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An *In vitro* investigation of Antibacterial Effect of Bark Root Extracts of *Solanum incanum* and Croton macrostachyus

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ABSTRACT

Background: The increasing antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. An in vitro experimental study was conducted with the aim to evaluate antibacterial effect of bark root extracts of S. incanum and C. macrostachyus against S. aureus and E. coli. The experimental study was carried out from November 2018 to April 2019 in Jimma University. The crude extracts of S. incanum and C. macrostachyus were extracted with petroleum ether, ethanol and distilled water using maceration methods. The antibacterial assay was carried out with agar well diffusion and minimum inhibition concentration.

Results: C. macrostachyus bark root ethanol extracts have generated antibacterial effect against E. coli and S. aureus with maximum zone of inhibition of 15 ± 0.58 mm and 17.33 ± 0.89 mm respectively. However, bark root C. macrostachyus aqueous extracts were showed lowest mean zone of inhibition (9 ± 0.33 mm and 9 ± 0.58 mm) against E. coli and S. aureus respectively. Petroleum ether bark root extract of S. incanum was showed highest zone of inhibition against E. coli (21.33 ± 0.33 mm) than S. aureus (14.67 ± 0.33 mm). S. incanum bark root ethanol extract was generated highest zone of inhibition of 22.33 ± 0.89 mm and 21.33 ± 0.33 mm against S. aureus and E. coli respectively. Aqueous bark root extract of S. incanum and C. macrostachyus had showed lowest zone of inhibition against E. coli and S. aureus. Bark root ethanol extract of S. incanum was generated minimum inhibitor concentration against S. aureus (6.25 mg/ml) and E. coli (12.5 mg/ml) than other solvents. There was statistical difference ($p<0.05$) between the concentrations of bark root ethanol and petroleum ether extract of Solanum incanum and Croton macrostachyus against S. aureus and E. coli. However, there was no statistical significant difference ($p>0.05$) between the concentration of S. incanum and C. macrostachyus aqueous extract against E. coli and S. aureus.

Conclusion: Bioactive compounds of medicinal plant extracts have been used to overcome the challenges of antimicrobial resistance. The current experimental study showed that, the bark root extract of Solanum incanum and Croton macrostachyus have high potent of antibacterial activities against E. coli and S. aureus. This study therefore substantiates the use of Solanum incanum and Croton macrostachyus as an antimicrobial medicinal plant.

Keywords: Antibacterial; *C. macrostachyus* extract; *E. coli*; *S. incanum* extract; Solvents; *S. aureus*

Introduction

Antimicrobial resistance in bacterial pathogens is a worldwide challenge associated with high morbidity and mortality. Multidrug resistant patterns in Gram-positive and negative bacteria have resulted in difficult to treat with conventional antimicrobials. Broad spectrum antibiotics are liberally and mostly unnecessarily used and result in emergency of resistance bacteria [1]. The emergence of resistant infections caused by most bacteria has led to mortality and morbidity and there is an urgent need to find solutions to combat bacterial resistance [2]. The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections; this is due to excessive use of antimicrobial, incorrect antimicrobial dosage and unregulated access to drugs (WHO, 2011). The reservoir of resistant bacteria in food animals implies a potential risk for transfer of resistant bacteria, or resistance genes, from food animals to humans [3,4].

The increasing antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [5]. Medicinal plant has great role in care of primary health of humans and animals due to its biological and medicinal activities, high safety margins and ability to overcome drug resistance action of pathogens [6]. Medicinal plants are an important source of traditional drugs, modern medicines, folk medicines, nutraceuticals, pharmaceutical intermediates and entities for synthetic drugs since plant extracts contain many medicinal metabolites such as alkaloids, glycosides, terpenoids, flavonoids and lignins (Tiwar et al., 2011). Antimicrobial compounds of medicinal plants differ from antibiotics as they have fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature [7]. Ethno-veterinary

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practice to animal health care is as old as the domestication of various livestock species [8]. There are so many medicinal plants used to treat food borne diseases which associated with gastroenteritis in humans and animals, among plants *Solanum incanum* and Croton macrostachyus are the common for treatment of food borne diseases associated with diarrhea [9].

