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Research Article

PHARMACOGNOSTIC PROFILE AND PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *ACHYRANTHES ASPERA*

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ABSTRACT

Objective: Medicinal plants have played a significant role in ancient traditional systems of medication in many countries. *Achyranthes aspera* Linn is one of the important medicinal plants having many therapeutic uses. The present study deals with pharmacognostic profile and preliminary phytochemical investigations of *Achyranthes aspera* Linn.

Method: Various parameters like fluorescence analysis of powdered as well as its extractives and phytochemical screening of different extractives were studied.

Result: Phytochemical screening of the plant revealed the presence of phenolics, flavonoids, tannins, alkaloids, proteins and carbohydrates.

Conclusion: The present study revealed that *Achyranthes aspera* Linn is an important source of many therapeutically and pharmacologically active constituents. The plant has been widely studied for its pharmacological activities and finds its position as a versatile plant having a wide spectrum of medicinal activities.

Keywords: *Achyranthes aspera*, Pharmacognosy, Phytochemical analysis, Flavonoids.

INTRODUCTION

Nature is a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources [1]. Plant-derived substances have recently become a great interest owing to their versatile applications. Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [2]. Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals [3]. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [4]. According to the WHO the first step for identification and purification of herbal drugs is the pharmacognostic (macroscopic and microscopic) studies which are essential for any phytopharmaceutical products used for standard formulation [5]. Recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species. In the present era of drug development and discovery of newer drug molecules, many plant products are evaluated on the basis of their traditional uses. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different compositions which occur as secondary metabolites [6].The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [7].

Achyranthes aspera Linn belongs to the family Amaranthaceae. It is an annual, stiff erect herb, and found commonly as a weed throughout India and is one of the important medicinal plants having many therapeutic uses as Odontalgic, Rheumatism, Bronchitis, skin disease and rabies [8]. Leaf extracts were reported to possess thyroid stimulating and anti-peroxidative properties [9]. The aqueous and methyl alcohol extracts of the plant also decreased blood glucose levels in normal and alloxan diabetic rabbits [10]. The main aim of the present investigation was to study the pharmacognostic profile and phytochemical constituents of *Achyranthes aspera*.

MATERIALS AND METHODS

Collection and identification of plant material

The specimen was collected from Coimbatore and authenticated by Botanical Survey of India, Coimbatore, India. The leaves of the plant (*Achyranthes aspera*) were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter. After complete drying, leaves were powdered well using a mixer. Then the powdered material was weighed and kept in air tight container and stored in a refrigerator for future use. About 10g of this powdered sample was refluxed with petroleum ether, chloroform, methanol, ethanol and water in the ratio of 1:10 (w/v). The crude extracts were collected in amber coloured sample bottles and stored. All chemicals and reagents used including the solvents were of analytical grade.

Pharmacognostic Profile

Extractive values

Extract of the powdered leaves were prepared with different solvents for the study of extractive values.

Fluorescence Analysis

A small quantity of dried and finely powdered leaf was placed in a clean grease-free microscopic slide, treated with 1 - 2 drops of the freshly prepared reagent solution, mixed gently by tilting the slide and waited for 2 - 4 minutes. The slide was then viewed day light and ultraviolet radiations (365 nm). The colours observed on application of different reagents in different radiations were recorded.

Phytochemical Analysis

Phytochemical analysis was carried out in the petroleum ether, chloroform, ethanol, methanol and water extracts of the leaves of *Achyranthes aspera* using standard procedures to identify constituents, as described by Harborne (1984), Trease and Evans (1979), and Sofowara (1993) [11, 12, 13].

Test for alkaloids

Dragendroff's test

To 5 mL of the extract few drops of Dragendroff's reagent was added for the formation of orange coloured precipitate.

Mayer's test

To 5 mL of the extract few drops of Mayer's reagent was added for the formation of cream coloured precipitate.

Wagner's test

To 5 mL of the extract few drops of Wagner's reagent was added for the formation of reddish brown coloured precipitate.

Hager's test

To 3 mL of the extract few drops of Hager's reagent was added for the formation of prominent yellow precipitate.

Test for flavonoids

To 3 mL of the extract few magnesium ribbons are dipped and conc. HCl was added over them and observed for the formation of magenta (brick red) colour indicating the presence of flavonoids.

Test for proteins

Biuret test

To 3 mL of the extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple color. On addition of alkali, it becomes dark violet.

Millon's test

To 3 mL of the extract few drops of Millon's reagent was added for the formation of red colour.

Test for carbohydrates

Molisch's test

To a small amount of the extract few drops of Molisch's reagent was added followed by the addition of conc. H_2SO_4 along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 mL of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates.

