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Potential Antibacterial Activity of Crude Extracts from *Aloe vera*, *Zingiber officinale* and *Vinca major* Medicinal Plants

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Abstract: Antibiotics are probably the most successful family of drugs so far developed for improving animal and human health. Because of increasing resistance to antibiotics of many bacteria, plant extracts are of new interest as antiseptics and antimicrobial agents in medicine. The methanolic extracts of *Aloe vera*, *Zingiber officinale* and *Vinca major* medicinal plants were evaluated for theirantibacterial activity on the Gram negative (*S.* Typhimuriumand *E. coli)* and Gram positive (*S. aureus* and *S. agalactia*) bacteria. The test was done by using disc diffusion method and minimum inhibition concentration determination. The results of this study showed that the compounds from *A. vera* and*Z. officinale*medicinal plants have an activity against the selected Gram-negative and Gram-positive bacteria. The extract isolated from *A.vera* has high antibacterial activity against *E. coli* (18.2±0.91) mm diameter and *S. aureus* (14.5±0.48) mm diameter zone of inhibition. But extracts from *V.major* has showed poor antibacterial activity inthis study. The minimum inhibitory concentrations were performed on *A.vera* and *Z. officinale*. The result showed that the MICs ranged from 2µg/mLto 420µg/mL. The lowest MIC (2µg/mL) values were obtained from the extracts of *A.vera*against *E. coli* microorganisms. The obtained result forms a good basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. So that continues progressing researches to test the safety efficiency of these plants are needed to be conducted and scientifically proved.

Key words: Antimicrobial Activity · Microbial Sensitivity Tests · Methanol Extract · Medicinal Plants

INTRODUCTION

Herbal medicine has been widely used all over the world and formed an integral part of primary health care in many countries including Ethiopia [1]. The use of medicinal plants to treat infections is an old practice in a large part of the world especially in developing countries where there is dependence on traditional medicine to maintain human and animal health [2]. Recognizing this WHO had launched a policy of urging its member states to promote and integrate traditional medicine in to their national health care system [3].

Since ancient times, many plants species reported to have pharmacological properties as they are known to possess various secondary metabolites which utilized to combat the disease causing pathogens [4].

Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens [5]. Hence, researchers have recently paid attention to safer phyto-medicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs [6].

Ginger is the rhizome of the plant *Zingiber officinale*, consumed as a delicacy, medicine, or spice. It lends its name to its genus and family *Zingiberaceae* [7]. The most well-known member of *Zingiber* is *Zingiber officinale*. In many parts of the world, *Z. officinale* has medicinal and culinary values [8]. The volatile oil gingerol and other pungent principles not only give its pungent aroma, but are the most medically powerful because they inhibit prostaglandin and leukotriene formation, which are products that influence blood flow and inflammation [9; 8]. Zingerone is likely to be the active constituent against enterotoxigenic *Escherichia coli* heat-labile enterotoxin-induced diarrhea [10].

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A.vera leaves contain phytochemicals under study for possible bioactivity, such as acetylate mannans, polymannans, anthra quinone, glycosides, anthrones, emodinand various lectins [11]. A. vera gel is a clear, cool, transparent gel that used topically for a number of skin problems, including acne. It contains enzymes that relieve pain and reduce inflammations. It also decreases redness and swelling and have anti-fungal and antibacterial properties. A. verahas healing powers when it comes to severe burns, because it has the ability to prevent blistering andscarring [12]. It is used as a multipurpose skin treatment. This may be partly due to the presence ofsaponin, a chemical compound that acts as an antimicrobial agent [13].

Vinca major and *minor* (Periwinkle) are members of the *Apocynaceae* family in the flowering herbs mostly found in tropical regions of the world [14]. Both *V. major* and *V. minor* are considered to be interchangeable in their medicinal uses [15]. Vinca is used to extract its alkaloids which have multifarious medicinal properties. Vincristine sulfate and vinblastine sulfate act as antineoplastic agents and also used in treatment of hodgkin's diseases, other lymphomas and choriocarcinoma. Vinca also exhibits hypotensive andantidiabetic property [16]. Theethanolic extracts of *V. major* aerial parts possess antidiarrheal activities [17]. The aqueous extract of the root antimalarial action exerted in chickens infected with *Plasmodium gallinaceum* orally 4.42g/kg dose. It has been shown antibiotic activity of benzene extract of flowers of various bacteria [18].

