Review Article

Physico-chemical and Phyto-chemical Standardization of *Gul-e-Banafshah* (Flowers of *Viola odorata* Linn.)

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ABSTRACT

Background

Banafshah, a powerful Unani medicine, needs to be standardized in order to maintain and evaluate its quality and safety and achieve the intended therapeutic effect. As a result, the perfect monograph that establishes its authenticity, quality, safety, and reproducibility will be prepared. This study investigates the phytochemical, physicochemical and pharmacognostic characteristics of *Banfashah* as well as their Total phenolic content.

Aim

The goal of the project is to compile the perfect *Banafshah* monograph.

Methodology

The WHO's criteria were followed in the physical-chemical and preliminary phytochemical investigations.

Result

The powdered *Banafshah* was gritty, Yellowish brown in color, Slightly aromatic, Slightly pungent and bitter, and pleasant. The results showed that the total ash, acid insoluble ash, water soluble ash, weight loss after drying, and pH (1% and 10% solution) were, in order, 11.196±0.113, 3.126±0.017, 2.336±0.121, 12.366± 0.154,7.12±0.062, and6.01±0.060respectively. The hydroalcoholic extract had a lower TPC value of 12.894±0.513, indicating that the aqueous extract contains a higher concentration of phenolic compounds. The total phenolic content of the aqueous extract was found to be 23.063±0.646. The results of the phytochemical screening showed the presence of volatile oil, alkaloids, proteins, carbohydrates, phenols, terpenes, and glycosides, including cardiac glycosides. TLC spots in different solvent systems have been noted in day light and UV light, based on TLC examinations of diverse drug extracts obtained in different solvent systems.

Conclusion

Pharmacopoeial standards could be established based on the results mentioned above. There are no comparable data sets in this regard, thus our conclusions can be used as standard for upcoming references.

Keywords

Banafshah; Physicochemical; Phytochemical; Standardization; Total phenolic content.

INTRODUCTION

The unani medicine is the source of primary health care due to its cultural acceptability and better compatibility with the human body and lesser side effects. *Banafshah* refers to the plant's blossoms. *Viola odorata* Linn. also known as "sweet violet," is a member of the Violaceae family and has been used for many years to cure a variety of illnesses in both Ayurvedic and Unani medical systems. The quality assurance of these conventional medications is intimately linked to their efficacy and safety. There has been many questions raised about the safety and

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DOI: https://doi.org/10.3329/bjms.v25i1.86404

effectiveness of herbal medications due to the massive global expansion in medicinal plants. Herbal medicine is susceptible to quality deterioration during all stages of production, storage, and cultivation, which can lead to a reduction in the medication's efficacy. The problems with Unani medicine standardization at every level are essential. For better therapeutic use, it's critical that real, legitimate medications are available. Before putting a medicine through pharmacological screening, its physicochemical parameters must be determined in order to verify its legitimacy, as the key factors influencing its efficacy are its physical and chemical characteristics. *Viola odorata* has a great therapeutic value, but not much is known about its physicochemical characteristics, which would help determine its authenticity and purity and prevent adulteration.

Thus, the goal of the current study was to standardize the phytochemical and physicochemical profiles of *Banafshah* (*Viola odorata*) using pharmacopeial recommendations for the standardization of herbal medications.

Sweet violet is used to treat cancer, diabetes, postoperative tumor metastases, common digestive issues, and pneumonia. From distinct species of this genus, several classes of chemicals, including cyclotides, flavonoids, alkaloids, and triterpenoids; have been isolated phytochemically. [1]

MATERIALS AND METHODS

Collection and Identification

The *Banafshah* (*Viola odorata* Linn.) flower was identified by the taxonomist at the National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, which were bought from Khari Baoli, Old Delhi (Authentication No.**NIScPR/RHMD/ Conult/2023/4318-19).**

Organoleptic Parameters: The test sample's organoleptic characteristics, including appearance, color, taste, and smell, were noted.

Sample preparation

- *Gule-Banafsha* coarse powder was extracted over a 6-hour period using a Soxhlet device and several solvents were used, like petroleum ether, chloroform, methanol and hydro-alcoholic solvent (ethanol and distilled water in ratio of 50:50).
- The 250 mL round bottom flask containing the coarse powder (20 gm) was used. To it, 200 mL of the appropriate solvent were added to make different extracts. The resulting extract were filtered with Whatman filter paper and then dried on water bath by evaporation for three hours, before being weighed. Once the extract was dried, it was then properly covered and stored in the refrigerator.

