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Standardization of *Berberis aristata* extract through conventional and modern HPTLC techniques

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ABSTRACT

Objective: *Berberis aristata* (Berberidaceae) is an important medicinal plant, found in the different region of the world. It has significant medicinal value in the traditional Indian and Chinese system of medicine. The aim of the present investigation includes qualitative and quantitative analysis of *Berberis aristata* extract. **Methods:** Present study includes determination of phytochemical analysis, solubility test, heavy metal analysis, antimicrobial study and quantitative analysis by HPTLC method. **Results:** Preliminary phytochemical analysis showed the presence of carbohydrate, glycoside, alkaloid, protein, amino acid, saponin, tannin and flavonoid. Solubility in water and alcohal were found to be 81.90% in water and 84.52% in 50% in alcohal. Loss on drying was found to be 5.32%. Total phenol and flavonoid content were found to be 0.11% and 2.8%. Level of lead, arsenic, mercury and cadmium complies the standard level. E. coli and salmonella was found to be absent whereas total bacterial count, yeast and moulds contents were found to be under the limit. Content of berberine was found to be 13.47% through HPTLC techniques. **Conclusions:** The results obtained from the present studies could be used as source of valuable information which can play an important role for the food scientists, researchers and even the consumers for its standards.

1. Introduction

Berberis aristata (Berberidaceae) is an important medicinal plant, also known as 'daruharidra', found in the Himalaya and other part of the world. Rasaut, which is a very valuable preparation, is made from this plant and are used for the treatment of diseases such as ophthalmic, jaundice and skin diseases. The plant is a valuable medicine for the treatment of remittent fevers, oxidative stress and used as a cooling laxative to children and as a tonic remedy for liver and heart. The plant contains berberine, oxyacanthine, berbamine, and palmatine among which berberine exhibits multiple pharmacological activities. It has febrifugal, hypotensive, immuno-stimulating, antiinflammatory, antimicrobial, antiprotozoal, anticholinergic and antiarrhythmic activities. It also has antiamoebic, antifungal, antihelminthic, leishmanicidal, tuberculostatic properties and some central nervous system activity as well. Bacteria related diarrhea, parasitic intestinal infections and ocular infections are the most prominent clinical uses of berberine. It has been reported that berberine exhibits local anesthetic, enzyme inhibitory, antipyretic and antiamnesic activities [1-4]. In the present study, we have evaluated the selective phytochemical parameters including quantification of berberine through HPTLC techniques in *Berberis aristata* extract. Further we have also evaluated heavy metal analysis and microbial content in the crude extract.

2. Material and methods

2.1. Chemicals and reagents

The hydroalcoholic extracts of *Berberis aristata* was procured from Garlico Herbal Concentrate, (M. P.) India. HPTLC precoated plates Silica Gel Merck 60F254 was used as a stationary phase. All the other chemical and reagents used in the present investigation are analytical grade.

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2.2. Method development

Priliminary phytochemical analysis was performed through standard official procedure [5]. Thin layer chromatography technique was adopted for the identification of different classes of components present in extract based on the methods described earlier [6, 7]. Solubility, loss on drying, heavy metal and microbiological analysis were performed according to the IP, 1996 and WHO guidelines [8, 9]. Total phenol and flavonoid content were also determined [10, 11].

2.3. HPTLC standardization

Further berberine content was determined through advance high performance thin layer chromatography (HPTLC) method. The chromatographic conditions for the HPTLC analysis used in the present investigation are as follows.

2.3.1. Preparation of standard

For the preparation of the calibration curv in the quantitative analyasis different concentration of the standard stock solution were prepared in the HPLC grade methanol.

