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Pharmacognostic Study and Development of Quality Control Parameters for Certain Traditional Antidiabetic Herbs

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HUMAN

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ABSTRACT

The objective of the present work is to study the pharmacognostic and phytochemical characteristics of some antidiabetic medicinal plants. Pharmacognostic and phytochemical investigation of *Momordica charantia, Azadirachta indica and Eugenia jambolana* describing its morphological, microscopical characterization, powder analysis, physicochemical evaluation, fluorescence analysis, preliminary phytochemical screening and TLC profiling has been studied in detail so as to develop a reference for academic and commercial purpose. Further, it can be used for the standardization and pharmacopoeial parameters development. The present findings are associated with standardization of parameters like macroscopic and microscopic characters, phytochemical screening, fluorescent analysis and physicochemical quantification of the plants. Ash values added more strength to crude drug standardization with prominent results indicating the involvement or non-involvement of irrelevant matter. Such study on the macro and microscopic anatomy, preliminary phytoconstituent screening and physicochemical parameters are important informations which may be useful in verification and contamination for quality control of this therapeutic plant afterwards. Thus, it is evident that the present study of the plant material provides various resourceful information in relation to pharmacognostical identification of this plant material. It would also help scientists to utilize such needful information regarding the plants identity and characteristics in building new polyherbal formulations.

INTRODUCTION

Nowadays there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is always supposed to be safe. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. The advantage of natural drugs is their easy availability, economic and less or no side effects but the disadvantage is that they are the victims of adulteration. The more effective the natural drug more is its demand and the chances of non-availability increases. To meet the growing demand, the natural drug is easily adulterated with low grade material. Adulteration or substitution is nothing but replacement of original plant with another plant material or intentionally adding any foreign substance to increase the weight or potency of the product or to decrease its cost. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. The most common error is one common vernacular name is given to two or more entirely different species.

All these problems can be solved by pharmacognostic studies of medicinal plants. It is very important and in fact essential to lay down pharmacognostic specifications of medicinal plants which are used in various drugs.[1]

India is popular for its rich natural resources. In India, there are about 6,000 plants which are used in herbal medicine. Over 1500 plants identified by Indian system of medicines of which 500 plants are commonly used for different ailments[2].

Diabetes mellitus, one of the major public health problems worldwide, is a metabolic disorder of multiple etiologies distinguished by a failure of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism as a result of defects in insulin secretion and/or insulin action[3,4]. According to International Diabetes Federation (IDF) report, elevated blood glucose is the third uppermost risk factor for premature mortality, following high blood pressure and tobacco use globally[4].

Diabetes mellitus in Ayurveda is covered under the heading of Prameha. Several Ayurvedic formulations have been used in the treatment of Diabetes mellitus for centuries. In addition to herbs, minerals find wide application in Ayurvedic prescription for diabetes. Medicinal herbs like *Aegle marmelos, Allium cepa, Allium Sativum, Momordica charantia, Gymnema*

sylvestre, Enicostemma littorale, Pterocarpus marsupium, Coccinia indica and Trigonella foneum graceum, phyllanthus amarus and glycyrrhiza glabra, Eugenia jambolana, Azadirachta indica are prescribed as single powder drugs or in combination (poly-herbal). Scientists have studied the chemical composition of the antidiabetic medicinal herbs used in Ayurveda.

Pharmacognosy literally means knowledge of drugs or pharmaceuticals, which deals with the drugs of vegetable, animal and mineral origin. It may be defined as an applied science that deals with "Biological, biochemical and economical features of natural drugs and their constituents". Pharmacognosy helps to study the identification of the source of the material forming drug, description of its morphology and anatomy, investigation of its potency, purity and freedom from admixture, devising the methods of cultivation, prescribing the details of collection and preparation processes and studying the constituents of the drug and investigation of their physicochemical properties[5].

The pharmacognostical studies of herbal drugs have become imperative for several reasons. As per the WHO norms, every drug has to undergo botanical standardization, particularly macroscopic and microscopic characterization which constitutes the major part of Pharmacognosy. This primary step enables the researcher in phytodrugs to affirm. The botanical standardization is based upon the tenet that certain microscopic characters are specific and restricted in distribution[6]. Microscopic parameters, though limited in their application under certain circumstances, have still highly reliable diagnostic values and play appreciable role in the herbal drugs.

