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CALLUS INDUCTION AND EFFECT OF L-PHENYLALANINE ON BIOSYNTHESIS OF PSORALEN AND BERGAPTEN IN CALLUS CULTURES OF *RUTA GRAVEOLENS* L., A MEDICINAL PLANT

B L MANJULA[1] and S MANJULA[2]

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Author/s

[1] Associate Professor,
Department of Botany,
Sri Jagadguru Renukacharya College of Science, Arts & Commerce, Race course Road, Bengaluru-560009, Karnataka, India
manjulasrivats@gmail.com

[2] Assistant Professor,
Department of Botany,
Government First Grade College, B M road, Ramanagara-562159, Karnataka, India, manjularaj1983@gmail.com

Abstract

Ruta graveolens L., commonly called Rue is known to contain furanocoumarins which have gained wide applications in pharmaceutical industry. *In vitro* callus production is an alternative source to produce furanocoumarins. In the present study, callus tissue was raised from the nodes of *Ruta graveolens* on MS medium supplemented with growth regulators and amino acids. L-Phenylalanine was used as a precursor to increase the production of Psoralen and Bergapten in the callus cultures. NAA (10.74µM) + BAP (4.44µM) supplemented with L-Phenylalanine of various concentrations yielded more callus. HPLC analysis revealed that the nodal extract contained less amount of Psoralen and Bergapten whereas Psoralen was not observed in the nodal derived callus. It was observed that the highest amount of Bergapten of 27.45 mg/g DW was produced in the callus raised on MS medium supplemented with NAA (10.74µM) + BAP (4.44µM) + L-Phenylalanine (2.42 mM).

Keywords: *Ruta graveolens*, callus culture, L-Phenylalanine, Psoralen, Bergapten

1. INTRODUCTION

Ruta graveolens L., a member of Rutaceae, an aromatic medicinal plant iscultivated in many Asian countries including China, India and Japan as it is native to Europe especially the Mediterranean region. The medicinal properties of this plant are attributed to the biologically active principles like flavonoids, acridone alkaloids, furanocoumarins and essential oils. The extracts of this plant was shown to have potent anticancer activity [1,2]. Psoralen, Bergapten, and Xanthotoxin which are found in *Ruta graveolens* have been identified as new Topoisomerase 1 inhibitors [3]. Cell proliferation is facilitated by DNA topoisomerase enzymes. Psoralen, Bergapten and Xanthotoxin have emerged as important biological targets for chemical intervention in the development of anticancer medicines due to their ability to suppress the activity of topoisomerase 1 enzymes. Currently, a few Topoisomerase 1 inhibitors are being tested in clinical trials as anticancer medicines. Natural compounds derived from plants can be important sources of new inhibitors because they have less negative effects.

In intact plants, these secondarymetabolites are generated in very small levels [4]. Because of their highly complicated structures and stereospecific chemical nature, chemical synthesis or semi synthesis of these metabolites is either extremely difficult or commercially unfeasible [5]. Precursor feeding has been shown to be an effective method for increasing the production of secondary metabolites from plant cells cultured *in vitro* [5, 6]. Several chemical substances can be added to the culture medium to boost the synthesis of secondary metabolites based on knowledge of the biosynthetic pathways. The underlying concept is that any compound that is an intermediate in or at the beginning of a secondary metabolite biosynthetic route has a good chance of increasing the yield of the final product [7]. Thus, adding a biosynthetic precursor to culture medium may increase the yield of the desired product. Several workers have used Phenylalanine as a precursor for the production of secondary metabolites [5]. L-Phenylalanine is catalyzed by the enzyme L– Phenylalanine ammonia lyase to form cinnamic acid and ammonia. Cinnamic acid is a precursor of furanocoumarins. Hence, in the present work L-Phenylalanine is used as a precursor to enhance the production of Psoralen and Bergapten in the nodal derived callus. For this reason, in this work the amounts of furanocoumarins such as Psoralen and Bergapten in nodes, node derived callus and callus grown on a nutrient medium supplemented with or without the precursor L- Phenylalanine and growth promoters is studied.

2. MATERIALS AND METHODS

Explant source: Healthy potted plants were maintained in polyhouse of Jnanabharathi campus, Bengaluru. Nodes of about 1cm excised from these plants were used as explants.

Surface sterilization: Tween-20 detergent was used to clean the explants for the first 5-10 minutes. After that, they were cleansed with running water for 30 minutes and treated with the fungicide Bavistin (0.1 percent) for 5 minutes. The explants were then surface sterilized with saturated chlorine water for 2 min and then with mercuric chloride (0.1 percent) for 2min. After each treatment the explants were thoroughly washed in sterile double distilled water.

