ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Phytochemical screening and evaluation of antibacterial activity of different extracts of Ruta graveolens L - a medicinal plant

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Manuscript Info

Manuscript History:

Received: 12 February 2015
Final Accepted: 22 March 2015
Published Online: April 2015

Key words:

antibacterial, secondary metabolites, flavanoid, phenolic compounds, phytochemical, disc diffusion,

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Abstract

Ruta graveolens L. is aromatic shrub belongs to family rutaceae. It is ornamental and medicinal plant used in the treatment of inflammation, ulcer, hypotension, reproductive disorders, menstrual problems, parasitic infection, wounds and injuries. The plant extracts showed good antibacterial and antifungal properties. Sixty days old seedlings were collected and leaves were shade dried and powdered. 5 grams of powder extracted with 25 ml different solvents, ethanol, methanol, chloroform and distilled water. Crude solutions were further diluted to $1/10^{th}$ and $1/100^{th}$ with DMS. Antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *E. coli* and *Staphylococcus aureus* cultures were evaluated by disc diffusion methods on Muller Hinton agar. In our study methanol and chloroform extract recorded better antibacterial activity than ethanol extract at higher dilution. Chloroform and methanol extracts showed more antibacterial activity than ethanol at lower concentration, water extract doesn't exert any activity. The phytochemical analysis of different solvent extracts of plant show considerable change in the nature of chemicals. Chloroform extract reported maximum number of secondary metabolites than remaining solvents.

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INTRODUCTION

Most of rural people even today depend on plants for medicines. In India, 95% of the traditional system prescriptions of Unani, Ayurveda, Homeopathy and Siddha are plant based chemicals (Satyavati et al., 1987). The plant based chemical compounds are classified into two classes; primary and secondary metabolites based on their chemical, biosynthetic origin and functional groups. Primary metabolites are involved in growth and development and secondary metabolites are involved in defense mechanism against harmful pests and infectious agents. The later class exhibit medicinal properties. Plant derived chemicals such as terpenoids, phenolics, alkaloids, flavanoids, glycosides, diterpenes, triterpenes and minor chemicals are having better compatibility with human body. It is estimated that 30% of the worldwide sales of drugs are based on plant products (Patwardhan et al., 2004). The increasing antibiotic resistant pathogens and failure of many chemotherapeutics has led the screening of medicinal plants for their antimicrobial activity (Mashobo and Bosisio., 1996). *Ruta graveolens* L. is aromatic shrub belongs to family rutaceae and is commonly known as rue, cultivated as ornamental and medicinal herb in gardens. The plant extract is used to treat inflammation (Ratheesh et al., 2009) and ulcers (Raghav et al., 2006). This plant extracts exerts cytotoxic, antihypotensive (Chiu and Fung., 1997), antibacterial (Farah Haddouchi et al., 2013), antihelminthic and phytotoxical (Asgarpanah and Khoshkam., 2012) properties. Plant extracts are also used in the treatment of reproductive disorders (Browner et al., 1985). Decoction of ruta graveolens is used to promote menstruation. Plant contains various volatile compounds and oils (Kostova et al., 1999; Masho et al., 2015). Oils are used to treat nervous nightmare and essential oils are also reported for their insecticidal properties. Fresh leaves used to relieve headache. In homeopathy this plant is used for the treatment of muscular pain, injuries, sprains, eye strain

joint and bone pain, arthritis, rheumatism, toothache, tennis elbow, back pain and head ache. The current study was conducted for phytochemical analysis and antimicrobial activity of different solvent extracts of *Ruta graveolens.* L.

Material and methods

Plant collection

The plants were collected from Sanjeevini Vatika Department of Horticulture, University of Agriculture Sciences, GKVK, Bangalore. Microbial cultures were obtained from the Department of Microbiology and Biotechnology, Jnanabharathi campus, Bangalore.

Plant Material Extraction and anti bacterial test

Sixty days old seedlings were collected and leaves were shade dried at room temperature for 10 days. The dried leaves were powdered and stored in air tight containers. 5 grams of powdered leaves were taken into each round bottom flask and extracted with 25 ml different solvents, ethanol, methanol, chloroform and distilled water through flash evaporator for 24 hours. The solution was filtered using a Whatman filter paper. The extract was evaporated at 40^{o}c. The dried crude extract was dissolved in dimethyl sulpoxide (DMS). 50mg/ml extract was prepared and used for further analysis. Crude solutions were further diluted to $1/10^{th}$ and $1/100^{th}$ with DMS. Antibacterial activity against *E. coli, Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, cultures were evaluated by disc diffusion methods on Muller Hinton agar. The sterilized media poured upto $3/4^{th}$ of petri dish and bacterial cultures were spread uniformly. The paper discs impregnated with each extract were placed on the inoculated agar plates and incubated for 24 hours at 37^{o}c. The inhibition zones were measured in cm.