In the present study, the recommended procedures were employed and data pertaining to morphological and anatomical characteristics of the selected medicinal plants were retrieved. Every essential observation was supplemented by supporting photographs. Customary parameters of Pharmacognosy such as powder drug analysis and powder microscopy were given due importance. These studies will offer the scope for easy and accurate identification of the specimen either in incomplete or fragmentary form.

MATERIALS AND METHODS

Collection and authentication of Plant materials

The plant material was collected from local market Buldana. The plants were authenticated from Department of botany, L.K.D. KBanmeruScience College, LonarDist-Buldana(MS) by Dr. M.R. Bhise having reference letter no. DOB/2018-19/01. The plant parts were separated, washed thoroughly with tap water, shade dried and homogenized to fine powder and stored in closed container for further studies.

Morphological evaluation[7]

Macroscopic identity of medicinal plant materials is based on shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface. However, since these characteristics are judged subjectively and substitutes or adulterants may closely resemble the genuine material, it is often necessary to substantiate the findings by microscopy and/or physicochemical analysis. Microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials; the specimen may have to be treated with chemical reagents. An examination by microscopy alone cannot always provide complete identification, though when used in association with other analytical methods it can frequently supply invaluable supporting evidence.

Microscopical evaluation[8,9]

Once the material has been examined and classified according to external characteristics, inspection by microscopy can be carried out as the next step.

Microscopy is used to determine the structural, cellular and internal tissue features of botanicals. It is usually used to identify and differentiate two herbals that are similar. This is the commonly used technique, convenient, quick and can be applied to proprietary medicines too. Microscopic inspection alone can’t always provide complete identification but when used in the association with other analytical methods.

Transverse section

Plant part understudy usually taken in the form of appropriate (longitudinal or transverse / cross) section to study the presence or absence of type (shape) of cells or tissues. Some of the

chemicals like phloroglucinol, chloral hydrate, safranine, methyl orange *etc*., use for clear visualization of cellular content. Using microscope detecting various cellular tissues, trichomes, stomata, starch granules, calcium oxalate crystals and aleuronic grains are some of important parameters which play important role in identification of crude drug.

Crude drug can also be identified microscopically by cutting the thin TS (transverse section), LS (Longitudinal section) especially in case of wood and by staining them with proper staining reagents.

Powder microscopy [10]

Dried powder usually taken for studying the presence or absence of cellular contents (type/ shape) by using microscope.

Microscopic evaluation also includes study of constituents in the powdered drug by the use of chemical reagents. These reagents used due to abundance of cellular contents, presence of coloring matters, shrinkage or collapse of cell wall which creates hurdles in microscopic evaluation.

For powder microscopy, a pinch of fine powder was taken on a glass slide, treated separately with water, chloral hydrate and iodine solution. The microscopic observations were accomplished using 45X and 10X objective lenses. The detected fragments of the powder were identified and drawn on paper.

Physicochemical evaluation[8, 11, 12]

Loss on drying (LOD)

LOD is the loss in weight in % (w/w) resulting from water and volatile matter of any kind that can be driven off under specified conditions. 1g sample is transferred to a shallow bottle and weighed. Sample was distributed evenly and dried in a hot air oven at 105°C for 1h with the stopper open. After 1h, the stopper was closed and cooled at room temperature and the bottle was weighed.

Determination of Foreign Matter

Herbal drug should be free from mould, insects and animal facial matter *etc* such as earth stone, extraneous material. Accurately weighed about 100g of crude drugs was spread in a

form of single layer on clean surface by visual in section for any possible determination of foreign matters were detected. Then the crude drugs were weighed and percentage of foreign matter was calculated.

Determination of Foaming Index

The saponins possess high molecular weight containing phytoconstituents which having the detergent activity. Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant material and their extracts is measured in terms of a foaming index.

Accurately weighed1g of coarse powder was reduced to fineness by passing through a sieve. The fine powder was weighed, transferred to 500ml conical flask containing 100ml of boiling water, maintained at moderate boiling for 30 minutes. Flask was cooled and the contents were filtered in100ml volumetric flask, sufficient water was added to make up the volume. The decoction was poured into10 ml of volumetric flask in successive portions of 1, 2, 3 ml *etc* upto 10 ml, and the volume of the liquid in each tube was adjusted with water upto 10 ml. The volumetric flask was stoppered and shaken in a lengthwise motion for15 seconds. They were allowed to stand for 15 minutes and the height of the foam was measured. The height of the foam in every tube was less than 1 cm, therefore foaming index was less than 100.

