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GC-MS analysis of Methanolic Extracts of *Ruta Graveolens* L. for Bioactive Compounds.

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ABSTRACT

The present study was undertaken to identify the active principles in leaf, Stem and Root extracts of *Ruta graveolens* L. using gas chromatography-mass spectrometry (GC-MS). The plant material was extracted in methanol by using Soxhlet apparatus for 24 hours and the extracts were analyzed using GC-MS. The mass spectra of the compounds observed were matched with the National Institute of Standards and Technology (NIST) library. The results revealed the presence of 17, 12 and 6 chemical compounds in leaf, stem and root extracts respectively. The major chemical constituents were kokusaginine (39.9%), Bergaptene (25%),2-Undecanone (14.97%),1-(1,3-Benzodioxol-5-ylmethyl)-3-Nitro-1(12.69%),Pyrrole-3-carboxaldehyde,1-(4-methoxyph(9.36%).Some of these compounds have been reported to possess various bioactivities such as anti-microbial, anti-viral, anti-oxidative, anti-proliferative, anti-inflammatory, anti-tumour,etc. The study is a base to understand the richness of bioactive components in methanolic extracts from different parts of *Ruta graveolens* L. Their isolation would have potential applications in drug designing as well as in the medicine and healthcare industries.

Keywords: *Ruta graveolens*, active principles, Gas Chromatography-mass spectrometry (GC-MS).

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INTRODUCTION

Ruta graveolens L. known as Rue or Sudab or Sadab in Hindi is the most common medicinal plant of the family Rutaceae. The plant is indigenous to South Europe and North Africa and it grows on waste stony ground but is distributed throughout the world. It is an ornamental evergreen shrub of up to one meter tall and has considerable medicinal importance. Compounds mainly including acridone alkaloids, coumarines, essential oils, flavonoids, and furoquinolines have been found in the roots and aerial parts of the plant[1],and is the main source of furanocoumarins such as psoralen, xanthotoxin (8-methoxypsoralen; 8-MOP) and bergapten (5-methoxypsoralen; 5-MOP) [2]. Two species of Ruta (genus) are reported to grow in India, of which *Ruta graveolens*(garden rue) is well known for its aromatic and medicinal uses.[4] In traditional system of medicine it is used as stimulant, emmenagogue, diuretic and abortefacient, resolvent[3-12].

In European herbal medicine, rue has also been taken to treat conditions as varied as epilepsy, vertigo, poisoning and eye problems. Infact for the eye problems, an infusion is used as eyewash, brings quick relief to strained and tired eyes and reputedly improves the eye sight [14]. It causes uterine haemorrhage and inflammation of uterus [15]. The flavonoids are a part of primary chemical components of *Ruta graveolens* L. The most important analyzed flavonoids are rutin (quercetin-3-β- rutinoside) that belongs to flavonol glycoside. It acts as a venotonic and capillary protector. Rutin helps increase visual sharpness and benefits other visual problems, and it was used against edema, thrombo-genesis, inflammation, spasms, and hypertension [15]. Quercetin is other major flavonoid found in *Ruta graveolens* and can also be obtained by rutin hydrolysis [16].The essential oil is spasmolytic, anti-inflammatory and antihistaminic and is a vermifuge [17]. It also has a depressing effect on the central nervous system. At high doses it works as a narcotic poison, provoking violent intestinal inflammation, tongue and larynx tumefaction, excitement, followed by fatigue, vertigo, mental confusion, trembling, nephritis with uterine swelling and abortion, liver damage, intestine damage, and eventually death [15].Different chemical compounds in the plant material can be detected and analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). Hence, the present work was intended to identify the presence of bioactive principles in the Methanolic extracts of *Ruta graveolens* L. by GC-MS.

MATERIALS AND METHOD

Plant Material

The plants of *Ruta graveolens* L. were bought from Melghat forest located at 21°26′45″N 77°11′50″E in northern part of Amravati District of Maharashtra State in India and

were established in Botanical garden, Department of Botany, Sant Gadge Baba Amravati, University, Amravati.

