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Microscopic Determination of Adulterations Through Powder Microscopy in Medicinal Plants Kasni and Anjbar (*Cichorium intybus* L. and *Polygonum aviculare* L.) Collected from the Local Market of Okara City

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Abstract

Herbal plants are essential foundation of medicine and without them there is no existence of life on Earth. In the present research work the powder of two medicinal plants *Cichorium intybus* L. (common name: kasni) and *Polygonum aviculare* L. (common name: anjbar) were analyzed under microscope to set the quality standards for adulteration in medicinal plants. These plants were collected from market of Okara. Besides microscopic characters pharmacognostic, physico-chemical and proximate analysis were also found out. Organoleptic study reveals the presence of Anomocytic stomata, helical thickenings, trichome, multicellular fiber, epidermal cells and cortical cells in *Cichorium intybus* L. plant. While in *Polygonum aviculare* L. scalariform thickening, lignified fiber and multicellular trichomes were observed. The proximate analysis of both plants revealed high percentage of dry matter (95.54% in *cichorium intybus*) which highlights its nutritional value. Fiber content was also high 37% in *cichorium intybus*) quality and can be used to treat constipation and bowl movements. Values of nitrogen free extracts are noticeable (59.58% in *polygonum aviculare*) indicating the presence of starch which is important source of energy for humans. Fluorescent analysis of these plants displays different colors at different wavelengths due to different magnitude of photon emitted in powder of sample. Ash values of both plants *cichorium intybus* 7.4% and *polygonum aviculare* 6.6% are also in safe range representing low contamination in crude samples. This study is a benchmark and provides baseline data to compare the results of the collected plant species to confirm and verify on the basis of their anatomical features.

Keywords: Standardization; Authentication; Pharmacognostic Analysis; Herbal Plants

1. Introduction:

The present research will aid to resolve two botanical confusions between two medicinal plants Kasni and Anjbar on the bar of phytochemical and anatomical markers. This study will also prevent two herbal adulterations and authenticate commercial quantification Kasni and Anjbar.

The research pinpoint objectives were to check nutritional importance by Proximate analysis first, then to determine the purity and authentication of drug plant by florescence analysis and ash value determination. Also, it included the powder microscopic study of drug plants by chloral hydrate.

Plants are natural living chemical laboratories. Mankind is utilizing plants for medicinal purpose i.e. for the treatment and prevention of diseases. About 10,000 to 12,000 plant species are discovered for their

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medicinal value. Herbs, shoots, roots, seeds, fruit, flower are the raw materials that are used medicinally (Yusupova *et al*., 2023). From mankind development to the advancement, some medicinal plants with their curative properties were recognized, noted, and conveyed to the descendants. The ancient written data of medicinal plant was found approximately 5000 years from Naagpur. Numerous spice plants, aromatic plants, medicinal plants were discovered in early 20th century. Arabs, Asians, Americans, Chinese, Persians unlocked a new world with discovery of medicinal plants (Petrovska, 2012).

Cichorium intybus L. (Kashmiri name: Kasni) is an herb and the member of Asteraceae family. Every part of this plant has medicinal important. (Jangra *et al*., 2018). Asteraceae family has worldwide distribution. About 33,000 plant species are of this family. Their renowned taxa (including lettuce, chicory, dandelions and daisy) are used as food and medicine for centuries. (Sharma *et al*., 2022). The name of the *Cichorium intybus L.* (common name: Chicory) is derived from Greek and Latin words. *Cichorium* is Latin word meaning "field", while *intybus* means "to cut" or "tube" which refers to structure of leaves and stem. In old era Rome, Greece and Egypt used this plant for enhancing metabolism and digestion. The plant was used as a vegetable as well as a paddock plant. This plant is used by Avicenna and Unani system of medicine. In Europe, India, South Africa chicory is used for insulin extraction and as substitute of coffee manufactured from the plant's taproot. Moreover, many secondary metabolites present in this plant indicated health importance. This plant has also been used as culinary in soups, syrups, food coloration, beer, meet dishes, as spice, vegetable and many other applications (Janda *et al*., 2021).

