

PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACT OF *AESCULUS INDICA*

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ABSTRACT

The aim of the study was to evaluate the phyto-constituents present in *Aesculus indica* leaves. The present study on *Aesculus indica* leaves shows that various phyto-constituents like saponins, flavonoids, tannins, amino acids, carbohydrates, triterpenoids and steroids were present but alkaloids and glycosides were found to be absent in both extracts in this plant and this plant found to be useful as traditional medicine against various human ailments. This article describes methods for performing qualitative and quantitative analysis of both the aqueous and ethanolic extracts of medicinal plant *Aesculus indica*. The amount of the saponins and tannins in ethanolic extract (13.4% and 5% respectively). These findings suggest that the rich phytochemical content of *Aesculus indica* may be responsible for its popular and wide traditional use.

Keywords: *Aesculus indica*, leaves, Phytochemical analysis.

INTRODUCTION

Various plant species has served as a source of medicine for people all over the world, for year's plants is one of the most intense area of natural product research yet the field is far for being exhausted¹. Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect². The medicinal values of these plants lie in their component phytochemicals, which produce definite physiological actions on the human body. The most important of these phytochemicals of plants are alkaloids, saponins, tannins, glycosides, flavonoids and phenolic components³. The detection of these active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude plant extracts and is very important in regards to their potential pharmacological effects⁴.

Aesculus indica (Hippocastanaceae), known as Himalayan chest nut or Indian horse chestnut is a large tree, distributed in the Himalayas from Kashmir to Nepal. It is one of the common plants seen in the Himalayan forest, lying at elevations between 2000 and 3000 meters⁵. It is generally known for its medicinal properties. Seeds are used as astringent, nutritious, while the oil is used in the treatment of skin disease and rheumatism, fruits are used in colic disorders⁶ and roots are used in leucorrhoea treatment⁷. The plant is reported to contain a mixture of saponins, one of which is described as aescin. It also contains flavonoids, glycosides, tannins and phenolic substances⁸. *Aesculus indica* is generally known for its medicinal properties. *Aesculus indica* plant pharmacologically to have anti-oxidant⁹, anti-viral⁹, immune-modulatory⁷, anti-inflammatory¹⁰, spasmolytic¹¹ and neurodepressive¹² activities. Hence, this study mainly dealt with phytochemical screening of *Aesculus indica* leaves.

MATERIALS AND METHODS

Collection and Authentication of Plant materials

Leaves of *Aesculus indica* were collected from Kullu, in September 2010 and dried in shade, then crushed to coarse powder. *Aesculus indica* was identified by a senior scientist, Dr.R.Raina and voucher specimen of *Aesculus indica* (5586) was deposited in the Herbarium of Forest Products Department, University of Horticulture & Forestry, Nauni, Solan (H.P.) India.

Preparation of extracts

Preparation of aqueous extract

Decoction

The dried leaves of *Aesculus indica* (15g) was subjected to decoction with distilled water (300ml) for 1-2 hrs. It was continuously stirred at an interval of 4-5min. The extract was filtered using a whatmann

filter paper and then concentrated on a hot water bath. Then extract was kept in refrigerator at 5°C for experimentation.

Preparation of alcoholic extract

Soxhlation

The dried leaves of *Aesculus indica* (40g) was placed in a thimble. The thimble was placed in the extraction chamber, which was suspended above a flask containing the solvent (ethanol (500ml) respectively) and below a condenser. The flask was heated and the solvent evaporated up into the condenser that trickled into the extraction chamber containing the plant material and then trickled back down into the boiling flask. A number of cycles were repeated for about 36hr. The extract was then filtered through the whatmann filter paper and concentrated on a hot water bath. Then extract was kept in refrigerator at 5°C for experimentation.

Qualitative Phytochemical Screening

The leaves extracts of *Aesculus indica* was analysed for the presence of alkaloids, saponins, tannins, cardiac glycosides, anthraquinone glycosides, flavonoids, amino acids, steroids, terpenoids, and carbohydrates according to standard methods^{13,14}.

Test for Alkaloids

To 1ml of acidic aqueous solution of samples few drops of Mayer's reagent was added. Formation of white or pale precipitate showed the presence of alkaloids.

Test for Saponins

1gm of extract was dissolved in 10 ml of distilled water in a test tube and shaken vigorously for 1-2 min. The presence of saponins was indicated by characteristic froth at least 1 cm in height, which persisted for 30 min.

Test for Tannins

Ferric chloride test: To 5 ml of extract solution, few drops of ferric chloride test reagent were added. An intense green, purple, blue or black color was taken as an evidence for the presence of tannins.

Lead acetate test: 5 ml of the extract and a few drops of 10% lead acetate were added. Precipitate was formed, indicates the presence of tannins.

