ASIAN JOURNAL OF PHARMACOGNOSY *Research Article*
Asian J. Pharmacogn 3(3): 29-35

eISSN-0128-1119

Physicochemical, phytochemical, and nutritional profiles of root powder of *Asparagus racemosus* (Willd) of Sri Lankan origin

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Abstract: *Asparagus racemosus* Willd is one of the well-known plants which has abundant medicinal effects. The present study aimed to standardize the roots of Sri Lankan grown *A. racemosus* for the first time by the determination of physicochemical, phytochemical and nutritional profiles using WHO guidelines and standard protocols. The analysis of physicochemical parameters showed that the roots of *A. racemosus* contained 9.7 ±0.2% total ashs, 4.4 ±0.2% water-soluble ash and 0.5 ±0.0% acid insoluble ash. The levels of tested heavy metals were below the WHO acceptable limits and harmful microorganisms were not present. Saponins, tannins, flavonoids, terpenoids, phenols and steroids were present in the root extract. Twelve prominent spots bearing R_f values of 0.03, 0.05, 0.08, 0.11, 0.17, 0.19, 0.27, 0.41, 0.49, 0.67, 0.81 and 0.93 were present in the TLC fingerprint profile of dichloromethane extract of *A. racemosus* root. Percentage of crude protein was 7.8 ± 0.2%, the total fat was less than 1%, crude fiber percentage was 28.9 ± 0.6% and carbohydrate was 37.2%. Energy value of *A. racemosus* was 180 kcal/100g. In conclusion, this is the first report on physicochemical, phytochemical and nutritional profiles of Sri Lankan grown *A. racemosus*. Further data gathered from this study can be used as a reference standard for the roots of *A. racemosus* of Sri Lankan origin.

Keywords: *Asparagus* racemosus, phytochemical, nutrition, thin layer chromatography

INTRODUCTION

Plant-based drugs are used worldwide as aphrodisiacs since time immemorial and present era the use of ethnopharmacological methods for impaired reproductive potential has dramatically increased globally. Even though one of the drawbacks in the acceptance of the herbal extracts among the scientific world is lack of chemical standardization. *Asparagus racemosus* Willd is one of the well-known medicines in Ayurveda which consider as the "Queen

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of the herbs" (Bopana and Saxena, 2007) due to its abundant medicinal effects. Its medicinal usage has been reported in Indian and the British Pharmacopoeias and also in indigenous systems of medicine in Sri Lanka. The genus *Asparagus* consisted of about 300 species around the world, out of them 22 species are found in India (Singla and Jaitak, 2014). Only 3 species are available in Sri Lanka. It has broad economic importance worldwide as a medicinal plant as well as research-oriented plant mainly due to its aphrodisiacs property. *A. racemosus* belongs to family Asparagaceae and genius *Asparagus*. According to the Ayurveda, roots of *A. racemosus* are used as medicine for a variety of disorders and discomforts. It has an adventitious root system. The roots are tuberous and finger-shaped, found in clusters, 5-15 cm in length and 2 cm in thickness. Roots are silvery-white. They are smooth when fresh and develop longitudinal wrinkles when dried (Amarasinghe *et al.,* 2015). *A. racemosus* possess several physical, chemical, and biological properties as antioxidants, immunostimulants, antihepatotoxic, antibacterial, antidiabetic, anticarcinogenic, antidiarrheal, antiulcerogenic and antioxytocic other than aphrodisiacs property (Gogte, 2000, Singh and Geetanjali, 2016). The main bioactive constituents of *A. racemosus* are the group of steroidal saponins. The steroidal saponins present in *A. racemosus* known as shatvarins. Shatvarin I to VI have been identified in previous research studies (Gogte, 2000). Moreover, immunosides (Handa *et al.,* 2003) asparagamine (Gogte, 2000), racemosol (Gogte, 2000), racemofuran (Wiboonpun *et al.,* 2004) and flavonoids (Gogte, 2000) are some other phytochemicals found in *A. racemosus*. Up to date no standardization parameters including physicochemical, phytochemical and nutritional profiles were established for the roots of *A. racemosus* grown in Sri Lanka. Chemical composition and therapeutic properties of medicinal plants can vary with the nutrient composition of the soil, climatic season, development stage of the plant, natural association with other plants. Furthermost of traditional physicians used only the Sri Lankan grown *A. racemosus* for their preparations. Thus, the need to differentiate *A. racemosus* grown in Sri Lanka from others. The present investigation was to standardize the roots of Sri Lankan grown *A. racemosus* for the first time by the determination of physico-chemical, phytochemical and nutritional profiles using WHO guidelines (2002) and standard protocols.

