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Microscopic Authentication of Weeds of Rice Fields Collected from Adjoining Areas of District Kasur

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Abstract

Weeds are unwanted plants but in other context also used for medicinal purposes in various localities but the problem of admixturing and adulteration of these medicinal plants is directly proportional to the increasing demand of herbal medicinal products. In this study six weed species which are medicinally important were collected form rice fields i.e., *Cyperus conglomeratus* Hochst., *Cyperus difformis* Blanco., *Paspalidium flavidum* A. Camus., *Juncellus pygmaeus* C.B. Clarke., *Alternanthera sessilus* R.Br. and *Eclipta alba* Hassk. The macroscopic, microscopic, florescence analysis and ash values were determined. *Eclipta alba* showed the highest water insoluble ash and acid insoluble ash of 21.4%w/w and 5.1%w/w respectively. *Cyperus difformis* showed the highest value of sulphated ash value of 5.3%w/w. These observations are set for the comparison of structures and level of adulterants can be found out by comparing. From this research work it can be suggested that these methods are quite significant, useful and cheap for the correct identification and determination of authenticity and purity of medicinal plants in pharmaceutical companies.

Keywords: Adulteration; Florescence Analysis; Ash Value; Authenticity; Pharmaceutical Companies

1. Introduction:

Since the ancient times, the plants are used to treat different diseases and are still used to this day. The method "trial and error" was used to determine whether the plant is capable to treat disease, in this way, to differentiate the harmful plant from beneficial plant with beneficial effects. The use of herbal plants is refined over the generation, which are called as traditional medicine sin many contexts. It is true that all the civilization has developed their medicinal structure based on the plants that are available in their habits. (Manzano *et al*., 2020).

The *cyperaceae* family (sedge family) mainly consist of 5000 species that contain 104 genera. It is the third largest family of monocots and are present in the tropics. The cyperaceae having regional importance of basket, weaving mats and sandals due to presence of strong fibrous leaves and stem (Mishra *et al*., 2015). *Cyperaceae family* having major pharmacological activities is used in the treatment of many diseases like fever, dysmenorrhea cancer, vesical and renal calculi, dyspepsia, skin diseases and diarrhea (Jyoti *et al*., 2018). The *cyperaceae* having such medicinal importance is due to presence of flavonoids, alkaloids, sesquiterpenoids, polyphenol, glycoside and saponinsin it (Kumar *et al*., 2017). Weeds are mostly neglected as their medicinal use is not considered to be on the very large scale. Plant plays a very important role in human life viz the food, fibre, fuel and medicine.

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Some of the ancient literature stated that each and every plant resent on earth has an importance in industry, allelopathy and medicine. Plant are the largest reservoirs of traditional medicines, phytochemicals and chemicals used for syntheitic drugs. Weeds has large number of tannins, glycolsides and alkaloids. Weeds also show antimicrobial activity. Saponins present in the weeds have important significance as cardiac depressant, antihypercholesterol and hypotensive properties. Due to presence of tannins weeds also act as antihaemorrhagic and antidiarrhoec agent. Phenol presents in the weeds have importance significance as antiaging, antiinflammation, cardiovascular protection, antipoptosis, anticarcinogen, antiatherosclerosis, inhibition of angiogenesis, improvement of endothelial function and inhibition of cell proliferation. Due to presence of glycosides in weeds it acts as antiulcer and antisecretory agent. The cardiac glycosides act as a stimulant in case of cardiac failure over the centuries. The plant glycosides inhibit chloride transport to the stomach and are not harmful when ingested orally (Chavan *et al*., 2013).

The main factor that caused the attention toward the green medicines are due to deleterious effect of synthetic drugs on human and environment (Chanda, 2014). The main advantages of the herbal medicines are the inexpensive, availability, effective, harmless, easily obtained and mainly doesn't cause many side effects. The herbal medicine uses are broad enough to treat different ailments therapeutically. The use of herbal medicines is able to treat different diseases including liver diseases, tumors, heart diseases, diabetes mellitus, cancer and many others. Some of the major pharmaceutical industries use the weeds as the raw material for different chemicals (Krishna *et al*., 2009).

