

Effect of growth hormones in induction of callus, antioxidants, and antibacterial activity in *Nerium odorum*

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ABSTRACT

Nerium odorum is an imperative species with worldwide therapeutic and commercial uses. *N. odorum* has been considered as a potentially important plant for industrial and pharmacological applications. Callus induction potential of *N. odorum* was assessed from leaf explants cultured on Murashige and Skoog's medium using different plant hormonal treatments. A range of different concentrations of 6-benzylaminopurine, 1-naphthaleneacetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), and kinetin (0.5–5.0 mg/L) have been incorporated in the culture medium to investigate the biomass, polyphenols production and oxidizing and antibacterial activity of the callus. The results showed highest callus induction at 2.0 mg/L in growth hormone combination of IAA and 2,4-D. The total phenolic content was 92.14 mg GA/g dry weight (DW). In addition, the 2,2-diphenyl-1-picrylhydrazyl activity and 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) activities were 46.7 µg/mL DW and 19.9 µg/mL DW, respectively. Interestingly, the explants produced hard calli using combination of IAA and 2,4-D presented a higher phenolic content and antioxidant capacity when compared to individual growth hormones of either IAA or 2,4-D. Altogether, these results demonstrate the extraordinary effect of different growth hormones on polyphenols production, antioxidant, and antibacterial activities in *N. odorum*.

1. INTRODUCTION

Nerium odorum Linn., commonly identified as rose ray, is an evergreen shrub, used as ornamental plant belongs to family Apocynaceae, omnipresent in temperate and subtropical regions of Africa, Europe, and South East Asia. The plant is widely grown in Indian gardens and found in humid and coastal areas including Assam, Arunachal Pradesh, Himachal Pradesh, Rajasthan, Karnataka, and in all parts of India. *N. odorum* plant is one of the most toxic plants of this family and it is effective in snakebite cure. The crushed leaves, twig, root, and bark are used as a rat poison and as an insecticide too. The root is suggested for external appliance to skin diseases, and the plant is popular medication for mental or venereal diseases as abortifacient in traditional medicine. The oil extracted from the root bark is suggested for medication against leprosy, epilepsy, and scaly nature of skin/skin diseases. The *N. odorum* leaves consist of minute quantity of latex which is used to make rubber [1].

N. odorum produces several secondary metabolites among these alkaloids are having a number of pharmacological attention and even primary compounds such as cardenolides, flavonoids, and terpenes have their own attention. Oleandrin has been recognized as effective antitumor

compound; in addition, it is used indigenously as a cardiac tonic and diuretic. *N. odorum* is recognized to be toxic against extensive range of tumor cells, and this plant also recognized as antioxidants, the leaves of *N. odorum* contain two novel cytotoxic pentacyclic and trans-Karenin and potential secondary metabolites [2]. Similar reports are also indicated the occurrence of oleandrin, folinerin, adynerin, and digitoxigenin cardiac glycosides in *N. odorum* [3]. The plant seeds consist of about 12% of 9-hydroxy-isoricinoleic acid. Methanolic extract of the leaves was found to be anticonvulsant, central nervous system depressant, and analgesic. *N. odorum* extracts have been reported to for oleanolic acid. Lipid peroxidation inhibitory activity from flowers of *N. odorum* was reported earlier [4]. *N. odorum* plants with milky sap have a high medicinal value; the enlarge demands of *N. odorum* plant for industrial and commercial exploitation require a substitute rate of proliferation.

During these decades, *in vitro* practice of callus culture is being extensively useful to produce indistinguishable quality of callus and disease-free plants. In observation of this evidence, the study was conducted for *in vitro* propagation of *N. odorum*. The cultured plant callus is extensively accepted as capable alternative for the production of secondary metabolites [5-7]. The opportunity of housing cell and tissue cultures for secondary metabolites production has been investigated, but the yield has not been optimized to reach higher levels for commercial application [8,9].

