

Evaluation of qualitative phytochemical analysis of water extract of *Achyranthes aspera* and *Achyranthes aspera* 30

Usha Kushwaha*

Department of Physiology and Biochemistry, Bakson Homoeopathic Medical College and Hospital, Greater Noida, Uttar Pradesh, India

Abstract

Introduction: Plant sources are a valuable starting material for drug development. These plants are the potential therapeutic agents, which provide maximum benefits with minimum adverse effects. **Objective:** The objective of the study is to evaluate the qualitative phytochemical analysis of water extract of whole plant excluding root of *Achyranthes aspera* and 30 potency of the same drug. **Methodology:** The qualitative phytochemical analysis of water extract of whole plant excluding root of *Achyranthes aspera* and *Achyranthes aspera* 30 has been performed to confirm the presence of alkaloid, saponin, phenolic compound, carbohydrate and proteins. Physicochemical constants such as ash, extractive values and moisture content were also determined. **Results:** The physicochemical analysis showed that the parts of this plant contained total ash value of 9.59% in which the acid-insoluble ash is 2.27%. The extractive values percentage of water-soluble extract is 20.91%. The qualitative phytochemical analysis reveals the presence of carbohydrates, protein, alkaloids, saponins and phenolic compounds in water extract of *Achyranthes aspera* and *Achyranthes aspera* 30. The analysis also shows that various bioactive phytochemicals are retained with dilution while preparation of Homoeopathic medicines. **Conclusion:** *Achyranthes aspera* in homoeopathic potency 30 contains its bioactive phytochemicals even after being a high dilution (with alcohol) of the original plant.

Keywords: *Achyranthes aspera*, Qualitative phytochemical screening, Water extract

INTRODUCTION

Plant sources are a valuable starting material for drug development. These plants are the potential therapeutic agents, which provide maximum benefits with minimum adverse effects.^[1] The World Health Organization has developed and launched the traditional medicine strategy 2014–2023 in response to the World Health Assembly resolution on tradition medicine (62.13). The strategy aims to support member states in developing proactive policies and implementing action plans that will strengthen the role traditional medicine plays in keeping the population healthy.^[2] *Achyranthes aspera* L. belongs to the family *Amaranthaceae* and is commonly known as ‘prickly chaff flower’. It is the herb of 1 cm in height is found throughout India.

Achyranthes aspera stem is angular, showing a single layer of epidermis with thin cuticle, uniseriate multicellular hairs, having crystal of calcium oxalate in the cortex and pith. Leaves are opposite, 2.5–12.5 cm, extremely variable, generally thick, pubescent tomentose velvety, rarely glabrate

petiole short. Flowers are 4–8 mm longer than the bracteoles, sepals sub-equal.^[3] The mother tincture is prepared using the whole, fresh plant, dried [Figure 1]. The drug strength is 1/10. Clinically, *Achyranthes aspera* is used in chickenpox, encephalitis, glomerulonephritis, infectious diseases, kidney abscess, measles, meningitis, neuritis, psittacosis, pyelonephritis, rubella, scarlet fever, septicaemia and typhoid fever.^[4]

In the present study, the extract of whole plant excluding root of *Achyranthes aspera* was prepared, and qualitative phytochemical analysis of water extract and in dilution 30 was carried out.

***Address for correspondence:** Dr. Usha Kushwaha, Bakson Homoeopathic Medical College and Hospital, Greater Noida, Uttar Pradesh, India. E-mail: kushwaha_usha2000@yahoo.com

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METHODOLOGY

Procurement of plant

Achyranthes aspera plant was collected from village Ladapura, Greater Noida, Uttar Pradesh, in the 1st week of August 2016. *Achyranthes aspera* 30 was procured from Bakson Drug and Pharmaceutical Private Limited Lot number P1222, M. L. Number: H. P. 2/H. All other used chemicals have been procured from Metropolitan Scientific Company, Delhi.

Processing of plant material

Parts of *Achyranthes aspera* plant were dried under shade for 15 days at physiology laboratory of Bakson Homoeopathic Medical College and Hospital, Greater Noida, U.P., India. Dried parts of the whole plant excluding root were homogenised to fine powder using mortar and pestle. The fine powder is stored in an amber colour glass bottle to carry out further studies. The water extract was prepared by taking 10 g of fine powder in 100 ml of water, stir for 6 h and leave it overnight then filtered it through Whatman filter paper no. 1.

Physiochemical parameters

Physiochemical parameters of the powdered drug such as ash values and extractive values loss on drying were performed.

