Physico-chemical and Phyto-chemical Standardization of a Unani Drug Banafshah (*Viola odorata* Linn.)

***Sumbul Rehman and Abdul Latif**

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

Abstract

In view of the growing demand of Ayurvedic and Unani drugs in domestic and global market, there is a need to ensure their quality, efficacy and safety through scientitific evaluation and laying down pharmacopoeial standards. In the present work, Banafshah (*Viola odorata* L.) has been standardized for its physico-chemical and phyto-chemical parameters as per WHO pharmacopoieal guidelines. The parameters evaluated includes: ash values: total ash, acid insoluble ash, water soluble ash, sulphated ash; moisture content, loss on drying; pH value at 1% solution and at 10% aqueous solution; melting range; solubility: water soluble extractive and alcohol soluble extractive; bulk density; crude fibre content and total alkaloid content. On phytochemical analysis it was found that Banafshah contains alkaloids, carbohydrates, flavonoids, glycosides, phenols and proteins. Besides this, determination of organoleptic characters of powder drug, extractive values in different organic solvents using soxhlet extractor, thin layer chromatography and fluorescence analysis of successive extracts of powder drug had been done. The study will help in laying down pharmacopoeial standards to determine the quality and purity of Unani drug *Viola odorata* Linn. for wider use in the manufacture of genuine herbal medicines.

Keywords: *Viola odorata* Linn., Physico-chemical, Phyto-chemical, Standardization.

Introduction

Herbal remedies derived from plants represent a substantial proportion of the global drug market and in this respect internationally recognized guidelines for their quality assessment are necessary. WHO has therefore stressed the need to ensure quality control of medicinal plant products for global consumption by using modern techniques and applying suitable standards (Iyengar, 2002).

Unani system of medicine is entirely based on the drugs of natural source and majority of the drugs are of herbal origin. And like any other system of medicine the efficacy of Unani system also depends on the efficacy and purity of drugs used. With the tremendous increase in the global use of medicinal plants, several concerns regarding the efficacy and safety of the herbal medicines have also been raised (Latif and Rehman, 2014). Hence it has become priority to standardize the Ayurvedic and Unani drugs to have uniform efficacy and safety measures so as to ensure regular supply of authentic medicinal plants and raw drugs. Present work is based on this rationale and deals with the pharmacopoeial standardization of a Unani drug Banafshah, *Viola odorata* L. in an attempt to

*Author for correspondence

ensure its identity, purity and genuineness to manufacture quality herbal medicines.

Viola odorata L. (Family-Violaceae) has been in use since ancient times by Greeks. Baitar (1985) has also mentioned about its medicinal uses. Native doctors consider the purple flowered variety to be the best; they use the flower separately and also the entire plant (Dymock, 1890). The herb is well known in India for its medicinal virtues and has been in use since ancient times. It is used for several diseases in Ayurvedic and Unani medicines.

Morphology and distribution

It is a glabrous or pubescent herb, rarely more than 15 cm. in height, arising from a rootstock, found in Kashmir and the temperate Western Himalaya at an altitude of 1500-1800m, above 5000 ft (Anonymous, 1976; Chopra *et al*., 1958; Ghani, 1921; Hooker, 1875; Nadkarni, 2000) in north temperate zone Nepal, Mishmi, and Khasi hills, China (Bhattacharjee and De, 2005; Dymock, 1890), temperate climates, Europe, North America (Khory and Katrak, 1985). *V. odorata* from Kashmir is considered to be of finest in quality (Anonymous, 1976).

Therapeutic effect

It is especially valued as a diuretic and expectorant, as a purgative in bilious infections; it is seldom given alone, but is prescribed along with other drugs, which also have an aperiant action such as tamarind, myrobalan. 'Banafshah' is recommended generally in those diseases where cooling treatment is thought to be indicated by the Unani physicians (Anonymous, 1976; Dymock, 1890; Khory and Katrak, 1985; Ibne Sina, 1887). Its leaves are said to relieve pain possibly due to cancerous growths, particularly in the mouth and throat (Anonymous, 1976). The fresh flowering herb is used in the homeopathy for the treatment of the diseases of skin and eyes, and for relief from pain in the ear. In folk medicine, it is used as a blood purifier. In large doses, the leaves as well as the roots are used as cathartic. The seeds are purgative and diuretic, they contain salicylic acid (Anonymous, 1976).