Determination of Swelling Index

Many medicinal plant materials are specific of therapeutics value or pharmaceutical utility because of their swelling properties, especially gums and those containing a specified amount of mucilage, pectin or hemicelluloses. The swelling index is measured in volume (in ml) taken.

Accurately weighed 1g of powder and transferred into 50ml of measuring cylinder added successive amount of 25 ml of water then the mixture was vigorously shaking after every 10 minutes interval for period of 1hr. Finally, the mixture was allowed to stand for 3 hr at room temperature. The volume in ml taken was measured which was occupied by the plant material including any sticky mucilage.

Total ash value

It depicts the total amount of material produced after the complete incineration of the ground drug above 400°C to remove all the carbon atoms. 2 g of powdered drug was weighed and placed in the crucible and heated at about 400°C. The crucible was cooled and the % of the total ash with reference to the air-dried sample of the crude drug was calculated.

Acid insoluble ash

Total ash obtained was dissolved in 1N HCl solution and heated for 5 min. The insoluble matter was filtered in whatman filter paper; the filter paper was further dried at 70°C and then cooled. The residue was weighed and the percentage of insoluble ash of the crude drug w.r.t. the air-dried sample of crude drug was calculated.

Water soluble ash

To the total ash crucible, 25ml double distilled water was added and boiled for about 5min. Insoluble matter was collected on an ashless filter paper in a crucible, washed with hot water and ignited for about 15 min above 45°C. The weight of the residue is subtracted from the weight of the total ash. Content of water-soluble ash in mg/g of the air-dried material was calculated.

Extractive value[11,12]

Extractive value determines the amount of active constituents extracted with solvent from a given amount of medicinal plant. It gives an idea about the nature of the chemical constituents present.

Determination of alcohol soluble extractive value

About 5 gms of air-dried course powdered drug was weighed and macerated with 100 ml of 90% alcohol in a closed flask for 24 hours, shaking frequently during the first 6 hrs and these allowed standing for 18 hrs.

Thereafter it was filtered rapidly taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105°C and weighed. The percentage of the alcohol soluble extractive values was calculated with reference to the air-dried drug.

Determination of water-soluble extractive value

About 5 gm of air-dried powdered drug was taken & macerated with 100 ml of chloroform water in a closed flask for 24 hrs shaking frequently during the first 6 hrs and then allowed to stand for 18 hrs.

Thereafter, it was filtered rapidly taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105°C and weighed. The percentage of the water-soluble extractive value was calculated with reference to the air-dried drug.

Extraction of plant material

***Momordica charantia*[13]**

Fruits were cut into slices and shade dried ground to a coarse powder and passed through a 80 mesh sieve. The powdered plant (250 g) was defatted with petroleum ether, chloroform, and ethanol (90%) successively using Soxhlet apparatus and later extracted using 50% ethanol and water by maceration. All the above extracts were also tested for the identification of phytoconstituents. The semisolid aqueous extract (6.79% W/W) was suspended in distilled water and employed for anti-diabetic activity.

***Azadirachta indica*[14]**

About 500 g of powdered materials were extracted using ethanol (50°C) using soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extract is then concentrated by distillation process and dried under reduced pressure. The solvent free semisolid mass thus obtained is used for the experiment. This semisolid mass contains the active compound Nimbidin.

***Eugenia jambolana*[15]**

The required amount of seed and fruit pulp of *E. jambolana* were collected and dried in room temperature. About 500 gm of both the seed and pulp were ground separately into powdered form and packed separately into the percolator along with 90% ethyl alcohol for 48 hrs. Materials were collected from the percolater and alcohol was allowed to evaporate.

Preliminary phytochemical screening[16]

The extracts were subjected to qualitative phytochemical analysis to detect the presence of carbohydrate, amino acids, cardiac glycosides, alkaloids, saponins, phenols, flavonoids, steroids, terpenoids, tannins, anthraquinones, quinones, fats and volatile oils.

