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**PHYLLANTHUS NIRURI LINN GROWN IN SRI LANKA:
EVALUATION ON PHYTO AND PHYSICO-CHEMICAL
PROPERTIES OF THE WHOLE PLANT**

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ABSTRACT

Phyllanthus niruri Linn is a small, erect annual herb belonging to the family Euphobiaceae. It is widely distributed in Asia and used for the treatment of jaundice, asthma, hepatitis, diabetes, fever causing by malaria. Though it is commonly used in Ayurveda and Traditional Systems of Medicine in Sri Lanka, very few scientific experiments were carried out using *P. niruri*. Therefore, an attempt was made to evaluate phyto and physico-chemical properties of *P. niruri* grown in Sri Lanka. According to the results, $7.7 \pm 0.2\%$ of total ash, $3.4 \pm 0.1\%$ of water soluble ash and $0.9 \pm 0.0\%$ of acid insoluble ash were present in the whole plant of *P. niruri*. Phytochemical screening reveals the presence of tannins, flavonoids, steroid glycosides, coumarins, saponins and cardiac glycosides in both hot water and hot methanolic extracts of *P. niruri*. TLC fingerprint profile of the methanolic extract

of *P. niruri* consists of 8 and 9 prominent spots at 254 nm and 366 nm respectively. Heavy metals such as Cd, Hg and As were not detected in the *P. niruri* grown in Sri Lanka.

However, 0.5 mg/kg of Pb was detected in the plant. In conclusion, physico and phytochemical properties of *P. niruri* grown in Sri Lanka was investigated for the first time and these results can be used as a reference standard for quality control of *P. niruri* grown in Sri Lanka.

KEY WORDS: *Phyllanthus niruri* Linn, phytochemical and physico-chemical parameters, fingerprint profiles, heavy metals.

INTRODUCTION

Menstruation is an expression of physiological events occurring in the female reproductive system. Scientifically menstruation is the monthly out flow of blood that starts at teenage and continues till menopause. In healthy woman menstruation occur at the interval of 28 days and duration varies from 3-7 days and According to the modern science it is to vary from 50-60 ml.^[1] If increase the frequency or volume of the flow, it consider as an abnormality. According to Ayurveda when menstrual blood is excessive in quantity or and duration it is called as *Raktapradara*.^[2] *Raktapradara* is a broadly classified disease; include various diseases condition of menstrual cycle can correlate menorrhagia in modern medicine. Dysfunctional uterine bleeding (DUB) is considered as the most common cause of menorrhagia. DUB has been defined as abnormal uterine bleeding not caused by pelvic pathology, medications, systemic disease or pregnancy.^[3] According to the WHO statistics this condition is a common problem, affecting one in every five women and it was estimated that at least 60% of woman who presence with heavy menstrual bleeding are more prone to do hysterectomy which is associated with significant complications in a majority of cases.^[4]

Plant based drug have been used worldwide in traditional medicine as well as Ayurveda medicine for treatment of various disease. According to World Health Organization (WHO), medicinal plants would be the best source to obtain verity of drugs. About 80% of individuals from developed countries use traditional medicines. About 13,000 plant species have been used as drugs throughout the world.^[5] In this respect, *Phyllanthus niruri* Linn is usually used as decoction (*Kashaya*) and powder (*Churna*) in traditional as well as Ayurveda medicine of system for health maintenance. Specially prescribe for *Rakthapradara* in Ayurveda text book of *Yogarathnakara*.^[6]

P. niruri is a small, erect annual herb belonging to the family Eupobiaceae. It grows 30 - 40 cm high and bears ascending herbaceous branches. The bark is smooth and light green Stem

is angular with numerous distiches, elliptic-oblong leaves and Flowers are yellow and very numerous. Fruits very small, tiny, smooth capsules containing seeds and seeds 3-gonous, the flowering time is July to August.^[7] It is highly distributed in most tropical and sub-tropical countries such as India, China and it is one of the most important medicinal plants used in different regions in the world for the treatment of various diseases such as jaundice, asthma, hepatitis, diabetes, fever causing by malaria.^[8] In the present study, an attempt was made to evaluate phyto and physico-chemical properties of *P. niruri* grown in Sri Lanka.