Many researches has been done on plant extract of Croton macrostachyus and *Solanum incanum* against both *E. coli* and Staphylococcus aureus, but there is no petroleum ether and ethanol bark root extracts of *Solanum incanum* and Croton macrostachyus were conducted. Therefore, the objective of this study was:

To evaluate antibacterial activity of ethanol, petroleum ether and aqueous bark root extractions of *Solanum incanum* and Croton macrostachyus against Diarrheogenic Escherichia coli and Staphylococcus aureus.

Material and Methods

Study design

An *in vitro* experimental study was conducted in Jimma University, Ethiopia from November 2018 to April 2019 to evaluate antibacterial effect of bark root extracts of S. incanum and C. macrostachyus against *S. aureus* and *E. coli*. The bark root plants were collected and the antimicrobial effect of the plants was conducted in the veterinary laboratory of the Jimma University.

Antimicrobial activities were determined by using agar well diffusion and minimum inhibitor concentrations (broth macrodilution) method. The extraction of plants were done using three different solvents; petroleum ether, ethanol and distilled water. Negative control (DMSO) and positive control (gentamicin) were used to monitor antibacterial activities of bark roots extracts of plants against isolated bacteria in all assays. For this study *E. coli* were isolated from fecal sample of diarrheic calf and Staphylococcus aureus were isolated from mastitis cow.

Plant collection

Bark root of *Solanum incanum* and Croton macrostachyus were collected for experimental study from West Shoa, Tokke Kutaye Woreda, Lencha and Berodo kebeles (Table 1). Fresh root of the plants were harvested from the field by digging and picking off the roots. The roots were carefully picked off and washed with tap water, and then the barks were removed and chopped into piece. The bark roots were collected and dried under shade for 20 days. Then, dried bark was packed in plastic bag and transported to Jimma University, department of Organic chemistry, at Analytic organic and inorganic chemistry research laboratory for extraction. The collected barks were grinded to powder by using mortar and pestle, and the powder was collected and stored at room temperature until extraction procedures undergone.

Table 1: The medicinal plants

Local name	Scientific name	Collected part of the plant	Site where plant collected
Hiddii loonii (O), Embuay (A)	Solanum incanum	Bark root	Tokke Kutaye Wereda, West Shoa, , Ethiopia
Bakkaniisa (O), Bisana (A)	Croton macrostachyus	Bark root	Tokke Kutaye Wereda, West Shoa, Ethiopia
Note: O=Afaan Oromo language, A=Amharic language			

Procedures of crude extraction of c. macrostachyus and s. incanum

The extraction methods for this study were carried out by maceration. Maceration method is the most common and easy procedure for crude extraction of medicinal plant. Extracted powdered bark root of *Solanum incanum* and Croton macrostachyus were mixed with 500 ml of 99.9% of petroleum ether in a flask. The mixture was shaken gently twice a day for three days. The solution was filtrated by six fold of gauze followed by Whitman No.1 filter paper. The filtered extraction was kept under Rota vapor at 40°C for evaporation of petroleum ether from solution to obtain the powder of extracts. The extracts were kept under room temperature and placed in dry oven at 40°C when we expect that solvent remained in the powder. This procedure was also done in the same way for ethanol and distilled water as a solvent.

Bacteria inoculum preparation

The inoculums of bacteria were done according to National Committee for clinical laboratory standards (CLS, 2009), Three to five pure colonies of bacteria were taken by touching the top of colonies and transferred into 5 ml of tryptophan broth and incubated at 37°C for 24 hrs. Then after bacterial inoculum suspensions were prepared by serially dilution and standardized with sterile saline to turbidity equivalent to 0.5 McFarland (1.5 x 108 CFU/ml). The suspension was adjusted to 0.5 McFarland turbidity according to the guidance of Roopshree and Balunas and Kinghorn using UV Visible spectrophotometer at 625 nm equal to (OD:0.01-0.08) [10,11].

