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Microscopic Characterization and Analysis of Ayurvedic Herbal Products Using Light Microscopy

Research Article

Pratima Dutta[1], Bhaskar Chaurasia[2*]

1. Reseach Scholar, 2. Faculty, Department of Rural Technology and Social Development, Guru Ghasidas Vishwavidyalaya (A Central University), Koni-495009, Bilaspur C.G., India.

Abstract

Objective: Today sophisticated modern research tools for assessment of the plant drugs are achievable but microscopic method is still one of the ingenious and procurable methods to start for substantiate the correct recognition of the source material. Powder microscopy helps to find out the impurities and also helps in quality assessment of the drug. To standardize and evaluate the readymade as well as homemade herbal powder and thus provides means for assessing the authenticity and morality of herbal drugs. Materials and Methods: Microscopic detailed examination of herbal plant parts such as *G. glabra*, *P. emblica*, *P. nigrum*, *P. longum* was established with different reagents such as acetic acid, iodine, sulphuric acid and hydrochloric acid followed by observation of slide under LEICA Software Capture and Display Software and photographs of each slide was captured for evaluation of the drug. Observation and Results: Microscopic assessment of the homemade and readymade herbal parts of *G.glabra* and *P.emblica* shows original cellular structures while unidentified cellular structure were observed in readymade powder of *P. longum* which perhaps the growth of fungal mycelium and leaf part were observed in *P. longum*, thus Microscopy method permits more detailed examination of a drug and it can be used to identify the organized drugs by their known histological characters. Conclusion: Powder microscopic evaluation of herbal powder is one of the simplest and authenticated methods for the proper identification of the drug. It helps in purity assessment of the readymade herbal powder. Microscopic study and physiochemical standards can be useful to substantiate and authenticate the drug.

Key Words: *Glycyrrhiza glabra, Phyllanthus emblica, Piper nigrum, Piper longum,* Powder micrscopy, Herbal products.

Introduction

Herbal drugs have significant role in the fields of pharmaceutical preparation and manufacture controls in order to moderate the quality and quantity of the herbal products. They are believed to restrain rich source of bioactive compounds which are essential to cure various human ailments. A Pharmacognostical study of *G.glabra* was established for the standardization of its stem (1). The microscopic tests, physiochemical evaluation, thin layer chromatography, spectrophotometric assays and fourier transform infrared spectroscopy techniques are the helpful parameters for quality assessment (2). Emblicanin is a bioactive compound of *P.emblica* rich in antioxidants, its powder form revealed all the characters of the fruit part. The presence of ascorbic acid, tannins and gallic acid suppress the oxidative stress leading atherosclerosis (3). Pharmacognostical standards of *E.officinalis* showed the impendence of lignified tissues, aleurone grains and prismatic crystals. Parameters such as macroscopy, cytomorphology, physical constants and phytochemical study helps in quality demarcation of *P. nigrum* under pharmacopoeial guidelines (4). The botanical standard of *P. longum* was mentioned in the previous description which shows its actual morphological characters before and after maturation of the fruit of *P. longum* along with other parameters such as extractive value, ash value, macro and microscopic characters, physiochemical tests, moisture percent, fluorescence assessment, and phytochemical determination (12). Some evidential investigations reported the interchangeable plant parts with the original root of *G. glabra* with *A. precatorius*. Thus it is challangable to aquire the correct botanical identity in order to differentiate the physiochemical character of the plant (9). Amla or Indian gooseberry is a green, pulpy, juicy fruit perform various countless healing effects. Moreover the multiple products of amla such as amla juice, hair oil, murabba, triphala, chyawanprash enlisted healing medication against several health illness (14). Quantitative analytical microscopy and macromorphology is convenient and inexpensive methods to establish immaculate identity of the source materials. However, some studies challenges in quality control laboratory. These are expensive, time dissipating and require skilled human to operate

