In vitro propagation, Antibacterial and Antioxidant activities of *Coleus Zeylanicus* (Benth.) L.H. Cramer

Kanagaraj K.[1], Nandagopal S.[2]* and Ganesh Kumar A.[3]*
1. Department of Botany, Periyar University, Salem-600 119, TN, INDIA
2. Department of Botany, Government Arts and Science College, Hosur-635 110, TN, INDIA
3. Department of Microbiology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-602 105, TN, INDIA
*nandagopalbdu@gmail.com; drgkanbazhagan@gmail.com

Abstract
The present study is an in vitro micropropagation of Coleus zeylanicus, a potential therapeutic plant utilizing shoot tip and nodal explants by following plant tissue culture methods. BAP and KIN blends brought about a normal shoot length of 4cm per explant. This blend likewise delivered 13 shoots most extreme for each explant with 90% shooting proficiency. Numerous shoots raised were additionally stretched on MS medium containing IAA (0.5-2.0 mg/ml) and IBA (0.5-2.0 mg/ml).

Established plantlets were then moved to plastic pots containing autoclaved garden soil, yard soil and sand in the proportion of (1:2:1) for solidifying measure (hardening). The endurance pace of plantlets changed under acclimatization and plants looked sound with no noticeable distinguishable phenotypic varieties. This is the primary report on plant recovery through organogenesis of Coleus zeylanicus. The examination likewise presents accessible information for all out phenolic and flavonoid content, its antioxidant and antibacterial action against five bacterial strains of in vivo and in vitro plant.

Keywords: *Coleus zeylanicus*, Micropropagation, GC-MS analysis, Antibacterial, Antioxidant activities.

Introduction
Coleus zeylanicus is an important medicinal herb of Lamiaceae family which is perennial, branched and aromatic. It generally grows in warm temperate climatic hill slopes upto 1800m altitude. It grows up to a height of 30 cm to 60 cm and has square stems, branched and the nodes are often hairy. It is generally vegetatively propagated through cuttings[4]. This plant contains various therapeutic constituents such as alkaloids, tannins, flavonoids, phenols, volatile compounds and bioactivities like anti-inflammation, anti-thrombotic, anti-oxidant and anti-carcinogenic activities which have major role in preventing number of chronic diseases in human beings and animals[10,20,33].

The essential oils in Lamiaceae are known as Nature's chemical reservoir, which are non-toxic and have shown good fighting potential against drug resistance pathogens[24,29,39]. In traditional medicinal practice, the plant leaves are used as a single drug remedy for children's cough and cold[12,13]. The juice of stem and leaves of the plant is mixed with honey and taken as a remedy for diarrhea[38]. Many floral members of the family are widely cultivated for their aromatic qualities and ease of its cultivation. This plant is to propagate by stem cuttings[44]. The essential oil of *Coleus zeylanicus* has been reported to show an inhibitory effect on enteric pathogens like *Salmonella typhi* and also against genitourinary infection causing fungi *Candida albicans*[14,18,19].

In recent days, there is an increased interest towards in plants *in vitro* technique, as it accelerates the multiplication of various threatened species of plant and also assists in germplasm conservation[18]. Further, improvement in genetic diversity is another way to increase the therapeutic drug-producing ability of the plants[42]. Therefore, it is very important to create a highly efficient micropropagation method for broad clonal production of *C. zeylanicus* and to further increase the yield of its bio-active photo-compounds. In spite of the fact that there are a couple of reports on micropropagation of various types of Coleus[11,36], yet at the same time there was no legitimate development strategy for this plant and further no reports on the tissue culture practice of *C. zeylanicus* are accessible.

Accordingly, there is an expanded need to build up an optimized technique for quick recovery of plants through tissue culture[1]. *In vitro* recovery technique for the creation of plants is requested, on the grounds that field developing plants might be exposed to different seasonal varieties, disease with plants microscopic organisms and growths too as ecological contaminations that can change the chemical substance constituents and therapeutic properties of the reaped plant tissues[32,37,40]. Micropropagation is one of the important methods in plant biotechnology that explains the capability of plant cells. Significant feature of this technology is its significant proliferate capacity in relative short time (useful in conservation strategy), for the generation of healthy and non-diseaseplants[15,21].

Micropropagation through shoot culture is often preferred to maintain clonal fidelity. An undisturbed exploitation of plants is to fulfil the increasing requirement by the Indian pharma industry with sustaining nature[2,34]. Our present examinations are outlined by following plant tissue culture conventions to recover great quality somaclones of this plant for volatile oils production. In this current work, we have depicted the progressive foundation of recovered plantlets in

soil with fast rate and supportable technique for shoot expansion from shoot tip and nodal explants interestingly. The convention detailed here could be a valuable for the protection, proliferation in enormous scope creation.

