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Original Article

Preliminary pharmacognostical and phytochemical evaluation of *Stachys tibetica* Vatke

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ABSTRACT

Stachys tibetica Vatke (Lamiaceae) is an important medicinal plant in the folk medicine of Ladakh, India and Tibet for the treatment of various mental disorders. Infusion and decoction of the whole plant is used as a cup of tea for a severe fever, headaches and to relieve tension. The recent study is aimed to evaluate the preliminary pharmacognostical and phytochemical nature of *Stachys tibetica* Vatke. The whole plant material was subjected to successive soxhlet extraction with petroleum ether (40 - 60°C), chloroform, ethyl acetate, methanol and finally decocted with water to get the respective extracts. The fluorescence characteristics of the powdered materials were analysed under ultraviolet light and ordinary light. Different physicochemical parameters such as ash value, extractive value, foaming index, pH values, loss on drying and determination of foreign matter were carried out as per WHO guidelines. The total fat, flavonoid, saponin and volatile contents were also determined. Macroscopical studies revealed the authentication of the plant drug. Physicochemical parameters helped to standardize the plant material while preliminary qualitative chemical tests of different extracts showed the presence of Glycosides, Carbohydrates, Phytosterols/triterpenoids, Saponins, Fixed oils, Fats and phenols/tannins. Quantification of the total flavonoids and saponins and contents were determined as 54.66 ± 0.58 mg/g and 75.42 ± 0.48 mg/kg respectively, while the volatile and fat contents were 6.5% and 0.7% respectively. Results may lay the foundation for the standardization of the drug and discovery of new molecules from *S. tibetica* for the treatment of various diseases.

Keywords *Stachys tibetica*, Lamiaceae, physicochemical, phytochemical, extraction

INTRODUCTION

The use of medicinal plants for the treatment of human diseases has increased considerably worldwide. Evaluation of the effects of these plants on organs and systems has contributed to the development of the scientific basis for their therapeutic applications and also has enriched considerably the therapeutic arsenal for the treatment of a number of diseases (Kumar et al., 2011). People of all ages in both developing and undeveloped countries use plants in an attempt to cure various diseases and to get relief from physical sufferings. Today, several medicinal plants and their products are still in use, being employed as home remedies, over the counter drugs as well as raw materials for the pharmaceutical industry and they represent a substantial proportion of the global drug market. However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines. Therefore, it has become extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies (Mulla and Swamy, 2010).

Stachys L. (Lamiaceae) is a large genus of herbs and shrubs comprising 300 species distributed in temperate and tropical regions of the world, with the exception of Australasia (Mabberley, 1997). Plants of this genus have been used in folk medicine for centuries to treat genital tumors, sclerosis of the spleen, inflammatory diseases, coughs and ulcers (Hartwell, 1982). *S. tibetica* is a herb distributed in tropical and subtropical regions of the world including Tibet, China, and India. In India it is found in the cold desert regions of Kargil, Ladakh Valley and in the mountains of Himachal Pradesh. The traditional practitioners in the Kargil and Ladakh regions of India use the drug for treatment of various mental disorders and phobias. In traditional practice, the whole plant is boiled and made into a decoction. A tea cup of the decoction is given twice a day to treat a fever (Ballabh and Chaurasia, 2007).

The present study is aimed to evaluate the preliminary pharmacognostical and phytochemical nature of *Stachys tibetica* Vatke. Investigations of various standardization parameters such as organoleptic, physico-chemical paramenter, fluorescence analysis, powdered drug reaction with different reagents, phytochemical screening and TLC analysis, saponin, fat, volatile and flavonoid contents can be helpful in the authentification of *Stachys tibetica Vatke.* The present study will also serve as reference material in preparation of the monograph.

