Physico-chemical and Phyto-chemical Analysis of Market Sample of Banafshah (*Viola odorata* Linn.)

***Sumbul Rehman**
and
Abdul Latif

Department of Ilmul Advia,
A.K. Tibbiya College,
Aligarh Muslim University,
Aligarh-202002

Abstract

Present study provides an updated standardization profile of market sample of a well known Unani drug Banafshah (*Viola odorata* Linn.) used for its efficacy in bilious affections, lung troubles, kidney diseases, liver affections, in fevers and as blood purifier. A review of literature, revealed that only few pharmacopoeial parameters like total ash value, acid insoluble ash, alcohol soluble and water soluble extractives, thin layer chromatography (TLC) of petroleum ether extract are reported in the Unani pharmacopoeia. These known parameters were matched with the market sample of Banafshah and were found to be within range with slight acceptable variation. However, data on water soluble ash value, sulphated ash value, extractive value in different solvents, organoleptic features, moisture content, bulk density, melting range, pH value, total alkaloid content, crude fiber content, qualitative analysis, phyto-chemical analysis with different reagents, IR spectral studies, FTAR analysis of different extracts, TLC of other extracts are unreported. These parameters were investigated by us for the first-time as per the pharmacopoeial guidelines. This communication provides updated pharmacopoeial parameters of Banafshah that will help to match its future market samples for evaluation of their identity and purity so as to have uniform therapeutic efficacy of manufactured products.

Keywords: Banafshah (*Viola odorata* Linn.), Standardization, Pharmacopoeial guidelines

Introduction

Standardization of bio-resources is mandatory to ensure their identity, quality, safety and efficacy for their proper use in health ailments; this is a very important in present scenario where there is highly increasing trend of utilizing medicinal plants specifically in developing countries, where they are accepted due to their safety, efficacy, cultural acceptability and lesser side effects (Kamboj, 2000). It is more specific for the traditional medicines which are based on natural drugs as Ayurveda, Unani, Siddha and Homeopathy (AYUSH) medicine. Diverse biological flora and fauna; geographical and climatic diversity provide us with variety of different species of same plant at a time collected from different places; moreover change in the environmental condition of a place after a regular interval of time also lead to difference in their quality. So there are upmost chances of variation in their physicochemical or phytochemical parameters that also affect their therapeutic efficacy and provide a very strong reason behind non-uniform results in pharmacological effect of same plant nowadays.

* Author for correspondence; Email : dr.sumbulrehman@gmail.com

To ensure the authenticity and quality of pharmaceutical material used in Unani formulations, Central Council for Research in Unani Medicine (CCRUM) (Anonymous, 2006) and Ministry of AYUSH in the Unani Pharmacopoeia of India (Anonymous, 2007) has laid down standardization profile monographs of Unani drugs along with the standard methods to evaluate the physic-chemical and phytochemical profile as per Drugs and Cosmetic Act, 1940.

The present study was carried out with a view to analyze physico-chemical and phyto-chemical parameters of a well known Unani drug –Banafshah (*Viola odorata* Linn.) collected from the local area of Kashmir (where it is said to be of the best quality as per literature, and is supplied throughout the country for consumption) (Ghani, 1921) and were matched with standards available in Unani pharmacopoeia while many new parameters which are not reported earlier were done as per the guidelines available to ensure its uniform quality. The attempt is to present an updated standardization profile of this important drug to help ISM drug manufacturers to produce quality medicine.

Review of literature

Viola odorata Linn. (Family-Violaceae) - a glaborous or pubescent perennial herb about 10-15 cm in height arising from short stout rootstocks, leaves are in radial/terminal tufts with bluish purple or white flowers borne singly on axillary peduncles, scented, tastes nauseous, bitter and mucilaginous (Anonymous, 2006). Commercially plant and flowers are used in medicine (Dymock, 1890; Khory and Katrak, 1985) while the native healers consider the purple flowered variety to be the best; they use the flower separately and also the entire plant (Dymock, 1890) and both are sold in the market in the name of 'banafshah'. Native of Kashmir and temperate western Himalaya, found at an altitude of 1500-1800 m, above 5000 ft., distributed over north and west Asia, North Africa, Europe, Nepal, Mishmi, and Khasi hills and China (Anonymous, 1976). *V. odorata* from Kashmir is considered to be of finest in quality (Ghani, 1921), which is often planted. In northern India *Viola cineria* Bioss. and *Viola serpenes* Wall. are used as substitute for *Viola odorata*, and are called as Banafshah (Dymock, 1890) and commercial drug available in the Indian market is generally highly adulterated with other *Viola* spp. as *V. biflora, V. canescens, V. cinerea, V. pilosa, V. sylvestris* (Anonymous, 1976).

