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Pharmacognostical Studies on Market Samples of *Pashanbheda*

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ABSTRACT

According to Ayurvedic Pharmacopoeia of India, (A.P.I.) *Pashanbheda* consists of rhizomes of *Berginia ciliata (Haw.) Sternb., syn. Berginia ligulata* (Haw.) Sternb. (Family. Saxifragaceae). Ayurvedic Formulary of India, Part-I has mentioned *Areva lanata* Juss as its substitute. Looking to the importance and existing controversies on *Pashanbheda*, we thought it will be interesting to work on the phytochemical and quality control aspects of *Pashanbheda*. It is therefore of paramount importance to investigate the basic source of drug supply i.e., crude drug market. Present study was planned to work out the market trend, to evaluates quality standards, and to find out the botanical identity of adulterants in market samples by using microscopic, HPTLC, Phytochemical analysis etc., of *Pashanbheda* with respect to the parameters mentioned in Ayurvedic Pharmacopeia of India (API).

Key Words *Pashanbheda, Crude Drug, Substitute, Botanical Name, HPTLC, API*

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INTRODUCTION

Pashanbheda is a highly reputed classical Ayurvedic drug, which possesses Urolithiatic2 property. A wide range of formulations are there, in which Pashanbheda is one of the ingredients e.g., *Pashanbhedayadi churna*[1], *Varunayadi kwatha* churn, *Elayadi kwatha* etc. Adulteration and substitution of crude drugs is one of the biggest drawbacks in promotion of *Ayurvedic* formulations. The present study "**Pharmcognostical Studies on Market Samples of *Pashanbheda***" was undertaken to find out market trends regarding supply of the crude drug *Pashanbheda* and work out its genuineness. *Pashanbheda* is one of the focus drugs in *Ayurveda*. It shares its habitat with many similar looking herbs and therefore chances of its adulteration with other species or genera of family Bryophyllum[2], Saxifragaceae etc is likely to happen. Sometimes, crude drug collectors and crude drug suppliers, for the sake of financial gains, mix with it poor quality, botanically different and exhausted herbs. The chief methods employed in evaluating drugs are -

- Organoleptic i.e., practical and on the spot tools.

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- Microscopic, physicochemical, and phytochemical – Lab based tools.
- Experimental and Clinical - final confirmative tools.

The market samples of the drug under study i.e., *Pashanbheda* were collected from four raw drug markets of India namely Haridwar, Lucknow, Pilibhit, and Delhi along with one Reference sample of the drug (*Bergenia ciliata* (Haw.) Sternb.) procured from Digoti (Latitude: 29.58, Longitude: 80.20, Elevation/ Altitude: 345meters) on the way of Ranikhet. The market samples of the drug were labelled, packed, stored, and subjected to ***pharmacognostical*** studies. All the crude drugs samples procured were dried and studied organoleptically, with naked eye & magnifying lens, with the help of Pharmacognostical procedure i.e., Appearance, size, shape, colour, odour, taste, fracture, and findings were recorded. Market samples were compared with the reference sample and effort was made to establish the identification features with the help of the photographs.

MATERIALS AND METHODS

BOTANICAL SPECIFICATION:

Out of our samples collected from the market, sample no.1,2,3,4 and 5 were identified as *Bergenia ciliata* (*Pashanbheda*) by organoleptic, microscopic, physicochemical, phytochemical analysis and TLC and HPTLC. Botanical specification of all the five samples is represented in following (shown in Table 1).

Table 1 Botanical Specification of Market Samples of Pashanbheda

SAMPLE NO.	PLACE of COLLECTION	BOTANICAL SPECIFICATION
Sample no. 1	Lucknow Market	*B. ciliata* (Haw.) Sternb.
Sample no. 2	Pilibhit Market	*B. ciliata* (Haw.) Sternb.
Sample no. 3	Haridwar Market	*B. ciliata* (Haw.) Sternb.
Sample no. 4	Delhi Market	*B. ciliata* (Haw.) Sternb.
Sample no. 5	Digoti (Ranikhet,Uttarakhand)	*B. ciliata* (Haw.) Sternb.

RESULTS AND DISCUSSION

Organoleptic Study of powder

***Microscopic study*:**

All five samples present, microscopically peeled root tuber of all market sample and reference sample. Inner zone of Phellem or cork is made of tangentially elongated cells and outer zone with compressed cells. Phelloderm consists of inter - cellular spaces, starch grains, few calcium oxalate crystals and cortical cells contain rosette crystals. Endodermis and pericycle are not seen. Vascular bundles are arranged in a ring, and they are conjoint, collateral, and open. Phloem tissue is composed of sieve elements and parenchyma, 2 -3 layered of cambium is present (shown in Table 2).

