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Research Article

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In vitro plant regeneration and metabolite profiling of an aromatic medicinal plant *Ruta graveolens* L. by using GC-MS

Malik Aabid Hussain*[1], Varsha Nitin Nathar[1], Roohi Mushtaq[2]

[1]Department of Botany, SGB Amravati University, Amravati, Maharashtra–444602, India, [2]Department of Biotechnology, Sri Pratap College, Srinagar, Jammu and Kashmir–190001, India

ABSTRACT

Ruta graveolens L. is an endemic plant of the Mediterranean region. It has been used for centuries as a medical preparation and has a variety of roles because of its varied chemical composition. *In vitro* culture is a useful tool for both multiplication and study of important secondary metabolites. The present study was aimed to develop an effective and reproducible protocol for callus induction and indirect plant regeneration of *Ruta graveolens* (L.) by using leaf explants and to analyze chemical components present in different extracts of *Ruta graveolens*. The leaf explants were cultured on Murashige and Skoog's medium (MSM) augmented with different combinations and concentrations of auxins and cytokinins for callus induction, shoot multiplication and rooting. The optimum plant growth regulator concentration for callus induction, shoot multiplication and root formation was recorded in MSM+2,4-D(1.5mg/L)+NAA(1.5 mg/L), MSM+ BAP (1.5mg/L) +IBA (1.0 mg/L) and half strength MSM+IBA(0.50 mg/L) respectively. The rooted Plantlets were successfully acclimatized and established in earthen pots. The leaves, stem, roots and callus of *Ruta graveolens* were extracted by using Acetone and Ethyla cetate solvents followed by volatile compound analysis using GC-MS. The phytochemical assay showed that extracts of *Ruta graveolens* contain various phytoconstituents having potential bioactivity. The major compounds found were 1, 3-Dioxolane-4-propanol, 2, 2-Dimetheyl-, kokusaginine, Bergaptene, 2 Undecanone, 3-Hexene-2-one, Alpha, - 1- Arabinopyranose, 1, 2:3,4-bis-o, and 1- (1,3-Benzodioxol-5-ylmethyl)-3-Nitro-1 which might be primarily contributing in the biological activity of the plant. The results of this study will make a way for the production of herbal medicines for various ailments by using callus cultures of *Ruta graveolens* L.

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***Corresponding author:**
Malik Aabid Hussain
maabid1990@gmail.com

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INTRODUCTION

Nature is a large reservoir of medicinal plants for centuries and a number of drugs have been isolated from natural resources on the basis of their use in traditional medicine (Farombi, 2003). Increasing knowledge of metabolic processes and the effects of plants on human physiology have enlarged the range of application of medicinal plants. Thousands of bioactive chemicals have been reported which are all different and have a restricted distribution in the plant kingdom. In spite of our dependence on modern medicine and tremendous advancement in synthetic drugs, a large part of the world population still uses drugs from plants. So it is need of the hour to identify plants with higher medicinal value (Balandrin *et al*., 1985). The demand of bioactive compounds is increasing day by day and has lead to over exploitation of many medicinal plants. The plant tissue culture technology is an efficient and useful tool for the preservation of plant species while providing an alternative source for the production of plant products (Nagata & Ebizuka., 2013).

Ruta graveolens L. (Rue) belonging to family Rutaceae is a hardy, evergreen shrub of upto one meter tall, greenish in colour and sharp unpleasant odour. The plant has been used since ancient times for medicinal purposes and is presently used for the treatment of various disorders such as pain, rheumatism, eye problems and dermatitis. Rue extracts have potent anti-cancer activity exhibited through strong anti-proliferative and anti-survival effects on cancer cells (Fadlalla *et al*., 2011). It's essential oils are used as fungicides and have a phytotoxic activity (Astelbaur *et al*., 2012). Metabolite profiling is an analytical method for the relative quantitation of a number of metabolites from biological samples (Fiehn *et al*., 2000). Gas

Chromatography/Mass Spectrometry (GC/MS) can be used to detect and analyze the wide variety of chemical compounds in plant materials. The GC-MS instrument separates chemical mixtures and identifies the components at a molecular level. Therefore the present work was aimed to develop an effective and reproducible protocol for callus induction and indirect shoot multiplication of *Ruta graveolens* (L.) by using leaf explants and to characterize metabolite profile of *Ruta graveolens* by using GC-MS.

MATERIALS AND METHODS

Collection and Maintenance of Experimental Material

The plants of *Ruta graveolens* L. were collected from Melghat forest located at 21°26′45″N 77°11′50″E in northern part of Amravati District of Maharashtra State, India and were established in Botanical garden, Department of Botany, Sant Gadge Baba Amravati, University, Amravati. The callus and plantlet regeneration was established from leaf explants on Murashige and Skoog's media supplied with different combinations and concentrations of phytohormones.

