

EXAMINATION OF *IN VITRO* WOUND HEALING ANTIMICROBIAL ACTIVITY OF EXTRACT OF SELECTED MEDICINAL PLANTS

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ABSTRACT

Objective: The objective of this study was to discover and examine the *in vitro* wound healing activity of selected medicinal plants against common wound infecting microorganisms.

Methods: *Ziziphus rugosa* and *Hemidesmus indicus* plant parts were used for aqueous and solvent extract preparation, maceration technique was followed. *In vitro* antibiotic test has been done using disc diffusion method.

Results: Maximum yield for aqueous extract was observed in *Z. rugosa* bark sample (50.6%) and minimum yield was noted in *H. indicus* leaf extract in (45.7%) and maximum yield in solvent extract was observed in methanol extract of *Z. rugosa* (37.5%) whereas minimum yield was observed in acetone extract of *Z. rugosa* (25%). For all sample extracts tested, leaf extract of acetone and methanol extract of *Z. rugosa* and *H. indicus* showed the highest antibiotic zone of inhibition of 15 mm and 12 mm, whereas lowest zone of inhibition was observed in aqueous bark extract of *Z. rugosa* (7 mm).

Conclusion: Crude aqueous and solvent extract of selected plant were showed that promising results have a wound healing aid, efficacy of which could be further improved by studying and practicing more advanced extraction procedure for future prospectus.

Keywords: Extraction, *Hemidesmus indicus*, Wound healing, *Ziziphus rugosa*, Zone of inhibition.

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INTRODUCTION

In India, there are about 47,000 plant species and they are distributed in various vegetation zones. The history of using medicinal plants in the herbal treatment of various contagious diseases goes back to medieval times which also possesses active components which can be used as a replacement to potent and beneficial herbal drugs against common bacterial infections [1]. Phytomedicine is used for primary health care around 80% of the world population. In 1991, the World Health Organization developed general instructions for assessment of phytomedicine, the turnover of phytomedicine in India as standard formulations and home remedies of Ayurvedic medicine system. Various microbial infections render health issues all over the mankind and other organisms where plants act as a valuable source of antimicrobial agents [2].

Ziziphus rugosa Lam. (Rhamnaceae), a large spreading climber armed shrub with large elliptic usually subcordate leaves, paniculate flowers and wood is reddish, moderately hard and fruit is small drupe, glabrous, white when ripe. The bark contains quercetin, betulin, betulinic acid, vanillic acid, myricetin, kaempferol, apigenin-7-O-glucoside, and apigenin [3]. The bark also contains several N-formyl cyclopeptide alkaloids. The isolation of triterpene saponins from the bark showed analgesic and tranquilizing activity in albino rats and produced no hepatotoxicity. Bark possess strong astringent and antidiarrheal properties various works posted that this plant shows anti-inflammatory, anxiolytic, central nervous system (CNS) depressant, antifungal, cytotoxic, antibacterial, analgesic, antioxidant, and antimicrobial properties. *Hemidesmus indicus* is commonly called as Indian sarsaparilla which belongs to the family of Periplocaceae thus known as *Periploca indica* [4,5]. *H. indicus* has been used as a folk medicine for a long time and found to be an important part of Ayurvedic and Unani medicine. It is a perennial climber found in the eastern and southern parts of India [6]. It is a slender, laticiferous, and endangered

shrub that is specifically known for its immense medicinal values; for example, antivenom, antiarthritic, antimicrobial, antiulcer, antileprotic, anticancerous, hepatoprotective, immunomodulatory, wound healing property, etc. The immense medicinal values of *H. indicus* can be considered as a royal source of herbal medicine in India [7,8].

In the present study, various crude aqueous and solvent extract of two selected medicinal plants samples of *Z. rugosa* and *H. indicus* were prepared and tested for *in vitro* antimicrobial sensitivity against commonly wound infecting organisms by isolating microbes from wound infected samples by disc diffusion method and measurement of inhibition zone if found [9,10].

MATERIALS AND METHODS

Material

The two medicinal plants *Z. rugosa* and *H. indicus* were selected for the antimicrobial study based on their literature study and their usage as home remedies for wound healing and anti-inflammatory property across various parts of Western Ghats of Uttara Kannada district of Karnataka from where they have been collected for the study. Their botanical identities were determined and authenticated in the Botany Department of Shivagangothri Campus located at Tolahunase, Davangere University, Davangere.

Preparation of plant extract

Maceration technique was followed to prepare the aqueous and solvent extract of selected plants. For the extract preparation, selected plant parts such as leaves and bark were isolated from a whole plant and were further cleaned using running water to remove dirt and grit. The cleaned plant parts were sterilized and air-dried. The dried plant samples were grinded in electronic mixer blender to obtain dried powdered samples for further preparation of plant extract.

