INVESTIGATION OF NUTRITIONAL PROPERTIES OF STANDARDIZED
WATTAKAKA VOLUBILIS LINN. F. STAPF: A GREEN LEAFY VEGETABLE

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ABSTRACT
Wattakaka volubilis Linn. F Stapf (Family: Asclapadaceae) is a leafy vegetable and a medicinal plant in Asian countries. In the present study, an attempt was made to (a) standardize and (b) investigate nutritional properties of W. volubilis grown in Sri Lanka. W. volubilis leaves were standardized by (a) determination of physicochemical parameters, (b) screening for phytochemicals and (c) development of TLC fingerprint. Nutritional properties were investigated in terms of protein, crude fat, carbohydrates, dietary fiber, magnesium, iron and calcium percentage. Antioxidant potential was investigated by (a) DPPH assay and (b) estimation of total polyphenolic and flavonoid contents. Results revealed that W. volubilis leaves consist of 16.5% of total ash, 0.8% of acid insoluble ash, 6.1% of water soluble ash, 32.6% of protein, 3.3% of crude fat, 17.8% of carbohydrates, 24.8% of dietary fiber, 0.5% of magnesium, 0.4% of iron and 1.0% of calcium. Antioxidant potential of W. volubilis leaves (IC₅₀: 7.06 ± 0.40 µg/mL) was comparable to that of L-ascorbic acid (IC₅₀: 6.40 ±0.21 µg/mL) and found high amounts of falvonoids and phenols. In conclusion, nutritional properties of W. volubilis leaves was established for the first time and observed physicochemical and phytochemical parameters may be used as tools to standardize W. volubilis grown in Sri Lanka.

KEYWORDS: Wattakaka volubilis, physico-chemical and phytochemical parameters, nutritional values.

INTRODUCTION
Wattakaka volubilis Linn. F Stapf; (Family: Asclapiadaceae) is a commonly consuming, freely available leafy vegetable and a valuable medicinal plant (Fig.1) which is used in Ayurveda and traditional medicine in Asian countries. W. volubilis is named as Madhumalati in Sanskrit, Kirianguna or as Behetanguna in Sinhalese and Kurinja or Kamal in Tamil. Morphological features are: large twining shrubs, with long glabrous branches. Older branches ash colour and very long, young branches green, slender and smooth. Leaves 6.3 – 15 by 4.5 – 11.5 cm, broadly ovate or acuminate, glabrous or more or less softly. Flowers numerous, green or yellowish green [1].
People used to prepare dishes from tender leaves of *W. volubilis* mixing with grated coconut and consume it with or without cooking as a traditional food in Asian countries. These palatable dishes are specially prepared for lactating mothers due to the trust on its galactagogue property. *W. volubilis* is often used in Sri Lankan traditional systems of medicine to treat burning sensation of the body, neurological diseases and respiratory diseases [2]. *W. volubilis* leaves are credited with many medicinal properties such as laxative, antipyretic, phlegm expectorant, emetic and use for hemorrhoids [3]. Normally physicians use to prepare fresh juice from the tender leaves [4] and powder form medications and decoctions from dry roots of *W. volubilis*. In Sri Lanka, a traditional paste named “Buddharaja kalka” is given as a remedy for asthma and chronic cough along with fresh juice mixture containing *W. volubilis, Adatoda vesica, Solanum melongena, Zingiber officinale, Vitex nigundo* and *Allium sativum* [2]. This is a very popular therapy among the traditional and Ayurvedic physicians in Sri Lanka. Similarly, “Maha koladaunda”, a pill recommend for fever is given along with a decoction containing *W. volubilis* as a one of major ingredients [2]. In addition, Wel dehi churna is a medicated powder used as a remedy for various skin diseases and which containing dry roots of *W. volubilis* as an ingredient [5].

Scientific experiments revealed the wound healing [6], anti-inflammatory, analgesic [7], antiarthritic [8], antioxidant [9], antiulcer [10], antidiabetic and antihyperlipidaemic [9] activities of *W. volubilis* grown in Sri Lanka, India, Taiwan, Cambodia, and Nepal [11]. Though *W. volubilis* is used in Ayurveda and traditional medicine, no scientific experiments were carried out to investigate the nutritional properties of *W. volubilis* grown in Sri Lanka. Therefore, in the present study, an attempt was taken to (a) standardize and (b) investigate nutritional properties of *W. volubilis* grown in Sri Lanka.

