ANTIFUNGAL ACTIVITY OF PHYTOCONSTITUENTS OF RUTA GRAVEOLENS L.

AJIT KENGAR AND GOVIND PARATKAR
Department of Botany, KET's V.G.Vaze College of Arts, Science & Commerce,
Mithagar Raod, Mulund (E.), Mumbai, Maharashtra, India.400 081
*Author for correspondence: (ajitkengar@gmail.com)
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INTRODUCTION:
Plants and their preparations have been used for thousands of years in indigenous cultures around the world for treating infectious diseases (Romagnoli et al, 2005). Many of these traditionally used plants have been scientifically evaluated with results yielding today's valuable drugs such as aspirin, digitoxin, morphine and quinine (Butler 2004). Most of developing countries are still relied upon plants and their preparations as a primary source of medicine. It is estimated that over 65% of the world population relies directly on plants as their main source of medicine with 75-90% of the world's rural communities (Fowler, 2006). The WHO reports that 80% of the people of Africa, 40% of China and Asia, and 40% of South America use medicinal plants for their primary care (WHO, 2002). Much of the scientific efforts was made in past few decades with medicinal plants and has focused on documenting the uses of traditional medicine, effectiveness of particular remedies, characterizing medicinal plant to their compounds, and testing plant compounds in vitro (Fowler, 2006; Gertsch, 2009). Based on the past history of success in finding new compounds, additional valuable discoveries will be made.

Infectious diseases accounts for high proportion of health problems in the developing countries including India. Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created (Davies, 1994). The resistance of the organisms increased due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases. Fungal infections remain a significant cause of death despite advances in medicine and the emergence of new antifungal agents (McNeil et al., 2001). Candida albicans is a major concern worldwide (Nolte et al., 1997). C. albicans, the agent of candidiasis, is an increasingly important disease that has a worldwide distribution due to the fact that it is a frequent opportunistic pathogen in AIDS patients (De Pavia et al., 2003). It is a common commensal of the gastrointestinal and urogenital tracts of human and is also the cause of Candidiasis in women (Black, 1996). Cryptococcus neoformans is the cause of the most common life-threatening meningitis in HIV-positive patients (Michaels et al., 1999). Aspergillus niger is an opportunistic human pathogen and a strong air pollutant. Since strains of fungal pathogen with multiple antibiotic resistances are increasing worldwide, it is of great importance to find effective treatments for these pathogens. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Srinivasan et al., 2001). To overcome this alarming problem of microbial resistance to antibiotics, the discovery of novel active compounds against new targets is a matter of urgency. The available antifungal drugs produce many adverse effects, show recurrence, or lead to the development of resistance. There is general agreement that new antifungal agents without these disadvantages are strongly needed.

Essential oils are known to be complex mixtures of monoterpenes, sesquiterpenes and volatile phenolics as well as alcohols, aldehydes, ethers, hydrocarbons and ketones. Phenols have been credited as being the most active components with the broadest spectrum of antimicrobial activity followed by aldehydes, ketones and alcohols. Recent literature indicates that essential oils have been tested for activity against many types of organisms known to cause human disease (Anthony et al, 2005; Edris, 2007). However, most of the commonly used plants have not been thoroughly analyzed, leaving potential bioactivity undocumented. The evidence in these recently published research findings indicates that essential oils and their
components have potential to be valuable resources in the production of new drugs useful against human diseases. Consequently, this study was undertaken to examine the activity of essential oils and extracts of *R. graveolens* L. on different fungus.

### MATERIALS AND METHODS:

**Plant Materials:** Fresh leaves of *R. graveolens* were collected from Botanic garden of V. G. Vaze College Mulund, Mumbai and shed dried. It was made into fine powder and stored in air tight plastic container.

**Preparations of Methanolic leaf extracts:** A crude extract of leaves was prepared from a fine powder of dried leaves. 50 g powder was refluxed with Methanol in a Soxhlet extractor for 72 hour. The extract was then filtered and concentrated under vacuum to obtain the crude extract.

**Extraction of essential oil:** Fresh leaves were thoroughly washed twice with distilled water and leaf material subjected to a hydro distillation for five hours using a Clevenger-type apparatus. The oils obtained were separated from the distilled water. Essential oils are volatile and therefore stored in sealed glass vials in a refrigerator at 4-5°C in order to prevent changes in chemical composition for further use.

**Chemicals and cultural media:** Mueller Hinton Agar (MHA), Nutrient Agar (NA), Sabourauds Dextrose agar, Barium Chloride (BaCl2), Sulphuric acid (H2SO4), Dimethylsulfoxide (DMSO) were used to conduct the experiments.

**Fungal organisms:** The pathogenic fungal cultures of *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus niger* were used for antifungal studies procured from Department of microbiology laboratory, K.E.M Hospital, Mumbai and maintained on potato dextrose agar (Hi-Media, Mumbai) in Biology laboratory of V.G. Vaze College Mulund, Mumbai.

