Proceedings of the First Science Symposium – December, 2002
Faculty of Science, University of Ruhuna, Matara, Sri Lanka
35-38

Preliminary screening of solvent extracts of leaves, stem and root of *Hemidesmus indicus* for different types of secondary metabolites

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Abstract

Identification of the classes of secondary metabolites found in different parts of *Hemidesmus indicus* (in Singhala. 'Eramusu') is presented here. It was found that roots of the plant contain alkaloids, saponins, steroids, triterpenoids, flavanoids and cardiac glycosides, the leaves contain saponins, steroids and triterpenoids and the stem contains steroids and triterpenoids.

Introduction

Procurement of herbal drugs and their use to alleviate human sufferings are perhaps as old as human civilization. Plants with medicinal properties enjoyed the highest reputation in the indigenous system of medicine throughout the world. In fact, it has been estimated that the traditional system of medicine in Sri Lanka meets the basic health needs of about 70% of the population (WHO 1978). *H. indicus* is widely used as a medicinal herb especially in Asia (Jayaweera 1981). This plant is slender, latiferous, twining and prostrate or semi-erect shrub occurring over the greater part of India and in the low country of Sri Lanka. The whole plant of *H. indicus* is believed to be a demulcent, alterative, diaphoretic, diuretic and tonic. This is used in treatment of loss of appetite, fever, skin diseases, syphilis and inflammation of urinary passages (Jayaweera 1981).

Several active components from roots of *H. indicus* have been isolated and characterized (Chatterjee and Bhattacharya 1988). Some phytochemical screening results of the bark of this plant have also been reported (Gunatilaka and Sotheeswaran 1980) but not many studies have been carried out on leaves of *H. indicus*. Objective of this study was to find out the secondary metabolites present in roots, stem and leaves of *H. indicus*. For this purpose, initially extractions were carried out for leaves, stems and roots separately using sequential extraction methods. Afterward phytochemical screening for each extract and chemical screening for extracts were carried out.

Materials and methods

Source of plant material

Fresh plants of *H. indicus* were collected from nearby areas of Matara. Leaves, stems and roots were separated, washed, air dried and used immediately for the extraction.

Extraction of plant material

Sequential extractions with solvents of increasing polarity were carried out. Ground roots (150 g) were extracted with hexane (300 mL) followed by ethyl acetate (300 mL) and then with methanol (300 mL) using a Soxhlet extractor for four hours. The same procedure was followed for leaves (75 g of leaves and 150 mL of each solvent) and stems (75 g of stems and 150 mL of each solvent). Extracts were concentrated using a rotary evaporator.

Phytochemical screening tests for crude extracts of roots, leaves and stems of Hemidesmus indicus

Screening for alkaloids
A volume of extract equivalent to 25 g of plant material was evaporated to dryness using a rotary evaporator. 2N HCl (5 mL) was added and the mixture was heated on a steam bath for 5 minutes with stirring. This was filtered and transferred into four test tubes in equal volumes.

Mayer's Test
A few drops of Mayer's reagent were added to one of the test tubes and formation of turbidity or precipitate was noted.

Wagner's Test
A few drops of Wagner's reagent were added to the second test tube and observations were recorded as in Mayer's test above. Alkaloids are assumed to be absent in the plant extract, if no precipitate or turbidity is observed in either test tube. If precipitates were noted then the two remaining fractions were combined, basified with concentrated ammonia and the resulting solution was extracted with chloroform. The combined chloroform extracts were dried and concentrated. The resultant solution was subjected to a thin layer chromatographic (TLC) examination. The TLC plates were sprayed with Iodoplatinate or Dragendorff reagent.

Screening for saponins(Froth Test)
The crude extract of *Hemidesmus indicus* (100 mg) was taken in a test tube and water (10 mL) was added. The mixture was shaken vigorously and the froth formation was monitored with respect to its height above the surface of the liquid level. If the height of the froth remain at 3 cm or greater for more than 30 minutes the test is considered positive.

Screening for Steroids and Triterpenoids (Salkowski Test)
A volume of extract equivalent to 10 g of plant material was evaporated to dryness. The residue was stirred with light petroleum (10 mL) and the organic layer was discarded. The residue was dissolved in chloroform (10 mL) and was divided into three test tubes in equal volumes. One test tube was held at an angle of 45° and concentrated H_2SO_4 (2 mL) was allowed to run down the inner wall of the test tube. When the solution was gently mixed and if a cherry colour is formed, that is considered to indicate the presence of unsaturated steroids. The solution in the second test tube was used as a reference solution.

