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IN-VITRO ANTIOXIDANT ACTIVITY PROFILING OF *INDIGOFERA ASTRAGALINA* DC. EXTRACTS ALONG WITH ESTIMATION OF ITS TOTAL PHENOLIC AND FLAVONOID CONTENT

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ABSTRACT

Indigofera astragalina (Silky Indigo) a rare herbaceous weed of Leguminosae family has shown astringent, diarrhea and toothache treating properties. The present study involves the in-vitro determination of antioxidant activity by DPPH free radical scavenging method, Total Phenolic Content by Folin's ciocalteu method and Total Flavonoid Content by quantitative aluminum chloride method (using Quercetin and Catechin standards) for various extracts of I. astragalina. From the study it was observed that the leaf methanolic extract (with 73.70%inhibition rates on free radicals at 50µg/ mL) showed highest antioxidant activity along with highest phenolic content 40.02 mg GAE / gm extract whereas stem Methanolic and leaf Ethyl acetate extracts (90.40 & 50.43 mg QE & CEs / gm extract) showed highest flavonoid content.

KEY WORDS

Ascorbic acid, Catechin, DPPH, Gallic acid, Quercetin.

1. INTRODUCTION

Indigofera astragalina is an uncommon weed belongs to Indigofera genus of family Fabaceae. It grows like a herb or woody plant and grows up to 1.5 meters height. It is commonly called as Silky Indigo due to presence of soft brownish hairs on leaves and legumes. It is found in West Indies, Bhutan, Myanmar, Pakistan, Bangladesh, Africa and India (Maharashtra, Karnataka, Kerala Tamil Nadu and Andhra Pradesh) [1-3].

This plant is reported to have different chemical constituents like oxalates, phytates, tannins, Vit-C, cyanogenic and cardiac glycosides, aromatic aminoacids, leucine, lysine, arginine, glutamic and aspartic acids [4, 5], alkaloids, glycosides, polyphenolics, and saponins [6].

I. Astragalina reported to exhibit a various pharmacological activity like Cytotoxic against (human cervical, lung, liver, breast and dermal cancer cell lines), anti-inflammatory, antibacterial, anticanceral activities, anti-helmintic and antifungal activities [6].

Due to the presence of diverse chemical compounds with different pharmacological activities and less work done, the plant *I. astragalina* was selected for the quantitative estimation studies by *in-vitro* antioxidant activity, total Phenolic and Flavonoid content estimation in various extracts of leaf and stem.

2. MATERIALS AND METHODS

2.1. Plant collection:

The leaf and stem of *Indigofera* were collected from Nellore district of Andhra Pradesh, India in the month of November 2017. The plant was taxonomically identified and authenticated by Dr. Sateesh Suthari, Scientist, Department of Plant Sciences, University of Hyderabad, Telangana. A voucher specimen (2575) stored in CIMAP

research centre, Hyderabad. Leaves and stem were shade dried, milled into a coarse powder by a pulverizer.

2.2. Extraction:

The leaf and stem powder (100 gms each) were successively extracted with hexane, ethyl acetate, methanol in ascending order of polarity by ultrasonication extraction method for 30 mins (30 min x 4 times) each and for 30% aq. Methanol extraction was left 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.

2.3. Estimation *of In-Vitro* Anti-Oxidant activity (*DPPH assay method)*

The extracts ability to scavenge DPPH radical was determined by using DPPH radical scavenging method. Various concentrations like 50, 25, 20, 15, 10 and 5 µg/mL in methanol were prepared from the sample stock solutions (1.0 mg/mL). 1 ml of a 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standard and allowed to react at room temperature for 30 min. The absorbance of the sample mixture was measured at 517 nm 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. From the obtained absorbance values the percentage antioxidant activity was calculated by following formula [7] (Table 1, Fig 1).

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

2.4. Estimation of Total Phenolic Content (*Gallic acid calibration assay method)*

Extract ample preparation: 500 mg of all the extracts dissolved separately in 50 mL of methanol and centrifuged for 5 mins in order to get pure and clear extract as liquor.

Sample preparation: extract sample (1 mL) withdrawed into 25 mL capacity conical flask, to it added deionized water (9 mL), Folin's reagent (1 mL) was added. Later 7% Na_2CO_3 (10 mL) was added after 5 mins time interval and shaked well for efficient mixing. Then final volume was made upto 25 mL with deionized water and gone for dark incubation for about 90 mins.The blank was also prepared by adding all the above mentioned ingredients without any extract sample in it. Finally, absorbance was checked at 750 nm in UV spectroscopy

The absorbance of all the samples are correlated with Standard concentrations ((50, 100, 150, 200 and 250 mg/ lit) of Gallic acid and unknown concentration of sample was calculated from the standard graph (Table 3, Fig 2).

