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 of acetone extract of leaves 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.

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]. Phytotherapy 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 in various vegetation zones. The history of using medicinal plants property across various parts of Western Ghats of Uttar 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.

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 Uttar 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 10^6 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 1–2×10^7 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 extract and 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.

| Plant species | Part of plant | Yield of extraction (%) |
|---------------|---------------|-------------------------|
|               |               | Aqueous | Methanol | Acetone |
| Z. rugosa     | Leaves        | 7.2 ml/15 g=48 | 3 ml/9 g=37.5 | 2 ml/8 g=25 |
| Z. rugosa     | Bark          | 7.6 ml/15 g=50.6 | 7 ml/20 g=34 | - |
| H. indicus    | Leaves        | 3.2 ml/7 g=45.7 | 2.9 ml/10 g=29 | 3.1/10 g=31 |

Z. rugosa: Ziziphus rugosa

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 bacteria 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 no trigger various side effects and accumulation of drugs which can be replaced by plant-based organic and natural antibiotic compounds.
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) |
| Ziziphus rugosa     | Leaf                   | Methanol      | 12            | 10            | 7             |
|                     |                        | Acetone       | 15            | 11            | 12            |
|                     |                        | Water         | 8             | 9             | 8             |
|                     | Bark                   | Methanol      | 9             | 8             | 9             |
|                     |                        | Water         | 7             | 8             | 7             |
| Hemidesmus indicus  | Leaf                   | Methanol      | 10            | 8             | 11            |
|                     |                        | Acetone       | 12            | 10            | 11            |
|                     |                        | Water         | No significant inhibition | No significant inhibition | No significant inhibition |

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                        |
|        |                   | Ziziphus rugosa | Hemidesmus indicus | Ziziphus rugosa | Hemidesmus indicus |
| 1.     | Staphylococcus aureus | 0.750 | 0.200 | 0.600 | 0.100 |
| 2.     | Pseudomonas aeruginosa | 0.625 | 0.090 | 0.700 | 0.085 |
| 3.     | Escherichia coli    | 0.700 | 0.250 | 0.675 | 0.250 |
| 4.     | Klebsiella pneumoniae | 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                                                                 |
|---------------|-------------------|-------------|---------------|---------------------------------------------------------------------------------------|
| Z. rugosa     | Rhamnaceae        | Zunna berry | Leaf          | Analgesic, anti-inflammatory, antibacterial, antifungal, anti-inflammatory               |
| Z. rugosa     | Rhamnaceae        | Zunna berry | Bark          | CNS depressant, cytotoxic, antimicrobial, antineural, anti-inflammatory                   |
| H. indicus    | Periplocaeeae      | Anantmool   | Leaf          | Antiarthritic, antimicrobial, antiseptic, antiviral, anti-inflammatory                    |
| H. indicus    | Periplocaeeae      | Anantmool   | Bark          | Immunomodulatory, hepato protective, wound healing activity                             |

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 hepato protective, CNS depressant, antiarthritic, analgesic, anti-inflammatory, antimicrobial, antifungal, and antivenom property by its various phytochemical constituents which need to be 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. 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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