WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 12, 1195-1210. Research Article ISSN 2277– 7105

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS IN CALLUS EXTRACTS OF *RUTA GRAVEOLENS* L

Malik Aabid Hussain[1*], Varsha Nitin Nathar[1] and Javid Iqbal Mir[2]

[1]Plant Tissue Culture Laboratory, Department of Botany, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India.

[2]Central Institute of Temperate Horticulture (CITH), Rangreth, Srinagar, J and K India.

Article Received on
21 August 2017,
Revised on 11 Sept. 2017,
Accepted on 01 October 2017
DOI: 10.20959/wjpr201712-9733

***Corresponding Author**
Malik Aabid Hussain
Plant Tissue Culture Laboratory, Department of Botany, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India.

ABSTRACT

The production of plant secondary metabolites by in vitro culture is one of the most challenging and thrilling field of recent scientific researches. The tremendous increase in demand of phytochemicals by pharmaceutical companies has lead to over exploitation of medicinal plants. Therefore in present study an efficient protocol for callogenesis was developed by using various plant growth regulators and a detailed Gas chromatography- Mass Spectrometry (GC-MS) analysis was carried out to identify the presence of bioactive principles in the callus extracts by using solvents with different polarities. The different explants of *R.graveolens* were cultured on MS medium with various concentrations of auxins (Indole Butyric Acid (IBA), 2, 4-Dichlorophenoxy acetic acid (2, 4-D) and Napthalene Acetic Acid (NAA) and cytokinins (Benzyl Amino Purine (BAP) and Kinetin) singly and in combinations for callus induction. Among them 2, 4-D (1.5 mg/l) +NAA (1.5 mg/l) responded well by giving the maximum percentage of callus induction (97.22±2.54). For the extraction of secondary metabolites 5-6 weeks old yellow compact callus was dried at room temperature and extracted with organic solvents through soxhlet extractor. The GC-MS analysis revealed the presence of various bioactive constituents with a wide range of biological activities (anti-microbial, anti-viral, anti-oxidative, anti-proliferative, photobiological, anti-inflammatory, anti-tumou, anti-platelet aggregation).

KEYWORDS: Callus, GC-MS, *Ruta graveolens*, Bioactive compounds.

1. INTRODUCTION

Ruta graveolens L. commonly known as bitter herb or garden rue is an important medicinal plant of family Rutaceae, Native to Europe, specially the Mediterranean region, but widely distributed in the temperate and tropical regions. It is grown for ornamental and medicinal purposes. It is a reservoir of different plant constituents belonging to four major chemical classes which may be present in variable amounts in the aerial parts of the plant, namely Alkaloids of quinoline and quinolinone(graveoline), furoquinoline (dictamine, skimmianine), pyrano-quinoline (rutalinium) and acridone(funacridone); Coumarins, like dicoumarins, furacoumarins (bergaptene), pyranocoumarins; Flavonoids (rutin) and essential oils [1]. *R. graveolens* has been used since ancient times for the relief of pain, eye problems, rheumatism and dermatitis.[2] The furanocoumarins like 5-methoxypsoralen (bergapten), 8-methoxypsoralen (xanthotoxin) and 5,8- dimethoxypsoralen (isopimpinellin) are currently used for the treatment of skin deseases like vitiligo and psoriasis.[3] Its extracts posses potent anti-inflammatory and anti-cancer activity.[4,5,6] *R.graveolens* has also shown antibacterial, analgesic, anti-inflammatory,antidiabetic and insecticidal activities. *R. graveolens* extracts have also been used as an antidote for toxins such as snake and scorpion venom.[7]

Due to the numerous biological activities, this plant has become medicine in many countries especially in Mediterranean region. A number of studies have been carried out to investigate different pharmacological activities due to the wide application of this plant in traditional medicine. The tremendous increase in the demand of these phytochemicals has lead to over exploitation of medicinal plants. The biotechnological approach by utilizing plant cell tissue and organ culture technology is an efficient and useful tool for the preservation of endangered species[8], while providing an alternative source for the production of phytochemical products [9]. Gas Chromatography/Mass Spectrometry (GC/MS) can be used to detect and analyse the wide variety of chemical compounds in plant materials. The GC-MS instrument separates chemical mixtures and identifies the components at a molecular level [10]. Therefore the present work was aimed to standardise the protocol for callogenesis and carry out a detailed GC-MS analysis to identify the presence of bioactive compounds in the callus extracts of the plant by using solvents with different polarities.

2. MATERIALS AND METHODS

2.1. 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. Different explants like leaves, Nodes and internodes were obtained from *Ruta graveolens* L.

2.2. Callus Induction

The explants were washed with running tap water followed by soaking in 2% Tween 20 solution for 7 min. After washing, explants were surface sterilized with (0.1%) mercuric Chloride for 3 min which was followed by dipping of explants in 70% ethyl alcohol for 2 minutes. Then explants were washed with distilled water 3 times in order to remove the traces of surface sterilants. The sterilized explants were inoculated on full strength Murashige and Skoog's, (1962) basal medium (MS medium) supplemented with different concentrations and combinations of growth regulators such as 2, 4-D, 2, 4-D +NAA NAA+BAP and BAP+IBA containing 3% (w/v) sucrose and 0.8% (w/v) agar. The pH of the medium was adjusted to 5.8 and it was autoclaved at 121ºC under 15 psi for 20 minutes. Cultures were incubated at 25 ±2ºC under 16 hours photoperiod and 55±5% relative humidity for callus induction.

2.3. Growth measurement

The growth measurement of callus was determined by following the method as described by.[11] Growth of the callus and its biomass was measured in terms of fresh (FW g/l) and dry weight (DW g/l). Fresh weight of callus was measured after removing the excess moisture and agar adhering to the callus surface using blotting paper. Dry weight of callus was determined by drying the callus in hot air oven at 40ºC until constant weights were achieved and was expressed in g/l DW.

