Screening of Antimicrobial, Antioxidant and Anticancer Activity of Ruta graveolens

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Abstract: In the present study antimicrobial and antioxidant activity of Ruta graveolens was investigated. The methanolic extract of was Ruta graveolens prepared and the extract was tested for antibacterial and antifungal activity by agar well diffusion method in laboratory condition. The extract of Ruta graveolens showed antibacterial activity against Gram-negative bacteria such as Klebsiella pneumoniae, Salmonella typhi, Escherichia coli and Gram-positive bacteria such as Methicillin-resistant Staphylococcus aureus and Bacillus subtilis however Pseudomonas fluroscence and Micrococcus luteus was found to be resistant. Antifungal activity of the extract was also analyzed which showed positive activity against Cryptococcus sps, Candida albicans, Penicillium sps, Aspergillus niger, Trichoderma viridae and Alternaria sps. Antioxidant activity of methanolic extract of Ruta graveolens was characterized using 1, 1,-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The EC50 of the extract was found to be 9 µg/ml. The anticancer activity of the extract was determined by MTT assay against MCF7 breast cancer cell line, the extract showed moderated activity and IC50 was found to be 85µg/ml. The findings of this study indicates the medical properties of Ruta graveolens, further screening of useful metabolites from Ruta graveolens could be exploited for new drug discovery.

Keys words: Antimicrobial • Antioxidant • Ruta graveolens • DPPH Assay • MTT Assay

INTRODUCTION

Plants are the most important source for all kind of food and medicine. From ancient time to modern world it is not possible to manufacture the medicine without plant or it’s photochemical. They are valuable source of natural active constituents that are used to maintain human health and also used for the treatment of many human diseases [1]. They are good source of economically important compounds such as phenol compounds, nitrogen containing compounds, vitamins and minerals which have anti-oxidant, anti-tumor, anti-mutagenic, anti- carcinogenic and diuretic activities.

Ruta graveolens (Fig 1 a, b) is one of the oldest known medical plants which is used in traditional medicine in ancient countries it is a native to Mediterranean region of southern Europe and Northern Africa. It is a well known medical plant used for treating many diseases such as Seizure, cough, hypertension, inflammatory conditions, eczema, ulcers, arthritis, antidote for venoms, insect repellent and even as an abortifacient [2-5]. Antimicrobial effects of extracts of this plant on fungi, protozoa, worms and bacteria are reported in several studies but the mechanisms are not well known. Phytochemical investigations have demonstrated the presence of more than 100 chemical compounds have been found in all parts of the plant, including fats, oils, flavonoids, furiquinolone, alkaloids, Glycosides, essential oils, Terpenoids, Sterioids, Sterols, coumarins, Tannins, Phenols, Saponin, pyranocoumarin Cardioglycosides, Carbohydrates, Amino Acids, Protein and others. All parts of the plant contain the active compounds, although they are mostly encountered in leaves [6-10]. Various chemical compounds has been reported to be present in R. graveolens which incude furanocumarins, carotenoids, chlorophyll, furanouquinolones [2, 11, 12]. Khalda [13] has reported that the extract of R. graveolens induces DNA damage and block Akt activation to inhibit cancer.
cell proliferation and survival. The main objective of the present investigation is to screen for antimicrobial, antioxidant and anticancer activity of the methanolic extract of *Ruta graveolens*.

**MATERIALS AND METHODS**

**Collection of Sample:** *Ruta graveolens* was collected from Shimoga district of Karnataka in the month of August 2014, the taxonomic studies and identification was done using the Keys of Gamble [14]. The aerial part of the plant before blooming was used in this study. The plant was dried, powdered and preserved for further studies.

**Preparation of Alcoholic Extract from *Ruta graveolens***: The aerial part of the plant were collected, washed thoroughly in tap water, rinsed in distilled water and shade dried at room temperature for 5 days. The air dried leaves were powdered in a grinding machine and stored in an airtight container. The powdered leaves were used to do a crude extract of the Ruta plant by using methanol at room temperature for 24-48 hours. The content was then filtered through the Whatman No.4 filter paper and the filtrate was dried. The extracts were recovered by filtration and kept at 40°C in a rotary vacuum evaporator. The residue was collected and store at 40°C for further use [15].

