Antioxidant activity and production of secondary metabolites of adult plant and in vitro calli of *Anodendron paniculatum*

K. Nagendra Prasad  
Department of Botany K.T.S. Government Degree College, Rayadurg, Anantapur (Andhra Pradesh), India  
G. Kranthi Kumar  
Department of Botany, KBN College, Vijayawada (Andhra Pradesh), India  
M. Raghu Ram*  
Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur (Andhra Pradesh), India  
*Corresponding author. E-mail: mraghuram2002@gmail.com

Abstract  
The members of the family Apocynaceae are the rich in alkaloids, terpinoids, flavonoids, phenolic compounds and hydrocarbons. *Anodendron paniculatum* Roxb. is a woody climber, the roots of which have been used to control vomiting and cough. In the present study, adult plant and tissue culture plant extracts of *A. paniculatum* collected from Eastern Ghats, Araku, Andhra Pradesh were used for the production of their secondary metabolites and antioxidant activity. Methanol extracts of calli showed high contents of total phenol and alkali from 25.53 and 25.98 mg/g respectively. Methanol extract of tissue culture plant at higher concentrations showed better antioxidant activity with reference to standard ascorbic acid. For this study different concentrations (12.5, 25, 50, 100 and 200 µg/ml) of extracts were prepared using four different solvents. Tissue culture extracts from methanol showed maximum scavenging activity with 231.9 ± 1.39 % at 200 µg/ml of concentration, while that of adult plant extracts showed the scavenging activity of 189.1 ± 0.74% only. These results on high antioxidant activity of the methanol extract of *A. paniculatum* calli can be attributed to the presence of high phenolic and alkali contents in calli when compared to that of adult plant extract.

Keywords: Alkaloids, *Anodendron paniculatum*, Antioxidant activity, Apocynaceae, Phenols

INTRODUCTION  
Genus *Anodendron* belongs to the subfamily Apocynoideae, family Apocynaceae (Takhtajan 2009) with 17 species (Middleton 2007). It is naturally distributed in India, Japan and South China. *Anodendron* consists of 19 species covered a large area of India, a few species of *Anodendron* namely *Anodendron benthamianum*, *A. howii*, *A. punctatum*, *A. affine*, and *A. formicinum* (Flora of China Editorial Committee 1995). Much of the earlier works on antioxidant activity from members of the family Apocynaceae were concentrated on extracts from aerial parts of the plant while the work related to callus extracts was very meagre. The previous records on antioxidant activity of the members of Apocynaceae include Anbukkarasi et al, (2016) on *Tabernaemontana divaricata* leaves; Bhadane and Patil, (2017) on *Carissa carandas* leaves; James et al (2011) on flower extract of *Alstonia scholaris*; and Ganapathy et al., (2011) on plant extract of *Holarrhena antidysenterica*. Hence, in the present study an attempt was made to compare the antioxidant activity in adult plant and callus extract of *A. paniculatum*.

MATERIALS AND METHODS  
Plant material: *Anodendron paniculatum* (Roxb.) A. DC. plants were collected from higher altitudes of Eastern ghats, Araku, Visakhapatnam, Andhra Pradesh.  
Preparation of crude extract: The aerial parts of the plant were shade dried and coarsely powdered. The powdered plant material (200mg) was extracted using soxhlet extractor by the (1000ml) solvents viz., petroleum ether (60-80°C), chloroform, ethylacetate, and methanol according to their polarity. The extracts were filtered and evaporated to dryness in a rotary vacuum evaporator. Five different concentrations (12.5, 25, 50, 100 and 200 µg/ml) these extracts were prepared using Dimethyl sulfoxide DMSO (Harborne, 1973).  
Callus induction: Leaf discs of *A. paniculatum* from members of the family Apocynacea include...
were used for callus induction in the present study. MS medium supplemented with 2 mg/ml of 2-4 D + 0.5 mg/ml Kinetin was used as callus induction medium (CIM). Leaf discs, after sterilization, were cut into 2-3 mm pieces using a sterile scalpel. The cultures were incubated for 6 weeks in 16 h light and 8 h dark at 25±2°C and tissues developed were observed for callus production at 3 week intervals.