Antibacterial susceptibility test

Agar well diffusion: Antibacterial susceptibility tests were carried out in JUCAVM Veterinary Microbiology Laboratory by two methods included minimum inhibitor concentration and agar well diffusion according to National Committee for Clinical Laboratory Stands (CLSI, 2009). Antibacterial susceptibility test for *E. coli* and *S. aureus* was determined using agar well diffusion method on Muller-Hinton agar. Petroleum ether, ethanol and aqueous bark roots extracts of Croton macrostachyus and S. incanum were used to test bacterial susceptibility test. The prepared inoculums of bacteria, which have 0.5 McFarland standards, were used for the process of susceptibility test. The sterile cotton swabs were dipped into the adjusted suspension of inoculum by pressing and rotating the swabs firmly against the inside of tubes above fluid level. Then, the swab was streaked over the surface of the Muller-Hinton agar plate repeatedly in three directions over surface of agar. Then the well or holes of 6 mm diameter were prepared into seeded Muller-Hinton agar using sterilize glass pipette.

The working solutions were prepared from the prepared stock solutions in twofold serial dilution methods. The concentrations of extracts were started from 100 mg/ml and ends at 3.125 mg/ml. 0.01 ml of the extracts of bark roots of each plant were filled into the prepared well using syringe. The gentamicin disc (10 µg/disc) and DMSO (0.01 ml) were used as positive and negative control respectively. Then, the inoculated plates were allowed to stand in safety cabinet for one hour to allow the extracts to diffuse into seeded Muller-Hinton agar and then incubated at 24 hrs for 37°C. After the incubation, the bacterial growth was determined by measuring the diameter of zone of inhibition around the well including well diameter. The zone of inhibition value was measured using caliper meter in millimeter for three replications of extracts against *E. coli* and *S. aureus*.

Minimum inhibitor concentration determination by broth micro dilution: Minimum inhibitor concentration is the lowest concentration of the extracts that inhibited the visible growth of bacteria. The least concentration of the plant extract that did not permit any visible growth of inoculates bacteria in broth culture as indicated by lack of turbidity was regarded as visual MIC in each case (Michael et al., 2003). The MIC was determined using broth dilution methods according to the description of Sambrook and Russell [12]. The MIC of bark roots extracts of Croton macrostachyus and *Solanum incanum* were carried out using two-fold broth dilution methods started from 100 mg/ml to 3.125 mg/ml of concentrations. The concentrations of extracts were prepared from stock solution. 1 ml of 200 mg/ml of each extracts were added to test tube containing 1 ml of nutrient broth in each tube-using syringe and serially diluted in two-fold dilution techniques to get concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml of plant extract.

The mixture of plant extracts with nutrient broth was inoculated with 0.01 ml of bacterial inoculum suspension, which has turbidity of 0.5 McFarland standards. The nutrient broth inoculated with sterile distilled water and nutrient broth with bacterial inoculum was used as negative control. Then, the inoculated test tube was caped using sterile cotton and incubated at 37°C for 24 hours. The minimum inhibitor concentration was observed after 24 hrs. and the presence of growth was evaluated by comparing turbidity of culture containing test tubes with the negative control. The lowest concentration in which there was no turbidity formation was regarded as MIC value.

Statistical data analysis

The data was analyzed with the aid of Microsoft excel 2016 and SPSS version 20. The data were collected in Microsoft excel and subjected to one-way ANOVA to determine statistical difference between different concentrations of extracts against *E. coli* and *S. aureus*. The post-hoc analyses with least significant difference (LSD) were employed to find the specific significant difference between the concentrations of extracts. Mean values of zone of inhibition were expressed as Mean ± Standard error and $p<0.05$ were regarded as statistically significant difference.