2.3.2. Preparation of samples

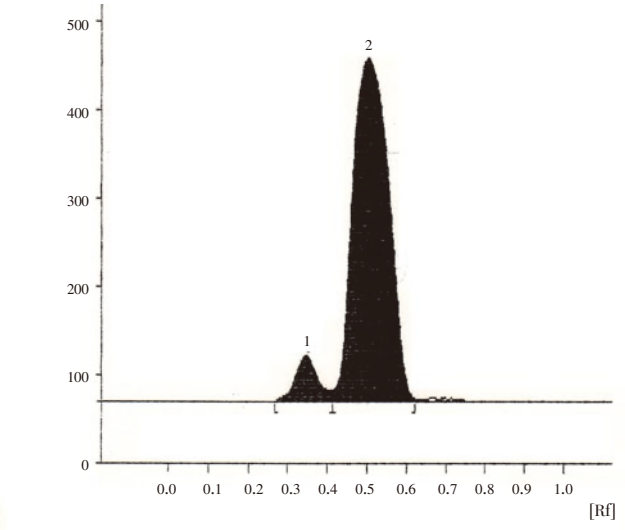
For the preparation of the samples solution, extract was dissolve in the methanol and then sonicated for 10 minutes and the final volume of the solutions was made up to 5 ml to get stock solutions. All the needed concentration of the samples was prepared from the stock solution by suitable dilution.

2.3.3. HPTLC Chromatographic analysis parameters

Analysis	Estimation of Berberine in *Berberis aristata* extract.
Plate material	HPTLC Precoated plates Silica Gel Merck 60F254
Solvent system	n-Propanol : Formic acid : Water (90: 1: 9)
Syringe	100 μ L Hamilton (Bonadzu, Switzerland)
Application mode	CAMAG Automatic TLC Sampler III
TLC Chamber	CAMAG, AMD 2 automatic developing chamber
Development mode	Ascending
Scanning	CAMAG TLC scanner 3 with Cats software
Experimental conditions	Temperature 25 ± 2 ℃, relative humidity 40 %

3. Results

Organoleptic study revealed that the extract has brown colour and bitter test. Preliminary phytochemical analysis of *Berberis aristata* showed the presence of carbohydrate, glycoside, alkaloid, protein, amino acid, saponin, tannin and flavonoid. TLC analysis incorporating solvent system (Benzene: ethyl acetate: diethyl amine (6: 3: 1)) showed to contain three prominent spots with Rf (0.02, 0.05, 0.01) and in (n-propanol: formic acid: H2O (90:1:9)) showed to contain four prominent spots with Rf (0.12, 0.07, 0.35, 0.07) respectively. Parameter such as solubility in water and alcohal were determined and was found to be 81.90% in water and 84.52% in 50% in alcohal. Loss on drying was found to be 5.32%. Total phenol and flavonoid content were also determined and found to be 0.11% and 2.8% in respect to standard gallic acid and rutin. Heavy metal analysis was performed and result showed that the level of lead, arsenic, mercury and cadmium complies the standard level i.e. lead<10 PPM, arsenic and mercury <1 PPM and cadmium<0.1 PPM. Microbiological assay was also performed in the current task and the result showed that E. coli and salmonella was found to be absent whereas total bacterial count, yeast and moulds contents were found to be under the limit. Optimization of HPTLC solvent system for quantitative analysis was done using combination of solvent system of varying polarity and the most suitable solvent system was found to be n-propanol : formic acid : water (90: 1: 9). Quantitative analysis of berberine in the *Berberis aristata* was performed through HPTLC techniques and result showed that content of berberine in the *Berberis aristata* was found to be 13.47% through HPTLC techniques compared to standard berberine. The respective HPTLC chromatograms of berberine and *Berberis aristata* were presented in the Figure 1(A, B and C). In the present analysis calibration curve of standard berberine was found to be linear ($y = 613.9x + 1059.1$, $R^2 = 0.996$), which was presented in the Figure 2. HPTLC photograph of standard berberine and *Berberis aristata* extract were presented in the Figure 3 (A and B). The interpretation of results suggests that the sample contained considerable amount of berberine.

