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In-Vitro Assessmentof Antioxidant Activity,Total Phenolic Andflavonoid Content For Various Extracts Of *Vitex Peduncularis Wall,Ex.Schauer*

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ABSTRACT

Vitexpeduncularis wall(Family: Verbenaceae) exhibit some bioactivities likeAnti-bacterial, Anti-fungal, Anti-inflammatory, Anti-pyretic, treatment of black water fever etc. The present study was focused on evaluation of Phenolic, Flavonoid and Antioxidant activity of different extracts from the leaves of *V. peduncularis*.The data revealed that the ethyl acetate extract showed significant anti-oxidant activity(56.92% at 15µg/ mL) *invitro*DPPH assay at 517nm along with good presence of the total Phenolic and total flavanoidalcontent (33.08 mg GAE/g,222.2mgQE/g and 86.38mg CE/g).The results suggested that *V.peduncularis* has the potential to be source of alternative medicine due to its reportedly good Anti-Oxidant activity.

KEYWORDS: *Vitex peduncularis*, Antioxidant, Flavonoid, Phenolic,DPPH method.

INTRODUCTION

Vitexpeduncularis belongs to a Verbenaceae family, found in many countries like India, Bangladesh.[1] It is a weedy shrub which grows about 1.5 meters, leaves are palmately compound with 3 foliolate, leaflets lanceolatoblong or narrow elliptic leaflets base acuminate, flowers are bisexual.[2] Traditional the plant is used as a folklore curative for various biological disorders. The leaves of it possess Anti-bacterial, Anti-fungal, Anti-inflammatory, Anti-pyretic, treatment of

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black water fever, Cytotoxic activity Anti-malarial, Anti-diabetic properties due to presence of complex phytochemicals.[3]This plant is rich in diverse chemical constituents likeAgnuside,[4]Peduncullariside, Limoniside, 4'-acetoxy-5-hydroxy-6,7 dimethoxyflavone, [5]Cirsimaritin, Genkwanin,[6]3 alpha friedelinol, 3 Beta Friedelinol, Pachypodol, Peduncularison, Vitexin, Ursolic acid,[7]2 Alpha-Hydroxy Ursolic Acid,[8]Epifriedelinol, Cis-3-Hexenoic acid, Cyclooctane, n-Hexadecanoic acid, 2-Nitrothiophene, 3,3-Dimethylacrylic acid, 2,6-Dimethyl-3-thioxo-3,4-dihydro-1,2,4-triazin5(2H)-one and pachypodol.[9]Some special pharmacological activities reported on *V.peduncularis*were Antiheamolytic agent,[10]Treatment of black water fever, Antibacterial activity,[11]Antipyretic, Cytotoxic activity, Antifungal, Anti- inflammatory activities in various articles.[12]In view of its variedpharmacological activity and phytochemicalreports estimation of phytochemicals and biological activityscreening of*V.peduncularis*leaf part was taken to the present work.

MATERIALS AND METHODS

Plant collection:

*Vitex peduncularis*plant material (Leaves) was collected from CSIR-CIMAP Research Centre, Mallapur, Boduppal, Telangana. The plant was taxonomically identified and authenticated byDr. A.Sabitha Rani, Assistant Professor, Department of Botany, Osmania University, college for Women, Koti, Hyderabad. A voucher specimen (CIMAP-2019/VP) was stored at CIMAP Research Centre, Hyderabad.

Extraction:

The leaf powder (100 gms) extracted using various solvents like hexane, ethyl acetate, methanol by ultrasonication for 30 mins (30 min x 4 times) and 30% aq. Methanol by overnight maceration, filtered and concentrated under reduced pressure by Rota evaporator. These extracts were used further for *in-vitro* antioxidant, Total Phenolic and Flavonoid content estimation studies.

*In-Vitro*Anti-Oxidantactivity

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The antioxidant activity of the extracts determined on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical method.[13]1mL of 0.3mM DPPH solution in methanol was mixed with 2.5mL of plant extract or standard solutions of varying concentrations (50, 40, 30, 20 and 10 µg/mL)and allowed to react at room temperature for 30 min. The absorbance of the sample mixture was measured at 517 nm using UV-Vis Spectrophotometerand compared with the absorbance values of Ascorbic acid standard 1 mL of 0.3 mM DPPH plus methanol (2.5 mL) was used as a blank and the percentage antioxidant activity (AA %) using the formula.

$$\textbf{Percentage of Inhibition (\%)} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100$$

Estimation of Total Phenolic Content

The total phenolics of *V.Peduncularis* extracts were determined using the Folin and Ciocalteu reagent.[14]An aliquot (1 mL) from each prepared extract samples and prepared standard solutions of Gallic acid in concentrations (100, 200, 300, 400 and 500mg/lit) were added to 25 mL volumetric flask, already containing 9 mL of distilled deionized water. 1 mL of Folin-Ciocalteu phenol reagent was added after 5 min, 10 mL of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (25 mL) with deionized water and shaked well. The reaction was kept in the dark for 90 min at room temperature, the blue color developed was checked for the absorbance against prepared reagent blank by determining at 750 nm with an UV-Visible spectrophotometer. The phenolic content was calculated as Gallic acid equivalents GAE/g of dry plant material on the basis of a standard curve of Gallic acid.

