

ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *RUMEX NEPALENSIS* LEAVES

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ABSTRACT

The aim of the present investigation was to evaluate the antimicrobial efficacy of methanolic extract of the leaves of *Rumex nepalensis* (MERN). The methanolic extract of *Rumex nepalensis* were tested for antibacterial efficacy against four bacterial strains and antifungal activity against three fungal strains by well diffusion method. In present study Ampicillin 10µg/well (antibacterial) and Fluconazole 10µg/well (antifungal) used as a reference standard. The methanolic extract possessed some antimicrobial activity against all the test organisms at concentration of 400µg/well. This study scientifically supports the usage of plant as a remedy for various superficial bacterial and fungal infections in traditional medicine.

Keywords: *Rumex nepalensis*, Methanolic extract, Antibacterial activity, Antifungal activity.

INTRODUCTION

The genus *Rumex* comprises of about 200 species of herbs. *Rumex nepalensis* Spreng. (Polygonaceae) commonly known as "jungli palak" in Hindi. It grows abundantly in many parts of India. It is widely distributed in the temperate Himalayas, Western Ghats, Nilgiri and Palni Hills at altitudes between 1200-4300m. The use of *Rumex nepalensis* for various therapeutic purposes is well known in Indian traditional medicine. Many of them used for its astringent qualities. Leaf extract is applied to cure skin sores; leaf infusion is given in colic and applied to syphilitic ulcers. The pounded root is given to animals in case of diarrhoea and dysentery. Leaf powder mixed with butter is applied to treat scabies.¹ Aqueous extract used as wash for reducing body pain.²

According to the research and as other species from genus *Rumex* shows purgative,³ analgesic, antipyretic,⁴ anti-inflammatory,⁵ psychopharmacological,⁶ antibacterial⁷ and antifungal⁸ activities on the roots of *Rumex nepalensis*. In the present work is to evaluate the antibacterial and antifungal activity of the methanolic extract of leaves of *Rumex nepalensis*.

MATERIALS AND METHODS

Plant material

The mature green leaves of *Rumex nepalensis* (Polygonaceae) were collected from Summer-Hill, Shimla (H.P.), India, in the month of September 2010. The leaves were identified and authenticated by Dr. R. Raina and voucher specimen number 5583 was deposited in the Herbarium of Forest Products Department, University of Horticulture & Forestry, Nauni, Solan (H.P.), India.

After authentication, the fresh leaves were collected in bulk, dried under shade and pulverized in a grinder. The coarse powder was used for further studies.

Preparation of methanolic extract

For the preparation of methanolic extract of leaves of *Rumex nepalensis* (MERN), the dried coarse powdered of leaves were extracted with methanol (95%) in a Soxhlet apparatus. The extract was concentrated and dried using Rotary flash evaporator and stored in a refrigerator at 5°C for experimentation.

Antimicrobial assay

Antibacterial activity of the plant extract

The methanolic extract of 400µg/well was tested against four bacterial pathogens namely *Bacillus cereus*, *Bacillus subtilis* (Gram-positive), *Escherichia Coli* and *Pseudomonas aeruginosa* (Gram-negative) for antibacterial activity. It was demonstrated by well diffusion method. Standard Ampicillin (10µg/well) was used as the positive control in the experiment.

Antifungal activity of the plant extract

The methanolic extract of 400µg/well was tested against three different fungal pathogens, *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* for antifungal activity. The standard reference antibiotic Fluconazole (10µg/well) was used. It was demonstrated by well diffusion method.

Well diffusion method^{9,10}

Antibacterial and antifungal activities of the plant extract were tested using well diffusion method. The prepared culture plates were inoculated with different selected strains of bacteria and fungi using streak plate method. Wells were made on the agar prepared surface with 6mm cork borer. The extract was poured into the well using sterile syringe. The plates were incubated at 37°C±2°C for 24 hours for bacterial and 25±2°C for 48 hours for fungal activity. The plates were observed for the zone clearance around the wells.

The methanolic extract of the dried leaves of *Rumex nepalensis* was used for the study. The methanolic extract was dissolved in sterile distilled water and applied to sterile wells at a concentration of 400µg/well and tested against different bacterial and fungal pathogens. It was demonstrated by well diffusion assay. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm). The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

RESULTS AND DISCUSSION

Antimicrobial activity of methanolic extract of *Rumex nepalensis* was investigated on seven clinical isolates and standard strains by well diffusion assay. The well diffusion method for antimicrobial activity showed significant reduction in microbial growth in terms of zone of inhibition around the well. The results of the test for antimicrobial activity of *Rumex nepalensis* are summarized in Table 1 and Fig. 1, 2. The methanolic extract possessed some antimicrobial activity against all the test organisms at concentration of 400µg/well. The inhibitory effect of the extract was found to be maximum against *Escherichia Coli* (12mm) for antibacterial activity and *Aspergillus niger* (11mm) for antifungal activity.

Preliminary phytochemical screening reveals the presence of anthraquinone, steroids, saponins, reducing sugar and tannins in the plant extract.⁶ Therefore, the observed antimicrobial activity may be attributed to these compounds. Aloe-emodin, an anthraquinone, has been isolated from the roots of *Rumex nepalensis* Spreng. This compound, i.e. aloe-emodin, has been reported to have antibacterial effects.⁷ Therefore, the antibacterial activity of *Rumex nepalensis* may be due to the presence of anthraquinone. However, the exact mechanism of action is yet to be determined.

