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PROXIMATE COMPOSITION FROM SELECTED MEMBERS OF ORDER MYRTALES

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ABSTRACT

Herbal drugs are one of the very well-known medicines adapted by millions of people worldwide since ancient time. The plant kingdom is a treasure of potential phytochemicals that can be utilized to treat variety of medical conditions. With the advent of increase in demand and knowledge of herbal medicines quality of these herbal medicines has grab the attention of various pharma industries. Thus, certain standard parameters to determine the quality of raw material are described in the present article. In the current study the quality of raw material 16 members of order Myrtales constituting 5 families *i.e.* Myrtaceae, Lythraceae, Lecythidaceae, Melastomataceae and Combretaceae from different regions of Maharashtra were carried out. Allied variety of plants from the region of Mumbai and Lonavala were considered for the study. Physicochemical parameters such as Moisture content, Total Ash Value, Acid soluble Ash, and Water-soluble Ash were determined. Values of proximate analysis of raw material were found to be within permissible limit as specified in pharmacopeia.

Keywords: Proximate Analysis, Myrtales, Moisture content, Total Ash, Insoluble Ash, Water soluble Ash.

1. INTRODUCTION

One of the oldest forms of healthcare known to humanity, used in all cultures throughout the history is the use of herbal medicine [1]. The knowledge of plantbased drugs developed gradually and then passed on for laying the foundation of traditional medicine systems all over the world. In some communities' herbal medicine is still believed as an epicenter of their medical system. The inclination towards the herbal medicine has gain popularity due to multiple notable side effects from western medicine [2-7]. The trust in herbal medicine has once again captivate attention towards its effective results against disease and ailments. One of the biggest examples for tremendous shift for the use of herbal and ayurvedic medicine was during COVID-19, where worldwide Ayurveda was rejuvenated within no time. Since the important phytoconstituents from plants are widely distributed and are directly or indirectly derived from plants, the discovery of these chemical compounds led to the identification of new potentially viable drugs [7, 8].

Quality control has been a critical operation in the pharmaceutical sector. Herbal medicine has a poor scientific foundation in its recognition as evidence-based medicine. Plants undoubtedly are one of the most fascinating categories because, they are the sources of bioactive molecules [4, 9] but can be toxic if preparation isn't authentic or as per the standard protocol. The therapeutic effect derived from the plant extracts accounted for medicinal use, rests on the principle of bioactive compounds [10]. The most evident quality of secondary metabolites is the enormous structural diversity. These are classified according to their chemical structures in different classes namely Alkaloids, Flavonoids, Phenolics, Saponins, and Terpenes [11]. For instance, due to the diverse active molecules in plants, the challenges in availability and quality of the raw materials circulation are often a major problem. There are countries identified, where the herbal product is in the market without any proper scientific assessment, safety, and toxicological data due to lack of effective regulation to manufacturing practices and quality standards [12]. Consumers can buy those products without prescription, with an unknown consequence post administering of such medicine. A well-defined and standard composition of the raw materials is therefore, one of the most important prerequisites to produce a quality drug. Considering products obtained naturally, of

plant origin, which are generally considered as unstable owing to various chemical/physical factors, although correctly authenticated ensuring the consistent quality of products is critical to the sustainability of the industry [13, 14]. It is also important to understand that variations in the quality could be possible even if different batches of the same herbal ingredient are used, factor affecting these qualities are based on i) Country origin ii) Environmental factors iii) Time of harvesting. iv) Plant part used and v) post-harvesting factors [12].

The interrelationship between biotic and abiotic factors in plants, has thus evolved itself to intricate alternative defense strategies which involve an enormous variety of Natural products. These chemical metabolites or secondary metabolites are produced as a by- product of regular metabolic pathways which produced primary metabolites needed for normal growth and development. Synthesis of specific secondary metabolites are restricted to plant families [10]. These complex secondary metabolites often contain more than one functional group and often exhibit multiple functionalities and bioactivity [15]. With all these complex and structural diversity, the synthesis of secondary metabolites is limited from the primary metabolism [16].

