Research Article

Curing Properties of Some Weeds With Respect To WHO Guidelines

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ABSTRACT

The present study was intended to evaluate the pharmacognostical properties of *Achyranthes aspera and Xanthium strumarium* which are very common along road side as weeds with medicinal properties. The various pharmacognostical parameters were carried out as per WHO guidelines in different plant parts as stem, leaf, root and fruit. Results of Histochemical , Microscopical pharamagnostic studies of *A. aspera & X. strumarium* will help identification of the raw material. Presence of citric acid and oxalic acid reflects antioxidant potential. Results of foaming index, swelling index and haemolytic acitivity refelects presence of saponin which is very vital phytochemical having versatile biological role. *A. aspera* contents acid insoluble ash (6.55-1.25%), total ash (19.2-7.60%), water soluble ash (12.65-6.35%), foaming index (80-30 Unit), dry matter content (58-35%), swelling index (1.4-1.2 ml), crude fibre content(22.45-33%) where as *X. strumarium* possesses acid insoluble ash (3.4-1.1%), total ash (35.3-3.95%), water soluble ash (39.9-2.85%), foaming index (50-20 Unit), drying matter (46.0-.31.18%), swelling index (1.5-1.2mL%),and crude fibre content(28.10-11.15%).

Keywords: *A. Aspera*, Histochemical , Microscopical, pharamagnostic *& X. Strumarium.*

INTRODUCTION

Plant materials are used throughout developed and developing countries as home remedies, over-the-counter drug products and raw materials for the pharmaceutical industry, and represent a substantial proportion of the global drug market. It is therefore essential to establish internationally recognized guidelines for assessing their quality. The World Health Assembly — in resolutions WHA31.33 (1978), WHA40.33 (1987) and WHA42.43 (1989) — has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards. In the present study common road side weeds *Achyranthes aspera and Xanthium strumarium* was studied on the guidelines recommended by WHO to ensure safety, efficiency and quality of product. A series of tests for assessing the quality of medicinal plant materials particularly haemolytic activity, swelling index, foaming index for commonly occurring road side weed was carried out.

Many herbal materials, contain saponins which may cause haemolysis:(when added to a suspension of blood, saponins produce changes in erythrocyte membranes) causing haemoglobin to diffuse into the surrounding medium. The swelling index is the volume in ml taken up by the swelling of 1 g of herbal material under specified conditions. The foaming ability of an aqueous decoction of herbal materials and their extracts is measured in terms of a foaming index.

Achyranthes aspera is a *common* weed found thought India up to 3000 ft, along roadsides, waste land and also as an undergrowth along forest borders during rainy season[1,2]. Since time immemorial, *A. aspera* used as folk medicine, holds a reputed position as medicinal herb in different systems of medicine in India. *A. aspera* is one of the major ingredients in Ayurvedic Preparation such as Apamarga Taila, Agnimukha *etc*. The plant is also reputed to be a laxative, stomachic, depurative, pectoral and astringent; its juice is administered in diarrhoea, dysentery, monorrhagia, piles, rheumatism, inflammation of internal organs, coughs, enlarged cervical glands, eruptions, boils, *etc. A. aspera* possess abortifacient activity[3,4,5,6], hypoglycemic[7], Hypolipidemic activity[8], anti-inflammatory activity[9,10,11], Antifungal[l2], Anti – feedant Activity[13] and antibacterial properties [14,15], gynecological disorders[16,17,18,19,20], estrogenic and pregnancy interceptory effects[21], diabetes mellitus[22]. Other uses of *A. aspera* includes usefulness for reclamation of wastelands, consummation of leaf as potherb

where as seeds cooked and eaten as they are rich in protein. *A. aspera* also found its uses in religious ceremonies in India. The branches of the herb are also used as toothbrush.