In the literature, no reports are available on the effect of growth hormones and their interactions toward secondary metabolites

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production, antioxidant, and antibacterial activities in callus cultures of *N. odorum*. In this connection, the present study aims for to compare the effects of different growth hormones, on the production of total phenols, antioxidant, and antimicrobial activities of *N. odorum* callus.

2. MATERIALS AND METHODS

2.1. Collection of Explants, Implantation, and Culture Conditions

The *N. odorum*, plant materials, was brought from plants grown in the Botanical Garden of University of Mysore, Mysore, Karnataka, India. An authentic sample was identified by taxonomist and the voucher specimen was submitted to the herbarium of the Department of Studies in Biotechnology, University of Mysore, Mysore. Leaves of 8–12 cm length were collected from *N. odorum* plants growing at an altitude of 2400 m at location 12°18'29.45"N and 76°38'18.83"E in the area of Mysore region, Karnataka, in June 2015 and were used for callus initiation.

2.2. Initiation of Callus Cultures

The newly formed young leaves excised from *N. odorum* were further sterilized in 0.5% (w/v) mercuric chloride used for 15 min and washed for 5 times for 5 min each in sterile H₂O. *N. odorum* leaves were cut into small pieces and inoculated to a sterile Murashige and Skoog's (MS) medium for callus initiation [10], supplemented with 8% agar, along with 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), 6-benzylaminopurine (BAP), kinetin (Kin), and 1-naphthaleneacetic acid (NAA) (Hi-Media, Bangalore, India) at different concentration of 0.5–5 mg/L. The sucrose was used as a single carbon source; the pH optimized to 5.8 before autoclaving with 1N NaOH and HCL. The explants on the medium were incubated at 24°C ± 2°C under diffuse fluorescent light (72/71 intensity) in an 18-h photoperiod. Calli were maintained with regular subculturing on a monthly basis which was originated from the explants within 21 days.

2.3. Determination of Callus Fresh/Dry Weight (FW/DW) and Callus Moisture Content

The callus was collected from tissue culture laboratory after 40 days of inoculation, and its media was completely washed with sterile distilled water. The calluses were placed beneath a fan (on blotting paper) to remove water and the weight of callus was weighed in a balance. Subsequently, the calli were positioned on Petri dishes and heated in an oven for 10 min at 65°C for drying. DW of callus was determined in an electronic balance. The moisture content was determined using the FW and DW of callus by the following procedures of Rashmi and Trivedi [11].

A=Weight of empty Petri dish

B=Weight of Petri dish with fresh calli

C=Weight of Petri dish with dried calli

Moisture content %=(B-A)-(C-A)/(B-A)×100

2.4. Extract Preparation

Calli on diverse growth hormones were harvested, 200 mg of DW in each callus was successively extracted successively utilizing 500 ml of non-polar, moderately polar, and polar solvents (Merck, Bangalore, India) in increasing polarity (hexane < ethyl acetate < methanol < water) using a Soxhlet apparatus by continuous hot percolation (boiling point, 52–62°C) until the solvent became colorless. The resultant solvent extracts were concentrated in a rotary evaporator (Thermo Scientific,

Germany) under controlled pressure. For the studies undertaken, the required amount of extract was weighed and solubilized in dimethyl sulfoxide (1 mg/ml) and was further diluted as indicated in the sections below.

2.5. Determination of Antioxidant Activity

2.5.1. Total phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, and 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay

The total polyphenols of the Soxhlet extracts were determined by Folin–Ciocalteu method as described by Pasko *et al.* [12]. The free radical scavenging capacity of Soxhlet extract was determined by DPPH and ABTS method following the procedures of Brand-Williams *et al.* [13] and Re *et al.* [14]. The experiments consisted of three replicates and were repeated 3 times.

2.6. Antibacterial Activity

The Gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella*, and *Shigella* and the Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* were obtained from stock culture unit of the Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore, Karnataka, India, and used for the assay. The extracts were screened for antibacterial activity using well diffusion method. The Soxhlet extracts from callus (100 µg/ml) were added to wells seeded agar plates. The plates were incubated at 37°C ± 2°C for 16 h. Chloramphenicol and methanol were used as positive and negative controls, respectively.