Material and Methods

Collection of plant material and explant preparation: Young nodal explants (1-1.5cm) were taken out from stock plants (3 months old) of *C. zeylanicus* which were developed in the Botanical Garden of Sathyabama University, Chennai, India. Gathered plants were completely washed with 10% (v/v) Dettol for 30s, trailed by washing three to multiple times in deionized water. It was then further surface disinfected with 0.1% (w/v) aqueous mercuric chloride (1-6 min) and lastly washed with deionized water. At that point, the surface disinfected explants were cut at the edges before inoculation.

Media composition and Culture conditions: Surface disinfected shoot tips (4-5mm) and nodal sections (10-12mm) were continued to culture on MS basal medium enhanced with supplement of 3% (w/v) sucrose (Himedia, Mumbai, India) for callus commencement. The pH of the MS medium was acclimated to 5.7 utilizing 1N NaOH or 1N HCl before gelling with 0.7 % agar. The medium was then sterilized (105 KPa and 121 ˚C for 15 min) and moved into culture vessels. The explants were then positioned upward on the MS medium and with firmly stopped non-permeable cotton. Every one of the mediums were incubated (25±1˚C) under 16/8 (light/dull) photoperiod of 45-50 µM 143-2s^{-1} irradiance utilizing cool white fluorescent cylinders with relative dampness (RH) of 55-60%. The subcultures were completed at each multi week time spans.

Multiple shoot induction and plantlet regeneration: Shoot tips with leaflets and nodal sections eliminated from 35 days old plants were set upward on MS medium containing 0.7% (w/v) agar, 3% (w/v) sucrose, 0.1 g/l myo-inositol with various fixations. For every treatment, a sum of ten recreates (each with two explants-20 explants per treatment) were tried. A benchmark group (MS basal medium without chemicals) was kept up to screen and look at the recurrence of reaction.

Rooting: To start root development, singular shoots with 6.8–10.8 cm long were chosen and moved to MS medium with different known concentrations of auxins (0.5mg/ml-2.0 mg/ml). One cluster of culture was set on basal MS medium without the auxins and regarded as control.

Acclimatization of plantlets and transfer to soil: The instigated roots from new plantlets were extracted from the MS medium and after that roots were washed to eliminate the followed MS medium. The washed plantlets were then positioned to new plastic pots (10 cm measurement) containing sterile nursery soil, farmstead soil and sand in the proportion of 2:1:1. Each pot was watered with faucet water routinely. At first the plantlets were kept up inside the room conditions for 5 weeks. The plantlets were relocated at Botanical Evaluation Garden and saved for additional development and improvement after the 45 days. The plantlets morphological, other development qualities and endurance rate were observed and noted.

Extraction of phytochemicals: 1g fresh weight of the plant material (*in vitro* and *in vivo* grown) was ground with 5 ml of ethanol with mortar and pestle. The contents were filled into a centrifuge tube. The mixture was ultrasonicated and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected into a separate tube. The pellet was re-suspended in 5 ml ethanol. The mixture was again centrifuged and the supernatant obtained was collected in the same tube. This extract was further subjected to antimicrobial and antioxidant activity.

Antimicrobial activity: The antimicrobial action of the concentrate was surveyed against five bacterial strains *Staphylococcusaureus*, *Escherichiacoli*, *Bacillussubtilis*, *Pseudomonasaeruginosa*, *Enterococcusfaecalis* utilizing agar diffusion strategy (Kirby Bauer susceptibility test). Sterile discs of 5mm were utilized for adding the plant extracts. Yard culture of the individual microorganisms was added in various Petri plates with Mueller-Hinton agar medium. Various concentrations of the concentrate 10, 20 and 30 µl were put on the medium and incubated (37C for 24-48 hrs). After incubation, the diameters of zone of inhibition were estimated. Streptomycin disc was considered as control. The test was repeated three times and the mean of the three readings for each disc was determined as the zone of inhibition.

Antioxidant activity: DPPH was utilized to decide the radical-scavenging activities of *C. zeylanicus* essential oil. To a methanolic solution of DPPH (200µm), 0.05ml of test samples was dissolved in distilled water with 20, 40, 100, 125 and 250 µg/ml of different concentrations of test samples. Equivalent measure of deionized water was added to the control and kept as blank. Following 20 minutes, the test blends were read at 517nm. The rate inhibition was determined by utilizing the equation:

$$\text{DPPH scavenging activity (\%)} = [(A_a - A_b)/A_a] \times 100$$

where A_a is the absorbance of the control (without oil) and A_b is the absorbance of the sample. The assay was done for both *in vitro* and *in vivo* plant extracts. The percentage of the scavenging activity was measured and a linear regression graph of percentage of scavenging activity against concentration was plotted to calculate IC_{50} value[19,32,35].