Xanthium strumarium, is a woody annual found abundantly throughout hotter parts of India usually near the outskirts of villages and ascends in the Western Himalayas up to an altitude of 7,000 ft above the sea level. *X. strumarium* has a history of safe use in folk medicine and show biological activity such as effective in long standing cases of malarious fevers[2,23], antileucodermic activity[24], antitrypanosomal activity[25], and anti-inflammatory and anti-nociceptive activities[26,27], Anti -feedant Activity[28], antifungal and antibacterial activity [15],[29] and Diuretic Activity[30].

MATERIALS AND METHODS

Plant Material

Plants of *A. aspera* were collected fresh in bulk from village Neeri Mankar 25 Km away from Nagpur. Specimens collected were identified by Prof. Alka Chaturvedi, Incharge of the herbarium at Department of Botany, RTM Nagpur University, Nagpur where Herbarium specimen with voucher number RTMB 5878 & RTMB 5879 was deposited. The leaves, roots were collected separately from plants dried under shade was then powdered using mechanical grinder.

The guidelines issued by the World Health Organization, Geneva (WHO) in WHA31.33 (1978), WHA40.33 (1987) and WHA 42.43 (1989 and 1991) recommendation; were followed for the assessment of herbal medicine.

Microscopic and Histochemical studies

Microscopic and histochemical studies were done on Fresh plant parts as per given guidelines of WHO[31-32].

Pharmacognostic Standardization

The plant parts of the selected herbs under study were dried under shade, powdered, stored in airtight containers and used for powder study, physico-chemical evaluation. The crude plant material was subjected to the physical evaluation. The various parameters evaluated such as dry matter, moisture content, ash value including acid insoluble and water soluble ash, crude fibre, foaming index, swelling index and haemolytic activity as per given guidelines of WHO for quality control methods for Herbal plants.

Plant Organic Acids

Preliminary as well as confirmatory screening for plant acid was carried out as per methods given by Khandelwal[33].

RESULTS

Microscopic and Histochemical studies

Xanthium strumarium

Stem

The Transverse Section of the *X. strumarium Stem* is almost hexagonal in outline. The Stem studied as young and mature Stem differently as it shows difference in structure. Epidermis consists of a single layer of tangentially elongated cells with few multi cellular hairs and shown presence of stomata. A thin cuticle is present over the epidermal cells. Cortex is a few to many layered deep and consists of a collenchyma and parenchyma. Collenchyma forms the hypodermis lying just below the epidermis. It is about 3-5 layered deep. Parenchyma lies below the collenchyma and extends up to the endodermis. Numerous intercellular spaces are also present. Endodermis is single layered which separates the cortex from the vascular tissues. The cells lack casparian strips but indicate presence of starch. Sclerenchymatous Pericycle follows endodermis. Sclerenchymatous patches are situated over the phloem groups of vascular bundles which are called as hard blast. Vascular tissue system is represented by vascular bundles, which are arranged in a ring. Each vascular bundle is conjoint, collateral, endarch and open. Xylem consists of vessels, trachieds, xylem parenchyma and fibers. Phloem consists of sieve tubes, companion cells and phloem parenchyma. A few layers of cambium are present between xylem and phloem elements. The *X. strumarium* young Stem shows higher content of Ergastic inclusions and central part of the section is occupied by parenchymatous pith. The mature Stem shows lower content of Ergastic inclusions and central part of the section is occupied by hollow pith.

Root

The root shows single layered epidermis followed by parenchymatous cortex having air spaces. Secondary growth takes place by phellogen and cortex get disintegrated. Endodermis and pericycle also get disintegrated after secondary growth starts. Vascular tissue is seen as continuous zone. Primary phloem is destroyed, therefore not seen. To the inner side of vascular cambium and its derivative are present. A broad zone of secondary xylem which consists of prominent radial rows of vessels and trachieds;

interfascicular regions mainly have trachieds. Primary xylem is to the inner side of secondary xylem although reduced. In this region Ergastic inclusions are present. Pith is extremely reduced or absent.