Ethnopharmacological Reports: Flowers are astringent, demulcent, diaphoretic, diuretic, laxative, refrigerant and expectorant (Nadkarni, 2000); flower and leaves are used in bilious affections, lung troubles, prolapse of the rectum and uterus and in restraining suppuration, kidney diseases, for calculous affections and liver affections, fevers, syphilis, skin diseases, chronic diarrhoea and dysentery (Anonymous, 1976; Farooq, 2005; Khory and Katrak, 1985; Pandey and Chadha,

1996; Sharma, 2003). It is also used as blood purifiers (Bhattacharjee and De, 2005; Chopra *et al*., 1958; Farooq, 2005; Sharma, 2003). The herb shows antimycotic and antibacterial activity, and is considered quite effective in the treatment of eczema (Kiritikar and Basu, 1996).

Use in Traditional medicine: The literature review undertaken on the test drug Banafshah reveals that the drug has been traditionally used in many diseases as in bilious affections, lung troubles, prolapse of the rectum and uterus, kidney diseases, liver affections, in fevers and as blood purifier (Attar, 1888; Ghani, 1921; Ibne Sina, 1887; Ibne Baitar, 1885; Khan, 1892; Hakeem, 2002). Present study is an attempt to provide standardization profile of a market sample of banafshah matched with the standards available and to explore those which are not existing but are necessary, as per pharmacopoeial guidelines and could be set as standard. The study was done in Department of Ilmul Advia, A.M.U., Aligarh in the year 2009-2010.

Material and Methods

Plant Material: Dried herbal material of Banafshah (*V. odorata* Linn.) was procured from the local market of Kashmir and botanically identified with available literature and then confirmed by Prof. S.H.Afaq in Pharmacognosy Section. A herbarium sample (Voucher No. SC-0099/09-V) was prepared and submitted in the museum of the Department of Ilmul Advia for future reference. Leaves and flowers were handpicked to remove them from debris material that includes some sandy material and wood pieces (Fig.1). They were washed with DDW and dried at room temperature in a ventilated room, milled to coarse powder and stored in a close air tight container in dark until use. Strict aseptic precautions were followed throughout the process.

Physico-chemical analysis: The analysis include the study to determine ash value, melting point, moisture content, pH value at 1% and 10% solution, solubility, bulk density, loss on drying (Jenkins *et al*., 1967; Anonymous, 1968; Anonymous, 1970; Afaq *et al*., 1994).

Phytochemical analysis: The analysis include the determination of the extractive values in different organic solvents, qualitative analysis of the chemical constituents present in the drug sample, Fluroscence Analysis of the powdered drugs and successive extracts (FTAR Analysis), crude fiber content, alkaloid estimation, Thin Layer Chromatography (TLC) (Jenkins *et al*., 1967; Anonymous, 1968; Anonymous, 1970; Afaq *et al*., 1994; Peach *et al*., 1955).

Statistical Analysis: Results are expressed as Mean, Standard error of Mean with Standard deviation. All the tests done were carried out in triplicates in standard laboratory conditions following the guidelines of Good Laboratory Practices (GLP).

Results

Correct identification and quality assurance of the raw material is an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy. So, standardization of the selected market sample of Banafshah was done as an up most criterion of our study. As the efficacy of many drugs mainly depends upon its physical and chemical properties therefore, the determination of physico-chemical characters for the authenticity of a drug is necessary before studying any medicinal property. These studies of any phyto drugs are necessary for standardization, as it helps in understanding the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially to the determination of the purity and quality of the drugs and chemicals official to it as in National Pharmacopoeia. It is also more important, because it helps in characterization of constituents or group of constituents that frequently lead to establish the structure-activity relationship and the likely mechanism of action of the drug. Phyto-chemical constituents present in the drug vary, not only from plant to plant but also among different samples of same species, depending upon various atmospheric factors, storage and drying conditions; a little deviation from the normal in terms of quality and quantity of the constituents may alter the effect of drug. Apart from the degradation in the quality of the drugs that occurs due to above conditions, adulteration also contributes to variability. Thus, keeping in view the above consideration, physico-chemical studies on the drug under study were carried out to characterize the drug sample for the future reference. Organopleptic features are presented in Table-1.