MATERIALS AND METHODS

Collection of plant material and authentication

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Table 1. Solvent systems for Detection of chemical constituents/secondary metabolites in *stachys tibetica* vatke

Steroids and their glycosides		Flavanoids and their glycosides		Alkaloids		Saponins	
Solvents	**Proportion**	**Solvents**	**Proportion**	**Solvents**	**Proportion**	**Solvents**	**Proportion**
EA : Methanol : Water	100:13.5:10	Chloroform : Methanol	80:20, 70:30, 50:50	Toulene : EA : Diethylamine	70:20:1	Chloroform: GAA : Methanol: Water	64:32:12:8
EA : Methanol : Water	75:15:10	EA : Methanol : Water	81:11:8	Toluene : EA : FA	5:4:1	Hexane: EA : methanol	6:3:1
Chloroform : Methanol : Water	70:30:4	n-Butanol : AA : Water	4:1:5 (Upper phase)				
n-Butanol : Ethanol : Water	40:11:9	EA: FA: GAA : Water	100:11:11:26				
Benzene : Ethanol	4:1						

*where as EA= ethyl acetate, Glacial acetic acid = GAA, Acetic acid= AA, Formic acid = FA

The plant specimen of *Stachys tibetica* Vatke was collected from the cold desert of Kargil and Ladakh, Jammu and Kashmir, India. The plant was identified and authenticated by the Department of Pharmaceutical Sciences, University of Kashmir as Specimen Voucher number-KUST01 by Sr. Asst. Prof. (Dr) Zulfiqar Ali Bhat, Srinagar-190006, India.

Drying and size reduction of plant

The whole plant material of *Stachys tibetica* was subjected to shade drying for about 1 week. The dried plant material was further crushed to powder and the powder was passed through mesh 40 and stored in an air tight container for further analysis.

Organoleptic study of plant material

In some cases, the general appearance of the herb is similar to related species. Thus, a detailed study of the morphological characters can be helpful in differentiating them. The organoleptic study of the drug includes its visual appearance to the naked eye along with its characteristics likes odour, taste, and texture. For each particular organoleptic group, a particular systemic examination can be carried out.

Determination of physicochemical constants of plant materials

Extractive Values

Cold extractive values

4 g of air-dried coarse drug powder was macerated separately with solvents (petroleum ether, chloroform, ethyl acetate, methanol and water) of volume 100 ml in a closed flask for 24 h, shaking frequently during 6 h and allowed to stand for 24 h. It was filtered rapidly, taking precaution against loss of solvent, and the filtrate evaporated to dryness in a tarred flat bottom dish and dried on a water bath, to a constant weight.

Hot extraction values

100 g of powdered material of the drug was packed in a Soxhlet apparatus separately for each solvent like petroleum ether, chloroform, ethyl acetate and methanol, but in case of the water extract the drug was prepared by a decoction method. Each extract was evaporated to dryness and the constant extractive value recorded.

Successive extractive values

100 g of dried and coarsely powdered material was subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform ethyl acetate, methanol and in case of the water solvent, the drug was prepared by a decoction method. The extracts were evaporated to dryness and their constant extractive values recorded.

Ash values

Total ash

1 g of the ground drug was incinerated in a silica crucible at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed to get the total ash content.

Acid insoluble ash

Ash was boiled with 25 ml dilute HCl (2 N) for 5 min. The insoluble matter was collected on an ashless filter paper, washed with hot water and ignited at a temperature not exceeding 450°C to a constant weight.

Water-soluble ash

Ash was dissolved in distilled water and the insoluble part collected on an ashless filter paper and ignited at 450°C to a constant weight. By subtracting the weight of the insoluble part from that of the ash, the weight of the soluble part of the ash was obtained.

Foreign matter

Foreign matter in the crude drugs can be due to faulty collection or deliberate mixing. It was separated from the crude drugs and the percentages calculated.

Loss on drying

100 g of powdered drug sample without preliminary drying was placed on a tarred evaporating dish and dried at 105℃ for 6 h and weighed. The drying was continued until two successive readings matched each other or the difference between two successive weighings was not more than 0.25%. A constant weight was reached when two consecutive weighings after

Table 2. Organoleptic characters of *Stachys tibetica* Vatke

	Fresh Leaf	**Stem**	**Flower**	**Root**
Color	Green	light green	White pink	Brown
Odor	Aromatic	Aromatic	Aromatic	Muddy
Taste	Bitter	Bitter	Characteristics	Bitter
Texture	Characteristics	Acute and brittle	-	Horny and hard
Shape	Bipinnate, Margin of the leaf is entire and apex is short.	-	Corolla, calyx is tubular/ campanulate	Cylindrical and snake like
Size	0.8 to 4.8 cm in length and0.1 to 0.3 cm in breadth	-	-	-

Fig. 1. Plant *Stachys tibetica* Vatke.

drying for 30 min in a desicator showed not more than a 0.01 g difference.

