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EVALUATION OF *DARUHARIDRA* WITH SPECIAL REFERENCE TO KASHAYA AND GHANA – HPLC BASED ANALYTICAL STUDY

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ABSTRACT

Daruharidra is commonly used herb in traditional medicine. Selection of *Daruharidra* is bit difficult as many *Berberis* species are sold in the market including *Coscinium fenestratum (Gaertn.) colebr*. Though kashaya is indicated in many conditions, *Tiktha rasa* makes it difficult for consumption. So adopting newer dosage forms like *Ghana* which can be easily dispensed by filling in capsule or compressed in the form of tablet is the need of the day. It becomes essential to screen major phyto-chemical components before and after processing to evaluate possible alterations, where HPLC plays an important role. Chromatographical analysis (HPLC) of kashaya and ghna prepared out of both Berberis lycium and *Coscinium fenestratum (Gaertn.) colebr* revealed change in berberin content before and after processing. As per the HPLC data, Berberis lycium could be a better choice for kashaya where as *Coscinium fenestratum (Gaertn.)colebr*, a choice for Ghana with respect to berberin content.

KEYWORDS: Kashaya, Ghana, Berberine

INTRODUCTION

Daruharidra (*Berberis aristata* D C) is one among endangered[1] but commonly traded medicinal Plant, widely used for its multiple therapeutic utility. Identification of *Daruharidra* is bit difficult as many Berberis species are sold in the market including *Coscinium fenestratratum* (Gaertn.) colebr[2]. Though not specified in the classical literature, modern literature describes *Daruharidra* having two varieties, one being Berberis group and other *Coscinium fenestratum (Gaertn.) colebr*. One of the synonyms of *Daruharidra*, Kaliyaka has been related with *Coscinium fenestratum (Gaertn.)colebr*[3,4]. *Coscinium fenestratum (Gaertn.) colebr* belongs to family Menispermaceae and is distributed in Western ghats, Kerala, Tamil nadu and Ceylon. It is a woody climbing shrub with cylindrical stem. Branches covered with fuscous cinerous bark and yellowish. Leaves are shiny above and tomentose below. Flowers are yellow colored which give rise to globose drupe fruits with globose seeds. Main Chemical constituents are berberine, berberrubine, palmatine, berlambine and oxypalmatine[7]. It is having Tiktha Kashaya Rasa, laghu, ruksha Guna, ushna virya, katu vipaka and kaphapithahara karma. *Daruharidra* is also called by synonyms like

Darvi, Parjanya, Pachampacha, Nisha and Vishodhini.

Though *Daruharidra* is mainly indicated in urdhvajatrugata roga, it is also indicated in conditions like prameha, kandu and shwetapradara, where its kashaya is the preferred dosage form.[5] Kwatha (one among panchavidha kashaya kalpana) has been widely used in ayurveda, which has shelf life for 24 hrs[6]. Palatability of kashaya prepared out of Tikta rasatmaka dravyas is difficult for prolonged administration and hence different dosage forms like rasakriya, Ghana, and ghanavati are in use. It is necessary to evaluate phytochemical constituents in Kashaya and Ghana so that standardized palatable dosage form can be evolved. Hence phyto-chemical analysis of *Daruharidra* was undertaken with special focus on Berberin content using HPLC method.

MATERIALS AND METHODS

Berberis lycium sample was procured from herb vendor of Uttarakhand state (Figure 1). *Coscinium fenestratum (Gaertn.) colebr* plant was collected from its natural habitat at Kakkur, Calicut district, Kerala state. Both the samples were dried under sunlight followed by shade drying to prevent fungal growth. Dried samples were pulverized to obtain coarse as well as fine powder. Dried specimens were subjected for botanical authentication by Dr Shantha T R, Research officer (Botany), National Ayurveda Dietetics Research institute, Ashoka Pillar, Bengaluru, by comparing with voucher specimens and were confirmed to be *Berberis lycium* and *Coscinium fenestratum (Gaertn.) colebr* respectively.

