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Nutritional Evaluation and Phytochemical Screening of *Commelina diffusa*: An Underutilized Wild Edible Plant of Sri Lanka

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Abstract Sri Lanka is blessed with hundreds of wild edible species which are capable of improving healthy dietary patterns of human being. Though, *Commelina diffusa* (Girapala) is an important wild edible species, its nutritional properties are still unexplored. This study was carried out to determine the proximate composition, microelements and to assess phytochemicals *in C. diffusa.* Phytochemicals were tested in both qualitative and quantitative manner and in qualitative assessment it has been used different organic solvents including hexane, ethanol, chloroform and water. The proximate nutritional composition including, ash, moisture, crude protein, crude fat and crude fiber were determined using AOAC standards. Microelements were analyzed using Atomic Absorption Spectrophotometric method. Ca was the most abundant mineral element followed by Fe > Cu > Zn which have shown higher values than most of the prominent leafy vegetables in Sri Lanka. Moisture and ash contents were 89.78% and 7.03% of fresh weight, respectively. The corresponding values for crude fat, crude fiber and crude protein were 0.01%, 3.52% and 3.10% respectively in dry weight basis. The result of quantitative phytochemical analysis revealed the presence of bioactive compounds namely, flavonoids (3.37%), alkaloids (5.55%), saponins (2.82%), phenols (7.27%), steroids (0.28%) and tannins (2.94%). *In concluding,* the results indicate that the tender shoots of *C. diffusa* contain an appreciable amount of bioactive compounds and microelements.

Keywords: *Commelina diffusa, micronutrients, phytochemicals, proximate composition*

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1. Introduction

Diversifying of agricultural products has become necessity to overcome the problems associated with food insecurity and malnutrition with increasing the world population [1]. This expansion and diversification of food habits have been recently attracted by broader range of plant species including wild edibles. These species have been identified as important contributors to human diet since they contain important microelements, antioxidants and many other bioactive compounds [2,3,4]. Nowadays, due to the renewed interest in wild edible plants and the recent findings on the beneficial role of their phytochemical constituents, these species have been defined as "new functional foods".

Commelina diffusa is a wild edible plant which belongs to family *Commelinaceae* and commonly known as '*Girapala*' in Sri Lanka, Though the plant is identified as a common weed in Sri Lanka and other South Asian countries [5], it is widely used in medicinal purposes such as, urinary infections, sore throats, acute tonsillitis swellings, malaria and kidney ailments [6,7]. Further, leaves and tender stems of the plant are rich in nutritionally important bioactive compounds and micronutrients [8,9]. On the other hand, *C. diffusa* is highly adaptable to adverse environmental conditions and requires low input and management practices; hence can be used as a low cost food. However, *C. diffusa* has been categorized as a wild, neglected or underutilized species by the modern society, despite its use as a medicine.

Underutilized wild edible plants (UWEP) as noted by Salvi and Katewa [10] are rich sources of nutritional and bioactive compounds which facilitate proper functioning of human body. Based on several studies of UWEP, it has been reported that UWEP play a crucial role in developing countries by offering dietary nutrients for rural dwellers. Therefore, studying UWEP species, is the practical solution to facilitate a diverse and healthy diet to battle against micronutrient deficiencies among rural and vulnerable social groups. Worldwide local communities have used these plant species for generations, but recently their traditional uses are being forgotten due to the loss of local knowledge, awareness and because of the modern life style of man. Available limited resources clearly

indicate that proper authentication, scientific analysis for functional properties and traditional knowledge of UWEP have to be recorded since these species are a promising solution for global food security [11,12,13]. These specialties will lead researchers to tap in to the untouched potentials of UWEP to utilize them in future as "future food".

Therefore, present study was conducted to ascertain the nutritive potential of the *C. diffusa* from Southern province, Sri Lanka, which only had little information in literature. This is an attempt which has been made to stimulate studies on nutritional values of UWEP, since these species have significant potential to fight against food insecurity and malnutrition in future.

2. Materials and Methods

2.1. Sample Collection

The tender shoots of *C. diffusa* used for this study were harvested in *Baddegama*, Sothern province, Sir Lanka (6.1688° N, 80.1794° E). The taxonomical identification of the plant was done by National Herbarium, Peradeniya, Sri Lanka. Leaves (1.5 kg) were harvested manually, transported to the laboratory and stored at 15 °C until processed. Tender shoots were weighed, removed debris and unwanted parts, washed, and freeze-dried using a freeze dryer (BK-FD12P, China). Freeze-dried samples were ground using electric grinder to a fine powder [14], sieved (sieve size:10), packed in to airtight glass bottles and stored in the refrigerator until further analysis.