2.4. Callus extraction

The collected *Ruta graveolens* callus was washed several times with distilled water to remove the traces of media, dried at room temperature and coarsely powdered. The powder was extracted with Methanol, Chloroform and Petroleum ether (3gram in each solvent) using Soxhlet apparatus for 48 hours and concentrated using waterbath. These three 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 until used. In all experiments, the chemicals used were of analytical grade (HiMedia,SD Fine Chemicals India; and Sigma-Aldrich, USA).

2.5. Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis of three callus extracts was carried out using gas chromatography – high resolution mass spectrophotometer. 2 µl of each sample was employed for GC – MS analysis. The GC-MS analysis was carried out using Alegent Hp 7880 with column of 30 meter length, 0.25 mm ID, and 0.32 thicknesses. Helium gas was used as carrier gas at constant flow rate of 1ml/ minute. Injector temperature was set at 100ºC. The oven temperature were programmed from 50ºC to 280ºC at 10ºC/ minute to 200ºC then10ºC/3 minutes to 250ºC ending with a 5 minutes isothermal at 280 º C. The sample was injected in split mode as 50:1.

2.6. Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

3. RESULTS AND DISCUSSION

Green plants are a treasure of effective chemicals, therapeutants and can provide valuable sources of natural pesticides. During the present study, it was observed that the *Ruta graveolens* L. has been worked out in various parts of the world. The pharmaceutical value of this plant is because of some chemical substances that have a definite physiological action on the human body. Different concentrations of plant growth regulators had a significant effect on callus regeneration. Leaf, nodal and internodal explants of *Ruta graveolens* were cultured on MS media fortified with 2, 4-D, 2, 4-D+NAA, NAA+BAP, and BAP +IBA in different concentrations to test the efficiency of sprouting of various explants. It was observed that after 9–12 days of inoculation, the explants showed callus initiation in all the concentrations. The nodal explants were the first to show callus initiation. The callogenic response showed variations among the hormonal combinations/concentrations and types of explants used. The callus response varied from 52.75±0.45 to 79.40±1.70 in leaf explants as shown in Figure.1 (A, B and C), 63.30±0.80 to 97.20±1.25 in nodal explants as shown in Figure.1(D, E and F) and 54.85±0.80 to 90.00±0.80 in internodal explants as shown in Figure.1 (G and H). Among different types of explants used, nodal segments proved to be best for callus induction.

Highest callogenic response, i.e. 97.20±1.25, was noticed with nodal explants at 1.5 mg/L 2, 4-D+1.5 mg/L NAA and lowest callogenic response i.e. 52.75±0.45 was observed in leaf explants at 2, 4-D 3.0 mg/l (Table 1, Figure 1and 2). A total of 14 combinations of auxins and cytokinins were tried for callus biomass production. The hormone combination for callus biomass production was standardized, and the callus biomass was 146.31 g/L FW and 20.57 g/L DW in MS solid medium.

The production of plant bioactive compounds by *in vitro* culture is one of the most challenging fields of recent scientific researches. For the last few years, the world has witnessed an increased demand of phytochemicals. Therefore, in vitro plant protocols has to be carefully established and monitored by phytochemical investigation of their selected extracts in order to supply standardized raw material. The search for new plant derived chemicals should thus be a priority in current and future efforts toward sustainable conservation and rational utilization of biodiversity.[12] The identification of the phytocompounds was carried out based on the retention time and molecular formula. The names of identified compounds in the different callus extracts of *Ruta graveolens* L. with their retention time (RT), molecular formula (MF), molecular weight (MW) and peak area percentage has been given in the Tables.

The highest peak area (%) of 36.26 and the lowest peak area (%) of 0.04 in chloroform extract of callus was obtained by 9(10H)-Acridinone, 1-hydroxy-3-methoxy-10-methyl (RT 31.57) and Ethane, hexachloro (RT 5.38) respectively. The detailed table of GC-MS analysis has been given in Table 2. The total ion chromatograph (TIC) showing the peak identities of the compounds identified have been given in Figure 3. Likewise the highest peak area (%) of 31.68 and the lowest peak area (%) of 0.43 in Methanolic extract of Callus was obtained by 1H-Imidazo[4,5c]pyridine,2-(3,4-dimethoxyphenyl) (RT 31.82) and Octadecanoic acid (RT 22.43) respectively. The detailed table of GC-MS analysis has been given in Table 3. The total ion chromatograph (TIC) showing the peak identities of the compounds identified have been given in Figure 4.Similarly,the highest peak area (%) of 31.62 and the lowest peak area (%) of 0.34 in Petroleum ether extract of Callus was obtained by α-D-xylofuranose, cyclic 1,2:3,5-bis(butylboronate) (RT 34.41) and 3-Acetoxydodecane (RT 18.95) respectively. The detailed table of GC-MS analysis has been given in Table 4. The total ion chromatograph (TIC) showing the peak identities of the compounds identified have been given in Figure 5. Also, some of the compounds remain unidentified with total peak area percentage of 1.65

(chloroform extract), 3.29 (methanolic extract) and 10.87 (petroleum ether extract). The total number of compounds found in three extracts was 66 but some of them were similar so the total number of different compounds found in all the three extracts was 51.

The gas chromatogram shows the relative concentrations of various compounds getting eleuted 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[13], anti-inflammatory, anti-tumor[14,15], anti-platelet aggregation, estrogenic activity[16] among others 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.

Table 1: Callus induction from Leaf, Nodal and Internodal explants at different concentrations and combinations of plant growth regulators.