Aqueous extraction

The powdered samples were mixed with distilled water at a ratio of 1:10 (g/ml). The mixture was filtered and boiled to an extent that a dense liquid formed. The filtered liquid was then centrifuged at 6000 rpm for 10 min at 4°C and the supernatant was isolated and stored for further testing.

Solvent extraction

For solvent extraction, methanol and acetone were considered. Primarily prepared air-dried powdered plant samples were dissolved in solvent at a ratio of 1:8 (g/ml). The mixture was kept for incubation in shaker incubator for 38°C at 140 rpm for 72 h. The mixture was then filtered and boiled until the extract became dense. The supernatant was collected and stored [11].

Antimicrobial assay

Bacterial culture preparation

The wound infected dressing samples were collected from nearby hospitals which included dressings of diabetes patients infected wounds and accidental injury wounds. Using sterile swabs, nutrient broth has been inoculated with above-mentioned samples and incubated at room temperature to obtain 10⁸ CFU/ml of density.

Disc diffusion assay

For the disc diffusion assay, nutrient agar plates were prepared and spread plate method was followed for even distribution of bacterial culture prepared and isolated from wound infected samples on a solidified nutrient media. From a sheet of Whatman Filter paper, discs of 6 mm diameter were punched, sterilized and were made to absorb 20 µl each of 20 µg/ml and were air-dried at 37°C for 48 h and thereafter, the discs were placed on the surface of inoculated nutrient agar plates, and then incubated at 37°C for 24–48 h to observe formation of inhibition zones around the discs [12].

Determination of minimum inhibitory concentration (MIC)

The MIC of the plant extracts was determined by concentrated dilutes of various plant extracts. The extracts and nutrient broth were taken in equal volume and mixed in the test tube. Standardized inoculums of 1–2×10⁷ CFU/ml of 0.1 ml were added to each tube. The tubes were incubated aerobically at 37°C for 19–25 h. For each test batch, two control tubes were maintained. This is as follows: Tube containing extracts and the growth medium without inoculums (antibiotic control) and the tube containing the growth medium, physiological saline, and the inoculums (organism control). MIC was determined as the lowest concentration of the extracts permitting no visible growth (no turbidity) when compared with the control tubes [13].

Statistical analysis

The statistical analysis was done using one-way ANOVA (Microsoft Excel 2007), each experiment was set up with triplicates and the data were represented as mean±standard error. Data were analyzed by ANOVA (p≤0.05). p<0.05 was considered statistically significant.

RESULTS

Yield of extraction

Yield of extraction of various organic and solvent plant extracts was examined. The yields varied from different plant extracts during extraction. Maximum yield for aqueous extract was observed in *Z. rugosa* bark sample (50.6%) and minimum yield was noted in *H. indicus* leaf

extract in (45.7%) and maximum yield in solvent extract was observed in methanol extract of *Z. rugosa* (37.5%) whereas minimum yield was observed in of acetone extract of leaves of *Z. rugosa* (25%) [14]. Further yields of various plant extracts were tabulated in Table 1.

Extraction yield: The yield of extracts of different plants studied.

Yield of extract = Obtained extract/total solid sample used

Determination of antimicrobial assay and MIC

Aqueous and solvent extract samples of two selected plants were prepared and further tested for antibacterial property against bacterial cultures isolated from three different wound infected bandage samples. Disc diffusion method was followed for which in general organic solvent extract samples showed promising results compared to aqueous extract. For all sample extracts tested, leaf extract of acetone and methanol extract of *Z. rugosa* and *H. indicus* showed highest antibiotic zone of inhibition of 15 mm and 12 mm whereas the lowest zone of inhibition was observed in aqueous bark extract of *Z. rugosa* (7 mm) and various other zones of inhibition for plant extract against wound infected cultures were tabulated in Table 2 [15].

MIC test was also performed against selected commonly wound infecting organisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* which were identified and cultured from bacterial cultures isolated from wound infected samples and confirmed by biochemical methods. MIC values were obtained from aqueous and organic solvent plant extracts that show antibacterial activity, which range between 0.1 and 0.775 mg/ml, the best MIC values were noted in the *H. indicus* solvent extract against *P. aeruginosa* culture (0.090 mg/ml) and least, unsatisfactory MIC was noted against *S. aureus* for *Z. rugosa* plant extract (0.750 mg/ml); further, MIC values other plant extracts against each organism are shown in Table 3 [16].

Antimicrobial potential of plants has been used since ages as a cure for many contagious diseases in humans. The ethnobotanical information of these Indian plant species selected for antibacterial activity is given in Table 4 [17,18].

