COMPARATIVE STUDY OF MEDICINAL PLANTS AS POTENTIAL EMPHASIS ON PHYSICOCHEMICAL AND PHYTOCHEMICAL PARAMETERS

Surbhi Jain[1]*, Hemant Swami[2], Raghvendra Dubey[3]

Abstract

Medicinal plants are invaluable sources for the discovery of novel molecules in scientific research. The accurate identification, authentication, and collection of these plants are imperative prerequisites for research endeavors. In this investigation, we procured and subjected four medicinal plants, namely *Ocimum sanctum, Lawsonia alba, Trigonella foenum graecum, and Carissa carandus,* to meticulous extraction and subsequent assessment of their physicochemical and phytochemical parameters. The physicochemical evaluation entailed determining the percentage of total ash, acid-insoluble ash, water-soluble ash, loss on drying, alcohol-soluble extractive value, and water-soluble extractive value to ascertain the purity and quality of the plant materials. Additionally, phytochemical screening was conducted on freshly prepared extracts to ascertain the presence of chemical constituents responsible for the identified pharmacological activities. The outcomes of this investigation shed light on the purity and quality of the plant drugs through physicochemical characterization

Keywords: Extracts, Physicochemical, Phytochemical, Plant drug

[1*,2,3]Institute of Pharmaceutical Sciences, SAGE University, Indore (M.P.) India

***Corresponding author:** Surbhi Jain
*Research Scholar, Institute of Pharmaceutical Sciences, SAGE University, Indore (M.P.) India
Email: surbhijain2425@gmail.com

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1. INTRODUCTION

Nature is rehabilitation for individual ailments that have diverged around the World. Plants have created one of the most complicated established medicine systems and have been persistent for thousands of years dating reverse to early humans. They represent an effectual source of traditional and modern medicines and play an important role in health care programs (1). The majority of the pharmaceutical industry is highly needy of the wild population for the liberation of raw material for the extraction of medicinally important compounds.

The genetic multiplicity of medicinal plants in the world is receiving scarce at a frightening rate because of harmful harvesting practices and over-harvesting to produce medicines, with little or no regard for the future. In contemporary medicine, plants are used as sources of straight therapeutic agents, as representation for novel synthetic compounds, and as a taxonomic marker for the amplification of more complex semi-synthetic chemical compounds (2). Herbal medicines are a talented choice over modern synthetic drugs, they show minimum or no side effects and are considered to be safer relatively. Generally, herbal formulations engage the use of fresh or dried plant parts. Accurate knowledge of such crude drugs is a very important aspect in preparation; safety and efficacy of the herbal product. Plants have been used as folk remedies. The ethno-pharmacology provides an option to move toward the discovery of antimicrobial agents, specifically the study of medicinal plants with a history of traditional use as a potential source of substances with significant pharmacological and biological activities.

The extraordinary donation of plants to the drug industry was probably because of the large number of phytochemical and biological studies all over the world. The Indian subcontinent is endowed with rich and diverse local health tradition, which is equally matched by rich and diverse plant genetic foundation. A comprehensive examination and documentation of plants used in local health traditions and ethno-pharmacological assessment to confirm their efficacy and safety can lead to the development of priceless herbal drugs or isolation of compounds of therapeutic value. Out of four plants undertaken for this study *Ocimum sanctum* e.g. Tulsi is a prominent herb in Ayurveda that exhibits diverse pharmacological actions and address physical, chemical, metabolic as well as psychological stress. It protects organs from industrial pollutants, heavy metals, and physical strain, it normalizes blood glucose, blood pressure, and lipid levels. It works as an anxiolytic, anti-depressant, and cognitive-enhancer. Tulsi's broad-spectrum antimicrobial activity supports its use as a sanitizer, mouthwash, and water purifier. Never the less, it also aids in wound healing, food preservation, and animal rearing. Cultivation of tulsi serves spiritual and practical purposes, providing solutions for poverty, hunger, and environmental concerns (3). The next herb of the study is *Lawsonia alba*, also known as 'Mehndi'. It is a shrub cultivated in India, the Middle East, and along the African coast. It is popularly used for cosmetic purposes like hand staining and hair dyeing. The leaves possess prophylactic properties against skin diseases, while the bark is known for its potential in treating jaundice, spleen enlargement, calculus affliction, and skin ailments (4). Next is *Trigonella foenum-graecum* e.g. Fenugreek, it is a versatile medicinal plant with multiple therapeutic uses. Its seeds are golden-yellow rhomboidal-shaped, but the leaves and stem also possess medicinal properties. Fenugreek contains various phytochemicals such as alkaloids, saponins, tannins, and phenols.

