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IN VITRO ANTIMICROBIAL ACTIVITIES OF CRUDE EXTRACTS OF TWO TRADITIONALLY USED ETHIOPIAN MEDICINAL PLANTS AGAINST SOME BACTERIAL AND FUNGAL TEST PATHOGENS

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ABSTRACT

The aim of the present study was to assess the antimicrobial activities of crude extracts of *Solanum incanum and Datura stramonium L.* against four bacterial disease-causing species namely; *Staphylococcus aureus, Streptococcus agalactiae, Salmonella typhi, Escherichia coli*) and two fungal pathogenic species (*Aspergillus flavus* and *Aspergillus niger).* Crude extracts of roots of *S. incanum* and *leaves of D. stramonium L.* were prepared using absolute ethanol, methanol (99.8%) and distilled water. The potential efficacy resulting extracts were assessed by determining zone of inhibitions on cultures of six selected pathogens by applying disc diffusion method. The minimum inhibitory concentration (MIC) of the plant extracts against the same pathogens was determined using the broth dilution method. Chloroamphenicol and Tilt were used as positive controls for bacterial and fungal pathogens, respectively, while Dimethyl Sulfoxide was used as a negative control for both. The results showed that all the methanolic and ethanolic crude extracts, with the exception of the methanolic crude extract of *D. stramonium*, had the highest growth inhibitory effects as compared with the water crude extracts on all bacterial pathogens. The methanol crude extract of *S. incanum* had highest zone of inhibition (22±1.15 mm) against *S. agalactiae* with a minimum inhibitory concentration of 25mg/ml. Similarly, the methanol crude extract of *S. incanum* had shown significant antifungal activity against *A. niger* with inhibition zone of 25.13±0.4mm and a minimum inhibitory concentration of 125mg/ml. The results of the present study indicate the potential of these medicinal plants in treating some bacterial and fungal infection.

Contribution/Originality: This study contributes in the existing literature by providing information regarding the antimicrobial (selected bacterial and fungal pathogens) efficacy potential of *Solanum incanum and Datura stramonium* crude extract.

1. INTRODUCTION

People in impoverished as well as in developed countries use medicinal plants as a source of drugs or as a source of herbal extracts for various therapeutic purposes (Verma and Singh, 2008). The practice of using plant based natural compounds as part of herbal formulation and as another sources of medicine remains to play major role in maintaining the health of people around the globe. Majority of the world population relying on traditional medicinal plants for some aspects of primary health care. The applications of such medicinal plants may, however, varies in the way of application from taking the roots, barks, stems, leaves and seeds to the use of extracts and

decoction from the plants. Nowadays, due emphasis has been directed given in isolating and identifying biologically active compounds which are responsible for the activities of different plant species (Stepp, 2004). Report from World Health Organization (WHO) (2009) indicated that 80% of the people in under-developed countries rely on traditional medicinal plants for primary health care. Nevertheless, among the estimated 250,000 species of higher plants, only 5-15% has been studied scientifically for their possible healing values (Stepp, 2004; Goel and Sharma, 2014).

Plants have the capacity to produce aromatic substances such as phenolics (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, and tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids) and additional metabolites. Research has also shown that many of these secondary metabolites exhibit *in vitro* antimicrobial properties and happened to treat a variety of infectious diseases (Terrier *et al*., 2007). Furthermore, the importance of medicinal plants and their derivatives becomes enormous at a time when antibiotic resistance has turn out to be a concern globally. These days, there is growing occurrence of various resistances in human pathogenic microorganisms, mainly usage of commercial antimicrobial without prescriptions used to treat infectious diseases (Cos *et al*., 2006). Due to fast spreading drug and multidrug resistant of bacterial and fungal pathogens the need for new drugs with less toxic effects are a highly demanding public interest. Therefore, intensive research activities are required to find out alternative solutions against drug resistant microorganisms. This needs evaluation of crude extracts and active constituents of traditionally used medicinal plants against human bacterial and fungal pathogens.

