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Research Article

# IN-VITRO ANTIOXIDANT ACTIVITIES, TOTAL PHENOLICS AND FLAVONOID CONTENTS OF WHOLE PLANT OF Hemidesmus indicus (Linn.)

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**ABSTRACT**

The objective of the present investigation was to evaluate the *in-vitro* antioxidant activity of total phenolics and flavonoids compounds extracted from the whole plant of *Hemidesmus indicus*. The aqueous extract of whole plant of *Hemidesmus indicus* was showed significant free radical scavenging activity than that of standard. Higher amount of phenols and flavonoids were found in aqueous extract of whole plant of *Hemidesmus indicus*. The radical scavenging activity was found to be concentration dependent manner. The results obtained from this study indicate that *Hemidesmus indicus* is a potential source of antioxidants and thus could prevent many radical diseases.

**Keywords**: *Hemidesmus indicus, in-vitro*, antioxidant, phenolics, flavonoids.

## INTRODUCTION

Free radicals produced from oxygen to form reactive oxygen species such as the singlet oxygen, superoxide, peroxyl, hydroxyl and peroxynitrite radicals, are constantly produced within living cells for specific metabolic purposes[1]. Living cells have complex mechanisms that act as antioxidant systems to counteract the damaging effects of reactive species. Oxygen radicals induce oxidative stress that is believed to be a primary factor in various diseases as well as normal process of ageing. However, there have been concerns about synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) because of their possible activity as promoters of carcinogenesis[2]. There is a growing interest towards natural antioxidants from herbal sources[3-5].

*Hemidesmus indicus* (Linn.) (Family: Apocynaceae)[6], commonly referred to as Indian sarsaparilla, Anantamool or Nannari is a commonly available perennial climbing plant, which has been used as folk medicine and as ingredient in Ayurvedic and Unani preparations against diseases of blood, inflammation, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism etc[7,8]. It has also been used in combination with other drugs for snake bite[9, 10]. The root is said to be tonic, diuretic, and alterative. Root decoction helps in skin diseases, syphilis, elephantiasis, loss of appetite, blood purification and for kidney and urinary disorders[11]. Several biological activities like hepatoprotective, antithrombotic, anti-ulcerogenic, antiinflammatory, immunomodulatory, antidiabetic etc. have been reported from various root extracts[12-16].

Lack of scientific support revealed that the antioxidant and free radical scavenging activity of aqueous extract of *Hemidesmus indicus* (Linn.). In view of the above fact, in the present study, it is possible to evaluate the *in-vitro* antioxidant activity of total phenolic and flavonoids compounds extracted from the whole plant of *Hemidesmus indicus* (Linn.).

## MATERIAL AND METHODS

### Collection and identification of the Plant materials

The whole plant of *Hemidesmus indicus* (Linn.), were collected from Tirunelveli District, Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India, Palayamkottai. The whole plant of *Hemidesmus indicus* were dried under shade, seggregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

### Preparation of Extracts

The dried powder was extracted sequentially by hot continuous percolation method using Soxhlet apparatus[17], using different polarities of solvents like petroleum ether, ethyl acetate and methanol. The dried powder was packed in Soxhlet apparatus and successively extracted with petroleum ether by for 24 hrs. Then the marc was subjected to ethyl acetate for 24 hrs, and the marc was subjected to methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

### Evaluation of Antioxidant activity by *in vitro* methods

### Determination of Hydroxyl radical scavenging activity[18]

This was assayed as described by Elizabeth and Rao (1990). The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the $Fe^{3+}$-Ascorbate–EDTA–$H_2O_2$ system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM),0.1 ml EDTA (0.1 mM), 0.1 ml $H_2O_2$ (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml $KH_2PO_4$-KOH buffer, pH 7.4 (20mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37ºC. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

### Determination of Nitric oxide radical scavenging activity[19]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964).The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25ºC for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

### FRAP assay[20]

A modified method of Benzie and Strain (1996) was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mMHCl and 20 mM$Fecl_3.6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $Fecl_3.6H_2O$. The temperature of the solution was raised to 37ºC before using. Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm.

The standard curve was linear between 200 and 1000 µM $Feso_4$. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

**Iron chelating activity [20]**

The method of Benzie and strain (1996) was adopted for the assay. The principle is based on the formation of *O*-Phenanthroline-$Fe^{2+}$ complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

**Estimation of total phenol[21]**

0.5 ml of Folins phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

**Estimation of total flavonoids[22]**

0.5 ml of extract and 4 ml of the vanillin reagent (1% vanillin in 70% conc. $H_2SO_4$) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

**RESULTS AND DISCUSSION**

**Hydroxyl radical scavenging activity**

Table 1 illustrated the percentage of Hydroxyl radical scavenging activity of aqeous extract of *Hemidesmus indicus*. The $IC_{50}$ values of aqueous extract of *Hemidesmus indicus* were found to be 105µg/ml respectively. Whereas, the $IC_{50}$ value of standard ascorbate was observed 370µg/ml. Data presented in the above table revealed that the aqueous extract of *Hemidesmus indicus* was showed a significant hydroxyl radical scavenging activity when compared with standard ascorbate.