The prepared sample was used to perform fluorescence analysis, phytochemical analysis and chromatographic evaluation.

Physico-chemical analysis

Determination of ash value, moisture content, pH value at 1% and 10% solution, solubility, bulk density, crude fiber content, solubility, and extractive values in various organic solvents are all included in physico-chemical analysis. These were determined in accordance with standard protocols as mentioned in Ayurvedic Pharmacopoeia of India/ WHO [2, 3]

Phytochemical analysis

The extracts obtained from the research drugs were subjected to qualitative examination as per the Pharmacopoeia of India (IP). The qualitative chemical tests were performed to check the presence or absence of primary and secondary metabolites in extract[4,5]

Thin Layer Chromatography (TLC)

The test samples of aqueous and hydro-alcoholic extracts were put on precoated silica gel 60 F_{254} TLC plates using capillary tubes after being appropriately diluted in the relevant solvents, and they were then run in several previously saturated solvent systems, as mentioned in table 6.TheTLC plates were air dried at room temperature after development, and spots were seen at 254 nm and 366 nm in a UV chamber.

Quantitative Analysis of Total Phenolic Content.

Preparation of Folin-Ciocalteu's Phenol reagent

A 1500 ml flask containing 100 g of sodium tungstate and 25 g of sodium molibedate was filled with 800 ml of water, mixed with 50 ml of phosphoric acid and 100 ml of HCl, and allowed to reflux for ten hours. Once the mixture had cooled, add 150 grams of lithium sulfate, 50 milliliters of water, and four to six drops of bromine water. Let the mixture stand for two hours. The mixture was allowed to cool after a 15-minute boil, and then it was filtered. The reagent must be free of any greenish hue.[7]

Procedure for determination of total phenolic contents

Gallic acid was used as a standard, and the total phenolics in extracts were quantified as milligram per gram of gallic acid equivalents (GAE) with the Folin-Ciocalteu reagent[8] Concentration of 0.5, 1.0, 2.5, 5.0 and 10.0 mg/ml of gallic acid were prepared in methanol. Concentration of 0.5 ml of 10mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced into test tubes and mixed with 5ml of a 10 fold dilute Folin- Ciocalteu reagent and 4ml of 7.5% sodium carbonate. After the tubes were wrapped in parafilm and left to stand at room temperature for half an hour, the absorbance was measured spectrophotometrically at 716 nm[9]

RESULTS

Organoleptic characters: The powder of the dried flower of *Banafshah* (*Viola odorata*) was light purple in colour with characteristic odour and taste. Organoleptic and macroscopic characters were summarized in table-1 and 2.

Tab. 1 Organoleptic characters of fine powder of dried flowers

S.No	Parameters	Gul-e-Banafshah
	Colour	Yellowish brown
	Appearance	Rough
	Odour	Slightly aromatic
	Taste	Slightly pungent and bitter

Tab. 2 Macroscopic characters of flowers

S.No	Parameters	Gul-e-Banafshah
	Colour	Purplish blue
	Shape	Ovate
	Size	0.6-0.8 cm in diameter
	Surface	Smooth
	Fracture	Uneven

Physico-chemical constants: Three separate measurements of each physicochemical constant were made, with the average results shown in table 3.

Tab. 3 Physico-chemical parameters

S. No.	Physicochemical parameters	Results mean ± S.E.M.(S.D)
1.	**Moisture content (%)**	
	Loss of weight on drying	12.366± 0.154 (0.266)
2.	**Ash value (%)**	
	Total ash	11.196±0.113 (0.196)
	Acid insoluble ash	3.126±0.017 (0.030)
	Water soluble ash	2.336±0.121 (0.210)
3.	**pH values (%)**	
	pH at 1%	7.12±0.062 (0.107)
	pH at 10%	6.01 ±0.060 (0.104)
4.	**Bulk density (gm/ml)**	
	Poured density	0.543±0.020 (0.351)
5	**Successive extractive values in different organic solvents**	
	Petroleum ether	0.883±0.008 (0.015)
	Chloroform	0.906±0.018 (0.032)
	Ethanol	6.260±0.030 (0.052)
	Aqueous	18.300±0.041(0.072)

Phyto-chemical analysis: The presence of alkaloids, carbohydrates, proteins, phenols, sterols, glycosides, cardiac glycosides, flavonoids, terpenes/sterols, and volatile oil is revealed by qualitative analysis of the phytochemicals.

