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

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Abstract
The members of the family Apocynaceae are the rich in alkaloids, terpinoids, flavonoids, phenolic compounds and hydrocarbons. *Anodendron paniculatum* Roxb. is an 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 alkaloid with 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 by 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 alkaloid 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 covering a large area of India, a few speices 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 areal parts of the plant while the work related to callus extracts was very meagre. The previous records on antioxidant activity of the members of Apocynacea 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^{0}C), chloroform, ethylacetate, and methanol according to their polarity. The extracts were filtered and evaporated to dryness in a rotary vaccum evaporator. Five different concentrations (12.5, 25, 50, 100 and 200 µg/ml) these extracts were prepared using Dimethyl sulfoxide DSMO (Harborne, 1973).

Callus induction: Leaf discs of *A. paniculatum*

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^{0}C and tissues developed were observedfor callus production at 3 week intervals.

Callus extract: The callus was dried first by freeze drying using a dryier and then powdered. The dried powder was extracted with four different solvents, petroleum ether (60-80^{0}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 pistil. The fine poweder was packed tightly in a soxhlet extracter 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 ^{0}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 separatedto 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.

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:

DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100. …….Eq. 1

Where A0 was the Absorbance of control reaction and A1 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 ≤ 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, medicinally valuable, and specific to its habitat condition, and its indiscriminate usage for the production of secondary metabolites which may lead to the ex-

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

S. N.	Name of the extract	Adult Plant		Callus	
		Total alkaloid content (mg/gm)	Total Phenolic content (mg/gm)	Total alkaloid content (mg/gm)	Total Phenolic 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

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	Adult Plant extract	Tissue culture extract
12.5	143.3±0.26		56.3± 0.22	56.1±0.11	33.8±0.13	121.8±1.12	46.0±0.25	83.2± 0.29	75.4±0.78	133.5±0.15
25	205.1±0.54		75.0±0.32	30.0±0.29	48.7±0.67	115.9±0.56	59.6±0.31	94.0±0.02	90.2±0.26	146.3±0.23
50	298.3±0.25		80.4±0.17	14.5±0.22	59.4±0.11	88.2±0.37	75.3±0.77	88.27±0.25	125.5±0.77	180.4±0.55
100	368.2±0.33		88.5±0.97	09.2±0.10	72.1±0.52	74.6±0.18	82.1±0.81	77.59±0.59	138.7±0.63	195.7±0.23
200	485.7±0.42		95.7±0.55	0.50±0.10	86.6±0.33	51.7±0.27	95.4±0.38	45.65±0.65	189.1±0.74*	231.9±1.39*

* Statistically significant at p≤0.05

tinction of the species. Hence, *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 phenoloic 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 methanolic extract of *A. paniculatum* from both callus and adult plant exhibited a significant concentration-dependent free radical scavenging from 133.5%-231.9% (in callus) and 75-189.1% (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 invitro antioxidant activity of methanolic extract of either callus or adult plant is done to the 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 extractable 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.

Conclusion

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 amount of phenols and alkaloids and high DPPH scavenging activity of callus extract over the adult plant suggests a way for production of antioxidents to be used in medicines.

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