

Original Article

## PHYTOCHEMICAL INVESTIGATION, GC-MS PROFILE AND ANTIMICROBIAL ACTIVITY OF A MEDICINAL PLANT *RUTA GRAVEOLENS L.* FROM ETHIOPIA

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### ABSTRACT

**Objective:** This study was designed to screen the phytochemicals present in various solvents extracts of *Ruta graveolens* (Rue) and furthermore to investigate their antimicrobial activity.

**Methods:** The leaves, stems and seeds of Rue were extracted using four different solvents viz. ethanolic, methanolic, chloroform, and aqueous of varying polarity. The phytochemical screening was carried out qualitatively and Gas Chromatography-Mass Spectroscopy (GC-MS) analysis was performed to identify major phytoconstituents present in the methanolic leaf extract. The antimicrobial effect of extracts was evaluated against six microbial strains namely *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Candida albicans*, *Candida tropicalis* and *Micrococcus luteus* with disc diffusion method.

**Results:** Phytochemical analysis revealed the presence of various secondary metabolites such as flavonoids, alkaloids, terpenoids, saponins and carotenoid. The methanolic leaf extract showed the presence of both tannin and phenolic contents in the higher amount, whereas aqueous extract displayed in the least amount. GC-MS analysis of methanolic leaf extract revealed the presence of approximately 26 phytochemical constituents. The antimicrobial assay revealed that *B. subtilis* showed a high zone of inhibition (20 mm) at 200 mg/ml of methanolic extract. However, *E. coli* and *C. tropicalis* did not show any zone of inhibition against each solvent extract.

**Conclusion:** In conclusion, secondary metabolites present in the extracts have biological activities which warrant further to evaluate *in vivo* pharmacological studies.

**Keywords:** *Ruta graveolens*, Anti-microbial, Phytochemicals, GC-MS analysis

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### INTRODUCTION

Medicinal plants and their products have been imparted valuable role for human health for thousands of years. In regards to medicinal valuable plants; nature provides us a reservoir of millions of natural products and also their derivatives which contain antioxidant, anti-microbial, anti-inflammatory, anti-tumor, anti-mutagenic, anti-carcinogenic and anti-diabetic potentials [1]. According to recent data from World Health Organization (WHO), 70-80 % of developing countries populations are using traditional plant products as an alternative or complementary medicine [2]. Herbal medicines are relatively safe for human use, thus plant-based antimicrobials medicines have immeasurable remedial potential that can serve the purpose with lesser side effect and also work against pathogens, those are multidrug resistant.

In the present study, the test plant *Ruta graveolens* (Rue) was explored for its bioactive, pharmacological and nutraceutical values. Rue is an odoriferous medicinal herb belonging to the family Rutaceae. It is a perennial herb well-known for its anti-leucoderma, anti-inflammatory and anti-cancer properties and its common name is Rue. Rue is native to Europe and also Mediterranean-Balkan Peninsula region and it was brought to Mexico and Tropics of America by Spanish [3]. Rue is also widely distributed in West Indies, Mexico, East Africa (Ethiopia), central South America and India [4, 5]. In Ethiopia, the leaves and berries of Rue are used in special cuisine, which was made by adding a characteristic Ethiopian spice mixture known as berbere. Rue oil is also used as a flavoring in foods and beverages and as fragment ingredient in manufacturing soaps and cosmetics.

Not only a delicacy but Rue is also having the wide range of medicinal usages; common uses in fertility regulation, menstrual cramps, earache, headache, nosebleed, strengthens eyesight and

helps prevent fatigue and sore in eyes and insect repellent. Moreover, Rue is also known to be used as diaphoretic, emetic, digestive stimulation, treatment of measles, scarlet fever, and heart problems, a remedy for epilepsy, vermifuge, diaphoretic, treatment of ulcer and gum problems [6, 7]. Brushed leaves are placed on a tooth or in the ear for pain relief [8, 9]. Rue extract is also believed to improve blood circulation, regulate blood pressure and help to prevent varicose veins and atherosclerosis. Rue oil is a commonly used homeopathic medicine as a rubefacient, in certain dermatoses as eczemas and psoriasis, and as an antiviral agent [8].

Various active ingredients present in Rue extract, which imparts its medicinal values. Rue contains a di-glycoside of quercetin known as rutin, a flavonoid, which is an active compound to scavenge nitric oxide [10]. Rue extract was also reported to scavenge hydroxyl radicals and inhibit lipid peroxidation at low concentrations [8]. Two acridone alkaloids namely arborinine and evoxanthine and furanoacridones, isolated from Rue plant have shown potent anticancer activity [11]. However, research is remaining to analyze phytoconstituents and their biological investigation.

