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Preliminary Phytochemical Investigation of Methanol and Chloroform extract of a wild plant *Achyranthes aspera* Linn. (Amaranthaceae)

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Plants are rich source for herbal drug. *Achyranthes aspera* Linn is one the medicinal plant which has a huge potential for therapeutic uses. In the present investigation different sequential extract of methanol and choloform of leaves was carried out by following the standard procedure of phyto chemical screening. The study revealed methanolic extract contains alkaloids, tannins, sterols, glycosides, phenolic compound, sugar and while chloroform extract indicated the presence of tannins, glycosides, sugar mixes as phyto constituents. Observed result showed that the phyto chemical contents are high in different sequential extracts of methanol than sequential extracts of chloroform. As the plant contain substantial amount of secondary metabolite it can be used in herbal drug designing

Keywords : Phytochemicals, Methanol, Chloroform, *Achyranthes aspera* L.

INTRODUCTION

Plants have played a major role in ancient medicinal practices. They are rich source of bioactive compounds. Which are naturally synthesized in all parts of the plant body. These active compounds have therapeutic properties and are the chief source of drugs. [David *et al,* 2005]. Multiple drug resistance has developed due to indiscriminate use of synthetic drug [Umate *et al,* 2017]. Therefore there is a new resurgence for finding out natural products which are more safer in use. Plant contains phyto chemicals such as flavonoid, steroid, terpenoid, saponin and alkaloid which are the major constituents of herbal drug. The plant is used for treatment of bleeding, renal complications, scorpion and snakebite [Londonkar *et al,* 2011]. *Achyranthes aspera* Linn. (Amaranthaceae) is an important medicinal plant, commonly known as Chirchita in Hindi, an annual, pubescent stiff erect herb, found as weed throughout India, tropical Asia and other parts of the world . The inflorescences are pale to bright purple colour. The fruits are oblong cylindrical, yellowish brown, smooth and glabrous. From literature review it has been found all the parts of the plant are utilised. Roots of *Achyranthes aspera* is used for astringents to wounds, in stomach pain and in abdominal tumours. [Mishra,D., 2018] To initiate labour pains the paste of the roots is applied to outside genitalia. A paste made up by utilizing roots of Achyranthes aspera and buttermilk is taken internally as an antifertility drug. The benzene extract of the stem bark of *Achyrnathes aspera* shows abortifacient activity in the rat. Leaf extracts have thyroid stimulating and anti peroxidative properties . [Dey, A. 2011] The leaves is utilized for the treatment of rabies, nervous disorders, insanity, creepy craw. The flowering spikes or seeds grinded into paste with water, are used for bites of poisonous snakes and reptiles and also used in night blindness. Keeping in view of the above said facts a preliminary investigation was carried out to find out the presence of different secondary metabolites in the methanol and chloroform extract of leaf.

Fig-1: Achyranthes aspera L.

MATERIALS & METHODS

Plant material collection and processing : The fresh, healthy and disease-free plant leaves of *Achyranthes aspera* were collected from road side of Centurion University, Ramachandrapur, Jatni, Bhubaneswar. Fresh leaves were washed thoroughly under tap water following with distilled water and dried in shade under room temperature for 15-20 days. The dried leaf was grinded into coarse powder and stored in an air tight container for further use.

Plant extract preparation : The dried leaves powder of *Achyranthes aspera* was extracted by using Soxhlet apparatus using a polar solvent methanol (CH_3OH) and a non-polar solvent chloroform ($CHCl_3$). About 30gms of leaf powder was dissolved in 250ml of methanol and chloroform separately using soxhlet apparatus at 30ºC for 48-72 hours. Extracts were concentrated under reduced pressure and stored in an air tight sample bottle and kept in 4ºC for further study [Kushwaha *et al,* 2019].

Chemical reagents used:

- Molisch's reagent
- Hager's Reagent
- Wagner's Reagent
- Vanilline hydrochloride Reagent
- Anthrone reagent
- Biuret reagent
- Ninhydrin reagent
- Dragendroff's reagent
- Mayer's reagent
- Barfoed's reagent
- Seliwanoff's reagent:

PHYTOCHEMICAL SCREENING : Preliminary phytochemical examinations were carried out to find out the presence of different phytoconstituents for all the extracts as per the standard methods. This helps to identify the medicinally active substance. Preliminary phytochemical screening was performed for various compounds such as carbohydrates, proteins, alkaloids, tannin, saponins etc. Methods of phytochemical screening was given underneath [Singh *et al,* 2016].

