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Research Article

Micropropogation of wild Indian medicinal plant *Phyllanthus debilis*

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Abstract

An efficient micropropagation protocol was developed for *Phyllanthus debilis* (Euphorbiaceae) an important herb mainly known for medicinal purpose. Nodal segments grown on MS medium containing BA (4.44 µM) and NAA (0.14 µM) recorded 75 % shoot regeneration producing highest number of total shoots (8.33 ± 1.15) with an average shoot length of 6.43 ± 0.06 cm after 35 days. Roots were induced after transfer to half strength MS medium supplemented with IBA (2.46 µM) in terms of average number of roots (6.0± 1.0) with mean root length of 4.90 ± 0.26 cm per shoot with 75±5.0 % of rooting response recorded after 30 days of culture .The rooted plantlets were transferred for hardening, 80 % of plants were successfully established in the field. Antioxidant properties of mother plant, *in vitro* grown plant and callus culture were evaluated using DPPH assays. The methanolic extract from the leaf extract of mother plants and *in-vitro* grown plants recorded the most effective DPPH radical scavenging activity (70.67±3.06%) with the values being close to synthetic antioxidant (BHT) as positive control. Callus extract of *P. debilis* was recorded the most effective DPPH radical scavenging activity (56.4 %). These results establish the antioxidant potential of *P. debilis*, which could be used as natural antioxidant source.

Keywords: *Phyllanthus debilis*; Antioxidants; Scavenging activity; Free radicals; Micropropagation.

Introduction

Plant tissue culture means the in-vitro culture of plants plant organs like embryos, nodes, or leaves on nutrient media under aseptic conditions (Altman, 2000). Antioxidant activity is one of the most important medical advances of the last decades. The essence of all research conducted in the past is that the damage to our cells and tissues that is at the root of most diseases is caused by highly active and dangerous chemical groups called free radicals. Free radicals scavenge the human body to grab or donate electrons and damages cells, proteins and DNA. They contribute to more than one hundred disorders in human neural disorders, diabetes and ageing (Kumpulainen and Salonen, 1999).

Phyllanthus has a long traditional use in ayurvedic system of medicine and it used as a medicinal agent in cultures around the world from very long time. *Phyllanthus* has been used for the treatment of treat jaundice, gonorrhea, frequent menstruation, dysentery and diabetes. It is also used in treatment of sores, inflammation and skin rashes. Plant tissue culture is the most reliable and efficient method for and mass scale production of disease free and identical plants of *Phyllanthus*. The main constituents in *Phyllanthus* are lignans, alkaloids, bioflavonoids and repandusinic acid. Repandusinic acid has shown to have anti-viral properties, inhibiting HIV and HTLV-I replication. This compound also has HIV reverse transcriptase activity. Pharmacological activity of *Phyllanthus* includes anti-viral, anti-inflammatory and hepatoprotective activity. It also controls the ability to block DNA polymerase, the enzyme needed for the hepatitis B virus to reproduce (Meixa et al., 1995). *Phyllanthus* extract can inhibit angiotensin-converting enzyme (Mills and Bone, 2000).

Antioxidants are capable of counteracting the damaging effects of the physiological process of oxidation in animal tissue. Antioxidants can be vitamins, minerals or enzymes assist in chemical reactions. Antioxidant scavenges free radicals and plays an important role in the prevention of free radical-induced diseases. The primary radicals are reduced to non-radical chemical compounds by donating hydrogen radicals and

then converted to oxidized antioxidant radicals. Thus the antioxidant helps in protecting the body from degenerative diseases (Yamaguchi et al., 1998).

A conservative estimate shows that there are 2,50,000 to 500,000 species of plants on Earth (Borris, 1996). The herbal medicine is the first and the oldest system of human health care. Plant extracts were used as source of antioxidants for better health and food preservation.Plants have been extensively studied in the past few years for their antioxidant and radical scavenging components. Water extract of echinacoside from *Echinacea* root anthocyanin phenolic compounds (Rice Evans et al., 1996), water extracts of roasted Cassia tora (Yen and Chuang, 2000), and whey proteins (Allen and Wrieden, 1982). The plant available flavonoids has been reported to scavenge reactive oxygen containing species that are produced under severe stress conditions and protect plant cell metabolism from oxidative damages (Tattini et al., 2004) and protect animal cell metabolism and has been considered to play an important role in human health (Romani et al., 2005). Nevertheless, there is no report available on *in-vitro* propagation and antioxidant activity *of Phyllanthus debilis*. Hence, the present investigation was carried out with the objective of evaluating wild species of *Phyllanthus debilis* collected from Coutrallam, Tamil Nadu, and its potential to explore the Medicinal uses. The main aim of the present study is to establish protocols for micropropagation of disease free plants of *phyllanthus* so as to ensure the year round availability of identical, disease-free and high quality planting material.