The phenolic content was expressed in mg Gallic acid equivalents (GAE)/ g fresh weight [8].

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

2.5. Estimation of Total Flavonoid content (*$AlCl_3$ colorimetric assay method)*

It was performed to identify the total flavonoid content in the extract samples by employing Quercetin and Catechin standards.

Extract sample preparation: 500 mg of all the extracts dissolved separately in 50 mL of methanol and centrifuged for 5 mins in order to get pure and clear extract liquor.

Sample preparation: From the above prepared extract sample (10 mL) was withdrawn and deionized water (10 mL), 5% $NaNO_2$ (0.3 mL) were added. After 5 mins time interval 10% $AlCl_3$ (0.3 mL) was added and shake well. Later again after 5 mins, 1 M NaOH (2 mL) was added and final volume was made upto 10 mL with deionized water and absorbance was checked at 510 nm in UV spectroscopy.

Standard Quercetin and Catechin graph was established by preparing standard concentrations (100, 200, 300, 400 and 500 mg/lit) of it separately. Blank was prepared by adding all the above-mentioned constituents without any extract sample [9] (Table 5, Fig 3).

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

Inference: The darker the colour complex, higher is the complexation with aluminum and indicates the presence of more number of flavonoid principles.

3. RESULTS AND DISCUSSION

3.1. *In-Vitro* Antioxidant Activity

The leaf and stem (hexane, ethyl acetate, methanol and aq.methanol) extracts of *I astragalina*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 (by leaf and stem hexane, ethyl acetate, methanol and aq.methanol extracts) was depicted in (Table-1, Fig-1).

S. No	Concentration µg/ mL	Ascorbic Acid	Leaf Extracts (% Inhibition)				Stem Extracts (% Inhibition)			
			Hexane	Ethyl acetate	Methanol	Aq. Methanol	Hexane	Ethyl acetate	Methanol	Aq. Methanol
1	5	38.23	13.52	13.20	**37.57**	11.29	12.63	13.09	34.36	12.01
2	10	58.56	17.53	13.85	**42.63**	10.72	02.18	3.138	10.72	18.02
3	15	74.58	12.87	16.88	**59.65**	15.30	03.60	3.03	11.86	31.75
4	20	93.92	9.30	21.96	**70.77**	13.74	0.88	0.97	13.99	42.97
5	25	95.02	8.98	26.94	**72.68**	14.81	0.76	0.865	12.041	51.58
6	50	97.23	10.06	45.12	**73.70**	21.68	6.53	8.65	19.96	88.77

Table 1: Affect of various extracts of *I. astragalina* on DPPH radicals.

The above results shows that the % inhibition rate by antioxidant principles on DPPH free radicals found to be more in leaf methanol extract (73.70% at 50µg/ mL concentration) when compared to other extracts of *I. astragalina* with respect to ascorbic acid reference standard whose percentage inhibition was (97.23% at 50µg/ mL).The Order of Anti-oxidant activity of *I. astragalina* extracts by DPPH method was as follows: Leaf Methanol>Stem Aqueous Methanol>Leaf Ethyl acetate>Leaf Aqueous Methanol>Stem Methanol >Leaf Hexane >Stem Ethyl acetate >Stem Hexane extracts.

