In vitro micropropagation, total phenolic content and comparative antioxidant activity of different extracts of *Sesbania grandiflora* (L.) Pers.

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***Sesbania grandiflora* (L.) Pers. is a common traditional medicinal plant used in bronchitis, anaemia, headache, fever, ophthalmia, nasal catarrh, leprosy, inflammation, gout and rheumatism. The present study aimed to assess plant regeneration and plantlets development *in vitro* using explants of *S. grandiflora* together with the estimation of total phenolic content and antioxidative activity of various extracts obtained from the plant. Murashige and Skoog (MS) basal medium added with different concentrations of plant growth regulators (PGRs) was used for plant tissue culture, whereas ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to evaluate the antioxidant potential of different extracts of the plant. In the presence of 6-benzylaminopurine (BAP; 0.1 mg/l), the highest level (85.41%) of seed germination was achieved while the highest callus formation (96.6%) was recorded with 2,4-dichlorophenoxyacetic acid (2,4-D; 0.5 mg/l). In addition, the highest shoot induction, shoot formation and shoot elongation were observed with BAP (0.1 mg/l), indole-3-butyric acid (3 mg/l) and naphthaleacetic acid + BAP (0.4 + 0.2 mg/l) respectively. The extract of dried calluses showed highest contents of proline (110.94 mg/g), phenol (16.42 mg/g) and flavonoid (22.22 mg/g), and also highest antioxidant potential with FRAP and DPPH assays. From the present study, we may conclude that the MS basal medium supplemented with PGRs is effective for the commercial production of *S. grandiflora*.**

Keywords: Antioxidant activity, *in vitro* micropropagation, phenolic content, plant growth regulators, *Sesbania grandiflora*.

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SESBANIA GRANDIFLORA (L.) Pers., commonly known as hummingbird tree of the family Leguminosae, is a popular traditional medicinal plant used in bronchitis, anaemia, headache, fever, ophthalmia, nasal catarrh, leprosy, inflammation, gout and rheumatism[1]. The dried leaves are used as tea and also as an antibiotic, antitumour and contraceptive. The juice obtained from fresh leaves was found effective in bruises[2]. The seeds of the plant are rich in protein while seed oil is a potent antioxidant agent. Various studies confirmed the presence of phytosterols in the seed oil which shows anti-inflammatory, analgesic and antipyretic activities. The presence of high phytosterol content makes it an important plant for the commercial production of steroidal hormones and cosmetics[3]. The flowers of *S. grandiflora* contain tannin and other nutritious contents. Moreover, its astringent nature also contributes in antimicrobial activity[4].

The process of plant tissue culture is considered to be highly valuable in terms of commercial production of a medicinal plant, in which the aseptic culture of cells and organs can be achieved under *in vitro* conditions[5]. Several compounds with a varied array of structures influence the tissue growth and morphogenesis under exposed atmosphere. Besides, this technique is useful for conserving medicinal plants and enhancing their secondary metabolites of industrial importance through micropropagation.

Based on the medicinal properties and pharmaceutical demand, *S. grandiflora* was selected for tissue culture techniques. This study reports *in vitro* propagation and comparative antioxidant activity of different solvent extracts obtained from *S. grandiflora.*

Wild plant seeds of *S. grandiflora* were collected from Cuddalore, Tamil Nadu, India and identified from the Botanical Survey of India, Coimbatore where a voucher specimen (SC 5/23) of the plant is available for future records.

The seeds were surface sterilized to produce contamination-free plants that can be maintained under aseptic *in vitro* conditions. Seeds were checked for viability and washed thoroughly with tap water for 25 min. The seeds were washed with sodium hypochlorite (2%) solution followed by a treatment with mercuric chloride (0.5%). Thereafter, they were washed in distilled water at least five times. A sterile tissue paper was used to remove the excess water before culture in a semi-solid MS medium.

Freshly prepared 3% sucrose (30 g/l), 0.1% meso-inositol (100 mg/l) and obligatory amount of plant growth regulators (PGRs) were added to the Murashige and Skoog (MS) basal medium[6]. Before autoclaving at 121°C and 1.06 kg/cm^2 pressure for 20 min, the medium was buffered with 1N NaOH and 1N HCl to adjust its pH to 5.6–5.8. Thereafter, the medium was kept under refrigerator condition until further use. The cultures were incubated at 25°± 2°C with a relative humidity of 70–80% and 16/8 (L/D) h photoperiod under photon flux density of 50 μE mol/m^2/s^2.