2.7. Statistical Analysis

All experiments including DPPH, ABTS, measurements of total phenolic content, and antimicrobial activity assay were conducted in triplicates and repeated for 3 times. The reported value for each sample was calculated as the mean and standard deviation of three independent experiments.

3. RESULTS

3.1. Effects of Hormonal Treatments on Callus Induction

Plant growth regulators are synthetic molecules used in plant tissue culture and supplemented at relatively low concentrations to work as signaling compounds for plant growth and development. In the present study, stem (nodal) and leaf were used as explants, in which leaf part of explants appear to be the best for callus induction which represents the results in accordance with the earlier reports Rashmi and Trivedi [11]. The MS medium, without any growth hormones, was unable to induce callus. Among all the growth hormones, IAA and 2,4-D hormones exhibited more competence in callus induction in individual along with their different combinations which correlate the earlier reports [11,15]. The MS medium supplemented with different combination of enzyme, with different concentration varying from 0.5 to 5 mg/L of IAA, NAA, 2,4-D, BAP, and Kin exhibited stimulation and induction of callus. The highest maximum callusing retort of 89 % was observed at the 2.0 mg/L of IAA [Figure 1]. At 0.5 mg/L of IAA, the induction of callus response was very less; simultaneously, on increase in the hormonal concentration up to 2 mg/L, the induction of callus response was very good. Interestingly, at 2.5 mg/L, and onward, callusing response was reduced at 5 mg/L, no callusing or growth was observed.

It was pragmatic that the higher concentration of IAA in media had an inhibitory effect on the callus induction and proliferation. 2,4-D with various in different concentration ranging from 0.5 to 5 mg/L exhibited stimulatory effects on callus induction. Interestingly, there was no induction of callus formation was observed on leaf explants inoculated to MS media supplemented with 0.5–5 mg/L of Kin. With respect to BAP and NAA, maximum callusing response of 15% in leaves was noted on supplement of 3 mg/L. A decreased concentration of BAP and NAA of 0.5–1.5 mg/L was unable to produce callus and higher concentration of 3.5 mg/L and above of BAP up to 5 mg/L in the MS media had inhibitory effect on callus induction [Table 1]. 2,4-D and IAA with different concentrations (0.5–5 mg/L) showed stimulatory effects on callus induction. Maximum callusing response of 91% in leaf explants was noted at 2 mg/L in combination of IAA and 2,4-D [Figure 1]. At 3 mg/L of IAA and 1 mg/L 2,4-D, swelling of callus was observed. At 5–10 mg/L of IAA and 2,4-D, no callusing or growth was observed. Media supplemented with different concentration of 2,4-D combinations with BAP, Kin, and NAA showed stimulatory effects on callus induction with maximum

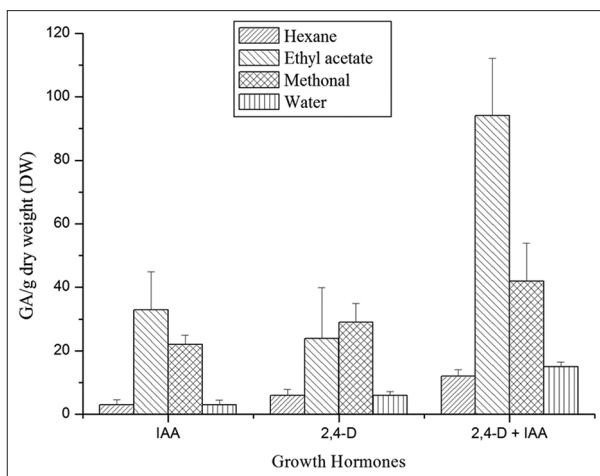


Figure 1: Callus induction of MS fortified with growth hormones. A; 2,4-D (2.5 mg/L), B; IAA (2.0 mg/L), C; Combination of 2,4-D and IAA at concentration 2.0 mg/L respectively.

