Antibacterial activity of *Acanthus sennii* extracts against *Staphylococcus aureus* and *Escherichia coli* pathogens

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ABSTRACT

Medicinal plants offer a major and accessible source of health care to people living in developing countries. Increasing drug resistant microbial infections intensified the search for new, safer, and more efficacious agents against microbial infections. *Acanthus sennii* is one of the medicinal plants used traditionally for the treatment of different infectious diseases in Ethiopia. Therefore, this study was carried out to evaluate antibacterial activity of *A. sennii* against pathogenic bacteria. Plant materials were extracted by maceration technique with chloroform, ethanol and water solvents. The antibacterial activities of the crude extracts of the plant were carried out by the agar well diffusion method. Broth dilution method was used to determine minimum inhibitory and streak plate bactericidal concentration of extracts. The results revealed that ethanol extracts of leaves revealed high antibacterial activity against standard strains of *Staphylococcus aureus* with inhibition zone of 14±0.6 mm at 25 mg/ml and 17±0.7 mm at 50 mg/ml. Ethanol extracts of buds showed high antibacterial activity against standard strains of *S. aureus* with inhibition zone of 25.7±0.7 mm at 100 mg/ml, also against standard strains of *E. coli* with inhibition zone of 16 mm at 50 mg/ml and 23.7 mm at 100 mg/ml. The mean minimum inhibitory concentration of 5.2±1.8 and 2.6±0.5 mg/ml was recorded for ethanol extract of leaves against standard strains of *E. coli* and clinical isolates of *S. aureus*; the mean minimum bactericidal concentration of 4.2±1.0 mg/ml with ethanol extract of leaves against standard strains of *S. aureus;* and the mean minimum bactericidal concentration of 12.5 mg/ml against standard strains and clinical isolates of *E. coli.* The result showed that *A. sennii* could be a candidate in the search for new antibacterial agents against these bacteria and its use in ethnomedicinal treatment of infectious diseases used by local communities may be validated. Isolating bio-active components and determining toxicity are future agenda.

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Keywords: *Acanthus sennii,* Antibacterial activity, *E. coli*, Minimum inhibition concentration, Minimum bactericidal concentration, *S. aureus*
DOI: https://dx.doi.org/10.4314/ejst.v13i2.2

INTRODUCTION

Infectious diseases are the continuous major causes of death in the world, with a great impact in developing countries (WHO, 2015). 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 defence (Jones *et al*., 2008). Prolonged use of antimicrobial agents led to microbial adaptation, resulting in the development of resistance in microbes and the consequent failure of antibiotic therapy, has led to hundreds of thousands of deaths annually (Palmer and Kishony, 2013). In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. *S. aureus* and *E. coli* were observed to be the most frequent resistant pathogenic bacteria (WHO, 2017).

Resistance to antimicrobial agents has important implications for morbidity, mortality, and health care. Statistics suggest irrational use of medicines costs approximately US$870 million to provide care and treatment for those who were admitted to the hospital due to adverse medical events in the UK (Błaszczyk *et al*., 2018). Either overuse or underuse of antibiotics can also result in serious antimicrobial resistance (Kumar *et al*., 2013). These developments and increasing in resistant microbial infections intensified the search for new, safer, and more efficacious agents to combat serious microbial infections (Vandeputte *et al*., 2011). In this context, traditional medicines, based largely on medicinal plants, offer a major and accessible source of health care to people living in developing countries.

Plants have played a central part in combating many diseases in human and domestic animal in many local communities, including Africa (Bussmann *et al*., 2011). They are good sources for new, safe, biodegradable and renewable drugs (Joy *et al*., 2012). Medicinal plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases (Kaur *et al*., 2013).