*** Corresponding Author:**

Bhaskar Chaurasia

Faculty, Department of Rural Technology and Social Development, Guru Ghasidas Vishwavidyalaya (A Central University), Koni-495009,
Bilaspur C.G., India.
Email Id: b.ch.myco@gmail.com

sophisticated instruments. Different analytical procedures are useful to for the examination of herbal contaminants (5,6). Ayurveda manifest reliable source of treatment alternative to allopathy as it treats the actual root cause, drug dependency, encourage self-healing, rejuvenation therapy, purification of toxicity, musculoskeletal treatment (31) and many more. Ayurveda system balanced the equilibrium and shows beneficial functions in modern lifestyle so it is indispensable to establish authenticated and substantiated herbal drugs. Herbal plants not only present in medications but is also added in diet supplements, cosmetics and spices. Consumption of herbal products contribute to the accomplishment but scientific disquisition reported that synthetic substances augmented in herbal diets cause toxic-effects to health (32). Studies proved that plant based products are comparatively safer, eco-friendly and free from side effects. Current status shows that 70% of 1.1 billion population depending on traditional medicine (7). Ayurvedic and herbal medication dependency was found in rural population to about 65%. WHO promotes and encourage the state members for documentation and to retain the traditional understandings of plants thereby assembles the knowledge of herbal plants accurately (30). The purpose of WHO in promoting traditional drugs is to advanced the well-being, potency and standard of the drug, to access and deliberate utilization of traditional medicine. The original herbal drugs have more potential to cure the genuine action of disease from root as compared the allopathy system. Insufficient supply of drugs, prohibitive cost of treatments, side-effects of diverse synthetic drugs and resistance to currently used drugs for various ailments have led to increase predominance on the utility of plant based products as a origin of medicines for the treatment of various illness (8). Advancement in the field of microscopy is beneficial in the field of biotechnology, biosciences, biochemistry, zoological sciences and pharmaceutical laboratories helps to study internal structure, composition, constitution and inclusions of plant and animal cells or other objects in detail. Size, shape, structure, dimensions, proportionate position of different cells and tissues, as well as the biochemical composition of the cell wall and the form and nature of the cell components are presumed during microscopic investigation of crude drugs. Microscopy helps to observe the internal structure, constitution, and inclusions of plant and animal cells and it serve to detect the adultrants and contaminants of the herbal preparations for assessing the authenticity and quality of herbal drugs.

Materials and Method

This study shows various cellular structures of plant parts by different chemical reagents such as HCl, CH_3COOH, H_2SO_4, I etc. Presently consciousness is being incrementally emphasized towards development of quality and efficacy of herbal products. In present work microscopic evaluation have been established with regards to *G. glabra, P. emblica, P. nigrum,* and *P. longum* each of them have their own pharmacological value in terms of various ailments. Size, shape, relative position of different cells and tissues as well as the chemical nature of the cell walls are considered during microscopic analysis of crude drugs.

Collection of Plant Material

The herbal plant products was considered after conducting the survey in various herbal shops. Thereafter observation of different brands with its consumption rate we purchased the specific 4 herbal plants. Furthermore, commercial plant products along with its specific plant parts were procured from the local herbal shops in the Bilaspur district of C.G.

1. Licorice powder of 'Basic Ayurveda' (Fig 1. C)
2. Amla powder of 'Swadeshi' (Fig 2. C)
3. Black pepper powder of '3 star' (Fig 3. C)
4. Long pepper powder of 'Divya' (Fig 4. C)

Figure 1 (A) Plant licorice. (B) Dry Stem part of licorice. (C) Powder of licorice. (D) Basic Ayurveda brand of licorice.

Figure 2 (A) Plant of Amla. (B) Dry fruit part of amla.(C) Powder of amla fruit. (D) Swadeshi brand of amla.

Figure 3 (A) Plant of Black pepper. (B) Dry fruit part of Black pepper. (C) Powder of black pepper. (D) 3, Star brand of Black pepper.

Figure 4 (A) Plant of long pepper. (B) Dry fruit part of long pepper. (C) Powder of long pepper. (D) Divya Brand of long pepper.

