



Pharmacognostical Study, Phytochemical and Physicochemical Evaluation and Establishment of Quality Standards for Certain Traditional Antidiabetic Medicinal Plants

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Authors' contributions

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ABSTRACT

Aim: The present work was aimed at evolving pharmacognostical and phytochemical quality standards for certain traditional herbs like *Phyllanthus amarus*, *Glycerrhiza glabra* and *Piper nigrum*. These three plants are reported to possess antidiabetic activity.

Study Design: The plants were collected, authenticated and Macro-morphological, qualitative and quantitative microscopic features as well as physicochemical, fluorescence analysis, phytochemical properties, and thin layer chromatography (TLC)

Place and Duration of Study: The study was carried out at College of Pharmacy, Dr. APJ Abdul Kalam University, Indore, during 2019-20

Methodology: The material were collected, authenticated and Macro-morphological, qualitative and quantitative microscopic features as well as physicochemical, fluorescence analysis, phytochemical properties, and thin layer chromatography (TLC) profile of *Phyllanthus amarus*, *Glycerrhiza glabra* and *Piper nigrum* were determined using standard methods.

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Results: The macroscopical and microscopical studies revealed useful diagnostic features. Phytochemical screening reveals the presence of secondary metabolites, physicochemical including fluorescence analysis of powdered drug proved useful to differentiate the powdered drug material. Thin layer chromatography analysis showed the presence of important phytoconstituents such Phyllanthin, hypophyllanthin, glycerrizin and piperine

Conclusion: The data generated from this study would serve as useful gauge for determining the quality of *Phyllanthus amarus*, *Glycerrhiza glabra* and *Piper nigrum* thereby correct identification and authentication of these plants.

It would also help scientists to utilize such needful information regarding the plants identity and characteristics in building new polyherbal formulations.

Keywords: *Phyllanthus amarus*; *Glycerrhiza glabra*; *Piper nigrum*; standardization.

ABBREVIATIONS

WHO : World Health Organisation
 T.S : Transverse section
 DM : Diabetes Mellitus
 IDDM : Insulin Dependent Diabetes Mellitus
 TLC : Thin Layer Chromatography
 HPTLC : High Performance Thin Layer Chromatography
 IDF : International Diabetes Federation
 LOD : Loss on Drying

1. INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal and minerals have been the basis of the treatment of human disease. Today estimate that about 80% of people in developing countries still relies on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day [1]. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments. The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. Herbal medicines are the major remedy in traditional system of medicine have been used in medical practices since antiquity. Currently 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects [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 marmelose*, *Allium cepa*, *Allium sativum*, *Momordica charantia*, *Gymnema sylvestre*, *Enicosterm malittorale*, *Pterocarpus marsupium*, *Coccinia indica* and *Trigonella foneum graceum* *phyllanthus amarus* and *glycerrhiza 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].

According to WHO (World Health Organization), the global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025 majorly in the developing countries. India

presently has the largest number of diabetic patients in the world and has been infamously known as the diabetic capital of the world [6]. Sulfonylureas, biguanide, thiazolidinedione, and glycosidase inhibitors are widely used to control the hyperglycemia, hyperlipidemia and insulin resistance of type 2 diabetes, but these drugs fail to significantly alter the course of diabetic complications and have limited use because of undesirable side effects and high rates of secondary failure. Moreover, they are not safe for use during pregnancy [7]. Thus, the management of diabetes without any side effects is still a challenge. There is continuous search for alternative drugs [8]. As a result of the global epidemic of diabetes, the limited potency and many side effects of medications currently in use, the need for new diabetes therapies is expected to grow dramatically during the next decade. An intense research has been conducted to identify new therapeutic targets and pharmacologic compounds that might correct the impaired glucose tolerance [9].

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 [10].

2. MATERIALS AND METHODS

2.1 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. K Banmeru Science 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.

