Pharmacognostic and phytochemical analysis of *Berberis aristata* stem and standardization of berberine by HPLC, HPTLC and IR Spectra

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***Abstract*: *Berberis aristata* (Berberidaceae) is an important medicinal plant which is mainly found in different region of the world. *Berberis aristata* has significant medicinal value in the traditional Indian and European system of medicine. The aim of the present investigation was undertaken to find out phytochemical analysis (qualitative and quantitative), Microscopic identification, HPLC, HPTLC, IR and antimicrobial activity of aqueous and alcoholic extract of *Berberis aristata*.**

***Keywords*: Pharmacognostic, Phytochemical, Berberine, *Berberis aristata*, HPLC, HPTLC, IR.**

Introduction: *Berberis* belongs to family Berberidaceae, represented by around 12 genera and 600 species. It is the major group with around 500 species [1]. It is widely distributed in temperate and sub-tropical regions of Northern hemisphere and temperate South-America [2]. These plants are known to have various medicinal components such as alkaloids having different pharmacological activities [3]. Some species are also used as a source of natural dye [4], [5]. The world health organization (WHO) has also recommended the evaluation of the effectiveness for various plant treatments of disease conditions where we lack safe of modern drugs. The decoction of the *Berberis aristata* is used as a wash for infected wounds and ulcers. The medicinal importance of the plant species especially root extract, reports on its antibacterial and antifungal activities [6]. In the present study, the herb was standardised by qualitative and quantitative techniques. Microscopic studies, physicochemical studies, preliminary phytochemical screening, HPTLC studies, IR spectral analysis and HPLC estimation of berberine in *Berberis aristata* were performed.

Materials and methods

Procurement & Authentication of Plant material

Procurement of the plant material

Berberis aristata (Stem) was purchased from Khari Baoli, Chandni chowk, Delhi-110006, Delhi, India. The stem sample of *Berberis aristata* plant were mechanically 5g of powdered and subjected to extraction using methanol and ethanol. The crude extract were collected in air tight plastic and glass containers and stored in cool condition.

Authentication of the plant materials

The collected plant material was authenticated by QC Department, AIMIL Pharmaceuticals (I) Limited, New Delhi.

Equipments

CAMAG Wincat V Switzerland [HPTLC Applicator], CAMAG TLC Scanner III, Switzerland [HPTLC Scanner], Perkin Elmer, Germany [IR Spectrophotometer], HPLC System Shimadzu, (Japan), HPLC Column Li Chrospher 100 RP-18e Column, AND Moisture Analyzer, Heating plate, Autoclave, and common glass wares.

Chemicals and Reagents

All the other chemical and reagents used in the present investigation were analytical grade such as Mayer's reagent, Dragendroff's reagent, Wagner's reagent, Hager's reagent and chemicals such as methanol, ethanol, sodium hydroxide, glycerine, phloroglucenol and acetone.

Pharmacognostic studies of *Berberis aristata* stem

Macroscopic studies

Macroscopic details of the *Berberis aristata* are given by observing it with naked eye or with the aid of a magnifying lens. All the description are observed on the basis of general condition of the sample i.e size, shape, outer surface, inner surface along with sensory characters like colour, odour and taste [7].

Microscopic studies

The stem powder is yellowish in color, fibrous in composition and bitter in taste. It shows fragments of cork cells, thick and spirally xylem vessels, group of stone cells, group of xylem fibres, simple fibre and sclereids fibre are also seen prominently [8].

Physicochemical Analysis

Preliminary Quantitative standard procedures were performed through standard official procedure like foreign matter, Moisture content, Ash value, Acid insoluble value, Water soluble extractive value and Alcohol soluble extractive value [9].

Qualitative Phytochemical Analysis

Qualitative chemical analysis were performed and observed in various extracts like chloroform, acetone, ethanol and water of *Berberis aristata* stem for detecting the secondary metabolites present in it. The extracts obtained by solvent extraction were subjected to various qualitative tests detect the presence of plant constituents Carbohydrates, Alkaloids, Flavonoids, protein, Tannin and steroids etc [10].

