



Preliminary Phytochemical Screening of Extracts of *Robinia pseudoacacia*

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Abstract

The Robinia pseudoacacia (Fabaceae) is one of the medicinal plants, Native to North America, commonly distributed in various regions of Kashmir. The plant is used as an antispasmodium, febrifuge, antioxidant; diuretic, emollient, antitumor etc. The present study was carried out to screen different solvent extracts of the Robinia pseudoacacia phytochemically for the presence of active constituents like Alkaloids, Flavonoids Saponins, Tannins, and Phenols. However, the positive results for the detection of Flavonoids, Tannins and phenols were obtained. The maximum yield of ethyl acetate extract of plant leaves 38.23% was obtained.

Keywords: *Robinia; Extract; Antioxidant; Diuretic; Antitumor; Alkaloids*

Introduction

Robinia pseudoacacia (Fabaceae or Leguminosae), commonly known as black locust, is a medium sized, melliferous tree which grows upto 6 metres commonly distributed in sandy and rocky soils. Leaves are pinnately compound, bluish green on top, pale underneath. They have a pair of short thorns at the base. Leaflets are oval shaped. Bark is light grey and deeply furred. Flowers are small, pear shaped, formed in droopy clusture. Flowering period occurs in late spring, from May to June. It is a native plant in North America. In Kashmir, *Robinia pseudoacacia* frequently occurs in district Kupwara and banks of Mansbal Lake, Ganderbal [1].

The plant is used as an antispasmodic, febrifuge, antioxidant, diuretic, emollient, laxative, antitumor, and antimicrobial [2]. Dried leaves are helpful in treatment of wounds caused by wounds. It acts as pain reliever. Used internally, it calms stomach burns, and is usually recommended to individuals who suffer from hyperacid gastritis and distensions. It is helpful in easing digestion. It has a sedating and calming effect and could be very useful in cases of headaches and stress. Infusion added

to baths can help young children who suffer from insomnia. Flower powder is used in cases of gastritis, duodenal and gastric ulcer. Four oligomeric flavonoids like Robinetinidol leucorobinetinidins, Robinetinidol-dihydro-robinetins, Robinetinidol-robinetin and robinetinidol-flavone have been reported. Five flavonoids like Acacetin, Secundiflorol, Mucronulatol, Isomucronulatol and Isovestitol have been isolated from the ethanolic extract of the whole plant. A bioactivity-directed fractionation of the ethanolic extracts of *Robinia pseudoacacia* has yielded robinlin, a novel homomonoterpene. It has shown strong bioactivity in the brine shrimp lethality test (BST) [3]. All parts of the plant (except flowers) and especially the bark are considered to be toxic [4]. However, the toxins have been reported to get destroyed by heat. Literature survey revealed that Linalool, phenyl ethyl alcohol, methyl anthranilate, 1-hexanol and 3-methyl pyridine has been isolated from the oil of the flowers of *Robinia pseudoacacia* [5,6]. It also contains polyphenolic compounds in leaves such as tannins [7] and taxon specific monoterpene (Robinlin) [8]. In addition, some natural compounds with antibacterial activity have been

identified in the leaves [9-11]. The compounds like Secundiflorol, Mucronulatol, Isomucronulatol and Isovestitol were identified by spectral analyses and were reported from this species in an ethanolic extract of leaves of the plant *Robinia pseudoacacia* [12]. The minor lectin was named as RpbA11 is a homotetramers compound was also isolated [13,14].

Results and Discussion

Extraction

The powdered plant material was subjected to successive solvent extraction using solvents in increasing order of their polarity. Various solvents used were petroleum ether, ethyl acetate and methanol. The shade dried plant material subjected to sequential extraction in petroleum ether, ethyl acetate and methanol. Maximum yield was found in ethyl acetate extract (38.23%). Total extractive values are tabulated in (Table 1, Figure 1).

Table 1: Extractive values of various extracts.

S. No	Extracts	Extract values (%)
1	Petroleum ether	6.45
2	Ethyl acetate	38.23
3	Methanol	13.69

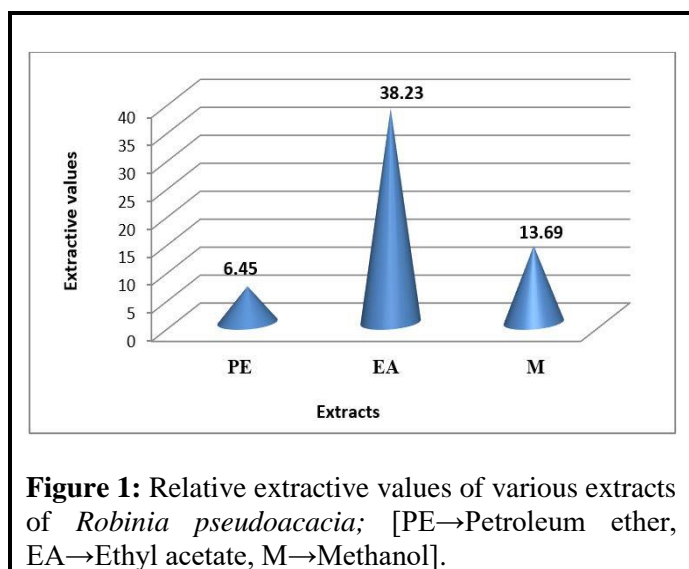


Figure 1: Relative extractive values of various extracts of *Robinia pseudoacacia*; [PE→Petroleum ether, EA→Ethyl acetate, M→Methanol].

