



# EVALUATION OF PHENOLIC, FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF *ASPIDOPTERYS CORDATA*: AN INDIGENOUS INDIAN PLANT

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## ABSTRACT

The swing towards herbal medicine is an amassing aspect in modern therapy to maintain social and community health referable to its safety margin and manifold assets. Polyphenolics such as tannins and flavonoids are evidenced as powerful antioxidants to resolve cellular stress, endure homeostasis in the body and avert stress-associated syndromes. Consistent consumption of antioxidants is a substitute to avoid serious illness. The current investigation aimed to measure the phenolic, flavonoid content and antioxidant activity of *Aspidopterys cordata*, an endemic plant to India. Total phenolic content was estimated by Folin–Ciocalteu colorimetric method taking gallic acid as standard. Whereas, total flavonoid content was determined by Aluminum chloride colorimetric assay using Quercetin as standard. The absorbance was measured at 760 nm and 510 nm respectively. Antioxidant activity was measured by two *In vitro* methods (DPPH and NO free radical scavenging assay) using standard protocols, where Ascorbic acid served as a reference standard. From the results, it was observed that the methanolic extract of *A. cordata* (106.24±0.75) was opulent with phenols. Similarly, flavonoid content in methanolic extract (88.17±1.35) was found higher than other extracts. In the DPPH assay, methanolic extract *A. cordata* (81.07%) was more effective to inhibit the free radicals with IC<sub>50</sub> values 39.704 µg/ml, higher doses were comparable to standard ascorbic acid (86.05%, IC<sub>50</sub>=34.29 µg/ml). Similarly, in the NO free radical scavenging assay, methanolic extract of *A. cordata* (79.39%) stood best among all to scavenge the free radicals with IC<sub>50</sub> values 46.53 µg/ml. Whereas, ascorbic acid was found to inhibit 84.22% with IC<sub>50</sub> values 39.08 µg/ml. The inhabitancy of various secondary metabolites such as alkaloids, carbohydrates, phenols, steroids, terpenoids, glycosides, saponins, especially tannins and flavonoids, either alone or in conjunction may account for the marked scavenging property. The extent of the phenolic compounds and flavonoids can ally directly to the displayed results.

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**KEYWORDS:** *Aspidopteryscordata*, Antioxidant, Total phenolic content, Total flavonoid content, Free radical scavenging.

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## I. INTRODUCTION

*Aspidopteryscordata* of the Malpighiaceae family called as bokadvel is a small climber with smooth, opposite, cordate-shaped leaves of size 5-9 cm×3-6 cm. In the flowering season (August-December), branches decorated with broadly oblong flowers, produced in the axils with 4-7 mm sized pale-yellow petals. Winged achene (samara) type fruits with dark brown color (Pullaiah, 2002; Saxena et al, 1994).

Antioxidant therapy is gaining importance due to the beneficial effects in the management of neurodegenerative diseases, cardiovascular diseases, aging, diabetes, etc. When the body is unable to balance the cellular stress with the scavenging mechanism, external support plays a key role to confront oxidative damage and antioxidants such as flavonoids, tannins, tocopherols and vitamins proved efficient for stress management and included in the majority of food supplements and nutraceuticals (Chakraborty, 2009; Joy and Mohan, 2020). Formation of free radicals is a protection mechanism to contest the infective access into the cellular atmosphere and the refurbishment of this stress freight copes with scavenging enzymes in the body (Pulivarthi et al, 2020). The distraction of balance between the creation and demolition of these reactive species produces cellular stress, progressed to be long-lasting and progressive neurodegenerative syndromes (Thakral et al, 2020; Venkatesan et al, 2020).

Herbs with polyphenolic compounds such as flavonoids, tannins and lignans are effective to combat stress in the body. Plants are being the source of drugs and the drug discovery towards natural products is gaining importance to develop safe and effective medicine for communicable and non-communicable diseases (Vardhini et al, 2010). India is a botanical hotspot for diversified medicinal and aromatic plants. The Himalayas, Eastern and Western ghats are the major regions with huge flora that need to be

explored for their phytochemical and pharmacological aspects. In the current study, *Aspidopteryscordata*, an endemic plant of Bhadrachalam forest was selected and total phenolic content, total flavonoid content and antioxidant potential of various extracts of *A. cordata* using *In vitro* models such as DPPH assay and nitric oxide free radical scavenging assay were performed. The extracts were also subjected to preliminary phytochemical screening to determine the presence of secondary metabolites.