Ethnobotanical investigations in Mecha *wereda*, west Gojjam zone of Ethiopia, have shown that *Solanum incanum*, *Datura stramonium* extracts have been used commonly and extensively in the folk medicine to treat different types of infectious diseases such as malaria, gastrointestinal infections, wound, dermatitis, chigger, toothache, ringworm, cardiovascular diseases, dandruff and other bacterial and fungal diseases (Getaneh *et al*., 2014). With this background information, this study was intended at elucidating the *in vitro* antimicrobial (antibacterial and antifungal) activities of the two widely used medicinal plants (*Solanum incanum*, *Datura stramonium L*.) on selected test pathogenic bacterial and fungal species, namely: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Salmonella* typhi, *Escherichia coli*, *Aspergillus flavus* and *Aspergillus niger* by using different solvent extraction methods.

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant

The plants, root parts of *Solanum incanum* and leaves of *Datura stramonium* were randomly collected from main campus of Haramaya University. The voucher specimens were identified and authenticated by a botanist at the Herbarium of Haramaya University. A voucher specimen was prepared and deposited in the University Herbarium.

2.2. Preparation of Plant for Solvent Extraction

After collection, the plants were separately cleaned with tap water to remove unnecessary particles. Then the cleaned plant parts were air dried at room temperature for five days for leaves and for two weeks for roots. The dried roots and leaves were separately pulverized using an electric grinder into fine powder (using 2 mm mesh size of sieve). Then they were stored in sterile airtight containers, finally kept in a refrigerator at 4^{O}C until use (Singh *et al*., 2007).

2.3. Preparation of Solvent Extracts

2.3.1. Preparation of Water Extracts

Fifty grams of the ground material (*Solanum incanum*, *Datura stramonium L*.) were soaked in 250 ml distilled water in a 1000 ml conical flask and permitted to stand for 24 hrs in a rotary shaker at 120 rpm. After 24 hrs, a muslin cloth was employed to filter the suspension, followed by Whatman No. 1 filter paper. Then, the filtrates

were kept in a water bath at 50 ^{O}C of temperature for evaporation and obtain the solid crude extracts. They were reserved in a refrigerator at 4^{O}C for further anti-microbial activities (Nduagu *et al*., 2008).

2.3.2. Preparation of Ethanol and Methanol Extracts

Fifty grams of air-dried powdered plant materials were put in 250 ml of absolute ethanol and 99.8% methanol kept in a conical flask and was shaken in a rotary shaker at 121 rpm for 24 hrs to help thorough mixing and enough maceration of the plant parts. After 24 hrs, the suspension was filtered separately with Whatman No. 1 filter paper. The resulting filtrate was concentrated using a vacuum rotary evaporator at 40^{O}C to remove the solvent. After the evaporation of solvents, the residual crude extracts were weighed and recorded. Finally, the crude extracts were stored in a refrigerator at 4^{O}C until tested for anti-microbial activities (Dewanjee *et al*., 2007).

2.4. Study Design

This study involved a laboratory based experimental design. The antimicrobial activities of the plant extracts against the test pathogens were determined using disk diffusion method. The minimum inhibitory concentrations (MIC) were determined for extracts against the selected pathogens using the broth dilution method. The treatments included two plants with one part each extracted by three solvents and each extract applied on six test pathogens in three replications (4x1x3x6x3). Chloroamphenicol and Tilt were used as positive controls for bacterial and fungal pathogens, respectively, while DMSO was used as a negative control for both.

2.5. Preparation of Culture Media and Sterilization

Potato Dextrose Agar, Nutrient broth, potato dextrose broth and Mueller-Hinton agar were prepared as per the manufacturer's instructions. Before usage, all media were first autoclaved at 121 ^{o}C and 15 psi for 15 minutes.

2.6. Test Pathogens

The bacterial test pathogens were brought from Ethiopian Public Health Institute (EPHI), Addis Ababa. These included *Staphylococcus aureus* ATCC 25923, *Streptococcus agalactiae* ATCC 12386, *Salmonella* Typhi ATCC 13311, *Escherichia coli* ATCC 25922. The fungal pathogens such as *Aspergillus niger* and *Aspergillus flavus* were gotten from infected ground nut in plant pathology laboratory of the School of Plant Sciences, Haramaya University. The bacterial cultures were sub-cultured using Muller Hinton Agar (MHA) (and incubated at 37^{o}C for 18-24 h) while fungal cultures were sub-cultured and maintained using Potato Dextrose Agar (PDA) and incubated at 27 ^{o}C for 5-7 days (Kesatebrhan, 2013).

