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Research Article

PHARMACOGNOSTICAL EVALUATION OF DIFFERENT PARTS OF *APAMARGA* (*ACHYRANTHES ASPERA* LINN.)

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ABSTRACT

Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants. Nowadays adulteration of crude herbal drugs is very common due to scarcity of drug and its high price prevailing in the market. These herbal medicines can stand in commercial market only if they are evaluated according to modern science. Evaluation of herb involves confirmation of its identity, determination of its quality and purity, and detection of nature of adulteration. So before utilizing a drug for therapeutic purpose, detail pharmacognostical study is essential as it not only helps in correct identification of the drug but also to get a clue for its phytochemicals, pharmacological activities and medicinal properties. API (Ayurvedic Pharmacopoeia of India) has provided standards for a number of plants. But for *Apamarga* standards are given only for roots and *Panchanga* (Whole plant), not for the other parts like seeds, leaf and stems which are also used on a large scale for different medicinal properties. Standards are required to be developed for each and every part of the *Apamarga.* Genuine samples of different parts of *Achyranthes aspera* Linn. was taken to set the standard for each and every part of *Apamarga* used for medicinal properties. The collected genuine samples were then subjected to macroscopic, microscopy, physicochemical, phytochemical and chromatographic study. Now a day most of the pharmaceutical companies are dealing with plant extracts and Ayurvedic *Vaidyas* too prefer using single drug extract instead of multiple drug formulations and are getting great response from the patients.

KEYWORDS: *Apamarga, Achyranthes aspera,* Microscopy, Phytochemical, Chromatography.

INTRODUCTION

The American society of Pharmacognosy defines pharmacognosy as the study of the physical, chemical, biochemical, and biological properties of drugs, drug substances or potential drugs of natural origin as well as the search for new drugs from natural sources.[1] According to the WHO more than 80 % of the world's population relies on traditional herbal medicine for their primary health care.[2] Most of the time we run after the drug which is rare and difficult to collect and neglect the herb which grows around us. *Apamarga* is one of them. Each and every part of the plant *Apamarga* has got great medicinal property. Like *Apamarga tandul* (seed) in *Bhasmak roga*[3], *Kaphaja Nadivrana*[4], *Gulma*[5], *Krimi Shiroroga*[6] etc.; *Apamarga beej kalka* in *Raktarsha*[7]; *Apamarga* root in *Arsha*[8], *Visuchika*[9], *Netra Abhisyanda*[10] etc.; *Apamarga patra swarasa* in *Sadyovrana*[11]; *Apamarga Kshaar* in *Plihodar*[12], *Badhirya*[13] etc. Because of the rapidly growing interest in herbal medicine in the western world and their attempts in the past to patent plants and plant products, it is being felt that there is an urgent need to bring to lime light the effectiveness of those herbs and related preparations which are being used in different Indian systems of medicine for thousands of years.

Material and Methods

Collection: The genuine samples of *Apamarga (Achyranthes aspera* Linn.*)* and its different parts viz. Root, Stem, Leaf, Seed and Whole plant were collected from Haridwar and its nearby area. A herbarium was also prepared for the plant and was authenticated at Botanical Survey of India (BSI), Dehradun.

Macroscopic and Microscopic evaluation: All the collected genuine samples were dried and studied macroscopically with naked eye, magnifying lens and measuring tape with the help of Pharmacognostical parameters i.e., shape, size, surface, colour, odour and taste and findings were recorded. The microscopic characters were studied through Transverse section (T.S) and Powder microscopy.

Physicochemical Study: Physicochemical parameters like moisture content, ph value, alcohol extractive value, water extractive value, total ash, acid insoluble ash etc. were recorded for different samples.

Phytochemical Study: Freshly prepared extracts were tested for the presence of various active phytocompounds like carbohydrates, alkaloids, amino acids, proteins, glycosides, phenolic compounds, saponins, flavonoids, tannins etc.

Chromatographic Study: Thin Layer Chromatography (TLC) for different samples was performed and different r_f values were recorded for different samples.

Observation and Results

1. Macroscopic characters of different parts of *Apamarga* (*Achyranthes aspera* Linn.)

(a) Root: Yellowish brown colour, cylindrical tap root, gradually tapering, slightly ribbed, rough due to presence of root scars, secondary and tertiary root present.

(b) Stem: Stem yellowish brown, erect, branched, cylindrical, hairy, solid and hollow when dry, 10-12 ridges present on outer surface.

(c) Leaf: Leaves simple, nearly sessile, stipule absent, opposite decussate, slightly wavy margin, obovate, slightly acuminates and pubescent due to presence of hairs.

(d) Seed: Seeds are brown colour, sub cylindric, truncate at the apex, round at the base, endospermic.

2. Microscopic characters of different parts of *Apamarga* (*Achyranthes aspera* Linn.) in transverse section (T.S) [Figure no. 1.1-1.4]

a. Root: TS of root showed single layered epidermis, followed by 8-16 layer rectangular, elongated, thin walled cork cells, followed by 5-7 successive, alternate more or less concentric rings of secondary vascular tissue and the conjunctive parenchyma. Ground tissue has phloem fibres, stone cells, oxalate crystals and starch grains. Secondary growth was more. Pith was absent.

b. Stem: TS of stem showed 8-10 prominent ridges on the outer most side. Single layered epidermis was seen with a thick cuticle. The cortex was composed of 8-10 layers of parenchymatous cells. Some of these parenchymatous cells consist of calcium oxalate crystals. A discontinuous ring of lignified fibres i.e., pericycle was seen. Vascular tissue showed anomalous secondary growth having incomplete ring of xylem and phloem. Cambium ring was present between secondary xylem and phloem. The central part of the stem was occupied by pith in which two medullary bundles were found fused together.