Plant Sample Extraction

The collected *Ruta graveolens* leaves, stem, and root were washed several times with distilled water to remove the traces of impurities, dried at room temperature until constant weights and coarsely powdered with an electric grinder. 3 gram powder from each part was extracted with Methanol for 24 hours using Soxhlet apparatus and concentrated using water bath. The extracts were then filtered through Whatman filter paper No. 42 to obtain free and clear extract. This extract was then concentrated to 5 ml and stored in refrigerator.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC–MS analysis was carried on a Varian Gas Chromatograph series 3800 fitted with a VF-5 ms fused silica capillary column (60 m × 0.25 mm, film thickness 0.25 µm) coupled with a 4000 series mass detector under the following conditions: injection volume 1.0µl with split ratio 1:60, helium as carrier gas at 1.0 ml/min constant flow mode, injector temperature 280ºC at 3ºC, oven temperature was programmed from 60 to 280ºC at 3ºC/min. Mass spectra: electron impact (EI+) mode, 70 eV and ion source temperature 250ºC. Mass spectra were recorded over 50–500 a.m.u. range.

Identification of components

The Interpretation of mass spectra obtained by the GC-MS method was conducted using the database of National Institute of Standards and Technology (NIST) having more than 62,000 patterns. The spectrums of the unknown components from the samples were compared with the spectrum of the known components stored in the NIST library. Name, molecular weight and structure of the components of the tested materials were ascertained.

RESULTS AND DISCUSSION

Plants have tremendous ability to synthesize aromatic substances, especially phenols or their oxygen substituted derivatives. Most of the compounds synthesised are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defence mechanisms against, insects and herbivores. In the present study, the presence of several biologically important components from all the three different parts was revealed. It was observed that the *Ruta graveolens* L. on which scientific studies has been carried out are validated in their uses in various parts of world. The medicinal value of this plant lies in some chemical substances that have a definite physiological action on the human body. The

presence of many coumarins, alkaloids, terpenes, and flavonoids from *Ruta graveolens* has been already reported [19]. The components of *Ruta sp.* are of great interest in medicinal chemistry as these compounds show a broad range of biological activity and a number of them are exploited in the pharmaceutical industries. Alkaloids have been obtained from leaves and stem extracts of *R.graveolens* and are common constituents of other *Rutaceae* plants as well [20]. The concentration of alkaloids in stems is very less compared to the leaves. The level of alkaloids in plants varies with the changing seasons, age and location [21]. In addition, alkaloids like dictamnine and methoxy dictamnine from *R. graveolens* tissues are well known antimicrobial factors [22]. The biological effects of furanocoumarins and furoquinolone alkaloids makes them attractive for pharmaceutical uses, hence the considerable interest is shown in their availability and sources. Hence the plant is potential in medicinal industry.

Alkaloids are potential and diverse as analgesics, anti-malarial, anti-spasmodic, anti-hypertensive and treatment for mental disorders. Coumarins as medicinal candidates for drugs with strong pharmacological activity, low toxicity and side effects, fewer drug resistance, high bioavailability, broad spectrum, better curative effects, etc., to treat various types of diseases are being actively studied [23]. Linear furanocoumarins present in the *Ruta graveolens* L., particularly xanthotoxin, bergapten, and isopimpinelin, have been applied in the treatment of skin diseases characterized by excessive cell proliferation (e.g., psoriasis, mycosis fungoides) or in pigmentation disorders (e.g., vitiligo)[24] and also in neurology[25]. Polyphenols possess anti parasitic activity and monoterpenes have been reported to constitute anti-protozoa, anti-plasmodic, anti-neoplasmic, anti-helminthic and anti-viral activities.

The GC-MS analysis of leaf, stem and root extracts showed 17, 12 and 6 chemical compounds respectively. In leaf extract the highest relative peak area percentage of 39.46 was shown by kokusaginine (4, 6, 7-trimethoxyfuroquinoline) an alkaloid and lowest relative peak area percentage of 0.81 by 2-Methoxy-4-vinylphenol. In stem extract, highest peak area (%) of 30.22 was again obtained by kokusaginine and lowest 0.83 by Dodecyl acetate. Similarly in root extract the highest peak area (%) of 55.46. was obtained by a compound which could not be identified and lowest relative peak area percentage of 0.65 by Methyl laurate. The total number of compounds found in all three parts was 36 but some of the compounds are present both in leaf and stem which makes the total number of different compounds found in all the three extracts 25, though some of them are found in both the extracts but the relative percentage varies from extract to extract. In case of Kokusaginine relative percentage is higher in leaf as compared to stem while as the relative percentage of Bergaptene is more in stem extract than the leaf. Both of these compounds were

found absent in root extract. One of the compounds which was found in both leaf and stem extracts is chloropyriphos which is an insecticide and is not found in plants naturally. It might have percolated to plant as it was sprayed externally to avoid insects.

