PHYTOCHEMICAL SCREENING AND ASSAY OF QUERCETIN IN ABUTILON INDICUM LINN

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Abstract—The leaves of Abutilon indicum (Linn) are said to be used by traditional practitioners in cases of inflammatory joint disorders. Polyphenols, flavonoids, and tannins are some of the major classes of phytoconstituents that are responsible for the analgesic activity present in Abutilon indicum (Malvaceae). Quercetin is isolated and purified from the dried leaves and flowers of Abutilon indicum (Malvaceae). Abutilon indicum, also known as Atibala, is used as one of the most important drugs in traditional medicine to treat a variety of ailments. One of the most prevalent dietary flavonoids, quercetin, has been studied in methanol with magnesium (II). Quercetin and magnesium (II) complex formation was investigated using UV-visible, infrared, RP-HPLC, and NMR spectroscopy methods. Using a calibration curve, the amount of quercetin in Abutilon indicum leaves and flowers was determined. Many people have been suffering from piles in recent years. The hemorrhoidal swelling is referred to as "piles."India has a long history of traditional medical systems that have been used to treat both rural and urban people, and it is known to be a storehouse of fragrant and medicinal plants. Swollen veins in the lower rectum or surrounding the anus are called hemorrhoids. By the time they are 50 years old, around half of adults have hemorrhoidal problems. There are two types of hemorrhoids: internal and external. Internal hemorrhoids form in the rectum or anus. The development of external hemorrhoids occurs outside the anus. Another name for hemorrhoids is piles. The most prevalent and problematic hemorrhoids are external hemorrhoids. Pain, intense itching, and trouble sitting are all possible symptoms of hemorrhoids. Thankfully, they can be treated by abutilon indicum.

Keywords: abutilon indicum (linn), hemorrhoids, quercetin, photochemical screening

INTRODUCTION

India is home to a vast array of therapeutic herbs. Up to 80% of people still primarily rely on traditional remedies, such as medicinal plants, for their medications, according to estimates from the World Health Organization (WHO). India offers a solid foundation for the use of several plants in general health care and the relief of common ailments among its people due to its mega-biodiversity and knowledge of rich historic traditional medical systems (Ayurveda, siddha, unani, and local health traditions). In clinical research, natural materials with therapeutic value are typically becoming more important because of their well-known lack of side effects when compared to medications. A rich and ancient method of healing that precedes the development of contemporary medications is herbal medicine, commonly referred to as phytotherapy.

ABUTILON INDICUM

Tamil : Nalla tutti, perundutti, Tutti

English : Country Mallow, Flowering maples

Malayalam : Katturam, Pitikkapattu, Tuvatti

Telugu : Adavibenda, Botlabenda, Dudi

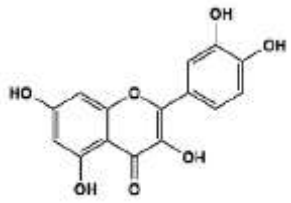
Taxonomic status:

Kingdom : Plantae

Subkingdom : Tracheobionta

Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Dilleniidae
Order : Malvales
Family : Malvaceae
Genus :Abutilon
Species :indicum

Native to tropical and subtropical areas, Abutilon indicum (Indian Abutilon, country Mallow) is a tiny perennial shrub in the Malvaceae family that is occasionally grown for its aesthetic qualities. In Bangladesh, India, Pakistan, and Sri Lanka, it is widely grown. Abutilon indicum is referred to as "Atibala" in Sanskrit in traditional medicine. Antioxidant, hepatoprotective, nephroprotective, wound-healing, antivenom, anticancer, antiarthritic, anti-Alzheimer's, hypoglycemic, antiulcer, aphrodisiac, anti-diarrheal, anticonvulsant, analgesic and sedative, diuretic, antimycotic, larvicidal, and antidiabetic properties. Additionally, the Ayurvedic Pharmacopeia of India recommends using root for hemorrhagic disorders, polyuria, and gout. The perennial shrub Abutilon indicum (Linn.) can grow up to 3 m tall and has a velvety tomentose appearance. Evergreen, base-cordate, stipulate, filiform, oval, acuminate, toothed, and infrequently subtrilobate, the leaves measure 1.9 to 2.5 cm in length. The cylindrical, hairy, stellate, yellowish-colored petiole is 1.5–1.70 cm long. The peduncles are joined above the center, and the flowers are yellow. Stipules are 9 mm long, pedicels are frequently 2.5-5 mm long, axillary solitary, joint very near to top, and the seeds are 3-5 mm long, kidney shaped, reniform, tubercled, or minutely stellate-hairy, black or dark brown. Petioles are 3.8-7.5 cm long.



