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Evaluation of Antibacterial Activities of *Ruta graveolens*, a Highly Medicinal Plant Against Human Pathogens

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Abstract: The present study was designed to make a comparative evaluation of the antimicrobial activities of petroleum ether, ethyl acetate, chloroform, methanol, ethanol and aqueous extracts of *Ruta graveolens* which is used medicinally in the treatment of different infectious diseases and disorders against several human pathogenic bacterial strains. The results showed that aqueous extracts had no antibacterial activity against any of the test strains. Rest all the solvents possessed activity against both Gram positive and Gram negative strains. MIC results revealed that *B. brevis, Bacillus lichenoformis* and *B. cerus* were inhibited at a concentration of 0.312 mg/l by methanol extracts. The study indicates that *Ruta graveolens* represents an untapped source of potentially useful antimicrobials and therefore worthy of further study.

Key words: Antibacterial activity; MIC; *Ruta graveolens.*

Introduction

Secondary metabolites of *Ruta* species are of great interest in medicinal chemistry as these compounds show a broad range of biological activity and a number of them are used in medicines [8,9]. This biological activity is attributed to the presence of coumarins and alkaloids, particularly furancoumarins, quinolines and furaquinolines [7]. Plant-derived psoralen and its derivatives: 5-methoxypsoralen (Bergapten) (5-MOP), 8-methoxypsoralen (Xanthotoxin) (8-MOP) and 5, 8-dimethoxypsoralen (Isopimpinellin) belonging to the furanocoumarin family (FCs) are well known as anti-fungal and anti-bacterial factors [6]. This group of secondary metabolites has been successfully and effectively used because of its photoreactive properties in the treatment of leucoderma, vitiligo and psoriasis diseases [1].

Ojala *et al*., [6] conducted antimicrobial screening against selected Gram-positive and Gram-negative bacteria, yeasts, mold, as well as plant pathogenic fungi, with emphasis on method optimization on methanol extracts prepared from seven coumarin containing plants, *Ruta graveolens* being one of them. Antibacterial properties of Tsunanian *R. graveolens* essential oil was reported by Fredj *et al*., [3]. The present investigation is an antibacterial screening study of different solvent extracts of *R. graveolens* against a battery of bacterial strains.

Materials and methods

Plant Material

Six month old *R. graveolens* plants grown in the experimental garden of Institute of Minerals and Materials Technology (IMMT) were used in

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the experiments. Different parts i.e. roots, shoots and fruits of *R. graveolens* were separated and were chopped into 0.5-1 cm long pieces. The plant materials were oven dried for one week at 60°C. Extraction was performed on dried and milled plant material of the *R. graveolens*. The dried plant material had been stored in the dark prior to extraction. About 100 gm of dried root, shoot and fruit materials were subjected to cold extraction with a series of solvents, i.e. petroleum ether, ethyl acetate, chloroform, methanol, ethanol and water successively in the order of increasing polarity. The extracts were then filtered and evaporated under reduced pressure using Rota evaporator and lyophilizer, after which they were freeze-dried and stored in -20°C until further use.

Preparation of crude extracts

The plant extracts that were stored at -20°C were dissolved in 50 ml of methanol to achieve a final concentration of 50 mg/ml of dried plant powder. Aqueous dilutions were kept in closed containers at 4°C for a maximum of 14 days.

Source of test microorganisms

The bacterial strains that were used in the present study has been enlisted in Table 1 .The bacterial strains were obtained from MTCC, IMTECH, Chandigarh, India, and American Type Culture Collection (ATCC, Rockville, MD).

Culture media and inoculum

Stocks were created by passing the original reference organisms once through Muller Hinton Broth (MHB) and plating on Muller Hinton Agar (MHA) plates. Colonies were chosen from plates for inoculation of slants that become working stocks. Slants were maintained at 4°C.

For inoculum preparations, bacteria were subcultured in Brain Heart Infusion (BHI) at 37°C for 8 hours and adjusted to a suspension of 1 X 10^6 to 2 X 10^6 CFU/ml.

Microplate broth dilution methods for assessment of Minimum Inhibitory Concentration (MIC)

Broth dilution technique was adopted using 96 well microtiter plates and tetrazolium salt, 2, 3, 5-Triphenlytetrazolium Chloride (TTC) (HiMedia, India) to assess the bacterial growth, determine the Minimal Inhibitory Concentration (MIC) according to Eloff [2]. A two fold serial dilution of plant extract made to a 5000 µg/ml stock solution was prepared in 96 well microtitre plates and 10 µl bacterial culture was added to each well. The presence of bacterial growth was detected by the addition of 0.5 % TTC. If the solution in the well remained clear after the addition of the indicator, bacterial growth was inhibited by that particular concentration of plant extract. The antibiotic neomycin (Sigma) at a concentration of 1250 µg/ml was included as a reference standard (positive control) in each assay and respective solvents were used as the negative control.

