

**Research Article****Phytochemical study and antioxidant property of *Hemidesmus indicus* (L) R. Br. roots**Jayalakshmi B.<sup>1\*</sup>, Kruthika L.<sup>1</sup>, Amruthesh K.N.<sup>2</sup><sup>1\*</sup>Post Graduate Department of Botany, Maharani's Science College for Women, Mysore-570006, Karnataka, India<sup>2\*</sup>Applied Plant Pathology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore-570006, Karnataka, India

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**Abstract**

**Objective:** Oxidative stress is a process due to generation of free radicals which trigger cell damage. Antioxidants significantly delay or prevent oxidation of oxidizable substrates. Plants have long been a source of exogenous antioxidants. In this contest the present work to investigate antioxidants properties and phytochemical constituents of *Hemidesmus indicus* a very important medicinal plant. **Material and methods:** Different solvent extracts namely petroleum ether, ethyl alcohol and methanol were prepared. These solvent extracts were subjected to Antioxidant assay by DPPH radical scavenging assay, Hydroxyl radical scavenging assay and Nitric oxide scavenging assay. Phytochemical analysis of petroleum ether, methanol, and alcohol extracts was carried out for the detection of active secondary metabolite or different constitutes such as tannins, alkaloids, flavonoids, terpenoids, steroids, carbohydrates, protein and saponins. **Results and conclusion:** The evaluation of DPPH radical scavenging activity ranged from 25- 34µg/ml. Hydroxyl radical scavenging showed that the methanol and ethanol extract having moderate scavenging activity (32-33 µg/ml) compare to the petroleum ether extract. Nitric oxide radical scavenging activity showed the methanol showed good scavenging activity with IC<sub>50</sub> values of 46µg/ml. Hence lower the IC<sub>50</sub> value, higher will be the scavenging activity.

**Keywords:** Antioxidant activity, Hydroxyl radical scavenging, Nitric oxide scavenging, DPPH radical scavenging, *Hemidesmus indicus*

**Introduction**

Free radicals can cause “oxidative stress”, a process that can trigger cell damage. Oxidative stress reflects an imbalance between the systemic manifestation of ROS and a biological system's ability to readily detoxify the reactive intermediates or result of an imbalance in pro-oxidant/antioxidant homeostasis that leads to the generation of toxic reactive oxygen species, such as hydrogen peroxide, organic hydro peroxide, nitric oxide (Adamu et al., 2005). Antioxidants significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate. Plants have long been a source of exogenous antioxidants. It is believed that two-thirds of the world's plant species have medicinal properties, and almost all of these have excellent antioxidant potential (Cowan, 1999).

The antioxidant potential of plants has received a great deal of attention because increased oxidative stress has been identified as a major causative factor in the development and progression of several life threatening diseases, including neurodegenerative and cardiovascular disease. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. In this contest the present work to investigate antioxidants properties and phytochemical constituents of *Hemidesmus indicus* a very important medicinal plant. It is commonly known as Indian sarsaparilla, it is found in South Asia. It belongs to the family Apocyanaceae It is a slender, laticiferous, twining, sometimes prostrate or semi-erect shrub. Roots are woody, slender, and aromatic. The stem and branches of *H. indicus* twine anticlockwise and are profusely laticiferous, elongate, narrow and deep purple or purplish brown colour with the surface slightly ridged at the nodes (The Wealth of India, 2005). *H. indicus* is used to make beverages and also used in traditional medicines. The extracts from the root are used as a

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coolant and a blood-purifier. The root is also administered in the fourth and ninth month of pregnancy to prevent miscarriage. They also claim its efficacy in treating ulcers, fever, loss of appetite, gastric, anorexia nervosa cough, excessive Menorrhagia, Diarrhea and Diabetes. It also help in increasing semen count, neutralizes poisons, works as a diuretic and emetic and has anti-inflammatory properties (Udayan and Balachandran, 2009).