2.2 Morphological Evaluation [11]

The plant material is categorized according to sensory characteristics. Organoleptic evaluation

provides the simplest and quickest means to establish the identity, purity and quality of a particular sample. Hence, this observation is of primary importance before any further testing can be carried out. 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.

2.3 Microscopical Evaluation [12,13]

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.

2.4 Transverse Section (T.S)

Plant part under study 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, safranin, 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.

2.5 Powder Microscopy [14]

The shade dried, powdered plant material was used for powder microscopic analysis. The

Organoleptic characters were observed and to identify the different characteristic features various staining reagent were used. Powder was stained with 1% phloroglucinol in 90% ethanol, concentrated hydrochloric acid and observed through microscope. All the lignified cells stained with pink colour.

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.

2.6 Physicochemical Evaluation [15,16]

2.6.1 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.

2.6.2 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.

2.6.3 Determination of foaming index

The saponins possess high molecular weight containing phyto constituents 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 weighed 1g 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 in 100ml volumetric flask, sufficient water was Added to make up the volume. The decoction was poured into 10 ml of volumetric flask in Successive portions of 1,2,3ml etc. upto 10 ml, and the volume of the liquid in each tube was adjusted with water upto 10ml. The volumetric flask was stoppered and shaken in a lengthwise motion for 15 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.

2.6.4 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.

2.6.5 Total ash value

Ash values are helpful in determination the quality and purity of a crude drug in the powdered form. The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring drug or adhering to it or deliberately added to it, as a form of adulteration.

2.6.6 Acid insoluble ash

The ash was boiled for 5 minute with 25 ml of distilled water. The insoluble matter was then collected in an ash less filter paper. It was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of the ash and the difference in weight represented the water soluble ash, the percentage of water soluble ash with reference to the air dried substances was calculated by the formula.

2.6.7 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 ash less filter paper in a crucible, washed with hot water and ignited for about 15min 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.

2.6.8 Extractive value [16]

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.

2.6.9 A determination of alcohol soluble extractive value

About 5gms of air-dried coarse powdered drug was weighed and macerated with 100ml 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 tared 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.

2.6.10 B determination of water-soluble extractive value

5gm of the powder drug was weighed and macerated with 100ml of chloroform water (95 ml distilled water and 5ml chloroform) in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish. 2ml of alcohol was added to the residue and it was dried at 105°C for 1 hour in the hot air oven and cooled in desiccators for 30 minute and weighed. The process was repeated till a constant weight was obtained; the percentage of water soluble extractive value with reference to the air dried drug was calculated.

2.7 Extraction of Plant Material

2.7.1 *Phyllanthus amarus*

Plant was cleaned out with water and cut into small pieces, dried and extracted with water or ethanol/water as following. Plant material (200 g) was soaked in water (2 L) and heated in a water bath at 80°C for 1 h for the aqueous extract. The same quantity was macerated 72 h in ethanol/water solution (2 L) for hydroalcoholic extract. The crude extracts were filtered with Whatman paper and evaporated in vacuum at 40°C using a rotary evaporator. The yield of the preparation was 16% (w/w) for Aqueous extract and 13% (w/w) for Hydroalcoholic extract.

2.7.2 *Glycyrrhiza glabra*

The dried liquorice roots were coarsely powdered. The liquorice powder was extracted with boiling water to isolate glycyrrhizin. The aqueous extract was concentrated dried and used as a liquorice extract.

2.7.3 *Piper nigrum*

Weigh sufficiently black pepper powder extracted with 150ml 95% ethanol in Soxhlet extractor for 2 hours. The solution was filtered and concentrated on the water bath at 60°C. 10 ml 10% of alcoholic potassium hydroxide was added to the filtrate with continuous stirring to separate precipitate and recrystallized by using cold water, keep overnight in refrigerator to isolate crystals of piperine.

2.8 Preliminary Phytochemical Screening [17]

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.

