



Phytochemical investigation of apamarga (*Achyranthes aspera* linn.) on flowers and fruits

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Abstract

Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently, may lead to drug discovery and development. Phytochemical investigation was carried out on the plant *Achyranthes aspera* which revealed the presence of medicinally important bioactive compounds. The presence of various phytochemical compounds in the plant *Achyranthes aspera* was evaluated in flowers and fruits. The extracts were subjected to qualitative screening test for various constituents. The extracts of *Achyranthes aspera* showed the presence of phytochemicals such as alkaloids, carbohydrates, flavonoids, proteins, and saponins.

Keywords: *Achyranthes aspera* linn., extracts, phytochemical investigation

Introduction

The term "medicinal plant" refers to a plant whose parts contain compounds that can be used to treat illnesses. The importance of medicinal plants to the wellbeing of people and communities is greater. Plant-based medicines are advantageous and well-known for their reliability, accessibility, and affordability. Whole plant parts that are primarily prepared from various plant parts may be included in herbal medicine. They are applied topically as well as orally and inhaled.^[1]

A wide variety of chemical compounds are produced by plants, and they are divided into primary and secondary metabolites according to their chemical class, biosynthetic origin, and functional groups. While secondary metabolites have been used as biocatalysts, they are not directly involved in growth and development like primary metabolites are. Primary metabolites can be found in all types of organisms and are widely distributed in nature. Similar to amino acids, nucleotides, carbohydrates, and chlorophyll, they play a crucial part in metabolic processes like photosynthesis, respiration, and nutrient assimilation.^[2]

The plant species *Achyranthes aspera* Linn., which belongs to the Amaranthaceae family, is also known as apamarga. It is a well-known medicinal plant that is found to be used in tropical African and Asian countries as herbal remedies. It thrives in humid climates and is typically found as a weed throughout India. Every single part of the plant is frequently used to treat a variety of illnesses, including stomatitis, asthma, piles, dysentery, etc. Additionally, it functions as an abortifacient, an anti-diabetic, and an anti-inflammatory.^[3]

Many Ayurvedic medicines' pharmacologically active components are currently being identified, and their value for drug therapy is being assessed^[4].

The major aim of this work is to perform collection, extraction of *Achyranthes aspera* and detection of phytochemical constituents such as flavonoids, carbohydrates, proteins, tannins, etc.

Materials and Methods

Collection and authentication of plant material

Plant materials *Achyranthes aspera* were collected from Chopda region of Jalgaon district (Maharashtra). The plants were verified by the Western Regional Centre of the Botanical Survey of India in Pune, and a herbarium was deposited at the Department of Pharmacognosy of the Smt. S. S. Patil College of Pharmacy in Chopda, District of Jalgaon.

Extraction of flowers and fruits of *Achyranthes aspera*

The collected flowers and fruits were carefully cleaned in the current study for extraction from *Achyranthes aspera* to remove foreign, earthy matter, and residual materials. Later, in the twilight, it withered. The leaves were spread in a tray and air dried for a period of 7 days. The parched leaves were then pulverized using a laboratory pulverizer and the powder (particle size approximately 0.4 mm) was used for extraction. Extraction is the basic step in herbal drug preparation and it helps the plant metabolites to get solubilised in solvents. The important factors that affect the efficiency of extraction process are solubility of metabolites in the menstruum, temperature of extraction, particle size of the plant materials etc. A molecule may be water soluble or water

insoluble based on its chemical makeup. The powdered dried leaves of *Achyranthes aspera* (100 gm of each) were successively extracted by using ethanol AR (Sigma Aldrich), in a Soxhlet extracting apparatus. Continued the process of extraction for a day or until the solvent gets decolourized. When the extraction was concluded, the extract was collected, strained using Whatman No.1 filter paper and the extract was concentrated under reduced pressure with a rotary vacuum evaporator. The weight of *Achyranthes aspera* (Linn. f.) sweet petroleum ether dried extract was determined and the product was calculated in comparison with the dried drug substance taken for extraction ^[5].

Phytochemical investigation of flowers and fruits of *Achyranthes aspera*

For the purpose of screening various types of secondary metabolites, preliminary phytochemical analysis was conducted. Different qualitative chemical tests, such as those for alkaloids, carbohydrates, glycosides, tannins, saponins, steroids, proteins, and flavonoids in various extracts of *Achyranthes aspera*'s flowers and fruits, were conducted as part of the phytochemical investigation. ^[6].

Carbohydrates detection

Molisch's test

On a 5 ml sample, a few drops of Molisch reagent were added. The test tube was then tilted, and 1 ml of sulphuric acid was gradually added through one side at the bottom. The violet ring that forms at the junction is a sign of the presence of carbohydrates. ^[4].

Fehling's test

1 ml of filtrate and 1 ml of each of Fehling's solutions A and B were boiled on a water bath. Reducing sugar is present when there is a red precipitate. ^[6].

Benedict's test

In a test tube, 2 ml of Benedict's reagent and test solution were combined. It was heated for five minutes in a bath of boiling water. The presence of reducing sugar in the test solution is indicated if the solution appeared to be green in colour. ^[7].

Proteins detection

Million's test

2 ml of Millon's reagent, which is mercuric nitrate in nitric acid with traces of nitrous acid, was added to the plant's extract. When heated gently, a white precipitate that initially appeared to contain amino acids turns red. ^[8].

Xanthoproteic test

A few drops of concentrated nitric acid were added to the extracts. Yellow colour formation indicates the presence of proteins. ^[4].