Figure 5 (A) border pitted xylem vessels (B) Parenchymatous cell (C) Raphides (D) Parenchymatous fibre. Observed in the homemade and readymade powder of *Glycyrrhiza glabra*

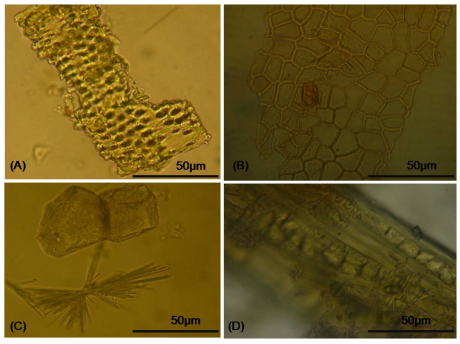
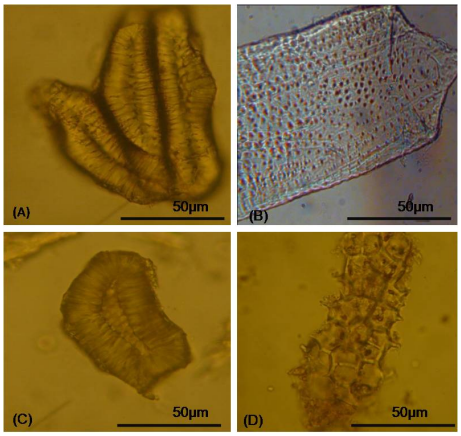


Figure 6 (A) Grouped stone cells (B) Pitted Scalar vessels (C) Single stone cell (D) Parenchymatous cell. observed in the homemade and readymade powder of *Phyllanthus emblica* L.



Processing of The Plant Material

The preferred plant parts bought were cleansed and washed in order to eliminate foreign particles externally. It is then dehydrated to about 1.5hr and pulverized to form fine powder with the help of pestle and mortar. The disintegrated powder is then sieved through 80µ sized mesh. This prepared herbal powder along with readymade herbal powder was examined under the light microscope.

Slide Preparation

The prepared and readymade herbal powder was stained with specific reagents named HCl, CH_3COOH, Iodine and H_2SO_4. In order to obtain clear and definite intracellular structures the slides should be thoroughly washed before use. Glass slides after staining with chemicals were observed carefully under the microscope.

Microscope

LEICA Capture and Display Software which gives standard parameters of the powder were used for identification and photography of cellular structures.

Results and Discussion

Microscopy method allows more detailed examination of a drug and it can be used to identify the organized drugs by their known histological characters. In the present study four important and common medicinal plants were selected for powder microscopic studies. The selected plant used in most of the household for various purposes. The results of present investigation will provide basic features and powder microscopic analysis of selected medicinal plant.

Powder microscopy observation of *Glycyrrhiza glabra* L.

Stem: Powder Microscopy of stem powder was carried out following standard guidelines. Stem powder of *G. glabra* treated with various reagents shows various structures of plants tissues in both homemade and readymade herbal powder Structures found in homemade powder of Mulethi were pitted xylem vessels (Fig 5. A), epidermis with parenchymatous cell (Fig 5. B), raphides (Fig 5. C) parenchymatous fibre (Fig 5. D), longitudinal cut mesocarp parenchyma, Tannin, sclerenchymatous cell, compound starch grains. Readymade powder of *G. glabra* shows structures such as parenchymatous fibre, xylem pitted vessels, tannin, compound starch granules, sclerenchymatous cell, trichome, criss-cross fibre. The observed pharmacognostical study may be useful to establish the botanical standards for identification and standardization of stem of *G. glabra*.

Table 1: Powder microscopy of homemade powder of *Glycyrrhiza glabra* L.

Sr. No.	Reagents	Different plant parts observed during study
1	HCl	Cork in surface view, Rhomboidal crystals, criss-cross fibre
2	CH_3COOH	Crystal fibres, starch grains in group, bundle of parenchymatous fibre, pitted mesocarp parenchyma, raphides.
3	Iodine	Border pitted xylem vessels, starch grains in group, bundle of fibres, trichome, fibres
4	H_2SO_4	Crystals, tannin, parenchymatous cell, fibre, resin

Table 2: Powder Microscopy of readymade powder of *Glycyrrhiza glabra*.L.

