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Extraction and Phytochemical Screening of Hibiscus Rosa – Sinensis

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ABSTRACT
Hibiscus rosa- sinensis Linn. is one of the important medicinal as well as ornamental plant, belongs to family Malvaceae. It is commonly called Gurhal. The extract (leaf) is largely used in the treatment of various diseases. In the present investigation aqueous extract and ethanol extract of the leaves were made using soxhlet apparatus. The qualitative phytochemical screening procedure was performed on extract. phytochemical study reveals that alkaloids, tannins, saponins, triterpenoids, flavonoids were present in the extract and TLC analysis.

Key words: Hibiscus rosa-sinensis Linn, Malvaceae, phytochemical screening, TLC, Phytoconstituents.

I. INTRODUCTION

Hibiscus rosa sinensis is also known as the "China rose" or the "Queen of the tropics" due to its widespread distribution in southeast China and some islands in the Pacific and Indian Oceans.(1) This plant belongs to the class Magnoliopsida and subkingdom Magnoliophyte; it is vascular and produces seeds.(2)It belongs to the family Malvaceae and is one of the 300 species in the genus Hibiscus. Furthermore, the juice from the leaves and blossoms has long been used as a complementary medicine to cure a variety of ailments and their unpleasant side effects.(3, 4)It is believed that the leaves and flowers encourage the growth of hair and aid in ulcer healing. (5, 6)

The "World Health Organization" advises that conventional methods of well-being and medication for individuals have shown to be more effective in treating medical conditions globally. India is among the nations endowed with a wealth of traditional medicinal systems and a diverse range of flora that serve to supplement the herbal remedies used in these customary medical systems. Ayurveda, Unani, and Siddha are recognized Indian medical systems that use common resources like herbs in their formulations.

1.1. About Hibiscus

1.1.1. Synonyms: China rose, Chinese Hibiscus, Shoeblack, Shoe flower, Blacking, Plant.(7)

1.1.2. Biological source:
(Hibiscus rosa-sinensis)

1.1.3. Family:
Malvaceae.

1.1.4. Taxonomic classification:

Kingdom:	Planate
Subkingdom:	Tracheobionta
Super division:	Spermatophytes
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Dilleniidae
Order:	Malvales
Family:	Malvaceae
Genus:	Hibiscus
Species:	Hibiscus rosa sinensis.(8)

1.2. Chemical constituents:

Hibiscus rosa-sinensis was found to include tannins, anthraquinones, quinines, phenols, flavanoides, alkaloids, terpenoids, saponins,

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cardiac glycosides, protein, free amino acids, carbohydrates, reducing sugars, mucilage, essential oils, and steroids, according to a preliminary phytochemical analysis. (4, 9)

In several Hibiscus rosa sinensis extracts, alkaloids, resin, glycosides, decreasing sugars, greasy components, sterols, and the lack of tannins and saponins were found. Researchers discovered four unknown chemicals in the leaves, including three sterols and an alkaloid, in addition to sit sterol and taraxeryl acetic acid derivative. The hydrocarbon content, unsaturated lipids and greasy liquid of Hibiscus rosa-sinensis leaves were also investigated. (4)

