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Evaluation of Antimicrobial Activity of *Ruta graveolens* Stem Extracts by Disc Diffusion Method

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Summary

In the present study, we evaluated the antimicrobial activity of ethanolic, methanolic, chloroform and water extracts of *Ruta graveolens* stem against *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424), *Klebsiella pneumoniae* (MTCC432), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 619), *Salmonella typhimurium* (MTCC 98) and *Aeromonas culicicola* (MTCC 3249) as well as fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium crysogenum*, *Rhizopus stolonifer* and *Fusarium oxosporium* by agar-disc diffusion and (MIC) method. The ethanolic stem extract showed pronounced inhibition of growth than other extracts. The ethanolic extract showed most susceptible activity against *S. aureus* and *B. subtilis* (zone of inhibition was 22.0±0.04mm and 19.4±0.24mm) where as *P. aeruginosa* (Zone of inhibition was 12.5±0.17mm) was the most resistant bacterial strain at a concentration of 100µg/ml. All the extract showed moderate antifungal activity except *Fusarium oxosporium*. Phytochemical analysis showed mainly the presence of alkaloids and steroids, which may be the active compounds.

Key Words: Antimicrobial, Agar-disc diffusion, Minimum Inhibitory Concentration, streptomycin, Phytochemical

Introduction

The discovery, development and clinical use of antibiotics during the nineteenth century have substantially decreased public health hazards resulting from bacterial infections. However, there has been a parallel and alarming increase in bacterial resistance to existing chemotherapeutic agents as a result of their injudicious use [1]. In many countries such as India and China, thousands of tribal communities still use folklore medicinal plants to cure sicknesses. The great interest in the use and importance of Indian medicinal plants by the World Health Organization in many developing countries has led to intensified efforts on the documentation of ethno medical data of medicinal plants [2, 3]. Biologically active compounds from natural sources have always been of great interest to working on infectious diseases. A number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plants [4, 5]. There is an ever-increasing demand for plant-based therapeutics in both developing and developed countries due to a growing recognition that they are natural products, non-narcotic and, in most cases, easily available at affordable prices; they also have no side effects.

Ruta graveolens commonly known as rue, is a dicot herb, belongs to *Rutaceae* family and native to Mediterranean region but widely distributed all over the tropical regions. The leaves are bipinnate or tripinnate with a feathery appearance and green to strongly glaucous blue-green in colour. This plant is used by the Jordanian populations, systemically for its antispasmodic and analgesic effects and externally for its antirheumatic activity [6]. The methanol, petroleum ether, ethyl acetate and water-methanol extracts of *R. graveolens* were

found to possess antimicrobial and cytotoxic activities [7]. *Ruta* in combination with $\text{Ca}_3(\text{PO}_4)_2$ is found to be effective in treatment of brain cancers, particularly glioma [8]. Leaf extracts also reported to possess strong anti-inflammatory activity [9]. However, no significant reports are available about the antimicrobial activity of *Ruta graveolens* stem; therefore, present investigation was undertaken to examine the antimicrobial activities of stem extract of *Ruta graveolens* using various pathogenic microbial strains.

Material and Methods

Collection and preparation of plant material

Stems of the plant material were collected from botanical garden of University campus and air dried for 7-10 days in a shade prevent the loss of active phytoconstituents and ground into fine powder using a mechanical grinder. The extraction of plant material was done by Maceration methods. The powder plant material (35 g) were soaked in 500 ml of 75% ethanol, methanol respectively while Distilled water and Chloroform was used as pure and kept for 72h in a shaker. The extract were filtered on filter paper, concentrated to dryness and stored at 4°C for further studies. The extract was dissolved in Dimethyl sulfoxide (DMSO) under aseptic condition to prepare the desired dilutions.

Preparation of test microorganisms

The pathogenic microbial strains namely *E. coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424), *Klebsiella pneumoniae* (MTCC432), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 619), *Salmonella typhimurium* (MTCC

98) and *Aeromonas culicicola* (MTCC 3249) were used for the antibacterial activity. Fungal strains namely *Aspergillus niger*, *Aspergillus flavus*, *Penicillium crysogenum*, *Rhizopus stolonifer* and *Fusarium oxosporium* were collected from the Institute of Microbial Technology, Chandigarh, India. All the microbial strains were transferred on nutrient agar slants and transferred in to nutrient broth and stored at 4°C until required for the study.