Results

Medicinal plants namely *Solanum incanum* and Croton macrostachyus collected from Tokke Kutaye Wereda, West Shoa of Ethiopia was investigated for their medicinal value (Table 1). The extracts of these plants had showed effective anti-bacterial activity against pathogenic *E. coli* and *S. aureus* in an *in vitro* trial. The antibacterial efficacy was related with the concentrations of extracts, as the concentration increased in two fold the mean zone of inhibition also relatively increased with little difference. When the concentration of extracts increased the zone of inhibition also increased, this showed that the crude extracts have direct proportion of dose to zone of inhibition. As this study showed that, the efficacy of antimicrobial activities of *Solanum incanum* and Croton macrostachyus were depending on the factors including; types of solvents, concentrations and medicinal plant. The bark root ethanol extracts are more effective than bark root petroleum ether extracts in inhibiting the growth of bacterial pathogens.

Antibacterial activities of bark roots extract of c. macrostachyus

In this study, Croton macrostachyus bark root extracts were performed *in vitro* against the growth of *E. coli* and *S. aureus* with different concentrations ranged from 100 to 3.125 mg/ml. Mean zone of inhibition of bark root of Croton macrostachyus extracts were recorded and generated as indicated in Table 2. The highest concentrations (100 mg/ml) of bark root Croton macrostachyus petroleum ether and ethanol extracts were showed highest mean zone of inhibition against *S. aureus* and *E. coli*. However, bark root Croton macrostachyus aqueous extracts were showed low mean zone of inhibition (9 ± 0.33 mm and 9 ± 0.58 mm) against *E. coli* and *S. aureus* at 100 mg/ml. Gentamicin and Dimethyl Sulphoxide (DMSO) were used as positive control and negative control respectively. Gentamicin (10 µgm/disc) had inhibited the growth of *S. aureus* and *E. coli* with recorded mean zone of inhibition about 24.67 ± 0.33 mm and 23 ± 0.58 mm respectively. The DMSO (5%) was not showed any inhibition effect on both *E. coli* and *S. aureus*. There was significant difference ($p<0.05$) in the mean zones of inhibition of the petroleum ether and ethanol bark root extracts of C. macrostachyus against *S. aureus* and *E. coli* among each concentration. However, aqueous extracts of C. macrostachyus showed non-significant difference ($p>0.05$) in the mean zone of inhibition among each of the concentrations against *E. coli* and *S. aureus* as indicated in Table 2.

Table 2: Mean Zone of inhibition of bark root extracts of C. macrostachyus against E. coli and S. aureus

Concentration (mg/ml)	Mean Zone of inhibition(mm), N=3					
	CBRPEE		CBRAE		CBRAE	
	E.coli	S.aureus	E.coli	S.aureus	E.coli	S.aureus
100	14 ± 0.58^{a}	14.33 ± 0.33^{a}	15 ± 0.58^{e}	17.33 ± 0.89^{d}	9 ± 0.33^{b}	9 ± 0.58b
50	11.33 ± 0.33^{b}	13 ± 0.58^{a}	11.67 ± 0.33^{b}	15.33 ± 0.89^{e}	--	--
25	10 ± 0.58^{b}	12 ± 0.58^{a}	10.67 ± 0.33^{b}	13 ± 0.58^{a}	--	--
12.5	--	10.67 ± 0.33^{b}	--	11 ± 0^{b}	--	--

6.25	--	9.67 ± 0.33[b]	--	10 ± 0.58[b]	--	--
3.125	--	--	--	--	--	--
G N T (1 0 µ m g / disc)	23 ± 0.58[c]	24.67 ± 0.33[c]	23 ± 0.58[c]	24.67 ± 0.33[c]	23 ± 0.58[c]	24.67 ± 0.33[c]
DMSO	--	--	--	--	--	--

Note: CBRPEE: Croton macrostachyus bark root petroleum ether extract, CBREE: Croton macrostachyus bark root ethanol extract, CBRAE: Croton macrostachyus bark root extract of aqueous extract, DMSO: Dimethyl Sulphoxide, GNT: Gentamicin, (--) no zone of inhibition N: number of replication with mean ± Standard error. Mean zone inhibition value with different superscripts in the same column are significantly different P<0.05.