Substitutes / Adulterants

Being so much of therapeutic importance, the drug *Viola odorata* L. is substituted with many adulterants. The commercial drug available in the Indian markets is generally highly adulterated with other *Viola* spp. These include *V. biflora*, *V. canescens*, *V. cinerea*, *V. pilosa*, *V. sylvestris* (Anonymous, 1976). In northern India *Viola cineria* Bioss. and *Viola serpenes* Wall. are used as substitute for *Viola odorata,* and are called as Banafshah (Dymock, 1890; Trease and Evans, 2009).

Therefore, present study was done to lay down standards on physico-chemical and phyto-chemical profile for *Viola odorata* as per WHO guidelines laid down for standardizing herbal drugs.

Material and Method

Collection of plant material

Whole herb of *Viola odorata* was procured from Kashmir and was identified by the Pharmacognosy Section, Department of Ilmul Advia, Aligarh Muslim University, Aligarh. The studied sample is preserved in the Herbarium of the Department, for future reference (Voucher No. SC-0099/09-V). Herb so obtained was dried at optimum temperature and further crushed and sieved to coarse powder mechanically and stored in air tight container for study (Fig-1).

Physico-chemical analysis

The analysis included the determination of ash value, melting point, moisture content, pH value at 1% and 10% solution, solubility, bulk density, loss on drying (Afaq *et al.*, 1994; Anonymous, 1968; 1970).

Fig. 1: Crude drug sample of *Viola odorata* Linn.

Phytochemical analysis

The analysis included the determination of the extractive values in different organic solvents, qualitative analysis of the chemical constituents present in the drug sample (Anonymous, 1987; Brewster and Ewen, 1971). Fluorescence analysis of the powdered drugs and successive extracts (FTAR Analysis), crude fibre content, alkaloid estimation (Farnsworth, 1966; Jenkins *et al*., 1967, Peach and Tracey, 1955).

IR spectroscopic study

For this, alcoholic extract of the drug was obtained by refluxing powdered drug (5.0 g) with absolute alcohol (50 ml) for 5 hrs and removing the solvent under reduced pressure. The IR spectrum of alcoholic extract was determined in KBr pellets with Perkin Elmer 1600 FTIR spectrometer (Peach and Tracey, 1955).

Thin layer chromatography

TLC analysis was conducted using different organic solvent systems in percolated silica gel 60F254 TLC plates. Thin Layer Chromatography of the extract of the test drug was carried out by spotted TLC plates were exposed to Iodine vapours in Iodine chamber and then heated at 105^0 C in oven for 10 minutes; plates were visualized in day light and UV short and long wavelength. The R_f value of spots was determined by the given formulae (Afaq *et al*., 1994; Anonymous, 1968; 1970).

$$R_f \text{ value} = \frac{\text{Distance travelled by the Spot}}{\text{Distance travelled by the Solvent}}$$

Observations and Results

Organoleptic characters: The powder of the dried herb of *V. odorata* was dark green with characteristic odourless and slightly taste, summarized in table-1.

Physico-chemical constants: Different physico-chemical constants were determined three times and then average values depicted in table-2.

Table 1: Organoleptic characters of powder of *Viola odorata* Linn.

S.No.	Parameter	Appearance
1.	Colour	Dark Green
2	Smell	Odourless
3.	Taste	Slightly bitter

Table 2: Physico-chemical analysis of *Viola odorata* Linn.