## Materials and methods

### Collection and extraction of plant material

Roots of *Hemidesmus indicus* were purchased from the local market pansari shop (Figure 1). Thoroughly washed sample of the test plant was dried in shade and powdered using a waring blender. Solvent extraction was carried out using soxhlet apparatus. Different solvents in the following order Petroleum ether, chloroform, ethyl acetate and methanol based on polarity from low to high. The solvent extract was concentrated separately under reduced pressure.



**Figure 1.** (a) *Hemidesmus indicus* whole plant (b) Roots of *H. indicus*

### Phytochemical analysis

Phytochemical analysis of petroleum ether, methanol, and alcohol extracts was carried out for the detection of active secondary metabolite or different constitutes such as tannins, alkaloids, flavonoids, terpenoids, steroids, carbohydrates, protein and saponins. The dried extracts obtained by soxhlet extraction were reconstituted in methanol and 1ml of each extract was subjected to standard phytochemical analysis according to the procedure described by Harborne (1998).

### Antioxidant assay

#### DPPH radical scavenging assay

Free radical scavenging activity of the different extract were measured in terms of hydrogen donating or radical scavenging ability using stable radical DPPH as described by Blois method (Blois, 1958). 3ml of methanol is taken in all test tubes. Different concentrations of solution (20, 40, 60, 80, and 100µg) were made using stock solutions (0.01g of extract dissolved in 1ml of methanol. Standard ascorbic acid solution was prepared by dissolving 0.001g of ascorbic acid in 1ml of methanol. For negative standard solution 3ml of methanol was taken in another

test tube. Then 1ml of DPPH was added to all the solutions. The mixture was vigorously shaken. Now all the mixture was kept for incubation in dark for 1hour. After incubation absorbance was measured at 517nm for all the mixtures including negative control and standard ascorbic acid solutions. Each experiment was run in triplicates and values are expressed as the mean ± standard deviation (SD). The DPPH scavenging ability was calculated using the following formula.

$$\text{DPPH scavenging effect (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where,  $A_{\text{control}}$  – absorbance of the negative control

$A_{\text{blank}}$  – absorbance of the sample

#### Hydroxyl radical scavenging assay

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. The scavenging ability of the compounds was determined according to the method Ruch et al. (1948). This method involves in vitro generation of hydroxyl radicals through Fenton system ( $\text{Fe}^{2+}/\text{Ascorbate}/\text{EDTA}/\text{H}_2\text{O}_2$ ). For this purpose, the stock solution was prepared by dissolving 0.001g of extract in 1ml of methanol. Reaction mixture was prepared using 0.36ml of 2-Deoxyribose (2.8Mm), 0.33ml of  $\text{KH}_2\text{PO}_4$  buffer (20Mm PH -7.4), 0.6ml of 30%  $\text{H}_2\text{O}_2$  (1.0Mm), 0.1ml ascorbic acid (100Mm). To this previously prepared  $\text{FeCl}_3$  –EDTA mixture [0.01ml  $\text{FeCl}_3$  (100Mm) + 0.1ml EDTA (100Mm) was added. Then to this mixture, stock solution was added in various concentrations such as 20, 40, 60, 80 and 100µl. This solution was incubated at 37°C for one hour. After incubation, 1ml of cold TCA (2.8%) and 1ml of TBA (1%) were added solutions of all the concentrations. Then the mixtures were heated at 100°C for 20 minutes to develop color. After development of color, the solutions were cooled and the absorbance was measured at 532nm. Each experiment was run in triplicates and values are expressed as the mean ± standard deviation (SD). The hydroxyl radical scavenging capacity was calculated using following formula:

$$\text{Hydroxyl radical scavenging capacity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where,  $A_{\text{control}}$  = in the absence of  $\text{FeCl}_3$  – EDTA and tested compound

$A_{\text{blank}}$  = in the absence of tested compound

#### Nitric oxide scavenging assay

The nitric oxide scavenging capacity of the compound is determined according to Green et al. (1982). The assay is based on generation of nitric oxide (NO) from sodium nitroprusside (SNP) and was measured by Griess reagent.

