoderma slight to severely necrotizing infections. It can be infected by several of microorganisms, such as bacteria, parasites and fungi. Gram positive bacteria such as S.aurous and streptococcus species are the most common causes of skin infection [22]. Antibiotic therapy is recommended after performing antibiotic susceptibility test [23].

The finding of our study showed that about 87% of wound samples were positive for *S.aurous* or *S. pyrogen* bacterial species and *staphylococcus aurous* was the most common (66.67%) isolates from the wound samples of *"Shimetere"* patients. The high prevalence might be because *S. aurous* is prevalent on the surface of skin that can easily enter into wounds as the disruption of the natural skin barrier. This is supported by the previous studies in different parts of the world, such as Egypt [24], Nigeria [25, 26], Bangladesh [27], Indonesia [28], and Italy [29].

According to our findings, all isolated *S. aureus* were susceptible to cloxacillin, so all isolate were methicillin susceptible *S. aureus* (MSSA). It is inconsistent from the previous study findings done in several part of the world reported prevalence of MRSA bacterial isolates from skin and soft tissues infection. MRSA from skin infection in Dessie referral hospital of Northeast Ethiopia (28.3%) [30], Sohag university hospitals of Egypt (89%) [31] and Birendra military hospital of Nepal (53.09%) [32]. The possible reason for absence of MRSA strain in this study is, patients don't believe in modern medicine, so the bacteria inside could not exposed to antibiotics to develop resistance. The small number of patient enrolled in might also lessen the prevalence MRSA strains.

There is a shift of focus to medicinal plants due to the increased antimicrobial resistance, severe adverse effects, high costs of synthetically available drugs and the community perception towards modern health care practice [33]. Many studies demonstrated that plant extracts are highly effective against bacterial microorganisms due to enormous kind of secondary metabolites [33, 34, 35].

In this study it was revealed that tannins and saponins were the most abundant secondary metabolites found as the two phytochemical test showed significantly visible positive results as compared to others

slightly available secondary metabolites in the methanol extracts of *Polygala sadebeckiana Gürke.* This result was slightly different from the previous study findings on the crude extracts of the plants with same family of *P. sadebeckiana* but different species. Study in Malaysia on *Polygala javana* plants revealed polyphenols, alkaloids, tannins, phytosterol and flavonoids were dominantly present but tannin was absent [36]. Similar study in Brazil on *Polygala boliviensis* showed significant presence of alkaloids, saponins, flavonoids, phenols, tanins and steroids [37]. The fact that different in species of the plant, maturity of the plant during collection, soil condition, fertilization, irrigation and pesticide use may be attributed for this disparity.

Plant extracts having chemicals with antimicrobial activity usually belong to alkaloids, flavonoids, polyphenol, saponins and tannins [38]. This study revealed Antibacterial activity of crude extract against *S. aurous* and *S. pyrogen* was associated with the high concentration of tannins and saponins as a secondary metabolite. This finding is supported by the previous study results such as: in Kenya the presence of saponin and tannins showed greater activity among the Gram positive bacteria than Gram negative bacteria [39], in Korea revealed saponin extract had strong antibacterial activities against gram positive bacteria by inhibiting cell wall [40] and in Nigeria saponin extract exhibited inhibitory effect on *S.aurous* but not on gram negative organism [41]. The mechanism of tannin and saponin might be the reason for their super antibacterial activity against gram positive bacterial strains.

For certain classes of antibiotics, the major killing effect against an organism is produced by either the time or the concentration of the drug at the binding site. In concentration-dependent bactericidal action, the rate of bactericidal activity will be depends on the magnitude of the maximum antibacterial concentration, whereas for drugs with time-dependent activity, the extent of bactericidal activity will depend mainly on the duration of drug exposure at concentrations great than the MIC [42].

The average IZ of the plant extract with 100, 75, 50 and 25 mg/mL concentration were 27, 20.33, 15.25, 11.96 mm ($p < 0.000$) for *S.aurous* and 30.02, 24.50, 19.07, 15.77 mm ($p < 0.000$) *S.pyrogen* bacteria. This result showed that the increase in concentration of *P. sadebeckiana* extract led to significantly increase in IZ diameter for both isolated bacteria strains. This finding was in agreement with the previous studies on antibacterial effects of medicinal plant extracts against similar bacterial species [38, 43, 44, 45]. However, the current study finding was different from other research work [19, 24, 43]. The possible reason may be antibacterial activity of the plant extract is concentration dependent.

MIC is defined as the lowest concentration of antimicrobials that inhibited the visible growth of microorganisms after overnight incubation. MBC is the lowest concentration antimicrobials that results in microbial death after sub culturing the organism in an antibiotic-free media [19].

