International Journal of Research
Available at https://journals.pen2print.org/index.php/ijr/
e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 06 Issue 11
October 2019

In-Vitro Assessmentof Antioxidant Activity, Total Phenolic Andflavonoid Content For Various Extracts Of *Bauhinia Purpurea.L*

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ABSTRACT

Bauhinia purpurea. Linn (Family: Fabaceae) has some important Traditional uses like laxative, anti-helmentic, dropsy, pain, rheumatism, convulsions, delirium and septicemia. The aim of the present study was evaluated Phenolic, Flavonoid and Antioxidant activity of different extracts of leaves of *Bauhinia purpurea.* The Antioxidant activity revealed that the Aq.methanolic extract (57.69% at 50 µg/ mL) showed significant anti-oxidant activity using the DPPH method, interestingly the Phenolic and flavanoidal assays the Aq. methanolic extract (33.08 mg GAE/g, 72.52 6mg QE/g and 36.30mg CE/g).The results suggested that *B. purpurea* has the potential to be source of alternative medicine due to its reportedly good Anti-Oxidant activity.

KEYWORDS:

Bauhinia purpurea, Total Phenolic Content, Flavonoid content, DPPH radical scavenging activity, Folin-ciocalteu.

INTRODUCTION

Bauhinia purpurea. Linn belongs to a Fabaceae family, is native to South China (which includes Hong Kong) and Southeastern Asia and it is also found throughout in India.[1] It is medium sized shrub or tree. This plant is used in the treatment of various traditional uses like wound-healing,[2] inflammation, [3] ulcers, pain, [4] diarrhoea etc.[5] The phytochemical screening of *B. purpurea* different reported the presence of flavonoids, steroids, glycosides etc., This plant is rich in diverse chemical constituents like Quercetin, Isoquercetin, Astragalin,[6] Monoterpenes (limonene, myrcene, linalool, citronellyl acetate and eugenol),[7]taxifolin, 6-(3''-oxobutyl)taxifolin,[8]

e-ISSN: 2348-6848
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Novel flavone glycoside 5,6-dihydroxy-7-methoxyflavone 6-O-beta-D-xylopyranoside,[9] Phytol fatty esters, lutein, β-sitosterol,[10] Dimeric flavonoids bis [3',4'-dihydroxy-6-methoxy-7,8-furano-5',6'-mono methylalloxy]-5-C-5-biflavonyl, (4'-hydroxy-7-methyl-3-C-α-L-rhamnopyranosyl)-5-C-5-(4'-hydroxy-7-methyl-3-C-α-D-glucopyranosyl) bioflavonoid,[11] Bauhiniastatins 1-4, pacharin, Bauhinoxepin C-J, Bauhibenzofurin A, Bauhispirorin A, Bauhinol E, (-)-Stropinin, Demethoxymatteucinol and bibenzyls.[12] The extracts showed Antinociceptive, anti-inflammatory, analgesic, antipyretic,[13] Antimalarial, antimycobacterial, antifungal, cytotoxicity,[14] Anti-diarrheal,[15] Anti-oxidant[16] and Anti-diabetics activities.[17] In view of the its varied pharmacological activity and phytochemical reports estimation of phytochemicals and biological activity screening of *B. purpurea* leaf part was taken to the present work.

MATERIALS AND METHODS

Plant collection:

B. purpurea leaf was collected from CSIR-CIMAP, Research Centre, Boduppal, Hyderabad, Telangana, India. The plant *B. purpurea* was taxonomically identified and authenticated by Dr. Sabitha Rani, Assistant Professor, Department of Botany, College for Women, Koti, Osmania University. A voucher specimen (CIMAP-19/BP) was deposited at CIMAP Research Centre, Hyderabad.

Extraction:

The leaf powder (100 gm each) 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-Oxidant activity

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.[18] 1 mL of 0.3 mM DPPH solution in methanol was mixed with 2.5 mL of plant extract or standard solutions of varying concentrations (50, 20, 15, 10 and 5 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 Spectrophotometer and 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.

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

Estimation of Total Phenolic Content

The total phenolic content of of *B. purpurea* extracts were determined using the Folin and Ciocalteu reagent.[19] An aliquot (1 mL) from each prepared extract samples and prepared standard solutions of Gallic acid in concentrations (50, 100, 150, 200 and 250 mg/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 colour 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 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 following formula.