Callus Induction

The leaf segments 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. After washing, the sterilized leaf segments were excised aseptically and transferred to culture tubes containing full strength Murashige and Skoog's, (1962) basal medium supplemented with different concentrations and combinations of plant growth regulators such as 2, 4-D, NAA, 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 121°C under 15 psi for 20 minutes. Cultures were incubated at 25 ±2°C under 16 hours photoperiod and 55±5% relative humidity for callus induction.

Growth Measurement

The growth measurement of callus was determined by following the method as described by (Rao & Ravishankar, 2000). The growth of the callus and its biomass was measured in terms of fresh weight (FW g/L) and dry weight (DW g/L). The fresh weight of callus was measured after removing the excess moisture and agar adhering to the callus surface using blotting paper. The dry weight of callus was determined by drying the callus in hot air oven at 40°C until constant weights were achieved and was expressed in g/L DW.

Indirect organogenesis

The yellowish compact calli formed after 5 weeks of culture were sub cultured for shoot differentiation on MS media supplemented with 30 g/L sucrose and 8 g/L agar containing different concentration/combination of BAP, Kinetin, NAA and IBA to induce multiple shoots.

Rooting of Shoots and Acclimatization

In vitro regenerated shoots were separated from cultures, washed thoroughly with sterile distilled water several times and were transferred to half-strength solid sterile MS medium supplemented with auxins alone and in combinations with BAP for *In vitro* rooting. The *In vitro* regenerated plantlets with sufficient roots were isolated from the culture tubes and washed with sterile double distilled water to remove adhering medium, transferred to the plastic cups containing a mixture of autoclaved soil, sand and vermicompost (1:2:1), irrigated with sterile water twice a day. The potted plantlets were initially maintained in a programmable environmental chamber for one month. The temperature and humidity were adjusted to 20±2°C and 50% respectively. The temperature of the programmable environmental chamber was raised by 1-°C every 5 days. After about 5 weeks the plants were transferred to bigger earthen pots containing normal garden soil in the greenhouse for further growth and development and were maintained under natural conditions of day length, temperature and humidity. The acclimatized plants were irrigated regularly. In all the above three types of inductions i.e. callogenic, shooting and rooting, MS media without plant growth regulators was used as control.

Statistical Analysis

20 replicates were raised for each experiment and were repeated thrice. The number of explants responding was recorded at intervals of 7days. All the investigated parameters were analyzed using analysis of variance (ANOVA) and significance was determined at $P<0.05$. The data were analyzed statistically using SAS System ('Local', W32_VSPRO) software and means were compared using Duncan's Multiple Range Test (DMRT). Variability in data has been expressed otherwise as mean±standard deviation.

Plant Sample Extraction

The collected *Ruta graveolens* leaves, stem, root and callus were washed several times with distilled water to remove the traces of impurities, dried at room temperature until constant weights were achieved and coarsely powdered with an electric grinder. 3 gram powder from each part was extracted with acetone and ethyl acetate separately for 24 hours using Soxhlet apparatus and concentrated using a 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 a refrigerator.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC–MS analysis was carried out 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 230ºC, 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. The Interpretation of mass spectra obtained by the GC-MS method was conducted by comparison of their mass spectra with those present in the IIIM library. Name, molecular weight and structure of the components of the tested materials were ascertained.

RESULTS AND DISCUSSION

The members of Rutaceae family are used in medicine of which *Ruta graveolens* (Rue) is at the forefront (Raghv *et al*., 2006). Rue contains various active compounds belonging to different groups like flavonoids, coumarin derivatives, furoquinolines, volatile oils etc (Pathak *et al*., 2003). The derivatives of medicinal plants are non-narcotic and have no side effects. The demand for these plants is increasing in both developing and developed countries (Sharma *et al*., 2008). Recent scenario shows that there is again revival of interest in herbal medicines. The World Health Organisation has a strong thrust upon evaluation of these herbal drugs for some major diseases against modern drugs (Ayyanar *et al*., 2008). Hence, traditional and herbal medicine is need of the hour (Arulrayan *et al*., 2007). *In vitro* plant multiplication protocols have many useful applications to medicinal plants like *Ruta graveolens*, e.g. true-to-type large scale propagation of superior genotypes and genotypic improvement via mutagenesis and/or genetic engineering, respectively (Ahmad *et al*., 2010).

Different concentrations of plant growth regulators had a significant effect on callus formation and plantlet regeneration. Leaf explants of *Ruta graveolens* were cultured on MS media supplied with 2, 4-D, NAA, 2, 4-D+NAA, NAA+BAP, and BAP+IBA in different concentrations to test the efficiency of sprouting of leaf explants. It was observed that after 9–12 days of inoculation, the explants showed callus initiation in all the concentrations. The callogenic response showed variations among the hormonal combinations/concentrations used. The callus response varied from 40.77±0.69 at 2, 4-D 0.5 mg/L to 78.11±1.26 at 1.5 mg/L 2, 4-D+1.5 mg/L NAA. It was observed that at lower concentrations of 2, 4-D (0.5 mg/l), only 40% of leaf cultures showed callus formation but with increased concentration (2.0) mg/L callus formation also increased from 40% to 75% (2.0 mg/L) and then decreased when the higher concentrations were used. None of the explants showed callus initiation when inoculated on media without plant growth regulators (control).The hormone combination for callus biomass production was also standardized and the highest callus biomass,135.00±1.73 g/L FW and 15.50±1.44g/L DW was observed at 2, 4-D+NAA (1.5+1.5) mg/L (Table 1; Figure 1a and b).Therefore 2, 4-D in combination with NAA (1.5+1.5) mg/L proved best for callus induction as well as for the highest biomass production in *Ruta graveolens* L.

