DOI: http://dx.doi.org/10.4314/star.v4i2.24
ISSN: 2226-7522(Print) and 2305-3372 (Online)
Science, Technology and Arts Research Journal
Sci. Technol. Arts Res. J., April-June 2015, 4(2): 199-203
Journal Homepage: http://www.starjournal.org/

Original Research

Evaluation of Antimicrobial Activity of Some Traditional Medicinal Plants and Herbs from Nekemte District against Wound Causing Bacterial Pathogens

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Abstract

Many infectious microorganisms are resistant or multi-resistant to synthetic drugs, which exert side effects to human health. In traditional medicines, a wide range of plants are used in the treatment of wounds and other diseases. The wound healing potential of some medicinal plant were traditionally used for wound treatment against wound-infecting bacteria, was assessed using disc diffusion and agar well diffusion. The traditional medicinal plants were extracted using diethyl ether, methanol and water and tested for their antimicrobial activities against clinically isolated bacterial that are known by attacking wound infection. All the extracts had antimicrobial activities, among which methanol extract was highly reactive all the tested organisms compared to the diethyl ether and aqueous extracts. Crude extracts of *C. edulis* (root), C.*longicenda* (leaf), *M. ferugunea* (bark), *B. antidyscenatrica* (root), *B. antidyscenatrica* (bark) and *E. longisetus A.Rich*.(root) showed various degrees of antimicrobial activity towards each standard and drug resistant microbial pathogen with mean zone of inhibition ranging from 8-20 mm against multidrug resistant *E.coli and P.aueroginosa respectively*. Of the different crude extracts, methanol extract of *carissa edulis* showed the highest mean zone of inhibition 10mm against *E. coli* while others did not show any inhibition zone against it. The potential antibacterial activity of this medicinal plant against both gram-positive and gram-negative bacteria justifies the use of the plants as the wound healing agent. The isolation of active principle of this plant may serve as a source and lead for the synthesis of either better or novel drugs in pharmaceutical industries.

Article Information

Article History:

Received : 15-04-2015

Revised : 23-06-2015

Accepted : 26-06-2015

Keywords:

Wollega administrative zones

Traditional medicinal plants

Wound healing

Antibacterial activity

Herbal extracts

Inhibition zone

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INTRODUCTION

Wounds, resulting from microbial infection, are the most common public health problems. Topical antimicrobial therapy is one of the most important methods of wound care. Some medicinal plants have been employed in folk medicine for wound care. Some of these plants either possess pro-wounding healing activities or exhibit antimicrobial and other related properties which are beneficial in overall wound care. Wound healing processes are well organized biochemical and cellular events leading to the growth and regeneration of wounded tissue in a special manner. Healing of wounds is an important biological process involving tissue repairs and regeneration. Wound healing are conveniently classified into any of three types, healing by first intention, healing by second intention and healing by third intention, depending on the nature of the edges of the healed wounds. The common wound pathogens includes bacteria, fungi, protozoa and viruses among which the most common are beta-haemolytic *Streptococci* [*Streptococcus pyogens* (*S.pyogens*)], *Staphylococcus au reus* (*S.aureus*), *Pseudomonas aeuruginosa (P. aeuruginosa), Escherichiacoli* (*E.coli*) and *Enterococcus,* Acinetobacter spp, *Klebsiella* spp, and *Coliforms* (Odimegwu *et al*., 2008; Mulu *et al*., 2006; Ranjan *et al*., 2010; Mehta *et al*., 2007; Ozumba , 2007; Ousey *et al*., 2009).

Although wounds may heal through the body's natural process of regenerating dermal and epidermal tissues, chronic forms cause significant impact on health and economic growth (Ousey *et al*., 2009). Topical antimicrobials may be indicated when the clinical signs and symptoms of an active infection are present. Complications of deep tissue infections such as bacteremia can be treated with systemic antibiotic (Werdin *et al*., 2009). However, the increase in antibiotic resistant strains together with the lack of and high cost of new generation antibiotics increased wound-related morbidity and mortality (Akinsulire *et al*., 2007).

Ethno botanical studies revealed that a wider range of Ethiopian plants are being used in the treatment of

wounds and other diseases in the traditional health care system of the country (Giday *et al*., 2007; Jayaraman *et al*., 2008; Teklehaymanot *et al*., 2010). Crude extracts of Ethiopian plants and others used elsewhere (Tadege *et al*., 2005) revealed strong antibacterial activities indicating that these plants can serve as sources of effective drugs against wound-causing bacteria. The objective of this study was, therefore, to evaluate the antimicrobial activity of selected traditional medicinal plants and herbs used in Nekemte district against wound causing bacteria.