Test for Alkaloids: To 2 ml of sample in clean test tube and add 3drops of picric reagent, formation of light yellow precipitate indicates the presence of alkaloids.

Test for Flavonoids: To 2 ml of sample add few drops of concentrated NH_3 solution, indication of yellow colour confirms the presence of flavonoids.

Test for Tannin: Take 2ml of sample in to dried test tube then add 3ml of distilled water in it and add few drops of 0.1% ferric chloride and observed for dark precipitate.

Test for Steroids: To 1ml of sample add 2ml of glacial acetic acid and 2ml of con. H_2SO_4 observed for colour change from violet to blue green.

Test for carbohydrates: Take 2 ml sample clean and dried test tube add 10ml water than add 2 drops of 20% ethanolic α-naphthol and 2 ml of Conc. H_2SO_4, reddish violet ring at the junction confirms the presence of carbohydrates.

Test for Protein: To 1ml of sample and 1ml of H_2SO_4 (con.) Change in colour precipitate from white to yellow on boiling indicates the confirmation of protein in extract.

Quantitative analysis

Total Steroids content estimation: 5gm of powdered sample is accurately weighed and extracted in 50ml of 10% aqueous HCl. Filter using Whatman's filter paper. The filtrate is transferred into separating funnel and added equal volume ethyl acetate. The solution is mixed well and allowed to stand till two layers separate. The aqueous layer is discarded. The solvent extract is collected in a beaker and dried at 100°C for 5 min on water bath. This was then heated with concentrated amyl alcohol to extract the steroid. The mixture becomes turbid at this point. It is allowed to cool, tared whatman's filter paper was used to filter the mixture and kept the filter paper at 60°C in oven for 5-10 min, the paper is cooled in a desiccator for half an hour and weighed [11].

Test for Flavonoids: To 5gm of powdered sample, added 50ml distilled water and 2ml of Concentrated Hcl. The mixture is boiled for 30 minutes on a water bath, cooled and filtered. 10ml of the ethyl acetate is added and shaken vigorously for about 2 minutes. The lower separated lower aqueous layer is discarded and filter the solvent extract into a tared filter paper. The filter paper is placed in an oven for drying at 60°C for 20-30 minutes. Cool and place in desiccator till constant weight [12].

Thin layer chromatography of *Berberis aristata* stem

Thin layer chromatography technique was adopted for the identification of different classes of components present in extract based on the methods described earlier [13].

Preparation of test sample: Extract 20gm of powdered sample in soxhlet apparatus with 150 ml n-hexane to defat for (5 to 6 h) then extract with 150 ml methanol (8 to 9 h). Filter and concentrate the extract. Dissolve 2mg of residue in 1ml of methanol.

Table 1: TLC analysis parameters

Analysis	Estimation of Berberine in *Berberis aristata*
Solvent system	Toulene: Ethyl acetate: Formic acid (5:4:1)
Plate material	Precoated silica gel 60 F_{254} (E. Merck)
Standard solution	5µl
Test solution	20µl
Visualization	Under UV 366nm Under UV 254nm

Quantitative analysis of *Berberis aristata* stem powder using HPLC

Estimation of Berberine content in *Berberis aristata* was determined through advance HPLC method [14]. The chromatographic conditions for the HPLC analysis used in the present investigation are as follows.

Preparation of reference solution: A 0.002 % w/v solution of berberine hydrochloride RS in 0.1% v/v strong ammonia solution in methanol.

Preparation of test solution: Boil under reflux 1g of the coarsely powdered stem of *Berberis aristata* with 40ml of 0.1 v/v solutions of strong ammonia solution in methanol for 30 minutes, cool and filter. Boil under reflux the residue with further 2 quantities, each of 30 ml, of 0.1% v/v strong ammonia solution in methanol, cool and filter. Combine all the filtrates and dilute to 100ml with 0.1%v/v solution of strong ammonia solution in methanol. Dilute 5 ml of this solution to 50ml with a 0.1% v/v strong ammonia solution in methanol.