**Table 1: Hydroxyl radical scavenging activity of aqueous extr acts of Hemidesmus indicus**

| S.No | Concentration (µg/ml) | % of activity(±SEM)* | |
|---|---|---|---|
| | | Sample (Aqueous extract) | Standard (Ascorbate) |
| 1 | 125 | 53.37±0.41 | 26.87±0.07 |
| 2 | 250 | 64.60±0.18 | 30.30±0.05 |
| 3 | 500 | 69.26±0.02 | 55.23±0.01 |
| 4 | 1000 | 73.85±0.01 | 60.64±0.02 |
| | | **$IC_{50}$ = 105 µg/ml** | **$IC_{50}$ = 370 µg/ml** |

***All values are expressed as mean ± SEM for three determinations**

**Nitric oxide radical scavenging activity**

Table 2 was shows the scavenging of nitric oxide radical by aqueous extract of Hemidesmus indicus. The $IC_{50}$ values of aqueous extract of Hemidesmus indicus were found to be 575µg/ml respectively. Whereas, the $IC_{50}$ value of standard ascorbate was observed 370µg/ml.

**Table 2: Nitric oxide radical scavenging activity of aqueous extracts of Hemidesmus indicus**

| S.No | Concentration (µg/ml) | % of activity(±SEM)* | |
|---|---|---|---|
| | | Sample (Aqueous extract) | Standard (Ascorbate) |
| 1 | 125 | 25.24±0.05 | 26.87±0.07 |
| 2 | 250 | 29.08±0.01 | 30.30±0.05 |
| 3 | 500 | 47.80±0.34 | 55.23±0.01 |
| 4 | 1000 | 63.49±0.43 | 60.64±0.02 |
| | | **$IC_{50}$ = 575 µg/ml** | **$IC_{50}$ = 370 µg/ml** |

***All values are expressed as mean ± SEM for three determinations**

**Iron chelating activity**

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components[25]. Iron binding capacity of the aqueous extract of *Hemidesmus indicus* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were summarized in Table 3. The $IC_{50}$ values of aqueous extract of *Hemidesmus indicus* were found to be 95µg/ml respectively. Whereas, the $IC_{50}$ value of standard EDTA was observed 130µg/ml.

**Table 3: Iron-chelating activity of aqueous extracts of *Hemidesmus indicus***

| S.No | Concentration (µg/ml) | % of activity(±SEM)* | |
|---|---|---|---|
| | | Sample (Aqueous extract) | Standard (EDTA) |
| 1 | 125 | 57.94±1.68 | 49.38±2.56 |
| 2 | 250 | 76.41±4.28 | 74.40±3.73 |
| 3 | 500 | 89.41±2.47 | 89.48±5.24 |
| 4 | 1000 | 92.02±3.41 | 96.41±4.79 |
| | | **$IC_{50}$ = 95 µg/ml** | **$IC_{50}$ = 130 µg/ml** |

***All values are expressed as mean ± SEM for three determinations**

Based on the data obtained from the present study was clearly indicated that the aqueous extract of *Hemidesmus indicus* were showed more effective metal chelating activity than that of standard. The results indicated that the plant extract possess iron binding capacity which might be due to the presence of polyphenols that averts the cell from free radical damage by reducing of transition metal ions[26].

**FRAP Assay**

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Table 4 was depicted the FRAP values of aqueous extracts of *Hemidesmus indicus* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml). The $IC_{50}$ values of aqueous extract of *Hemidesmus indicus* were found to be 160µg/ml respectively. Whereas, the $IC_{50}$ value of standard ascorbate was observed 370µg/ml. The aqueous extract of *Hemidesmus indicus* was showed significant antioxidant activity than that of standard.

**Table 4: FRAP Assay of aqueous extracts of Hemidesmus indicus**

| S.No | Concentration (µg/ml) | % of activity(±SEM)* | |
|---|---|---|---|
| | | Sample (Aqueous extract) | Standard (Ascorbate) |
| 1 | 125 | 44.32±0.05 | 26.87±0.07 |
| 2 | 250 | 65.13±0.03 | 30.30±0.05 |
| 3 | 500 | 67.29±0.07 | 55.23±0.01 |
| 4 | 1000 | 71.95±0.01 | 60.64±0.02 |
| | | **$IC_{50}$ = 160 µg/ml** | **$IC_{50}$ = 370 µg/ml** |

***All values are expressed as mean ± SEM for three determinations**

**Total phenol**

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups[27]. The phenolic compounds may contribute directly to antioxidative action[28]. The total phenolic content of aqueous extract of *Hemidesmus indicus* was presented in Table 5. Aqueous extract of *Hemidesmus indicus* was found higher content of phenolic components.

**Table 5: The total Phenolic content of aqueous extracts of root of *Hemidesmus indicus***

| S.No | Extracts | Total phenol content (mg/g of Catechol) (±SEM)* |
|---|---|---|
| 1. | Aqueous extract of *Hemidesmus indicus* | 3.45 ± 0.12 |

***All values are expressed as mean ± SEM for three determinations**

**Total flavonoids**

The total amount of flavonoids content of aqueous extract of root of *Hemidesmus indicus* was summarized in Table 6. Flavonoids present in food of plant origin are also potential antioxidants[29], [30]. The higher content of flavonoids was found in aqueous extract of *Hemidesmus indicus*.

**Table 6: The total flavonoids content of aqueous extracts of root of Hemidesmus indicus**

| S.No | Extracts | Total flavonoids content (mg/g) (±SEM)* |
|---|---|---|
| 1. | Aqueous extract of *Hemidesmus indicus* | 2.19 ± 0.03 |

***All values are expressed as mean ± SEM for three determinations**

**CONCLUSION**

The results of the present study was clearly indicated that the aqueous extract of *Hemidesmus indicus* can be used as easily accessible source of natural antioxidants and as a possible food supplement in pharmaceutical industry. *In vitro* study indicates that these plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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