## II. MATERIALS AND METHODS

### Plant material

*Aspidopteryscordata* was collected from Kinnerasani Wild Life sanctuary, Bhadrachalam District Telangana, and confirmed by Botanist Dr. K. Venkata Ratnam, Assistant Professor, Department of Botany, Rayalaseema University, Kurnool; leaf specimen has submitted (RU/BD/VSN-092) for future reference.

### Reagents and chemicals

All the chemicals and reagents were procured from Sigma Aldrich (laboratory grade).

### Preparation of extracts

*A. cordata* herb was collected and dried under shade. The dried leaves were powdered and subjected to defatting with petroleum ether. Later, it was extracted with chloroform and methanol independently on ultrasonicator at 40 kHz for 45 mins at a temperature of 40°C. The supernatants were separated, filtered, and dried under a vacuum. The extract concentrates were kept in a desiccator and percentage yield was calculated (Chandrika et al, 2021).

### Phytochemical screening

The preliminary phytochemical investigation of *A. cordata* extracts was carried out by employing standard protocols (Harborne, 1973).

### Estimation of total phenolic content

Folin-Ciocalteu (FC) assay was used to estimate the total phenolic content of extracts of *A. cordata*. 200µl of the extract

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solutions were mixed with 2.5 ml of FC reagent (diluted to 10 times) and 2 ml of Na<sub>2</sub>CO<sub>3</sub> solution (7.5% w/v) followed by proper mixing and incubation at 30°C for 90 minutes. Absorbance for all the samples was recorded at 765 nm and expressed in terms of mg equivalents of gallic acid. The calibration curve was constructed by plotting the absorbance against concentration. The experiment was done in triplicates (Singleton et al, 1965).

#### Estimation of total flavonoid content

The flavonoid content of the extracts was determined by a colorimetric method using Aluminium trichloride method (Zhishen method). A volume of 125 µL of the extract was added to 75 µL of a 5% Sodium nitrite (NaNO<sub>2</sub>) solution. After 6 min, 150 µL of AlCl<sub>3</sub> solution (10%) was added followed by the addition of 750 µL of NaOH (1M). The final volume of the solution was made to 2500 µL with distilled water. After 15 min of incubation, the mixture turned pink and the

$$\% \text{ Free radical scavenging activity} = [(A_0 - A_s)/A_0] \times 100$$

Where,

A<sub>0</sub> was the absorbance of blank (DPPH solution alone)

A<sub>s</sub> was the absorbance of extracts (DPPH + sample)

#### Nitric oxide radical scavenging assay:

All extracts of *A. cordata* were screened for nitric oxide radical scavenging activity using sodium nitroprusside. In this experiment, 2 mL of sodium nitroprusside (10 mM) and 0.5 mL of phosphate buffer (pH-7.4) was mixed with 0.5 mL of the test solution and incubated

$$\text{Percentage of nitric oxide radical scavenging assay} = [(A_0 - A_s)/A_0] \times 100$$

Where,

A<sub>0</sub> was the absorbance of control

A<sub>s</sub> was the absorbance of the treated sample

### III. RESULTS AND DISCUSSION

#### Percentage yield

The powdered *A. cordata* was subjected to Soxhlet extraction with petroleum ether, Chloroform, methanol and water. The percentage yields were calculated by using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of the extract obtained}}{\text{Weight of the leaf powder taken}} \times 100$$

#### Preliminary phytochemical screening

The preliminary phytochemical study of petroleum ether, chloroform, methanol and aqueous extracts of *A. cordata* unveiled that the extracts were comprised of various phytochemicals such as

absorbance was measured at 510 nm. The total flavonoid content was expressed as gram equivalence of Quercetin per gram dry weight (QE) (Chang et al, 2002).

#### In vitro antioxidant assay

##### DPPH radical scavenging assay:

The free radical scavenging activity of *A. cordata* extracts was estimated. Extracts of different concentrations were prepared using DMSO, whereas a solution of 25 mg/L DPPH was prepared by using methanol. In a 96-well plate, 5 µL of the extract solution followed by 195 µL of DPPH solution was added and incubated for 20 minutes at room temperature. The absorbance was measured at 515 nm for individual extracts and the free radical scavenging activity was recorded by comparing the absorbance values with the blank. The above procedures were repeated using ascorbic acid as positive controls in triplicates. The antioxidant activity was calculated using the formula given below (Sija et al, 2019).

for 150 min at 25°C. Ascorbic acid solution and DMSO served as standard and control respectively. Sulfanilic acid reagent (1 mL 0.33% of sulfanilic acid in 2% glacial acetic acid) was added to 0.5 mL of nitrite and kept for 5 min. Naphthyl ethylene diamine dihydrochloride (NEDD, 1 mL of 1%) was added and incubated for 30 min at 25°C (Prieto et al, 1999). The absorbance was recorded at 540 nm and the percentage of nitric oxide inhibition was calculated as:



alkaloids, flavonoids, phenols, steroids, terpenoids, glycosides, tannins, saponins, and carbohydrates (Table 1).

