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Preliminary phytochemical screening of *Iris kashmiriana* Baker collected from Budgam, Kashmir, India

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ABSTRACT

The present study was intended to discover the preliminary phytochemicals of *Iris kashmiriana* Baker collected from Kashmir region, India. The preliminary phytochemical analysis was conducted in methanolic and aqueous extracts which showed the presence of carbohydrates, tannins, flavonoids, phenols, phytosterols, saponins, diterpenes, cardiac glycosides. Among the various phytochemicals studied, alkaloids and proteins were found to be absent in both methanolic and aqueous extracts. From the results, it was noted that the extracts of *Iris kashmiriana* Baker was found to be a rich source of variety of active secondary metabolites. This report will lead to the further isolation and characterization of these active secondary metabolites for bioefficacy and bioactivity.

Keywords: Phytochemical, Bioactive compounds, *Iris kashmiriana*, Mazar mund, Kashmir.

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INTRODUCTION

Description

Iris kashmiriana Baker has the common name of 'Kashmir Iris'. It is known as 'Mazarmond', Mazarmund', or 'safed mazarmond' which is derived from 'Mazar' meaning graveyard, or cemetery, and 'Mond' meaning root or underground swollen portion, the rhizome. It has thick and stout rhizome, which is fibrous and creeps along the ground. It has ensiform (sword-like), yellowish-green, or glaucous (blue-green), straight, leaves. They have scarious (paper-like) margins and ribs. The herbaceous leaves can grow up to between 45–60 cm long, and between 3.5-4.5 cm wide. It has an oval (in cross section), thick stem or peduncle that can grow up to between 50-75 cm tall. Occasionally, it can reach up to 125 cm tall. It has 1-2 short, 1 cm long, branches (or pedicels).The branching habit distinguishes it from *Iris albicans* (another white flowering tall bearded iris), which does not have branches. The stem has long, green, spathes (leaves of the flower bud), They are 7–11 cm long. They appear from the middle of the stem, up towards the flowers, and they have a narrow scarious margin, the stems (and the branches) hold several clusters of flowers, normally between 2 to 3 flowers, early in the season, between April to June. Up to 4-6 flowers can be blooming at one time. It has a cylindrical, perianth tube that is white, with blue markings and yellow-green veins, or green tube. The tube is 2.2–2.5 cm long. The fragrant flowers also come in various shades. The most common are white, or creamy white. Or white tinged with blue. Other forms found include pale blue lilac lavender or blue-purple, the purple forms may not hybrids. The white forms are very similar to *Iris albicans*. Like other irises, it has 2 pairs of petals, 3 large sepals (outer petals), known as the 'falls' and 3 inner, smaller petals (or tepals), known as the 'standards'. The falls are obovate, rounded or cuneate (wedge-shaped), 6.5–9.5 cm (3–4 in) long and 2.5–3.9 cm wide. They often have blue markings, and yellow-green veining, especially on the hafts (section of petal near the stem). In the centre of the fall, is a dense, narrow, white beard of hairs, tipped with yellow. The standards are obovate, oblong or elliptic shaped, 6.5–9.5 cm long and 2.5–3.9 cm wide. They have a short yellowish haft, and sometimes have a sparse beard. It has style branch that is 5 cm long, with an entire stigma, and large and triangular crests. It has white, or cream, filaments that are 1.3–2 cm long. It has 1.4 – 1.7 cm long anthers, and cream pollen. It has a green ovary that has ridges and grooves, and is 1 – 1.2 cm long. After the *Iris* has flowered, it rarely, produces a seed capsule, which is about 3–4.3 cm long and 2.2 cm wide, with thick and woody capsule walls. Within the capsule, are wrinkled, globular, dark red-brown or red-brown seeds [1,2,3,4].

Distribution and habitat

It is native to tropical Asia. It is found in India, (within Kashmir, and Jammu,) Nepal, Afghanistan, and Pakistan, (or Baluchistan). It is thought to be the most easterly species in

the subgenus of *Iris* section. It is listed as an endemic ornamental garden plant with *Iris hookeriana* and *Iris duthiei* (a synonym of *Iris kemaonensis*) in Kashmir. It grows close to settlements at 2500 ft to 9500 ft .It can be found at an altitude of 1,500-2,200 m (4,900–7,200 ft) above sea level [5,6,7].

Traditional use

The use of underground parts of several species of Iris was well established in traditional European folk medicine for centuries. Different uses, modes of administration and dosage of the plant *Iris kashmiriana* have been reported from different parts of Kashmir Himalaya. In Kajinaag range of Kashmir Himalaya, the powder of whole plant was used for treatment of joint pains and after mixing it with oil it was used to cure the skin infections [8]. In Bandipora area, the dried rhizome was not only used to cure joint pains but also to treat eczyma and respiratory problems [9]. In traditional medicine the plant was not only used to cure the human related ailments, but also to treat animal ailments. e.g. a mixture of rhizome powder, water and sugar made into semi-solid balls was given as tonic against general body weakness [10], for hepatic disorders and dropsy in cattle [11].