A basic requirement of being safe and effective should be fulfilled irrespective of synthetic or plant-based products [17]. It thus, becomes the responsibility of regulatory bodies to ensure that consumers get medication, which guarantees purity, safety, potency, and efficacy and set up a standard for assessment. The regulatory authorities follow various standards of quality prescribed for raw materials and finished products documented in formularies, pharmacopoeias, and manufacturing through operation statutory imposed Good Manufacturing Practices (GMP). Currently there's no official standard methodology is available for the herbal preparation. Those manufactures who are testing the formulation prior to production, have standardized their own parameter, with most of them are only preliminary in nature. At present, the biggest challenges are to identify the presence of ingredients in any formulation. Thus, the concern with respect to standardization seems of great to be worrying hence, the first important task is to design such parameters by which the presence of the entire ingredient can be identified, for which biggest challenge is identification present in any formulation, chromatographic and Spectrophotometric various methods can be taken into consideration. Besides qualitative estimation certain quantitative method for evaluation of bioactive compounds might be helpful [12].

Having said this, the censorious to standardize the product remains same. Few notable challenges that are not applicable to synthetic drugs might influence the quality of herbal drugs. If quality is compromised, undesired harmful effect shall be recorded. These adverse effects are attributed either due to poor quality or the improper use. Hence, the commencement of guidelines with the view of monitoring the safety of medicines should be enhanced and broadened [18]. According to [19-21], standardization and quality control of herbals is majorly identified by evaluating physicochemical properties of crude drug, pertaining to selection of the plant, handling of crude material, safety aspects, efficacy and stability study of finished product, data on safety and risk based on experience, provision of product information to consumer and product promotion [12].

To achieve the desired quality of herbal drugs a resolute attention towards evaluating procedure for crude/raw herbal materials shall be considered. Hence, the preliminary steps shall cover the identification of the correct raw material, kind of adulterants and impurity that generally comes along, this can be achieved by carrying out Macro and microscopic analysis. Subsequently, the grade of the product can be determined by Ash value (i.e, Total ash, Water-soluble Ash and Acid- Insoluble Ash content). Similarly, evaluating foreign organic matter determine the matter present other than source plant. Moisture content determine the percentage of moisture present, so as to avoid microbial contamination at initial stage. Low moisture content evinces better stability against degradation of product. Extractive values to evaluate the chemical constituents extracted under different solvents this can be used to identify exhausted raw material in terms of their phytoconstituents. Identification and characterization of crude drug concerning phytochemical determined constituent, hence can be by physicochemical analysis [12, 7].

Quality has always been a concern for WHO, there by recognizing the growing need for standardization and maintaining the quality of herbal drugs [22, 23]. Some internationally recognized pharmacopoeias have provided monographs stating the standards and parameters for many herbal products. These pharmacopoeias are 1) Chinese Herbal Pharmacopoeia 2) British Herbal Pharmacopoeia 3) United States Herbal Pharmacopoeia 4) British Herbal Compendium 5) Japanese Standards for Herbal Medicine 6) The Ayurvedic Pharmacopoeia of India (API) has laid down monograph for herbs and herbal products to maintain quality in the respective nations.

The subject of herbal drug standardization has an immense potential yet, further research needs to be conducted to understand the effectiveness of drugs in human beings and to solve the intricacies related to contradictory theories about herbal medicines. For standardization of herbal formulations, a profound knowledge of important herbs, which is widely described in Ayurvedic formulation is of prime importance. With an advent of change in technology, evaluation of herbal medicine using advanced and modern techniques of standardization such as UV-visible, TLC, HPLC, HPTLC, GC-MS, spectrofluorimetric and other methods shall add more value to such work.

2. MATERIAL AND METHODS

2.1. Collection of plant material

The healthy plants were collected from the different regions. The study was conducted bycollecting the leaves from Mumbai (18.9787° N, 72.8351° E) and Lonavala (18.7557° N, 73.4091° E) Maharashtra, India pertaining to three different seasons *viz;* Winter, Monsoon and Summer. The collected leaves were thoroughly washed with water and were used for determining moisture content of the plants whereas, for the other parameters the materials were dried at room temperature for a day, further dried using a hot air oven at 55°C for 48 hours. The dried plant material was pulverized to a coarse powder using a grinder, sieved using a 180-micron mesh; then it was collected and stored in air-tight containers for the further analysis.