26% [Table 1]. Similarly, IAA and BAP, IAA and Kin, and IAA and NAA combinations showed stimulatory effects on callus induction. Maximum callusing response of 60% was recorded at 0.5 and 2 mg/L for IAA combined with BAP, Kin, and NAA, respectively [Table 1]. It was observed that 2,4-D and IAA had enhanced callus growth in the presence of auxins. Plant growth hormones can be classified into different types according to their molecular structures and physiological functions. The most extensively used and studied class of plant growth regulators in plant tissue cultures were auxins. 2,4-D and IAA were effectively induced callus formation in many plant species. The result from this study revealed that the presence of 2,4-D and IAA in the culture media was essentially required to induce callus formation in this species even though the cytokinin was absent. The effectiveness of 2,4-D and IAA in inducing the callusing is attributed to its main characteristic which can stimulate cell division of plant tissues and strongly suppress organogenesis. It is also considered to be the most potent among any other commonly used auxins.

MS media supplemented with varying concentration of 0.5–2.0 mg/L of NAA and BAP exhibited significant effect on callus induction. The maximum highest callusing response (25%) was recorded at 2.5 and 1 mg/L for BAP and NAA. Different concentrations of NAA and Kin did not show any stimulatory effects on callus induction. Maximum callusing response (17%) was recorded at 1 and 1.5 mg/L for Kin and BAP. At 2.5–5 mg/L of Kin and BAP, no callusing or growth was observed. In the present work, BAP, NAA, and Kin either individually or in combination could not induce callus significantly. In further experiments, BAP and NAA were supplemented to the MS media in combination with auxins (2,4-D and IAA). The optimum treatment for callus induction in this study was identified in MS medium supplemented with 2.0 mg/L 2,4-D and 2.0 mg/L IAA combination. The findings revealed that the supplementation of auxins at an optimum concentration and combination with 2,4-D and IAA is required to produce calli with the desirable morphology. The hormonal combination of 2,4-D and IAA was found effective in producing optimum callus induction in several plant species. The FW and DWs and moisture content showed good growth of callus on 2,4-D and IAA along with their combinations [11,15,16].

Table 1: Callus induction on leaf explants on MS medium supplemented with different concentrations and combinations of growth hormones (mg/L) in *Nerium odorum*.

Medium composition mg/L	% of callus induction	Degree of callusing	Day of callus induction	Fresh weight (mg)	Dry weight (mg)	Moisture content (%)	Nature of callus
MS	-	-	-	-	-	-	-
2,4-D	85	+++	21	1.56	0.35	77.56	Whitish
BAP	15	+	33	1.05	0.16	57.0	Light green
IAA	89	+++	24	1.82	0.59	67.5	Yellow
NAA	15	+	28	0.96	0.28	70.0	Yellow
Kin	-	-	-	-	-	-	-
2,4-D+IAA	91	+++	19	2.36	0.83	64.83	Yellow
2,4-D+NAA	16	++	28	0.95	0.36	62.10	Whitish
2,4-D+BAP	26	++	26	0.84	0.19	77.38	Whitish
IAA+NAA	27	+++	30	0.6	0.15	75.0	Yellow
IAA+BAP	61	+	32	1.23	0.46	62.0	Yellow

Callus growth observations by measuring % of callus induction, degree of callusing, day of callus induction, callus fresh and dry weight, and nature of callus of randomly selected samples from different concentrations (milligrams per liter) of growth hormones of *Nerium odorum* leaf. (-) indicates no regeneration and (+) indicates status of callus induction, +: Poor, ++: Good, +++: Excellent. MS: Murashige and Skoog's, 2,4-D: 2,4-dichlorophenoxyacetic acid, BAP: 6-benzylaminopurine, IAA: Indole-3-acetic acid, NAA: 1-naphthaleneacetic acid, Kin: Kinetin.