S.No.	Physicochemical Parameter	Results Mean±S.E.M. (S.D.)
1.	Moisture Content	
	Loss of Weight on Drying	12.28 ± 0.01 (0.02)
	Toulene Distillation Method	12.60 ± 0.01 (0.02)
2.	Ash Value (in %)	
	Total Ash	11.24 ± 0.01 (0.02)
	Acid Insoluble Ash	3.15 ± 0.00 (0.01)
	Water Soluble Ash	2.35 ± 0.07 (0.19)
	Sulphated Ash	0.59 ± 0.02 (0.05)
3	pH Values (in %)	
	pH at 1%	7.05 ± 0.01 (0.02)
	pH at 10%	6.02 ± 0.01 (0.02)
4	Bulk Density (in gm/ml)	0.54 ± 0.01 (0.02)
5	Melting Range	102-120^{0}C
6.	Solubility (in %)	
	Alcohol Soluble extractive	18.49 ± 0.02 (0.04)
	Water Soluble extractive	26.72 ± 0.02 (0.04)

Phyto-chemical analysis: The phyto-chemicals present in the drug were qualitatively analysed by different chemical tests and results are given in table-3.

Qualitative analysis of the Phyto-chemicals: Qualitative analysis of the phyto-chemical reveals the presence of alkaloids, carbohydrates, proteins, amino acids, phenols, sterols, glycosides, flavonoids, tannins, resins, sterols/ terpenes and volatile oil presented in table-4.

Table 3: Phyto-chemical analysis of *Viola odorata* Linn.

S.No.	Physicochemical Parameter	Results Mean ± S.E.M. (S.D.)
1.	Crude Fibre Content	7.33 ± 0.01 (0.02)
2.	Total Alkaloid Estimation	6.04 ± 0.08 (0.01)
3.	Extractive values in different organic solvent	
	Petroleum ether (60-80^0)	1.69 ± 0.02 (0.05)
	Diethyl Ether	0.85 ± 0.02 (0.03)
	Chloroform	0.76 ± 0.01 (0.03)
	Alcohol	9.53 ± 0.32 (0.56)
	Aqueous	11.88 ± 0.28 (0.49)

Table 4: Qualitative analysis of the phytochemicals of *Viola odorata* Linn.

S.No.	Chemical Constituents	Test Reagents	Results
1.	Alkaloids	Dragendorff's Reagent	+ve
		Wagner's reagent	+ve
		Mayer's reagent	+ve
2.	Carbohydrates	Molish Test	+ve
		Fehling Test	+ve
		Benedict Test	+ve
3.	Flavonoids	Mg Ribbon and dil. Hcl	+ve
4.	Glycosides	NaOH Test	+ve
5.	Tannins/Phenols	Ferric Chloride Test	+ve
		Liebermann's test	+ve
		Lead Acetate test	+ve
6.	Proteins	Xanthoproteic test	-ve
		Biuret test	+ve
7.	Starch	Iodine Test	-ve
8.	Saponins	Frothing with $NaHCO_3$	+ve
9.	Steroids/Terpenes	Salkowski Reaction	+ve
10.	Amino acids	Ninhydrin Solution	+ve
11.	Resins	Acetic anhydride test	+ve

Indications: ' -ve 'Absence and '+ve' Presence of constituents

Florescence analysis: Florescence analysis under UV light is sometime very characteristic for a drug. As many drugs and the constituents present in the drug emit specific colour when they are exposed to ultraviolet radiations because the radiant energy excites the solution which emits that particular colour known as fluorescence. Hence the fluorescence analysis of the successive extracts and the powdered drug of Banafshah treated with different chemical reagent was done and different change in the colour so appeared was observed and noted. The details are presented in table-5 & 6.

IR spectral study of the drug: Novel IR spectral study of the alcoholic extract of the drug was done by running the alcoholic extract in the IR range (3500-490 cm^{-1}) of the electro-magnetic spectra and major characteristic peaks were noted (Table 7).

Table 5: Fluorescence analysis of *Viola odorata* Linn.