2.1.1. Determination of moisture content [24]

The moisture content present in any plant product facilitates growth of microbes which further leads to crude drug destruction. The preparation of crude drug from the harvested drug plants involves cleaning to remove soil or other extraneous material followed by drying which plays important role in the quality as well as purity of the material.

The method referred from ASTM 2216 with slight modification. The moisture is the indicator of the amount of water present in the plant material. Moisture content is the defined as the ratio of the mass of water present in the sample to the mass of solids, expressed in percentage.

Empty crucible was weighed, and 10 gm of fresh plant material was added, followed by keeping it in oven for 6 hours at 60°C. The method of weighing the material

consecutively performed for a week until constant weight of the dried material was obtained. Further to this Moisture content was calculated.

Moisture content is Calculated as per the equation: -Moisture Content = W2- W3 *100; Where, Weight of crucible, W1 = A (g); Weight of crucible and plant Material, W2 = B(g) and Weight of oven dried material W3 = C (g).

2.1.2. Determination of Ash value [25]

Ash is measured as the residue of the crude drugs obtained after incineration, mostly inorganic salts, and non-volatile inorganic components. An ash value implies the naturally inherent inorganic salts or those imparted from external sources [7]. This value varies within wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs. Total ash usually consists of carbonates, phosphates, silicates and silica which includes both physiological ash- which is derived from the plant tissue itself and non-physiological ashwhich is the residue adhering material to the plant surface e.g, sand and soil. In certain drugs, the % variation of the weight of Ash sample to sample is very small and any marked difference indicates a change in quality. Direct contamination, such as sand or earth is forthwith detected by the ash value.

2.1.3. Total Ash

The process of oxidation of components is involved while enacting Ash content. Increase in a value indicates contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing. Total Ash is designed to measure the total amount of material produced after complete incineration of the ground drug with a 450°C temperature to remove all the carbons. The alkali chloride may be volatile and might get lost by this process. The total ash value generally gives an idea on the presence of Carbonates, Phosphates, Silicates, and Silica whichincludes both: Physiological Ash obtained from the plant tissue itself and Non- physiological Ash which is residue of the adhering material to the plant like sand and soil.

1gm leaf powder of 16 different plants were weighed and transferred to a pre weighed crucible. The powders were incinerated at a temperature exceeding 450°C until free from carbon. After incineration, the crucibles were cooled in a desiccator, weighed and percentage of total ash was determined and documented as mean of three reading for each plant material.

Total ash is Calculated as per the equation: - Total ash= $(W_1-W_2) \times 100/W_3$; Where, W_1 = weight of empty crucible (grams) W_2 = weight of crucible with ash and W_3 =weight of powder taken (grams).

2.1.4. Acid insoluble ash

Acid insoluble ash is the residue obtained after extracting the total ash with HCl, with reference to 100 gm of drug. Acid insoluble ash value particularly indicates contamination with silicious material e.g., earth and sand. Comparison of this with the total ash value of the same sample will differentiate between contaminating materials and natural ash of the drug. It is documented that the acid insoluble ash value should not exceed 2%.

Total ashes of leaf powder of all 16 plants were obtained using above-described procedure. 25ml of 2N HCL was added to the ash and the mixture was mixed for 5 minutes. The mixture was filtered through ashless filter paper (Whatman filter paper no. 41 and washed with hot water. This filter paper (with the insoluble matteri.e., the acid insoluble ash) was transferred to a pre weighed crucible and ignited till constant weight was obtained. The crucible was cooled in a desiccator, weighed and the percentage of acid insoluble ash of all the 16 plants was calculated. The experiment was designed in three sets for each plant material.

Acid insoluble ash is Calculated as per the equation: -Acid insoluble ash= $(W1-W2) \times 100/W3$ Where, W1= weight of empty crucible (gram)W2= weight of crucible with ash (gram) and W3 weight of powder taken for obtaining total ash (gram)

2.1.5. Water soluble ash

Water soluble ash is that part of the total ash content which is soluble in water. Water soluble ash value is the difference in between the total ash and the residue obtained after treatment of total Ash with water. It is good indicator of either previous extraction of the watersoluble salts in the drug or incorrect preparation of raw material.

