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COMPARATIVE PHYSICOCHEMICAL AND PRELIMINARY PHYTOCHEMICAL EVALUATION OF *SIGRU PATRA CHOORNA* AND *VARUNA TWAK KWATHA BHAVITHA SIGRU PATRA CHOORNA*

Brinda Krishnan[1]*, P. Y Ansary[2], Greeshma K C[3]

*[1]PG Scholar, [2]Professor and Head, [3]Assistant Professor, Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, Kerala, India.

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ABSTRACT

Bhavana is an important pharmaceutical process in Ayurveda in which a powdered drug is triturated with a liquid medium. The physicochemical and phytochemical characteristics of the drug may undergo qualitative and quantitative changes during processing. In the present study, *Sigru patra choorna* was subjected to *Bhavana* using *Varuna twak kwatha*. **Materials and Methods**: *Sigru patra choorna* and *Varuna twak kwatha bhavitha Sigru patra choorna* were subjected to physicochemical evaluation, qualitative analysis of ash, determination of water and alcohol-soluble extractives, successive solvent extraction and preliminary phytochemical screening. The observations obtained from the two samples were comparatively assessed. **Results**: Total ash values were 9.1% and 7.24% in *Sigru patra choorna* and *Varuna twak kwatha bhavitha Sigru patra choorna,* respectively. The acid-insoluble ash values were 3.2% and 3.8%, while water-insoluble ash values were 4.2% and 3.68%, respectively. Total sugar was 1.99% and 4.51%, and reducing sugar was 0.78% and 4.08%, respectively. Cold-water soluble extractive values were 24% and 28.4%, whereas cold-alcohol soluble extractives were 9.2% and 10.78%. Preliminary phytochemical screening of the cold-water extracts showed the presence of saponins, carbohydrates and proteins in two samples. Phenolic and tannin reactions showed differences in *Sigru patra choorna* and *Varuna twak kwatha bhavitha Sigru patra choorna* samples. Successive solvent extraction demonstrated variation in the extractive yield and qualitative phytochemical profile among the samples. **Conclusion:** The comparative analysis demonstrated measurable differences in physicochemical parameters, extractive values and qualitative phytochemical reactions between *Sigru patra choorna* and *Varuna twak kwatha bhavitha Sigru patra choorna*. These findings provide preliminary analytical data for the standardization of the *Bhavitha* preparation.

INTRODUCTION

Ayurvedic pharmaceutical preparations frequently involve processing procedures intended to modify the properties of the raw drug. *Bhavana* (trituration) is one such procedure in which a powdered drug is triturated with a specified liquid medium. The liquid used for *Bhavana* may influence the physicochemical and chemical profile of the processed material.[1]

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Sigru, botanically identified as *Moringa oleifera* Lam., is widely used in traditional medicine and has been investigated for its diverse phytochemical composition. The leaves have been reported to contain several classes of bioactive compounds, including phenolic compounds and flavonoids.[2] *Varuna*, identified as *Crateva nurvala* Buch.-Ham., is another important medicinal plant described in Ayurveda. Phytochemical investigations of *C. nurvala* have reported the presence of phenolics, flavonoids, saponins, tannins, phytosterols and triterpenoid constituents.[3-6] *Varuna twak kwatha* was used as the *Bhavana dravya* for *Sigru patra choorna*. The use of a decoction as the liquid medium provides an opportunity for interaction between the powdered leaf material and constituents extractable from the *Varuna*

bark decoction during trituration. Such processing may influence extractability and the qualitative phytochemical profile of the resulting preparation. Physicochemical parameters such as ash values, moisture content, extractive values and other quality-control parameters are important in the evaluation and standardization of medicinal plant materials.[7] Extractive values provide an indication of the amount of constituents soluble in particular solvents and can therefore be useful for comparative evaluation of raw and processed materials.

MATERIALS AND METHODS

Collection of Drugs

The leaves of *Moringa oleifera* Lam. (*Sigru patra*) and bark of *Crateva nurvala* Buch.-Ham. (*Varuna twak*) were collected for the present study and authenticated botanically prior to processing. The bark of *Crateva nurvala* Buch.-Ham. was collected from Pathanamthitta district, Kerala, while the leaves of *Moringa oleifera Lam.* were collected from Alappuzha district, Kerala and carefully examined for their identity and purity. The collected plant materials were cleaned to remove adhering foreign matter and extraneous materials. Botanical authentication of both drugs was carried out in the Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura and at Department of Botany, St. Albert's College, Ernakulam.