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 + NaOH Sol. (10%)	Dark Green	Dark Green	Black
5.	P. drug + Glacial Acetic acid	Green	Green	Black
6.	P. drug +dil. HNO_3	Green	Dark Green	Black
7.	P. drug + dil. H_2SO_4	Dark Green	Dark Green	Black
8.	P. drug + dil. Hcl	Dark Green	Green	Black
9.	P. drug +Wagner's reagent	Dark Green	Brownish Green	Dark Green
10.	P. drug + Benedict's reagent	Dark Green	Bright Green	Dark Green
11.	P. drug + Fehling Reagent	Very Dark Green	Dark Green	Dark Blue
12.	P. drug + Picric acid	Light Green	Light Green	Green
13.	P. drug + Lead Acetate (5%)	Dark Green	Light Green	Black
14.	P. drug +$CuSO_4$ (5%)	Light Green	Dark Green	Black
15.	P. drug + KOH (10%) methanolic	Very Light Yellow	Green	Dark Green
16.	P. drug + Glacial Acetic acid+ HNO_3	Green	Green	Dark Green
17.	P. drug +10%NaOH + $Conc^n$ HNO_3	Brown	Dark Green	Very dark Green
18.	P. drug + Dragendorff reagent	Brownish Green	Dark Green	Black
19.	P. drug + Ninhydrin (2%) in acetone	Dark Green	Dark Green	Black
20.	P. drug + Iodine sol. (5%) in alcohol	Gold Brown	Brownish Green	Black

P. drug = Powdered Drug

Table 6: Fluorescence analysis of the successive extracts of *Viola odorata* Linn.

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 7: IR Spectral study of *Viola odorata* Linn.

Test Drug	IR , υ (cm^{-1})
Banafshah (*V.odorata* Linn.)	3463.19, 2930.35, 2365.70

Thin layer chromatographic profile: Thin layer chromatographic analysis of the various extracts of *V. odorata* was carried out using different solvent systems methanol: acetic acid (45: 8: 4) as solvent system. R_f values were calculated after the development of chromatogram. The R_f values in the given solvent are used to characterize the drugs identity and purity. The results obtained are given in fig. 2; table-8.

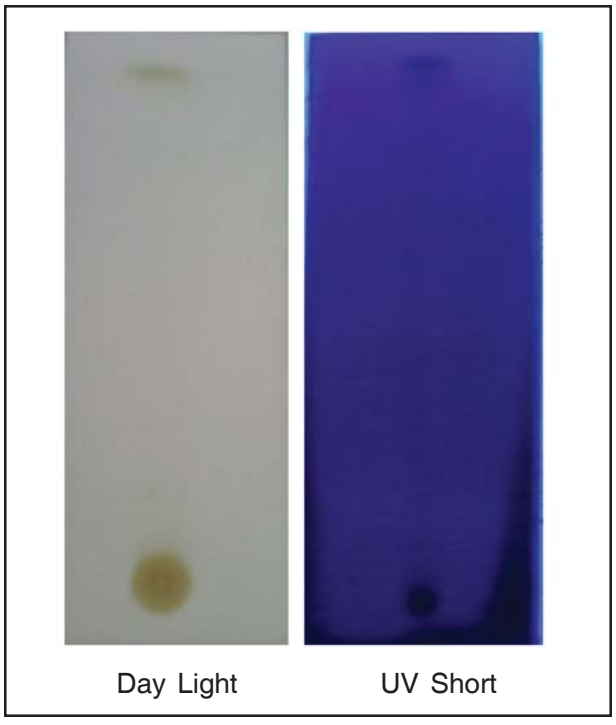

Fig. 2: TLC Banafshah- Petroleum ether extract

Table 8: Thin layer chromatography of *Viola odorata* Linn.

Extract	Solvent System	Treatment	Visualizing Agent	No. of Spots	R_f value
Petroleum ether	Benzene: Chloroform (8:2)	I_2 Vapour	Day Light UV Long UV Short	3 3 1	0.06, 0.10, 0.20 0.06, 0.10, 0.20 0.10(G)
	Petroleum ether: ether (8:2)	"	Day Light UV Long UV Short	4 3 1	0.07,0.15, 0.53, 0.61, 0.07, 0.53, 0.61 0.53 (D.G)
Chloroform	Benzene: Chloroform (4:1)	I_2 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)	I_2 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.)