The yellowish compact calli formed after 5 weeks of culture was sub cultured for shoot differentiation on MS media supplemented with 30 g/L sucrose and 8 g/L agar containing different concentration of BAP, Kinetin, NAA and IBA singly or in combinations to induce multiple shoots. Multiple shoot initiation started from the callus after two weeks when sub cultured onto fresh MS medium containing different cytokinins like BAP and kinetin and auxins like IBA.

The maximum shoot induction response and shoot length (mm) was recorded as 79.22±1.02 and 79.66±1.73 in BAP+IBA (1.5+1.0) mg/L respectively while as highest shoot number (23.00±1.77) was recorded in BAP+KIN (1.5+1.0) mg/L (Table 2, Figure 1c and d). The shoot induction, shoot number and shoot length showed an increase as the concentration of plant growth regulators was increased but further increase in plant growth regulator concentrations showed a decline in shoot induction response, shoot number and shoot length while none of the control cultures showed positive response for shoot regeneration. In our earlier studies, we have reported successful indirect regeneration of the same plant from Internodal explants (Hussain & Nathar, 2020). Similar results with different phytohormone combinations and concentrations have been reported by some earlier workers in *Ruta graveolens* using various explants like leaf discs (Gurudeeban *et al*., 2012; Al-ajlouni *et al*., 2015), nodal explants (Tejavathi & Manjula, 2010) and stem, leaf, shoot tip and root explants (Shabana *et al*., 2010).

Rooting of the *In vitro* regenerated shoots did not occur on full strength MS medium though the media was supplied with rooting hormones like IAA and IBA, so they were transferred to half-strength solid sterile MS medium supplemented with different auxin concentrations as well as cytokinin (BAP) in combination with auxins for *In vitro* rooting. Rooting was obtained in 3 weeks after transferring the shoots to rooting media containing hormones like IAA, IBA and BAP. The maximum percentage of root induction 85.22±1.71, the highest number of root formations 8.22±1.07 and the maximum mean root length (mm) 36.00±1.45 was achieved on the medium containing 0.5 mg/L IBA. (Table 3, Figure 1e). Though IAA also showed up to 82% root induction response but the mean root number and mean root length was lower than IBA. Plantlets significantly developed lengthy roots and root induction strengthened with time. IBA was found better in inducing the roots. No response for root induction was achieved in the media without any plant growth regulator (Control).

The plantlets with well-developed root and shoot systems were first maintained in a programmable environmental chamber for about 5 weeks and then isolated from the culture vessels and washed with sterile double distilled water to remove adhering medium. The hardened plants were then transferred to the earthen pots containing sterilized mixture of soil, sand and vermicompost (1:2:1) and were kept in the green house for acclimatization. The 85% of plantlets were successfully acclimatized. The 100% survival rate in *In vitro* raised plantlets of *T. indica* has been reported earlier by some workers (Faisal *et al*., 2005). No variation was observed among the acclimatized plants with respect to morphological characteristics (Figure 1f).

Table 1: Effect of plant growth regulators on Callus induction from Leaf Explants of *Ruta graveolens* L.