Based on the previous reports, this study was designed to evaluate the phytochemicals present in the various solvent extract of Rue plant and subsequently, identified a variety of phytocomponents in the methanolic extract of Rue by using Gas Chromatography-Mass Spectroscopy (GC-MS). In addition, the antimicrobial activity of Rue extract was also investigated.

### METHODS AND MATERIALS

#### Chemicals and reagents

Chloroform, DMSO, sulphuric acid (H<sub>2</sub>SO<sub>4</sub>), diluted ammonia, ferric chloride (FeCl<sub>3</sub>), hydrogen chloride (HCl), deionised distilled water, Folin-Ciocalteu reagent, Draggendorff's reagent, sodium carbonate

and gallic acid were used for phytochemical screening. Mueller-Hinton broth and agar media were used for the antibacterial assay. The entire reagents used were of Sigma and Himedia, India.

#### Instruments

Grinder, water bath, glassware (conical flasks, beakers, test tubes, Petri plates), micropipettes, gel puncher, digital balance, incubator, laminar flow hood, hot air oven, spectrophotometer, gas chromatography-mass spectrometry, refrigerator, autoclave and microwave oven.

#### Microorganism used

The organisms used are *Candida albicans* (MTCC 183), *Candida tropicalis* (MTCC 184), *Escherichia coli* (MTCC 443), *Proteus vulgaris* (MTCC 426), *Micrococcus luteus* (MTCC 1538) and *Bacillus subtilis* (MTCC 441). These were preserved at CSIR-Central Leather Research Institute, Adyar, Chennai (India).

#### Plant material

The experimental plant Rue was collected from a local garden of Addis Ababa, Ethiopia in the month of December and January. Rue plant is harvested in early summer shortly before flowering begins. Plant samples were identified as Rue by the taxonomist at School of Biotechnology, Lovely Professional University, India. A reference specimen (voucher No.) of this plant is LPU/PHAR/HRB/13/06. The whole plant and the seed were washed thoroughly with sterile distilled water in order to remove dirt and dust present on the surface. The plant and seeds were then dried in shade for 15 d. The leaves, stems and the seeds were separately made into a fine powder in the grinder, which passed through 40-micron mesh. The powder was kept in airtight amber colour bottle till used.

#### Plant extract preparation

The powdered samples were mixed with solvents like methanol, acetone, chloroform and distilled water individually in 1:20 ratio. In this method, 30 g of the sample powder was percolated with 600 ml of solvent in a screw capped conical flask separately and the entire flasks were placed in a shaker at 100 rpm at room temperature for 24 h. After 24 h of shaking, all the extracts were filtered by using a clean muslin cloth and then re-filtered by Whatman no. 1 filter paper. The filtrate was then collected in a fresh beaker and concentrated by evaporating the solvents in a water bath at 35-40 °C. The extracts were labeled accordingly and were kept in a refrigerator at 4 °C until use [12].

#### Phytochemical screening qualitative analysis

The preliminary phytochemicals (carbohydrates, fatty acids, proteins, saponins, tannins, flavonoids, alkaloids, terpenoids, cardiac glycosides, carotenoids, polyphenol, and amino acids) screening of various solvents extracts were carried out using standard procedure to identify the various phytoconstituents [13, 14].

#### Quantitative estimation of tannins

The quantitative determination of tannin in Rue sample was performed as per protocol [15]. Briefly, 500 mg of the dried extract was dissolved in 50 ml of distilled water in a screw capped conical flask and allowed to shake for 1 h. Approximately 5 ml of filtrate was mixed with 2 ml of 0.1 M FeCl<sub>3</sub> in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min by UV-visible spectrophotometer. All the experiments were carried out in triplicate.

#### Quantitative determination of total phenolic content

The total phenols content in Rue was determined by the spectrophotometric method as per protocol with minor modification [16]. The dried extract was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. A 5 ml of the extract was mixed with 10 ml of distilled water, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol into a 50 ml flask and incubated at room temperature for 30 min for color development. Absorbance was measured at 505 nm by a spectrophotometer. All the experiments were performed in

triplicate and assay of the total phenolic content was expressed in mg gallic acid equivalent per g of the extract by comparing with gallic acid as standard.