Sodium nitroprusside in aqueous solution at physiological PH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions which can be qualified by Griess reagent. Initially Griess reagent has to be prepared. For this purpose, 0.05g of Naphthyl ethylene diamine (0.1%) was dissolved in water. Both the solutions were mixed and made up to 50ml by using distilled water. The 1.5ml of sodium nitroprusside (5mM) in phosphate buffer (pH=7.0) was mixed with various concentrations (20, 40, 60, 80, and 100 $\mu$ l) of 1ml extract and the mixture was incubated at 25°C for 30min. After incubation, 1.5 Griess reagent was added to the incubated mixture. The reaction mixture was incubated again at room temperature for 10-15 min. The absorbance of the solutions was measured at 546nm. Each experiment was run in triplicates and values are expressed as the mean  $\pm$  standard deviation (SD). The nitric oxide scavenging percent was calculated using the following formula.

$$\text{Nitric oxide scavenging capacity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where,  $A_{\text{control}}$  = Absorbance of tested compound in absence of sample of the extract.

$A_{\text{sample}}$  = In presence of sample of the extract.

### Statistical Analysis

Statistical calculations like Mean and standard error were carried out using one way ANOVA.

### Results

#### Yield of extracts

Among the solvent extracts maximum yield was obtained by methanol extract (45.2 g/kg) followed by Ethanol (18.0g/Kg) and Petroleum ether (13.7 g/kg).

#### Antioxidant activity of *Hemidesmus indicus*

Free radical scavenging activity was evaluated by hydrogen donating ability using stable radical DPPH. The ability of the extracts to neutralize hydroxyl radical was expressed as 50% inhibitory concentration ( $IC_{50}$ ) in  $\mu$ g/ml. The Methanol and ethanol extract of *Hemidesmus indicus* was most active with  $IC_{50}$  value at 25 $\mu$ g/ml and 29 $\mu$ g/ml respectively. Above two extracts showed higher radical scavenging activity compared to standard ascorbic acid (27 $\mu$ g/ml). In hydroxyl radical scavenging assay, hydroxyl radical generated through Fenton system in aqueous media inhibited by the scavenging activity of the extract which is expressed as  $IC_{50}$  values and compared with the standard ascorbic acid. The antioxidant activity of *Hemidesmus indicus* was medium in methanol and ethanol extract with  $IC_{50}$  values 32 $\mu$ g/ml and 33 $\mu$ g/ml. Petroleum ether extract showed a least active with  $IC_{50}$  value at 41 $\mu$ g/ml. So all the extracts showed medium antioxidant activity compared to standard ascorbic acid activity (27 $\mu$ g/ml). The formation of nitrite by the reaction of

sodium nitroprusside with oxygen is inhibited by scavenging activity of the extract which is expressed as  $IC_{50}$  values and compared with standard ascorbic acid. The methanol and alcohol extracts showed moderate antioxidant activity with  $IC_{50}$  values at 46 $\mu$ g/ml and 52 $\mu$ g/ml respectively and petroleum ether showed least activity with  $IC_{50}$  value at 64 $\mu$ g/ml. Hence lower the  $IC_{50}$  value, higher will be the scavenging activity (Table 1).

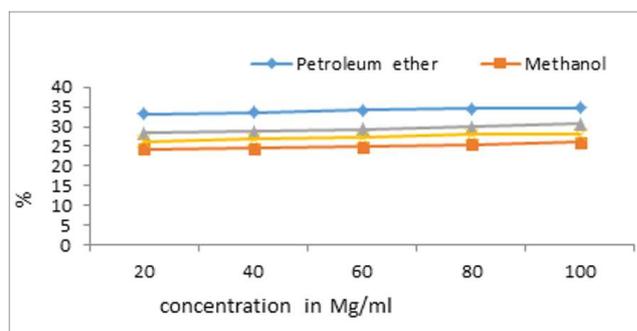
The antioxidant effects of *Hemidesmus indicus* by DPPH, hydrogen peroxide and nitric oxide radical scavenging methods increases with increase in concentration and the data are depicted in figure 2, 3 and 4, respectively.