PGR	Conc. (mg/L)	Mean RP (%)*	Fresh Weight (g/L)*	Dry Weight (g/L)*
Control	-	-	-	-
2,4-D	0.5	40.77 ± 0.69^{d}	98.50 ± 1.32^{de}	8.66 ± 1.15^{de}
	1.0	44.55 ± 0.19^{d}	94.83 ± 0.76^{f}	8.50 ± 0.50^{def}
	1.5	63.33 ± 1.67^{abc}	110.70 ± 0.81^{c}	10.66 ± 0.57^{c}
	2.0	75.55 ± 0.95^{a}	114.83 ± 0.76^{b}	12.00 ± 1.00^{b}
	2.5	54.99 ± 1.66^{bcd}	82.00 ± 1.00^{i}	7.66 ± 0.57^{efgh}
	3.0	52.77 ± 0.96^{cd}	78.83 ± 1.89^{j}	7.53 ± 0.05^{efgh}
NAA	0.5	52.77 ± 0.96^{cd}	73.16 ± 1.75^{k}	6.16 ± 0.28^{ij}
	1.0	54.99 ± 1.66^{bcd}	82.66 ± 1.52^{i}	7.46 ± 0.45^{efgh}
	1.5	63.33 ± 1.67^{abc}	97.00 ± 1.00^{def}	8.57 ± 0.39^{def}
	2.0	68.33 ± 1.92^{ab}	86.66 ± 1.15^{gh}	7.83 ± 0.28^{efg}
	2.5	63.33 ± 1.67^{abc}	87.00 ± 1.00^{gh}	7.50 ± 0.50^{efgh}
	3.0	52.77 ± 0.96^{cd}	64.33 ± 1.52^{m}	6.23 ± 0.25^{hij}
2,4-D + NAA	1.0 + 1.0	52.77 ± 0.96^{cd}	77.33 ± 1.52^{j}	7.43 ± 0.40^{fghi}
	1.5 + 1.5	78.11 ± 1.26^{a}	135.00 ± 1.73^{a}	15.50 ± 1.44^{a}
	1.5 + 1.0	68.33 ± 1.92^{ab}	101.66 ± 1.73^{d}	9.83 ± 0.86^{d}
NAA + BAP	1.5 + 2.5	68.33 ± 1.92^{ab}	89.33 ± 1.15^{g}	8.26 ± 0.69^{def}
	2.0 + 2.0	71.66 ± 1.66^{a}	71.33 ± 1.52^{k}	7.00 ± 0.50^{ghij}
	2.5 + 1.5	75.55 ± 0.95^{a}	65.33 ± 1.15^{l}	7.06 ± 0.60^{ghij}
	2.5 + 2.0	63.33 ± 1.67^{abc}	65.00 ± 1.00^{l}	6.06 ± 0.11^{j}
BAP + IBA	1.0 + 0.5	55.00 ± 1.66^{bc}	76.16 ± 0.76^{j}	6.44 ± 0.41^{hij}
	1.0 + 2.0	68.33 ± 1.92^{ab}	96.33 ± 1.73^{f}	8.33 ± 0.57^{def}
	2.0 + 1.0	55.00 ± 1.66^{bc}	48.00 ± 1.73^{n}	5.00 ± 0.50^{k}

*Values represent mean±standard deviation of 20 replicates per treatment in three repeated experiments. Means with the same letter within columns are not significantly different ($P<0.05$) using Duncan's multiple range test (DMRT).
PGR = Plant growth regulator; RP = Response Percentage; Conc. = Concentration

Table 2: Effect of Plant Growth Regulators on Indirect Shoot Regeneration from Leaf callus of *Ruta graveolens* L

PGR	Conc. (mg/L)	Mean RP (%)*	Mean Shoot number*	Mean shoot length (mm)*
Control	-	-	-	-
BAP	0.5	34.33 ± 1.15^{j}	5.33 ± 1.46^{i}	32.22 ± 1.17^{j}
	1.0	43.22 ± 1.07^{i}	9.22 ± 1.17^{hi}	38.66 ± 1.45^{i}
	1.5	61.67 ± 1.53^{e}	14.55 ± 0.84^{de}	55.55 ± 1.39^{f}
	2.0	50.77 ± 1.17^{gh}	11.55 ± 0.84^{f}	49.66 ± 1.67^{g}
KIN	0.5	31.77 ± 1.26^{k}	5.78 ± 1.35^{hi}	39.89 ± 1.50^{i}
	1.0	48.89 ± 1.26^{h}	7.66 ± 1.20^{gh}	43.00 ± 1.45^{h}
	1.5	61.55 ± 1.50^{e}	13.44 ± 0.70^{ef}	60.88 ± 1.57^{d}
	2.0	52.66 ± 1.20^{g}	8.99 ± 1.15^{g}	57.00 ± 1.85^{ef}
BAP + IBA	1.0 + 1.0	58.33 ± 1.15^{f}	13.55 ± 1.65^{ef}	56.44 ± 1.07^{f}
	1.5 + 1.0	79.22 ± 1.02^{a}	19.77 ± 1.02^{b}	79.66 ± 1.73^{a}
	2.0 + 1.0	73.78 ± 1.68^{b}	16.55 ± 1.02^{cd}	75.66 ± 1.34^{b}
	2.5 + 1.0	71.77 ± 1.64^{b}	15.11 ± 1.17^{de}	70.11 ± 1.02^{c}
BAP + KIN	1.5 + 1.0	57.44 ± 1.54^{f}	23.00 ± 1.77^{a}	59.22 ± 1.35^{de}
	2.0 + 1.0	66.22 ± 1.65^{d}	18.44 ± 0.77^{bc}	69.67 ± 1.53^{c}
	2.5 + 1.0	72.11 ± 1.26^{b}	16.55 ± 1.26^{cd}	77.66 ± 1.34^{ab}
	1.5 + 1.5	68.66 ± 1.34^{c}	15.11 ± 1.35^{de}	68.44 ± 1.02^{c}

*Values represent mean±standard deviation of 20 replicates per treatment in three repeated experiments. Means with the same letter within columns are not significantly different ($P<0.05$) using Duncan's multiple range test (DMRT).
PGR = Plant growth regulator; RP = Response Percentage; Conc. = Concentration

The GC-MS analysis is a valuable method which has been increasingly applied for the metabolite profiling of medicinal plants. In the present study, the very complex chemical profile of *Ruta graveolens* was characterized using GC-MS. The extraction of different plant parts was carried out to acquire valuable information about secondary metabolites present in *Ruta graveolens* L. The *In vivo* plant parts like leaf, stem, root and *In vitro* callus material was investigated for biologically active compounds by GC-MS analysis. Each extract showed the presence of many secondary metabolites. The GC-MS analysis revealed the occurrences of total 19 compounds in acetone extracts and 22 compounds from ethyl acetate extracts. In our earlier reports, the presence of 51 compounds in callus extracts of *R. graveolens* and 25 compounds in methanol extracts of the same plant with a wide range of bioactivities and potential economic importance have been documented (Malik *et al*., 2017a,b).