Blank preparation:To 25 mL volumetric flask, 9 mL distilled water, 1 mL Folin-Ciocalteu reagent was added after 5 min and 10 mL of 7% Sodium Carbonate (Na_2CO_3) solution was added to the mixture. The final volume was made upto (25 mL) with distilled deionised water and shaked, and incubated for 90 min in dark condition at room temperature.The phenolic

content expressed in mg Gallic acid equivalents (GEA)/100 g fresh weight. All samples were analysed in duplicates. Total phenolic content was calculated by

$$\textbf{Total Phenolic Content} = \text{Concentration} \times \frac{\text{Vol. of the Sample}}{\text{Weight of the Sample}}$$

Estimation of Total Flavonoid content

The aluminum chloride colorimetric method[15] was used for the determination of the total flavonoid content of different extracts of *V.peduncularis* leaf. For total flavonoid determination, Quercetin and Catechin was used to make the standard calibration curve

a. Colorimetric assay (Quercetin)

Total flavonoid content was measured with the Alcl3 colorimetric assay. An aliquot of extracts or standard quercetin solution (50, 100,150, 200, 250 and 300mg/lit) was positioned into test tubes and 4mL of distilled water and 0.3 ml of 5 % Sodium Nitrite(Na_2NO_3). After 5 minutes, 0.3 mL of 10 % Aluminum Chloride was added. At 6^{th}minute, 2 mL of 1 M Sodium Hydroxide was added. Finally, volume was making up to 10 mL with distilled water and mix well. The solution was mixed well and the absorbance (in UV-VIS spectrophotometer) was measured against prepared reagent blank at 510 nm. Flavonoid content expressed as mg Quercetin equivalents (QE) /100 g fresh mass.

b. Colorimetric assay (Catechin)

Total Flavonoid content was measured by the aluminum chloride colorimetric assay. An aliquot of extracts and standard solution of Catechin (50, 100,150, 200, 250 and 300mg/lit) was

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added separately to 10 mL volumetric flask, containing 4 mL of distilled deionized water. To the flask was added 0.3 mL of 5 % Sodium Nitrite ($NaNO_2$). After 5 min, 0.3 mL of 10 % Aluminum Chloride was added. At 6^{th}min, 2 mL of 1 M Sodium Hydroxide was added and total volume was made up to 10 mL with distilled deionized water. The solution was mixed well and the absorbance (in UV-VIS spectrophotometer) was measured against prepared reagent blank at 510 nm. Flavonoid content expressed as mg Catechin equivalents (CE) /100 g fresh mass.

***Blank preparation*:**To the 10mL capacity volumetric flask, 5% Sodium Nitrate and 10%Aluminum Chloride ($AlCl_3$)(0.3 mL each) were added one after the other with a gap of 5mins time interval.1M Sodium Hydroxide (2 mL) was added after 5 mins and final volume was made up to 10 mL with deionised water.

The darker the color complex, higher is the complexation with aluminum and indicates the presence of more number of flavonoid principles Acid labile complexes are also formed with the orthodihydroxyl groups in the A- or B-ring of flavonoids. The total Flavonoid content was calculated by following formula.

$$\textbf{Total Flavonoid content} = \text{Concentration} \times \frac{\text{Vol. of the Sample}}{\text{Weight of the Sample}}$$

RESULTS AND DISCUSSION

In-Vitro Antioxidant Activity (DPPH radical scavenging activity)

The leaf (hexane, ethyl acetate, methanol and aq.methanol) extracts of *V.peduncularis*determined for *in-vitro* antioxidant assay by DPPH method showed dose dependent inhibition of DPPH radicals.Percentage scavenging of DPPH radical examined at different concentrations (leaf hexane, ethyl acetate, methanol and aq.methanol extracts) was depicted in (Table-1, Fig-1).

Table 1
Affect of various extracts *V.peduncularis*on DPPH radicals.

