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Evalution of the different solvents for phytochemical constituents of *Hemidesmus indicus* root extracts

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Abstract: The root of *Hemidesmus indicus* Linn were extracted with four different solvents viz. chloroform, methanol, ethanol and water. Phytochemical screening showed the presence of protein, amino acid, lipids, carbohydrates, alkaloids, tannins, flavonoids, saponins, sterols, glycosides and resins in the root extracts. The present study identified the phytochemicals and phyto-constituents as primary and secondary metabolites in roots of *H. indicus* which can help for future research and drug manufacturers.

Keywords: *Hemidesmus indicus* Linn, phytochemical, bio-active compound, therapeutic

Introduction: *Hemidesmus indicus* Linn belonging to family Asclepiadacea is known as "Anantmool" (Sanskrit) which means 'endless root' (Gupta, 2006). Plant has two varieties black and white namely black variety is called 'Krishna Saarivaa' and white variety called is 'Saarivaa' (Gogte, 2000). Roots are woody, aromatic, cylindrical, 0.2-0.7 inch or more in thickness, somewhat tortuous, seldom branched, brownish or purplish in color. The surface of young roots is generally smooth but in older root surface is transversely cracked and longitudinally fissured. It has a characteristic fragrance and sweetish taste (Edward and Harris 1990; Kurian, 1995).

Phytochemicals are plant derived substances, beneficial and useful to human health and having the capability of disease prevention (Chen *et al*., 1998). Secondary metabolites are important drugs of this plant from ancient time. Secondary metabolites of plants viz. alkaloids, tannins, flavonoids, saponins, sterols, glycosides and resins are of indispensable importance. Chemical evaluation of the plants for secondary metabolites includes qualitative, quantitative and biochemical tests. In the present investigation qualitative chemical tests were carried out for identification of various phytoconstituents.

The roots of *H. indicus* are bitter, astringent, aromatic, refrigerant, anthelminitic, and tonic. They are also useful in burning sensation, leprosy, epileptic fits, diarrhoea, arthralgia. (Sharma *et al*., 2000).

H. indicus roots have been reported for antimicrobial and pharmacological activites (Pandey and Dwivedi 2001). The roots have terpenoidal fraction of antioxidant potential and antiacne activity. It is found that the antioxidant potential and antiacne activity could be attributed to non-polar terpenoidal fraction (Kumar *et al*,.2008).

Study on aqueous root extract of *H. indicus* for hypoglycemic activity on streptozotocin induced diabetic rats. The dose of drug exhibited significant antidiabetic activity (Kannabiran and Mahlingam 2008).

Antimicrobial activity of saponin fraction was determined from the roots of *H. indicus* against pathogenic bacteria and fungi in-vitro condition by agar diffusion assay (Khanna and Kannabiran 2008).

Phytochemical study and antioxidant property of *H. indicus* root presented for different solvent. It was carried out for the detection of active secondary metabolite or different constitutes such as tannins, alkaloids and flavonoids (Jayalakshmi *et al.*, 2018).

Material and Method

Sample Collection

The sample of *H. indicus* in the present investigation was collected from study site Dindori district of Madhya Pradesh. The plant was identified from the existing literature and was compared with herbarium specimen sheet available in State Forest Research Institute, Jabalpur and Tropical Forest Research Institute, Jabalpur. Authentic plant material was preserved as Herbarium specimen. no. 17699 and 1770.

Solvent Extract

The dried root samples were ground into the fine powder using a blender. The powder was dissolved in different solvents like Chloroform, Methanol, Ethanol and Water.

Extraction was carried out in soxhlet apparatus. The extract was then distilled for individual samples separately with the solvents and the filtrate was collected and concentrated by evaporating the solvent to get final stock.

Phytochemical Studies

Phytochemical screening was carried out using standard methods by Santhi *et al*. (2011), Savithramma *et al*. (2011) and Vaghasiya *et al*. (2011).

Test for Protein: In 1 ml of extract add 0.2 ml of nitric acid. White precipitate indicated presence of protein.

Test for Amino acid: Take 0.5 ml extract add 0.25% ninhydrin reagent and boiled for few minute. Formation of Blue color/ bluish black color indicates presence of amino acid.

Test for Lipid: In 1 ml of plant extract, few drops of Sudan III were added. Red colour indicated presence of lipid.