The present study was undertaken to investigate the effects of methanolic extracts of *Z. officinale*, *V. major* and *A.vera*againstdifferent Gram positive and Gram negative bacteria.

MATERIALS AND METHODS

Plant Collection: Fresh plant parts of *V. major* and *A.vera* were collected randomly from the gardens in and around Mekelle, whileroots ofginger purchased from market to test its potential antibacterial activity. The taxonomic identities of plants were confirmed by Pharmacology and Toxicology team, Department of Veterinary Medicine, Mekelle University. The collected plantswere washed with running tap water, air dried at room temperature, homogenized to a finepowder and stored in air-tight bottles at 4°C. Exposure to sunlight was avoided to prevent the loss of active compounds.

Organisms: Somepathogenic bacteria species of Gram negatives (*S.* Typhimuriumand *E. coli)* and Gram positive (*S. aureus* and *S. agalactia*) were obtained from National Veterinary Institute (NVI), Debre-zeit, Ethiopia, to test the susceptibility of the organism to the crude extracted medicinal plants. They were cultured in nutrient agar for 24 hours and the fresh inoculums were taken for the test.

Preparing of Extracts: After homogenized to a fine powder it weighed by using a sensitive balance and the crushed powder was macerated in 100 mL, 80% methanol in a beaker which was sufficient enough to cover all the plant powder for three days at room temperature after continues shacking. The maceration step done three times for eachsample. From extract sediments and plant residues were removedby Whatman no. 54 filter paper. The crude extract solutions were put to the glass and methanol was evaporated by rotary vacuum evaporator. Then the concentrate poured in to beaker and put in hot air oven at 40°c for complete drying. After complete drying the extracts were left at room temperature by sealing with aluminum foil until further processes were started. At the end the weight of each plant extract was measured by using sensitive balance and the percentage yield of each plant extract were calculated.

Study Design: The study was conducted by using the methanolic extracts of *A.vera*, *Z. officinale* and *V. major* medicinal plants. The *in vitro*antibacterial susceptibility testing of the three medicinal plant extracts against *S.* Typhimurium, *E. coli*, *S.aureus* and *S.agalactia* were done using disc diffusion method and minimum inhibition concentration (MIC) determination.

***In Vitro*antibacterial Susceptibility Testing of Medicinal Plant Extracts:** Stock solution of each crude extract was prepared by diluting 400 milligrams of each plant crude extract in 2 mL of dimethylsulfoxide (DMSO) to prepare a concentration of 200mg/mL. To obtain a dose dependent effects against the test bacteria and determine the minimum inhibitory doses of the extract, a serial dilution was done in micro tubes according to Samson [19] to prepare a final concentration of ranging from 12.5-200mg/mL.

Pure overnight inoculums of *E. coli*, *S.* Typhimurium, *S. aureus* and *S. agalactia* were prepared by suspending of colonies into the physiological saline 0.85%and adjusted to 0.5 McFarland standard. The bacterial

suspensions were spread evenly on the surface of Mueller Hinton agar for antimicrobial testing of medicinal plant extracts by using disc diffusion method with 6 mm diameter disc. Discs were impregnated by 200mg/mL and 100mg/mL medical plants extracts dissolved in DMSO. After the impregnation, discs were spread on the surface of Mueller Hinton agar evenly;antimicrobial susceptibility testing was read after 24 hours incubation at 37°C. The inhibition zones were controlled with discs impregnated with pure DMSO (control negative) and using antibiotic standard, tetracycline 30µg (control positive). After the incubation, inhibition zones were measured in mm by digital caliper.

Determination of MIC: MIC wasdeterminedby a serial dilution technique using micro titer plates. The different plant extracts were taken and serial dilutions of the extracts were done by DMSO and using nutrient broth for bacterial culture with respective inoculums. The micro plates were incubated for 24 hours at 37 °C. After 24 hours the micro plates were checked for bacterial growth and the lowest concentration of each plant extract without visible growth is defined as MIC of the corresponding one.