Leaf

The lamina is comparatively thin and midrib region is prominent- more bulging on lower side with a small bulge on the upper sides as seen in Transverse section. Because of differentiation of mesophyll into upper palisade and lower spongy parenchyma and restrictions of stomata to the lower epidermis. Xanthium Leaf can be considered to a dorsiventral Leaf. The lamina and midrib regions cellular details will be separately described Lamina is very thin. Epidermis is a thin layer of tangentially elongated cells, producing numerous hairs all over. Cuticle is a thin covering over epidermal cells. Hairs are usually uniseriate, a few- cell long (base may be multicellular). Stomata are restricted to lower epidermis. Mesophyll is differentiated into two distinct zones- upper palisade is a single layer of somewhat compact cells, cells elongated radially, with chloroplast; lower spongy parenchyma cells are loosely arranged with very distinct intercellular spaces; the walls of mesophyll cells may be wavy.Scattered vascular strands seen in lamina are cut in various planes- some obliquely some transversely, some longitudinally (depending upon vein's orientation in the lamina); a few xylem elements and phloem cells are seen, spiral thickenings prominent.

Achyranthes aspera.

Stem

The Transverse Section of the *A. aspera Stem* is almost square shaped in outline with ridges and furrows. Epidermis is the outermost layer of thickly cuticularised cells. With mulicellular and uni-or muliserate hairs. Cortex differentiated into collenchyma, chlorenchyma. Collenchyma occurs in patches just below the ridges. Chlorenchyma forms a few layers below the epidermis in the grooves or between the collenchymatous patches. In this cortex region Ergastic inclusions are present giving a black star shaped appearance. Plate No: 8 show the magnified view of these inclusions. In order to identify the Ergastic inclusions micro chemical tests for identification of Ergastic inclusions was carried out, which reveals that these are calcium oxalate crystals. Endodermis did not show distinct casparin strips. This layer is almost distinguishable even after the secondary growth. Pericycle lies immediately outside the vascular tissues and consists of 3 to 4 cells deep groups of sclerenchyma. Vascular Tissue System consists of secondary tissues and pith.The primary phloem forms groups of crushed tissues followed by a ring of cambium lies, which separates the underlying zone of secondary xylem. Secondary xylem consists of many vascular bundles embedded in prosenchyma. In the region, there is no differentiation between secondary xylem elements and prosenchyma. A few large vessel can, however, be seen prominently. Phloem groups of the embedded vascular bundles appear as embedded in the thick walled prosenchyma. These are called as included phloem or phloem island or interxylary phloem. Primary xylem lies near the pith. Protoxylem elements are endarch. The vascular bundles thus would be conjoint, collateral, endarch and open. Pith is a well developed, parenchymatous and present in the centre.Two medullary vascular bundles are present in the centre with their xylem facing each other.

Root

In root Secondary growth takes place by vascular cambium and phellogen at very early stage of life cycle. The epidermis is ruptured. Periderm is below the epidermis which is multiple layered Cortex- can be seen in primary structure but reduced due to formation of secondary vascular tissues on inner side .Endodermis and Pericycle gets crushed, therefore may or may not be seen.Vascular tissue is seen as alternating continuous zones. Primary phloem is destroyed, therefore not seen. To the inner side of vascular cambium and its derivative are present. A broad zone of secondary xylem which consists of prominent radial growth rows of vessels and trachieds; interfascicular regions mainly have trachieds. Primary xylem is to the inner side of sec xylem although reduced. In this region Ergastic inclusions are present. Pith is extremely reduced or absent.