**Callus extract:** The callus was dried first by freeze drying using a dryer and then powdered. The dried powder was extracted with four different solvents, petroleum ether (60-80°C), chloroform, ethyl acetate, and methanol in a Soxhlet apparatus.

**Preparation of callus crude extract:** The dried *A. paniculatum* plant material of 20g weight was cleaned and ground to fine powder using a mortar and pistol. The fine powder was packed tightly in a soxhlet extractor and extraction was done using 200 ml of petroleum ether. The extraction process was carried out for 6 hrs. Similarly using other solvents also the extractions were made and the final extracts obtained were evaporated by Roto evaporation under reduced pressure at 60 °C to get a solid dry product which was stored in dried bottles for further use.

**Quantification of total phenolic content:** The determination of the phenolic content followed the method of Singleton et al., (1999). The amount of phenols in the extract was expressed in terms of gallic acid equivalents (mg of GA/g of extract) by using the calibration graph and the absorbance recorded for the extract.

**Quantification of total alkaloid content:**

**Preparation of solutions:** Caffeine standard solution was prepared by dissolving 1 mg pure atropine (Sigma USA) in 10 ml distilled water (Shamsa et al., 2008).

**Standard curve:** Different standard Aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of atropine were prepared and separated into funnels. To this 5 ml of phosphate buffer and 5 ml of BCG solutions were mixed with 4ml of chloroform. The extracts were collected in a 10 ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of chloroform was measured by using spectrophotometer at 470 nm. Blank was prepared without atropine.

**Table 1.** Total phenolic and alkaloid contents (mg/gm) of the adult plant and callus extracts of *Anodendron paniculatum*.

| S. No. | Name of the extract | Adult Plant | Callus |
|-------|---------------------|-------------|--------|
|       |                     | Total content (mg/gm) | Total content (mg/gm) | Total content (mg/gm) | Total content (mg/gm) |
| 1     | Petroleum ether     | 9.02±3.52   | 8.11±0.33 | 9.02±3.52   | 8.11±0.33          |
| 2     | Ethyl acetate       | 11.62±0.48  | 12.41±1.20 | 11.62±0.48  | 12.41±1.20         |
| 3     | Chloroform          | 18.11±1.16  | 17.89±1.33 | 18.11±1.16  | 17.89±1.33         |
| 4     | Methanol            | 21.19±1.33  | 20.62±1.99*| 25.98±2.09*| 25.53±1.89*        |

* Statistically significant at p≤0.05

**Antioxidant activity of DPPH free radical scavenging:** The DPPH activity was carried out as described by Bidchol et al., (2009) with slight modifications. 0.1 ml of extract containing 3 ml of DPPH solution (0.1 mmol/L in 95% ethanol v/v) and this was shaken thoroughly, incubated for 30 minutes at room temperature. The readings were measured by using spectrophotometer at 517 nm with standard Ascorbic acid.

The radical scavenging activity was measured as decrease in the absorbance of DPPH and calculated using the following equation. The percent DPPH scavenging effect was calculated by using following equation:

\[
\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100.
\]

Where \(A_0\) was the Absorbance of control reaction and \(A_1\) was the Absorbance in presence of test or standard sample.

**Statistical analysis:** The data are presented as mean ± SE of 15 explants per treatment and it was taken from the average of three replicates. Data were statistically analysed using Duncan’s multiple range test and found significant if \(P \leq 0.05\).

**RESULTS AND DISCUSSION**

Phytochemical screening of *A. paniculatum* adult plant extract revealed that out of the four different solvents used, methanolic extract showed high content of alkaloids and phenolic compounds (Tables 1 and 2). Highest phenolic content was observed in callus extract with 25.53 mg/g of gallic acid equivalent of callus extract, when compared to that of adult plant extract with 20.62 mg/g. Similarly, highest alkaloid content of 25.98 mg/g of AE/g was observed in callus extract when compared to that of adult plant extract in which 21.19 mg/g of AE/g was recorded.

