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Original Research Article

Qualitative Phytochemical Analysis of Ethanolic Extracts of *Hibiscus laevis Flowers* and *Euryale ferox* Seeds

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Abstract

The purpose of the research was to determine whether or not *H. laevis* and *E. ferox* had any potential in the field of phytochemistry. Ethanol was the solvent of choice for the extraction process of both the flowers of *H. laevis* and the seeds of E. ferox. There is evidence of the presence of alkaloids, glycosides, carbohydrates, phytosterols, gum, and mucilage in the ethanolic extract of *H. laevis*. On the other hand, there was a lack of steroidal compounds, protein and amino acids, tannins, saponins, oil, and lipids. The ethanolic extract of E. ferox, on the other hand, reveals the presence of alkaloids, glycosides, tannins, carbohydrates, and phytosterols. On the other hand, there was a notable lack of steroids, proteins and amino acids, gum and mucilages, saponins, and oil and fats. Because of the presence of a variety of secondary metabolites, both of the plant extracts have the potential to be further investigated for appropriate pharmacological activity.

Keywords: Phytochemistry, Secondary metabolites, *H. laevis*, *E. ferox*

INTRODUCTION

Thousands of years of clinical use have demonstrated the efficacy of herbal medicine, often known as the study and practise of therapies based on plant extracts [4]. Several spices and plants have been investigated for their potential antiulcer effects, with conflicting findings. *Hibiscus laevis* and *Euryale ferox* are only two of the many plants and foods that have been shown to have gastro-protective properties [3]. The rose mallow with halberd-shaped leaves, scientifically known as *Hibiscus laevis*, is a native perennial of the eastern and central United States and Canada[8]. The herb known as makhana, or *Euryale ferox* Salisb. (Nymphaeaceae) is used to treat kidney illness, diarrhoea, and leucorrhea[1]. Ulcers are treated with a wide variety of plants and polyherbal concoctions, as attested by both written and oral history [7]. This emphasises the need to investigate the possible antiulcer effects of medicinal herbs traditionally used to treat gastrointestinal diseases. Because of this, both modern scientific procedures and traditional medical knowledge are required to successfully isolate, characterise, and standardise the active components from a herbal source. Safer and more effective antiulcer drugs can be developed by combining old knowledge with modern science[2].

Herbal treatments have shown promise in the treatment of stomach ulcers in the laboratory, but very few have made it to clinical trials or been made available to the

public. This shows that despite substantial investment of time, energy, and finances, medical research is not benefiting its intended recipients. Therefore, the protocol's goal is to discover whether or not the plant extracts include any phytomolecules. The halberd-leaf rose mallow (*Hibiscus laevis*) is a perennial plant native to the middle and eastern regions of North America. The tallest stems only bear a single flower or a cluster of flowers at their very tips. Although their inner throats are typically a deep crimson or a rich purple-pink, they are typically either white or a very pale pink [8]. The Halberd-leaved rose mallow is a tall plant that may grow to a height of 6 feet thanks to its erect stems and leaves. The large, deeply cut lobes on the leaf stems alternate with one another. Flowers develop from the base of the plant in the axils of the leaves. The massive cup-shaped flowers are often pink but can be white; their throats are rich crimson or purple. The five overlapping petals open widely during the day and contract tightly at night. It is widespread throughout the state and thrives in wet areas (Warner and Erwin, 2001). Each flower, in particular, draws attention to its core staminal column.

Similar to, and once known as, *Hibiscus militaris,* this plant got its name from the way its leaves seem like the handle of a gun. Commonly known as Makhana, or *Euryale ferox*, Traditional Oriental medicine relies on the Salisb. (Nymphaeaceae) plant to treat a wide range of conditions, including kidney illness, chronic diarrhoea, excessive leucorrhoea, and spleen hypo function [1]. High concentrations can be found in the Indian state of Bihar, specifically in the regions of Mithila, Darbhanga, and Madhubani. Ayurvedic and traditional Chinese medicine practitioners have used it for decades to treat renal failure, chronic diarrhoea, excessive leucorrhoea, and hepatic dysfunction. Antioxidant, antibacterial, anti-ischemic, anti-diabetic, immunomodulatory, anti-melanogenic, and cytotoxic are only a few of the many functions performed by its bioactive components[6]. Foxnuts are an aquatic perennial that may grow in water as deep as five metres and spread via rhizomes. Because it is a water lily, its mature rosette of large, spiny leaves floats on the water's surface. Both China and India cultivate the plant extensively for human consumption. All sorts of familiar and exotic edible plants (even tropical ones) are listed, along with their respective places around the world and how to acquire them. The Gorgon Plant (*Euryale ferox*) is the sole survivor of the perennial water lily species Euryale, which belongs to the Nymphaeaceae family. Its normal habitats are huge bodies of water like ponds and water gardens. It's simple to plant the seeds, but the plant isn't viviparous, so it doesn't produce offspring while still attached to its mother[5].