Liebermann-Buchardt Test
To the third test tube, about three drops of acetic anhydride was added and mixed. The detection of the colour change of the mixture was started immediately after the addition of one drop of conc. H_2SO_4 to the test tube. The colour change was monitored over a period of one hour. If the solution turns bluish green in colour that is considered to be an indicative of the presence of steroids while a red, pink or violet colour is considered to be an indicative of the presence of triterpenoids. A pale yellow colour is considered to indicate the presence of saturated steroids or triterpenoids in the extract.

Screening for Cardiac Glycosides
If Salkowski and Leibermann-Buchardt tests gave positive results, then Kedde Test (for the presence of unsaturated lactones) and Keller-Killiani Test (for the presence of 2-deoxy sugars) were performed. If the latter two tests are positive then the plant extract is considered to contain cardiac glycosides.

Kedde Test
On the center of a piece of chromatography paper, the plant extract (0.2 mL) was spotted and developed using chloroform as the mobile phase. The paper was dried and sprayed with Kedde reagent[4] and air-dried. The formation of purple spots on the paper is considered as a positive response to Kedde test.

Keller-Killiani Test
Plant extract (10 mL) was evaporated to dryness. The dried extract was defatted with light petroleum. Then the residue was treated with $FeCl_3$ reagent (3 mL). The test tube was held at an angle of 45° and conc. H_2SO_4 (2 mL) was added carefully along the inner wall of the test tube. Formation of a purple ring at the interphase is considered as a positive response to Keller- Killiani test.

Screening for Flavonoids
A volume of extract equivalent to 3 g of plant material was evaporated to dryness. The residue was defatted with light petroleum and was dissolved in rectified spirit (2 mL). The solution was divided into two test tubes in equal volumes. To one test tube conc. HCl (0.5 mL) and three magnesium turnings were added and it was shaken with octanol. If the colour of the solution turns orange, red or crimson within 10 minutes, it is considered as an indicative of the presence of flavanoids in the plant extract.

Chemical Tests
First, primary tests were carried out to identify the chemical nature of each extract. Then tests for functional groups were carried out. Presence of carbonyl group was tested using Brady's reagent. If an orange colour precipitate is formed it is considered as an indication of the presence of carbonyl compounds in the extract. Fehling's test was then carried out. If the colour of the precipitate with Fehling's reagent change from blue-green, yellow and then to red it is considered as an indicative of the presence of aldehydes in the plant extract. Presence of phenolic group was tested using $FeCl_3$ reagent. Formation of purple, red-purple or red coloured solution indicates the presence of phenolic compounds. The presence of phenolic compounds was further tested by carrying out dioazotization reaction.

Results and discussion
Phytochemical screening.
The inferences made based on the results of phytochemical screening for each extract of roots, leaves and stems of *H. indicus* are given in table 1.0. According to these results (Table 1.0), roots of *H. indicus* contain alkaloids, saponins, steroids, triterpenoids, flavanoids and cardiac glycosides. Leaves contain saponins, steroids and triterpenoids. Stem of the plant contains steroids and triterpenoids.

Table 1. Phytochemicals present in extracts of *H. indicus* (+ present, - absent)

Plant part	Solvent used for the extraction	Alkaloids	Saponins	Steroids and Triterpenoids	Flavanoids	Cardiac Glycosides
Root	Hexane	-	-	+	-	-
	Ethyl acetate	-	-	+	-	-
	Methanol	+	+	+	+	+
Leaf	Hexane	+	+	+	+	+
	Ethyl acetate	-	-	+	-	-
	Methanol	-	-	+	-	-
Stem	Hexane	-	-	-	-	-
	Ethyl acetate	-	-	+	-	-
	Methanol	-	-	+	-	-

Chemical analysis

The inferences made based on the results of the chemical analysis for each extract are given in the Table 2.

Table 2. Chemical analyses of extracts of *Hemidesmus indicus* (+ present, - absent)

Plant part	Solvent used for the extraction	Carbonyl Compounds	Phenolic Compounds
Root	Hexane	+	-
	Ethyl acetate	-	+
	Methanol	-	-
Leaf	Hexane	+	-
	Ethyl acetate	-	+
	Methanol	-	+
Stem	Hexane	-	-
	Ethyl acetate	-	+
	Methanol	-	+

According to the chemical tests carried out for the crude extracts, root and leaves of the plant contain both carbonyl compounds and phenolic compounds, while stem contains only phenolic compounds.

Conclusion

According to results of these analyses, it is clear that *H. indicus* contains a wide range of secondary metabolites including alkaloids, saponins, steroids, triterpenoids, flavanoids and cardiac glycosides.

Acknowledgement

Financial assistance by Ruhuna University (Ru/SF/RP/2000/04) is acknowledged.

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