c. Leaf: The lamina shows a single layered epidermis on the upper side composed of cubical cells. The upper epidermis shows mostly uni, bi and multi cellular hairs. The epidermis is followed by a layer of hypodermis which is usually 3-5 layered of cells, thick and is interrupted at places by the palisade layer. The cells of lower epidermis are cubical in shape mostly with uni-tricellular trichomes. Four vascular bundles are scattered in ground tissue consisting of thin parenchymatous cells. Vascular bundle consists of xylem vessels, tracheids and xylem parenchyma. Phloem consists of sieve tubes, companion cells, phloem parenchyma and pericycle. The pericycle is made of 2-3 layered of thick walled, non-lignified cells.

d. Seed: TS of seed showed outer most single layered testa consists of rectangular shaped compactly arranged parenchyma cells. Perisperm cells loosely arranged consists of oil globules, some of cells were filled with yellowish brown content and also prismatic crystals of calcium oxalate. Endosperm made up of compactly arranged parechymatous cells loaded by starch grains and oil globules.

3. Powder microscopy of different parts of *Apamarga* (*Achyranthes aspera* Linn.) [Figure no. 2.1-2.5]

Table 1: Microscopy of Different Parts of *Apamarga*

Features	Root	Stem	Leaf	Seed	Whole plant
Starch	-	-	-	-	-
Calcium Sulphate	-	+	-	-	+
Cellulose	+	-	+	+	+
Mucilage	+	+	+	+	+
Cutin	+	+	+	+	+
Cell nuclei	-	-	-	+	-
Lignine	+	+	+	+	-

4. Physicochemical study of different parts of *Apamarga* (*Achyranthes aspera* Linn.)

Table 2: Physicochemical Study of all Samples

Test	Root	Stem	Leaf	Seed	Whole plant	API (Whole plant)	API (Root)
Moisture Content	9.75%	9.21%	7.41%	7.38 %	10.49%	-	-
pH	6.8	5.3	7.1	5.9	6.7	-	-
Total Ash	12.48%	8.94%	5.78%	5.87%	14.54%	Not>17%	Not>9%
Acid Insoluble Ash	3.54%	2.56%	1.31%	1.54%	4.32%	Not>5%	Not>1%
Water Soluble Ash	7.84%	5.42%	2.58%	4.27%	6.75%	-	-
Aqueous Extractive Value	19.43%	18.94%	21.06%	15.43%	21.53%	Not<12%	Not<10
Alcohol Extractive Value	9.76%	8.64%	10.48%	8.94%	11.31%	Not<2%	Not<2%
Petroleum Ether Extractive	2.69%	3.43%	3.89%	2.67%	4.49%	-	-

5. Phytochemical study of different parts of *Apamarga (Achyranthes aspera* Linn.*)*

Table 3: Carbohydrate test for different samples

Sample	Name of Test	Aqueous extract	Alcohol extract	Petroleum ether extract
Root	Molisch test	-ve	+ve	-ve
	Benedict test	-ve	+ve	-ve
	Barfoed's test	+ve	+ve	-ve

	Fehling test	+ve	+ve	-ve
Stem	Molisch test	+ve	+ve	-ve
	Benedict test	+ve	-ve	-ve
	Barfoed's test	+ve	-ve	-ve
	Fehling test	+ve	+ve	-ve
Leaf	Molisch test	+ve	+ve	-ve
	Benedict test	+ve	+ve	-ve
	Barfoed's test	+ve	+ve	-ve
	Fehling test	+ve	+ve	-ve
Seed	Molisch test	+ve	+ve	-ve
	Benedict test	-ve	-ve	-ve
	Barfoed's test	-ve	+ve	-ve
	Fehling test	+ve	+ve	-ve
Whole plant	Molisch test	+ve	+ve	-ve
	Benedict test	+ve	+ve	-ve
	Barfoed's test	+ve	+ve	-ve
	Fehling test	+ve	+ve	+ve

Table 4: Alkaloid analysis in different samples

Sample	Name of Test	Aqueous extract	Alcohol extract	Petroleum ether extract
Root	Dragendorff test	+ve	+ve	-ve
	Mayer's test	+ve	-ve	-ve
	Wagner's test	-ve	-ve	-ve
	Hager's test	-ve	-ve	-ve
Stem	Dragendorff test	+ve	+ve	-ve
	Mayer's test	+ve	-ve	-ve
	Wagner's test	+ve	-ve	-ve
	Hager's test	+ve	-ve	-ve
Leaf	Dragendorff test	-ve	+ve	+ve
	Mayer's test	-ve	-ve	+ve
	Wagner's test	+ve	-ve	+ve
	Hager's test	-ve	-ve	-ve
Seed	Dragendorff test	+ve	-ve	-ve
	Mayer's test	+ve	-ve	-ve
	Wagner's test	-ve	-ve	-ve
	Hager's test	-ve	+ve	-ve
Whole plant	Dragendorff test	+ve	+ve	-ve
	Mayer's test	+ve	+ve	-ve
	Wagner's test	+ve	+ve	-ve
	Hager's test	+ve	+ve	-ve

Table 5: Amino acid analysis in different samples

Samples	Name of the Test	Aqueous extract	Alcohol extract	Petroleum ether extract
Root	Ninhydrin test	-ve	+ve	-ve
Stem		+ve	-ve	-ve
Leaf		+ve	-ve	-ve
Seed		+ve	+ve	-ve
Whole plant		+ve	+ve	+ve

Table 6: Analysis of Proteins in different samples

Samples	Name of the Test	Aqueous extract	Alcohol extract	Petroleum ether extract
Root	Biuret test	-ve	-ve	-ve
	Xanthoproteic test	-ve	-ve	+ve
	Millon's test	+ve	-ve	+ve
	Biuret test	+ve	+ve	-ve

