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Research article

Phytochemical investigation on leaves of *Achyranthes Aspera*

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ABSTRACT

Achyranthes aspera Linn is a potential folklore medicinal plant (amaranthaceae) used as an antioxident and antiinflammatory. The plant material was dried in shade, crushed and subjected to prepare different sequential and non-sequential extracts using soxhlet apparatus. Extracted ethyl acetate, methanol and other solvent fractions ware treated in phytochemical screening and show that identification of glycoside, Triterpene, diterpene, alkaloids, tannins - phenolic compounds, Saponins, steroids and sterols, protein and amino acids as the major chemical groups. Extract ware also studied for physical evaluation, fluorescence characteristic, ash value, moisture content. The results exhibited the presence of different phytochemical. All these phytochemical have potential therapeutic or physiological actions on human system, for that the leaves of A. Aspera can stand as a potential source of some vital drugs.

Keywords: *Achyranthes aspera*, Antioxidants, Phytochemical.

INTRODUCTION

Achyranthes Aspera Linn belonging to family Amaranthaceae, is commonly found as a weed on way side and at waste places throughout India. It is known as Apamarg in Sanskrit, Aghedo and Aghedi in Gujarati, Chirchira and Chirchitta in Hindi and Prickly chaff flower in English. Plant is 1meter erect or subscandent annual herb. Leaves are opposite, elliptic or obovate, form an acute or obtuse base, acuminate or rounded at apex. Flower: Deflexed, congested near the apex of the axis, perfect, bracts and bracteoles subequal. It is widely used for asthmatic cough, snakebite, hydrophobia, urinary calculi, rabies, influenza, piles, bronchitis, diarrhea, renal dropsy, gonorrhea and abdominal pain. The seeds are employed as an emetic, purgative, and cathartic, in gonorrhoea, for insect bite and in hydrophobia cough including whooping cough as an anti-asthmatic. The leaves are used in wounds, injuries and in intermittent fever, as an antiasthmatic, for urination, dog bite and in typhoid. The root is used in whooping cough, tonsilitis, haemorrhage, cough and hydrophobia, as an antiasthmatic diuretic, diaphoretic, and antisyphilitic [1].

MATERIAL AND METHOD

Procurement of plant material

The leaves of Achyranthes aspera was collected from Bhopal, Madhya Pradesh (India). The plants were authenticated and the voucher specimen was deposited in the department of Sanjay nikunj Gyaraspur dist. Vidisha.

Drying and size reduction

After identification and authentication leaves were subjected to drying in normal environmental condition under shade. The dried leaves were powdered by pulverization and were stored in air tight container [2].

Extraction by fractionation

By Petroleum Ether

About 1.5 kg of shade dried powder of leaves of Achyranthes aspera was extracted with petroleum ether (60°- 80°) for 24 hrs by using soxhlet apparatus. After completion of extraction the solvent was removed under reduced pressure and the extractive was determined.

By Methanol

The marc left after petroleum ether extraction was dried and extracted with methanol for 24hrs. After completion of extraction, the solvent was removed under reduced pressure and the extractive value was determined. The crude methanol extract, after removal of the

solvent, was dissolved in 10% sulfuric acid solution and partitioned with chloroform, ethyl acetate and n-buatnol successively to give chloroform , Ethyl acetate , n-Butanol and water soluble fractions respectively.

Pharmacognostic Evaluation

The above extracts were used for further studies such as Colour, Consistency and Percentage of eield, Fluorescence analysis, Ash values, Moisture containet, and phytochemical investigation.

Physical Evaluation

Colour, consistency and extractive values of different extracts of leaves of Achyranthes aspera well performed and result shown in table no. one [3].

Fluorescence Characteristic

The fluorescence characteristic of different extracts was studied by observing them under UV Light at 365nm. The tests and observations are recorded in the table below table no. two.

Fluorescence Characteristic with Different Chemical Reagent

Plant extracted powder is treated with different chemical reagents and seen in the UV cabinet under 254nm, different colours will be produced. Therefore it can be used for the identification of the drug. The tests and observations are recorded in Table No. three.