Test for Carbohydrates: 1 ml of extract was added to 1 ml of Benedict's reagent and heated for 5 min. Formation of orange precipitate indicated the presence of carbohydrate.

Test for Alkaloids: 1 ml of extract + 1 % HCl and 6 drops of Mayer's reagent and few drops of Dragendorff's reagesnt. Orange precipitate indicated the presence of alkaloids.

Test for Tannins: In 5 ml of extract was added few drops of 1 % lead acetate. A yellow precipitate indicates the presence of tannin.

Test for Flavonoids: 5 ml of dilute ammonia solution were added to a portion of filtrate of extract followed by addition of cons. H_2SO_4. A yellow coloration is observed which confirmed the presence of flavnoids.

Test for Saponins: 5 ml of extract was added to 20 ml of distilled water and agitated in graduated cylinder for 15 min. The formation of a layer of foam indicated the presence of saponin.

Test for Sterols: In 0.5 ml of the extract, 2 ml of cons. Sulphuric acid was added from the side of the test tube. The test tube was shaken for few minute. The development of red colour indicated the presence of sterols.

Test for Glycosides: 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of $FeCl_3$ solution. This was then underplayed with 1 ml cons. H_2SO_4. A brown ring at the interface indicates a deoxy sugar characteristic of glycosides.

Test for Resins: In a dry test tube 2 ml of acetic acid and 2 drops of sulphuric acid were added to 0.5 ml of plant extract. A purple colour was obtained which changed to violet within 10 min this indicated the presence of resins.

Results and Discussion

Root extracts of *H. indicus* were prepared in various solvents viz. Chloroform, Methanol, Ethanol and Water. A number of phytochemical tests were performed using standardized methods and the results are presented in the table.

Table Comparative analysis of presence of different metabolites in chloroform, methanol, ethanol and water extracts of *Hemidesmus indicus* Root.

Compounds	Chloroform	Methanol	Ethanol	Water
Protein	+	+	+	+
Amino acids	-	+	+	+
Lipids	-	+	+	-
Carbohydrate	-	+	+	+
Alkaloids	-	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+

Saponins	-	+	+	+
Sterols	+	+	+	+
Glycoside	+	+	+	+
Resin	+	+	+	+

(+) - Indicates the presence of the phyto-constituent

(-) - Indicates the absence of the phyto- constituent

The qualitative phytochemical analysis of the extracts, prepared using Chloroform; Methanol, Ethanol and Water, were performed to identify the presence of different beneficial compounds in the studied samples. The above four types of extracts of root of *H. indicus* showed the presence of phyto-constituents the primary metabolites viz. protein, amino acid, lipids and carbohydrates and the secondary metabolites viz. alkaloids, tannins, flavonoids, saponins, sterols, glycosides and resins. The roots of *H. indicus* have been used since long in the traditional system of medicine in India (Bhadane *et al.,* 2008). It may be due to the existence of various bioactive phyto-constituents in the roots of this medicinally important plant species.

Chloroform extract of roots showed the presence of primary metabolites viz. protein but failed to show amino acid, lipids and carbohydrates, It also showed the presence of secondary metabolites viz. tannins, flavonoids, sterols, glycosides and resin but showed absence of alkaloid, and saponin. Methanolic extract of root showed the presence of all phyto-constituents tested viz. protein, amino acids, lipids, carbohydrates, alkaloids, tannins, flavonoids, saponins, sterols, glycoside, and resins. Similarly Ethanolic extract of root also demonstrated the presence of all the phyto-constituents listed in Table. Aqueous extract of leaf showed the presence of all phyto-constituents tested except lipids. The results of phytochemical analysis of *H. indicus* (roots) from different extracts are similar to the past reports of Ganatra *et al.* (2013) and Nutan *et al.* (2019).

The result of present investigation clearly indicates that the phytochemical screening of qualitative estimation of plant roots, studied is rich source of medicinally active metabolites. Solvent plays major role in the identification and extraction of phytochemicals in plants. Out of the four solvents used, the Methanolic and Ethanolic extracts proved more suitable then other extracts. Truong *et al.* (2019) also used Methanol as optimal solvent for the identification of phytochemical compounds in an important medicinal plant species *Severinia buxifolia.*

The results obtained during this investigation will provide a base for the future application of *H.indicus* in the treatment of various diseases caused by pathogenic fungi or bacteria. The study also opens window for future research on this medicinally important plant species.

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