According to the WHO the 80% of the people around the world depend upon herbal medicine to treat different types of diseases. The 11% of the essential drugs are produced for the plants. About 70-95% in the current developing countries use traditional medicine for daily life care. Pakistan due to its climatic conditions has about 6000 of herbal plants in it. Approximately 600- 700 of these species are used to treat different diseases and also exported to different countries. 60% of the population in Pakistan use traditional medicine for daily health care (Alamgeer *et al*., 2018).

1.1. Objectives:

- To determine the purity and authentication of weeds by florescence analysis and ash values determination**.**
- To determine the anatomical characters through powder microscopy.

2. Materials and Methods:

2.1. Collection of Material:

The weeds, *Cyperus conglomeratus, Cyperus difformis, Paspalidium flavidum, Eclipta alba, Alternanthera sessilis and Juncellus pygmaeus* were collected from the rice field during October 2021 and dried under the shade for 30-35 days until all the moisture is dried up. The stem, root and leaves were separated from each other then they are subjected to anatomical and chemical standardization by using Phloroglucinol analysis in which powder microscopy, florescence analysis as well as estimation of ash value were carried out.

2.2. Macroscopic Characters:

Macroscopic examination of different plant species reveals distinct characteristics. *Eclipta alba* displays an upright, branched, cylindrical structure marked by longitudinal ridges and a brown hue. Its leaves are broad, undivided, and sessile. In contrast, *Alternanthera sessilis* presents fleshy, wide, oblong leaves with rounded tips. The stem of *Alternanthera sessilis* is cylindrical, occasionally slightly quadrangular, and light brown.

In *Cyperus conglomeratus*, leaves are arranged alternately, boasting smooth margins and a simple, elongated lanceolate shape with parallel venation. The stem stands erect, is devoid of hair, and possesses a woody texture. In the case of *Cyperus difformis*, the leaves are concise and sleek, while the stem is tall, upright, and hairless.

Juncellus pygmaeus showed a towering, erect stem densely clustered in tufts. Its numerous leaves are lengthy, although shorter than the stem itself. In the context of *Paspalidium flavidum*, the stem achieves height, bears a slight compression, and forms tufts. Its leaves are elongated, flat, smooth, and exhibit a linear lanceolate shape with a pronounced keel.

2.3. Microscopic Characters:

Each of dried leaf, root and stem were macerated using a mortar and pestle. A few drops of Chloralhydrate were added using a dropper to achieve a homogeneous solution. Subsequently, a few drops of

this solution were placed onto a slide and covered with a cover slip. Then put the slid under the light microscope to observe under different objective lens.

2.4. Florescence Analysis:

A gram of dried powder was subjected to treatment with various chemical reagents including 50% HNO3, 1N NaOH, 50% H2SO4, 1N HCl, and distilled water. Following this treatment, the mixture was allowed to settle at room temperature before being filtered using Whatman filter paper. This mixture visualized under the ordinary light, long UV light (366) under UV chamber, short UV light (254nm), obtained different colours.

2.5. Phytochemical Analysis:

In the determination of ash values, a sample comprising 5g of plant powder was subjected to ignition within an electric furnace set to a temperature range of 500-550°C. The process continued until a consistent weight of ash was attained. For the assessment of sulphate soluble ash value, a portion of the powder was combined with 1ml of sulphuric acid. Subsequent ignition at temperatures of 260-800°C ensued until a stable weight was achieved. To determine water soluble ash value, a segment of the ash was mixed with 25ml of distilled water and boiled for 5 minutes. Filtering this mixture through ashless filter paper and rinsing with hot water occurred before ignition and weighing. The calculation of crude drug percentage involving water insoluble ash was made possible. In the context of acid insoluble ash value, a combination of 25ml of distilled water and dilute HCl was prepared, to which the total ash value was added. Boiling for 10 minutes followed, after which filtration through Whatman 1 filter paper was conducted. The remaining residue underwent ignition in a furnace to ensure a constant weight was reached.

3. Results:

The present study focuses on anatomical standardization of six weeds found in rice fields, namely *Cyperus conglomeratus, Cyperus difformis, Paspalidium flavidum, Juncellus pygmaeus, Alternanthera sessilis, and Eclipta alba*.