Determination of Ash Values

Total Ash

3 gm of powdered crude drug were accurately weighed in a silica crucible. The powdered drug was gradually increased the heat until free from carbon and after cooling it was kept in a desiccator. Then with reference to the air dried sample ash is weighed and percentage was calculated.

Acid Insoluble Ash

The total ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. The insoluble matter was filtered and collected on ash less filter paper and washed the filter papers with hot water. Then cool the crucible and kept in desiccator. The acid insoluble ash was weighed and calculated with reference to the air dried drug.

Water Soluble Ash

The ashes were boiled for 5 minutes with 25 ml of water, filtered ash ignite for 15 minutes at a temperature not exceed 450 degree centigrade. The weight of the insoluble matter was subtracted from the pre weighed ash and the difference represented as water soluble ash.

Sulphated Ash

1 gm of drug powder was taken in the crucible and the content was accurately ignited gently at first gently, until the substance is thoroughly charged. The residue was cooled and moistened with 1 ml of Sulphuric acid with gentle heat until the white fumes are no longer evolved. Then ignite the sample residue at 800º ± 25º until all the black particles were disappeared. The crucible was allowed to cool and weigh. The ash value of the powdered drug was recorded in table no. four.

Moisture Content

The presence of moisture in a crude drug can lead to its deterioration due to either activation of certain enzymes or growth of microbes. Karl- fischer method is a standard procedure for determining moisture content.

Phytochemical Investigation

Different extracts obtained from the above extraction were analyzed for different phytoconstituents investigation qualitative. The following chemical tests were carried out and the results were tabulated in table no. five [4].

Tests for Alkaloids

Wagner's Reagent Test

With alkaloid it shows reddish brown precipitate. It is prepared by dissolving 1.27 gm of Iodine and 2 gm of Potassium Iodide in 5ml of water and the final volume is made up to 200 ml.

Mayer's Reagent Test

To prepare the reagent, 1.36 gm of mercuric chloride is dissolved in distilled water. In another part dissolve 5gm of potassium iodide in 60 ml of distilled water. Then both the parts were mixed and the volume was adjusted to 200 ml. With alkaloids it shows white to buff precipitate.

Dragendroff's Reagent Test

With alkaloids this reagent gives orange- brown colored precipitate. To prepare this reagent, 14 gm of sodium iodide was boiled with 5.2 gm of bismuth carbonate in 50 ml glacial acetic acid for few minutes. Then it was allowed to stand for overnight and the precipitate of sodium acetate was filtered out. To 40 ml of filtrate 160 ml of acetate and 1 ml of water was added. The stock solution was stored in amber-colored bottle.

During experiment; to 10 ml of stock solution 20 ml of acetic acid was added and the final volume was made up to 100 ml with water [5].

Hager's Reagent Test

This reagent shows characteristic crystalline precipitate with many precipitates. In this case a saturated aqueous picric acid was used for detection of alkaloids.

Tests for Carbohydrates

Benedict's test

In this method of test for monosaccharide, 5 ml of Benedict's reagent and 3 ml of test solution when boiled on a water bath and brick red precipitate appears at the bottom of the test tube confirms the presence of the compounds.

Fehling's Test

In this method 2 ml of Fehling 'A', 2 ml of Fehling 'B' and 2 ml of extract were boiled. The presence of reducing sugar is confirmed if yellow or brick red precipitate appears at the bottom of the test tube confirms the presence of the monosaccharide [6].

Moloch's Test

When the aqueous or alcoholic solution of the extract and 10% alcoholic solution of α- napthol were shaken and concentrated

Sulphuric acid was added along the side of the test tube, a violet ring at the junction of two liquids confirms presence of carbohydrates.

Tests for Glycosides
Test for Cardiac Glycosides
Keller-Killiani Test

To an extract of the drug in glacial acetic acid few drops of Ferric Chloride and concentrated Sulphuric acid is added. A reddish brown colour is formed at the junction of the two layers and upper layer turns bluish green [7].