MATERIALS AND METHODS

Plant material

Whole plants of *P. niruri* were collected from home gardens in Western Province of Sri Lanka between the periods of April to July 2015. The plant material was identified and authenticated by Senior Scientist, Bandaranayaka Memorial Ayurveda Research Institute, Navinna, Maharagama, Sri Lanka. A voucher specimen (specimen number: PN 1501) was deposited at Bandaranayaka Memorial Ayurveda Research Institute, Navinna, Maharagama, Sri Lanka. Whole plants of *P. niruri* were shade dried, crushed and powdered by using a domestic grinder and kept in an air tight container until used.

Hot water extract

Sample (5 g) was taken into a round bottom flask and distilled water (100 mL) was added. The contents were shaken well and a reflux condenser was attached to the flask and boil gently for 2 h, allowed to cool, and filtered rapidly using a dry filter paper (Qualitative filter paper, 90 mm Diameter Whatman ®). Then the filtrate was transferred to a round bottom flask and evaporated to dryness under reduced pressure (at 70 °C) using a rotor vapor and stored at 4 °C until use.

Hot methanol extract

Sample (5 g) was taken into around bottom flask and methanol (100 mL) was added. The contents were shaken well and a reflux condenser was attached to the flask and boiled gently for 2 h, allowed to cool and filtered rapidly using a dry filter paper (Qualitative filter paper, 90mm Diameter Whatman ®). 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.

Investigation of physico-chemical parameters of *Phyllanthes niruri*

Physico-chemical parameters such as total ash, water soluble ash and acid insoluble ash content of *P.niruri* were determined according to the WHO guide lines.^[9]

Total ash content

The powdered material (2 g) was accurately weighed, in a previously ignited and tared crucible. The material was spread in an even layer and ignites it by gradually increasing the heat to 500-600 °C using muffle furners until it turned into white ash, indicating the absence of carbon. The crucible was cooled in a desiccators and weighed. The content of total ash in the dried material was calculated as:

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

Acid insoluble ash content

HCl (2M, 25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min using a hot plate. The watch glass was rinsed with 5 mL of hot water and the rinsed contents added to the crucible. The acid insoluble matter was collected on an ash less filter paper 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.

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

Water soluble ash content

Water (25 mL) was added to the crucible containing the total ash and boiled for 5 min. The water insoluble matter was collected on an ash less filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The weight of this residue was subtracted from the weight of total ash and the content of water soluble ash calculated.

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

Phytochemical screening studies of *Phyllanthus niruri*

Presence or absence of phytochemicals such as tannins, flavonoids, steroid glycosides, coumarins and saponins were screened according to the standard protocols.^[10] using hot water and hot methanolic extracts of the plant.

Determination of the presence/absence of tannins

Sample was diluted with water and added to diluted FeCl_3 solution. Blackish blue or green blackish color in the presence of ferric chloride was taken as an indication for tannins.

Determination of the presence/absence of flavonoids

Sample was dissolved in methanol (50 %, 1 - 2 mL) by heating. Then metal magnesium and 5 - 6 drops of con. HCl were added. Appearance of a red color was taken as confirmation of flavonoids.

Determination of the presence/absence of steroid glycosides

Sample was dissolved in equal volumes of acetic anhydride and CHCl_3 . The mixture was transferred to a dry test tube and con. H_2SO_4 acid was introduced to the bottom of the tube. Formation of a reddish brown or violet – brown ring at the interface of the two liquids was taken as an indication for steroids.

Determination of the presence/absence of coumarins

Coumarins form a yellow color with 1% KOH in absolute ethanol. 1 mL of portions of 1% solutions of the extract in test tube was treated with 3-4 drops of 1% KOH in absolute ethanol.

Determination of the presence/absence of saponins

Sample was mixed with 5 mL of distilled water in a test tube and it was shaken vigorously. Formation of a stable foam was taken as an indication for the presence of saponins.

Determination of the cardiac glycosides

Sample was mixed with glacial acid and FeCl_3 and con. H_2SO_4 acid was added to the mixture along the side of the tube. Appearance of green blue color indicates the presence of cardiac glycosides.

Development of Thin Layer Chromatography (TLC) fingerprints in *Phyllanthus niruri*

Methanol extract was redissolved in 20 mL methanol and 4 μL was spotted on TLC plate.

Absorbent: Silica gel-GF₂₅₄

Solvent system: methanol: ethyl acetate: dichloromethane: cyclohexane (0.5:1:4:6 v/v/v).

DETECTION

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

Heavy metal analysis

Quantitative determination of Arsenic.^[11] Mercury.^[12] Cadmium.^[13] and Lead.^[11] were carried out according to relevant methods described in AOAC methods.