Plant- based human and domestic animal health care persists and remains as the main alternative treatment for different diseases in Ethiopia (Damtew Bekele *et al*., 2012). The majority of Ethiopian people especially in rural

communities still depend on traditional medicinal plants to treat several diseases because their derived products can be exploited with sustainable, comparative and competitive advantage including reduced cost, less dangerous, more effective and readily available (Moorthy *et al*., 2007). *A. sennii*is medicinal plant that has been used traditionally for the treatment of different infectious diseases. It is endemic to Ethiopia with different local names; i.e. *Kusheshile* (Amharic) (Ermias Lulekal *et al*., 2011; Muthuswamy and Solomon Mequanente, 2009), *Chocha* (Kem-batigna), (Melesse Maryo *et al*., 2015), *Sokoro* (Oromifa) (Rainer *et al*., 2011). It is traditionally used for treating scorpion sting with root decoction given orally and to treat bleeding and stabbing pain leaf paste (Mirutse Giday *et al*., 2007; Tesfaye Awas and Sebsebe Demissew, 2009; Muthus-wamy and Solomon Mequanente, 2009). The leaves are used as a medicine with butter and applied to wounds (Rainer *et al*., 2011). Despite such advantages *A. sennii* has, the use of locally made medicines were prepared as infusions in hot water or mixed with food to treat infection and the majority of the evidence on the antimicrobial activity of this plant was anecdotal and lacked scientific validity. Moreover, increasing the resistance of microorganisms towards the present-day drug leads to failure in the treatment of infectious diseases that demand a new antimicrobial drug with selective action against new targets in the microbial cells, without irreversible side effects in the host (Vandeputte *et al*., 2011). Therefore, this study was aimed to evaluate antibacterial activity of *A. sennii* extracts against *S. aureus* and *E. coli* pathogens.

MATERIALS AND METHODS

Study design, area and period

Laboratory experimental design was conducted to evaluate the antibacterial activity of *A. sennii* extracts against selected pathogenic bacteria at Bahir Dar University post graduate Microbiology laboratory, Bahir Dar town from May to July 2018.

Plant collection and preparation

A. sennii leaves and buds were collected from around Bahir Dar town in May 2018.These plant parts were washed with tap water, dried in open air, separately powdered to suitable size and made ready for extraction (Sukhdev *et al*., 2008).

Extraction of plant materials

Plant materials were extracted by maceration technique with chloroform, ethanol and water solvents with occasional shaking at room temperature for three days. Ground plant materials were soaked with each solvent separately at 10:1 solvent-to sample ratio (v/w). The extracts were separately filtered by Whatman No. 1 filter paper and concentrated with dry oven at 80 °C for 4 h (Pham *et al*, 2015). Further, fresh solvents were added to the residue at the same ratio until required amount of extracts were obtained. The dry extracts were stored in sample bottles at refrigerator for further use (Sukhdev *et al*., 2008).

Antibacterial activities of *A. sennii* extracts

Source of the test bacterial pathogens and inoculums preparation

Representative gram negative and gram-positive bacteria (standard reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923) and clinical isolates of *S. aureus* and *E. coli* were obtained from Amhara Public Health Institute. The bacteria were activated by streaked on nutrient agar (Hi media) and incubated at 37 °C for 24 h. The bacteria used for the study was prepared by inoculating isolates into nutrient broth and incubated at 37 °C for 24 h. The culture turbidity was adjusted to a 0.5 McFarland standard using sterile normal saline (CLSI, 2012).

The antibacterial assays

The antibacterial activity of the crude extracts of *A. sennii* against *S. aureus* and *E. coli* were carried out by the agar well diffusion method (Gauniyal and Uday, 2014). The standardized bacterial broth cultures prepared before were swabbed on Mueller-Hinton Agar (Himedia) plates using sterile cotton swabs. After thirty minutes, five equidistant wells were made on each plate with a 6 mm diameter sterilized corkborer. Stock solution of each plant extract was prepared using sterilized distilled water at a different concentration (25, 50 and 100) mg/ml and 100 µl of each plant extracts were added into the wells aseptically. The plates were allowed to diffuse at room temperature for 2 h and incubated at 37 °C for 18-24 h in triplicates. Sterile distilled water without plant extract used as negative control and Gentamicin (0.01 mg/ml) was used as a positive control, respectively (Cheruiyot *et al*., 2009). The antibacterial activity was evaluated by measuring the diameter of the zone of inhibition in mm using ruler (Gauniyal and Uday, 2014).

