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Proximate analysis and bioactive compounds analysis of *Gymnema lactiferum*

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Abstract
The plant, *Gymnema lactiferum* has been gradually utilized as an herbal remedy to cure various ailments, even though they have not been explored clinically. The present investigation focused mainly on the phytochemical investigation of the leaves of this plant, employing standard procedures and qualitatively and quantitatively investigation of selected phytochemicals. Results showed the presence of various phytochemicals, particularly the Alkaloids, Flavonoids, Saponins, Phenolic compounds, Protein, and Carbohydrate in *Gymnema lactiferum*. The quantitative test results revealed that Total Phenolic Compounds (17.2242±0.17 mg/GAE/100g), Alkaloids (0.0991±0.00187 mg/g), Saponins (0.01377±0.00532 mg/g) and IC_{50} value for Antioxidant (9.6685 mg/ml). According to the proximate analysis; Moisture, Crude fat, Crude fiber, Crude protein, Total ash and carbohydrate contents had values of 77.5174±0.7718%, 1.0518±0.9466%, 3.2525±0.2570%, 1.9146±0.5731% and 1.9464± 0.7376% respectively. *Gymnema lactiferum* is a rich source for Ca, K, Na minerals. This plant contained Calcium (583.99 mg/100g), Potassium (529.28 mg/100g), Sodium (246.99 mg/100g) and Iron (14.64 mg/100g). Based on the findings of the study, *Gymnema lactiferum* leaves have both medicinal and nutritional potentials.

Keywords: *Gymnema lactiferum*, DPPH, proximate analysis, TPC, alkaloids

1. Introduction
In developing countries, ethnomedicine is most popular among people. In Ayurveda medicine, lots of plants are used to prepare many drugs. Therefore, the knowledge of the plants is gaining attention of indigenous practitioners. In Sri Lanka, various green leaves are consumed to fulfil both nutritional and medicinal remedies for some ailments. Leaves of *Gymnema lactiferum* are often consumed to prevent higher blood sugar level. *Gymnema lactiferum* belongs to Asclepiadaceae family (milkweed family) and which grown as a twining or straggling plant. Commonly this plant can be found in Sri Lanka, Assam, Malaysia, and India [1]. This plant is distributed in both dry and wet zone of Sri Lanka and leaves are 3-8 cm long blade shape and flowering has been detected from July to November [2]. In Sinhala, common names of this plant are "Kurincha" / "Kurighghan" and in Sanskrit known as "Kshirakakoli".

Leaves of this plant are consumed as cooked form and raw form with coconut crapes as well as herbal gruels [3]. Several studies on this plant have been reported glycation induced protein cross-linking inhibitory effects [4] anti-diabetes activity, antioxidant activity [5]. In Ayurveda, it has been reported that usage of leaves to treat cough, ulcers, pitta, kapha and pain in eyes and formulated drug is used to improve the function of heart, jaundice, piles, urinary calculi [6]. In adenopathy, this often uses to treat asthma, cough, bite, obesity, constipation, boil, fever, snake bite, cardiopathy, and water retention [7]. This study aim was to analysis the dietary potentials of *Gymnema lactiferum* as a nutraceutical.

2. Materials and methods
2.1 Collection and Identification of plant material
Fresh healthy leaves were collected from Kadawatha, Gampaha district between 8-10 a.m. in June. Plant sample was identified and authenticated by National Herbarium, Peradeniya Botanical Garden, Sri Lanka.

2.2 Preparation of Methanolic Extract
The leaves were washed using distilled water and chlorinated water and shade dried for 10 days at room temperature. Then well dried leaves were pulverized into coarse powder. It was extracted using 80% methanol using Soxhlet apparatus.

2.3 Qualitative analysis of phytochemical
Qualitative analysis of bioactive compounds was conducted according to the methods described by Banu and Cathrine [8], Singh *et al*., [9] and Cynthia [10].

Screening of alkaloids
Mayer's test
A few drops of Mayer's reagent were added to 1ml of extract solution. Yellow colour precipitate or turbidity indicates presence of alkaloids.

Wagner's test
To 1ml of extracted solution, 3-4 drops of Wagner's reagent were added. The sample was observed yellow colour precipitation as positive for alkaloids

Screening of flavonoids

Ferric chloride test

To 1 ml of extracted solution, 0.5 ml of 10% FeCl3 solution was added. Formation of a woody brownish colour precipitate indicates the presence of flavonoids.

Screening of saponins

Frothing test

To 100 mg of powder, 20 ml of distilled water was added and shaken well for 20 min. Persistence of foam indicates the positive results for saponins.

Screening of tannins

Ferric chloride test

To 1 ml of extracted solution, 3-4 drops of 5% $FeCl_3$ solution was added. Green colour precipitate indicates the presence of tannins.

