



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

***In-vitro* antioxidant activity of flavonoids rich fraction of aerial parts of *Hemidesmus indicus* Linn, R. BR.**

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ABSTRACT

The aerial parts of plant contain a number of medicinally important compounds. The present study was carried out to extract the flavonoid rich fraction from the aerial parts of *Hemidesmus indicus* Linn. R. Br. and identified the phytochemicals presents in the flavonoid rich fraction of aerial parts of *Hemidesmus indicus* Linn. R. Br. & determined the total flavonoid contents and antioxidant potential of obtained favonoid rich fraction of *Hemidesmus indicus* Linn. R. Br. Total flavonoid content was estimated by Aluminium Chloride colorimetric method and the flavonoid content was 0.406 % TFC (Total Flavonoids Contents) in grams of Quercetin equivalent. Antioxidant activity was evaluated by DPPH method and IC₅₀ value of flavonoid rich fraction of aerial parts of *Hemidesmus indicus* Linn. was found to be 21.39 µg/ml.

Key words: Total flavonoids, Aluminium chloride, Antioxidant activity, DPPH, IC₅₀ value.

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Received 28 August 2012, Accepted 03 September 2012

Please cite this article in press as: Bokade BM *et al.*, *In-vitro* antioxidant activity of flavonoids rich fraction of aerial parts of *hemidesmus indicus* linn, R. BR. American Journal of PharmTech Research 2012.

INTRODUCTION

Hemidesmus indicus Linn R. Br. is a diffusely twinnig undershrub having numerous slender wiry laticiferous branches with purplish brown bark. Stems and branches which twine anticlockwise are profusely laticiferous, elongate, narrow, terete and wiry having a deep purple or purplish brown colour with the surface slightly ridged at the nodes. Leaves: simple, petiolate, exstipulate, opposite, entire, apiculate acute or obtuse, dark green above but paler and sometimes pubescent below. Leaves of the basal parts of the shoots are linear to lanceolate. *Flowers*: Greenish yellow to greenish purple outside, dull yellow to light purplish inside, calyx deeply five lobed, corolla gamopetalous, about twice the calyx, stamens five, inserted near the base of corolla with a thick coronal scale. The plant is found throughout India growing under mesophytic to semi dry conditions in open shrub jungles, hedges, uncultivated soil etc. It is found in Sri lanka, Pakistan, Bangladesh and Moluccas.^{1,2}

The different parts of the plant is used by the tribal people in india for the treatment of body heat and cuts, asthma and cough, dysentery, rheumatism and toothache and skin diseases. Tribal people of Andhra Pradesh use this plant to treat asthma, skin diseases, conjuntives and renal disorders.^{1,2}

Hence the present study was carried out to extract the flavonoid rich fraction and to determine the Total flavonoid content and Antioxidant potential of the flavonoid rich fraction.

MATERIALS AND METHODS:

Collection and processing of plant material

Aerial parts of the plant *Hemidesmus indicus* Linn. R. Br. were collected from the Hill region of Jawahar Nagar Bhandara, Maharashtra in the month of October, 2011 and authenticated with the help of herbarium sheet by Dr. N. M. Dongarwar, Department of Botany, R. T. M. Nagpur University, Nagpur, the specimen numbers given to the authenticated sheet was 9284.



Figure 1: Plant of *Hemidesmus indicus*

The aerial parts were cleansed and shade dried for a week and grounded into small size powders. 60g of plant material was defatted with the petroleum ether (60⁰-80⁰) in soxhlet extractor. The defatted marc was refluxed with 80% ethanol for about 3-4 hrs to get desired flavonoid rich fraction.

Phytochemical analysis

Phytochemical analysis involves the qualitative and quantitative analysis of herbal plants. The preliminary qualitative and quantitative tests have been attempted in aerial parts of *Hemidesmus indicus* Linn. R. Br. to find out the presence or absence of bioactive compounds.

Phytochemical Screening³

The preliminary screening was carried out by using standard procedures described by C. K. Kokate.

Quantitative determination of the chemical constituents

Determination of total flavonoids contents^{4,5}

The flavonoid rich fraction of *Hemidesmus indicus* Linn R. Br. were subjected for the estimation of total flavonoid contents by Aluminium chloride Colorimetric Method as follow:

The aluminium chloride colorimetric method was performed as given by chang, *et al.* Quercetin was used to make the calibration curve. Ten milligrams of Quercetin (Natural Remedies Ltd.) was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-1800 spectrophotometer. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of ethanol extracts (0.1g in 25ml ethanol) were reacted with aluminium chloride for determination of flavonoid content as described above.