Polygonum aviculare L. (Pakistani name: Anjbar) known as "knotgrass weed" in English and it is a member of polygonaceae family. Genus Polygonum has 300 species distributed world widely, mostly in temperate zone. This plant has been traditionally used as medicine against respiratory disorders, skin diseases and women's disorders. It was used in form of tonic as diuretic, trenchant, constringent and, hypotensive. It is traditionally used in food as salad in Korea. In Prehistoric times Native Americans used the seeds used as source of food due to their emetic and therapeutic effect. In the Middle Ages in Poland *P. aviculare* used in flour. The plant has a strong soothing effect when applied to the outside wounds. The diluted juice of this plant can reduce inflammation (Benrahou *et al.,* 2023). Numerous studies have confirmed the Anti-inflammatory, Antimicrobial and Antifungal effects of plants (Muresan *et al*., 2021).

Commercially prepared herbal drugs arise the issue of impurities. Owing to the fact, addition of non-medicinal plants in the form of adulterants to the medicinal drug slower their action, thus it urges to take actions for authentic raw material, drug standardization and quality control parameters (Kamboj, 2012). Unfortunately, the natural drugs have become the victim of adulteration. In this method foreign substances are added on purpose to increase the weight or strength of the product by decreasing its cost. Chicory is used as adulterant in coffee which causes stomach disorder, giddines and joint pain (Awasthi *et al.,* 2014). Mostly root of chicory is used as adulterant in coffee which is rich in alkaloids. (Shaikh *et al.,* 2010) Seeds of chicory has been used for a long time in ayurvedic medicine and used as adulterant in coffee that is healthy and reduce gastrointestinal problems (Rahman *et al*., 2016). Adulterations in polygonaceae members like *fallopia multiflora* has been seen. There is the shortage of medicinally important plants. Hence the plants like *Polygonum aviculare* are adulterated with *Polygonum aubertii, Pteroxygonum giraldii*, and *Polygonum ciliinerve* which resembles with *fallopia multiflora* (Liu *et al*., 2011). *Rheum palmatum* is cathartic drug plant belongs to polygonaceae family is adulterated with less lively variabilities of *Rheum* plants species like *Rheum rhaponticum* (Mohiuddin & Katti, 1933).

Pharmacognostical study includes macroscopic study, microscopic study, powder study, phytochemical analysis, physico chemical analysis (%moisture content, loss on drying, extractive values), fluorescence analysis, ash values, and organoleptic characters of plants. Adulteration and substitution can be prevented by pharmacognostic study as it set parameters for standardization and authentication of medicinal plants (Chanda, 2014).

1.1. Objectives:

The main objectives were:

- To check nutritional importance by Proximate analysis.
- To determine the purity and authentication of drug plant by florescence analysis and ash value determination.
- To study the powder microscopy of drug plant by chloral hydrate.

2. Materials and Methods:

2.1. Collection of Plant Samples:

Plants were collected from a store in Okara. The powder of these plants was made by herbalist. He made fine powder with mastery. 300-gram powder was taken in polythene bags and stored in our research laboratory in GCU.

2.2. Preparation of Reagents:

For powder study concentrated Chloral hydrate was prepared i.e. 50 gram in 20 ml distilled water. For fluorescence and physicochemical analysis solutions like 1N NaOH (40 g in distilled water and volume make up to 1000 ml), 1N HCL (36.5ml in distilled water and volume make up to 1000ml), 50% H_2SO_4 (50ml in distilled water and volume making up to 100 ml) and 50% HNO_3 (50 ml in distilled water and volume makeup to 100 ml).

