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Pharmacognostic Characterization and Development of Standardization Parameters for the Quality Control of *Berberis Pachyacantha* Bien. ex koehne

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Abstract

Berberis pachyacantha commonly known as Barberry is the member of the genus *Berberis* belonging to family (Berberidaceae). The genus *Berberis* is an important drug in Indian and European system of traditional medicine due to the presence of biologically active alkaloid. In spite of its numerous medicinal attributes, no published work is available till date on pharmacognostical standardization and developments of its quality control on its root part. So, the present study was undertaken to evaluate pharmacognostic characters of stem of *Berberis pachyacantha*. **Methods:** The studies were carried out in terms of morphological, microscopic characters and physicochemical parameters using standard methods. **Results:** Microscopy of the stem revealed vascular bundle, Prismatic calcium oxalate crystals, stone cells, fibers, pitted and spiral xylem vessels were also identified in the microscopy of powdered samples. Preliminary phytochemical screening showed the presence of alkaloids, glycosides, saponins, tannins, flavonoids, coumarins and sterols. Physiochemical parameters such as the moisture content, ash content, heavy metal and mineral content, extractive values, fluorescence analysis and pH of extracts were determined. Quantitative estimation of flavanoids and Phenolic. **Conclusion:** The current results provide necessary information for the correct identification and quality assessment of *Berberis pachyacantha* crude drug or herbal products containing the plant.

Keywords

Berberis pachyacantha; Standardization; Alkaloids; Berberidaceae

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INTRODUCTION

Natural products from plants, animals and minerals are the basis for treating human diseases (1). Medicinal plants are presently in demand and their acceptance is increasing progressively. Herbals especially medicinal herbs more than a tenth of the plant species (over 50,000 species) are used in pharmaceutical and cosmetic products (2,3). Therefore, there is a need to evaluate phytoconstituents obtained from traditional medicines, based on various phytochemical screening, analytical and pharmacological methods (4). The primary focus in this direction is the herb characterization or detailed pharmacognostic evaluation that gives external appearance, microscopy and physical characteristic of the crude drug (5).

Berberis pachyacantha (Berberidaceae) belonging to the genus *Berberis* is an important drug in Indian and European system of traditional medicine. Due to the presence of biologically active alkaloid berberine it has been used from ancient times for curing rheumatism, fever, jaundice, vomiting during pregnancy, eye disease, kidney and various other ailments. The root extract of few species of *Berberis* are known as powerful drug in the treatment of malarial fever as quinine (6). The Plant *Berberis pachyacantha* Koehne is distributed in Pakistan, North-West Himalayas and Kashmir, India (7,8,9, 10). The plant is a shrub 2-3 meters tall, deciduous, glabrous, stem is dark red to pale brownish. Leaves are usually 3-6cm long. Roots and bark of various species are used as folk remedy for the treatment of various inflammatory diseases such as lumbago, rheumatism and to reduce fever (7). The plants of genus *Berberis* have also been used as a folk medicine for the treatment of rheumatic and other chronic inflammatory disorders (11), antimicrobial properties (12), antiarrhythmic and sedative effects (13), antihypertensive activity (14), Choleretic action, gallbladder stones (10) and Hepatoprotective activity (15). In spite of its numerous medicinal attributes, no published work is available till date on pharmacognostical standardization. Therefore, this study was done to establish important pharmacognostical parameters and quality parameters of this plant that could be beneficial in the identification of this plant.

2. MATERIALS AND METHODS

2.1 Procurement and authentication of plant material

The root part of *Berberis pachyacantha* Koehne were collected in the month of July 2013, at an altitude of 2850-2900 m above the sea level, from the Gulmarg, Jammu and Kashmir, India. Herbarium specimen was prepared, and the plant was authenticated and identified by the Prof Naqshi Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir under specimen voucher No KU/BP/13. A sample specimen of collected material was deposited in herbarium for future references.