		Leaf explant	**Nodal explants**	**Internodal explants**
PGR	**Concentration (mg/l)**	**RP (%)**	**RP (%)**	**RP (%)**
2,4-D	1.5	63.33±1.66	90.11±1.66	71.66±1.66
	2.0	75.55±0.96	86.11±2.54	86.11±2.54
	2.5	55.00±1.66	71.66±1.66	68.33±1.66
	3.0	55.00±1.66	68.33±1.66	63.33±1.66
2,4-D +NAA	1.0+1.0	52.77±0.96	68.33±1.66	63.33±1.66
	1.5+1.5	75.55±0.96	97.22±2.54	90.00±1.66
	1.5+1.0	68.33±1.66	86.11±2.54	83.44±1.01
NAA+BAP	1.5+2.5	68.33±1.66	88.88±0.96	75.55±0.96
	2.0+2.0	71.66±1.66	90.00±1.66	71.66±1.66
	2.5+1.5	78.11±1.26	86.11±2.54	75.55±0.96
	2.5+2.0	63.33±1.66	90.11±1.01	71.66±1.66
BAP+IBA	1.0+0.5	55.00±1.66	83.44±1.01	71.66±1.66
	1.0+2.0	68.33±1.66	92.22±1.89	83.44±1.01
	2.0+1.0	55.00±1.66	63.33±1.66	55.00±1.66

Values are mean ± SD of three replicates.

PGR = Plant growth regulator; Conc. = concentration, RP = response percent

Table 2: Chemical Compounds identified in Chloroform extract of Callus of *Ruta graveolens* L.

Peak No	RT	Name of Compound	Peak area %	MW	MF
1	4.73	Benzyl Alcohol	2.58	108	C_7H_8O
2	5.38	Ethane,hexachloro	0.04	236	C_2Cl_6
3	12.80	Phenol,2,4-bis(1,1-dimethylethyl)-	0.79	206	$C_{14}H_{22}O$
4	13.92	8-Oxabicyclo[5.1.0]octane	1.05	112	$C_7H_{12}O$
5	16.75	1-Nonadecene	0.86	266	$C_{19}H_{38}$
6	19.24	Dictamnine	1.40	199	$C_{12}H_9NO_2$
7	19.60	n-hexadecanoic acid	8.29	256	$C_{16}H_{32}O_2$
8	20.96	Heptadecanoic acid	1.47	270	$C_{17}H_{34}O_2$
9	21.19	Xanthotoxin	0.61	216	$C_{12}H_8O_4$
10	21.64	Barbituric acid,5-allyl-5-Phenyl- (Alphenal)	5.39	244	$C_{13}H_{12}N_2O_3$
11	21.99	2H-Benzopyran-2-one,3-(1,1-dimethyle-2-propenyl)-7-hydroxy-6-methoxy	4.64	260	$C_{15}H_{16}O_4$
12	22.83	Hexanenitrile, 3-methyl-	6.32	111	$C_7H_{13}N$
13	23.15	Octadecanoic acid	1.22	284	$C_{18}H_{36}O_2$
14	23.36	1,3-Bis(trimethylsiloxy) benzene	1.80	254	$C_{12}H_{22}O_2Si_2$
15	23.65	Phenyl cyanide,2,4,6-triisopropyl-	6.80	229	$C_{16}H_{23}N$
16	24.09	Pyrrole-3-carboxaldehyde,1-(4-methoxyphenyl)-2,5-dimethyl-	1.92	229	$C_{14}H_{15}NO_2$
17	24.40	Isopimpinellin	3.48	246	$C_{13}H_{10}O_5$
18	26.57	Indolin-3-one, 2, 2-di-t-butyl-1-methyl-	1.57	259	$C_{17}H_{25}NO$
19	27.15	Furo[2,3-b]quinoline, 4,6,8 trimethoxy-	1.85	259	$C_{14}H_{13}NO_4$
20	27.31	4-(1,1-Dimethylallyl)-9-methoxy-7H-furo [3, 2-g] [1] benzopyran-7-one	1.97	284	$C_{17}H_{16}O_4$
21	27.66	Benzene, 5-isothiocyanato-1, 2, 3-trimethoxy-	5.80	225	$C_{10}H_{11}NO_3S$
22	29.26	Cholest-5-en-3-ol, 24-propylidene-, (3β)	0.86	426	$C_{30}H_{50}O$
23	31.57	9(10H)-Acridinone,1-hydroxy-3-methoxy-10-methyl-	36.26	255	$C_{15}H_{13}NO_3$
24	32.27	Propanamide,N-(n-decy)l-n-methyl-2,2-dimethyl	0.30	255	$C_{16}H_{33}NO$
25	36.23	Acetamide,N-ethenyl-N-methyl-	0.94	99	C_5H_9NO

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

Table 3: Chemical Compounds Identified in Methanolic extract of Callus of *Ruta graveolens* L.

Peak No.	RT	Name of Compound	Peak area %	MW	MF
1	3.33	2-Furancarboxaldehyde,5-methyl-	0.99	110	C_6H_6O2
2	18.24	Dictamnine	0.51	199	$C_{12}H_9NO_2$
3	18.44	n-hexadecanoic acid	2.75	256	$C_{16}H_{32}O_2$
4	19.88	Heptadecanoic acid	0.48	270	$C_{17}H_{34}O_2$
5	20.63	Xanthotoxin	2.24	216	$C_{12}H_8O_4$
6	21.04	2H-1-Benzopyran-2-one,3-(1,1-dimethyl-2-propenyl)-7-hydroxy-6-methoxy	2.57	260	$C_{15}H_{16}O_4$
7	21.93	1-Hexene,3-methyl-	2.84	98	C_7H_{14}