Gas Chromatography Mass Spectrometry (GC-MS) Analysis: The volatile components of both the *in vitro* and *in vivo* plant materials were analysed by GC-MS using the same parameters. The spectrum of the compounds was compared with the spectrum of the known compounds deposited in the NIST and Wiley Library[22-25,31].

Statistical analysis: 10-20 explants were chosen according to the treatment for every replication study. Immensely significant qualities were noted down. The Analysis of Variance (ANOVA) was determined to distinguish the importance contrasts among the analyses and Duncan's multiple range test (DMRT) was completed for correlation by keeping 5% as significance level[18].

Results and Discussion

***In vitro* establishment:** The explant (node) was decontaminated by treating with 0.1% $HgCl_2$ at fluctuated time spans between 4 minutes to 15 minutes. The openness length of the explants to the sterilizing chemicals is most significant advance. The disinfecting medicines were even not powerful in disposing of microorganisms when the explants sterilization time was less than 10 min[5-9].

Essentially the treatment was deadly to the followed microorganisms as well as that end up being deadly when the term of openness was above for 12 min[16]. A 12 min surface disinfecting period was chosen to be an ideal and the equivalent is continued in this work. The surface sanitized explants were sliced at the edges to eliminate the $HgCl_2$ (Table1). Hence the explant culture started in the medium (Figure 1).

Multiple shoot induction and plantlet regeneration: Shoot tips (4-5mm) and nodal portions (10-12 mm) of *C. zeylanicus* were cultured on MS medium which was enhanced with B5 nutrients and different convergences of N6-benzylamino purine BAP (0.2-2.0 mg/l) and KIN (0.2-1.0 mg/l) to test shoot regenerating proficiency.

Following fourteen days, cultured explants which showed shoot bud commencement were noticed (Figure 2). Shoot induction recurrence was recorded to be more in tip of shoot explants than the nodal portions. The examination was completed in three unique methods of chemical supplementation (Table 2). At the point when BAP was enhanced separately, the greatest normal length of shoots per explant was discovered to be 2.5cm. KIN enhanced medium 12.6 shoots were noticed. BAP was discovered to be acceptable accordingly than KIN (Table 2). The cytokinin, BAP has been regularly utilized for organogenesis induction[17,27,28]. A correlation of the general viability of different cytokinins for various shoot enlistment revealed adequacy (BAP > KIN > Zeatin > Adenine)[29].

Table 1
Surface sterilization of nodes and shoot tip explants with 0.1% mercuric chloride for culture initiation *in vitro*.

S.N.	Treatment period (minutes)	Number of explants	Number of contamination free cultures	Percentage of contamination free cultures	Percentage of responded cultures
1	4	10	0	0	-
2	5	10	4	40	100
3	7	10	5	50	100
4	10	10	7	70	85.7
5	**12**	**10**	**8**	**80**	**87.5**
6	15	10	10	100	20.0

This data represents the mean number of 10 replicates repeated twice, recorded after 4 weeks period in culture.

Table 2
Effect of cytokinins on shoot multiplication from shoot tip and node explants of *Coleus zeylanicus*

Concentration of growth regulators (mg/l)	Shoot tip		Node	
	Percentage of response (%)	Mean number of shoots/explant (Mean±SE)	Percentage of response (%)	Mean number of shoots/explant (Mean±SE)
Control	100	6.33±0.5	100	3.3±0.2
BAP				
0.2	80	8.5±0.4	50	1.0±0.2
0.5	**90**	**10.0±1.5**	60	1.1±0.2
1.0	70	8.3±1.5	65	1.4±0.2
1.5	60	7.3±1.5	**70**	**2.0±0.3**
2.0	68	8.3±1.5	60	2.5±0.3
KIN				
Control	100	6.3±1.5	100	1.2±0.5
0.2	70	7.0±1.0	50	1.0±0.2
0.4	75	7.6±1.5	50	1.2±0.2
0.6	85	9.3±0.5	**70**	**2.3±0.3**
0.8	**90**	**9.7±1.5**	50	1.8±0.3
1.0	40	0.6±3.5	50	1.5±0.3

Table 3
Effect of different concentrations of BAP and KIN on shoot multiplicationfrom shoot tip and node of *Coleus zeylanicus*.