RESULTS AND DISCUSSION

Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal, whole plant of *P. niruri* was subjected to systematic physicochemical and phytochemical evaluation. Results of the physicochemical parameters of *P. niruri* are shown in Table 1. Total ash is particularly important in the evaluation of purity and quality of a plant. The ash value was determined by 3 different methods, which measured total ash, acid insoluble ash, and water soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition.^[14] The total ash usually consists of carbonates, phosphates, silicates, and silica, which include both physiological ash and non physiological ash. The physiological ash comes from the mineral components of the plant itself. However, the plant may contain foreign matter adhered to it by contact with the soil and sand. This foreign matter is called non-physiological ash. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the leafy vegetable for marketing.^[14] Acid insoluble ash indicates contamination with silica, for example, earth and sand. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the plant.^[15] In the present study, very low amount of acid insoluble ash content indicates the purity of *P. niruri*.

Table 1. Physico-chemical parameters of *Phyllanthus niruri* whole plant.

| Physico-chemical parameters | Percentage (%) in dry weight basis |
|-----------------------------|------------------------------------|
| Total ash | 7.7±0.2 |
| Water soluble ash | 3.4±0.1 |
| Acid insoluble ash | 0.9±0.0 |

Values are presented as Mean ± SEM, n=6.

A similar research was carried out by Khatoon and co-workers^[16] using the whole plant of *P. niruri* grown in India. The values obtained for total ash content (6.23 ± 0.41) and acid insoluble ash content (0.23 ± 0.08) were almost same as the results of the present study. However, the water soluble ash content was significantly higher in *P. niruri* grown in Sri Lanka compared to that of *P. niruri* grown in India (0.32 ± 0.08).

Phytochemical screening reveals the presence of tannins, flavonoids, steroid glycosides, coumarins, saponins and cardiac glycosides in both hot water and hot methanolic extracts of *P. niruri*. TLC fingerprint profile of the methanolic extract of *P. niruri* consists of 8 and 9 prominent spots at 254 nm and 366 nm respectively. Moreover, two densitograms were obtained (Fig. 1) for the TLC fingerprint profiles of *P. niruri* methanolic extract. In the present investigation, Cd, Hg and As (minimum detection limits of Cd, Hg and As: 0.1, 0.05 and 0.5 mg/kg respectively) were not detected in the *P. niruri* grown in Sri Lanka. However, 0.5 mg/kg of Pb was detected in the plant.

In conclusion, physico and phytochemical properties of *P. niruri* grown in Sri Lanka was investigated for the first time and these results can be used as a reference standard for quality control of *P. niruri* grown in Sri Lanka.

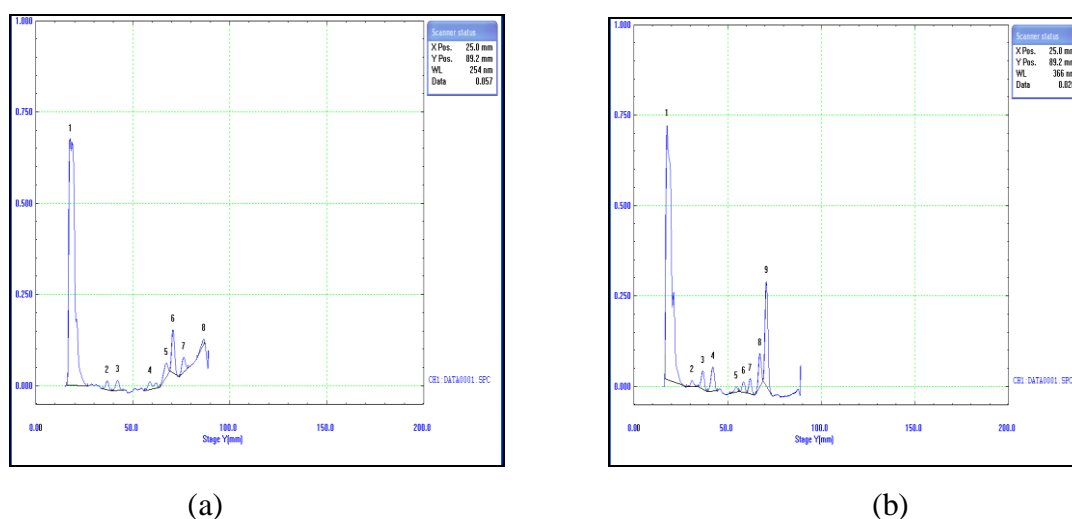


Fig 1. Densitogram of *Phyllanthus niruri* methanolic extract at (a) 254 nm and (b) 366 nm.

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