MATERIALS AND METHODS

Collection of Asparagus racemosus: Roots of *A. racemosus* were collected from three different locations (n = 3 per location) from Hambantota (Southern Province) Anuradhapura (North Central Province) and Colombo (Western Province) in Sri Lanka between the period of February to March 2017. The plant material of Sri Lankan origin was identified and authenticated by the Curator of Botanical Garden, Peradeniya (ARS1). Roots of *A. racemosus* were shade dried for 7 days at room temperature, crushed and powdered by using a commercial grinder and kept in an airtight container until used.

Moisture content: To calculate physicochemical parameters in dry weight basis moisture content was measured as described in WHO (2002). The dry weight of the crucible was taken after keeping in the calibrated oven at 105°C for one hour. Then plant material (2 g) was taken into the dried crucible and weight of the sample and crucible was measured. Then crucible with the sample was kept in the calibrated oven at 105°C for 4 hours until it gets a constant weight and cooled to room temperature using desiccators. The final weight of crucible containing the sample was measured.

$$\text{Moisture percentage} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

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eISSN-0128-1119

Determination of physicochemical parameters of roots of Asparagus racemosus: Physicochemicalal parameters such as the total ash content, acid insoluble and water-soluble ash contents were determined for *A. racemosus roots* according to methods described in guidelines of WHO (2002).

Total ash content: The powdered material (2 g) was accurately weighed, in a previously ignited and tarred crucible and weight of the sample and crucible was measured. The material was spread in an even layer and ignites it by gradually increasing the heat to 500-600°C using muffle Furner (Nabertherm GmbH, Bahnhofstr.20, Germany) until it gets a constant weight (completely turned to white ash), indicating the absence of carbon. The crucible was cooled to room temperature using desiccators. The final weight of crucible containing total ash was measured.

$$\% \text{ Total Ash} = \frac{\text{Total Ash Weight}}{\text{Weight of Sample}} \times 100$$

Acid insoluble ash content: Hydrochloric acid (2M, 25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 minutes using a hot plate. The watch glass was rinsed with 5 mL of hot water and then rinsed contents added to the crucible. The acid-insoluble matter was collected on a Whatman Grade 1 ashless filter paper (Sigma-Aldrich Chemie GmbH, Eschenstrasse 5, Germany) and washed with hot water until the filtrate was neutral. The filter paper containing the acid-insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight in the muffle furnace (Nabertherm GmbH, Bahnhofstr.20, Germany) for 15 minutes at a temperature not exceeding 450°C. Then the residue was allowed to cool in desiccators and weighed without delay.

$$\% \text{ Acid Insoluble Ash} = \frac{\text{Acid Insoluble Ash Weight}}{\text{Weight of Sample}} \times 100$$

Water-soluble ash content: Distilled water (25 mL) was added to the crucible containing the total ash and boiled it for 5 minutes and it was filtered by using Whatman Grade 1 ashless filter paper (Sigma-Aldrich Chemie GmbH, Eschenstrasse 5, Germany). Then the water-insoluble matter was collected on an ashless filter paper and washed with hot water. Then the filter paper containing water-insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight in the muffle furnace (Nabertherm GmbH, Bahnhofstr.20, Germany) for 15 minutes at a temperature not exceeding 450°C. Then the residue was allowed to cool in desiccators and weighed without delay. The weight of this residue was subtracted from the weight of total ash and the content of water-soluble ash calculated.

$$\% \text{ Water Soluble Ash} = \frac{\text{Total Ash Weight- Water Insoluble residue}}{\text{Weight of Sample}} \times 100$$

Determination of nutritional values of roots of Asparagus racemosus: Crude protein, total fat and crude fiber contents were determined according to standard protocols. Based on the above data carbohydrate content and energy value were calculated.