Concentration of growth regulators (mg/l)	Shoot tip		Node	
BAP + KIN	**Percentage of response (%)**	**Mean number of shoots/explant (Mean±SE)**	**Percentage of response (%)**	**Mean number of shoots/explant (Mean±SE)**
0.2 + 0.2	30	4.0± 0.2	40	1.0 ± 0.2
0.5 + 0.2	40	4.0± 0.2	50	1.4 ± 0.2
1.0 + 0.4	50	4.2± 0.2	**60**	**2.1 ± 0.3**
1.5 + 0.6	60	5.2 ±0.2	50	2.4 ± 0.3
2.0 + 0.8	**70**	**7.1± 0.3**	50	1.8 ± 0.3
BAP + KIN				
0.2 + 0.2	40	5.0 ± 0.2	60	2.0 ± 0.3
0.5 + 0.5	60	10.0 ± 0.4	60	2.5 ± 0.3
1.0 + 1.0	60	11.0 ± 0.4	65	3.1 ± 0.4
1.5 + 1.5	**90**	**13.0 ± 0.5**	**90**	**4.0 ± 0.5**
2.0 + 2.0	80	12.0 ± 0.5	80	3.0 ± 0.4

Evaluation was determined after 4 weeks. Treatment means followed significantly different from each other at 5% significance level (P <= 0.05) based on DMRT.

Accordingly, with our investigation, BAP has been utilized as inclination to different cytokinins to prompt shoots in *Coleus forskohlii*. Consequently, the medium has BAP (1.0-2.0 mg/l) normalized as the ideal mechanism for huge creation and extension of *Coleusforskohlii*[11]. It is shown that BAP is exceptionally powerful for *in vitro* engendering in plants[43]. At the point when BAP and KIN mixes were contemplated, the reaction was still better. Various mixes of BAP and KIN were tried (Table 3). Of the different blends, we endeavored 1.5mg/l of BAP and 1.5mg/l KIN. Elevated 13.3 shoots per explant with 90% shoot recovering recurrence were recorded (Figure 1, Table 3).

In *Coleus blumei*, presence of BAP 1.5 mg/l enhanced in MS medium guarantees a lot higher level of recovery than the one without phytohormones[22]. In *Coleus forskohlii*, expansion of shoot tip was acquired most noteworthy within the sight of BAP[11]. The main shoot tip culture is that in a moderately brief timeframe, an enormous number of infection free plants was delivered. The best morphogenetic reaction (15-20 shoots for every explant) was noted when shoot apices were cultured on MS medium with 2.0 mg/l BAP. Higher number of morphologically changed numerous shoots were seen from the shoot tips in MS medium with consolidated BAP and KIN. In confirmation of our current investigation, BAP and KIN blends were finished up preferably to deliver a greater number of plantlets.

Effect of auxins on rooting of shoots: To root establishing, extended shoots were cultured on half and full-fortified MS medium provided with different sorts of auxins like IAA and IBA (0.5-2.0 mg/ml) individually. A benchmark (control) group was additionally included (Table 4). Plantlets noticeably created extensive roots and were strengthened within 10 days. IAA was noted to be more preferably satisfactory than IBA. Roots were bare inside 5–10 days after the exchange of shoots to the established MS medium. Following fourteen days, created plantlets (Figure 1e) and recovering limit of rhizogenesis were nearly seen as 98%.

IAA was referred to as expected auxins for root acceptance in various medicinal plants[43]. Expanded grouping of IAA above 1.5mg/l slowly continued to an abatement in the limit of root recovery. An equivalent reaction was seen in *Coleusblumei*[22]. The normal number of roots started per shoot was going from 3-11 and the most extreme root length noted as 2.76 cm (Table 3, Figure 1). In any case, IBA created more slim roots. Rather than our report, IBA was considered for delivering exceptionally fanned extrinsic roots in *Coleusforskohlii*[41]. Shoots likewise delivered roots when refined utilizing basal medium with no development controllers, yet the root acceptance was higher with IAA (0.5 mg/ml).

Acclimatization of rooted plantlets and examination of morphological characters: A fundamental piece of *in vitro* proliferation is to acquire recuperated plants that are prepared for getting the sterile and *in vitro* environment. The nursery and field have extensively lower relative mugginess, higher light level and septic environment that are upsetting to miniature proliferated plants from *in vitro* conditions. The upside of any micropropagation structure can, in any case, simply be totally recognized by the productive trade of plantlets from tissue-culture vessels to the incorporating conditions found *ex vitro*[23,26,30].

