

Original Article

ANTIMICROBIAL ACTIVITY OF SOME FOLK MEDICINAL PLANTS USED IN RAJASTHAN AGAINST SELECTED PATHOGENIC MICROORGANISMS

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ABSTRACT

Objective: To investigate the antibacterial efficacy of methanol extracts of leaves and roots of *B. diffusa*, *Eclipta alba*, *Phyllanthus niruri* and *Ricinus communis*.

Methods: The antimicrobial efficacy of methanol extracts of some medicinal plants was evaluated by agar well diffusion method against selected pathogenic bacterial strains. Gram+ve strains (*S. aureus*, *B. subtilis*) were tested and Gram-ve strains tested were (*E. coli*, *S. typhii* and *K. pneumoniae*). Antifungal activity against was tested.

Results: *B. diffusa* and *P. niruri* leaf extract showed highest antibacterial activity against *S. aureus* and *S. typhii*. Leaf extract of *P. niruri* and *R. communis* showed highest antifungal activity against *A. niger* and *C. albicans* respectively

Conclusion: The methanolic leaf extracts of *B. diffusa* and *P. niruri* were highly active against *S. aureus* and *S. typhii*.

Keywords: Antibacterial, Medicinal plants, Plant extract

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INTRODUCTION

Plants produce an incredible array of secondary metabolites and many of these have been developed into economically important products including; oils, gums, resins, tannins, rubber, waxes, pigments, flavors, fragrances, surfactants, preservatives, pesticides, and pharmaceuticals [1]. Thus medicinal plants are under tremendous pressure all across the globe, especially in India. More than 90% of the medicinal plants for herbal industries in India and for export are drawn from the natural habitats thus challenging their existence [2, 3]. The structure of flavonoid compounds is a key determinant of their radical scavenging and metal chelating activity and this is referred to as structure-activity relationships [4]. Historically plants have provided a good source of anti-infective agent [5, 6]. Medicinal plants are finding their way into pharmaceuticals, nutraceuticals, cosmetics and food supplements. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action because there have been an alarming increase in the incidence of new and re-emerging infectious diseases [7]. Natural products of higher plants may give a new source of antimicrobial agents with the possibly novel mechanism of action. Contrary to the synthetic drugs, antimicrobial of plant origin not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases [8]. Use of traditional medicine among the tribal local people and medicinal healers (hakims) is a significant part of Rajasthan's tradition and it is widely practiced till date.

Eclipta alba (Linn.) Hassk., is commonly known as Bhingaraj, commonly used for the treatment of gastrointestinal disorders, respiratory tract disorders (including asthma), fever, hair loss and greying of hair, liver disorders (including jaundice), skin disorders, spleen enlargement, and cuts and wounds. Pharmacological activities of plant extracts have revealed anticancer, hepatoprotective, anti-inflammatory, and antimicrobial properties [9]. *B. diffusa* L. (Nyctaginaceae) fresh or dried is the source of the drug punarnava which is official in Indian Pharmacopoeia as a diuretic. The plant is bitter, astringent, cooling, anthelmintic, diuretic, aphrodisiac, cardiac stimulant, diaphoretic, emetic, expectorant, anti-inflammatory, febrifuge and laxative besides being an active ingredient as a tonic [10, 11]. It is useful in all types of inflammation,

strangury, leucorrhoea, lumbago, myalgia, cardiac disorders, jaundice, anaemia, dyspepsia, constipation, cough, bronchitis and general debility, dyspepsia, oedema, jaundice, cough, hemorrhoids, pulmonary cavitations, anaemia, enlargement of spleen, abdominal pain, abdominal tumours, cancers [12, 13] and acts as an anti stress agent.

Phyllanthus niruri Linn. Belongs to Euphorbiaceae is often used in the traditional system of medicine for a variety of ailments including dropsy, diabetes, jaundice, asthma and bronchial infections [2]. In the Ayurvedic system of medicine, it is used in problems of the stomach, genitourinary system, liver, kidney and spleen. It is bitter, astringent, stomachic, diuretic, febrifuge and antiseptic. The whole plant is used in gonorrhoea, menorrhagia and other genital affections [14]. *Ricinus communis* Linn belongs to family Euphorbiaceae, popularly known as 'castor plant'. In the Indian system of medicine, the leaf, root and seed oil of this plant have been used for the treatment of inflammation and liver disorders. The plant has been found to be useful in hepatoprotective [15], anti-filarial [16], antioxidant [17], antiasthmatic [18] and antimicrobial [19] activities. Roots of this plant showed anti-inflammatory and free radical scavenging, anti-fertility, antidiabetic, and antimicrobial properties.

MATERIALS AND METHODS

Collection of plant material

Fresh plants were collected from regional areas of Jaipur and authenticated by taxonomist the leaves were shade dried then coarsely powdered.

Solvent extraction

The dried leaves were powdered with the help of waring blender then powder was filled in a thimble and extracted successively with methanol solvent in a soxhlet extractor for 48 h. The crude extracts were concentrated using vacuum evaporator.