**Antifungal bioassay:** Antifungal activity of *R. graveolens* was studied by the agar well diffusion method. The Methanolic extract and Essential oil samples were tested in dose levels of 10-100μg/ml and 10-100μl/ml respectively. Stock solutions of essential oils were prepared in dimethylsulfoxide (DMSO). The Sabouraud's Agar medium was prepared and inoculated with 0.5ml of aqueous suspension of the above cited test organisms. The suspension was prepared from 48 hour old cultures. The cultures were then transferred into sterile petridishes for growth of fungi. The Mueller Hinton agar medium in the plates were allowed to set at room temperature for about 10 minutes and allowed to solidify in a refrigerator for 30 minutes. 3 cups of 6mm diameter were made in each petri plate at equal distance. Stock solutions were prepared in concentrations of 10 - 100μg/ml and 100 - 1000μg/ml of residual extract, Methanolic extract and Essential oil respectively. Each prepared concentration was poured in the cups with sterile micropipettes with a control. The Methanol was used as negative control. The culture petri plates were incubated for 16-48 hrs at 30-37°C and examined by measuring the zones of inhibition with the zonal scale and the results were tabulated.

### RESULTS AND DISCUSSIONS:

All the extracts showed varying degrees of antifungal activity on the tested fungal strains. *In vitro* antifungal activities of methanolic and essential oils of *R. graveolens* are tabulated (Table.1 and 2). The methanolic extract of *R. graveolens* was active against all tested fungi. Furthermore, it was observed that *A. niger* was more susceptible than *Candida albicans*. The Methanolic extract at higher concentration of 100μl showed 26 mm zone of inhibition in the *A. niger* (Fig.1.1). At the same concentration *C. neoformans* showed 24 mm zone of inhibition (Fig.1.2). In *Candida albicans*, the methanolic extract was least effective than other two fungal strains. In 100μl methanolic extract, 21 mm zone of inhibition was recorded in *C. albicans*. In the essential oil studies the oil was more effective on the *C. albicans* than other two strains. The maximum zone of inhibition was observed on *C. albican*. At the higher concentration (100 μl) the zone of inhibition recorded was 26mm and lowest concentration of 10 μl of dosage showed 17 mm. In *A. niger*, the oil was least effective. The maximum zone of inhibition observed was 15 mm at 100 μl concentration of oil. The oil showed moderate antifungal activity in *C. neoformans*, than the *Candida* and *Aspergillus*. The maximum zone of inhibition was recorded 20 mm at 100 μl concentration of oil and minimum 13 mm at the 10 μl concentration.

The results of the antifungal assay of both the extracts of *R. graveolens* indicated that the plant exhibited dose dependent antimicrobial activity against the tested microorganisms at three different concentrations of 50, 100 and 200μg. The potential sensitivity of the extracts was recorded against all the tested fungal organisms. All the extracts showed varying degrees of antifungal activity with tested microorganisms. The findings were in close agreement with the earlier work carried out by Oliva and Meepagala(2003) in the ethyl acetate extract of *R. graveolens* leaves and shown fungicidal activity against several agriculturally important pathogenic fungi like *Colletotrichum fragariae*, *C. gloeosporioides*, *C. acutatum*, *Botrytis cinerea* and *Fusarium oxysporum*. According to Hashemi Karouei. et al (2011) ethanolic extract from *R.graveolens* roots had good antifungal effects on *Saprolegnia* while, ethanolic and methanolic extract of *Euphorbia tirucalli* are inhibitory to *Aspergillus flavus* (Jadhav et al, 2010)

The zone of inhibition recorded was dose dependent and methanolic extract was found to be the most effective antifungal agent as compared to the oil. This may be due to better solubility of the active components in organic solvent (Lin, et al., 1999). These reports can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay.

### CONCLUSION:

The investigation supports the traditional usage of the studied plants and suggests that *R.graveolens* extracts and essential oils possess compounds with antifungal properties that can be used for development in new useful drugs and compounds as antifungal agents for the treating infectious diseases.
Table 1. Antifungal Activity of Methanolic Extract of *R. graveolens* L.

<table>
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<tr>
<th>Conc. of Methanolic Extract (µg/ml)</th>
<th>Zone of inhibition (mm)</th>
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<td></td>
<td><em>Candida albicans</em></td>
<td><em>Aspergillus niger</em></td>
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Table 2. Antifungal Activity of Essential Oil of *R. graveolens* L.

<table>
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<tr>
<th>Conc. of Essential oil (µl/ml)</th>
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<td><em>Candida albicans</em></td>
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**Fig. 1.1** : Effect of different conc. Of Methanolic Extract on *Aspergillus niger*

**Fig. 1.2** : Effect of different conc. of Methanolic Extract on *Cryptococcus neoformans*

**Fig. 1.3** Effect of different conc. of Essential Oil of *Ruta graveolens* on *Candida albicans*

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**REFERENCES:**


Meepagala. K. (2005); Algalcidal and antifungal compounds from the roots of Ruta graveolens and synthesis of their analogs, phytochemistry, 66(22), 2689-2695.