2.7. Preparation of Inoculum

Three to five well isolated bacterial colonies were selected from the plates and transferred into test tubes containing sterile normal saline solution and agitated with a vortex mixer to get a suspension accustomed to 0.5 McFarland turbidity standard of 1.5 x 10^{8} CFU/ml by using UV-Visible spectrophotometer at 625 nm (Roopashree *et al*., 2008).

Similarly, spore suspensions of *A. niger* and *A. flavus* from PDA medium were extracted using water free of microorganism and adjusted to 10^{6}spores/ml using a hemacytometer (Bazie *et al*., 2014).

2.8. Yields of Crude Extracts

After having extracted the plant part with appropriate solvents the filtrates were transferred in to container and concentrated to dryness using a vacuum rotary evaporator. The concentrated extracts were weighed. All extracts were prepared in three replicates. The percentage yield of the crude extracts was calculated based on the following formula.

$$\text{Percentage yield} = \frac{x1 - x2}{y} \; x\,100$$

Where, x1 = Weight of container with crude extract, x2 = Empty container and y= the weight of the soaked powder (Parekh and Chanda, 2007).

2.9. Determination of Antimicrobial Activity

2.9.1. Determination of Antibacterial Activity

The antibacterial activities of the different solvent crude extracts of the two plants were assessed by disc diffusion method according to Kirby-Bauer (Bauer *et al.*, 1966). Diffusion discs with about 6 mm diameter were set from Whatman No. 1 filter paper by using puncher and placed in a beaker and decontaminated by oven at 180 °C for 30 minutes. A stock solution of 500 mg of each extract per ml of 99% DMSO was prepared. And 20µl of each plant extract was then transferred onto a sterile filter paper disc and allowed to dry for 30 minutes. Five milliliters of a culture suspension of bacteria with a concentration of 1.5×10^8 CFU/ml was added to 250 ml of MHA medium agar (maintained at 45-50 °C in a molten state) (Bauer *et al.*, 1966). The medium was later transferred into Petriplates and permitted to harden. The impregnated discs were then put using forceps aseptically on the surface of the pre-inoculated MHA medium. Paper discs that were prepared in similar manner and handled but without plant extract (DMSO) served as negative control and paper discs impregnated with Chloroamphenicol (at a concentration of 0.1 mg/ ml) were used as positive control. The antibiotic was reconstituted by getting dissolved 50 mg of powder in 500 ml DMSO to get a concentration of 0.1 mg/ml (Arekemase *et al.*, 2013). The plates were then left at room temperature for 30-60 minutes for proper diffusion and incubated at 37°C for 24 hours. Plates were scrutinized for zone of inhibitions (zones showing no microbial growth) around the discs after the incubation period and then the resulting diameters of zones of inhibition were determined using a ruler. Then zones of inhibition of the tested bacterial test pathogens were recorded by subtracting diameter of disc (6 mm) from final zone of inhibition. All plates were prepared in three replicates.

2.9.2. Determination of Antifungal Activity

The efficacy test of the crude extracts against fungi (*A. flavus* and *A. niger*) were similarly conducted using disc diffusion method. Sterile filter paper discs (6 mm in diameter) were impregnated with 50 µl of each plant extract (at a concentration of 1000 mg/ml) and allowed to dry for 30 minutes. Five milliliters of a culture suspension with a concentration of 10^6 spores/ml was added to 250 ml of PDA medium which had been previously cooled down to 45°C-50°C (Bazie *et al.*, 2014). The medium was then poured into Petriplates and allowed to solidify. The impregnated discs were placed with sterile forceps aseptically on the surface of the pre-inoculated PDA medium. Paper discs that were handled in similar manner but devoid of plant extract (DMSO) considered as negative control and paper discs impregnated with commercially produced anti-fungi, Tilt (at the concentration of 0.1 mg/ml), considered as a positive control. The plates were incubated at 27°C for 4 days to determine zones of inhibition of tested fungal pathogen by subtracting diameter of disc (6 mm) from final zone of inhibition. All plates were prepared in triplicates.