Determination of minimum inhibitory concentration

The two-fold broth dilution method was used to determine minimum inhibitory concentration (MIC) of extracts, which showed high antimicrobial activity with the agar well diffusion method (CLSI, 2012). The plant extract solutions (100 mg/ml) were serial dilution with nutrient broth as 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256 to bring 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, 1.56 mg/ml, 0.78 mg/ml and 0.39 mg/ml concentrations, respectively and 20 µl of a standard suspension of test organisms were added to each concentration of the extract. Extra test tubes containing peptone water and peptone water plus the test organisms were prepared to serve as a negative and positive control, respectively. The broth tubes were incubated at 37 ℃ for 24 h. The MIC value of the extract was taken as the lowest concentration that showed no visible growth or turbidity in the test tube (CLSI, 2012).

Minimum bactericidal concentration

From all tubes showed no visible signs of growth/turbidity (100, 50, 25, 12.5, 6.25, 3.125 and 1.56) mg/ml, loop full of inoculums were streaked on nutrient agar plates and incubated at 37 ℃ for 24 h. The least concentration that showed no visible growth after incubation was considered the MBC value of the tested extracts against the tested bacterial species (CLSI, 2012).

Data analysis

The statistical differences of the mean zone of inhibition, MIC and MBC of extracts for individual bacterium and among bacteria was carried out by employing one-way analysis of variance followed by Duncan's multiple comparison tests using the Statistical Package for the Social Sciences (SPSS) Version 20 software. P-values less than 0.05 were considered statistically significant.

RESULTS

Antibacterial activity of *A. sennii* extracts

The highest antibacterial activity was seen in ethanol extracts of bud against standard strains of *S. aureus* with inhibition zone of 25.67±0.67 mm at 100 mg/ml and leaves extracts with inhibition zone of 14±0.58 mm and 17±0.67 mm at 25 mg/ml and 50 mg/ml, respectively. The lowest antibacterial activity was seen in ethanol extracts of buds against clinical isolates of *E. coli* with

inhibition zone of 5.67±0.33mm,5mm and 10±0.33 mm at 25 mg/ml, 50 mg/ml and 100 mg/ml, respectively. However, water extract of *A. sennii* leaves were devoid of an antibacterial activity against any of the tested bacterium. Chloroform extracts of buds also devoid of an antibacterial activity against standard strains and clinical isolates of *E. coli* at 25 mg/ml. Negative control exhibited no zone of inhibition as expected whereas, positive control (Gentamicin, 0.01 mg/ml) exhibited zones of inhibition against all strains studied (Table 1).

The mean zone of inhibition among bacteria showed statistically significant difference at all extracts of buds and leaves of the plant ($P<0.05$) except water extracts at 25 mg/ml. The mean zone of inhibition all extracts of bud and leaf of the plant among bacteria showed statistically significant difference ($P<0.05$) at 50 and 100 mg/ml. The statistical analysis of the data showed that there were significant differences among positive control, water, ethanol and chloroform extracts of *A. sennii* buds and leaves on mean zone of inhibition within each tested bacterium at all concentrations ($P<0.05$).

Minimum inhibitory concentration of *A. sennii* extracts

As shown from the Table, ethanol extract of *A. sennii* leaves showed the lowest MIC value against standard strains and clinical isolates of *S. aureus*; while water extract of *A. sennii* bud and leaves showed the highest MIC value of 50 mg/ml against all test bacteria except standard strains of *E. coli* (Table 2).The MIC values of ethanol extracts of leaves and chloroform extracts of buds and leaves showed statistically significant difference among bacteria at $P<0.05$. The statistical analysis of the data showed that there were significant differences among water, ethanol and chloroform extracts of *A. sennii* buds and leaves on MIC within each tested bacterium (Table 2).

Minimum bactericidal concentration of *A. sennii* extracts

Ethanol extract of *A. sennii* leaves showed the lowest MBC value against standard strains of *S. aureus*; while water extract of *A. sennii* bud and leaves showed the highest MBC values 100 mg/ml against both the tested bacteria (Table 3).

The MBC values of ethanol extracts of leaves and chloroform extracts of buds and leaves showed statistically significant difference among bacterial strains at $P<0.05$. The statistical analysis of the data showed that there were

significant differences among water, ethanol and chloroform extracts of *A. sennii* buds and leaves on MBC within each tested bacterium.