Leaf

It is a thick and leathery with xerophytic characters. A T.S of the Leaf differentiated into Lamina and midrib region. Epidermis is present on both the surfaces of Leaf. It is multiple layered on both the surfaces. The upper epidermis is 2-3 layered thick; the cells of outermost layer are small with their outer walls prominently convex; the inner layer cells are larger and unequal with slightly thick cuticle on outer side of epidermis. The lower epidermis is unique –it is also multiple being 3-4 cell thick; the continuity of the inner layers is

broken by typical infoldings of the outermost layer in the mesophyll tissue; the infolded parts appear as cups and are the sites of stomata being called stomatal crypts or pits; several unicellular elongated hairs arises from the epidermal cells of the crypt; the stomata are slightly elevated above the crypt epidermis. This epidermis is also surrounded by thick cuticle and the nature of cells is similar to that of upper epidermis. The entire peculiarity of the epidermis is helpful in preventing the excessive loss of Water (to reduce the transpiration). At the end of lamina the upper and lower epidermis meet. In the midrib region the upper epidermis is a prominent concavity; in the region it is almost double in thickness than on the lamina part. The lower epidermis is prominently bulged out; the inner layer cells are somewhat rounded and thick-walled.

Mesophyll is present between the abaxial and adaxial epidermis of lamina. It is differentiated into upper palisade parenchyma and spongy parenchyma below it (near lower epidermis). The number of palisade layers may be 1-4; the outermost layer consists of compactly arranged; chloroplast- containing cells. The spongy parenchyma cells are thin-walled loosely- arranged with numerous air spaces between them. The number of chloroplasts in these cells is less; a distinct substomatal chamber is present to the inner side of each stomata. Calcium oxalate crystal united to form druces (sphaeraphides) are present in some enlarged cell near inner palisade layer.In the laminar part smaller vascular bundles are seen. Each Vascular bundle is surrounded by parenchymatous bundle sheath. The xylem is towards palisade side and consists of small trachieds and small amount of parenchyma where as the phloem is on the lower side consisting of a few sieve tube members and small amount of parenchyma.The epidermis over midrib is multiple almost double the thickness of that of lamina on upper side and almost of same thickness of that on the lower side. The mesophyll is lacking in midrib. The conjunctive tissue parenchyma replaces mesophyll on either side near the epidermis. The druces are present in some cells. Intercellular spaces are absent. The center of the midrib is occupied by one large distinct prominent vascular bundle and four lateral vascular strands. The xylem consists of trachieds and vessels. Distinct xylem (vessels, trachieds, parenchyma) on upper side and phloem on lower side; cambium –like zone in between xylem and phloem.

Histochemical studies

Presence of cellulose was detected by blue colour formation as cellulose dissolves in cold concentrated sulphuric acid and is precipted as amyloid on dilution. Yellow color of the outermost deposition on epidermis indicates that it is composed of cutin – a fat like substance. Cutin content in *X. Strumarium* compares more when compared with *A. Aspera.* The suberised portion becomes red stained indicating the presence of suberine in the cell wall of root of both the plants. Presence of Lignin indicated by, red violet color prominent mainly in root. The cells with mucilage are stained bright blue indicating that the material possesses mucilage. Table No [1]

Pharmacognostic Standardization

Table No [2] The hemolytic activity of the plant extracts increases as the concentration of the plant material increases. It is at minimum at 0.1 mL concentration where as maximum at 1.0 mL concentration. At 0.1 mL concentration it is maximum in *X. strumarium* Root followed by Stem and Fruit. It follows the same trand at 1mL concentration. The results are expressed in Table No: 24. The scale used to express results of foaming index is that if the highest of the foam in test is 0.1 mL the foaming index is 10 and likewise. *A. aspera* Stem shows the highest Foaming index 80, followed *A. aspera* Root and Fruit showing the same value of Foaming index as 50. *X. strumarium* show maximum Foaming index as 50 in Root and 20 in Fruit.