3. 2. Effects of Growth Hormones on Nature and Moisture Content of Callus

The FW and DW of the callus was measured after various periods, the significant difference in FW and DW ($P = 0.01$) was observed in the value of weight from callus initiation up to 6 months, whereas no significant differences were found at 6–9 months. The time involved of callus growth in its optimization of media and a uniform callus was obtained after 5 months with frequent subculturing. The values for FW and DW after 1 month were 256 and 53 mg, respectively. These FW and DWs increased rapidly to 3622 and 198, respectively, after 6 months by frequent subculturing. The FW and DW of callus from 6 to 9 months exhibited 268 mg of callus increased to an average weight of 357 mg every month in FW. Figure 1 depicts the callus cultures of *N. odorum*. Leaf callus was loose in texture and friable. It was white and yellow in color with different growth hormone treated with combinations of 2,4-D. Callus was compact and non-friable, light yellow to light green in color with different growth hormone in combinations of IAA [Table 1]. The moisture content varied in the callus derived from explants under the influence of various 2,4-D and IAA. It was observed that moisture content varied from 69% to 80% which supports good growth of callus.

3.3. In Vitro Antioxidant Assays

Three *in vitro* tests (total phenolic content, DPPH, and ABTS) were used to characterize antioxidant property of an isolated callus of *N. odorum*. The phenolic content of *N. odorum* callus cultures under different growth hormonal conditions is presented in Figure 2. The results show that the production of polyphenols was affected by the type of growth hormone used during the growth of callus. The total phenolic content of ethyl acetate extract was between 37.81 and 23.96 mg GA/g DW for IAA and 2,4-D, respectively, except these for the all other growth hormone (BAP and NAA) the phenolic content have no significant effect. Interestingly, when comparing with the combination of IAA and 2,4-D, the production of polyphenols by callus of *N. odorum* is superior to the production of polyphenols by individual growth hormones IAA and 2,4-D, which exhibited a total phenolic content of 92.14 mg GA/g DW [Figure 2].

The highest antioxidant potential was demonstrated by an ethyl acetate extract obtained from IAA and 2,4-D callus, whose radical scavenging activity, expressed as EC, was 19.9 $\mu\text{g}/\text{mL}$ (in ABTS test) and 46.7 $\mu\text{g}/\text{mL}$ (in DPPH assay) [Figure 3]. The lowest scavenging activity, with EC (ABTS test) and 164 $\mu\text{g}/\text{mL}$ IC50 values of about 45.6 $\mu\text{g}/\text{mL}$ (DPPH test), was demonstrated by the methonolic extract of 2,4-D callus [Figure 3]. Based on our studies, the antioxidant activities of this plant from leaves and stem and roots have been reported extensively [5,6]. Even then, there were no reports available by *in vitro* growth of callus, slight differences in the antioxidant activities do occur that solely depend on varieties, location, and growth conditions [17]. Overall, in the studied experiments, the estimation of the antioxidants capacity exhibited positive results by scavenging free radicals. The inhibition in the DPPH and ABTS activity was found more in leaf extraction callus developed in growth hormones IAA and 2,4-D combination. The ethyl acetate extracts of *N. odorum* from this family showed antioxidant properties through hydroxyl scavenging ability. *In vitro* studies indicated the ability to scavenge free radicals (hydroxyl ions) [18].

3. 4. Antibacterial Activity

The ethyl acetate extracts of callus from *N. odorum* were highly inhibiting the growth of both Gram-positive and Gram-negative

bacteria. The variation with respect to concentration of extract, the zone of inhibition was observed from 10 to 16 mm from the extracts of IAA and 2,4-D growth hormones [Table 2]. The extracts of 2,4-D were most effective, exhibiting a zone of inhibition ranged from 19 to 24 mm for *B. subtilis*, *K. pneumonia*, and *S. aureus*, whereas the inhibition zone for *E. coli* ranged from 13 to 17 mm. The extract of IAA showed zone of inhibition ranging from 8 to 16 mm against

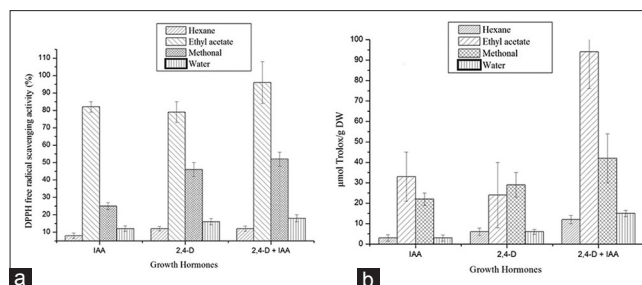


Figure 2: Total phenolic content in callus cultures of *Nerium odorum* under different growth hormonal conditions in different solvent extraction. The values are means of three individual experiments with three replicates. Bars indicate standard errors.