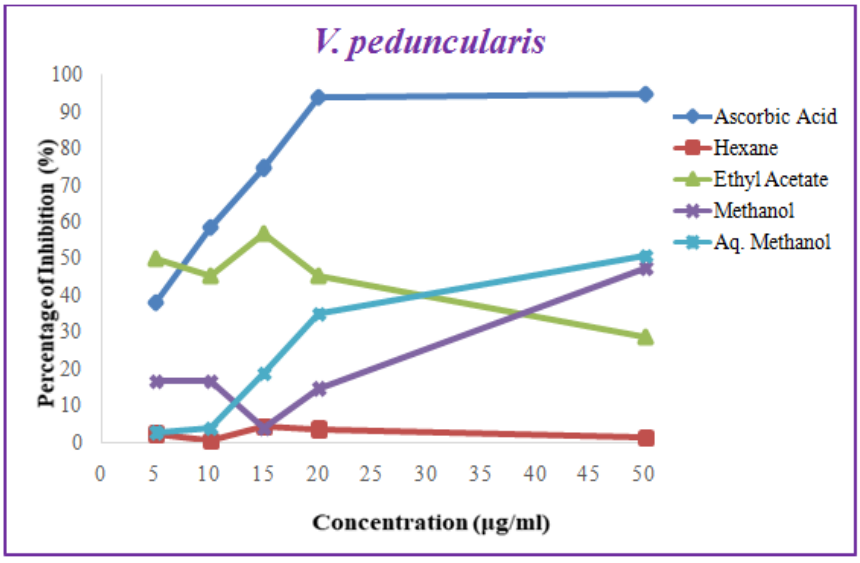
Concen	Ascorbic	% Inhibition by Leaf Extracts

tration	Acid	Hexane	Ethyl acetate	Methanol	Aq. Methanol
10	76.46	1.99	50.05	16.77	2.42
20	152.92	0.44	45.31	16.77	3.82
30	229.38	4.43	56.92	4.10	18.75
40	305.84	3.32	45.23	14.39	35.18
50	382.3	1.33	28.82	47.30	50.78

The *In-Vitro* Anti-oxidant activity using DPPH method was observed for various extracts and they were to be **(**50.05, 45.31, 56.92, 45.23, 28.82% at 10, 20, 30, 40 and 50µg/ mL). From the obtained above results it was showed that the percentage (%)inhibition rate by antioxidant principles on DPPH free radicals found to be more in leaf ethyl acetate extract with 56.92% at 30µg/ mL concentration when compared to other extracts of *V.peduncularis* with respect to Ascorbic Acid reference standard whose percentage inhibition was (382.3% at 50µg/ mL).

Figure 1

Graph of Extracts by DPPH method



Total Phenolic Content

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The total phenolic content of *V.peduncularis*leaf extract was estimated by Folin-Ciocalteu assay method. The plant leaf extract (Hexane, Ethyl acetate, Methanol and 30% aq. Methanolic extracts) the total phenolic content found to be 14.13, 31.46, 33.08, 20.44mg respectively as Gallic acid equivalents/100 grams of extract and is depicted in (Table-3). Standard graph of Gallic acid was depicted in (Table 2, Fig2).

Table 2

Absorbance values of Gallic acid standard

S. No	Concentration (µg/ml)	Absorbance (nm)
1	0	0
2	100	0.516
3	200	0.99
4	300	1.485
5	400	2.014
6	500	2.406

Figure 2
Standard graph of Gallic acid.

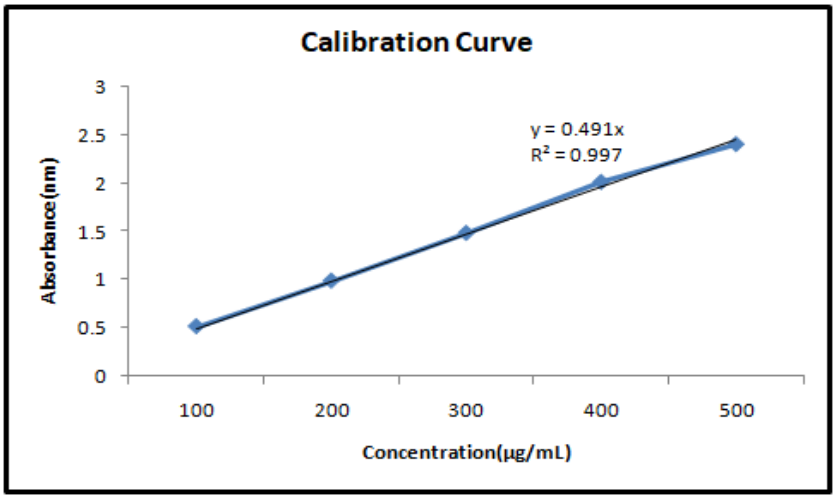


Table 3

mg/100gm Gallic acid equivalents of different extracts of *V.peduncularis*

S. No	Extracts	mg/100 gm Gallic acid equivalents
1	Hexane	14.13
2	Ethyl acetate	33.08
3	Methanol	20.44
4	Aq.Methanol	31.46