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

Estimation of Total Flavonoid content

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p-ISSN: 2348-795X
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The aluminum chloride colorimetric method[20] was used for the determination of the total flavonoid content of extract of *B. purpurea* leaf. For total flavonoid determination, Quercetin and Catechin was used to make the standard calibration curve.

a. Colorimetric assay (Quercetin)

Aluminum chloride colorimetric assay method is used for determination of flavonoid content. An aliquot of extracts or standard solution of Quercetin (100, 200, 300, 400 and 500 mg/lit) was added to 10 mL volumetric flask, containing 4 mL of distilled deionized water. To the flask was added 0.3 mL of 5 % Sodium Nitrite (Na_2NO_3). 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 Quercetin equivalents (QE) /100 g fresh mass.

b. Colorimetric assay (Catechin)

Aluminum chloride colorimetric assay method is used for determination of flavonoid content. An aliquot of extracts and standard solution of Catechin (100, 200, 300, 400 and 500 mg/lit) was 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 10 mL capacity volumetric flask, water (10 mL), 5% Sodium Nitrate and 10% $AlCl_3$ (0.3 mL each) were added one after the other with a gap of 5 mins time interval. 1 M 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 ortho dihydroxyl 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 extracts (hexane, ethyl acetate, methanol and aq.methanol) of *B.purpurea* 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 of the extracts was depicted in (Table-1, Fig-1).

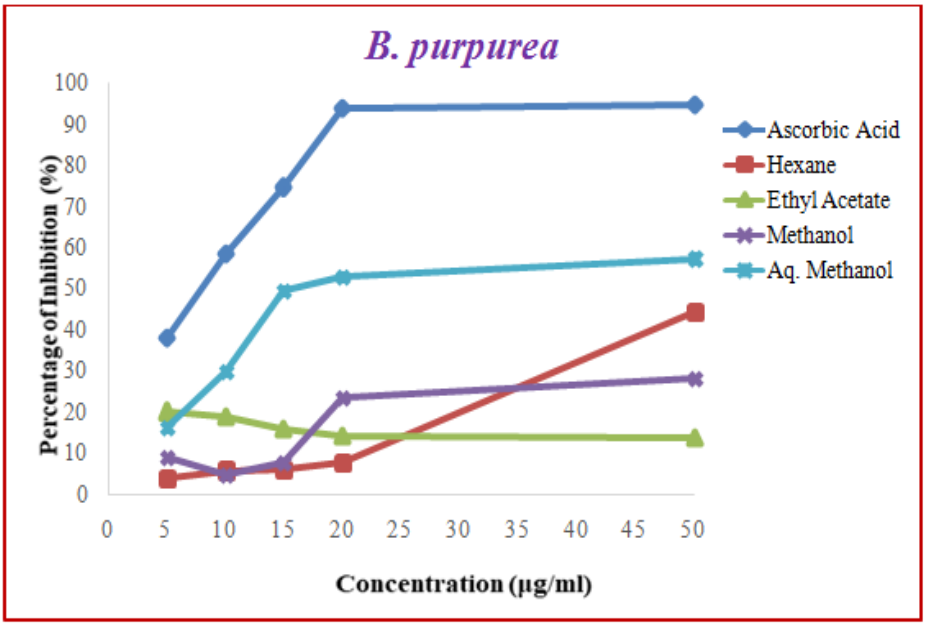
Table 1
Affect of various extracts *B. purpurea* on DPPH radicals.

Concentration	Ascorbic Acid	% Inhibition by Leaf Extracts			
		Hexane	Ethyl acetate	Methanol	Aq. Methanol
5	38.21	3.92	19.92	9.13	**16.39**
10	58.59	5.44	18.88	4.86	**30.12**
15	74.54	5.98	15.78	7.71	**49.72**
20	93.96	7.67	14.21	23.55	**53.11**
50	97.25	44.46	13.67	28.33	**57.69**

The *In-Vitro* Anti-oxidant activity of 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 Aq. Methanol extract with 57.69 % at 50 µg/ mL concentration when compared to other extracts with respect to ascorbic acid reference standard whose percentage inhibition was (97.23% at 50 µg/mL).

Figure 1

Graph of Extracts by DPPH method



3.1. Total Phenolic Content

The total phenolic content of *B. purpurea* leaf extract (Hexane, Ethyl acetate, Methanol and 30% aq. Methanolic extracts) found to be 14.13, 31.46, 20.44, 33.08 mg GAE respectively as Gallic acid equivalents/100 grams of extract was revels that aq. Methanol extract (33.08 mg GAE**)** rich in this contents (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	50	0.256
3	100	0.517
4	150	0.763
5	200	1.032
6	250	1.313

Figure 2

Standard graph of Gallic acid.