8	22.33	Octadecanoic acid	0.43	284	$C_{18}H_{36}O_2$
9	22.93	Phenyl cyanide,2,4,6-triisopropyl-	3.71	229	$C_{16}H_{23}N$
10	23.43	Pyrrole-3-carboxaldehyde,1-(4-methoxyphenyl)-2,5-dimethyl-	0.75	229	$C_{14}H_{15}NO_2$
11	23.77	Isopimpinellin	1.56	246	$C_{13}H_{10}O_5$
12	24.64	5,8-Dimethoxy-2,3,10,10a-tetrahydro-1H,4aH-phenanthrene-4,9-dione	0.87	274	$C_{16}H_{18}O_4$
13	25.06	2-Methyl-3-heptyl-4-[ethoxycarbonyloxy]-7-methoxyquinoline	0.71	359	$C_{21}H_{29}NO_4$
14	26.12	2-Butanone,4-(2,6-dimethyl-4-mercapto-3-quinolyl)-	0.57	259	$C_{15}H_{17}NOS$
15	26.41	1H-Indazol-4-amine,1-(4-fluorophenyl)-4,5,6,7-tetrahydro-6,6-dimethyl-	0.63	259	$C_{15}H_{18}FN_3$
16	27.19	Furo[2,3-b]quinoline, 4,6,8 trimethoxy-	2.52	259	-$C_{14}H_{13}NO_4$
17	27.70	9(10H)-Acridone,1-hydroxy-10-methyl	2.98	225	$C_{14}H_{11}NO_2$
18	28.15	Campesterol	8.24	400	$C_{28}H_{48}O$
19	28.53	Phorbol	1.05	364	$C_{20}H_{28}O_6$
20	30.40	3,5-Diacetyl-4-(2-furyl)-1-phenyl-1,4-dihydropyridine	28.09	307	$C_{19}H_{17}NO_3$
21	31.82	1H-Imidazo[4,5c]pyridine,2-(3,4-dimethoxyphenyl)-	31.68	256	$C_{14}H_{13}N_3O_2$
22	35.58	Stigmasta-3,5-diene-7-one	1.12	410	$C_{29}H_{46}O$

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

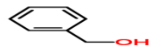
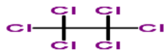
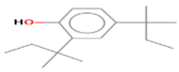
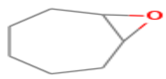
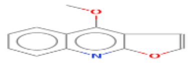
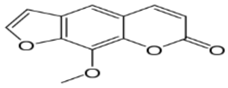
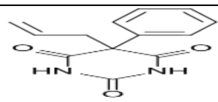
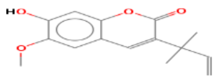
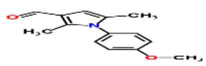
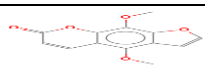
Table 4: Chemical Compounds Identified in Petroleum ether extract of Callus of *Ruta graveolens* L.

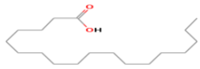
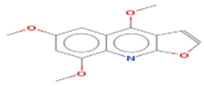
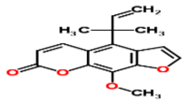
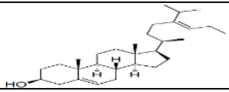
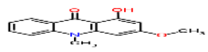
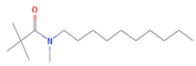
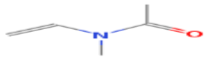
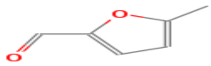
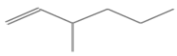
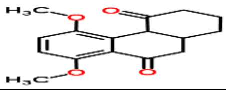
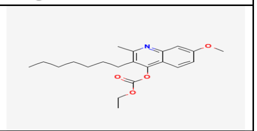
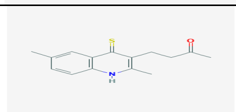
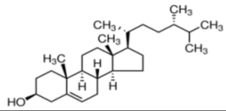
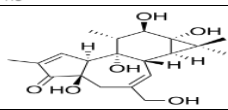
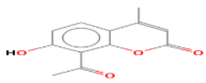
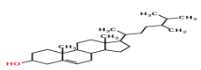
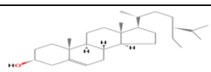
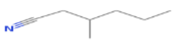
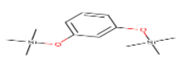
Peak No	RT	Name of Compound	Peak area %	MW	MF
1	3.31	Cyclopropane,1,1,2,3-tetramethyl-	1.18	98	C_7H_{14}
2	3.68	3-tert-Butyl-5-chloro-2-hydroxybenzophenone	0.47	288	$C_{17}H_{17}ClO_2$
3	16.85	1,3-Dioxolane, 2-phenyl-	0.92	150	$C_9H_{10}O_2$
4	18.44	Benzofuran-2-one,4-amino-2,3-dihydro	5.03	149	$C_8H_7NO_2$
5	18.95	3-Acetoxydodecane	0.34	228	$C_{14}H_{28}O_2$
6	20.66	Coumarin,8-allyl-7-hydroxy-6-ethyl-4-methyl-	2.41	244	$C_{15}H_{16}O_3$
7	21.03	2H-1-Benzopyran-2-one,3-(1,1-dimethyl-2-propenyl)-7-hydroxy-6-methoxy	1.11	260	$C_{15}H_{16}O_4$
8	21.91	5-Hexenenitrile, 2-methyl-	1.68	109	$C_7H_{11}N$
9	22.60	Cyclopropa[3,4]cyclohepta[1,2-a]napthalen-10-ol,1,1a,1b,2,3,7b,8,9,10,10a-decahydro-5-methoxy-10-methyl-	0.67	272	$C_{18}H_{24}O_2$
10	23.07	Propanamide,N-(1-ethyl-1,2,3,4-tetrahydro-2,2,4-trimethyl-7-quinolinyl)-	1.78	274	$C_{17}H_{26}N_2O$
11	23.78	Isopimpinellin	1.57	246	$C_{13}H_{10}O_5$
12	25.29	Campesterol	0.90	400	$C_{28}H_{48}O$
13	25.66	Ergost-5-en-3-ol, (3β)	1.86	400	$C_{28}H_{48}O$
14	27.96	3,5-Diacetyl-4-(2-furyl)-1-phenyl-1,4-dihydropyridine	11.14	307	$C_{19}H_{17}NO_3$