Parameters used for the physico-chemicals study of the test drugs were: (i) Ash value; (ii) Moisture Content; (iii) pH value; (iv) Melting range; (v) Solubility; (vi) Bulk density; (vii) Crude fiber estimation; (viii) Alkaloid estimation. Results are expressed as Mean value in Table-2.

Parameters used for the phyto-chemicals study of the test drugs were: (i) Extractive value (Table-2); (ii) Qualitative analysis of the phyto-chemicals (Table-3); (iii) Fluorescence Analysis of Powdered drugs (Table-4); (iv) Fluorescence Analysis of the Successive extracts of the test drug (Table-5); (v) IR Spectral Analysis of the test drugs (Table-6); (vi) Thin Layer Chromatography (Table-7).

Fresh sample - *V.odorata*

Parts Used - *V. odorata*

Market sample- *V. odorata*

Fig.1: Banafshah (*Viola odorata* Linn.)

Day Light

UV Short

TLC Banafshah - Petroleum ether extract

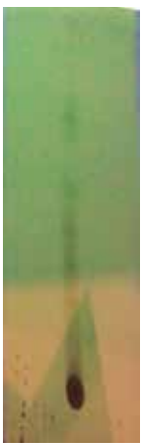
Iodine Vapour

UV Long

TLC Banafshah - Chloroform extract

Day Light

Iodine Vapour



UV Long

UV Short

TLC Banafshah- Ethanolic extract

Fig 2: Thin Layer Chromatography of Banafshah (Viola odorata Linn.)

Table 1: Organoleptic Characters of Banafshah (Viola odorata Linn.)

Parameters	Banafshah
Colour	Dark Green
Smell	Odourless
Taste	Slightly Bitter

Table 2: Physicochemical study of market sample of Banafshah

S. No	Parameters		*V.odorata* Linn. (Violaceae) Mean ± S.E.M. (S.D) (Market Sample)	*V.odorata* Linn. (Violaceae) (Percentage / Gram) (CCRUM, 2006)	*V.pilosa* Blume. (Violaceae) (Percentage / Gram) (UPI, 2007)
1	Percentage of Ash Value (w/w)	Total Ash	11.24 ± 0.01(0.02)	Not more than 14.25%	Not more than 13%
		Acid Insoluble Ash	3.15 ± 0.00(0.01)	Not more than 1.7%	Not more than 3%
		Water Soluble Ash	2.35 ± 0.07(0.19)	-------	-------
		Sulphated Ash	0.59 ± 0.02(0.05)	-------	-------
2	Moisture Content (v/w)	Loss of weight on Drying at 1050C	12.28 ± 0.01(0.02)	-------	-------
		Toulene Distillation Method	12.60 ± 0.01(0.02)	-------	-------
3	pH Value	1% solution	7.05 ± 0.01(0.02)	-------	-------
		10% solution	6.52 ± 0.01(0.02)	-------	-------
4	Solubility (% in w/w)	Alcohol Soluble	18.49 ± 0.02(0.04)	Not less than 5.25%	Not less than 2%
		Water Soluble	26.72 ± 0.02(0.04)	Not less than 18.31%	Not less than 11%
5	Extractive Value (% in w/w)	Petroleum Ether	1.69 ± 0.02(0.05)	-------	-------
		Diethyl Ether	0.85 ± 0.02(0.03)	-------	-------
		Chloroform	0.76 ± 0.01(0.03)	-------	-------
		Ethanol	9.53 ± 0.32(0.56)	-------	-------
		Aqueous	11.88 ± 0.28(0.49)	-------	-------
6	Melting Range		102-1200C	-------	
7	Bulk Density (% in w/w)		0.54 ± 0.01(0.02)	-------	
8	Crude Fiber Content (% in w/w)		7.33 ± 0.01 (0.02)	-------	
9	Total Alkaloid estimation (% in w/w)		6.04± 0.08(0.01)	-------	

