



Phytochemical screening of *Hemidesmus indicus* root

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Abstract

The present study showed that plant have been used as green medicine from ancient days due to the presence of some secondary metabolites in it. Medicinal plants play a major part in all types of medicinal system all over the world. In ancient India, traditional healers used medicinal plants as a major part of medicine along with other components. Medicinal plants cure major diseases without any side effects. Medicinal plants are used by many ethnic groups for the treatment of infectious diseases. About 25% of modern medicines obtained directly or indirectly from plants (Pallab *et al.*, 2009)^[1]. India has a rich flora that is widely distributed throughout the country. Indian medicinal plants have a vast source of several pharmacologically active compounds. They are commonly used in home remedies against multiple ailments. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body.

Keywords: *Hemidesmus indicus* root, phytochemical test, secondary metabolites, flavonoids

Introduction

Medicinal plants are considered to be greatly important for each and every one of living beings. The medicinal value of the plant is determined by the effective secondary metabolite it holds upon it and so it has the capacity to treat the people and also produce a characteristic physiological action. Medicinal plants have the preference to produce a large variety of chemical compounds. They are classified as primary metabolites and secondary metabolites. The primary metabolites are nucleic acids, lipids, proteins whereas the secondary metabolites include alkaloids, flavonoids, saponins, cardiac glycosides and so on. Plant uses these phytochemicals for their defence mechanism against pathogens.

Hemidesmus indicus L Root, commonly called as Nannari, used to screen the phytochemical activity. It belongs to the *Asclepiadaceae* family. The presence of secondary metabolites has been considered to be cured many kind of infections to mankind. Phytochemicals are the compound found in plants which is essential for colour, taste odour and nutritional effects. It is also defined as the biologically active compounds found in the plants. Phytochemical are classified into primary metabolites and secondary metabolites. Primary metabolites are compounds directly involved in growth and development of plant whereas secondary metabolites are involved in metabolic pathway.

The study about the phytochemical investigation of *Hemidesmus indicus* root was very important for knowing the biological activities. The important phytoconstituents of the medicinal plants are alkaloids, flavonoids, tannin, phenols, and cardiac glycosides (Mir *et al.*, 2013)^[2]. In view of the above fact, it is possible to assess the phytochemical activity of *Hemidesmus indicus* root using standard methods.

Materials & Methods

Plant Material

Hemidesmus indicus root was collected along with the aerial parts and authenticated by Dr. John Britto, the Department

of Botany, Rapinet Herbarium, St. Joseph's College, Trichy-620002.

Processing of plant

The fresh roots were collected in bulk during summer. The roots were washed, shade dried for getting the moisture free nature and then milled into coarse powder using a mechanical grinder. The powder was stored in sterile container for further studies.

Preparation of extracts for the biological and phytochemical screening

The powdered plant material (150gm) was mixed with water and alcohol (1500mL). The root powder mixed with water was boiled and filtered with a muselin cloth and it was condensed in hot air oven at 50°C. Likewise coarsely powdered root material was soaked in ethanol for 3 days, filtered and allowed to condense at 50°C. The aqueous and alcoholic extract were stored in a container and refrigerated for future use (Jonathan, 2009)^[5].

Phytochemical Investigation

The alcoholic extracts of *Hemidesmus indicus* root were studied for their phytoconstituents using different phytochemical tests. (Ayoola *et al.*, 2008)^[10].

Qualitative phytochemical screening

Freshly prepared extracts were tested for the presence of phytochemical constituents using standard methods (Anonymous, 2001; Lala, 1981)^[3, 7].

Test for Alkaloids

Dragendorff's test: To 1 ml of the extract, 1 ml of Dragendorff's reagent (potassium bismuth iodide solution) was added. An orange-red precipitate indicates the presence of alkaloids.

Mayer's test: To 1 ml of extract, 1 ml of Mayer's reagent (Potassium mercuric iodide solution) was added. Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

Test for steroids

2 ml of acetic anhydride was added to 0.5 g phenolic extract with 2 ml of H₂SO₄. The colour change from violet to blue or green indicates the presence of steroids.

Test for terpenoids

Salkowski test: To 0.5 g of the extract, 2 ml of chloroform was added and carefully add 3 ml concentrated H₂SO₄ to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

Test for flavonoids

- 1. Alkaline reagent test:** Few drops of dilute ammonia and Con. HCl were added to the portion of extract. A yellow colouration indicates the presence of flavonoids.
- 2. Zinc Hydrochloride test:** To few drops of extract, zinc dust and con. HCl was added, the presence of red colouration indicates the presence of flavonoids.
- 3. Aluminium test:** To few drops of extract, 1 % Aluminium solution was added, yellow colour indicates the presence of flavonoids.