Stem	Xanthoproteic test	+ve	+ve	-ve
	Millon's test	+ve	+ve	+ve
Leaf	Biuret test	+ve	+ve	-ve
	Xanthoproteic test	-ve	-ve	-ve
	Millon's test	-ve	-ve	-ve
Seed	Biuret test	+ve	+ve	-ve
	Xanthoproteic test	-ve	+ve	-ve
	Millon's test	-ve	-ve	-ve
Whole plant	Biuret test	+ve	+ve	-ve
	Xanthoproteic test	+ve	+ve	+ve
	Millon's test	+ve	+ve	+ve

Table 7: Analysis of Saponin in different samples

Samples	Name of the Test	Aqueous extract	Alcohol extract	Petroleum ether extract
Root	Foam test	+ve	+ve	-ve
Stem		+ve	-ve	-ve
Leaf		+ve	-ve	-ve
Seed		-ve	-ve	-ve
Whole plant		+ve	+ve	-ve

Table 8: Analysis of Glycosides in different samples

Samples	Name of the Test	Aqueous extract	Alcohol extract	Petroleum ether extract
Root	Borntrager's test	+ve	+ve	-ve
Stem		+ve	+ve	-ve
Leaf		+ve	-ve	-ve
Seed		+ve	+ve	+ve
Whole plant		+ve	+ve	-ve

Table 9: Analysis of Phenolic compounds in different samples

Samples	Name of the Test	Aqueous extract	Alcohol extract	Petroleum ether extract
Root	Phenolic test	+ve	+ve	-ve
Stem		-ve	+ve	-ve
Leaf		+ve	+ve	-ve
Seed		+ve	+ve	-ve
Whole plant		+ve	+ve	-ve

Table 10: Analysis of Steroids in different samples

Samples	Name of the Test	Aqueous extract	Alcohol extract	Petroleum ether extract
Root	Salkowski reaction	+ve	+ve	+ve
Stem		+ve	+ve	-ve
Leaf		+ve	+ve	-ve
Seed		+ve	+ve	-ve
Whole plant		+ve	+ve	+ve

Table 11: Analysis of Tannin in different samples

Sample	Name of Test	Aqueous extract	Alcohol extract	Petroleum ether extract
Root	$Fecl_3$ test	+ve	+ve	-ve
	Lead acetate test	+ve	+ve	-ve
	Potassium dichromate test	+ve	+ve	-ve
	Gelatin test	+ve	+ve	-ve
Stem	$Fecl_3$ test	+ve	+ve	-ve
	Lead acetate test	+ve	-ve	-ve
	Potassium dichromate test	-ve	-ve	-ve
	Gelatin test	-ve	-ve	-ve
Leaf	$Fecl_3$ test	+ve	+ve	-ve
	Lead acetate test	+ve	+ve	-ve
	Potassium dichromate test	-ve	-ve	-ve

	Gelatin test	-ve	-ve	-ve
Seed	$Fecl_3$ test	+ve	-ve	+ve
	Lead acetate test	+ve	+ve	-ve
	Potassium dichromate test	-ve	-ve	-ve
	Gelatin test	-ve	-ve	-ve
Whole plant	$Fecl_3$ test	+ve	+ve	-ve
	Lead acetate test	+ve	+ve	-ve
	Potassium dichromate test	+ve	+ve	-ve
	Gelatin test	+ve	+ve	-ve

Table 12: Analysis of Flavonoid in different samples

Samples	Name of the Test	Aqueous extract	Alcohol extract	Petroleum ether extract
Root	Shinoda test	+ve	+ve	+ve
Stem		-ve	+ve	-ve
Leaf		-ve	+ve	-ve
Seed		+ve	-ve	-ve
Whole plant		+ve	+ve	-ve

6. Chromatographic study of different parts of *Apamarga* (*Achyranthes aspera* Linn.)

Table 13: Thin Layer Chromatography (TLC) [Figure no. 3]

Samples	No. of Spots	R_f Value
Root	5	0.19, 0.20, 0.45, 0.50, 0.66
Stem	6	0.19, 0.45, 0.50 , 0.66, 0.83, 0.87
Leaf	6	0.45, 0.50, 0.58, 0.66, 0.83, 0.91
Seed	6	0.13, 0.45, 0.58, 0.61, 0.66, 0.83
Whole plant	8	0.19, 0.20, 0.45, 0.50, 0.58, 0.60, 0.66, 0.83

Table 14: High Performance Thin Layer Chromatography (HPLC) for Oleanolic acid [Figure no. 4.1-4.5]

Sample	Total no. of Peak	Peak for Oleanolic acid	Ret. Time	Peak Area	Oleanolic acid %
Root	8	1	2.078	684769	0.051 %
Stem	6	0	0	0	Absent
Leaf	28	0	0	0	Absent
Seed	7	1	2.078	211370	0.016 %
Whole plant	10	1	2.078	1448	0.0001094 %

DISCUSSION

Achyranthes aspera has yellow brown colour root with tap root system. Stem of the plant comprises of 10-12 ridges on the outer surface. Leaves are pubescent due to presence of hairs on them. Seeds are subcylindric, brown in colour. Transverse section of root showed the presence of 5-7 successive, alternate, more or less concentric rings of secondary vascular tissue. In stem pith consists of two medullary bundles fused together. In leaf around four vascular bundles are found scattered in the ground tissue.

In powder microscopy it was noticed that Cellulose and Lignin were present in all the samples except in the stem of *Achyranthes aspera*. Calcium sulphates were present only in stem and whole plant of *Achyranthes aspera*. Cell nuclei were present only in seed and whole plant of *Achyranthes aspera*.

Phytochemical study of different parts of *Apamarga* showed the presence of carbohydrates, proteins, alkaloids, saponin, tannins etc. In Thin Layer Chromatography number of spots in root, stem, leaf, seed and whole plant were 5,6,6,6 and 8 respectively. In High Performance Thin Layer Chromatography it was found that Oleanolic acid was absent in stem and leaf. It was found to be maximum in root of *Achyranthes aspera* i.e., 0.051 %.