2.3. Microscopic Evaluation:

The internal structures of plants like vessels, tracheids, cells and fibers are revealed in leaves of *Cichorium intybus* and root of the *Polygonum aviculare* with the help of powder microscopy, proximate, fluorescence and physico-chemical analysis.

2.4. Powder Microscopy:

The macerated powder was taken into pestle and mortar and mixed with few drops of chloral hydrate until a translucent and homogenous solution is made. From this solution we took a drop and placed it on glass slide. We covered it with cover slips and observed it under Light Microscope with different powers 10x, 20x, 30x and 40x. The anatomical structures become prominent in labomed microscope under 40x power

2.5. Fluorescent Analysis:

Dried powder of about 1gram was treated with different chemical reagent like 1Normal NaOH, 1Normal HCL, 50 percent H_2SO_4, 50 percent HNO_3 as well as with distilled water, then allowed the moisture to settle down at room temperature by using whatmann filter paper #1. Filtered the mixture. This mixture was visualized under ordinary light, short UV light (253 nm) and long UV light (366 nm) under UV chamber, obtained different colors. Some constituents show fluorescence in daylight. To check all necessary constituents, we treated solutions with different wavelengths for their luminance. (Chanda, 2014).

2.6. Proximate Analysis:

The proximate analysis (Dry matter, moisture, carbohydrate, fat, ash and nitrogen free extract) was performed from University of Veterinary and Animal Sciences. Proximate analysis shows the presence of biochemical and other organic matter. The moisture and carbohydrate value were determined by weight difference method. Nitrogen free extract values were determined by kjeldahl method (Hussain *et al*., 2009). The ash content was determined by burning 1 gram of oven-dried sample in a crucible in a muffle furnace at 550°C for 24 h (Khalil *et al*., 2012).

2.4. Physico-chemical Analysis:

Physico-chemical analysis like Total ash, Sulphuric soluble ash, Water soluble ash and Acid insoluble ash were determined to check impurities present in the sample. Protocols matched with Indian Pharmacopoeia (Kumar *et al.*, 2013).

3. Results:

3.1. Powder Microscopy:

When plant powder mixed with chloral hydrate was seen under 40x power of light microscope. Following Anatomical structures were seen.