Test for Saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and again shaken vigorously and observed for the formation of emulsion.

Test for tannins

- A little quantity of test solution was taken and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.
- To 1 ml of the extract, add few drops of ferric chloride solution. Formation of a blue- black or brownish green colour product shows the presence of tannins.
- A little quantity of the extract was treated with aqueous ammonia solution. A deep green colour indicates the presence of tannins.

Test for Coumarins

0.5 g of the moistened plant extract was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Quantification of metabolites

Secondary metabolites like flavonoids, tannins and phenols were quantified employing standard methods (Ayoola *et al.*, 2008) [10]. Coumarins was estimated by making use of the method described by Kuster and Rocha (2004) [6].

Determination of total tannins

The total tannin content in the plant extract was determined by modification of a previous method (Polshettiwar *et al.*, 2007) [9]. The water and phenolic extracts (0.1 ml) was

mixed with 0.5 ml of Folin- Denis reagent followed by 1ml of Na₂CO₃ (0.5% w/v) solution and made upto 10 ml with distilled water. The absorbance was measured at 755 nm within 30 minutes of the reaction against the reagent blank. Standard curve was prepared using 20, 40, 60, 80 and 100 µl of tannic acid. Total tannins in extracts were expressed as equivalent to tannic acid (g TE/g extract).

Determination of total flavanoids

Aluminum chloride colorimetric method was used for flavonoids determination (Chang *et al.*, 2002) [4]. The root extract (0.5ml of 1:10g/ml⁻¹) in ethanol was mixed with 1.5ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 minutes; the absorbance of the reaction mixture was measured at 415nm with a single beam Systronics UV/Visible spectrophotometer (India). The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100g/ml⁻¹ in methanol.

Determination of total phenols

Total phenols were determined by making use of the method given by McDonald *et al.*, (2001) [8]. The diluted root extract (0.5 ml of 1:10 g/ml⁻¹) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na₂CO₃ (4 ml, 1M). The mixtures were allowed to stand for 15 minutes and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L⁻¹ solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g⁻¹ of dry mass), which is a common reference compound.

Result

Phytochemical potentials

Phytochemical Analysis of the drug

Preliminary phytochemical screening of aqueous and alcoholic extract answered positively for steroids, flavonoids, phenolic compounds, tannins, lignin, and negatively for alkaloids, saponins, proteins, carbohydrates, terpenoids, aminoacids and reducing sugars. Gopiesh *et al.*, 2007 also reported similar results. Inulin and cardiac glycosides were present in alcoholic extracts. Alkaloids, Saponins, phlobatannins, fat are absent in both the extracts. (Table-1)

Table 1: Qualitative phytochemical analysis of *Hemidesmus indicus* root extract

S. No	Secondary metabolites	HIRAE	HIREE
1	Steroids	-	-
2	Flavonoids	+	+
3	Phenolic compounds	+	+
4	Tannins	+	+
5	Lignin	-	+
6	Proteins	+	+
7	Carbohydrates	+	+
8	Aminoacids	+	+
9	Reducing sugars	+	+
10	Terpenoids	+	+
11	Alkaloids	-	+
12	Saponins	+	-

Quantitative analysis of phytoconstituents

Considerable quantities of phenolic compounds, flavonoids and tannins were detected from the aqueous and alcoholic extracts of *Hemidesmus indicus* root. These components were available in higher quantities in aqueous extracts than alcoholic extracts. Aqueous extracts showed 81.60±12.66mg/g, flavonoids, 111.00±11.53 mg/g, tannins & 166.33±12.66mg/g phenolic compounds whereas alcoholic extract yielded 41.33±10.26mg/g flavonoids, 74.66±5.03mg/g tannins & 160.66±6.02mg /g phenolic compounds. Available phenolic compounds were expressed in terms of Quercetin equivalent, flavonoids in terms of gallic acid equivalent and tannins in terms of tannic acid equivalent (Table 2).

Table 2: Quantitative determination of important phytoconstituents of *Hemidesmus indicus* root

S. No	Phytoconstituents	Concentration in mg/g	
		HIRAE	HIREE
1	Flavonoids	41.33±10.26**	81.60±12.66**
2	Tannins	74.66±5.03**	111.00±11.53**
3	Phenolic Compounds	160.66±6.02**	166.33±12.66**

Summary and Conclusion

Hemidesmus indicus root is considered to have the good potential source of bioactive compounds. Both the extracts are the richest source of steroids, flavonoids, phenolic compounds, tannins, lignin. Other than this, HIREE had inulin and cardiac glycosides. When compared to HIRAE, HIREE hold upon many secondary metabolites and for this reason, Ethanolic extract possess more activity.

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