Table 2 Organoleptic characters of powder of reference sample and market samples of Pashanbheda

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Texture	Coarse	Coarse	Coarse	Coarse	Coarse
Color	Light blackish brown	Dark brown	Dark brown	Light yellowish brown	Light yellowish brown
Odor	Slight	Slight	Slight	Slight characteristic	Slight characteristic

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	aromatic odor	characteristic	characteristic		
Taste	*Tikta, -Kashaya*	*Tasteless*	*Tikta, Kashaya*	*Tikta Kashaya*	*Tikta Kashaya*
Touch	Rough	Rough	Rough	Rough	Rough

***Powder Microscopy*:** All five samples of drug under powder microscopy study found:

Fragments of polygonal cork cells are seen in surface view. Plenty of simple and compound starch grains, Fragments of xylem vessels with simple pits and spiral thickening, **Tracheid is w**ith helical thickenings, Rosette crystals of calcium oxalate and brownish stanniferous **Pigment** cells are seen (shown in Table 3).

Table 3 Powder Microscopy

	Sample No 1	**Sample No 2**	**Sample No 3**	**Sample No 4**	**Sample No 5**	**Standard as Per API**
Foreign Matter	0.2	0.1	Nil	Nil	nil	Not more than 2%
Loss of dryness	8.07	7.56	8.08	6.54	8.16	-
Total Ash	11.6	6.87	10.9	11.6	8.37	Not more than 13 %
Acid Insoluble Ash	1.03	1.1	0.3	0.3	0.9	-
Alcohol Soluble Extractive Value	12.1 %	20 %	12.4 %	12.3 %	9.03 %	Not less than 9 %
Water Soluble Extractive Values	16.9	23	18.9	19	18.8	Not less than 15%
Ether Soluble Extractive Values	1.6	1	1	0.9	0.5	-

PHYTOCHEMICAL STUDY:

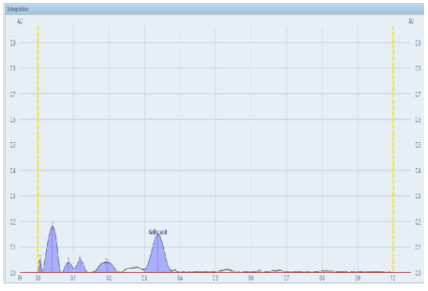
Phytochemical examination of all the samples (five samples – one Reference sample and four market samples) reveals presence of Starch, Sugar, Phenol, Tannins and Flavonoids.

Table 4 Qualitative Phytochemical Analysis study

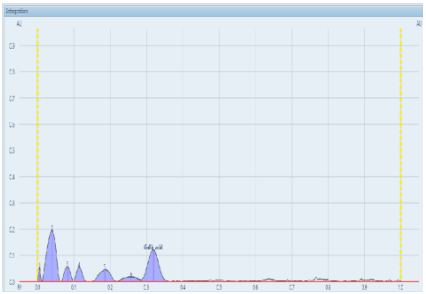
SAMPLES	**Sample.1**	**Sample.2**	**Sample.3**	**Sample.4**	**Sample.5**
Total Sugar present in 1mg	0.50	0.80	0.81	0.99	0.93
Total Starch present in 1mg	0.34	0.30	0.42	0.38	0.44
Total Tannin present in 1mg	0.53	0.39	0.35	0.32	0.21
Total Phenol present in 1mg	0.002	0.002	0.002	0.002	0.002
Flavonoid present in 1mg	0.095	0.098	0.11	0.093	0.090

Phytochemical analysis of all the five samples (one reference sample and four market samples) showed the presence of sugar, starch, tannin, phenol and flavonoids. **(**Shown in **Table 4)** Quantitative phytochemical analysis was not done due to financial constraints.