Phytochemical analysis

For phenol test 5 mg of leaf extract was treated with 2% ferric chloride, for glycosides 2ml of glacial acetic acid and 2 drops of 2% ferric chloride, for triterpenoids 5 mg of dry leaf extract was treated with 2ml of chloroform, 1 ml of acetic anhydride and 1 ml of concentrated sulphuric acid. Phenolic compounds were tested by 3% lead acetate; glycolipids by glacial acetic acid and ferric chloride, steroids were tested by chloroform, saponins by distilled water, flavonoids by sodium hydroxide and tannins by acetic anhydride solution.

Results and discussions

Results

Antibacterial activity

Antibacterial activity of chloroform extract showed the zone of inhibition of 2.4 cm for *E. coli*, 1.8 cm for *Bacillus subtilis*, 1.4 cm for *Staphylococcus aureus* and 0.8 cm for *Pseudomonas aeruginosa.* $1/10^{th}$ and $1/100^{th}$ dilutions of the crude extract showed the zone of inhibition as in table 1. Antibacterial activity of methanol extract and its dilutions were measured by zone of inhibition and recorded in table 2. Zone of inhibition of ethanol extract and its dilutions were reported as in table 3. And distilled water extract showed no zone of inhibition. The results clearly showed that the different extraction methods have different degree of antibacterial activity on same bacteria and also on different strains. The phytochemical analyses of Ruta graveolens for different extracts were recorded in table 4.

Table 1 Zone of inhibition of chloroform extract

Organism	Zone of inhibition in cm		
	Crude extract	$1/10^{th}$ dilution	$1/100^{th}$ Dilution
E coli,	2.4	1.6	0.6
Bacillus subtilis	1.8	1.4	0.9
Staphylococcus aureus	1.4	1.3	1.3
Pseudomonas aeruginosa	0.8	0.7	0.4

Table 2 Zone of inhibition of methanol extract

Organism	Zone of inhibition in cm		
	Crude extract	$1/10^{th}$ dilution	$1/100^{th}$ Dilution
E coli,	1.7	1.31	O.7
Bacillus subtilis	1.7	0.8	0.7
Staphylococcus aureus	1.3	1.0	1.2
Pseudomonas aeruginosa	1.1	1.2	1.3

Table 3 Zone of inhibition of ethanol extract

Organism	Zone of inhibition in cm		
	Crude extract	$1/10^{th}$ dilution	$1/100^{th}$ Dilution
E coli,	1.9	1.1	1.0
Bacillus subtilis	1.5	0.9	0.8
Staphylococcus aureus	1.5	1.2	0
Pseudomonas aeruginosa	1.6	0.7	0

Table 4 Phytochemical analysis of *Ruta graveolens*

Chemicals present	Chloroform extract	Methanol extract	Ethanol extract	Distilled water
Phenols	+	-	-	-
Glycosides	+	+	+	-
Triterpenoids	-	+	-	-
Phenolic compounds	+	-	+	+
Glycolipids	+	+	+	+
Flavanoids	-	-	-	-
Saponins	-	-	-	-
Tannins	-	-	-	-
Ferric chloride	-	-	-	-
Steroids	-	-	-	+

+ = presence, - = absence

Discussion

The biological activity of the plant depends on many factors like, plant part, geographical source, soil conditions, time of the harvest, moisture and post harvest process methods. For example high temperature during tissue grinding may denature certain chemical constituents. Different concentrations of solvent or different solvents alone or in combinations are used for the maximum recovery of bioactive compounds, because different plants constitute different compositions of active compounds. Different extraction protocols are followed in herbal medicine preparation, ethanol – water mixture extraction protocols are used in majority of herbal medicine industries (Ganora, 2008). Anti-microbial properties of plant extracts have been treated as new classes of antibiotics (Ali et al., 2001). *Ruta graveolens* plant extracts showed more effect on gram positive bacteria than gram negative bacteria (Alzoreky et al., 2003), antibacterial activity reported on *Staphylococcus aureus* (Ojala et al., 2000), *Bacillus subtilis* (Al-Bakri, and Afifi., 2007). Guarrera et al (1999) reported anti parasitic activity. Both hydro and hydroalcholic (ethanol 70%) extracts of this plant shows no antibacterial effect on main human pathogens (Ahmadi jalali et al., 2012).