Table 1. *In vitro* seed germination efficiency of *Sesbania grandiflora*

Type of hormone	Concentration (mg/l)	Regeneration efficiency (%)		
		10 day	20 day	30 day
BAP	0.1	37.49 ± 4.1^{d}	85.41 ± 3.6^{a}	79.16 ± 4.8^{b}
NAA	0.1	29.16 ± 4.1^{e}	70.83 ± 4.2^{b}	77.08 ± 2.7^{b}
IAA	0.1	35.41 ± 0.3^{d}	81.24 ± 4.3^{a}	54.00 ± 2.8^{c}
2,4-D	0.1	20.83 ± 0.5^{e}	77.08 ± 4.3^{b}	70.83 ± 2.4^{b}

[a] and [b] are considered significant; [c–e] are considered as non-significant.

For callus induction, the seeds were surface sterilized and placed on the MS semi-solid basal medium. The medium was incubated at 25° ± 2°C under dark for 5 days and thereafter, kept under 16/8 h photoperiod. Well-grown callus pieces were transferred to MS medium containing altered concentrations of 6-benzylaminopurine (BAP) and kinetin (KIN) in combination with indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), naphthaleneacetic acid (NAA) as well as individually. The organogenic calluses were subcultured at an interval of every 20 day with the same treatment as applied for fresh medium until shoot regeneration began. The cultures were incubated at 25° ± 2°C in photoperiod (16/8 h) for four weeks and calluses were harvested for further studies.

Well-developed parts were transferred into MS medium containing IAA, BAP, NAA and 2,4-D (0.1–0.4 mg/l). After three weeks of culture, the shoots produced roots that were sub-cultured with a combination of hormones (Table 1). The individual root length was measured after six weeks. The regenerated plantlets were removed from the culture and washed with sterile distilled water. Thereafter, the plantlets were transferred into plastic cups containing sterilized vermiculite with soil (2 : 1 w/w) mixture. The plantlet cups were initially covered with polythene bags to prevent excess transpiration and to also preserve high relative moisture (80%). The potted plants were kept under 16/8 h photoperiod for two weeks and half strength MS salt solution was poured regularly. Thereafter, the plants were carefully transferred to the greenhouse. A survival percentage of 90 was achieved during hardening.

The seeds and calluses of *S. grandiflora* were shade-dried, powdered and extracted with hexane, ethyl acetate, chloroform, acetone and methanol at room temperature for 3 day with occasional stirring. The extraction mixture was filtered using Whatman filter paper and the solvent was removed under high pressure and low temperature (40°–45°C) using a rotary evaporator.

Proline was quantified according to the method described by Malaisamy and Mohan[7] with minor modifications. Briefly, seeds and calluses (internode and cotyledon) were homogenized with 2 ml of cold ethanol (40%) in a mortar containing small quantity of washed disinfected sand. After agitating for 10 min, the extracts were filtered using Whatman filter paper, and 2 ml of an aliquot of each extract was used. A spectrophotometer was used to measure absorbance at 528 nm and the values were compared with a standard curve of proline. The results were expressed as proline μM per mg of the fresh and dried matter.

Total phenolic content (TPC) was estimated using the personalized Folin–Ciocalteau technique[8] with few modifications. The plant sample (0.05 g) was ground with acetone : water (1 : 1, v/v) at 4°C. Next, 9 μl of the extract aliquot was mixed with Folin–Ciocalteau reagent (109 μl) and left for 3 min at 25°C. Thereafter, Na_2CO_3 solution (180 μl; 7.5%, w/v) was added to the extract and mixed thoroughly. The solution thus obtained was allowed to stand for 5 min at 25°C and the absorbance was measured at 760 nm. TPC was calculated by preparing a standard curve of gallic acid and expressed as mg/g gallic acid equivalents (GAE).