DISCUSSION

An examination to determine microscopic and histochemical Characteristics of Herbal materials is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. Present Quality Control Tests, clearly indicates presence of saponin in both the herbs under study, by values of foaming index, swelling index and haemolytic activity. The foaming ability of an aqueous decoction of the plant materials and their extracts is measured in terms of a foaming index. Many medicinal plants materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin or hemicelluloses (WHO Geneva, 1998). The Swelling index is the volume in mL taken up by the swelling of 1 g of the plant material under specific conditions. Its determination is based on the addition of the water or a Swelling agent as specific in the test procedure for pulverized

material. The characteristic property of saponins is their ability to cause haemolysis; when added to a suspension of blood, saponins produce change in erythrocyte membranes, causing hemoglobin to diffuse into the surrounding medium.

Saponins are often bitter to taste, and so can reduce plant palatability (e.g., in livestock feeds), or even imbue them with life-threatening animal toxicity In plants, saponins may serve as anti-feedants, and to protect the plant against microbes and fungi. Some plant saponins (e.g. from oat and spinach) may enhance nutrient absorption and aid in animal digestion. some saponins are toxic to cold-blooded organisms and insects at particular concentration. Most saponins, which readily dissolve in water, are poisonous to fish. Therefore, in ethno-botany , they are primarily known for their use by indigenous people in obtaining aquatic food sources. Saponins are used widely for their effects on ammonia emissions in animal feeding. Saponines anti-inflammatory potential is well recorded in *Carthamus tinctorius*[34], *Madhuca longifolia*[35], *Sapindus mukorossi*[36], *Melilotus elegans*[37]. The identification and development of saponins have greatly contributed to medical treatment of cancer and many of these compounds are now being used in clinical practice. Almost all saponins induce apoptosis in tumor cells; they are preferable drugs for the treatment of cancer, because eliminating tumor cells by apoptosis is helpful to lower side effects in patients by avoiding necrosis. There are more than 11 mainly distinguished classes of saponins including dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes,cycloartanes, lanostanes, cucurbitanes, and steroidals. Among these saponins, cycloartanes, dammaranes, oleananes,lupanes and steroids showed strong antitumor effect on kinds of cancers[38].

Because of organic acid importance in intermedediary metabolism and plant respiration, a multitude of methods have been devised for determining these organic acids. Organic acids are water soluble, colorless liquids or relatively low melting solids. *X.strumarium* and *A.aspera* show accumulation of Citric acid and oxalic acid showed in Vacuoles in various plant parts where as there was no accumulation of Malic and Tartaric acid. Zander[39], reported presence of organic acids and resins in *X. strumarium* plant. The organic acids studied above are the part of the ergastic substances present in various tissue of the plant Stem, Root and Leaf. Their study is of vital importance to know the chemical basis of ergastic substances.

CONCLUSION

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacopoeia, these standards must be established. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy and physico–chemical parameters. The present investigation of *X. strumarium* and *A.aspera* can be concluded that the pharmacognostical study and yielded a set of qualitative and quantitative parameters or standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies for the first-rate pharmacological activity.

As there is no record on pharmacognostical work on different parts of plants under study, therefore the present work is undertaken to produce some pharmacognostical standards and this founding may help to proper identification and ensures the quality of the drug and also help this amazing plant grown on commercial basis for better use in pharmaceutical herbal formulations.