The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. Different phytochemicals which have been identified from extracts have been found to possess a wide range of activities, anti-microbial, anti-viral, anti-oxidative, anti-proliferative[26], anti-inflammatory, anti-tumour[27, 28]anti-platelet aggregation, estrogenic activity [29] among other with potential economic importance. The identification and isolation of these active compounds could lead to the new drug discovery at a cheaper cost which would be useful in medicine.

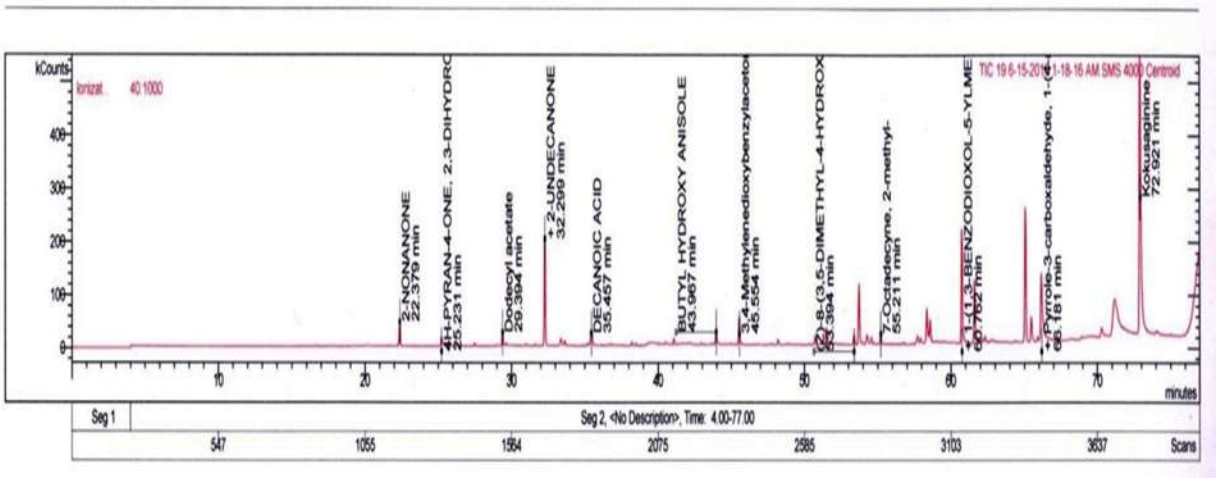


Figure 1: GC-MS chromatogram of the Methanolic extract of Leaf of *Ruta graveolens* L.

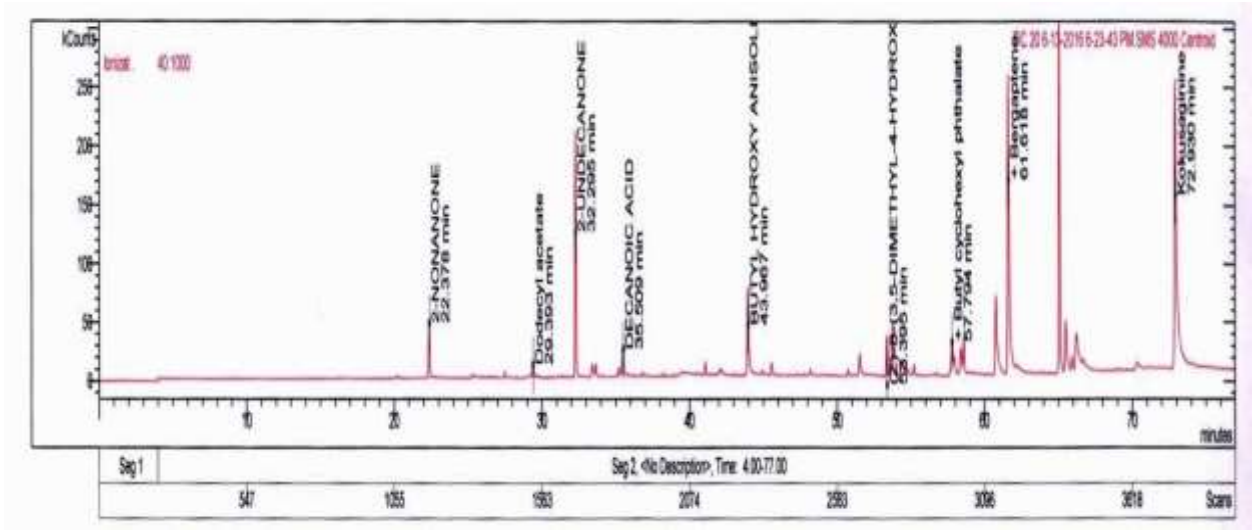


Figure 2: GC-MS chromatogram of the Methanolic extract of Stem of *Ruta graveolens* L.