Traditionally, it has been used for labor assistance, lactation stimulation, and as a laxative. Modern research supports its therapeutic effects, making fenugreek a promising candidate for new drug development (5). And the last plant included in the study is *Carissa carandas*, also known as karonda, which is an evergreen thorny shrub from the Apocynaceae family. Its small berry-shaped fruits are commonly used in pickles and spices in northern India. It thrives in various soil types and exhibits drought-resistant qualities. With over 25 known Carissa species, five are native to India. The fruit is highly valued for its rich content of iron, vitamin C, and pectin, making it a sought-after ingredient in jams, jellies, and syrups. *Carissa carandas* is utilized in Ayurvedic preparations, with root extracts used for chest pain and leaf extracts employed in fever treatment (6).

Natural products are the source for bioactive compounds and has possible for developing some novel therapeutic agent (7). Authentication and standardization are precondition steps, predominantly for herbal drugs and their formulations in traditional systems of medicine. The aim of the study is to accentuate (emphasizing/ underlining) on the phytochemicals and their standardization to access the quality parameters of the undertaken medicinal plants.

2. EXPERIMENTAL WORK

2.1 Preparation of Plant Material

The leaves of plants *Ocimum sanctum, Lawsonia alba, Trigonella foenum graecum and Carissa carandus* were collected, washed with tap water and then rinsed with distilled water. Afterwards the leaves were dried in shade at room temperature until there is no evidence of loss of weight of leaves.

The dried material is then mechanically powdered, sieved (using 80 mesh) then stored in air tight container and used for further physicochemical and phytochemical analysis.

2.2 Preparation of Plant Extracts

The coarse powder of plant's leave was successively extracted in a soxhlet extractor by using dissimilar solvents such as petroleum ether, methanol, and water. The extracts so obtained were further subjected to (drying or concentration) in vacuum desiccators, finally the residue so obtained was used for further studies by preserving it in refrigerator.

2.3 Phytochemical Screening

The freshly prepared different extracts were qualitatively assessed for the presence of specific chemical constituents. These constituents were identified by characteristic colour changes and precipitation reactions using standard procedures (8-11).

2.3.1 Alkaloids

Wagner's test: Extracts were dissolved in dilute hydrochloric acid and filtered. Then the filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of brown/reddish precipitate indicated the presence of alkaloids

2.3.2 Glycosides

Keller-Kiliani test: 5ml of extract was dissolved in 2ml chloroform. To that few drops of dilute H2SO4 was added to form a layer. A brown colour indicated the presence of glycoside.

2.3.3 Proteins and amino acids

Xanthoproteic test: The extracts were treated with few drops of conc. Nitric acid. The formation of yellow colour indicated the presence of protein.

2.3.4 Tannins

Lead acetate test: To few drops of 1% lead acetate 2 ml of extract was added. The presence of yellow color indicated the presence of tannins.

2.3.5 Flavonoids

H_2SO_4 test: A fraction of extract was treated with concentrated sulfuric acid and observed for the formation of reddish or orange colour.

2.3.6 Saponins

Frothing test: 5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. The formation of foam indicated the presence of saponins.