Crude protein: Crude protein was determined by the Kjeldahl digestion as described in AOAC (2012a).

Total fat: Total fat was determined according to the method described in AOAC (2012b).

Crude fibers: Crude fibers were determined using the method described in AOAC (2012c).

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Carbohydrate: Carbohydrate was determined by the calculation using crude protein, fat, crude fat, ash, and moisture content.

Energy value: Energy value was calculated by using the calorie values of protein, fat, and carbohydrates.

Determination of presence/absence of selected heavy metals of roots of Asparagus racemosus: Quantitative determination of Arsenic, Mercury, Cadmium, and Lead were carried out according to relevant methods described in AOAC methods (|2000).

Microbiological limits: *Staphylococcus aureus* (2013), *Escherichia coli* (1991), *Coliforms* (2013), *Salmonella* (2013) and yeast and molds (1992) were determined for the powder of *A. racemosus* according to the methods described in standards issued by Sri Lanka Standards Institution, Sri Lanka.

Preliminary phytochemical screening of roots of Asparagus racemosus: Presence or absence of phytochemicals such as saponins, tannins, alkaloids, flavonoids, terpenoids, phenols, and steroid glycosides were screened according to the standard protocols (Yadav and Agarwala, 2011) using dichloromethane extracts of *A. racemosus* roots.

Tests for saponins: Frothing test: Water (5 mL) was added to the 5 mL of the extract and shaken vigorously. Persistence of froth for at least 5 minutes indicates the presence of saponins.

Tests for Tannins: A Few drops of $FeCl_3$ solution (0.1%) was added to the extract (2 mL) and mixed well. Blue-black precipitate indicates the presence of tannins.

Tests for alkaloids: A Few drops of HCl (1%) and 6 drops of Mayer's reagent were added to the extract (2 mL) and mixed well. Creamish, brownish-red or orange precipitate indicates the presence of alkaloids.

Tests for flavonoids: Dilute (5 folds) ammonia solution (5 mL) was added to the 3 mL of extract followed by the addition of concentrated H_2SO_4. The yellow color formed, disappear on standing indicates the presence of flavonoids.

Tests for terpenoids: Salkowski test: Extract (5 mL) was mixed with 2 mL of chloroform and added to 3 mL of concentrated H_2SO_4 along the sides to form a layer. A reddish-brown color indicates the presence of terpenoids.

Test for monoterpenes: To 1 mL of extract, a few drops of 10% Vanillin in Ethanol were added. Then add a few drops of concentrated H_2SO_4. A red color indicates the presence of terpenoids.

Tests for phenols: (a) A Few drops of $FeCl_3$ (10%) were added to 2 mL of extract and mixed well. A green or blue color indicates the presence of water-soluble phenols. (b) To 2 mL of extract, a few drops of lead acetate were added. Yellow precipitate indicates the presence of flavonols and flavones.

Tests for steroid: Acetic Anhydride (2 mL) was added to 1 mL of extract followed by 2 mL of H_2SO_4. A color change from violet to blue or green indicates the presence of steroids.

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Development of Thin Layer Chromatography (TLC) fingerprint profile for the root of Asparagus racemosus: Development of TLC fingerprints was done using the dichloromethane extract of the root samples.

Preparation of dichloromethane extract: Sample (20 g) was taken into a round bottom flask and dichloromethane (100 mL) was added. The contents were shaken well and a reflux condenser was attached to the flask and boiled gently for 2 hours, allowed to cool and filtered rapidly using a dry filter paper. Then the filtrate was transferred to a round bottom flask and evaporated to dryness under the reduced pressure (at 40°C) using a rotor vapor and stored at 4°C until use. About 8 µL of the extract was spotted on a TLC plate and fingerprint developed in methanol: ethyl acetate: dichloromethane: cyclohexane in a ratio of 0.2:1:4:6 v/v/v.