Preparation of *Sigru patra Choorna*

The collected *Sigru* leaves were carefully cleaned to remove extraneous matter and dried under suitable conditions. The dried leaves were pulverized and passed through an appropriate sieve to obtain a fine, uniform powder. The prepared *Sigru patra choorna* was stored in a clean, dry, airtight container until further use.

Preparation of *Varuna twak kwatha*

The bark of *Crateva nurvala* Buch.-Ham. was cleaned thoroughly, dried and reduced to a coarse powder. One kilogram of the coarse powdered *Varuna twak* was taken in a suitable vessel and mixed with 8 liters of potable water, maintaining a drug-to-water ratio of 1:8. The mixture was subjected to gentle boiling and reduced to approximately one-eighth of the initial volume, yielding about 1 liter of aqueous decoction. The decoction was allowed to cool and was filtered through a clean filter medium to obtain a clear filtrate. The freshly prepared *Varuna twak kwatha* was used as the *Bhavana dravya* for the preparation of *Varuna twak kwatha bhavitha Sigru patra choorna*.

Preparation of *Varuna twak kwatha bhavitha Sigru patra choorna*

For preparing *Varuna twak kwatha bhavitha Sigru patra choorna*, 1kg of *Sigru patra choorna* was subjected to *bhavana* with *Varuna twak kwatha*. The required quantity of *Varuna twak kwatha* was added to the *Sigru patra choorna* and triturated thoroughly until the liquid was uniformly incorporated into the powder. The material was then dried completely. This process of addition of *Varuna twak kwatha,* trituration and drying were repeated for seven consecutive *Bhavana* cycles. After completion of the seventh *Bhavana,* the dried material was pulverised and passed through a suitable sieve to obtain a uniform powder. The final *Varuna twak kwatha bhavitha Sigru patra choorna* was stored in a clean, dry, airtight container and used for further physicochemical and phytochemical evaluation.

Physicochemical Evaluation

The two samples were evaluated for foreign matter, total ash, acid-insoluble ash, water-insoluble ash, moisture content, volatile oil, crude fibre, tannin, total sugar, reducing sugar, phenol and pH. The observations were obtained from the experimental data recorded in the study.

Qualitative Analysis of Ash

The ash obtained from the powdered drug was subjected to specific qualitative tests for carbonate, phosphate, chloride, sulphate and potassium. Characteristic colour changes or precipitate formation were recorded as positive reactions.

Test for Carbonate

A small quantity of ash obtained from the sample was transferred into a test tube and treated with dilute hydrochloric acid. Brisk effervescence was observed with the evolution of a colourless, odourless gas, which turned lime water milky, indicating the presence of carbonates.

Test for Phosphate

A small quantity of ash obtained from the sample was treated with concentrated nitric acid in a test tube and heated. The mixture was then filtered, and a few drops of the filtrate were added to approximately 4ml of ammonium molybdate solution in a separate test tube. The formation of a bright yellow precipitate upon cooling indicated the presence of phosphate.

Test for Chloride

A small quantity of ash obtained from the sample was treated with dilute nitric acid in a test tube until effervescence ceased. The mixture was then boiled to expel carbon dioxide and filtered when necessary. Silver nitrate solution was added to the resulting filtrate. The formation of a white, curdy precipitate that dissolved in ammonium hydroxide confirmed the presence of chloride.

Test for Sulphate

A small quantity of ash obtained from the sample was treated with dilute hydrochloric acid and boiled to expel carbon dioxide. The mixture was then filtered, and the filtrate was treated with barium chloride solution. The formation of a white precipitate that remained insoluble in mineral acids indicated the presence of sulphate.

Test for Potassium

The ash obtained from the sample was diluted with approximately 3ml of distilled water and filtered. The resulting filtrate was treated with a saturated solution of picric acid and shaken thoroughly. The formation of a yellow precipitate indicated the presence of potassium.

Extractive Values

Cold Water-Soluble Extractive

Approximately 5g of accurately weighed *choorna* was transferred into a conical flask, and 100ml of chloroform water (prepared by adding 2.5ml of chloroform to 1000ml of distilled water) was added. The mixture was shaken occasionally during the first 6 hours and subsequently allowed to stand undisturbed for 18 hours. It was then rapidly filtered, taking care to minimise solvent loss. The resulting filtrate was transferred to previously weighed beakers and evaporated to dryness, followed by drying at 110°C. The residue was weighed and calculated as the percentage of water-soluble extractive.