MATERIALS AND METHODS

Procurement of Plants Part:

In the month of November 2021, specimens of *Hibiscus laevis* and *Euryale ferox* were retrieved from the herbarium at Bilwal medchem and research laboratory Pvt. Ltd., in Jaipur. These items were deposited on November 20th, 2021 with the reference number BMRL/AD/PA-128/2021. For the purpose of future research and evaluation, the department has kept a representative sample of the same thing as a voucher. During the course of the experiment, both the flower of the *Hibiscus laevis* and the seeds of the *Euryale ferox* were utilised.

Extraction of *Hibiscus laevis* Flowers:

Using a grinder, the flowers were reduced to a powder form. In order to remove the particles, the ethanol was heated to a temperature of sixty degrees for three hours. The extract was filtered using cheesecloth prior to being gravity-filtered through a P8 coarse filter and then vacuum-filtered using a 0.45 μm filter. The ethanolic extract was first flash frozen at

80 degrees Celsius, and then freeze dried using a lyophilizer, and finally reconstituted to achieve the desired final stock concentration of 100 milligram per millilitre.

Extraction of *Euryale ferox* Seeds:

The E. ferox plants were harvested and the seeds were extracted from those plants. In a nutshell, one hundred gram of E. ferox seeds were dehydrated, ground, and then extracted using the solvent. Ethanol, at a concentration of 90%, was utilised as the extraction solvent. After shaking the combination for sixteen hours with a mechanical device, the extract was strained through a filter. After that, the extract was put into an evaporator with a vacuum and heated to 700 degrees Celsius under a lowered pressure. After that, it was dried off and put away in a desiccant until it was needed again.

PHYTOCHEMICAL ANALYSIS

The samples of the medicine were analysed for the presence of phytoactive components. These included tannins, phenols, saponins, flavonoids, proteins, carbohydrates, reducing sugars, lipids, and alkaloids.

Tests For Alkaloids

- **Dragondroff's Reagent Test:** 1 ml of Dragendorff's reagent also known as Potassium Iodide and Bismuth Sub Nitrate Solution was added to 2 ml of test extracts in a test tube, an orange-colored precipitate indicate the presence of alkaloids.
- **Wagner's Test:** Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water (5 mL) and the solution was diluted to 100 mL with distilled water. A few drops of this solution were added to the test extracts; a brown-colored precipitate indicates the presence of alkaloids.

Test For Glycosides

- **Legal Test:** To the extracts of test sample equal volume of water and 0.5 ml of strong lead acetate solution was added, shaked and filtered. Filtrate was extracted with equal volume of chloroform and the chloroform extract was evaporated to dryness. The residue was dissolved in 2 ml of pyridine and sodium nitropruside 2 ml was added followed by addition of NaOH solution to make alkaline. Formation of pink colour indicates the presence of presence of glycosides or aglycon moiety.
- **Keller-Killiani Test:** Test extracts (0.5 g) were shaken with distilled water (5 ml). To this, glacial acetic acid (2 mL) containing a few drops of ferric chloride was added, followed by H_2SO_4 (1 mL) along the side of the test tube. The formation of brown ring at the interface gives positive indication for glycoside and a violet ring may appear below the brown ring.

Test For Steroids

- **Salkowski Reaction Test:** Ethanolic extracts were evaporated to dryness and extracted with chloroform; add 2 ml conc. H_2SO_4 from sidewall of test tube to the chloroform extract. Formation of yellow coloured ring at the junction of two liquid, which turns red after 2 min, indicate the presence of steroid moiety.

Tests For Carbohydrates

- **Molisch's Test:** Few drops of molisch reagent were added to 2 mL of test extracts. Later, a few drops of concentrated H_2SO_4 were added along the walls of a test tube. At the junction of two liquids, a violet colour ring appeared, indicating that carbohydrates were present.
- **Fehling Solution Test:** To 2 ml of test extracts, an equal volume of Fehling's (A & B) solution was added and heated for five minutes, the resulting red/dark

red precipitate indicating the presence of carbohydrates.

Test For Flavonoids

- **Shinoda Test:** Ten drops of dilute HCl and a piece of magnesium were added to 1 ml of test extract, the resulting deep pink colour indicating the presence of flavonoids.

Test For Proteins And Amino Acids

- **Ninhydrin Test:** Two drops of 0.2% freshly prepared ninhydrin solution added to 1 ml of test extracts. Production of purple colour shows the presence of proteins.
- **Biuret Test:** Two drops of 3% copper sulphate and few drops of 10% sodium hydroxide were added to 1 ml of test extracts. The presence of protein is indicated by a colour either violet or pink.

Test For Tannins And Phenolic Compound:

- **Lead Acetate Test:** 1 ml of lead acetate solution was treated with 0.5 ml of test extracts; the formation of precipitation can be interpreted as an indicator of presence of tannin and phenolic compounds.
- **Ferric Chloride Solution Test:** 2 ml of 5% neutral ferric chloride solution were added to 1 ml of test extracts. Black or blue-green coloration or precipitate was taken as positive result for the presence of tannins and phenolic compounds.

