ANTIOXIDANT ACTIVITY OF ROOTS OF *HEMIDESMUS INDICUS* VAR. *PUBESCENS* – AN *IN VITRO* STUDY

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Summary

The antioxidant property of roots of *Hemidesmus indicus* (L.) R. Br. var *pubescens* (W. & A.) Hk. f., was evaluated by *in vitro* methods. Methanol (HIRM) and aqueous (HIRA) extracts of the drug were evaluated for *in vitro* antioxidant activity against DPPH, ABTS, hydrogen peroxide, nitric oxide and superoxide radicals. HPTLC fingerprinting studies were also performed on both extracts. Both extracts exhibited similar scavenging effects against ABTS and superoxide radicals whereas against DPPH and nitric oxide, the methanol extract was more effective. The study revealed that extracts of *H. indicus* var. *pubescens* roots possess antioxidant and free radical scavenging effects. Further studies can be undertaken to evaluate the *in vivo* antioxidant potential of these extracts in various animal models.

Key words: H*emidesmus indicus* var. *pubescens*; *in vitro* antioxidant activity; DPPH assay; ABTS assay; nitric oxide radical scavenging; superoxide radical scavenging; hydrogen peroxide scavenging assay.

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Introduction

Sarsaparilla is an important drug used in ayurveda, endowed with many medicinal properties. The official European sarsaparilla is derived from *Smilax china* L. (1) and Australian sarsaparilla from *Smilax glycyphila* Sm. (2).The accepted botanical source of Indian sarsaparilla is *Hemidesmus indicus* (L.) R.Br. (Periplocaceae), which is known as Sariva in Ayurveda. *Hemidesmus indicus* (L.) R.Br. var. *pubescens* (W. & A.) Hk. f. is a taxonomic variety of *Hemidesmus indicus* var. *indicus*, found in South India (3). Tannins and β- sitosterol are the reported phytoconstituents in *H. indicus* var. *pubescens* (4).

Indian Sarsaparilla is used as antipyretic, anti-diarrhoeal, astringent, diaphoretic, diuretic, refrigerant and tonic (5, 6, 7). Roots are useful in biliousness, blood disorders, dysentery, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, leprosy, leucoderma, leucorrhoea, itching, bronchitis, asthma, eye diseases, epileptic fits in children and kidney stones (5, 6, 8, 9, 10).

Toxicity (11, 12, 13) and anti ulcer activity (14, 15) studies have been reported so far on *H. indicus* var. *pubescens*. While antioxidant studies have been carried out on European (*S. china*) (16), Australian *(S. glycyphila)* (2) and Indian (*H. indicus* var. *indicus)* Sarsaparilla (17), no work has been reported on roots of *H. Indicus* var. *pubescens* so far (18, 19). Hence the present work has been undertaken.

Materials and Methods

Plant material

The roots of *H. Indicus* var. *pubescens* were collected from the vicinity of Thuthukudi District, Tamil Nadu, India, during November 2008. The plant material was identified and authenticated by Dr. S.N. Yoganarasimhan, Taxonomist and Research Coordinator at M. S. Ramaiah College of Pharmacy, Bangalore, Karnataka, India. The taxonomic identification was carried out following local flora (20), and the herbarium specimen (*Anita Murali No.034*) along with crude drug sample has been deposited at the herbarium and crude drug museum of PG Department of Pharmacognosy, M. S. Ramaiah College of Pharmacy, Bangalore.

Preparation of extracts

The roots of *H. indicus* var. *pubescens* were washed thoroughly, cleaned, dried at room temperature and coarse powdered. The powdered drug was successively extracted with solvents of increasing polarity, by soxhlation. Finally, the marc was macerated with chloroform- water for 24 h to obtain the aqueous extract. The methanol (HIRM) and aqueous (HIRA) extracts were concentrated under reduced pressure and were subjected to preliminary organic analysis (21).

Chromatographic studies

TLC and HPTLC studies were carried out on HIRM and HIRA following prescribed methods (22). HPTLC studies were performed using Camag HPTLC system equipped with Linomat V applicator, Camag TLC scanner 3 and WinCATS- 4 software for interpretation of data. An aluminum plate (10×10 cm) precoated with silica gel $60F_{254}$ (E Merck) was used as adsorbent. All the solvents used were of HPLC grade, obtained from MERCK.

In vitro antioxidant activity

Free radical scavenging effects of HIRM and HIRA against different free radicals were evaluated by *in vitro* methods. The results are expressed in terms of IC_{50}, which is the concentration of sample required to cause 50% inhibition of free radicals. Ascorbic acid was used as the standard antioxidant.

