

Diversity of Bioactive Components of *Achyranthes aspera* Linn leaves.

Pawar S.G. ^{*1}, Kamble V.M. ²,

Department of Botany,

Yashwantrao Mohite College, Erandwane, Pune.

Bharati Vidyapeeth Deemed University, Pune (India) – 411 038.

Abstract: *Achyranthes aspera* Linn is well known medicinal plant and found enormous information about drug in ancient literature such as Ayurvedic, Unani, Tibba, Siddha, Allopathy, Homoeopathy, Neuropathy and Home remedies. *Achyranthes aspera* Linn is widely used for stomachic, bronchitis, heart diseases, abdominal pains, dysentery, dyspepsia etc. It has traditionally used in cough and asthma. The present communication deals with the diversity of bioactive components of *Achyranthes aspera* leaves and evaluation of soluble extractive percentage using three different solvents.

Soluble extractive percentage of *Achyranthes aspera* leaves has been found more in aqueous extract (3.75%) followed by methanolic extract (1.5%) and acetone extract (0.3%). Soluble extractive percentage is useful for evaluation of crude drug and nature of chemical constituents present in crud drug. Phytochemical analysis of *Achyranthes aspera* revealed presence of carbohydrates, proteins, amino acids, saponin glycosides, anthraquinone glycosides, alkaloids and steroids. The present study is clearly indicates the *Achyranthes aspera* leaves have important source of bioactive and therapeutically active constituents and exhibiting the diversity and variation in bioactive components.

Keywords: *Achyranthes aspera*, Soluble extractive percentage, Phytochemical analysis, Bioactive components.

Introduction:

Medicinal plants and their properties were written by sages in ancient literature such as Ayurvedic, Unani, Tibba, Siddha etc. and have been handed down by generation to generation from thousands of years (Bown, 2001). The medicinal plants are richest source of bioactive components which play crucial role in therapeutic and pharmacological properties. Ayurvedic drugs are secured than synthetic one because synthetic drugs have many side effects; they are harmful and unsafe for human being. Study of phytochemical analysis and evaluation of soluble extractive percentage are most important because they give an idea about the bioactive components of that plant. Soluble extractive percentage is useful for evaluation of crude drug and nature of chemical constituents present in crud drug. Phytochemical analysis is useful for discovery of potent remedies on various diseases (Pawar et.al, 2015).

Achyranthes aspera is well known herbaceous medicinal plants and traditionally used in asthma and cough. It is found throughout the India and treated as a weed in the field. The vernacular name of the *Achyranthes aspera* is aghada and belongs to family Amaranthaceae. It is a sacred herb and used in Lord Ganesh and Gouri patra pooja. *Achyranthes aspera* has therapeutic and medicinal properties such as spermicidal (Paul et.al, 2010), antiparasitic (Zahir et.al, 2009), hypoglyceamic (Akhtar and Iqbal, 1991), antimicrobial (Khan et.al, 2010), anti-

inflammatory (Kumar et.al, 2009), antipyretic (Sutar et.al, 2008), hepatoprotective (Bafna and Mishra, 2004), anti-oxidant (Malarvili and Gomathi, 2009), anti-allergic activity (Datir et.al, 2009) etc. The many plants and their products have been evaluated on the basis of their traditional uses for the purpose of drug development and discovery of new drug (Srivastav et.al, 2011).

Materials and Methods:

A. Collection and Identification:

Achyranthes aspera Linn fresh plants have been collected from the Pune region and have been identified and authenticated from the Botanical Survey of India (BSI), Pune. The specimen (VMK-06) has been deposited in herbarium department, Botanical Survey of India, Pune, (Maharashtra).

B. Determination of Extractive value of *Achyranthes aspera* Linn, leaves and seed powder:

The soluble extractive percentage has been calculated by using following formula and recorded.

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

C. Extraction Process

Maceration:

In maceration (for fluid extract), whole or coarsely powdered plant material is subjected to solvent extraction in a stoppered container for a definite period with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermo labile drugs.

Preparation of solvent extract:

Three different solvent extracts were used for phyto-chemical study aqueous, methanol and acetone. 50 gm of the powdered plant material was taken and subjected to the maceration solvent extraction. The extraction was carried out for 24 hrs with 250 ml each solvents. After this extracts were concentrated to dryness by keeping for complete evaporation of solvents. Standard protocols were followed for studying phyto-constituents.

Phytochemical screening of plant extract: (Khandelwal K.R. 2002)

1. **Test for carbohydrates: Molisch's test** – 3 ml of extract was taken in a test tube 2 drops of alcoholic α -naphthol solution was added, shaken well and then 1ml of concentration sulfuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.
2. **Test for proteins: Millon's test** – About 3 ml of sample extract was treated with 5 ml of Million's reagent. White precipitate was obtained. The mixture was then warmed, precipitate turned to brick red. It indicates the presence of proteins.
3. **Test for amino acids: Ninhydrin test** - About 3 ml of plant extract solution was heated followed by addition of 3 drps of 5% ninhydrin solution. The test tubes with this solution were kept in boiling waterbath for 10 minutes. The purple color was observed. It indicated the presence of amino acids.
4. **Test for steroids: Leibermann- Burchard reaction** – To 3 ml extract 10ml chloroform was added followed by 2 ml of acetic anhydride. Then 2 drops of conc. Sulfuric acid was added from the side of the test tube. The blue green color indicated the presence of steroids.
5. **Test for glycosides :**
 - a. **Cardiac Glycosides (Legal's Test)** – To the 3 ml extract 1ml pyridine was added by frequent shaking followed by 1ml sodium nitroprusside. Pink to red color appeared. It indicates the presence of cardiac glycosides.
 - b. **Anthraquinone glycosides (Borntrager's test)** – To 3 ml extract dil. Sulfuric acid was added. The

solution was then filtered. Then equal volume of chloroform was added to the filtrate. After shaking organic solvent was separated. Finally equal volume of ammonia solution was added. No bright pink, red or violet color was developed in the upper layer which indicates the absence of anthraquinones.