Biuret test

A few drops of a 1 percent copper sulphate solution and a 4 percent sodium hydroxide solution were added to 0.5 ml of plant extract. The appearance of violet colour indicated the presence of protein. ^[8].

Alkaloids detection

Dragendorff's test

Dragendorff's reagent should be added to 0.5 ml of plant extract (Potassium bismuth iodide solution). Reddish-brown precipitate's appearance confirms the presence of alkaloid and signals a positive test result. ^[8].

Hager's test

Hager's reagent was added to a small amount of filtrate, and the presence of a strong yellow colour indicated that the test was successful. ^[9].

Mayer's test

The sides of the tube containing a few ml of filtrate were used to add a few drops of Mayer's reagent. A creamish-yellow precipitate's formation indicated a successful outcome. ^[9].

Wagner's test

Wagner's reagent was added in small amounts along the sides of a test tube that contained a small amount of filtrate. Reddish-brown precipitate formation was viewed favourably. ^[9].

Tannins detection

Lead acetate test

5 ml of 45 percent ethanol was heated with 2 g of test solution for 5 min. The filtrate from the filtering of the chilled solution was used in the subsequent test. Three drops of lead acetate mixture were added to one millilitre of filtrate, and the presence of tannins was detected in a cream gelatinous precipitate. ^[10].

Saponin detection**Foam test**

With vigorous shaking, 5 ml of distilled water was added to 5 ml of filtrate (aqueous extract). Saponins are an indication of a stable froth. ^[11].

Flavonoids detection**Shinoda test**

2-3 ml of the filtrate from the (methanol extract) were added to a piece of magnesium ribbon and 1 ml of concentrated HCl. Flavonoids are present when the solution is pinkish-red or red in colour. ^[11].

Shinoda test for flavanols

3 ml of the resulting solution was combined with 4 ml of aluminium chloride in methanol. The formation of a yellow colour proves the presence of flavanols. ^[12].

Sulphuric acid test

5 ml of diluted ammonia and then concentrated sulfuric acid were added to 1 ml of extract. The presence of flavonoids is indicated by the appearance of a yellow colour. ^[8].

Tannins detection**Lead acetate test**

To 0.5 ml of the plant extract, a few drops of lead acetate at 10% were added. The presence of tannins was indicated by the precipitate's formation. ^[8].

Iodine test

Add a few drops of iodine solution to the 3 ml extract solution. Tannins can be detected by a blue colour that changes when something is boiled and then returns when it is cooled. ^[13].

Potassium dichromate test

10% aqueous potassium dichromate solution gives yellowish brown precipitate ^[14].

Steroid detection**Liebermann-Burchard test**

Add a few drops of acetic anhydride to 0.5 ml of plant extract, then boil and cool the mixture. Concentrated sulfuric acid was poured into the test tube and added to the sides. Steroids are indicated by the development of a brown ring at the intersection of two layers and by the green colour of the upper layer, whereas triterpenoids were indicated by the development of a deep red colour. ^[8].

Salkowski test

The plant extract was mixed with a few drops of concentrated sulfuric acid and 0.5 ml of chloroform. After shaking and standing for a while, the lower layer began to turn red, indicating the presence of steroids, and the lower layer began to turn yellow, indicating the presence of triterpenoids. ^[8].

Result and Discussion**Phytochemical investigation of flowers and fruits of *Achyranthes aspera***

The identification of bioactive principles in the flowers and fruits of *Achyranthes aspera* is crucial for the discovery of new sources of industrially and therapeutically valuable compounds that could result in the development of novel drugs. The results of a number of qualitative extract tests revealed that the flowers of *Achyranthes aspera* contained sugars, alkaloids, flavonoids, and saponins. The fruits of *Achyranthes aspera* contained alkaloids, flavonoids, saponins, proteins, and carbohydrates.

Table 1: Phytochemical investigation of *Achyranthes aspera* of flowers and fruits

| Tests | <i>Achyranthes aspera</i> Flowers | <i>Achyranthes aspera</i> Fruits |
|------------------------|-----------------------------------|----------------------------------|
| Test for carbohydrates | | |
| Molisch's test- | + | ++ |
| Fehling's test | + | ++ |
| Benedict's test | + | +++ |
| Test for proteins | | |
| Million's test | - | - |
| Xanthoproteic test | - | + |
| Biuret test | - | + |
| Test for alkaloids | | |
| Dragendorff's test | + | + |
| Hagers's test | + | + |

| | | |
|----------------------------|-----|-----|
| Mayer's test | - | - |
| Wagner's test | ++ | + |
| Test for flavonoids | | |
| Shinoda test | ++ | ++ |
| Shinoda test for flavanols | - | - |
| Sulphuric acid test | ++ | ++ |
| Test for tannins | | |
| Lead acetate test | - | - |
| Dil. Iodine test | - | - |
| Pot. dichromate test | - | - |
| Test for saponins | | |
| Foam test | +++ | +++ |
| Test for steroids | | |
| Libermann-Burchard test | - | - |
| Salkowski test | - | - |

+: Positive, -: Negative

Conclusion

Numerous phytochemical components have been isolated from the plant, according to the study. According to the current study, the phytochemicals that were extracted are very valuable. Additionally, the phytochemicals' isolation, purification, and characterization will yield interesting studies. Alkaloids, carbohydrates, flavonoids, proteins, and saponins are the main chemical components. This plant has a rich source of phytochemicals that make it useful for food, drugs, and herbal medicine.

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