CONCLUSION

The plant *Apamarga* (*Achyranthes aspera* Linn.) belongs to family Amaranthaceae. In the present study macroscopic, microscopic and physicochemical study of different parts of *Apamarga* was done to establish the standards for each and every part of *Apamarga*. Preliminary phytochemical screening of extract of different parts of *Apamarga* showed the presence of secondary metabolites, which may be responsible for its different pharmacological action. Oleanolic acid possesses anti-inflammatory and anti-oxidant property. Chromatographic study of different parts showed that Oleanolic acid is found to be maximum in root of *Apamarga*. Therefore root will possess maximum anti-inflammatory and anti-oxidant property as compared to other parts of *Apamarga*.

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Study Photographs

Transverse Section (T.S) of different parts of *Achyranthes aspera* Linn. [Figure no. 1.1-1.4]

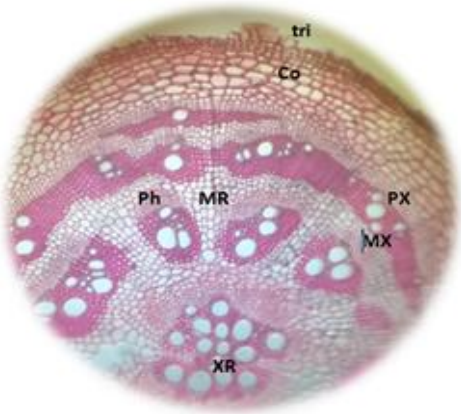


Figure 1.1 T.S of Root of *Achyranthes aspera*

Figure 1.2 T.S of Stem of *Achyranthes aspera*

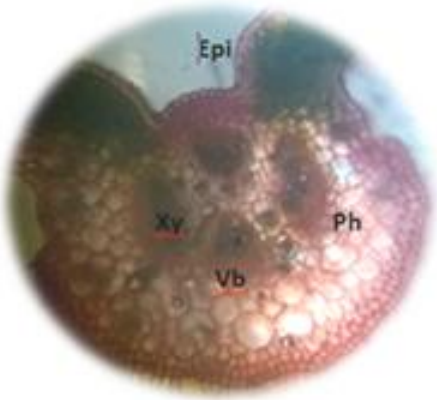


Figure 1.3 T.S of midrib of Leaf of *Achyranthes aspera*

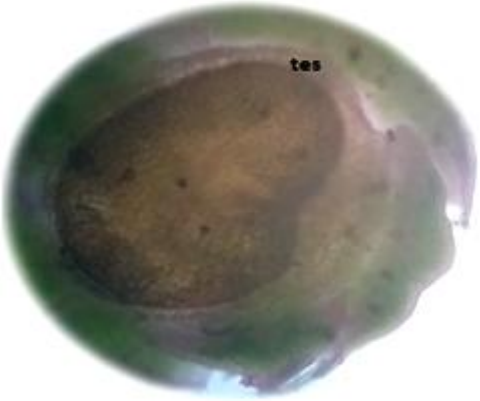


Figure 1.4 T.S of Seed of *Achyranthes aspera*

Note:
Co- cortex,Xy- xylem, PX- protoxylem, MX- metaxylem, Ph- phloem, MR- medulary ray, XR- xylem ring, Epi- epidermis, Per- peridermis, Mb- medullary bundle , XPR- xylem and phloem ring, Vb- vascular bundle, tes- testa

Powder microscopy of different parts of *Achyranthes aspera* Linn. [Figure no. 2.1-2.5]

Figure no. 2.1: Powder microscopy of Root of *Achyranthes aspera*

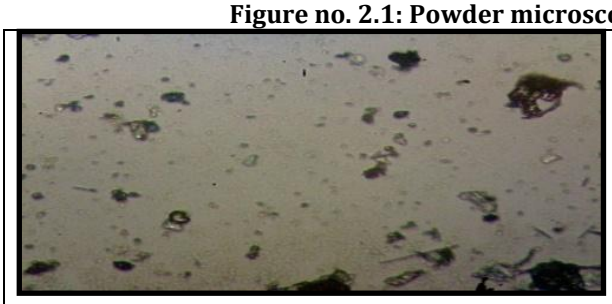
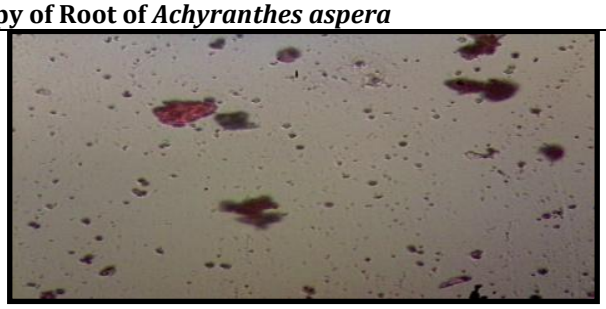
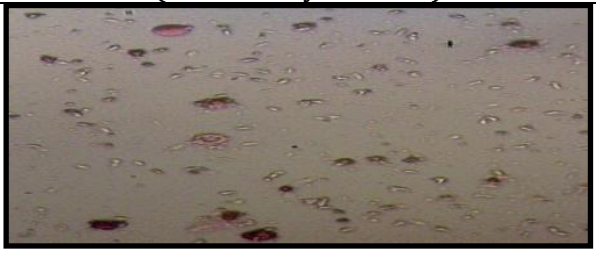
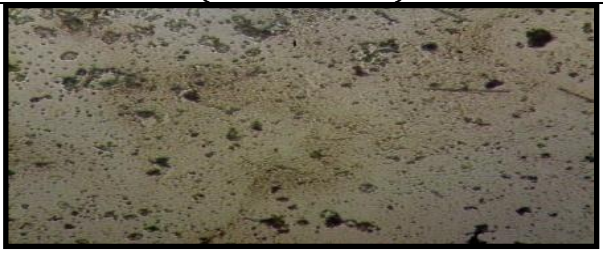
	