**Table 1. Percentage yield of *A. cordata***

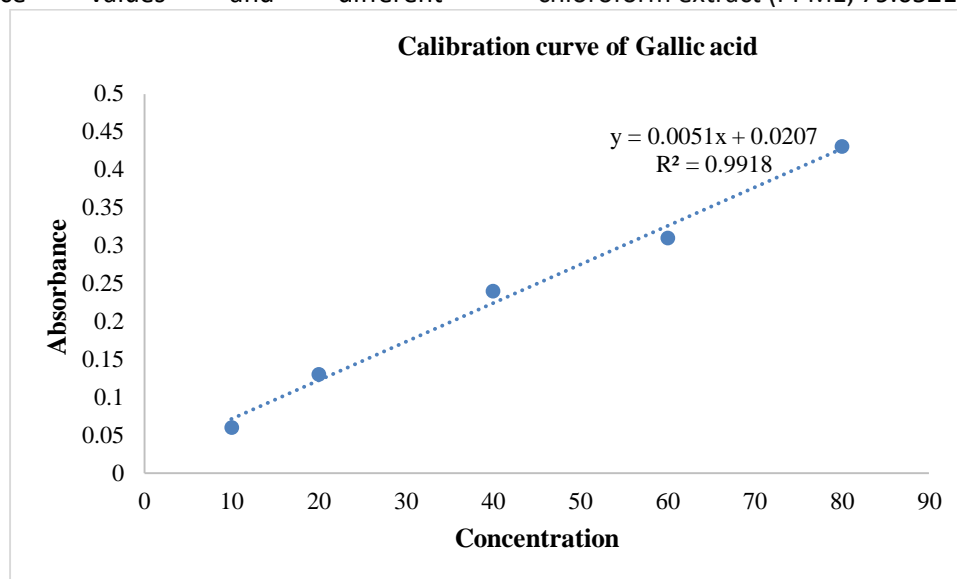
Extract	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
Percentage yield	1.27	4.17	11.34	2.15

### Total phenolic content

Phenolic compounds are metabolic end products of plants and they are having a linear relation with shielding oxidative stress. Since they are the first line protective agents against oxidative damage to the tissue. The total phenolic content of *A. cordata* was evaluated by Folin–Ciocalteu method taking gallic acid as the reference standard. A calibration curve was plotted against the absorbance values and different

concentrations of gallic acid (Figure 1). Total phenolic content of the extracts was calculated from the regression equation of calibration curve ( $y = 0.0051x + 0.0207$ ,  $R^2 = 0.9918$ ) and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) (Table 2). Total phenolic content values were observed high in methanolic extract of *A. cordata* (CMME,  $106.24 \pm 0.75$ ) followed by chloroform extract (PPME,  $79.05 \pm 1.42$ ).

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**Fig-1: Calibration curve of Gallic acid**

**Table 2. Preliminary phytochemical screening results of prepared extracts**

Phytochemicals	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	-	+	+	-
Flavonoids	-	+	+	+
Terpenoids	+	+	-	-
steroids	+	-	-	-
Saponins	-	+	+	+
Tannins	-	+	+	+
Proteins	-	-	-	+



Carbohydrates	-	-	-	+
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(+) present, (-) absent

### The flavonoid content

Flavonoids are an important class of polyphenols to quench excess free radicals generated in the cells. They proved to delay aging and give numerous health benefits towards neurodegenerative diseases, cardiovascular diseases and cancer. The flavonoid content of the extracts of *A. cordata* was determined by a colorimetric method using the Zhishe technique and was found to be  $88.17 \pm 1.35$  and  $73.04 \pm 1.28$  of gram equivalence of Quercetin at 510 nm

(Table 3 and Figure 2) for methanolic extract followed by chloroform extract. The calibration curve was made by linear regression and the results represented the average of three determinations to each concentration. Total flavonoid content of the extracts was calculated from the regression equation of calibration curve ( $y = 3.46x + 0.0327$ ;  $R^2 = 0.9947$ ) and expressed as mg Quercetin equivalents (QE) per gram of sample in dry weight (mg/g).