Total ash of the powder of the 16 plants was obtained using the procedure described. Where, 25ml of water was added to the ash and the mixture was boiled for 5 minutes. The mixtures were filtered through Whatman filter paper no 41 and washed with hot water. This filter paper (with insoluble matter) was transferred through a pre weighed crucible and ignited at a temperature not exceeding 450°C till constant weight was obtained. The crucible was cooled and weighed. The percentage of water-soluble ash in all these plants was calculated a below. The results are expressed as mean of three sets of reading.

Total ash is Calculated as per the equation: - Total ash $(W4) = (W1-W2) \times 100/W3$; Where, W1= weight of empty crucible (gram)W2= weight of crucible with ash (gram) and W3= weight of powder taken for obtaining total ash (gram).

After addition of water and filtering the solution, Water insoluble ash (W7) =W5-W6; Where, W5=weight of crucible with ash (gram) W6=weight of empty crucible (gram); Water soluble ash=W4-W7 X100/W3

3. RESULTS

The above data represent consolidate result of Moisture content in Mumbai and Lonavala region, pertaining to three seasons i.e. Monsoon, Winter, and Summer. The highest moisture content in the region of Mumbai recorded from Lagerstroemia speciosa followed by Terminalia catappa, Getonia floribunda and Couroupita guanensis during monsoon. However, in the region of Lonavala no Major changes in the values of moisture content was recorded and following plant species for their highest moisture contents were recorded from Lagerstroemia speciosa followed by Getonia floribunda and Couroupita guanensis during Monsoon. Whereas, in the season of summer the highest moisture content was recorded from the plant of Terminalia crenulata as followed by Getonia floribunda, Couroupita guianensis, Combretum indicum and Eucalyptus globulus. In the region of Lonavala, the moisture content recorded highest from Terminalia crenulata, Couroupita guianensis. Syzygium cuminii and Psidium guajava. The data recorded for winter season for moisture content represents highest moisture value from Lagerstroemia speciosa, Syzygium jambos, Getonia floribunda, Terminalia crenualata and Couroupita guanensis. For the Lonavala region Syzygium jambos, Terminalia cremulata, Couroupita guanensis and Terminalia catappa supported the data with highest moisture content.

A comparative study on the elite plants of order Myrtales in monsoon season for different regions were considered for determination of the Ash content. The data for Mumbai region obtained shows the maximum ash content value observed in *Combretum indicum* 12.97%, *Terminalia catappa* 12.59%, *Terminalia mantaly* 13.22% and *Pimenta dioica* 10.26%. Whereas the data obtained for Lonavala region shows the maximum ash content in *Pimenta dioica* 13.84%, *Terminalia bellirica* 14.19%, *Psidium guajava* 7.68% and *Terminalia catappa* 10.12%.



Fig. 1: Representation of Moisture content of Mumbai region in different seasons studied.



Fig. 2: Representation of Moisture content of Mumbai region in different seasons studied.



Fig. 3: Representation of Total Ash value from the region of Mumbai in different seasons studied.





For summer season, the data for Mumbai region shows the maximum ash content value observed in *Terminalia mantaly* 10.32%, *Combretum indicum* 9.92%, *Memecylon umbellatum* 9.573%, *Lagerstroemia speciosa* 9.39% and *Terminalia crenulate* 8.33%. Whereas for Lonavala the highest Ash content observed in *Pimenta dioica* 14.01%, *Terminalia bellirica* 11.51%, *Terminalia catappa* 10.72% and *Combretumindicum* 11.15%. For Winter, in the region of Mumbai the total ash contents in *Combretum indicum* was 15.24% followed by *Terminalia catappa* 12.17%, *Pimenta dioica* 10.6%, *Terminalia bellirica* 10.11%. whereas, for the Lonavala region the highest value of ash content was observed in *Combretum indicum* with 12.51%, *Terminalia bellirica* 13%, *Terminalia mantaly* 11.88% and *Terminalia catappa* 10.96%.



Fig. 5: Representation of Insoluble Ash Content value from the region of Mumbai in different seasons studied.