Culture medium: Murashige and Skoog's medium [8] supplemented with various concentrations of auxins (NAA, 2, 4-D, IAA and IBA), cytokinins (BAP and Kin) alone and BAP in combination with NAA along with amino acids (L-Phenylalanine, L-Glycine and L-Glutamine) was used as a culture medium. Sucrose (3%) was used as a carbon source while bacteriological grade agar was used as the gelling agent (0.8 percent). The pH of the medium was fixed to 5.6 and it was autoclaved for 15 minutes at 109 kpa pressure. On this medium surface sterilized explants were inoculated.

Culture conditions: The cultures were incubated at 25°C with a 16:8h light: dark cycle under fluorescent tube lamps. Each experiment was repeated three times to ensure the accuracy of the results. When necessary, subculture was done on a regular basis.

The nodes were placed on MS media supplemented with NAA, 2,4-D, IAA, IBA and cytokinins like BAP, kinetin of different concentrations and a combination of NAA and BAP in different concentrations to induce callus formation. The callus raised on MS + NAA (10.74µM) + BAP (4.44µM) was sub cultured on to the same media composition supplemented with various concentrations of L-Glutamine (0.68mM, 1.36 mM, 2.05mM, 2.73mM), L-Glycine (1.33mM, 2.66mM, 3.99mM, 5.32mM) and L-Phenylalanine (0.6mM, 1.20 mM,1.8mM, 2.42mM, 3.0mM and 3.60mM). The node, node derived callus raised on MS medium with growth regulators and the callus raised on MS medium supplemented with growth regulators and an amino acid L-Phenylalanine at various concentrations were collected, shade dried for 15 days and crushed into powder.

Sample I = Nodal explant
Sample II = Node derived callus on MS + NAA (10.74µM) + BAP (4.44µM)
Sample III = Node derived callus on MS + NAA (10.74µM) + BAP (4.44µM) +L- Phenylalanine (0.60mM).
Sample IV = Node derived callus on MS + NAA (10.74µM) + BAP (4.44µM) + L- Phenylalanine (1.20mM)
Sample V = Node derived callus on MS + NAA (10.74µM) + BAP (4.44µM) +L- Phenylalanine (1.8mM)
Sample VI = node derived callus on MS + NAA (10.74µM) + BAP (4.44µM) +L- Phenylalanine (2.42mM)
Sample VII= node derived callus on MS + NAA (10.74µM) + BAP (4.44µM) +L- Phenylalanine (3.0mM)
Sample VIII= node derived callus on MS + NAA (10.74µM) + BAP (4.44µM) +L- Phenylalanine (3.60mM)

HPLC analysis: HPLC studies were carried out for both qualitative and quantitative estimation of Psoralen and Bergapten present in nodal extract and node derived callus extract. The

standards such as Psoralen, Bergapten and solvents such as HPLC grade methanol and acetonitrile were procured from Sigma. Methanol and deionized sterile triple distilled water were procured from SD Fine Chemicals.

Preparation of crude plant extract: Shade dried plant material (node and node derived callus) was powdered. 95% ethanol was added into the powdered material as given in pharmacopoeia. The mixture was sonicated for 30 minutes. After sonication the mixture was filtered and filtrate was concentrated using Roto Vaccum Evaporator. The concentrated extract was transferred into a pre weighed Eppendorf tube and evaporated to dryness.

Preparation of reference standard solution–Psoralen and Bergapten: Standard stock solutions of Psoralen and Bergapten were prepared in methanol to get theconcentration of 1 mg/ml and HPLC analysis was carried out and the chromatograms of the same are shown in Fig. 1 and Fig. 2.

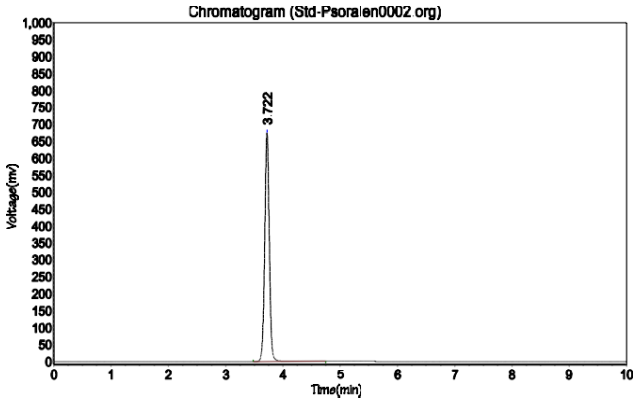

Fig. 1. Separation of Psoralen standard by isocratic method of HPLC (acetonitrile: water (70: 30 v/v) peak identified Psoralen (3.7 min) Auto-scaled chromatogram.