Table 3: Qualitative Analysis of the Phytochemicals

S.No	Chemical Constituents	Test Reagents	Banafshah
1.	Alkaloids	Dragendorff's reagent	+
		Wagner's reagent	+
		Mayer's reagent	+
2.	Carbohydrates	Molish Test	+
		Fehling Test	+
		Benedict Test	+
3.	Flavonoids	Mg Ribbon and dil. Hcl	+
4.	Glycosides	NaOH Test	+
5.	Tannins/Phenols	Ferric Chloride Test	+
		Liebermann's test	+
		Lead Acetate test	+
6.	Proteins	Xanthoproteic test	_
		Biuret test	+
7.	Starch	Iodine Test	_
8.	Saponnins	Frothing with NaHCO3	+
9.	Steroids/Terpenes	Salkowski Reaction	+
10.	Amino acids	Ninhydrin Solution	+
11.	Resins	Acetic anhydride test	+

Indications: '_'Absence and '+' Presence of constituents

Table 4: Fluorescence Analysis of the Test Drugs with chemicals of Banafshah

S.No.	Powdered drug	Day Light	UV Short	UV Long
1.	P. drug + Con. HNO_3	Light Orange	Light Green	Green
2.	P. drug + Con. Hcl	Dark Green	Light Green	Light Green
3.	P. drug +Con. H_2SO_4	Dark Brown	Black	Black
4.	P. drug +Iodine sol. (5%) in alcohol	Gold Brown	Brownish Green	Black
5.	P. drug + Glacial Acetic acid	Green	Green	Black
6.	P. drug + Gl. Acetic acid+ HNO_3	Green	Green	Dark Green
7.	P. drug + NaOH Solution (10%)	Dark Green	Dark Green	Black
8.	P. drug +10%NaHO+Concn HNO_3	Brown	Dark Green	Very dark Green
9.	P. drug +dilute HNO_3	Green	Dark Green	Black
10.	P. drug + dilute H_2SO_4	Dark Green	Dark Green	Black

S.No.	Powdered drug	Day Light	UV Short	UV Long
11.	Powdered drug + dilute Hcl	Dark Green	Green	Black
12.	P. drug + Drangendorff reagent	Brownish Green	Dark Green	Black
13.	P. drug +Wagner's reagent	Dark Green	Brownish Green	Dark Green
14.	P. drug + Benedict's reagent	Dark Green	Bright Green	Dark Green
15.	P. drug + Fehling Reagent	Very Dark Green	Dark Green	Dark Blue
16.	P. drug + KOH (10%) methanolic	Very Light Yellow	Green	Dark Green
17.	P. drug +$CuSO_4$ (5%)	Light Green	Dark Green	Black
18.	P. drug + Ninhydrin (2%) in acetone	Dark Green	Dark Green	Black
19.	P. drug + Picric acid	Light Green	Light Green	Green
20.	P. drug + Lead Acetate (5%)	Dark Green	Light Green	Black

Table 5: Fluorescence Analysis of the successive extracts of Banafshah

Extracts	Day Light	UV Short	UV Long
Petroleum ether	Brown	Light Green	Dark Brown
Diethyl ether	Dark Green	Dark Brown	Black
Chloroform	Black	Green	Dark Black
Alcohol	Brown	Green	Greenish Brown
Aqueous	Brown	Dark Green	Black

Table 6: IR Spectral Details of Alcoholic Extract of Drug

Test Drug	IR, υ (cm-1)
Banafshah	3463.19, 2930.35, 2365.70

Table 7: Thin Layer Chromatography of Banafshah

Extract	Solvent System	Treatment	Visualizing Agent	No. of Spots	Rf value
Petroleum ether	Benzene: Chloroform (8:2)	I2 Vapour	Day Light	3	0.06, 0.10, 0.20
			UV Long	3	0.06, 0.10, 0.20
			UV Short	1	0.10(G)
	Petroleum ether: ether (8:2)	”	Day Light	4	0.07, 0.15, 0.53, 0.61
			UV Long	3	0.07, 0.53, 0.61
			UV Short	1	0.53(D.G)