**Antimicrobial Activity of *Ruta graveolens***: The antimicrobial activities of the extract were determined by the Kirby-Bauer agar diffusion method according to NCCLS standards [16, 17]. All the bacterial cultures were grown in nutrient broth and incubated at 37°C for 24 hours whereas, the fungal cultures were grown in Dextrose agar and incubated at room temperature for 48 hours. Nutrient agar and Potato Dextrose agar of about 20ml were poured into each sterilized petriplate aseptically and allowed to solidify. With the help of sterilized cork borer a well of 6mm diameter was made on the surface of the media. The bacterial and fungal suspensions were made and it was swabbed on the solidified media using sterilized swabs in the respective plates. The crude extract was dissolved in Dimethylsulfoxide and the suspension was sterilized by filtration through a membrane filter [18]. The crude extract of concentration about 100µg/ml was filled into the wells of the agar plates were incubated at 37°C for 24 to 48 hours. The fungal plates were incubated at 28°C for 3-4 days. Inhibitory activity DMSO was also tested. Nystatin was used as reference disc for fungi. Tetracycline, Methicillin and Ampicillin were used as the reference disc for bacteria and Nystatin was used as standard antibiotic for fungi. After the period of incubation the zone of inhibition was measured, tabulated.

**Microorganisms Used:** The pure culture of bacteria were procured from NCL Pune, following were the bacterial culture used: *Bacillus subtilis*(2655), *Staphylococcus aureus*(2127), *Escherichia coli*(2685), *Micrococcus flavu*(2376), *Salmonella typhi* (2257),*Pseudomonas fluroscence*, *Klebsiella pneumonia*(2706), *Streptococcus mitis* (Clinical isolates), *Cryptococcus sps* (3349), *Candida albicans*(3100), *Aspergillus niger*(Clinical isolates), *Trichoderma sps* (Clinical isolates), *Penicillium* (Clinical isolates), *Alternaria sps* (Clinical isolates).

**Antioxidant Activity:** 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay:
The antioxidant activities of the methanol extract were measured on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical [19]. Various concentrations of 1ml of the test extract were added to 4ml of a 0.004% (w/v) methanol solution of DPPH. After 30 minutes of incubation period at room temperature, the absorbance was measured against blank at 517nm in spectrometer. Inhibition of free radical DPPH in percent (%) was calculated.

\[ 1\% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100 \]

Where \( A_{\text{blank}} \) is the absorbance of the control reaction (Containing all reagents except the test compound) and \( A_{\text{sample}} \) is the absorbance of the test sample. \( IC_{50} \) was calculated from the graph plotted inhibition percentage against extract concentrations.

**Anticancer Activity**

**MTT Assay:** (3-(4, 5-dimethythiazol-2-yl)- 2, 5- diphenyl tetrazolium bromide)

The MCF7 breast cancer cell line was procured from National Centre for Cell Sciences Pune, India, was used in this study. Seed 50,000 cells / well of MCF7 in a 96 well plate and incubate for 24 hrs at 37°C, 5% CO₂ incubator. Compounds from crude extract to be tested from 0-320 µg/ml in DMEM media without FBS and are to be incubated for 24 hr. 100 µl/well of the MTT was added in to each well, a working solution to the respective wells and incubate for 3 to 4 hours. After incubation with MTT reagent, was discarded by pipetting without disturbing cells and 100 µl of DMSO was added to rapidly solubilizing the formazan. The microplate was then read with an ELISA reader at wavelength 570 nm within 1hour. The half maximal inhibitory concentration (IC50) was calculated as suggested by Mosmann [20].

**RESULT AND DISCUSSION**

**Antimicrobial Activity of Ruta graveolens:** The antibacterial and antifungal activity of the methanolic extract of *Ruta graveolens* has been represented in Fig. 02, 03 and 04 respectively.