2.8.1 Qualitative chemical tests

- **Test for alkaloids**

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

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

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

- **Test for glycosides**

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

- **Test for flavonoids**

(a) Shinoda test: To 2ml test solution, few fragments of Magnesium ribbon were added and to it conc. H₂SO₄ was added drop wise. Pink scarlet or crimson red colour appears.

(b) Zinc chloride reduction test: To 2ml 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 2ml test solution, sodium hydroxide solution was added to give a yellow or red colour.

- **Test for tannins**

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

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

- **Test for proteins and amino acids**

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

(b) Ninhydrin test: To 2ml 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 0.5N 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 2ml chloroform was added with few drops of conc. Sulphuric acid (3ml), 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

2.9 Thin Layer Chromatography [18, 19]

Chromatographic methods are important analytical tool in the separation, identification and estimation of components present in the plant. It is one of the most important and uncomplicated chromatographic technique used of separation of compounds. TLC is often used for the investigation of herbal medicines since various pharmacopoeias still use TLC to provide first feature 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.

3. RESULTS AND DISCUSSION

3.1 Collection and Authentication of Plant Materials

The plant material was selected on the basis of exhaustive literature survey and collected from local market Buldana. The plant parts were prepared for study by separating, washing with tap water, drying under shade and make it to fine powder and stored in air dried well closed container for further analysis.

3.2 Morphological Evaluation

The fresh of material of selected plants were collected and the characters were analysed for its external appearance like colour, odour, taste,

shape, size which is shown in table 1 and Figs. 1,2,3. The morphological study of a medicinal plant was helpful in quick detection of plant material and also plays an important role in equality of drug.

3.3 Microscopical Evaluation

The microscopical studies plays a key factor in establishing the authenticity of the plant materials. The botanical identity of the plant parts was established by examining its morphological, anatomical features as well as the WHO recommended physiochemical chemical studies. The microscopic study is the study of internal structure which is done by taking appropriate section of the plant parts under microscope. Each distinctive character can be noted down, some of which are retained in the powder study also. Different chemicals are used in study are phloroglucinol, chloral hydrate, safranine, methyl orange, etc.

3.3.1 T.S. of *Phyllanthus amarus*

Transverse section of plant part shows the presence Circular shape of stem cortex surrounds with several layers of parenchymatous cells. Then below that radially arrange medullary rays, lignified xylem and phloem fibers with fairly wide lumen. Cambium and pith consist numerous calcium oxalate crystals and starch grains.



Fig. 1. *Phyllanthus amarus*



Fig. 2. *Glycyrrhiza glabra*



Fig. 3. *Piper nigrum*

Table 1. Morphological characters

Plants	<i>Phyllanthus amarus</i>	<i>Glycyrrhiza glabra</i>	<i>Piper nigrum</i>
Colour	Dark green	Brown	blackish-brown
Odour	Characteristic	None	Characteristic
Taste	Characteristic, bitter	Sweet	Bitter
Shape	Long, palmate	Solitary	Spheroidal
Size	12-25cm	5cm long, 1.2 cm diameter	3.5-5.5 mm

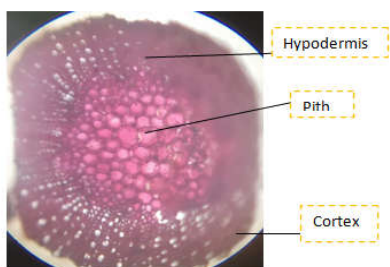


Fig. 4. T.S. of *Phyllanthus amarus*

3.3.2 T. S. of *Glycyrrhiza glabra*

Transverse section of root shows the presence of thick walled parenchyma from the cortex, Cork, groups of fibers surrounded by calcium oxalate crystals prism shape and spherical starch granules. thick walled polygonal cells, medullary rays and pith, lignified border pitted vessels, radially arranged xylem patches, separated from each other by medullary rays which are continuous through the cambium,