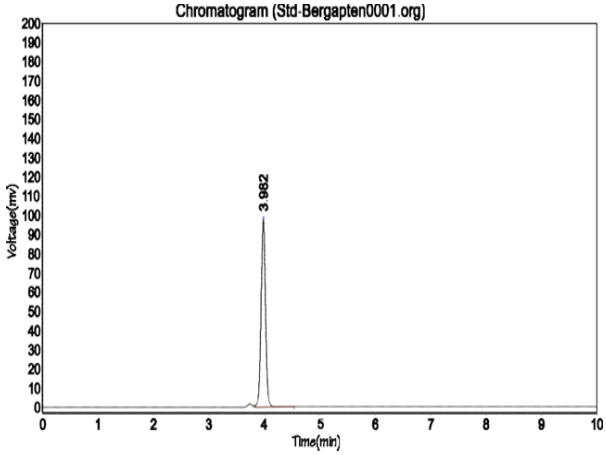

Fig. 2. Separation of Bergapten standard by isocratic method of HPLC (acetonitrile:water (70: 30 v/v) peak identified Bergapten (3.9 min) Auto-scaled Chromatogram

Extraction of Psoralen and Bergapten: The methanolic crude extract of each sample was diluted in methanol to a concentration of 1 mg/ml. All materials were analyzed using a Waters 515, Model- 2998, with a photodiode array detector and a Reversed phase column Nova- Pack C18, 4 µm, 39×150 mm. An isocratic elution method with a flow rate of l µl/10 minutes was utilized for separation. The column temperature was kept constant at 28°C and the detection wavelength of the photodiode array was 254nm. Acetonitrile and water (70:30 v/v) is used as the mobile phase to separate Psoralen and Bergapten from *R. graveolens* node and node derived callus extract. Injection volume taken was 5µl. Using acetonitrile and water (70:30 v/v) as a mobile phase, Psoralen and Bergapten was isolated from *R. graveolens* extract of node and node derived callus. Analysis was done using Empower software. The result is shown as the means of three replicates. Operation was started by injecting 5µl of each standard solution one by one and then followed by samples. The run time for each injection was 30 min.

Analysis of Psoralen and Bergapten: 5µl of reference standard of Psoralen and Bergapten and the samples were injected separately. The chromatograms were recorded and the analyte peak was measured. The content of Psoralen and Bergapten was calculated in different samples from declared content of Psoralen and Bergapten in reference sample.

Statistical Methods: In the present study, descriptive statistical analysis has been carried out and results are presented as Mean ± SD (Min-Max). Significance is assessed at 1 % level of significance.The following assumptions on data were made.

1. Dependent variables should be normally distributed.
2. Samples drawn from the population should be random; cases of the samples should be independent. One-way Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of samples and Tukey Post hoc test has been used to find the pair wise significance [9].

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, Med Calc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs and tables etc**.**

3. RESULTS & DISCUSSION

The node explants were inoculated onto MS medium supplemented with growth regulators either alone or in combination to induce callus formation. When an explant was inoculated onto MS medium supplemented with MS+IAA, MS+IBA, MS+BAP and MS+Kinetin of various concentrations individually there was no callus or scanty callus formation. When an explant was inoculated onto MS medium supplemented with NAA (10.74 µM), there was yellow friable callus formation after 6 weeks of culture. When the callus obtained on NAA (10.74 µM) was sub cultured on to MS+NAA (10.74 µM) + BAP (4.44 µM) / MS+NAA (10.74 µM) + BAP (13.20 µM), pale greenish yellow friable callus and pale greenish yellow nodulated callus were produced respectively after 8 weeks of culture (Fig. 3 and Fig. 4).

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The maximum callus was produced on MS+NAA (10.74µM) + BAP (4.44µM) within 60 days of inoculation with fresh weight of 898.95 ± 7.18mg. Fresh and dry weight of callus was recorded after 8 weeks of culture in combination of auxins and cytokinins i.e., NAA+BAP in the present study (Table-1 and Table-2). Callus proliferation in tissues of most dicotyledonous plants is often thought to require the presence of both auxin and cytokinin in the growth medium. The enhancement of callus induction depends on different concentrations of combination of a cytokinin with an auxin [10] as reported in *Psophocarpus*, *Vigna aconitifolia* and a few other legumes [11,12,13].