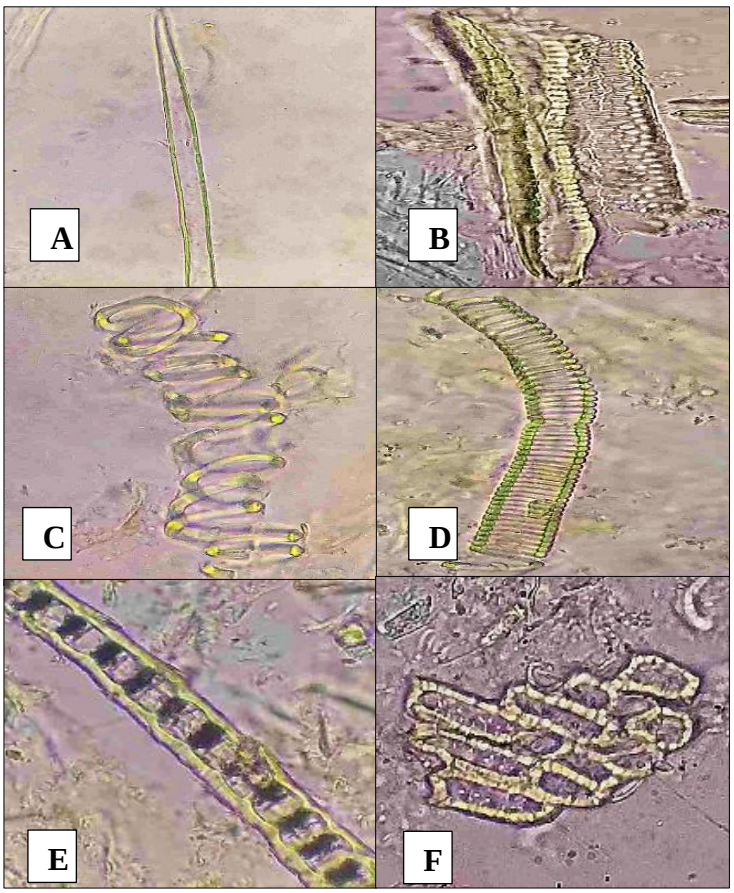

Figure 1: *Cichorium intybus* L. Leaves; A. Trichome. B. Scalariform thickening. C. Spiral thickening. D. Spiral thickening. E. Fiber. F. Thick wall fiber (40x)

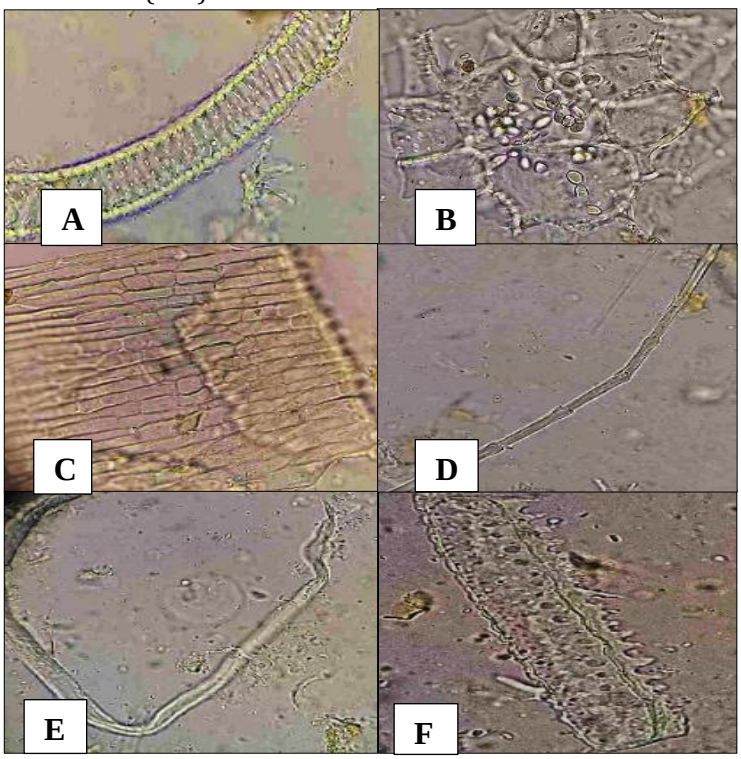

Figure 2: *Polygonum aviculare L.* Root; A. Scalariform thickening. B. Cortical cells showing starch. C. Epidermal cells. D. Fiber. E. Fiber F. Lignified perforated vessel (40x)

Table 1: Proximate analysis of powders of *Cichorium intybus* L. and *Polygonum aviculare* L

	Type of test	***Cichorium intybus* L. %**	***Polygonum aviculare* L. %**
1	Dry Matter (g/10 g)	95.54 ± 1.66	94.48 ± 1.4
2	Moisture (g/10 g)	4.93 ± 1.01	5.65 ± 1.20
3	Crude Protein (g/10 g)	11.14 ± 1.70	7.46 ± 1.17
4	Crude Fiber (g/10 g)	37.53 ± 1.66	19.63 ± 1.21
5	Fat (g/10 g)	5.3 ± 1.13	6.35 ± 1.1
6	Ash (g/10 g)	7.4 ± 1.50	6.6 ± 1.01
7	Nitrogen Free Extract (g/10g)	38.19 ± 0.98	59.58 ± 1.51