Fig. 5. T. S. of *Glycyrrhiza glabra*

3.3.3 T. S. of *Piper nigrum*

It shows the presence of Mesocarp consisting of parenchymatous cells, scattered with oil cells and vascular bundles. Exocarp consisting of 1 layer of epidermal cells and 2-3 layers of stone

cells. Testa consists of 2-3 layers of brown to dark brown compressed cells on the outer side, hyaline layer consists of 1 layer of transparent cells on the inner side. Endocarp consists of 1 layer of stone cells (beaker cells), inner tangential wall strongly thickened, and sometimes containing crystals of calcium oxalate. Perisperm consists of thin-walled polygonal cells, filled with small starch granules.

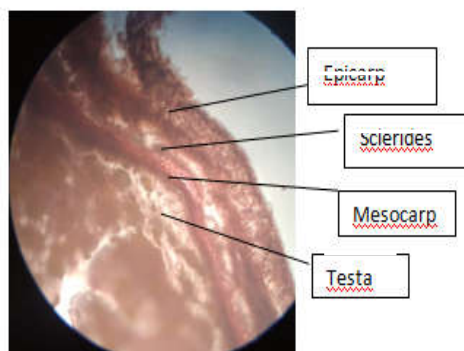


Fig. 6. T. S. of *Piper nigrum*

3.4 Powder Microscopy

Powder study is used for identification of internal characters present in the plants. It is similar to microscopic evaluation, but difference is that, only dried powder is taken instead of section of the plant. All the reagents and chemicals used for the analysis are also same as used in the above study.

3.4.1 Powder characters of *Phyllanthus amarus*

It shows the presence of Polygonal epidermal cells containing mucilage, anisocytic stomata, oval shape starch granule, glandular and multicellular trichomes are present.



Fig. 7. Multicellular Trichomes



Fig. 8. Glandular Trichomes

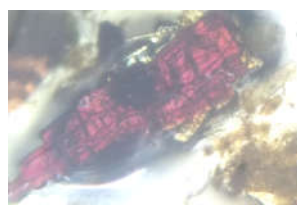


Fig. 9. Fibers



Fig. 10. Epidermal cells, starch grains



Fig. 11. Lignified fibers



Fig. 12. Tannins content

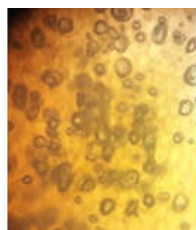


Fig. 13. Starch granules



Fig. 14. Calcium oxalate crystal



Fig. 15. Sclerides



Fig. 16. Stone cells



Fig. 17. Epicarp



Fig. 18. Oil cells

3.4.2 Powder Characters of *Glycerrhiza glabra*

It shows presence of characteristic features such as oval shape starch grains, Prism shape calcium oxalate crystals and xylem vessel, cork cells, tannins, lignified fibers.

3.4.3 Powder characters of *Piper nigrum*

It revealed Creamish-yellow; shows stratified cork, pieces of phloem fibres, stone cells and prismatic crystals of calcium oxalate.

3.5 Physicochemical Parameter

Many Physicochemical parameters have been studied 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 consists mainly of silica and indicates contamination with earthy material. Moisture content of drugs should be at a 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 extraction of any crude drug with a particular solvent yields 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.