Fig. 6: Representation of Insoluble Ash Content value from the region of Lonavala in different seasons studied.

The result for Acid insoluble ash content for Mumbai shows maximum Acid insoluble ash content in Terminalia mantaly 1.39%, Combretum indicum 1.36%, Terminalia catappa 1.38%, and Pimenta dioica 1.26%. For Lonavala region the results showed variation in the where, Terminalia catappa recorded with 9.49% of acid insoluble content followed by Syzygium cumini 9.92%, Psidium guajava 7.25%, and Syzygium jambos 7.19%.

The result for Acid insoluble ash content for Mumbai (summer), *Terminalia catappa* 1.40% of insoluble followed by *Pimenta dioica* 1.27%, and *Couroupita guianensis* 1.03%. for Lonavala region, *Pimenta dioica* 2.55% followed by *Terminalia crenulata* 1.51%, *Memecylon umbellatum* 1.34%, likewise *Callistemon citrinus* 1.20% and *Lagerstroemia speciosa* 1.05%.

The result for Acid insoluble ash content for Mumbai and Lonavala (Winter), For Mumbai region, it shows *Terminalia catappa* 1.37% and others less than 1% of acid insoluble ash. for Lonavala region, *Pimenta dioica* 2.65%, *Lagerstroemia speciosa* 2.36%, *Couroupita guianensis* 2.4% followed by *Terminalia catappa* 1.54%, and *Terminalia bellirica*. 1.15%.

Determination of water-soluble ash value for Mumbai and Lonavala (Monsoon) region represents Lagerstroemia *speciosa* with 4.06% the highest value as compared to the other studied plants, *Couroupita* guianensis 3.77% and Combretum indicum 3%. For the region of Lonavala in monsoon, Memecylon umbellatum showed 16.44% the highest value for water soluble ash content whereas, Pimenta dioica 14.17%, Terminalia catappa 10.42% and Combretum indium 0.37%.

The result obtained for summer season in Mumbai and Lonavala region, for Mumbai region it shows *Terminalia catappa* with 3.75% of water-soluble ash whereas, *Lagerstroemia speciosa* shows 3.60%, *Careya arborea* 3.54%, *Combretum indicum* 3.23% and *Pimenta dioica* 2.94%. For Lonavala region in summer, *Memecylon umbellatum* 4.59%, *Couroupita guianensis* 4.37%, *Terminalia catappa* 3.45%, *Psidium guajava* 3.38% and *Terminalia crenulata* 2.43%.

The result obtained for winter season in Mumbai and Lonavala, Mumbai region shows *Lagerstromia speciosa* with 4.48% of water-soluble ash whereas, *Eucalyptus globulus* shows 4.08%, *Terminalia catappa* 3.85%, *Combretum indicum* 3.78%, *Terminalia crenulata* 2.96% and *Memecylon umbellatum* 2.54% of water-soluble ash content. For Lonavala region in winter, *Lagerstroemia speciosa* 5.30%, *Eucalyptus globulus* showed 5.28% of water insoluble ash content, followed by *Terminalia catappa* 4.76%, *Terminalia crenulata* 4.48% and *Callistemon citrinus* 3.07% of water-soluble ash content.



Fig. 6: Representation of Water-Soluble Ash Content from the region of Mumbai in different seasons studied.



Fig. 7: Representation of Water Soluble Ash Content from the region of Lonavala in different seasons studied.

4. DISCUSSION AND CONCLUSION

The study of herbal drug standardization is colossal and vast [26]. The safety and efficacy of herbal products are dependent upon the standardization of these herbal drugs. [27, 28]. Subsequently the herbal industries are growing tremendously with many prevalent contradictory theories about herbal medicines on its efficacy on human health [26]. Narrowing down to ideal standard method would be great in terms of sustaining the quality which may bring down adverse effects of some medicinal herbs. This study provides insights on the legitimate comparison on the quality of the product which are aligned with the standards specified in the monographs. The method is thus, used to generate the data on product properties/purity when used in food, cosmetics, pharmaceutical and nutraceutical industries.

Conflict of Interest

No Conflict of Interest

5. REFERENCES

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