2.2 Reagents

All the reagents used were of analytical grade obtained from central drug house (P) LTD. Bombay, India.

2.3 Macroscopic analysis

Macroscopic evaluation is an important parameter to establish the identity of the crude drugs. Thus, detailed morphological study of the characters could be beneficial in distinguishing them. The macroscopic evaluation of root of *Berberis pachyacantha* was done as per the method described earlier (16).

2.4 Microscopic analysis

The microscopic examination of root of *Berberis pachyacantha* are not only essential to identify the adulterants but also indispensable in the correct identification of the plant. Various cellular structures like calcium oxalate crystals, starch granules, lignified tissues were carefully examined with their size and shape was also performed by various staining reagents (16). The dried root was powdered, and the microscopic characters were examined by using various staining reagents (17).

3. Proximate analysis

Proximate analysis was performed on powdered root of *Berberis pachyacantha* for the determination of various physicochemical parameters like total ash, acid insoluble ash, water soluble ash [Pharmacopoeia I 1996]. Extractive values, loss on drying, swelling index, (18).

3.1 Determination of pH

The pH of aqueous solution (1% and 10%) of the powdered drug was carried out using a calibrated glass electrode (19).

3.2 Determination of fat content (20).

3.3 Extractive values (hot, cold and successive) (21).

3.4. Fluorescence analysis

Several plant materials exhibit fluorescence on exposure to UV light and this property is utilized in plant identification. The fluorescence study of the samples was carried out in daylight and UV light (254 nm and 366 nm) both. Likewise, after treatments with different chemical reagents for instance nitric acid, sodium hydroxide, iodine, acetic acid, picric acid, hydrochloric acid, ferric chloride, etc. were also analyzed (22). Behavioral analysis of powdered plant material with various chemical reagents was also conducted.

4. Preliminary Phytochemical Investigation

Qualitative phytochemical screening was carried out on the extracts of *Berberis pachyacantha* to determine the presence of various phytoconstituents. to identify the presence of various primary and secondary metabolites like alkaloids, glycoside, tannins, flavonoids, , steroids and sterols, terpenes and terpenoids, phenolic compounds, coumarins, mucilage, resins, carbohydrates, protein, amino acids, saponins, and fats and oils, etc (18).

5. Heavy metal and Mineral Content

Heavy metal analysis of powdered plant material was analyzed using AtomicAbsorption Spectrometry (AAS) for iron, chromium, copper, zinc, cobalt, manganese, nickel, lead, cadmium and mercury. The heavy metals analysis of powdered plant material is an important tool to evaluate the quality of crude drugs. Heavy metal evaluation for root of *B. pachyacantha* was done by wet digestion method described by (23).

RESULTS

The macroscopic character has always served as a useful key in faster and early identification of plant material and also serves as an important standardization parameter. The macroscopic features of *Berberis pachyacantha* are**:**

Macroscopical evaluation

Berberis pachyacantha is a shrub with 0.60-1.21 m in height, sometimes attaining height of 2.43m, twigs and young shoots are glabrous. Scan dent branches bear numerous slender leafy twigs, wood is very tough, bright yellow in color. Leafy twigs are green or purple, grooved and annular, studded with spines in the axils of which are tufts of leaves. Leaves obovate, entire or with a few spiny teeth, glabrous, 2.5 to 6.35 cm. Umbel flowers, pedicle slender, red 1.2 cm, petal notched. Berry sausage-shaped when young, eventually top-shaped, 0.84 cm by 0.44 cm, purplish red, turning to dark blue with glaucous bloom, with the dry style and large round stigma still attached.