2.10. Determination of Minimum Inhibitory Concentration (MIC)

2.10.1. MIC against Bacterial Test Pathogens

The crude extracts obtained from various solvents and found to have potential activities against the selected pathogens using disc diffusion were further designated for evaluating of MIC using broth dilution method. The initial concentration of the plant extract (200mg/ml) was diluted using two-fold serial dilution by transferring 1ml from the plant extract (stock) solution into 1ml of sterile nutrient broth kept in a vial and mixing the contents thoroughly and then serially diluting in 3 other vials. The serial dilutions gave concentrations of 100mg/ml,

50mg/ml, 25mg/ml, 12.5mg/ml (Hadia *et al.*, 2012). After obtaining various concentrations of the extract, each given concentration was inoculated with 0.02 ml of the standardized bacterial cell suspension and incubated at 37ºC for 24 hours. The pathogenic bacterial growth in the broth was determined by turbidity or cloudiness of the broth and the lowest concentration of the extract which prohibited the growth of the test organism were considered as the Minimum Inhibitory Concentration (MIC).

2.10.2. MIC against Fungal Test Pathogens

The MIC of the crude extracts against fungal test pathogens was similarly determined by the broth dilution method. The primary concentration of the plant extract (1000mg/ml) was made dilute with the technique double fold serial dilution by relocating 2ml from plant extract (stock) solution into 2ml of sterile potato dextrose broth and mixing the contents in a vial and then serially diluting in 3 other vials. The serial dilutions gave concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml (Hadia *et al.*, 2012). Each vial was inoculated with 0.02ml of the standardized fungal spore suspension and incubated at 27ºC for 72 hours (Hadia *et al.*, 2012). Turbidity or cloudiness of the broth were considered as the indicators for the growth of the pathogenic fungi and the lowest concentration of the extract which repressed the growth of the fungal test pathogen were considered as the Minimum Inhibitory Concentration (MIC).

2.11. Data Analysis

For getting reliable data, all the experiments were performed in three replications. Statistical analysis was done using computer software SPSS, version 20. All results of the antimicrobial activity studies were expressed as mean values of zone of inhibition ± standard error of mean. Statistical tests were also done using analysis of variance (one-way ANOVA) post-hoc test at $p<0.05$ coupled with least significant difference (LSD) to compare results. All data were analysed at 95% degree of confidence ($\alpha = 0.05$) and $p<0.05$ values were considered to indicate statistically significant differences.

3. RESULTS

3.1. Percentage Yield of Plant Extracts from Dried Plant Materials

The yield of crude extracts of the two selected traditional medicinal plants is calculated in Table 1. Accordingly, the yield (amount) of the crude extracts ranged from 4.33% to 7.07% on dry mass basis. Highest yield was obtained from methanol crude leaf extract of *Datura stramonium L* (13.14±0.30%). The minimum percentage yield was obtained from water crude root extract of *Solanum incanum* (4.33±0.176%) Table 1.

Table-1. The percentage yields of crude extracts of the selected plants (values are shown as mean ±SEM, n=3).

Plant species	Plant Part	Weight and percentage yield of crude extracts by extraction solvents					
		Ethanol		Methanol		Water	
		Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)
D. stramonium	Leaves	4.71±0.1212	9.42 ± 0.243^{Ab}	6.57±0.1501	13.14 ± 0.30^{Ba}	3.53±0.0656	7.07 ± 0.131^{Ac}
S. incanum	Root	2.56±0.0872	5.12 ± 0.174^{Cb}	3.53±0.1453	7.07 ± 0.291^{Da}	2.17±0.0882	4.33 ± 0.176^{Cc}

n = number of experimental replicates, SEM: standard error of the mean, values with different superscripts in the same row (lower case) show significant difference between the performance of solvents in yielding crude extract and values with different superscripts in the same column (upper case) are significant difference between plant species in yielding crude extract.

3.2. Antimicrobial Activities of the Crude Extracts

A total of six (6) crude extracts (ethanol, methanol and water extracts) prepared from the two selected plants and the antibiotics used as control were assessed for their effectiveness against the test microorganisms Table 2-4.

3.2.1. Antimicrobial Activities of S. incanum against Test Pathogens

The *in vitro* activities test of the ethanol, methanol and water crude extracts of against *S. incanum* showed that the zone of inhibitions of the ethanol and methanol root extracts were in the range of 5.5-17.5 mm and 10.69 - 25.0 mm, respectively Table 2. However, the water crude extract of root of the plant was inhibitory only to *A. niger* and showed a zone of inhibition of 14.17±0.6 mm. The diameters of zone of inhibitions observed due to the antibiotics on the tested bacteria and fungi ranged from 18.67 - 47.67 mm and 29.5-38.5mm for Chloroamphenicol and Tilt, respectively.