Deep Blue colour of Mucilage (Stain: Methylene Blue)	**Red colour of Cutin (Stain: Sudan III)**
	
Red colour of Lignine (Stain: Phlorogucinol + HCl)	**Pale yellow colour of Cellulose (Stain: Iodine Solution)**

Figure no. 2.2: Powder microscopy of Stem of *Achyranthes aspera*

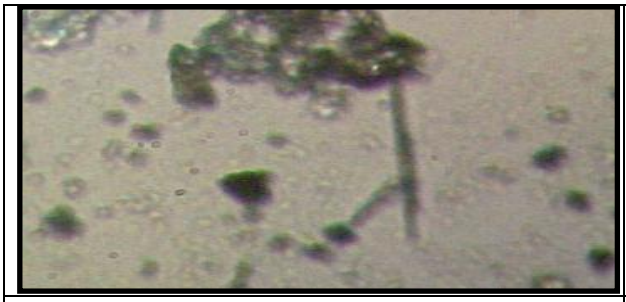
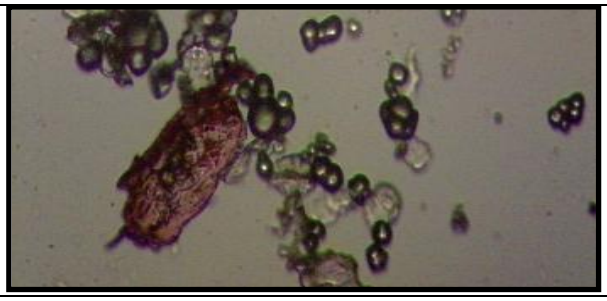
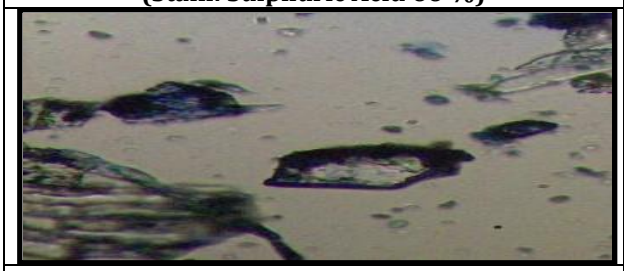
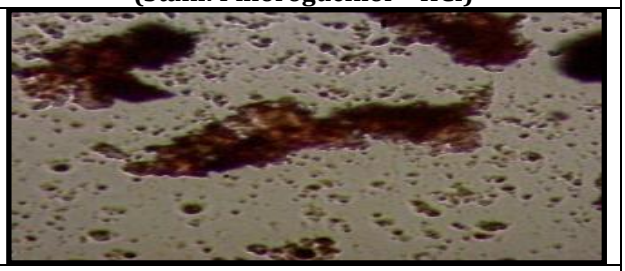
	
Needle Shaped Crystals of Calcium Sulphate (Stain: Sulphuric Acid 66 %)	**Red colour of Lignine (Stain: Phloroguicinol + HCl)**
	
Deep Blue colour of Mucilage (Stain: Methylene Blue)	**Red colour of Cutin (Stain: Sudan III)**

Figure no. 2.3: Powder microscopy of Leaves of *Achyranthes aspera*

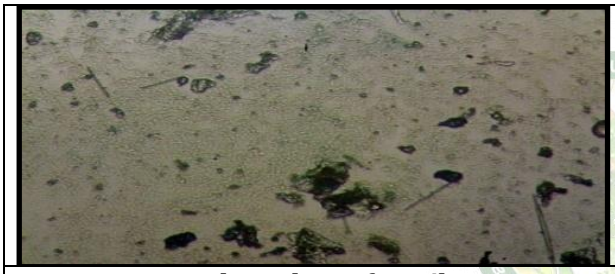
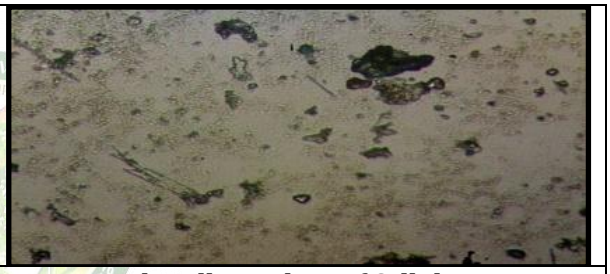
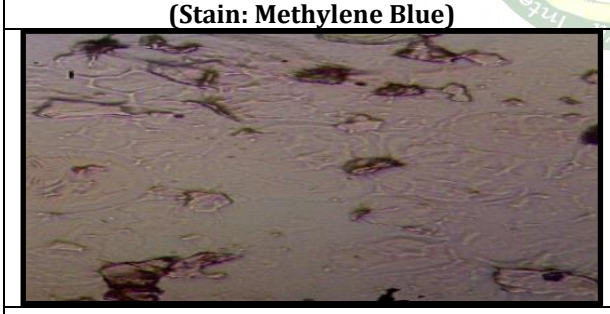
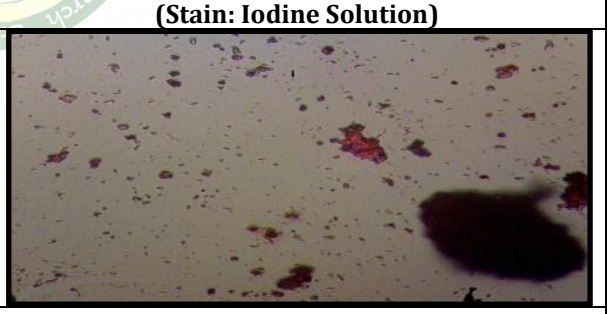
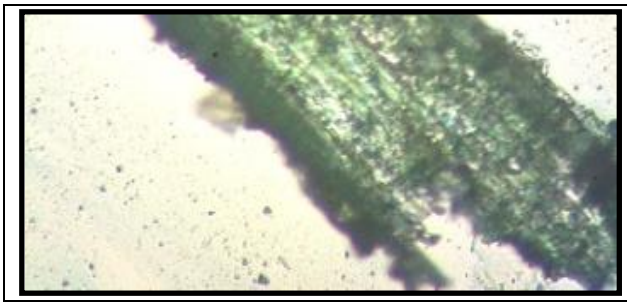
	