Quercetin

Quercetin is a flavonoid with a molecular structure that includes two aromatic rings, an oxygen heterocycle, and five hydroxy groups.

Molecular formula : $C_{15}H_{10}O_7$

Molecular weight : 302.23g/mol

IUPAC Name : 2-(3,4-dihydroxyphenyl)-3,5,7- trihydroxychromen-4-one

Synonyms : Quercetin,117- 395,Meletin,Sophoretin,Quercetine,Xanthaurine,Quercetol

Description : Quercetin appears as yellow needles or yellow powder.Converts to anhydrous form at 203-207 °F. Alcoholic solutions taste very bitter.

Physical description

Color form :Yellow needles

Boiling point :Sub lines

Melting point :601 to 603^0F

Solubility :less than 1 mg/mL at 70 °F Very soluble in ether, methanol; soluble in ethanol, acetone, pyridine, acetic acid. Soluble in alcohol and glacial acetic acid; insoluble in water. In water, 60 mg/L at 16 °C.

Hemorrhoids, sometimes referred to as piles, are vascular structures found in the anal canal. They are cushions that aid in controlling the stool in their normal state. They become a disease when they swell or become inflamed; the disease is commonly referred to by the unqualified term hemorrhoid. Depending on the type of hemorrhoids, there are different signs and symptoms. Defecating with bright red, painless rectal bleeding is a common symptom of internal hemorrhoids. In the vicinity of the anus, external hemorrhoids frequently cause pain and swelling. It is typically darker if bleeding happens. It is common for symptoms to improve within a few days. Following the healing of an external hemorrhoid, a skin tag might still be present. Although the precise etiology of hemorrhoids is still unknown, several variables that raise abdominal pressure are thought to be related. Constipation, diarrhea, and prolonged toilet sitting are a few examples of this. Additionally, hemorrhoids are more prevalent during pregnancy. The area is examined in order to make a diagnosis. Serious causes of the symptoms should not be ruled out, as many people mistakenly call any discomfort that appears around the anal area hemorrhoids. It makes sense to perform a colonoscopy or sigmoidoscopy to confirm the diagnosis and rule out more serious causes.

Causes

- **Pregnancy:** During pregnancy, the increasing weight of the developing fetus and uterus put pressure on the pelvis. Hemorrhoids may occur in up to 35%Trusted Source of pregnant people.
- **Aging:** Hemorrhoids are most common among adults ages 45–65 years. However, young people and children can also get them.
- **Diarrhea:** Hemorrhoids can occur after cases of chronic diarrhoea.
- **Chronic constipation:** Straining to move a stool puts additional pressure on the walls of the blood vessels, which may result in hemorrhoids.
- **Sitting for too long:** Spending a long time in a seated position, especially on the toilet, can cause hemorrhoids.
- **Diet:** Eating low fiber foods may contribute to hemorrhoids.
- **Heavy lifting:** Repeatedly lifting heavy objects can lead to hemorrhoids.
- **Anal intercourse:** This can cause new hemorrhoids or worsen existing ones.

MATERIALS AND METHODS

EXTRACTION OF ABUTILON INDICUM

1.Collection of plant sample

The fresh leaves were collected from Trichy district, Tamil Nadu, India.

2.Method

The abutilon indicum leaf powder is extracted with the solvent of acetone by the Soxhlet extraction method.

3.Preparation of the leaf powder

The leaves of Abutilon indicum were washed thoroughly in tap water to remove dust particles. The leaves were then dried in shade at room temperature and coarsely powdered by a mechanical grinder.

4.Solvent of acetone extraction

Crude plant extract was prepared by Soxhlet extraction method. About 20gm of powdered plant material was uniformly packed into a thimble and extracted with 250 ml of solvents. Acetone used as solvent. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and then heated at 30-40^{0}c till all the solvent got evaporated. Dried extract was kept in refrigerator at 4^{0}c for further analysis.

Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds using following standard methods.