Qualitative chemical tests

Test for alkaloids

(a) Mayer's test: To 2 ml test solution, 2N HCl was added. The aqueous layer formed was decanted and Mayer's reagent was added to it. A cream coloured precipitate indicates the presence of alkaloids.

(b) Dragendroff's test: To 2 ml test solution and Dragendroff's reagent was added to it. A reddish-brown precipitate indicates the presence of alkaloids.

(c) Wagner's test: To 2 ml test solution and Wagner's reagent was added to it. A reddish-brown precipitate indicates the presence of alkaloids.

(d) Hager's test: To 2 ml test solution and Hager's reagent was added to it. A yellow coloured precipitate indicates the presence of alkaloids.

Test for glycosides

(a) To 2 ml test solution, equal quantity of Fehling's solution A and B was added and solution was heated. A brick red precipitate indicates the presence of glycosides.

(b) Legal's test: To 2 ml test solution, pyridine and alkaline sodium nitroprusside was added to obtain a blood red colour.

Test for flavonoids

(a) Shinoda test: To 2 ml test solution, few fragments of Magnesium ribbon was added and to it conc. H_2SO_4 was added dropwise. Pink scarlet or crimson red colour appears.

(b) Zinc chloride reduction test: To 2 ml test solution, a mixture of zinc dust and conc. HCl was added. A red colour is obtained after few minutes.

(c) Alkaline reagent test: To 2 ml test solution, sodium hydroxide solution was added to give a yellow or red colour.

Test for tannins

(a) Gelatin test: To 2 ml test solution, 1% Gelatin solution containing 10% sodium chloride was added to obtain a white precipitate.

(b) Ferric chloride test: To 2 ml test solution, ferric chloride was added to give a blue green colour.

Test for proteins and amino acids

(a) Millon's test: To 2 ml test solution, Millon's reagent is added which gives a white precipitate, which on heating changes to red.

(b) Ninhydrin test: To 2 ml test solution, ninhydrin solution was added and the solution was boiled. Amino acids and proteins when boiled with 0.2% ninhydrin reagent show a violet colour.

Test for fats and fixed oils

(a) Stain test: Small amount of the extract was pressed between two filter papers; the stain on the filter paper indicates the presence of fixed oils.

(b) Saponification test: Few drops of 0.5 N alcoholic potassium hydroxide was added in small quantity to the extract solution with a drop of phenolphthalein and heated on a water bath for 1-2h. The formation of soap or partial neutralization for the alkali indicates the presence of fats and fixed oils.

Test for Sterols

(a) Liebermann-Burchard test: To the test solution, 3-4 drops of acetic anhydride were added, the solution was boiled cooled and conc. sulphuric acid (3 drops) was added. A brown ring appears at the junction of the two layers. The upper layer turns green showing the presence of steroids.

Test for triterpenoids

(a) Salkowski test: To the test solution 2 ml chloroform was added with few drops of conc. sulphuric acid (3 ml), and shaken well. Appearance of reddish-brown colour at lower layer indicates presence of steroids and that of yellow colour shows the presence of triterpenoids.

Thin layer chromatography [17, 18]

Thin layer chromatography is simply known as TLC. It is one of the most popular and simple chromatographic technique used of separation of compounds. TLC is frequently used for the analysis of herbal medicines since various pharmacopoeias still use TLC to provide first characteristic fingerprints of herbs. TLC has the advantages of being simple and can be employed for multiple sample analysis. In the phytochemical evaluation of herbal drugs, TLC is being employed extensively for the following reasons:

It enables rapid analysis of herbal extracts with minimum sample clean-up requirement,

It provides qualitative and semi quantitative information of the resolved compounds.

It enables the quantification of chemical constituents.

For each plate, more than 30 spots of samples can be studied simultaneously in one time. In summary, the advantages of using TLC to construct the fingerprint of herbal medicines are its simplicity, versatility, high velocity, specific sensitivity, simple sample preparation and its economy.

Fluorescence analysis[18]

The drug powder was treated with acids such as 1 N HCL and 50% H_2SO_4; and alkaline solutions such as aqueous sodium hydroxide, alcoholic sodium hydroxide; and other solvents such as nitric acid, picric acid, acetic acid, ferric chloride, and nitric acid with ammonia. They were subjected to fluorescence analysis in daylight and in the ultraviolet (UV)-light (254 nm and 365 nm).