Deep Blue colour of Mucilage (Stain: Methylene Blue)	**Pale yellow colour of Cellulose (Stain: Iodine Solution)**
	
Red colour of Lignine (Stain: Phloroguicinol + HCl)	**Red colour of Cutin (Stain: Sudan III)**

Figure no. 2.4: Powder microscopy of Seeds of *Achyranthes aspera*

	
Deep Blue colour of Mucilage (Stain: Methylene Blue)	**Pale yellow colour of Cellulose (Stain: Iodine Solution)**

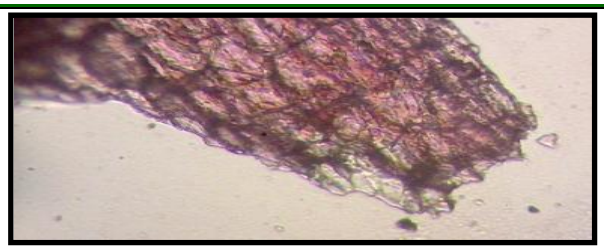
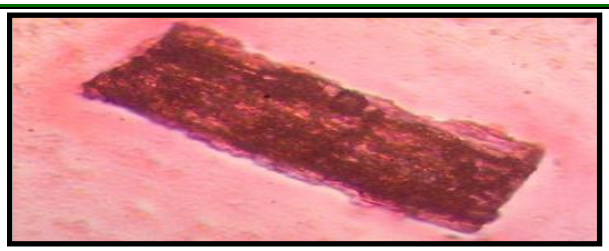
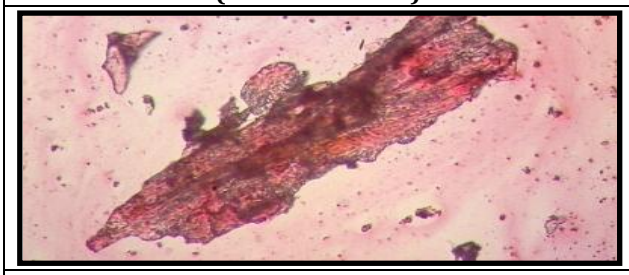
	
Red colour of Cutin (Stain: Sudan III)	**Red colour of cell nuclei (Stain: Safranin)**
	
Red colour of Lignine (Stain: Phloroglucinol + HCl)	

Figure no. 2.5: Powder microscopy of Whole plant of *Achyranthes aspera*

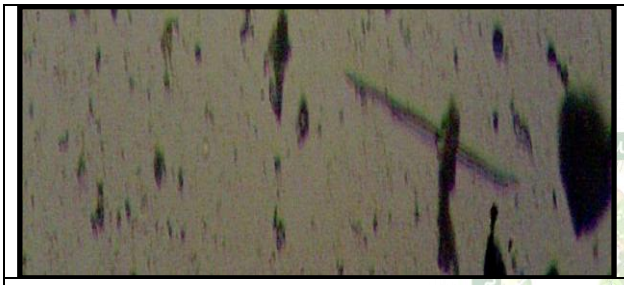
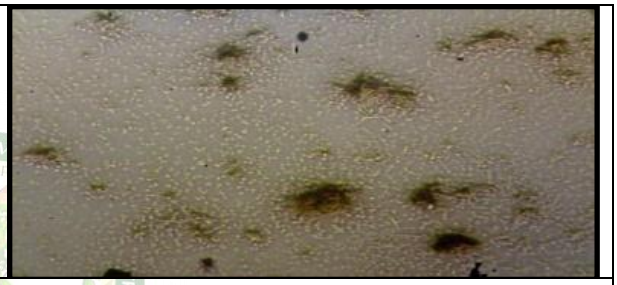
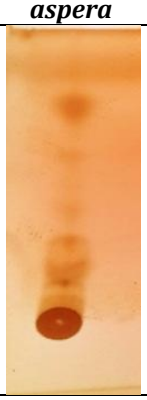
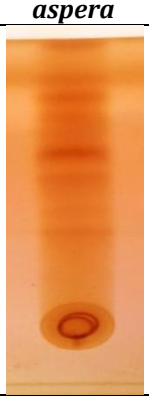
	
Needle Shaped Crystals of Calcium Sulphate (Stain: Sulphuric Acid 66 %)	**Pale yellow colour of Cellulose (Stain: Iodine Solution)**
	
Deep Blue colour of Mucilage (Stain: Methylene Blue)	**Red colour of Cutin (Stain: Sudan III)**

Figure no. 3: TLC (Thin Layer Chromatography) of different parts of *Achyranthes aspera*

Samples	Root of *Achyranthes aspera*	Stem of *Achyranthes aspera*	Leaf of *Achyranthes aspera*	Seed of *Achyranthes aspera*	Whole plant of *Achyranthes aspera*
TLC Plate					
R_f Value	0.19, 0.20, 0.45, 0.50, 0.66	0.19, 0.45, 0.50, 0.66, 0.83, 0.87	0.45, 0.50, 0.58, 0.66, 0.83, 0.91	0.13, 0.45, 0.58, 0.61, 0.66, 0.83	0.19, 0.20, 0.45, 0.50, 0.58, 0.60, 0.66, 0.83

HPLC for different parts of *Achyranthes aspera* Linn. [Figure no 4.1-4.5]