Antimicrobial screening

All bacterial strains of (*S. aureus*, *B. subtilis*, *E. coli*, *S. typhii* and *K. pneumoniae*), were obtained from S. M. S. Medical college and fungal

strains (*A. niger*, *C. albicans*, *F. oxysporum*) Microbiology lab, Deptt of botany, university of Rajasthan, Jaipur respectively. The bacteria were maintained on nutrient broth (NB) at 37 °C and fungus were maintained on potato dextrose agar (PDA) at 28 °C.

Preparation of inoculum

The gram +ve (*S. aureus*, *B. subtilis*) and gram -ve bacteria (*E. coli*, *S. typhii* and *K. pneumoniae*) were incubated in nutrient broth in a rotary shaker at 37 °C, centrifuged at 10,000 rpm for 5 min, then pellet was suspended in double distilled water and cell density was standardized spectrophotometrically (A_{610} nm). The fungal inoculum was prepared from 6 to 12 d old culture grown on PDA medium. Then petri dishes were flodded with 5 to 10 ml of distilled water and the conidia were scraped using a sterile spatula. The spore density of each fungus was adjusted with a spectrophotometer (A_{595} nm) to obtain a final concentration of 10^5 CFU/ml.

Anti-bacterial activity

The antibacterial activity of the extracts was determined by the disc diffusion method. Different concentration of the extracts (100 µg/ml) was prepared by reconstituting with methanol. The test microorganisms were seeded into the respective medium by spread plate method 10 µl (10^6 CFU/ml) with the 24 h cultures of bacteria grown in nutrient broth. After solidification filter paper discs of Whatmann no. 1 (6 mm diameter) were impregnated with the extracts were placed on test organism-seeded plates. These bacterial species (*S. aureus*, *B. subtilis*, *E. coli*, *S. typhii* and *K. pneumoniae*) were used for the antibacterial test. Streptomycin (10µg/ml) used as positive control, methanol solvent (100 µg/ml) used as negative control. The plates were incubated at 37 °C for 24 h. The diameter of the inhibition zones was measured in mm.

The anti-fungal activity

The antifungal activity was tested by disc diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 d old) by point inoculation. The filter paper discs (6 mm diameter) impregnated with 100 µg/ml concentrations of the extracts were placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Black discs impregnated with methanol followed by drying off was used as negative control and Nystatin (10µg/ml) used as positive control. The activity was determined after 72 h of incubation at 28 °C. The diameter of inhibition zones were measured in mm.

Statistical analysis

The data of all the parameters were statistically analyzed (statistical software used Minitab 14-state college, PA, USA) and zone of inhibition diameter values are expressed as Mean Diameter±SEM (n= 3)

RESULTS AND DISCUSSION

Table 1 summarizes the microbial growth inhibition of methanol extract of *B. diffusa*, *E. alba*, *P. niruri* and *R. communis* against selected bacterial species. Methanolic leaf extract of *B. diffusa* showed significant activity against *S. aureus*, *B. subtilis* around 14 mm. The highest antibacterial activity of 19 mm in *S. aureus* and least activity recorded in *K. pneumoniae* measured 10 mm. Leaf extract of *B. diffusa* exhibited the highest activity against *S. aureus* and lowest in *E. coli*. *E. alba* leaf extract possessed maximum activity against *S. aureus* (17 mm) and 15 mm against *S. typhii* and *K. pneumoniae*. Bark/root extract of this plant showed highest inhibitory activity against *E. coli* and *B. subtilis* and least activity observed in *S. typhii*. *P. niruri* leaf extract showed a similar zone of inhibition against *S. aureus* and *S. typhii* (18 mm). Bark and root extract of *P. niruri* showed varied activity in the zone of inhibition from 10-16 mm against all the tested bacteria. Leaf and root/bark extract of *R. communis* showed almost similar antibacterial activity against all the tested bacteria. Leaf extract of *R. communis* showed highest antibacterial activity against *S. aureus* (18 mm) and least activity against *E. coli* and *K. pneumoniae* (14 mm). Bark root extract of this plant showed significant activity against *S. aureus* and *B. subtilis* (14 mm) and least against *S. typhii*. The root and leaf extract of *B. diffusa*, *E. alba*, *P. niruri* and *R. communis* showed significant

activity against these bacterial species. Leaf extract showed significant antibacterial activity when compared with bark root extract of all the tested plant extracts. Bark root extract of all medicinal plants extract was almost similar or higher activity when compared with Streptomycin antibiotic.

Antifungal activity of these plant leaf extracts showed significant activity when compared with bark or root extract (table 2). *B. diffusa* bark and leaf extract showed antifungal activity against *A.niger* (10 mm) and 11 mm respectively. Similar results were obtained with *E. alba*, *P. niruri* and *R. communis*. All these plants showed less antifungal activity when compared with Nystatin antibiotic.

CONCLUSION

The current investigation proved the antibacterial efficacy of the plant against different bacterial strains and thus validating the traditional use of the plant against different diseases. Further research is needed toward isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

CONFLICT OF INTERESTS

Declare none

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