Sr. No.	Reagents	Different plant part observed during study
1	HCl	Parenchymatous fibre, parenchymatous cell, rhomboidal crystals, bordered pitted vessels, sclerenchymatous cells, reticulate vessels, tannin, tracheid vessels with parenchyma of upper epidermis
2	CH_3COOH	Epidermis with parenchyma cells, parenchymatous fibre, group of starch, longitudinal cut mesocarp parenchyma, bordered pitted vessels, raphides.
3	Iodine	Calcium oxalate crystals, starch in groups
4	H_2SO_4	Crystals, fibres, parenchyma

Investigation results in previous studies on *G. glabra* shows similar structures such as starch grains, crystal fibres, tannin, bordered pitted vessels (1) (Fig 5 A). Some studies on *G. glabra* root reveals almost similar microscopic structures that present in stem portion of this plant such as starch grains, fragments of pitted xylem vessels, crystals, parenchymatous cells (9) (Fig 5 B). In this present work the actual structures of this plant were observed and thus there were no contaminants or other parts obtained during pharmacognostic study. Pharmacology findings confirmed proper identification of the herbal drugs which is essential for the quality control and purity of the drug.

Powder microscopic observation of *Phyllanthus emblica* L.

Fruit: Microscopic observation of fruit part of amla shows different plant parts when stained with different reagents. Observation of homemade powder shows mainly grouped stone cells (Fig 6. A), pitted scalar vessel (Fig 6. B), single stone cell (Fig 6. C), parenchymatous cell (Fig 6. D), raphides, sclerenchymatous cells, resinous cell, starch granules, oil droplets, parenchymatous fibre, reticulate fibre, starch granule, cut mesocarp parenchyma, parenchymatous cell, group of pitted vessels, bundles of stone cells, crystals over parenchyma, fibre.

In readymade powder of *P. emblica* fruit shows structures such as different types of stone cells, parenchymatous fibre, parenchymatous cells, raphides, pitted mesocarp parenchyma, fibre, starch granules, fragments of sclereid fibre, fragment of sclereid fibre, sclereid fibre, tannin cells, sclerenchymatous cells, group of fibres.

Table 3: Powder microscopy of homemade powder of *Phyllanthus emblica* L.

Sr. No.	Reagents	Different plant parts observed during study
1	HCl	Bundles of stone cells, crystal, crystal over parenchyma, group of fibres, group of pitted vessels, oil droplets, parenchyma, parenchymatous fibre, reticulate fibre, trichome, xylem pitted vessels, group of stone cells
2	CH_3COOH	Brachysclereid type stone cell, brown content, cortical parenchyma with starch, criss-cross fibres, elongated stone cells, fibre, packed stone cells, parenchymatous fibre, resin, stone cells with highly lignified thickening and broad lumen, tannin, xylem pitted vessels, trichome, raphides.
3	Iodine	Stone cell, parenchyma, starch cells
4	H_2SO_4	Packed stone cells, crystals, tannin, oil droplets, stone cells, fibres, lignified thick walled parenchymatous cells, parenchymatous cells

Table 4: Powder microscopy of readymade powder of *Phyllanthus emblica* L.

Sr. No.	Reagents	Different plant parts observed during study
1	HCl	Cortical parenchyma with starch, fibres, pitted mesocarp parenchyma, group of starch, raphides, sclerenchymatous cell, tannin, resin, bordered pitted vessels
2	CH_3COOH	Brachysclereid type stone cell, brown content, cortical parenchyma with starch,criss-cross fibre, elongated stone cells, fibre, packed stone cell, bundle of parenchymatous fibre, resin, sclereid, bordered pitted vessels, stone cell with broad lumen, trichome, raphides.
3	Iodine	Brachysclereid type stone cell, bundle of parenchymatous fibre, group of starch grains, mesosclereid stone cell, packed stone cells, xylem pitted vessels
4	H_2SO_4	Brown content, crystals, group of fibres, lignified xylem vessels, osteosclereid type stone cell, parenchymatous cells, pitted scalar vessels, resin, sclerenchymatous cell, starch granule, tannin

Microscopy of amla powder showed all the characteristic structures of amla fruit when compared to previous findings. Structures such as tannin cells, starch grains, different types of stone cells (3) (Fig 6 A and C) were inspected in amla powder with different reagents. Standardization of *P. emblica* shows diagnostic features such as xylem vessels, group of elongated stone cells, group of stone cells (Fig 6 A), pitted xylem vessels, parenchyma cells (10) (Fig 6 D). Powdered examination of fruit part of *P. emblica* confirms the anatomical parts vascular strand, ground tissue, different crystal masses (11). In this way amla powder was standardized which can be used in ayurvedic pharmacopoeial drugs as well as in food supplements.

