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PHYSICO-CHEMICAL ANALYSIS OF THE LEAVES OF *DILLENIA BRACTEATA* AND *PRIVA CORDIFOLIA*

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ABSTRACT

The demand for herbal medicines is increasing because of fewer toxicity and side effects of the medicines. But, they also possess significant untoward activity if used without standardization. The present work was focused on standardizing the plant powders by assessing the quality parameters of same. The plants *Dillenia bracteata* (Dilleniaceae) and *Priva cordifolia* (Verbenaceae) are widely distributed throughout South India and are traditionally used for the treatment of various ailments. Physico-chemical parameters analyzed to the selected plants include Ash values, Extractive values, Moisture content and Fluorescence analysis, which were carried out as per WHO guidelines.

INTRODUCTION

Herbal medicines are widely used in the developing world, as a substitute to the pharmaceutical drugs. Increase in herbal medicines popularity brought concerns and fears over the professionalism of practitioners, and quality, efficacy and safety of their treatment methods and products from herbal and natural sources available in the market. The need for biological drugs from plant sources are increasing day by day for its comparably safer to synthetic drugs, less toxic, reliable and easy availability. Therefore the demand for promising drugs from plant sources is growing continually [1]. Physico-chemical evaluation is meant for identification, authentication and detection of adulteration and also assemblage of quality control standards of crude drugs. Physico-chemical parameters include Ash values which find out the quality and purity of a crude drug, especially in the powdered form. Hence ash determination provides a basis for judging the identity and quality of the drug and gives information about its adulteration with inorganic matter. Extractive values are used for perseverance of the nature of the chemical constituents present. The taxonomical identification of plant material and Pharmacognostic evaluation is important to provide the standards and to avoid adulteration of drugs [2]. The physicochemical evaluation helps in

formulating pharmacopoeial standards, while fluorescence analysis helps in distinguishing the drug in powder form. The physico chemical constants like moisture content, ash values, extractive values and fluorescence analysis are rarely constant for crude drugs, but they may help in evaluation. Ash value is a criterion to judge the identity and purity of crude drug. The extract obtained by exhausting crude drug is indicative of approximate measure of their chemical constituents. Determination of the moisture content helps prevent degradation. The fluorescence analysis is an appreciated, easy and direct method for the identification of fluorescent compounds [3]. Different compounds give fluorescence when exposed to short and long wavelength Ultraviolet light. Hence the aim of the study was to set physicochemical standards for the plants *Dillenia bracteata* and *Priva cordifolia* which are known to possess a wide range of therapeutic activities.

The plant *Dillenia bracteata*, commonly known as *Racemed fish-bone tree* is locally known as chiruthaeku in Telugu, bettadakanagalu in Kannada and colikkay in Tamil and belongs to the family *Dilleniaceae* and is widely distributed throughout South India[4].

The plant *Priva cordifolia* is commonly known as *Heart-Leaf Velvet Bur*. The local name of *P.cordifolia* in Telugu is Magalingaku and in Tamil it is called Ottu urinji and belongs to the family *Verbenaceae* and is widely distributed throughout South India.

MATERIALS AND METHODS

Fresh leaves of *Dillenia bracteata* were collected from Tirumala hills, Chittoor District, Andhra Pradesh, in the month of February. Fresh leaves of *Priva cordifolia* were collected from Talakona forest, Chittoor District, Andhra Pradesh, in the month of September.

The taxonomical identification and authentication of both the plants were done by Dr. K. Madhava Chetty, Assistant Professor, S.V. University, Tirupati, Andhra Pradesh. The leaves were collected and dried under shade at room temperature for 5 days. Later the leaves were grinded into powder coarsely and passed through mesh size no. 50. The powdered sample was stored in a closed container free from pollution and environmental contaminants [5]. Voucher specimens of both the plants have been kept in the herbarium (DB/SNS/SVCP/2016/04 and PC/SNS/SVCP/2017/12) at the Department of Pharmacognosy, Sri Venkateswara College of Pharmacy.

Determination of Physico-chemical characteristics

The moisture content, total ash content, water soluble ash content and acid insoluble ash content, water soluble extractives, alcohol soluble extractives and ether soluble extractive and Fluorescence analysis were the parameters of determining the physicochemical characteristics. The physicochemical studies were done as per WHO guidelines [6,7,8]. The results are depicted in Tables 1 - 4.

Ash values

Ash values of the plant material were calculated according to standard procedures [9].

