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Berberis ceylanica schneider Stems: A Rich Source of Berberine with Antimicrobial Properties

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Abstract

Background: *Berberis ceylanica Schneider* is an endemic plant to Sri Lanka belonging to the family Berberidaceae. Even though *B. ceylanica* is used in traditional medicinal systems in Sri Lanka, very few research work has been carried out for this plant. There are no records on detailed pharmacognostic evaluation on *Berberis ceylanica*.

Methods: Aim of the present study was to carry out following experiments using stems of *B. ceylanica* (a) quantification of berberine content by TLC-densitometric technique (b) antimicrobial activity by well diffusion method against *Staphylococcus aureus, Streptococcus pyogenes* and *Candida albicans* (c) investigation of antioxidant factors and phytochemical screening.

Results: Hot water extract of *B. ceylanica* stems contained 4.1 ± 0.3% of berberine and it has shown potent antimicrobial activity against three microorganisms including *S. aureus* (27.3 ± 1.3 mm), *S. pyogenes* (26.0 ± 1.0 mm) and *C. albicans* (20.3 ± 0.3 mm). Furthermore, IC_{50} of hot water extract of *B. ceylanica* against DPPH assay was 24.8 ± 0.5 µg/ml. The total phenolic and flavonoid contents were 15.3 ± 0.40 mg gallic acid equivalents/g extract and 10.8 ± 0.20 quercetin acid equivalents/g extract respectively in *B. ceylanica*. Alkaloids, flavonoids, glycosides, phenols, saponins, cumarins and sterols were present in the hot water extract.

Conclusion: Findings revealed the antimicrobial and antioxidant properties and presence of high content of berberine in hot water extract of *B. ceylanica* stems for the first time.

Keywords: *Berberis ceylanica*; Berberine; Antimicrobial activity; Antioxidant activity

Introduction

Plant family Berberidaceae comprises 14 genera and 701 species of perennial herbs and shrubs. Its members occur in most temperate regions of the world. The most important and largest genus is *Berberis*, with about 500 species. *Berberis aristata* is one of the most important plants used in Ayurveda system of medicine which belonging to the genus *Berberis*. At present, this plant is critically endangered species due to extensive collections by pharmaceutical industries due to high content of isoquinoline alkaloid berberine. In recent decades, berberine has intrigued increasing interest in its significant bioactivities, such as antioxidant, antmicrobial and anticancer effects. There are three Berberis species found in Sri Lanka. They are *Berberis ceylanica Schneider* (Figure 1), *Berberis tinctoria Leschen* and *Berberis wightiana Schneider.* Among them morphological characters of *B. ceylanica* is very closer to that of *B. aristata.*

Figure 1: *Berberis ceylanica Schneider* (i) Plant (ii) Stem (iii) Flowers

B. ceylanica distributed specially in mountain forests and forest borders up to about 2200 m height. It is a shrub 3 m or more tall. Stem minutely pubescent, angled, Spines1-2 (-3.25) cm long, furrowed, concolorous. Leaves are lamina 1.5-5 × 1-2.5 mm, ovate, elliptic or obovate, margin subserrulate or 1-3 spinose with spines 0.5-1.5 mm long. Venation somewhat reticulate, petiole 2 - 4 mm long. Pedicle 7-16 mm long, bracts 1-2 mm long. Outer sepals 3.25 × 2.5 mm, oblong – ovate, inner sepals 6.5 mm long, ovate, Petals 6 × 4.5 mm, obovate, entire, Berries 12 × 5 mm, ellipsoid to obovoid excluding very short style [1]. Even though *B. ceylanica* is very similar to *B. aristata*

morphologically, it is an unexploited plant. Therefore, aim of the present study was to carry out following experiments using stems of *B. ceylanica* (a) quantification of berberine content by TLC-densitometric technique (b) antimicrobial activity by well diffusion method against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Candida albicans* (c) investigation of antioxidant factors and phytochemical screening.

Material and Method

Collection of plant material

Samples of *B. ceylanica* stems were collected from Department of Ayurveda, Pattipola in Uva Province, Sri Lanka during the period of August-September 2017. Plant material was identified and authenticated by Senior Scientist, Botany Division, (specimen number 1445 E) and deposited at Bandaranayaka Memorial Ayurvedic Research institute, Navinna, Maharagama, Sri Lanka. Shade dried stems were cut into small pieces and ground using a domestic grinder. Powdered plant material was kept in an airtight container until used.