Table 2: HPLC Chromatographic analysis parameters

Analysis	Estimation of Berberine in *Berberis aristata*

Plates material	HPTLC Precoated plates Silica Gel Merck 60F254
Drying device	Oven
Test, standard sample spots	5 µl and 10 µl
Solvent name	Methanol
Scanning	CAMAG TLC scanner 3 with Cats software
Conditions	Temperature 25± 2 ◦C, relative humidity 40 %
TLC Chamber, mode	TLC Chamber, CAMAG Automatic TLC Sampler III
Instrument:	CAMAG Linomat 5 application parameters
Spray gas:	Nitrogen Gas
Sample solvent:	n Propanol: Water: Formic Acid (9:8.0:0.4)
Pre-dose	0.2µl
Size of Syringe (µl):	100
Application (mm):	10
Tracks:	4
Band (mm):	1

Qualitative analysis of *Berberis aristata* stem powder using HPTLC

Qualitative analysis of Berberine content in *Berberis aristata* was determined through advance High performance thin layer chromatography (HPTLC) method [15]. The chromatographic conditions for the HPTLC analysis used in the present investigation are as follows.

Preparation of reference solution: 1mg of Berberine standard was weighed and dissolved in 1ml HPTLC grade acetone.

Preparation of test solution: 5g of the dried powdered stem was soaked in methanol (4 × 20 ml, each for 1 h). The extracts were combined, filtered, and evaporated to dryness by rotary evaporation. Accurately weighed methanol extract (10 mg) was dissolved in methanol (10 ml).

Table 3: HPTLC Chromatographic analysis parameters

Analysis	**Estimation of Berberine in *Berberis aristata* Extract**
Plates material	HPTLC Precoated plates Silica Gel Merck 60F254
Test and standard sample spots	5 µl and 10 µl
Solvent system	n-propanol: water: formic acid (9:8:0.4 v/v)
Syringe	100µL Hamilton (Bonadzu, Switzerland)
Development mode	Ascending
Scanning	CAMAG TLC scanner 3 with Cats software
Experimental conditions	Temp. 25± 2 ◦C, relative humidity 40 %
Application mode	CAMAG Automatic TLC Sampler III
TLC Chamber	TLC Chamber

IR Spectral Analysis

IR Spectra of test sample i.e *berberis aristata* stem powder was compared with IR Spectra of reference berberine. The IR Spectra were compared on the basis of different peaks obtained at different Wavenumber [cm^{-1}] representing different functional groups [16].

Results

Macroscopic studies

Stem pieces yellow coloured are irregularly cut, variable in length and thickness about 2cm, nearly cylindrical, variable in length and thickness about 15 to 20 mm., bark about 0.4-0.8 cm thick, pale yellowish brown, soft, closely and deeply furrowed, surface rough, brittle, wood portion yellow, more or hard radiate with xylem rays. Pith present very small. Stems also branched; bark thin, fracture surface short and gets period off at places exposing the inner dark yellow wood. The herb has bitter taste and no specific odour.

Figure 1: *Berberis aristata* Tree

Figure 2: *Berberis aristata* Stems

Microscopic studies

- Xylem vessels: sections of reticulate and spirally thickened, fibrous sclereids related with stone cells, groups of xylem fibres associated with parenchyma containing prismatic crystals of calcium oxalate
- Cork: Surface view shows fragments of cork
- Fibres: radially cut medullary rays crossing the fibres, prismatic crystals of calcium oxalate and simple starch grains dissipated accordingly and in the parenchymatous cells.