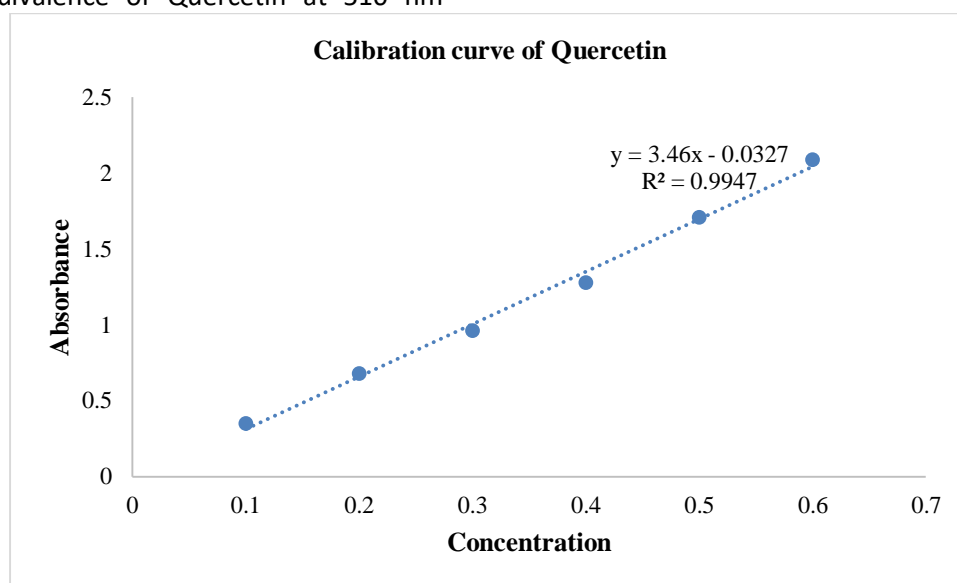


Fig-2: Calibration curve of Quercetin

Table 3. Total Phenolic content of *A. cordata* extracts

Extracts	mg GAE/mg dry weight
<i>A. cordata</i> petroleum ether extract (ACPE)	$22.1 \pm 1.14$
<i>A. cordata</i> Chloroform extract (ACC)	$79.05 \pm 1.42$
<i>A. cordata</i> methanol extract (ACM)	$106.24 \pm 0.75$
<i>A. cordata</i> aqueous extract (ACAQ)	$61.18 \pm 2.39$

### In vitro Antioxidant activity

#### DPPH radical scavenging assay

Screening of antioxidant potential is an essential parameter to understand the ability of the plant to conquer cellular stress and

protection credibility. *A. cordata* was compiled to have potent antioxidant activity in a dose-dependent manner, compared with standard Ascorbic acid. Methanol extract of *A. cordata* has displayed substantial free radical



scavenging activity in a dose-dependent manner followed by aqueous and chloroform extracts. A standard curve was plotted (Figure 3) using various concentrations of ascorbic acid. At a higher concentration (100 µg/mL), the *A. cordata* extract with 81.07% of inhibition stood high among the extracts next to Ascorbic acid (86.05%) (Table 5 and Figure

4). Methanolic extracts of *A. cordata* (IC<sub>50</sub> = 39.704) were inferred to be a potent antioxidant among the others (Figure 5). The ability of these extracts to scavenge DPPH could also reflect its ability to impede stress and subsequently the protective effects in the body.