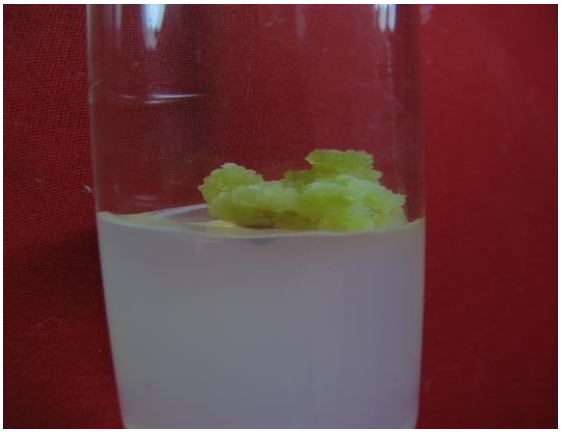

Fig 3. Pale greenish yellow friable callus

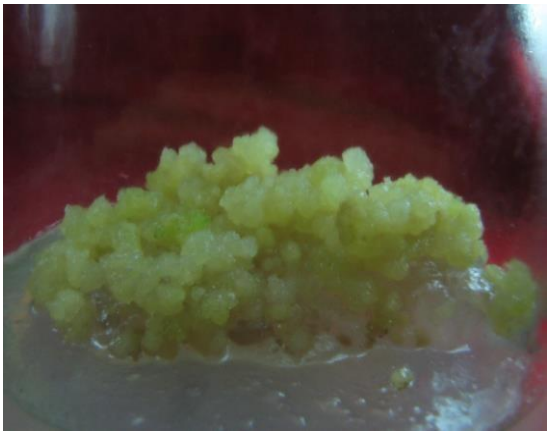

Fig 4. Pale greenish yellow nodulated callus.

Even in several medicinal plant tissue cultures, presence of an auxin along with kinetin produced profuse callus as reported in *Cephaelis ipecacuanha* [14], *Coptis* [15], *Solanum viarum* [16] *Evolvulus alsinoides* [17], *Andrographis paniculata* [18] and compact or watery friable callus in *Physalis angulata* [19]. In tissue culture, cytokinins appear to be necessary for cell division. In their absence, metaphase of mitosis was found to be considerably protracted and it was suggested that cytokinins are probably required to regulate the synthesis of proteins involved in the formation and function of mitotic spindle apparatus. Likewise , even in the

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present study, combination of an auxin with cytokinin favoured callus growth from nodal explants.

When all of the necessary amino acids are consistently synthesized in cultures, adding an amino acid or a blend of amino acids to boost cell proliferation and facilitate plant regeneration can still be beneficial [20].

In the present investigation, nodal explants showed a good response in terms of callus initiation on media containing NAA (10.74 µM) + BAP (4.44 µM) with different concentrations of L-Glutamine, L-Glycine and L-Phenyl alanine. Fresh and dry weight of callus was recorded after 8 weeks of culture (Table-1 and Table-2). Among the media supplemented with amino acids, the media supplemented with L-Phenylalanine at a concentration of 2.42 mM was the best for maximal creamish nodular callusing with fresh weight of 35640.50 ± 121.26 mg (Fig. 5), followed by the media supplemented with L-Glutamine at a concentration of 1.36mM which induced greenish yellow nodular callus formation with fresh weight of 29280.00 ±15.62 mg (Fig. 6) and L-Glycine at a concentration of 1.33mM which induced Creamish friable callus formation with fresh weight of 8595.00 ± 195.95 mg (Fig. 7).

Fig .5 Creamish nodular callus

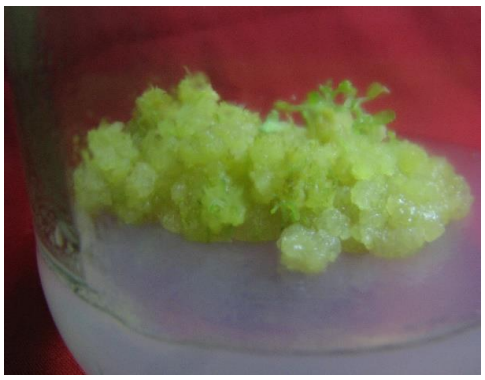


Fig. 6 Yellow nodular callus

Fig .7 Creamish friable callus

Table 1: Effect of growth regulators and amino acids on callusing from nodal cultures of *Ruta graveolens* L.