Antibacterial activities of bark roots extracts of s. incanum

The experimental outcome of this study was generated from the triplicate trials with the mean of inhibition against *E. coli* and *S. aureus* with different concentrations of plant extracts (petroleum ether, ethanol and aqueous extracts). The bark root extracts of *Solanum incanum* were performed against the growth of *E. coli* and *S. aureus*. The mean zone of inhibition of bark root of *Solanum incanum* extracts were recorded and generated as Table 3. The *Solanum incanum* bark root ethanol extracts were generated highest zone of inhibition (22.33 ± 0.89 mm) at 100 mg/ml and lowest zone of inhibition (≤ 11 ± 0.58 mm) at 6.25-3.125 mg/ml against *E. coli*. However, *Solanum incanum* bark root ethanol extract had generated the highest zone of inhibition (22.67 ± 0.89 mm) at 100 mg/ml and lowest zone of inhibition (≤ 12 ± 0.58 mm) at 6.25-3.125 mg/ml against *S. aureus*.

Table 3: Mean Zone of Inhibition of bark root extracts of S.incanum against E. coli and S. aureus

Concentration (mg/ml)	**Mean Zone of inhibition (mm), N:3**					
	SBRPEE		SBREE		SBRAE	
	E.coli	S.aureus	E.coli	S.aureus	E.coli	S.aureus
100	21.33 ± 0.33[a]	14.67 ± 0.33[e]	22.33 ± 0.89[a]	22.67 ± 0.89[a]	9.33 ± 0.33[d]	9.33 ± 0.33[d]
50	18.67 ± 0.33[b]	13.67 ± 0.33[e]	19 ± 0.58[b]	20.33 ± 0.89[b]	--	--
25	16.67 ± 0.67[c]	12 ± 0.57[e]	17 ± 0.58[c]	17.67 ± 0.33[c]	--	--
12.5	11.67 ± 0.89[d]	10 ± 0.57[d]	14.33 ± 0.33[e]	15 ± 0.58[e]	--	--
6.25	10 ± 0.58[d]	7 ± 0.58[f]	11 ± 0.58[d]	12 ± 0.58[d]	--	--
3.125	--	--	--	--	--	--
G N T (1 0 µ m g / disc)	23 ± 0.58[a]	24.67 ± 0.33a	23 ± 0.58a	24.67 ± 0.33[a]	23 ± 0.58[a]	24.67 ± 0.33[a]
DMSO	--	--	--	--	--	--

Note:SBRPEE: Solanum incanum bark root petroleum ether extract, SBREE: Solanum incanm bark root ethanol extract, SBRAE: Solanum incanum bark root aqueous extract, DMSO: Dimethyl Sulphoxide,GNT: Gentamicin, --: no zone of inhibition. N: number of replication with mean ± Standard error. Mean zone inhibition value with different superscripts in the same column are significantly different P<0.05.

MIC of c. macrostachyus and s. incanum extract against bacteria

MIC of petroleum ether, ethanol and aqueous extracts of Croton macrostachyus and *Solanum incanum* bark root were determined with two-fold serial broth dilution method against E. coli and *S. aureus*. The results for MIC of Croton macrostachyus and *Solanum incanum* bark root were indicated in Table 4. No any inhibition activities were showed within distilled water which was used as negative control.