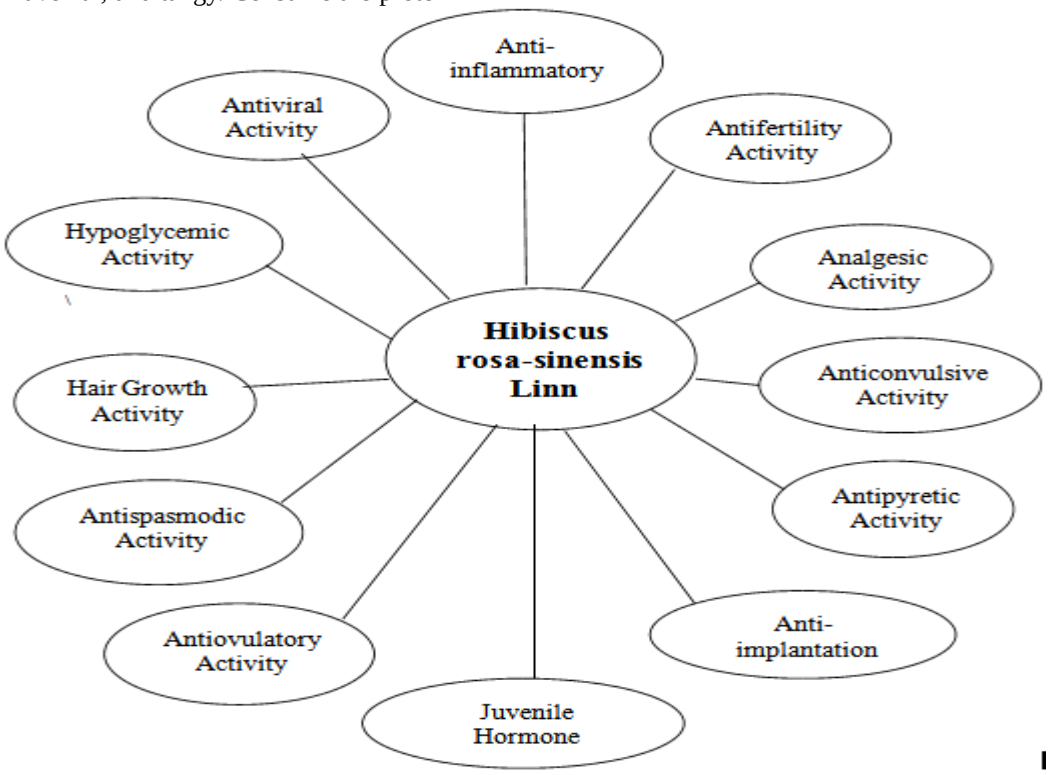
Additionally, malvalic and streptacic cyclic acids have been found. Flowers include vitamins, cyaniding di-glucoside, ascorbic acid, flavonoids, riboflavin, niacin, and thiamine. From deep yellow flowers, 3-7-di-glucoside, cyanidin-3-sophoroside-5glycosides, quercetin-3-diglucoside, cyanidin-3, 5-diglucoside, and 3-7-di-glucoside have been separated. (10)

1.3. Uses of Hibiscus

In many different countries, hibiscus flower tea is either hot or cold. The beverage is colorful, flavorful, and tangy. Consume the protein-rich, antioxidant-rich seed after meals or in place of coffee. Hibiscus seed oil is extracted and used to food while cooking. Research indicates that extracts from the flowers and roots of hibiscus have potent contraceptive properties. Indian Ayurveda also makes extensive use of hibiscus blossoms.

1.4. Pharmacological activities of Hibiscus rosa-sinensis

Hibiscus rosa-sinensis is a naturally occurring source of many chemicals with a wide range of pharmacological actions that can aid in the creation of innovative medicinal formulations. Studies on the pharmacological activity of Hibiscus rosa-sinensis leaves, barks, roots, and flowers have been carried out; these materials have been utilized to treat a variety of illnesses, including cancer, diabetes mellitus, wound healing, hypertension, and aphrodisiac.[18] Research has demonstrated that distinct plant components may possess a range of characteristics, including anti-inflammatory, antimicrobial, antioxidant, and anti-ulceration effects.(11)In addition to its traditional uses in herbal mixes and beverages, this plant has also been utilized as an oral contraceptive and to manage dysfunctional uterine haemorrhage.(12)



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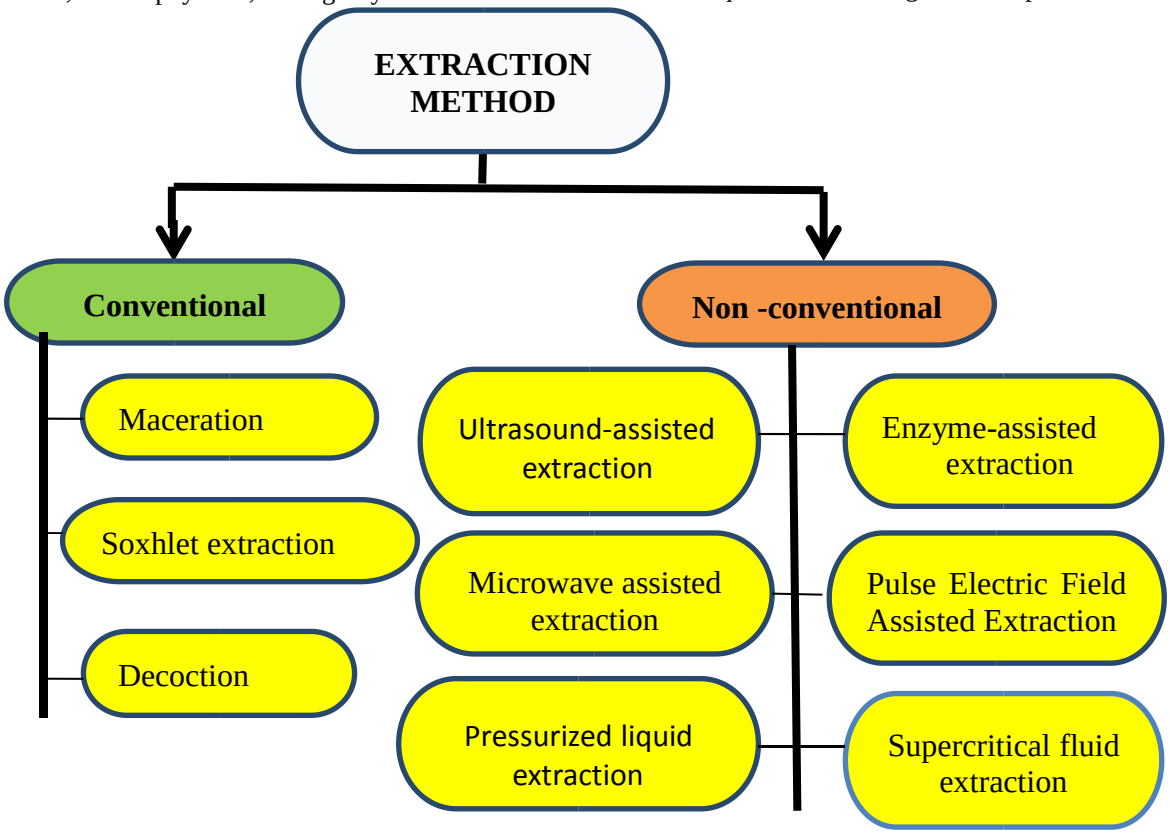
II. EXTRACTION