MS + growth regulators (µM) + aminoacids (mM)	Fresh weight of Callus from Nodal Cultures (mg)	
	Mean ± SD	95%CL
MS + NAA (5.37)	99.10 ± 1.59	98.36 - 99.84
MS + NAA (10.74)	500.70 ± 4.81	498.45 - 502.95
MS + NAA (16.11)	449.80 ± 4.50	447.69 - 451.91
MS + NAA (24.47)	419.85 ± 2.80	418.54 – 421.16
MS + NAA (26.83)	390.50 ± 2.24	389.45 – 391.55
MS + NAA (32.19)	349.75 ± 3.80	347.97 – 351.53
MS + NAA (37.55)	309.75 ± 2.55	308.56 – 310.94
MS + NAA (42.91)	280.25 ± 2.63	279.02 – 281.48
MS + NAA (48.27)	260.00 ± 2.81	258.68 – 261.32
MS + NAA (53.63)	199.75 ± 3.43	198.14 – 201.36
MS + 2,4-D (4.52)	199.75 ± 3.43	198.14 – 201.36
MS + 2,4-D (9.00)	250.50 ± 3.20	249 – 252
MS + 2,4-D (13.53)	300.50 ± 6.26	297.57 – 303.43
MS + 2,4-D (18.03)	281.00 ± 5.53	278.41 – 283.59
MS + 2,4-D (22.5)	272.75 ± 1.80	271.91 – 273.59
MS + 2,4-D (27.03)	268.55 ± 1.57	267.81 – 269.29
MS + 2,4-D (31.53)	261.65 ± 2.11	260.66 – 262.64
MS + 2,4-D (36.0)	225.25 ± 2.55	254.06 – 256.44
MS + 2,4-D (40.53)	240.00 ± 0.92	239.57 – 240.43
MS + 2,4-D (45.03)	150.20 ± 1.44	149.53 – 150.87
MS + NAA (10.74) + BAP (4.44)	898.95 ± 7.18**	895.59 – 902.31
MS + NAA (10.74) + BAP (13.20)	700.00 ± 6.10**	695.50 – 698.20
MS + NAA (10.74) + BAP (4.44) + L-Gln (0.68)	21705.00 ± 19.87**	21695.7 – 21714.3
MS + NAA (10.74) + BAP (4.44) + L-Gln (1.36)	29280.00 ± 15.62**	29273.37 – 29282.63
MS + NAA (10.74) + BAP (4.44) + L-Gln (2.05)	19417.00 ± 8.65**	19412.95 – 19421.05
MS + NAA (10.74) + BAP (4.44) + L-Gln (2.73)	19300.50 ± 9.45**	19296.08 – 19304.92

MS + NAA (10.74) + BAP (4.44) + L-Gly (1.33)	14262.50 ± 39.35**	14244.08 – 14280.92
MS + NAA (10.74) + BAP (4.44) + L-Gly (2.66)	10458.50 ± 6.71**	10455.36 – 10461.64
MS + NAA (10.74) + BAP (4.44) + L-Gly (3.99)	8595.00 ± 195.95**	8503.29 – 8686.71
MS + NAA (10.74) + BAP (4.44) + L-Gly (5.32)	6780.50 ± 8.87**	6776.35 – 6784.65
MS + NAA (10.74) + BAP (4.44) + L-Phe (0.60)	32500.50 ± 6.86**	32497.29 – 32503.71
MS + NAA (10.74) + BAP (4.44) + L- Phe (1.20)	33450.50 ± 5.10**	33448.11 – 33452.89
MS + NAA (10.74) + BAP (4.44) + L-Phe (1.81)	34500.00 ± 2.81**	34498.68 – 34501.32
MS + NAA (10.74) + BAP (4.44) + L-Phe (2.42)	35640.50 ± 121.26**	35583.75 – 35697.25
MS + NAA (10.74) + BAP (4.44) + L-Phe (3.0)	33450.55 ±5.10**	33448.10-33452.80
MS + NAA (10.74) + BAP (4.44) + L-Phe (3.6)	32400.00±6.80**	32397.00-32403.00

Significance - F=1660680; P<0.001,95% Confidence Limits**

Note: Results are represented as Mean ± SD. Data within a column followed by the symbol (**) represent 1% level of significance compared to all treatments by Tukey post-hoc test.

Table 2. Effect of growth regulators and amino acids on callusing from nodal cultures of *Ruta graveolens* L.