Table 4: Minimum inhibition concentration of extracts against S.aureus and E. coli

Bacteria spp.	**MIC of Solanum incanum bark roots extract(mg/ml)**		**Control**		**MIC of Croton macrostachyus bark roots extract (mg/ml)**			**Control**
	PEE	EE	AE	SDW	PEE	EE	AE	SDW
E.coli	25	12.5	100	---	50	25	100	---
S. aureus	12.5	6.25	100	---	25		12.5	100

Note: PEE: Petroleum ether extract; EE: Ethanol extract; AE: Aqueous extract; SDW: Sterile distilled water; (---) No inhibition effect

Discussion

Antibacterial effect of croton macrostachyus extracts

In this study, bark root Croton macrostachyus extracts showed antimicrobial activities against gram positive and gram negative bacteria. In this study, the extractions of Croton macrostachyus bark root were performed with different solvents (petroleum ether, ethanol and distilled water). Croton macrostachyus bark root ethanol extract had showed maximum mean zone inhibition (17.33 ± 0.89 mm) and minimum mean zone inhibition (10 ± 0.58 mm) against *S. aureus*. Habtom and Kebede, (2017) reported that, crude extracts of Croton macrostachyus had showed the maximum zone of inhibitions (17 mm) against Staphylococcus aureus. Aylate et al., (2017) also indicated that, methanol leaf extract of Croton macrostachyus had showed the lowest zone of inhibition (9.25 ± 0.54 mm) and highest inhibition zone (21.63 ± 0.02 mm) against *E. coli* and *S. aureus* respectively. Wagaw et al., (2015) showed that, the ethanol leaves extract of C. macrostachyus against *E. coli* was 13.33 ± 1.53 mm and also Jepkoech and Gakunga (2017) reported that, methanolic extract of Croton macrostachyus was showed zone inhibition (12 mm) against S. aurous at a concentration of 100 mg/ml.

In this study, petroleum ether extract of Croton macrostachyus showed antibacterial activity against the growth of S. aurous and *E. coli*. Petroleum ether extract of *Solanum incanum* had showed mean zone of inhibition ranges from 10 ± 0.58 -14 ± 0.58 mm and 9.67 ± 0.33 -14.33 ± 0.33 mm against *E. coli* and S. aurous respectively. S. aurous was more susceptible to petroleum ether extract of Croton macrostachyus than *E. coli* with equal concentration. This is due S. aurous have only one peptidoglycan layer and lack of lipopolysaccharide. But, gram negative bacteria have outer membrane lipopolysaccharide which endows the bacterial surface with strong hydrophobicity and acts as strong permeability barrier (Jolly and Menon, 2015). Therefore, the bioactive compounds of Croton macrostachyus can easily penetrate into the cell of S. aurous and inhibit its growth.

Aqueous bark root extract of Croton macrostachyus were showed lower zone of inhibition against *E. coli* and *S. aureus*. As Jepkoech and Gakunga (2017) reported that, Aqueous Croton macrostachyus leave extract had showed the zone inhibition (9.25 mm) against *S. aureus* at 100 mg/ml. Therefore, it supported with this current study which was recorded as root of Croton macrostachyus generated mean zone of inhibition (9 ± 0.33 mm) against *S. aureus* and E. coli. Aqueous extract of Croton macrostachyus was generated low zone of inhibition; this may be due to the effect of temperature on bioactive compounds during extraction procedures. The phytochemical constituents of aqueous root extract of Croton macrostachyus may be denatured and removed from the crude extraction while the evaporation procedures. Some phytochemical compounds are thermolabile, easily evaporated and denatured. Therefore this is the reason why aqueous extraction usually generates low antimicrobial activities. Croton bark root ethanol extract pronounced more antibacterial effect against both *E. coli* and S. aureus when compared to petroleum ether extract. This may be due to the presence of polar and non-polar, or lipophilic and lipophobic bioactive in ethanol extract. The antibacterial activities of bark root extract of Croton macrostachyus had showed high zone of inhibition (17.33 ± 0.089 mm and 15 ± 0.58 mm). But, when compared the extracts of Croton macrostachyus is lesser than gentamicin 23 ± 0.58 mm and 24.67 ± 0.33 mm.