Test for Alkaloids [Singh *et al,* 2016]

Alkaloids are naturally occurring organic compounds. These are generally containing basic nitrogen atoms. They have heterocyclic structure.

The plant extract was warmed with 2% H_2SO_4 for two minutes, the addition of reagent indicates the presence of Alkaloids.

- **Mayer's Test :** To 5ml of extract few drops of Mayer's Reagent were added, the formation of creamy white colour precipitate indicates the presence of Alkaloids.
- **Dragendroff's Test** : To 5 ml of extract few drops of Dragendroff's Reagent were added, the appearance of orange colour precipitate indicates the presence of Alkaloids.
- **Wagner's Test :** To 5ml of extract few drops of Wagner's Reagent added, the formation of reddish-brown precipitation indicates the presence of Alkaloids.
- **Hager's Test :** Few drops of Hager's Reagent added to 3ml of extract, the formation of prominent yellow colour precipitate indicates the presence of Alkaloid.

Test for Phenolic compounds [Kamble *et al,* 2018]

Phenols are phenyl group bonded to hydroxyl group and also aromatic compounds. These are crystalline solid that is volatile. These are found in almost all parts of plant. They improve disease resistance capacity in plant.

- **Phenol Test :** 1 ml of extract was added with Ferric chloride solution, formation of yellow precipitate indicates the presence of phenols.
- **Ellagic acid Test :** 1 ml extract was added with few drops of 5% (v/v) Glacial acetic acid and 5% (w/v) sodium carbonate solution. The formation of muddy yellow, olive brown, and chocolate colour indicates the presence of phenols.
- **Hot water test :** Hot water was taken in a test-tube, leaf mixture was dip into it. Warm it for few minutes. The development of black or brown colour ring at the junction of dipping indicates the presence of phenols.

Test for Tannins [Sharma *et al,* 2013] [Singh *et al,* 2016]

Tannins are polyphenolic biomolecules. Tannin cause dry and pucker feeling in mouth due to the astringency from (for example consumption of wine, raw fruit, and tea etc.)

- Gelatin test:1ml of extract was taken in a dry test tube. 1% gelatin solution was added to it followed by the addition of 10% NaCl. Appearance of white precipitation indicates the presence of Tannins.
- Vanillin hydrochloride test: Addition of few drops of vanillin hydrochloride reagent to the extract, formation of purplish-red colour indicates the presence of Tannin.
- Ferric chloride Test:Small quantity of extract and 5ml of 45% ethanol was boiled separately for 5min. each of the mixture is cooled and filtered. 1ml of each filtrate was diluted with distilled water and added with two drops of ferric chloride. The formation of greenish to black colour indicates the presence of Tannin.

Test for Saponins [Kamble *et al,* 2018]

Saponins are chemical compounds found in different plant species. Saponin consist of an aglycone link to one or

more carbohydrate chain. These are plant steroid compounds or triterpenoids having bitter taste. These are form foam in aqueous solution and lyse erythrocytes.

* Foam Test : Addition of 1 ml extract to water and shaken well. Formation of honey comb like foam indicates the presence of Saponins.

* Blood Test : Few drops of blood added to 1 ml of extract and observe under microscope. The lyse of blood cells under microscope indicates the presence of Saponins.

Test for Sterols [Kamble *et al,* 2018]

These occurs widely in plants and animals. These are characterized by the presence of 1,2-cyclopentanophenenthrane ring system. Examples: Steroids

- Salkowski Test: 1 ml extract added to Conc. H_2SO_4. Formation of wine-red colour indicates the presence of Sterols.

- Liebermann- Burchard's Test: To 1 ml of extract acetic Anhydride and Conc. H_2SO_4 was added along the sides of test tube. Formation of red ring at the junction of two layers indicates the presence of Sterols.

Test for Glycosides [Kamble *et al,* 2018]

Glycosides are made up of one or more sugar combined with alcohol, phenol or combined molecules. Those of sugars known as glucosides or fructo-sides.

- Keller- Killiani Test: 1 ml extract was mixed with few drops of glacial acetic acid and boiled for a min and cooled. To this solution 2 drops of ferric chloride solution was added. The contents were transferred to another tube containing Conc. Sulphuric acid. At the junction of 2 layers, Formation of reddish-brown ring indicates presence of Glycosides.