Total ash

The powdered material (5 g) was accurately weighed and placed in a china dish. The material was then spread in an even layer and it was ignited by gradually increasing the heat to 500–600°C until it was white indicating the absence of carbon. The residual ash was allowed to cool in a desiccators. The content of total ash (w/w) of air-dried material was calculated with the following formula.

$$Percentage\ total\ ash = \frac{weight\ of\ ash\ (g)}{weight\ of\ sample\ (g)} \times 100$$

Determination of acid insoluble ash content

Acid insoluble ash was determined by adding 25 mL of 2N HCl into china dish containing previously weighed ash. Covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsed contents were added to the china dish. The acid insoluble ash was collected on an ash less filter paper. The filter paper containing acid insoluble ash was transferred to the previously used china dish, dried in oven. The residue was allowed to cool in a desiccator and weighed. The content of the acid insoluble ash (w/w) of air-dried material was calculated as follows;

$$Percentage\ acid\ insoluble\ ash = \frac{weight\ of\ ash\ (g)}{weight\ of\ sample\ (g)} \times 100$$

Determination of water soluble ash content: Water insoluble ash was determined by adding 25 mL water into china dish containing previously weighed dry ash. Covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsed contents were added to the china dish. The water insoluble ash was collected on an ash less filter paper. The filter paper containing water insoluble ash was transferred to the previously used china dish. The china dish was dried in oven. The residue was allowed to cool in a desiccator and weighed. The content of the water insoluble ash (w/w) of air-dried material was calculated.

UV Fluorescence analysis

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, acetic acid, ferric chloride, and nitric acid with ammonia. They were subjected to fluorescence analysis in daylight and UV light of short wavelength (254 nm) and UV light of long wavelength (365 nm) for their characteristic colour [12].

$$Percentage\ water\ soluble\ ash = \frac{weight\ of\ ash\ (g)}{weight\ of\ sample\ (g)} \times 100$$

Extractive values

Extractive values were calculated according to the procedure described by Arambewela and Arawwawala (2010) [10]. Accurately weighed 5 g of powdered plant material was taken. Then 100 mL of each solvents of 50% chloroform-water, ethanol and petroleum ether was procured. Maceration of the drug was carried out within a closed container for 24 h with the aid of mechanical shaker for 6 h. Then let it stay further as it is for next 18 h. The extract was then filtered. The filtrate 25 mL in pre-weighed petridish was evaporated to dryness in oven at 105 °C and after then weighed it again. The percentage w/w extractive values of all solvents with reference to drug was calculated

$$Percentage\ extractive\ value = \frac{weight\ of\ residue\ (g)}{weight\ of\ sample\ (g)} \times 100$$

Moisture Content – By the Procedure of Loss on drying.

Accurately weighed 1 g powder of leaves was placed in china dish. Then it was placed in an oven at a temperature of 100°C for an hour. The powder was weighed again and compared with the original weight of the powder. The process was continued with an interval of half an hour till two successive weights were concordant. The loss on drying was calculated by the following expression [11].

$$Percentage\ Moisture\ content = \frac{Final\ weight\ of\ sample\ (g)}{Initial\ weight\ of\ sample\ (g)} \times 100$$

Statistical analysis: All the results were calculated in triplicates. The data are represented as mean ± SEM. Microsoft Excel 2013, is used for statistical analysis.

RESULTS AND DISCUSSIONS

Physicochemical parameters are important parameters in detecting adulteration and are adopted to confirm the purity and quality of drug. The results of Ash values, Extractive values, Moisture content and Fluorescence analysis are depicted in **Table 1, 2, 3 & 4.** Ash value is particularly important parameter as it shows the presence and absence of foreign matters like metallic salts or silica.

Physico-chemical parameters

Ash values

Table 1. Ash values of powdered leaf of *Dillenia bracteata* (*Dillenaceae*) and *Priva cordifolia* (Verbenaceae)

Ash values	*D. bracteata* leaf powder (%w/w)	*P. cordifolia* leaf powder (%w/w)
Total Ash value	9.8 ± 0.0586	9.21 ± 0.0573
Acid insoluble ash value	3.2 ± 0.0577	2.49 ± 0.0152
Water soluble ash value	5.26 ± 0.0371	5.14 ± 0.0881

Extractive values

Table 2. Extractive values of powdered leaf of *Dillenia bracteata* (*Dillenaceae*) and *Priva cordifolia* (Verbenaceae)

Extractive values	*D. bracteata* leaf powder (%w/w)	*P. cordifolia* leaf powder (%w/w)
Alcohol soluble extractive	19.67 ± 0.0315	22.7 ± 0.0182
Ether soluble extractive	6.91 ± 0.0278	4.11 ± 0.0421
Water soluble extractive	15.86 ± 0.0824	18.62 ± 0.0632

Moisture Content

The percentage moisture content of powdered leaf of *Dillenia bracteata* and *Priva cordifolia* were found to be 5.70 ± 0.1000 % w/w and 4.29 ± 0.0989 % w/w respectively.

