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PRELIMINARY PHYTOCHEMICAL SCREENING OF STEM AND LEAVES EXTRACT OF DIPLOCYCLOS PALMATUS (L.) JEFFRY-SHIVALINGI

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Abstract

Diplocyclos palmatus Linn. commonly known as Shivalingi is a lesser heard and perennial climber having diversified medicinal values. So far no systematic studies were reported on phytochemical screening profile of stem and leaves extract of the selected plant. Hence, the present attempt was undertaken with an objective to investigate the presence of active phytochemicals.

Introduction

Diplocyclos palmatus (Family: Cucurbitaceae) is a perennial climber with thin stems growing up to 6m long. Native to Australia, it is more commonly known as the Lollipop Climber or Striped Cucumber (E), Shivalingi (H). The plant is native to Australia, Malesia, Papua New Guinea and Tropical Africa. It is largely distributed in warmer rain forests.1 It has been recorded in India as growing and spreading in the wild. Shivalingi is a perennial climber with hairless stem, becoming thickened and white dotted on the ridges when older. Leaves are broadly ovate, 3.5-14 x 4-14.5 cm, palmately lobed. Lobes are linear-lanceshaped to elliptic, hairless. Leaf stalk os 1.5-9.0 cm long. Flowers are small, white or yellowish, male in stalkless clusters of 2-8, along with 5 female flowers in the same axil. Sepal cup is 3-4 mm long in male, 1.5-2.5 mm long in female, sepals smaller than tube. Flower of male larger than female. Fruit is solitary, or in clusters of 2-5. It is ovoid-round, 1.5-2.5 cm. When ripe, it is red with longitudinal white stripes, and reminds one of lollipop, hence the common name. It is found in India, including the Himalayas, at altitudes of 200-1500 m. Flowering: August-October. It is used in India for its medicinal properties in the treatment of rheumatic pain, cough, flatulence and various skin diseases.2-3 Keeping in view the medicinal attributes of species as mentioned in folklore investigation of phytochemical aspects are required to reveals the presence of active phytochemicals in the extracts, therefore, the present work was undertaken.

Material and Methods

Selection, collection and authentication of plant/plant material

The plant parts were collected in the months of July-September 2016 from the various local sites of Rewa, M.P. and identified & authenticated by Dr. S. N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, M.P. and was deposited in our Laboratory, Voucher specimen No. PCog/DP/156.

Successive Extraction of Plant Material

Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered stem and leaves of D. palmatus (250gms) were loaded in Soxhlet apparatus and was extracted with petroleum ether (60-62°C), Chloroform, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined.

Preliminary Phytochemical Screening of Extract

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedure were adopted to perform the study.4-6

Tests for carbohydrates

Molisch's test

To the Sample 2-3 drops of 1% alcoholic - napthol solution and 2 ml of conc. sulphuric acid was added along the sides of the test tube. Appearance of purple to violet ring at the junction of two liquids shows the presence of carbohydrates.

Fehling test
To the sample add fehling reagent, appearance of brick red precipitate shows presence of carbohydrates.
Test for glycosides

Legal's test
To the sample add 1 ml of pyridine and few drops of sodium nitropruside solutions and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Borntrager's test
Sample was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

Baljet's test
To the sample add picric acid, orange color shows presence of glycosides.

Test for alkaloids
A small portion of the sample was stirred separately with few drops of dilute hydrochloric acid and was tested with various reagents for the presence of alkaloids.

The reagents are
Dragendroff's reagent - Reddish brown precipitates
Wagner;s reagent - Reddish brown precipitates
Mayer's reagent - Cream color precipitates
Hager's reagent - Yellow color precipitates

Test for proteins and free amino acids
Small quantities of the sample was dissolved in few ml of water and treated with following reagents.
Million's reagent: Appearance of red color shows the Presence of protein and free amino acid.
Ninhydrin reagent: Appearance of purple color shows the Presence of Proteins and free amino acids
Biuret's test: Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added. Appearance of pink or purple color shows the presence of proteins and amino acids.

Test for tannins and phenolic compounds
A small quantity of the sample was taken separately in water and test for the presence of phenol compounds and tannins was carried out with the following reagents.
Dilute Ferric chloride solution (5%) - Blue color or green color
10% lead acetate solution - White precipitates

Test for flavonoids
Alkaline reagent test
To the test solution add few drops of magnesium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicates presence of flavonoids.