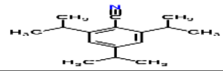
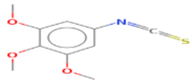
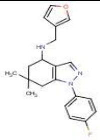
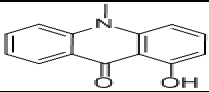
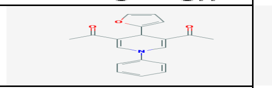
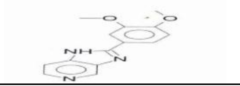
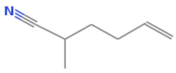
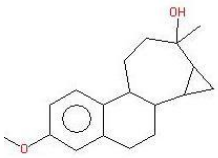
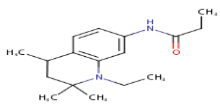
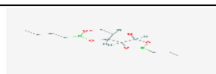
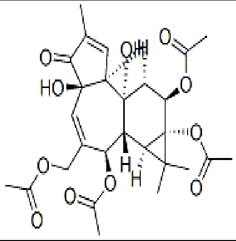
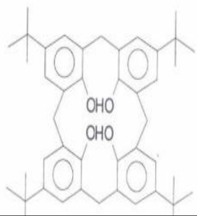
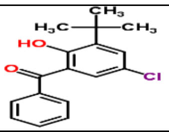
15	30.70	γ-Sitosterol	4.98	414	$C_{29}H_{50}O$
16	31.71	1H-Imidazo[4,5-c]pyridine,2-(3,4-dimethoxyphenyl)-	1.02	255	$C_{14}H_{13}N_3O_2$
17	34.41	α-D-xylofuranose,cyclic 1,2:3,5-bis(butylboronate)	31.62	281	$C_{13}H_{24}B_2O_5$
18	35.55	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,2,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-4a,7b-dihydro	1.21	548	$C_{28}H_{36}O_{11}$
19	37.10	Pentacyclo[19.3.1.1(3,7).1(9,13).1(15,19)]octacosa1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-25,26,27,28-tetrol, 5,11,17,23-tetrakis(1,1-dimethylethyl)	19.13	648	$C_{44}H_{56}O_4$
20	38.89	Unidentified	0.69	-	-

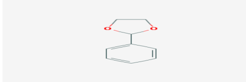
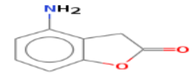
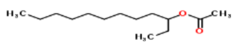
RT=Retention Time, MW=Molecular Weight, MF=Molecular Formula

Table 5: Summery of the Chemical Compounds identified in Callus extracts of *Ruta graveolens* L.

Peak No	Name of Compound	Nature of compound	Structure
1	Benzyl Alcohol	Aromatic alcohol	
2	Ethane,hexachloro	Hydrocarbon	
3	Phenol,2,4-bis(1,1-dimethylethyl)-	Phenol	
4	8-Oxabicyclo[5.1.0]octane	Diazepine epoxide	
5	1-Nonadecene	Alkane hydrocarbon	
6	Dictamnine	furoquinoline alkaloid	
7	n-hexadecanoic acid	Palmitic acid	
8	Heptadecanoic acid	Fatty acid	
9	Xanthotoxin	furanocoumarin	
10	Barbituricacid,5-allyl-5-Phenyl-	Barbiturate	
11	2H-Benzopyran-2-one,3-(1,1-dimethyle-2-propenyl)-7-hydroxy-6-methoxy	Quinazoline alkaloids	
12	Pyrrole-3-carboxaldehyde,1-(4-methoxyphenyl)-2,5-dimethyl-	Aldehyde	
13	Isopimpinellin	Coumarin	

14	Octadecanoic acid	Fatty acid	
15	Furo[2,3-b]quinoline, 4,6,8 trimethoxy-	furoquinoline alkaloid	
16	4-(1,1-Dimethylallyl)-9-methoxy-7H-furo [3, 2-g] [1] benzopyran-7-one	Furanocoumarin	
17	Cholest-5-en-3-ol, 24-propylidene-, (3β)	Steroid	
18	9(10H)-Acridinone,1-hydroxy-3-methoxy-10-methyl-	Acridone alkaloids	
19	Propan amide,N-(n-decy)l-n-methyl-2,2-dimethyl	Amide	
20	Acetamide,N-ethenyl-N-methyl-	Amide	
21	2-Furancarboxaldehyde, 5-methyl-	Aldehyde	
22	1-Hexene, 3-methyl-	Ester	
23	5,8-Dimethoxy-2,3,10,10a-tetrahydro-1H,4aH-phenanthrene-4,9-dione	Anthraquinone	
24	2-Methyl-3-heptyl-4-[ethoxycarbonyloxy]-7-methoxyquinoline	Quinolone	
25	2-Butanone,4-(2,6-dimethyl-4-mercapto-3-quinolyl)-	Quinolone	
26	Campesterol	Steroid	
27	Phorbol	Diterpene	
28	Stigmasta-3,5-diene-7-one	Steroid	
29	Coumarin, 8-allyl-7-hydroxy-6-ethyl-4-methyl-	Coumarin	
30	Ergost-5-en-3-ol, (3β)	Steroid	
31	γ-Sitosterol	Steroid	
32	Hexanenitrile, 3-methyl-	Others	
33	1,3-Bis(trimethylsiloxy) benzene	-	

