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Physicochemical Characterization of *Berberisaristata*, Simm (*Daaruharidra* According to Ayurveda)

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Abstract

Daruharidra (Berberisaristata) has been considered as a very significant herb in many eye and skin diseases for external application. The bark of the roots is the main part of the plant that is used, in its crude form as powder or decoction. The present study is an attempt to standardize the drug for quality control and safety evaluation as it is used in many formulations of Ayurveda. It is also used as *rasaut, rasanjan*, which is prepared with the help of goat's milk. Standardization of *Berberis aristata* was necessary to ensure its safety and efficacy after using it in the formulation. Macroscopic and microscopic studies were carried out to identify the root bark. The medullary ray cells and xylem fibres observed in the TS of the rootbark can be called as the anatomical markers. Physical constant values like moisture content, Ash values, Extractive values, were estimated. *Berberine* the primary phytoconstituent(marker compound) was identified in the HPTLC of the root bark extract.

Keywords

Berberis aristata, Standardization, Herbal drug, Phytoconstituents

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INTRODUCTION

The need of standardization of herbal drugs is important in the present era for quality control and safety evaluation[1]. *Berberisaristata* is one of the most popular drugs of Ayurveda. It is present along with Turmeric (*Curcuma longa*) in majority of the formulations of Ayurveda. According to Wealth of India the root and the stem bark contain a number of alkaloids of which the chief active alkaloid is Berberine, its concentration being higher in plants growing at lower altitudes[2]. This drug regulates the fat metabolism. Studies done on *Berberisaristata* show that the phytoconstituent Berberine as the most important active principle.

Chemical composition-The root bark of *Berberisaristata* contains berberine, quaternary ammonium salt of isoquinolone alkaloid. Berberine has antibacterial, antifungal, antiviral and antioxidant, antiinflammatory, antitumor and anti diabetic activities.

Berberine has been found to produce long lasting, dose related fall in blood pressure of anesthetized rabbits. They are also useful in oriental sores. It is used in drugs for cholera,diarrhoea, dysentery and eye complaints. Berberine is found to be useful in acute cholera and tuberculosis, its sulphate is found to be antibacterial against Staphylococcus aureus. Thiophosphamide derivative is found to be antitumor. It has also found to be useful in lesion and sores[3].

Ayurvedic description-*Daarvi (Berberisaristata)* is included under many groups of herbs in classical Ayurveda texts as follows-

Charaka- Arshoghna-Group of herbs used to treat haemorrhoids

Kandughna-group of drugs used to treat itching,

Lekhaniya-group of herbs that has scraping,cholesterol reducing quality.

Charaka has mentioned this as one of the herbs used in powder massage,useful in pruritus,acne and urticarial.(charak,sutra,3)[4]. *Sushruta*[5] and *Vagbhata*[6] have mentioned the drug in *Haridradi, Mustadi, Lakshadi* group of drugs.In Raja Nighantu it is explained to have the following properties it has *Tikta rasa*, and *Katu,Ushna* Properties. It acts on Diabetes, heals wounds earlier, and used in various skin diseases[7].

MATERIALS AND METHODS

Standardization was carried out as per the pharmacopeia guidelines. Present investigation is a detailed report for quality control of the herb *Berberisaristata*, which

includes physicochemical parameter determination, safety evaluation, microscropical evaluation, and chromatographic fingerprinting as well.

Estimation of heavy metals, pesticides, and aflatoxins was carried out to ascertain the presence of any contaminant in the sample. HPTLC of the methanol extract of Berberis aristata was carried out using isopropanol, formic acid and water in the ratio of 4.5:0.1:0.4 (v/v/v).

Collection of the Drug-Daruharidra available at the local market was bought and stored in an air tight polythene bag at room temperature (28-32◦C) and given for authentication .The sample was authenticated both macroscopically and microscopically. The plant material (bark) was bought from the local market and authenticated from the Total Herb Solutions Institute, Mumbai. Sample no THS/13/10/19/test 916 was generated tested and authenticated as Berberis *aristata Sim*.

ANALYSIS OF THE SAMPLE OF BERBERISARISTATA-

Determination of physico-chemical parameters

Foreign organic matter (FOM), loss on drying, ethanol soluble extractive, water soluble extractive, acid-insoluble ash was determined as per the procedure described in Indian Pharmacopoeia 2007(8).