Legal Test

To a solution of glycoside in pyridine, sodium nitropruside solution and sodium hydroxide solution were added. A pink to red colour will confirm the presence of glycosides.

Test for Anthroquinon Glycosides
Borntrager's test

To perform Borntrager's test, 0.1gm of the powdered drug was boiled with 5 ml of 10% Sulphuric acid for 2 minute. Filtrate was shaken with equal volume of benzene. The benzene layer was allowed to separate, then half of its volume of aqueous ammonia (10%) was added and shaken gently and the layers were allowed to separate. The lower ammonia layer will show red pink colour due to presence of free Anthraquinone.

Modified Borntrager's test

When 0.1gm of the drug, 5ml of dilute HCl and 5 ml of 5% solution of ferric chloride were added and boiled for few minutes and then subsequently cooled and filtered part is shaken with benzene; the separated benzene layer and equal volume of dilute solution of ammonia shows pink colour [8].

Tests for Gums and Mucilages
Ruthenium Red Test

In this test 0.08 gm of ruthenium red when dissolved in 10 ml of 10% solution of lead acetate, it stains the mucilage to red colour.

Molisch's Test

When the aqueous or alcoholic solution of the extract and 10% alcoholic solution of α- napthol were shaken and concentrated Sulphuric acid was added along the side of the test tube, a violet ring at the junction of two liquids confirms presence of carbohydrates, gums and mucilage.

Test with 95% Alcohol

When 95% alcohol added to the extract, gums get precipitated out. The precipitate is insoluble in alcohol.

Tests for Proteins and Amino Acids
Biuret Test

When 2ml of the extract, 2 ml of 10% NaOH solution and 2-3 drops of 1% CuSO4 solution were mixed, the appearance of violet or purple colour confirms the presence of proteins.

Ninhydrin Test

When 0.5 ml of ninhydrin solution is added to 2 ml of the extract and boiled for 2 minute and then cooled. The appearance of blue colour confirms the presence of proteins.

Xanthoproteic Test

When 2ml of the extract and 1 ml of conc. HNO3 were boiled and cooled, subsequently 40% NaOH solution added drop by drop to it. Appearance of coloured solution indicates the presence of proteins.

Millon's Test

2ml of the extract and 2 ml of millon's reagent were boiled, subsequently cooled, and then few drops of NaNO2 were added to it. Appearance of red precipitate and red coloured solution indicates the presence of proteins.

TESTS FOR TANNINS AND PHENOLIC COMPOUNDS
Test with Lead Acetate
Tannins get precipitate with lead acetate.
Test with Ferric Chloride

Generally phenols were precipitated with 5% w/v solution of ferric chloride in 90% alcohol and thus phenols are detected.

Test with Gelatin Solution

To a solution of tannins (0.5 - 1%) aqueous solution of gelatin (1%) and sodium chloride (10%) were added. A white buff precipitate confirms the compounds.

Tests for Steroids and Sterols
Salkowski's Test

To 5ml of the solution of the extract in chloroform in a dry test tube, equal volume of conc. H2SO4 was added along the side of the test tube. Upper chloroform layer showing a play of colours first from bluish red to gradually violet and lower acid layer showing yellow colour with green fluorescence [9].

Libermann Burchard Reagent Test

About 2 ml of the solution of extract in chloroform was placed in a dry test tube. Then 2 ml of acetic anhydride and 2-3 drops of conc. H2SO4 was added to it and allowed to stand for few minutes. An emerald green colour develops if steroid or sterols are present.

Tests for triterpenoids
Test with Tin and Thionyl Chloride

For detection of triterpenoids the extract was dissolved in chloroform. A piece of metallic tin and 1 drop of thionyl chloride was added to it. Pink colour confirms the result.

Tests for saponins
Foam Test

About 1 ml of alcoholic and aqueous extract was diluted separately with distilled water to make the volume up to 10 ml, and shaken in a graduated cylinder for 15 minutes and kept aside. 1 cm layer of foam after standing for 30 minutes indicates the presence of Saponins [10].