The methanolic extract of was *Ruta graveolens* prepared and the extract was tested for antibacterial and antifungal activity by Agar well diffusion method in laboratory condition. The extract of *Ruta graveolens* showed antibacterial activity against Gram-negative bacteria such as *Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli*, the extract showed maximum activity against *Klebsiella pneumoniae* followed by *Salmonella typhi* and *E.coli* however *Pseudomonas fluorscence* showed resistance against the extract it also showed minimum activity against other standard antibiotics such as Methicillin, Amoxicillin and Tetracycline. Among Gram-positive bacteria the extract was found to be sensitive to *Staphylococcus aureus*, *S. myitis* and *Bacillus subtilis*, *Micrococcus flavus* was found to be resistant towards the extract, this investigation reveals that Gram-negative bacteria are found to be more susceptible than Gram-positive bacteria towards that of methanolic extract of *Ruta graveolens*. Similar kind of reports has been reported by França Orlanda et al. [21], Saeed Al-Sokari and Aly El Sheikh.
Fig. 3: Antimicrobial activity of *Ruta graveolens* A. *Staphylococcus aureus*; B. *Salmonella typhi*; C. *Bacillus subtilis*

Fig. 4: Antifungal activity of *Ruta graveolens*.

[22], Hashemi Karouei [23], Harish Kumar *et al.* [24], Ivan G. Ivanov *et al.* [25], Pinkee Pandey *et al.* [26], Dragana Pavloviæ *et al.* [27] and Meepagala *et al.* [28]. However [29] has reported that the inhibitory effects on the growth of Gram-positive bacteria was more when compared to that of Gram-negative bacteria.

The extract was also analyzed for its antifungal activity six different fungal isolates and Nystatin was used as standard antifungal agent. Penicillium spp. and Trichoderma species showed maximum activity followed by *A.niger*, *Candida albicans* and Cryptococcus. The *Ruta* extract showed comparatively more activity towards Cryptococcus spp than Nystatin. The activity of the extract was comparatively lesser than the standard antifungal agent Nystatin. Pinkee Pandey *et al.* [26] and Issa and Masoud [30] has also reported the positive activity of *Ruta graveolens* against various fungi such as *A.niger*, *A.flavus*, Penicillium crysogenum, *Rhizopus stolonifer*. Pinkee Pandey *et al.* [26] has reported the negative activity of *Ruta* against *Fusarium oxysporium*. However the above mentioned results reveals the moderate activity of *Ruta graveolens*, Ivan G. Ivanov *et al.* [25] has reported that 7-metoxi comarin, 4-hydroxi comarin extracted from the plant showed the better activity. Oliva *et al.* [4] has reported the presence of antifungal agent in *R.graveolens* against agriculturally important fungi in ethylacetate extrace; similarly [28] has demonstrated the fungicidal activity against *Colletotichum fragariae*, *C.gloeosporioides*, *C.acutatum*, *Botrytis cineara* and *Fusarium oxysporium*.They found that Rutacridone epoxide was the bioactive constituent from the ethylacetate extract of *R. graveolens* roots which showed fungicidal activity, Rutacridone epoxide also showed significantly higher fungicidal activity than commercial fungicides, captan and benomyl). Hashemi Karouei *et al.* [29] reported the antifungal activity of ethanolic extract of *R.graveolens* against *Saprolegnia spp*. In *Ruta graveolens*, the existence of saponin, tannin, alkaloid and glycosid has been proved. Saponin has soap characteristics and its anti-fungal effect has been tested [31].