DISCUSSION

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobial agent to replace synthetic ones. Photo chemicals derived from plant products serve as a sample to develop less toxic and more effective medicines in controlling the growth of microorganism (Joy *et al*., 2012).

Antibacterial activity of *A. sennii* extracts

The results showed that water extract of *A. sennii* showed good antibacterial activity against tested bacterial species, however, water extracts did not show antibacterial activity against any of the tested bacteria at 25 mg/ml.

Reason for the lack of antibacterial activity may be attributed to drying method used and the polarity potential of the solvents to extract the secondary metabolites of the plant. Abeysinghe and Weeraddana (2011) reported that water extracts of *Avicennia marina* did not inhibit *Staphylococcus* spp. and *Proteus* spp. Water extract of *Moringa oleifera* leaves was not active against *S. aureus* and *E. coli* (Abdallah, 2015). Obeidat (2011) also reported that aqueous leaf extracts of *Ecbalium elaterium* appeared to exhibit neither antibacterial nor antifungal activities. According to Koohsari *et al*. (2015) aqueous extracts of *Lippia citriodora, Plantago major, Althaea officinalis, Tilia bengonifolia* and *Adiantum capillus-veneris* showed no significant antibacterial effect.

On the other hand, Obeidat (2011) reported that water extracts from *Eminium spiculatum* leaves exhibited antibacterial activity against *E. coli* and extracts of *Lupinus varius* leaf exhibited antibacterial activity against methicillin-resistant S. *aureus* (MRSA) and *E. coli*. According to the study conducted in Algeria, *Punica granatum* bark aqueous macerate was a potent inhibitor against *S. aureus* (ATCC 25922) and *E. coli* (ATCC 25923) with disc diffusion method (Kadi *et al*., 2011).

Table 1. Antibacterial activity of *A. sennii* extracts against *S. aureus* and *E. coli* using agar well diffusion method.

		Mean zone of inhibition (mm)				
		Staphylococcus aureus		*Escherichia coli*		
Concentration	**Plant extracts**	**Standard**	**Clinical**	**Standard**	**Clinical**	***P* value**
25 mg/ml	Buds+ Water	0.0±0.0^{a1}	0.0±0.0^{a1}	0.0±0.0^{a1}	0.0±0.0^{a1}	-
	Leaves+ Water	0.0±0.0^{a1}	0.0±0.0^{a1}	0.0±0.0^{a1}	0.0±0.0^{a1}	-
	Buds+ Ethanol	13.7±0.3^{d3}	12.0±0.0^{c3}	7.3±0.7^{b2}	5.7±0.3^{a2}	<0.001
	Leaves+ Ethanol	14.0±0.6^{b3}	11.0±0.6^{a3}	11.3±0.7^{a4}	10.7±0.3^{a4}	0.01
	Buds+ Chloroform	9.0±0.6^{c2}	7.0±0.6^{b2}	0.0±0.0^{a1}	0.0±0.0^{a1}	<0.001
	Leaves+ Chloroform	13.3±0.3^{d3}	11.0±0.6^{c3}	8.7±0.3^{b3}	7.0±0.0^{a3}	<0.001
	Gm (0.01 mg/ml)	21.7±0.3^{b4}	19.7±0.3^{a4}	24.7±0.3^{c5}	22.7±0.3^{b5}	<0.001
50 mg/ml	Buds+ Water	7.0±0.6^{b1}	5.3±0.3^{a1}	5.7±0.3^{a1}	5.0±0.0^{a1}	0.024
	Leaves+ Water	14.3±0.3^{c2}	12.3±0.3^{b2}	11.3±0.3^{b2}	8.3±0.3^{a2}	<0.001
	Buds+ Ethanol	17.3±0.3^{c4}	14.3±0.3^{a3}	16.0±0.6^{b5}	13.7±0.3^{a6}	<0.001
	Leaves+ Ethanol	17.7±0.3^{d4}	16.0±0.6^{c4}	13.3±0.3^{b34}	11.7±0.3^{a4}	<0.001
	Buds+ Chloroform	16.7±0.3^{c34}	14.3±0.3^{b3}	14.0±0.6^{b4}	12.7±0.3^{a5}	0.001
	Leaves+ Chloroform	15.7±0.7^{c3}	13.3±0.9^{b33}	12.7±0.3^{b3}	10.7±0.3^{a3}	0.003
	Gm (0.01 mg/ml)	21.7±0.3^{b5}	19.7±0.3^{a5}	24.7±0.3^{c6}	22.7±0.3^{b7}	<0.001
100 mg/ml	Buds+ Water	11.3±0.3^{ab1}	10.3±0.3^{a1}	12.0±0.6^{b1}	10.3±0.3^{a1}	0.052
	Leaves+ Water	17.7±0.3^{c2}	14.3±0.3^{b2}	14.7±0.3^{b2}	12.7±0.3^{a2}	<0.000
	Buds+ Ethanol	25.7±0.7^{c5}	21.3±0.3^{a56}	23.7±0.3^{b5}	20.3±0.3^{a5}	<0.000
	Leaves+ Ethanol	22.0±0.6^{b3}	20.3±0.3^{b55}	20.7±0.3^{b4}	17.3±0.7^{a4}	0.001
	Buds+ Chloroform	23.7±0.3^{c4}	22.3±0.3^{b6}	21.7±0.3^{b4}	18.3±0.3^{a4}	<0.001
	Leaves+ Chloroform	18.7±0.9^{b2}	17.7±0.3^{b3}	16.3±0.9^{ab3}	14.3±0.7^{a3}	0.021
	Gm (0.01 mg/ml)	21.7±0.3^{b3}	19.7±0.3^{a4}	24.7±0.3^{c5}	22.7±0.3^{b6}	<0.001