Figure 1. Tender shoots of *Commelina diffusa* (Girapala)

2.2. Proximate Composition Analysis

Those prepared laboratory samples were used for proximate composition analysis (moisture, ash, crude fat, crude protein and crude fiber), in triplicate using Association of Official Analytical Chemist (AOAC) approved methods [15].

2.3. Quantification of the Microelements

The microelement analysis by an Atomic Absorption Spectrophotometer (AAS) was conducted according to Machado *et al*., [16] with some minor modifications. For determination of total concentration of the microelement in the samples, microwave-assisted acid digestion was carried out for 1 g of the freeze dried samples after mixing with 3 mL of concentrated HNO_3 and 2 mL of H_2O_2 in a digestion vessel of the microwave digestion oven (MARS 6240/50, USA). The program was operated under; 400 to 1800 W power, a 15-minute ramp time until 180°C. Obtained solution after the digestion, made up to 100 mL with de-ionized water and filtered with 0.22 µm nylon membrane filters. Filtered samples were used for analytical determinations of microelement, namely, calcium (Ca), copper (Cu). iron (Fe) and zinc (Zn) using AAS (iCE 3400 AAS, USA).

2.4. Qualitative Phytochemical Analysis

2.4.1. Preparation of Extracts

Table 1. Qualitative Phytochemical Analysis Procedure

Phytochemical	Testing method
Alkaloids	Concentrated hydrochloric acid (2 mL) was added to 2 mL of extract and few drops of Mayer's reagent were added. Green color or white precipitate indicates the presence of alkaloids
Tannins	2 mL of 5% ferric chloride was added to 1 mL of extract. Formation of dark blue or greenish black indicates the presence of tannins.
Saponins	Distilled water (3 mL) were added to 2 mL of extract and shaken in a graduated cylinder for 15 min lengthwise. It resulted in the formation of 1 cm layer of foam that indicated the presence of saponins.
Flavonoids	To 2 mL of extract, 1 mL of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.
Quinones	To 1 mL of extract, 1 mL of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.
Phenols	2 mL of distilled water followed by few drops of 10% ferric chloride was added to 1mL of the extract. Formation of blue or green color indicates presence of phenols.
Terpenoids	0.5 mL of the extract was treated with 2 mL of chloroform and conc. sulphuric acid. Formation of red brown colour at the interface indicates the presence of terpenoids.
Coumarins	1 mL of 10% sodium hydroxide was added to 1mL of the extract. Formation of yellow colour indicates the presence of coumarins.
Anthraquinones	To 1 mL of extract few drops of 10% ammonia solution was added, appearance of pink color precipitate indicates the presence of anthraquinones.
Steroids	To 1 mL of extract equal volume of chloroform is added and a few drops of concentrated sulphuric acid added appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of steroids.

In vitro *phytochemical screening was done for extracts including ethanol (99.5%,* AR grade, Sigma Solvents Pharmaceuticals Ltd., Germany), *hexane (99.5%,* AR grade, Sigma Solvents and Pharmaceuticals Ltd., Germany), *chloroform* (99.0–99.4%, AR grade; Sigma Solvents and Pharmaceuticals Ltd., Germany) *and water. Each freeze dried samples (1 g/20 mL) were vigorously shaken in a shaker (SSL1, China) for 4 hrs. and*

*centrifuged (*Z 216 M, Germany*) at 3000 rpm for 10 min. The supernatant was collected to a centrifuge tubes and filtered through polyether sulfone (0.45µm) filter membrane. Finally, the extracts were subjected to various qualitative testes for the identification of phytochemicals present in the extracted samples.*

2.5. Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was conducted according to Santhi and Sengottuvel [17] with slight modifications.

2.5.1. Alkaloid Determination

5g of the freeze dried sample were weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered to stand for 4 hours. This was filtered and the extract was concentrated using a water-bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the preparation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed.

2.5.2. Tannin Determination

Freeze dried sample (500 mg) was mixed with 50 ml of distilled water and mixture was shaken for one hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a tube and mixed with 3 ml of 0.1M FeCl3 in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120nm wavelengths, within 10 minutes. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured.

2.5.3. Flavonoid Determination

100g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed.