- c. **Coumarin glycosides:** Moistened dry powder was taken in test tube. Test tube was covered with filter paper soaked in dil NaOH. Kept in water bath. After some time filter paper was exposed to ultra-violet light. Yellowish fluorescence was observed for positive test.
- d. **Saponin glycosides (Foam test)** – About 50mg of extract was diluted in the successive solvents and made up to 20 ml. The suspension was shaken for 15min. A 2cm layer of foam appeared. That is saponins are present.

6. Test for flavonoids: Sodium hydroxide test – To 3 ml of extract increasing amount of sodium hydroxide was added it showed colouration, which was decolorised after addition of dil. hydrochloric acid.

7. Test for alkaloids: Solvent free extract was stirred with 10 ml of dilute hydrochloric acid and filtered. The filtrate was tested with following alkaloidal reagents as follows:

- a. **Mayer's test** – To 3 ml filtrate two drops of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate was observed. It indicated positive test.
- b. **Hager's test** – To 3 ml of filtrate, 1ml of Hager's reagent was added. A prominent yellow precipitate occurred. It indicated the presence of alkaloids.

8. Test for Tannins and phenolic compounds:
5% FeCl₃ Solution: Deep blue black colour.

Results and Discussion:

The crude aqueous, methanolic and acetone extract of *Achyranthes aspera* leaves was subjected to various phytochemical tests for confirmation of different phytochemical constituents. The result of soluble extractive percentage is summarized in Table No.1. Soluble extractive percentage of *Achyranthes aspera* leaves has been found maximum in aqueous extract (3.75%) as compared to methanolic and acetone extracts. Soluble extractive percentage of methanolic extract has been found comparatively lower than aqueous extract that is 1.5% followed by acetone extract that is 0.3%. The bioactive compounds of *Achyranthes aspera* leaves investigated and

summarized in Table No. 2. The qualitative phytochemical analysis of *Achyranthes aspera* leaves confirmed the presence of carbohydrate,

anthraquinone glycoside, saponin glycoside, flavonoids, alkaloids, proteins, amino acids and steroids in three various extracts.

Table No. 1. Soluble extractive percentage of *Achyranthes aspera* Linn

Solvents	Weight of Plant material (g)	Soluble Extractive percentage (%) of <i>Achyranthes aspera</i> (L) Medik leaves
Methanol	2	1.5
Acetone	2	0.3
Aqueous (Water)	2	3.75

Table No. 2. Phytochemical screening of aqueous, methanolic and acetone extracts of *Achyranthes aspera* Linn.

Sr. No.	Secondary metabolites	Phytochemical tests	<i>Achyranthes aspera</i> Linn leaves		
			Methanol	Acetone	Aqueous
1.	Carbohydrates	Molisch's Test	++	++	++
2.	Proteins	Millon's Reagent Test	++	--	++
3.	Amino acid	Ninhydrin Test	++	--	++
4.	Steroid	Liebermann Burchard Reaction	++	--	--
5.	Glycosides	Legal's Test	--	--	--
	a)Cardiac glycosides	Borntreger's Test	++	++	++
	b)Anthraquinone glycosides	Fluorescence Test	--	--	--
	c)coumarin glycosides	Foam Test	++	--	++
6.	Flavonoids	Sodium hydroxide test	--	--	++
		Mayer's Test	--	--	++
7.	Alkaloids	Hager's Test	--	--	++
		Dilute Nitric acid Test	--	--	--
8.	Tannins & phenolic compounds		--	--	--

Biochemical compounds like carbohydrates and anthraquinone glycosides have been detected in all extracts of *Achyranthes aspera* leaves. Most physiologically important compounds like proteins, amino acids and saponin glycosides have been observed in methanolic as well as aqueous extracts but have been found absent in acetone extracts. Flavonoids, alkaloids have been recorded in aqueous extract and steroids in methanolic extract. Tannins, phenolic compounds, glycosides like cardiac and coumarin had been found totally absent in all extracts. Hossain et.al (2013) also reported presence of steroids in methanolic extract of *Achyranthes aspera* leaves and Rafiqkhan et.al (2013) found presence of flavonoids, proteins, carbohydrates, saponins, steroids, alkaloids in methanolic as well as aqueous extracts. Flavonoids have good antioxidant, antibacterial properties and free radical scavenging activity (Harborne, 1973 and Aquil et.al, 2006). Neogi et.al (1970) observed water soluble alkaloid 'Achyranthine' and reported better cardiovascular activity. Saponins are useful to lower the cholesterol level in blood and also show expectorant property (Harborne, 1973).

Conclusion:

The present study have been given very promising results of phytochemical analysis and soluble extractive percentage of *Achyranthes aspera* leaves crude extracts using three different solvents. Soluble extractive percentage is useful for evaluation of crude drug and nature of chemical constituents present in crud drug. *Achyranthes aspera* leaves crude extracts showed the presence of bioactive phytochemical constituents like carbohydrates, proteins, amino acids, saponin glycosides, anthraquinone glycosides, alkaloids and steroids, which showing diversity and variation. Number of bioactive phytochemical constituents is responsible for therapeutic and pharmacological properties which may give idea to develop new drug from *Achyranthes aspera* plant on various disease.

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