### Phytochemical studies

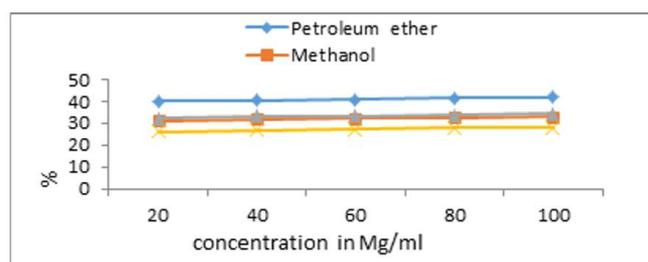
Petroleum ether showed presence of flavonoids, terpenoids, tannins and proteins. Ethanol extracts showed the presence

**Table 1.**  $IC_{50}$  value of DPPH, hydrogen peroxide and nitric oxide radical scavenging by different of *Hemidesmus indicus*

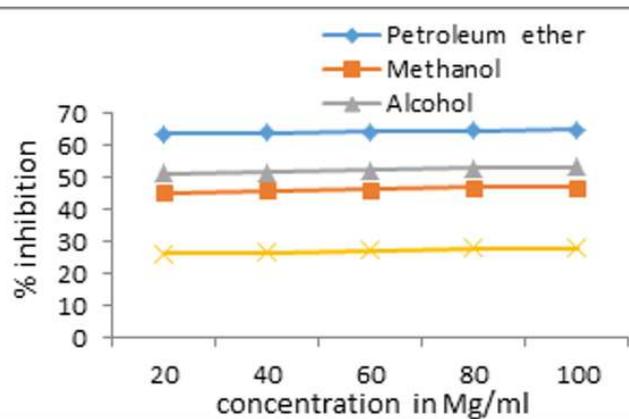
Solvent extract	$IC_{50}$ ( $\mu$ g/ml)		
	DPPH	H2O2	NO
Petroleum ether	34 $\pm$ 0.63	41 $\pm$ 0.64	64 $\pm$ 0.4
Methanol	25 $\pm$ 0.62	32 $\pm$ 0.5	46 $\pm$ 0.6
Alcohol	29 $\pm$ 0.8	33 $\pm$ 0.7	52 $\pm$ 0.8
Ascorbic acid	27 $\pm$ 0.6	27 $\pm$ 0.5	27 $\pm$ 0.6



**Figure 2.** DPPH radical scavenging of different extracts of *H.indicus* and standard ascorbic acid at different concentration (20Mg-100Mg)



**Figure 3.** Hydroxy radical scavenging of different extracts of *H.indicus* and standard ascorbic acid at different concentration (20Mg-100Mg)



**Figure 4.** Scavenging effect of nitric oxide radical scavenging of different extracts of *H. indicus* and standard ascorbic acid at different concentration(20Mg-100Mg)

**Table 2.** Phytochemical composition of solvent extracts of *H. indicus*

Phytochemical compounds	Petroleum ether extracts	Ethyl acetate extracts	Methanol extracts
Flavonoids	+	+	+
Terpenoids	+	-	+
Tannins	+	+	+
Steroids	+	-	+
Carbohydrates	-	+	+
Proteins	+	+	+
saponins	-	-	+

(+) Present (-) Absent

of flavonoids, tannins, carbohydrates, and saponins. Methanol extract showed the presence of flavonoids, terpenoids, tannins, steroids, carbohydrates, proteins and saponins (Table 2).