**MATERIALS AND METHODS**

**Plant material**

*W. volubilis* were collected from home gardens in Western Province of Sri Lanka between the periods of May to July 2013. The plant material was identified and authenticated by Senior Scientist, Bandaranayaka Memorial Ayurveda Research Institute, Navinna, Maharagama, Sri Lanka. A voucher specimen (specimen number: W 2561) was deposited at Bandaranayaka Memorial Ayurveda Research Institute, Navinna, Maharagama, Sri Lanka.

**Determination of physico-chemical parameters of Wattakaka volubilis leaves**

Hot and cold water extractable matter, hot and cold ethanolic extractable matter, ash contents (total ash, acid insoluble ash and water soluble ash) were determined according to the methods described in WHO [12].
Screening of preliminary phytochemical compounds in *Wattakaka volubilis* leaves
Presence or absence of phytochemical compounds such as phenolic compounds, saponins, glycosides and flavanoids were screened in water extract and ethanolic extract (both hot and cold) [13].

Development of Thin Layer Chromatography (TLC) fingerprints in *Wattakaka volubilis* leaves
Sample (4 g) was added to a beaker containing methanol (50 ml) and stirred well for 30 min. The extract was filtered and evaporated the filtrate using a rotovapour (Buchi, B-480). After that, the residue was redissolved in 10 mL of methanol and spotted (2 and 4 µL) on TLC plate.

Absorbtent: Silica gel-GF$_{254}$
Solvent system: ethyl acetate: dichloromethane: cyclohexane (0.5:3.5:1 v/v/v).

Detection
Direct visualization: Anisaldehyde was sprayed to the TLC plate and heated at 105 °C for 5 min.
Scanning: Densitometer (CS–9301PC, Shimadzu, Japan at 254 nm)

Determination of protein, crude fat, carbohydrates, dietary fiber, magnesium, iron and calcium contents in *Wattakaka volubilis* leaves
Amounts of protein, crude fat, carbohydrates, dietary fiber, calcium, magnesium and iron were quantified according to AOAC methods [14].

Determination of Antioxidant activity of leaves of *Wattakaka volubilis* 2, 2–diphenyl - 1 – picrylhydrazyl (DPPH) scavenging assay in *Wattakaka volubilis* leaves
In this experiment, known concentrations of (0 - 100 µg/mL) methanolic extract of *W. volubilis*, butylated hydroxyl toluene (BHT) and L – ascorbic acid were prepared in different test tubes by adding MeOH up to 1.5 mL (both BHT and L – ascorbic acid served as positive controls). Three milliliters of methanolic solution of DPPH (2 mg/100 mL in MeOH) 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 $\lambda$ 517 nm. Control was prepared as above by adding MeOH instead of test solution. This experiment was done in triplicates. The percentage of radical scavenging activity and IC$_{50}$ values were calculated [15,16].
Quantitative determination of total polyphenolic content
The total polyphenolic content was estimated as described by Spanos and Worlstad [17] and expressed as Gallic acid equivalents (mg gallic acid/g extract).

Quantitative determination of total flavonoid content
The total flavonoid content was estimated as described by Meda and co-workers [18] and expressed as quercetin equivalents (mg quercetin/g extract).

Statistical analysis
Data were analyzed by using Mann Whitney test and findings of P < 0.05 was considered to indicate statistical significance. All data were presented as Mean ± SEM. All the values were express as dry weight of the sample and they were performed in triplicates.

RESULTS AND DISCUSSION
The quantitative determination of some pharmacognostic parameters is useful for setting standards for medicinal plants. Results of the physicochemical parameters of W. volubilis 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 [19]. 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 [8]. 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 [8]. In the present study, very low amount of acid insoluble ash content and moderate level of total ash content indicates the purity of W. volubilis leaves. Extractive values are representative of the presence of the polar or non polar extractable compounds in a plant material. In the present study, amount of water extractable matter (cold and hot) was significantly higher than that of ethanol extractable matter (cold and hot) which indicates the presence of polar compounds in the plant. Five prominent spots bearing Rf values of 0.17, 0.37, 0.50, 0.57, 0.69 were observed in the TLC profile of W. volubilis. Phytochemical screening of water and ethanol extracts (both cold and hot) revealed the presence of phenolic compounds, tannins, saponins, coumarin, and glycosides. In addition, W. volubilis hot water extract contained
alkaloids and flavonoids. Moreover, similar studies have been carried out to establish the physicochemical and phytochemical parameters of some other medicinal plants grown in Sri Lanka [20, 21, 22].

