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PRELIMINARY PHYTOCHEMICAL INVESTIGATIONS OF *GARDENIA FLORIDA*

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ABESTRACT
Plant *Gardenia florida* Family Rubiaceae is extensively used in clinical symptomes but it has various pharmacological activities have been investigated. It is a very familiar plant almost available in the perfect world. In the traditional system of medicine, many plants were used in the treatment of convulsive disorders. Classically, the root of the plant *Gardenia florida* is used in the Ayurvedic system of medicine for the treatment of convulsions. The root of *Gardenia florida* was collected, authenticated, dried in shade and powdered. The 95% alcoholic extract of the powered root was prepared by microwave assisted extraction and hot percolation method (yield 3.96%w/w). Solvent of the alcoholic extract was evaporated in a vacuum rotary evaporator and then it was kept in the desiccators at reduced pressure for complete drying. n-Hexane and chloroform fractions were prepared by solvent-solvent extraction method.

KEYWORDS: *Gardenia florida.*

INTRODUCTION

Gardenia florida commonly known as Gandhraj flower, belongs to the family Rubiaceae. This plant has also been reported for some other important biological activities like- antiperiodic, cathartic, anthelmentic, antiseptic antispasmodic, antidysentric and antifungal.[1,2] Various chemical constituents are reported in this plant such as glycosides, genipin, gardenoside, geniposidic acid, oleanolic acid, D-mannitol, stigmasterol, cerbinal, etc.[3] Anticonvulsant activity of stigmasterol is reported in some other plants. The root of this plant contains stigmasterol, which favors its traditional use. However, no systemic and scientific study has been done to evaluate the anticonvulsant effectiveness of the root of this plant till date. Therefore, this plant has been selected for the scientific study to validate the folk lore claims of anticonvulsant action.[4,5]

About the Plant

Gardenia florida

Common name -	Gandhraj
Kingdom	Plantae
Order	Gentianales
Family	Rubiaceae
Genus	*Gardenia*
Species	*florida*

Synonyms

Gardenia angstifolia, Gardenia angusta, Gardenia grandiflora, Gardenia pictorum, Gardenia radicans, Janipa florida, Janipa grandiflora.

Morphology

This is an evergreen shrub and small tree growing to 1–15 meters tall. The leaves are opposite or in whorls of three or four, 5–50 cm long and 3–25 cm broad, dark green and glossy with a leathery texture. The flowers are solitary or in small clusters, white, or pale yellow, with a tubular-based corolla with 5-12 lobes (petals) from 5–12 cm in diameter. Flowering season is from about mid-spring to mid-summer.

Habits

Gardenia florida is originated in Asia and is most commonly found growing wild in Vietnam, Southern China, Taiwan, Japan, Myanmar and India.

Chemical constituents

- Fruit-Pigment, quinic acid, 3, 4-di-*o*-cafferoyl quinic acid, chlorogenic acid, coumaroylgeninipin gentiobioside (III).
- Stem and Root- Acetyl oleanolic acid, D-mannitol and stigmasterol.
- Flower- Triterpenoids, gardenolic acid, etc.

Traditional use

Anticonvulsant, Antiperiodic, cathartic, anthelmentic and antiseptic.[1,4,6,7]

Phytochemical Investigations

Phytochemicals are naturally occurring chemical compounds found in plants. Most of the phytochemicals have been found to possess biological activity which, provide health benefits for humans other than those attributed to macro and micronutrients. Phytochemical investigations include various steps like-

- Collection, identification and authentication of plant material.
- Preparation of extract and various fractions.
- Phytochemical screening for identification of chemical component.

Collection, identification and authentication of plant material

Collection of Plant Material

The root of *Gardenia florida* was collected from the local area of Jaunpur District (U.P.).

Identification and Authentication of Plant Material

Root of *Gardenia florida* was identified and authenticated by Priyanka Agnihotri, Scientist Plant Diversity systematic and Herbarium Division NBRI (Lucknow). The plant specimen was preserved in herbarium and museum, NBRI. (Lucknow) with Ref. No. LWG-54.

Drying of Plant Material

The plant material was dried in shade.

Course powder of Plant Material

The dried root of *Gardenia florida* was crushed and then powdered with the help of a hand homogenizer.

Extraction

Extraction is an important process for separating and isolating the medicinally active compounds from plants by adopting standard procedures. Extraction is also used for the qualitative and quantitative analysis of drug/herbal medicine and its active constituents.[8]

General methods for extraction

- Maceration.
- Infusion.
- Decoction.
- Percolation.
- Counter current extraction.
- Pressurized solvent Extraction.
- Microwave assisted extraction.