Powder microscopy examination of the leaf, stem, and root of *Cyperus conglomeratus* revealed the presence of cortical cells, Stomata embedded in epidermis, fibers, bundles of vessels and tracheids, starch crystals with scalariform vessels, pith cells, and phloem fibers (Figure 1). The Fluorescent analysis of *Cyperus conglomeratus* showed the pale-yellow color with 1N NaOH solution in day light and yellow and green color in 254nm and 360nm respectively. It showed the Raddish yellow color in 50% HNO3in day light and brown and dark brown color in 254nm and 360nm respectively (Table 1). The total ash value of *Cyperus conglomeratus* is 24.82%w/w. It has 4.32%w/w and 2.05%w/w in water insoluble and Acid insoluble ash respectively (Table 3).

For *Cyperus difformis*, the microscopic analysis showed Stomata embedded in epidermis, phloem fibers, helical vessels, cortical cells, thickening of reticulate vessels, unicellular and multicellular trichomes, Scalariform with perforations and elongated unicellular fibres (Figure 2). The florescence analysis showed the pale brown with 1N NaOH in daylight and light brown and green color in 254nm and 360 nm respectively. It also showed the Reddish-brown color with 50% HNO3 in day light and brown and dark brown color in 254nm and 360 nm respectively (Table 2). The ash value of *Cyperus difformis* shows the total ash value of 21.2%w/w and 11.3% & 3.9% w/w of water insoluble ash and acid insoluble ash respectively (Table 3).

The powder microscopy of *Paspalidium flavidum* showed phloem fibers, groups of cortical cells, filiform fibers, and Stomata embedded in epidermis (Figure 2). The florescence analysis of *Paspalidium flavidum* showed the light yellow color with 1N NaOH in day light and pale brown and green color in 254nm and 360nm respectively. It showed the yellow green with 50% HNO3in day light and dark yellow and dark brown color in 254nm and 360nm respectively (Table 2). The total ash value of *Paspalidium flavidum* has 27.3%w/w. It shows 21.3%w/w and 2.1%ww in water insoluble ash and acid insoluble ash value respectively (Table 3).

In *Juncellus pygmaeus*, stomata were present in epidermal cells, along with groups of cortical cells, scalariform thickening in vessels, leaf traces, reticulate vessels, fibers, and epidermal cells with longitudinal shapes (Figure 1). The florescence analysis of *Juncellus pygmaeus* showed the Reddish-brown color with 1N NaOH in day light and light brown and light green in 254nm and 360nm respectively. In 50% HNO3 solution showed Raddish green color in day light and brown colour in 254nm and 360nm respectively (Table 1). The ash value of *Juncellus pygmaeus* showed the 37.2%w/w and 21.1% w/w and 1.9%w/w in water insoluble ash and Acid insoluble ash respectively (Table 3).

Eclipta alba showed characteristics like anisocytic stomata, unicellular trichomes, helically thickened vessels, cortical cells, Scalariform with perforations and epidermal cells with longitudinal and cylindrical shapes in its leaf, stem, and root. (Figure 1). The florescence analysis of *Eclipta alba* showed the pale brown with IN NaOH in daylight, it shows the light

brown and green color in 254nm and 360nm respectively. It showed reddish brown color in 50% HNO3 in daylight. It showed the light brown and green color in 254nm and 360nm respectively (Table 1). The total ash value of *Eclipta alba* is 22.4% w/w. it shows the 21.4% w/w and 5.2%w/w respectively (Table 3).

Lastly, *Alternanthera sessilis* displayed features such as unicellular trichomes, helically thickened vessels, paracytic stomata, starch crystals, and clusters of fibers in its leaf, stem, and root under powder microscopy (Figure 2). The florescence analysis of *Alternanthera sessilis* showed the pale-yellow color with 1N NaOH in day light and light yellow and green color in 254nm and 360nm respectively (Table 2). The total ash value of *Alternanthera sessilis* is 19.21% w/w. It showed the 2.31% and 1.74%w/w of water insoluble ash and acid insoluble as respectively (Table 3)

The following anatomical structures had seen under microscope at 40x.

