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Antimicrobial Potential of Hydro-Alcoholic Extract of *Ruta graveolens* Leaves

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Abstract: In the present study, the antibacterial activity of hydro-alcoholic extract of *Ruta graveolens* leaves was evaluated against some bacterial pathogens namely *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus subtilis,* using the agar-well diffusion method. *E. coli* was found to be the most susceptible bacterial strain (zone of inhibition: 19 mm at a concentration of 200 mg/ml), while that of streptomycin (the standard drug) which showed 22 mm diameter zone of inhibition). Phytochemical analysis showed the presence of phenolics, alkaloids and steroids, which may the active compounds in the hydro-alcoholic extract of *Ruta graveolens* leaves. The results suggest that *R. graveolens* can be used as an herbal drug in the treatment of bacterial diseases.

Key words: Hydro-alcoholic extract, *Ruta graveolens,* Streptomycin, Antibacterial property.

Introduction:

Microbial infections pose a health problem throughout the world, and past twenty years, there has been a lot of interest in the investigation of natural materials as a source of new antibacterial agents [1]. Medicinal plants contain bioactive principles which can be used as an alternative to cheap and effective herbal drugs against common bacterial infections. *Ruta graveolens* is a medicinal plant which is a native to the Mediterranean region, however, widely distributed into all over the tropical regions. It is commonly known as Rue, a dicot herb and belongs to the family *Rutaceae*. This is a hardy, evergreen shrub of up to one meter tall, with a characteristic grayish color. The plant is used as contraceptive [2], relieves symptoms of hangover and applied externally as a poultice against rheumatic pain [3]. Rue's active ingredients may have antifungal property [4]. Aqueous, methanolic and ethanolic extract of *Ruta graveolens* have shown anti-inflammatory activity [5]. In the present study, the work was undertaken to explore the antimicrobial activity of ethanol extract of *R. graveolens* leaves against some medically important bacterial pathogens.

Materials and Methods

Collection and identification of plant material

Leaves of *R. graveolens L.* were collected from the local area of Sagar (M.P) in the month of September - October and authenticated at the Botany Department, Dr. H.S. Gour University Sagar (M.P). India.

Preparation of crude drug extract

The plant material was identified and authenticated by Departmental herbarium Incharge and voucher specimen of the collected sample was deposited in our institutional herbarium for the reference. After collection of plant material, leaves were washed in running tap water to remove dust material. Plant material was air dried for 6-7 days at room temperature to prevent the loss of active phytoconstituents. The air-dried leaves (35 g)

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were powdered using a mechanical grinder and soaked in 500 ml of 75 % ethanol and kept in a shaker for 72 h. The crude extract was collected by filtration and evaporated under reduced pressure to give a blackish green amorphous mass of leaves with a yield of 2.120 (w/w).

Preliminary phytochemical analysis of extract

Preliminary phytochemical analysis was carried out according to the methods suggested by Trease and Evans (1989) [6] as described in Table 1.

Test microorganisms

Pure cultures of human pathogenic bacteria, including *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424), *Klebsiella pneumonia* (MTCC 432), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 619) and *Salmonella typhimurium* (MTCC 98) clinical isolates were collected from the Institute of Microbial Technology, Chandigarh, India and preserved as slant agar cultures at 4°C.

Drugs and chemicals

The following chemicals and drug were used: Mueller Hinton agar (HIMEDIA), ethanol (Qualigens), tween 80 and streptomycin (R.K. Pharmacy, Sagar).

Agar well diffusion assay

The agar well diffusion method was used to evaluate the antimicrobial activity of the subjected extracts [7]. Inoculums of 10 µl suspension containing 10^6 CFU/ml of bacteria were spread on Muller Hinton Agar medium. Five wells were prepared in the plates with the help of a sterilized cork-borer. A 50 µl of ethanolic extract, DMSO (negative control) and streptomycin (positive control) were placed in the wells and allowed to diffuse at room temperature for two hours [8]. The experiments were conducted in triplicate and the test plates were incubated 24 h at 27°C. The diameters of zone of inhibition measured in mm [9].

Result and discussion

The antibacterial activity of *R. graveolens* leaf extract is shown in Table 2. The extract showed antibacterial activity against all the test organisms in a concentration dependent manner. Maximum antibacterial activity was shown against *E. coli* (zone of inhibition was 19 mm) followed by *B. subtilis* (17.9 mm) and S. *aureus* (17 mm) while

Table1. Preliminary phytochemical screening of ethanolic extract of *R. graveolens* leaves

Plant	Tannins	Flavonoids	Alkaloids	Anthraquinones	Saponins	Steroids	Terpenoids
R. graveolens	+	+	+	-	-	+	-

+ Present: - Absent

Table 2. Results of antibacterial screening of the different concentrations of crude ethanolic extract of *R. graveolens* leaves

Concentration of extract (mg/ml)	Zone of inhibition (mm)					
	E. coli	*P. aeruginosa*	*K. pneumonia*	*S. aureus*	*B. subtilis*	*S. typhimurium*
200	19.0	12.4	15.0	17.0	17.9	16.7
150	17.2	08.5	13.1	14.4	14.9	14.1
100	14.0	06	10.0	12.4	12.2	12.0
50	11.1	NI	08	09	10	08.5

*Values are means of triplicate readings

NI: No inhibition

test extract showed no or moderate antibacterial activity against *P. aeruginosa* at a concentration of 50 mg/ml. *S. aureus* was found to be one of the most susceptible bacteria due to the presence of single membrane of the organism, which makes it more accessible to permeation by active principles of the extract.

Among the 6 bacterial strains investigated in the study, *P. aeruginosa* and *K. pneumonia* were found to be the most resistant pathogens to the test extract. This may be due to the fact that *P. aeruginosa* contains restrictive outer membrane barrier and trans-envelope multidrug resistance pumps (MDRs). These results also proved that Gram-negative bacteria were more resistant than Gram-positive bacteria [10]. The phytochemical analysis of the *R. graveolens* leaf extract revealed the presence of tannins, alkaloids and flavonoids which may the active compounds for the observed antimicrobial activity. Herbs that have tannins as their main components, are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [11], thus exhibiting antimicrobial activity. One of the largest groups of chemical produced by plants is the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications [12].

Conclusion

The antimicrobial activity of leaf hydro-alcoholic extract of *R. graveolens* was confirmed in the present study. Results proved the importance of plant extracts to inhibit growth of bacteria, which may be useful to combat against bacterial disease. Further studies are required to determine its active compounds responsible for the antimicrobial activity.

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