RESULTS AND DISCUSSION

Collection and authentication of plant materials

The plant material was collected from local market Buldana. The plant parts were separated, washed thoroughly with tap water, shade dried and homogenized to fine powder and stored in closed container for further studies.

Morphological evaluation

The macroscopic study of a medicinal plant was helpful in rapid identification of plant material and also plays an important role in standardization of drug. The fresh of material medicinal plants were collected and the characters were analysed for its morphological characters like colour, odour, taste, shape, size which is shown in table 1 and figure no. 1, 2, 3.

Figure 1- *Momordica charantia*

Figure 2- *Azadirachta indica*

Figure 3- *Eugenia jambolana*

Table no.1- Morphological characters

Plants	***Momordica charantia***	***Azadirachta indica***	***Eugenia jambolana***
Colour	Green	Green	Cream
Odour	Bitter	bitter	Characteristic
Taste	Bitter	bitter	Astringent
Shape	Elongated longitudinally grooved	Serrate margin, glabrous and apex acumnate	Oval shape
Size	2.6 to 25 cm long 2.6 cm in diameter	14 – 30 cm long alternate, estipulate	1-2 cm diameter

Microscopical evaluation

The microscopic study is the anatomical study which is done by taking appropriate section of the plant parts under study. Each distinguishing character can be noted down, some of which are retained in the powder study also. Some of the chemicals which are used in obtaining clear sections are phloroglucinol, chloral hydrate, safranine, methyl orange, *etc.*

T.S. of *Momordica charantia*

Outer mesocarp encircling the inner whitish, pithy made up of cholenchymatous tissues, contain calcium oxalate crystals. The cells of the inner mesocarp are smaller in size and contain numerous small crystals of calcium oxalate and starch. Anastomosing bicollateral vascular bundles and latex tubes traversed throughout the mesocarp tissue; endocarp consists of thin walled more or less tangentially elongated cells often adhering to the seed. Seeds are oblong with grooved margins and a sculptured surface.

T. S. of *Azadirachta indica*

TS of neem leaf shows a biconvex outline; epidermis on either side covered externally with thick cuticle; below epidermis 4-5 layered collenchyma present; stele composed of one crescent-shaped vascular bundle towards lower and two to three smaller bundle towards upper surface; rest of tissues composed of thin-walled, parenchymatous cells having secretory cells and rosette crystals of calcium oxalate; phloem surrounded by non-lignified fibre strand; crystals also present in phloem region.

T. S. of *Eugenia jambolana*

The transverse section of Eugenia Jambolana leaves showed the two to three layered epidermis, containing rounded cells, mesophyll composed of isodiametric thin walled parenchymatous ground cells which are packed with simple starch grains. Mid-rib region the vascular bundles, xylem and protoxylem towards upper epidermis and phloem on the lower side. Palisade cells, Starch grains, oil globules, tannin cell and stone cells also present and vascular bundles not fully developed.

