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TLC profiling and phytochemical screening of *Podophyllum hexandrum* Royle– an endangered medicinal plant

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ABSTRACT

Plants are a tremendous source for the discovery of new products of medicinal value for drug development. Today several distinct chemicals derived from plants are important drugs currently used in one or more countries in the world. *Podophyllum hexandrum* contains several lignans which possess antitumor activity. Podophyllotoxin is the most active cytotoxic natural product. It is used as starting compound for the synthesis of anticancer drug etoposide and tenoposide. These drugs are used for lung cancer, testicular cancer, neuroblastoma, hepatoma and other tumours. The plant is an endangered species and is included in the Red Book. Preliminary TLC profiling and phytochemical screenings were carried out on the rhizome and leaf extracts of this plant. The qualification of podophyllotoxin was performed by thin layer chromatography (TLC) with R_f values of 0.85 (leaf) and 0.94 (root) when compared with the standard. Phytochemical screening revealed the presence of alkaloids, carbohydrates, phenols, glycosides, flavonoids, saponins and terpenes in seed, leaf and rhizome extracts (ethyl acetate and methanolic). However seed and leaf extracts of *P hexandrum* showed the absence of saponins.

Keywords: *P hexandrum*; flavonoids; saponins; podophyllotoxin; phytochemical

INTRODUCTION

Podophyllum hexandrum is a moisture and shade loving erect, glabrous, succulent herb thriving from Kashmir to Sikkim in Himalayas at altitudes ranging from 2500-4000 m. The common names of *P hexandrum* are Indian Mayapple, Bankakri, Papri, Banwangan etc. The plant grows to 46 to 61 cm (1.5 to 2 feet) in height with petiole bearing one or two leaves with round blades up to 30 cm in diameter. Plant with two leaves bears a single pendulous small white flower in May (hence the name Mayapple) the odour of which has been described as 'nauseous'. The fruit which ripens in mid-summer is about 2.5 to 5 cm in length and is pulpy, lemon yellow and berry-like. The fruit is the only edible part of plant but insipid (Chatterjee 1952, Hutchison 1959, Rix 1982, Dewick and Shaw 1988) all other are considered as poisonous. Mature fruits contain up to 25 seeds each in a mucilaginous aril. The seeds are dark brown, oval, flattened and tapered at the apex with a dull brown rough surface

and an oval inconspicuous hilum. As the seeds age they become wrinkled (Krochmal et al 1974). *P hexandrum* has been extensively exploited in Ayurvedic system of medicine for treatment of ailments like constipation, cold, biliary fever, septic wounds, inflammation, burning sensation, mental disorder, genital warts, monocytoid leukemia, Hodgkin's and non Hodgkin's lymphoma (Singh and Shah 1994). Podophyllotoxin is one such secondary metabolite and is produced by *P hexandrum*. Podophyllotoxin a lignan is a plant-based pharmacologically active compound which has been shown to possess cytotoxic activities (Imbert 1998). It is currently isolated from the rhizomes of *P hexandrum.* In the search of novel, effective and non-toxic radio protectants a number of plant products have been evaluated for the plant protection against lethal dose of radiation including *P hexandrum* (Goel et al 1998, Rajesh et al 2005) and it was found that pre-radiation administration of the extracts of *P hexandrum* mitigated radiation induced postnatal and physiological alternations and proved highly effective in control of both planned and unplanned radiation exposure (Goel et al 2002). Recently *P hexandrum* extracts have been reported to offer radioprotection by modulating free radical flux involving the role of lignans presents (Chawla et al 2006). Due to its anticancerous property podophyllotoxin is in increased demand throughout the world. Total synthesis of podophyllotoxin is an extensive process and availability of compound from natural resource is an important issue for pharmaceutical companies that manufacture these drugs (Canel et al 2000).

MATERIAL and METHODS

Collection of plant material

The plant samples of *P hexandrum* were collected from Dr Yashwant Singh Parmar University of Horticulture and Forestry, Medicinal and Aromatic Plants Research Station, Rahla, Manali, Kullu, HP. The authentic seeds were procured from Jammu and Kashmir Medicinal Plant Introduction Centre, Srinagar, J&K. The plant parts (roots and leaves) were shade-dried for some days and ground into powder with the help of an electric grinder and later stored in air tight bottles for further use.

Preparation of extracts

Solvent extract: The 50 g of each powdered plant material was filled in the thistle funnels of two separate soxhlet extractors and extracted with 250 ml 99 per cent methanol (MERCK) up to 48 hours. After filtration extract was concentrated to dryness at 40°C. Since the fresh plant material contains water the extracts were further concentrated to dryness by means of a freeze-dryer to extract any excess water from the sample. It was stored at 4°C until further use.

Thin layer chromatography (TLC) analysis

Thin layer chromatography of the samples of root and leaf methanol extract of *P hexandrum* was done using the solvent chloroform and methanol (9:1) and the R_f value was calculated. R_f value was 0.85 for leaf and 0.94 for root sample.