Hot Water-Soluble Extractive

About 1g of *Choorna* was taken in a round-bottom flask and 100ml of distilled water was added. The flask was connected to a water condenser and heated in a water bath for 1 hour. The mixture was then allowed to stand for some time and filtered rapidly into pre-weighed beakers, taking care to prevent loss of solvent. The residue was dried at 110°C and weighed and expressed as the percentage of hot water-soluble extractive.

Cold Alcohol-Soluble Extractive

About 5g of accurately weighed *Choorna* was placed in a sterilized conical flask. To this, 100ml of 99.9% isopropyl alcohol was added, and the flask was tightly closed. The contents were shaken intermittently during the first 6 hours and subsequently allowed to stand undisturbed for 18 hours. The mixture was then rapidly filtered, ensuring minimal loss of solvent. The filtrate was collected in a pre-weighed, sterilized beaker and evaporated to dryness. The residue obtained was dried at 100°C, and successive heating and weighing were carried out until a constant weight was achieved. The percentage of cold alcohol-soluble extractive was calculated.

Hot Alcohol-Soluble Extractive

About 1g of accurately weighed *Choorna* was transferred into a round-bottom flask, and 100ml of isopropyl alcohol was added. The flask was fitted with a water condenser and heated in a water bath for 1 hour. After heating, the contents were allowed to cool for a short period and then rapidly filtered into a beaker, taking necessary precautions to minimize solvent loss. The filtrate was evaporated to dryness in a sterilized, pre-weighed beaker and further dried at 100°C. The dried residue was weighed, and the percentage of hot alcohol-soluble extractive was calculated.

Successive Solvent Extractive

The powdered drug was successively extracted with solvents of increasing polarity, such as petroleum ether, chloroform, ethyl acetate, alcohol and water, using a Soxhlet extraction apparatus. Each solvent extract was concentrated separately, dried to constant weight and weighed. The percentage yield of each successive extract was calculated with reference to the weight of the drug taken.

Preliminary Phytochemical Screening

Cold-water extracts of the two samples were screened qualitatively for alkaloids, flavonoids, saponins, carbohydrates, proteins, phenols, steroids and tannins. The successive solvent extracts were separately examined for alkaloids, flavonoids, phenols and steroids.[6]

Test for Alkaloids

About 0.5ml of the extract of *Choorna* was taken in a test tube. To this, 2.0ml of hydrochloric acid solution was added, followed by 1.0ml of Dragendorff's reagent. The development of an orange-red precipitate immediately after the addition of the reagent indicated the presence of alkaloids.

Test for Carbohydrates

For the detection of carbohydrates, Fehling's test and Benedict's test were performed using the extract of *Choorna*. In Fehling's test, 2ml of the extract was taken in a test tube, and equal quantities of Fehling's solutions A and B were added. The mixture was heated to boiling for a few minutes, and the formation of a red to brick-red precipitate indicated the presence of carbohydrates. In Benedict's test, 0.5 ml of the extract was mixed with 5ml of Benedict's reagent and heated to boiling for 5 minutes. The development of a bluish-green colour indicated the presence of carbohydrates.

Test for Proteins

The extract was subjected to Ninhydrin test. Development of characteristic blue or purple colour indicated the presence of proteins.

Test for Phenolic Compounds

For the detection of phenolic compounds, two qualitative tests were performed. About 1ml of the extract of *Choorna* was taken in a test tube, to which 2 ml of distilled water and a few drops of 10% aqueous ferric chloride solution were added. The development of blue or green colouration indicated the presence of phenolic compounds. In the lead acetate test, 1ml of the extract was treated with 5ml of distilled water followed by a few drops of 1% aqueous lead acetate solution. The formation of a yellow-coloured precipitate indicated the presence of phenolic compounds.

Test for Tannins

For the detection of tannins, ferric chloride and lead acetate tests were performed. About 1–2 ml of the extract of *Choorna* was treated with a few drops of 5% aqueous ferric chloride solution. The development of a bluish-black colour, which disappeared on the addition of dilute sulphuric acid followed by the formation of a yellowish-brown precipitate, indicated the presence of tannins. In the lead acetate test, about 5ml of the extract was treated with a few drops of 1% lead acetate solution. The formation of yellow or reddish precipitate indicated the presence of tannins.

Test for Flavonoids

The extract was subjected to the Shinoda test by treatment with magnesium turnings and concentrated hydrochloric acid. Development of a characteristic pink colour indicated the presence of flavonoids.