Tests for flavonoids
Test with NAOH

For the detection of flavonoids, the extract was first dissolved with water. It was filtered and the filtrate was treated with sodium hydroxide. A yellow colour confirms the presence of flavonoids.

Test with Sulphuric Acid: A drop of H2SO4 when added to the above, the yellow colour disappears.

Shidona test

Extract was dissolved in methanol (1 mL). A pinch of magnesium powder was added followed by conc. HCl. Appearance of pink colour indicates the presence of flavonoids, bioflavonoid. All Phytochemical investigation data are shown in table no six [11].

REFERENCES

1. Dhand Vivek, Tripathi A K, Manhas R K, 2003. Estimation of Carbon Content in Some Forest Tree Species. Journal of Nat. medicine. 129, Pages 918-922
2. Gragg GM, Newman DJ, 2001. Natural Product Drug Discovery in the Next Millennium Pharm. Biol. 39, Pages 8 - 17. Doi: 10.1076/phbi.39.s1.8.0009.
3. Wealth of Indian. 2002. A Dictionary of Indian Raw materials and Industrial Products, National Institute of Science Communication, Council of Scientific and Industrial Research, New Delhi. Raw material. 10, Pages 100-107.
4. Fire A, Xu S, Montgomery MK, et al, 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 391, Pages 806-811. Doi: 10.1038/35888.
5. S D Bonde, L S Nemade, 2011. Murraya koenigii (Curry leaf): Ethnobotany, Phytochemistry and Pharmacology - A Review. J Pharm Phytopharmacol Res. 1(1), Pages 23-27.
6. Jyoti M, Vihas TV, Ravikumar A, Sarita G (2002). Glucose lowering effect of aqueous extract of Enicostemma Littorale Blume in diabetes: a possible mechanism of action. J. Ethnopharm. 81, 317-20. Doi: 10.1016/s0378-8741(02)00095-8
7. Thilahgavani Nagappan, Perumal R, 2011. Biological Activity of Carbazole Alkaloids and Essential Oil of Murraya koenigii Against Antibiotic Resistant Microbes and Cancer Cell Lines. Molecules. 16, Pages 9651-9664. Doi: 10.3390/molecules16119651.
8. Johannes Reisch, Olav Go, Anura Wickramasinghe, 2006. Induction of apoptosis by carbazole alkaloids isolated from Murraya koenigii. Phytomedicine. 13. Pages 359–365. Doi: https://doi.org/10.1016/j.phymed.2005.03.010.
9. Nykanen A, Haley B, Zamore PD, 2001. ATP requirements and small interfering RNA structure in the RNA interference pathway. Cell. 107, Pages 309-321. Doi: 10.1016/s0092-8674(01)00547-5.
10. Victor M, Bei Y, Gay F, 2002. HAT activity is essential for CBP-1-dependent transcription and differentiation in Caenorhabditis elegans. EMBO Rep. 3, Pages 50-55. Doi: https://doi.org/10.15252/msb.20145857.
11. A.R.Ivan, 2006. Medicinal plant of World. Chemical Constituents, Traditional Uses and Modern Medicinal Uses, Human Press Totowa. New Jersey. Pages 283-289.
12. Tiwari DK, 2001. Imbalance in Antioxidant Defence and Human Diseases: Multiple Approach of Natural Antioxidants Therapy. Curr Sci. 81, Pages P1179-1187.
13. M W Chase, J Reveal, 2009. A phylogenetic classification of land plants to accompany APG III. Botanical Journal of Linnean Society. 161, Pages 122-127. Doi: https://doi.org/10.1111/j.1095-8339.2009.01002.x.
14. Halliwell B, Gutteridge J M C, Aruoma O I, 1987. The Deoxyribose Method A Simple Test Tube Assay for Determination of Rate Constants for Reactions of Hydroxyl Radicals. Analytical Biochemistry. 165, Pages 215- 219. Doi: https://doi.org/10.1016/0003-2697(87)90222-3.