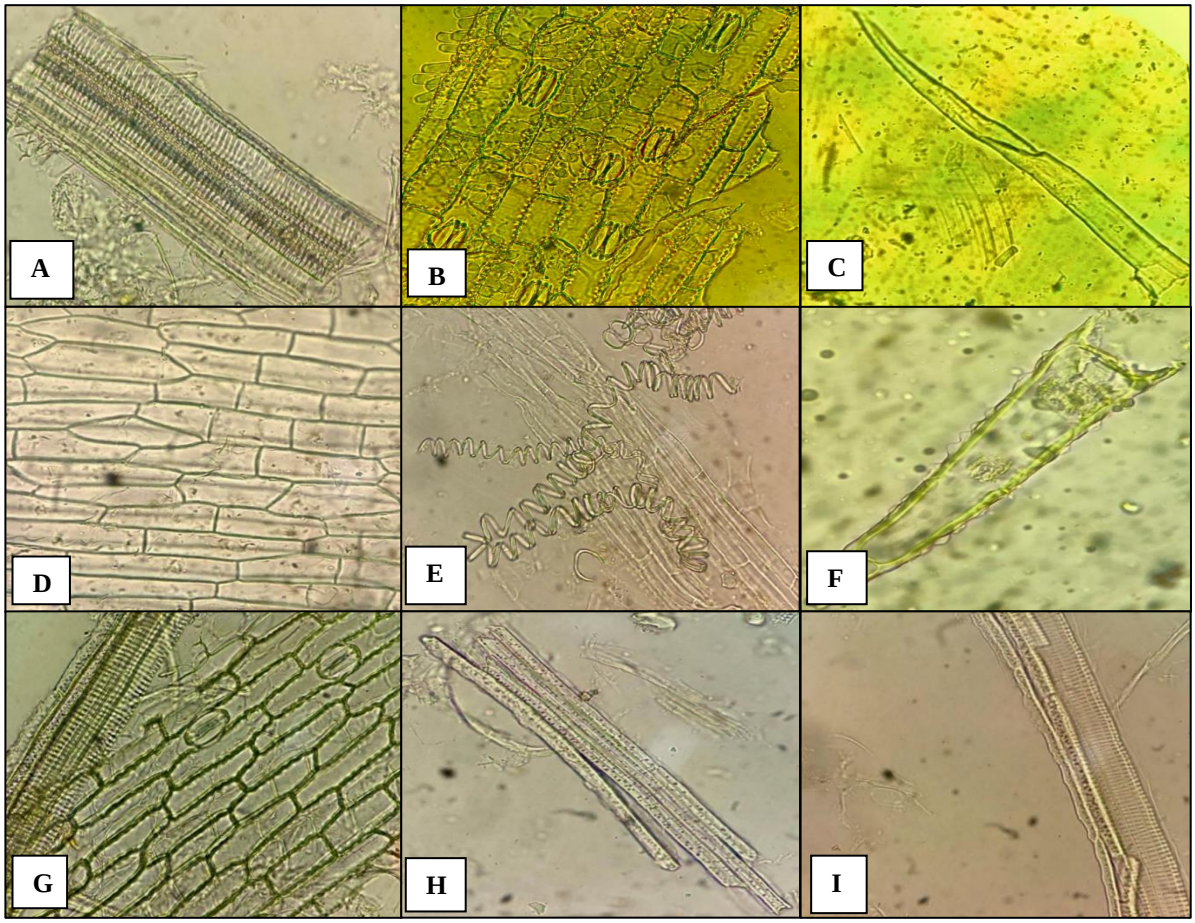

Figure 1**: A.** Bundle of vessels and tracheid in leaf of *Cyperus conglomeratus*. **B**. Dumbbell Stomata present in epidermis of stem of *Cyperus conglomeratus*. **C**. Trichome present in root of *Cyperus conglomeratus*. **D**. Epidermal cells with longitudinal shape in stem of *Eclipta alba*. **E**. Spiral thickening in stem of Eclipta alba. **F**. trichome present in Leaf of *Eclipta alba*. **G**. Stomata present in epidermal cell in leaf of *Juncellus pygmaeus*. **H**. Reticulate vessel in root of *Juncellus pygmaeus*. **I**. Scalariform vessel in stem of *Juncellus pygmaeus.* (40x).