Determination of total ash

For determination of the total ash^[5] value, 2 gms of air dried drug powder, was placed as a uniform layer in crucible silica. It was ignited on the burner and heated at 550±50°C in muffle furnace until it was white, indicating the absence of carbon. It was allowed to cool down in a desiccator and weighed to determine the percentage of ash with reference to air-dried drug. Silica crucible was placed again in furnace for 30 minutes and cooled in desiccator and weighed. This process was repeated until the constant weight of crucible was obtained.

Determination of acid-insoluble ash

For determination of acid-insoluble ash, the ash was boiled with 25 ml of dilute HCl for 5 min and it was filtered through an ash-less Whatman filter paper and washed with hot distilled water until it became acid-free. Then, the paper was placed in silica crucible and burnt in a muffle furnace. When it became carbon-free, it was placed in a desiccator to cool down, and weight was taken and calculated.

Determination of solvent extractive values

Ten grams of air-dried drug powder was taken in a closed flask, and 100 ml of water was added. It was stirred for 6 h. Then, it was allowed to stand overnight. Then, it was filtered through Whatman filter paper. 5–10 ml of filtrate was taken in a pre-weighed petri dish and dried at 105°C till the constant weight achieved. The petri dish was placed in the desiccator and allowed to cool; the weight was measured and the solvent extractive value was calculated.

Qualitative phytochemical analysis

Preliminary phytochemical screening was performed for several compounds such as carbohydrates, steroids, alkaloids, saponins and phenolic compounds.^[6,7] These compounds

are termed as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of metabolites, the samples were subjected different chemical tests as described below.

Test for carbohydrates

- i. Fehling's test – 34.66 g of copper sulphate was dissolved in distilled water and made up to 500 ml (Fehling A). 173 g of potassium sodium tartrate and 50 g of sodium hydroxide were dissolved in distilled water and made up to 500 ml (Fehling B). 1 ml of Fehling's A and 1 ml of Fehling's B in a test tube were mixed. 2 ml of testing solution in the test tube was added. The red precipitate formed which showed the presence of reducing sugar (glucose and fructose).
- ii. Molisch's test – 5 ml of testing solution was taken in a test tube. Two drops of Molisch reagent was added and mixed thoroughly with a dropper. 3 ml of concentrated sulphuric acid was added along the sides of the test tube. A reddish violet ring appeared at the junction of two liquids. Hence, monosaccharides were present.
- iii. Barfoed's test – 5 ml of Barfoed's reagent was taken in a test tube. 0.5 ml of testing solution was added, mixed well and boiled for 2 min. The tube was kept in a test tube rack and examined for precipitate after 10–15 min. A red precipitate clinging to the bottom-most part of the test tube forms showed the presence of monosaccharide.
- iv. Seliwanoff's test – 5 ml of Seliwanoff's reagent was taken in a test tube. 5 drops of the testing solution was added and heated the contents to just boiling. The presence of red colour indicated that fructose was present in the testing solution.

Test for protein

- i. Xanthoprotein test – 1 ml of concentrated nitric acid was added to 2–3 ml of test protein solution and heated to boil. The solution was cooled. 40% NaOH was added. A white precipitate formed on adding nitric acid which on heating turns yellow and then dissolved to impart yellow colour to the solution. On adding alkali, the colour deepened to attain orange colour.
- ii. Biuret test – 2–3 ml of testing solution was taken in a test tube. An equal volume of 10% sodium hydroxide solution was added and mixed thoroughly. Then, 0.5% copper sulphate solution was added drop by drop and mixed until a purplish violet colour is obtained. Positive reaction indicated that the given protein solution contained at least two peptide bonds.

Test for alkaloid

- i. Wagner's test – Test solution was treated with Wagner's reagent (iodine-potassium iodide solution) which showed reddish brown precipitate.

Test for saponins

- i. Foam test – Test solution was shaken which showed foam formation and it was stable for at least 15–20 min; positive for the presence of saponins.

Test for phenolic compound

- i. Ferric chloride test – The solution was treated with a few drops of ferric chloride solution to show dark colour.

RESULTS

Physiochemical parameters

Determination of total ash

The total ash was 9.59% and acid-insoluble ash was 2.27% as shown in Table 1.

Extractive values

The percentage yield of water-soluble extract was 20.91%.



Figure 1: Dried *Achyranthes aspera*

Qualitative phytochemical analysis

The water extract and dilution 30 showed the presence of carbohydrate, protein, saponins, phenolic compound and alkaloid as shown in Table 2 and Figure 2.