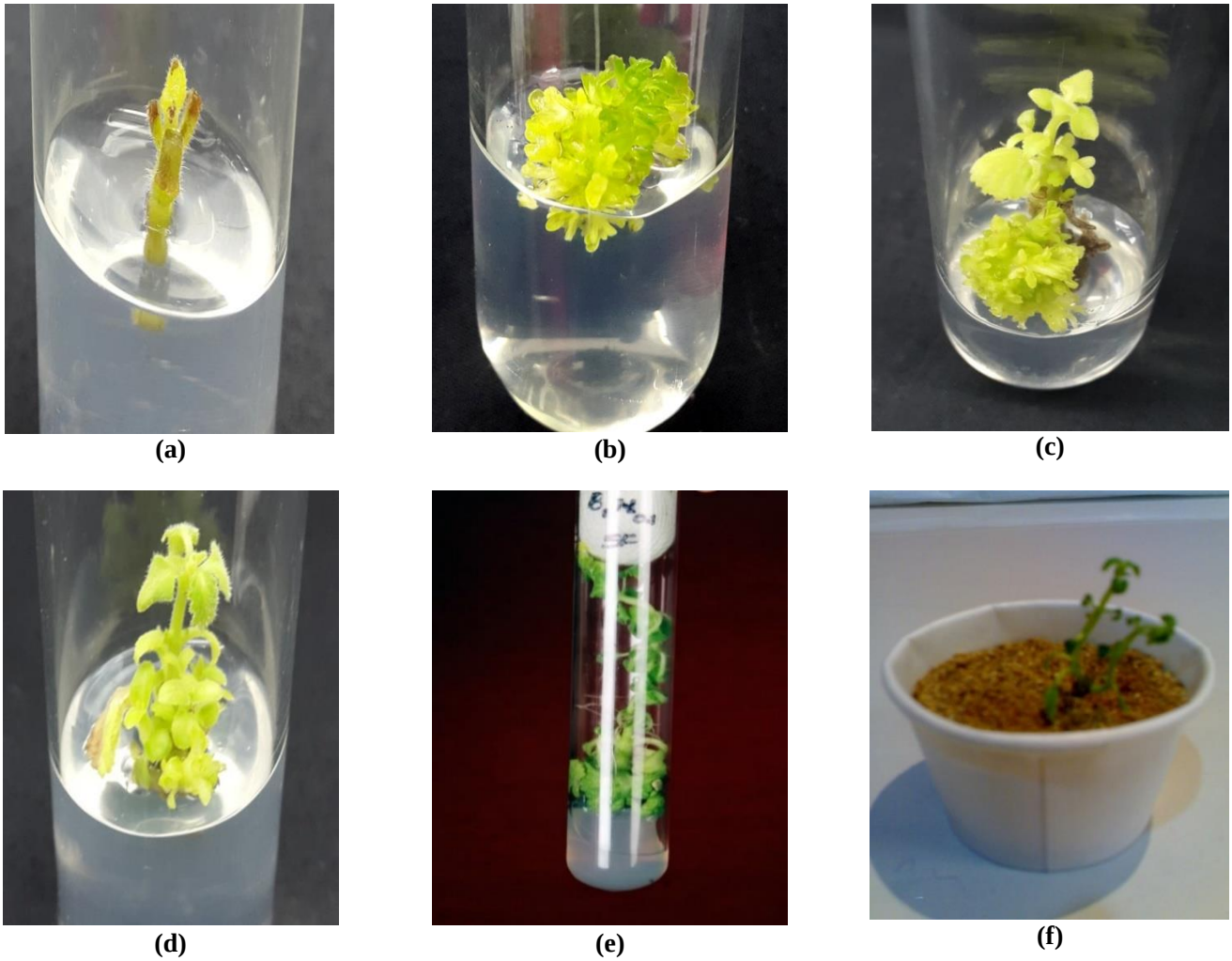
The viably joined plantlets were moved to plastic cups containing sterile nursery soil, yard soil and sand (2:1:1) for solidifying reason. Plantlets were kept up in the method of culture room (25±1˚C) conditions from the outset for 5

weeks and kept in lab conditions for around a month. Finally, the plantlets were moved to organic assessment nursery. The fundamental advancement speeds of plant height were 12.1-29.3 cm during the underlying 5 weeks of acclimatization. The perseverance rate lessened from 90% and 80%, independently following 10-20 weeks of acclimatization (Table 4). From the start, a couple of leaves were made bordering the guideline shoot. The stem was especially flimsy and improvement of hairs on the stem was taken note. The number of leaves extended after 8-20 weeks of acclimatization.

Table 4
Effect of auxin on rooting of *in-vitro* raised shoots of *Coleus zeylanicus*.

Concentration of Auxin (Mg/L)	Percentage of response (%)	Number of lateral roots	Length of the root (cm)
Basal	20	2.10±0.12	1.1±0.09
0.1	70	4.80±0.25	1.80±0.23
0.5	**90**	**11.25±0.42**	**2.67±0.23**
1.0	80	9.0±0.40	1.83±0.24
0.1	65	3.7±0.22	1.3±0.20
0.5	**80**	**6.5±0.34**	**1.7±0.41**
1.0	70	5.7±0.24	1.5±0.21

Each value addresses the treatment methods for twenty free replicates. Treatments followed by various letters are essentially not quite the same as one another at 5% degree of significant (P<= 0.05) as indicated by DMRT.