The MIC and MBC values of the plant extracts were 1.67 and 10 mg/ml for *S. aureus* and 0.98 and 4 mg/ml for *S.pyrogen.* The results in the present study revealed that the methanol extract of *P. sadebeckiana* root was more potent against *S. pyrogen* than *S. aureus* isolate. The plant extract in our study was relatively more potent to inhibit the growth of *S. pyrogen* and/or *S. aureus* than several plant extracts on the previous studies [19, 22, 35, 38, 46]. However, it was less potent than plant extracts on

other study findings [35, 37]. The difference in potency of the plant extracts might be due to the diversity in susceptibility patterns of isolated *S. pyrogen* and *S. aureus* bacterial strains.

The MBC/MIC ratios of the methanol extracts were 5.99 and 4.08 against *S. pyrogen* than *S. aureus* isolate. The MBC/MIC ratio greater than 4 is usually considered to be a bacteriostatic effect; whereas values less than 4 show bactericidal effects [19]. So, the methanol extracts of *P. sadebeckiana* was determined to bacteriostatic for both bacterial isolates. The bacteriostatic nature the plant extract might contribute for the recurrence of *"Shimetere"* because of reinfections in spite of the patient use roots of the plant as a traditional remedy.

CONCLUSION

S. aurous and *S. pyrogen* were the two bacterial strains isolated from *"Shimetere"* which were susceptible for the reference antibiotics. Tannins and saponins were the most abundant secondary metabolites found in the crude extract though other tested secondary metabolite were slightly available. *Polygala sadebeckiana Gürke* extract had antibacterial activities against diameter for both isolated bacteria strains, which was increased significantly by increasing concentration of the plant extract.

Abbreviations

DMSO-Dimethyl sulfoxide, MBC-Minimum Bactericidal Concentration, MDR-Multi-Drug Resistant, MIC-Minimum Inhibitory Concentration, MRSA-Methicillin-Resistant Staphylococcus Aurous, MSSA-Methicillin-Susceptible Staphylococcus Aurous, IZ-Zone of Inhibition

RECOMMENDATION

Further studies needed to be conducted on *"Shimetere"* and other effects of *Polygala sadebeckiana Gürke* plant extracts.

LIMITATION OF THE STUDY

As bacteria were isolated from wound sample of only five patients which might decreases the possibility of identifying other bacterial species and resistant strains.

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from Wolkite University ethical review board. Written informed consent was obtained from study participants before collecting patient samples. Patient identifiers were not used to confirm the privacy of study participants and all data taken during the data collection process was kept confidential and used only for our research purpose. Generally, Experimental procedures was

conducted in accordance with the World Medical Association (WMA) Declaration of Helsinki and Collection of plant material complied with Convention on international trade in endangered species of wild fauna and flora.

Consent to Publish:

It is not applicable because the manuscript cannot contain individual person's data in any form (including individual's details images or videos).

Availability of data and materials:

The datasets analyzed during this study are available from the corresponding author on reasonable request.

Competing Interests: Author declares that there is no competing of interest.

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Author's Contribution:

BZ participated in the design of the study, supervised and checked the data collection, analysis and helped by drafting and checking the manuscript. Other authors participated in the design of the study, supervised and checked the data collection and helped to draft the manuscript. All authors read and approved the final manuscript.