Antibacterial effect of *Solanum incanum* extracts

In this study, bark root of *Solanum incanum* extracts showed an antibacterial broad spectrum activity against Gram-negative and Gram positive bacteria. The difference in the antimicrobial effect *Solanum incanum* extracts against *S. aureus* and *E. coli* may be due to differences in permeability barriers. In this study, the antibacterial activities of *Solanum incanum* petroleum ether and ethanol extract was more pronounced than aqueous extract. Tewelde and Ghebriel (2017) reported that, Petroleum ether fruit extract of *Solanum incanum* had showed mean zone of inhibition of 6.7 ± 0.58 mm and 5.5 ± 0.5 mm at 100 mg/ml against *E. coli* and *S. aureus*. In contrast to Tewelde and Ghebriel (2017), this study showed that *Solanum incanum* bark root petroleum ether extract generated antibacterial effect against both *E. coli* and *S. aureus* at 100 mg/ml with mean zone of inhibition of 21.33 ± 0.33 mm and 14.67 ± 0.33 mm respectively. This contradicted result may be due to the part of plant extract and the chemical constituents of bark root and fruit of *Solanum incanum*. Therefore, bark root extracts of *Solanum incanum* have high antimicrobial effect than that of fruit extract against *E. coli* and *S. aureus*. But, this study revealed that ethanol extract of bark root extracts of *Solanum incanum* was indicated mean zone of inhibition (22.33 ± 0.89 mm) against growth of *E. coli*. This result is in agreement with the result of Tewelde and Ghebriel (2017) with mean zone of inhibition against *E. coli* (24 ± 0.04 mm).

This study showed that, ethanol extract of *Solanum incanum* had showed maximum mean diameter zone of inhibition (22.67 ± 0.33 mm) against *S. aureus* which disagree with the reported finding of Tewelde and Ghebriel (2017) which was about low mean zone of inhibition (10 ± 0.91 mm) against *S. aureus*. The finding result of Dakone and Zeleke (2018) showed that, ethanol root extract of *Solanum incanum* had showed antibacterial effect against *E. coli* and *S. aureus* with mean diameter zone of inhibition 14.0 ± 1.0 mm and 15.40 ± 0.6 mm respectively. This result showed that, great variation with the result of this study with the difference of 8.33 mm for *E. coli* and 7.27 mm for S. aureus. Therefore, ethanol extract of *Solanum incanum* had showed more inhibition effect against gram negative and gram positive than the finding of Dakone and Zeleke (2018). In this experimental study, aqueous extracts of bark root of *Solanum incanum* showed the minimum of low zone of inhibition (9.33 ± 0.33 mm) against *E. coli* and *S. aureus*. This result agreed with Jepkoech and Gakugna (2017) as they reported that, the aqueous fruit extracts of *Solanum incanum* had generated zone of inhibition ranged from 6-9.25 mm at 0.01-100 mg/ml against the growth of *S. aureus*. But, Dakone and Zeleke (2018) showed that, the root of *Solanum incanum* aqueous extract pronounced to inhibit the growth of *S. aureus* with mean zone of inhibition (14.70 ± 4.6 mm) which contrast to this study.

The antibacterial activities of bark root extracts of *Solanum incanum* were compared with standard antibiotics (gentamicin), and ethanol and petroleum ether generated maximum mean diameter zone of inhibition (22.33 ± 0.89 mm and 21.33 ± 0.33 mm) respectively against *E. coli* as gentamicin (23 ± 0.58 mm) generated. Ethanol bark root extract of *Solanum incanum* was showed antibacterial activities than petroleum ether and aqueous extract with maximum mean zone of inhibition (22.33 ± 0.89 mm) against *E. coli* and mean zone of inhibition (22.67 ± 0.89 mm) against *S. aureus*. This is due to, ethanol and petroleum ether solvents have the ability to extract lipophilic and lipophobic bioactive compounds, and lipophilic phytoactive agents respectively. The lipophilic phytochemical agents have the ability to penetrate the peptidoglycan layer of gram positive and gram negative bacteria [13].