Preparation of hot water extract of *Berberis ceylanica* stems

Stem powder of *B. ceylanica* (50 g) was added to a round bottom containing 250 ml of distilled water and refluxed for 4 h, filtered and filtrate was concentrated using a rotor vapor and stored at 4°C until use (yield: 15% dry weigh basis).

Quantification of berberine content in hot water extract of *Berberis ceylanica* stems

Test solution: Stock solution was prepared by dissolving 300 mg of hot water extract of *B. ceylanica* in 20 mL of deionized water.

Standard solution: Standard solution was prepared by dissolving 20 mg of berberine in 10 mL (2 mg/mL) of methanol.

Calibration curve

The calibration curve for berberine was drawn with 3 data points using the berberine standard. Different amounts (2, 4, 6 µL) of the standard solution were applied on the pre-coated TLC plate of uniform thickness of 0.2 mm. The plate was developed using Butanol: Ethyl acetate: Acetic acid: Water in a ratio of (4:4:1:1v/v/v/v). The plate was scanned densitometrically (CS-9301PC, Shimadzu, Japan) at 366 nm. The peak area under the curve was recorded and the calibration curve for berberine was plotted [2].

Estimation of berberine in test samples

From the test solution 2, 4 and 6 µl were spotted on a pre-coated TLC plate (as mentioned above) to a distance of 80 mm. The plate was developed using the same solvent system used for the berberine standard. Densitogram was recorded as described above for the calibration curve. The amount of berberine present in the sample was calculated from the calibration curve.

Determination of Antimicrobial Activity

The antibacterial activity [*Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615)] and antifungal activity [*Candida albicans* (ATCC 90028)] of *B. ceylanica* was investigated by well diffusion method [3,4] using hot water extract of the stem. The suspension was prepared in sterile normal saline and turbidity adjusted to 0.5 McFarland standard. The suspension of organisms was used to inoculate blood agar plates to obtain a confluent growth. Wells (diameter: 7 mm and height: 4 mm) were cut in the agar surface using a cork borer. Each well was filled with 100 µl (400 µg/well) of hot water extract of *B. ceylanica*. All the plates were incubated 24 h at 37°C before measuring the inhibition zone sizes. Any zone of inhibition around the wells containing either plant extract or reference drugs was considered as sensitive. Cefoxitin (30 µg), penicillin (10 µg) and Ketaconazole (10 µg) were used as reference drugs. All experiments were performed in triplicates to ensure reproducibility of results.

Investigation of antioxidant factors

DPPH radical scavenging ability, total polyphenol and total flavonoid contents were evaluated for hot water extract of *B. ceylanica* stem.

DPPH (1,1-diphenyl-2-picrylhydrazyl assay) radical scavenging assay

In this assay, known concentrations of (0-100 µg/mL) the extract and L-ascorbic acid were prepared in different test tubes by adding methanol up to 1.5 mL. Three milliliters ofmethanolic solution of DPPH (2 mg/100 ml) were added to these tubes and shaken vigorously. The tubes were allowed to stand at room temperature for 5 min. and the absorbance was measured at 517 nm. Control was prepared as above by adding methanol instead of test solution. L- ascorbic acid served as the positive control. This experiment was done in triplicates. IC_{50} value was calculated based on the percentage of radical scavenging activity [5,6].

Quantification of total polyphenol and flavonoid contents

The total polyphenoilc content was estimated according to the Folin-Ciocalteu method [7] and total flavonoid content was determined using the Dowd method [8]. Gallic acid and quercetin were taken as standards for express the results of polyphenol and flavonoid contents respectively.

Screening of secondary metabolites

Secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, cumarins and sterol were screened for hot water extract of *B. ceylanica* stems according to methods described by Yadav and Agarwala [9].

Statistical analysis

All data were presented as Mean ± SEM. All the values were expressed as dry weight of the sample and they were performed in triplicate.