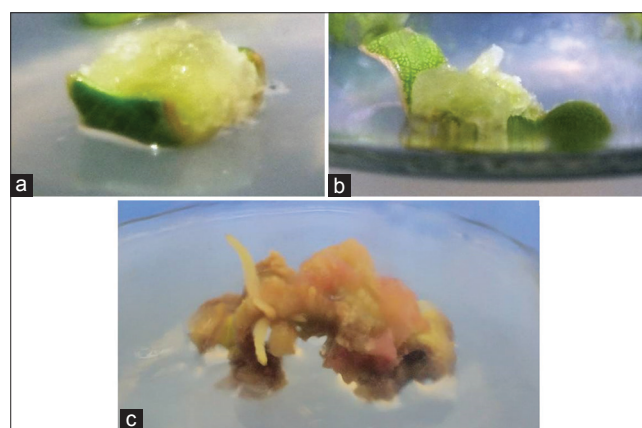


Figure 3: The DPPH and ABTS capacity of callus cultures of *Nerium odorum* under different growth hormonal conditions in different solvent extraction. The values are means of three individual experiments with three replicates. Bars indicate standard errors.

Table 2: Antimicrobial activity and the zone of inhibition of the ethyl acetate extracts from callus supplemented with different concentrations and combinations of growth hormones.

Bacterial pathogens	Zone of inhibition (mm)		
	2,4-D	IAA	2,4-D+IAA
<i>Escherichia coli</i>	10	12	12
<i>Klebsiella pneumoniae</i>	19	15	16
<i>Bacillus subtilis</i>	22	16	20
<i>Staphylococcus</i>	24	16	21
<i>Salmonella</i>	18	13	12
<i>Shigella</i>	12	16	11

Extracts were screened for antibacterial activity using well diffusion method. The overnight culture was centrifuged and the pellet was resuspended in sterile water. The suspension of bacteria was plated on nutrient agar plates. The Soxhlet extracts from callus (100 $\mu\text{g}/\text{mL}$) were added to wells seeded agar plates. The plates were incubated at 37°C for 16 h. 2,4-D: 2,4-dichlorophenoxyacetic acid, IAA: Indole-3-acetic acid.

K. pneumonia, *B. subtilis*, and *E. coli* [Table 2]. The antibacterial activity of the tuber may be due to the presence of phenolic active compounds in *N. odorum*. Antibacterial effect against Gram-negative and Gram-positive bacteria could be as natural source for producing pharmacological products [19,20]. The results of the current study supported the traditional treatment by medicinal plants and proposed antibacterial agents from plant extracts with antibacterial properties. The maximum activity was observed against Gram-negative and Gram-positive bacteria from ethyl acetate extract grown on 2,4-D compared with IAA and along with the combination of IAA and 2,4-D, respectively. Antimicrobial properties of medicinal plants are being increasingly stated from various parts of the world. Based on the World Health Organization report, the plant active constituents are used as folk medicine in traditional therapies of 80% of the world's population [20,21]. In this study, the extracts obtained showed strong activity against most of the tested bacterial strains. The results were compared with standard drug. The effect of antibacterial in medicinal plants varies intensely depending on the phytochemical features of plant [19,20].

4. CONCLUSION

Finally, it can be concluded that combinations of IAA and 2,4-D in MS media exhibited the highest calli formation from leaf explants of *N. odorum*. Our data revealed that calli subculture on IAA and 2,4-D was effective in increasing phenolic content and antioxidant property of IAA and 2,4-D exudation. This study also showed that ethyl acetate extracts from *in vivo* gave higher results for antioxidant activity and inhibition zone against Gram-negative and Gram-positive bacteria compared to the same concentration of callus extract from individual growth hormones (IAA/2,4-D). Further and more specific studies toward characterization of compounds, *in vivo* or *in vitro*, are recommended to determine the characteristics of secondary metabolites.