D: Dark L: Light Br.: Brown Bl: Blue G: Green
Y: Yellow O: Orange B: Black Fl.: Fluorescent

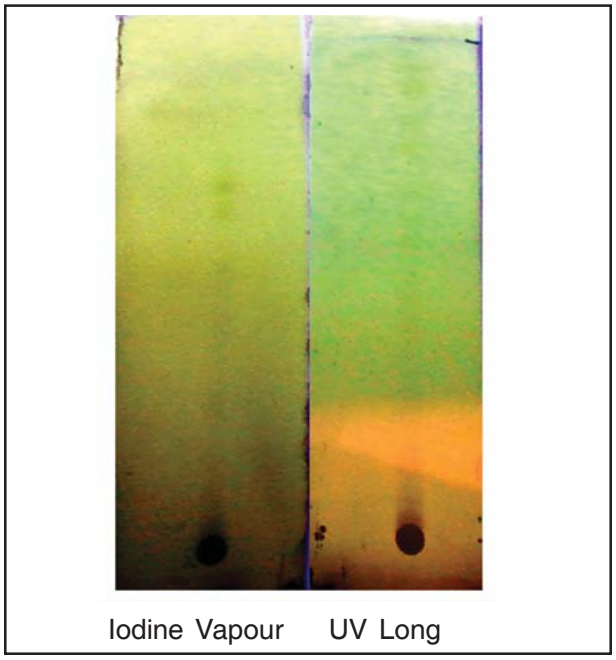

Fig. 3: TLC Banafshah-Chloroform extract

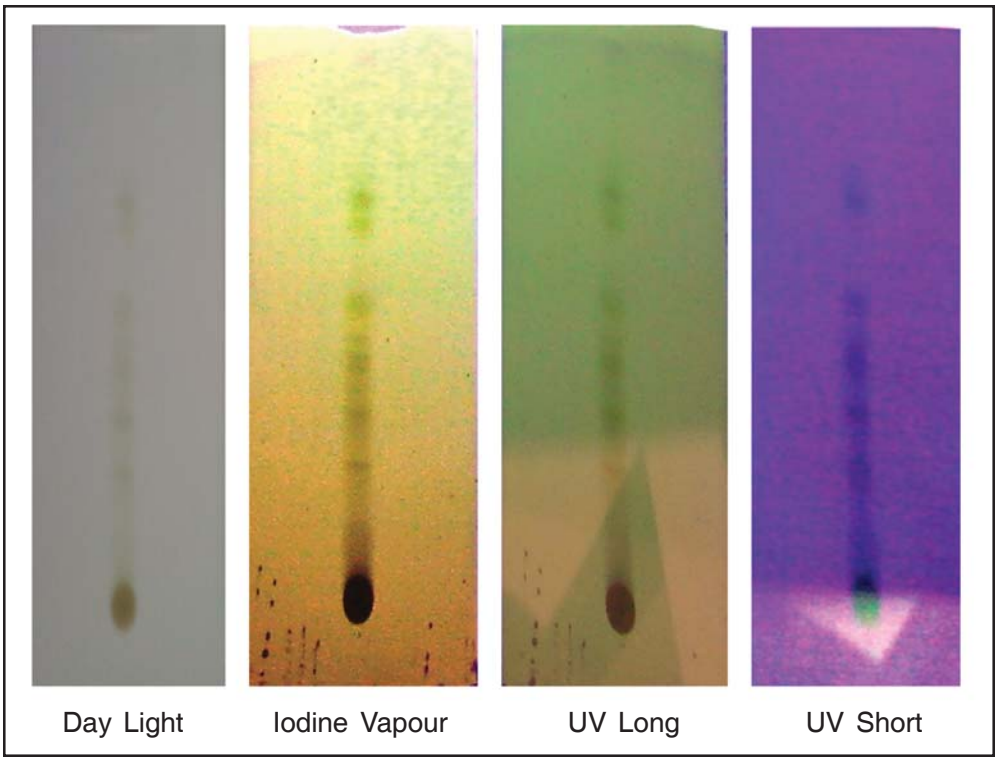

Fig. 4: TLC Banafshah- Ethanolic extract

Conclusion

The physico-chemical evaluation of the powder drug reveals the standard parameters for the quality and purity of herbal drugs and also gives information regarding the authenticity of crude drug. The data generated in the present study for Banafshah (*Viola odorata* L.) will be helpful in future for determining the quality and purity of this drug so as to ensure its therapeutic efficacy.