Evaluation of antimicrobial activity

The antimicrobial assay of different solvent extract was performed by agar disc diffusion method [10, 11]. The bacterial strain was activated by inoculating a loopful culture in the nutrient broth (30 ml) where as fungal strain in PDB and incubated for 6 h to maintain McFarland standard turbidity (10⁸cells/ml). 0.1 ml of inoculums was inoculated into the molten Muller Hinton agar media (Hi-media) and PDA media, spread uniformly into the Petri plate. The test compound (40µl) was introduced on the disc (6mm) (Hi-Media) and allowed to dry. Then the disc was impregnated on the seeded agar plate. Dimethyl sulfoxide (DMSO) was used as a negative control where as Streptomycin and Nystadin was used as a positive control. The plates were done in triplicates and were incubated for 24h at 37°C for antibacterial activity while for antifungal activity the plates were incubated for 48-72 h at 28°C. The antimicrobial activity was taken on the basis of diameter of zone of inhibition, and the mean of three readings is presented.

Minimum Inhibitory concentration

The agar dilution method was used to determine the MIC of all the extract. 1 ml each of the extracts was added to sterile molten nutrient agar and potatoes dextrose agar media, a loop full of the standardized bacterial and fungal culture were used to inoculate the plates which were incubated at 37°C for 24 h. Growth of organisms on each concentration was checked to determine the minimum concentration that inhibits growth of test organism.

Minimal bactericidal concentration (MBC)

Samples were taken from the nutrient agar plates that showed no visible growth after 24 h incubation and sub cultured into freshly prepared sterile nutrient agar. The least

concentration that did not produce growth after 24 h was regarded as the MBC.

Results and Discussion

Disc diffusion methods are extensively used to investigate the antibacterial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of substances to be examined. All the concentrations of the plant extracts showed strong activity against all the test organisms on concentration dependent manner (Fig. 1,2,3,4,5,6,7 and 8). The results showed that increase in concentration of extract increased the zone of inhibition. Ethanolic extract of *Ruta graveolens* stem showed most potent antibacterial activity against *S. aureus* and *B. subtilis* (zone of inhibition was 22.0±0.04mm and 19.4±0.24mm) while *P. aeruginosa* (Zone of inhibition was 12.5±0.17mm) was the most resistant bacterial strain at a concentration of 100µg/ml. This may be due to the fact that *P. aeruginosa* has intrinsic resistance from a restrictive outer membrane barrier. All the extract showed moderate antifungal activity except *Fusarium oxosporium*. *A. niger* was the most resistant fungi (Zone of inhibition was 4.7±0.35mm) where as *Fusarium oxosporium* was the most susceptible fungi (Zone of inhibition was 16.0±0.22mm) at a concentration of 100µg/ml. *P. chrysogenum* did not showed any growth of inhibition against all the tested extract. Some of the phytochemical compounds e.g. glycoside, saponin, tannin, flavonoids, terpenoid, alkaloids, have variously been reported to have antimicrobial activity. The extract of this plant also possess alkaloidal and phenolics substances which also have biological activities. The presence of flavonoids, tannins and alkaloids in the extract may responsible for activity against pathogenic microorganisms. In our study, some of the fungal strains did not respond to crude extracts, might be due to masking of antimicrobial activity by the presence of some inhibitory compounds or factors in the extract or synergism by the presence of some compounds or factors in the extract.

In conclusion, the antimicrobial activity of this plant would help for development of a new alternative medicine system which has no side effects. *R. graveolens* stem extracts possess a broad spectrum of activity and open the possibility of finding new clinically effective antimicrobial compounds.