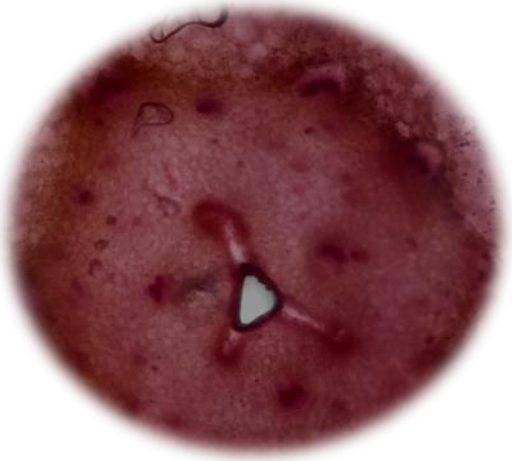


Fig. 4: Mesocarp region of *Momordica charantia*

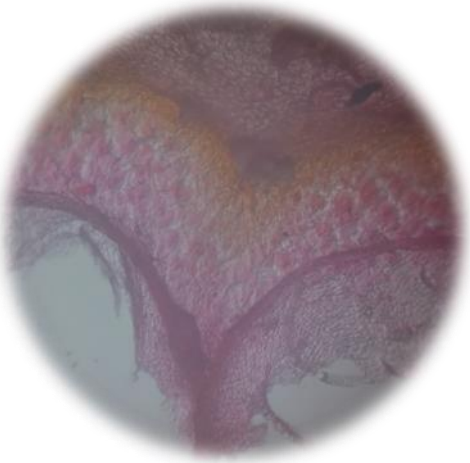


Fig. 5: Outer region of *Momordica charantia*

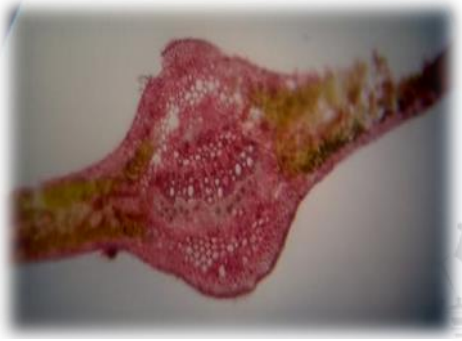


Fig. 6: T.S. of *Azadirachta indica*

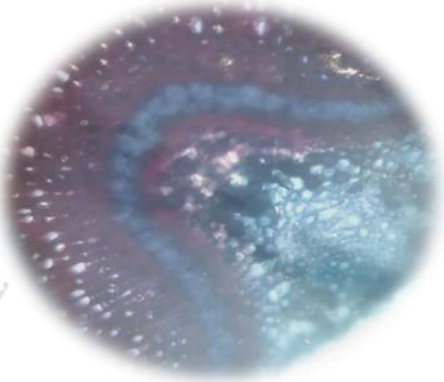


Fig. 7: T. S. of *Eugenia jambolana*

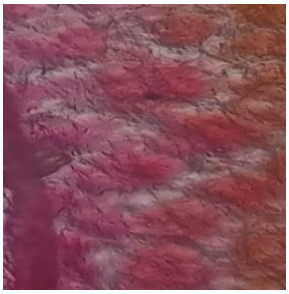
Powder microscopy

Powder study is similar to microscopic study except here dried powder is taken instead of section of the plant. All the reagents used are also same like above.

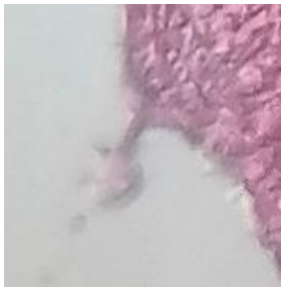
Powder characters of *Momordica charantia*

Trichomes small and glandular with multicellular stalk, latex cells, multicellular head, simple starch grains, epidermal cells and calcium oxalate prismatic crystals and palisade like cells.

Epidermis



Sclerenchymatous cells

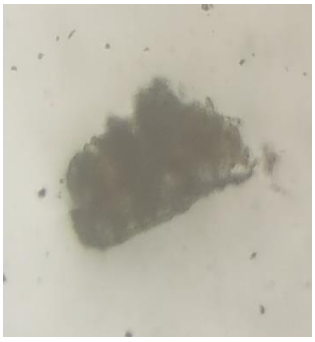


Glandular trichome

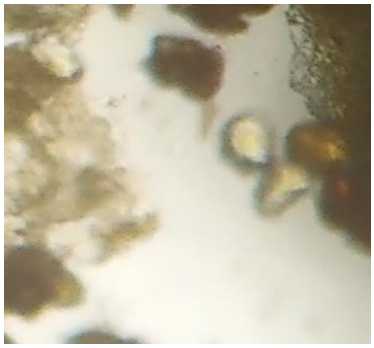
Fig. 8, 9, 10: Powder characters of *Momordica charantia*

Powder characters of *Azadirachta indica*

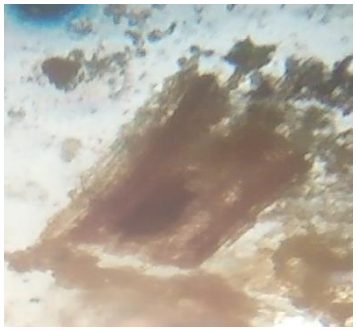
Reddish-brown; shows cork cells; numerous prismatic crystals of calcium oxalate, phloem fiber, starch grain.