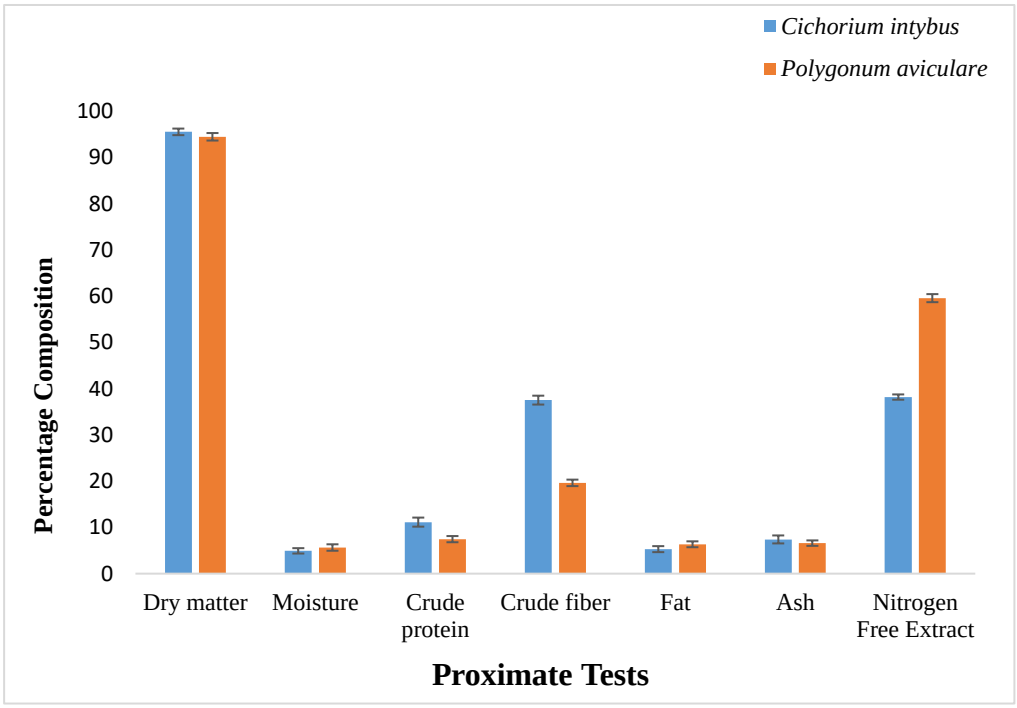

Figure 3: Graph showing comparative proximate composition of *Cichorium intybus* and *Polygonum aviculare*.

The standard error in test of *Cichorium intybus* % dry matter is 95.54± 0.71, in % moisture 4.93± 0.58, in % crude protein 11.14± 0.98, in % crude fiber 37.53± 0.96, in % fat 5.3± 0.65, in % ash value 7.4±0.86 and in nitrogen free extract it is 38.19± 0.57. The standard error in test of *Polygonum aviculare* L.: % dry matter is 94.48± 0.81, in % moisture 5.65±0.70, in % crude protein 7.46±0.67, in % crude fiber 19.63±0.70, in % fat 6.35±0.63, in % ash value 6.6± 0.59 and in nitrogen free extract 59.58±0.87.

In *Cichorium intybus* the % dry matter was 95.54± 0.71, % moisture 4.93± 0.58, % crude protein 11.14± 0.98, % crude fiber 37.53± 0.96, % fat 5.3± 0.65, in % ash value 7.4±0.86 and nitrogen free extract it was 38.19± 0.57. In *Polygonum aviculare* L.% dry matter was 94.48± 0.81, % moisture 5.65±0.70, % crude protein 7.46±0.67, % crude fiber 19.63±0.70, % fat 6.35±0.63, % ash value 6.6± 0.59 and nitrogen free extract 59.58±0.87.

Table 2: Fluorescent Analysis of powder of *Cichorium intybus* and *Polygonum aviculare* under visible and UV light.