Total flavonoid (TFC) content was measured according to Kim *et al.*[9]. Briefly, the plant sample (0.05 g) was homogenized with acetone : water (1 : 1, v/v) at 4°C. Distilled H_2O (100 μl) was first added to the microplate wells (96-well plate) and then a sample (25 μl) and $NaNO_2$ (7.5 μl; 5%) were added. The mixture was allowed to stand for 5 min and then $AlCl_3$ (7.5 μl; 10%) was added to it. The mixture was again allowed to stand for an additional 6 min and thereafter, 1M NaOH (50 μl) and H_2O (60 μl) were added to it. The mixture was kept for 5 min and the absorbance was read at 510 nm with the help of a microplate reader (Zenyth 200rt Microplate Reader UK-Biochrom Ltd.). TFC was calculated with a standard curve of quercetin and expressed as mg/g quercetin equivalents.

A slightly modified method of Xu and Chang[10] was used to perform the ferric reducing antioxidant power (FRAP) assay. The FRAP solution was prepared with acetate buffer (300 mM; pH 3.6), 2,4,6,-tri(2-pyridyl)-s-triazine (TPTZ) (10 mM) in HCl (40 mM) and $FeCl_3 \cdot 6H_2O$ (20 mM) (10 : 1 : 1). The FRAP reagent (150 μl) was added to the sample (20 μl) into 96-well plates, incubated at room temperature under dark condition for 8 min and then the sample was read at 600 nm. The results of the regression equation were calculated for the FRAP values (units, mM Fe(II)/g) of the sample ($R^2 = 0.962$).

The free radical scavenging activity was calculated by 2,2-diphenyl-1-picrylhydrozyl (DPPH) method as described by Nagarajan *et al.*[11]. Briefly, a solution of DPPH (0.1 mm) was prepared with MeOH (1.0 ml). The plant extracts, i.e. hexane, ethyl acetate, chloroform, acetone and methanol were prepared with different concentrations ranging from 50 to 250 µg/ml. After incubating for 30 min, the absorbance was measured at 517 nm. Lesser absorbance indicates higher scavenging activity. Ascorbic acid was used as a standard antioxidant. The percentage of DPPH scavenging ability at different concentrations was estimated using the formula $[(A_{c1} - A_{s2}/A_{c1}) \times 100]$, where A_{c1} is the absorbance of the control and A_{s2} is the absorbance of the sample.

One-way analysis of variance (ANOVA) was used to evaluate the significance of the difference of means of data from various experiments using SPSS statistical software package (version: 10). The values are presented as mean ± SD and $P < 0.05$ is considered as significant.

Seed germination and establishment of seedlings of *S. grandiflora* are less susceptible to ecological stress and produce more number of plants in tissue culture. *In vitro* seed germination efficiency was observed with all hormones, i.e. IAA, NAA, BAP and 2,4-D at the same concentration (0.1 mg/l). Results (Table 1 and Figure 1) showed that from 10th to 30th day, the highest germination was recorded with BAP (37.49–85.41%), followed by IAA (35.41–81.24%). The lowest germination was observed with 2,4-D (20.83–77.08%). Germinated plantlets growing vigorously were selected for further micropropagation clonally by splitting and sub-culturing after every four weeks. The growth of plants was fast and produced ample *in vitro* callus biomass. Cotyledons, nodes and internodes were collected from a seedling of *in vitro* explants and sub-cultured for the production of more biomass.

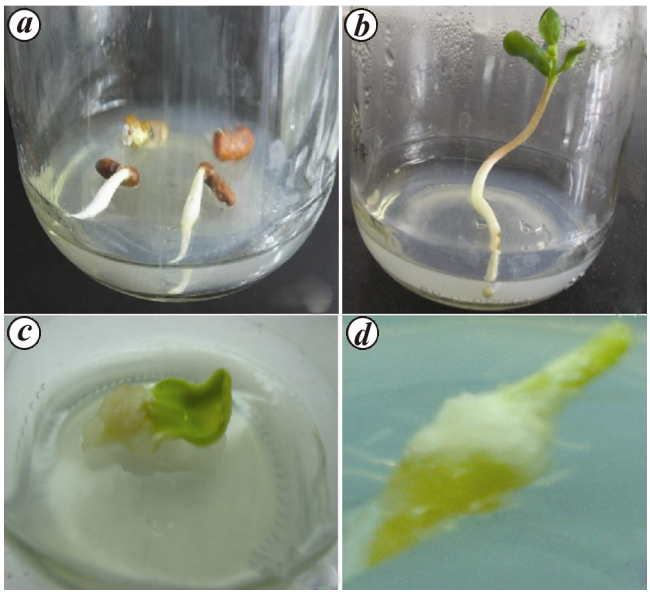

Figure 1. *In vitro* seed germination and callus formation in *Sesbania grandiflora*. ***a***, Seed germination; ***b***, shoot elongation; ***c***, callus formation from *in vitro* cotyledon; ***d***, callus formation from node.