Table-2. Antimicrobial activities of crude extracts of S. incanum and standard antibiotics against test pathogens (mean ± SEM, n = 3) at concentration of 500mg/ml for bacteria pathogen and 1000mg/ml for fungal pathogen.

	Zone of inhibition					
Test pathogen	**Ethanol**	**Methanol**	**Water**	**Chloroamphenicol**	**Ceftriaxone**	**Tilt**
S.agalactiae	11.67±0.882^{Bc}	22.0±1.15^{Ab}	0.00±0.00	33.0±1.53^{Ba}	10.0±0.58^{Cc}	-
E.coli	7.66±0.193^{Cc}	10.69±0.44^{Bb}	0.00±0.00	18.67±1.45^{Ca}	20.0±0.58^{Aa}	-
S. typhi	5.50±0.289^{Dd}	12.0±1.155^{Bc}	0.00±0.00	36.0±1.15^{Ba}	22.3±0.88^{Ab}	-
S. aureus	16.61±0.20^{Ad}	21.77±0.15^{Ab}	0.00±0.00	47.67±1.33^{Aa}	18.67±0.88^{Bc}	-
A. niger	17.50±0.29^{Ac}	25.13±0.40^{Cb}	14.17±0.6^{Ad}	-	-	38.5±0.29^{Aa}
A. flavus	0.00±0.00	0.00±0.00	0.00±0.00	-	-	29.5±0.29^{Ba}

Key: EtCE=Ethanol crude extract, MtCE=Methanol crude extract, WCE=Water crude extract, Chl= Chloramphenicol, n=number of experimental replicates, SEM = standard error of the mean, ND = Not determined, means with the same small letter superscript in the same row are not significantly different; means with the same capital letter superscript in the same column do not have significant difference ($p<0.05$).

3.2.3. Antimicrobial Activities of D. stramonium *Against Test Pathogens*

The leaf extracts of Datura stramonium were also tested for their antimicrobial properties against the test pathogens. As shown in Table 3, the diameters of the zone of inhibition of the ethanol and water extracts were in the range of 19.5 – 8.87 mm and 17.5 – 4.47 mm respectively. The methanol extract of D. stramonium did not exhibit antibacterial activity against all tested bacteria.

Table-3. Antimicrobial activities of crude extracts of D. stramonium L. and standard antibiotics against test pathogens (mean ± SEM, n = 3).

	Zone of inhibition (mm)							
Test pathogen	**EtCE**		**MtCE**		**WCE**		**Chl**	**Tilt**
	500mg/ml	1000mg /ml	500mg /ml	1000mg /ml	500mg /ml	1000mg /ml	0.1mg /ml	0.1mg /ml
S. agalactiae	8.87±0.23^{Ba}	ND	0.00±0.00	ND	0.00±0.00^{Db}	ND	33.0±1.53	ND
E.coli	0.00±0.00^{Cb}	ND	0.00±0.00	ND	4.47±0.24^{Ca}	ND	18.67±1.45	ND
S. Typhi	9.50±0.29^{Ba}	ND	0.00±0.00	ND	6.23±0.145^{Bb}	ND	36.0±1.15	ND
S. aureus	14.7±0.145^{Aa}	ND	0.00±0.00	ND	9.5±0.29^{Ab}	ND	47.67±1.33	ND
A. niger	ND	19.5±0.28^{b}	ND	23.5±0.26^{a}	ND	17.5±0.29^{c}	ND	38.5±0.29
A. flavus	ND	0.00±0.00	ND	0.00±0.00	ND	0.00±0.00	ND	29.5±0.29

Key: EtCE = Ethanol crude extract, MtCE = Methanol crude extract, WCE = Water crude extract, Chl = Chloramphenicol, n = number of experimental replicates, SEM = standard error of the mean, ND = Not determined, means with the same lower case superscript letters in the same row are not significantly different; means with the same capital letter superscript in the same column do not have significant difference ($p<0.05$).