MATERIALS AND METHODS

Plant Sample Collection, Identification and Extraction

Selected traditional medicinal plants and herbs that are used for antimicrobial activity of wound infection causing pathogens were collected and taxonomically identified at Wollega University Biology Department Herbarium. Plant materials were washed using tap water; air dried in shade and powdered using wooden-made pestle and mortar and stored in sterile bottle at 4°C for analysis. The powdered plant materials were extracted with distilled water, diethyl ether and 99% methanol after three days soaking each with frequent agitation until the soluble matter has dissolved**.** The extracts were filtered by passing through Whitman's filter paper. The solvent extracts were concentrated under reduced pressure using Rota Vapor and preserve at 4^0C in air tight bottle for further investigation.

Plant extracts were reconstituted with extracted solvents make a solution of 100 mg/mL and then filtered with a membrane of pore size of 0.2 µm in a sterile. Sterility of the filtered extracts was checked by plating them on Muller Hinton agar (Akinsulire *et al*., 2007).

Test Microorganisms

The test bacteria were *S. aureus*, *E. coli*, *P. aeuruginosa* and all the clinical isolates were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI) Microbiology Department.

The test organisms were grown in 10 mL Nutrient broth at 37 ^{0}C, authenticated and maintained in Muller Hinton agar medium (Tadege *et al*., 2005). Muller-Hinton agar was prepared according to the manufacturer's instruction, autoclaved and dispensed on sterile plate.

Antimicrobial Activity Assay

Agar disc diffusion method was used to determine the antimicrobial activity of plant extracts. Twenty ml Muller-Hinton Agar (MHA) were poured in Petri plates and allowed to solidify and dry. Bacterial culture was swabbed by medical cotton swap on the surface of MHA and allowed to dry for 15 min. Each piece of sterile 6-mm assay discs was then distributed on the surface of the agar. The discs were impregnated with each plant extract of different solvents. One of remaining discs was impregnated with tetracycline or Erythromycin for bacteria as positive control while the other disc was impregnated with 40 mL sterile distilled water and standard solution as negative control. The plates were then left at room temperature for 30-60 minutes to let the extract and controls diffuse to the agar and incubated at 37^0C for 24 hours for each bacterium. Plates were observed for inhibition zones (zones showing no microbial growth) around the discs after the incubation period (Jayaraman *et al*., 2008).

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the extract inhibiting the visible growth of any microorganism. The MIC of different concentrations of the extracts was determined using two-fold dilutions method: 100, 50, 25 and 12.5 mg/mL. The half strength (50µl of 50 mg/mL) and quarter strength (25µl of 25 mg/mL) of extracts were used in addition to the normal strength (100 µl of 100 mg/mL) of diethyl ether and methanol which were used as negative control. The 6 mm cork borer was used to bore wells in the already solidified media inoculated with the microorganisms. The different extract concentrations were dispensed into each well and properly labeled. The preparation was left to diffuse for 30 minute before incubating at 37^oC for 24 hours for the bacterial strains. The lowest concentration of antimicrobial agent that completely prevented the growth of the microorganisms was taken as the minimum inhibitory concentration (MIC) of the extract. The zone of inhibition was observed and recorded.

RESULTS

Background Information of the Medicinal Plants

In the last two decades the attention given to natural medicinal compounds from plants and animals has shown significant increasing trends. Based on this, the selected medicinal plants from east Wollega were subjected to study for their biological activity against disease causing bacteria. The communities under the study areas were used traditional medicinal plants and herbs to treat different aliments/diseases by following the prescription given from the traditional healers. The plants and herbs collected for this purpose were summarized their traditional use in Table 1. The name of the selected plants and herbs were written in the local language afan oromo (O) and scientific name.

The inhibition zones of the extracts against selected bacterial strains depicted in the (Table 2 and 3) with the corresponding extracting solvents. The diethyl ether and methanolic extracts of these plants and herbs showed inhibition zones against bacteria strains from 7 mm up to 20 mm in diameter. *Clematis longicenda* (leaf) extract showed good biological activity (10 mm inhibition diameter) only against *Pseudomonas aeruginosa* for methanol extract while the diethyl ether extract was not observed for its growth inhibiting properties against all strains used in this study. This significant biological activity may attribute to the use of this plant for tonsillitis treatment.

Plant samples like *P. lanceolata* (whole), *R. communis* (root), *R.absinicus* (root), *M. ferugunea* (root), *S. abssinica* (leaf) and *J. schnimperriana* (root) did not show any biological activities against all clinical isolates for methanol extracts with the exception of *P. lanceolata* (whole) which showed better growth inhibition against *S. aureus* for diethyl ether extract. The present result may support the use of this plant for the treatment of wounds by the traditional healers.