The callus formation of the cotyledon, node and internode explants was observed on MS medium amended with different hormones (2,4-D, IAA and NAA) at a concentration of 0.5 mg/l. The callus formation was first noticed on node and internode explants after one week of incubation. On the 10th day, highest callus formation was observed from internode with 2,4-D (53.3%), followed by node (49.4%) and cotyledon (33.4%) in the presence of NAA. On the 30th day, the highest callus formation was observed from internode (96.6%) with 2,4-D, followed by node (92.6%). The callus formation increased steadily with increasing number of days (Table 2 and Figure 2).

Previous studies revealed that a combination of NAA (0.4 mg/l) and BAP (0.2 mg/l) was efficient for callus proliferation and produced higher callus formation (96–98%) in *Peganum harmala* cytokinin than a combination of IAA and BAP[12]. The addition of 2,4-D (0.5 mg/l) and KIN (0.5 mg/l) with MS medium was also found effective for callus induction in internode of *Tribulus terrestris*[13].

In the present study, callus growth was observed up to 40 days. Callus cultures were harvested at every 10, 20, 30 and 40 days. The fresh as well as dry weight of the callus was found to increase up to 60 days, which suggests that biomass accumulation increased steadily. Highest friable callus formation was observed with 2,4-D (0.5 mg/l), while compact callus was recorded with BAP (0.5 mg/l). The nodular callus was induced from the initial callus (Figure 2). The callus grown in MS medium was greenish in colour, fast-growing and granular in the culture medium. The callus was subcultured at every four weeks interval for further proliferation, multiple shoot and root formation, and induction of organogenesis of *S. grandiflora*.

The maximum shoot induction was recorded with BAP (0.1 mg/l; 96.5%), followed by NAA (0.1 mg/l; 73.2%) and 2,4-D (0.1 mg/l; 56%) (Table 3). The highest amount of shoot formation (76.6%) and multiple shoot formation (64.3%) were observed with IBA (3 mg/l). Shoot formation with NAA + BAP (0.4 + 0.2 mg/l) was recorded as 59.4%, whereas multiple shoot formation was 52.3%. Similarly, the highest shoot elongation was observed with a combination of NAA + BAP (0.4 + 0.2 mg/l; 19.55 cm), followed by NAA + BAP (0.2 + 0.4 mg/l; 12.43 cm) and least with 2,4-D (0.1 mg/L; 6.21 cm).

The results (Figure 3) showed that indirect root formation from organogenic callus by different types of phytohormones can be useful in the isolation of root-specific compounds which can conserve the ecological balance through plant tissue culture. In root induction, auxins were responsible for forming adventitious roots within three days culture of *S. grandiflora*. The highest amount of root induction was observed with IBA (2.5 mg/l; 76.3%), whereas lowest root induction was observed with

Table 2. Callus formation of *in vitro* cotyledons (C), node (N) and internodes (In) of *S. grandiflora*