3.3. Minimum Inhibitory Concentration (MIC) of the Crude Extracts

The minimum inhibitory concentration (MIC) assay was used to determine the potential efficacy of the extracts that exhibited activities against the microbes in the preliminary tests. The data revealed that the MIC of ethanol, water and methanol crude extracts of plants against the tested bacterial pathogens ranged from 25 to 100 mg/ml Table 4. At 25 mg/ml, the methanol extract (against *S. aureus* and *S. agalactiae)* and ethanol extract of *S. incanum* (against *S. aureus)* shared lower MIC. For fungal pathogen MIC values also determine using broth dilution method. The data revealed that the MIC of ethanol, water and methanol crude extracts of plant against the tested fungal pathogen ranged from 125 to 500 mg/ml.

Table-4. Minimum inhibitory concentration (MIC) of plant extracts against bacterial and fungal test pathogens.

Test pathogen	EESI	MESI	WESI	EEDS	MEDS	WEDS
S. agalactiae	50	25	_	50	_	_
E. coli	100	100	_	_	_	100
S. typhi	100	50	_	100	_	100
S. aureus	25	25	_	50	_	100
A. niger	250	125	500	250	125	500

Key:-= not tested, EESI = Ethanol extract of *S. incanum* , MESI = Methanol extract of *S. incanum*, WESI = Water extract of *S. incanum*, , EEDS = Ethanol extract of *D. stramonium*, MEDS = Methanol extract of *D. stramonium*, WEDS = Water extract of *D. stramonium*. MIC = Minimum inhibitory concentration.

4. DISCUSSION

The present study evidently revealed that the percentage yield of the crude extracts of the two different plants varied from solvent to solvent. This could be due to the difference in polarity and extraction potential of methanol, ethanol, and water. The present study revealed that methanol was the most effective solvent to produce relatively higher yield of crude extract from medicinal plants for the purpose of producing antimicrobial substances compared to ethanol and water. As Cowan (1999) indicated that known major antimicrobial agents that have been from plants are soluble in organic solvents and this indicates improved efficiency of non-polar as extracting solvent than polar solvents. Extraction from leaf of *D. stramonium* and root of *S. incanum* using water showed no significant difference in yield of crude extracts. The crude extract of *Solanum incanum* had an inhibitory effect on both Gram negative and Gram-positive bacterial pathogens but the zone of inhibitions was greater in Gram positive pathogens than in Gram negative ones. The most affected Gram-positive pathogen was *S. agalactiae*, while the least affected was the gram-negative pathogen, *S. typhi*. This might be because of the morphological differences. Gram-positive bacteria are more vulnerable due to their outer peptidoglycan layer which is not an effective permeability wall, whereas Gram-negative bacteria have an external membrane (Nikaido, 1996). From the tested crude extracts, the ethanol extract of *Solanum incanum* exhibited maximum zone of inhibition against *S. aureus* and a minimum zone of inhibition against *S.* Typhi. Indhumathi and Mohandass (2014) reported that the ethanol extract of *Solanum incanum* had maximum zone of inhibition (20mm), a finding which is in complement with the present study.

Methanol extract of *Solanum incanum*, in the present study, showed maximum zone of inhibition against *S. agalactiae* and minimum zone of inhibition against *E.coli*. Biruhalem *et al.* (2011) reported that the methanol leaf extracts of *Solanum incanum* showed 8.0 mm and 9.0 mm zone of inhibitions against *S. aureus* (clinical isolate) and *S. pyogenes*, respectively, indicating that their extracts were less effective than the methanol extracts of the present study. Moreover, in the present study, variations in antibacterial activities were not only seen between the sources of the extracts, but also between the extraction solvents.

The methanol extract of *D. stramonium* did not exhibit antibacterial activity against all tested bacteria. This may show that, the active compounds which are responsible for the activity against the test bacteria were unable to be extracted by the aforementioned solvents or the plant extracted with methanol might not comprise bioactive compounds that repress the growth of bacteria. Eftekhar *et al.* (2005) reported similar result in that antibacterial activity of the methanol extracts of the leave parts of the *D. innoxia* and *D. stramonium* extracts revealed no anti-bacterial activity against *E. coli*.