The GC-MS analysis of acetone extracts of leaf, stem, root and callus showed 13, 10, 11 and 8 chemical compounds respectively (Table 4, Figures 2-5). Most of the components found were overlapping but the peak area percentage varied from one extract to other which means that 19 different

Figure 1: *In vitro* callus formation and plant regeneration of *Ruta graveolens* L. from leaf explants. (a) Callus formation at NAA 2.5mg/L + BAP 1.5 mg/L, (b) Callus formation at 2,4-D 1.5mg/L + NAA 1.5 mg/L, (c) Multiple shoot formation at BAP 1.5mg/L +IBA 1.0mg/L, (d) Profuse multiple shoot clusters developed at BAP 1.5mg/L + IBA 1.0 mg/L., (e) Rooting of the *In vitro* regenerated shoots at IBA 0.5mg/L., (F) Acclimatized Plant

Table 3: Effect of Plant growth regulators on *In vitro* root induction of regenerated shoots of *Ruta graveolens* L.

Plant Growth Regulator	Concentration (mg/L)	Mean Response Percent (%)*	Mean Root number*	Mean Root length (mm)*
Control	-	-	-	-
IAA	0.25	72.66 ± 1.21^{e}	4.33 ± 1.15^{cd}	16.67 ± 1.15^{e}
	0.50	80.89 ± 1.83^{b}	5.11 ± 1.02^{bc}	26.44 ± 1.26^{c}
	1.0	82.33 ± 1.20^{b}	7.44 ± 1.26^{a}	29.22 ± 1.17^{b}
	1.5	75.78 ± 1.35^{cd}	4.78 ± 1.35^{bc}	21.55 ± 1.65^{d}
IBA	0.25	74.66 ± 1.15^{de}	6.55 ± 1.35^{ab}	31.33 ± 1.53^{b}
	0.50	85.22 ± 1.71^{a}	8.22 ± 1.07^{a}	36.00 ± 1.45^{a}
	1.0	77.55 ± 1.39^{c}	7.44 ± 1.39^{a}	35.22 ± 1.50^{a}
	1.5	69.33 ± 1.86^{f}	4.89 ± 0.96^{bc}	24.22 ± 1.34^{c}
IBA + BAP	1.0 + 0.5	18.33 ± 1.20^{g}	2.51 ± 0.0^{de}	9.88 ± 1.35^{f}
	1.5 + 0.5	16.33 ± 1.15^{gh}	2.33 ± 1.00^{de}	7.00 ± 1.45^{g}
	2.0 + 0.5	15.99 ± 1.53^{gh}	1.51 ± 0.46^{e}	7.33 ± 1.15^{g}
IAA + BAP	1.0 + 0.5	14.33 ± 1.20^{h}	2.22 ± 1.26^{de}	7.44 ± 1.17^{g}
	1.5 + 0.5	15.33 ± 1.53^{h}	3.99 ± 1.15^{cd}	10.11 ± 1.5^{f}
	2.0 + 0.5	15.33 ± 1.15^{h}	3.89 ± 1.02^{cd}	11.66 ± 1.21^{f}

*Values represent mean±standard deviation of 20 replicates per treatment in three repeated experiments. Means with the same letter within columns are not significantly different ($P<0.05$) using Duncan's multiple range test (DMRT).

compounds were found in four acetone extracts. These phytoconstituents largely contributes to the pharmacological activity of plant. The major compound found in all the extracts with variable peak area was 1,3- Dioxolane-4-propanol,2,2-Dimetheyl- (Peak area 78.5% in leaf extract, 85.4% in stem extract, 72.6% in root extract and 78.2% in callus extract). A previous study has also reported the presence of several flavonoids, alkaloids, ketones and phenolic compounds in leaf, stem and fruit parts of Rue extract indicating that it could be useful for the production of potent antioxidants to cure various human ailments (Mancuso *et al.*, 2015). Due to the presence of various secondary metabolites in the extracts, rue plant has the potential application in various pharmaceutical industries (Sharma, 2006).

The Gas Chromatography–Mass Spectrometry analysis of ethyle acetate extracts of leaf and stem revealed the presence of 20 and 22 chemical constituents respectively (Table 5, Figures 6 and 7). Among which 20 similar compounds were found in both the ethyl acetate extracts with variable peak area percentages. The major constituents in ethyle acetate extracts were kokusaginine, Bergaptene, 2 Undecanone and Dotriconane.