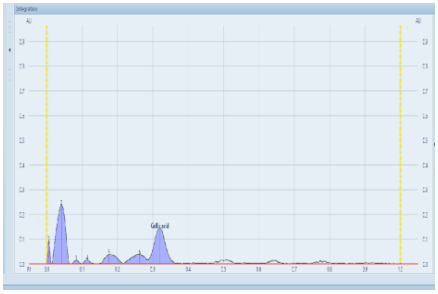
HPTLC fingerprinting –



Sample 1



Sample 2



Sample 3

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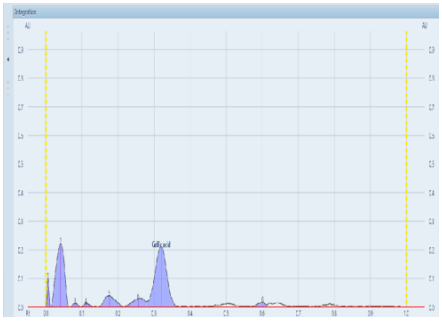
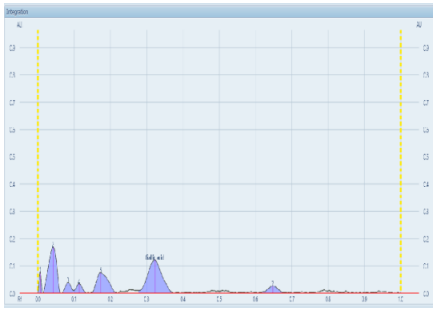


Sample 4



Sample 5

Sample 1-5 R_f values in HPTLC Fingerprinting of all five samples of *Pashanbhed* under UV 254 nm of wavelength

Table 5 HPTLC fingerprinting of all five samples of *Pashanbhed*

Stationary Phase	TLC Aluminium sheet silica gel ^{60} F 254 plate				
Mobile Phase	Toluene – Ethyl Acetate -Formic Acid (7:3 :0.5)				
Rf. Value of spots Visualized UV long 254 nm					
S.N.	**Sample no.1 Lucknow (7 spots)**	**Sample no. 2 Pilibhit (7 spots)**	**Sample no.3 Haridwar (7 spots)**	**Sample no. 4 Delhi (7 spots)**	**Sample no. 5 Digoti (7 spots)**
1.	0.01	0.01	0.01	0.01	0.01
2.	0.03	0.03	0.03	0.03	0.03
3.	0.05	0.05	0.05	0.05	0.05
4.	0.18	0.18	0.18	0.18	0.18
5.	0.31	0.31	0.31	0.31	0.31
6.	0.62	0.62	0.62	0.62	0.62
7.	0.98	0.98	0.98	0.98	0.98

R_f Values in HPTLC Fingerprinting of Market samples of *Pashanbhed* under UV 366 nm of wavelength

Table 6 HPTLC fingerprinting of all five samples of *Pashanbhed*

Stationary Phase	TLC Aluminium sheet silica gel ^{60} F 254 plate				
Mobile Phase	Toluene – Ethyl Acetate -Formic Acid (7:3 :0.5)				
Rf. Value of spots Visualized UV long 366 nm					
S.N.	**Sample no.1 Lucknow (8 spots)**	**Sample no. 2 Pilibhit (8 spots)**	**Sample no.3 Haridwar (8 spots)**	**Sample no. 4 Delhi (8 spots)**	**Sample no. 5 Digoti (8 spots)**
1.	0.00	0.00	0.00	0.00	0.00
2.	0.02	0.02	0.04	0.04	0.04
3.	0.18	-	-	0.18	0.18
4.	-	0.19	0.19	-	-
5.	0.22	-	-	-	0.22
6.	-	0.24	0.24	0.24	-
7.	0.48	-	-	-	-
8.	-	0.49	0.49	-	-
9.	0.68	0.68	0.68	0.68	0.68
10.	0.87	0.87	0.87	0.87	0.87
11.	0.98	0.98	0.98	0.98	0.98

Comparative Analysis: -

With respect to the pharmacognostical specifications standards of official botanical source drug for *Pashanbheda*, published in Data base on Medicinal plant used in Ayurveda and Siddha, were taken into consideration for comparison with market samples. As the same plant species were found to be sold in the market

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by the name of *Pashanbheda* with entirely same botanical specifications, comparison of external morphological features and microscopic findings come exactly same as Pashanbhed (the one which is published in data base). Though, also an effort was made to compare bioactive molecules present in the market samples with that of official drug.

DISCUSSION

After screening out of five market samples, four samples were botanically identified as *B. ciliata* (Haw.) Sternb. It is a matter of satisfaction that none of the samples collected for study were adulterated with other herbs except some earthy material and remains of aerial part of the drug. *B. ciliata* (Haw.) Sternb. has been accepted as official drug for *Pashanbheda* by API (Part1 vol.1.57)[3] During study, it was noticed that all market samples (Haridwar, Lucknow, Delhi and Pilibhit) have same pharmacological attributes as that of *Pashanbheda*. *B. ciliata* R(Haw.) Sternb. has been accepted as botanical source of *Pashanbheda*.

PHYSICOCHEMICAL STUDY

The physicochemical studies were carried out on all the five samples of the drug following the validation method as per API.