Microwave assisted extraction

The powdered plant material (10g root of *Gardenia florida*) was extracted using a microwave synthesizer, at a power 280 W for 20 min, using 95% alcohol as solvent. The solvent: sample ratio was 1:10 and the pre-leaching time was 10 min. The crude extract so obtained was collected, cooled at room temperature, filtered and concentrated under reduced pressure with controlled temperature using a vaccum rotary evaporator **(Yield - 5% w/w).** This procedure was repeated many times to obtain sufficient quantity of the extract for its pharmacological evaluation and fractionation.[9]

Extraction by soxhlet apparatus

The powdered plant material (100g) was packed in a soxhlet apparatus and subjected to hot percolation, continuous for 10 hours using 400 ml 95% ethanol as the solvent. The extract was concentrated to a semisolid mass under vaccum and completely dried in a desiccator (yield 3.96% w/w).

Preparation of aqueous extract

Approximately 100g of the shade dried powder of the root of *Gardenia florida* was taken in a 1 L beaker and water was added up to a sufficient level to immerse the drug completely. This set up was placed aside for 72 hours, with stirring at alternate intervals. Finally, the extract from the beaker was vaccum filtered to get a clear watery brown colored extract. The extract was concentrated under high vaccum and completely dried in a desicator (yield 4.23% w/w).

Fractionation of the alcoholic extract

About 50g of the alcoholic extract was suspended in 200ml of distilled water and extracted several times by taking 20 ml solvent of decreasing polarity (n-hexane and chloroform) in a separating funnel. Residue obtained from the n-hexane partition was allowed to warm on a boiling water bath to remove the remaining portion of n-hexane before partitioning it with chloroform. All the fractions were concentrated by vacuum and placed in desiccators at reduce pressure for complete drying. (Yield of n-hexane fraction 0.023% w/w and chloroform fraction 0.05% w/w).

Determination of the percentage yield of crude extract and fractions

The percentage yields of the crude extracts and fractions were calculated by using the formula given below-

$$\text{Percentage yield} = \frac{\text{Weight of extract (gm)}}{\text{Weight of dry powder (gm)}} \times 100$$

Preliminary Phytochemical Studies[10,11]

The crude extract and the various fractions were subjected to different chemical tests separately, for the identification of various active phytoconstituents. The tests were as follows-

Tests for Alkaloids

1. Dragendroff's Test

Extract/fraction was mixed with Dragendroff's reagent (potassium bismuth iodide solution). Formation of a reddish brown precipitate confirmed the presence of alkaloids.

2. Wagner's Test

Extract/fraction was mixed with Wagner's reagent (iodine potassium iodide solution). Formation of a reddish brown precipitate confirmed the presence of alkaloids.

3. Mayer's Test

Extract/fraction solution/suspension (1mL) was mixed with one drop of Mayer's reagent (mixture of mercuric chloride and potassium iodide). Formation of a cream coloured precipitate confirmed the presence of alkaloids.

4. Hager's Test

Extract/fraction solution/suspension was mixed with Hager's reagent (saturated solution of picric acid). Formation of a yellow precipitate confirmed the presence of alkaloids.

2. Tests for Steroids

1. Libermann Burchard Test

Extract/fraction solution/suspension was treated with a few drops of acetic anhydride, boiled and cooled. Then, concentrated sulphuric acid was added from the side of the test tube. A brown coloured ring was formed at the junction of the two layers and the upper layer turned green, which showed the presence of steroids, and formation of a deep red colour indicated the presence of triterpenoids.

2. Salkowski Test

Extract/fraction solution/suspension was treated with 1 mL of chloroform. Then, 1 mL of concentrated sulphuric acid was added carefully and shaken gently. A reddish brown colour in the lower layer indicated the presence of a steroidal ring, i.e. glycone portion of the glycoside.

3. Tests for Flavonoids

1. Extract/fraction solution/suspension was mixed with 20% sodium hydroxide solution, A yellow colour was formed which disappeared on addition of dilute HCl.

4. Tests for Saponins

1. Foam Test

Extract/fraction solution/suspension was mixed with 5 ml of distilled water in a test tube and then it was shaken vigorously. The formation of a stable foam was taken as the indication for the presence of saponins.

5. Tests for Glycosides

1. Legal Test

Extract/fraction solution/suspension was treated with pyridine and alkaline sodium nitroprusside solution, Appearance of blood red colour indicated the presence of glycosides.