MS + growth regulators (µM) + aminoacids (mM)	Dry weight of Callus from Nodal Cultures(mg)	
	Mean ± SD	95%CL
MS + NAA (5.37)	9.33 ± 0.44	9.12 – 9.53
MS + NAA (10.74)	49.00 ± 0.65	48.7 – 49.3
MS + NAA (16.11)	44.80 ± 0.38	44.62 – 44.98
MS + NAA (21.47)	42.00 ±0.65	41.7 – 42.3
MS + NAA (26.83)	39.00 ± 0.65	38.7 – 39.3
MS + NAA (32.19)	35.00 ± 0.65	34.7 – 35.3
MS + NAA (37.55)	30.90 ± 0.6	30.62 – 31.18
MS + NAA (42.91)	28.00 ± 0.65	27.7 – 28.3
MS + NAA (48.27)	26.00 ± 0.65	25.7 – 26.3
MS + NAA (53.63)	20.00 ± 0.46	19.79 – 20.21
MS + 2,4-D (4.52)	19.84 ± 0.08	19.8 – 19.88
MS + 2,4-D (9.03)	24.86 ± 0.13	24.8 – 24.92
MS + 2,4-D (13.53)	30.00 ± 0.65	29.7 – 30.3
MS + 2,4-D (18.03)	28.00 ± 0.46	27.79 – 28.21
MS + 2,4-D (22.53)	27.20 ± 0.11	27.15 – 27.25
MS + 2,4-D (27.03)	26.12 ± 0.18	26.04 – 26.2
MS + 2,4-D (31.53)	26.40 ± 0.22	26.29 – 26.51
MS + 2,4-D (36.03)	25.40 ± 0.18	25.31 – 25.49
MS + 2,4-D (40.53)	23.55 ± 0.28	23.42 – 23.68
MS + 2,4-D (45.03)	14.98 ±0.11	14.93 – 15.03
MS + NAA (10.74) + BAP (4.44)	85.00 ± 1.26**	84.41 – 85.59
MS + NAA (10.74) + BAP (13.20)	70 ± 1.10**	69.40 – 70.49
MS + NAA (10.74) + BAP (4.44) + L-Gln (0.68)	630.00 ± 6.49**	626.96 – 633.04
MS + NAA (10.74) + BAP (4.44) + L-Gln (1.36)	920.00 ± 6.49**	916.96 – 923.04
MS + NAA (10.74) + BAP (4.44) + L-Gln (2.05)	569.50 ± 4.26**	567.51 – 571.49
MS + NAA (10.74) + BAP (4.44) + L-Gln (2.73)	520.00 ± 2.81**	518.68 – 521.32
MS + NAA (10.74) + BAP (4.44) + L-Gly (1.33)	241.00 ± 3.48**	239.37 – 242.63

MS + NAA (10.74) + BAP (4.44) + L-Gly (2.66)	677.00 ± 7.33**	673.57 – 680.43
MS + NAA (10.74) + BAP (4.44) + L-Gly (3.99)	400.00 ± 9.32**	395.64 – 404.36
MS + NAA (10.74) + BAP (4.44) + L-Gly (5.32)	120.00 ± 5.62**	117.37 – 122.63
MS + NAA (10.74) + BAP (4.44) + L-Phe (0.60)	1000.00 ± 3.63**	998.3 – 1001.7
MS + NAA (10.74) + BAP (4.44) + L- Phe (1.20)	1147.50 ± 25.52**	1135.56 – 1159.44
MS + NAA (10.74) + BAP (4.44) + L-Phe (1.81)	1382.00 ± 11.52**	1376.61 – 1387.39
MS + NAA (10.74) + BAP (4.44) + L-Phe (2.42)	1500.00 ± 4.59**	1497.85 – 1502.15
MS + NAA (10.74) + BAP (4.44) + L-Phe (3.0)	1147.50 ± 25.52**	1135.56 – 1159.44
MS + NAA (10.74) + BAP (4.44) + L-Phe (3.6)	1000 ± 3.60 **	998.2 – 1001.7

Significance - F=113270.9; P<0.001,95% Confidence Limits**

Note: Results are represented as Mean ± SD. Data within a column followed by the symbol (**) represent 1% level of significance compared to all treatments by Tukey post-hoc test.

Hussein *et al*., in 1994 studied the effect of some amino acids on the growth of *Datura stramonium* cultured on MS medium with 1 mg/l of both 2, 4-D and kinetin and sub cultured on fresh medium containing some amino acids [21]. They found that the fresh and dry weight of callus was reduced. However, in the present study there was gain in the fresh and dry weight of the callus that was raised on MS+NAA (10.74 µM) + BAP (4.44 µM) supplemented with different concentrations of amino acids like L-Glutamine, L-Glycine and L-Phenylalanine. HPLC analysis of node showed that 1g dry weight of the sample contained 0.004 mg of Psoralen and 6.41 mg of Bergapten (Fig. 8). The concentrations of furanocoumarins were higher in *Ruta* species than those found in other families known to produce the same compounds (Moraceae, Apiaceae and Fabaceae) [22]. Innode derived callus, Psoralen was absent whereas 9.06 mg of Bergapten was present in one gm dry weight of the sample. (Fig. 9). Trillini and Ricci in 1998 reported that the HPLC analysis of the callus tissues of *Heracleum sphondylium* showed the absence of psoralen while various O-alkyl furanocoumarins such as bergapten, imperatorin, isoimpinellin and xanthotoxin were present in much more substantial amounts [23]. This fact supports the hypothesis that the biosynthesized Psoralen is first hydroxylated and a transmethylation reaction completes the biosynthetic pathway to its O-alkyl derivatives [24]. In the present study also, Psoralen was absent in the callus.