Materials and methods

Collection of Phyllanthus debilis

Healthy plants of *Phyllanthus debilis* collected in Cutrallam (shenbagadevi falls) were raised in pots containing soil and farm yard manure (1:1) under green house condition at .The explants were prepared from the above plants for our experiment.

Explant preparation

The explants were surface sterilized following the procedures as described by (Pati et al., 2001). The plant specimens were collected from healthy potted plants kept in the green house. The explants namely node, inter node, juvenile leaf and shoot tips were washed thoroughly in running tap water and then washed with 70% ethanol. The explants were then immersed in 0.1% (w/v) mercuric chloride ($HgCl_2$) for 8 minutes for surface sterilization and then washed repeatedly in sterile double distilled water three to five times to remove traces of $HgCl_2$ adhering to the explants. The cut ends of the explants were again trimmed with the help of sterile blade to eliminate any possible residue of sterilant and the explants were used for culturing.

Preparation of tissue culture media

The MS medium (Murashige and Skoog, 1962) was used as sole basal medium. The composition of media is given in -iron and vitamins were prepared and stored. Stock solutions of salts were prepared using double distilled water and the required volume made up to with standard volumetric flask. Iron stock solution was stored in amber bottle to prevent photolysis. All the stock solutions were stored at 4°C and meso-inositol, cytokinin and auxin stock solutions were freshly prepared and used every month. For preparation of medium, all four stock solutions were mixed thoroughly with required amounts of sterile distilled water. Sucrose 3% (30 g/L), 0.01% meso-inositol (100mg/L) and required amount of plant growth regulators (PGRs) were added and the pH was adjusted to 5.6 ± 0.2 with 1N HCl or 1N NaOH prior to autoclaving. The gelling agent agar (Himedia Grade 301) 0.9% (w/v) was added to the prepared media constituents and mixed well before dispensing into glasswares. The contents were labelled and sterilized in an autoclave at 15 lb pressure for 15-20 minutes at 121°C. After inoculating with explants, all the culture vials were kept under 16/8h (light/dark) photoperiod at 25 ± 2 °C.

Stock solution

Plant growth regulator used with basal medium contains auxins and cytokinins.

Preparation of auxins

The auxins namely 2,4-D, IAA, IBA and NAA were prepared separately by dissolving 100 mg in 1mL of 1N NaOH and the volume was then made up to 100mL in a standard flask by adding double distilled water and stored at 4°C.

Preparation of cytokinins

The cytokinins namely benzyl adenine (BA) and kinetin (KN) were prepared separately by dissolving 100mg in 1mL of 1N HCl and the

volume was then made up to 100mL in standard flask by adding double distilled water and stored at 4°C.

Culture conditions

The cultures were incubated in culture chamber at 25 ± 2°C for the light condition, the culture vials were placed on the rack at a distance of 25 cm from the light source. A 16/8 h (light / dark) photoperiod of cool white light was provided from 2000 Lux.

In-vitro micropropagation of Phyllanthus debilis shoot proliferation

Explants from node and shoot tip of *Phyllanthus debilis* plant were inoculated on MS basal medium supplemented with individual concentrations and combination of BA (1.11, 2.22, 4.44, 8.88 and 11.10 µM) and NAA (0.054, 0.14, 0.27 and 0.54 µM) for shoot multiplication. At the end of the experiment, percentage of shooting, shoot length and the number of shoots per explant were recorded. After six weeks, the micro shoots were transferred to rooting medium.

Root proliferation

The healthy shootlets were transferred to half strength MS basal medium supplemented with individual concentrations of IBA (0.49, 0.98, 2.46, 4.92 and 12.30 µM) for root initiation. The percentage of rooting, root length and the number of roots per individual shoots were documented.