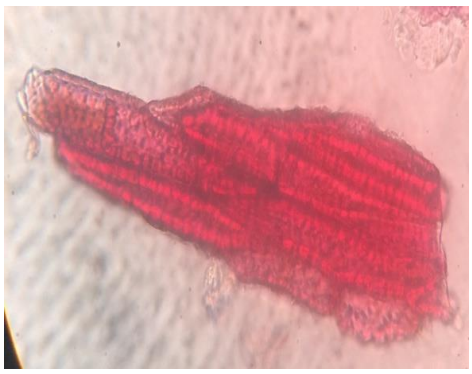
Macroscopical characters of Root (Figure-1)

The dried mature roots are slightly curved, cylindrical, about 0.3-1.2 cm in dia. It possesses bitter taste, outer surface smooth, longitudinally striated, brown in color, inner surface yellow, and fracture hard, incomplete and uneven.

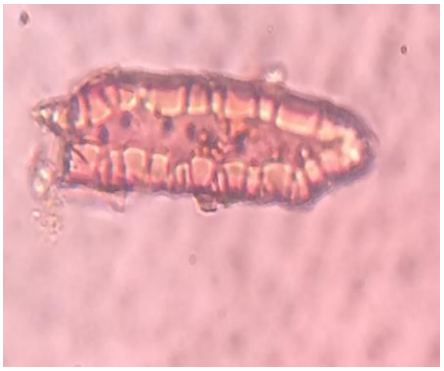
Color: Brown (Externally), Yellow (internally).
Odor: Characteristic
Taste: Bitter
Size: The dried mature roots are slightly curved, cylindrical, about 0.3-1.2 cm in diameter and it varies according to size.
Shape: Slightly curved.
Texture: Fibrous and rough.
Extra markings: Prominent striations and ridges.

Microscopical characters of Root

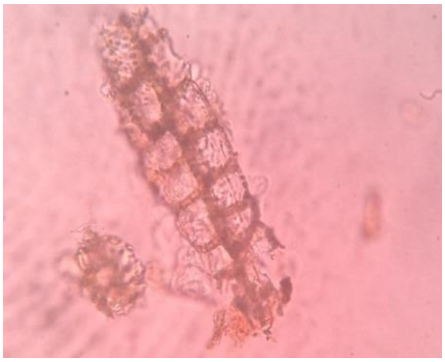
Powder of root bark when examined under microscope shows numerous fragments of cork with conspicuous granular, dark reddish contents (**Figure-2**). In surface view, the cells are polygonal with highly thickened walls; in sectional view the cells are arranged in alternating layers of thinner-walled cells with pale, brownish contents and thicker walled lignified cells with dense, reddish brown contents. The vessels were highly lignified, usually occur in bundles and were in abundance. The powder was yellow brown in colour, with no distinct odor and a bitter taste. It was easy flowable with a fine to coarse texture. Microscopic powder features revealed presence of prismatic crystals of calcium oxalate, starch granules, fragment of pitted vessel, thick walled sclereids.



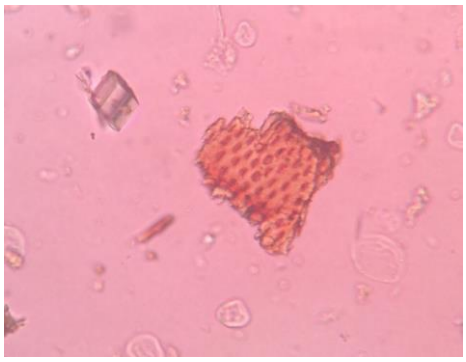
Group of Lignified Vessels



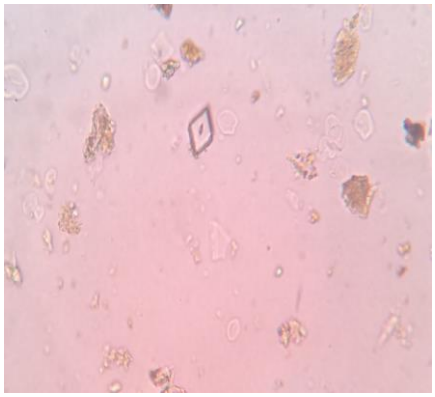
Thick walled Sclereid



Crystal sheath



Fragment of Pitted vessel



Prismatic crystal

Lignified Cork Cells

Microscopical characters of Root (Figure-2)

Physicochemical evaluation

The proximate analysis was used for the pharmacognostical analysis of powdered root of *Berberis pachyacantha* (Table-1). The fluorescence characteristics of powdered drug was studied under U.V and visible light after treating with different chemical reagents is reported (Table-2). Also, the preliminary phytochemical investigation of **methanolic and aqueous** extracts revealed various phytoconstituents (Table-3).