Test for Saponins

The extract was vigorously shaken with 1–3 drops of sodium bicarbonate solution. Persistent frothing or formation of a stable foam indicated the presence of saponins.

Test for Steroids

The extract was treated with concentrated sulphuric acid was gently introduced along the sides of the test tube. The emergence of red colouration in the chloroform layer signifies the presence of steroids.

RESULTS

Table 1: Physicochemical parameters of *Sigru patra choorna* and *Varuna twak kwatha bhavitha Sigru patra choorna*

S.No	Parameters	*Sigru patra choorna*	*Varuna twak kwatha bhavitha Sigru patra choorna*
1	Foreign matter	Nil	Nil
2	Total ash	9.1%	7.24%
3	Acid Insoluble ash	3.2 %	3.8%
4	Water insoluble ash	4. 2%	3.68%
5	Moisture content	13%	13%
6	Volatile oil	Nil	Nil
7	Crude fibre	7.80%	7.80%
8	Tannin content	0.16%	0.12%
9	Total sugar	1.99%	4.51%
10	Reducing sugar	0.78%	4.08%
11	Phenol	0.71%	0.66%

The observations showed that total ash decreased from 9.1% in plain *Sigru patra choorna* to 7.24% in the *bhavitha* sample. Acid-insoluble ash increased from 3.2% to 3.8%, whereas water-insoluble ash decreased from 4.2% to 3.68%. Tannin content decreased from 0.16% to 0.12%, while total sugar increased from 1.99% to 4.51% and reducing sugar increased from 0.78% to 4.08%. Phenol content changed from 0.71% to 0.66%. The pH increased from 5.28 to 5.82.

Qualitative Analysis of Ash

Table 2: Qualitative analysis of ash

S.No	Experiment	*Sigru patra choorna*	*Varuna twak kwatha bhavitha Sigru patra choorna*
1	Carbonate	+	+
2	Phosphate	_	+
3	Chloride	+	+

4	Sulphate	+	+
5	Potassium	–	–

The *Bhavitha* sample showed positive reactions for carbonate, phosphate, chloride and sulphate, whereas potassium was not detected.

Extractive Values

Table 3: Water- and alcohol-soluble extractive values

S.No.	Type of extractives	*Sigru patra choorna*	*Varuna twak kwatha bhavitha Sigru patra choorna*
1	Cold water soluble	24%	28.4%
2	Hot water soluble	28.1%	25.1%
3	Cold alcohol soluble	9.2%	10.78%
4	Hot alcohol soluble	10.6%	10.7%

The *Bhavitha* sample showed a higher cold-water-soluble extractive value than *Sigru patra choorna*. Cold-alcohol-soluble extractive was also higher in the *Bhavitha* sample.

Successive Solvent Extractive Values

Table 4: Successive solvent extractive values

S.No.	Solvents	*Sigru patra choorna*	*Varuna twak kwatha bhavitha Sigru patra choorna*
1	Petroleum ether	5.38%	4.48%
2	Cyclohexane	0.41%	0.73%
3	Acetone	1.64%	2.80%
4	Alcohol	5.11%	6.37%

The *Bhavitha* sample showed higher extractive values than plain *Sigru patra choorna* in cyclohexane, acetone and alcohol fractions, whereas the petroleum ether extractive was lower.

Preliminary Phytochemical Screening

Cold-Water Extract

Table 5: Qualitative phytochemical analysis of cold-water extracts

S.No	Experiment	*Sigru patra choorna*	*Varuna twak kwatha bhavitha Sigru patra choorna*
1	Test for alkaloids		
	1) Dragendroff' s test	–	–
	2) Meyer's test	–	–
2	Test for flavonoids	–	–
3	Test for saponins	+	+
4	Test for carbohydrates		
	1) Fehling's test	+	+
	2) Benedict' s test	+	+
5	Test for proteins	+	+
6	Test for phenols		
	1) Test for chloride test	+	+
	2) Lead acetate test	–	+
7	Test for steroids	–	–
8	Test for tannins		
	1) Ferric Chloride test	–	+
	2) Lead Acetate test	+	+

The two samples showed positive reactions for saponins, carbohydrates and proteins. The *bhavitha* sample showed additional positive reactions for the lead acetate test for phenols and ferric chloride test for tannins compared with *Sigru patra choorna.*