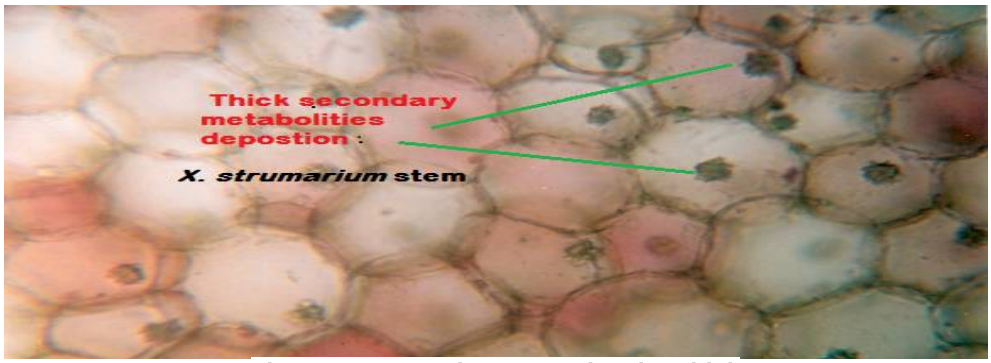

Fig. 1: X. strumarium stem showing thick depostion of secondary metabolites

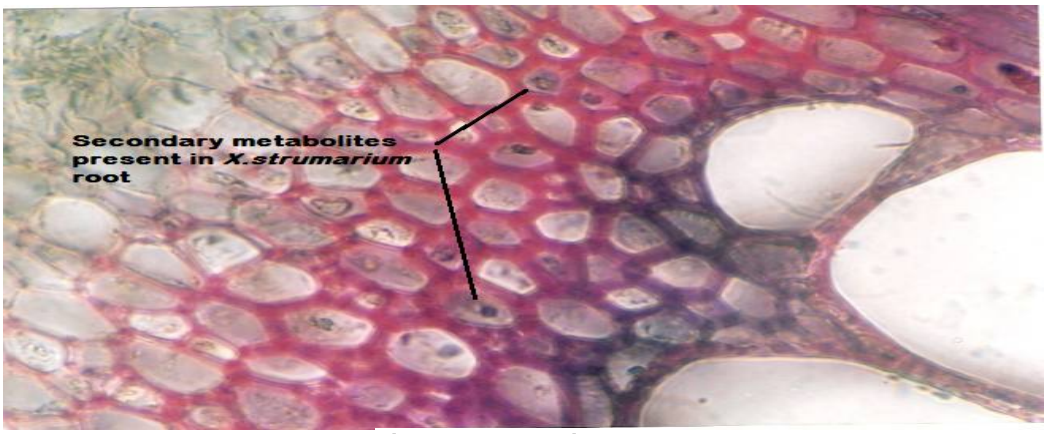

Fig. 2: x.strumarium root

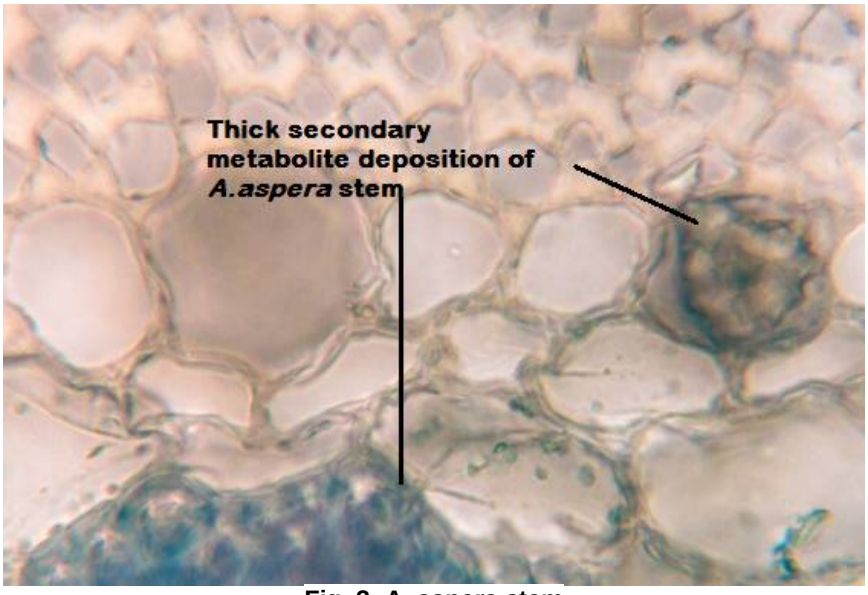

Fig. 3: A. aspera stem

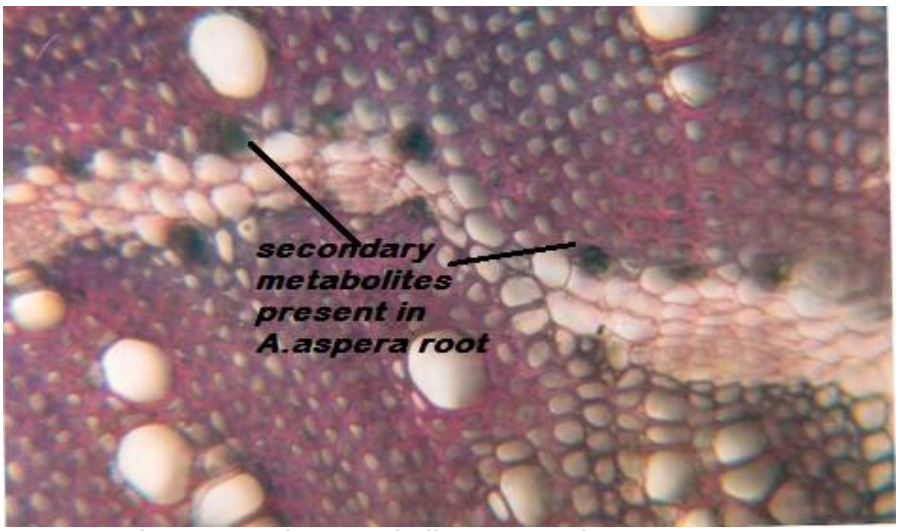

Fig. 4: secondary metabolites present in A.aspera root

Table 1: Histochemical Studies

Plant Name	***X. strumarium***				***A. aspera***			
Chemical compound	Leaf	Stem	Root	Fruit	Leaf	Stem	Root	Fruit
Cellulose	+	+	+	+	+	+	+	+
Cutin	-	+	+	-	-	+	+	-
Suberine	-	-	+	-	-	-	+	-
Lignin	-	-	+	-	-	-	+	-
Mucilage	+	+	-	-	-	-	-	-
Citric acid	+	+	+	+	+	+	+	+
Oxalic acid	-	+	+	-	+	+	+	+
Malic acid	-	-	-	-	-	-	-	-
Tartaric acid	**-**	**-**	**+**	**-**	**-**	**-**	**-**	**-**

Table 2: Pharmacognostical Properties of *Achyranthes aspera And Xanthium strumarium*

Plant Name	***X. strumarium***				***A. aspera***			