Acknowledgement

Authors are thankful to DRS-I (UGC), Department of Ilmul Advia, A.K. Tibbiya College, AMU, Aligarh for providing financial assistance during the study.

References

Anonymous, 1970. Pharmacopoeia of India. 2nd edition. Ministry of Health & Family Welfare, New Delhi, pp 238-239.

Anonymous, 1976. The Wealth of India: A dictionary of Indian Raw materials & Industrial Products. Vol. X. CSIR, New Delhi, pp 514-517.

Afaq, S.H., Tajuddin., Siddiqui, M.M.H., 1994. Standardization of Herbal Drugs. AMU Publication Division, Aligarh, pp 33-34, 41-42, 100, 143-146.

Anonymous, 1968. British Pharmacopoeia. General Medicine Council. Pharmaceutical Press, Bloomsbury square, London, pp 1276-77.

Anonymous, 1987. Physiochemical Standards of Unani Formulations, Part 2. CCRUM, New Delhi, pp. 274-278.

Baitar I., 1985. Al-jamiul-mufridat-ul-advia-wa-ul-aghzia. (Urdu Translation), Part-I. CCRUM, New Delhi, pp. 287-288.

Bhattacharjee, S.K. and De, L.C., 2005. Medicinal Herbs and Flowers. Aavishkar Publishers, Jaipur, pp 267-268, 398-399.

Brewster, R.C. and Ewen, M.C., 1971. Organic Chemistry, 3rd Ed. Prentice Hall of India Pvt. Ltd., New Delhi, p. 604.

Chopra, R.N., Chopra, J.C., Handa, K.L. and Kapur, L.D., 1958. Indigenous drugs of India. U.N. Dhur and Sons Pvt. Ltd., Calcutta, pp 98, 529, 676.

Dymock, W., 1890. Pharmacographica Indica-A History of The Principal Drugs of Vegetable Origin, Met with in British India, Vol. I. The Institute of Health and Tibbi Research, Pakistan, pp 16-17, 43-44.

Farnsworth, N.R., 1966. Biological and Phytochemical screening of plants. *J. Pharm. Sci.* 55: 225.

Ghani, H.N., 1921. Khazainul advia, Vol. II. Idara Kitabul Shifa, New Delhi, pp 99, 397-398.

Iyengar, M.A., 2002. Quality Control and Standardization of Drugs. In : R.U. Ahmad and Rajeev Kr. Sharma (Eds.) Proceedings of WHO Training-cum-Workshop on Production of ISM&H Drugs with Current Good Manufacturing Practices. Pharmacopoeial Laboratory of Indian Medicine, Ghaziabad, Dept. of ISM&H, New Delhi, pp. 71-74.

Hooker, J.D., 1875. Flora of British India, Vol. I. Bishen Singh Mahendra Pal Singh under the authority of the Secretory of state for India in Council, London, p. 184.

Ibn-e-Sina, 1887. Al-Qanoon-fi'l-tib, Vol. II. (Urdu translation by Ghulam Hussain Kantoori). Matba Nawal Kishore, Lucknow, p. 55.

Jenkins, G.L., Knevel A.M. and Digangi, F.E., 1967. Quantitative Pharmaceutical Chemistry. 6th edition. The Blackiston Division, McGraw Hill Book Company, U.S.A, pp. 225, 235, 379, 425, 463, 492.

Khory, R.N. and Katrak, N.N., 1985. Materia Medica of India and Their Therapeutics. Neeraj Publishing House, New Delhi, pp. 10, 54, 412-413.

Latif, A. and Rehman, S., 2014. Standardization of a Herbal Medicine-*Swertia chirayita* Linn. *Pharmacophore* 5 (1): 98-108.

Nadkarni, K.M., 2000. The Indian Materia Medica, Vol. I. Bombay Prakashans Pvt. Ltd., pp. 1274-75.

Peach, K. and Tracey, M.V., 1955. modern methods of Plant Analysis, Vol. III. Springer-Verlag. Berlin-Guttingen-Heidelberg, pp. 626-27.

Trease and Evans, 2009. Pharmacognosy, 15th edition. Elsevier publication, Delhi, pp. 429, 456, 481.