Phytochemical screening

The Phytochemical screening of plant extract (seed, rhizome and leaf) samples was carried out by using the procedures of Kokate (1994) and Kokate et al (1995).

Detection of alkaloids

Mayer's test: To a few ml of filtrate a drop or two of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate indicated the test as positive.

Mayer's reagent: Mercuric chloride (1–35 g) was dissolved in 60 ml of water and potassium iodide (5.0 g) was dissolved in 10 ml of water. The two solutions were mixed and made up to 100 ml with water.

Wagner's test: To a few ml of filtrate few drops of Wagner's reagent were added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.

Wagner's reagent: Iodine (1.27 g) and potassium iodide (2 g) were dissolved in 5 ml of water and made up to 100 ml with distilled water.

Detection of carbohydrates

Fehling's test: One ml of filtrate was boiled on water bath with 1 ml each of Fehling solutions A and B. A red precipitate indicated the presence of sugar.

Fehling's solution A: Copper sulphate (34.66 g) was dissolved in distilled water and made up to 500 ml distilled water.

Fehling's solution B: Potassium sodium tartarate (173 g) and sodium hydroxide (50 g) were dissolved in water and made up to 500 ml.

Benedict's test: To 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic coloured precipitate indicated the presence of sugar.

Benedict's reagent: Sodium citrate (173 g) and sodium carbonate (100 g) were dissolved in 800 ml distilled water and boiled to make it clear. Copper sulphate (17.3 g) dissolved in 100 ml distilled water was added to it.

Detection of phenolic compounds and tannins

Ferric chloride test: The extract (50 mg) was dissolved in 5 ml of distilled water. To this few drops of neutral 5 per cent ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Lead acetate test: The extract (50 mg) was dissolved in distilled water and 3 ml of 10 per cent lead acetate solution was added. A bulky white precipitate indicated the presence of phenol compounds.

Detection of glycosides

To 2 ml extract glacial acetic acid few drops of 5 per cent $FeCl_3$ and conc H_2SO_4 were added. Reddish brown colour at the junction of two liquid layers and upper layer that appeared bluish green indicated the presence of glycosides (Trease and Evans 1989).

Detection of flavonoids

Few drops of 10 per cent concentrated sulphuric acid were added to the extract followed by 1 ml of ammonia. Formation of greenish yellow precipitate indicated the presence of flavonoids (Siddiqui and Ali 1997).

Detection of terpenes

To 2 ml of extract 5 ml chloroform and 2 ml conc H_2SO_4 were added. Reddish brown colouration of interface indicated the presence of terpenes (Harborne 1973).

Detection of saponins

Foam test: The extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of saponins (Kokate 1999).

RESULTS and DISCUSSION

Aim of the present study was to investigate the TLC profiling and phytochemical of *P hexandrum*. It was found that high content of alkaloids, carbohydrates, flavonoids, glycosides, terpenes and phenols was present in the seed, rhizome and leaf extracts of *P hexandrum*. However seed and leaf extracts of *P hexandrum* showed the absence of saponins except rhizome extract (Table 1). The phytochemical analysis of methanolic extract of *P hexandrum* revealed that plant extract contained glycosides, flavonoids, saponins and terpenes (Wani et al 2012a). Phytochemical screening in two different rhizome extracts (aqueous and methanolic) of *P hexandrum* revealed the presence of diverse groups of phyto–constituents (Wani et al 2012b). Thin layer chromatography was performed on two different extracts (methanolic and aqueous) using the solvent system chloroform:methanol (9:1). After performing TLC of both the extracts R_f values were calculated for all the spots seen under container containing iodine chamber.

Qualitative analysis performed on the root and leaf extracts of *P hexandrum* showed the presence of podophyllotoxin performed by thin layer chromatography (TLC) with R_f values of 0.87 (leaf) and 0.94 (root) when compared with the standard. Phytochemical screening indicated the presence of alkaloids,

Table 1. Phytochemical analysis of *Podophyllum hexandrum* extract

Phytochemical test	Plant part		
	Seed	Rhizome	Leaf
Detection of alkaloids			
Mayer's test	+	+	+
Wagner's test	+	+	+
Detection of carbohydrates			
Fehling's test	+	+	+
Benedict's test	+	+	+
Detection of phenolic compounds			
Ferric chloride test	+	+	+
Lead acetate test	+	+	+
Detection of glycosides	+	+	+
Detection of flavonoids	+	+	+
Detection of terpenes	+	+	+
Detection of saponins by foam test	-	+	-

Fig 1. Leaf extract

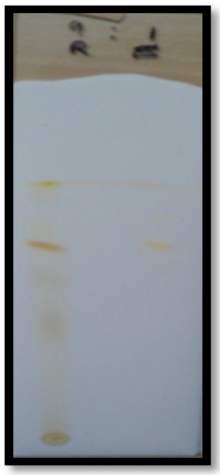

Fig 2. Root extract

carbohydrates, phenols, glycosides, flavonoids, saponins and terpenes in seed, leaf and rhizome extracts (methanolic). However, seed and leaf extracts of *P hexandrum* showed the absence of saponins.

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