Research & Reviews

Research and Reviews: Journal of Botanical Sciences

Berberine content in *Coscinium fenestratum* grown in Sri Lanka

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Short Communication

Received: 05/03/2012
Revised: 16/08/2012
Accepted: 01/11/2012

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Keywords: *Coscinium fenestratum*, berberine. TLC fingerprints

ABSTRACT

Coscinium fenestratum (Gaertn.) Colebr. (Family: Menispermaceae) is one of the most important plants used in traditional systems of medicine. The major compound in the *C. fenestratum* is berberine. In the present study, berberine content was quantified using *C. fenestratum* grown in Sri Lanka. Results revealed that hot extraction method was more suitable for getting high yield of berberine from *C. fenestratum.* In conclusion, this information is useful as guidance for setting a specification of raw materials and extracts of *C. fenestratum* for pharmaceutical preparations.

INTRODUCTION

Medicinal plants are used as a major source of drugs for the treatment of various health disorders. There are total of some 250,000 species of higher plants in the world, much less than the species of animals (5-10 million). However, plants contribute to our lives more than animals mainly due to their extraordinary array of diverse classes of biochemical with a variety of biological activities[1]. Plants with medicinal properties, the gift of mother nature to mankind, are in use for centuries in the traditional systems of Ayurveda, Siddha, Unani etc. The plant kingdom has immensely contributed to the health needs of man when no concept of surgical management existed. Even today almost 25 % of all prescribed medicines in the developed world contain ingredients derived from medicinal plants [2]. *Coscinium fenestratum* (Gaertn.) Colebr. is one of the most important plants used in traditional systems of medicine. *C. fenestratum* is belonging to family Menispermaceae. The vernacular name of the plant is Venivel in Sinhala, False calumba or Tree Turmeric in English and Atturam or Kadari in Tamil [3].

This species occurs in the hills of Malabar region, particularly Western Ghats[2] and in the jungles of South India, Malacca, Singapore Sumatra and Sri Lanka. It is common in the moist low-country forests in Sri Lanka [4-5]. Berberine is one of the major alkaloid constituent in *C. fenestratum*. Berberine has broad spectrum of pharmacological activities. The drug is useful in vitiated conditions of, inflammations, wounds, ulcers, jaundice, burns, skin diseases, abdominal disorders, diabetes, fever and general debility. An infusion, tincture and concentrated liquor are also prepared to wash wounds and skin rashes. Stem pieces are boiled and one cup is given in case of a fresh, deep cut, being the most common use against tetanus. It purifies the blood. Decoction of stem is given internally in cases of bites from monkeys, snakes, brahmin-lizards and geckos. The root bark is used for dressing wounds, ulcers and in cutaneous leishmaniasis [6]. It is known to treat influenza and eye diseases. Simply boiling the pieces and bathing with the water relieves body pain. *Coscinium* is also used to treat bleeding piles and excessive bleeding during menstruation. For snakebite poisoning, paste of *Coscinium* and turmeric is applied. For quick healing of ulcers, *Coscinium* powder is applied after mixing with ghee. Many traditional healers of Chhattisgarh use the bark in their treatments. A combination of the bark and honey is taken internally for the treatment of jaundice. Bark is also used in the treatment of leucorrhoea and other gynecological troubles. According to them its aqueous extract is more useful but due to non-availability of fresh bark, a decoction by boiling the bark in water is used

by taking it in empty stomach daily morning. It has also been applied in a complex decoction after childbirth in Peninsular Malaysia. In Vietnam, tablets made from crude alcoholic *C. fenestratum* extracts are prescribed to cure dysentery [4,6,7,8].

MATERIALS AND METHODS

Collection of plant materials

Samples of *Coscinium fenestratum* stems were collected from the local market in Western Province, Sri Lanka and plant material was identified and authenticated by Dr. Chandima Wijesiriwardhane, Senior Research Officer, Herbal Technology Division, Colombo 07, Sri Lanka.

Hot methanolic extract

Ten grams of finely powdered *C. fenestratum* was refluxed with methanol for 3 h. The extract was filtered and the filtrate was evaporated to dryness under reduced pressure at 50 °C. The extract was weighed and dissolved in 10 mL of methanol and stored under 4 °C until use.

Cold methanol extract

Ten grams of finely powdered *C. fenestratum* was soaked in methanol for 24 h. The extract was filtered and the filtrate was evaporated to dryness under reduced pressure at 50 °C. The extract was Weighed and dissolved in 10 mL of methanol and stored under 4 °C until use.

Quantification of berberine content in *Coscinium fenestratum* methanolic extracts

Test solutions: Test solutions were prepared by dissolving each methanolic extract in 10 mL of methanol.

Standard solution: Standard solution was prepared by dissolving 20 mg of berberine in 10 mL of methanol.

Procedure: Test solutions (2 µL) and standard solution (2, 5 10, 15 µL) were spotted on a pre-coated TLC plate.

Adsorbent: Silica-gel 60 F_{254}

Solvent system: n- butanol: ethyl acetate: acetic acid: water at a ratio of 2.5: 5: 1.5: 1

Detection : Before spraying - Under UV light (at 254 nm and 366 nm).
After spraying - Dragendorff's reagent was sprayed to the TLC plate and observations were carried out without heating the plate. Dragendorff's reagent was used to detect the alkaloids present in the extracts including berberine.

Scanning: Densitometer (CS – 9301PC, Shimadzu, Japan at 254 nm (before spraying)

Calibration curve

The calibration curve for berberine was drawn with 4 data points; 2, 5, 10, 15 µL of the standard solutions was applied on the pre-coated TLC plate of uniform thickness of 0.2 mm. The plate was developed in the solvent system (mentioned above) to a distance of 80 mm. The plate was scanned densitometrically at 366 nm. The peak area under the curve was recorded and the calibration curve for berberine was plotted.