Among the crude extracts, Ethanol extract was produced maximum zone of inhibition against *S. aureus* and minimum zone of inhibition against *S.agalactiae* (8.87±0.23 mm). In previous studies by Solomon (2015) the ethanol extract of *D. stramonium* was evaluated its antibacterial activities against *S. typhi*, *E. coli*, *S. aureus* and *B. subtilis* using disc diffusion method. The results indicated that, ethanol leaf extracts of *D. stramonium* showed 11.20, 12.57, and 15.50 mm inhibition zone against *S. typhi*, *E. coli* and *S. aureus* at 40 mg/ml respectively. This report was in agreement with the present study, in that ethanol leaf extracts of *D. stramonium* exhibited inhibition activities against *S.typhi* and *S. aureus* with inhibition zone of 9.50±0.29 and 14.7±0.145 mm. but *E.coli* did not inhibit by

ethanol extracts of *D. stramonium*. The difference between these results might be related to strain difference between tested pathogens and solvent concentration.

Similarly, the water extract of leaves of *D. stramonium* showed maximum zone of antibacterial activity against *S. aureus* and minimum zone of inhibition against *E. coli*. According to Akharaiyi (2011) the water extract showed inhibitory zones against eight clinical bacterial isolates *Streptococcus b hemolytic*, *P. aeruginosa*, *E.coli*, *S. aureus*, *K. pneumoniae*, *B. cereus* and *S. dysenteriae*. Johnson *et al.* (2011) have also reported that water extract of *D. stramonium* showed the antibacterial activity against *S. aureus* and *E. coli* which is conformity to these findings. The methanol extracts of *D. stramonium* showed no activity against all the tested bacterial pathogens. This study also revealed that the nature of the micro-organism had effect on the inhibition potential of plant extracts. The data generally suggested that *A. niger* was more sensitive than *A. flavus* to all types of crude extracts obtained from *S. incanum*. Ethanol, methanol and water crude extracts of *S. incanum* resulted in inhibition zones of 17.5±0.29, 25.13±0.40 and 14.17±0.60 mm, respectively, on *A. niger* at concentrations of 1000 mg/ml while no zone of inhibition was detectable on *A. flavus* at the same concentration of all three types of crude extracts. However, according to Dalal and Alkhalifah (2016) the methanol extract of *S. incanum* displayed moderate to good activity against *A. flavs* and *A. niger* with zone of inhibitions of 11±1.0 and 17±0.8 mm, respectively.

In this study, the crude extracts of the root of *Solanum incanum* were found to be even more effective on *A. niger* than on all the tested bacteria. The differences between the effect of *Solanum incanum* on *A. niger* and the tested bacteria could be possily due to the variation in concentrations of the extracts used (500mg/ml and 1000mg/ml for bacteria and fungi, respectively) and the nature of the pathogens.

The three solvent extracts of leaves of *D. stramonium* showed inhibition activity against the tested fungi pathogen. *A. niger* was more sensitive organisms to ethanol, methanol and water crude extract of *D. stramonium* while *A. flavus* was resistant to the extracts obtained using all three types of crude extracts. Hadia *et al.* (2012) reported that ethanol extract of *D. stramonium* exihibit antifungal activity against *A. niger* with zone of inhibition of 10±0.3 mm. However, this report has great variation with the present study, in which ethanol extract of *D. stramonium* showed 19.5±0.28mm inhibition zone against *A. niger*. The difference of the results encountered might be related to factors like the geographic location, the season and time of harvest and the method of preparation. Solomon (2015) also report that ethanol extract of *D. stramonium* has 14.03±0.46 mm zone of inhibition against *A. niger*.

In addition, among the three solvent extracts of *D. stramonium* against the bacterial strain, the ethanol extract, which is relatively more non-polar solvent, was found to be higher in its antibacterial activity than that of water and methanol extract, indicating that the bioactive compounds are efficiently extracted with relatively non-polar solvent than water and methanol. Crude extract of *D. stramonium* show high activity on *A. niger* than bacterial test pathogen.