2.3.7 Terpenoids

Salkowski test: 1ml of the extract was added to 1ml of chloroform and filtered. To the filtrate 1ml of acetic acid was added and then a few drops of conc. Sulphuric acid run down the side of test tube. Brown colour showed the presence of terpenoids.

2.3.8 Steroids

Salkowski test: 1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The brown colour indicated the presence of steroids.

2.4 Evaluation of Physicochemical parameters

Physicochemical parameters such as percentage of total ash, acid insoluble ash, water soluble ash loss on drying, alcohol soluble extractive value and water-soluble extractive value were calculated based upon standard procedures (12-14).

2.4.1 Determination of total ash

Two grams of each plant's drug powder (*Ocimum sanctum, Lawsonia alba, Trigonella foenum graecum and Carissa carandus*) was placed in a previously ignited (350^0C for 1 hour) and tarred crucible. The dried material was spread in an even layer in the crucible then weights the crucible to find out the accurate weight of the sample taken. The material ignited by gradually increasing the heat to 550^0C for 5 hours in a muffle furnace until it turns white, indicating the absence of carbon. The material is then Cooled in a desiccator and weighed. Total ash content was calculated in mg/g of air-dried material.

2.4.2 Determination of Acid insoluble ash

The total ash obtained above was boiled with 25 ml of dilute hydrochloric acid (2N) for 5minutes. The insoluble ash was collected on ash-free filter and washed with hot water, then transferred into a pre-weighed silica crucible, ignited, cooled, and weighed. The same procedure was repeated until the constant weight was obtained. The percentage

of acid-insoluble ash was calculated with reference to the air-dried powder sample.

2.4.3 Determination of Water-soluble ash

Water-soluble ash is a good indicator of either previous extraction of the water-soluble salts in the drug or incorrect preparation. The ash was washed from the crucible into a 100 ml beaker using 25 ml of chloroform water and it was boiled for 5 min over a bunsen burner and filtered through ash less filter paper (Whatmann No: 42). The residue was washed with hot water twice, ignited to ash cooled and weighed. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug.

2.4.5 Determination of loss on drying

1 gm of leaves of each plant (*Ocimum sanctum, Lawsonia alba, Trigonella foenum graecum and Carissa carandus*) was transferred into a petri-dish plate and the contents was distributed evenly to a depth not exceeding 10 mm. The loaded plate was subjected to heat at 105^0C in hot air oven for 1 hr and then cooled in desiccators, loss in weight was recorded as moisture content. Respective moisture content percentage of the samples was calculated.

2.4.6 Alcohol soluble extractive value

A 5.0 g of each plant material drug (*Ocimum sanctum, Lawsonia alba, Trigonella foenum graecum and Carissa carandus*) was weighed accurately and placed in a stoppered conical flask. A 100 ml of 90 % alcohol was added to the flask and the stopper of the flask was replaced firmly. The flask's contents were shaken mechanically for about 6 hours and it was allowed to macerate for another 18 hours before filtration. The filtrate was collected and evaporated to dryness, and then the residue so obtained was dried to a constant weight at 105^0 C.

2.4.7 Water soluble extractive value

A 5.0 g of each plant material drug (*Ocimum sanctum, Lawsonia alba, Trigonella foenum graecum and Carissa carandus*) was weighed accurately and placed in a stoppered conical flask. A 100 ml of chloroform-water was added to the flask and the stopper of the flask was replaced firmly. The flask and its contents were shaken mechanically for 6 hours and were allowed to macerate for another 18 hours before filtration. The filtrate was collected and evaporated to dryness and then the obtained residue was dried to a constant weight at 105^0 C.

3. RESULTS

Studies of physicochemical characterization can serve as a precious foundation of information and are usually applied in judging the purity and quality of the plant drug. The extractive values give an idea about the chemical constitution of the drug. The findings were shown in **Table 1**.