Test for Cardiac Glycosides

Keller Killiani's test: To 0.5g of plant extract, add 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride. Transfer to a small test tube; add carefully 0.5 ml of concentrated Sulphuric acid by the side of the test tube, blue color appears in the acetic acid layer.

Test for Anthraquinone Glycosides

Borntrager's test: 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red or violet coloration in the ammonical phase indicated the presence of anthraquinone.

Test for Flavonoids

Shinoda Test: To the test solution add few fragments of magnesium ribbon and add concentrated Hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

Test for Amino acids

Amino acids and proteins when boiled with few drops of 5% solution of nin-hydrin, violet color appears.

Test for Sterols and Triterpenoids

Libermann-Burchard test: Extract treated with few drops of acetic anhydride, boil and cool, concentrated Sulphuric acid is added from the side of the test tube, shows brown ring at the junction of two layers and the upper layer turns green which shows the presence of sterols and formation of deep red color indicates the presence of triterpenoids.

Tests for Carbohydrates

Molisch's test: Treat the test solution with few drops of alcoholic α -naphthol. Add 0.2ml of conc. H_2SO_4 , purple ring is seen at the junction.

Fehling's test: Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents are mixed and few drops of sample is added and boiled, a brick red precipitate of cuprous oxide forms, if reducing sugars are present.

Table 1: Qualitative analysis of the phytochemical of leaf extracts of *Aesculus indica*

S/No.	Phytoconstituents	Inference	
		Aqueous Extract	Ethanolic Extract
1.	Alkaloids	-	-
2.	Saponins	+	+
3.	Tannins	+	+
4.	Glycosides		
	Borntrager's test	-	-
	Keller-killiani test	-	-
5.	Flavonoids	+	+
6.	Amino acids	+	+
7.	Steroids & Terpenoids	+	+
8.	Carbohydrates		
	Molisch's test	+	+
	Fehling test (reducing sugar)	+	+

+ indicate the presence of constituents and – indicate the absence of constituents

Quantitative Phytochemical Screening

Determination of Saponins

20g of sample powder was dispersed in 200ml of 20% aqueous ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous NaCl. The remaining solution was heated in a water bath. After evaporation, the samples

were dried in the oven to constant weight and the saponin content was calculated in percentage¹⁵.

Determination of Tannins

Samples are analyzed by adding 1 ml sample and 5 ml indigo carmine to the 500 ml flask and adding 200 ml water. Titrate this against the Potassium permanganate solution (N/40 or 0.005M) until the royal blue fades to a light green. Then titrate drop-wise until the lime green changes to yellow. Record this value as X ml.

A blank titration using 5 ml of indigo carmine alone in 200 ml water should also be carried out. The blank value should be 1 ml and should be recorded as Y ml.

Total Tannin (%) = $(X-Y)/10$ expressed as 'tannic acid' equivalents¹⁶.

Table 2: Quantitative analysis of the phytochemicals of ethanolic leaf extracts of *Aesculus indica*.

S.No.	Phytoconstituents	% Yield
1.	Total Saponin content	13.4%
2.	Total Tannin content	5%

RESULTS

The results of qualitative analysis of *Aesculus indica* leaves in aqueous and ethanolic extracts are shown in Table 1. The results obtained showed the presence of saponins, tannins, flavonoids, amino acids, steroids and terpenoids are present in both extracts. Alkaloids and Glycosides were found to be absent in both extracts, while absence of reducing sugar in ethanolic extract of *Aesculus indica*. Results of quantitative estimation of the saponins and tannins in the ethanolic extract of leaves of *Aesculus indica* (% and % respectively) shown in Table 2.

DISCUSSION

In this study, the results of the investigations showed that the plant material possessed almost all the important secondary metabolites. *Aesculus indica* leaves showed positive results for all the constituents analyzed, except for one i.e., alkaloids and glycosides. [Tab.1]

Medicinal plants have been used as remedies for human diseases for centuries.

Biological activity of crude drug is mainly due to the active chemical constituents¹⁷. The medicinal value of plants lies in some chemical substances (usually secondary metabolites), that produce a definite physiological action on the human body.

Saponins are glycosides occurring widely in plants. They are abundant in many foods consumed by animals and man¹⁸. In medicine, it is used as antibiotic¹⁹, antiviral²⁰, anti-inflammatory²¹ and anti-ulcer²². Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action²³.

Triterpenoids and Sterols from plants and fungi possess antitumor-promoting and anti-inflammatory activities²⁴. Tannins are known to possess general antimicrobial and antioxidant activities²⁵. Recent reports show that tannins may have potential value as cytotoxic and antineoplastic agents²⁶. Other compounds like saponins also have antifungal properties^{27, 28}.

Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity^{29, 30}.

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