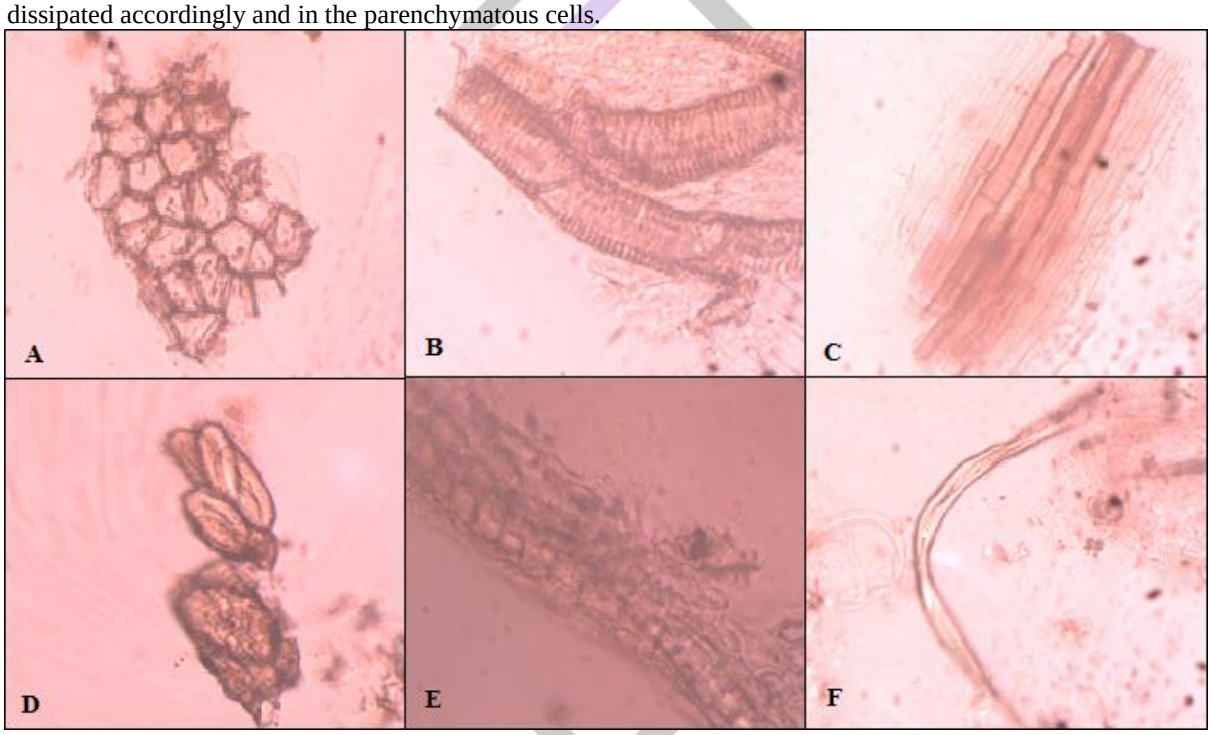

Figure 3: Powder microscopy of *Berberis aristata* (A: Cork cell, B: Thick and Spirally xylem vessels, C: Group of Xylem Fibres, D: Group of stone cells, E: Sclereids fibre. F: Simple fibre)

Physicochemical Analysis

Table 4 shows the results for Physicochemical Analysis of herb.

Table 4: The Physicochemical data obtained on analysis of *Berberis aristata* stem

Parameter	Result (percent w/w)
Foreign matter	0.32
Total ash	0.81
Acid insoluble	0.08
Water soluble extractive value	25
Alcohol soluble extractive value	20

Qualitative Phytochemical Analysis

Dried powder was extracted in Soxhlet with various solvents chloroform, acetone, ethanol and water successively and tested for different constituents and the Results of preliminary phytochemical studies are detailed in the Table 5.

Table 5: Qualitative-Chemical Analysis of *Berberis aristata* stem

Qualitative test	Chloroform	Acetone	Ethanol	Water
Alkaloids	-ve	+ve	+ve	-ve
Flavonoids	+ve	+ve	-ve	+ve
Protein	+ve	+ve	+ve	+ve
Tannin	+ve	-ve	+ve	+ve
Steroids	+ve	+ve	+ve	+ve
Carbohydrates	+ve	+ve	-ve	+ve

- Implies absent; + implies present

Thin layer chromatography of *Berberis aristata* stem

Thin layer chromatography profile of *Berberis aristata* at 366nm and 254 nm is illustrated in Figure 4 and 5.