## Discussion

Antioxidants are compounds that hold back the oxidation of essential biological macromolecules by inhibiting the propagation of the oxidizing chain reaction (Mandal et al., 2009). Oxidative stress is initiated by free radicals which are generated constantly and seek stability through electron pairing with biological molecules such as proteins, lipid and DNA of healthy human cells and can cause various disease. Natural antioxidant is very effective to control the oxidative stress and hence prevent the imitation of disease. These antioxidant are present in number of green leafy vegetables, free radical scavenging capacity, inhibition of lipid peroxidation, metal ion chelating ability and reducing ability.

DPPH, hydroxyl radical and nitric oxide scavenging methods are selected for the determination of antioxidant activity in the current study. The scavenging ability of the samples is the measure of antioxidant activity. In DPPH method, a stable

radical is used as a substrate to evaluate the antioxidant activity of *H. indicus* extracts. The antioxidant capacity was determined by the DPPH radical scavenging activity. It is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecules. In the presence of hydrogen donors, DPPH is reduced and a free radical is generated from the scavengers. The reaction of DPPH is monitored by measuring the decrease of the absorbance of its radical at 517nm. The methanol (25µg/ml) and alcohol (29µg/ml) extract of *H. indicus* have medium scavenging activity.

The hydroxyl radical is the most reactive radical known to initiate lipid peroxidation and damage of biochemicals (Ravikiran et al., 2016). The scavenging effect of the extracts of hydroxyl radicals generated by Fenton's system is quantified spectrophotometrically at 532nm. In methanol and alcohol extract of *H. indicus* showed a good scavenging activity, while petroleum ether showed a moderate scavenging activity.

Nitric oxide radical scavenging activity was determined by the ability of the extract to inhibit the formation of nitric ions that can be produced by the interaction of nitric oxide generated by sodium nitroprusside in aqueous solvent at physiological pH (Kumara et al., 2013). The reduced nitrite ions are quantified spectrophotometrically at 540nm. *Hemidesmus indicus* have showed medium scavenging activity with least IC<sub>50</sub> values.

Petroleum ether extracts have exhibited good inhibition of free radicals generated by DPPH, hydrogen peroxide and nitric oxide when compared to the standard ascorbic acid. In hydroxyl radical scavenging shows the methanol and ethanol having moderate activity compare to the petroleum ether. In nitric oxide shows all the three extracts have moderate scavenging activity.

Methanolic extract by DPPH assay method shows appreciable activity comparable to standard ascorbic acid, which contains large amounts of flavonoids and phenolic compounds exhibits reasonable antioxidant and free radical scavenging activities (Mandal et al., 2009). There is scanty reports on antioxidant of *H. indicus* but in the present shows methanol and alcohol extract shows the good scavenging activity compare to the petroleum ether extract.

Phytochemical analysis of the solvent extracts of *H. indicus* roots revealed that flavonoids and tannins were commonly present in all the studied extracts except alcohol extract. The ethanolic extract of *H. indicus*, which contains all the phytochemicals that were investigated like

alkaloids, flavonoids, tannins, steroids, and phenols which was also reported by Nogat et al., (2016). Phytochemical investigations revealed that roots of *H. indicus* showed presence of flavonoids coumarino-ligans and tannins (Baheti et al., 2006). Literature reports antioxidant property of aqueous, methanol and alcoholic extracts. But in the present study three solvents like petroleum ether, methanol, and alcohol extracts were used and all of them have shown good result. But the activity of petroleum ether extract has not be reported and the present study confirms one more solvent which showed good activity.

Totally, the scavenging activities of phenolic substances might be due to reactive hydrogen donating ability of hydroxyl substitute, since phenol substance present in the extract are good electron donors and may accelerate the conversion of hydroxyl to water (Mathew and Abraham, 2006). These are several methods for the determination of antioxidant activities. The chemical complexity of extracts which is often a mixture of group of compounds with different functional groups (Karadag et al., 2009).

Free radical scavenging is an important aspect to maintain good health using antioxidants. The potent antioxidant activity from plant source in the present study have validated and proved to be good source of plant based antioxidant.

**Conflicts of interest:** None

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