Based on the knowledge of biosynthetic pathways, several organic substances can be added to the culture medium to increase the synthesis of secondary metabolites. The amount of bergapten increased in the callus which was raised on MS+NAA (10.74 µM) + BAP (4.44 µM) supplemented with L-Phenylalanine of 0.60 mM, 1.20 mM, 1.81 mM 2.42 mM, 3.0 mM and 3.60 mM concentrations than the callus which was raised on MS+NAA (10.74 µM) + BAP (4.44 µM) without L-Phenylalanine (Table 3).

The highest amount of Bergapten of 27.45 mg/g dry weight of the sample was produced in the callus raised on MS+NAA (10.74 µM) + BAP (4.44 µM) supplemented with L-Phenylalanine of 2.42mM (Fig. 10) whereas the reduced amount of Bergapten of 15.23 mg/g dry weight of the sample was produced in the callus raised on MS+NAA (10.74 µM) +BAP (4.44 µM) supplemented with L-Phenylalanine of 3.6mM (Fig. 11). It is very much important to determine

the appropriate precursor concentration in the precursor feeding test as excess precursors may cause feedback inhibition to the metabolic pathway [25]. When Phenylalanine was increased to 10mM, Diadzein and Genistein levels were greatly inhibited [26].

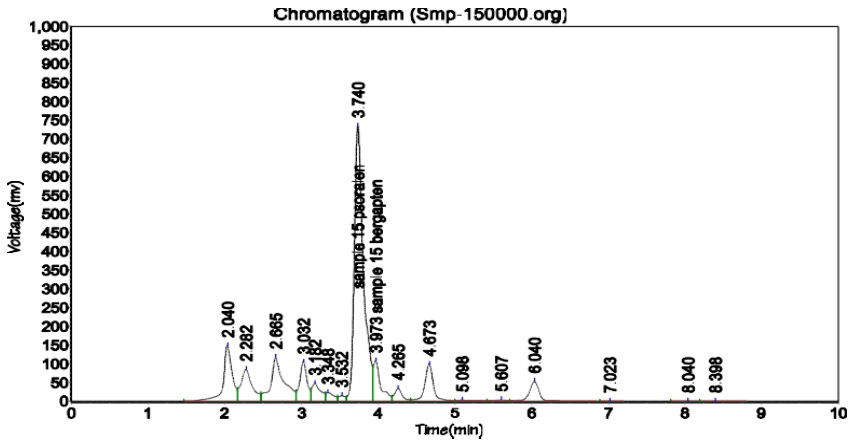
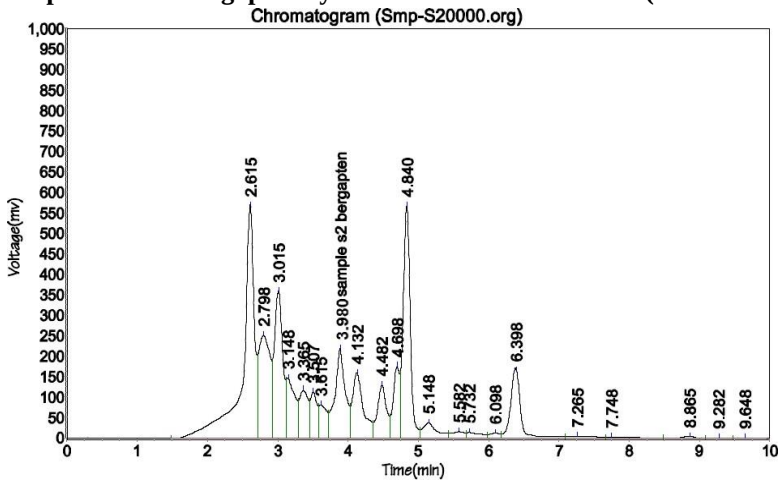


Fig. 8. Separation of Psoralen and Bergapten by isocratic method of HPLC (acetonitrile:water (70: 30 v/v) peak identified Psoralen (3.7 min) and Bergapten (3.9 min) Auto- scaled Chromatogram