#### Gas chromatography-mass spectrometry (GC-MS) analysis

In this method, 5 g of powdered Rue were soaked in methanol overnight and then filtered through a Whatman no. 41 filter paper. The filtrate was then concentrated by bubbling nitrogen gas into the solution. GC-MS analysis of the extract was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer equipped with an Elite-5MS fused silica capillary column. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.99 %) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 3 ml was employed (split ratio of 10:1). The injector temperature was at 250 °C and ion source temperature was adjusted at 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min) with an increase of 10 °C/min to 200 °C, then 5 °C/min to 280 °C ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV, a scan interval of 0.5 sec and fragments from 45 to 450 Da. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adapted to handle mass spectra and chromatograms was a TurboMass Version 5.2.0. Interpretation on mass-spectrum GC-MS was conducted using the database of National institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library and the molecular weight and structure of the compound.

#### Antimicrobial susceptibility test

Screening for antimicrobial activity of different solvent extracts of Rue was carried out by commonly known disc diffusion method accordingly with minor modification [17]. The sterile empty disc was prepared from Whatman no.1 filter paper of 6 mm in diameter. In two screw cap tube, 100 mg and 200 mg of each dried extract were dissolved in phosphate buffer saline (PBS). A 10 µl of the respective solvent of Rue extract was added to the discs (1 mg/disc and 2 mg/disc respectively) individually and aseptically. Discs were then allowed to dry in laminar air flow hood at room temperature, in order to maintain sterile condition. After drying, these discs were used for screening the antibacterial activity. In disc diffusion analysis, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37 °C for 18 h in an incubator. Agar plates of the Muller Hilton's Agar media were prepared. Each plate was then inoculated by swabbing with bacterial suspension or with 18 h fresh culture (10<sup>5</sup>-10<sup>6</sup> colony forming unit "CFU"/ml), which was swabbed evenly on the surface of solid agar media by the help of sterilized cotton swab. After 20 min, previously prepared discs of Whatman no. 1 paper with different extracts concentration were placed on the surface of agar medium. Standard antibiotic discs were used as a positive control to compare the antibacterial activity. All the plates were incubated at 37 °C for 24 h and then the diameter of the zone of inhibition was measured in mm. All types of equipment and media were proper labeled and sterilized before performing the experiments.

#### Statistical analysis

The phytochemicals and antimicrobial tests were conducted in triplicate and the results were expressed as mean±standard deviation (SD). Analysis of variance (ANOVA), Tukey's HSD, and least significant difference (LSD) tests were applied using Statistical Package for the Social Sciences (SPSS version 19.0, IBM Corp., Armonk, NY, USA) for comparisons of variance and means, and differences were considered significant at  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

#### Phytochemicals containing tannins and phenolic contents

Table 1 represents the qualitative analysis of phytochemicals present in the various solvent extract of Rue. The qualitative analysis of methanolic Rue extract showed the presence of more phytochemicals as compared with other solvent extracts of the leaf, stem and seed samples. The methanolic extract of the leaf also

showed the presence of high amount of tannin. However, seed extracts in all four solvent showed a very low amount of tannin (table 2). The previous study also supported the presence of

different phytochemicals viz. alkaloids, flavonoids, terpenoids, saponins and polyphenols present in different parts of plant extracts of Ruta plants [18-20].

**Table 1: Qualitative analysis of phytochemicals present in Rue with various solvent extract**

Phytochemical analysis of the plant using ethanolic extract												
Phytochemical constituents	Carbohydrate	Fatty acid	Proteins	Saponins	Tannins	Flavonoids	Alkaloids	Terpenoids	Glycosides	Carotinoide	Polyphenols	Amino acid
Leaf extract	-	-	-	-	+	+	+	+	+	+	-	+
Stem extract	-	-	-	-	+	+	+	+	+	+	+	+
Seed extract	-	-	-	-	+	+	+	+	+	-	-	+
Phytochemical analysis of the plant using methanolic extract												
Leaf extract	-	-	-	-	+	+	+	+	+	+	+	+
Stem extract	-	-	-	-	+	+	+	+	+	+	+	+
Seed extract	-	-	-	-	+	+	+	+	+	+	+	+
Phytochemical analysis of the plant using chloroform extract												
Leaf extract	-	-	-	-	+	+	+	+	+	-	-	+
Stem extract	-	-	-	-	+	+	+	+	+	-	-	+
Seed extract	-	-	-	-	+	+	+	+	+	-	-	+
Phytochemical analysis of the plant using aqueous extract												
Leaf extract	-	-	-	-	+	+	+	+	-	-	-	+
Stem extract	-	-	-	-	+	+	-	-	+	+	+	+
Seed extract	-	-	-	-	+	+	+	+	-	-	-	+