Swelling index

3 g of a specified quantity of plant material previously reduced to the required fineness and accurately weighed was put into a 25 ml glass stoppered measuring cylinder. 25 ml of water was added and the mixture was shaken thoroughly every 10 min for 1 h. It was allowed to stand for 3 h at room temperature. The mean value of the individual determination was calculated related to 1 g of plant material.

Foaming index

About 1 g of the plant material was reduced to a coarse powder, weighed accurately and transferred to a 500 ml conical flask containing 100 ml of boiling water. It was maintained at moderate boiling for 30 min, cooled and filtered into a 100 ml volumetric flask. Sufficient water was added through the filter paper to dilute to volume. The decoction was poured into 10 stoppered test tubes in successive portions of 1 ml, 2 ml, 3 ml up to 10 ml. The volumes in these test tubes were mixed with water up to 10 ml. The test tubes were stoppered and shaken in a lengthwise motion for 15 s, 2 shakes per second. It was allowed to stand for 15 min., the height of the foam measured and foam index calculated (Anonymous, 1998).

Determination of pH

pH 1% solution

Dissolved accurately weighed 10 g of the drug in accurately measured 100 ml of distilled water, filtered and pH of the filtrate checked with a standardized glass electrode.

pH 10% solution

Dissolved accurately weighed 10 g of the drug in accurately measured 100 ml of distilled water, filtered and the pH of the filtrate checked with a standardized glass electrode (Chaudhari, 1996; Anonymous, 1996; Mukherjee, 2002; Anonymous, 1998).

Table 3. Extractive Values of *Stachys tibetica* Vatke

Solvents	Cold extractive values (Individual) % yield (w/w)	Hot extractive values (Individual) % yield (w/w)	Successive extractive values % yield (w/w)
Petroleum ether (40 - 60°C)	01.18	07.57	07.08
Chloroform	02.60	05.54	03.98
Ethyl acetate	02.26	04.47	02.02
Methanol	11.56	19.54	16.40
Water	15.79	30.06	21.53

*Average of 5 readings were taken

Powdered drug reaction with different reagents

The powdered drug was treated separately with different reagents and acids including picric acid, hydrochloric acid, nitric acid, iodine, ferric chloride, and sodium hydroxide. The colour shown after the treatment was noted (Sama et al., 1994).

Extraction of plant materials

Whole plant was successively extracted by continuous hot extraction method

Whole plant material was dried in shadow and powdered. The powdered material was passed through sieve no. 40 mesh, weighed & then used for extraction. The weighed powder was successively extracted with petroleum ether, chloroform, ethyl acetate, methanol in a soxhlet apparatus and the final mark was extracted with water using a decoction method. The resulting extracts were concentrated under reduced pressure using a rotary vacuum evaporator to get the syrupy viscous masses. The viscous masses were transferred to porcelain dishes and dried. The amount of extract was weighed and stored in amber colored airtight bottle at 5 - 7°C (Evans, 2000).

Florescence analysis

Many herbs show fluorescence when the cut surface or powder is exposed to UV light and this can be useful in their identification. The fluorescence character of the plant powders (40 mesh) was studied both in daylight and UV light (254 and 366 nm) and after treatment with different reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, and ferric chloride (Chase and Pratt, 1949; Kokoshi et al., 1958).

Phytochemical investigation

After collection and authentication, the plant material was

Table 4. Powdered drug reaction with different reagents

S.No.	Chemical treatment	Observation
1.	Iodine	Light yellow
2.	Glacial acetic acid	Light green
3.	Ferric chloride 5%	Buff color
4.	Lead acetate	Yellowish green
5.	Potassium hydroxide 1%	Light yellow
6.	Picric acid	Yellow
7.	1N HCl	Buff color
8.	1N H_2SO_4	Reddish brown
9.	50% HNO_3	Light yellow

*Average of 5 readings were taken

Fig. 2. Stems, leaves, Flower buds, Flowers, Roots of *Stachys tibetica* Vatke.

shade dried and powdered. It was passed through sieve no. 40 and subjected to extraction. The weighed quantities of plant material were extracted separately with petroleum ether, chloroform, ethyl acetate, methanol and water by a cold extraction method. The plant material was also successively extracted with different solvents like petroleum ether, chloroform, ethyl acetate, methanol in a soxhlet apparatus while the water extract was prepared by decoction. The extracts were evaporated to dryness under reduced pressure and controlled temperature (40 - 50℃) (Harborne, 1989). Total methanol extract was also prepared from the drug material by a continuous hot extraction method. The extracts were subjected to preliminary phytochemical investigation for the detection of the following compounds: carbohydrates, protein, amino acids, fats and oils, sterols and steroids, glycoside, coumarins, flavonoids, alkaloids, tannins and phenolic compounds, acidic compounds, saponins, terpenes and terpenoids, mucilage resins and lipids/ fats **(**Harborne, 1998; Trease and Evans, 1985).