Total Flavonoid Content:

Total Flavonoid content of *V.peduncularis*leaf extract (Hexane, Ethyl acetate, Methanol and aq. Methanol) were found to be 0.00, 86.38, 55.52and 69.92mg QE/100 gramextract respectively and was depicted in (Table-5). Standard graph of Quercetin is depicted in (Table-4, Fig-3)

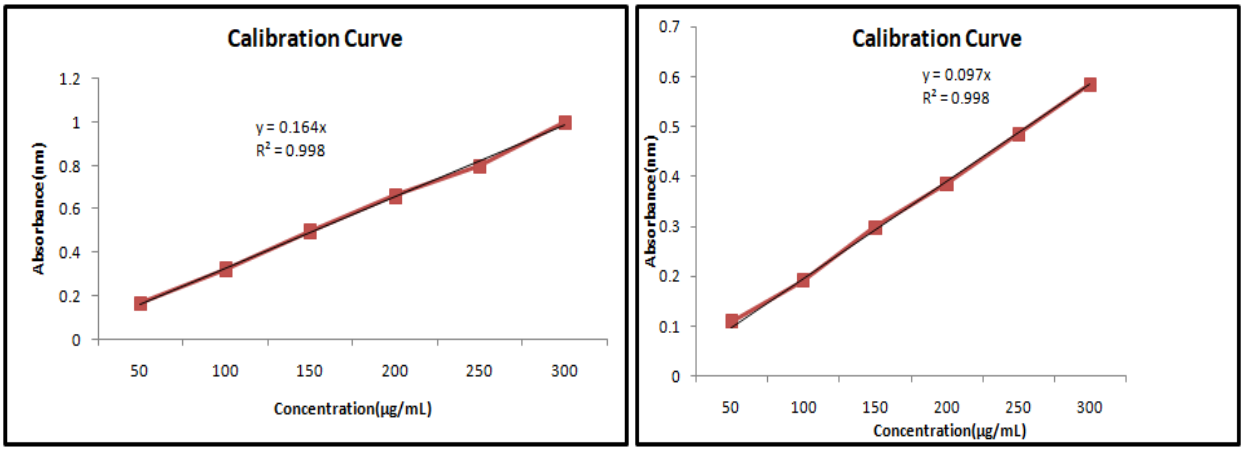
Table 4:

Absorbance values of Flavonoid content using Quercetin and Catechin as standards

S. No	Concentration (µg/mL)	Absorbance	
		Quercetin	Catechin
1	0	0	0
2	50	0.11	0.168
3	100	0.192	0.324
4	150	0.298	0.498
5	200	0.385	0.661
6	250	0.485	0.798
7	300	0.584	0.998

Figure3

Standards graph of Quercetin and Catechin



Total flavonoid content of *V.peduncularis* leaf extract (Ethyl acetate, Methanol and aq. Methanol)were found to be 222.2, 157.4and 129.9mg QE/100gram extract respectively and was depicted in (Table-5).Standard graph of Catechin depicted in (Table-4, Fig-3).

From the obtained overall results which were depicted in (Table-5 & Fig-3) it was inferred that the total Flavonoid content was found to be more in Ethyl acetate extract with222.2mg QE/ 100 gm extract.

Table 5
mg/100gm Quercetin and Catechin equivalents different extracts of *V.peduncularis* leaf

Extract	Flavonoid Content mg Catechin/gr. Extract	Flavonoid Content mg Quercetin/gr. Extract
Ethyl acetate	86.38	222.2
Methanol	55.52	157.4
Aq.methanol	69.92	129.9

SUMMARY AND CONCLUSION

From the overall studies conducted on various extracts of*V.peduncularis*, the antioxidant activity performed using DPPH method showed leafethyl acetate extract as the most active one with percentage inhibition rate of about 56.92µg/ml at 15µg/mL concentration indicating the

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presence of more number of electron rich chemical constituents. Similarly the total phenolic content performed by Folin-Ciocalteu assay methodusing Gallic acid as a standard showed that the leafethylacetateextract contains high phenolic content of about 33.08 mg GAE/ 100 gr. extract. Likewise the total Flavonoidalcontent performed by aluminum chloride colorimetric assay method using Quercetin and Catechin as a standards visualized that the leaf ethyl acetateextract contains high amount of Flavonoidal content in Quercetin and Catechin standard (222.2mg QE/ 100g and 86.38mg CE/ 100g) respectively.

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