The calli of *Anodendron* showed similar results as of aerial parts with high contents of total phenol and alkaloid. Methanol extracts of calli showed high contents of total phenol and alkaloid with 20.62 mg/gm and 21.19 mg/gm respectively, followed by chloroform, ethyl alcohol and petroleum ether respectively. As this plant is rare, medicinal-ally valuable, and specific to its habitat condition, and its indiscriminate usage for the production of secondary metabolites which may lead to the ex...
In vitro means of producing secondary metabolites will be fruitful alternative, which was proved in prior experiments of in vitro calli establish and production of secondary metabolites by dual culture system. In the present study, the reported high amount of total phenols and total alkaloids in callus extracts than adult plant extract may be due to accumulation of more phenolic and alkaloid compounds. The high antioxidant activity of callus tissues observed in this study may be attributed to the high phenolic content. Similar reports were reported from members of Leguminoseae (Elnour et al., 2015). Methanolic extract of callus with high antioxidant activity than leaf extracts correlated with more phenolic contents in callus when compared to leaf extract of Justicia gendarusa was reported by Amid et al., (2008). However, Ganapathy et al., (2011) reported that high SOS scavenging activity in Holarrhena antidysenterica was due to high total phenol content of the plant.

The antioxidant activity of extracts from callus developed from leaf disc explants and adult plant concerning DPPH assay was tested at different concentrations ranging from 12.5 to 200 µg/ml using four different solvents (Table 1 and 2). The antioxidant activity of methanolic extract of A. paniculatum from both callus and adult plant exhibited a significant concentration-dependent free radical scavenging from 133.5% to 231.9% (in callus) and 75.4% to 189.1% (in adult plant) compared with ascorbic acid. The highest percentage of scavenging activity was recorded in callus methanolic extract with 231.9% than in methanolic extract of adult plant 189.1%, while that of ascorbic acid was 487.5%. This in vitro antioxidant activity of methanolic extract of either callus or adult plant is due to neutralization of free radicals (DPPH), by transfer of an electron or hydrogen from secondary metabolites present in them.

Majority of the Apocynaceae members produce secondary metabolites like flavonoids, phenolic compounds and Alkaloids. These compounds can be easily extracted in methanol basing on their polarity. All these compounds have high antioxidant activity (Iqbal et al., 2017). In fact, the A. paniculatum methanolic extracts also have good amount of flavonoids, phenols and Alkaloids and this could be reason for the use of this plant extract in the treatment of various disorders. A. paniculatum is an important medicinal plant which was included in the list of endangered plants. Hence, the regeneration of this species is of immense importance. In addition, the highest scavenging activity of callus extract over the adult plant suggests a way for production of antioxidants to be used in medicines.

Table 2. DPPH free radical scavenging activity (% inhibition) of adult plant and tissue culture extracts of Anodendron paniculatum.

| Conc. (µg/ml) | Ascorbic acid (100µM) | Petroleum ether | Ethyl acetate | Chloroform | Methanol |
|--------------|-----------------------|----------------|--------------|------------|----------|
|              | Adult Plant extract   | Tissue culture extract | Adult Plant extract | Tissue culture extract | Adult Plant extract | Tissue culture extract | Adult Plant extract | Tissue culture extract |
| 12.5         | 143.3±0.26            | 56.3±0.22       | 33.8±0.13    | 46.0±0.25  | 75.4±0.78 | 133.5±0.15 |
| 25           | 205.1±0.54            | 75.0±0.32       | 48.7±0.67    | 83.2±0.29  | 90.2±0.26 | 146.3±0.23 |
| 50           | 298.3±0.25            | 80.4±0.17       | 59.4±0.11    | 75.3±0.77  | 125.5±0.77 | 164.0±0.55 |
| 100          | 368.2±0.33            | 88.5±0.97       | 72.1±0.52    | 82.1±0.81  | 138.7±0.63 | 195.7±0.23 |
| 200          | 485.7±0.42            | 95.7±0.55       | 86.6±0.33    | 95.4±0.38  | 189.1±0.74* | 231.9±1.39* |