Table-1: Physicochemical analysis of root *Berberis pachyacantha*

Physico-chemical Parameters	Results
Total ash value (% w/w)	7.72
Acid insoluble ash value (% w/w)	1.02
Water soluble ash value (% w/w)	2.80
Swelling index (% w/v)	2
Fat content (% w/w)	2.5
Foreign matter (% w/w)	0.02
Loss on drying (% w/w)	4.16

Foaming index	NMT 100
pH 1 % solution	6.2
pH 10 % solution	6.4

Table-2: Successive extractive values of root of *Berberis pachyacantha*

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
Aqueous	15.79	30.06	21.53

Fluorescence analysis

Fluorescence analysis was performed with various reagents to observe the difference in the physical characteristics of the crude drugs. The fluorescence analysis results of this plant are presented in Table-3.

Table-3: Fluorescence analysis of powdered root of Berberis *pachyacantha*

Powder + Reagent/ Solvent	Visible light	UV 254 nm	UV 365 nm
Powder	Greyish brown	White green	Woody brown
Powder treated with dist. H_2O	Light yellow	Light green	Light bluish
Powder treated with 10% aq. NaOH	Yellow red	Light grey	Greenish
Powder treated with NH_3	Light brown	Light green	Green
Powder treated with conc. H_2SO_4	Reddish brown	Dark brown	Green
Powder treated with conc. H_2SO_4 + H_2O	Dark brown	Dark green	Dark green
Powder treated with conc. HCl	Golden yellow	Light green	Light green
Powder treated with conc. HCl + H_2O	Buff color	Light green	Light green
Powder treated with conc. HNO_3	Light yellow	Light green	Light green
Powder treated with conc. HNO_3 + H_2O	Light yellow	Light green	Light green
Powder treated with 5% Iodine	Light yellow	Green	Light green
Powder treated with 5% Ferric chloride solution	Greenish black	Light green	Black
Powder treated with Picric acid	Yellow	Greenish yellow	Light green
Powder treated with Glacial acetic acid	Light grey	Light green	Light pink
Powder treated with petroleum ether	Yellow	Yellow	Yellow
Powder treated with chloroform	Light yellow	Light grey	Light yellow
Powder treated with ethyl acetate	Light yellow	Light grey	bluish
Powder treated with methanol	Lightyellow	Light green	Light pink

Preliminary Phytochemical Screening of various Extracts root of *Berberis pachyacantha*

The preliminary phytochemical screening was done to identify the constituents of the plant and the results have been tabulated in table 3 which indicated the presence of various phytoconstituents like carbohydrates, alkaloids, flavonoids, polyphenolic compounds, tannins and terpenoids.

Table-4: Phytochemical screening of Cold extracts of *Berberis pachyacantha* root part

Test	Inference	Pet. Ether	Chloroform	Ethyl acetate	Methanol	Aqueous
Carbohydrates						
Molish's test	Violet ring	-	-	+	+	+
Fehling's test	Brick red precipitate	-	-	-	+	+
Benedict's test	Orange red precipitate	-	-	-	+	+
Tannins						