- Molisch's Test: 1 ml extract was added with Molisch reagent, 1 ml of Conc. sulphuric acid was added along the sides of the tube. At the junction of 2 layers, Formation of reddish violet ring indicates the presence of Glycosides.

- Baljet's Test: To 5ml of extract 5 drops of sodium picrate were added. Change in yellow to orange colour indicates the presence of glycosides.

Test for Terpenoid [Tiwari *et al,* 2018]

Terpenoids are also called as isoprenoids. Terpenoids add flavour to plants.

- Salkowski test: To 5ml of extract 2ml of chloroform, and 3ml concentrated H2SO4 was added. Formation of reddish-brown colouration of the interface indicates the presence of terpenoids.

Test for Carbohydrate [Kushwaha *et al,* 2019]

These are the substances with general formula of $(CH_2O)_X$. These are found in wide variety of natural and processed foods.

- Fehling's test: 1 ml of Fehling A and 1 ml of Fehling B was mixed in a test tube. 2 ml of extract was added to the test tube. The formation of red precipitate indicates the presence of carbohydrate (Reducing Sugar).

- Molisch's test: 5 ml of sample was taken in a test tube. Two drops of Molisch reagent was added to it and mixed thoroughly, 3 ml of concentrated sulphuric acid was added along the sides with a dropper. Formation of reddish violet ring at the junction of two liquids indicates the presence of Monosaccharide.

- Barfoed's test: In a test tube 5 ml of Barfoed's reagent was taken. 0.5 ml of extract was added to it, mixed well and boiled for 2 min. The tube was kept in a test tube rack and examined for precipitate after 10-15 min. Formation of red precipitate clinging to the bottom?most part of the test tube indicates the presence of monosaccharide.

- Seliwanoff's test: In a test tube 5 ml of Seliwanoff's reagent was taken. 5 drops of the extract were added and heated the contents to just boiling. The formation of red colour indicates the presence of fructose.

Test for Protein [Parveen S.,2018][Umate S.K and Marath V.R.]

Proteins are complex nitrogenous compounds, occurs in plant and animal cells. Proteins on hydrolysis with strong inorganic acids or by enzymes produce a mixture of amino acids.

- Biuret test : To 3ml of extract few drops of 10% sodium chloride solution and 1% copper sulphate was added. Formation of violet/purple colour indicates the presence of protein. And On adding alkali it becomes dark purple in colour.

- Xanthoprotein test : To 3ml of extract few drops HNO_3 was added. Formation of intensely yellow colour indicates the presence of proteins in the sample extract.

- Ninhydrin test : To the 1ml of extract 2% ninhydrin solution was added. Formation of violet colour indicates the presence of protein.

RESULT AND DISCUSSION

Plants are the phenomenal centrality to the prosperity of individuals and organizations from a long time ago. Plant realm gives a huge gracefully of various phytochemical with likelytherapeutic properties. The data in table - 1 portrayed the preliminary phytochemical examination reports of the

Table 1 : Preliminary phytochemical analysis of the leaves of *Achyranthes aspera*

Sl No.	Phytochemicals	Chloroform extract	Methanol extract
01	Alkaloid	-	+
02	Phenolic	-	+
03	Tannin	+	+
04	Saponin	-	-
05	Sterol	-	+
06	Glycoside	+	+
07	Terpenoid	-	-
08	Carbohydrate	+	+
09	Protein	-	-
10	Fixed oil	-	-
11	PH	6	7

+ indicates the presence and - indicates absence

different concentrates, demonstrates that the methanolic extract from the leaves of *Achyranthes aspera* was found to contain alkaloids, tannins, sterols, glycosides, phenolic compound, sugar and while chloroform extract indicated the presence of tannins, glycosides, sugar mixes as phytoconstituents. The major phytochemical of intrigue present in the methanolic concentrate of leaves of plant *Achyranthes aspera*. The blends as known to be naturally elements, in this manner help the antimicrobial exercises through different systems. The PH of the chloroform and methanol concentrates of the leaves was discovered to be 6 and 7 respectively.

CONCLUSION

The present study suggested that extracted phytochemicals are very valuable . Further their isolation, purification and characterization will pave a new way in drug designing. The methanolic extract from the leaves of *Achyranthes aspera* was found to contain alkaloids, tannins, sterols, glycosides, phenolic compound, sugar and while chloroform extract indicated the presence of tannins, glycosides, sugar mixes as phytoconstituents .Methanol extract of leaves possesses higher phytochemical contents than chloroform extracts.

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