DISCUSSION

Plants contain many bioactive phytochemical substances that produce definite physiological and biochemical action on the human body. The phytochemical research approach is considered to be effective in discovering bioactive profile of plants for therapeutic importance. The total ash was 9.59% and percentage yield of water soluble extract was 20.91%.

This experiment shows the evaluation of qualitative phytoconstituents of water extract of whole plant excluding root of *Achyranthes aspera* and *Achyranthes aspera* 30.

The phytochemical analysis of water extract of whole plant excluding root of *Achyranthes aspera* and *Achyranthes aspera* 30 shows that various bioactive phytochemicals such as carbohydrate, protein, alkaloid and phenolic compounds are retained even with dilution in Homoeopathic 30 potency of *Achyranthes aspera*. The foam test of *Achyranthes aspera* 30 is negative whereas of water extract is positive.

CONCLUSION

The qualitative phytochemical analysis shows that various bioactive phytochemicals are retained even with dilution while preparation of Homoeopathic medicines. *Achyranthes aspera* 30

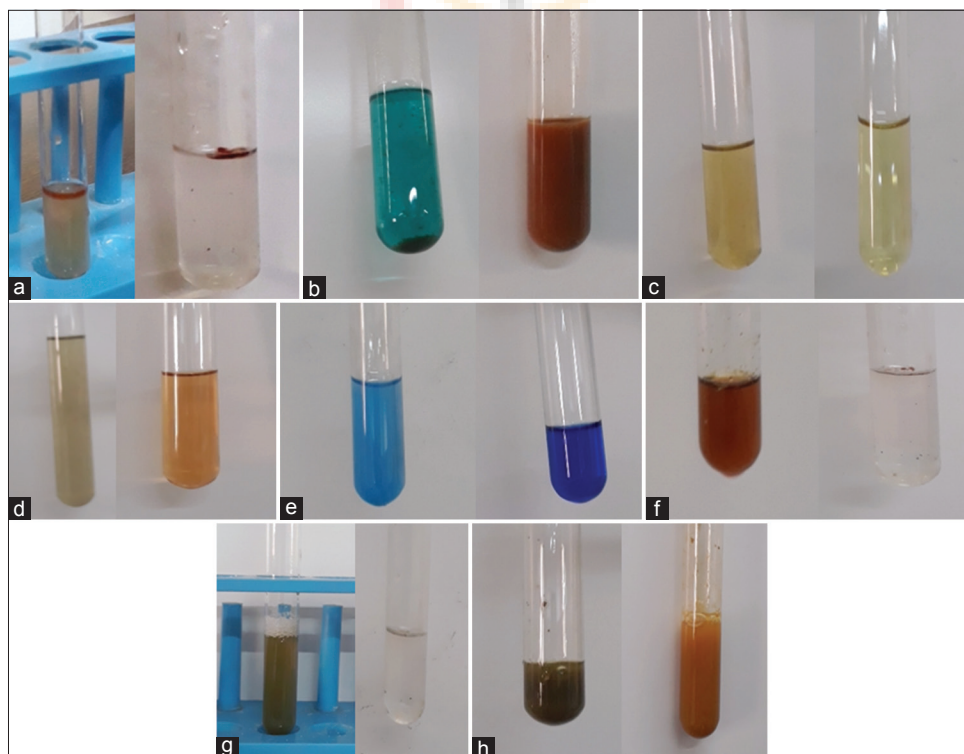


Figure 2: Phytochemical analysis by colour reactions. (a) Molisch test, (b) Fehling's test, (c) Seliwanoff's test, (d) Xantoprotein test, (e) Biuret test, (f) Wagner's test, (g) foam test, (h) ferric chloride test (first figure under each heading is of water extract and second is of dilution 30)

Table 1: Ash value

Type of ash	Result (%)
Total ash	9.59
Acid-insoluble ash	2.27
Acid-soluble ash	7.32

Table 2: Phytoconstituents present in water extract of whole plant excluding *Achyranthes aspera* and *Achyranthes aspera* 30

Test and phytoconstituent	Water extract	<i>Achyranthes aspera</i> 30
Test for carbohydrates		
Molisch's test	Positive	Positive
Fehling's test	Positive	Positive
Seliwanoff's test	Negative	Negative
Test for protein		
Xantoprotein test	Positive	Positive
Biuret test	Positive	Positive
Test for alkaloids		
Wagner's test	Positive	Positive
Test for saponins		
Foam test	Positive	Negative
Test for phenolic compound		
Ferric chloride test	Positive	Positive

contains its bioactive phytochemical even on dilution with alcohol.

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Conflicts of interest

None declared.

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