1.Test for proteins:

- **Millon's test :**Crude extract when mixed with 2ml of million's reagent.While precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.
- **Ninhydrin test :** Crude extract when boiled with 2ml of 0.2% solution of ninhydrin violet colour appeared indicated the presence of amino acids and proteins

2.Test for carbohydrates

- **Fehling's test :** Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars**.**
- **Benedict's test :** Crude extract when mixed with 2ml of benedict's reagent and boiled,areddish brown precipitate formed which indicated the presence of the carbohydrates.
- **Molisch's test :**Crude extract was mixed with 2ml of molisch's reagent and the mixture was shaken properly.After that,2ml of concentrated H_2SO_4 was poured carefully along the side of the test tube.Appearance of a violet ring at the interphase indicated the presence of the carbohydrate.

- **Iodine test :** Crude extract was mixed with 2ml of iodine solution.a blue or purple coloration indicated the presence of the carbohydrate.

3.Test for phenols and tannins

Crude extract was mixed with 2ml of 2% solution of $FeCl_3$.a blue-green or black coloration indicated the presence of phenols and tannins.

4.Test for flavonoids

- **Shinoda test:** Crude extract was mixed with few fragments of magnesium ribbon and concentrated Hcl was added drop wise, apink scarlet colour appeared after few minutes which indicated the presence flavonoids
- **Alkaline reagent test :** Crude extract was mixed with 2ml of 2% solution of NaOH an intese yellow colour was formed which turned colourless, On addition of few drops of diluted acid which indicated the presence of flavonoids

5.Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

6.Test for glycosides

- **Liebermann's :** Crude extract wasmixed witheach of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added.Acolour change from violet to blue to green indicated the presence of steroidal nucleus.i.e., glycone portion of glycoside.
- **Salkowski's test**: Crude extract was mixed with 2ml of chloroform .Then 2ml of concenrtrated H_2SO_4 was added carefully and shaken gently.A reddish brown colour indicated the presence of steroidal ring,i.e.,glycone portion of the glycoside**.**
- **Keller-kilani:** Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4.A brown ring at the interphase indicated the presence of cardiac glycosides.

7.Test for steroid

Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower choloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform . Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture.T he development of a greenish coloration indicated the presence of steroids.

8.Test for terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

9.Test for alkaloids

Crude extract was mixed with 2ml of 1% HCL and heated gently. Mayer's and Wagner's reagent were then added to the mixture Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Result and discussion:

The qualitative phytochemical examination of Abutilon indicum leaf extract confirms the presence of alkaloids, glycosides, carbohydrates, saponins, phenolic compounds, tannins, flavonoids, proteins, amino acids, and terpenoids. This study's phytochemical features will be helpful in developing new drugs, including traditional dosage forms, to treat a variety of human conditions.

1	**Test for protein**	
	Millon's test	+
	Ninhydrin test	-
2	**Test for carbohydrate**	
	Fehling's test	+
	Benedicts' s test	++
	Molisch's test	-
	Iodine test	-
3	**Test for phenol and tannin**	+
4	**Test for flavonoids**	
	Shinoda test	+
	Alkaline reagent test	+
5	**Test for saponins**	-
6	**Test for glycosides**	
	Liebermann's test	+
	Salkowski's test	+
	Keller-Kilani test	-
7	**Test for steroids**	+
8	**Test for terpenoids**	-
9	**Test for alkaloids**	+

Table 1:Qualitative phytochemical analysis

Anti-inflammatory activity

The albumin denaturation assay method. Additionally, the assay for the standard medication (quercetin) and the sample (leaf extract) to cure hemorrhoids was established.

Extract	Concentration µg/ml	Protection% Mean ± SD
S2 Test sample	2.5	58.51 ± 1.12
	5	62.18 ± 1.14
	7.5	67.19 ± 1.54
	10	71.28 ± 1.41
Standard(quercetin)	5	79.56 ± 1.65

Table 2:Assay Determination of Anti inflammatory activity

Conclusion

The albumin denaturation assay method's phytochemical screening and anti-inflammatory properties suggest that the plant Abutilon indicum may have a number of bioactive compounds that help treat hemorrhoids. Abutilon indicum leaf extract has anti-inflammatory effects on hemorrhoids at different concentrations (2.5,5,7.5,10 µg/ml). While the normal medication of quercetin concentration shows 79.56+ 1.65 protection mean + SD, the nearby sample shows 71.28+1.41 at concentration 10 µg/ml, indicating anti-inflammatory effect to treat hemorrhoid illness.

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