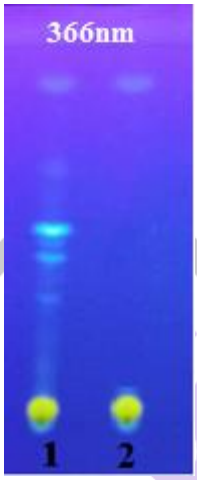

Figure 4: TLC profile of test solution of *Berberis aristata* (1), Berberine standard (2) at 366 nm

Figure 5: TLC profile of test solution of *Berberis aristata* (1), Berberine standard (2) at 254 nm

Table 6: Rf values of the spots obtained in Test solution of *Berberis aristata* stem

UV 366nm	UV 254nm
0.27 *Blue* 0.35 *Blue* 0.38 *Blue* 0.41 *Blue* 0.54 *Blue* 0.85 *Blue*	0.85 *Blue*

A band (0.85) corresponding to berberine is visible in both test and standard solution tracks under UV 366nm and UV 256nm.

Quantitative analysis

Table 7 shows the amount of flavonoids, proteins and steroids present in the herb.

Table 7: Quantitative Phytochemicals of *Berberis aristata*

Sample	Flavonoids	Steroids
Berberis aristata stem	1.992%	2.67%

Identification of *Berberis aristata* using HPTLC

HPTLC fingerprinting of *Berberis aristata* stem powder was performed to show the presence of berberine as berberine hydrochloride in the herb as shown in figure 6 and 7. (Table 8)

Table 8: HPTLC densitometric estimation of berberine in *Berberis aristata* stem powder

Component Name	Peak	X-Position	Y-Position	Area	Area (%)	Height	Rf
Berberine Standard	1	10.0 mm	32.4mm	287.20	100.0	230.86	0.25
Berberis aristata stem	1	20.0 mm	32.9mm	7.73	100.0	11.18	0.25