34	Phenyl cyanide,2,4,6-triisopropyl-	-	
35	Indolin-3-one, 2, 2-di-t-butyl-1-methyl-	-	
36	Benzene, 5-isothiocyanato-1, 2, 3-trimethoxy-	-	
37	1H-Indazol-4-amine,1-(4-fluorophenyl)-4,5,6,7-tetrahydro-6,6-dimethyl-	-	
38	9(10H)-Acridone,1-hydroxy-10-methyl	-	
39	3,5-Diacetyl-4-(2-furyl)-1-phenyl-1,4-dihydropyridine	-	
40	1H-Imidazo[4,5c]pyridine,2-(3,4-dimethoxyphenyl)-	-	
41	5-Hexenenitrile, 2-methyl-	-	
42	Cyclopropa[3,4]cyclohepta[1,2-a]napthalen-10-ol,1,1a,1b,2,3,7b,8,9,10,10a-decahydro-5-methoxy-10-methyl-	-	
43	Propanamide,N-(1-ethyl-1,2,3,4-tetrahydro-2,2,4-trimethyl-7-quinolinyl)-	-	
44	α-D-xylofuranose,cyclic 1,2:3,5-bis(butylboronate)	-	
45	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,2,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-4a,7b-dihydro	-	
46	Pentacyclo[19.3.1.1(3,7).1(9,13).1(15,19)]octacosa1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-25,26,27,28-tetrol,5,11,17,23-tetrakis(1,1-dimethylethyl)	-	
47	Cyclopropane, 1, 1, 2, 3-tetramethyl-	-	
48	3-tert-Butyl-5-chloro-2-hydroxybenzophenone	-	

49	1,3-Dioxolane, 2-phenyl-	-	
50	Benzofuran-2-one,4-amino-2,3-dihydro	-	
51	3-Acetoxydodecane	-	

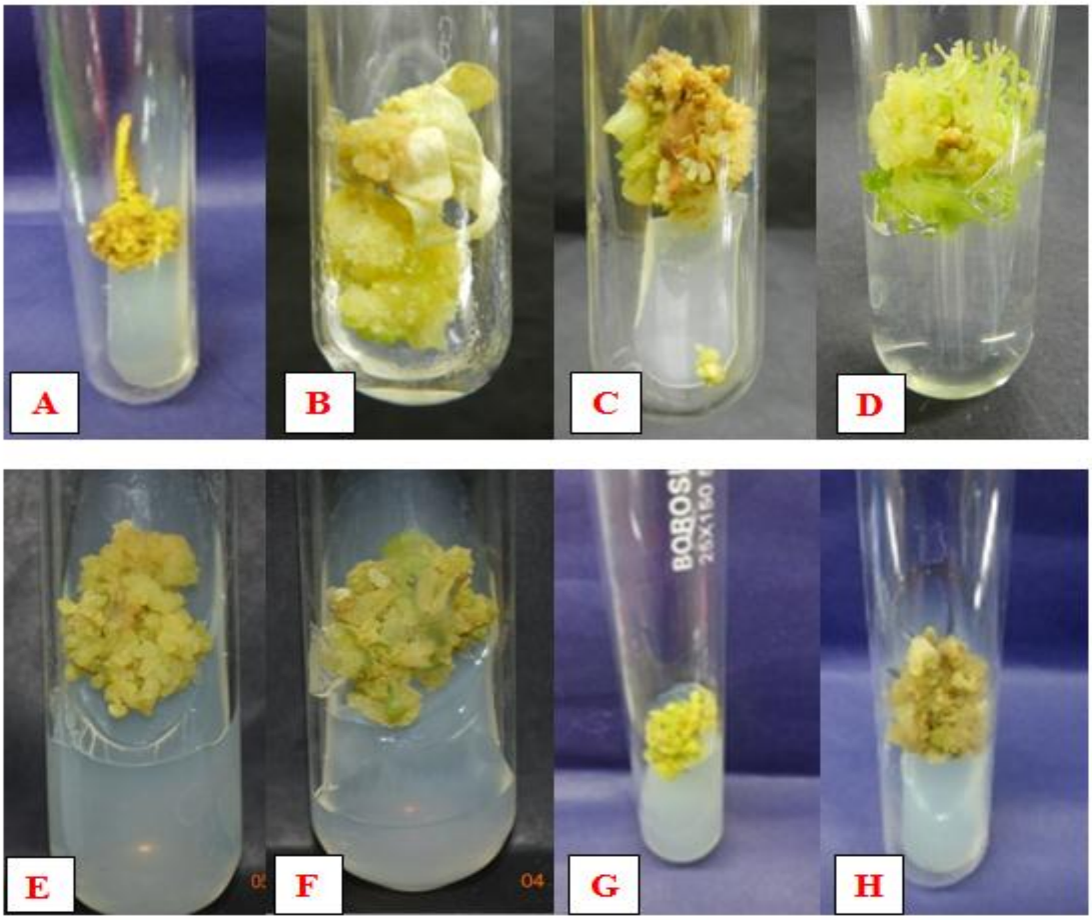


Figure.1. Callogenic response to different plant growth regulators using various explants of *Ruta graveolens L.*

A, B and C = Callogenic response from leaf explants at 2, 4-D (3.0) mg/l, 2, 4-D + NAA (1.5+1.5)mg/l and BAP+IBA (1.0+0.5)mg/l respectively.

D, E and F = Callogenic response from nodal explants at 2, 4-D+NAA (1.5+1.5)mg/l, NAA+BAP (1.5+2.5) mg/l and BAP+IBA (2.0+1.0)mg/l respectively.

G and H = Callogenic response from internodal explants at BAP+IBA (2.0+1.0)mg/l and 2, 4-D (2.0) mg/l respectively.