Results and Discussion

B. ceylanica extract was shown strong antimicrobial effect (Table 1) against all three tested microorganisms with the inhibition zones of *S. aureus*, (27.33 ± 1.33 mm) *S. pyogenes* (26.00 ± 1.00 mm) and *C. albicans* (20.33 ± 0.33 mm). However, inhibition zones of Cefoxitin, penicillin and Ketaconazole, the reference drugs were comparatively higher than that of *B. ceylanica* extract. Similar results were observed with *B. aristata against S. aureus* [10] and *C. albicans* [11].

Table 1: Antimicrobial activity of *Berberis ceylanica Schneider* stem

Microorganism	Inhibition zone (mm)	Antibiotics	Inhibition zone (mm)
Staphylococcus aureus	27.3 ± 1.3	Cefoxitin	33.3 ± 2.0
Streptococcus pyogenes	26.0 ± 1.0	Penicillin	40.7 ± 2.9
Candida albicans	20.3 ± 0.3	Ketaconazole	28.3 ± 1.3
Values are expressed as Mean ± SEM, n=6			

Detection and quantification of berberine were performed by TLC densitometric method and showed yellow fluorescence under UV 366 nm. In generally, visualization of orange color spots after spraying the Dragendorff's reagent confirms the presence of alkaloids in a TLC fingerprint. Apart from the spot correspond to berberine (Rf value of 0.63), three orange color spots were visualized in the TLC fingerprint of *B. ceylanica* after spraying the Dragendorff's reagent. These may be one or more alkaloids such as palmatine, berbamine, aromaline, oxyberberine or karachine [12]. Berberine is a well-known isoquinoline which reported to be the major alkaloid in genus Berberis [13]. Berberine containing plants such as *B. aristata* and *Coscinium fenestratum* are used in Ayurvedic and traditional medicinal systems as an antibiotic and used as a remedy for gastrointestinal disorders, diabetes, hypercholesterolemia, blood pressure, etc [14-16]. In the present study, 4.1 ± 0.3% of berberine was found in hot water extract of Sri Lankan grown *B. ceylanica*. According to Nawarathna and co-workers [17], 1.68 ± 0.07% and 0.20 ± 0.02% of berberine were found in methanol extract and methanol: dichloromethane (1:1 v/v) extract respectively. Therefore, polar solvents have more ability to extract berberine than that of non polar solvents. In a similar study [18], it was revealed that 3.55 % of berberine was present in *B. aristata* which belongs to the same plant family and genus. In both experiments, hot methanolic extract of the stem was used to investigate the percentage of berberine content. Further, berberine content was investigated using *C. fenestratum* grown in Thailand [16]. In this experiment, ethanolic extracts of *C. fenestratum* were prepared using three different extraction techniques (soxhlet, percolation, maceration) to investigate the berberine content in stems. According to results, berberine was present in the range of 2.67 ± 0.27% to 3.37 ± 0.30%. Furthermore, berberine content of Sri Lankan grown *C. fenestratum* was investigated using hot methanolic (2.00 ± 0.01%) and cold methanolic (1.64 ± 0.01%) extracts of the stem [2]. Therefore, *B. ceylanica* grown in Sri Lanka is headed among the berberine bearing plants and thereby can be used as a promising candidate for pharmaceutical industry.

DPPH is a stable free radical which gives a deep violet at 517 nm. When presence of hydrophilic antioxidants such as proton radical scavengers or hydrogen donors deep violet color of DPPH [19]. IC_{50} value of hot water extract for DPPH assay was 24.8 ± 0.5 µg/ml). The ability of *B. ceylanica* hot water extract to scavenge DPPH free radicals was more powerful than that of *B. aristata* (33.31 µg/ml) [18]. However, DPPH scavenging ability of *B. ceylanica* was lower than that of L- ascorbic acid, standard which used in this experiment (IC_{50}: 6.40 ± 0.21 µg/ml). Both polyphenolic and flavonoid contents of *B. ceylanica* were 15.3 ± 0.40 mg gallic acid equivalents/g extract and 10.8± 0.20 mg quercetin acid equivalents/g extract respectively.

Conclusion

In the present study, we have investigated the berberine content, antimicrobial and antioxidant factors in the hot water extract of *B. ceylanica* stem for the first time. Further *B. ceylanica* can be used as a substitute for berberine bearing plants as it contain high amount of berberine.

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