'+'-Present, '-'-Absent

**Table 2: Quantitative estimation of phenols and tannins in various solvent extracts of rue**

Tests	Solvent for extraction	Plant part		
		Leaf (%)	Stem (%)	Seed (%)
Estimation of Tannins	Ethanolic extract	15.15±0.30	15.25±0.41	4.25±0.51
	Methanolic extract	18.25±0.11	15.15±0.21	4.24±0.10
	Chloroform extract	3.10±0.10	2.25±0.32	0.20±0.05
	Aqueous extract	1.04±0.20	1.25±0.11	0.28±0.14
Estimation of Total Phenolic content	Ethanolic extract	15.62±0.01	12.25±0.15	4.25±0.11
	Methanolic extract	16.25±0.16	12.12±0.12	5.04±0.38
	Chloroform extract	15.28±0.12	15.25±0.08	2.15±0.21
	Aqueous extract	10.05±0.14	9.24±0.22	2.25±0.61

'±'-Standard Deviation

### Analysis of GC-MS profile

GC-MS analysis is an interesting tool to evaluate a number of active bio components used in cosmetic, pharmaceutical and food industry [21]. In the present study, we characterized the chemical profile of Rue using GC-MS. Fig. 1 represents the gas chromatogram showing the relative concentrations of various compounds eluted as a function of retention time. The height of peak indicates the relative concentrations of the components present in the plant. Mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to the appearance of peaks at different m/z ratio. These mass spectra are a fingerprint of that compound which can be identified from the data library. As per previous literature survey, this is the first report to analyze the chemical constituents of Rue by GC-MS. Table 3 represents the complex chemical composition present in Rue extract. Some of the GC-MS peaks remained unidentified because of lack of authentic samples and library data of corresponding compounds.

The methanolic extract of Rue has shown about 26 active phytochemicals which contribute to the pharmacological activity of plant. The major constituents were namely Cyclohexene, 3,4-diethenyl-3-methyl (peak area.21 %), Acetic acid, dec-2-yl ester (peak area 2.08 %), 2-Undecanone (peak area 0.58 %), Acetoxytetradecane (peak area 1.21 %), 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R) (peak area 0.60 %), Methoxybenzylamine, N,N-diethyl- $\alpha$ -methyl (peak area 0.65 %), 1,4-Naphthalenedione, 2-hydroxy-3-methyl (peak area 0.85 %),

1,3-Benzodioxole, 5-(2,2-dimethylethyl) (peak area 0.61 %), 4-Methoxy-benzoic acid cyclopentylidene-hydrazide,(peak area 0.49 %), Isopsoralen,(1.34), Ficusin (peak area 0.91 %), 3-Acetamido-4-hydroxy-2(1H)-quinolinone, (peak area 0.25 %), 4-Methyl-2-(phenylacetyl) phenol, (peak area 0.74 %); 7H-Furo(3,2-g) (1) benzopyran-7one, 4-methoxy, (peak area 10 %), 7H-Furo(3,2-g) (1) benzopyran-7one, 4-methoxy phytol, (peak area 1.38 %),4-Butoxy-2-hydroxybenzoxazole, (peak area 0.17 %); p-Dimethylaminobenzylidene p-anisidine (peak area 7.73 %),10H-Phenothiazine, 1-methoxy, (peak area 1.66 %), 6,8-Dimethyl-3-carbotheoxyquinoline, (peak area 3.33 %),2,2-Dihexylmalonic acid, diethyl ester; (peak area 0.25 %), Imindazo (2,1-b) thiazole,6-phenyl, (peak area 0.29 %), Furo[2,3-b]quinoline, 4,6,7-trimethoxy-(peak area 7.05 %), 2(1H)-Phenanthrenone, 3,4,4a,9,10,10a-hexahydro-6-methoxy-1,1,4a-trimethyl-7-(1-methylethyl)-(4aStrans,(53.47); 6-Azaester-1,3,5(10),6,8-pentaen-1,7-one,3-methoxy (peak area 0.55 %), 9(10H)-Acridinone, 1-hydroxy-2,3-dimethoxy-10-methyl (peak area 1.84 %) and Vitamin E (peak area 1.76 %). A previous study has reported the presence of several flavonoids, alkaloids, ketone and phenolic compounds in leaf, stem and fruit parts of Rue extract [22].

