

## RESEARCH PAPER

# A comparative study of phytochemical screening in plant *Hemidesmus indicus* (L.) R.Br. collected from different geographical regions of Telangana state

R. SUMAN KUMAR\*, P. RAMCHANDRA REDDY, S. GANGADHAR RAO AND K. NETHAJI

Department of Botany, University College of Science, Osmania University, HYDERABAD (TELANGANA) INDIA

**ABSTRACT**

*Hemidesmus indicus* belongs to the family Apocynaceae, which is used widely as an important medicinal plant. The extracts from the root are used as a coolant and a blood-purifier and is used in many forms. In the present study, emphasis was given on phytochemical screening of root extract and to select an elite species of the above plant with more number of phytochemicals. The plant material was collected from six geographical locations viz., Mahabubnagar, Warangal, Nalgonda, Nizamabad, Karimnagar and Adilabad, which are with different edaphic and climatic conditions of Telangana State, India are designated as site I to VI, respectively. Fourteen phytochemicals were tested for their maximum and minimum presence and the extractions were prepared in aqueous, acetone, ethanol, petroleum ether and chloroform. The results are very clear that the root extracts of plants collected from site IV, V and VI are showing maximum number of phytochemicals and on the other hand plants from site III is showing minimum number of phytochemicals. The present study helps future researchers and the academicians to collect the plant which yield more number of phytochemicals and further it opens a new era of research to quantify and extract the pure compound from the above site plants.

**Key Words :** *Hemidesmus indicus*, Apocynaceae, Medicinal plant, Phytochemical screening

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Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug. In India, plants have been traditionally used for human and veterinary health care. According to the reviews, world population uses around 35,000-70,000 species of plants for medicinal, nutraceuticals and/or cosmetic purpose (Shanti *et al.*, 2010). There is an increased interest in plant drug extracts, and this is due to several reasons, specifically, synthetic medicine can be inefficient, abusive and or incorrect use of these drugs results in deleterious side effects whereas drugs obtained from natural plant origin

**\* Author for correspondence**

**R. Suman Kumar**, Department of Botany, University College of Science, Osmania University, HYDERABAD (TELANGANA) INDIA (Email: sumanvandy2008@gmail.com)

are non-narcotic, having no or fewer side effects and are cost effective (Nagashayan *et al.*, 2000 and Srikanth *et al.*, 2013).

*Hemidesmus indicus* R. Br. (Family: Apocynaceae), commonly known as Indian sarsaparilla or Anantmool is a slender, laticiferous and twining shrub, occurs over the greater part of India (Anonymous, 1997). It is widely recognized in folk medicine and prepared against disease of biliousness, blood diseases, diarrhea, skin diseases, respiratory diseases, fever, bronchitis, eye diseases, burning sensation, rheumatism and gastric disorders (Shete and Bodhankar, 2010). The roots served as remedy for leprosy, syphilis, leucoderma, asthma, dysentery, kidney and urinary diseases and root extracts have been found to exhibit various pharmacological properties (Ambasta, 1992). The major chemical constituents are coumarin, hemidesmine, hemidine, hemidesine and rutin (Anonymous, 1976) Thus, the present study was aimed at phytochemical screening and identification of an elite ecotype of *Hemidesmus indicus* collected from different geographical areas.

## RESEARCH METHODOLOGY

### Plant material :

The leaves of the plant species were collected from six different geographical areas of Mahabubnagar (Site-I), Warangal (Site-II), Nalgonda (Site-III), Nizamabad (Site-IV), Karimnagar (Site-V) and Adilabad (Site-VI) of Telangana State, India. The plants were identified using available flora at the Department of Botany, Osmania University, Hyderabad, India (Chopra, 1956 and Harborne, 1973).

### Preparation of extracts :

Preparation of extract and screening for phytochemicals were done by following the standard protocol which is explained in detail as below.

To prepare the acetonic, methanolic, chloroformic and petroleum ether extracts, 150 g of each plant material was collected, dried in the oven at 70°C for 4 hrs and grinded to powder. It was separately macerated with the above solvents and allowed to stand for 72 hrs and then filtered. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored at 5°C in the refrigerator. For the aqueous extraction, 50 g of the plant powder was weighed into 50 ml Erylen-Mayer flask and

to this was added 400 ml of distilled water. This was heated to boil using hot plate. The mixture was stirred at regular intervals (3-5 min) for one hour after which it was filtered with No. 1 Whatman filter paper. The filtrate was then filtered, sterilized using a membrane filter of pore size 0.45 cm diameter. The extracts were concentrated in a hot water bath at 80°C for 5 hrs during which 0.5 g charcoal was added to decolorize it. Sterile decolorized filtered extract was then refrigerated at 5°C.

### Phytochemical screening :

#### Test for flavonoids :

0.5 g of various extracts was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80 per cent ethanol and filtered. The filtrate was used for the following tests: 3 ml of the filtrate was mixed with 4 ml of 1 per cent potassium hydroxide in a test tube and the colour was observed. A dark yellow colour indicated the presence of Flavonoids.

#### Test for alkaloids :

0.5 g of various extracts were mixed in 8 ml of 1 per cent HCl, warmed and filtered. 2 ml of the filtrate was treated separately with both reagents (Maeyer's and Dragendorff's), the alkaloids were observed by presence or absence of the turbidity or precipitation.