Epidermal cells



Starch granules

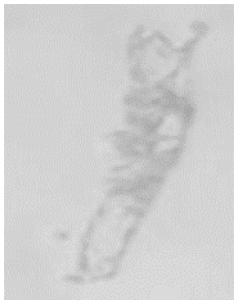


Phloem fiber

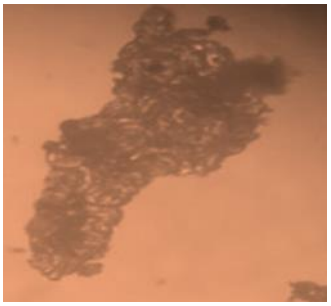
Fig. 11, 12, 13: Powder characters of *Azadirachta indica*

Powder characters of *Eugenia jambolana*

The dry leaf powder showed the multicellular and lignified trichomes, calcium oxalate crystals that were prismatic and cluster form.



Fibers



Epidermal cells with stomata

Multicellular Trichomes

Fig. 14, 15, 16: **Powder characters of *Azadirachta indica***

Physicochemical Parameters

Physicochemical parameters such as Loss on drying, Foreign organic matter, Foaming, swelling index, total ash value, acid-insoluble ash, water soluble ash, extractive values, alcohol soluble extractive, water soluble extractives of selected medicinal plants were determined according to standard procedures.

Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water-soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not. The result of the parameter is shown in table 2.

Table no. 2- Physicochemical Parameter

Plants/ Parameter	***Momordica Charantia %***	***Azadirachta indica%***	***Eugenia jambolana%***
Loss on drying	3.21	15.85	4.0
Foreign organic matter	4.5	0.24	8.21
Foaming index	12.0	10.0	8.0
Swelling index	4.5	20.0	16.9
Total Ash value	6.8	3.47	4.9
Acid insoluble ash	0.25	1.12	1.8
Water soluble ash	3.56	4.2	3.0
Water soluble Extractive value	36.9	18.12	22.0
Alcohol soluble Extractive value	19.0	14.32	13.7

Phytochemical Screening

A systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents and to establish a chemical profile of a crude drug for its proper evaluation. Extracts of various plant parts like root, stem and leaf were taken by using different extraction methods. These extracts were then subjected to preliminary phytochemical screening for the identification of various classes of active chemical constituents using the various standard procedures. The result of preliminary phytochemical screening shows the presence of various phytochemicals which is shown in table 3.

Table no. 3- Phytochemical Screening

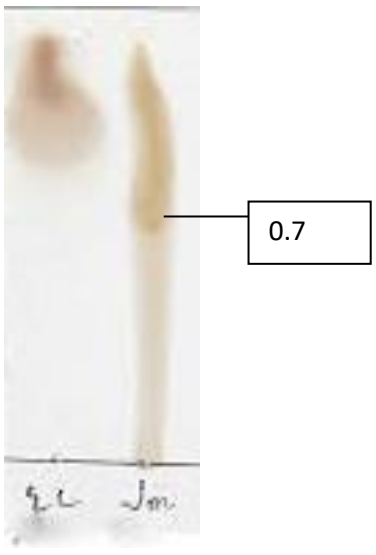
Plants	***Momordica charantia***	***Azadirachta indica***	***Eugenia jambolana***
Alkaloids	+	+	+
Glycosides	+	+	+
Flavonoids	+	+	+
Tannins	+	-	+
Proteins	+	-	+
Fixed oil	+	-	+
Steroids	+	+	+
Terpenoids	+	-	+
Carbohydrate	+	+	-

TLC Profile

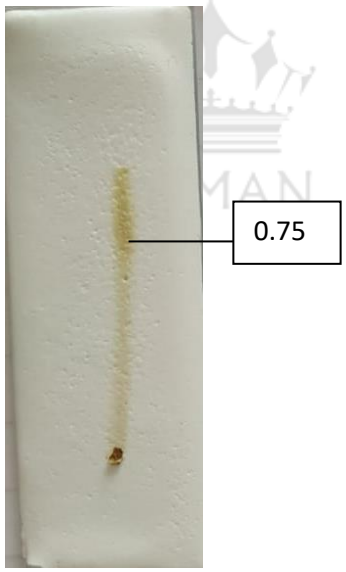
TLC analysis of extracts was done by using different solvent system which revealed the different Rf value which indicate the presence of different chemical compound present in the extract. The result of TLC shown in table 4 and figure 17, 18, 19.