Type of hormones	2,4-D	IAA	NAA	BAP
Concentration (mg/l)	0.5	0.5	0.5	0.5
10 day				
C	33.4 ± 2.1^{f}	35.6 ± 2.6^{f}	42.3 ± 1.4^{e}	38.1 ± 6.7^{f}
N	49.4 ± 2.8^{d}	32.3 ± 2.2^{f}	45.2 ± 2.1^{e}	36.9 ± 2.3^{f}
In	53.3 ± 3.8^{d}	46.3 ± 3.1^{e}	44.5 ± 2.8^{e}	40.6 ± 3.3^{e}
20 day				
C	59.6 ± 3.7^{e}	60.2 ± 3.2^{d}	66.6 ± 1.2^{d}	67.8 ± 2.1^{d}
N	65.9 ± 4.7^{d}	58.7 ± 3.3^{e}	64.8 ± 1.3^{d}	61.3 ± 2.9^{d}
In	79.9 ± 4.7^{c}	68.7 ± 4.4^{d}	69.9 ± 1.0^{d}	75.2 ± 2.3^{c}
30 day				
C	78.9 ± 2.7^{c}	86.2 ± 3.2^{b}	87.7 ± 1.8^{b}	89.1 ± 2.3^{b}
N	92.6 ± 2.3^{a}	83.4 ± 4.2^{b}	88.1 ± 1.3^{b}	91.1 ± 2.4^{a}
In	96.6 ± 3.3^{a}	83.7 ± 5.0^{b}	89.4 ± 1.2^{b}	90.0 ± 2.3^{a}
40 day				
C	71.4 ± 2.2^{c}	75.8 ± 2.8^{c}	76.5 ± 3.5^{c}	72.9 ± 1.9^{c}
N	79.6 ± 2.2^{b}	74.8 ± 4.8^{c}	78.8 ± 1.1^{b}	71.4 ± 1.1^{c}
In	86.6 ± 2.8^{a}	78.8 ± 5.8^{b}	76.3 ± 4.1^{b}	72.9 ± 1.6^{b}

$^{a–c}$ are considered significant; $^{d–f}$ are considered as non-significant.

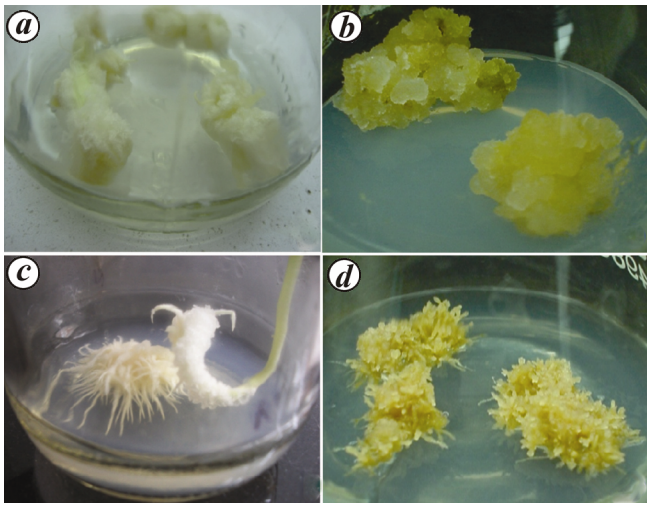

Figure 2. Callus, organogenic callus and multiple root formation in *S. grandiflora*. ***a***, Callus formation from internode; ***b***, organogenic callus; ***c***, multiple roots from internode; ***d***, multiple roots from callus.

GA_3 (1 mg/l; 40.2%). Gurel and Gurel[14] reported that auxins are healthy to induce adventitious root formation in several plants species. They found that silver nitrate (2.0 mg/l) can effectively form roots on the leaf explants in sugar beet (*Beta vulgaris*). NAA and BAP were found effective for *in vitro* regeneration in *Echinacea pallida* and *Solanum laciniatum*[15]. Moreover, *in vitro* regeneration in *S. grandiflora* shoots was successfully achieved in the presence of BAP[16].

Proline acts as a signalling molecule and influences defence pathways, allows multifaceted metabolic and developmental processes. It offers supplementary opportunities for plants development[17]. With adequate knowledge about the importance of proline, the seed and fresh and dried calluses (cotyledon, node and internode) of *S. grandiflora* were tested for the presence of proline. Results (Table 4) showed that the highest proline content was observed from the dried node and internode calluses (110.94 mg/g), followed by dried cotyledon callus (62.73 mg/g). The lowest proline content was observed from fresh cotyledon callus (10.51 mg/g).

TPC in different callsues of *S. grandiflora* was found to range between 3.68 and 16.42 mg/g (Table 4). The highest TPC (16.42 mg/g) was observed in dried calluses (node and internode) followed by wild plant seeds (16.05), whereas the lowest amount of TPC (3.68 mg/g) was obtained in fresh calluses (node and internode). The TPC values were intended using the gallic acid standard curve ($y = 0.0025x - 0.0786$, $R^2 = 0.9997$) and expressed as mg/g GAE, FW and DW.