Microscopic characters

The bark and stem of the given sample of Berberisaristata was analysed microscopically and the findings were as follows. Circular in outline with outer well developed, cork narrow pericycle traversed by stone cells, central narrow pith surrounded by xylem and medullary rays. Transverse section shows the multi-layered cork consisting of 3-45 rectangular to squarish radically arranged suberized cells, lignified, yellow coloured and thin walled arranged radially. Cortex narrow composed of tangentially elongated parenchymatous tissue containing stone cell isolated or in group and starch grains. Pericycle characterized by discontinuous band of isolated or group of 2-5, lignified fibres. Sieve elements irregular in shape, thin walled a few cells containing yellowish brown contents; Phloem fibre contained in tangential row.(Ref-Figure 1:Microscopic sketch of Berberis aristata)

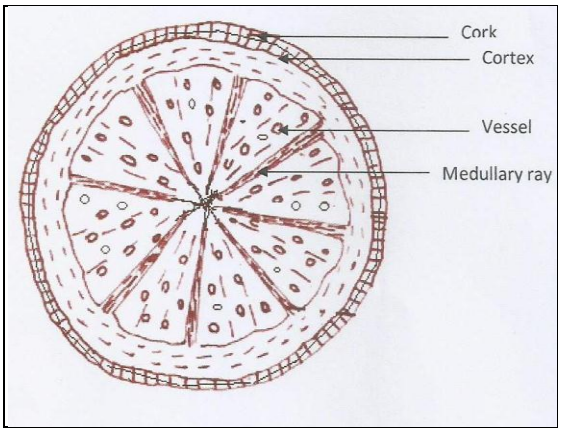

Figure 1 Microscopic sketch of Berberis aristata

Powder studies-Yellow colour, shows mostly fragments of cork cells, yellow coloured phloem fibres entire or in pieces, stone cells in singles or in groups, numerous prismatic crystals of calcium oxalate, xylem vessels, xylem fibres and ray cells.

Physicochemical characterization-

Alcohol extractive, Water extractive, pH value,Total ash acid insoluble ash, Moisture content, Density, Foreign matter were determined and found to be identical with Berberisaristata.Qualitative and Quantitative estimation displayed presence of Carbohydrates (0.658%),Protein content (0.021%),Flavanoids content (0.056%),Phenol content (0.006%).

Thin layer chromatographic fingerprinting

The thin layer chromatographic fingerprinting was developed according to the protocol suggested by Tamboli *et al*(9). HPTLC is being employed extensively as it enables rapid analysis of herbal extracts with minimum sample clean-up requirement.

Sample preparation for Chromatographic fingerprinting–

Test Solution-1 g of accurately weighed powder of Berberisaristata and 25 ml of methanol was added. The solution was sonicated for 15 minutes and the contents of the flask were filtered. The filtrate was evaporated on water bath and reconstituted in 10 ml methanol.

Standard solution-Dissolved 10mg of standard berberine in 10 ml methanol (Stock solution). One ml of the stock solution was diluted to 10 ml.

Solvent system-Isopropanlol: Formicacid: Water(4.5:0.1:0.4 v/v/v)

The plates were developed with an ascending technique. Test samples and 1,2,4,6,8,10 µl of standard solutions were applied (25µl) separately on a precoated silica gel 60 F_{254}

TLC plate (E. Merck) of uniform thickness of 0.2 mm. The plate was developed in the solvent system in a twin trough chamber to a distance of 8.5 cm.

Later the plates were evaluated at 254nm and 366nm.(Ref-Figures 2-7 show different chromatograms of Berberis aristata)