2.5.4. Saponin Determination

Freeze dried sample (1 g) was mixed with 20% ethanol (10 mL). The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n -butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage.

2.5.5. Total Phenolic Content Determination

TPC was determined according to the Folin – Ciocalteu procedure by using gallic acid as the standard [16]. Methanolic extract of the freeze dried sample (1 g/10 mL) were prepared stirring the sample over-night with methanol in a universal tube. The reaction mixture (1 mL of methanol extract and 5 mL of 10% of Folin-Ciocalteu reagent) was allowed to stand for 5 min at room temperature (28°C) before adding exactly 4 mL of a 7.5% sodium carbonate aqueous solution. Samples were left for 60 min at room temperature before measurement at 765 nm using UV-vis spectrophotometer (G10S, USA). TPC values were calculated using gallic acid standard curve within the range of 0.02-0.1 mg/mL.

2.6. Statistical Analysis

All experiments were carried out in triplicates and the data expressed as mean ± SD using the Microsoft Excel 2010 spreadsheet.

3. Results and Discussion

Data on proximate composition and micrelement analysis of the extracts of *C. diffusa* are shown in Table 2 and Table 3.

Table 2. Proximate Composition of *C. diffusa*

Parameters	Percentage (%)
Moisture	89.78±0.10
Crude fat	0.01±0.12
Crude fiber	3.52±0.13
Crude protein	3.10±1.15
Ash	7.03±0.01

The percentage of moisture and ash contents are given per 100 g of fresh weight. Detected values for moisture (89.78%) was higher than the reported values of *C. diffusa* variety which was found in Nigeria (19.88%) [17] and also these values found to be very similar to moisture contents of commercial leafy vegetables in Sri Lanka [18].

Ash content (7.03%) of this species is found to be at a significant level. Moreover, ash content is also considered as a good indicator to predict the mineral composition of a plant, which means *C. diffusa* might possess a significant mineral content.

The percentage of fiber and protein in *C. diffusa* were higher than *Alternanthus asessilis* (*Mukunewenna*), *Centella asiatica* (*Gotukola*) and *Trianthema portulacatrum* (*Sarana*), which are identified as highly consuming leafy vegetables in Sri Lanka [19]. According to recommended daily allowance (RDA) of protein, 100 g of *C. diffusa* could contribute to the daily protein requirement of adults (4%) and pregnant women (20%). [20]. Dietary proteins are responsible for the manufacturing and safeguarding of certain organic materials which are necessary for the proper functioning of human body. Furthermore, fiber is a main nutritional component in human body which facilitates proper functioning of digestive system. Since *C. diffusa* is rich in fiber, this has been used as an herb in traditional medicine system to treat complications in digestive system and to cleanse the digestive tract [21].

Comparatively lower fat level was detected in *C. diffusa* than the other leafy vegetables and this value is lower than the reported value of *C. diffusa* variety which was studied by Nadeeshani *et al*., [2]. As source of nourishment, consumption of *C. diffusa* can improve human health by providing cheap and accessible protein and other nutrients in rural communities.

Edible tender *C. diffusa* collected from Sri Lanka has relatively high microelement contents which are similar to most of the mineral constituent of UWEP worldwide [22,23,24].

Referring to Table 3, Ca is the most abundant micronutrient (63.72 mg/100 g) which is comparatively higher than the reported values of prominent leafy vegetables as *Sesbania grandiflora* (29.65 mg/100 g) and *Trianthema portulacatrum* (34.43 mg/100 g) [19].

Table 3. Microelements of *C. diffusa*

Element	Amount (mg/100 g)
Ca	63.72±3.01
Fe	10.31±0.34
Zn	5.43±0.24
Cu	6.72±2.21

Ca is required for the proper functioning of human body including the functioning of cardiac muscles, blood coagulation, milk clotting and the regulation of cell permeability. Moreover, Ca is the main building block of bones and contains in human blood and in extracellular fluids [25]. Fe, Zn and Cu levels of *C. diffusa* are higher than the detected values of prominent leafy vegetables in Sri Lanka, namely, *Alternanthus asessilis* (Fe:5.5; Zn:1.2; Cu:0.31 mg/100 g), *Centella asiatica* (Fe:9.53; Zn:2.28; Cu:0.24 mg/100 g), *Amaranthus viridis* (Fe:9.14; Zn:1.41; Cu:0.21 mg/100 g) and *Dregea volubilis* (Fe:4.82; Zn:0.69; Cu:0.25 mg/100 g) [2]. Therefore, if use as a supplement in diet, *C. diffusa* can be a good source of dietary Fe and it could contribute 58% of the RDA of 18 mg/day needed by adults [26] to overcome the nutritional deficiency. Fe availability is crucial in cellular function including regulating the balance between Fe uptake, storage and utilization. Furthermore, dietary intake of Fe is essential to replace the loss in form of human excreta (stools, urine and from the skin). This loss was calculated as 0.9 and 0.8 mg/day for adult male and female, respectively [27].