Gelatin test

Pre-weighed 50mg of extract was mixed with 5 ml of distilled water and 2 ml of 1% gelatin solution. Then a few ml of 10% of sodium chloride were added and presence of white precipitate indicates the tannins.

Screening of phenolic compounds

Lead acetate test

A few drops of 1% lead acetate was added to 1 ml of the extract. Formation of white colour precipitate indicates the presence of phenolic compounds.

Screening of carbohydrates

Fehling's test

To 2ml of methanol extract, 2ml of distilled water and 2ml of Fehling's A and B were added and heated in a water bath until it boiled. Brick red colour indicates the presence of carbohydrates.

Bradford's test

Few drops of Bradford's reagent were added into 2ml of methanol extract. Presence of blue colour precipitate indicates the carbohydrates

Screening of protein

Millon's test

To 2ml of filtrated extract, 6 drops of Millon's reagent was added and formation of white colour precipitate indicates the protein.

2.4 Quantitative analysis

Quantitative analysis of total phenol content

The total phenol content of the sample was determined using Folin-ciocalteu's phenol reagent in accordance to ISO 14502-1 method [11]. 1.0 ml of extract (70% methanol extract) was added into a test tube and then 5ml of 10% Folin-ciocalteu's phenol solution and 4 ml of 7.5% sodium carbonate solution were mixed and heated for 10 min at 40 ^{0}C. Then absorbance was measured at 765 nm. Gallic acid was used to construct the standard curve and amount of total phenol content was expressed as mg/GAE/100g.

Quantitative analysis of total alkaloid content

The total alkaloid content was determined using gravimetric method described by J.B. Harbone [12]. 5 g of dried powder of sample was mixed with 200ml of 10% acetic acid in ethanol and kept it for 8 hours. Then filtered sample was concentrated to one –fourth of volume at 80 ^{0}C. Then conc. NH_4OH was added drop-wise until the precipitation was completed. Finally, the settled solution was filtered and precipitate was collected and weighed. Total alkaloid content was expressed as (mg/g).

Quantitative analysis of total saponin content

The method described by Obadoni and Ochuko [13] was followed to analyze the total saponin content as (mg/g). 20g of dried powder was mixed with known amount of 20% ethanol and heated in a water bath at 55 ^{0}C for 4 hours. The residue was re-extracted and concentrated to 40 ml using previous solvent system and then diethyl ether was added and shaken vigorously. 60 ml of n-butanol was added to recovered aqueous layer and 5% aqueous sodium chloride was mixed with pooled sample. After evaporation of the sample, final weight of sample was measured.

Quantitative analysis of antioxidant content

In-vitro antioxidant activity was determined using DPPH radical scavenging activity. The ability of selected plant extract to scavenge the DPPH was assessed by the method of Blois with some modification [14]. The series of methanol extract (2.5 – 40 mg/ml) was mixed with 2.5 ml of DPPH in a dry test tube and then it was covered with Al foil and allowed to stand for 30 min in a dark place. The standard calibration curve was constructed using Gallic acid concentration series. The absorbance of the samples was measured at 517 nm using UV-Vis spectrophotometer. IC_{50} value was calculated as follows.

$$\% \text{ Inhibition} = (\text{Blank absorbance} - \text{Sample absorbance}) / (\text{blank absorbance}) \times 100 \quad (1)$$

Proximate analysis

Standard procedures of Association of Official Analytical Chemist [15] were followed for the proximate analysis. Moisture content of the sample was analyzed according to the oven drying method at 105 ^{0}C; Crude fat content of powdered *Gymnema lactiferum* was estimated by extracting the sample using petroleum ether as a solvent in a Soxhlet apparatus; The defatted sample was digested with 1.25% (w/v) of sulphuric acid and the same concentration of sodium hydroxide to determine the amount of crude fiber. Dry ashing method, 923.03 specified in AOAC was carried out to determine the ash content of the sample

Determination of Mineral content

The amount of calcium, potassium, sodium and iron of the samples were analyzed by dry ashing and Atomic Absorbance Spectrophotometer (AAS) [15]. Pre-weighed 3 g of dried sample was incinerated and then kept in a muffle furnace at 550 ^{0}C for 3 hours. Hot 10% HCl and HNO_3 (3:1) were added to sample to dissolve and after that topped up to 100 ml with distilled water in a volumetric flask. The absorptions of

solution were read and amount of mineral content was calculated as mg/100g.

2.5 Statistical analysis

The collected data were statistically evaluated by one-way analysis of variance (ANOVA) by using Minitab 17 software and significant differences between means were determined by Tukey's multiple comparison. All test data were analysed at 5% significance level. Linearity of standard curve in TPC, and Antioxidants analysis was determined by regression analysis and linearity by using Minitab 17 software. All the analysis was triplicated and expressed with means ± standard deviation.