DPPH Assay

DPPH assay is based on the measurement of the scavenging ability of an antioxidant towards the stable DPPH radical (23). Methanolic solution of DPPH free radical is purple in color and it is reduced to the corresponding yellow colored hydrazine, on reaction with any hydrogen donor. This decoloration assay was evaluated by addition of the antioxidant to methanolic solution of DPPH and the decrease in absorbance was measured at 490 nm (24).

Radical Scavenging of ABTS

The ABTS assay is based on inhibition of the absorbance of radical cation, ABTS+ at 734 nm, by antioxidants. In this assay, the ABTS cation radical was generated using potassium persulphate. The absorbance of the formed chromophore was measured at 734 nm. The colored radical when mixed with antioxidant, is converted to the colorless ABTS. The percentage inhibition was calculated after measuring the absorbance at 734 nm (25).

Scavenging of Hydrogen peroxide

Hydrogen peroxide is generated *in vivo* by several oxidase enzymes and is scavenged via its reduction product hydroxyl radical (OH•). When an antioxidant is incubated with hydrogen peroxide, the loss of hydrogen peroxide is measured spectrophotometrically at 230 nm (26).

Nitric Oxide Radical Inhibition

Sodium nitro prusside generates nitric oxide in aqueous solution. This NO is converted into nitric and nitrous acids on contact with dissolved oxygen and water. The liberated nitrous acid was estimated using the modified Griess-Illosvoy method, where nitrous acid reacts with Griess reagent, to form a purple colored azo dye. In presence of scavengers, the amount of nitrous acid decreases. The degree of decrease in the formation of purple azo dye reflects the extent of scavenging. Absorbance of the chromophore formed was measured at 540 nm (27).

Scavenging of Superoxide Radical by Alkaline DMSO Method

In this method, superoxide radical was generated by addition of sodium hydroxide to dimethyl sulfoxide (DMSO). The generated superoxide reduces nitro blue tetrazolium (NBT) into a red colored formazan dye at room temperature. This was measured at 560 nm. Superoxide scavenger inhibits the formation of red colored formazan dye (28).

Results and Discussion

Phytochemical analysis and HPTLC studies

Preliminary phytochemical analysis of the extracts revealed the presence of glycosides, phenolic compounds, tannins and coumarins. The presence of these phytoconstituents and the reported steroidal sapogenins, smilagenin and sarsapogenin were confirmed by HPTLC fingerprinting studies.