Estimation of berberine in test samples

From each test solution 2 µL were spotted on a pre-coated TLC plate of uniform thickness of 0.2 mm. The plate was developed in the solvent system (mentioned above) to a distance of 80 mm and densitogram was recorded as described above for the calibration curve. The amount of berberine present in each sample was calculated from the calibration curve.

RESULTS AND DISCUSSION

Coscinium fenestratum (Gaertn.) Colebr. (Family: Menispermaceae) is widely used in the traditional medicinal systems in Sri Lanka. In the present study, an attempt was made to quantify berberine content in the market samples of *C. fenestratum* stems. As

shown in the Table 1, the yield of the crude extracts of each extraction method was not significantly different from each other. However, the highest amount of berberine (2.00 ± 0.01% dry weight basis) was obtained from hot methanolic extract (Table. 2). Rojsanga and Gritsanapan[9] were reported that berberine content present in *C. fenestratum* grown in Thailand ranging from 1.17 ± 0.25 % to 2.88 ± 0.21% dry weight basis. Similar results were obtained in our investigation also. TLC fingerprint of hot methanolic extract is comparable to the TLC fingerprint of cold methanolic extract and the major component was berberine with the R_f value of 0.62 (Table 3).

In generally, visualization of orange color spots after spraying the Dragendorff's reagent conform the presence of alkaloids in a TLC fingerprint. Apart from the spot corresponds to the berberine, two other orange color spots were visualized in the TLC fingerprints of *C. fenestratum* after spraying the Dragendorff's reagent. These may be one or more alkaloids such as palmatine, berbamine, aromaline, oxyberberine or karachine [10]. Detection and quantification of berberine were performed by TLC densitometric method at the wavelength of 366 nm. Calibration curve for berberine was constructed by plotting peak area against various concentrations of berberine. It was found to be linear and correlation coefficient (r) was 0.986 (Fig. 1).

TLC densitometric method was found to be accurate and precise for quantification of berberine in the stem extracts of *C. fenestratum.* This method has several advantages over the other analytical procedures, such as high- performance liquid chromatography (HPLC) and spectrophotometry, such as simple pretreatment of samples, low cost, and a large number of samples that can be screened in parallel. However, some disadvantages were found such as the lower precision and sensitivity of the method compared to the HPLC [11].

In conclusion, this information is useful as guidance for setting a specification of raw materials and extracts of *C. fenestratum* for pharmaceutical preparations.

Table.1. Yields of crude extracts of *Coscinum fenestratum* by hot and cold extraction methods

Type of extraction method	%Yields of crude extracts (Dry weight basis)
Hot methanol extract	9.3±0.3[a]
Cold methanol extract	7.7±0.7[a]

The values marked with the same letters are not significantly (P >0.05) different with each other.

Table. 2. Berberine content of *Coscinum fenestratum* by hot and cold extraction methods

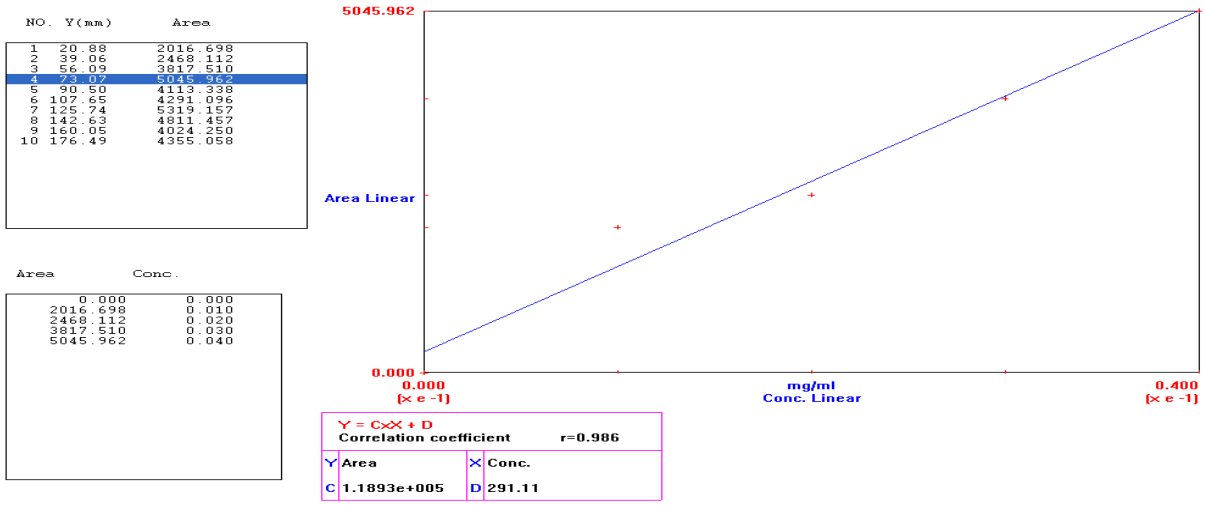
Type of extraction method	Berberine percentage in powdered sample (dry weight basis)
Hot methanol extract	2.00 ± 0.01[a]
Cold methanol extract	1.64 ± 0.01[b]

The values marked with the different letters are significantly (P < 0.05) different with each other.

Table 3. TLC fingerprint profiles of *Coscinum fenestratum* methanolic extracts after spraying Dragendorff's reagent

	R_f values and respective colors of the spots
Coscinium fenestratum	0.53(orange), **0.62(dark orange),** 0.96(orange).
Standard (berberine)	**0.62(dark orange)**

Fig. 1. The calibration curve for berberine (standard)



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