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Figures

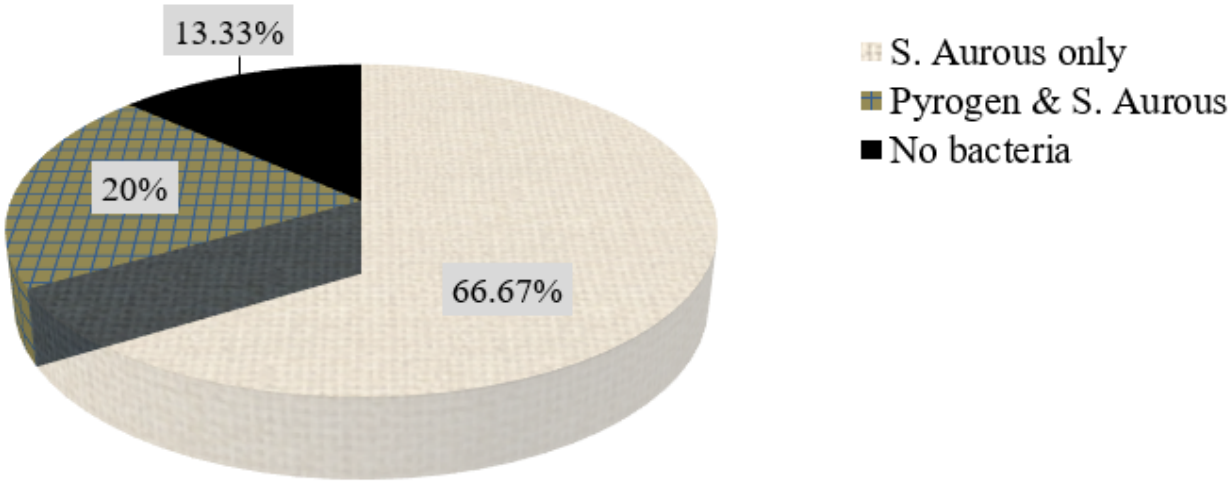

Figure 1

Type and percentage of identified bacteria among the total number of wound samples collected from patients with "*Shimetere*"

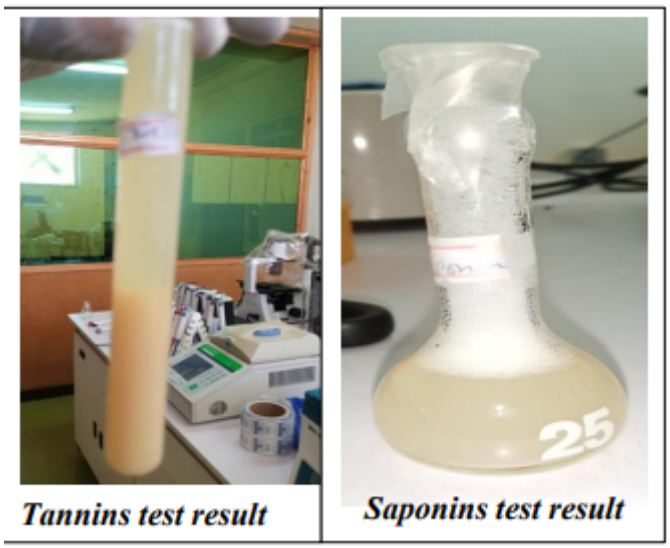

Figure 2

Moderately positive test results from phytochemical screening

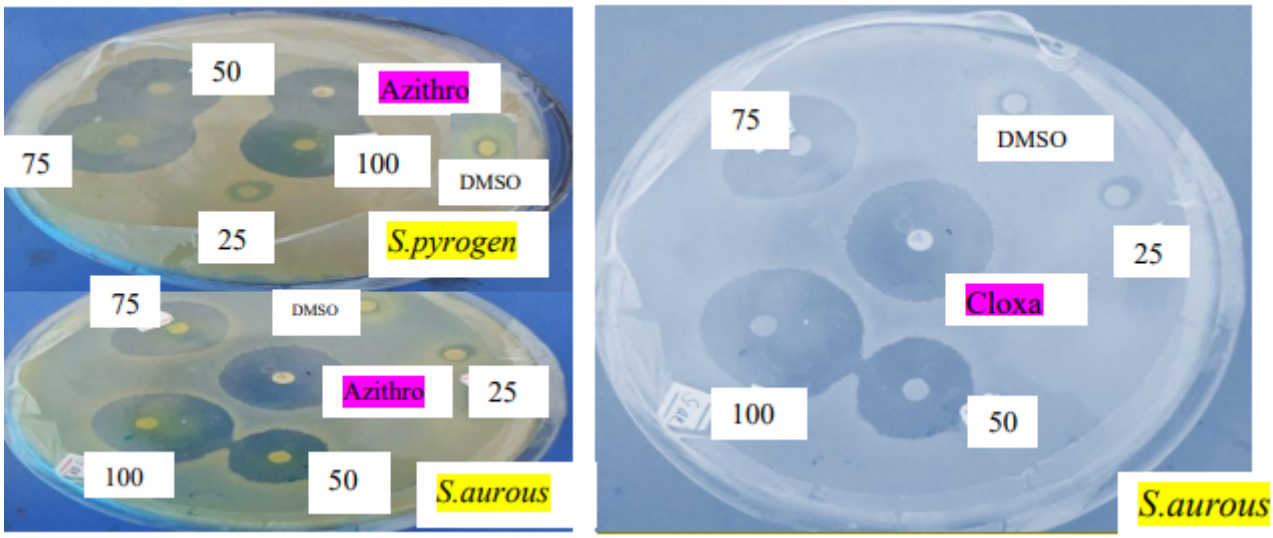

Figure 3

Inhibition zone of extracts with different concentration against *S.aurous* and *S. pyrogen*