CONCLUSION

The results of the above study revealed that the methanolic extract of *Rumex nepalensis* was exhibit antimicrobial activity, which might be helpful in preventing the bacterial and fungal infections and can

be used in alternative system of medicine. However, further studies are necessary to find the exact mechanism of antimicrobial efficacy and to isolate the active compound(s) responsible for this biological activity.

Table 1: Antimicrobial activity of methanolic leaf extract of *Rumex nepalensis*

| Test microorganisms | Zone of inhibition (in mm) | |
|-------------------------------|----------------------------|-------------------------------|
| Gram-positive bacteria | <i>MERN</i> (400µg/well) | |
| <i>Bacillus cereus</i> | 8 | Ampicillin (10µg/well) 20 |
| <i>Bacillus subtilis</i> | 10 | 20 |
| Gram-negative bacteria | <i>MERN</i> (400µg/well) | |
| <i>Escherichia Coli</i> | 12 | 19 |
| <i>Pseudomonas aeruginosa</i> | 9 | 20 |
| Fungi | <i>MERN</i> (400µg/well) | |
| <i>Candida albicans</i> | 10 | Fluconazole (10µg/well) 19 |
| <i>Aspergillus niger</i> | 11 | 20 |
| <i>Aspergillus flavus</i> | 10 | 20 |

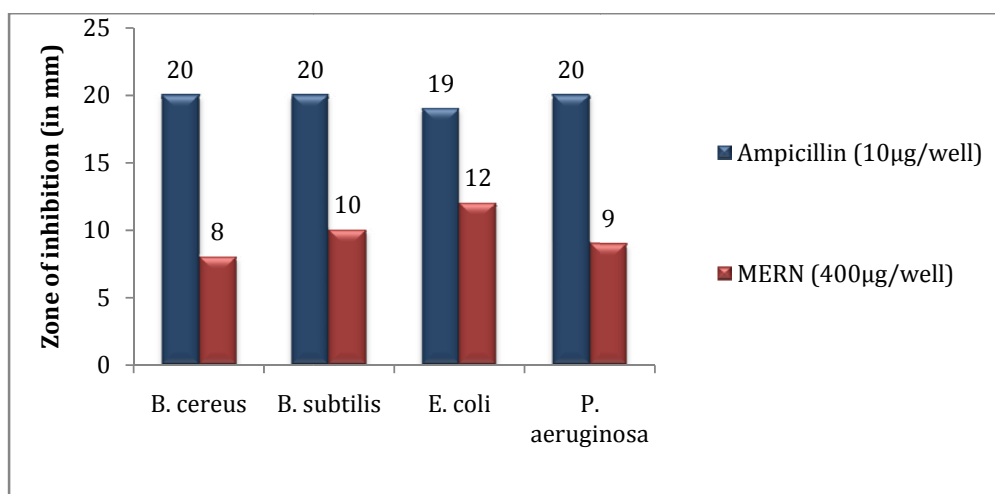


Fig. 1: Antibacterial activity of *MERN* in different bacterial strains

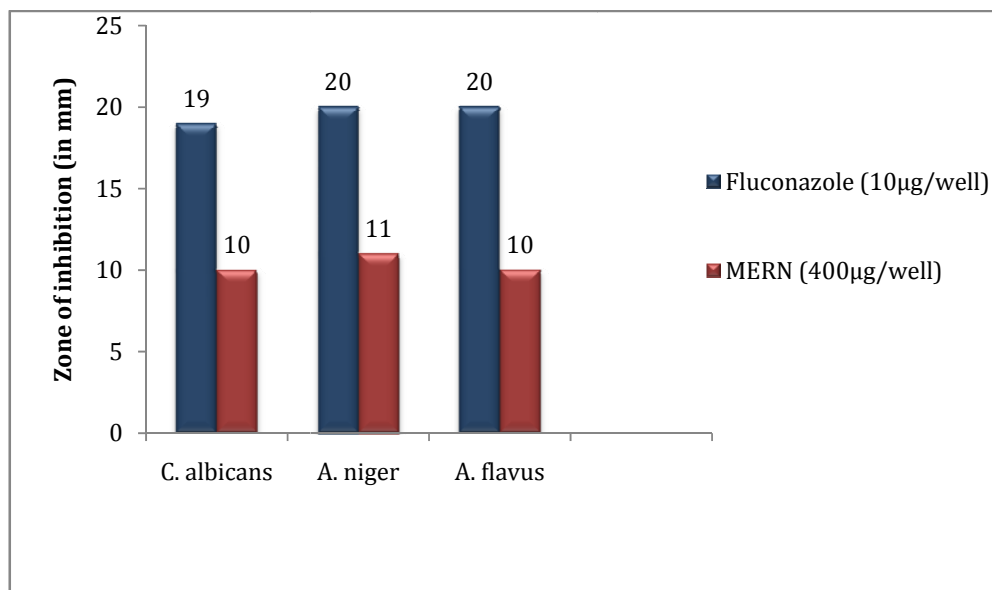


Fig. 2: Antifungal activity of *MERN* in different fungal species

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