1. Total ash value: The reference range for total ash value can vary depending on the specific plant and its parts (leaves, stems, etc.). Generally, a higher total ash value indicates a higher inorganic content. Comparing the values in the table, P2 (*Lawsonia alba*) has the highest total ash value (7.10%), followed by P4 (*Carissa carandus*) with 6.80%. P1 (*Ocimum sanctum*) has the lowest value of 5.25%, and P3 (*Trigonella foenum graecum*) falls in the middle with 5.50%.
2. Acid insoluble ash: The acid insoluble ash values should ideally be low, indicating minimal amounts of insoluble inorganic matter. In this case, all the plants have relatively low acid insoluble ash values, with P4 having the lowest value of 1.25%, followed by P3 (1.32%), then P2 (1.50%) and highest P1 (2.0%).
3. Water-soluble ash: The water-soluble ash values represent the solubility of inorganic matter in water. Higher values can indicate a higher presence of water-soluble minerals. In this table, P4 (*Carissa carandus*) has the highest water-soluble ash value of 4.55%, followed by P3 (4.55%), P1 (3.50%).and P2 (3.25%),
4. Loss on drying: Loss on drying measures the moisture content in the plant samples. Comparing the values, P4 (*Carissa carandus*) has the highest loss on drying value of 24.50%, indicating a relatively higher moisture content. P2 (*Lawsonia alba*) has the second-highest value of 22.25%, followed by P3 (20.50%) and P1 (18.5%).
5. Alcohol soluble extractive value: The alcohol soluble extractive value represents the percentage of plant constituents that can be extracted using alcohol as a solvent. Higher values indicate a higher concentration of alcohol-soluble compounds. In this table, P4 (*Carissa carandus*) has the highest value of 9.10%, followed by P1 (8.50%), P3 (8.20%), and P2 (7.50%).
6. Water-soluble extractive value: The water-soluble extractive value indicates the percentage of plant constituents that can be extracted using water as a solvent. Comparing the values, P3 (*Trigonella foenum graecum*) has the highest water-soluble extractive value of 9.60%,

followed by P2 (8.25%), P4 (7.80%) and P1 (7.30%).

Table: 1 Finding of Physicochemical parameters of selected plants

Parameters	**Results % (w/w)**			
	P1	**P2**	**P3**	**P4**
Total ash value	5.25%	7.10%	5.50%	6.80%
Acid insoluble ash	2.0%	1.50%	1.32%	1.25%
Water-soluble ash	3.50%	3.25%	3.90%	4.55%
Loss on drying	18.5%	22.25%	20.50%	24.50%
Alcohol soluble extractive value	8.50%	7.50%	8.20%	9.10%
Water soluble extractive value	7.30%	8.25%	9.60%	7.80%

P1= *Ocimum sanctum,* P2= *Lawsonia alba,* P3=*Trigonella foenum graecum,* P4=*Carissa carandus*

The Phytochemical screening of medicinal plants is very much important to evaluate the possible medicinal utilities of a plant and also to determine the active principles responsible for the known pharmacological activities exhibited by the plants. The data shown in **Table 2** shows the screening of three different extracts, i.e., Petroleum ether extract, methanol extract and Aqueous extract of four plants (*Ocimum sanctum, Lawsonia alba, Trigonella foenum graecum and Carissa carandus*) based on phytochemical tests. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes.

S. N.	**Phytoconsti -tuents**	**P1**			**P2**			**P3**			**P4**		
		Pet ether	Methanol	Water	Pet Ether	Methanol	Water	Pet ether	Methanol	Water	Pet Ether	Methanol	Water
1	Alkaloids	-	+	+	-	+	+	-	+	+	-	+	+
2	Glycosides	-	-	-	+	-	-	-	-	-	-	+	+
3	Proteins and amino acids	-	-	-	+	-	-	-	+	-	-	-	-
4	Tannins	-	+	+	-	+	-	-	-	-	-	+	+
5	Flavonoids	-	+	+	-	+	+	-	+	+	-	-	+
6	Saponins	-	+	+	-	+	+	-	-	+	+	-	-
7	Terpenoids	+	-	-	-	+	+	-	--	+	-	-	+
8	Steroids	-	-	-	-	-	-	+	-	-	+	-	-