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***Powder microscopic observation of Piper nigrum* L.**

Fruit: Powder of fruit of black pepper stained with different reagent shows different plant parts. Powder microscopy of homemade powder of black pepper shows Reticulate vessels (Fig 7. A), trichome (Fig 7. B), annular vessels (Fig 7. C), oil droplets with resin (Fig 7. D), stone cells, starch granules, oil ducts, readymade powder of black pepper shows raphides, stone cells, parenchymatous cells, starch granules, base of trichome, pointed apex of trichome, fungal mycelium (Fig 8 A and B), algal filament (Fig 9 A and B).

Figure 7 (A) Reticulate vessels (B) Trichome (C) Annular vessels (D) Oil droplets with resin observed in the homemade and readymade powder of *Piper nigrum*

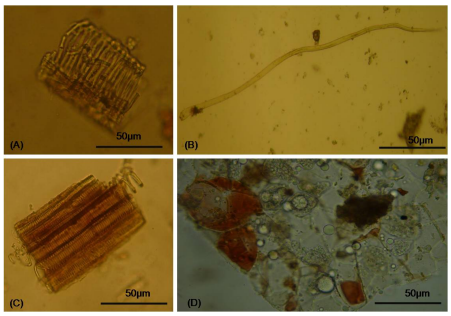

Figure 8 (A) and (B) Fungal mycelium growth observed in the readymade powder of *Piper nigrum*

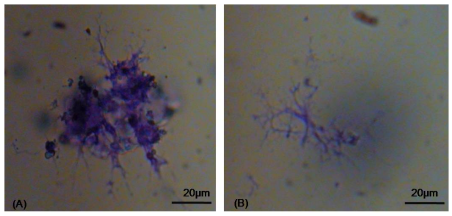

Figure 9 (A) and (B) Algal filaments observed in the readymade powder of *Piper nigrum*

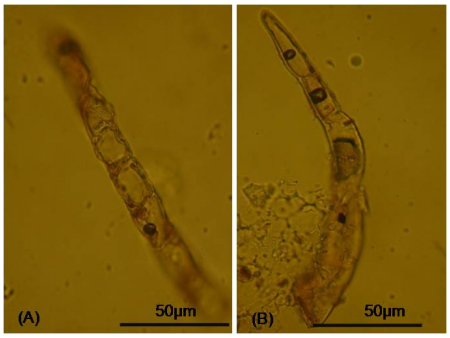

Table 5: Powder Microscopy of homemade powder of *Piper nigrum* L.

Sr. No.	Reagents	Different plant parts observed during study
1	HCl	Stone cells, crystals, fibre, vessels, group of stone cells
2	CH_3COOH	Brown content, stone cells, fibres, resins, criss-cross fibres, raphides.
3	Iodine	Starch granules, crystals,
4	H_2SO_4	Crystals, sclerenchymatous cells

Table 6: Powder Microscopy of readymade powder of *Piper nigrum* L.

Sr. No.	Reagents	Different plant parts observed during study
1	HCl	Pitted vessels, parenchyma, crystals, starch grains
2	CH_3COOH	Lignified thick walled Parenchymatous cells,pentagonal parenchymatous cells, starch grains, pitted vessels, raphides.
3	Iodine	Starch grains, fungus
4	H_2SO_4	Brachysclereid stone cells, stone cells, lignified thick walled parenchymatous cells, criss-cross fibre, annular vessels, reticulate vessels, crystals

The results from the above evaluation of drug reported similar microscopic structures as shown in past few papers. Structures of oil droplets (Fig 7 D), stone cells, annular vessels (Fig 7 C), sclereids, pitted fiber, calcium oxalate crystals (22) seen in this study. Microscopic characteristics examination demonstrate the presence of starch, sclereids, stone cell, epicarp, mesocarp (4). Additionally other than real plant structures some algal and fungal mycelium growth was detected in the commercial herbal powder of *P. longum*. which may grow due to improper storage condition as fungal and algal growth need moisturized environment for its survival. Existence of such undesirable growth may deteriorate the quality and efficacy of the herbal product.