5% $FeCl_3$	Yellow color	-	-	+	+	+
Lead acetate	White precipitate	-	-	-	+	+
Gelatin test	White precipitate	-	-	+	+	+
Flavonoids						
Shinoda test	Pink colour	-	-	+	+	+
Lead acetate	Yellow precipitate	-	-	+	+	+
Phenolic						
1% FeCl3	Bluish color	-	-	+	+	+
Phytosterols						
Salkowski	Golden yellow ring at junction	+	+	-	+	-
Libermann's	Brown ring at junction	+	+	-	+	-
Saponins						
Foam test	Foaming	-	-	-	-	+
Froth test	Frothing	-	-	-	-	+
Fats and Oils						
Filter paper	Emerald green color	+	+	+	-	-
Filter paper or Stain test	Permanent stain on filter	+	+	+	+	-
Cardiac Glycosides						
Keller killani	Brown ring at junction	-	-	-	+	+
Alkaloids						
Dragendroff's	Orange precipitate	-	-	-	+	+
Wagners	Reddish brown precipitate	-	-	-	+	+

Table -5: Phytochemical screening of hot successive of *Berberis pachyacantha* root part

Test	Inference	Pet. Ether	Chloroform	Ethyl acetate	Methanol	Aqueous
Carbohydrates						
Molish's test	Violet ring	-	-	+	+	+
Fehling's test	Brick red precipitate	-	-	-	+	+
Benedict's test	Orange red precipitate	-	-	-	+	+
Tannins						
5% $FeCl_3$	Yellow color	-	-	+	+	+
Lead acetate	White precipitate	-	-	-	+	+
Gelatin test	White precipitate	-	-	+	+	+
Flavonoids						
Shinoda test	Pink colour	-	-	+	+	+
Lead acetate	Yellow precipitate	-	-	+	+	+
Phenolic						
1% FeCl3	Bluish color	-	-	+	+	+
Phytosterols						
Salkowski	Golden yellow ring at junction	+	+	-	+	-
Libermann's	Brown ring at junction	+	+	-	+	-
Saponins						
Foam test	Foaming	-	-	-	-	+
Froth test	Frothing	-	-	-	-	+
Fats and Oils						
Filter paper	Emerald green color	+	+	+	-	-
Filter paper or Stain test	Permanent stain on filter	+	+	+	+	-

Cardiac Glycosides						
Keller killani	Brown ring at junction	-	-	-	+	+
Alkaloids						
Dragendroff's	Orange precipitate	-	-	+	+	+
Wagners	Reddish brown precipitate	-	-	+	+	+

Table-6: Heavy metal of *Berberis pachyacantha* root part along with their pharmacopoeial limits

Heavy metal	Heavy metal in percentage of Drug (ppm)	Percentage limit (ppm)
Lead (Pb)	1.01	NMT 10.0
Cadmium (Cd)	0.04	NMT 0.3
Mercury (Hg)	0.44	NMT 1.0
Arsenic	0.46	NMT 10.0
Chromium (Cr)	0.027	NMT 2.0
Nickle (Ni)	0.01	NMT 1.63

Table-7: Mineral content of *Berberis pachyantha* (ppm) root part along with their pharmacopoeial limits

Mineral	Mineral content in percentage of Drug (ppm)	Percentage limit
Cobalt (Co)	0.060	No regulatory limits by WHO
Manganese (Mn)	0.004	No regulatory limits by WHO
Magnesium (Mg)	0.047	No regulatory limits by WHO
Iron (Fe)	0.400	NMT 20.0 ppm
Zinc (Zn)	0.266	NMT 27.4 ppm
Copper (Cu)	0.109	NMT 3.00 ppm

5. DISCUSSION

The standardization is a vital step in establishing the correct identity, purity, safety and quality of crude drug and it should be established before the inclusion of crude drug in herbal pharmacopoeia (24). Physicochemical and phytochemical analysis are used to check the genuine nature of the crude drug; thus, it plays an important role in preventing the possible steps of adulteration (25). Despite its ethno medicinal importance, no attempt has been made for the pharmacognostic standardization of root part of *Berberis pachyacantha*.