Determination of fat content

A weighed quantity of the sample (3 g) was extracted with anhydrous ether in a continuous extraction apparatus for 6 h, and then the extract was filtered into a clean dry weighed flask. The extraction flask was rinsed with a small quantity of ether, filtered and added to the weighed flask. The solvent was evaporated and dried to a constant weight at 105℃ (Mukherjee, 2002).

Flavonoid determination

10 g of the crude drug powder was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 m). The filtrate was later transferred into a crucible and evaporated to dryness and weighed to a constant (Boham and Kocipal-Abyazan, 1994).

Saponin determination

20 g of the crude drug was put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55℃. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ℃. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath to remove the solvent. After evaporation, the sample was dried in an oven to a constant weight and the saponin content calculated (Obadoni and Ochuko, 2001).

Determination of volatile content

100 g of the *Stachys tibetica* drug was subjected to hydro distillation in a Clevenger apparatus according to the method recommended in British Pharmacopoeia (1988).

Thin layer chromatographical profile of *Stachys tibetica*

Steroids/ terpenoids and their glycosides, flavanoids and their glycosides, alkaloids, saponins were detected by using different solvent systems and detecting reagents on precoated TLC plates as mentioned below (Stahl and Sies, 2003, Wagner and Bladt, 1996).

Detection of Steroids/triterpenoids and their glycosides

Solvent system used for the detection of steroids and their glycosides are shown in Table 1 and the detecting agent (spraying agent) used are as below.

Spraying reagents:

(i) Vanillin – Sulphuric acid (VS) reagent:
Solution I: 5% methanolic sulphuric acid
Solution II: 1% methanolic vanillin
The developed TLC plate was sprayed with 10ml of solution 1, followed immediately by 5 - 10 ml of solution II, and then heated for 5 - 10 min at 110℃ under observation. Steroids/triterpenoids and their glycosides give blue, blue-violet or pink colored spots.
(ii) Vanillin – Phosphoric acid (VPA) reagent:

Table 5. Fluorescence analysis of *Stachys tibetica* Vatke

S. No.	Treatment	Day light	UV light 254nm	UV 366 nm
1.	Powder	Greyish white	White green	Woody brown
2.	Powder treated with dist. H_2O	Very light	Light green	Light green
3	Powder treated with 10% aq. NaOH	Yellow green	Light green	Green
4.	Powder treated with NH_3	Light brown	Light green	Green
5.	Powder treated with conc. H_2SO_4	Reddish brown	Dark brown	Green
6.	Powder treated with conc. H_2SO_4 + H_2O	Dark brown	Dark green	Dark green
7.	Powder treated with conc. HCl	Golden yellow	Light green	Light green
8.	Powder treated with conc. HCl + H_2O	Buff color	Light green	Light green
9.	Powder treated with conc. HNO_3	Light yellow	Light green	Light green
10.	Powder treated with conc. HNO_3 + H_2O	Light yellow	Light green	Light green
11.	Powder treated with 5% Iodine	Light yellow	Green	Light green
12.	Powder treated with 5% Ferric chloride solution	Greenish black	Light green	black
13.	Powder treated with Picric acid	Yellow	Greenish yellow	Light green
14.	Powder treated with Glacial acetic acid	Light green	Light green	Light pink
15.	Powder treated with petroleum ether	colorless	colorless	Colorless
16.	Powder treated with chloroform	Light green	Light green	Light yellow
17.	Powder treated with ethyl acetate	Light green	Light green	Pink
18.	Powder treated with methanol	Light green	Light green	Light pink