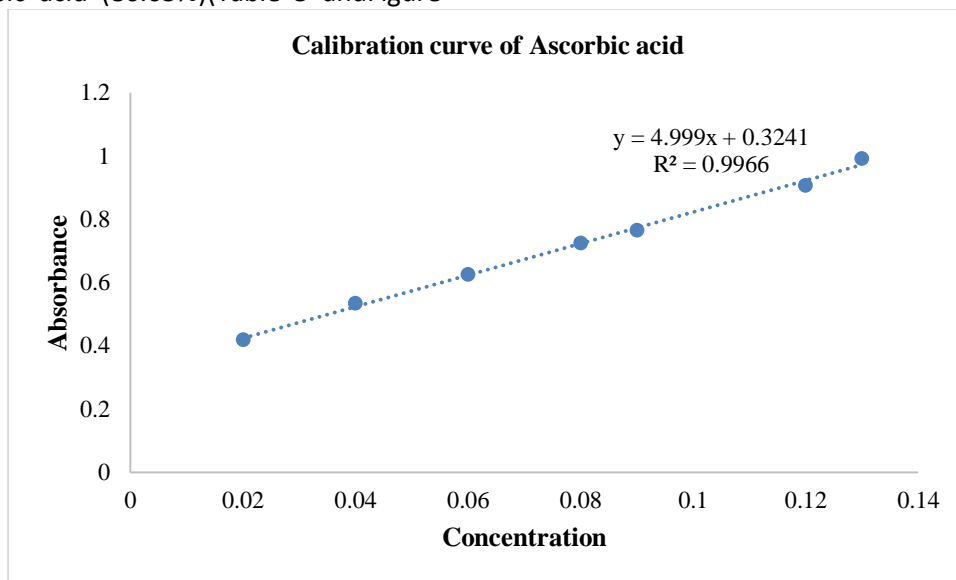


Fig-3: Calibration curve of Ascorbic acid

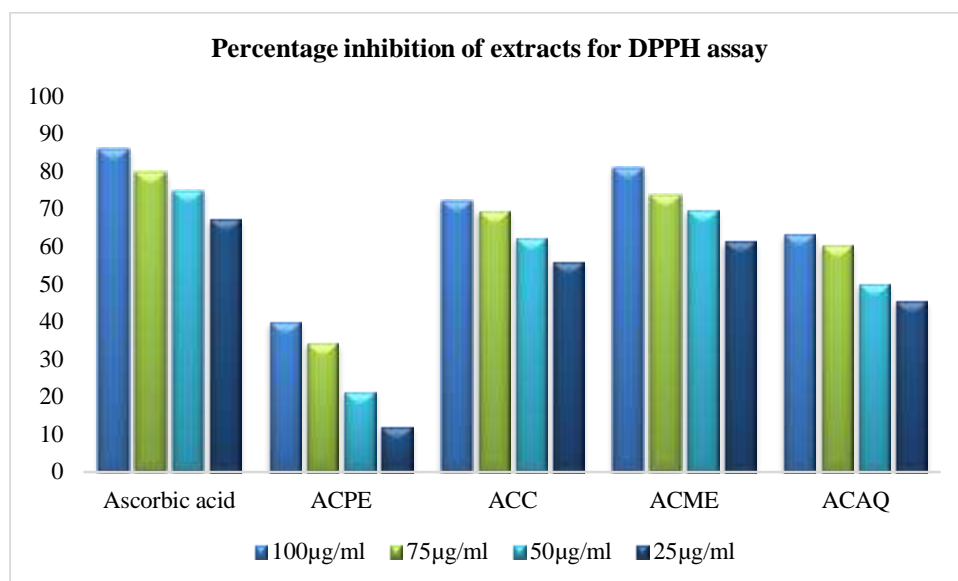
Table 4. Total flavonoid content of *A. cordata* extracts

Extracts	mg QE/mg dry weight
<i>A. cordata</i> petroleum ether extract (ACPE)	25.04±1.07
<i>A. cordata</i> Chloroform extract (ACC)	73.04±1.28
<i>A. cordata</i> methanol extract (ACM)	88.17±1.35
<i>A. cordata</i> aqueous extract (ACAQ)	67.22±2.17

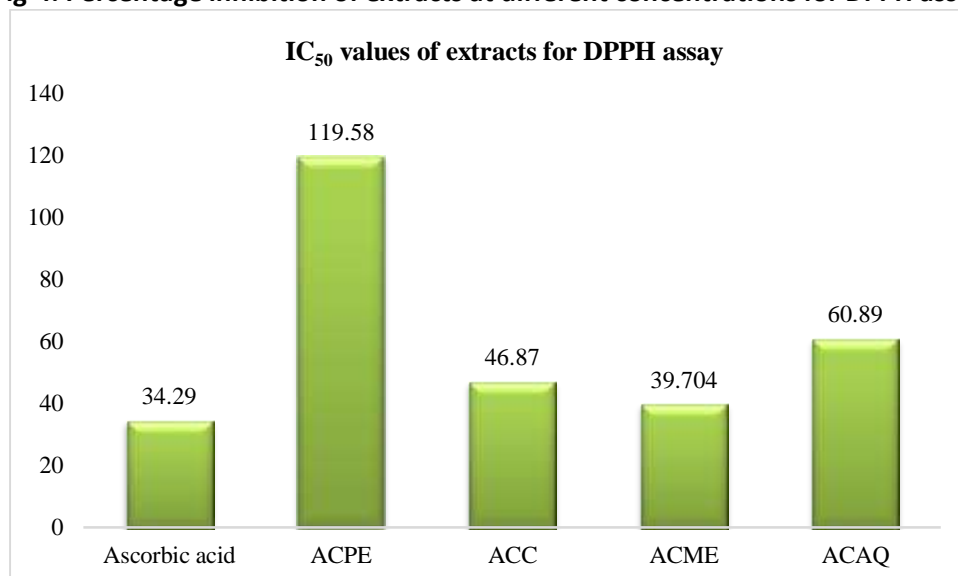
Table 5. DPPH Scavenging activity of *A. cordata* extracts with IC<sub>50</sub> values.