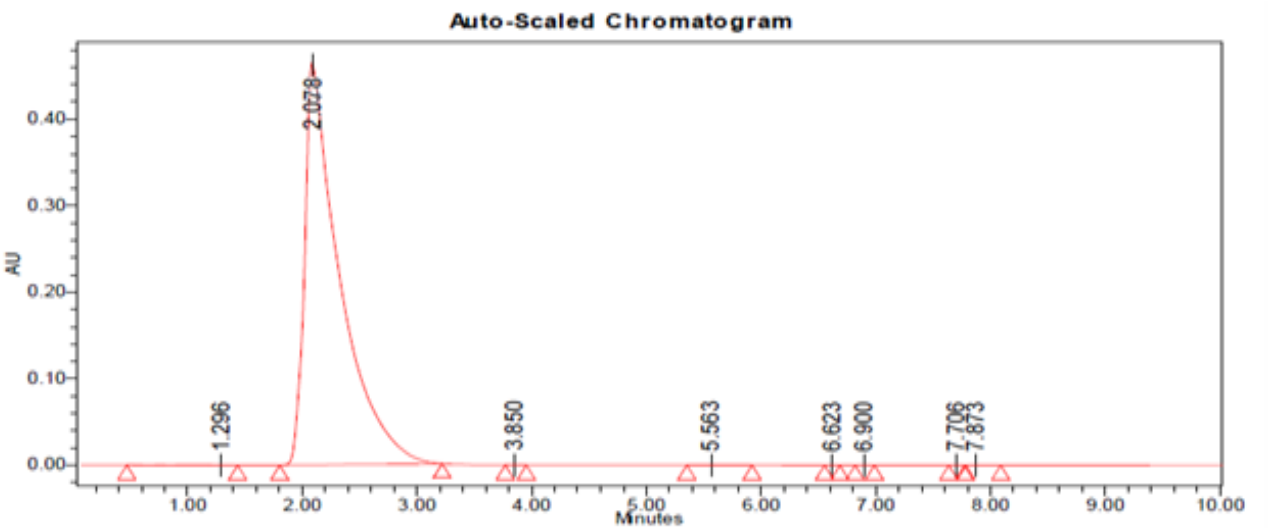


Figure no. 4.1: HPLC for root of *Achyranthes aspera*

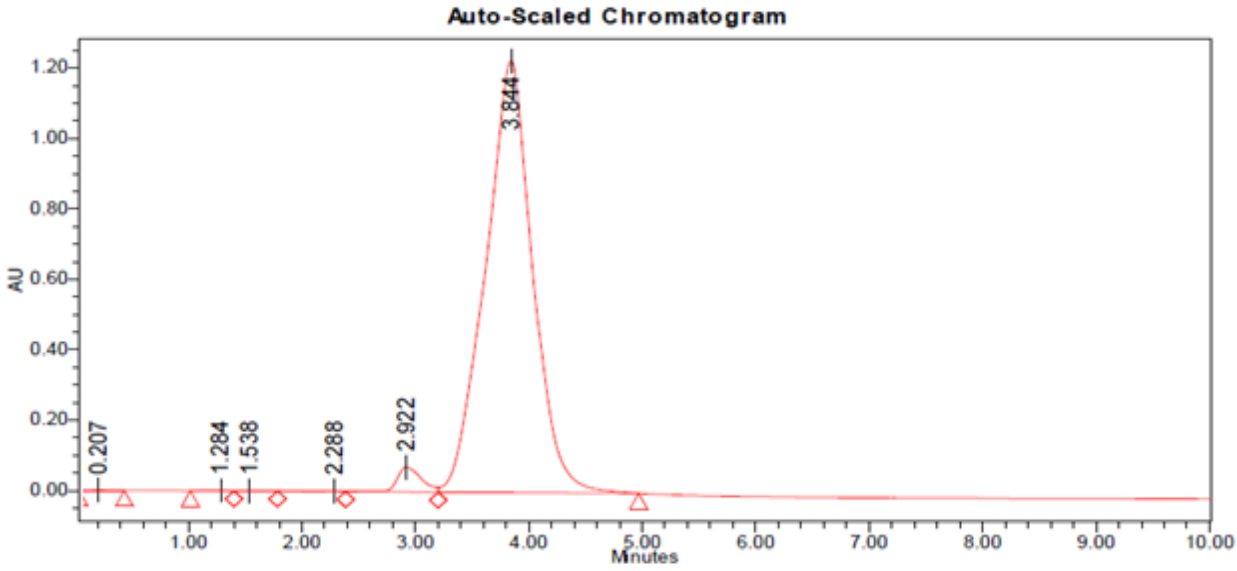


Figure no. 4.2: HPLC for stem of *Achyranthes aspera*

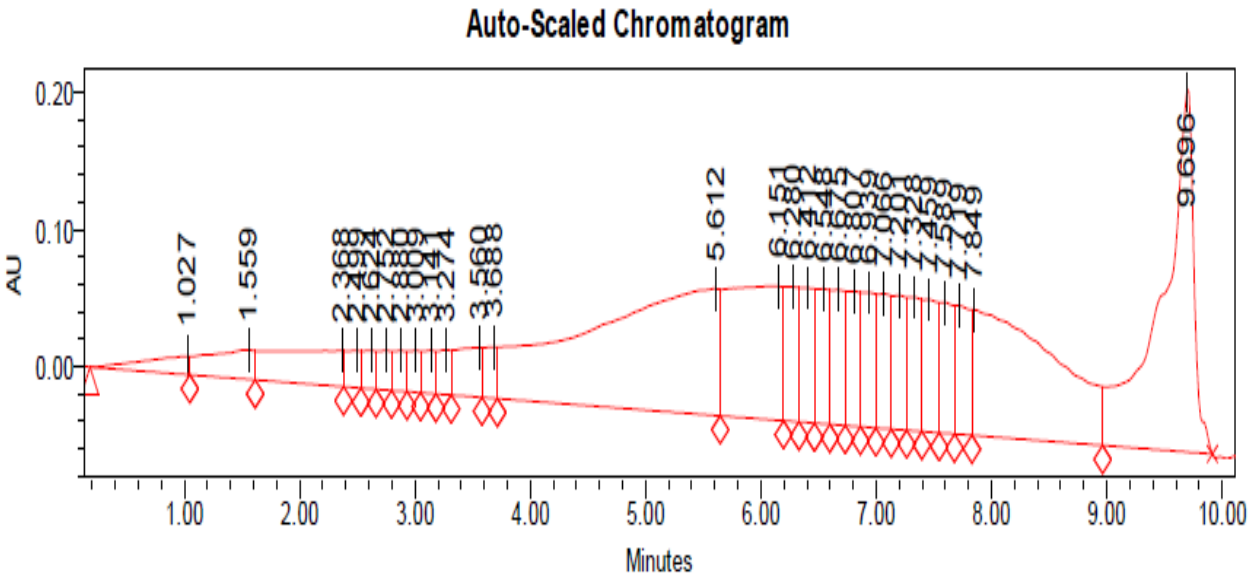