Comparative analytical study was conducted as follows.

A.Pharmacognostic study:

1. Macroscopy (Appearance, Foreign matter)
2. Microscopy (Powder microscopy)

B.Physico-Chemical Study

1. Total ash
2. Acid insoluble ash
3. Water soluble ash
4. Alcohol soluble extractive
5. Water soluble extractive

B. Preparation of Kashaya and Ghana

Kashaya and Ghana of both *Berberis lycium* and *Coscinium fenestratum (Gaertn.)colebr* were prepared as per Standard protocol of Sharangadhara Samhita[8]. Kashaya of both *Berberis lycium* and *Coscinium fenestratum (Gaertn.) colebr* were dried by two methods.

1. Vacuum drying using Rotary flash evaporator to prevent deterioration of thermo labile compounds
2. Drying by direct heating (Rasakriya and Ghana). Kashaya (Decoction) was further boiled using mandagni (mild fire) to convert it to Rasakriya and then dried over water bath to dry completely to obtain Ghana (Dried mass), which was further moulded in to 1cc pallet. Both kashaya and Ghana were subjected for phyto-chemical analysis and HPLC.

C. Preliminary phyto-chemical studies:

Qualitative analysis of both Kashaya and Ghana of *Berberis lycium* and *Coscinium fenestratum (Gaertn.) colebr* was carried out to detect presence of phyto-chemicals such as Alkaloid, Phenolics, Flavonoids, Glycoside, Mucilage and amino acid as per standard protocol[9].

D. Chromatographic analysis:

Chromatography was carried out for both qualitative and quantitative analysis with respect to the presence of Berberin.

1. Thin layer Chromatography:

Thin layer chromatography was done as per Rojsanga et al[10]. Sample for TLC analysis was prepared by using ethanol. 1gram each of both samples was refluxed with 10 ml of Ethanol over water bath. Solution was filtered through whatman No 1 fileter paper and centrifuged to obtain sediment free solution. Preheated silica plate was used for spotting of samples. Solvent system was prepared by using ethyl acetate: butanol : formic acid : water (50:30:12:10). Developed chromatogram plate was dried and visualized under UV (366 nm). RF Value was calculated by using formula and compared with standard Rf value of Berberin.

Rf value - Distance travelled by solute / Distance travelled by solvent

1. HPLC (High performance/pressure liquid chromatography)

HPLC study was carried out to estimate the quantity of Berberine in given samples. LC 2010A from SHIMADZU, consisting quartenary pump, UV detector, auto injector, column oven and supported by LC-Solution software was used during HPLC analysis.

Chromatographic conditions:

Mobile phase preparation : Filtered and degassed mixture of 50 volumes of buffer and 50 volumes of Acetonitrile.

Buffer preparation : 78mg of sodium Lauryl sulfate and 5.5g of Tartaric acid dissolved in 250 ml of water collected from Sartorius water purification system. Mix the buffer properly.

Column : Silica CN 5µsize, 250 × 4.6mm (Merck)

Detector : SPD- M 10 Avp Photo diode array detector/UV detector

Wavelength : 343nm

Flow rate : 1.8ml/min

Injection volume : 20µl

Berberin was used as standard reference sample for the study

Results:

Macroscopic features:

Berberis lycium stem pices are of the size of 1 to 2 inches long having yellow colour on both internal and external surface (Figure 1). Striations are seen on both surfaces. Stem pieces are odourless and bitter in tase. ***Coscinium fenestratum (Gaertn.)colebr*** cut stem shows unique wheel like medullary rays, which is deep yellow in colour. They diverge from centre to the periphery with thick cortex. Pith in the centre surrounded by xylem and phloem vessels. Stem pieces are yellowish brown externally and dark yellow internally. Stem pieces are odourless and bitter in taste.