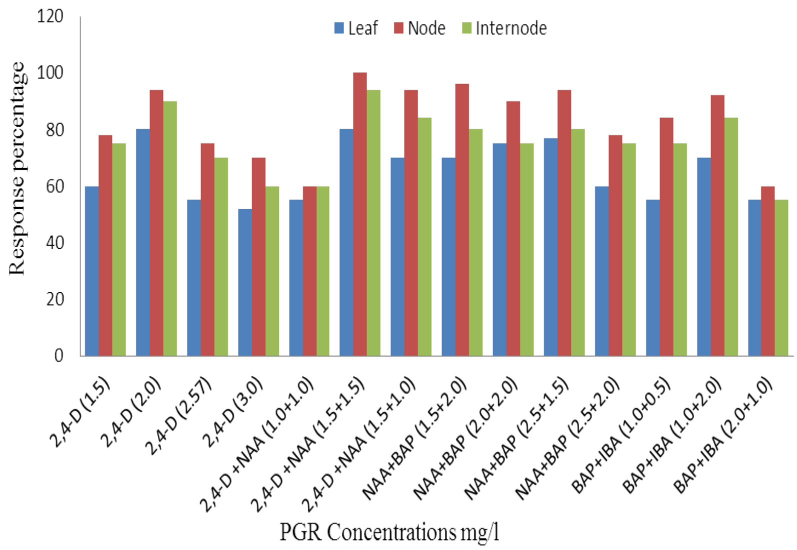


Figure 2. Callogenic response from Leaf, Nodal and Internodal explants.

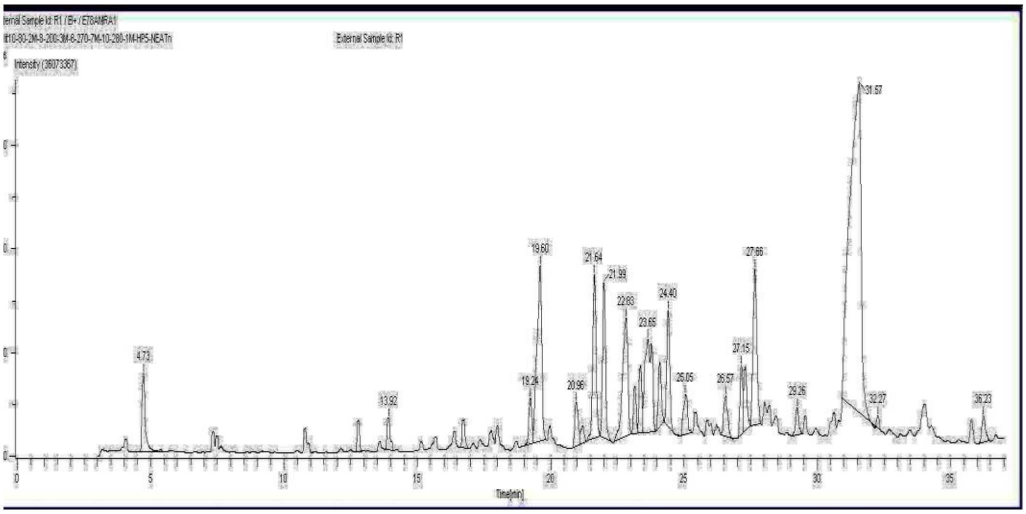


Figure. 3: GC-MS chromatogram of the chloroform extract of Callus of *Ruta graveolens* L.

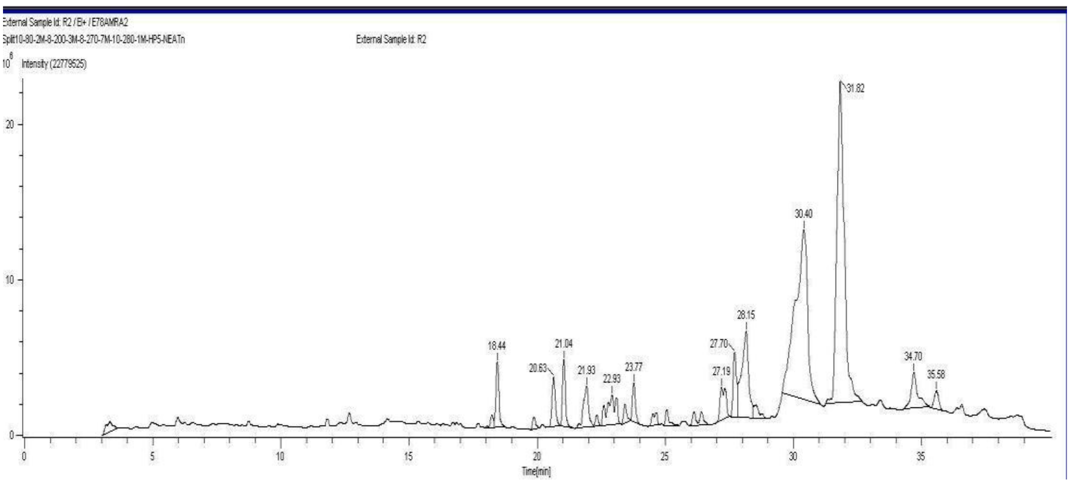


Fig. 4: GC-MS chromatogram of the Methanolic extract of Callus of *Ruta graveolens* L.