Then, a total flavonoid content of the flavonoid rich fraction was calculated using following calculation:-

$$\text{TFC} = \frac{R \times D.F. \times V \times 100}{W}$$

Where,

R – Result obtained from the standard curve

D.F. – Dilution factor

V – Volume of stock solution

100 – For 100g dried plant, W – Weight of plant used in the experiment

Determination of Antioxidant Activity^{6,7}

The flavonoid rich fraction of *Hemidesmus indicus* Linn. R. Br. were subjected for the estimation of in-vitro Antioxidant activity by DPPH Radical scavenging activity as follow:

The free radical scavenging activity was determined *in vitro* by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay using Blois's method. About 0.1 mM solution of DPPH (2.2 mg in 100mL methanol) was prepared and 2 ml of this solution was added to 3 ml of the extract dissolved in methanol and ascorbic acid which is taken as standard at different concentrations (10–50 mcg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min for the complete reaction to occur and the absorbance was measured at 517 nm using a spectrophotometer (UV 1800 PC). The IC₅₀ values of the extracts were compared with that of ascorbic acid, which was used as the standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radicals was calculated using the following formula,

$$\text{DPPH Scavenged(\%)} = \frac{(A_{\text{cont.}} - A_{\text{test}})}{A_{\text{cont.}}} \times 100$$

Where,

A_{cont} is the absorbance of the control.

A_{test} is the absorbance of extract / ascorbic acid (standard) at different concentrations.

The antioxidant activity of the extracts was expressed as IC₅₀ values.

The IC₅₀ value is defined as the concentration (in µg/ml) of extract/ ascorbic acid (standard) that inhibits the formation of DPPH radicals by 50 %.

RESULTS AND DISCUSSION

Phytochemical screening:

The preliminary phytochemical screening of 80% ethanolic extract(i.e. flavonoid rich fraction) of *Hemidesmus indicus* Linn. Showed the presence of flavonoids and phenolics as shown in table1.

Table 1: Phytochemical constituents of flavonoid rich fraction (i.e. 80% ethanolic extract) of *Hemidesmus indicus* Linn. R. Br.

Tests	80% ethanolic extract (flavonoid rich fraction)
Alkaloids	-
Terpenoids	-
Saponins	-
Tannins	-
Sugars	-
Phenolics	++
Flavonoids	+++
Cardiac Glycoside	-

Note: '+' represents presence and '-' represents absence.

Total flavonoids contents:

The total flavonoids content of flavonoid rich fraction of *Hemidesmus indicus* Linn. was found to be 0.406 % TFC (total flavonoid content) in grams of Quercetin equivalent (QE) as shown in table 2 & 3. The calibration curve of Quercetin is shown in the figure 2.

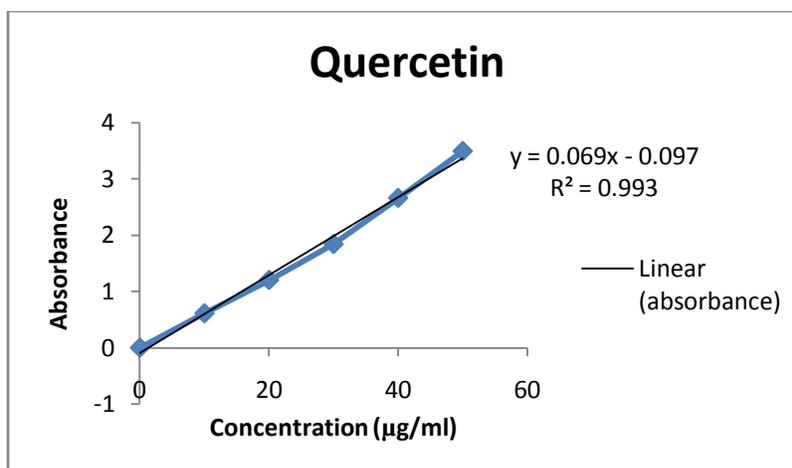


Figure 2: Standard calibration curve of Quercetin

Table 2: Results from calibration curve.

Type of the Extract	Absorbance	Concentration, µg/ml
80% Ethanolic Extract of <i>Hemidesmus indicus</i> Linn. R. Br.	1.023	16.23

Table 3. % Total flavonoids Contents of flavonoid rich fraction

Type of the Extract	% TFC in grams
80% Ethanolic Extract of <i>Hemidesmus indicus</i> Linn. R. Br.	0.406

Antioxidant activity:

The antioxidant activity of flavonoid rich fraction of *Hemidesmus indicus* Linn. by DPPH radical scavenging activity was carried out and IC₅₀ value was found to be 21.93 mcg/ml. The results of antioxidant activity shown in table 4 and figure 3 & 4.