Due to the presence of various phytochemicals in the extract, rue plant has the potential application in various pharmaceutical industries. Alkaloids are well known for their potential and diverse pharmacological activities including analgesics, anti-malarial and anti-hypertensive. Flavonoids are renowned for anti-oxidant properties including anti-cancer and anti-inflammation [23]. Polyphenols are liable for antibiotic against microbial agents [24].

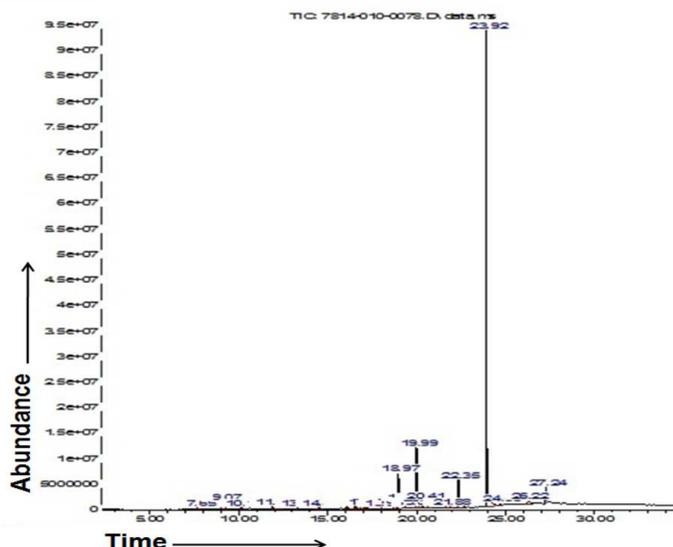


Fig. 1: Gas chromatography-mass spectroscopy chromatogram of rue plant

Table 3: Phytochemicals identified in the methanolic extract of Rue by GC-MS

S. No.	Retention time	Name of the compounds	Molecular formula	Mol.Wt.	Area%
1.	7.646	Cyclohexene, 3,4-diethenyl-3-methyl	C <sub>11</sub> H <sub>16</sub>	148	.21
2.	9.069	Acetic acid, dec-2-yl ester	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200	2.08
3.	10.173	2-Undecanone	C <sub>11</sub> H <sub>22</sub> O	170	0.58
4.	11.901	-Acetoxytetradecane	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	1.21
5.	13.281	: 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	180	0.60
6.	14.486	-Methoxybenzylamine, N,N-diethyl- $\alpha$ -methyl	C <sub>13</sub> H <sub>21</sub> NO	207	0.65
7.	16.055	1,4-Naphthalenedione, 2-hydroxy-3-methyl-	C <sub>11</sub> H <sub>8</sub> O <sub>3</sub>	188	0.85
8.	16.505	1,3-Benzodioxole, 5-(2,2-dimethylethyl)-		178	0.61
9.	16.592	4-Methoxy-benzoic acid cyclopentylidene-hydrazide	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	207	0.49
10.	16.883	Isopsoralen	C <sub>13</sub> H <sub>12</sub> O <sub>3</sub>	186	1.34
11.	17.042	Ficusin	C <sub>11</sub> H <sub>6</sub> O <sub>3</sub>	186	0.91
12.	18.015	3-Acetamido-4-hydroxy-2(1H)-quinolinone	C <sub>11</sub> H <sub>6</sub> O <sub>3</sub>	222	0.25
13.	18.727	4-Methyl-2-(phenylacetyl)phenol	C <sub>14</sub> H <sub>6</sub> O <sub>3</sub>	192	0.74
14.	18.974	7H-Furo(3,2-g)(1)benzopyran-7one, 4-methoxy	C <sub>11</sub> H <sub>16</sub> N <sub>2</sub> O	216	10
15.	19.293	7H-Furo(3,2-g)(1)benzopyran-7one, 4-methoxy phytol	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	210	1.38
16.	19.816	4-Butoxy-2-hydroxybenzoxonitrile	C <sub>14</sub> H <sub>12</sub> NO	92	0.17
17.	19.991	p-Dimethylaminobenzylidene p-anisidine	C <sub>4</sub> H <sub>14</sub> NO	254	7.73
18.	20.179	10H-Phenothiazine, 1-methoxy	C <sub>16</sub> H <sub>18</sub> N <sub>2</sub> O	303	1.66
19.	20.412	6,8-Dimethyl-3-carbotheoxyquinoline	C <sub>16</sub> H <sub>22</sub> NH <sub>3</sub>	161	3.33
20.	21.632	2,2-Dihexylmalonic acid, diethyl ester	C <sub>9</sub> H <sub>11</sub> N <sub>3</sub>	251	0.25
21.	21.879	Imindazo(2,1-b)thiazole,6-phenyl	C <sub>18</sub> H <sub>18</sub> NH <sub>3</sub>	179	0.29
22.	22.343	Furo[2,3-b]quinoline, 4,6,7-trimethoxy-	C <sub>11</sub> H <sub>3</sub> N <sub>2</sub> O	259	7.05
23.	23.912	2(1H)-Phenanthrene, 3,4,4a,9,10,10a-hexahydro-6-methoxy-1,1,4a-trimethyl-7-(1-methylethyl)-(4aStrans	C <sub>14</sub> H <sub>13</sub> O <sub>4</sub>	314	53.47
24.	24.696	6-Azaestra-1,3,5(10),6,8-pentaen-1,7-one,3-methoxy	C <sub>21</sub> H <sub>30</sub> O <sub>2</sub>	399	0.55
25.	26.221	9(10H)-Acridinone, 1-hydroxy-2,3-dimethoxy-10-methyl		285	1.84
26.	27.238	Vitamin E	C <sub>19</sub> H <sub>15</sub> NO <sub>6</sub>	430	1.76