Values are expressed as mean of three replicates± S.E.M. Values with different letters in the same row and numbers in the same column at each concentration indicate statistically significant differences (Duncan's test, $P<0.05$), GM=*Gentamicin,* **-** *= no bacterial activity.*

Koohsari *et al*. (2015) also reported that aqueous extract of *Sambucus ebulus* had effects against *S. aureus* with inhibition zone diameter of 14 mm at 500 mg/ml in the agar well diffusion method. According to the study conducted in Algeria, *Punica granatum* bark ethanol macerate was found to be potent inhibitor against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) (Kadi *et al*., 2011). Ethanol extracts of *Lippia citriodora* and *Plantago major* showed a remarkable antibacterial effect against *S. aureus* with the inhibition zone diameter of 26 mm and 33 mm, respectively, at 500 mg/ ml (Koohsari *et al*., 2015). A previous study conducted in Bangladesh also documented that ethanol extract of *Mimosa pudica* and *Lawsonia inermis* twig showed the highest effect against *S. aureus* with zone of inhibition 11.2 ± 0.14 mm and 17.1 ± 0.14 mm, respectively, whereas, *Lawsonia inermis* showed the zone of inhibition 7.20 mm against *E. coli* in disc diffusion method (Akter *et al*., 2010) which were lower than the result of our study.

On the other hand, ethanol extract of *Mimosa pudica* twig (Akter*et al*., 2010), *Lippia citriodora* and *Plantago major* (Koohsari*et al*., 2015) and *Ecbalium elaterium* (Obeidat, 2011) were not active against *E. coli*.

A study conducted by Abdallah (2016) reported that chloroform extract of *Moringa oleifera* leaves showed antibacterial activity against *S. aureus* (11.0±0.5 mm) at 200 mg/ml which was lower than the result of the present study. Murugan *et al*. (2013) also reported that chloroform extracts of *Cassia auriculate* leaves showed good activity against *S. aureus* (12 mm) and *E. coli* (14 mm).

On the other hand, Akter *et al*. (2010) reported that chloroform extract *Lawsonia inerm* is not active on *E. coli* and extract of *Mimosa pudica* twig did not have antibacterial activity against *S. aureus* and *E. coli* in disc diffusion method which did not agree with the results of the present study. Antibacterial activities of plant extracts in the current study differed from studies reported before by almost all authors. The probable reason for this difference may be attributed to concentration of extracts, types of plants, methods of extraction, methods of test as well as bacterial strains used.

Minimum inhibitory concentration of *A. sennii* extracts

The present study showed that the lowest MIC value (2.6±0.52 mg/ml) was obtained with ethanol extracts of leaves against *S. aureus* and the highest MIC value (50 mg/ml) of was obtained with water extracts of buds and leaves against all tested bacteria.