Berberis pachyacantha is a well-known drug and used to cure various disorders (26). *Berberis pachyacantha* belongs to the family *berberidaceae*. The medicinal properties of plants material are mainly due to the presence of various phytoconstituents (27). The presence of different phytoconstituents such as flavonoid, tannin, saponins, alkaloid and glycoside in the phytocohemical tests justifies their therapeutic potential (28, 29, 30). These phytoconstituents have been reported to have multiple biological effects such as anti-inflammatory, anti-allergic, antioxidant, antidiabetic, analgesic, antispasmodic, antibacterial, anti-viral and anti-cancer activities (31).

Pharmacognostical and physicochemical studies, being reliable and inexpensive, play an important role in quality control issues of the crude drug samples (32). The morphological and microscopical characters observed in the root of Berberis pachyacantha serve as basis for the identification of right sample of the plant.

Recently, there has been an emphasis in standardization of medicinal plants of therapeutic potential. In spite of modern techniques, pharmacognostic evaluation is still more reliable for identification and evaluation of plants. World Health Organization (WHO) recommends that the macroscopic and microscopic evaluation is most important in establishing the identity and purity of plants (33). Phytoconstituents obtained from natural sources have been gaining importance in the day by day due to the health promoting activity. So it is necessory to check the quality safety and efficacy of herbal drugs before its consumption (34,35).

The powder study reveals the presence prismatic calcium oxalate crystals; liginified annular vessels, parenchymatous cells and cork cells. Total ash, acid insoluble ash and water-soluble ash parameters indicate the presence of inorganic and silica components in the sample studied (36). The total ash value, acid insoluble ash and water-soluble ash of root of *B. pachyacantha* was observed 7.72 %, 1.02 % and 2.80 % respectively.

The results of the extractive studies reveal the presence of secondary metabolite in the powder sample (36). The extractive values were used to find

out the amount of active principles. The successive hot and cold extraction yield calculated for petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of root of *B. pachyacantha* showed that aqueous extract registered higher percentage of yield 30.06 and 15.79 % respectively as compared with other solvents.

Loss on Drying (LOD) determines the amount of moisture as well as volatile components present in a drug. Higher moisture content in the drug sample may causes hydrolysis of active ingredients of the drug and decreases its quality and efficacy. The final dryness of the drug and rate of moisture removal are equally important, and it was observed that the moisture content in root of *B. pachyacantha* was found to be 4.16 %. The pH of the root (1% and 10% solutions) was found to be 6.2 and 6.4. The pH of the extract reveals the concentration of acidic and basic compounds. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. 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 pharmacognostical evaluation (37).

The compounds that are responsible for therapeutic effect are usually the secondary metabolites. The results of preliminary phytochemical screening of root of *B. pachyacantha* showed the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins, sterols and tannins. This preliminary phytochemical screening may be useful in the detection and further quantitative analysis of such compounds.

Quality evaluation and standardization of the herbal preparation is a fundamental requirement of industry and other organization dealing with ayurvedic and herbal products. According to WHO guidelines, an herbal product needs to be standardized with respect to safety before releasing it into the market (38). These bioanalytical parameters can be utilizing for the simultaneous analysis of different phytoconstituents present in the *B. pachyacantha* plant material. In future, this information may be useful as a standard to identify and to differentiate from its adulterants and other related species.

CONCLUSION

In conclusion, the parameters which are evaluated here can be considered as unique enough to identify and decide the authenticity of *B. pachyacantha* which will serve in the development of pharmacopoeial standards for future studies.

The phytochemical studies reported in the present study need further scientific investigation to ascertain its identity up to compound level. Study on various biological activities of *B. pachyacantha* needed to substantiate the usage of as folk healers. Pharmacognostic characters studied will be helpful in quantitative and qualitative standardization of *B. pachyacantha*. However, detailed differential studies using molecular and chemical makers are required for *B. pachyacantha,* for their authentication especially in their drug form.

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Conflicts of interest

The authors report no conflict of interest.

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