On the other hand, TFC in calluses was found to range between 2.05 and 22.22 mg/g, in which the highest value (22.22 mg/g) was recorded in dried calluses (node and internode), followed by wild plant seeds (20.10 mg/g), dried calluses (cotyledon; 12.51 mg/g) and fresh callus (cotyledon; 2.05 mg/g) (Table 4). TFC was used as a model curve of quercetin and expressed as mg/g quercetin equivalents FW and DW of *S. grandiflora*.

Phenolics and flavonoids are naturally occurring compounds in fruits, grains and vegetables. They show a broad variety of physiological properties and possess the capability to decrease oxidative break associated with numerous diseases, including cardiovascular, cataract, cancer, immune deficiency, diabetes, atherosclerosis, hepatitis, asthma, arthritis, liver injury and ageing[18]. The

Table 3. Development of shoots and roots from *in vitro* callus of *S. grandiflora* with different plant harmones

Hormone	Concentration (mg/l)	Shoot induction	Shoot formation	Multiple shoot formation	Shoot elongation (cm)	Root induction	No. of roots (%)
BAP	0.1	96.5 ± 1.25^{a}	–	–	7.50 ± 1.6^{c}	–	–
NAA	0.1	73.2 ± 3.2^{b}	–	–	12.21 ± 2.2^{b}	–	–
IAA	0.1	71.8 ± 1.80^{b}	–	–	8.0 ± 2.4^{c}	–	–
2,4-D	0.1	56.0 ± 2.98^{c}	–	–	6.21 ± 1.4^{c}	–	–
IBA	1.0	–	53.0 ± 1.6^{c}	42.7 ± 0.2^{d}	–	–	–
IBA	2.0	–	64.2 ± 1.4^{b}	51.8 ± 2.7^{c}	–	–	–
IBA	3.0	–	76.6 ± 3.2^{a}	64.3 ± 2.5^{b}		–	–
IAA + BAP	0.2 + 0.4	–	20.2 ± 2.2^{f}	30.7 ± 2.2^{e}	6.24 ± 2.5^{c}	–	–
IAA + BAP	0.4 + 0.2	–	33.3 ± 2.1^{e}	40.4 ± 2.7^{d}	9.36 ± 2.1^{c}	–	–
NAA + BAP	0.2 + 0.4	–	46.7 ± 2.7^{d}	42.5 ± 2.2^{d}	12.43 ± 1.7^{b}	–	–
NAA + BAP	0.4 + 0.2	–	59.4 ± 2.4^{c}	52.3 ± 2.4^{c}	19.55 ± 2.1^{a}	–	–
2,4-D + BPA	3+1	–	–	–	–	49.5 ± 1.6^{c}	28 ± 1.8^{e}
IBA	0.5	–	–	–	–	58.3 ± 2.8^{b}	32 ± 2.1^{d}
IBA	1.5	–	–	–	–	70.4 ± 2.8^{a}	41 ± 2.1^{c}
IBA	2.5	–	–	–	–	76.3 ± 2.5^{a}	46 ± 3.0^{c}
GA_3	1.0	–	–	–	–	40.2 ± 2.7^{c}	26 ± 2.3^{e}

$^{a–c}$ are considered significant; $^{d–h}$ are considered as non-significant; IBA, Indole-3-butyric acid; GA_3, Gibberellic acid.

Table 4. Quantification of proline, phenol, flavonoid and ferric reducing antioxidant power (FRAP) in calluses and seeds of *S. grandiflora*

Samples	Proline (mg/g)	Phenol (mg/g GAE)	Flavonoid (mg/g)	FRAP mg (Fe(II)/g)
Fresh callus (node and internode)	12.62 ± 2.1^{g}	3.68 ± 0.3^{h}	2.45 ± 1.3^{h}	129 ± 3.7^{c}
Dried callus (node and internode)	110.94 ± 4.5^{d}	16.42 ± 2.7^{g}	22.22 ± 2.3^{f}	230 ± 4.2^{a}
Fresh callus (cotyledon)	10.51 ± 1.2^{h}	2.65 ± 2.0^{h}	2.05 ± 0.5^{h}	105 ± 3.1^{d}
Dried callus (cotyledon)	62.73 ± 3.6^{e}	7.62 ± 1.6^{h}	12.54 ± 1.4^{g}	165 ± 4.6^{b}
Seeds	11.37 ± 1.8^{g}	16.05 ± 2.2^{g}	21.51 ± 2.2^{f}	101 ± 2.4^{d}

$^{a–c}$ are considered significant; $^{d–h}$ are considered as non-significant; GAE, Gallic acid equivalent.