Table 2. MIC of *A. sennii* extracts against *S. aureus* and *E. coli* using broth dilution.

Solvent	Plant parts	MIC (mg/ml)				Control		*P* value
		Staphylococcus aureus		*Escherichia coli*		Positive	Negative	
		Standard	Clinical	Standard	Clinical			
Water	Bud	50.0±0.0^{3}	50.0±0.0^{3}	50.0±0.0^{3}	50.0±0.0^{3}	+	-	-
	Leaves	50.0±0.0^{3}	50.0±0.0^{3}	41.7±14.4^{3}	50.0±0.0^{3}	+	-	-
Ethanol	Buds	5.2±1.0^{a2}	5.2±1.0^{a2}	6.3±0.0^{a1}	8.3±3.6^{a1}	+	-	0.596
	Leaves	2.6±0.5^{a1}	2.6±0.5^{a1}	5.2±1.8^{b1}	6.3±0.0^{b1}	+	-	<0.001
Chloroform	Buds	4.2±1.0^{a12}	5.2±1.0^{a2}	50.0±0.0^{b3}	50.0±0.0^{b3}	+	-	<0.001
	Leaves	3.1±0.0^{a1}	3.1±0.0^{a1}	12.5±0.0^{b2}	25.0±0.0^{b2}	+	-	<0.001

Values are expressed as mean of three replicates± S.E.M. Values with different letters in the same row and -numbers in the same column at each solvent indicate statistically significant differences (Duncan's test, $P<0.05$), +=growth, - = no growth.

Table 3. The MBC (mg/ml) of *A. sennii* extracts against *S. aureus*. and *E. coli* pathogens.

Solvent	Plant parts	MBC				*P* value
		Staphylococcus aureus		*Escherichia coli*		
		Standard	Clinical	Standard	Clinical	
Water	Buds	100.0±0.0^{3}	100.0±0.0^{3}	100.0±0.0^{2}	100.0±0.0^{2}	-
	Leaves	100.0±0.0^{3}	100.0±0.0^{3}	100.0±0.0^{2}	100.0±0.0^{2}	-
Ethanol	Buds	10.4±2.8^{a2}	10.4±2.8^{a2}	16.7±0.0.0^{a1}	12.5±0.0^{a1}	0.33
	Leaves	4.2±1.0^{a1}	5.2±1.0^{a1}	12.5±0.0^{b1}	12.5±0.0^{b1}	<0.001
Chloroform	Buds	10.4±2.8^{a2}	12.5±0.0^{a2}	100.0±0.0^{b2}	100.0±0.0^{b2}	<0.001
	Leaves	5.2±1.0^{a1}	6.3±0.0^{a1}	12.5±0.0^{ab1}	16.7±4.1^{b1}	0.017

Values are expressed as mean of three replicates± S.E.M. Values with different letters in the same row and numbers in the same column at each solvent indicate statistically significant differences (Duncan's test, $P < 0.05$).

Obeidat (2011) reported that MIC value of water extract of *Ecbalium elaterium* fruits was 64 mg/ml against *E. coli* which was higher than our study Obeidat (2011) reported that ethanol extracts of *Mandragora autumnalis* fruits showed very strong activity against MRSA with the best MIC (4 mg/ml) and 8 mg/ml against *E. coli*. MIC values reported by the author were higher than MIC values of *A. sennii* leaves (*S. aureus*) of the current study and in line with *A. sennii* buds (*E. coli*). He also reported that MIC of ethanol extracts of *Ecbalium elaterium* fruits was 64 mg/ml against *E. coli* which was higher than our study.

Salama and Marraiki (2010) reported that chloroform extracts of *Polygonum aviculare* stem and leaf extracts showed MIC value of 18 mg/ml and 20 mg/ml against *S. aureus* and 15 mg/ml and 18 mg/ml against *E. coli*, respectively, which did not agree with the current study. The probable reason for difference may be attributed to plant type and parts, concentration of bacteria, methods of extraction, methods of antibacterial test as well as bacterial strains used.