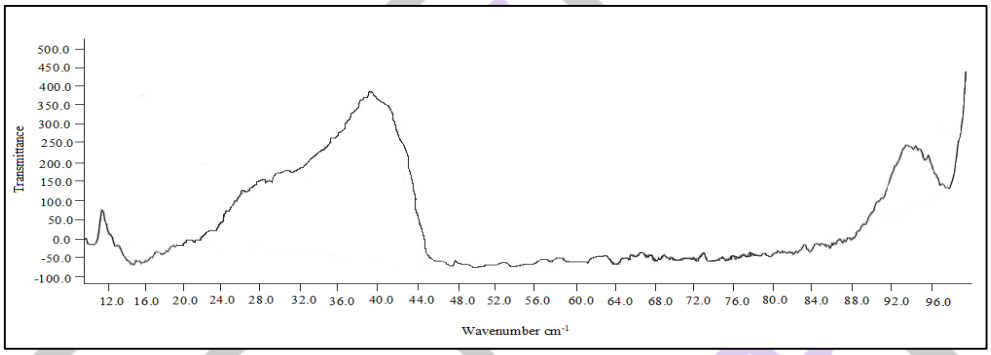

Figure 6: HPTLC densitometric estimation of berberine

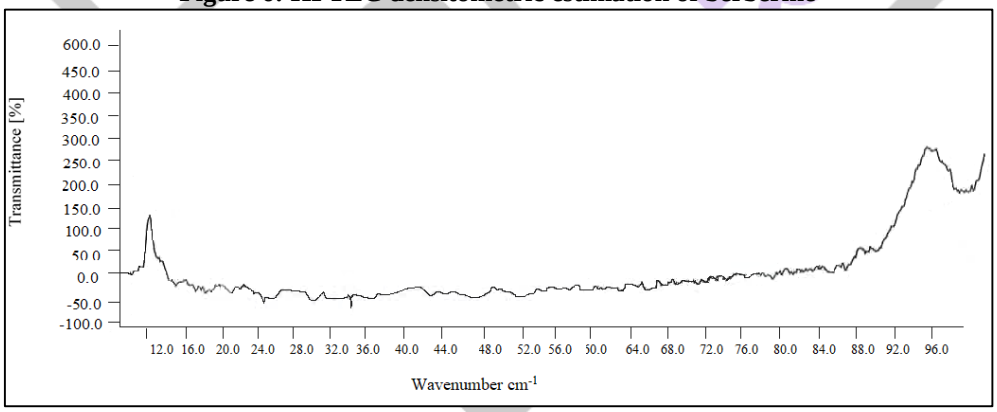

Figure 7: HPTLC densitometric scan of *Berberis aristata*

Quantitative analysis of *Berberis aristata* stem powder using HPLC

Berberine content was estimated in *Berberis aristata* through advance HPLC method (Table 9).

Table: 9 HPLC of Berberine in the *Berberis aristata* stem powder

SAMPLE	PEAK NUMBER	RETENTION TIME	AREA	AREA %
Blank	-	-	-	-
Berberis Powder	1	10.04	27442360	100.0
Standard	1	10.06	28753348	100.0