C. diffusa contains satisfactory levels of Zn (5.43 mg/100 g) and Cu (6.72 mg/100 g) which were identified as essential micronutrients that cannot be formed by human body. Zn has been playing a vital role in biological processes such as, immunity development, regulating intracellular signaling pathway and important in the function of lipid and glucose metabolism [28]. In this regard, 100 g of *C. diffusa* can be used to fulfill the RDA of Zn level for an adult which is 4–14 mg/day [29].

The Cu content of *C. diffusa* is higher than the RDA of 0.7, or 1.1 mg/day which is required for children and adults, respectively [27]. Cu stimulates the immune system to fight against infections, repair tissues, and promote healing. Beyond that, Cu bioavailability is important since it is involved in proper functioning of organs and metabolic processes [30].

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties which are considered to be beneficial to human health. The presence of bioactive ingredients and the quantitative determination of the percentage crude yield of bioactive compounds of the current study showed that the *C. diffusa* shoots are rich in phytochemicals [31]

The qualitative phytochemical screening was done to four different extracts of *C. diffusa*. Ethanol extract found to have more bioactive constituents followed by hexane > chloroform > water. In the preliminary screening, ethanolic extract detected the presence of wide range of phytochemicals including, alkaloids, saponins, flavonoids, tannins, phenols, steroids, quinone, terpinoids, coumarine and anthraquinone. *Hexane and chloroform extracts showed negative results for*, quinone, *and anthraquinone while* water extracts showed negative results for, quinone, *steroids*, coumarine *and anthraquinone.*

Parallel to the qualitative detection, quantitative phytochemical composition was determined in tender shoots of *C. diffusa* (Table 4).

Table 4. Quantitative Phytochemicals Composition in Tender Shoots of *C. diffusa*

Composition	Amount (%)
Alkaloids	5.55±0.03
Flavonoids	3.37±0.02
Phenols	7.27±0.05
Saponins	2.82±0.02
Tannins	2.94±0.01
Steroids	0.28±0.01

The quantitative estimation of primary metabolites revealed that the various phytochemical constituents present in the plant extract. *The high levels of alkaloids (5.55%) and flavonoids (3.37%) were found and that level is significantly contributing to the improvement of human biological activities. Moreover,* Alkaloids have a wide range of pharmacological properties including antimalarial, antiasthma, anticancer properties as reported by Prakash *et al*., [31].

C. diffusa contains 2.82% saponin level which are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions [32]. Some researchers have also reported that some saponins have anti-cancer and immune modulatory properties [33,34].

Obtained results revealed a good recovery of total phenolic content (TPC) in *C. diffusa* as, 7.27%. Selected daily consuming leafy vegetables, namely, *Centella asiatica* (gotukola), *Gymnema lactiferum* (kuringan), *Sesbania grandiflora* (kathurumurunga) and *Passiflora edulis* (passion fruit) were analyzed for their TPC [35]. All identified values were lower than the TPC of selected UP species in the study. According to Zeba and Nacoulma [36] amount of bioactive compounds as phenolic compounds present in plant leaves has a relationship with the habitat of the plants that grown and it has been reported that temperature, rainfall and relative humidity like factors have a direct impact on availability of bio active compounds in plant species.

Above findings indicate that *C. diffusa* has higher levels of protein and fiber content and also rich in microelements as Ca, Fe, Cu and Zn. Therefore, *C. diffusa* can serve as a plant food supplement which can be used in nutrition and

pharmaceutical industries to prevent malnutrition or hidden hunger. Studies on micronutrient and antioxidant bioaccessibility, quantitative phytochemical evaluation and toxicity are ongoing to ascertain its possibility to be promoted as “future food”.

4. Conclusions

In the present study important phytochemicals have been identified from the tender shoots of *C. diffusa* by quantitative and qualitative phytochemical analysis. Microelements including, Ca, Fe, Zn and Cu were assessed and further studies are ongoing to identify the bioaccessibility of microelements in *C. diffusa* tender shoots.

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