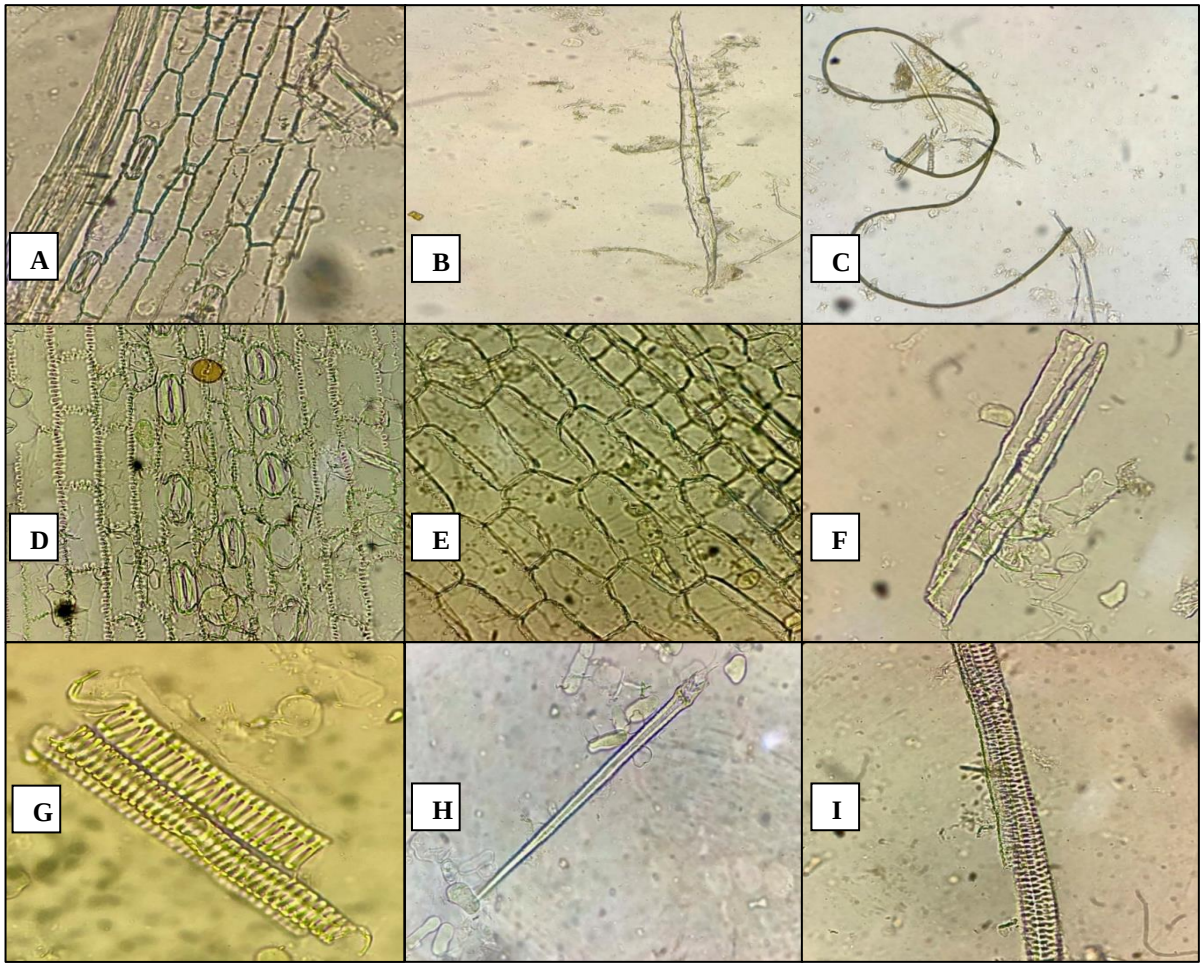

Figure 2: **A**. Stomata present in epidermal cells with fibers in leaf of *Paspalidium flavidum*. **B**. Phloem fiber present in stem of *Paspalidium flavidum*. **C**. Elongated fiber present in the root of *Paspalidium flavidum*. **D**. Stomata present in epidermis in stem of *Cyperus difformis*. **E**. Epidermal cell in leaf of *Cyperus difformis*. **F**. Trichome present in the root of *Cyperus difformis*. **G**. Spiral vessel that is uncoiling in Leaf of *Alternanthera sessilis*. **H.** Trichome present in the stem of *Alternanthera sessilis*. **I**. Scalariform vessel present in the root of *Alternanthera sessilis*. (40x).