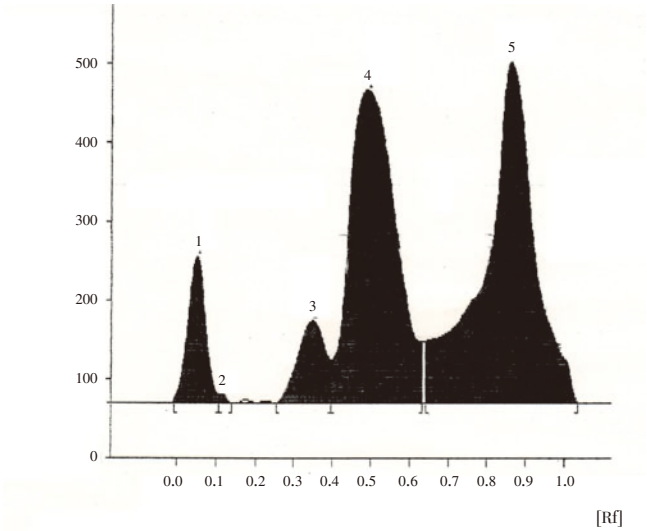


Wavelength: 254 nm
Track: 1, noise level: 0.473AV, raw data file: DINBER_2
V4.06 S/N:0612A004 CAMAG SOFTWARE (c) 1998 SCANNER 3: 061121
Track 1, Analysia a: be5mcl

Peak	Start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.27	0.2	0.25	51.8	11.82	0.41	12.4	2120.8	7.73
2	0.41	12.4	0.50	386.8	88.18	0.62	3.0	25325.9	92.27

Total helght=438.7 total area=27446.7

Figure 1(A): HPTLC chromatogram of standard berberine marker compound.

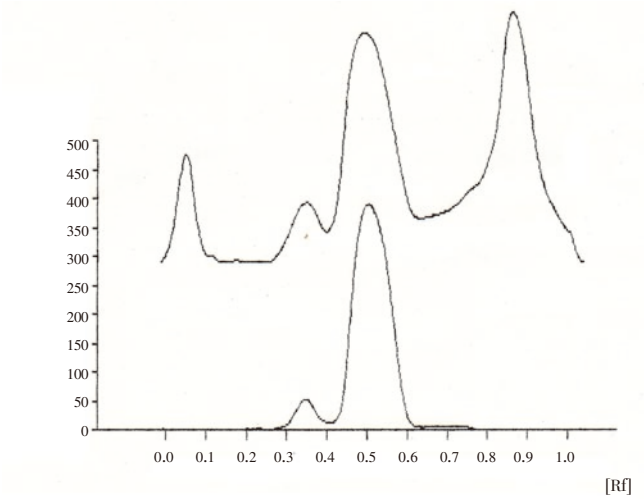


Wavelength: 254 nm
Track: 2, noise level: 0.473AV, raw data file: DINBER_2
V4.06 S/N:0612A004 CAMAG SOFTWARE (c) 1998 SCANNER 3: 061121
Track 2, Analysia a: ba5mcl

Peak	Start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	−0.01	5.5	0.05	186.2	16.48	0.11	11.0	6019.8	7.03
2	0.11	11.0	0.11	12.6	1.11	0.14	0.1	142.7	0.17
3	0.25	0.0	0.35	103.9	9.20	0.40	54.1	5292.0	6.18
4	0.40	54.1	0.49	396.8	35.04	0.63	76.1	34138.3	32.88
5	0.64	76.9	0.87	431.1	38.16	1.04	2.4	40043.3	4676

Total helght=1129.5 total area=85636.1

Figure 1(B): HPTLC chromatogram of *Berberis aristata* plant extract.



Wavelength: 254 nm
File name: DINBER_2 track 1 to 3
V4.06 S/N:0612A004 CAMAG SOFTWARE (c) 1998 SCANNER 3: 061121

Figure 1(C): Comperative HPTLC chromatogram of berberine with *Berberis aristata* plant extract.

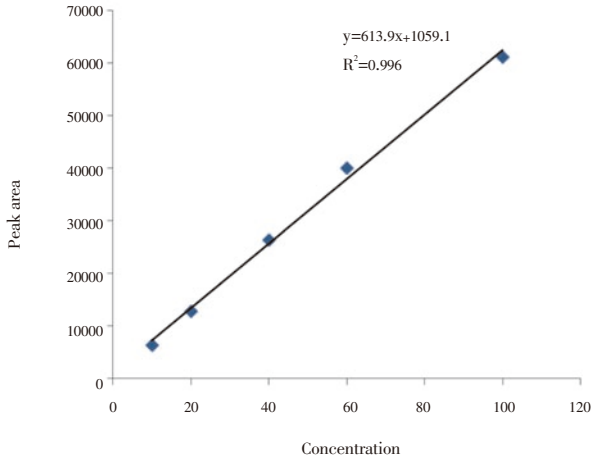


Figure 2: Standard calibration curve of berberine.