### Antimicrobial susceptibility activity

Rue exhibited a broad spectrum of antibacterial activity as represented in table 4. Aqueous extract of Rue exhibited least antibacterial activity against the bacterial pathogens. Whereas, chloroform extract did not show any inhibitory effect against bacterial pathogens. This difference is attributed to the solubility of the active component in a different solvent [25]. The resistance observed by *B. subtilis* in methanolic extracts is more than ethanolic extract, while in the case of gram-negative bacteria *E. coli* was not sensitive to any of the extracts. While *C. albicans* showed resistance to most of the extracts used in this study except aqueous extract. A study has shown that increasing the dose of extracts from 100 mg/ml to 200 mg/ml, increased the susceptibility towards

microorganism. However, the methanolic extract of Rue has greater antimicrobial activity as compared to other solvent extracts. Various photo components present in Rue extract including Fatty acid, saponins, tannins, flavonoids, alkaloids, terpenoids, glycosides, carotenoids, polyphenols and tannins are secondary metabolites which serve a protective mechanism against various microorganisms [26]. Flavonoids are hydroxylated phenolic compounds which are responsible for protection against microbial infection [27]. Saponin leads to leakage of proteins and some enzymes from the microbial cell and thus responsible for antimicrobial activity [28]. A previous study has shown that flavonoids and phenolic components present in the plant extract have potential antimicrobial activity [29]. Hence, we could hypothesize that the antimicrobial activity of Rue plant might be due

to the presence of the above-mentioned phytoconstituents in the extract. Interestingly, some researchers reported the antibacterial

activity of Rue plant and its cytotoxic activity which also supported the present study [30, 31].

**Table 4: Antibacterial activity of true with diameter of the zone of inhibition**

Test organisms	Zone of Inhibition in diameter(mm) inhibition zone include the diameter of disc (06 mm)							
	Ethanolic extract		Methanolic extract		Chloroform extract		Aqueous extract	
Dose (mg/ml)	100	200	100	200	100	200	100	200
<i>B. subtilis</i>	16±0.35	17±0.30	18±0.17	20±0.08	-	-	07±0.22	09±0.43
<i>E. coli</i>	-	-	-	-	-	-	-	-
<i>P. vulgaris</i>	15±0.65	16±0.25	10±0.06	11±0.18	-	-	10±0.26	11±0.18
<i>C. abicans</i>	-	-	-	-	-	-	07±0.22	09±0.45
<i>C. tropicalis</i>	-	-	-	-	-	-	-	-
<i>M. lutees</i>	14±0.20	15±0.42	12±0.28	13±0.15	-	-	07±0.04	09±0.12

'±'-Standard Deviation, '-'-Absent

## CONCLUSION

In conclusion, the phytochemical analysis revealed the presence of various secondary metabolites namely flavonoids, alkaloids, terpenoids, saponins and carotenoid in Rue extract and approximately 26 phytoconstituents as analyzed by GC-MS study. This study also revealed the antibacterial properties of Rue extract, which can be used as antimicrobial agents in drugs development of infectious disease. Further research on this plant can pave the way to newer innovation in herbal medicine.

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## CONFLICT OF INTERESTS

No potential conflict of interest was reported by the authors

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