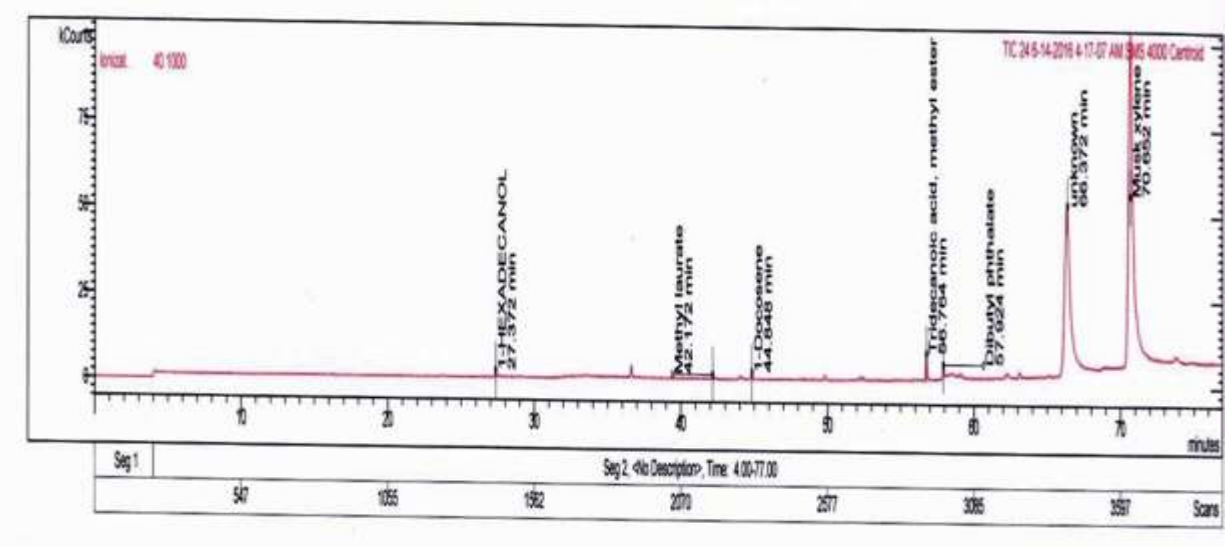
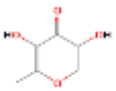
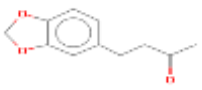
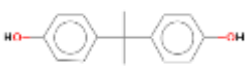
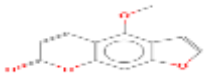
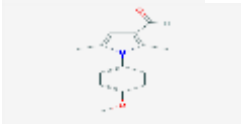
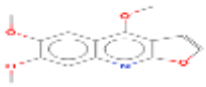


Figure 3: GC-MS chromatogram of the Methanolic extract of Root of *Ruta graveolens* L.

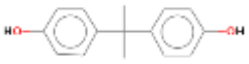
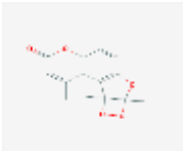
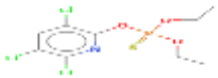
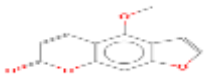
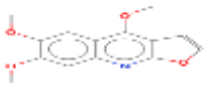
Table 1: Chemical Compounds identified in Methanol extract of leaf of *Ruta graveolens* L.

Peak No	RT	Name of Compound	Peak area %	MW	MF	Structure
1	22.37	2-Nonanone	2.12	142.23	$C_9H_{18}O$	
2	25.23	4H-pyran-4-one,2,3-dihydro-3,5-dihydrox	1.10	144.1253	$C_6H_8O_4$	
3	29.39	Dodecyl acetate	1.23	228.37	$C_{14}H_{28}O_2$	
4	32.29	2-Undecanone	9.60	170.29	$C_{11}H_{22}O$	
5	33.38	2-Methoxy-4-vinylphenol	0.81	150.17	$C_9H_{10}O_2$	
6	35.45	Decanoic acid	2.05	200.31	$C_{12}H_{24}O_2$	
7	43.96	Butyl hydroxyl anisole	1.20	180.24	$C_{11}H_{16}O_2$	
8	45.55	3,4Methylenedioxybenzylacetone	2.73	192.21	$C_{11}H_{12}O_3$	
9	53.39	(z)-8-(3,5-Dimethyl-4-hydroxyphenyl) -2-0	0.96	228.28	$C_{15}H_{16}O_2$	
10	55.21	7-octadecyne,2-methyl-	1.14	264.48	$C_{19}H_{36}$	
11	58.36	3H-[1,2] Dioxeto[3' ,4':4, 5]furo[3,2-f] 1]benzopyran-3-one,7a,9a-dihydro-1,7a,9a-trimethyl-	3.31	260.44	$C_{14}H_{12}O_5$	
12	58.58	Chlorpyrifos	1.84	350.58	$C_9H_{11}Cl_3NO_3PS$	