Plant part	**Leaf**	**Stem**	**Root**	**Fruit**	**Leaf**	**Stem**	**Root**	**Fruit**
Dry matter	42.25	46.00	31.18	34.20	58.40	49.30	35.21	38.00
Moisture %	57.75	54.00	68.82	65.8	41.60	50.70	64.79	62.00
Ash%	35.3	22.15	9.75	3.95	19.2	11.3	16.9	7.60
Acid insoluble ash content%	3.4	7.75	1.6	1.1	6.55	1.85	4.45	1.25
Water soluble ash content%	31.9	14.4	8.15	2.85	12.65	9.45	12.45	6.35
Crude fiber content%	11.15	28.10	16.95	26.40	26.70	29.10	22.45	33.00
Foaming index%	30	40	50	20	30	80	50	50
Swelling index	1.5 mL	1.3 mL	1.2 mL	1.2 mL	1.4 mL	1.3 mL	1.2 mL	1.1 mL
(Plant extract used in ml)	**Haemolytic Activity**							
0.1	+	+ +	+ + +	+ +	+	+	+	+
0.2	+ +	+ + +	+ + + +	+ + +	+ +	++	+ +	+ +
0.5	+ + +	+ + + +	+ + + + +	+ + + +	+ + +	+ + +	+ + +	+ + +
1.0ml	+ + + +	+ + + + +	+ + + + + +	+ + + + +	+ + + +	+ + + +	+ + + +	+ + + +

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