Tab. 4 Qualitative analysis of the phyto-chemicals present in test drugs

S.No	Chemical constituents	Test/Reagent	Present/absent
1	**Alkaloids**	Dragendroff's test	**+ve**
		Hager's test	**-ve**
		Wagner's test	**-ve**
2	**Carbohydrates**	Molisch's test	**+ve**
		Fehling's solution test	**+ve**

S.No	Chemical constituents	Test/Reagent	Present/absent
3	Flavonoids	Mg. ribbon test	+ve
4	Cardiac Glycosides	Baker's yeast test	+ve
5	Tannins	Ferric chloride test	+ve
6	Proteins	Millon's reaction	-ve
		Biurette's test	-ve
		Xanthoproteic test	-ve
7	Starch	Iodine test	-ve
8	Phenols	Lead acetate test	-ve
9	Sterols/ terpenes	Liebermann-burchard's test	-ve
		Hosse's reaction	-ve
10	Saponins	Frothing	+ve

Florescence analysis: When exposed to UV radiation, a number of drugs and their constituents release a distinctive color known as fluorescence. This is because the radiant energy in the pharmaceuticals excites the solution, which produces the specific color. The fluorescence analysis of the powdered drug treated with different chemical reagents was done and change in the colour so appeared was observed and mentioned in the table no. 5

Tab. 5 Fluorescence analysis of powdered drug *Banafshah* (*Viola odorata*) in different chemical reagents

Treatment of powder with different chemical reagents	Short UV light (254 nm)	Long UV light (366 nm)	Day light
Powder alone	Willow green	No Fluorescence	Skin color
Powder + 50% H_2SO_4	Black	Lime green	Blackish Brown
Powder + glacial acetic acid	Dark green	Dark yellow	Brown
Powder glacial acetic acid + Conc.HNO_3	Yellow*	Yellowish brown	Reddish brown
Powder + 50% HNO_3	Greenish	Green	Light brown
Powder + 50% HCl	Olive green	No Fluorescence	Light brown
Powder + Conc. H_2SO_4	Black	Blackish brown	Brown
Powder + 5% KOH	Dark Green	No Fluorescence	Brown
Powder + 10% NaOH	Dark green	No Fluorescence	Light brown
Powder +10% NaOH+Conc. HNO3	Pea green	No Fluorescence	Light orange
Powder + Dragendroffs reagent	Dark green	Black	Red
Powder + Wagners reagent	Dark brown*	Dark brown	Blackish brown
Powder + Hagers reagent	Kelly green	No Fluorescence	Sun yellow color
Powder + Benedicts reagent	Pea green	No Fluorescence	Dark green
Powder + Fehlings solution	Sea green	No Fluorescence	Sky blue
Powder + 5% Iodine solution	Brown*	Darkbrown	Reddish brown
Powder + Picric acid	Yellowish*brown	Dark brown	Light brown
Powder + 2% Ninhydrin	Yellow*	Dark brown	Brown
Powder + Lead acetate	Yellowish*brown	Brownish black	Brown
Powder + 10% methanolic KOH	Purple*	Dark purple	Purple
Powder + 5% $CuSO_4$	Light brown*	Dark brown	Brown

Tab.6 Thin Layer Chromatography of *Viola odorata* Linn.

S.No	Extracts	Mobile Phase	Ratio
1	Aqueous	Toluene: Ethylacetate: Formicacid	5:4:1
2	Hydro-alcoholic	Toluene: Ethylacetate: Formicacid	5:4:1