#### Test for glycosides :

ml each of various extracts were hydrolyzed separately with 5 ml each of conc. HCl and boiled for few hours on a water bath and hydrolysates were subjected to the following test: A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous 10 per cent sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

#### Test for steroids :

0.5 g of the various solvent extracts fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green indicated the presence of steroids.

#### Test for phenols :

To 1ml of various solvent extracts, 2ml of distilled water followed by a few drops of 10 per cent aqueous ferric chloride solution were added. Formation of blue

or green colour indicated the presence of phenols.

*Test for terpenoids (Salkowski test) :*

5 ml of various solvent extracts were mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated ( $H_2SO_4$ ). A layer of the reddish brown colouration was formed at the interface thus, indicating a positive result for the presence of terpenoids.

*Test for saponins :*

0.5 g of various solvent extracts was dissolved in boiling water in a test tube. Test cooling aqueous extracts were mixed vigorously to froth and the height of the froth was measured to determine the saponins contents in the sample. 2.0 g of the powdered plant material was boiled in distilled water in a test tube in boiling water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion thus, a characteristic of saponins.

*Test for resins :*

1 ml of various solvent extracts was treated with few drops of acetic anhydride solution followed by 1 ml of conc.  $H_2SO_4$ . Resins give colouration ranging from orange to yellow.

*Test for tannins :*

0.25 g of various solvent extracts were dissolved in 10 ml distilled water and filtered. 1 per cent aqueous Iron chloride ( $FeCl_3$ ) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples.

*Test for cardiac glycosides (Keller-Killani test) :*

5 ml of various solvent extracts were mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride ( $FeCl_3$ ) solution, followed by the addition of 1 ml concentrated sulphuric acid. Brown ring was formed at the interface which indicated the presence of deoxysugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer.

*Test for carboxylic acids :*

1 ml of the various extracts was separately treated with a few ml of sodium bicarbonate solution. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acid.

*Test for coumarins :*

0.5 g of the moistened various extracts were 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.

*Test for quinones :*

1 ml of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinones give coloration ranging from red to blue.

*Test for xanthoproteins :*

1 ml each of the various extracts was treated separately with few drops of concentrated  $HNO_3$  and  $NH_3$  solution. Formation of reddish orange precipitate indicates the presence of Xanthoproteins.

## RESULTS AND REMONSTRATION

The present study has shown remarkable variations in the number of phytochemicals present in the plants collected from different sites. The maximum solubility of the phytochemicals from the plants of all the sites was in methanol, acetone solvents and water. The details of phytochemical screening in the plants of *Hemidesmus indicus* are given in Table 1. The results from the present study are as follows.

### Site I :

Out of 14 phytochemicals studied, only flavonoids, alkaloids, glycosides, steroids, phenols, saponins, coumarins, quinones, Xanthoproteins are observed to be maximum and the other phytochemicals terpenoids, resins, tannins, cardiac glycosides, carboxylic groups are minimum.

### Site II :

In the site-II, steroids, terpenoids, cardiac glycosides, carboxylic groups, quinones are seen minimum

**Table 1 : Comparative phytochemical screening in root extracts of *Hemidesmus indicus***

Phytochemicals	Site I	Site II	Site III	Site IV	Site V	Site VI
Flavonoids	+++	+++	+++	+++	+++	+++
Alkaloids	+++	+++	+++	+++	+++	+++
Glycosides	+++	+	+	+	+++	+++
Steroids	+++	+	+	+++	+	+++
Phenols	+++	+++	+++	+++	+++	+++
Terpenoids	+	+	+	+++	+++	+++
Saponins	+++	+++	+	+++	+++	+++
Resins	+	+++	+	+++	+++	+++
Tannins	+	+++	+++	+++	+++	+++
Cardiac glycosides	+	+++	+	+++	+++	+++
Carboxylic acid	+	+	+	+++	+++	+++
Coumarins	+++	+	+	+++	+++	+++
Quinones	+++	+	+++	+++	+++	+++
Xanthoproteins	+++	+++	+	+++	+++	+++

whereas the other nine phytochemicals are maximum.

#### Site III :

There is a remarkable observation is noteworthy that only flavonoids, alkaloids, phenols, tannins and quinones are maximum while the other nine phytochemicals are observed to be minimum.

#### Site IV :

Except the glycosides, all the other 13 phytochemicals are in maximum of their presence.

#### Site V :

All the phytochemicals are observed to their maximum except steroids are in minimum.

#### Site VI :

The root extract from the plants of this site have shown a worth noting observation is that all the 14 phytochemicals studies were shown to their maximum presence (Table 1).

#### Conclusion :

From the present work on comparative studies of phytochemical screening in plant *Hemidesmus indicus* collected from different geographical areas shown considerable variations in presence of number of phytochemicals. The plants collected from site-VI are showing maximum presence of phytochemicals than the

other five sites. Production of some phytochemicals in one site and absence of the same in another site may be due to environmental induced production of certain phytochemicals or may be due to activation or suppression of certain genes producing the phytochemicals in particular environmental conditions. Hence, out of the plants collected from the above six sites, the plants of site-VI can be used to extract maximum number of phytochemicals.

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