Carissa edulis (root) extracts (methanol and diethyl ether) revealed high inhibition capacity towards *P. aeruginosa* strain with inhibition zones of 20mm and 10 mm respectively. In addition to this, its methanol extract showed high reactivity against *E.coli* (10 mm). Its methanol extracts caused higher growth inhibition compared with its diethyl ether extract against clinical isolated bacterial pathogens.

Table 1: Plants used for treatment of common diseases with single-species prescription in East Wollega

No	Local name	Scientific name	Family	Habit (site of growth)	Plant parts used	Indigenous use	Method of preparation	Route of Admini-stration
1	Edda fitie (O)	*Clematis longicauda steud ex.A. rich*	Ranunculaceae	Climber (wild)	Leaf	Tonsillitis	The juice of freshly squeezed leaf is mixed with melted butter.	Oral
2	Korxobi (O)	*Plantago lanceolata L.*	Plantaginaceae	Herb (wild)	whole	Herpes wounds	The juice of freshly squeezed leaf is pasted with butter and made to ointment.	Topical
3	Kabosimbiro (O)	*Ricinus communis L.*	Euphorbiacea	Shrub (domestic)	Root	Rabies	The root is pounded, well spiced, and mixed with food.	Oral
4	Agamsa (O)	*Carissa edulis Vahl.*	Apocynaceae	Shrub (wild)	Root	Rabies	The root is powdered and mixed with food.	Oral
5	Komogno (O)	*Brucea antidysenterica J.F. Miller*	Simaroubiaceae	Shrub (wild)	Root	Toothache	The root is chewed or held between teeth.	(Buccal) topical
6	Dhangago (O)	*Rumex abyssinicus Jacq.*	Ploygonaceae	Herb (wild)	Root	Gonorrhea	The root is powdered and mixed with honey.	Oral
7	Dhumuga (O)	*Justicia schimperiana (Hochest. Ex Nees) T. Anders*	Acanthaceae	Shrub(domestic)	Root	Seizure	The root is dried, pounded, and pasted with butter and put over head.	Topical
8	Sotalloo (O)	*Millettia ferruginea (hochst.) Baker.*	Fabaceae	Tree (wild)	Bark	Tooth-ache	The bark is chewed or held between teeth.	(buccal) topical
					Root	Gangrene	The powdered root is mixed with rancid butter or dispersed in perforated area.	Oral/ topical
9	*Mata bokke(O)*	*Echinops longisetus A.Rich.*	Asteraceae	Shrub(wild)	Root	Migraine, diarrhea, heart pain, different forms of infections	The root is powdered and mixed with meal and oral taken or applied on external surfaces infected.	Oral/ topical

Table 2: Antimicrobial activity of diethyl ether against wound infection causing bacterial pathogens

Test plants/antibiotics	Solvent	Inhibition Zone in Diameter		
		E. coli	*S.aures*	*P.aueroginosa*
Clematis longicenda(leaf)	E	**	**	**
Stephanie abssinica(leaf)	E	**	**	**
Plantago lanceolata(whole)	E	**	8 mm	**
Ricinus communis (root)	E	**	**	**
Carissa edulis(root)	E	**	**	10 mm
Brucea antidyscenatrica(root)	E	**	**	**
Brucea antidyscenatrica(bark)	E	**	10 mm	7 mm
Echinops longisetus A.Rich. (root)	E	**	**	8 mm
Rumex absinicus(root)	E	**	**	**
Justicia schnimperriana(root)	E	**	**	**
Millettia ferugunea(root)	E	**	**	**
Millettia ferugunea(bark)	E	**	**	10 mm
Tetracycline	M	10 mm	30 mm	10 mm
Erythromycin	M	8 mm	15 mm	15 mm

**= not detected, E= diethyl ether

Table 3: Antimicrobial activity of methanol extracts against wound infection causing bacterial pathogens

Test plants/antibiotics	Solvent	Inhibition Zone in Diameter		
		E. coli	*S.aures*	*P.aueroginosa*
Clematis longicenda(leaf)	M	**	**	10mm
Stephanie abssinica(leaf)	M	**	**	**
Plantago lanceolata(whole)	M	**	**	**
Ricinus communis (root)	M	**	**	**
Carissa edulis(root)	M	10mm	**	20mm
Brucea antidyscenatrica(root)	M	**	10mm	10mm
Brucea antidyscenatrica(bark)	M	**	**	15mm
Echinops longisetus A.Rich. (root)	M	**	**	18mm
Rumex absinicus(root)	M	**	**	**
Justicia schnimperriana(root)	M	**	**	**
Millettia ferugunea(root)	M	**	**	**
Millettia ferugunea(bark)	M	**	**	15mm
Tetracycline	M	10mm	30mm	10mm
Erythromycin	M	8mm	15mm	15mm

**= not detected, M=methanol

B. antidyscenatrica (root and bark) except the diethyl ether extract of the root, it showed medium antibacterial activity which ranges from 7-15 mm for *S.aures*. *and P.aueroginosa*. This result indicates that the plant is rich in biologically active compounds which can be function at high concentration. *E. longisetus A.Rich.*(root) showed high reactivity against *P.aueroginosa* with inhibition zones of 18 mm in methanol and 8 mm in diethyl ether extracts respectively. Many studies reveal that this plant is the promising source of antibacterial agents in the future.