	Cichorium intybus			***Polygonum aviculare***		
Solvent used	**Day light**	**254nm (UV light)**	**360nm (UV light)**	**Day light**	**254nm (UV light)**	**360nm (UV light)**
1	Powder +1N NaOH (Aqueous)	Pale brown	Light yellow	Light green	Pale yellow	Light brown	Green
2	Powder + 1N HCL	Reddish brown	Light brown	Green	Dark brown	Reddish brown	Green
3	Powder + 50% H_2SO_4	Brown	Light brown	Green	Brown	Light brown	Green
4	Powder + 50% HNO_3	Reddish green	Red	Brown	Reddish orange	Red	Dark brown
5	1% KOH	Yellowish brown	Brown	Green	Yellowish brown	Chocolate brown	Light green
6	Powder as such	Light green	Brown	White	Light green	Brown	White

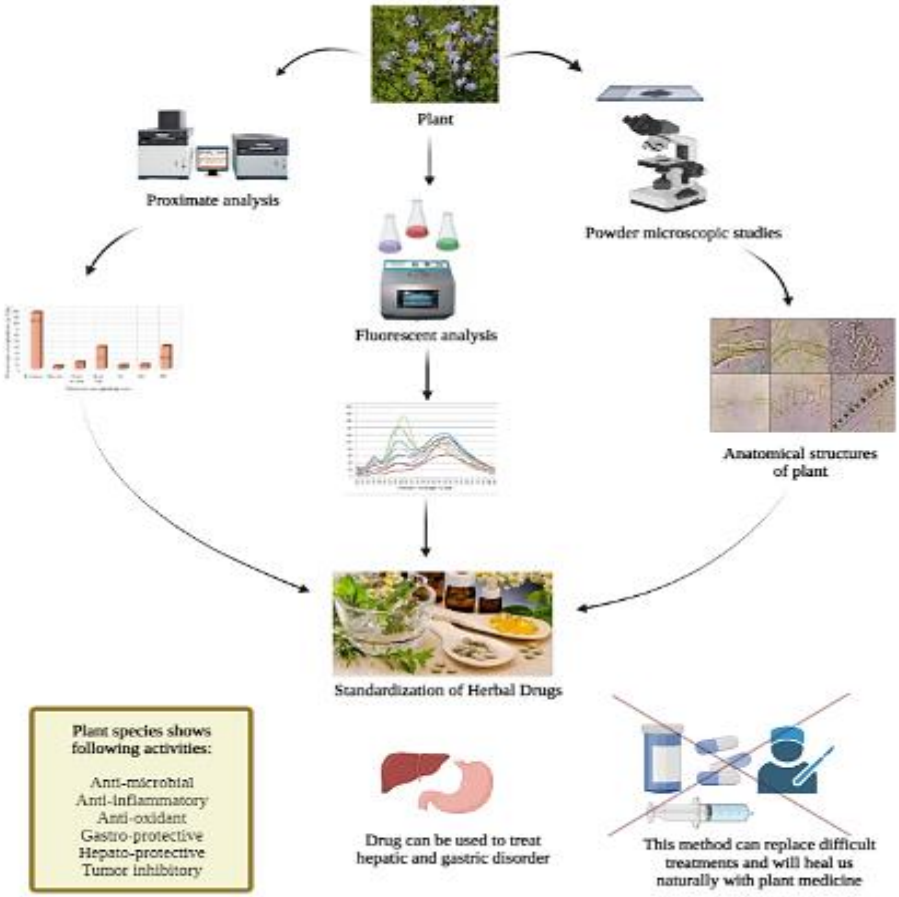

Figure 4: Outlines showing techniques used in research work for determination and standardization of adulteration

Table 3: Physico-chemical analysis of powdered samples.