MATERIALS AND METHODS

Plant Material Collection

In the present study, the fresh rhizomes of *Iris kashmirina* were collected in May, at an altitude of 1655 m from Rangrate, Budgam, Kashmir. The plant was identified and authenticated by Prof. Akhtar H. Malik, KASH Centre for Biodiversity and Taxonomy University of Kashmir under specimen voucher number 2229-KASH

Preparation of extracts

After collection and authentication, rhizomes of the plant material were air dried and powdered. The powdered sample was then passed through sieve no. 40 and subjected to extraction. A weighed quantity of the powdered drug was extracted in a soxhlet with methanol. Aqueous extract was prepared by decoction method. The extracts were evaporated to dryness under reduced pressure and controlled temperature 40-50ºC [12]. The extracts were then kept in desiccators to remove remaining moisture, and finally stored in air tight containers at 4ºC for further use.

Preliminary phytochemical screening of the extracts

The methanolic and aqueous extracts so obtained were subjected to preliminary phytochemical screening. Phytochemical studies were performed to identify the presence of various constituents according to the standard methods [13].

Test for carbohydrates

Extracts were dissolved individually in 5 mL distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Benedict's test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars [14].

Test for Tannins

Ferric chloride test: To 2 mL of aqueous extract 2 mL of 5 % $FeCl_3$ was added and observed for the formation of yellow brown precipitate [15].

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of white precipitate indicates the presence of tannins.

Test for Anthraquinone glycosides

Borntrager's test: 1 mL of sulphuric acid was added to the test sample in a test tube and boiled for 5 minutes. Filtered and cooled the filtrate. The filtrate was then shaken with equal quantity of carbon tetrachloride. Separated the organic layer, added ammonia to it and observed for pink color [14].

Test for Cardiac glycosides

Keller Killiani test: To 2 mL alcoholic filtrate, 1 mL glacial acetic acid and 12 drops of $FeCl_3$ was added followed by 1 mL of concentrated H_2SO_4. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer [13].

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides [14].

Test for saponins

Froth Test: Extracts were diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 g of extract was shaken with 2 mL of water. If foam produced persists for ten minutes it indicates the presence of saponins [13].

Test for phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols [16].

Test for phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols [15].

Test for Flavonoids

Shinoda test: To 2 mL extract few drops of concentrated hydrochloric acid 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 [15].

Test for alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide) and observed for the formation of a cream precipitate.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide) and observed for the formation of brown/reddish precipitate.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide) and observed for the formation of orange precipitate.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution) and observed for the formation of yellow precipitate [16].

Test for proteins and amino acids

Xanthoproteic Test: The extracts were treated with few drops of conc. nitric acid and observed for the formation of yellow color.

Ninhydrin Test: To the extract, 0.25% *w/v* Ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid [16].

RESULTS AND DISCUSSION

In the preliminary phytochemical screening of methanolic and aqueous extracts of *Iris kashmiriana* Baker secondary metabolites like carbohydrates, tannins, flavonoids, phenols, phytosterols, proteins, saponins, diterpenes, cardiac glycosides, anthraquinones and alkaloids were tested. Thus, out of 44 tests conducted, 32 tests were found to be positive for carbohydrates, tannins, flavonoids, phenols, phytosterols, saponins, diterpenes, and cardiac glycosides. Among the various phytochemicals studied, alkaloids and proteins were found to be absent in both methanolic and aqueous extracts (Table-1).

Table 1: Preliminary phytochemical analysis of metanolic and aqueoua extracts *of Iris kashmiriana* Baker

Tests	Inference	Extracts	
		Methanolic	Aqueous
Carbohydrates			
Molisch's test	Violet ring	+	+
Fehling's test	Brick red ppt.	+	+
Benedict's test	Orange red ppt.	+	+
Tannins			
5% $FeCl_3$ test	Yellow color	+	+
Lead acetate test	White ppt.	+	+
Gelatin test	White ppt.	+	+
Flavonoids			
Shinoda test	Pink color	+	+
Alkali reagent test	Intense yellow color Which becomes colorless on addition of dil. acid	+	+
Lead acetate test	Yellow color ppt.	+	+
Phenols			
1% $FeCl_3$	Bluish color	+	+
Phytosterols			
Salkowski test	Golden yellow ring at junction	+	_
Libermann's test	Brown ring at junction	+	_
Proteins			
Xanthoproteic test	Yellow color	_	_
Biuretic test	Blue color	_	_
Saponins			
Foam test	Foaming	+	+
Ftoth test	Frothing	+	+
Diterpenes			
Copper acetate test	Emerald green color	+	+
Cardiac glycosides			
Keller killiani test	Brown ring at junction	+	+
Legal test	Pink color	+	+
Alkaloids			
Mayer's test	Cream ppt.	_	_
Hager's test	Yellow ppt.	_	_
Dragendroff's test	Orange ppt.	_	_
Wagner's test	Reddish brown ppt.	_	_

CONCLUSION

From the present study, it was concluded that *Iris kashmiriana* Baker showed the presence of a number of active secondary metabolites such as carbohydrates, tannins, flavonoids, phenols, phytosterols, saponins, diterpenes, cardiac glycosides. From the results, it was noted that the extracts of *Iris kashmiriana* Baker was found to be a rich source of variety of active secondary metabolites. This report will lead to the further isolation and characterization of these active secondary metabolites for bioefficacy and bioactivity.

CONFLICT OF INTEREST:

We declare that we have no conflict of interest.

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