(a) **(b)** **(c)**

(d) **(e)** **(f)**

Figure 1: (a) Direct regeneration of *Coleus zeylanicus* from nodal explant, (b) Multiple shoot induction after 10 days of culture, (c) Shoot multiplication after 15 days, (d) Shoot multiplication after 20 days, (e) Shoot elongation and rooting after 30 days and (f) Hardened plantlet in foam cup.

Antimicrobial property: The antimicrobial activity was carried out with five human pathogenic bacterial strains namely *Staphylococcus aureus, Enterobacter aerogenes, Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa*. The experiment was carried out with *in vitro* and *in vivo* plant extracts at different concentrations of 10 µl, 20 µl and 30 µl. The zones of inhibition for both the samples were recorded and compared.

From the results, it was observed that the antibacterial property of the extract showed an increasing tendency with respect to the concentration. The maximum zone of inhibition was obtained at the highest concentration of both the extracts at 30 µl (Figure 2).

Moreover, *in vitro* plant sample has more antimicrobial activity than *in vivo* plant sample against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* whereas against *Enterobacter aerogenes* and *Pseudomonas aeroginosa*, the *in vivo sample* showed larger zone of inhibition than *in vitro* sample.

GC-MS analysis: The *in vitro* and the *in vivo* plant samples extracted in solvent, were subjected to GC-MS analysis. The compounds present in both the samples were compared. Out of the number of compounds present, 8 compounds matched in both the samples. They are Linoleic acid, Widderine, Naphthalene, Gamma Patchouline, Gurjunene, Levomenol, Gamma Himachalene and Eicosane. Cubenol and copaene were present in *in vitro* sample but not in the *in vivo* sample whereas diuron, quinoxaline and citronella are present in *in vivo* samples but not in the *in vitro* samples (Table 6, Figure 3). These compounds are of great importance in pharmaceutical and cosmetic industry[42,25].

GC-MS investigation of the hexane root concentrate of *Coleusforskohlii* showed the presence of α-Cedrene, γ-cadinene, citronellal, labdane derivative, ß-citronellol[38]. The fundamental oil from the leaves of *Coleusparvifolius* Benth (Labiatae) was distinguished by gas chromatography and mass spectrometry (GC-MS). The significant constituents were discovered to be (E)-phytol, eicosatrienoate, n-tetradecanoic corrosive, octoil2-methyl-7-octadecyne, nonadecane, germacrene-D and α-humulene[19].

Antioxidant assay (DPPH scavenging assay): Plants are by and large wealthy in optional metabolites creation like phenolics, flavonoids and carotenoids, which have cell antioxidant action (Figure 4). Flavonoids are known for scavengers of most oxidizing atoms including singlet oxygen and other free radicals[27,28,30,45].

Flavonoids are known to smother responsive oxygen species, chelate minor components of free-radical creation and shield the cells from stress[7]. Likewise, phenolics in plants normally have oxidative pressure resilience. Crude concentrates of organic products, spices, vegetables, oats and other plant materials are rich in phenolics and are profoundly utilized in the food business for their intense oxidative qualities and other medical advantages. The ethanol extracts of leaves of *in vivo* and *in vitro* plants of *coleus zeylanicus* were analysed for antioxidant potential assessed by DPPH scavenging assay. Results presented as IC_{50} values of *in vivo* and *in vitro* plant extract were calculated to be 59.76 g/ml and 28.22 g/ml respectively. This increase in the antioxidant property is due to the increased phenolic and flavonoid components in the plant extract. The extract showed strong antioxidant activity against the free radicals.