Table no. 4- TLC Profile

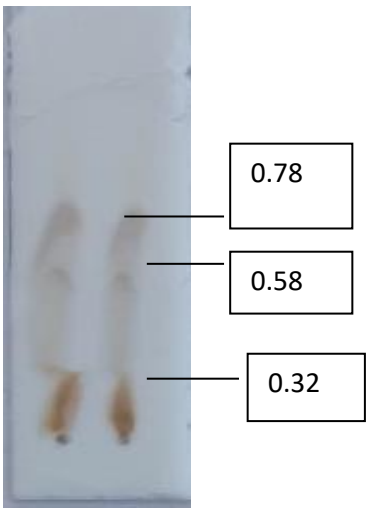
Plants	Extract	Solvent system	Rf Value
Momordica charantia	Methanolic extract	Ethyl acetate: Methanol: Water (8:1.2:0.8) Spraying reagent: conc. H2SO4	0.7 (brown spot of charantin)
Azadirachta indica	Methanolic extract	Methanol: Tolune (8:2)	0.74(Brown) 0.75 (Green)
Eugenia jambolana	Methanolic extract	n- butanol: water (1: 1)	0.32, 0.58, 0.78 (brown)



TLC of *M. Charantia* fruit methanolic extract



TLC of *A. indica* leaves methanolic extract



TLC of *E. Jambolana* fruit methanolic extract

Fig 17, 18, 19: TLC Plates

Fluorescence analysis

Crude drugs show their own characteristic fluorescence when exposed to ultraviolet radiation and is dependent on its chemical constituents. Fluorescent analysis of the drug powder with different solvents is an important pharmacognostic tool in checking adulterants. The

powdered drugs were boiled with different solvents according to their increasing polarity. The boiled powders with solvents were examined under short UV (254nm), long UV (365nm) and visible light and the results are shown in table 5.

Table no. 5- Fluorescence analysis of powder

Plant material	Observation under	Dry powder	Powder + Water	Powder +HCL	Powder +HNO3	Powder +H2SO4	Powder +NAOH	Powder + KOH	Powder + Alc. NAOH	Powder + Alc. KOH	Powder + Ammonia
M. charantia (Fruit)	Normal light	Brown	Dark brown	Golden	Golden	Yellow	Brown	Brown	Cream	Golden	Brown
	UV Light	Yellowish brown	Light green	Light green	Light green	Light green	Greenish brown	Greenish brown	Greenish yellow	Light green	Greenish brown
A. Indica (leaves)	Normal light	Yellowish brown	Gold	Dark golden	Light golden	Green	Dark golden	Golden	Golden	Golden	Dark golden
	UV Light	Dull yellow	Greenish brown	Light green	Greenish yellow	Dark brown	Dark brown	Light green	Dark green	Dark green	Brown
E. jambolana (Seed)	Normal light	Cream	Golden	Golden	Golden	Yellow	Brown	Brown	Cream	Golden	Brown
	UV Light	Light brown	Light green	Light green	Light green	Light green	Greenish brown	Greenish brown	Greenish yellow	Light green	Greenish brown

CONCLUSION

Pharmacognostic studies and phytochemical screening can serve as a basis for proper identification of a plant. Before any drug can be included in the pharmacopoeia, these standards must be established. A systematic approach is necessary in pharmacognostic study which helps in confirmation and determination of identity, purity and quality of a crude drug. The present findings provide the pharmacognostic, physicochemical, and phytochemical information about the *Momordica charantia, Azadirachta indica and Eugenia jambolana* and this might be useful by providing additional support with regard to its identification and standardization parameters. The present findings are associated with standardization of parameters like macroscopic and microscopic characters, phytochemical screening, fluorescent analysis and physicochemical quantification of the plants. Ash values added more strength to crude drug standardization with prominent results indicating the involvement or non-involvement of irrelevant matter. Such study on the macro and microscopic anatomy, preliminary phytoconstituent screening and physicochemical parameters are important information which may be useful in verification and contamination for quality control of this therapeutic plant afterwards.

This study will be helpful in the future pharmacognostic standardization of these important plants. Furthermore, information regarding physicochemical characteristics of trunk bark and nature of chemical constituents present in them would also be useful for standardization of

such herbal drugs of folk medicinal practice of present era and enrichment of Ayurvedic Pharmacopoeia. It would also help scientists to utilize such needful information regarding the plants identity and characteristics in building new polyherbal formulations.

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