Fluorescence Analysis

Table 3.Fluorescence analysis of powdered leaf of *Dillenia bracteata (Dilleniaceae*)

Treatments (*D. bracteata* leaf powder)	Observations		
	Visible/ Day light	Short UV (254 nm)	Long UV (365 nm)
Powder as such	Green	Green	Dark Green
Powder + 1 N Aqueous NaOH	Yellowish green	Pale blue	Dark blue
Powder + 1N Alcoholic NaOH	Yellowish green	Dark green	Dark brown
Powder + 1 N HCl	Green	Greenish yellow	Greenish brown
Powder + Conc. H_2SO_4	Brown	Dark Green	Dark brown
Powder + Conc. HNO_3	Pale green	Fluorescent green	Dark brown
Powder + Conc. HCl	Green	Pale green	Dark Green
Powder + 50% H_2SO_4	Brown	Fluorescent green	Dark Green
Powder + 50% HNO_3	Greenish yellow	Fluorescent green	Violet
Powder + 40% NaOH + 10% lead acetate	Pale green	Fluorescent green	Dark Green
Powder + Acetic acid	Yellowish green	Pale green	Dark Green
Powder + Ferric chloride	Brown	Dark Green	Dark blue
Powder + NH_3	Green	Fluorescent green	Bluish green
Powder + Benzene	Dark Green	Yellowish green	Blue
Powder + Petroleum ether	Green	Yellowish green	Dark Blue
Powder + Acetone	Yellowish green	Fluorescent green	Dark brown
Powder + Chloroform	Dark Green	Pale Yellow	Blue
Powder + Methanol	Yellowish green	Yellowish green	Brown
Powder + Ethanol	Yellowish green	Fluorescent green	Dark brown

Table 4.Fluorescence analysis of powdered leaf of *Priva cordifolia* (Verbenaceae)

Treatments (*P.cordifolia* leaf powder)	Observations		
	Visible/ Day light	Short UV (254 nm)	Long UV (365 nm)
Powder as such	Dark Green	Pale Green	Dark Green
Powder + 1 N Aqueous NaOH	Greenish yellow	Pale Blue	Dark blue
Powder + 1N Alcoholic NaOH	Yellowish green	Dark green	Dark brown
Powder + 1 N HCl	Dark Green	Greenish yellow	Dark brown
Powder + Conc. H_2SO_4	Pale Brown	Pale Green	Brown
Powder + Conc. HNO_3	Pale green	Fluorescent	Dark Green

		green	
Powder + Conc. HCl	Green	Pale green	Dark Green
Powder + 50% H_2SO_4	Pale Brown	Green	Dark Green
Powder + 50% HNO_3	Yellowish green	Fluorescent green	Violet
Powder + 40% NaOH + 10% lead acetate	Pale green	Fluorescent green	Dark Green
Powder + Acetic acid	Yellowish green	Pale green	Dark Green
Powder + Ferric chloride	Brown	Yellow	Dark yellow
Powder + NH_3	Green	Fluorescent green	Dark Green
Powder + Benzene	Green	Fluorescent green	Blue
Powder + Petroleum ether	Pale green	Fluorescent green	Blue
Powder + Acetone	Yellowish green	Pale green	Blue
Powder + Chloroform	Green	Dark Green	Blue
Powder + Methanol	Pale Yellow	Pale Blue	Blue
Powder + Ethanol	Yellow	Fluorescent yellow	Blue

CONCLUSION:

Since time immemorial plants have been playing an important role in the treatment of various ailments. Herbal plants are considered to be a source of varied phytoconstituents exhibiting various pharmacological activities. Therefore it becomes a necessity to study the phytochemical constituents and physico-chemical characteristics before its use in the field of research and pharmaceutical formulation [13]. The extractive values are an integral part of physicochemical analysis and these quantitative values are determined for further standardization of the plant powder. They are indicative weights of chemical constituents from the crude drug. A high extractive value indicates the better extraction of phytochemicals from the plant material with the particular solvent being used for the procedure. These values also aid in the selection of best solvent that will further assist in having maximum yield [14]. The ash values for the powdered plant are calculated to figure out the amount of siliceous material left over in the residue and to determine the extraneous material adhered to the plant while its collection. A plant which hasn't undergone garbling process usually shows highest ash values and is rendered as of poor quality. On the other hand, loss on drying tells about the moisture content present in the dried or collected plant material, as presence of excess moisture than stated leads to microbial degradation. The presence of excessive moisture is also one of the leading causes for the deterioration of the constituents within the plant as it brings about the hydrolysis of the constituents [15]. A low level of moisture helps in the prevention of microbial contamination [16]. The current investigation describes the physicochemical quantifications of *Dillenia bracteata* (Dilleniaceae) and *Priva cordifolia* (Verbenaceae). It was found that Ethanol had maximum extractive yield. Ash values added more strength to crude drug standardization with prominent results indicating minimal

involvement of extraneous matter. Such study on the physicochemical parameters is important information which may be useful in confirmation and quality control of these medicinal plants.

Declaration of interest

We declare no conflict of interests regarding the publication of this paper.

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