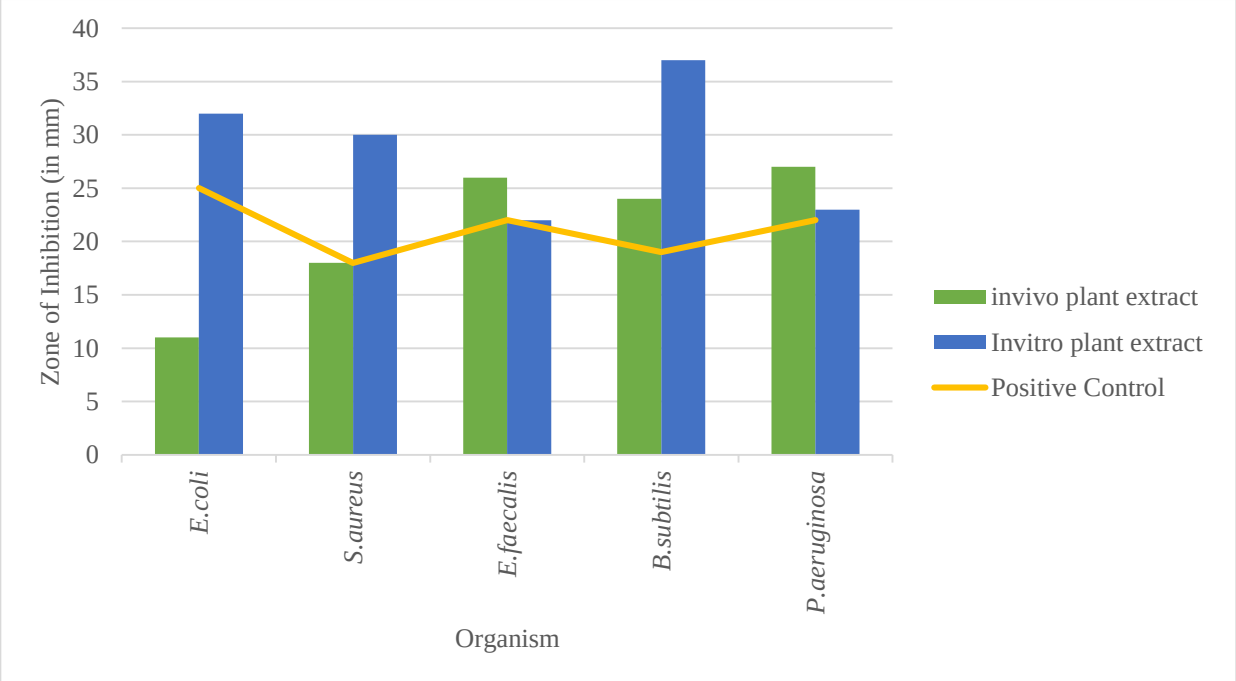
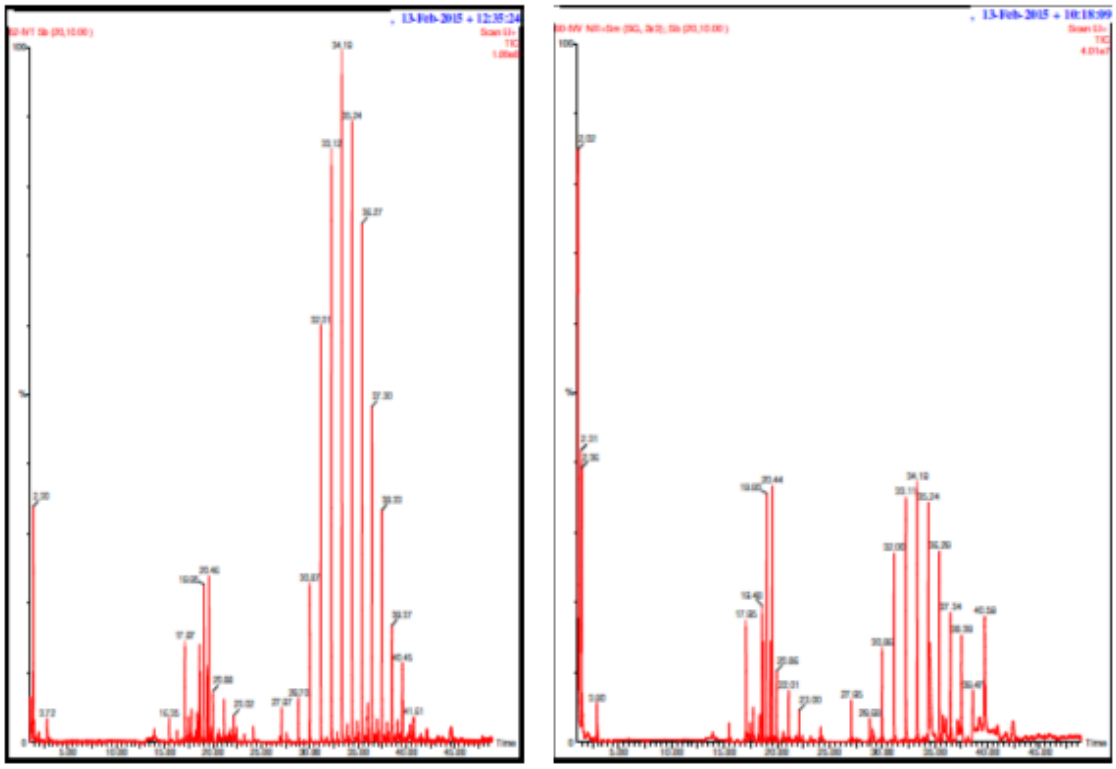