The methanol and diethyl ether extracts of *M. ferugunea* bark showed significant inhibition activity against *P. aueroginosa* with minimum inhibition zone of 15mm and 10mm respectively. This implies that the bark of this plant extract contains broad spectrum compounds of natural origin. Hence, further study is suggested to identify and characterize these biological active compounds since the community uses this plant for the treatment of toothache and gangrene.

The MIC value of plant extracts against the tested bacteria ranged from 100% (100 µl) to 25 %(25 µl) (methanolic and diethyl ether) extracts. The most frequent MIC value of the extracts was 25% of the extract (25µl). All bacterial strains were highly susceptible against each standard antibiotics starting from 8mm to 30mm diameter, but *E.coli* is the most resistant bacteria while *S.aures* was the most susceptible.

DISCUSSION

Antimicrobial activity investigations have been found to offer important clues in the identification and development of traditionally medicinal plants used medicinal activity into discover modern drugs for treatments of different animal and human illness. So, contribution of the field has also been reflected in the current study. In this current study Methanolic and Diethyl ether extracts of different family and species of medicinal plants showed strong antibacterial activity against clinical isolated bacterial strain and this study is supported by study conducted by Tadege *et al* (2005). The inhibition zone of these medicinal plants (*C. edulis*, *B. antidyscenatrica*) shown greater inhibition zone and therefore they have high antimicrobial activity antagonist these clinical isolated bacterial pathogens (Woldeamanuel *et al*., 2005). This result shows that the plant might have important compounds that can be used for the treatment of wound-causing bacteria and viruses.

Especially Methanolic extract of *C. edulis* (root) (20mm) and *B. antidyscenatrica* (bark) (15mm) showed high inhibition zones at a concentration of 100mg/ml against *P*. aeuruginosa in agar well diffusion method. This was similar to the methanolic extract of *B. antidyscenatrica(root)* on *S. aureus*. Most of the MIC values of these medicinal plants as well as other plant extracts were almost similar with 25% MIC. The similarity or closeness of the MIC values of the plant extracts could be due to the sensitivity of the micro titration method in detecting minimum amount of turbidity which was the indicator of the growth of the test organisms than visual inspection. Methanolic extract of the root of *Carissa edulis* was the first strong plant for its antibacterial activity. This indicates that the active principle which inhibits the growth of susceptible bacteria may dissolve better in methanol than in water. The diethyl ether extract of bark *B. antidyscenatrica* had strong activity against *S. aureus* comparable to the *P. lanceolata* (whole). The *P. aeuruginosa* was the most inhibited bacteria by most of the plant extracts while the most resistant was *E*. coli, which was only inhibited by the methanolic extract of *C. edulis* (root). Gram-negative bacteria are frequently reported to have developed multi-drug resistance to many of the antibiotics currently available in the market of which *E. coli* is the most prominent (Mandal *et al*., 2010; Kaur *et al*., 2010).

In general, most of the methanolic and diethyl ether extracts of the plants showed antibacterial activities. This indicates the potential of the plants to be used as antibacterial agents against wound causing pathogens. However, further studies should be conducted with different extraction solvents and toxicity and photochemical analysis should also be performed on these plants to use them as sources and templates for the synthesis of drugs to control wound and other disease-causing bacteria.

CONCLUSIONS

From the overall study, it can be concluded that, the results obtained confirm the therapeutic potentials of the methanol and diethyl ether extracts the medicinal plants particularly the first one, which are currently used by traditional healers for treatment of various wound causing pathogen and affirm the traditional usage of these plants as an alternative medicine in the local community is reasonable. The results can also provide baseline information for the future studies about isolation, identification and characterization of the pure and active components which are responsible for antibacterial activities of these plants and it has shown a promising potential for being used as starting material in new drug discovery. Generally, outcomes of this research have been shown as plant extracts contain compounds that have antibacterial activities and it is important to propose the plants in the control of resistant pathogens.

Acknowledgements

The authors are grateful to Ethiopian Public Health Institute (EPHI) for providing us with human pathogens, and also Wollega University for funding the project.

Conflict of Interest

Authors declared no conflict of interest.

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