It is described as the process of treating plant or animal tissues with a solvent so that the medicinally active ingredients dissolve while the majority of the inert stuff stays undissolved.
The extraction solvent that is utilized is called **"menstruum."**
"Marc" is the term for the inert, insoluble substance that is left behind following extraction.

2.1. METHOD OF EXTRACTION

The general methods used to extract medicinal plants are distillation methods (water, steam, and phytonic; using hydrofluorocarbon solvents), maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueousalcoholic extraction by fermentation, counter current extraction, microwave-assisted extraction, ultrasound extraction (sonication), and supercritical fluid extraction.

There are a several methods that may be used for aromatic plants, including steam and water distillation, hydrolytic maceration, expression, and effleurage (cold fat extraction). Micro distillation, protoplast extraction, solid phase micro extraction, and headspace trapping are a few of the most recent techniques for extracting aromatic plants.



2.1.1. Maceration:

In maceration (for fluid extract), the plant medicine, either whole or coarsely powdered, is maintained in contact with the solvent in a stoppage container for a predetermined amount of time while being frequently agitated to dissolve any soluble materials. When it comes to thermo labile medications, this approach works well.(13)

2.1.2. Infusion

This is a diluted mixture of the crude medications' easily soluble ingredients. The process of making fresh infusions involves macerating the contents in either cold or hot water for a brief duration .(13)

2.1.3. Percolation

This method makes use of a percolator with a thin, cone-shaped vessel that is open at both

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ends. (14) The plant material is placed in a percolation chamber after being moistened with the solvent. After that, the plant material is repeatedly washed with the solvent in order to extract the active component. Up to the degree of saturation, the solvent can be utilized.(15)

2.1.4. Decoction

The process of decoction involves boiling a crude medicine in water for 15 minutes, letting it cool, straining it, and then running enough cold water through it to get the desired amount.(16) This approach extracts the heat- and water-soluble components from the drug.

2.1.5. Soxhlet Extraction

The process of soxhlet extraction is necessary only in situations when an impurity is insoluble in a solvent and the target component has a restricted solubility in that solvent. A straightforward filtering process may be utilized to separate the desired chemical from the insoluble material if the desired compound has a high solubility in a solvent. This system's benefit is that just one batch of solvent is recycled, as opposed to several parts of heated solvent being passed through the sample. Since prolonged heating may cause compound degradation, this approach cannot be applied to thermo labile compounds.(17)

2.1.6. Microwave assisted extraction

In this method microwave energy facilitate the separation of active ingredients from the plant material into the solvent. Microwaves possess electric and magnetic fields which are perpendicular to each other. The electric filed generates heat via dipolar rotation and ionic conduction. As high as the dielectric constant of the solvent, the resulting heating is fast. Unlike the classical methods, microwave assisted extraction heats the whole sample simultaneously. During the extraction, heat disrupts weak hydrogen bonds due to dipole rotation of molecules and the migration of dissolved ions increases the penetration of solvent in to the sample or matrix.(18)