Table 1: Fluorescent Analysis of powder of *Cyperus conglomeratus,* Juncellus pygmaeus and *Cyperus difformis* under visible and UV light.

		Cyperus conglomeratus			***Juncellus pygmaeus***			***Cyperus difformis***		
S.#	**Solvent used**	**Day light**	**254nm (UV light)**	**360nm (UV light)**	**Day light**	**254nm (UV light)**	**360nm (UV light)**	**Day light**	**254nm (UV light)**	**360nm (UV light)**
1	Powder + 1N NaOH (Aqueous)	Pale yellow	Light brown	green	Raddish brown	Light brown	Light green	Pale brown	Light brown	Green
2	Powder + 1N HCl	Dark brown	Raddish brown	green	Pale brown	Light yellow	Green	Dark yellow	Raddish brown	Green
3	Powder + 50% H_2SO_4	Brown	Light brown	green	Brown	Light brown	Green	Orange	Light brown	Green
4	Powder + 50% HNO_3	Reddish orange	Red	Dark brown	Reddish green	Brown	Brown	Reddish Brown	Brown	Dark brown

5	1% KOH	Yellowish brown	Chocolate brown	Light green	Yellowish green	Red	Green	Yellowish Green	Chocolate brown	Light green
6	Powder as such	Light green	Brown	White	Light yellow	Brown	white	Light brown	Brown	White

Table 2: Fluorescent analysis of powder of *Paspalidium flavidum*, *Alternanthera sessilis* and *Eclipta alba* under visible and UV Light.

S. #	Solvent used	Paspalidium flavidum			*Alternanthera sessilis*			*Eclipta alba*		
		Day light	254nm (UV light)	360nm (UV light)	Day light	254nm (UV light)	360nm (UV light)	Day light	254nm (UV light)	360nm (UV light)
1	Powder + 1N NaOH (Aqueous)	Light yellow	Pale brown	Dark green	Pale yellow	Light yellow	Green	Pale brown	Light brown	Green
2	Powder + 1N HCl	Light yellow	yellowish brown	Black	Dark green	Raddish green	Green	Dark yellow	Raddish brown	Green
3	Powder + 50% H_2SO_4	Yellowish brown	Light green	Dark green	Green	Light brown	Green	Orange	Brown	Green
4	Powder + 50% HNO_3	Yellowish green	Dark yellow	Dark green	Reddish yellow	Brown	Dark brown	Reddish brown	Light brown	Green
5	1% KOH	Dark purple	Light green	Dark brown	Yellowish white	Chocolate brown	Light green	Yellowish brown	Green	Dark brown
6	Powder as such	Dark brown	Light purple	Black	Light green	Brown	White	Light yellow	Chocolate brown	White

Table 3: Physio-chemical analysis of powdered plant samples.

S. No.	Plant Name	Loss of weight on drying (w/w)	Total ash value (w/w)	Water insoluble ash (w/w)	Acid insoluble ash (w/w)	Sulphated ash (w/w)
1	*Alternanthera sessilis*	41.24%	19.41%	2.31%	1.74%	2.13%
2	*Cyperus difformis*	21.3%	21.2%	11.3%	3.9%	5.3%
3	Juncellus pygmaeus	37.2%	18.3%	21.1%	1.9%	3.1%
4	*Cyperus conglomeratus*	31.25%	24.82%	4.32%	2.05%	2.37%
5	*Paspalidium flavidum*	37.1%	27.3%	21.3%	2.1%	2.1%
6	*Eclipta alba*	47.2%	22.4%	21.4%	5.1%	2.1%