Powder microscopy of ***Berberis lycium*** revealed presence of single and group of fibres, Rectangular and elongated stone cells, Prism shaped crystals, Presence of tannins and Fragments of tissues where as ***Coscinium fenestratum (Gaertn.) colebr*** showed Abundance of fibres, Tannins containing cells, Stone cells with tannin in lumen, Presence of tracheids, Parenchymatous cells with tannin, Xylem with pits and Fragments of tissues (Figure 2).

Observations made during Reagent tests are illustrated in Table 1. Thin layer chromatography study revealed intense

greenish yellow florescent spots at in both samples of *Daruharidra* (*Berberis lycium* and *Coscinium fenestratum (Gaertn.) colebr*) having Rf value 0.66 (Figure 3). Intensity of florescent spot is observed to be more in *Berberis lycium* compared to *Coscinium fenestratum (Gaertn.)colebr*. HPLC analysis revealed more berberin content in *Berberis lycium* Kashaya compared to *Coscinium fenestratum (Gaertn.) colebr* and when compared between Ghana, *Coscinium fenestratum (Gaertn.) colebr* showed more presence of berberin (vide table No 2, figure 4)

DISCUSSION

Berberis aristata DC is considered as an endangered species of *Daruharidra*. So its allied spiecies Berberis *asciatica* Roxb, *Berberis lycium* Royle, *Berberis chithra*, *Berberis vulgaris* Linn are being used as *Daruharidra* in North Indian market[11]. The synonyms such as *kaliyaka* or *pita chandana* of *Daruharidra* can be taken as *Coscinium fenestratum (Gaertn.) colebr* and it is being substantiated by physicians in south India[12]. As contraversy prevailed, both Berberis *lycium* Royle and *Coscinium fenestratum (Gaertn.)colebr* (Gaertn.) colebr were taken for analytical study to ascertain quality of kashaya and Ghana with respect to its berberin content.

Abundance of Fibres, Tannins, Stone cells, Tracheids, Prism shaped crystals and fragments of tissue were found in powder microscopic study of *Berberis lycium* Royle and *Coscinium fenestratum (Gaertn.) colebr* (Gaertn.) colebr, which suggest that members of Menispermaceae probably have affinities with woody genera of Berberidaceae. “In the respective system of Bentham and Hooker, engler and hacitichinson, Menispermaceae is placed closed to Berberidaceae [13]**.** The occurence of *Berberine* in both families shows the affinity of Menispermaceae with Berberidaceae. Physicochemical investigation is important in knowing the purity and genuinity of the drug. The physicochemical parameters observed of both *Coscinium fenestratum (Gaertn.) colebr* and *Berberis lycium* Royle were within the API standards which signifies genuinity of raw materials used for the study . Since *Coscinium fenestratum (Gaertn.)colebr* was personally collected from its natural habitat, presence of foreign matter was negligible. Market samples are not always free from adulteration and hence few pieces of Morinda pubescence species were found in *Berberis lycium* Royle sample. Ash value of both *Berberis* and *Coscinium* samples were found within normal limits, which indicates the complete oxidation of organic matter as well as absence of foreign matter. Alcoholic extract, Kashaya and Ghana of *Berberis lycium* Royle and *Coscinium fenestratum (Gaertn.) colebr* showed the presence of Alkaloids, Flavonoids Tannins, Phenols,and Saponins. These phytochemicals probabaly contribute bitter taste to *Daruharidra*. Alcoholic extract, *Kashaya* and *Ghana* did not show presence of mucilage and amino acids as both get precipitated mostly due to heat. Intensity of yellow colour is observed more in alcoholic extract due to the solubility of pigments as well as Berberine in alcoholic media. Test for carbohydrates and protein remain negative in *Coscinium fenestratum (Gaertn.) colebr* while slight

presence was detetected in *Berberis lycium* which might have contributed to its density.