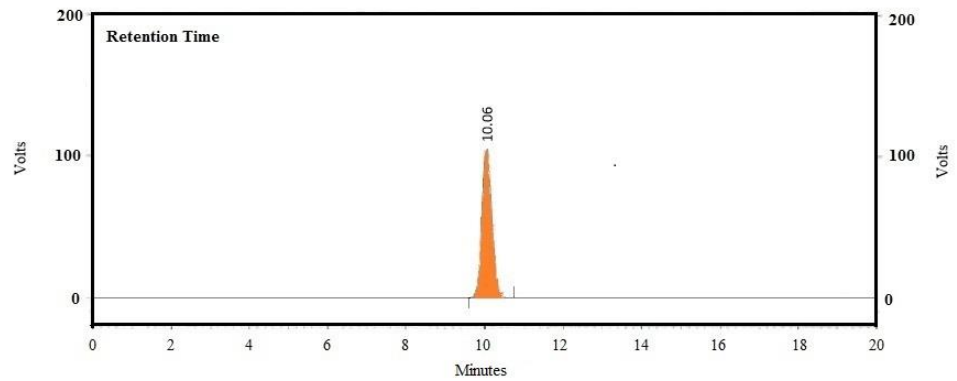

Figure 8: HPLC profile of Standard Berberine

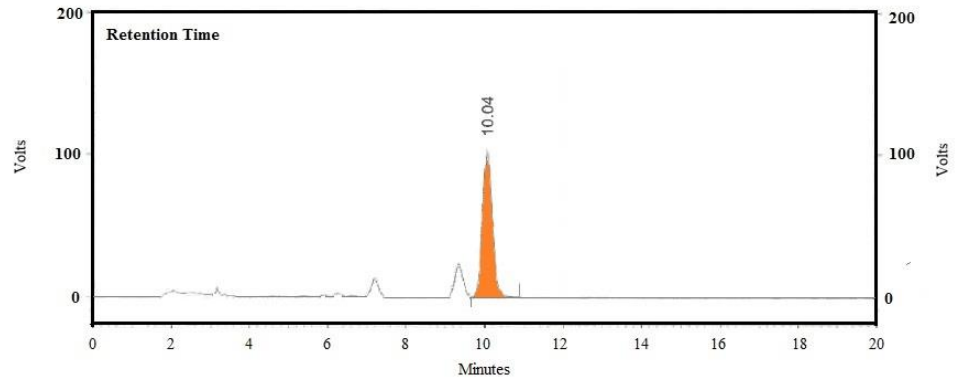

Figure 9: HPLC profile of Berberis aristata Stem Powder

IR Spectral Analysis of *Berberis aristata* stem powder with standard berberine

The IR spectra of *Berberis aristata* powder confirmed the presence of berberine (Figure 10 and 11). The IR spectrum gave absorption at 1032 cm^{-1} and small peak is observed at 1103 cm^{-1} indicating the presence of ($O-CH_2-O$) group. A peak at 1442.75 cm^{-1} in *Berberis aristata*, showed CH bending. A major peak was seen at 1597.6 cm^{-1} in *Berberis aristata* powder indicates aromatic C-C stretching. A small peak was observed at 2826.8 cm^{-1} in powder indicates C-H stretching in aromatic functional group. A standard berberine hydrochloride sample was also analyzed and it showed a similar spectrum indicating the presence of berberine in the sample.

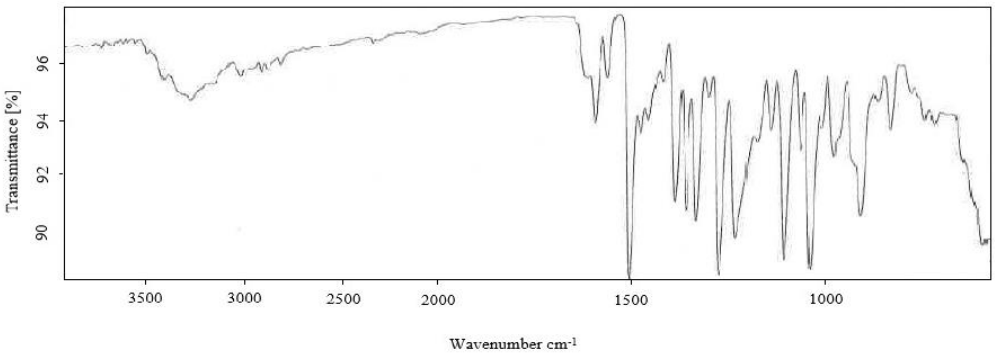

Figure 10: IR Spectra of standard Berberine

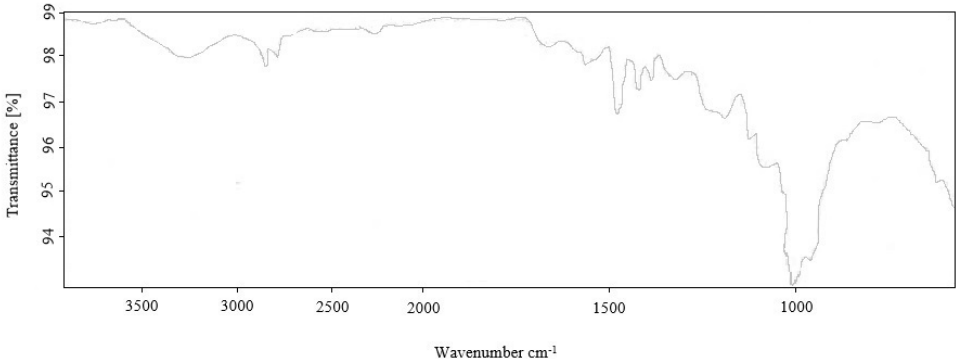

Figure 11: IR Spectra of *Berberis aristata* powder

CONCLUSION

Berberis aristata is an erect spiny shrub native to northern Himalaya region and used extensively in Ayurvedic system in medicine. Present studies has shown the various identifiable characters in powder microscopy of the herb like fibers, cork cells, sclerieids fibers. Physico-chemnical studies for identity and purity of the herb were performed. Preliminary phytochemical screening was performed to confirm the presence of secondary plant metabolites. Quantitative estimation of flavonoids, and steroids and were performed and were found to be 1.992%, and 2.67% respectively. Qualitative analysis was performed by HPTLC shows the presence of berberine in the herb. Quantitative estimation by HPLC showed the presence of berberine as 1.07 percent. IP spectral analysis of berberine and herb were also performed.

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