Figure 2: Antibacterial activity of *in vitro* and *in vivo* leaf extract (30µl) of *C. zeylanicus*

Table 6
GC-MS analysis of bioactive compounds with similar peaks in *in vitro* and *in vivo* plant extract of *Coleus zeylanicus*

Retention time (minutes)	Compounds present	
	***In vitro* sample**	***In vivo* sample**
2.31	Cyclohexane	Cyclohexane
6.80	-	4-Pyridinamine, 3,5-dibromo
7.74	-	Diuron
11.48	-	Quinoxaline
17.97	Linoleic acid	Linoleic acid
19.49	Widderine	Widderine
19.59	Cubenol	-
20.31	Copaene	Y langene
20.46	Naphthalene	Naphthalene
30.86	Gamma Patchouline	Gamma Patchouline
34.19	Gurjunene	Gamma Gurjunene
35.23	-	Citronella
36.28	Levomenol	Levomenol
38.33	Gamma himachalane	Gamma Himachalene
40.59	Eicosane	Eicosane



In vitro sample *In vivo* sample

Figure 3: GCMS report of *in vitro* and *in vivo* leaf extract of *Coleus zeylanicus*

The comparative studies of *in vitro* and *in vivo* plants were reported in another genus. The efficient protocol for *in vitro* micropropagation of *Salvia hispanica* (Chia seeds) was developed. Micropropagation and multiplication of Chia were highly dependent on the type of cytokinin used[3,31]. Such micropropagation techniques thus proved to enhance the growth of elite plant material in a short span of time. *Coleus* species have long been exploited for their aesthetic and medicinal values. The interest for various Coleus species for various purposes creation of illness free plants, quick expansion of somaclones and quicker presentation of novel cultivars with attractive hereditary characteristics. Our investigation was based to create framework for mass propagation and aseptic culture of *Coleus zeylanicus*an intense therapeutic plant. In this current investigation, shoots were recovered from extracted shoot tips and nodal sections of *C. zeylanicus* on cytokinin enhanced medium.

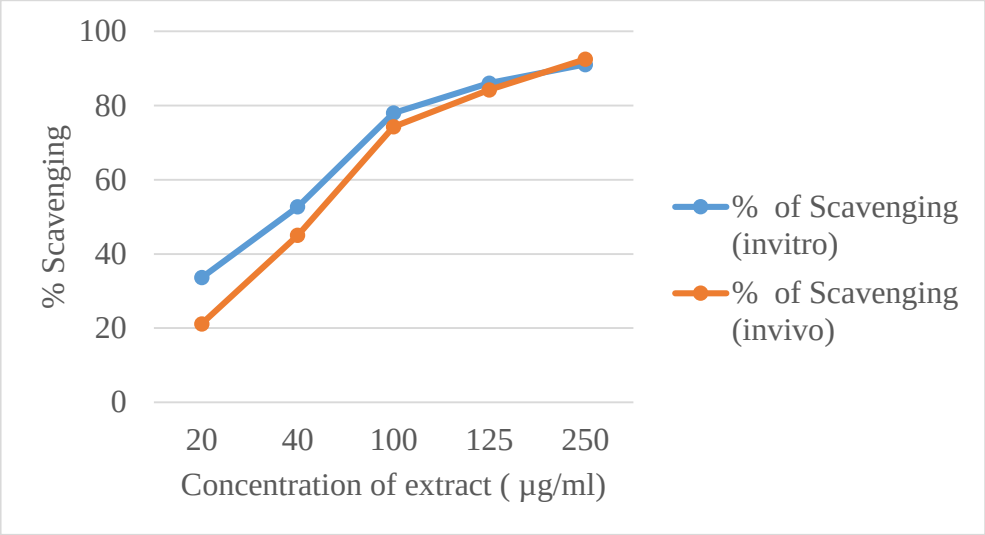


Figure 4: Comparitive antioxidant activity of *in vivo* and *in vitro* leaf extract of *C. zeylanicus*

The writing survey uncovers that diverse recovery frameworks are accessible for mass propagation of numerous Coleus species for different purposes. Coleus species are prominently known to deliver numerous optional metabolites. *C. blumei* produce rosmarinic corrosive (RA) and *C. forskohlii* structure forskolin[46], which are known for their promising natural and pharmacological exercises.

Conclusion

In this current investigation, a productive recovery convention for *Coleus zeylanicus* has been set up of interest. *Coleus zeylanicus* is a restorative plant with numerous bioactive mixtures. Accordingly, an enormous duplication of this plant is enthusiastically recommended. This examination consequently advances an effective micropropagation strategy which is helpful for preservation of this significant therapeutic plant. Plants delivered from *in vitro* recovery on extracted tissues will be valuable for yield improvement.

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