Hardening

The healthy rooted plantlets were carefully removed from the medium without disturbing the root and shoot and washed thoroughly in sterile distilled water in order to remove the medium sticking on to the roots. The plantlets were then transferred to pots (6 cm dia) containing red soil, vermiculite and farm yard manure in 1:1:1 ratio for hardening. The hardened plantlets were closed with polythene bags to prevent transpiration and to maintain relative humidity (RH) of 80%. They were maintained at 25 ± 2°C in 16/8 hrs photoperiod for two weeks. The plantlets in paper cups were fertigated with half strength MS salt solution every 3 days. The plantlets were slowly stepped down to field condition. After hardening, they were transferred to earthen pots and kept in field conditions.

Preparation of the plant extract

Preparation of the extracts was done according to a combination of the methods used by (Lu and Foo, 2001). The sample extract was obtained from the dried plant material of 15 grams with 150 mL mixture of methanol, ethanol and water (Merck, extra pure) for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rotavator at 40 °C to a constant weight and then dissolved in methanol, ethanol and water. The dissolving rate of the crude extracts was approximately 100 %. The solution was stored at 18 °C until use.

Antioxidant Assay

Antioxidant assay on plant extracts of *Phyllanthus debilis* plant were estimated for their free radical scavenging activity by using DPPH (1,1-Diphenyl-2,Picryl-Hydrazyl) free radicals, (Ozkan et al., 2004; George et al., 1996).

Screening method

50 µL of leaf extracts of *Phyllanthus debilis* medicinal plant were taken in microtiter plate. 100 µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for their discoloration, from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative assay of antioxidant activity

Selected samples of 100 µl from qualitative assay were mixed with 2.7 ml of methanol and then 200 µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT).

Results and discussions

Callus induction from explants of *Phyllanthus debilis*

The response for the growth and development of calli varied with different explants. Callus was developed from when the leaf explants *Phyllanthus debilis* were cultured on 2, 4-D. Lower concentrations callus induction medium with 2, 4–D showed better callus induction and proliferation. Whereas higher concentration of 2, 4-D (22.62 µM) did not show

much effect on callus induction. Callus in the 2, 4- D (4.52μM) supplemented medium was found well developed, albino, spongy, and loosely arranged. The moisture content of callus was also high as compared to other auxins supplemented media. In NAA supplemented medium the callus was pale, yellowish green in color, more friable, hard and granular. Callus grown on medium supplemented with BA was green in color more compact, hard and granular.

Among three plant growth regulators which were studied, the response of callus was good in 2, 4-D (4.52 μM) compared to others (Table 1; Fig 1). The rate of callus induction and the percentage of callus response varied depending upon the 2, 4-D concentrations used. The response of callus was 75% on MS media supplemented with 2, 4 –D (4.52 μM) and the rate of proliferation was recorded from 12th day after inoculation upto 40 days with four day intervals.

Figure 1. Initiation of callus from basal medium with 2,4-Diphenyl picryl hydroxyl

In-vitro plant regeneration of *Phyllanthus debilis*

Multiple shoots developed from nodal explants cultured on MS medium supplemented with different concentration and combinations of BA (1.11, 2.22, 4.44, 8.88 and 11.10 μM) and NAA (0.054, 0.14, 0.27 and 0.54 μM). Initiation of multiple shoots in most of the treatments was observed within 3 weeks of culture.

Nodal explants callus transferred to MS medium containing BA alone (1.11-8.88 μM) grew well, but, simultaneously developed microshoots. The 4.44 μM BA produced 5 –6 small shoots with an average length of 5.3 cm after 35 days. Whereas higher concentration of BA (8.88μM) produced dark green callus in base of the cut end and compact structures which ultimately turned brown and failed to develop into normal shoots (Table. 2).

Table 1. Impact of auxin and cytokinin on callus induction from explants of *phyllanthus debilis*

PGR Concentration (μM)			Juvenile leaf % Response	Node % Response
2,4-D	NAA	BA		
0.45	-	-	40 ± 10	25 ± 5.0
2.26	-	-	50 ± 0.0	35 ± 10
4.52	-	-	75 ± 5.0	25 ± 5.0
11.31	-	-	55 ± 0.0	20 ± 5.0
22.62	-	-	45 ± 0.0	-
-	1.34	-	25 ± 5.0	15 ± 5.0
-	2.69	-	40 ± 0.0	20 ± 8.6
-	5.37	-	30 ± 0.0	15 ± 5.0
-	13.43	-	25 ± 5.0	15 ± 5.0
-	26.85	-	15 ± 5.0	-
-	-	1.11	20 ±5.0	15 ± 5.0
-	-	2.22	25 ±0.0	25 ±13.2
-	-	4.44	35 ± 0.0	15 ± 5.0
-	-	8.88	30 ± 0.0	-
-	-	13.32	20 ±0.0	-

Results represent mean ± SD of six replicates. Data were recorded after 40 days of culture

Among the various concentrations of NAA tested, NAA (0.14 μM) recorded 35% response for shoot proliferation producing 2.33 ± 1.15 shootlets with an average shoot length of 1.60 ± 0.2 cm after six weeks of culture (Table.2). Higher concentrations of NAA (0.54 μM) in the culture medium showed the inhibition in the growth of the shoots.