Data Analysis: To determine mean inhibitory zone of inhibition produced by each plant extracts on bacteria, the data was subjected to one way analysis of variance (ANOVA) and the experimental results were expressed as mean +/- standard deviation and different between different concentrations. *P*-value < 0.05 were regarded as significant and *P*-values < 0.01 as very significant.

RESULTS

Percentage yields of the crude extract; better yields were obtained from *V. major (*1.9%) and *A.vera* (2.19%).

Antibacterial Activities of the selected medicinal plant extracts*;*asshown in table 2 below, the selected medicinal plant extracts showed different antibacterial activities on the tested microorganisms. The experimental results expressed as mean ± standard deviation of the mean in different concentrations of the medicinal plant extracts. The results of this study showed that the extracts isolated from *A.vera* has high antibacterial activity against *E. coli* (18.2±0.91) mm diameter, which is found to be resistant for the standard antibiotic (Tetracycline) and against*S. aureus* (14.5±0.48) mm diameter zone of inhibition but *V. major* has poor antibacterial activity.

MIC was performed using *A.vera* and *Z.officinale* methanolic extracts by a serial dilution technique using micro titer plates with the standard drug chloramphenicol. The minimum inhibitory concentration on *V.major* is not tested because it has poor antibacterial activity on the agar disc diffusion antibacterial test. The minimum inhibitory concentration test is done threetimes for each

Table 1: Information overview about collected medical plants

Medicinal plants	Family	Used parts	Amounts of the Crashed parts used in g	Yield (g)	% Yield
A.vera	*Xanthorrhaeceae*	Leaf	180	5.292	2.l9%
Ginger	*Zingiberaceae*	Root	213	2.164	1.02%
V. major	*Apocynaceae*	Root, steam, leaf and flower	221.65	4.32	1.9%

g: gram, %= percent

Table 2: Mean of zone of inhibition of *A. vera*, *Z. officinale* and *V. major*± standard deviationof the mean with tetracycline as a reference standard and DMSO as a control.

	Zone of inhibition (mm) (mean ± SD)								
Medicinal plants	Conc. mg/mL	*S.* Typhimurium	P-value	*E. coli*	P-value	*S. aureus*	P-value	*S. agalactia*	P-value
A. vera	100	9.13±1.2	0.00	12.6±2.39	0.00	9.0±0.97	0.00	8.9±1.5	0.00
	200	12.63±1.9		18.2±3.07		14.5±1.35		13.6±2.6	
Z. officinale	100	8.3±1.86	0.02	9.7±1.16	0.03	8.8±1.16	0.00	8.7±0.88	0.02
	200	10.61.89		12.6±2.34		12.3±2.34		10.4±1.41	
V. major	100	6.4±0.69	0.22	6.00±0.00	0.04	6.00±0.00	0.02	6.4±0.69	0.32
	200	6.95±1.58		6.7±0.75		6.7±0.75		6.8±0.76	
Tetracycline	30 µg	23.3±1.7		7.2±1.33		12.5±1.93		16.5±1.97	
DMSO		6.0±0.00		6.0±0.00		6.0±0.00		6.0±0.00	

mg= milligram, mm= millimeter, mL= milliliter, SD= standard deviation

N.B: As the diameter of paper disc used was 6 mm, 6 mm diameter included in the table is indicative of no activity.

Table 3: Minimum inhibitory concentration of *A.vera, Z. officinale* and thestandard drug chloramphenicol against the selected Gram positive and Gram negative bacteria.

	Minimum inhibition concentration (MIC) in mg/mL		
Organisms	*A.vera*	*Z. officinale*	Chloramphenicol
S. Typhimurium	0.098	0.093	0.036
E. coli	0.02	0.05	0.2
S. aureus	0.42	0.16	0.05
S. agalactia	0.1	0.19	0.026

mg= milligram, mL= milliliter

bacterium and takes the mean as the lowest concentration inhibited bacterial growth. A representative data set is shown in Table 3.

DISCUSSION

The main factors that determine the antimicrobial activity are the type and composition of the plant extract, amount used, type of microorganism, pH value and temperature of the environment [20, 21]. The solvent that used to extract the components of the medicinal plant also affect antimicrobial activity of the extract [22]. Several reports had been published that describe the antibacterial properties of different herbs and spices [21]. However, still there is little information about the exact mechanism of their antimicrobial action [23- 28].