Table: 2 Phytochemical composition of different extracts of selected plants

+ Present –Absent

P1= *Ocimum sanctum,* P2= *Lawsonia alba,* P3= *Trigonella foenum graecum,* P4= *Carissa carandus*

4. DISCUSSION

In the study four different medicinal herbs were examined and evaluated based on their physiochemical and phytochemical properties. Leaves of the plants were used for the assessment after drying and crushing, to aid in the process of extraction. To ensure extraction of all phytochemicals different solvents were used including water, ethanol and pet ether. Physiochemical analysis is used to identify and characterize the physical and chemical properties, control quality, detect adulterants, and set standards. On the other hand, phytochemical analysis is employed to identify bioactive compounds, determine medicinal properties, evaluate quality, aid in drug development, and optimize formulations. These analyses are essential for understanding the therapeutic potential of medicinal plants, ensuring their safety and effectiveness, and maintaining quality control.

In the study, the careful extraction of bioactive compounds and comprehensive analysis of their chemical composition, provides valuable insights into the potential therapeutic properties of these plants. The phytochemical screening revealed the presence of alkaloids, glycosides, proteins and amino acids, tannins, flavonoids, saponins, terpenoids, and steroids in the extracts, highlighting their potential pharmacological activities. The investigated plant species, namely *Ocimum sanctum* (Holy Basil), *Lawsonia alba* (Henna), *Trigonella foenum graecum* (Fenugreek), and *Carissa carandus* (Carissa), play a crucial role in traditional medicine due to their diverse therapeutic applications.

In a similar study by Borah R and Biswas S. P. phenol, carbohydrates, tannins, flavanoids, saponin, glycosides, terpenoids and alkaloids were found to be present in *ocimum sanctum* plant (15), and in another study done by S. Archna et.al,

physiochemical parameters such as total ash content found was 8.6% , acid insoluble ash was 0.8% and water soluble ash was 3.6% for the leaves of *ocimum sanctum* (16). Higher ash content indicates high content of minerals relatively.

For the plant *lawsonia inermis*, Jain VC et.al found the total ash value is 14.60% (comparatively double than *lawsonia alba*), acid insoluble ash value is 4.50 %, and water-soluble ash value is 3.0 %. Loss on drying was found to be (4.5 %) w/w. Alcohol soluble extractive value and aqueous extractive value were 3.8 % w/w and 5.0 % w/w respectively (17).

A study performed by Chanchal kumar mishra et.al on the fruit of *carissa carandas* releals the values of total Ash 20 %, Acid insoluble Ash 18 %, Water Soluble Ash 16 %, Alcohol soluble Extractive 1.2 %, water soluble extractive 2.0 % , and Loss on drying 14% (18).

These plants have been recognized for their potential health benefits, including anti-inflammatory, antimicrobial, antioxidant, anti-diabetic, and many other properties. Moreover, the evaluation of physicochemical parameters ensures the quality and purity of the plant materials used in the study. Parameters such as total ash, acid insoluble ash, water-soluble ash, loss on drying, alcohol soluble extractive value, and water-soluble extractive value serve as indicators of the plants' quality and reliability. The results obtained from this research contribute to the scientific understanding and validation of the traditional uses of these plants. The identification of specific phytochemicals and their potential therapeutic properties opens avenues for further research and exploration, including the development of natural product-based formulations or potential drug candidates.

5. CONCLUSION

The physicochemical parameters were established in the study which can be important for the detection of adulteration and mishandling of the crude drug. The generated information of the present study will provide data that is helpful in the identification and authentication of these medicinal plants and may help in preventing their adulteration. The obtained results also assumed that the plant extracts could be used for the new formulations of natural origin.

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