Presence of Greenish yellow fluoroscent spot with R f value 0.66 in both *Berberis lycium* Royle and *Coscinium fenestratum (Gaertn.) colebr* is suggestive of common chemical component *Berberine* in ethanolic extract, which is in accordance with chromatography conducted in earlier studies[14]. Presence of berberine in higher concentration among *Berberis lycium* Royle *kashaya* can be attributed to the density of sample compared to C*oscinium fenestratum (Gaertn.) colebr*, which is fibrous and lighter. Detection of berberin in slightly higher concentration in the Kashaya of *Berberis lycium* can be related to density as well as natural habitat of *Berberis lycium* Royle. Heating leads to caramelization[15] process of sugars present and further prevent berberine to be released in to solution during sample preparation, which might have occurred with *Berberis lycium* Royle during HPLC analysis. Bio degradation is quite common during processing phase[16] in both *Berberis lycium* Royle and *Coscinium fenestratum (Gaertn.) colebr* (Gaertn.) colebr. Phenolics are known to attack other active principles[17] during processing phase, which might have reduced active berberin content in Ghana.

CONCLUSION

Comparative HPLC analysis of both *Berberis lycium* Royle and *Coscinium fenestratum (Gaertn.) colebr* (Gaertn.) colebr in *Kashaya* and *Ghana* form is suggestive of selectivity of specific dosage form of different species. It can be suggested that *Kashaya* of *Berberis lycium* Royle and *Ghana* of *Coscinium fenestratum (Gaertn.) colebr* may be effective as far as berberine concentration is concerned.

Table 1

PHYTOCONSTITUENTS	**Alcoholic extract (Bl & C f)**	***Kashaya* C f**	***Kashaya* B l**	***Ghana* C f**	***Ghana* B l**
Alkaloids **Dragendroff** **Wagner** **Mayers**	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Phenols	+	+	+	+	+
Glycosides	-	-	+	-	+
Carbohydrates	-	-	-	-	-
Mucilage	-	-	-	-	-
Amino acid	-	-	-	-	-

Table No 2			
Sl No	**SAMPLES**	**DESCRIPTION**	**BERBERINE%(W/W)**
1	B l Hot Aqueous	Brown colour dried mass	0.60
2	C f Hot Aqueous	Brown colour dried mass	1.21
3	B l *Kashaya*	Brown colour dried mass	2.02
4	C f *Kashaya*	Brown colour dried mass	1.41
5	B l *Ghana*	Dark brown dried mass	0.79
6	C f *Ghana*	Dark brown dried mass	1.04

Fig. 1

Coscinium fenestratum Berberis lycium

Fig. 2

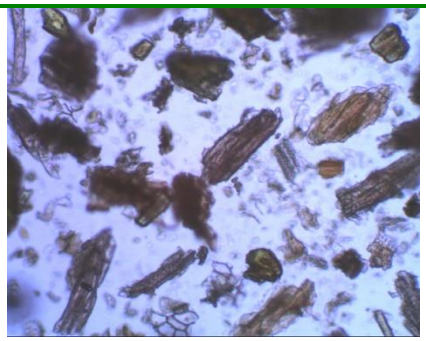
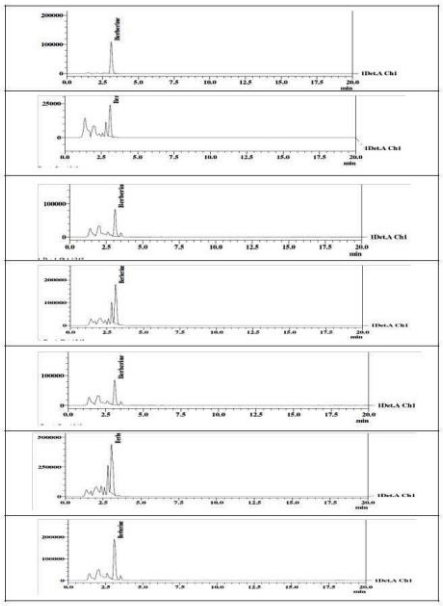


Fig. 3

Fig. 4



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