* Statistically significant at p≤0.05
ACKNOWLEDGEMENTS

First author, K. Nagendra Prasad is thankful to the Department of Botany, K.T.S. Government College, Rayadurg, Anantapur, and second author G. Kranthi kumar is thankful to KBN College, Vijayawada. The authors are thankful to the Dept. of Botany & Microbiology, Acarya Nagarjuna University for providing necessary facilities to complete this research work.

REFERENCES

1. Amid, A., Johan, N.N., Jamal, P. and Zain, W.N.W.M., (2011). Observation of antioxidant activity of leaves, callus and suspension culture of Justicia gendarusa. African Journal of Biotechnology, 10(81):18653-18656. DOI: 10.5897/AJB11.2734
2. Anbukkarasi, M., Thomas, P. A., Sundararajan, M., and Geraldine, P. (2016). Gas chromatography-mass spectrometry analysis and in vitro antioxidant activity of the ethanolic extract of the leaves of Tabernaemontana divaricata. Pharmacognosy Journal, 8(5) : 451–458.
3. Bhadane, B. S., & Patil, R. H. (2017). Isolation, purification and characterization of antioxidative steroid derivative from methanolic extract of Carissa carandas (L.) leaves. Biocatalysis and Agricultural Biotechnology, 10, 216–223.
4. Bidchol, A.M., Wilfred, A., Abhijna, P. and Harish, R., (2011). Free radical scavenging activity of aqueous and ethanolic extract of Brassica oleracea L. var. italica. Food and Bioprocess Technology, 4(7):1137-1143. DOI 10.1007/s11947-009-0196-9
5. Chi, V.V. (1997). Dictionary of Vietnam Medicinal Plants. Medical Publishing House, Hanoi.
6. Elnour, M.E., Ali, A.M. and Saeed, B.A.T. (2015). Effect of different concentrations of auxins and combination with kinetin on callus initiation of Trigonella Foenum-Graecum. L. Int. J. Technic. Res. Appl, 3(2):117-122.
7. Flora of China Editorial Committee (1995). Flora of China (Gentianaceae through Boraginaceae). Volume 16. Flora of China Press & Missouri Botanical Garden Press, Beijing and St.Louis.
8. Ganapathy, P. S., Ramachandra, Y. L., & Rai, S. P. (2011). In vitro antioxidant activity of Holarrhena antidysenterica Wall. methanolic leaf extract. Journal of Basic and Clinical Pharmacy, 4(4), 175.
9. Harborne, J.B. and Williams, C.A. (2000). Advances in flavonoid research since 1992. Phytochemistry 55 : 481-504. DOI.org/10.1016/S0031-9422(00)00235-1
10. Iqbal, Z., Iqbal, M.S. and Mishra, K., (2017). Screening of antioxidant property in medicinal plants belonging to the family Apocynaceae. Asian J Pharm Clin Res, 10(12):415-418. DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i12.22303
11. James, J., Veettil, A. K. T., Pratyush, K., Misra, C. S., Sahadevan, L. D. M., & Thankamani, V. (2011). In vitro antioxidant activity of flowers and fruits of Alstonia scholaris. International Journal of Phytomedicine, 3(4), 475–479.
12. Middleton D.J., Flora Malesiana. (2007). Series I, Volume 18. Foundation Flora Malesiana, Netherlands.
13. Shamsa, F., Monsef, H., Ghamooshi, R. and Verdian-rizi, M., (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J Pharm Sci., 32:17-20.
14. Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M., (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in Enzymology., 299: 152-178. doi.org/10.1016/S0076-6879(99)99017-1
15. Takhtajan, A. (2009). Flowering plants. Springer, St. Petersburg.