3. Results and analysis

Quantitative analysis of phytochemicals

Analysis of phytoconstituents is vital for different purposes such as synthesis of drugs etc. This study was carried out to identify and quantify of specific phytochemical in methanolic extract of *Gymnema lactiferum* leaves. The results of secondary metabolites screening of *Gymnema lactiferum* leaves tabulated in table 01. According to the results, the different types of phytochemical tests emphasize presence of alkaloids, flavonoids, phenolic compounds, saponins, carbohydrates and protein. These bioactive compounds elicit various medicinal properties [16].

Table 1: Qualitative analysis of *Gymnema lactiferum* leaves

Secondary metabolites	Test/s	Methanol extract
Alkaloids	Mayer's test	+
	Wagner's test	+
Flavonoids	Ferric Chloride test	+
Phenolic compounds	Lead acetate test	+
Tannins	Ferric chloride test	-
	Gelatin test	-
Saponins	Frothing test	+
Carbohydrates	Fehling's test	+
	Bradford's test	+
Protein	Millon's test	+

(+) Presence, (-) Absence

Quantitative analysis

After preliminary screening of phytochemical constituents; quantitative determination of plant extract is used for isolation and quantitation of specific secondary metabolites.

Polyphenols are the most abundant secondary metabolites synthesis by plants. They possess some health benefits which include antioxidant property, anti-inflammatory properties and anti-carcinogenic properties [17]. These compounds contain an aromatic ring and one or more hydroxyl moieties. The content of total phenolic was conducted according to the absorbance values of the extract solutions, reacted with Folin–ciocalteu reagent and compared with the standard solutions of Gallic acid. The test result revealed that 17.2242 ± 0.17 (mg/ GAE/ 100g) of phenolic compounds contained methanolic extract of *Gymnema lactiferum* leaves.

Total alkaloids and total saponins content were determined using the gravimetric methods of previously mentioned. Alkaloids are the vast single class of phyto constitutes which defined as substances which comprise one or more nitrogen atoms, in combination as part of a cyclic system [18]. It has been reported that they are important in the genotype for cell activity and gene code realization and biologically significant as active stimulators for endogenous security [19]. Saponins are glycosides which act as surface active agents. It was reported that it has anti-fungal and antibacterial properties [20]. Table 2 indicates the amount of saponins and alkaloids present in methanolic extract.

Table 2: Quantitative analysis results for alkaloid and saponin contents

Secondary metabolites	Methanol extract (mg/g)
Alkaloid	0.0991±0.0019
Saponin	0.0138±0.0053

Valves expressed as means ± SD

Proximate and Mineral analysis

Table 03 indicates the proximate composition of *Gymnema lactiferum* leaves, according to the results that *Gymnema lactiferum* leaves show higher fiber content (3.2525± 0.2570%) rather than the crude fat (1.0518± 0.9466%) content. Higher fiber content aids for absorbing trace element in gut within the digestive system and prevent diverticulosis [21]. The results obtained from the analysis revealed that *Gymnema lactiferum* leaves have higher amount of carbohydrates and it can be cognized as rich energy source.

Table 3: Proximate analysis of *Gymnema lactiferum* leaves

Parameters	% of dry weight
Moisture	77.5174±0.7718
Crude fiber	3.2525±0.2570
Crude fat	1.0518±0.9466
Crude protein	1.9146±0.5731
Ash	1.9464± 0.7376
Carbohydrate	14.3173

Valves expressed as means ± SD

The amount of calcium had the highest value among the tested minerals, (583.9909mg/100g). Calcium is necessary for building and maintaining teeth and bones, blood coagulation and regulation of cell permeability [22]. Rickets, osteoporosis, back pain and indigestion can be caused due to the deficiency of calcium [23].

Table 4: Mineral composition of *Gymnema lactiferum* leaves

Ion	Ion concentration (mg/ 100g)
Na	246.9939
Ca	583.9909
Fe	14.6474
K	529.2824

According to the table 4 that *Gymnema lactiferum* leaves are a rich source of calcium and potassium (529.2824mg/100g). Potassium and sodium play a role in maintaining lower blood pressure levels, reduce the adverse effects of sodium chloride intake on blood pressure and reduce bone loss [24]. *Gymnema lactiferum* leaves contain less sodium than potassium (246.9939 mg/100g). Iron is noted for formation of hemoglobin, transport oxygen around the body [25]. Deficiency of Fe causes anemia, depression and decreases the infection

resistance [26]. Among tested minerals, the least amount of ion concentration was showed Fe which was recorded as (14.6474 mg/100g) in *Gymnema lactiferum* leaves.

4. Conclusion

According to the observed results from this study, methanolic extract of *Gymnema lactiferum* leaves contained secondary metabolites such as alkaloids, flavonoids, phenolic compounds and saponins which have several beneficial diseases curing properties. Mineral analysis results revealed that *Gymnema lactiferum* leaves were rich in Ca, K, Na and contained significant amounts of Fe. It can be concluded that *Gymnema lactiferum* leaves have potential as a neutraceutical.

5. References

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