Antibacterial activity was evaluated by disc diffusion method against *S.* Typhimurium, *E. coli*, *S. aureus* and *S. agalactia*, using tetracycline as a standard drug and DMSO as a control positive and negative respectively. The results of this study showed that the extracts isolated from *A.vera* and *Z. officinale* was found to have significant antibacterial activity against both the selected Gram positive and Gram negative bacteria, similar findings has been reported by Kaithwas*etal*. [29] on ethanol extracts of *A.vera* and Omoya and Akharaiyi [8] on ethanol and methanol extracts of *Z. officinale*. The zone of inhibition showed that both *A.vera* and *Z. officinale*were active than the standard drug tetracycline (30µg) against*E.coli*. The data in this study also indicate that*V. major* extract had poor effect againstall tested bacteria.The result is in contrary to Mehrab *et al*. [18], whohad reported antimicrobial activity of benzoic extract of flower part of *Vinca*against *S.* Typhiand *S. aureus* and ethanolic extracts of leaf on *S. aureus*. This difference might be due to the different technique employed to extract the plant material.

The results clearly demonstrated that *A.vera* and *Z. officinale* possesses antimicrobial activity than *V. major*. It could be theorized that the presence of greater amount of the anthraquinones and phenolic antioxidants in the *A.vera* extract could be responsible for the observed antimicrobial activity [30] and the ginger extracts having chemical compounds such as saponin, alkanoids and flavonoids have been reported to have antifungal and antibacterial activities *in vitro* [31]. Extracts from *A.vera* have more potent action on Gram-negative *E. coli* bacteriathan the other tested bacterial speciesand also from the standard antibiotic (Tetracycline). This result has similarity with the research of Pratibha, *et al*. [32] who reported that the ethanol extracts of *A.vera* has high activity on *E.coli* and *S. aureus* and they reported 11mm and 14mm diameter activity against *E. coli* and *S. aureus*respectively in their research.

The 200mg/mL concentration of *V. majora* extract produced insignificant ($p>0.05$) zone of inhibition on *S.*Typhimuriumand *S. agalactia* compared to the 100mg/mL concentration. Compared with the 100mg/mL concentration of *Z. officinale* and *A.vera* extract, with the 200mg/mL concentration dose dependently and significantly ($p<0.05$) produced zone of inhibition on the tested bacteria. Similar reports was statedby Renisheya *et al*. [33], who mentionedthat the anti-bacterial property of DMSO gel extracts of *A.vera* against the selected strains of human pathogenic bacteria and the degree of inhibition varied depending upon the concentration of the extract. Highest concentration of *A.vera* displayed maximum zone of inhibition.

Using the microplate bioassay minimum inhibitory concentration of *A.vera*and *Z. officinale*against Gram positive and Gram negative bacteria was investigated. The results show the MICs were various ranged from 2µg/mL to 420µg/mL. The lowest MIC (2µg/mL) values were obtained from the extracts of *A.vera* on *E. coli* microorganisms on the test followed by the standard antibiotic chloramphenicol (2.6µg/mL). Fractionation of this plant extract containing fractions of saponin in the *A.vera* extract and flavonoids fractions in *Z. officinale*extract which inhibits the growth of bacteria in methanol extract [22].

CONCLUSION

A. vera and ginger extract tested showed antimicrobial activity against all the tested microbes. However,there is poor antimicrobial activityof *Vinca* extract.The results obtained in this study justify the use of Aloes and ginger in ethno veterinary medicine. In the research the observations determined that medicinal plant extracts were more effective to some antibiotic resistant bacteria. It is an important step for the treatment of bacterial infections caused by antibiotic resistant bacteria. This is particularly of urgent interest when the growth rate of multi-resistant drug strains of bacteria worldwide is considered. The obtained result forms a good basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. The practice of using plants as supplementary or alternative medicine in developing countries will reduce not only the clinical burden of drug resistance development but also the side effects and cost of the treatment with allopathic medicine.

From the present study the following recommendations are forwarded:

- Developing these medicinal plants specially *A.vera* for the treatment of susceptible bacteria to fight treatment failure of the present antibiotics.
- A continues progressing researches to test the safety efficiency of these plants need to be conducted and scientifically proved.
- The government should create awareness in the population to keep the medicinal plant for sustainable use.

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