In UV 254 nm

Figure 3(A): HPTLC photograph of standard berberine and *Berberis aristata* extract at 254 nm.

In UV 365 nm

Figure 3(B): HPTLC photograph of standard berberine and *Berberis aristata* extract at 365 nm.

4. Discussion

Physicochemical standards are generally used for determining the identity, purity and strength of the drug source. These characters are also used to check the genuine nature of the crude drug, thus it plays an important role in preventing the possible steps of adulteration. There are various physiochemical parameters developed which are incorporated for the evaluation of crude drugs. The phytochemical analysis conducted on Berberis aristat extracts revealed the presence of carbohydrate, glycoside, alkaloid, protein, amino acid, saponin, tannin, flavonoid and other phytoconstituents. The TLC analysis revealed the presence of different phytoconstituents including steroidal, triterpenoidal, phenolic and flavonoidal compounds. The curative properties of medicinal plants are due to the presence of various classes of secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, etc [12]. Tannins are dietary anti-nutrients with strong astringent property and are known to be useful in the treatment of inflamed or ulcerated tissues, cancer, mild anti-septics, diarrhea as well as to check small hemorrhages. Flavonoids have a membrane permeability effect and are considered as potential antioxidants and have protective action against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumor [13-16]. Also, the plant extract were reported to contain saponins, which have a potential anti-inflammatory, coagulant, antidiabetic, antioxidant, aldose reductase inhibitory activity, haemolytic and cholesterol binding properties [17-20]. Phenols and phenolic compounds are greatly used in skin infections, wound healing, inflammation; antioxidant, immune enhancers, anti clotting and hormone modulators [15].

TLC or HPTLC is an inexpensive method for separation, qualitative identification, or semi-quantitative analysis of samples. TLC and HPTLC techniques can be used to solve many qualitative and quantitative analytical problems in a wide range of fields, including medicine, pharmaceuticals, chemistry, biochemistry, food analysis, toxicology and environmental analysis. The use of TLC/HPTLC has expanded considerably due to the development of gradient TLC methods, improved stationary and mobile phase selection and as new methods of quantitation methods [21]. Different concentration of standard solution of berberine (standard marker compound) was applied on HPTLC plates along with methanolic extract of *Berberis aristata*. The HPTLC plates were developed in a suitable solvent system and dried in air and scanned densitometrically at 254 nm. The method was validated in terms of precision and accuracy. The peak areas were recorded and relationship between the concentration and peak response was found to be linear within the range of used concentration for standard marker compound. Correlation coefficient of the respective marker compound was found to be 0.996. The HPTLC method was developed for simultaneous quantification of berberine in presence of other plant constituents in the sample solutions. The proposed method was found to be precise, simple, specific and sensitive in the tested concentration.

Quality evaluation of herbal preparation is a fundamental requirement of industry and other organization dealing with ayurvedic and herbal products. The growing use of botanicals (drug and other products derived from plants) by the public is forcing moves to assess the health claims of these agents and to develop standards of quality and manufacture. It is evident that the herbal industry needs to follow strict guidelines and such regulations are necessary. Herbal drugs regulations in India as well as an overview of regulatory status of herbal medicine in USA, China, Australia, Brazil, Canada and Germany has been reported. According to WHO guidelines, an herbal product needs to be standardized with respect to safety before releasing it into the market [22].

5. Conclusion

In the present study we have evaluated and presented the phytochemical standardization of hydroalcoholic extract of *Berberis aristata*. This information may be useful as a standard in the future to identify and to differentiate from its adultrants and other related species of berberis.

Conflict of interest statement

The authors report no conflict of interest.

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