Figure 2. *In vitro* plant regeneration of *Phyllanthus debilis*

The synergistic effect of BA (1.11, 2.22, 4.44, 8.88 and 11.10 μM) and NAA (0.054, 0.14, 0.27 and 0.54 μM) in varying concentrations was studied for shoot multiplication of *Phyllanthus debilis*. Among the various concentrations and combinations of BA and NAA, MS medium

supplemented with BA (4.44 µM) and NAA (0.14 µM) recorded 75 % shoot regeneration producing highest number of total shoots (8.33 ± 1.15) with an average shoot length of 6.43 ± 0.06 cm from nodal explants after six weeks of culture (Table. 3). The combination of BA(4.44 µM) with NAA (0.27 µM) was found to show 50% response producing 4.00 ± 1.00 shootlets per explants with an average shoot length of (3.87 ± 0.21cm).

At lower concentration of BA (1.11 µM) and NAA (0.27 µM), the results showed only 40% response, producing (4.33 ± 1.15) shootlets per explants with an average length of (3.33 ± 0.15cm). When the concentration of BA (11.10 µM) and NAA (0.54 µM) was increased, a gradual fall in the number of shoots per explants was recorded (Tale.3). Similarly on increasing the concentration of NAA (0.27 µM and 0.54 µM), the response was low due to the basal calli formation in the cut ends of the explant. Nodal explants on MS medium without plant growth regulators did not show any response.

Table 2. Individual effect of auxin and cytokinin on shoot induction of *Phyllanthus debilis*

Plant growth regulator (µM)	% Response	No. of Shoots / explant	Shoot length (cm) average
NAA			
0.054	-		-
0.14	10	2.6±1.2	1.5±0.5
0.27	-	-	-
0.54	-	-	-
BA			
1.11	25.0±5.0	3.2 ±0.2	2.0±0.0
2.22	30.0±5.0	3.3±1.1	2.0±0.1
4.44	35.0±5.0	4.0±1.0	3.3±0.2
8.88	20.0±0.0	2.2±0.0	2.0±0.0

Results represent mean ± SD of six replicates. Data were recorded after 35 days of culture.

For root induction individual microshoots (4.6 cm) taken from the *in vitro* proliferated shoots were placed in half strength MS medium supplemented with various concentrations of IBA (0.49, 0.98, 2.46, 4.92 and 12.30 µM). Rooting occurred in all concentrations but with different rooting percentages, and the optimal response was observed on half strength MS medium supplemented with IBA (2.46 µM) in terms of average number of roots (6.0± 1.0) with mean root length of 4.90 ± 0.26 cm per shoot with 75±5.0 % of rooting response recorded after 30 days of culture (Table. 4).

Increasing the concentration of IBA (4.92 and 12.30µM) resulted in a decrease in the percentage of root induction response with decreased number of roots and root length. The excised shoots did not show rooting on culture medium without plant growth regulators.

Hardening of *in vitro* plantlets of *Phyllanthus debilis*

Rooted plantlets of *Phyllanthus debilis* was transferred to paper cups containing sterile soil, sand and vermiculite (1:1:1), kept at 25 ± 2^{o}C for one week (vide Materials and Methods) and transferred to the shade house after 35 days of normal growth.

Figure 3. Hardened of *in vitro* plantlets of *Phyllanthus debilis*

Phyllanthus debilis, more than 71% of plantlets survived hardening in red soil, vermiculite and farmyard manure (1:1:1) for one week. However, the survival rate decreased by 15-20 % after 2- 3 weeks of acclimatization. It was observed that gradual acclimatization of *in vitro* grown plants to external environment was most essential for *Phyllanthus debilis* plant. More than 55% of the plants transferred to pots survived and resumed growth.