In vitro antibacterial efficiency of different extracts *R. graveolens* were assayed based on their zone of inhibition. Chloroform extract of *R. graveolens* showed antimicrobial activity on both Gram positive and Gram negative bacteria. Chloroform extract observed maximum activity against *E coli* with 2.4cm zone of inhibition and minimum against *Pseudomonas aeruginosa with* 0.8 cm zone of inhibition. And moderate against *B. subtilis and S. aureus* with zone of inhibition of 1.8 cm and 1.4 cm respectively. Antibacterial activity decreased for $1/10^{th}$ and $1/100^{th}$ dilution. Increasing dilutions observed maximum decreased activity against E coli, and moderate against *B. subtilis* and *P. aeruginosa*, and no variation in the activity against *S. aureus*. This study indicated that, at lower concentration of chloroform extract showed maximum activity on *S. aureus* and minimum activity against *P. aeruginosa*. As the concentration increased its activity increased maximum against *E. coli*. Methanol extract exert similar antibacterial activity on *E. coli, B. subtilis* with 1.7 cm of zone of inhibition and lower activity on *S. aureus* and *P aeruginosa* with 1.3 cm and 1.1 cm of zone of inhibition respectively. $1/10^{th}$ dilution recorded reduced activity on E. coli, B. subtilis and *S. aureus*, but retained same activity against *P aeruginosa* culture. $1/100^{th}$ dilution recorded maximum decreased activity against *E. coli* and narrow decreased activity against *B. subtilis* and no change against *S. aureus* and *P. aeruginosa*. This indicates that at lower concentration methanol extract showed maximum activity against *S. aureus* and *P. aeruginosa* and minimum on remaining organisms and vice versa at higher concentrations. Ethanol extract reported maximum antibacterial activity against *E. coli* with 1.9 cm zone of inhibition and same type of antibacterial activity against remaining organisms with 1.5 cm of zone of inhibition. Antibacterial activity against all organisms gradually decreased for $1/10^{th}$ dilution. $1/100^{th}$ dilutions were observed no zone of inhibition for *S. aureus* and *P. aeruginosa*. Aqueous extract of plant was not shown zone of inhibition against any of four organisms. It indicated that aqueous extraction doesn't contain the secondary metabolites of antimicrobial activity. Methanol extract reported faster wound healing property in experimental rats (Hayder et al., 2014). Methanol extracts were also reported for their antifungal property (Issa Gholampour Azizi et al., 2012). Harish Kumar et al (2014) reported maximum zone of inhibition for methanol extracts against *K. pneumonia* and *S. aureus*. Ethanol extract of Ruta graveolens have more antifungal activity than methanol extract (Issa Gholampour et al., 2012). Olia et al observed antibacterial effect against *Pseudomonas aeruginosa* for hydroalcholic extract of the plant (Olia et al., 2004).

Crude aqueous or alcoholic extracts are generally using in the initial screening of plants for antimicrobial activities (Cowan et al., 1999). The current phytochemical analysis of different solvent extracts of *Ruta graveolens* observed considerable change in the nature of chemical composition. The chloroform extract constitute more class of chemicals such as phenols, glycosides, phenolic compounds and cardiac glycolipids. Methanol extract constitute glycosides, triterpenoids and cardiac glycolipids, ethanol is general extraction solvent for many plant extracts, constitute glycosides, phenolic compounds and cardiac glycolipids, water extract contains phenolic compounds, cardiac glycolipids and steroids. Chemicals like, alkaloids, coumarins, terpenoids, volatile substances, furoquinolines and flavonoids have been isolated from *Ruta graveolens* (kuzovkina et al., 2004). Presence of saponin, tannins and glycosides are reported by Hashemi et al (2011). Volatile oils contain high content of aliphatic acids, ketones and alcohols (Ivanovaa et al., 2003). Flavonoids such as rutin and quercetin isolated from the leaves of *R. graveolens* (Pathak et al., 2003). The phenolic compounds, alkaloids and terpenoides extracted from *ruta graveolens* reported antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* (Al-Bakri et al., 2007). Furanocoumarins of this plant reported as potent antioxidants (Karagozler et al., 2008; Piao et al., 2004). Flavonoids are one of the widespread groups of natural compounds and the most important natural phenolics present in *R. graveolens* they possess broad spectrum free radical scavenging properties (Liu et al., 2008; Hollman and katan., 1999).

Crude extracts of all methods show good results but on higher dilution methanol extract recorded maximum zone of inhibition against all four organisms. Chloroform extract was also recorded considerable antibacterial activity for all four organisms. Crude ethanol extract was recorded better antibacterial activity for all organisms but its antibacterial effect decreased with increased dilution. $1/100^{th}$ dilution of ethanol extract had no antibacterial effect on *S. aureus* and *P. aeruginosa.* In our study methanol and chloroform extract recorded better antibacterial activity than ethanol extract at higher dilution.

Conclusion

The medicinal plants are the rich source of various bioactive compounds that made them traditional medicine to combat and cure various ailments. The medicinal properties are due to the high steroids, flavonoids, phenols, tannins, terpenoids and saponins. Further investigations are required for specific activity evaluation and downstream processing technology.

Acknowledgement

The author expresses thanks to the chairman of the department of microbiology and biotechnology and Bangalore University for extending his cooperation for carrying this study.

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