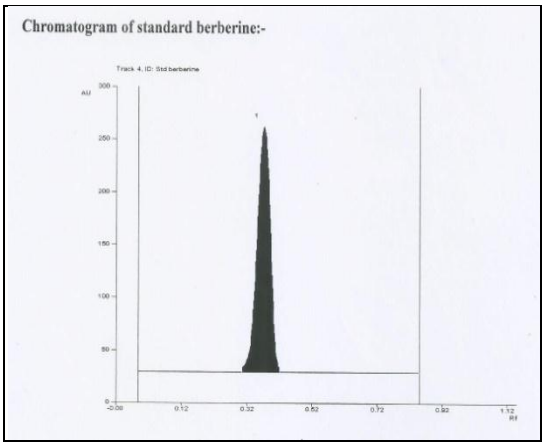

Figure 2 Chromatogram of Standard berberine

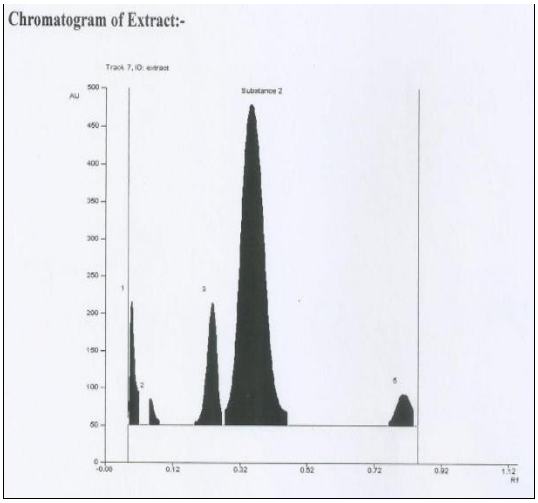

Figure 3 Chromatogram of Extract

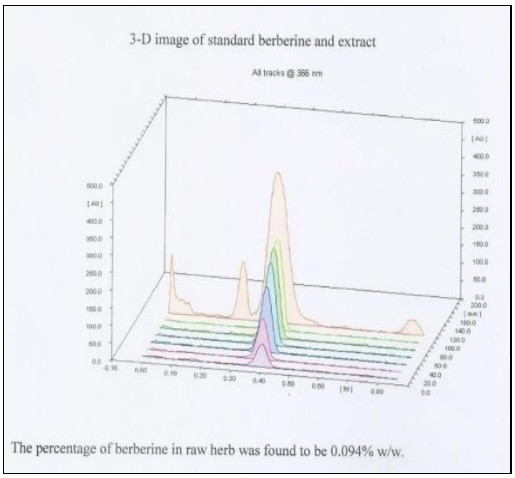

Figure 4 *3D* image of standard berberine and extract

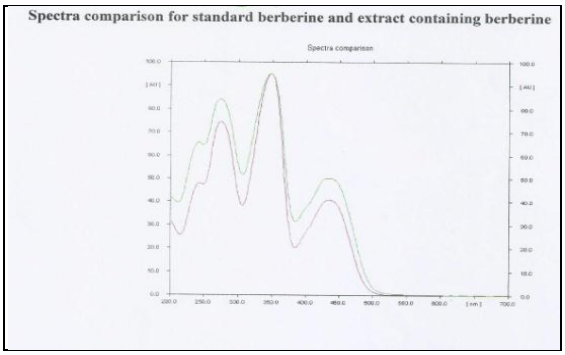

Figure 5 Spectra comparison for standard berberine and extract containing berberine

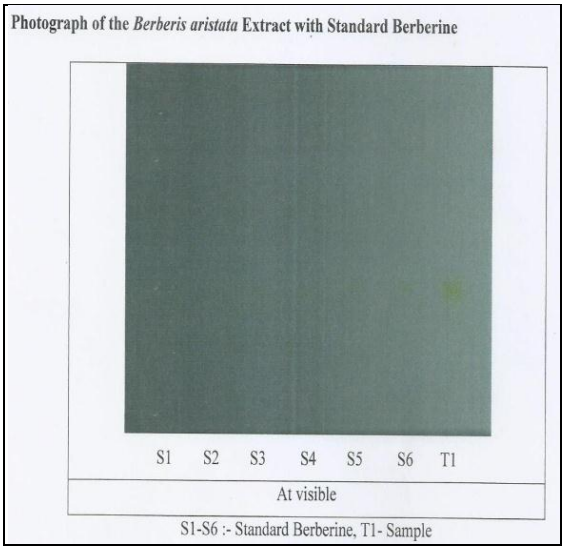

Figure 6 Photograph of the Berberisaristata extract with Standard berberine

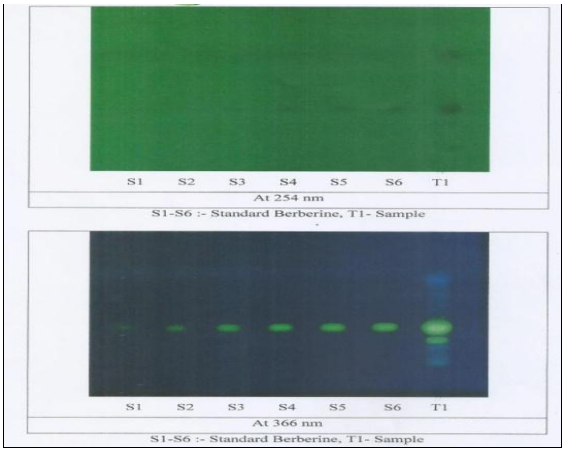

Figure 7 Chromatogram of standard berberine and extract

Evaluation of contaminants

Pesticide determination

Pesticides were analysed in Liquid chromatography-mass spectroscopy (LC-MS).A total of 25 organophosphorous pesticides were tested for the toxicological studies, but they were found to be beneath the level of quantification.

Aflatoxins determination

No alfatoxins were detected in the tested sample.

Heavy or toxic metal analysis

The heavy metal analysis for the presence of lead, cadmium, mercury and arsenic was performed for the Berberisaristata sample. It was carried out by using atomic absorption spectrometer. Standard linear calibration curve was prepared with absorbance against concentration and concentration of respective metal was calculated in the samples. Heavy metals were not detected.

Microbial contamination-

Salmonella, Eschericheacoli, Staphylococcusaureus and Pseudomonas aeringuosa were tested to negative while yeast and moulds were found in the decoction of Berberisaristata in acceptable limits.

DISCUSSION AND RESULTS

The test solution was compared with the standard solution of Berberine during the HPTLC studies. The chromatogram of the extract showed multiple peaks and the standard peak identifying presence of the marker compound Berberine. The standard solution showed only the presence of berberine.The photographs and 3D images show the presence of Berberine in a significant amount in the extract. The physico-chemical characterization also helps identify the extract as Berberisaristata.

CONCLUSION

The present investigation is an emphasized report on quality control and safety evaluation of Berberisaristata (Root bark).The report is enriched with scientific data obtained after following various standard testing procedures suggested by official books.In addition the report also has summarized information of microscopic evaluation of the root bark of Berberisaristata and its powder. The present paper is fulfilling the basic needs for quality control of the raw materials of herbal origin drugs.

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