2. Baljet Test

Extract/fraction solution/ suspension, was treated with picric acid. Formed a an orange colour which indicated the presence of glycosides.

6. Tests for Amino Acids

1. Ninhydrin Test

Extract/fraction solution/suspension when boiled with 0.2% solution of ninhydrin, formation of violet color indicated the presence of amino acids.

7. Tests for Proteins

1. Millon's Test

Extract/fraction solution/suspension, when mixed with 2 mL of Millon's reagent (solution of mercuric nitrate and nitrous acid), gave a white precipitate which turned red upon gentle heating confirming the presence of proteins.

2. Biuret Test

To the extract/fraction solution/suspension, biuret reagent (solution of sodium hydroxide, hydrated copper (II) sulphate, together with potassium sodium tartrate) was added; formation of a violet colour indicated the presence of proteins.

8. Tests for Phenolic Compounds

Extract/fraction solution/suspension was treated with ferric chloride solution. The formation of a blue or a green colour indicated the presence of phenolic compounds.

9. Tests for Fixed Oils

1. Spot Test

A small quantity of extract/fraction was pressed between filter papers. Oil stains on the paper indicated the presence of fixed oils.

10. Tests for Carbohydrates

1. Molisch's Test

Extract/fraction solution/suspension was mixed with a few drops of Molisch's reagent (alcoholic α-naphthol) and the mixture was shaken properly. A few drops of concentrated sulphuric acid were added carefully, along the sides of the test tube. Appearance of a violet ring at the inter-phase indicated the presence of carbohydrates.

2. Fehling's Test

Equal volumes of Fehling A and Fehling B reagents were mixed together and 2ml of extract/fraction solution/suspension was added to it and boiled gently. A brick red precipitate appeared at the bottom of the test tube indicating the presence of reducing sugars.

3. Benedict's Test

Extract/fraction solution/suspension, when mixed with 2 ml of Benedict's reagent and boiled, gave a reddish brown precipitate indicating the presence of carbohydrates.

RESULT

Phytochemical analysis of crude extract and various fraction of root of *Gardenia florida*.

S.No.	Name of the test	Ethanolic Extract	Aqueous Extract	Chloroform fraction	n-Hexane fraction
1	**Tests for alkaloids**				
	Hager's test	+ + +	+ + +	+ + +	+ +
	Mayer's test	+ + +	+ + +	+ + +	++
	Wagner's test	+ + +	+ + +	+ + +	+ +
	Dragendroff's test	+ + +	+ + +	+ + +	+ +
2	**Test for carbohydrates**				
	Molisch's test	++	+ + +	-	-
	Benedict's test	+ + +	++	++	+
	Fehling's test	-	-	++	+
3	**Test for phenolic compounds**	+ + +	_	_	_
4	**Test for saponins**				
	Foam Test:	_	+ + +	+ + +	_
5	**Test for proteins**				
	Millon's Test:	+ +	+ + +	_	_
	Biuret Test:	_	_	_	_
6	**Test for steroids**				
	Libermann Burchard Test	+ + +	_	+ + +	_
	Salkowski Test	+ + +	_	+ + +	+ +
7	**Test for glycosides**				
	Legal Test	+ + +	+ +	+ +	+
	Baljet Test:	+	+ + +	_	+ +
8	**Test for amino acids**				
	Ninhydrin Test	_	_	_	_
9	Test for Flavonoid	+ + +	+ + +	+ +	+ +

(+) Present in traces, (++) Present in adequate quantity, (+++) Present in abundance, (-) Absent

Phytochemical screening

The root of *Gardenia florida* was collected, authenticated, dried in shade and powdered. The 95% alcoholic extract of the powered root was prepared by microwave assisted extraction and hot percolation method (yield 3.96%w/w). Solvent of the alcoholic extract was evaporated in a vacuum rotary evaporator and then it was kept in the desiccator at reduced pressure for complete drying. n-Hexane and chloroform fractions were prepared by solvent-solvent extraction method, using a separating funnel and the yield obtained was 0.05% w/w and 0.023% w/w respectively. Aqueous extract of the powered root was prepared by cold maceration method (yield 4.23% w/w).

Preliminary phytochemical screening of all the extracts/fractions was done by the analysis of their chemical constituents. Alkaloids, carbohydrate, protein, steroid, glycosides and flavonoides were identified in the alcoholic extract/fractions while aqueous extract gave positive tests for all the above compounds except steroids.

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