Successive Solvent Phytochemical Screening

Table 6: *Sigru patra choorna*

S.No	Experiment	Extracts			
		Petroleum ether	**Cyclohexane**	**Acetone**	**Alcohol**
1	Test for alkaloids				
	1) Dragendroff' s test	–	–	–	–
	2) Meyer' s test	–	–	–	–
2	Test for flavonoids	–	–	+	+
3	Test for phenols				
	1) Ferric chloride test	+	+	+	+
	2) Lead Acetate test	+	+	+	+
4	Test for steroids	–	–	+	–

Table 7: *Varuna twak kwatha bhavitha Sigru patra choorna*

S.No	Experiment	Extracts			
		Petroleum ether	**Cyclohexane**	**Acetone**	**Alcohol**
1	Test for alkaloids				
	1) Dragendroff' s test	–	–	–	–
	2) Meyer' s test	–	–	–	–
2	Test for flavonoids	–	–	+	+
3	Test for phenols				
	1) Ferric chloride test	+	+	+	+
	2) Lead Acetate test	+	+	+	+
4	Test for steroids	–	–	+	–

The successive solvent screening showed that alkaloids were not detected in any of the tested fractions. Flavonoids were detected in the acetone and alcohol fractions of *Sigru patra choorna* and *Varuna twak kwatha bhavitha Sigru patra choorna*. Phenolic reactions were observed in all tested fractions of the *Bhavitha* sample. Steroid reactions were observed in the acetone fraction.

DISCUSSION

The present study provides a comparative analytical profile of *Sigru patra choorna* before and after *Bhavana* with *Varuna twak kwatha* included as the source drug for the *Bhavana* medium. A noticeable change was observed in the physicochemical profile following *Bhavana*. Total ash decreased from 9.1% to 7.24%, while acid-insoluble ash increased from 3.2% to 3.8%. The pH changed from 5.28 in *Sigru patra choorna* to 5.82 in the *Varuna twak kwatha bhavitha Sigru patra choorna* sample. These observations indicate that the processing procedure was associated with changes in the measurable physicochemical characteristics of the powder. The carbohydrate-related parameters showed a prominent difference. Total sugar increased from 1.99% to 4.51%, while reducing sugar increased from 0.78% to 4.08%. The changes in the *Bhavitha* sample may reflect contribution from the *Bhavana* medium and changes occurring during processing. However, the present data alone cannot establish direct transfer or chemical transformation of individual constituents. The extractive values also demonstrated changes after *Bhavana*. Cold-water-soluble extractive increased from 24% to 28.4%, while cold-alcohol-soluble extractive increased from 9.2% to 10.78%. The successive solvent extraction further demonstrated increased extractive values in cyclohexane, acetone and alcohol fractions of the *Bhavitha* sample compared with *Sigru patra choorna.*

The preliminary phytochemical analysis provides additional comparative information. Saponins, carbohydrates and proteins were detected in two samples. Alkaloids, flavonoids and steroids were not detected in the cold-water extracts. The *Bhavitha* sample showed positive phenolic and tannin reactions

that were not observed in some corresponding tests of *Sigru patra choorna.* In the successive solvent extracts, flavonoids were detected in acetone and alcohol fractions of both *Sigru patra choorna* and *Bhavitha Sigru patra choorna*. Phenolic reactions were detected across all four fractions of the *Bhavitha* sample. These qualitative differences may indicate alteration in the extractable phytochemical profile following *Bhavana*.

Thus, the main analytical significance of the present study is the demonstration that *Sigru patra choorna* and *Varuna twak kwatha bhavitha Sigru patra choorna* possess distinguishable physicochemical and qualitative phytochemical profiles.

CONCLUSION

The present study demonstrated comparative differences in the physicochemical and preliminary phytochemical characteristics of *Sigru patra choorna* and *Varuna twak kwatha bhavitha Sigru patra choorna. Bhavana* was associated with changes in total ash, acid-insoluble ash, extractive values, sugar content, pH and selected qualitative phytochemical reactions. The *Bhavitha* sample showed increased cold-water-soluble extractive and higher extractive values in selected successive solvent fractions compared with *Sigru patra choorna*. The appearance of additional positive reactions for selected phenolic and tannin tests in the *Bhavitha* sample provides preliminary evidence of a change in the qualitative phytochemical profile following *Bhavana*. The physicochemical and preliminary phytochemical parameters documented in the present study may therefore serve as reference analytical data for the standardization and quality control of *Varuna twak kwatha bhavitha Sigru patra choorna.*

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***Address for correspondence**
Dr. Brinda Krishnan
PG Scholar,
Department of Dravyaguna Vigyana,
Government Ayurveda College,
Tripunithura, Kerala.
Email: kashimon009@gmail.com

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