3.6 Phytochemical Screening

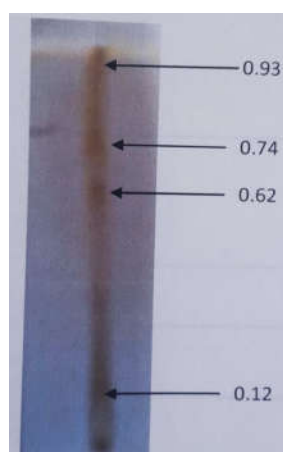
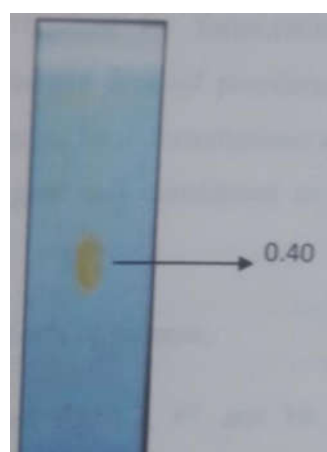
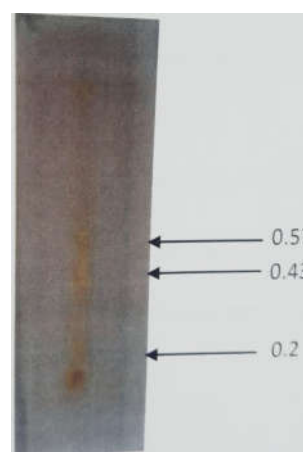
A lot of analytical techniques have developed for the quality control of drugs from plant origin. Therefore, it is very important to undertake the phytochemical investigations along with biological screenings to understand the therapeutic efficacy of medicinal plants and also to develop quality parameters. A preliminary phytochemical analysis of extract is essential for identifying plant constituents and to establish a chemical summary 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 analysis 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 2. Physicochemical parameter

Plants/ Parameter	<i>Phyllanthus amarus</i> %	<i>Glycerrhiza glabra</i> %	<i>Piper nigrum</i> %
Loss on drying	5.9	3.8	9.34
Foreign organic Matter	0.038	2.0	1.4
Foaming index	166.66	250	110
Total Ash value	21.2	7.5	9.33
Acid insoluble ash	6.48	1.5	0.05
Water soluble ash	12.50	1.7	2.07
Water soluble Extractive value	35.26	9.95	19.6
Alcohol soluble Extractive value	30.84	13.13	9

Table 3. Phytochemical screening

Plants	<i>Phyllanthus amarus</i>	<i>Glycerrhiza glabra</i>	<i>Piper nigrum</i>
Alkaloids	-	+	+
Glycosides	-	+	-
Flavonoids	+	+	-
Tannins	+	+	-
Proteins	-	-	+
Fixed oil	+	-	+
Steroids	+	+	+
Terpenoids	-	+	-
Carbohydrate	+	+	-
Saponins	+	+	-

Fig. 19. TLC of *Phyllanthus amarus*Fig. 20. TLC of *Piper nigrum*Fig. 21. TLC of *Glycerrhiza glabra*

3.7 TLC Profile

TLC study of extracts shows different compounds which was done by using different mobile phase, which revealed the different Rf

value which indicate the presence of different chemical constituents present in the extract. The result of TLC shown in Table 4. and Figs. 19, 20, 21.

Table 4. TLC profile

Plants	Extract	Solvent system	Rf Value
<i>Phyllanthus amarus</i>	Ethanolic extract	Chloroform: Methanol (6:4)	0.12, 0.62,0.74,0.93
<i>Glycerrhiza glabra</i>	Aqueous extract	Chloroform: Methanol (8:2)	0.2, 0.43, 0.57.
<i>Piper nigrum</i>	Ethanolic extract	Toluene: diethyl ether :dioxane(9.4:3.2:2.4)	0.40

4. CONCLUSION

In conclusion, the pharmacognostic features examined in the present study that includes morphological, anatomical, physico-chemical and TLC studies, may serve as a tool for identification/ validation of the raw material and standardization of its formulations in fixing quality control parameters as well as answer to the latest GMP norms and FDA guidelines on standardization of herbal drugs. In present investigation various standardization parameter such as organoleptic, physico- chemical parameter, fluorescence analysis, powdered drug reaction with different reagents, phytochemical screening and TLC analysis. The result of present study will also serve as a reference material in preparation of monograph.

NOTE

The study highlights the efficacy of " Siddha, Ayurveda, Unani and Allopathy " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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