Figures 2-7 represent the gas chromatograms which show the relative concentrations of various compounds eluted as a function of retention time. The height of the peak indicates 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 bioactive compounds which have been revealed in the present study have been found to possess anti-microbial, anti-viral, anti-

Table 4: GC-MS Analysis of Different Acetone Extracts of *Ruta graveolens* L.

S. No	RT	Name of Compound	*Peak area %				MW	MF
			Leaf extract	Stem extract	Root extract	Callus extract		
1	5.16	1-Acetyl-1-methoxyiminoethane	-	-	0.63±0.02	-	115	$C_5H_9NO_2$
2	5.87	Toluene	0.36±0.04	0.36±0.04	0.36±0.04	0.36±0.04	92	C7H8
3	7.05	3-Hexene-2-one	7.67±0.19	8.22±0.21	14.81±0.13	13.64±0.13	98	$C_6H_{10}O$
4	9.10	1,3-Dioxolane-4-propanol, 2,2-Dimetheyl-	78.51±0.51	85.40±0.45	72.65±0.50	78.26±0.40	132	$C_6H_{12}O_3$
5	10.18	Methyyllaurate	0.27±0.03	0.29±0.01	0.53±0.01	0.30±0.01	214	$C_{13}H_{26}O_2$
6	10.71	o-Xylene	1.74±0.02	2.73±0.02	2.25±0.02	2.71±0.15	106	C_8H_{10}
7	11.83	p-Xylene	0.57±0.02	0.81±0.30	0.71±0.02	0.74±0.01	106	C_8H_{10}
8	13.07	2,2,6-Trimethyl-4-Methylene-1-oxo-5-cycl	-	0.14±0.04			198	$C_{14}H_{30}$
9	25.14	3-(isopropyle) -1,2,4-Triazole	0.84±0.01	0.69±0.05	0.77±0.06	1.76±0.06	-	-
10	27.53	Napthalene	5.42±0.42	-	-	-	128	$C_{10}H_8$
11	27.59	1,6-Methano (10) Annulen-11-one	-	0.16±0.03	-	-	156	$C_{11}H_8O$
12	29.40	1-Undecanol	0.50±0.05	-	-	-	172	$C_{11}H_{24}O$
13	32.31	2-Undecanone	1.88±0.06	-	-	-	170	$C_{11}H_{22}O$
14	36.73	Alpha, - 1- Arabinopyranose, 1,2:3,4-bis-o	-	-	6.67±0.08	-	230	$C_{11}H_{18}O_5$
15	38.27	1,2:3,5-di-o-Isopropylidene-D-xylofurano	-	-	0.59±0.06	-	230	$C_{11}H_{18}O_5$
16	57.96	Dibutyl phthalate	0.85±0.03	-	0.56±0.04	1.90±0.04	278	$C_{16}H_{22}O_4$
17	57.99	Cyclohexyl butyl phthalate	-	0.54±0.05	-	-	304	$C_{18}H_{24}O_4$
18	58.59	Chlorpyrifos	0.43±0.05	-	-	-	350	$C_9H_{11}Cl_3NO_3PS$
19	60.83	1-Adamantane carboxylic acid, morpholide	0.84±0.04	-	-	-	249	$C_{15}H_{23}NO_2$

* Values represent mean±standard deviation of three replicates
- = Absent; RT = Retention time; MW = Molecular Weight; MF = Molecular formula

Table 5: GC-MS Analysis of Ethyl acetate Extracts of *Ruta graveolens* L.