Sundar and Kolpillaie (2015) showed that, petroleum ether extract of *Solanum incanum* showed antimicrobial activity against

E. coli (18 mm) than *S. aureus* (14 mm) which agreed with this current experimental study. In this *in vitro* experimental study, *Solanum incanum* bark root petroleum ether extract had showed the maximum zone of inhibition against gram negative (*E. coli*) than gram positive (*S. aureus*). These antibacterial activities may be due to the phytochemical compounds of *Solanum incanum* which extracted with petroleum ether solvent. Petroleum ether is non-polar solvent which have ability to extract nonpolar bioactive compounds. Non polar bioactive agents have ability to penetrate lipopolysaccride and directly enter into the cell of gram negative bacteria. However, Ethanol extract had showed high zone of inhibition against both *E. coli* and *S. aureus* with equal distribution of concentrations. The high antibacterial efficacy of *Solanum incanum* bark root ethanol extract may be due to the ability of ethanol in extracting the polar and non-polar bio active compounds. Both polar and non-polar bioactive compound may act as broad spectrum activities against gram negative ad gram positive bacteria. Bark root *Solanum incanum* aqueous extract were showed lowest zone of inhibition (9.33 ± 0.33 mm) against both *S. aureus* and *E. coli* at 100 mg/ml. The bark root extracts of *Solanum incanum* had showed the inhibition efficacy on *S. aureus* than *E. coli* except of petroleum ether extract which against *E. coli* than *S. aureus* with relatively equal concentrations. As the result indicated that, the *Solanum incanum* ethanol extract is more efficacies against both *E. coli* and *S. aureus* than petroleum extract.

Minimum inhibitor concentration assay against isolated bacteria

Dichloromethane/methanol (1:1) leave extract of C. macrostachyus was showed minimum inhibitor concentration against *E. coli* and *S. aureus* at 25 mg/ml was reported by Belayhun et al., 2018. This result is in agreement with current study and showed that the leave and bark root extracts of Croton macrostachyus have relatively equal MIC against *S. aureus* and *E. coli* reported that, the bark root ethanol extract of C. macrostachyus had MIC of 50 mg/ml against *S. aureus* and 100 mg/ml against *E. coli* [14]. The result of Tensay is disagree with the result of this study that, ethanol bark extract of *Solanum incanum* had MIC at 12.5 mg/ml against S. aureus and at 25 mg/ml against *E. coli* [14]. However, this result is disagreeing with the current result of this study. Habtom and Kebede, (2017) showed that, ethanol extract of *Solanum incanum* had MIC against *E. coli* and *S. aureus* at 100 and 25 mg/ml. The finding result of Habtom and Kebede (2017) is contradicted to this current study. This study revealed that bark root of *Solanum incanum* had showed the lowest minimum inhibitor concentration against *S. aureus* at 6.25 mg/ml and at 12 mg/ml against *E. coli*. Aqueous extracts of Croton macrostachyus and *Solanum incanum* had showed minimum inhibitor concentration against *E. coli* and *S. aureus* with high concentration at 100 mg/ml. This indicated that, the aqueous extracts had low antimicrobial activities against E. coli and *S. aureus* [15-18].

Conclusion

Bioactive compounds of medicinal plant extracts have been used to overcome the challenges of antimicrobial resistance. The current experimental study showed that, the bark root extract of *Solanum incanum* and Croton macrostachyus have high potent of antibacterial activities against *E. coli* and *S. aureus*. This study therefore substantiates the use of *Solanum incanum* and Croton macrostachyus as an antimicrobial medicinal plant.

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