Conc µg/ml	Percentage of inhibition				
	AA	ACPE	ACC	ACME	ACAQ
100	86.05±0.38	40.01±0.84	72.05±1.11	81.07±1.08	63.29±1.17
75	79.82±0.24	34.29±1.05	69.44±1.28	73.67±1.33	60.37±1.52
50	74.94±0.67	21.33±1.64	62.31±1.19	69.74±1.91	50.04±1.64
25	67.22±0.52	12.06±0.87	56.04±2.07	61.42±2.04	45.48±1.18
IC <sub>50</sub> µg/ml	34.29	119.58	46.87	39.704	60.89





**Fig-4: Percentage inhibition of extracts at different concentrations for DPPH assay**



**Fig-5: IC<sub>50</sub> values of extracts for DPPH assay**

#### Nitric oxide radical scavenging activity

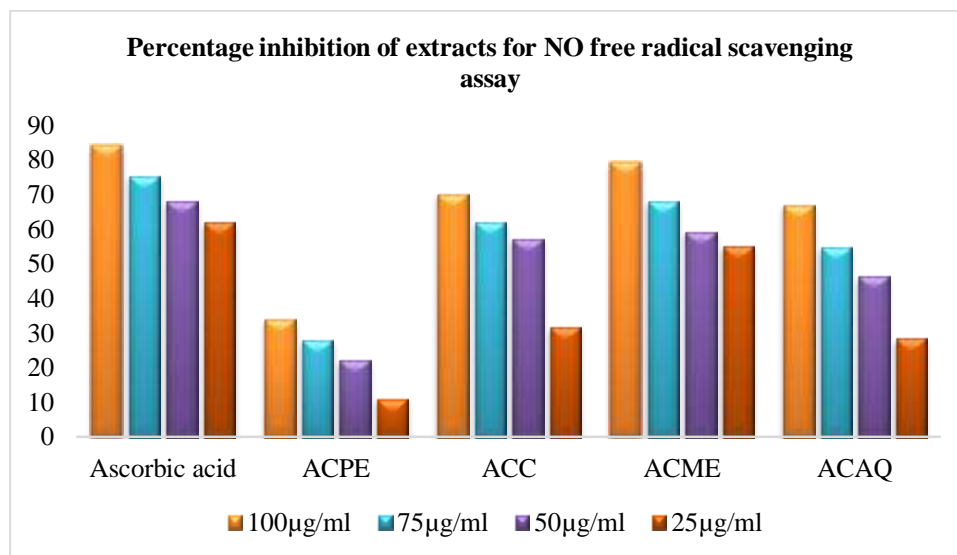
The extracts in sodium nitroprusside solution inhibited the accumulation of nitrite, a stable oxidation product of NO liberated from sodium nitroprusside in the reaction medium with time compared to the standard Ascorbic acid. When compared to the standard Ascorbic acid, *A. cordata* methanol extract exhibited high NO free radical scavenging activity in a dose-dependent manner (Table 6 and Figure 6). At a higher concentration of 100 µg/mL, the methanolic *A. cordata* extract was showing 79.39% of inhibition with IC<sub>50</sub> values

46.53 µg/ml followed by Chloroform extract 70.13% with IC<sub>50</sub> values 58.42 µg/ml and aqueous extract 67.14% with IC<sub>50</sub> values 66.61 µg/ml. Whereas for ascorbic acid it was found to be 84.22% with IC<sub>50</sub> values 39.08 µg/ml (Figure 7). The polyphenolic compounds such as tannins and flavonoids present in the *A. cordata* may neutralize the free radicals liberated by the nitroprusside in the given procedure and may offer protection in the animal body related to cellular stress.