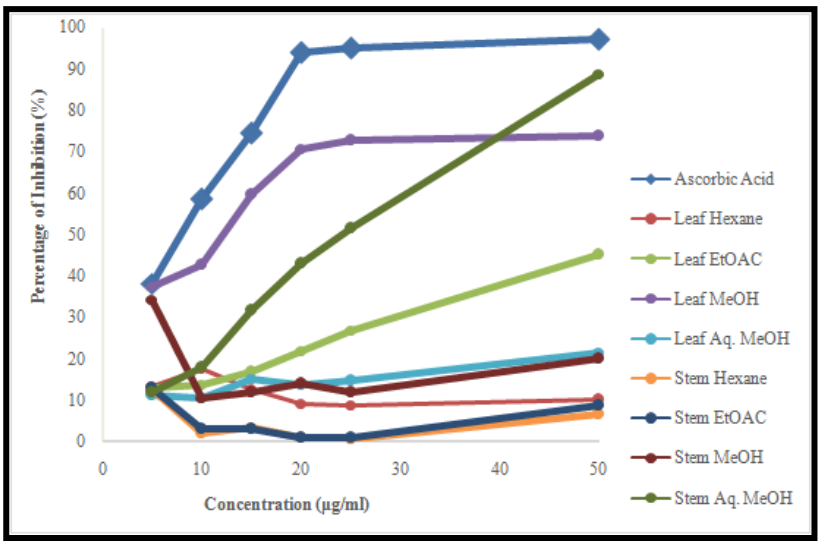

Figure 1: Graph of Extracts by DPPH method

3.2. Total Phenolic Content

The total phenolic content of *I. astragalina* leaf and stem extracts were estimated by Folin-Ciocalteu assay method. The plant leaf and stem extracts (Hexane, Ethyl acetate, Methanol and 30% aq. Methanolic extracts) the total phenolic content found to be 9.58, 36.97, 40.02, 39.60, 8.56, 9.64, 24.82 and 25.78mg respectively as Gallic acid equivalents/ gram of extract and is depicted in Table-3. Standard graph of Gallic acid was depicted in (Table 2, Fig2).

S. No	Concentration (µg/ml)	Absorbance (nm)
1	0	0
2	50	0.258
3	100	0.519
4	150	0.761
5	200	1.034
6	250	1.310

Table 2: Absorbance values of Gallic acid standard

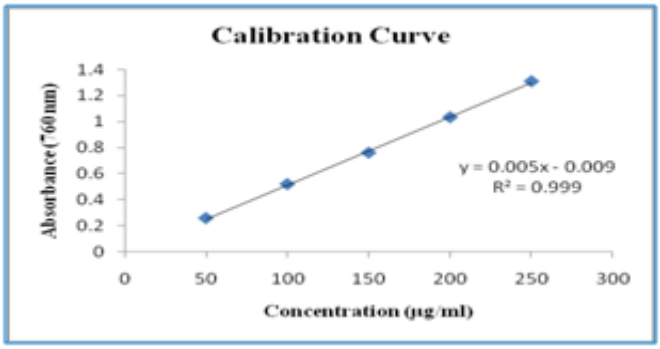

Figure 2: Standard graph of Gallic acid.

S. No	Plant Part	(Extracts)	Unknown Concentration (µg/mL)	mg/ gm Gallic acid equivalents
1	Leaf	Hexane	95.831	9.58
2		Ethyl acetate	369.77	36.97
3		Methanol	**400.29**	**40.02**
4		Aq. Methanol	396.072	39.60
5	Stem	Hexane	85.657	8.56
6		Ethyl acetate	96.40	9.64
7		Methanol	248.25	24.82
8		Aq. Methanol	257.85	25.78

Table 3: mg/gm Gallic acid equivalents (GAEs) of different extracts of *I. astragalina*

Based on the results obtained, the total Phenolic content showed the presence of highest phenolic content in leaf methanolic extract with (40.02 mg GAE/gr. Ext.) compared to other extracts. The Order of Phenolic content of plant *I. astragalina* leaf and stem extracts

Leaf methanol>Leaf Aq. Methanolic >Leaf Ethyl acetate >Stem Aq. Methanol>Stem Methanol > Stem Ethyl acetate>Leaf Hexane >Stem Hexane extracts

3.3. Total Flavonoid Content

Quercetin Standard method: Total Flavonoid content of *I. astragalina* leaf and stem extracts (Hexane, Ethyl acetate, Methanol and aq. Methanol) were found to be 26.21, 35.08, 115.17, 11.44, 3.66, 19.65, 90.40 and 19.48 mg QE/gram extract respectively and was depicted in (Table-5). Standard graph of Quercetin is depicted in (Table-4, Fig-3).

S. No	Concentration (µg/mL)	Absorbance	
		Quercetin	Catechin
1	0	0	0
2	100	0.200	0.336
3	200	0.380	0.407
4	300	0.509	0.509
5	400	0.622	0.600
6	500	0.814	0.814
7	600	0.972	1.071

Table 4: Absorbance values of Flavonoid content using Quercetin and Catechin as standards.

From the obtained overall results, it was shown that the total Flavonoid content were found to be more in stem methanolic extract of *I. astragalina* with 90.40mg QEs/ gm extract followed by Stem Methanol> Stem Hexane > Leaf Aq. Methanol > Stem Aq. Methanol > Stem Ethyl acetate > Leaf Hexane > Leaf Methanolic extracts.