Test For Gum & Mucilage:

- **Ruthenium Test:** Take a small quantity of dried test extracts powder, mount it on a slide with ruthenium red solution, and observe it under microscope; the formation of pink colour shows the presence of mucilage.

Test For Saponin:

- **Foam Test:** 1ml solution of test extracts were diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. The formation of frothing, which persists on warming in a water bath for 5 min, shows the presence of saponins.

Test For Oils And Fats

- **Spot Test:** Dried extracts sample to be tested is rubbed between the folds of filter paper. The appearance of translucent spot confirms the presence of fats in the test sample.

Test For Phytosterols

- **Salkowski's Test:** Small quantity of test extracts dissolved in 5 ml of chloroform. On adding a few drops of conc. sulphuric acid and allowing the solution to stand, formation of brown ring indicated the presence of phytosterols in test extract.
- **Liebermann Burchard's Test:** The test extracts were treated with few drops of acetic anhydride, boiled and cooled. On adding conc. sulphuric acid, formation of a bluish green colour solution confirmed the presence of phytosterols.

RESULTS AND DISCUSSION

Phytochemical Analysis of *Hibiscus laevis* extract

Qualitative chemical test are performed to analyze the presence of chemical constituents present in the extract of *Hibiscus laevis* (Table 1). Ethanolic extract shows presence of alkaloid, glycosides, carbohydrate, phytosterols and gum and mucilage. However, steroids, protein and amino acids, tannins, saponins and oil and fats were absent.

Table 1: Preliminary Qualitative Tests for *Hibiscus laevis* Extract:

TESTS	OBSERVATIONS
TEST FOR ALKALOIDS	
Dragendorff's test	**+ ve**
Wagner's test	**- ve**
TEST FOR GLYCOSIDES	
Legal test	**- ve**
Keller- kiliani test	**+ ve**
TEST FOR STEROIDS	
Salkowski test	**- ve**
TEST FOR CARBOHYDRATES	
Molisch's test	**- ve**
Fehling test	**+ ve**
TEST FOR FLAVONOIDS	
Shinoda Test	**- ve**
TEST FOR PROTEIN AND AMINO ACIDS	
Ninhydrin test	**- ve**
Biuret test	**- ve**
TEST FOR TANNIN & PHENOLIC COMPOUND	
Lead acetate test	**- ve**
Ferric chloride test	**- ve**
TEST FOR GUM & MUCILAGE	
Ruthenium Test	**+ ve**
TEST FOR SAPONINS	
Foam test	**- ve**
TEST FOR OILS & FATS	
Spot test	**- ve**
TEST FOR PHYTOSTEROLS	
Salkowski's Test	**+ ve**
Liebermann Burchard test	**- ve**

(+) Present, (-) Absent

Phytochemical Analysis of *Euryale ferox* extract. Qualitative chemical test are performed to analyze the presence of chemical constituents present in the extract of *Euryale ferox* (Table 2). The ethanolic extract shows presence of alkaloid, glycosides, tannins, carbohydrate and phytosterols. However, steroids, proteins and amino acids, gum and mucilages, saponins, and oil and fats were absent

Table 2: Preliminary Qualitative Tests for *Euryale ferox* Extract:

TESTS	OBSERVATIONS
TEST FOR ALKALOIDS	
Dragendorff's test	**+ ve**
Wagner's test	**- ve**
TEST FOR GLYCOSIDES	
Legal test	**+ ve**
Keller- kiliani test	**- ve**
TEST FOR STEROIDS	

Salkowski test	**- ve**
TEST FOR CARBOHYDRATES	
Molisch's test	**+ ve**
Fehling test	**- ve**
TEST FOR FLAVONOIDS	
Shinoda Test	**- ve**
TEST FOR PROTEIN AND AMINO ACIDS	
Ninhydrin test	**- ve**
Biuret test	**- ve**
TEST FOR TANNIN & PHENOLIC COMPOUND	
Lead acetate test	**+ ve**
Ferric chloride test	**- ve**
TEST FOR GUM & MUCILAGE	
Ruthenium Test	**- ve**
TEST FOR SAPONINS	
Foam test	**- ve**
TEST FOR OILS & FATS	
Spot test	**- ve**
TEST FOR PHYTOSTEROLS	
Salkowski's Test	**+ ve**
Liebermann Burchard test	**- ve**

(+) Present, (-) Absent

CONCLUSION

Alkaloids, glycosides, carbohydrates, phytosterols, gum, and mucilage have all been identified in an ethanolic extract of H. laevis. On the other hand, E. ferox has a wide variety of bioactive compounds, including alkaloids, glycosides, tannins, polysaccharides, and phytosterols. The presence of many secondary metabolites suggests that both plant extracts need further study for potential pharmacological action

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