Serial No.	Plant Name	Loss of weight on drying	Total ash value	Water insoluble ash	Acid insoluble ash	Sulphated ash
1	*Cichorium intybus* L.	33.85%w/w	19.82%w/w	4.56%w/w	3.05%w/w	2.47%w/w
2	*Polygonum aviculare* L.	36.4%w/w	49.3%w/w	25.4%w/w	5.5%w/w	7.2%w/w

4. Discussion:

Mankind has been closely coordinated to its natural environment. Since the beginning, man has found everything from nature like food, medicine, clothing etc. Medicinal plants were used as disinfectant, aromatic products, pharmaceutical and cosmetic products. Different part of plant has different active compounds but mostly used in medicines for synergistic actions (Jamshidi-Kia *et al.*, 2018). Herbal drugs prepared should be wholly free from molds, insect, excreta, sand, stones, poisonous and harmful foreign matter and chemical residues. Insects and invisible matters are microbial impurities, which can produce venoms and possible toxins (Naifu *et al*., 2017).

Herbal drugs are being really popular across the globe due to its effectiveness and people consider green medicine safe. Large proportion of the world is dependent on them so, that increase in trade leads to adulteration of these herbal products (Ekor, 2014). Current extraordinary growth in medicinal plants require methods for standardization to evaluate the safety, efficacy and quality of these medics. There are several physical and chemical parameters given by WHO for standardization of medicinal plants (Nafiu *et al*., 2017). By sticking to guidelines, we selected some of their techniques standardize our crude powder.

Anatomical structures i.e. Trichome, Scalariform and spiral thickenings, thick wall fibers, cortical cells, fiber, epidermal cells, stone cell, Anomocytic stomata and vessels, perforated vessels, lignified tracheid, and Shizogenous cell have been seen in the powder of *Cichorium intybus*. It provides data for forensic identification of plants species. Hence, valid identity of plants prevents us from selecting non-medicinal plants. Moreover, the characters like fibers that are medically important were seen in much more quantity in microscope. So, it added more reasons to use anatomical characterization or drug identification. All these structures were seen in leaves powder under 40x power of microscope. The leaves are petiolate, pinnate, reticulate, glabrous, exstipulate and dentate having obtuse apex with distinct midrib. The shape of the leaves is spathulate. Morphological characterization clarifies the taxonomic confusion between two closely related species. All these anatomical and physical characters were reported by (Eltaher *et al.*, 2023) during their characterization of *Cichorium* taxa by using morphological traits. Similarly, the powder of *Polygonum aviculare* showed scalariform thickenings, cortical cells with starch, collenchyma cells, epidermal cells, lignified perforated vessels, scalariform and reticulate vessels, and multicellular trichome. The same structures of root were observed by (Liang *et al.*, 2014) during their quality assessment of *Polygonum multiform* plant. Their powder contains starch granules, vessels, calcium oxalate crystals when treated with chloral hydrate. As a result, anatomy of plants is effective for the factual proof of presence and foresee of potential drug constituents

Proximate analyses were performed to analyze drug quality. If the values of nutritional content in a sample are up to the mark i.e. in the normal ranges, then plant can be used as medicine. To elaborate this, proximate analysis was perfomed and *Cichorium intybus* provides us information about dry matter, moisture content, crude protein, crude fiber, ash values, fixed carbon, volatile matter, nitrogen free extract and mineral content (Vadiya *et al*., 2017). Our results show Dry matter 95.54%, moisture 4.93%, crude protein 11.14%, crude fiber 37.53%, fat 5.3% and ash 7.4%. Similar results were reported by (Essiett & Akpan, 2013) during proximate analysis of two members of asteraceae. According to (Owoyele *et al.*, 2004) unsaturated fatty acids have beneficial health role. High moisture can't form drugs. Normal range was 20% by (Ibrahim *et al.,* 2010). Total ash was 7.4% which is low than accepted range of ash value by British pharmacopeia is 22% indicating low inorganic matter. So, our sample indicated the values within safe ranges. The proximate analysis of *Polygonum aviculare* show that content of dry matter was very high 94.45% which is applicable as food source. Moisture content was 5.55%. Crude protein content was 7.70%. Crude fiber was 19.56% which is good enough to take it as dietary supplement. Fat content was low 6.25%. Ash

values were also low 6.80% indicating its purity. Nitrogen free extract has 58.76% which highlights the presence of starch. Same results were reported by (Hameed *et al*., 2008) in which the stem of *Persicaria maculosa* has ash content 12%, crude protein 7.7%, crude fiber 13.75% and moisture 5.4%.