DISCUSSION

Wound healing of cut wounds, burn wounds, and diabetic wounds which are type of cutaneous wounds generally exposed to environment and readily get infected by various types of microbes. In general, wound infected samples were isolated to find out major contributors of microbial infection such studies evidenced to found a common group of bacterial load such as *E. coli*, *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*. Burn injuries are open wounds and render a dangerous threat to burn victims, mostly those with wide areas exposed [19]. This is the foremost cause of dehydration, systemic infection, and other hurdles experienced by burns affected patients. One of the crucial factors for successful treatment of various open wounds in victims is to lock the injury at the earliest. Contamination, either by bacteria or fungi, can give on to worsening of the wound healing process and several other problems [20]. The use of plant-based extract which possesses notable antibacterial agents locally or systemically can contribute to wound healing, especially for cutaneous wounds. Most modern antibiotics trigger various side effects and accumulation of drugs which can be replaced by plant-based organic and natural antibiotic compounds.

Table 1: Yield of extract of aqueous and solvent extraction of plant samples

Plant species	Part of plant	Yield of extraction (%)		
		Aqueous	Methanol	Acetone
<i>Z. rugosa</i>	Leaves	7.2 ml/15 g=48	3 ml/8 g=37.5	2 ml/8 g=25
<i>Z. rugosa</i>	Bark	7.6 ml/15 g=50.6	7 ml/20 g=35	-
<i>Hemidesmus indicus</i>	Leaves	3.2 ml/7 g=45.7	2.9 ml/10 g=29	3.1/10 g=31

Z. rugosa: *Ziziphus rugosa*

Table 2: Plant extract antimicrobial activity and length of zone of inhibition

Plant species	Part of the plant used	Plant extract	Antibiotic inhibition zone (bacteria culture isolated from different wound infected samples)		
			Culture 1 (mm)	Culture 2 (mm)	Culture 3 (mm)
<i>Ziziphus rugosa</i>	Leaf	Methanol	12	10	7
		Acetone	15	11	12
		Water	8	9	8
<i>Hemidesmus indicus</i>	Bark	Methanol	9	8	9
		Water	7	8	7
		Methanol	10	8	11
<i>Hemidesmus indicus</i>	Leaf	Acetone	12	10	11
		Water	No significant inhibition zone found	No significant inhibition zone found	No significant inhibition zone found

Table 3: Minimum inhibitory concentration values for plant extract against wound infecting microbes

S. No.	Microorganisms	Minimum inhibitory concentration (mg/ml)			
		Aqueous extract		Organic solvent extract	
		<i>Ziziphus rugosa</i>	<i>Hemidesmus indicus</i>	<i>Ziziphus rugosa</i>	<i>Hemidesmus indicus</i>
1.	<i>Staphylococcus aureus</i>	0.750	0.200	0.600	0.100
2.	<i>Pseudomonas aeruginosa</i>	0.625	0.090	0.700	0.085
3.	<i>Escherichia coli</i>	0.700	0.250	0.675	0.250
4.	<i>Klebsiella pneumoniae</i>	0.660	0.220	0.700	0.190

Table 4: Therapeutic importance of various parts of *Z. rugosa* and *H. indicus*

Plant species	Family	Common name	Part of plant	Therapeutic importance
<i>Z. rugosa</i>	Rhamnaceae	Zunna berry	Leaf	Analgesic, anti-inflammatory, antibacterial, antifungal, anxiolytic
<i>Z. rugosa</i>	Rhamnaceae	Zunna berry	Bark	Central nervous system depressant, cytotoxic, antimicrobial
<i>H. indicus</i>	Periplocaceae	Anantmoool	Leaf	Antiarthritic, antimicrobial, antiulcer, antivenom, antileprotic
<i>H. indicus</i>	Periplocaceae	Anantmoool	Bark	Immunomodulatory, hepatoprotective, wound healing activity

Z. rugosa: *Ziziphus rugosa*, *H. indicus*: *Hemidesmus indicus*

Their utilization hinders microbial growth on site of infection or around the injury and renders a need able microenvironment for healing [21].

Further, more refined plant extraction procedure needs to be followed to produce pure antibiotic samples for stronger zone of inhibition and better wound healing of burns and wounds. Although *in vitro* examination was successful to an extent, *in vivo* acceptability needs an in-depth study of various aspects of raw material selection, selection of organic solvent, solvent-sample ratio, and different stages of purification procedures in large scale production [22].

CONCLUSION

Plant-based phytochemical extraction procedures have various challenges in *in vivo* treatment of wound healing applications. In general, selected medicinal plants such as *Z. rugosa* and *H. indicus* have already showed various property of hepatoprotective, CNS depressant, antiarthritic, analgesic, anti-inflammatory, antimicrobial, antifungal, and antivenom property by its various phytochemical constituents which need to isolated in manner which is economical and with more efficacy for successful usage and large scale sustainable production of plant extract as antimicrobial compounds for wound healing applications.

AUTHORS' CONTRIBUTIONS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ms. Samskrathi D contributed to the collection of the plant sample; Ms. Samskrathi D and Ms. Srujana TL have carried out extraction and antibiotic testing to measure the diameter of inhibition zone; and Mr. Chandukishore T contributed to

the study guide and coordinated the manuscript writing, editing, and finalization of results. All authors discussed the results and contributed to the final manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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