S. No	RT	Name of Compound	*Peak area %		MW	MF
			Leaf extract	Stem Extract		
1	5.85	Toluene	1.42±0.03	1.40±0.13	92	C_7H_8
2	17.43	Decane	0.50±0.03	0.57±0.04	142	$C_{10}H_{22}$
3	22.35	2-Nonanone	2.71±0.18	1.33±0.06	142	$C_9H_{18}O$
4	27.50	Napthalene	0.46±0.04	0.46±0.04	128	$C_{10}H_8$
5	27.77	Oxalic acid, isobutylnonyl ester	3.46±0.18	4.35±0.24	272	$C_{15}H_{28}O4$
6	29.37	1-Undecanol	1.46±0.04	0.30±0.06	172	$C_{11}H_{24}O$
7	32.27	2-Undecanone	11.19±0.18	4.64±0.19	170	$C_{11}H_{22}O$
8	36.96	Nonadecane	7.20±0.26	9.37±0.23	268	$C_{19}H_{40}$
9	43.95	T-Butylhydroxyanisole	0.93±0.25	4.63±0.23	180	$C_{11}H_{16}O_2$
10	45.13	Dotriacontane	6.52±0.19	9.08±0.06	450	$C_{32}H_{66}$
11	52.47	Dotriacontane	4.51±0.14	6.46±0.10	450	$C_{32}H_{66}$
12	53.36	p-Anisic acid, 2, 6-dimethylnon-1-en-3-yn	1.49±0.29	1.77±0.06	300	$C_{19}H_{24}O_3$
13	53.71	2-octylcyclopropene-1-heptanol	6.90±0.30	3.52±0.04	266	$C_{18}H_{34}O$
14	59.12	Hexatriacontane	2.79±0.04	3.94±0.04	506	$C_{36}H_{74}$
15	60.74	1-(1,3-Benzodioxol-5-ylmethyl)-3-Nitro-1	7.87±0.19	1.58±0.07	122	$C_7H_6O_2$
16	61.59	Bergaptene	3.87±0.08	15.32±0.29	216	$C_{12}H_8O_4$
17	65.21	2-Hexyl-1-octanol	8.20±0.04	2.47±0.15	214	$C_{14}H_{30}O$
18	65.46	7-(2,3-Dihydroxy-3-Methylbutoxyl)-4,8-Dimethoxypsoralene	-	2.83±0.07	229	$C_{13}H_{11}NO_3$
19	66.17	Pyrrole-3-carboxaldehyde, 1- (4-methoxyph	4.67±0.18	3.78±0.05	229	$C_{14}H_{15}NO_2$
20	66.66	5,8-Dimethoxypsoralene (Isopimpinellin)	-	0.48±0.06	246	$C_{13}H_{10}O_5$
21	70.83	Hexatriacontane	1.04±0.45	1.53±0.04	506	$C_{36}H_{74}$
22	72.89	kokusaginine	24.60±0.52	20.11±0.35	259	$C_{14}H_{13}NO_4$

* Values represent mean±standard deviation of three replicates
- = Absent; RT = Retention time; MW = Molecular Weight; MF = Molecular formula

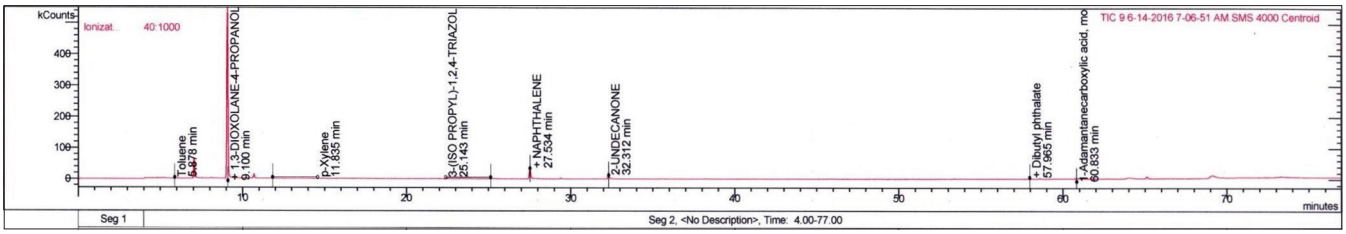

Figure 2: GC-MS Chromatogram of acetone extract of leaf of *Ruta graveolens* L.

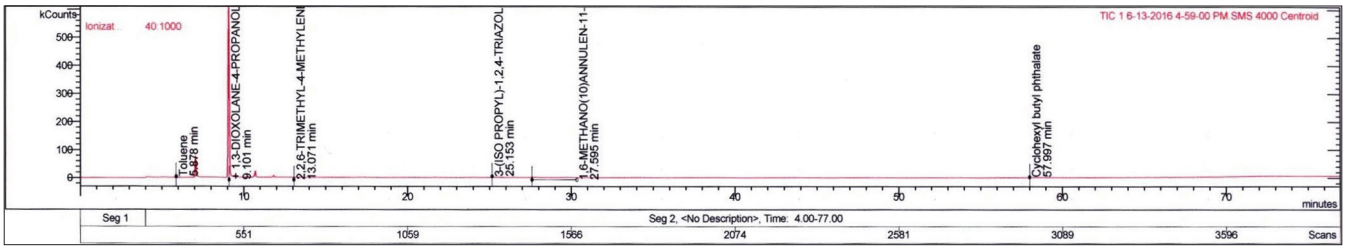

Figure 3: GC-MS Chromatogram of acetone extract of stem of *Ruta graveolens* L.

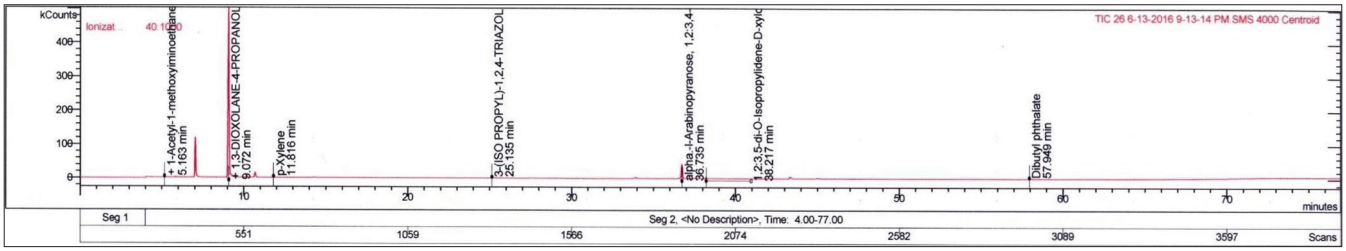

Figure 4: GC-MS Chromatogram of acetone extract of root of *Ruta graveolens* L.