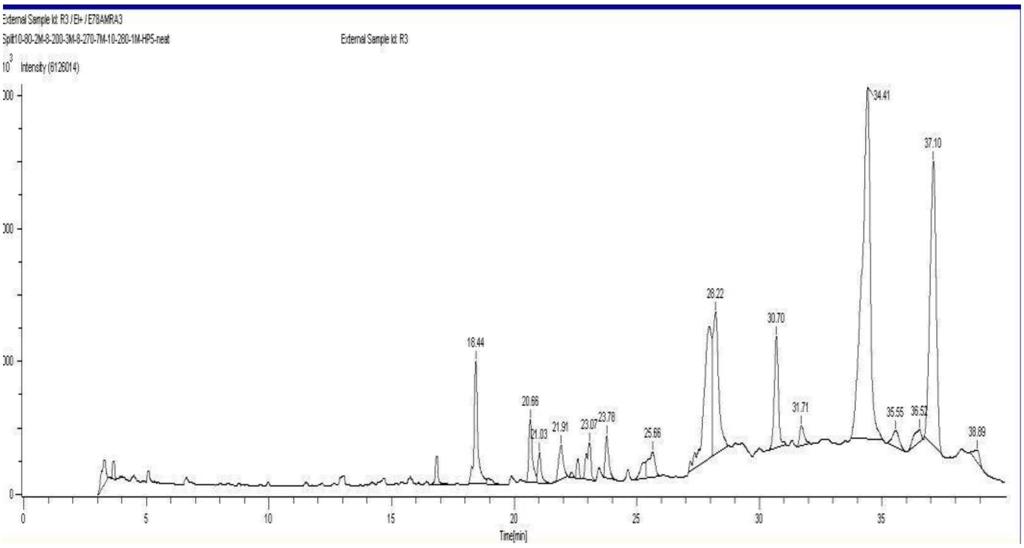


Figure. 5: GC-MS chromatogram of the Petroleum ether extract of Callus of *Ruta graveolens* L.

4. CONCLUSION

This GC-MS analysis of callus cultures of *Ruta graveolens* L. have shown presence of many bioactive constituents belonging to different chemical groups like Alkaloids, Phenols, Steroids, Coumarins, Furanocoumarins etc, with a wide range of biological activities (anti-microbial,anti-viral,anti-oxidative,anti-proliferative,photobiological,anti-inflammatory,anti-tumour,anti-platelet aggregation). These results showed that in-vitro plant cell cultures have potential for commercial production of secondary metabolites. Thus, it should be a priority to search for new plant derived chemicals by using biotechnological approaches, specifically, plant tissue cultures for the sustainable conservation and rational utilization of biodiversity. Further studies should be carried out for large scale production and isolation of these compounds in pure form.

5. ACKNOWLEDGMENT

The authors express sincere thanks to Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT) Mumbai for supporting instrumental analysis for this research work.

6. REFERENCES

1. The European Agency for the evaluation of Medicinal products, Committee for veterinary medicinal products "Ruta graveolens", EMEA-542, 1999.

2. Ratheesh M, Helen A. Anti-inflammatory activity of *Ruta graveolens* Linn on carrageenan induced paw edema in wistar male rats. Afr. J. Biotechnol, 2007; 6(10): 1209-1211.
3. Edelson R. Les médicaments photoactivés. Pour la science, 1998; 132: 64–72.
4. Pathak S, Multani A, Banerji P. Ruta 6 selectively induces cell death in brain cancer cells but proliferation in normal peripheral blood lymphocytes: a novel treatment for human brain cancer.International journal of oncology, 2003; 23: 975-982.
5. Preethi K, Kuttan G, Kuttan R. Anti-tumour activity of Ruta graveolens extract.Asian Pacific Journal of Cancer Prevention, 2007; 3: 439-443.
6. Diwan R, Malpathak N. Furanocoumarins: Novel topoisomerase I inhibitors from *Ruta graveolens* L. Bioorganic and Medicinal Chemistry, 2009; 17: 7052-7055.
7. Sallal AJ, Alkofahi A. Inhibition of the hemolytic activities of snake and scorpion venoms in Vitro with plant extracts. Biomed. Lett, 1996; 53: 211-215.
8. Kayser O, Wim Q. Medicinal Plant Biotechnology: From Basic Research to Industrial Applications, 2007; 2.
9. Nagata T, Ebizuka Y. Medicinal and aromatic plants XII. In: Biotechnology in Agriculture and Forestry. Springer Verlag, Heidelberg, USA, 2002.
10. Helmja K, Vaher M, Gorbatsova J, Kaljurand M. Characterization of bioactive compounds contained in vegetables of the Solanaceae family by capillary electrophoresis. Proc.Estonian Acad. of Sci. Chem., 2007; 1721-86.
11. Rao R. Ravishankar GA. Biotransformation of Protocatechuic Aldehyde and caffeic acid to vanillin and capsaicin in freely suspended and immobilized cell culture of *Capsicum rutescens*. J. Biotechnol, 2000; 76: 1371-146.
12. Philipson JD. Plants as source of valuable products. In: B.V. Chalwood and M.J. Rhodes (Eds.), Secondary products from plant tissue culture, Oxford, Clarendon Press, 1990; 1-21.
13. Kim Y, Kim Y, Ryu S. Antiproliferative effect of furanocoumarins from the root of angelica dahurica on cultured human tumour cell lines. Phytotheraphy Research, 2007; 21: 288-290.
14. Matsumoto K, Alkao Y, Kobayashi E, Ito T, Ohguchi K, Tanaka T, linuma M, Nozawa Y. Cytotoxic benzophenone derivatives from Garcinia species display a strong apoptosis-inducing effect against human leukemia cell lines.Biological and pharmaceutical Bulletin, 2003; 26: 569.

15. Chen J, Shen Y, Qian L, Chen L, Zheng K. 3D-QSAR of benzothiazole derivatives as potent anticancer agents. Chinese Journal of Chemical Physics, 2007; 20: 27.
16. Harris C, Henttu P, Parker M, Sumpter J. The estrogenic activity of phthalate esters in vitro. Environmental Health Perspective, 1997; 105: 802-811.