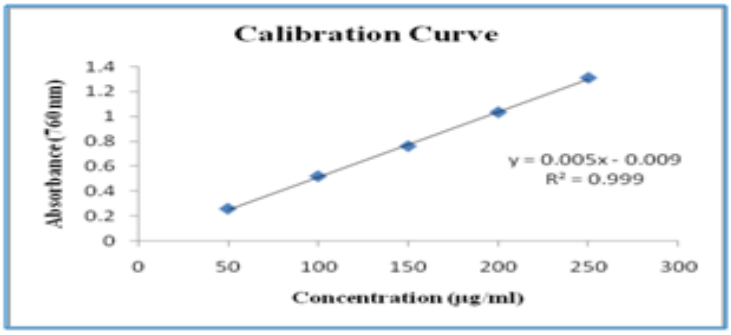


Table 3

mg/100gm Gallic acid equivalents of different extracts of *B. purpurea*

S. No	Extracts	mg/100 gm Gallic acid equivalents
01	Hexane	14.13
02	Ethyl acetate	31.46
03	Methanol	20.44
04	Aq. Methanol	**33.08**

Total Flavonoid Content

Total Flavonoid content of *B. purpurea* leaf extracts (Hexane, Ethyl acetate, Methanol and aq. Methanol) were found to be 10.27, 51.22, 50.89 mg and 72.52 QE/100 gram extract respectively and was depicted in (Table-5). Standard graph of Quercetin is depicted in (Table-4, Fig-3).

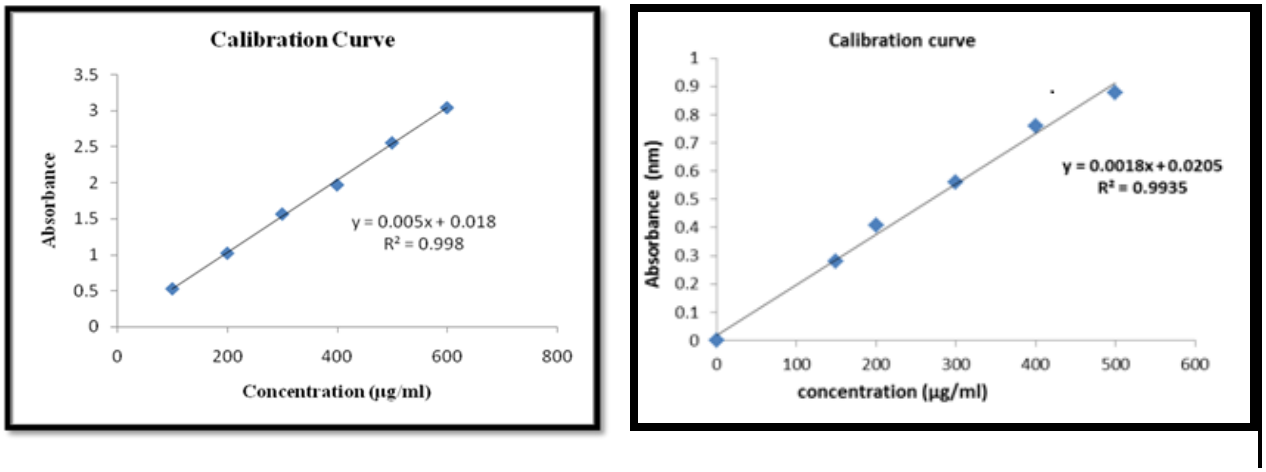
Table 4

Absorbance values of Flavonoid content using Quercetin and Catechin as standards

S. No	Concentration (µg/mL)	Absorbance	
		Quercetin	Catechin
1	0	0	0
2	100	0.202	0.335
3	200	0.383	0.409
4	300	0.511	0.507
5	400	0.623	0.603
6	500	0.811	0.812
7	600	0.974	1.073

.**Figure 3**

Standards graph of Quercetin and Catechin



Total flavonoid content of *B.purpurea* leaf extract (Hexane, Ethyl acetate, Methanol and aq. Methanol)were found to be 1.66, 13.82, and18.00mg 36.30 CE/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 Aq.methanolic extract with72.52mg CE/ 100 gm extract.

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

Extract	Flavonoid Content	
	mg Catechin/gr. Extract	mg Quercetin/gr. Extract
Hexane	1.66	10.27
Ethyl acetate	13.82	51.24
Methanol	18.00	50.89
Aq.methanol	**36.30**	**72.56**

SUMMARY AND CONCLUSION

From the overall studies conducted on various extracts of *B. purpurea*, the antioxidant activity performed using DPPH method showed Aq. methanolic extract as the most active one

e-ISSN: 2348-6848
p-ISSN: 2348-795X
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with percentage inhibition rate 57.69 µg/mL at 50 µg/mL concentrations indicating the presence of more number of electron rich chemical constituents. Similarly the total phenolic content performed by Folin-Ciocalteu assay method using Gallic acid as a standard showed that the Aq.methanolic extract contains high phenolic content of about 33.08 mg GAE/ 100 gr. extract. Likewise the total Flavonoidal content performed by Aluminum chloride colorimetric assay method using Quercetin and Catechin as a standards visualized that the Aq. methanolic extract contains high amount of Flavonoidal content in Quercetin and Catechin standards (72.52 mg QE/ 100 gr and 36.30 mg CE/ 100 gr respectively).

ACKNOWLEDGEMENT

Authors thank, Professor Dr. G. Krishna Mohan, Centre for Pharmaceutical Sciences, Institute of Science and Technology, JNTU, Hyderabad, India for his constant encouragement and support.

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