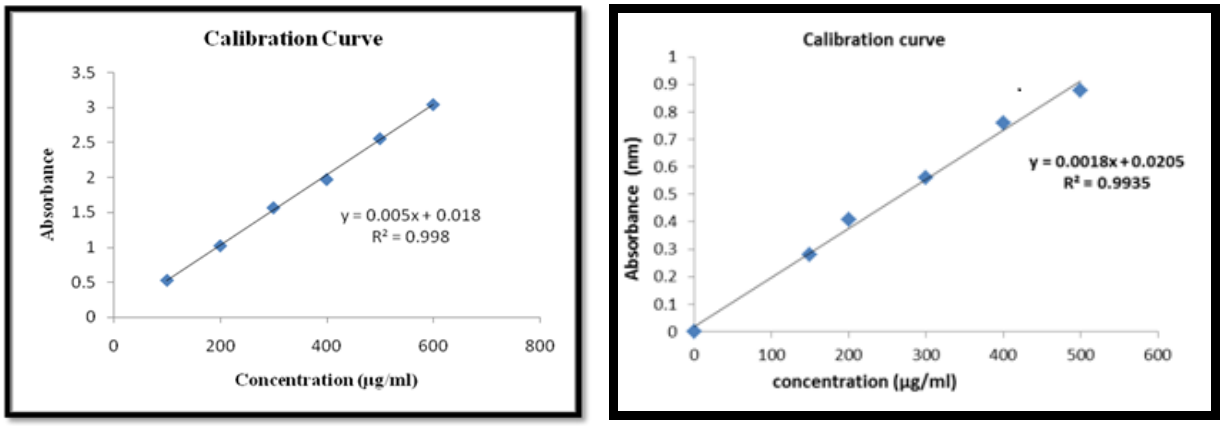

Figure 3: Standards graph of Quercetin and Catechin

Catechin Standard method: Total flavonoid content of *I. astragalina* leaf and stem extracts (Hexane, Ethyl acetate, Methanol and aq. Methanol) were found to 29.93, 55.43, 49.76, 18.87, 11.40, 13.22, 14.85 and 15.29 mg CE / gram 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 leaf ethyl acetate extract of *I. astragalina* with 55.43 mg CE/ gm extract.

The Order of Flavonoid content of plant extracts using Catechin as standard.

Leaf Ethyl acetate>Leaf Methanol >Leaf Hexane >Leaf Aq. Methanol>Stem Aq. Methanol >Stem Methanol >Stem Ethyl acetate> Stem Hexane extracts

Plant Part	Extract	Quercetin		Catechin	
		Unknown Concentration (µg/ml)	Flavonoid Content mg QEs/ g Extract	Unknown Concentration (µg/ml)	Flavonoid Content mg CEs/ gr. Extract
Leaf	Hexane	262.180	26.21	299.316	29.93
	Ethyl acetate	350.830	35.08	554.340	55.43
	Methanol	1151.73	115.17	497.62	49.76
	Aq. Methanol	114.430	11.44	188.764	18.87
Stem	Hexane	36.6040	3.66	114.016	11.40
	Ethyl acetate	196.513	19.65	132.23	13.22
	Methanol	904.072	90.40	148.56	14.85
	Aq. Methanol	194.871	19.48	152.96	15.29

Table 5: mg/gm Quercetin and Catechin equivalents (QEs & CEs) of Hexane, Ethyl acetate, Methanol and 30% Aq. Methanol extracts of *I. Astragalina* leaf and stem.

4. SUMMARY AND CONCLUSION

Based on the studies conducted on various extracts of *I. Astragalina*, the antioxidant activity performed using DPPH method showed leaf methanolic extract as the most active one with percentage inhibition rate of 73.70% at 50µg/mL concentration indicating the presence of more number of phenolic chemical constituents. Similarly, the total phenolic content performed by Folin-Ciocalteu assay method using Gallic acid as a standard showed that the leaf methanolic extract contains high phenolic content of about (40.02 mg GAE/ gr. Extract). Likewise, the total Flavonoid content performed by aluminum chloride colorimetric assay method using Quercetin and Catechin as a standard visualized that the stem methanolic extract contains high amount of Flavonoid content in Quercetin standard method (with 90.40 mg QE/gr extract) where as in Catechin standard method, the leaf ethyl acetate extract showed high amount of Flavonoid content with (50.43 mg CE/ gr Extract).

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