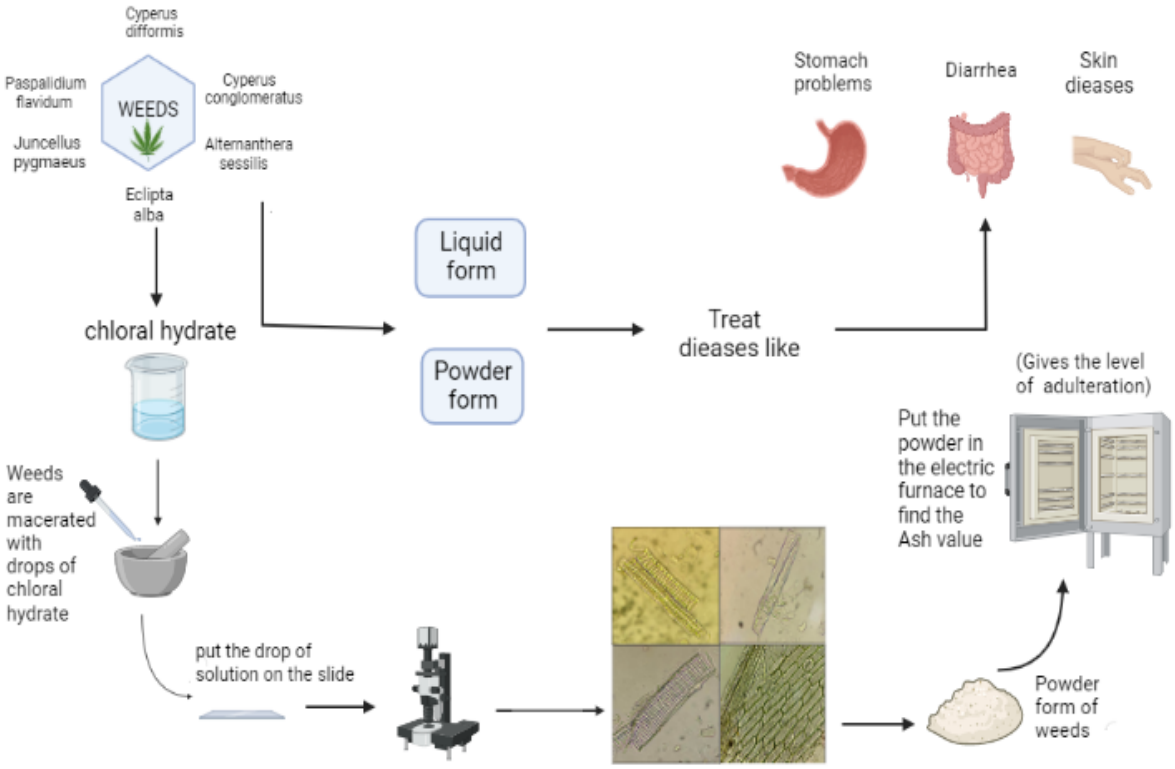

Figure 3: Standardization holds immense significance in the pharmacognostic evaluation of a crude drug. Pharmacognostic studies plays a vital role in both authenticating and identifying plant materials. These studies also function as a tool to uncover adulterants and alternatives, aiding in the maintenance of therapeutic efficacy.

4. Discussion:

The international trade of global economy of herbal products has been annually increasing up to 15%. The south and south east Asian countries provide most of the need of raw material for herbal products. The 8000 species are harvested from wild for medicines from which 960 are in the active trade. Due to increase in demand of herbal products there is increasing concern about the admixtures of species and adulteration. The adverse effects of herbal medicines caused due to adulterations has been recognizes and documented lately which provide the review of magnitude and nature of adulteration. These articles also listed the biological and chemical equivalence of species that can be used as substitutes and adulterants and their consequences on consumers health. In order to avoid the adulteration, the microscopy to authenticate the herbal products are much needed practice (Srirama *et al*., 2017).

Cyperaceae is also called as sedge family which is monocot having graminoid flowering. They resemble grasses only distinguishable character is their triangular cross section and the spirally arranged leaves. *Cyperaceae* is used as medicines around the globe (Jyoti *et al*., 2018).