Table1. MIC values of alcoholic, Chloroform and aqueous extracts of *Ruta graveolens* stem against tested bacterial strain

BACTERIA	*Minimum Inhibitory Concentration (µg/ml)			
	Ethanolic	Methanolic	Chloroform	Aqueous
<i>E. coli</i>	7.5	7.5	10.5	12.5
<i>S.typhimurium</i>	5	6.5	6.5-7.5	ND
<i>K. pneumoniae</i>	7.5	10	7.5	ND
<i>S. aureus</i>	5.5	6.5	5.5	10.5
<i>B. subtilis</i>	5-6	7.5	10.5	12
<i>P. aeruginosa</i>	ND	ND	ND	ND
<i>A. culicicola</i>	5.5	6.5	7.5	12.5

Table2. MIC values of alcoholic, Chloroform and aqueous extracts of *Ruta graveolens* stem against tested fungal strain

FUNGUS	*Minimum Inhibitory Concentration (µg/ml)			
	Ethanolic	Methanolic	Acetone	Aqueous
<i>A. niger</i>	ND	ND	ND	ND
<i>A. flavus</i>	ND	ND	ND	ND
<i>P. chrysogenum</i>	ND	ND	ND	ND
<i>R. stolonifer</i>	ND	ND	ND	ND
<i>F. oxosporium</i>	7.5	7.5	ND	ND

*= Mean of 3 determinations;
 ND = Not Determined since there was no inhibitory activity