The drug powder was treated with various solutions like 1N NaOH, 1N HCL, 50% H_2SO_4, 50% HNO_3, 1% KOH by using methanol or water as solvent in order to perform fluorescence analysis to check the constituents of crude drug. It is the most important technique is analyzing pharmaceutical drugs (Siddiqui *et al.*, 2017). Our results collaborate with work of (Ritesh & Gopalkrishnan, 2023) who checked the fluorescence of an asteraceae member at 254nm and 365nm. Different colors in sample arises by the excitation of photons at different wavelengths. Since wavelengths are co-related with energy present in the sample then, it determines antioxidant activity of plant. The plant shows yellow color in visible light, water green color at 254 nm and green color at 365 nm when treated with 1N HCL solution. Table 4.2 illustrated the fluorescent behavior of *Cichorium intybus* plant. The plant shows light brown color at 254 nm and green color at 365 nm. Similarly, the fluorescent analysis of *Polygonum aviculare* showed various colors with solvents. Powder shows light green color in daylight, brown color in 254 nm UV light and white color in 360 nm light. This work collaborates with work of (Deshpande & Joshi, 2016) who acquired the same results in polygonaceae family member *Antigonon leptotus* when treated with 1N HCL showed dark brown color at 254 nm and brownish yellow at 366 nm. Such difference in colors indicate different kind of energies present in sample and we can analyze enzymatic activity with them (Zhu & Hamachi, 2020). Hence, the plants with high antioxidant activity are the best plant to be utilized as drug.

Performing physiochemical analysis indicating the the presence of foreign matter like carbonate, silicate and oxalate. The high value of ash indicates the presence of substitution and negligence in drug preparation. About 6% to 9% ash value is considered in safe zone with less contamination (Aziz *et al.*, 2019). The sample of *Cichorium intybus* indicates the ash value of 19.82%. The Water insoluble ash is 4.56% w/w. The Acid insoluble ash was 3.05% w/w. The Sulphated ash value is 2.47%. This work collaborates with (Momin & Kadam, 2011) who found same ash values in *sesbania grandiflora* whose ash values are 7.5% and water insoluble ash was 4.2%. The value of Total ash in *Polygonum aviculare* was 49.3% w/w. Water insoluble ash was 25.4%. Acid insoluble ash was 5.5% w/w. Sulphated ash was 7.2% w/w. Similar results were given by (Vermani *et al*., 2010) performing physico-chemical analysis in uttarakhand area plants. *Cedrela Toona* has % acid insoluble ash 5.59% and water insoluble ash was 76%. Sulphated ash in sample reports substances which are not volatized. Sulphated ash in our sample was 7.2% w/w and similar results were reported by (Nesanthine & Manoharam, 2020) during their physico-chemical analysis of a medicine *Maruthampattai chooranam.*

5. Conclusion:

Plant anatomical studies have been playing a vital role in describing and identifying qualities of medicinal plants, recently. In our work the powder of plants revealed different anatomical structures like stomata, trichome, epidermal and cortical cells. The powder studies play a significant role in revealing structures, cellular features and botanical origin of plants. Moreover, powder studies visualize the presence of adulterants in the plant. Our aim was to determine adulteration in crude sample which was fulfilled with help of microscopy and some physico-chemical analysis. Results confirmed the absence or very minute quantity of adulterants in plant powder. As each plant has its own unique structure so, one can confirm their specimen with these plant structures for anatomical identification. This research work will be helpful to other researchers for comparison of same plant species. Moreover, they can use the same standardization techniques to their plants in order to find adulterant in plants. These standardization techniques are cost effective, reliable, quick and convenient.

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