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6. REFERENCES

- Zibbu G, Batra A. A review on chemistry and pharmacological activity of *Nerium oleander* L. J Chem Pharm Res 2010;2:351-8.
- Siddiqui BS, Khatoon N, Begum S, Farooq AD, Qamar K, Bhatti HA, et al. Flavonoid and cardenolide glycosides and a pentacyclic triterpene from the leaves of *Nerium oleander* and evaluation of cytotoxicity. Phytochemistry 2012;77:238-44.
- Bandara V, Weinstein SA, White J, Eddleston M. A review of the natural history, toxinology, diagnosis and clinical management of *Nerium oleander* (common oleander) and *Thevetia peruviana* (yellow oleander) poisoning. Toxicon 2010;56:273-81.
- Dey P, Chaudhuri TK. Pharmacological aspects of *Nerium indicum* Mill: A comprehensive review. Pharm Rev 2014;8:156.
- Lipsky KA. Problems of optimization of plant cell culture processes. J Biotechnol 1992;54:93-108.
- McDonald KA, Jackman AP, Thorup JE, Dandekar AM. Plant callus as a source for biochemicals. Appl Biochem Biotechnol 1997;54:89-108.
- Dix PJ. Plant Cell Line Selection. Weinheim: VCH; 1990. p. 4-17, 30-7, 187-211.
- Yeoman MM, Yeomen CL. Manipulating secondary metabolism in cultured plant cells. Plant Cell Rep 1996;134:553-69.
- Profumo P, Gastado AM, Riboldi CU. Formation of cardiac glycosides in calli from leaf explants of *Nerium oleander* L. Plant Med Phytother 1993;26:346-9.
- Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 1962;15:473-97.
- Rashmi R, Trivedi MP. Effect of various growth hormone concentration and combination on callus induction, nature of callus and callogenic response of *Nerium odorum*. Appl Biochem Biotechnol 2014;172:2562-70.
- Pasko P, Bartoń H, Zagrodzki P, Gorinstein S, Fołta M, Zachwieja Z. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. Food Chem 2009;115:994-8.
- Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebenson Wiss Technol 1995;28:25-30.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
- Sehrawat AR, Sanjogta U, Chowdhury JB. Establishment of plantlets and evaluation of differentiated roots for alkaloids in *Rauwolfia serpentina*. J Plant Biochem Biotechnol 2002;11:105-8.
- Phelan S, Hunter A, Douglas GC. Effect of explants source on shoot proliferation and adventitious regeneration in 10 *Buddleia cultivars*. Sci Hortic 2009;120:518-24.
- Chidambaram U, Pachamuthu V, Natarajan S, Elango B, Suriyanarayanan, Ramkumar KM, et al. *In vitro* evaluation of free radical scavenging activity of *Codariocalyx motorius* root extract. Asian Pac J Trop Med 2013;6:188-94.
- Farzinebrahimi R, Mat Taha R, Rashid KA, Ali Ahmed B, Danaee M, Rozali SE. Preliminary screening of antioxidant and antibacterial activities and establishment of an efficient callus induction in *Curculigo latifolia* Dryand (Lemba). Evid Based Complement Altern Med ECAM 2016;2016:6429652.
- Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A. Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two Gram-negative and Gram-positive bacteria. Int J Microbiol 2013;2013:746165.
- Oroojalian F, Kasra-Kermanshahi R, Azizi M, Bassami MR. Phytochemical composition of the essential oils from three *Apiaceae* species and their antibacterial effects on food-borne pathogens. Food Chem 2010;120:765-70.
- Prakasha A, Grice ID, Kumar KV, Sadashiva MP, Shankar HN, Umeha S. Extracellular polysaccharide from *Ralstonia solanacearum*; A strong inducer of eggplant defense against bacterial wilt. Biol Control 2017;110:107-16.

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