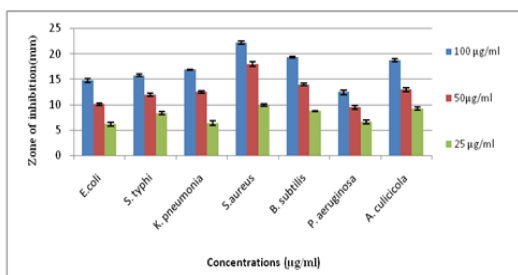


Fig 1: Antimicrobial activity of Ethanolic extract of *Ruta graveolens* stem

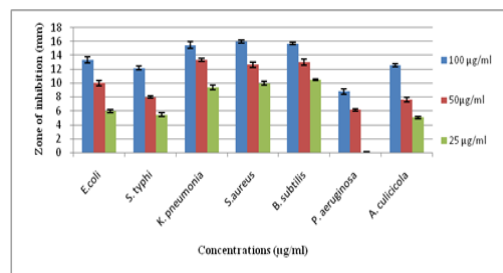


Fig 4: Antimicrobial activity of aqueous extract of *Ruta graveolens* stem

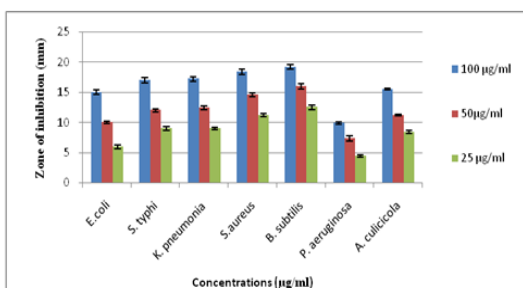


Fig 2: Antimicrobial activity of Methanolic extract of *Ruta graveolens* stem

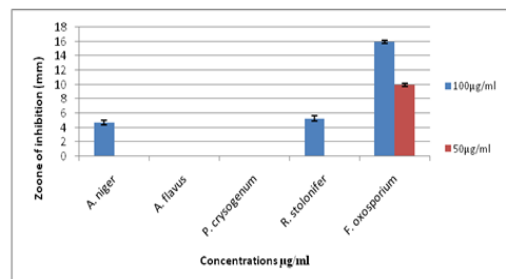


Fig 5: Antifungal activity of ethanol extract of *Ruta graveolens* stem

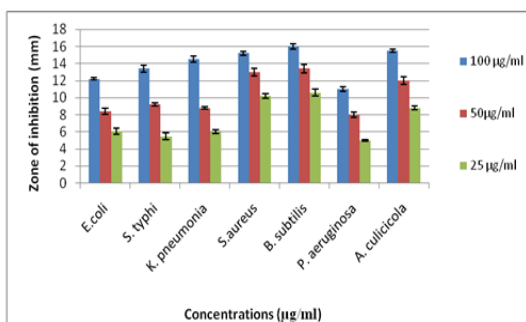


Fig 3: Antimicrobial activity of Chloroform extract of *Ruta graveolens* stem

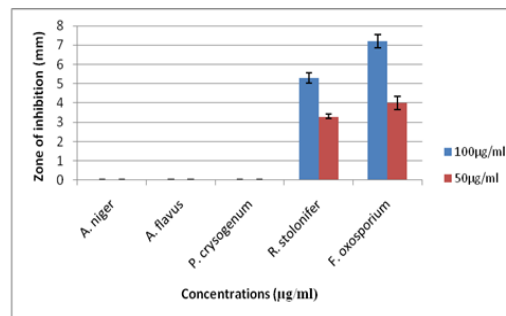


Fig 6: Antifungal activity of methanol extract of *Ruta graveolens* stem

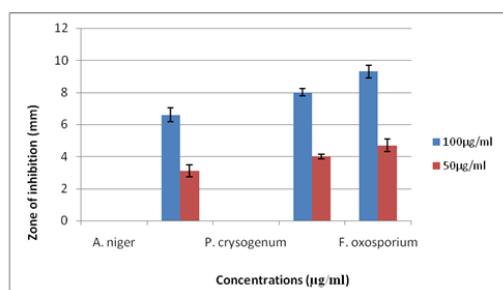


Fig 7: Antifungal activity of Chloroform extract of *Ruta graveolens* stem

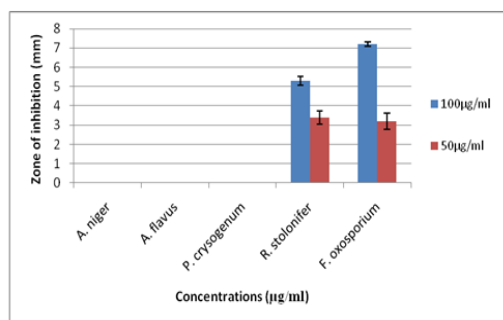


Fig 8: Antifungal activity of aqueous extract of *Ruta graveolens* stem

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References

[1] Service, R. F. 1995. Antibiotics that Resist Resistance. *Scien.* 270:724-727.

[2] Waller, D. P. 1993. Methods in Ethnopharmacology. *J. Ethnopharmacol.* 38:189-195.

[3] Perumal-Samy R and D. Patricraja. 1996. Antibacterial Activity of Aqueous Extracts of Some Selected Weeds. *Acta Bot. Indi.* 24:113-114.

[4] Brantner, A and E. Grein. 1994. Antibacterial Activity of Plant Extracts Used Externally in Traditional Medicine. *J. Ethnopharmacol.*, 44:35-40.

[5] Perumal-Samy R and S. Ignacimuthu. 1997. Antibacterial Activity of Some Medicinal Plants from Eastern Ghats, South India, Solai. *Bull. Ethnopharmacol.* 39- 41.

[6] Khouri, N.A., and Z.E. Akawi. 2005. Antiandrogenic Activity of *Ruta graveolens* L in Male Albino Rats with Emphasis on Sexual and Aggressive Behavior. *Neuroendocrino.* 26:823-829.

[7] Ivanova, A., B. Mikhova, H. Najdenski, I. Tsvetkova and I. Kostova. 2005. Antimicrobial and Cytotoxic Activity of *Ruta graveolens*. *Fitotera.*76:344-7.

[8] Pathak, S., A. S. Multani, P. Banerji and P. Banerji. 2003. *Ruta* 6 Selectively Induces Cell Death in Brain Cancer Cells but Proliferation in Normal Peripheral Blood Lymphocytes: A Novel Treatment for Human Brain Cancer. *Interna. Oncol.* 23:975-82.

[9] Ratheesh, M and A. Helen. 2007. Anti-inflammatory Activity of *Ruta graveolens* Linn on Carrageenan Induced Paw Edema in Wistar Male Rats. *Afr. J. Biotechnol.* 6: 1209-1211.

[10] Bauer, A.W., W. M. M. Kirby., J.C., Sherris and M. Turck. 1966. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am. J. Clin. Pathol.* 45: 493-496.

[11] Parekh, J and S. Chanda. 2007. *In vitro* Antimicrobial Activity of *Trapa natans* L. Fruit rind Extracted in Different Solvents. *Afr. J. Biotech.* 6:766-770.