Shinoda's test
Small quantities of the sample was dissolved in alcohol, to them piece of magnesium followed by conc. hydrochloric acid drop wise added and heated. Appearance of pink, crimson red, green to blue color shows the presence of flavonoids.

Tests for fixed oils and fats

Spot test
A small quantity of sample was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

Saponification test
Few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of sample along with a drop of phenolpthlein, the mixture was heated on a water bath for 1-2 hours, formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Tests for steroids and triterpenoids
Libermann-burchard test
Treat the sample with few drops of acetic anhydride, boil and cool. Then add con. sulphuric acid from the side of test tube, brown ring is formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoid.

Salkowski test
Treat the sample with few drop of conc. sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

Test for mucilage and gums
Small quantities of sample was added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitates was dried in oil and examined for its swelling property for the presence of gum and mucilage.
To the sample add ruthenium red solution, pink color shows presence of mucilage.

Test for waxes
To the test solution add alcoholic alkali solution, waxes get saponified.

Results and Discussions
The shade dried coarsely powder of stem and leaves and was successively extracted with petroleum ether, chloroform, ethanol and water in a soxhlet apparatus. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator. The percentage yields of various extract along with their color, nature and pH were presented in table 1.

Table 1: Percentage yield of various extracts of D. palmatus (L.) Jeffry

S./No.	Extract	Estimated percentage (%w/w)	Color of extract	Nature of extract	pH
1.	PEEDPS	1.90	Light Green	Semi Solid	7.12
2.	CEDPS	1.61	Green	Semi Solid	7.09
3.	EEDPS	9.89	Brown	Solid Powder	6.99
4.	AEDPS	10.59	Brown	Solid Powder	7.01
5.	PEEDPL	2.67	Green	Solid	7.05
6.	CEDPL	1.98	Dark Green	Semi solid	7.10
7.	EEDPL	12.84	Light Brown	Solid Powder	7.01
8.	AEDPL	15.43	Brown	Solid Powder	7.0

Abbr.: PEEDPS: Petroleum ether extract of D. palmatus Stem, CEDPS: Chloroform extract of D. palmatus Stem, EEDPS: Ethanolic extract of D. palmatus Stem, AEDPS: Aqueous extract of D. palmatus Stem, PEEDPL: Petroleum ether extract of D. palmatus Leaves, CEDPL: Chloroform extract of D. palmatus Leaves, EEDPL: Ethanolic extract of D. palmatus Leaves, AEDPL: Aqueous extract of D. palmatus Leaves. All values are Mean, n=3

Preliminary Phytochemical Screening of Extract
The extract obtained after extraction of plant material were subject to phytochemical screening

which revealed the present of various active phytoconstituents. The results were presented in table 2-3.

Table 2: Preliminary phytochemical screening of stem of D. palmatus

S/No.	Constituents	Stem Extract			
		PEEDPS	CEDPS	EEDPS	AEDPS
1.	Carbohydrates	+	+	+	+
2.	Glycosides	-	-	-	-
3.	Alkaloids	-	-	+	+
4.	Protein & Amino acid	-	-	-	+
5.	Tannins & Phenolic compounds	-	-	-	-
6.	Flavonoids	-	+	+	+
7.	Fixed oil and Fats	-	-	-	-
8.	Steriods & Triterpenoids	+	-	+	+
9.	Waxes	-	-	-	-
10.	Mucilage & Gums	-	-	-	-

Abbr. - = Absent, + = Present

Table 3: Preliminary phytochemical screening of leaves of D. palmatus

S/No.	Constituents	Leaves Extract			
		PEEDPL	CEDPL	EEDPL	AEDPL
1.	Carbohydrates	+	+	+	+
2.	Glycosides	-	-	-	-
3.	Alkaloids	-	+	+	+
4.	Protein & Amino acid	-	-	+	+
5.	Tannins & Phenolic compounds	-	-	-	-
6.	Flavonoids	-	+	+	+
7.	Fixed oil and Fats	-	-	-	-
8.	Steriods & Triterpenoids	+	-	+	+
9.	Waxes	-	-	-	-
10.	Mucilage & Gums	+	-	+	+

Abbr. - = Absent, + = Present

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