***Powder microscopic observation of Piper longum* L.**

Observation of long pepper under the microscope shows following plant structures when treated with different reagents. Powder microscopy of homemade powder of long pepper shows plant structures such as sclerenchymatous cell (Fig 10 A), spiral vessels (Fig 10 B), stone cells (Fig 10 C), starch granules (Fig 10 D), annular ring, raphides, tannin cells, starch granule, trichome with bulbous base, calcium oxalate crystal . In readymade powder of long pepper different structures such as starch granules, brown content, raphides, annular rings, sclereid fibre, stone cells, fibre. trichomes, leaf part (Fig 11).

Figure 10 (A) Sclerenchymatous cell (B) Spiral vessels (C) Stone cells (D) Starch granules observed in the homemade and readymade powder of *Piper longum*

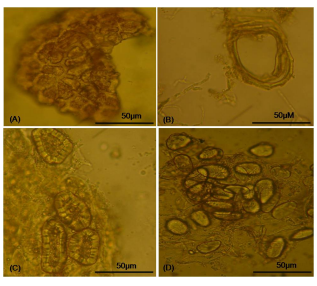

Figure 11. Leaf part observed in the readymade powder of *P. longum*

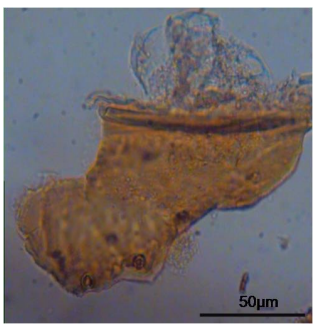

Table 7: Powder Microscopy of homemade powder of *Piper longum* L.

S. No.	Reagents	Different plant parts observed during study
1	HCl	Acicular piperine crystal, stone cells, oil droplets, parenchyma cells
2	CH_3COOH	Starch grains, raphides
3	Iodine	Starch granules, fibre, crystals, stone cells,
4	H_2SO_4	Mesocarp, oil ducts, annular ring

Table 8: Powder Microscopy of readymade powder of *Piper longum* L.

S. No.	Reagents	Different plant parts observed during study
1	HCl	Resin cells, pitted mesocarp parenchymatous cells,
2	CH_3COOH	Stone cells, starch granules, group of starch granules, parenchymatous fibre, crystals, pitted vessels, raphides.
3	Iodine	Compound starch grains, resin cells, starch grains, group of starch grains, pitted vessels, crystals
4	H_2SO_4	Crystals, leaf part, oil droplets, sclerenchymatous cells, tannin, trichome with bulbous base, single trichome, crystals, annular rings.

Practical exploration of *P. longum* exhibit cellular structures such as oleo resin, calcium oxalate, brown content, stone cells (12) (Fig 10 C). Other microscopical studies of *P. longum* shows starch grains, calcium oxalate crystals, oil globules, lignified sclerenchymatous cells (29). This preliminary phytochemical study is essential in improving human health by standardizing the superiority and capacity of herbal formulated drugs. Instead of such characteristic structures leaf part (Fig 11) were also noticed in the readymade powder of *P. longum.*