Minimum bactericidal concentration of *A. sennii* extracts

The present study also showed that the lowest MBC value was found with ethanol extracts of leaves (4.2±1.04 mg/ml) against standard strains of *S. aureus* and the highest MBC value was found with water extracts of bud and leaves (100 mg/ml) against all tested bacteria. Obeidat (2011) reported that MBC of water extract of *E. elaterium* fruit was (128) against *E. coli* which was higher than our study. Obeidat (2011) reported that ethanol extract of *Mandragora autumnalis* fruits showed MBC value of 16 mg/ml and 8 mg/ml against *E. coli* and MRSA, respectively. He also reported that ethanol extracts of *Ecbalium elaterium* fruits was 64 mg/ml against *E. coli.* MBC value of ethanol extract of *Lippia citriodora* was 62.5 and 250 against *S. aureus* and *E. coli*, while MBC value of *Plantago major* was 31.25 and 125 against *S. aureus* and *E. coli* (Koohsari *et al*., 2015). Salama and Marraiki (2010) reported that chloroform extracts of *Polygonum aviculare* stem and leaf showed MIC value of 20 mg/ml against *S. aureus* and 15 mg/ml and 18 mg/ml against *E. coli*, respectively, which are not in line with the current results. The probable reason for difference may be attributed to plant type and parts, concentration of bacteria, methods of extraction, methods of antibacterial test as well as bacterial strains used. The results obtained during this investigation elucidated clearly that ethanol extract exhibited higher activity followed by chloroform against all the tested bacteria. The results obtained during

this investigation elucidated clearly that, Gram-positive bacteria were more sensitive than Gram-negative bacteria towards plant extracts tested. These are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria, with Gram-negative outer membrane acting as a barrier to antibiotics (Burt, 2004). This result is in agreement to that previously recorded by Lopez *et al*. (2005) who reported that Gram-negative bacteria were not susceptible to plant extracts when compared to Gram-positive bacteria, and this has been attributed to the external lipopolysaccharide (LPS) wall that surrounds the peptidoglycan cell wall of the former. Among the test bacteria, the most susceptible bacterium at all concentration of plant extracts was standard strains of *S. aureus* and the most resistant was clinical isolates of *E. coli* with different mean zone of inhibition depending on plant parts, type of solvent and concentration of extracts. This data is in close agreement with Abeysinghe and Weeraddana (2011) who report that out of seventy-five extracts; almost all extracts exhibited the highest antibacterial activity against *S. aureus.* On the other hand, Akter *et al*. (2010) reported that ethanol extract of *Lawsonia inermis* was found to exhibit most effective antibacterial activity against Gram-negative organisms. Plant extracts have higher zone of inhibition against standard strains than clinical isolates. These differences in the potency of the plants extract against the strains of the same bacterial species might be associated to the susceptibility differences between the strains in which the clinical isolates could have a higher chance of developing a resistance mechanism of decreasing the access of the bioactive metabolites to the target sites since they had been isolated from the clinic settings in which resistant strains are common (Cantón *et al*., 2013).

CONCLUSION

The results of present study clearly indicated that *A. sennii* extracts have potential antibacterial activity against standard and clinical isolates of *S. aureus* and *E. coli* in concentration dependent manner. The antibacterial activities exhibited by *A. sennii* in this study validate its use in ethno medicinal treatment of infectious diseases caused by these bacteria by the local communities. Thus, this study conducted on antibacterial activity of the crude extracts of the plant against *S. aureus* and *E. coli* further studies are needed to test their fractionation, study toxicology, phytochemical analysis and isolate the bio- active components from these valuable plants on *S. aureus*, *E. coli* and other bacteria to innovate new natural antibiotics and minimize the spread of drug resistant bacteria.

ACKNOWLEDGEMENTS

The authors would like to thank Bahir Dar University, Department of Biology, for the supply of growth media and reagents in the conduct of this research and Dr. Baye Sitotaw for his support during laboratory work.

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Abbreviations

ATCC	American type cell collection
CLSI	Clinical and Laboratory Standards Institute
E. coli	*Escherichia coli*
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
NCCLS	National Committee for Clinical Laboratory Standards
S. aureus	*Staphylococcus aureus*
WHO	World Health Organization