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Studies on Phyto Chemical Constituents and Antibacterial Activity of Endemic Medicinal Plant *Anodendron paniculatum*

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Abstract
For the present study, *Anodendron paniculatum* plants were collected from higher altitudes of Eastern Ghats, Araku, Andhra Pradesh India. Highest yields of chemical constituents like alkaloids, terpinoids, phenols and flavonoid were obtained in methanolic extracts. Among them terpinoids were recorded in high amounts ((41.19+1.33 mg limalool/g)). Of the four different extracts of *A. paniculatum* tested for antimicrobial activity, Methanolic extracts showed maximum antimicrobial activity for the test organisms. Methanolic extracts were proved to have high antimicrobial activity against *Vibrio cholera* when compared to positive control.

Keywords
Anodendron paniculatum, Apocynaceae and Phytochemical analysis.

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INTRODUCTION
The plants of family Apocynaceae are native throughout India, Pakistan, China, Bangladesh, and Sri Lanka (Mahmood *et al*., 2011). *Anodendron paniculatum* (Roxb.) A. DC was an important medicinal plant used by tribal folklore for many health ailments. The roots of this plant have been used in traditional folk medicine as remedy for vomiting and cough in India (Chi, 1997). Moreover, its latex is used to cure the poison of snake and centipede bites (Forster, 1993). In Andhra Pradesh, *A. paniculatum* was included in the list of medicinal plants with the International Union for Conservation of Nature (IUCN) status as endangered or threatened species (Ved *et al*. 2002).

Family members of Apocynaceae are well known for their secondary metabolites viz. Terpens, sterols, saponins, alkaloids (Seigler, 1998, Gunatilaka, 1986) in addition triterpenes and sterols are also reported from the members of Apocynaceae (Bhadene *et al*., 2018, El-Kashef *et al*., 2015). Previous reports from literature indicated that root, leaf and stem extracts of the members of the family Apocynaceae showed antimicrobial activity against *E. coli, Pseudomonas aeruginoase* and *B. subtilis* (Agyare *et al*., 2013) *Proteus vulgaris* (Dehghansi *et al*., 2012). Very little information was available in the literature on species of *Anodendron*. Antibacterial compounds were identified from stem material of *Anodendron fermim* (Qin *et al*., 2014). There were no reports on

biochemical constituents from plant material of *A. paniculatum* so far. This was the first report on chemical compounds like terpinoids, alkaloids, flavanoids and phenols from *A. paniculatum* methanol extracts.

MATERIALS AND METHODS

Plant Material

The *Anodendron paniculatum* (Roxb.) A. DC. Plants growing in higher altitudes of Easter ghats, Araku, Visakhapatnam, Andhra Pradesh were collected for the study.

Preparation of extracts

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^{o}C), chloroform, acetone, and ethanol according to their polarity. The extracts were filtered and evaporated to dryness in a rotary vacuum evaporator. Two different concentrations 5% and 10% of these extracts were prepared using DSMO (Harborne, 1973).

QUANTITATIVE ESTIMATION OF PHYTO CHEMICAL CONSTITUENTS

Quantification of Total Phenolic content

The determination of the phenolic content followed the method of Singleton *et al.* (1999). Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic extract, 2.5 ml of 10% Folin- ciocalteu reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was prepared with 0.5ml of methanol in place of methanolic extract. The samples were incubated at 45^{o}C for 45 min. The absorbance was determined at 765nm using spectrophotometer. Triplicates were maintained for each sample and the same procedure was repeated for standard solution of Gallic acid and calibration line was constructed. 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

BCG (Bromo Cresol Green) solution (1X10^{4}) was prepared by heating 69.8 mg BCG with 3 ml of 2N NaOH and 5 ml of distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water. Phosphate buffer (pH 4.7) was prepared by adjusting the pH of 2M Sodium Phosphate (71.6 g Na_2HPO_4 in 1 lit distilled water) to 4.7 with 0.2M Citric acid (42.02 g citric acid in 1 L distilled water) caffeine standard solution was made by dissolving 1 mg pure atropine (Sigma USA) in 10 ml distilled water (Shamsa *et al*., 2008).