2.1.7. Ultrasound-assisted extraction

This is a sophisticated method that can extract a significant number of bioactive chemicals in a shorter length of time. The primary benefit of this method is the increased solvent penetration into the matrix as a result of the acoustical cavitations that break down the cell walls. Additionally, because it operates at low temperatures, it is better suited for extracting chemicals that are thermally unstable.(19)

III. 3. IDENTICATION TEST

3.1. Qualitative techniques for the determination of phytochemicals

3.1.1. Alkaloids Test

1.Mayer's Test

A little quantity of plant extract is put to the test tube's sidewalls, along with two drops of Mayer's reagent. A white, creamy precipitate is a sign that alkaloids are present.(20)

2. Hager Test

To the extract is added a little quantity of Hager's reagent. It is evident that alkaloids are present when a yellow precipitate form.(21)

3.1.2. Flavonoids Test

1. Alkaline Reagent Test for Flavonoids

The presence of flavonoids is demonstrated by treating a little quantity of the extract with a few drops of sodium hydroxide and seeing if the solution's bright yellow becomes colourless when diluted acid is added.(22)

3.1.3. Saponin Test

5mL of extract place in a test tube along with a drop of Na2CO3 solution. It was given a good shake and then allowed to rest for five minutes. The presence of saponins is indicated by the creation of a 2 cm thick foam.(23)

3.1.4. Triterpenoids Test

1. Horizon examination.

1mL of extract is mix with 2mL of trichloroacetic acid. By forming a red precipitate, terpenoids are verified to be present.

3.2. QUALITATIVE TECHNIQUES

3.2.1. Thin Layer Chromatography (TLC)

In particular, thin layer chromatography is useful for qualitative analysis. Using the TLC method, a solute is distributed between two phases: a liquid mobile phase and a stationary phase that acts by adsorption(24).

The most often used adsorbent is a comparatively thin, homogeneous coating of dry, finely powdered substance put to a glass or plate. In addition, partitioning or combining partition and adsorption can be used to achieve separation, depending on the specific types of support, how they are prepared, and which solvent system they are used with.

Thin layer chromatography of the leaves and flowers of Hibiscus rosa sinensis. In this work,

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crude ethanolic flower and leaf extracts of Hibiscus species were analyzed using thin layer chromatography (TLC) to determine the composition of the chemicals.

These were scored 10 x 20 centimeter stationary phase silica gel G plates.

In an iodine vapour-filled atmosphere, 0.2% ninhydrin in acetone is sprayed, plates are sprayed with a vanillin-sulphuric acid reagent, and the results are seen under a UV lamp. The following formula was used to determine each spot's R_f value

$$\mathbf{Rf} = \frac{\textbf{Distance travelled by solute}}{\textbf{Distance travelled by solvent}}$$

IV. RESULTS AND DISCUSSION

4. Collection of plants materials

4.1.Plant Material:

Leaves of Hibiscus rosa sinensis were collected from the herbal garden of Sachdeva college of pharmacy (Ludhiana Chandigarh State Highway ,Gharuan, Mohali, Punjab, National Highway 95, PB 140413, Mohali, India) in the month of October November, 2023.

4.2. Method of extraction:

China rose, Hibiscus rosasinensis the leaves were properly cleaned, dried for 24 hours in the shade, and then dried again in an oven set at 30 to 40 degrees Celsius. The grinder helped to minimize size. After being put through sieve number 22, powdered leaves were utilized for further analysis.

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4.3. Soxhelt extraction procedure: The extraction was done by soxhlet apparatus using solvent i.e., ethanol.

4.3.1. Requirements:

4.3.2. Chemical: Ethanol, Petroleum gel, Distil water, powder of hibiscus leaves.

4.3.3. Apparatus: Soxhelt apparatus, heating mental, Round bottom flask (RBF), Condenser.

4.3.4. Procedure:

The finely ground leaves were put in a bag made of filter paper and put into a soxhlet device. After heating the extraction solvent in the flask, the vapors condensed. The filter paper bag with the powdered leaves was filled with the condensed extract by drips. The liquid contents of the chamber siphon were collected into a flask when the liquid level reached the top of the siphon tube. This process was continued and carried out until the siphon tube was emptied. The collected extract was then evaporated by rotavapor to remove the solvent completely and crude extract were obtained in the gummy or semisolid form.

Soxhlet apparatus assembly.