Standardization of a compound ayurvedic formulation is a critical and essential issue to be considered in assuring the therapeutic efficacy and safety and to rationalize their use in the health care. Organoleptic parameters such as color, odor, taste, shape and size were analyzed and recorded which relates the previous findings on various herbal drug parts and this phytochemical examination will further beneficial in separation and purity control parameters in future studies. Some earlier comparative investigations between two herbal drugs *A. precatorius* and *G. glabra* was distinguished. *G. glabra* shows the characteristic cellular features that differentiate it from *A. precatorius* on the basis of surface appearance, presence of stone layer in the cortex, vascular rays, presence of pith, organoleptic characters (9). Histological examination of *G. glabra* shows actual characteristic feature which authorized the microscopic character authentication (1). Some studies on *E. officinalis* shows the appearance of essential features sclereids, prismatic crystals, stone cells, pitted vessels, starch grains, fibres which might be helpful for the further studies in order to differentiate contaminants and exact identification of the plant (3). The detailed pharmacognostic standardization on *P. emblica* was well-established between 3 growth stages of fruit. The tender fruit consist of epidermal cells, parenchyma cells, minute crystals, starch grains, elongated stone cells. The middle fruit stage shows elongated and regular type stone cells which was not found in the tender fruit, about 9-11 stone cells were observed in single slide. On the other hand, mature fruit exhibit elongated and regular type stone cells, about 3-6 stone cells was remarked per slide, spiral and pitted xylem vessels. The three stages of seasonally grown fruit significantly differ in chemical constituents, this ensures the authentication and genuineness of the actual herbal plant which might be further useful for ayurvedic and herbal sectors to accept the purity and efficacy of amalaki as rasayana drug for the treatment of several diseases (10). Exploratory inspection, micrometry, physiochemical analysis, phytochemical screening, macro morphology, cytomorphology, fluorescence assay was carried out in order to standardized the herbal drugs which might be advantageous and perform behavioral therapy for the treatment of various health illness. Such methods are economical and reasonable for describing the exact and accurate identity of the drug (4). Observational experiments displays detectable internal cellular features of *P. longum* which is significantly decisive for enhancing standardization of the drugs (12). This pharmacognostic study may be beneficial to establish authenticated standards for drug identification and standardization. Presence of other plant part to the original plant part drug may reduce the effectiveness or phytochemical effect of the main drug. Earlier information reported that about 1.1 billion humans (7) and 65% inhabitants of rural areas (30) entirely depends on traditional treatment and thus such data can be used for the drug development so as to ensure the purity of herbal formulations and is beneficial for strengthening the health of the population.Thus standard parameters with quality control and purity of the drug must be analyzed before they introduce in the pharmaceutical or ayurvedic companies. Microscopical evaluation is very important in the initial identification of ingredients as well as in the detection of contaminants and adulterations. Identification of original drug is the first step to maintain the quality of the final product. But there is a need of further investigation on the basis of chemical evaluation, physical evaluation and biological evaluation which was not consolidated in this study.

Conclusion

The various pharmacognostical, physiochemical and phytochemical standards, thus obtained from this study will help in establishing, the identity, purity, quality, safety, and efficacy of each drug. Some of the herbal samples were found to confirm the quality standards while others did not comply probably due to adulteration or being spurious or substandard raw material. In case of the standardization of herbal raw material proper botanical identification has prime

importance to utilize them as a raw material for herbal as well as cosmeceutical industries. Sometimes spoiled or adulterated material, improper cultivation, collection, harvesting or adverse effects during processing may cause severe health problems. In this situation the key step is to confirm the authenticity of the required raw material by physicochemical methods (quantitative and qualitative) of determination. The real cell components explain the specific features of the plant part which dissociate it from adulterants and are with pure therapeutic effect with different mechanism of action inside the cellular matrix which in turn regulates the metabolic activity of the body. This study is planned to reveal the contaminants on actual herbal drugs by actual herbal drug. Although, the quality, safety, efficacy, and standard measure of traditional drugs are important to supply the safer drugs to public demand. Present study is aimed to examine the quality and purity of herbal product of selected plants. The quality of commercial products has been compared with homemade herbal powder of the same plant. Essentially, this study provide significant data to create awareness about quality testing of herbal raw material among herbal, cosmeceuticals and pharmaceutical industries of the country that would be beneficial for the therapeutic outcomes of commercially available herbal products. Thus it is recommended that the quality of the herbal products should be carefully and strictly monitored although patients and general public should also be conscious about the conceivable adulteration and trustability of the product before use.

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