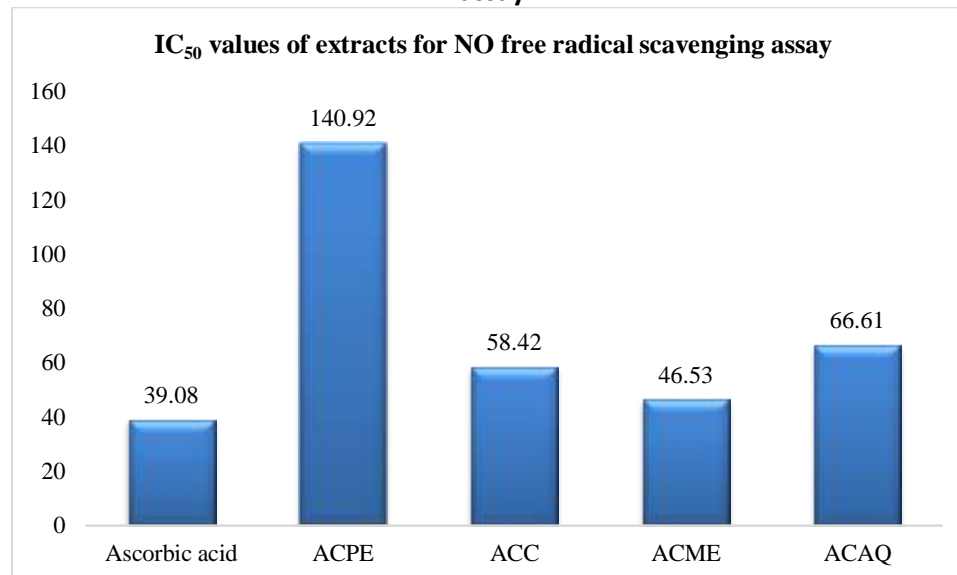
**Table 6. NO free radical Scavenging activity of *A. cordata* extracts.**



Conc $\mu\text{g/ml}$	AA	ACPE	ACC	ACME	ACAQ
100	84.22 $\pm$ 0.41	34.04 $\pm$ 0.76	70.13 $\pm$ 1.06	79.39 $\pm$ 1.84	67.14 $\pm$ 1.17
75	75.23 $\pm$ 0.56	28.06 $\pm$ 1.4	62.04 $\pm$ 1.08	68.01 $\pm$ 1.82	54.82 $\pm$ 1.53
50	68.03 $\pm$ 0.13	22.17 $\pm$ 0.72	57.35 $\pm$ 1.17	59.37 $\pm$ 1.23	46.28 $\pm$ 1.41
25	62.16 $\pm$ 0.27	11.08 $\pm$ 1.37	31.74 $\pm$ 1.02	55.12 $\pm$ 1.19	28.37 $\pm$ 1.06
IC <sub>50</sub> $\mu\text{g/ml}$	39.08	140.92	58.42	46.53	66.61



**Fig-6: Percentage inhibition of extracts at different concentrations for NO free radical scavenging assay**



**Fig-7: IC<sub>50</sub> values of extracts for NO free radical scavenging assay**

All the vascular plants are the sources of flavonoids with varied concentrations either in the free form or as glycosidic form. Flavonoids are avowed to be an important class of protective agents to maintain proper homeostasis by impeding the cellular stress in the tissue. These polyphenolic compounds

deliquesce the assembled free radical scavengers complemented with intrinsic mechanisms to reinforce the antioxidant property (Robert, 2001).

It is imperative to study the antioxidant potential of the plants to comprehend their effectiveness in protecting against cellular



stress. Several *In vitro* & *In vivo* measures were implemented to screen the antioxidants such as DPPH assay, nitric oxide free radical assay, etc. (Krishnaiah et al, 2011). Total phenolic, total flavonoid content of *A. cordata* were evaluated using standard protocols. *In vitro* antioxidant screening in DPPH and NO free radical assay was performed for all extracts. To condense the results, it can be perceived that methanolic extract of *A. cordata* was opulent in phenols and flavonoids and can be interrelated to the high antioxidant activity observed in both methods. Whereas, petroleum ether, chloroform and aqueous extracts, although rich in phytochemicals, the lower concentrations of the protective agents may be a reason to display the reduced scavenging potential. Further investigation is in progress to estimate the complete phytochemical and pharmacological profile of the plant to validate its traditional claims and the reported antioxidant activity.