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Extract of Hibiscus Rosa Sinensis

V. TABLE FOR IDENTIFICATION TEST.

S.No	Identification Test	Observation	Inference
1. (a)	**Test for Alkaloids** **Mayer's test**Alkaloids in natural products can be found using Mayer's reagent, an alkaloid precipitating reagent. Mayer's reagent, newly made by dissolving 1.36 grams of chloride with 5.00 grams of potassium iodide in 100 milliliters of water. We took 3 milliliters of extract in a test tube. Mayer's reagent was applied in little drops down the test tube's sides.	 It gives creamy precipitate.	It shows the presence of alkaloid.
(b)	**Hager's test**Hager's reagent is used to perform the picric acid test. 1g of picric acid is added to 100ml of distilled water to create Hager's reagent, and 3ml of the extract is collected and placed in a test tube. A few drops of Hager's reagent were applied to the test tube's sidewalls.	 It gives yellow precipitate.	Slight amount of Alkaloid is present.

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2.	**Tests for Saponins** In a test tube containing 5mL of extract, a drop of Na2CO3 solution was added. It was given a good shake and then given five minutes to rest.	 Foam formation observed.	It shows the presence of saponin.
3.	**Test for Triterpenoids** **Horizon test.** 1mL of extract was mixed with 2mL of trichloroacetic acid.	 It gives red precipitate.	It shows the presence of Triterpinoids.
4. (a)	**Tests for Flavonoids** **Alkaline reagent test**Add two or three drops of sodium hydroxide to two milliliters of extract.	 It gives a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL.	It shows the presence of Flavonoid.

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5.1. PHYTOCHEMICAL SCREENING BY TLC

5.1.1. Material and methods

In October and November of 2023, leaves of Hibiscus rosasinensis were taken from the herbal garden of Sachdeva College of Pharmacy, which is located near Ludhiana, Chandigarh State Highway, Gharuan, Mohali, Punjab, National Highway 95, PB 140413, Mohali, India. A "Soxhlet Extraction apparatus" was used to extract 100 grams of shade-dried plant material with 500 milliliters of 95% ethanol in order to create an ethanolic extract. The prepared plant was then macerated in water for a whole day. to get an extract in water. Distilling out the solvent allowed for the concentration of the extract. After that, the extract was put through TLC screening in order to identify different phyto ingredients using TLC techniques.

5.1.2. Phytochemical screening

a) flavnoids: For the purpose of detecting alkaloids in ethanolic (leaf) extracts of

Hibiscus rosa sinensis Linn morphotypes, TLC analysis uses For the purpose of detecting alkaloids in ethanolic (leaf) extracts of Hibiscus rosa sinensis Linn morphotypes, TLC analysis uses Ethyl acetate: Formic acid: Glacial acetic acid: H2O (100:11:11:26) as the mobile phase. Tested morphotype leaf extracts exhibit separation on TLC plates. as the mobile phase. Tested morphotype leaf extracts exhibit separation on TLC plates.

$$Rf = \frac{\text{Distance travel by solute}}{\text{Distance travel by solvent}}$$

Distance travelled by sample is 3.8
Distance travelled by solvent is 4.5 **Rf value obtain is 0.84**

2. TABLE FOR THIN LAYER CHROMATOGRAPHY

Phytoconstituents	Solvent	Spot colour	R_f value
Flavnoids	Ethyl acetate: Formic acid: Glacial acetic acid: H2O (100:11:11:26)	Yellowish green	0.84

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5.2. ASH VALUE

The inorganic residues found in herbal medications, such as phosphates, carbonates, and silicates, are often represented by the ash values. These are crucial indicators that show the potency and purity of herbal medication.

5.2.1. Total ash: To the weighted crucible (weight of sample), 2 g of the air-dried plant material was added. The sample was gradually heated to 500–600° in an electrical muffle furnace until it became white, signifying the lack of carbon. It was then reweighed (ash weight) after being cooled in a desiccator. % total ash was used to compute the total ash content.

Ash value obtain is **0.158**

$$\% \text{ Total ash} = \frac{\text{Ash weight}}{\text{Weight of sample}} \times 100$$

%Total ash value is 7.9 %.

VI. CONCLUSION

Based on the examination of the literature Part of the Malvaceae family, Hibiscus rosa sinensis is utilized in pharmacological products. This investigation used a crude extract of H. rosasinensis leaves, and the outcomes were similar to those of the standard. The plant's ability to treat medical conditions is mostly due to the presence of flavonoids, alkaloids, saponin, and triterpenoids. In conclusion, Hibiscus rosa sinensis leaf extract extracted with ethanol has superior flavonoid. following a successful investigation of the plant leaf using TLC's phytochemical screening method.

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