Fig. 9. Separation of Bergapten by isocratic method of HPLC (acetonitrile: water



(70:30 v/v) peak identified Bergapten (3.9 min) Auto-scaled Chromatogram

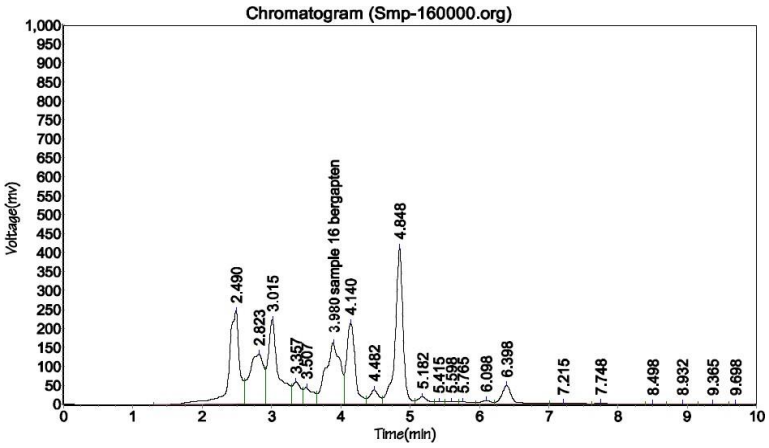


Fig. 10. Separation of Bergapten by isocratic method of HPLC (acetonitrile: water (70:30 v/v) peak identified Bergapten (3.9 min) Auto-scaled Chromatogram

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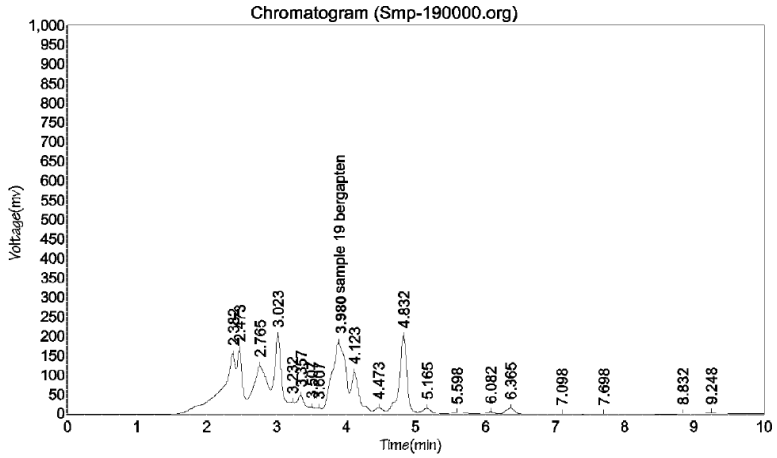

Fig. 11. Separation of Bergapten by isocratic method of HPLC (acetonitrile: water (70: 30 v/v) peak identified Bergapten (3.9 min) Auto-scaled Chromatogram

Table 3. The content of Psoralen and Bergapten in node, node derived callus raised on MS medium supplemented with growth regulators and L-Phenylalanine.

Sl. No.	Sample	Media Composition	Psoralen		Bergapten	
			%	mg/g (DW)	%	mg/g (DW)
1.	Node	-	0.0004	0.004	0.65	6.41
2.	Node derived callus	MS + NAA (10.74 µM) + BAP (4.44 µM)	Absent	-	0.90	9.06
3.	Node derived callus	MS + NAA (10.74 µM) + BAP (4.44 µM) + L – Phe (0.60 mM)	Absent	-	1.43	14.23
4.	Node derived callus	MS + NAA (10.74 µM) + BAP (4.44 µM) + L – Phe (1.20 mM)	Absent	-	1.97	19.64
5.	Node derived callus	MS + NAA (10.74 µM) + BAP (4.44 µM) + L – Phe (1.81 mM)	Absent	-	2.69	26.88
6.	Node derived callus	MS + NAA (10.74 µM) + BAP (4.44 µM) + L – Phe (2.42 mM)	Absent	-	2.75	27.45
7.	Node derived callus	MS + NAA (10.74 µM) + BAP (4.44 µM) + L – Phe (3.0 mM)	Absent	-	2.00	20.54
8.	Node derived callus	MS + NAA (10.74 µM) + BAP (4.44 µM) + L – Phe (3.60 mM)	Absent	-	1.5	15.23

4.CONCLUSION

The nodal explants showed a good response in terms of callus initiation on media containing NAA (10.74 µM) + BAP (4.44 µM) supplemented with different concentrations of amino acids like L-Glutamine, L-Glycine and L-Phenylalanine. Callus culture raised on MS+NAA (10.74 µM) + BAP (4.44 µM) with appropriate concentration of L-Phenylalanine can be used for more production of Bergapten which can be a commercially feasible alternative for Bergapten production as the plant contains less amount of Bergapten.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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