#### IV. CONCLUSION

The current investigation aimed to measure the phenolic, flavonoid content and antioxidant activity of *Aspidopterys cordata*, an endemic plant to India. Total phenolic content was estimated by Folin–Ciocalteu colorimetric method taking gallic acid as standard. Whereas, total flavonoid content was determined by Aluminum chloride colorimetric assay using Quercetin as standard. Antioxidant activity was measured by DPPH and NO free radical scavenging assay *In vitro* methods using standard protocols, where Ascorbic acid served as a reference standard. From the results, it was observed that the methanolic extract of *A. cordata* ( $106.24 \pm 0.75$ ) was opulent with phenols. Similarly, flavonoid content in methanolic extract ( $88.17 \pm 1.35$ ) was found higher than other extracts. In the DPPH assay, methanolic extract *A. cordata* (81.07%) was more effective to inhibit the free radicals with  $IC_{50}$  values  $39.704 \mu\text{g/ml}$ , higher doses were comparable to standard ascorbic acid (86.05%,  $IC_{50} = 34.29 \mu\text{g/ml}$ ). Similarly, in the NO free radical scavenging assay, methanolic extract of *A. cordata* (79.39%) stood best among all to scavenge the free radicals with

$IC_{50}$  values  $46.53 \mu\text{g/ml}$ . Whereas, ascorbic acid was found to inhibit 84.22% with  $IC_{50}$  values  $39.08 \mu\text{g/ml}$ . The inhabitancy of various secondary metabolites such as alkaloids, carbohydrates, phenols, steroids, terpenoids, glycosides, saponins, especially tannins and flavonoids, either alone or in conjunction may account for the marked scavenging property.

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#### REFERENCES

- Pullaiah., 2002. Flora of Eastern Ghats.1, Regency publications, New Delhi.
- Saxena, H.O., Brahmam, M., 1994. Flora of Orissa.1, Orissa Forest Development Corporation, Orissa.
- Chakraborty, G.S., 2009. Antioxidant activity of the successive extracts of *Aesculus indicaleaves*. *International Journal of Pharmaceutical Sciences and Drug Research*, 1(2), pp.121-123.
- Joy, N., and Mohan, M., 2020. Evaluation of *In vitro* antioxidant and cytotoxic effect of methanol extract from aerial parts of *Myristicabeddomei King S.* *International Journal of Pharmaceutical Sciences and Drug Research*, 12(2), pp.115-21.
- Pulivarthi, V., Josthna, P., and Naidu, C.V., 2020. *In vitro* antidiabetic activity by glucose uptake of yeast cell assay and antioxidant potential of *Annona reticulata* L. leaf extracts. *International Journal of Pharmaceutical Sciences and Drug Research*, 12(3), pp.208-13.
- Thakral, J., Borar, S.R., and Kalia, A.N., 2020. Antioxidant potential fractionation from methanol extract of aerial parts of *Convolvulus arvensis* Linn. (Convolvulaceae). *International Journal of Pharmaceutical Sciences and Drug Research*, 2(3), pp.219-23.
- Venkatesan, S., Somasundaram, A., and Rengaraj, S., 2020. Evaluation of *In vitro* antioxidant activity and HPTLC finger printing analysis of *Physalis peruviana* fruits. *International Journal of Pharmaceutical Sciences and Drug Research*, 12(4), pp.384-9.



Vardhini, Vidya., Anuradha, Sanduni., Edupuganti, Sujatha., and Rao, S.S.R., 2010. Role of brassinosteroids in alleviating various abiotic and biotic stresses-a review. *Plant Stress*, 4, pp.55-61.

Udaya, Chandrika, P., and Sunitha, K., 2021. Pharmacognostic evaluation, estimation of phenolic, flavonoid composition and antioxidant activity of *Aspidopterys indica* (Willd.) W. Theob: An endemic plant to peninsular India. *Int J Res Pharm Sci*, 12(2), pp.1-10.

Harborne, J.B., 1973. *Phytochemical Methods*. Chapman and hall Ltd., London: U.K., pp.49-188.

Singleton, V.L., and Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *The American Journal of Enology and Viticulture*, 16, pp.144-158.

Chang, C.C., Yang, M.H., Wen, H.M., and Chern, J.C., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10, pp.178-182.

Sija, S.L., Athulya, A.S., Mahima, M.R., and Vidhya, A., 2019. Antioxidant and antimicrobial activity of different plant parts of *Anacardium occidentale* L. and *Mangifera indica* L.: A Comparative Study. *International Journal of Pharmaceutical Sciences and Drug Research*, 11(4), pp.111-5.

Prieto, P., Pineda, M., and Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, 269, pp.337-341.

Robert, J., and Nijveldt., 2001. Flavonoids: a review of probable mechanisms of action and potential applications. *The American Journal of Clinical Nutrition*, 74(4), pp.418-425.

Krishnaiah, D., Sarbatly, R., and Nithyanandam, R.R., 2011. A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing*, 89, pp.217-233.