**Antioxidant Activity of *Ruta graveolens***: The methanolic extract of the *Ruta graveolens*was subjected to DPPH assay for its antioxidant activity. DPPH is a stable free radical, used to study the radical scavenging effect of the extract. Free radical scavenging capacity of the extract measured by DPPH assay has been shown in Fig. 06. The concentration required for 50% scavenging of DPPH (EC50) was found to be 9µg/ml. This result reveals the strong antioxidant activity of *Ruta graveolens*. This experimental result is accordance with that of the previous findings of Renuka Diwan *et al.* [32] has reported the antioxidant activity of *in vitro* cultures were comparatively more than that of *in vivo* plant materials.
Mohammadi Motamed et al. [33] has also reported the antioxidant activity of \textit{R. graveolens} they have reported that the IC$_{50}$ of methanol extract was 200.5 µg/ml which is found to be quite high when compared to that of our findings. This may be due to the variation of the chemical composition which depends on various factors namely, climatic condition, season, geographic condition, harvest period, part of the plant used, extraction techniques etc [34-36]. Phenolics, flavonoids and cumarines are the most probable ones which could be able to have scavenging activity of DPPH radical. The flavonoid which is on the most divers and wide spread group on natural compounds which acts as primary antioxidants [37]. Furanocoumarins are the main active constitute of \textit{Ruta graveolens} which is a potent antioxidant [38, 39].

\textbf{Anticancer Activity of \textit{Ruta graveolens}}: The anticancer activity of methanolic extract of \textit{Ruta graveolens} was investigated using MTT assay on MCF7 breast cancer cell line. The cells were treated with increasing concentration of extract. A mitochondrial enzyme in living cells, succinate dehydrogenase, cleaves tetrazolium ring, converting the MTT to an insoluble purple Formazan. Therefore, the amount of Formazan produced is directly proportional to the number of viable cells. The graph No. 06 shows the decrease in percentage of the viable cells with the increase in the concentration of the extract. The death of MCF-7 cell increased by 50% when the concentration of the extract was (IC$_{50}$ 160µg/ml, which indicates the moderate activity of the extract. The present investigation also supports the findings of Pathak et al. [40] and Preethi et al. [41]. Aljaiyash et al. [42] has also proved the cytotoxic activity of \textit{Ruta graveolens} against breast and colon cell lines MCF7 and HCT-116 respectively. Gamble [14] has reported the similar activity of \textit{Ruta graveolens} on MCF7 breast cancer cell lines. A lipid per oxidation is a free radical chain reaction and is known to cause 2 main steps of carcinogenesis initiation and progression. During carcinogenic process lipid peroxidation is increased and more complex and reactive compounds such as malondialdehyde and 4 hydorynonenal are produced. These products of lipid peroxidation are found to be mutagenic and carcinogenic. This indicates that the agent which can reduce the production of free radical \textit{in vivo} can be considering to the potential for anticancer therapy. It has been revealed that at higher concentration, the extract of \textit{Ruta graveolens} act as a pro-oxidant rather that antioxidant. At low concentration \textit{Ruta graveolens} extract act as antioxidant and scavenge free hydroxyl radicals and inhibits lipid per oxidation. However at high concentration it acts as prooxidants and is capable of changing the redox potential in the cell and affects the permeability of mitochondrial membrane which in turn leads to apoptosis.
Khalda [13] has also proved that the extract at higher concentration effects the mitotic division of the cell, this is due to formation tripolar or multipolar spindles, short or disarranged spindle fibers, lagging chromosomes at metaphase-anaphase transition and micronuclei formation and hence the cell cycle is stopped. It has also been reported that the extract of *Ruta graveolens* also induces the activation of p53 tumor suppressor protein, which regulates cell cycle, apoptosis, senescence and signaling pathways., it has also been proved by Khalda [13], that the extract also induces the activation of DNA damage response protein complex, which in turn activates the p53 protein, it has also been found that the concentration at higher range are found to be cytotoxic even to fibroblast. The kinetics of caspase has also been studied by Khalda, he suggested that apoptosis is also been induced by the extract due to the caspase activity. Hence the data suggests that the methanolic extract of the *Ruta graveolens* is found to be a strong anticancer agent.

**CONCLUSION**

The present study demonstrates the potential of *Ruta graveolens* which has got high medicinal properties. It has a valuable sources of flavonoids rutin, alkaloids, quinolone, furocoumarin, pyranocoumarin and essential oils like 2-nananone, 2-undecyl acetate [10] with high antibacterial, antioxidant and anticancer activity. Our results revel that these metabolites could be used in food and pharmaceutical industries.

**ACKNOWLEDGEMENT**

The authors would like to thank the management of Gokula Education Foundation for all the support and encouragement to carry out this work.

**REFERENCES**


