PHYTOCHEMICAL ANALYSIS OF LEAF AND STEM OF *Hydnocarpus pendandra* (Buch.-Ham.) Oken (ACHARIACEAE)

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ABSTRACT

The present study focuses on the preliminary phytochemical profile of leaf and stem of *H.pendandra*. The preliminary phytochemical screening showed the presence of Alkaloids, Flavonoids, Carbohydrates, Glycosides, Steroids, Phenols, Proteins, Tannis, Saponins, Terpenoids, and fixed oil.

Key words: *Hydnocarpus pendandra* Phytochemical profile, Plant extracts, Achariaceae and Kerala

INTRODUCTION

Medicinal plants possess potential chemical compounds which exhibit the properties of healing and pain relieving. The genus *Hydnocarpus* (Achariaceae) includes forty species that are spread across the globe. In the Indian System of Medicine, *Hydnocarpus pendandra* (Buch.-Ham.) Oken. is primarily used for treating leprosy and other skin disorders. It is known as "Chaulmoogra" and is also used to treat other indications including constipation, inflammation, blood disorders, and worm infestations. Species of Hydnocarpus are also used in traditional medicine in China, Thailand, Malaysia, and Myanmar for skin disorders. This article intends to validate the traditional uses of the selected species.

There is a spreading phenomena of antimicrobial resistance by pathogen there is an intensive search for natural compound from plant to combat diseases. Plants serve as the main source of food with copious nutrients content. Traditional societies around the world had inherited deep knowledge of various plants and their medicinal value, though they lacked knowledge on components present and their mode of action. Medicinal properties attributed to various medicinal herbs have paved way to the discovery of new drugs. The oil of *H.pendandra* play the greatest role in medicinal field.

MATERIALS AND METHODS

Collection and Authentication

The plant was collected from the Western Ghats, Kerala, India, during April 2017. The plant was identified by Dr. S.John Britto, Director and Head, The Rapinat Herbarium and Center for Molecular Systematics St. Joseph's College (*Autonomous*) Tiruchirappalli, India. The voucher specimen was deposited at The Rapinat Herbarium.

Extraction of plant material

Leaves were air dried under shade at room temperature, ground with electric grinder into fine powder and stored in air tight container for further use. 10 grams of powdered sample mixed in 150 ml of solvents (i.e. methanol, ethanol, acetone, Chloroform, Petroleum either and water) for extraction, was kept in rotary shaker for three days at room temperature. The extracts were filtered by using filter paper then air dried and stored for further usage. The crude extracts were further re-suspended in 1 ml of respective solvents for the investigation of phytochemical and antibacterial activities.

Phytochemical screening

Test for alkaloids

Wagner's Test: 2 ml of extract was treated with few drops Wager's reagent. Formation of reddish brown precipitate indicated the presence of alkaloids.

Hager's Test: 2 ml of extract was treated with few drops of Hager's reagent (saturated solution of picric acid). Formation of yellow color precipitate signified positive result.

Mayer's Test: 2 ml of extract was treated with few drops of Mayer's reagent. Formation of cream precipitate indicated the presence of alkaloids.

Test for proteins

Biuret Test: 2 ml of extract was treated with 2 ml 5% NaOH and 2 ml 1% $CuSO_4$ solutions. Violet or purple coloration indicated presence of proteins and free amino acids.

Xanthoprotetic Test: 2 ml of extract was treated with few drops of concentrated HNO_3. Formation of yellow colour indicated the presence of proteins.

Conc. H_2SO_4 Test: 2 ml extract was treated with few drops of conc. H_2SO4. Formation of white precipitate indicated the presence of proteins.

Xantho proteins Test: 2 ml of extract was treated with few drops of conc. HNO3 and NH3 solution. Formation of reddish orange precipitate indicated the presence of xantho proteins.

Test for amino acids

Ninhydrin test: 2 ml of extract was treated with 1ml of freshly prepared 0.25% ninhydrin reagent and boil it for few minutes. Formation of blue color indicated the presence of amino acids.

Test for flavonoids

Alkaline Test: 2-3 ml of extract was treated with few drops of NaOH solution. Formation of intense yellow color which turned colorless on addition of few drops of dilute HCl.

Pew's tests: 2-3 ml of extract was treated with zinc powder in a test tube, followed by drop wise addition of conc. HCl. Formation of purple, red or cherry color indicates the presence of flavonoids.

Lead acetate test: 1 ml extract was treated with 1 ml 10% lead acetate ($Pb(OAc)_4$) solution. Formation of yellow Color precipitate indicated the presence of flavonoids

Conc.H_2SO_4 test: 5ml of dilute ammonia solution was added to the extract followed by conc.H_2SO_4. Yellow color indicated the presence of flavonoids.

Test for fixed oils

$CuSO_4$ test: 2 ml of extract was treated with 1 ml of 1% $CuSO_4$ solution and 10% NaOH solution. Blue coloration indicated the presence of fixed oils.

Test for phenols and tannins

Ferric chloride test: 2 ml of extract was treated 2-3 drops of 5% ferric chloride solution. Formation of bluish-black color showed presence of phenols and black color shows tannins.

Potassium dichromate test: 2 ml of extract was treated with 5% potassium dichromate solution. Positive result was confirmed by a formation of brown precipitate (for phenol).