Results and discussion

Shoot extracts of *Ruta graveolens* were tested against fourteen pathogenic strains by Minimal Inhibition Concentration (MIC) method (Table 1). The interaction effect of microorganisms and plant extract concentrations seemed to be highly significant. Methanol extract exhibited maximum activity against most of the strains followed by ethanol extracts. Ethyl acetate extracts exhibited modest activity against the test organisms followed by chloroform extracts. Petroleum ether extracts exhibited considerably lower activity as compared to ethanol, methanol, chloroform and ethyl acetate extracts. Aqueous extracts however demonstrated almost no activity against most strains. The MIC values were in the ranges of 5000 to 312 µg/ml for all the tested bacterial strains (Table 1).

Methanol extract was most effective causing inhibition of all the test strains with the minimal inhibitory concentrations ranging from 2500 to 312 µg/ml. *K. pneumoniae* was the only exception with a MIC greater than 5000 µg/ml. Three bacillus strains i.e. *B. brevis*, *B. cerus* and *B. licheno-formis* and the two *Salmonella* strains i.e. *S. typhi* and *S. typhimurium* exhibited susceptibility to the methanol extracts at a minimum concentration of 312 µg/ml. *S. faecalis* was inhibited by methanol extract at a minimal concentration of 625 µg/ml. Methanol extract at a minimal concentration of 1250 µg/ml inhibited the growth

Table 1. Antibacterial activity of shoots of *in vitro* raised plants extracted with different solvents by MIC Method*

Bacterial strain	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Ethanol	Aqueous
Staphylococcus aureus ATCC	2500	2500	2500	1250	625	>5000
Staphylococcus epidermidis	2500	2500	1250	2500	1250	>5000
Streptococcus faecalis	1250	1250	625	625	625	>5000
Micrococcus leuteus	2500	1250	1250	1250	625	>5000
Bacillus subtilis	2500	1250	1250	1250	625	>5000
Bacillus brevis	1250	625	1250	312	625	>5000
Bacillus cerus	2500	312	1250	312	1250	>5000
Bacillus lichenoformis	2500	625	1250	312	1250	>5000
Listeria monocytogenes	1250	2500	1250	1250	2500	>5000
Salmonella typhi	2500	1250	1250	312	625	>5000
Salmonella typhimurium	625	1250	2500	312	312	>5000
Klebsiella pneumoniae	>5000	>5000	>5000	>5000	>5000	>5000
Pseudomonas aureginosa ATCC	2500	625	2500	2500	1250	>5000
Escherchia coli ATCC	2500	625	2500	1250	1250	>5000

of *M. leuteus*, *B. subtilis, L. monocytogenes* and *E. coli*. MICs for methanol extracts against *Staphylococcal* strains, *P. aureginosa* as well as *E. coli* were recorded to be 2500 µg/ml.

S. aureus exhibited maximum susceptibility towards ethanol extract (MIC-625 µg/ml) whereas *S. epidermidis* was inhibited by chloroform and ethanol extracts at a minimum concentration of (1250 µg/ml). *S. faecalis*, *M. leuteus* and *B. subtilis* were inhibited best by ethanol extracts at a minimum concentration of 625 µg/ml. Among all the bacterial strains tested against ethyl acetate extracts, the extract was most effective against *B. cerus* (MIC- 312 µg/ml). The chloroform and the petroleum ether extracts of *Ruta* aerial parts showed similar MIC values (ranging from 2500-1250 µg/ml) for most of the Gram positive and Gram negative bacterial strains.

The pure DMSO/methanol negative control showed no inhibitory activity. For positive control experiments the antibiotic neomycin (Sigma) at a concentration of 1250 µg/ml was used which revealed that the growth of bacterial strains was indicated by addition of TTC which showed clear and reproducible visualization of areas of dead (white) cells as indicated by no change in colour.

In classifying the antibacterial activity as Gram positive or Gram negative, it would generally be expected that a much greater number would be active against Gram positive than Gram negative bacteria [5]. However, in this study, almost all the extracts were equally active against both Gram positive and Gram negative bacteria. Ojala *et al*., [5] screened *R.graveolens* methanolic extract against selected Gram-positive and Gram-negative bacteria and reported that the extract has

Kumar *et al*. [4] reported that among the different solvent extracts investigated, methanolic extracts of *Ruta graveolens* L shows *in vitro* antibacterial activity against human pathogens like *Escherichia coli*, *Staphulococcus aureus* and *Klebsielle premonia*. This study is in corrobo-ration with our findings.

The activity against both the types of bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general toxins.

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