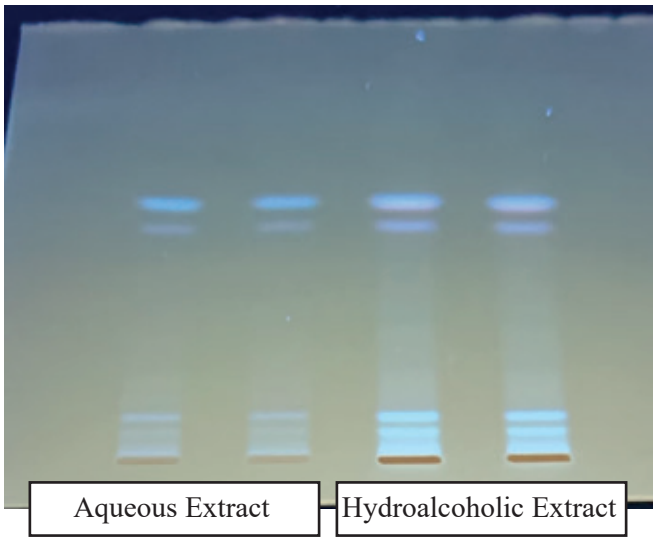

Figure 1: Thin Layer Chromatography of *Viola odorata* Linn.

Quantitative Analysis of Total Phenolic Content.

Tab. 7: Absorbance of Standard Compound (Gallic Acid)

S.NO	Concentration (µg/ml)	Absorbance (Mean) λmax=716 nm
1	0.5	0.117
2	1.0	0.155
3	2.5	0.192
4	5.0	0.286
5	10.0	0.290

Tab. 8: Total Phenolic Content of Viola odorata in Different Plant Extracts

S.NO	Extract Sample	Concentration (µg/ml)	Mean±SD
1	Aqueous Extract	100	23.063±0.646
2	Hydro-alcoholic Extract	100	12.894±0.513

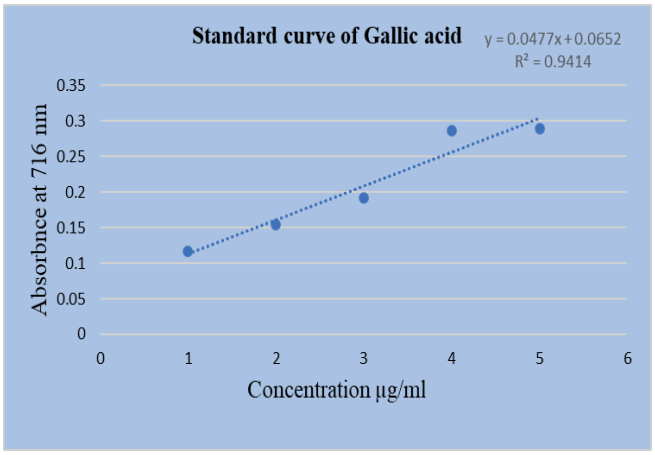

Figure 1: Standard curve of Gallic acid

DISCUSSION

These days, improving and safeguarding patient health and safety is mandated by all medical regulatory bodies, and the majority of nations are adopting ideas such as pharmacovigilance, cosmetovigilance, herbovigilance, hemovigilance and macro vigilance into their national health programs.[6] In quality control, identifying the real plant is crucial and difficult task. While a single plant may have two local names, two or more plants may have the same local name. It is quite likely that another plant is being gathered and combined with the real thing while gathering medicinal plants from the fields. To establish identity, the gross morphology will provide specific drug-related information. Microscopy and histology are useful for both powdered and non-powdered medications. The identity of variety of adulterants in pharmaceuticals can be determined or verified through the use of quantitative microscopy, stomatal characteristics, trichome features, calcium oxalate crystal analysis, and anomalous cross-sectional characteristics.

The quality control of Unani medications heavily relies on physicochemical studies. Because many medications' physical and chemical characteristics are correlated with their efficacy. Therefore, before examining a drug's medical qualities, its physicochemical characteristics must be established for legitimacy. It holds more significance as it helps in the characterization of individual constituents or groups of constituents that often determine the structure-activity relationship and the most likely molecular mechanism of action of the drugs.

CONCLUSION

The physicochemical, phytochemical, and antioxidant qualities of *Banafshah* are all thoroughly evaluated in this work. It confirms *Banafshah*'s use in Unani medicine and shows that it has a lot of potential. Standardization of Unani medications is therefore essential for preserving and evaluating their quality and safety. The standard parameters used for the Standardization of *Banafshah* (Viola odorata) include the following: preliminary phytochemical analysis; percentage of weight loss on drying; ash value; acid insoluble ash; water soluble ash; pH value; moisture content; fluorescence analysis of powdered drug and different extracts; TLC; all of which will aid in the preparation of the ideal monograph.

Source of support: None

Conflict of Interest: None

Ethical clearance: Not applicable

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