Braymer's Test: 2 ml of extract was treated with 2 ml H_2O and followed with 2-3 drops of $FeCl_3$ (5%). Green precipitate proved presence of tannins.

Test for Coumarins: 2 ml of extract was treated with 3 ml of 10% NaOH solution. Yellow coloration indicated the presence of coumarins.

Test for saponins

Foam Test: 2 ml extract was diluted with 10 ml of distilled water and warmed gently. It was shaken for 5 minutes. Persistent froth indicated the presence of saponins. The same extract was added with few drops of olive oil. Formation of a soluble emulsion, confirmed the presence of saponins.

Test for Glycosides

Keller kiliani Test (*Test for cardiac glycoside*)**:** 2 ml extract was treated with 1 ml glacial acetic acid, one drop 5% $FeCl_3$ and 1 ml conc. H_2SO_4. A brown ring of the interface indicated the presence of cardiac glycosides.

Glycoside Test: Small amount of extract was treated with 1 ml water and shake well. Then aqueous NaOH was added. Yellow color appeared that indicated the presence of glycosides.

Test for sterols

Salkowski's Test: 2 ml of extract was treated with 2 ml chloroform and 2 ml conc. H_2SO_4. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of sterols.

Keller killiani Test: (*Test for cardiac glycoside*)**:** 2 ml extract was treated with 1 ml glacial acetic acid, one drop. 5% $FeCl_3$ and 1 ml conc. H_2SO_4. A brown ring of the interface indicated the presence of cardiac glycosides.

Test for Terpenoids

Salkowski's Test: 2 ml of chloroform and 1 ml of conc.H_2SO_4 was added to 1 ml of extract and observed for reddish brown color that indicated the presence of terpenoids.

RESULT AND DISCUSSION

The results of qualitative screening of phytochemicals of *H.pendandra* leaf and stem showed the presence of Alkaloids, Carbohydrates, Glycosides, Flavonoids, Phenols, Tannins, and Fixed oils, Sponins, Sterols and Terpenoids. High concentrations of phytochemicals were found in methanolic, ethanolic, acetone and aqueous extracts while a very low concentration in chloroform and petroleum ether extracts (Table 1).

Table 1. Phytochemical screening of *Hydnocarpus pendandra* leaf and Stem sample

S.No.	Phytochemical constituents	Extracts											
		Acetone		Aqueous		Chloroform		Ethanol		Methanol		Petroleum ether	
		L	S	L	S	L	S	L	S	L	S	L	S
1.	Test for Alkaloids												
	Hager's Test	++	+	++	++	-	-	++	-	++	++	+	-
	Mayer's Test	++	++	+++	++	-	-	++	-	++	++	-	-
	Wagner's Test	++	++	-	++	-	-	++	+	++	++	-	+
2.	Test for Carbohydrates												
	Molisch's Test	+	++	++	+	-	-	+	++	+	++	-	-
	Fehling test	+	-	+	-	-	-	-	+	-	+	-	-
	Benedict's Test	+	+	+	-	-	-	+	+	+	+	-	-
3.	Test for Flavanoids												
	Alkaline Test	+	+	+	++	-	-	+	-	+	+	-	-
	Conc.H_2SO_4 Test	+	+	++	+	-	-	+	+	++	+	-	-
	Pew's Test	++	++	++	-	-	-	+	++	++	++	-	-
	Lead acetate	++	+	++	+	-	-	++	+	++	+	-	-
4.	Test for fixed oils												
	$CuSO_4$ Test	++	+++	++	++	+	+	++	++	+	++	+	+
5.	Test for Phenols												
	Ferric chloride Test	+++	+	++	-	-	-	++	++	++	++	-	-
	Potassium Dichromate Test	-	+	-	-	-	-	-	++	-	++	-	-
6.	Test for Tannins												
	Ferric chloride Test	-	+	+	-	-	-	+	+	+	+	-	-
	Braymer's Test	+	+	+	-	-	-	+	+	+	+	-	-
7.	Test for saponins												
	Foam Test	++	+	++	+	-	-	++	+	+	++	+	-
8.	Test for Glycosides												
	Keller kiliani Test	++	++	++	+	-	+	++	++	++	++	-	-
	Glycoside Test	+	+	+	+	-	+	+	+	+	+	-	-
9.	Test for Coumarins												
	10%NaOH Test	+	+	+	+	-	+	+	+	+	+	+	-
10.	Test for Sterols												
	Salkowshi's Test	+	++	+	-	+	+	+	++	+	++	-	-
	Keller killiani Test	++	++	++	+	-	+	++	++	++	++	-	-
11.	Test for Proteins												
	Biuret Test	-	-	-	-	-	-	-	-	-	-	-	-
	Xanthoproteic Test	+	+	+	+	+	+	+	+	+	+	-	+
	Conc.H_2SO_4 Test	-	-	-	+	+	-	+	+	+	+	-	-
12.	Test for Amino acids												
	Ninhydrin Test	-	-	-	-	-	-	-	-	-	-	-	-
13.	Test for Terpenoids												
	Salkowshi's Test	++	++	++	+		-	+		++	++	++	++

CONCLUSION

The study on the leaf and stem of *H.pendandra* for its phytochemical constituents has revealed the presence of secondary metabolites. Methanol, ethanol, acetone and aqueous are good extractive solvents. Further research on *H.pendandra* is necessary for elucidating the active principles and their mode of action.

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