Generally, the plant extracts gained from non-polar solvents (ethanol and methanol) exhibited higher activities than water extracts. This is because the nature of cell wall of the bacteria has a non-polar characteristic which could be easily degraded with the principle "like dissolves like". In addition, alcohols were found to have the capacity to pass through the cellular membrane easily to extract the intracellular constituents from the plant material and the presence of non-polar remains in the extracts, which had a potentially strong activity against the test bacteria. In their activity against pathogenic bacterial test, most of crude extracts of selected plant displayed comparatively the profound inhibitory activity against the Gram-positive bacteria when comparing with that of Gram-negative bacteria. This phenomenon could be possibly explained in associating the significant variations in the outer wall composition of Gram-positive bacteria and Gram-negative bacteria. The ability of Gram-negative bacteria to resist antibacterial substances is associated to the water loving surface of their outer layer which is made of lipopolysaccharide molecules, offering a barrier to various molecules in passing through and is also related with the enzymes in periplasm space, which are able of dissociating the molecules presented from outside (Nikaido, 1996;

Gao *et al*., 1999). Gram-positive bacteria are devoid of such an outer layer and cell wall composition. Therefore, this is in compliment with the phenomenon observed in the present study in that majority of the extracts have shown slight activity against *S. typhi* and *E. coli* than that of *S. aureus* and *S. agalactiae*. Although there are differences in absorbency, some of the tested extracts displayed satisfactory activities against Gram-negative bacteria.

The present results also revealed that different plant assayed possess different levels of antimicrobial activities. Among the extracts of the two different types of plant tested against the four bacterial pathogens, the root extracts of *S. incanum* showed the higher range of antibacterial activities followed by the leaves extracts of *D. stramonium* exhibited the lower zone of inhibition. In comparison, *S. aureus* and *S. agalactiae* was found to be more vulnerable bacterial strain, whereas *E. coli* and *S. typhi* were found to be less sensitive. Similarly, from the two fungal pathogens, the root extracts of *S. incanum* showed the highest range of antifungal activities. *A. flavus* was resistant fungal pathogens for all crude extract of selected plant. The variance in effectiveness of these different plant extracts may be due to the different type of plant species or bioactive compounds are not found uniformly among different plants, nature of the microorganism and potency of extracting solvent.

Methanol extract of *S. incanum* exhibited lower MIC value of 25mg/ml than ethanol extract of *S. incanum* (50 mg/ml) against *S. agalactiae*. Owino *et al.* (2013) reported that methanol extract of *S. incanum* repressed *S. aureus* and *S. agalactiae* at MIC of 4.7 and 37.5 mg/ml respectively. However, this report was in contrary with the present study, in which methanol extract of *S. incanum* showed strongest antibacterial activity against *S. agalactiae* with MIC value of 25mg/ml.

Methanol extract of *D. stramonium and S. incanum* were having the lowest MIC value (125 mg/ml) aganist *A. niger*. Hadia *et al.* (2012) analyzed antifungal activity of ethanol extract of *D. stramonium* with MIC value of 12.5mg/ml against *A. niger*. However, this report was contrary with the present study, in which ethanol extract of *D. stramonium* showed less antifungal activity against *A. niger* with MIC value of 250mg/ml. The comparatively low MIC values documented for the *S. incanum* extracts against the test pathogens confirmed the high activity of the extract at low concentrations. Extracts with lower MIC scores are very effective antimicrobial agents. The higher MIC observed for *E. coli* and *S. typhi* in this study could be due to increased resistance to some of the bioactive ingredients in the plant. MIC is vital because populations of bacteria and fungus exposed to an inadequate concentration of the extract can acquire resistance to antimicrobial agents. The higher activity of antimicrobial agents at smaller amount is very vital for chemotherapeutic purposes because of their toxicity to patients administered with such agents would be low.

5. CONCLUSION

The present study revealed that *S. incanum*, and *D. stramonium* exhibited significant antimicrobial effect by the crude extracts against the four bacterial pathogen (*E. coli*, *S. aureus*, *S. agalactiae* and *S.* Typhi) and two fungal pathogen (*A. niger* and *A. flavus*) which is an indicator for the occurrence of antimicrobial agents in it. The three solvents employed for the extraction process, that is; water, methanol and ethanol have exhibited various power in their extraction effectiveness which could be due to their variance in polarity. The traditional healers use mainly water as the solvent but in this study found that the plant extracts by alcohol (ethanol and methanol) provided more efficient antimicrobial activity compared to those extracted by water. Generally, the result of the current study confirmed the traditional practice of these medicinal plants for treatments of some microbial infections in folk medicines in Ethiopia.

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