Standard curve

Aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of atropine standard solution and transfer each to different separating funnels. Then add 5 ml pH 4.7 phosphate buffer and 5 ml of BCG solution and shake a mixture with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10 ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine.

Quantification of Total Flavonoid Content

The flavonoid content was performed according to the methodology of Kosalec *et al.* (2004). With slight modifications, using aluminum chloride ($AlCl_3$). Samples from each extract were prepared at an initial concentration of 20 µg/mL and diluted to 10, 5, 2 and 1 µg/mL concentrations. A total of 760 µL of methanol, 40 µL of potassium acetate (10%), aluminium chloride (10%) and 1.120 µL of distilled water were added to 50 µL of each extract concentration. For the blank assay, the volume of aluminum chloride and 10% potassium acetate was replaced by distilled water. Incubation took place at room temperature for 30 min and the spectrophotometer reading was performed at 415 nm. The calibration curve was performed with quercetin, the test was performed in triplicate and the mean value expressed in µgEq.Q/g.

Quantification of Total Terpenoid Content

The total terpenoid content of the plant extracts was determined based on an assay described by Ghorai *et al*., (2012) with some modifications. Linalool was used as the standard for estimation. An aliquot of the reaction mixture obtained after Salkowski test employed for the qualitative analysis of terpenoids in the extract was transferred to colorimetric cuvette. The absorbance was measured at 538 nm against blank *i.e*., 95% (v/v) methanol. For the standard curve, 200 µl of linalool solution in methanol was added with 1.5 ml chloroform and serial dilutions [dilution level-100 mg/200 µl to 1 mg/200 µl linalool Conc.] were prepared in which total volume of 200 µl was made up by the addition of 95% (v/v) methanol. Calibration curve of linalool was plotted and the total terpenoid content expressed as milligrams of linalool equivalents per gram of dry weight (mg linalool/g DW) was determined using the regression equation. Samples were analysed in triplicates.

Salkowski test – To 5 ml of the extract, added 2 ml of chloroform and mixed well. To the solution, added 3

ml of conc. H_2SO_4 carefully to form a layer. Formation of reddish brown coloration at interface indicates the presence of terpinoids.

ANTIMICROBIAL ACTIVITY

For this study, bacterial suspension with 1X 10^6 (CFU/ml) was prepared using 24h old cultures. The nutrient agar plates were then inoculated with bacterial suspensions using sterile swab (Doughari, 2007). Then four equidistant wells of 7mm diameter were punctured in each media plate using a sterile cork borer. Two different concentrations (5% and 10%) of the chloroform, ethanol, petroleum ether and methanolic extracts of *A. paniculatum* were poured into the wells of nutrient agar media. Separate plates were maintained for each extract.

Then the plates were incubated at 37^{o}C for 24h along with standard Ampicillin (10µg/ml). After incubation the diameter of the inhibition zone was measured in mm and the results were tabulated.

RESULTS AND DISCUSSION

Aerial parts of *A. paniculatum* were extracted with Petrolium Ether, Chloroform, Ethyl Acetate and Methanol. Highest yields of chemical constituents like alkaloid, terpene, and phenols and flavonoids were recorded in methanolic extract when compared to other solvents. The aerial parts of *A. paniculatum* contain more amount of terpinoids (Table-1). Alkaloids and phenols were recorded in equal concentration while flavinoids were recorded at slight low concentrations.

Though alkaloids and non-alkaloid constituents like terpenoids, sterols, flavonoids, phenolic acids and their constituents were reported in the Apocynaceae members, this is the first record of Alkaloid, flavonoid, phenols and terpinoids from *A. paniculatum*. Among these 4 constituents the terpinoids were reported in high concentration. Alkaloids and flavinoids are reported to be Responsible for antimicrobial activity in the members of *Apocynaceae* was previously reported by Suffradin *et al*., (2002). Sterols, tannins and triterpenols from Apocynaceae members are reported to be responsible for antimicrobial activity (EL-Kashef *et al*., 2015).