*Average of 5 readings were taken

Table 6. Phytochemical screening of *Stachys tibetica* Vatke

Solvents	Carbohydrates	Proteins & amino-acids	Lipids / fats and oils	Sterols and steroids	Glycosides	Coumarins	Saponins	Flavonoids	Alkaloids	Phenolics / Tannins	Acidic comp	Terpenes and terpenoids
Successive extracts												
Pet. ether	+	-	+++	++	-	-	-	-	-	-	++	+
Chloroform	+	+	++	+++	-	+++	-	+	+	+	++	+++
EA	+	-	-	+	+	+	-	+	-	-	+	+
Methanol	++	-	-	-	++	+	+	+++	++	+	-	-
Aqueous	+++	-	-	-	+++	-	+++	+	+	++	-	-
Cold extracts (Individual)												
Pet. ether	+	-	++	++	-	-	-	-	-	-	+	-
Chloroform	-	-	+	++	-	+	-	-	+	-	+	+++
EA	-	-	-	-	-	-	-	+	-	-	+	-
Methanol	++	+	-	+	+	+	++	++	+	+	+	+
Aqueous	++	-	-	-	-	-	+++	++	+	+	-	-
Extract by continuous hot extraction process												
Total methanolic extract	++	+	+	+++	++	+++	++	+++	+	+++	+	++

*Average of 5 readings were taken, (+++) Very strongly positive, (++) Strongly positive, (+) Positive test, (-) Negative test, Pet. Ether = Petroleum ether, Ethyl acetate = EA

Solution a: 1 gm vanillin dissolved in 100ml of 50% phosphoric acid.
Solution b: 2 parts 24% phosphoric acid and 8 parts 2% methanolic vanillic acid.
After spraying with either solution a or b, the plate was heated for 10 min at 100℃. A red-violet colour indicates the presence of steroids/ triterpenoids and their glycosides.
(iii) Antimony (III) chloride reagent:
20% solution antimony (III) chloride in chloroform.
The developed TLC plate was sprayed with the reagent and then heated for 5 - 6 min at 100℃. There was a red – violet colour in visible light and a red – violet, blue and green fluorescence in UV at 365 nm indicating the presence of steroids/triterpenoids and their glycosides.
(iv) Anisaldehyde – sulphuric acid reagent:
0.5 ml of anisaldehyde was mixed with 10 ml glacial acetic acid, followed by 85 ml of methanol and 5 ml of concentrated sulphuric acid, in that order. The developed TLC plate was sprayed with the reagent, heated at 100℃ for 5 - 10 min.
Steroids /triterpenoids and their glycosides give blue, blue-violet or pink coloured spots.

Detection of flavonoids and their glycosides
Solvent system used for detection of flavonoids and their glycosides glycosides are shown in Table 1 and the detecting agents used are as below.

Detection
The developed TLC plate was observed in visible light and in UV at 365 nm. Flavonoids and their glycosides appear as yellow, dark blue, orange zones /spots. The color gels intensified on exposure of the plates to ammonia vapors.

Detection of alkaloids
Solvent systems used for the detection of alkaloids are shown in Table 1 and the detecting agent used is as below.

Detection
Dragendroff's reagent: The developed TLC plate was sprayed with the reagent and then heated for 5 - 6 min at 100℃.

Detection of saponins
Solvent systems used for detection of saponins are shown in Table 1 and the detecting agent used is as below.

Detection
Anisaldehyde – sulphuric acid reagent: The developed TLC plate was sprayed with the reagent, heated at 100℃ for 5 - 10 min.

RESULTS

Pharmacognostical studies of *Stachys tibetica* Vatke
The herb is an erect perennial or diffuses annually, rarely sub shrubs or shrubs, sometimes rhizomatous and commonly known as Yakjak, Yagzes (Leh and Ladakh). This is used for the treatment of various diseases. The plant is shown in Fig.1.

Organoleptic study of *Stachys tibetica* Vatke
The organoleptic characteristics of *Stachys tibetica* Vatke are shown in Fig. 2 and Table 2.

Physicochemical constants

Cold extractive value
The cold extractive values of the whole plant material in different solvents (individual) are shown in Table 3.

Hot extractive values
The hot extractive values of the whole plant material in different solvents (individual) are shown in Table 3.

Successive extractive values
The successive extractive values of the whole plant material in different solvents (individual) are shown in Table 3.