According to API foreign matter in case of *Pashanbheda* should not be >2%. Since the reference sample was collected from an authentic source, foreign matter was within standard limit. Amount of foreign matter found in different market samples in increasing order (Reference sample = Delhi = Haridwar < Pilibhit < Lucknow). All the market samples have foreign matter within range (2%).

Moisture content found in market samples of Delhi is maximum and found very minimum in reference sample. Maximum moisture was found in the Reference sample (8.16 %). An excess of water in drug encourages microbial growth, a presence of fungi or insects and deterioration following hydrolysis.

The ash value is indicator of presence of inorganic and earthy material in the drug sample. Total ash content found in market samples of Pilibhit is maximum and found very minimum in Delhi sample.

Acid insoluble ash content found in market samples of Delhi is maximum and found very minimum in Pilibhit sample. Maximum percentage of acid insoluble ash is found in sample of Pilibhit (1.1%).

Estimation of extractive values determines the number of active constituents in each amount of plant material when extracted with a particular solvent. It also gives whether the crude drug is exhausted or not. Alcohol soluble extractive found in Reference sample is maximum and found very minimum in Pilibhit sample (Reference sample < Lucknow < Delhi < Haridwar < Pilibhit)**.** Maximum percentage of alcohol soluble extractive is found in Pilibhit sample (20.0%). Water soluble extractive found in market samples of Lucknow is maximum and found very minimum in Pilibhit sample.

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(Lucknow < Reference sample < Haridwar < Delhi < Pilibhit). Maximum percentage found in Pilibhit sample (23.0%)

The percentage of Ether soluble extractive found in different samples in increasing order (Reference sample < Delhi < Haridwar = Pilibhit < Lucknow). Maximum percentage found in Lucknow sample (1.6%).

PHYTOCHEMICAL STUDY

Phytochemical examination of all the samples (five samples – one Reference sample and four market samples) reveals presence of Starch, Sugar, Phenol, Tannins and Flavonoids.

HPTLC FINGER PRINTING

Based on Organoleptic (macroscopic) and microscopic study HPTLC finger printing was decided for all five market samples, at Pharmacognosy Lab. NBRI (National Botanical Research Institute,) Lucknow.

HPTLC fingerprinting of five market samples of *Pashanbheda* at 4µl of applied volume under UV 366nm wavelength showed presence of six (6) peaks in sample no. 1, 2 and 3. Five (5) peaks in sample no. 4 and 5.

HPTLC finger printing done under UV 254 nm showed seven (7) spots of same Rf values in all three samples (0.01, 0.03, 0.05, 0.18, 0.31, 0.62, 0.98) **and** under the wavelength UV 366 nm showed eight spots in sample no. 1, 2 and sample no. 3 (Market sample of Lucknow, Pilibhit and Haridwar) and seven spots in sample no. 4 and 5 (Market sample of Delhi and Reference sample). In all these samples five spots have same Rf values (0.00, 0.02, 0.68, 0.87, 0.98). it suggests that all these samples have similar phytoconstituents.

HPTLC fingerprint of five samples of *Pashanbheda* at 4µl of applied volume under UV 254 nm wavelength shows presence of six peaks respectively for sample no. 1, 2 and 3 and five peaks respectively for Sample no. 4 and Reference sample. This may be interpreted as least number of phytochemicals present in Reference sample. This is since Reference sample and sample no. 5 was quite young and maturity of the plant part used influences the quality and quantity of primary and secondary metabolites.

Sample no.1 (Lucknow market), Sample no.2 (Pilibhit market) and Sample no.3 (Haridwar market) shows presence of 6 phytochemicals and sample no.4 (Delhi market) shows presence of 5 phytochemicals.

CONCLUSION

Yogyattavam and *Sampataa* are the two essential classical properties of herbal drug. To achieve this there is a need to ascertain certain standards, quality and purity of raw drug as well as standards formulations to maintain their therapeutic efficacy for worldwide acceptance and globalization of Ayurveda. Adulteration and substitution of raw drugs makes the condition worse. From this study, it is evident that sample procured from Lucknow, Pilibhit and Haridwar market was found to be the best in terms of number of active metabolites present followed by

sample collected from Digoti and Delhi market. Thus, it may be concluded that Sample no. 1 (Lucknow), Sample no. 2 (Pilibhit), Sample no. 3 (Haridwar) and Sample no. 4 (Delhi) were of *Bergenia ciliata*. In the market surveyed *Bergenia ciliata* are being sold as *Pashanbheda*. Though no adulterants were detected in the market sample of the drug during the study.

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