Antioxidant activity

Determination of antioxidant activity in leaf and callus extract of Phyllanthus debilis

50 µl of each of the twelve extracts (methanol, ethanol and water extracts of mother and in vitro grown plant and callus of *Phyllanthus debilis*) were estimated for free radical scavenging

activity using Diphenyl-2-picryl hydrazyl (DPPH) assay. The samples observed for its bleaching from purple to yellow and pale pink were considered as strong positive and weak positive respectively.

Table 3. Effect of different concentration of cytokinin (BA) and auxin (NAA) on *in vitro* shoot multiplication from nodal explants of *Phyllanthus debilis*

Hormones (µM)		% response	No of shoots*	Shoot length (cm)*
BA	NAA			
1.11	0.054	28.33±2.9	1.67±0.58	1.40 ± 0.10
	0.14	40.00±5.0	3.0 ±0.00	2.10 ± 0.20
	0.27	43.33±5.8	4.00±1.00	2.33 ± 0.15
	0.54	21.67±2.9	1.67±0.58	1.50 ± 0.10
2.22	0.054	23.33±5.8	2.33±1.15	2.23 ± 0.06
	0.14	33.33±5.7	3.00±1.00	2.50 ± 0.20
	0.27	46.67±2.9	3.33±1.15	1.90 ± 0.10
	0.54	31.67±2.9	2.00±0.00	1.73 ± 0.12
4.44	0.054	45.00±8.7	2.67±1.15	1.40 ± 0.10
	0.14	71.67±2.9	8.00±1.00	4.37 ± 0.50
	0.27	50.00±5.0	2.67±1.15	2.53 ± 0.21
	0.54	38.33±2.8	3.00±1.00	1.43 ± 0.12
11.10	0.054	35.00±5.0	2.33±0.58	1.57 ± 0.21
	0.14	38.3 ±7.6	3.00±1.00	2.40 ± 0.10
	0.27	31.67±7.6	3.00±1.00	1.80 ± 0.20
	0.54	25.00±8.6	1.33±0.58	1.73 ± 0.15

Explants were cultured on MS based media supplemented with BA and/or NAA. Data were recorded after six weeks of culture. Results represent mean±SD of six replicated experiments.

Table 4. Root formation from *in vitro* grown shoots of *Phyllanthus debilis*

IBA (µM)	% response	No. of roots / shoot	Root length (cm)
0.49	30.0±5.0	3.3±0.2	3.2±0.2
1.23	80.0±0.0	4.3±1.0	4.8±0.2
2.46	45.0±0.0	3.3±0.1	3.3±0.3
4.92	30.0±5.0	3.2±0.2	2.0±0.1

Results represent mean ± SD of six replicates. Data were recorded after 30 days of culture.

Among the twelve extracts of *phyllanthus debilis*, the methanolic extract from the leaf extract of *phyllanthus debilis* mother plant (S1) recorded the most effective DPPH radical scavenging activity (70.67±3.06%, Figure 5) with the values being close to synthetic antioxidant (BHT) as positive control. In each case, methanolic extracts recorded higher percentage of free radical scavenging activity than ethanolic extractions. Callus extract of *phyllanthus debilis* was recorded the most effective DPPH radical scavenging activity (56.4%) than in vitro cultivated plants of *Phyllanthus debilis*. Least percentage of radical-scavenging activity (17.67 ± 0.58) was recorded in the callus extract (water) of *phyllanthus debilis.*

The rate of callus induction and the percentage of callus response varied depending upon the 2, 4-D concentrations used. The response of callus was 75% on MS media supplemented with 2, 4 –D (4.52 µM) and the rate of proliferation was recorded from 12th day after inoculation up to 40 days with four day intervals. The above findings related to the nature of the callus was confirmed with the reports of Hildebrandt et al (1963), Davey et al., (1971) and Kirkham and Holder (1981) in pea and tomato; *Atropha belladonna*. While the colour of callus changed with the concentration of 2, 4-D used, the callus was found to be creamish in 2, 4-D (4.52µM), and in the higher concentration it was appeared yellowish to brown in colour. These findings harmonized with Uddin et al., (2006) in *Stevia rebaudiana*. Multiple shoots developed from nodal explants cultured on MS medium supplemented with individual concentration of BAP (1.11-8.88 µM) and NAA (0.54-5.36 µM). Initiation of multiple shoots in most of the treatments was observed within 3 weeks of culture. Significant induction of shoot multiplication for *Phyllanthus debilis* was recorded on MS basal medium supplemented with combination of BA + NAA. The role played by cytokinins and auxins on the culture media in stimulating *in-vitro* multiplication and growth of shoots in several plant species is known. Also the synergistic combination of auxin and cytokinin combinations on organogenic differentiation has been well established in several systems (Pereira et al., 2000; Pretto and Santarem, 2000).