Ash value
The ash values of *Stachys tibetica* Vatke were found as total ash = 6.72%, acid insoluble ash = 1.09% and water soluble ash = 2.83.

Foreign matter analysis
The foreign matter in the whole plant material of *Stachys tibetica* Vatke was found as 0.01%.

Foaming index
Foaming index was calculated to be more than100.

Loss on drying
Loss on drying in *stachys tibetica* vatke powder was found as 8.1568%.

Table 7. Identification of compounds with Thin layer chromatography profile (TLC)

Compound	Rf value	Result				
		Pet. ether	Chloroform	Ethyl acetate	Methanol	water
Extracts of *Stachys tibetica* Vatke.						
Terpenes	0.39	+	+	+	+	+
Flavonoids	0.80	-	+	+	+	+
Alkaloids	0.52	-	+	-	+	+
Steroids	0.47	+	+	-	+	-
Saponins	0.55	-	-	-	+	+
Successive extracts of *Stachys tibetica* Vatke						
Terpenes	0.39	+	+	-	+	-
Flavonoids	0.80	-	-	+	+	+
Alkaloids	0.52	-	+	-	+	+
Steroids	0.47	+	+	-	-	-
Saponins	0.55	-	-	-	+	+

Swelling index
Swelling index was found to be 3.

pH Values
The pH values of 1% and 10% solution were 6.59 and 6.34 respectively.

Powdered drug reaction with different reagents
The powdered drug was reacted with reagents and the results are shown in Table 4**.**

Fluorescence analysis
The powder of the whole plant of *Stachys tibetica* Vatke (mesh size 40) was examined under daylight and UV light. The observations are shown in Table 5.

Phytochemical screening of successive extracts
The phytochemical screening of successive extracts of *Stachys tibetica* Vatke is shown in Table 6.

Phytochemical screening of cold extracts
The phytochemical screening of cold extracts of *Stachys tibetica* Vatke is shown in Table 6.

Phytochemical screening of Total methanol extract
The whole plant of *Stachys tibetica* Vatke was extracted with methanol using a soxhlet apparatus and subjected to phytochemical screening which results in the class of phytochemicals (Table 6).

Fat and volatile content of *Stachys tibetica* Vatke
The fat and volatile contents of *Stachys tibetica* Vatke were determined as 6.5% and 0.7% respectively.

Flavonoid and saponin content of *Stachys tibetica* Vatke
The flavonoid and saponin contents of of *Stachys tibetica* Vatke were determined as 54.7 mg/g and 75.4 mg/g.

Thin layer chromatography profile
The TLC of extracts and successive extracts of *Stachys tibetica* Vatke showed the presence of compounds (Table 7).

DISSCUSSION

The indigenous or traditional system of medicine has gained importance in the field of medicine. A large number of populations depend on traditional practitioners, who in turn are dependent on medicinal plants, to meet their primary health care needs. As the usage of these herbal medicines has increased, issues and the motto regarding their quality, safety, and efficacy in industrialized and developing countries have cropped up (Kumar et al., 2010). Increasing interest has forced the researchers to scientifically screen various traditional claims. Therefore, at present, both common users and healthcare professionals seek updated, alterative information regarding the safety and efficacy of any recommended medicinal plant as a drug prior to its use (Bhat et al., 2011).

The relevance of pharmacognosy in the standardization of herbal drugs was been long been stressed. Many monographs on pharmacognostic parameters have emerged as an aid in the pharmacognostic investigations. These studies help in the identification and authentication of the plant material. Medicinal plant materials are categorized according to sensory, macroscopic and microscopic characteristics. An examination to determine these characteristics is the first step towards establishing the identity and degree of purity of such materials.

The extractive value is used to determine the amount of active constituents extracted with solvents. Ash values are used to determine the extraneous matter, such as sand and soil, adhering to the plant surface. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range in many natural products (e.g., alkaloids like berberine), which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents, hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of the pharmacognostical evaluation (Ansari, 2006).

In the present investigation various standardization parameters such as organoleptic and physico-chemical parameters, fluorescence analysis, powdered drug reaction with different reagents, phytochemical screening and TLC analysis, saponin, fat, and flavonoid content were implemented which could be helpful in the authentification of *Stachys tibetica Vatke.* The results of the present study will also serve as reference material in the preparation of the monograph.

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CONFLICT OF INTEREST

The authors have no conflicting financial interests.

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