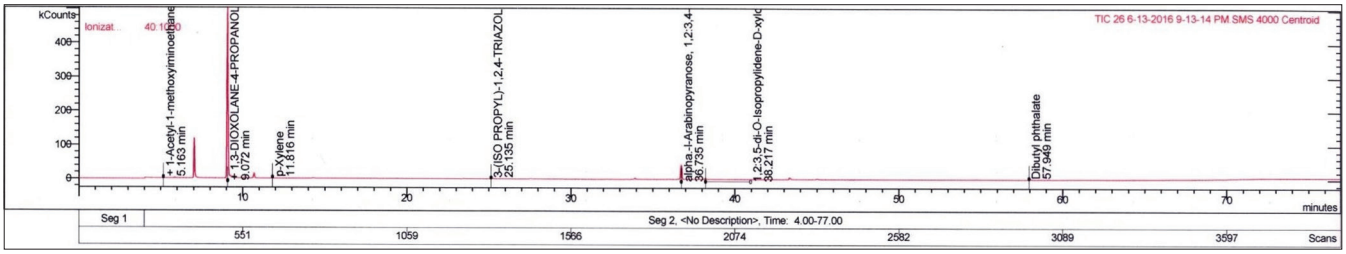

Figure 5: GC-MS Chromatogram of acetone extract of callus of *Ruta graveolens* L.

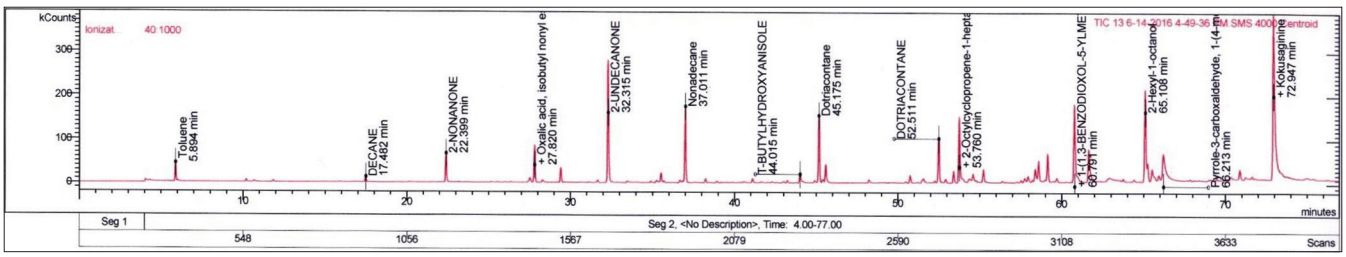

Figure 6: GC-MS Chromatogram of ethyl acetate leaf extract of *Ruta graveolens* L.

oxidative, anti-proliferative activities (Kim *et al.*, 2007) among others with potential economic importance. The isolation of these chemical constituents could lead to the new drug discovery at a cheaper cost which would be useful in medicine.

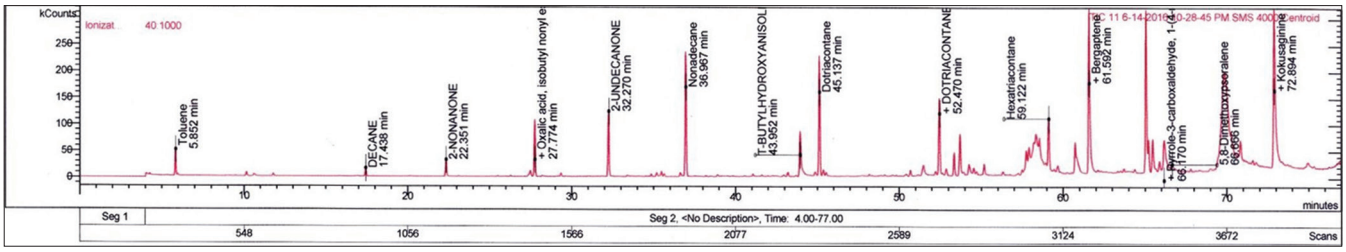

Figure 7: GC-MS Chromatogram of ethyl acetate stem extract of *Ruta graveolens* L.

CONCLUSION

In conclusion, a reproducible protocol for callogenesis and indirect plant regeneration was developed. The results showed that *In vitro* callus cultures have the potential for commercial production of secondary metabolites. The metabolite profiling revealed the presence of various secondary metabolites in different *Ruta* extracts as analyzed by GC-MS. The present study has brought to light, how non-targeted metabolite profiling can be undertaken to acquire and widen the knowledge regarding the chemical composition of any plant. Further research on this plant can pave the way to newer innovation in herbal medicine.

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