Among the four different extracts of *A. paniculatum* tested for antimicrobial activity, methanolic extracts showed maximum antimicrobial activity for the test organisms. However, ethanolic extracts of *Tabernamontana coronaria* exhibits considerable zone of inhibition against *E. coli* and *S. aureus* was reported by Latha *et al.,* (2012).

The results reveled that methanolic extracts exhibited maximum zone of inhibition against the four tested bacteria. Methanolic extract at 10 mg/ml concentration showed maximum zone inhibition for *Vibrio cholera* which was resistant to the standard ampicillin as no growth was observed in the plates (Table-2). Methanolic extracts of *A. paniculatum* were moderately active against *S. aures and P. aeuriginosa*. Similarly, Ganjewala and Gupta (2013) reported that the methanolic extracts of different parts of *Alstonia scholaris* Linn. (latex) showed potent antibacterial activity against the *S. aureus, B. subtilis, P. aeruginosa* and *E. coli* the standard Ampicillin. Qin *et al*., (2014) reported that methanolic extracts of *A. formicinum* stem showed moderate antimicrobial activity against *P. aeruginosa* and *S. aures* against the standard Gentamicin. However, showed remarkable antibacterial activity against *E. coli*.

Table-1 Quantitative estimation of Phyto chemical constituents from aerial parts of the *Anodendron paniculatum*

Sl no	Name of the extract	Total alkaloid content (mg of AE/g)	Total Flavonoids content (µgEq.Q/g)	Total Terpenoids content (mg linalool/g)	Total phenols content (mg of GA/g)
1	Petroleum ether	15.28±2.52	8.11±0.33	18.2±3.52	10.40±1.08
2	Ethyl acetate	16.62±0.48	12.41±1.20	21.62±0.48	14.22±1.44
3	Chloroform	24.42±1.16	17.89±1.33	28.11±1.16	23.24±1.40
4	Methanol	25.98±2.09*	20.62±1.99*	41.19±1.33*	25.53±1.89*

Table-2 Zone of Inhibition due to antimicrobial activity of compounds extracted from aerial parts of the *Anodendron paniculatum*

Extract (200 mg/ml)	Zone of inhibition (mm)							
	S.pyogenes		*P. aeruginosa*		*Vibrio cholera*		*S. aureus*	
	5%	10%	5%	10%	5%	10%	5%	10%
Petrolium Ether	0±0.0	10.2±0.2	0±0.0	9.5±0.3	0. ±0.0	8.5±0.05	10±0.0	12.3±0.36
Ethyla Acetate	9.3±0.0	11.5±0.09	7.5±0.5	10.3±0.5	8.3±0.4	9.3±0.33	8.8±0.5	14±0.0
Chloroform	10.0±0.33	13.5±0.3	9.1±0.45	13.5±0.45	10.0±0.33	13.3±0.9	8.5±0.81	15.5±0.5
Methanol	11.5±0.5	19.5±0.5	9.8±0.5	14.0±0.5	13.8±0.8	20.0±0.0	10.2±0.3	17.8±0.45
Ampicillin (10µg/ml)	65±0.5	65±0.3	30±0.7	30±0.6	_	_	60±0.5	60±0.7

(-- No antimicrobial activity)

CONCLUSION

Alkaloids, terpinoids, flavonoids and phenols are reported for the first time in *A. paniculatum* plant. Methanolic extract exhibited maximum antimicrobial activity than other solvent extraction. Methanol extraction of *A. paniculatum* showed antimicrobial activity against *Vibrio cholera* along with other bacteria tested. Ethanolic extracts showed moderate antimicrobial activity for *S.aureus* and *P.aeruginosa*.

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