Figure no. 4.3: HPLC for leaf of *Achyranthes aspera*

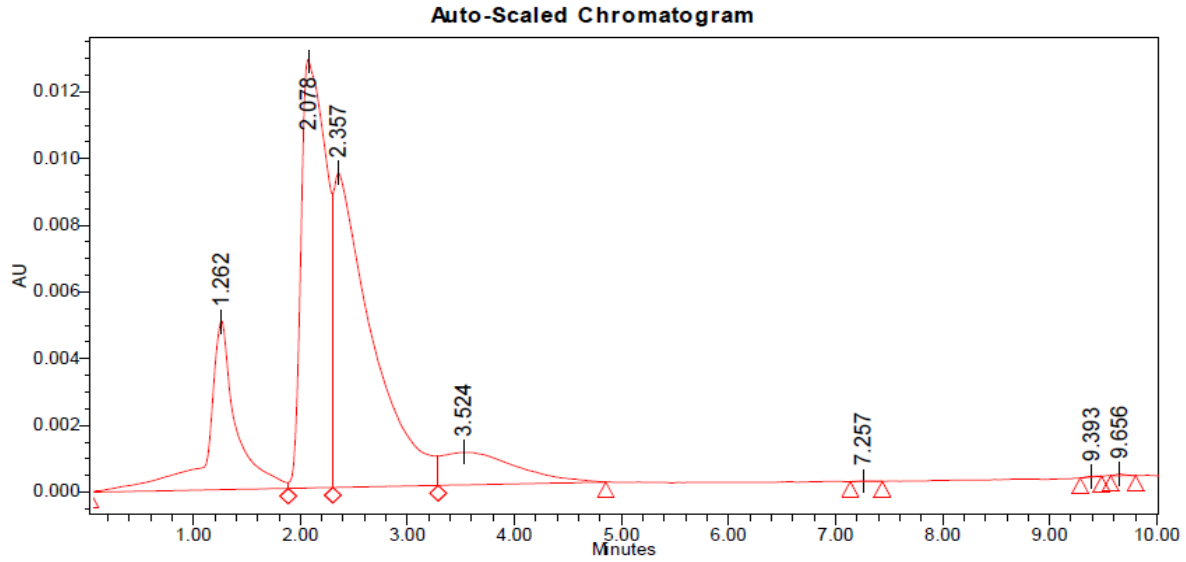


Figure no. 4.4: HPLC for seed of *Achyranthes aspera*

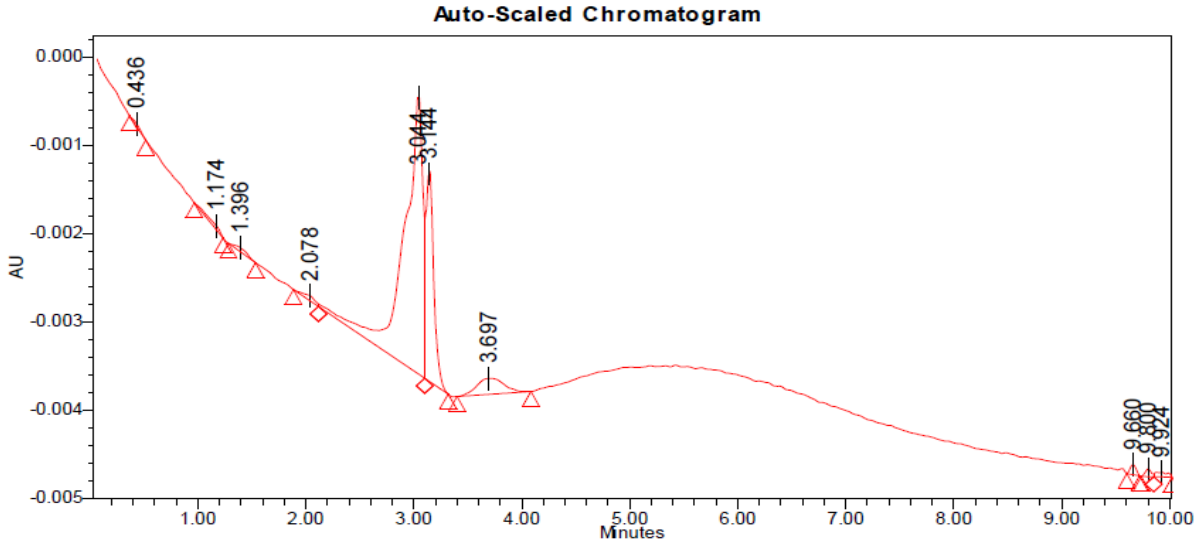


Figure no. 4.5: HPLC for whole plant of *Achyranthes aspera*