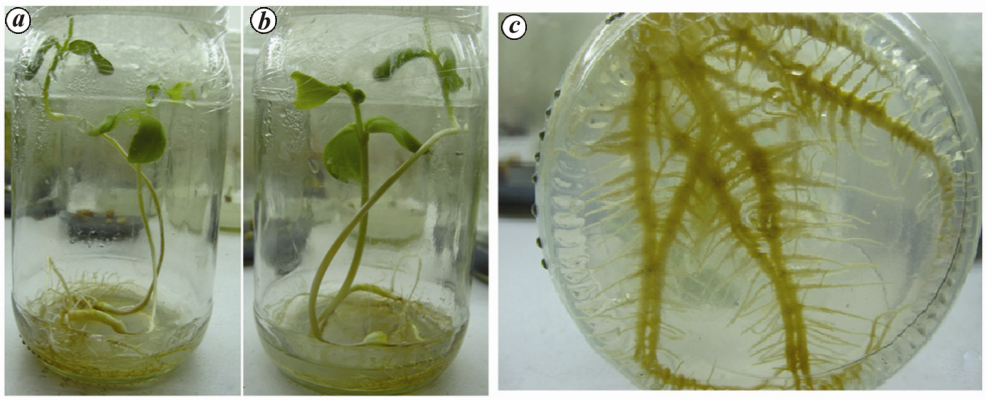

Figure 3. Shoot and multiple root formation and elongation in *S. grandiflora*. ***a***, Shoot formation; ***b***, multiple root formation; ***c***, multiple roots.

estimation of phenols in food and herbs is also important because of their antioxidant, antibacterial and antiproliferative efficacy[19].

FRAP assay measures tumbling possibility of an antioxidant to react with the ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex which produces a coloured ferrous tripyridyltriazine (Fe^{2+}–TPTZ) complex[20]. In the present study, the highest FRAP content (230 mg/g) was found in dried calluses (node and internode), followed by dried callus (cotyledon; 165 mg/g), fresh callus (129 mg/g) and seeds (101 mg/g). Regression equation results of FRAP for Fe^{2+} showed $y = 0.0019x + 0.0114$ and $R^2 = 0.9356$.

In the present study, the free radical scavenging activity was carried out with hexane, ethyl acetate, chloroform, acetone and methanol extracts of seeds and calluses of *S. grandiflora*. The highest scavenging activity was measured for the hexane callus extract (IC_{50}: 71.66 µg/ml), methanol callus extract (IC_{50}: 72.31 µg/ml) and chloroform seeds extract (IC_{50}: 72.75 µg/ml). An earlier study reported that the leaf extract of *Nelumbo nucifera* shows

free radical scavenging activity with IC_{50} values of 230.62 µg/ml for methanol, 231.91 µg/ml for hexane and 390.54 µg/ml for acetone extract[21]. The acetone extract obtained from *Helicteres isora* also showed strong radical scavenging activity with an IC_{50} value of 26.25 µg/ml (ref. 22). Similarly, the aqueous leaf and callus extracts of *Senecio candicans* exhibited free radical scavenging activity with IC_{50} value of 10.74 and 55.42 µg/ml respectively[23].

The modern concepts of free radical biology and medical science are inter-related, where reactive oxygen species (ROS) are causative agents for many health problems. Such health issues can be treated with a regular intake of antioxidants that reduce ROS production in the body[24]. Interestingly, the medicinal plants contain numerous bioactive compounds which exhibit antioxidative activity by reducing oxidative stress-induced injury[25].

In the present study, an efficient method for plant regeneration has been established for *S. grandiflora*. Plant tissue culture has often been considered to be an appropriate system for large-scale production of medicinal results. The present *in vitro* method was found effective for the large-scale production of calluses in *S. grandiflora*. Strong antioxidant activity as well as higher proline, phenol, flavonoid and FRAP contents upturn the demand of *S. grandiflora* in the herbal medicine industries. Hence, the present *in vitro* tissue culture method is highly important to fulfilling its market demand by increasing its production without disturbing its natural habitat.

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