In *Phyllanthus debilis,* the synergistic effect of BA (4.44µM) and NAA (1.34 µM) with MS medium recorded the highest percentage (80 % of nodal explants producing shoots (8 ± 1.0) with an average length of 4.56 ± 0.29 cm per shoot. The result corroborates with the findings of Sushmita et al., (1997) that the nodal explants of miniature rose "The Fairy" inoculated on MS medium supplemented with BA (0.5mg/ L) and NAA (0.2 mg/L) produced highest (7-8) shoots per explants after culture. It is also been clearly discussed that the addition of cytokinins to the

culture medium is essential for *in vitro* multiplication from shoot tips or lateral buds in many plants.

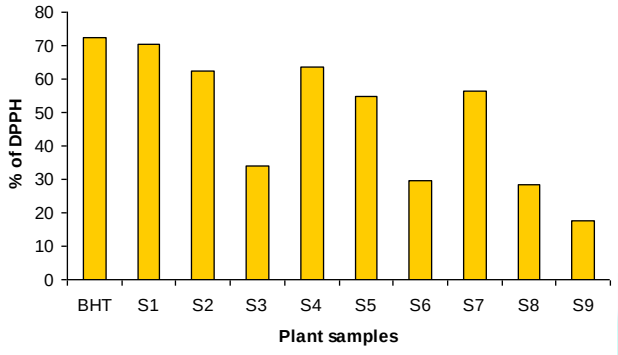

Figure 5. Antioxidant activity in leaf and callus extract of *Phyllanthus debilis*

BHT – Standard, S1 – Methanol leaf extract (mother plant), S2 - Ethanol leaf extract (mother plant), S3 - Water leaf extract (mother plant), S4 - Methanol leaf extract (in vitro grown plant), S5 - Ethanol leaf extract (in vitro grown plant), S6 - Water leaf extract (in vitro grown plant), S7 – Methanol callus extract, S8 – Ethanol callus extract and S9 – Water callus extract.

The first roots appeared after two weeks of culture, and after four weeks, the root system was well developed. The maximum rooting response was achieved on medium supplemented with 1.23 µM IBA, 80% response; 5.3±0.25 number rootlets per shootlets and an average root length of 4.8±0.2 cm in 30 days old cultures. The observation on the reduction of MS salts strength to one half to enhance the rooting frequency is in agreement with earlier finding in *P. niruri* and *S. rebaudiana* (Sivaram and Mukundan, 2003). In the present study root induction was obtained with lower concentration of IBA.

The successful acclimatization of micropropagated plants and their subsequent transfer to the field is a crucial step for commercial exploitation of *in vitro* technology.In the present study, *Phyllanthus debilis* recorded the survival rate of 80% on hardening the plantlets in red soil, vermiculite and farmyard manure (1:1:1). It was observed that gradual acclimatization of *in vitro* grown plants to external environment was most essential for *Phyllanthus debilis*. In spite of the decrease in the survival rate by 10% after 2- 3 weeks of acclimatization, 70% of the plants of *Phyllanthus debilis* transferred to pots survived. The results are in accordance with the reports of Asano and Tanimoto (2003) in which miniature rose “Shortcake” plantlets after the acclimation treatment, transplanted on the soil in a green house and resulted in a successful survival rate of 70% Scavenging activity for free radicals of DPPH (1,1-Diphenyl-2-picrylhydrazyl) has widely used to evaluate the antioxidant activity of natural products from plant and natural sources.In the present study the results demonstrated that, DPPH a free radical, stable at room temperature, produces purple colour solution in methanol. It is reduced in the presence of an antioxidant molecule, giving rise to yellow coloured methanol solutions. All the nine extracts (methanolic and ethanolic extract) from the leaf of mother plant, *in-vitro* plants and callus of *Phyllanthus debilis* were studied antioxidant activity. Most effective DPPH radical scavenging activity (70.6%) was recorded in the methanolic extract of mother plant of *Phyllanthus debilis* (S1) with the values (72.33%) being close to synthetic antioxidant (BHT) as positive control. Whereas callus methanol extract was recorded the average value (54.45%). The activity of the extracts was attributed to their hydrogen donating ability (Moore et al., 2001).

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