13	60.76	1-(1,3-Benzodioxol-5-ylmethyl)-3-Nitro-1	12.69	122.12	$C_7H_6O_2$	
14	61.62	Bergaptene	7.36	216.18	$C_{12}H_8O_4$	
15	65.48	7- (2,3-Dihydroxy-3-)-Methylbutoxy)-4,8-Dimethoxyfuro[2,3-β]Quinoline	2.96	229.00	$C_{13}H_{11}NO_3$	
16	66.18	Pyrrole-3-carboxaldehyde, 1- (4-methoxyph	9.36	229.27	$C_{14}H_{15N}O_2$	
17	72.92	kokusaginine	39.46	259.25	$C_{14}H_{13}NO_4$	

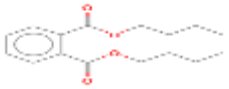
RT=Retention Time, MW=Molecular Weight, MF=Molecular Formula

Table 2: Chemical Compounds identified in Methanolic extract of stem of *Ruta graveolens* L.

Peak No	RT	Name of Compound	Peak area %	MW	MF	Structure
1	22.37	2-Nonanone	3.44	142.23	$C_9H_{18}O$	
2	29.39	Dodecyl acetate	0.83	228.37	$C_{14}H_{28}O_2$	
3	32.29	2-Undecanone	14.97	170.29	$C_{11}H_{22}O$	
4	35.50	Decanoic acid	2.11	200.31	$C_{12}H_{24}O_2$	
5	43.96	Butyl hydroxyl anisole	6.88	180.24	$C_{11}H_{16}O_2$	
6	53.39	(z)-8-(3,5-Dimethyl-4-hydroxyphenyl) -2-0	2.75	228.28	$C_{15}H_{16}O_2$	
7	57.79	Butyl cyclohexylphthalate	3.17	304.38	$C_{18}H_{24}O_4$	
8	58.38	3H-[1,2] Dioxeto[3' ,4':4, 5]furo[3,2-f] 1]benzopyran-3-one,7a,9a-dihydro-1,7a,9a-trimethyl-	1.68	260.44	$C_{14}H_{12}O_5$	
9	58.58	Chlorpyrifos	1.94	350.58	$C_9H_{11}Cl_3NO_3PS$	
10	60.77	1-(1,3-Benzodioxol-5-ylmethyl)-3-Nitro-1	6.95	122.12	$C_7H_6O_2$	
11	61.61	Bergaptene	25.00	216.18	$C_{12}H_8O_4$	
12	72.93	kokusaginine	30.22	259.25	$C_{14}H_{13}NO_4$	

RT=Retention Time, MW=Molecular Weight, MF=Molecular Formula

Table 3: Chemical Compounds identified in Methanol extract of Rootof *Rutagraveolens* L.

Peak No	RT	Name of Compound	Peak area %	MW	MF	Structure
1	27.37	1-Hexadecanol	0.86	242.44	$C_{16}H_{34}O$	
2	42.17	Methyl laurate	0.65	214.34	$C_{13}H_{26}O_2$	
3	44.84	1-Docosene	1.26	308.58	$C_{22}H_{44}$	
4	56.76	Tridecanoicacid,methyl ester	4.63	228.37	$C_{14}H_{28}O_2$	
5	57.92	Dibutyl phthalate	2.23	278.34	$C_{16}H_{22}O_4$	
6	66.37	unknown	55.46	-----	----------	----------
7	70.65	Musk xylene	34.88	297.26	$C_{12}H_{15}N_3O_6$	

RT=Retention Time, MW=Molecular Weight, MF=Molecular Formula

CONCLUSION

The present study would form a basis for understanding the richness of bioactive components in methanolic extracts in different plant parts of *Ruta graveolens L.* The abundance of these components and their isolation would have potential applications in drug designing as well as in the medicine and healthcare industries.

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