Fehling's test

The extract was treated with 5 ml of Fehling's solution (A and B) and kept in boiling water bath. The formation of yellow or red color precipitate indicates the presence of reducing sugar.

Test for tannins

A fraction of the extract was dissolved in water and then it was subjected to water bath at 37^0 C for 1 hour and treated with ferric chloride solution and observed for the formation of dark green colour.

Test for sterols

Liebermann-Burchard test

To a small amount of the extract few drops of chloroform, acetic anhydride and H_2SO_4 was added along the sides of the test tube to observe the formation of dark red or pink colour.

Test for glycosides

Baljet's Test

To 5 mL of the extract few drops of sodium picrate was added to observe yellow to orange colour.

Keller-Killiani test

To 5 mL of the extract few drops of ferric chloride solution was added and mixed, then sulphuric acid containing ferric chloride solution was added, it forms two layer showed reddish brown while upper layer turns bluish green indicates the presence of glycosides.

Test for phenols

Ferric chloride test

A fraction of the extract was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour.

Test for saponins

Foam test

To a small amount of the extract few drops of distilled water was added and shaken vigorously until persistent foam was observed.

Test for terpenoids

Chloroform test

To 5 mL of the extract few drops of chloroform and conc. H_2SO_4 was added carefully along the sides of the test tube to form a layer and observed for the presence of reddish brown colour.

RESULTS AND DISCUSSION

Extractive values

Extractive values of the successive extracts of *Achyranthes aspera* are given in Table 1.

Table 1: Percentage of successive extracts of *Achyranthes aspera*

Solvents	Extract values (% w/w)
Petroleum ether	2.3
Chloroform	5.24
Methanol	6.34
Ethanol	8.3
Water	9.7

Fluorescence analysis

The powdered sample of *Achyranthes aspera* was subjected to fluorescence analysis, results are tabulated in Table 2.

Table 2: Fluorescence analysis of *Achyranthes aspera*

Plant sample	Day light	UV light (365nm)
Powder	Green	Dark green
Powder+ NaOH	Dark green	Light green
Powder+Acetone	Pale green	Yellowish green
Powder+HCl	Light green	Green
Powder+HNO_3	Light green	Green
Powder+Acetic acid	Brown	Dark brown
Powder+$CHCl_3$	Yellowish green	Pale green

Phytochemical Analysis

Powdered leaves of *Achyranthes aspera* were subjected to various qualitative tests for the identification of phytochemical constituents includes tests for alkaloids (Dragendroff's test, Mayer's test, Hager's test, Wagner's test), saponins, glycosides (Baljet's test, Kellar-Killiani test), carbohydrates (Molisch's test, Fehling's test), proteins (Biuret test, Xanthoprotein test, Millon's test), tests for tannins, flavonoids, steroids (Liebermann-burchard test), phenols, terpenoids were performed using specific reagents and results are tabulated in Table 3.

Table 3: Phytochemical analysis of extracts

Phytochemicals	Petroleum ether	Chloroform	Methanol	Ethanol	Water
Alkaloids	-	-	+	-	+
Flavonoids	-	-	+	+	+
Proteins	-	+	+	+	+
Carbohydrates	-	+	+	+	+
Tannins	-	+	+	+	+
Sterols	-	-	+	-	+
Glycosides	-	+	+	+	+
Phenols	-	-	+	+	+
Saponins	-	-	+	+	+
Terpenoids	-	+	+	-	+

'+' present, '-'absent

Phytochemical screening results of the powdered sample of *Achyranthes aspera* extracted in water and methanol showed the presence of all the constituents whereas the chloroform extract showed the presence of proteins, carbohydrates and cardiac glycosides and the ethanol extract was positive for flavonoids, tannins, glycosides, carbohydrates, proteins, saponins, phenols. In the present study, the preliminary phytochemical screening of the various extracts revealed the presence of major bioactive compounds which may retain a wide range of actions.

CONCLUSION

Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals. The use of traditional medicine is widespread and plants still present a large source of novel active biological compounds with different activities, including anti-inflammatory, anti-cancer, antiviral, and antibacterial and cardio protective activities. Even today plant materials continue to play a major role in primary health care as therapeutic remedies in developing countries. Researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs. Results of phytochemical evaluation reveal the presence of alkaloids, flavonoids, proteins, carbohydrates, tannins, phenols, glycosides, saponins and terpenoids. The pharmacognostic profile and phytochemical screening of the present study showed favorable effects for the standardization parameters of plant parts. This established a significant scope to develop a broad spectrum use of *Achyranthes aspera* in herbal medicine and as a base for the development of novel potent drugs and phytomedicine.

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