Present study concerned with the standardization of six weeds present in the rice field i.e., *Cyperus conglomeratus, Cyperus difformis*, *Paspalidium flavidum, Juncellus pygmaeus, Alternanthera sessilis* and *Eclipta alba* are used as a medicine in terms of fever, cancer, diabetes, respiratory and inflammatory diseases. The aim of this study was to investigate anatomical features of these species through the powder microscopy to enhance their use in the pharmacognosy. The study involves the powder microscopy, florescence analysis and Ash value. The samples for this study were collected from Kasur.

Powder microscopy of leaf, stem and root of *Eclipta alba* shows the presence of Anisocytic stomata, unicellular trichomes, helical thickened vessels, cortical cells and epidermal cells with longitudinal and cylindrical shape. Similar structured are also reported by (Prasad *et al*., 2012). Powder microscopy of leaf, stem and root of *Alternanthera sessilis* show the Prescence of unicellular trichome, helical thickened vessel, paracytic stomata, starch crystals and clusters of fibres. Such structures are also reported by (Kumar

et al., 2015). Powder microscopy of leaf, stem and root of *Cyperus conglomeratus* show the presence of Cortical cells, fibres, Bundle of vessels and tracheid, Starch crystal with scalariform vessels, pith cells and phloem fibre. Such structures are also reported by (Jyoti *et al*., 2018). But his study shows the presence of gramineous stomata and no such structure seen here.

Powder microscopy of leaf, stem and root of *Cyperus difformis* show the presence of Stomata present with subsidiary cell, Phloem fibre, helical vessels, cortical cells, thickening of reticulate vessels, unicellular and multicelluar trichome and elongated unicellular fibre. Powder microscopy of leaf, stem and root of *Paspalidium flavidum* show the presence of phloem fibre, group of cortical cells, filiform fibre and stomata in epidermal cells. Powder microscopy of leaf, stem and root of *Juncellus pygmaeus* show the stomata present in epidermal cells, group of cortical cells, Scalariform thickening in vessels, leaf trace, reticulate vessels, fibres and epidermis cells with longitudinal shapes. There has not been any work on these species yet.

Florescent analysis of all the plant powders were carried out according to the methods of (Semwel *et al*., 2013). The reagent used for the florescence analysis were HCl and NaOH, H_2SO_4, 50%HNO_3 and 1%KOH. Florescence analysis of powder of all the plant sample were performed by using different chemical reagent which showed different coloration under UV light and Visible light. The florescence analysis of six species is shown in Table (3.1 and 3.2). The fluorescence analysis exhibits varying colors under distinct parameters, when subjected to UV light at wavelengths of 254nm and 360nm.

The physicochemical attributes of the powdered medication were assessed, encompassing ash values such as acid insoluble ash, water insoluble ash, and water-soluble ash. Additionally, the extractive values for both water-soluble and alcohol-soluble compounds were determined. Upon undergoing the drying process, the medication displayed weight loss, as outlined in (Table 3.3). The parameters of the ash value provide insight into the percentage of impurities within a plant sample. Impurities can alternatively be assessed by comparing the plant sample to an existing reference sample of the same plant.

(Fig. 3) depicts the transformation of weeds into powder using a pestle and mortar along with a choralhydrate solution. The solution drops are placed onto a slide and covered with a cover slip before observing the slide under a microscope. In determining the ash value, the powder undergoes ignition in an electric furnace until a consistent ash weight is attained. Furthermore, these weeds find application in the treatment of various diseases, such as stomach issues, diarrhea, and skin diseases, either in powder or solution form.

5. Conclusion:

The importance of anatomical studies is increasing steadily as they are crucial for understanding the qualitative and quantitative medicinal properties of plant species. The microscopy is helpful in the revealing of different structures such as types of stomata, types of trichomes, different types of vessels like helical vessels, scalariform vessels and reticulate vessels and other characteristic features for the elucidation of the medicinal plants. The physiochemical methods Viz. ash value, sulphated ash value and water-soluble ash value are helpful to find out the amount of adulteration and impurities present in the plant. The parameters of the ash value provide insight into the percentage of impurities within a plant sample. Impurities can alternatively be assessed by comparing the plant sample to an existing reference sample of the same plant. From above study we can suggest that the above used methods are very significant and useful for the correct identification and determination of authenticity and purity of medicinal plants in the pharmaceutical companies. The anatomical research will also help to manage weed control practices.

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