Extract	Solvent System	Treatment	Visualizing Agent	No. of Spots	Rf value
Chloroform	Benzene : Chloroform (4:1)	I2 Vapour	Day Light UV Long UV Short	1 2 1	0.08 0.13 0.13(L.G)
	Chloroform: Methanol (3:7)	”	Day Light UV Long UV Short	1 4 5	0.41 0.33. 0.5, 0.75, 0.83 0.50 (G), 0.54(D.G), 0.63(L.G), 0.83(G), 0.90(D.G)
Alcohol	Toulene: Ethyl acetate : Benzene : Acetic acid (4:1:2:2 drops)	I2 Vapour	Day Light UV Long UV Short	6 6 5	0.23, 0.30, 0.35, 0.38, 0.49, 0.52 0.23, 0.30, 0.35, 0.38, 0.49, 0.52 0.30(L.Br.), 0.35(Br.), 0.38(Br.), 0.49(G), 0.52(L.G)
	Benzene: Ethyl acetate: Di ethyl ether	”	Day Light UV Long UV Short	1 1 1	 0.54, 0.63 0.54 0.54(D.Br.)

Discussion

Standardization is considered as a prerequisite for any phyto-drug to assess its biological activity or determination of biological standards of the herbal material that provides the analytical characteristics which may prove to be useful in fixing the physicochemical standard.

In the present study it was observed from the descriptions available in Unani pharmacopoeia of India (2007) that the drug mentioned under the name of Banafshah is botanically a different species i.e. *Viola pilosa* of Violaceaeae family where they have mention of leaves of Banafshah, so it is obvious that the standard parameters available in UPI (Anonymous, 2007) are just for identity of leaves; and in our analysis of market sample (MS) of *V.odorata* , we have taken leaves and flowers of Banafshah , so there are upmost chance of slight variation and which are obvious due to change in species of the drug, but still it was seen that the variation was not so large or may be said to be none, as according to UPI (2007) total ash value /gm of the drug should not be more than 13% , and in MS it was of 11.24% . Similarly in acid insoluble ash value UPI (2007) report it to be not more than 3% and in MS it was found to be 3.15%. Other parameter for identification measurement was of solubility in UPI (2007); that alcohol solubility should not be less than 2% and water solubility to be not less than 11% and

in MS alcohol solubility was 18.49% and water solubility 26.72%, i.e as per the range of UPI, 2007. So, in spite of a different species and flowers combined in our drug sample the difference is not there in two different species or the study may also be taken as that when leaves and flowers both are used the values of above mentioned parameters should be as available in the present study.

And when these results are compared with other works of Ministry of AYUSH in Central Council for Research in Unani Medicine (Anonymous, 1992; Anonymous, 2006), it was observed that the drug sample mentioned is of same species i.e. *V.odorata*, but in that also the analytical parameters that are mentioned are for leaves of Banafshah. Total ash value mentioned for same species is not more than 14.25% , and in MS it was found to be 11.24% (below 14.25%) and acid insoluble ash is reported in Council research work is that it should not be more than 1.7%, and in MS it was 3.15% (above from 1.75%) that is a difference in the acid insoluble value. However alcohol solubility mentioned is not less than 5.25% and water solubility as 18.31% and in MS alcohol solubility was found to be 18.49% (i.e. above 5.25%) while aqueous solubility as 26.72% (above 18.31%). So, the same species is matched with this work, and can be taken as more approachable sample of Banafshah to match with the standard. And it was observed that market sample of Banafshah studied for physio-chemical and phytochemical analysis is of standard quality and authentic.

Conclusion

Sample of Banafshah available in the market is of standard value in terms of its physico-chemical and phyto-chemical standards as per the pharmacopoeial guidelines with a slight variation, as matched with reported parameters available for its identity and purity. Some new parameters were also studied that are not mentioned in the pharmacopoeia and reported for the first time viz., FTAR Analysis, IR Spectral study. Additional standardization parameters may supplement the existing parameters and provide a roadmap for further research analysis of 'Banafshah' to check for its identity and purity so as to have genuine, authentic, safe drug with uniform pharmaceutical efficacy (Shariq, 2008).

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