Fig. 1. HPTLC fingeprinting of HIRM for Smilagenin & Sarsapogenin at 366nm

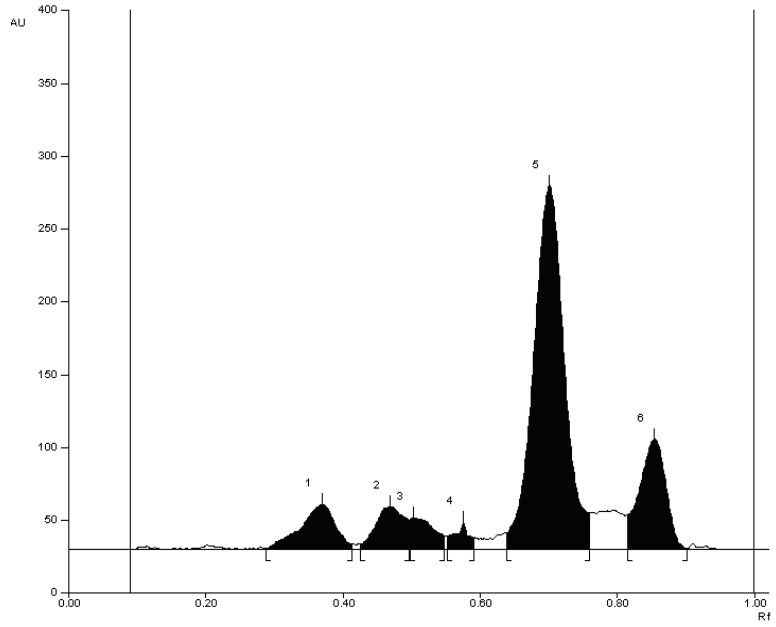


Fig. 2. HPTLC fingeprinting of HIRM for Phenolic compounds and Tannins at 366nm

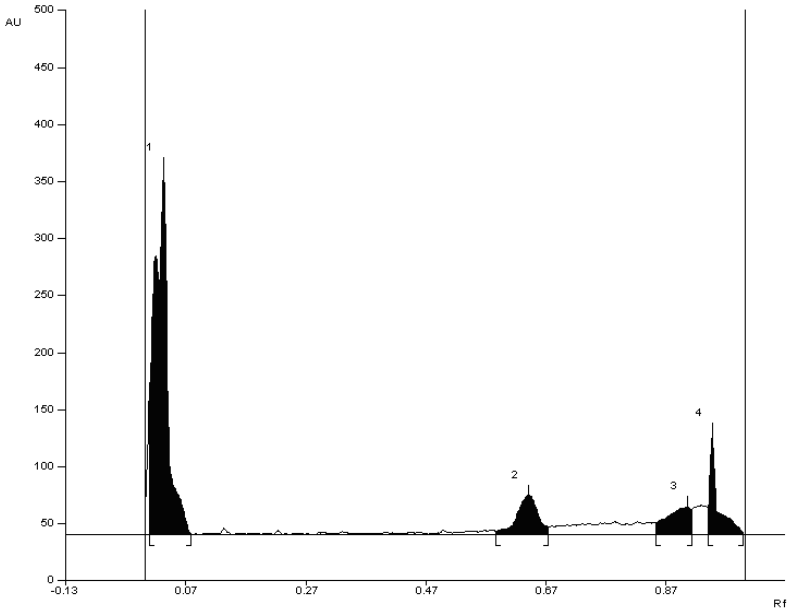
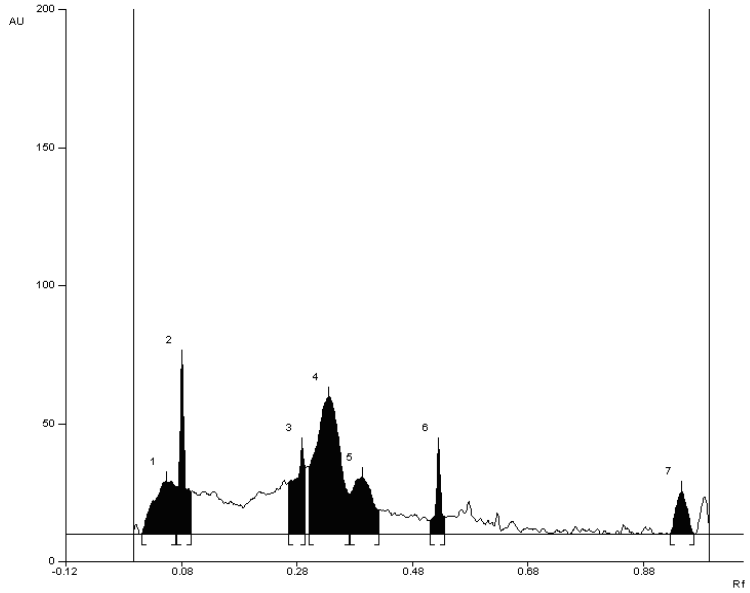


Fig. 3. HPTLC fingeprinting of HIRA for Coumarins at 366nm



***In vitro* antioxidant studies**

Methanol and aqueous extracts of *H. indicus* var. *pubescens* roots were subjected to *in vitro* antioxidant studies. Both HIRM and HIRA exhibited moderately potent free radical scavenging activities against DPPH (IC50 66.66 ± 0.33µg/ml for HIRM and 114.33 ±0.33 µg/ml for HIRA) and ABTS (IC50 29.43 ±0.88 µg/ml for HIRM and 30.0 ±0.57 µg/ml for HIRA) radicals. Both extracts exhibited similar scavenging effects against ABTS and superoxide radicals whereas against DPPH and nitric oxide, the methanol extract was more effective. In scavenging hydrogen peroxide, the aqueous extract was more effective than the methanol extract.

Table 1: In vitro antioxidant activity studies [IC_{50} values (µg/ml)]

Test	HIRM	HIRA	Standard
DPPH	66.6± 0.33	114.33±0.33	2.69± 0.14
ABTS	29.33± 0.88	30.0±0.57	4.25± 0.29
Hydrogen Peroxide	1071.12±0.75	580.0±5.87	197.5± 1.01
Nitric oxide	473.52±40.25	790.0±5.77	93.0± 0.61
Superoxide	350.66±2.18	337.66±1.45	>1000

HPTLC fingerprinting studies confirmed the presence of constituents like glycosides, phenolic compounds, tannins and coumarins in addition to the steroidal sapogenins- smilagenin and sarsapogenin. Polyphenols and tannins (29, 30, 31), coumarins (32, 33), and glycosides (34, 35) are reported to possess antioxidant activity. The presence of these phytoconstituents could have contributed to the antioxidant effect of *H. indicus* var. pubescens roots.

Conclusion

Our study demonstrates the ability of *H.indicus* var. *pubescens* roots to interact and scavenge a wide variety of free radicals responsible for oxidative damage. Further studies are needed to evaluate the *in vivo* antioxidant potential of this drug in various animal models.

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