Table 4. % Inhibition of DPPH radical and respective IC₅₀ values

Conc.	% inhibition [#]	
	Ascorbic acid	80% EtOH <i>Hemidesmus indicus</i>
10	91.26±0.76	53.78±0.58
20	93.45±0.67	60.34±0.62
30	95.15±0.65	65.78±0.49
40	99.03±0.67	69.21±0.57
50	99.27±0.78	72.78±0.51
IC ₅₀ Values	5.07	21.93

Note: [#] values expressed as mean ± SEM of three determinations

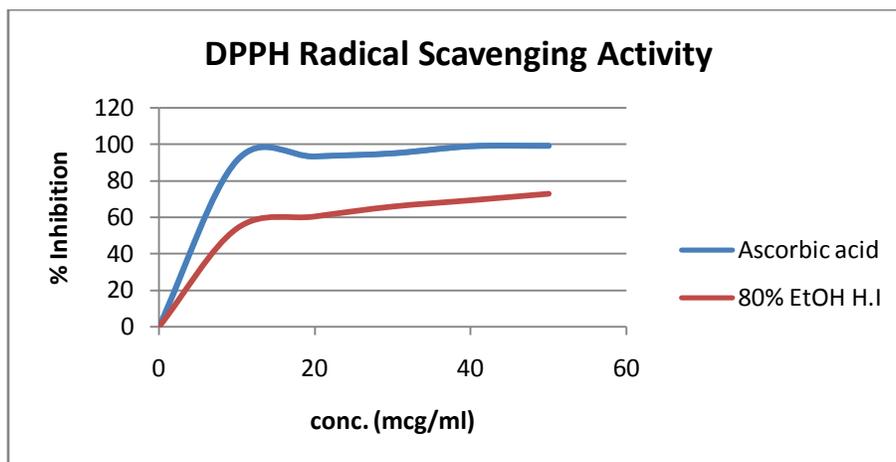


Figure 3: DPPH Radical Scavenging Activity of flavonoid rich fraction

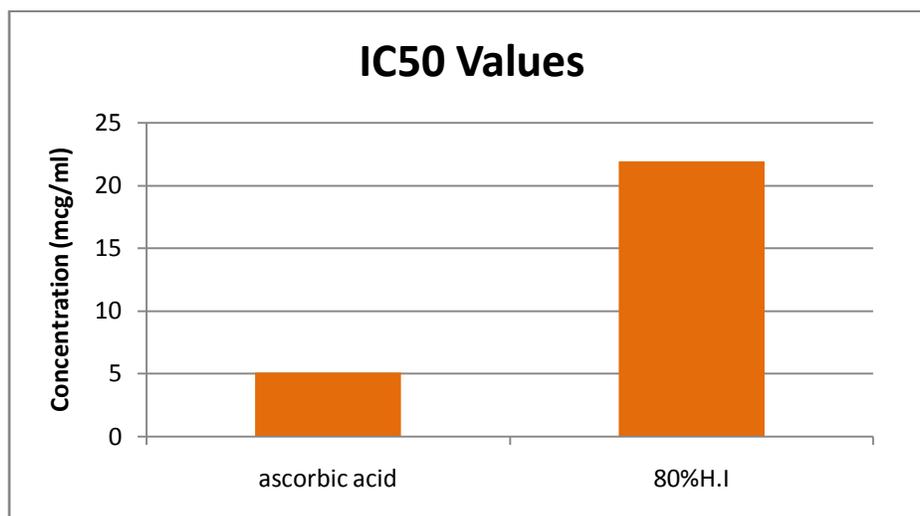


Figure 4: IC₅₀ Values of Ascorbic acid and Flavonoid rich fraction

CONCLUSION

The present study proved the significant antioxidant activity of 80% ethnaolic extract i.e. flavonoid rich fraction of aerial parts of *Hemidesmus indicus* Linn. probably due to the presence of rich amount of more polar flavonoids. This research provides information which could trigger further research in the direction of partial or full isolation and characterization of the flavonoid/s from the flavonoid rich fraction of aerial parts of *Hemidesmus indicus* Linn.

ACKNOWLEDGEMENT

Authors are thankful to the Department of Pharmaceutical chemistry and Department of Pharmacognosy, Gurunanak College of Pharmacy, Nari, Nagpur.

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