Petroleum ether extraction

60 g of the powdered material was extracted with petroleum ether (hot extraction) using soxhlet apparatus at boiling point temperature for 9-10 h. The extraction was collected and dried in oven.

Ethyl acetate

After defating the plant material of *Robinia pseudoacacia* with petroleum ether, the material was extracted with ethyl acetate (hot extraction) using soxhlet apparatus at boiling temperature for 15-20 h. The extract was carefully filtered using Whatmann's filter paper and concentrated in vacuum under reduced pressure using rotary evaporation and dried in oven at 40°C.

Methanol extraction

Ethyl acetate extraction residue was dried completely and then extracted with methanol (hot extraction) using soxhlet apparatus at boiling temperature for about 48 hours. The extract was carefully filtered using a Whatmann's filter paper and finally concentrated in vacuum under reduced pressure using rotary evaporation. Concentrated extract was stored in labeled sterile bottle till further evaluation.

Phytochemical Screening Procedure

The different extracts so obtained were subjected to preliminary phytochemical screening. Phytochemical studies were performed to identify the presence of various phytoconstituents as follows:

Alkaloids

To the 2 ml of methanolic filtrate, 1.5 ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Mayer's reagent/Wagner's reagent/Dragendroff reagent was added. Formation of orange precipitate was observed to detect the presence of alkaloids [15].

Flavonoids

2 g plant material was extracted in 10 ml alcohol or water. To 2 ml filtrate few drops of concentrated HCl followed by 0.5 g of zinc or magnesium turnings was added. The solution was observed for the appearance of magenta red or pink color after 3 minutes [16].

Terpenes

To 2 ml of aqueous extract, 5 ml chloroform, 2 ml acetic anhydride and concentrated H₂SO₄ were added carefully to form layer. Reddish brown coloration of interface was observed to detect the presence of Terpenes [17].

Tannins

To 2 ml of aqueous extract 2 ml of 5% FeCl₃ was added and observed for the formation of yellow brown precipitate [16].

Saponins

Aqueous extract of 2 g powder was made the solution was shaken vigorously and observed for a stable persistent froth. The froth was mixed with few drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion [18].

Steroids

In a small quantity of petroleum ether extract, 2 ml of acetic anhydride solution was added in CHCl₃. This was followed by addition of conc.H₂SO₄. A green colour was produced which turns to blue indicates the presence of steroids.

Phenols

To 2 ml of alcohol or aqueous extract, 1 ml of 1% ferric chloride solution was added. Blue or green color indicated phenols [19].

Glycosides

A small quantity of the extract was dissolved in 1 ml of water. Sodium hydroxide solution was added; yellow colour appeared that indicates the presence of glycosides.

Anthraquinones

0.5 g of the extract was boiled 10 ml of sulphuric acid (H₂SO₄) and filtered while hot. The filtrate was shaken with 5 ml of chloroform (CHCl₃). The chloroform layers was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour change [17].

Phytochemicals analyses of various extracts

Preliminary phytochemical analysis of various extracts showed the presence of certain phytochemicals (constituents) in the extracts. The tests were performed using above standard procedures. The qualitative analysis of successive extracts of leaves of *Robinia pseudoacacia* has revealed the presence of Terpenoids, Flavonoids, Glycosides, Phenols, Tannins, and Steroids. However, Alkaloids, Saponins, Proteins, Anthraquinones were found to be absent (Table 2).

Table 2: Phytochemical screening of various extracts.

S. No	Constituents	Observation
1	Alkaloids	Absent
2	Anthraquinones	Absent
3	Flavonoids	Present
4	Glycosids	Present
5	Phenols	Present
6	Proteins	Absent
7	Saponins	Absent
8	Steroids	Present
9	Tannins	Present
10	Terpenoids	Present

Conclusion

In summary, different extracts of leaves of *Robinia pseudoacacia* have been obtained. These extracts were phytochemically screened for different active chemical constituents like Terpenoids, alkaloids, tannins, steroids, saponins, proteins, flavonoids and glycosids. The presence of terpenoids, steroids, glycosids and flavonoids in the extracts of leaves of *Robinia pseudoacacia* may become a field of research for their isolation and exploration in future.

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Conflict of Interest

The authors have no actual or potential conflicts of interest to report.

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