Fig: 1. *Wattakaka volubilis* Linn. F Stapf : Creeper

Values are expressed as mean ± SEM., n=6

Fig: 2. Nutritional properties of *Wattakaka volubilis* leaves

Antioxidant activity of *W. volubilis* was determined by DPPH scavenging assay. DPPH• is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH• shows a strong absorption band at λ 517 nm. DPPH• radicals react with suitable reducing agents and then electrons become paired off and the solution loses color stochiometrically depending on the number of electrons taken up [23]. Therefore, change in absorbance produced by this reaction was used to test the ability of *W. volubilis* to act as a free radical scavenger. *W. volubilis* demonstrated a significant
(P < 0.05) and dose-dependent (r² = 0.98) DPPH radical scavenging activity indicated by the decolorization of DPPH (from a deep purple to a yellow color). Both synthetic antioxidants, L-ascorbic acid and BHT also demonstrated significant (P < 0.05) and dose-dependent (L-ascorbic acid: r² = 0.90; BHT: r² = 0.96) DPPH radical scavenging activities. Interestingly, the radical scavenging activity of W. volubilis (IC₅₀: 7.06 ± 0.40 μg/mL) was comparable to the L-ascorbic acid (IC₅₀: 6.40 ± 0.21 μg/mL) and significantly higher than BHT (IC₅₀: 12.00 ± 0.29 μg/mL).

Table 1. Physicochemical parameters of Wattakaka volubilis leaves

<table>
<thead>
<tr>
<th>Physico-chemical parameters</th>
<th>Percentage in dry weight basis</th>
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<tr>
<td>Hot water extractable matter</td>
<td>31.7 ± 0.3</td>
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<tr>
<td>Hot ethanol extractable matter</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Cold water extractable matter</td>
<td>7.0 ± 0.3</td>
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<tr>
<td>Cold ethanol extractable matter</td>
<td>1.4 ± 0.1</td>
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<tr>
<td>Total ash content</td>
<td>16.5 ± 0.1</td>
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<tr>
<td>Acid-insoluble ash content</td>
<td>0.8 ± 0.0</td>
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<tr>
<td>Water-soluble ash Content</td>
<td>6.1 ± 0.1</td>
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</table>

Values are expressed as mean ± SEM, n=6

Antioxidants with free radical scavenging activities of plants may have great relevance in the prevention diseases and therapeutic properties. Phytoconstituents like flavonoids and phenolic compounds, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity [24] and [25]. As evident by present study, W. volubilis was rich with flavonoids and phenolic compounds. The mean total polyphenolic content and mean total flavonoid contents of W. volubilis were 75.8 ± 0.40 mg gallic acid equivalents/g extract and 45.3 ± 0.20 mg quercetin acid equivalents/g extract respectively. Therefore, polyphenolic compounds and flavonoids contained in W. volubilis may play a major role in the observed antioxidant activity of the plant. Recent investigations suggest that the plant origin antioxidants with free-radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis, and aging process [26]. Therefore, consumption of W. volubilis will be beneficial to mankind.
The percentage of protein, fat, carbohydrate, dietary fiber, and minerals such as magnesium, iron, and calcium content in *W. volubilis* were illustrated in Fig.2. *W. volubilis* is rich in protein, dietary fiber and carbohydrate. These nutrients are essential for the physiological functions of human body. *Sesbania grandiflora* and *Centella asiatica* are frequently consuming leafy vegetables and use as medicinal plants in Sri Lanka. When comparing the *Centella asiatica* and *Sesbania grandiflora* [27] and [28] with *W. volubilis*, *W. volubilis* contain high percentage of dietary fiber. Fiber is an important nutrient of a meal to maintain the body weight and help to weight loss in over weight personals because it keeps feeling full and helps control the hunger. Fiber can also lower cholesterol and blood pressure, and help to temper blood-sugar swings by slowing the absorption of carbohydrates into the bloodstream after meals. This lowers the risk of cardiovascular disease and type 2 diabetes. Further, *W. volubilis* contained significantly high contents of protein (32.6 %) and carbohydrate (17.8%) than that of *Centella asiatica* (9.9%) and *Sesbania grandiflora* (0.4 %) respectively [27, 28]. In conclusion, present study reveals the nutritional properties of *W. volubilis* grown in Sri Lanka for the first time and observed physicochemical parameters may be used as tools to standardize *W. volubilis*.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest regarding the publication of this paper.

**REFERENCES**