Statistical analysis: MINITAB 19 was used for data analysis and calculation of mean values. Data were expressed as Mean ± SEM.

RESULTS AND DISCUSSION

Standardization of plant ingredients is essential to assess the quality and justify their acceptability for preparation of drugs (Baur, 1998). Furthermore, chemical and nutritional factors of a plant ingredient may vary from one country to another as those factors depend on the climatic and soil conditions and agricultural practices of the country (Mishra, 2016). Even though standardization parameters were established for *A. racemosus* grown in India (Pathak *et al.,* 2015; Kumari and Gupta, 2016; Kumar *et al.,* 2018) such an assessment has not been done for *A. racemosus* grown in Sri Lanka. The roots of *A. racemosus* are used as medicine for variety of disorders and discomforts (such as gastrointestinal, cardiovascular, neurodegenerative, etc) in Ayurveda and Traditional medicinal systems of Sri Lanka (Alok et *al,* 2013). Therefore, in the present study, standardization parameters were established for the roots of *A. racemosus*. Physicochemical parameters of *A. racemosus* roots are illustrated in Table 1.

Table 1. Physicochemical parameters of *Asparagus racemosus* grown in Sri Lanka

Physico-chemical parameters	**Percentage (%) by dry weight basis**
Total ash	9.7 ±0.2%
Water soluble ash	4.4 ±0.2%
Acid insoluble ash	0.5 ±0.0%

Results are presented as Mean ± SEM; n = 6

The total ash content of *A. racemosus* grown in Sri Lanka was significantly higher than that of *A. racemosus* grown in India. Table 2 illustrates the nutritional properties of *A. racemosus* grown in Sri Lanka. Values for protein, carbohydrates, crude fiber energy were significantly higher in *A. racemosus* grown in Sri Lanka than that of *A. racemosus* grown in India (Kumar *et al.,* 2018). Furthermore, energy value (180 kcal/100g) of *A. racemosus* grown in Sri Lanka was more than 8 folds higher than that of Indian grown *A. racemosus* (22 kcal/100g) (Kumar *et al.,* 2018). The levels of tested heavy metals (Hg, As, Cd and Pb) were below the WHO acceptable limits and microorganisms including *Staphylococcus aureus, Escherichia coli, Coliforms*, *Salmonella* and yeast, and molds were not present. It was revealed that saponins, tannins, flavonoids, terpenoids, phenols and steroids were present in the root extract. However, alkaloids were not present the extract and similar results were also obtained for *A. racemosus* grown in Indian (Pathak *et al.,* 2015; Kumar *et al.,* 2018). TLC is a simple and cheap

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eISSN-0128-1119

technique which is used to establish standard fingerprints for plant extracts. In the present study, 12 prominent spots bearing R_f values of 0.03, 0.05, 0.08, 0.11, 0.17, 0.19, 0.27, 0.41, 0.49, 0.67, 0.81 and 0.93 were present in the TLC fingerprint profile *A. racemosus* grown in Sri Lanka. Similar TLC fingerprints have been developed for many other plants to assure the purity and differentiate from substitutes or adulterants (Arawwawala and Arambewela, 2010; Arawwawala *et al.,* 2011; Hewageegana *et al.,* 2014).

Table 1. Nutritional properties of *Asparagus racemosus* grown in Sri Lanka

Nutritional Factors	**Percentage (%) by mass**
Crude Protein	7.8 ± 0.2
Total Fat	Less than 1
Carbohydrates	37.2± 0.5
Crude Fiber	28.9 ± 0.4
Energy value	180 kcal/100g

Results are presented as Mean ± SEM; n = 6

CONCLUSION

In conclusion, this is the first report on physicochemical, phytochemical and nutritional profiles of *A. racemosus* grown in Sri Lanka**.** Data of the present study can be used as the standard values of *A. racemosus* grown in Sri Lanka. Further, these results may benefit in carrying out further research of herbal medicines based on *A. racemosus* roots.

ACKNOWLEDGMENT

University Grant Commission, Ward Place, Colombo 7, Sri Lanka, is acknowledged for financial assistance.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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