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Research Article

Comparative Analysis of Qualitative and Quantitative Phytochemical Evaluation of Selected Leaves of Medicinal Plants in Jaffna, Sri Lanka

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Abstract

The traditional system of medicine in Sri Lanka has shown much better improvement, has fewer side effects, and is less expensive than modern synthetic drugs in the treatment of many diseases. The objective of the present study was to comparatively evaluate the qualitative and quantitative analysis of phytochemical constituents of leaves of *Murraya koenigii* (L.) Spreng., *Tinospora cordifolia* (Wild) Hook.f., *Enicostemma axillare* (Lam) A. Raynal, and *Gymnema sylvestre* R. Br. were collected from Jaffna District. The shade-dried leaves were powdered and extracted with ethanol using the cold extraction technique. These ethanolic extracts were subjected to phytochemical analysis using recommended laboratory techniques. The one-way analysis of variance (ANOVA) and Tukey's multiple comparisons at probability value ($p < 0.05$) were used in the statistical analysis of the data. Phytochemical screening showed the presence of alkaloids, flavonoids, tannins, terpenoids, steroids, saponins, phenols, and glycosides. *Murraya koenigii* shows the highest phenol and alkaloid contents (1960.71±66.88 and 19.42±0.26). *Enicostemma axillare* shows the highest flavonoid and tannin contents (22.27±0.86 and 1.26±0.017). Therefore, *E. axillare* and *M. koenigii* can be used as nutraceuticals in traditional medicine.

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INTRODUCTION

Medicinal herbs have been known for ages as a rich source of medicinal agents for the prevention of diseases and ailments worldwide[1]. According to the World Health Organization, up to 80% of the world's population still relies on traditional treatments such as herbs for their primary health care[2]. In Sri Lanka, four traditional medicinal systems have been adopted: Ayurvedic medicine, Siddha, Unani, and Deshiya Chikitsa[3]. Plants and herbal medicines are primarily employed in the Ayurveda and Deshiya Chikitsa medicinal systems to treat many diseases[4]. Even though synthetic pharmaceuticals are readily available and very successful in curing numerous diseases in today's society, some people still prefer to use traditional folk medicines since they have fewer side effects[5].

More than 13,000 secondary metabolites have been isolated from the medicinal plants. The secondary metabolites serve as defense molecules or perform specialized functions in plants. These secondary metabolites possess medicinal properties, including antidiabetic and antioxidant activity[6,7]. Alkaloids, phenolics, terpenoids, flavonoids, saponins, xanthones, polysaccharides, and other compounds have been reported to have antidiabetic activity[8].

Plants have medical value because they contain chemical compounds that have a specific physiological effect on the human body. Alkaloids, flavonoids, tannins, and phenolic compounds are essential bioactive molecules found in plants[9]. *Murraya koenigii* (L.) Spreng., *Tinospora cordifolia* (Wild) Hook.f., *Enicostemma axillare* (Lam) A. Raynal, and *Gymnema sylvestre* R. Br. are commonly available in the Jaffna district and used to treat many diseases in traditional folk medicine. The leaves of these

plants are high in bioactive chemicals such as polyphenols, alkaloids, and flavonoids, which have a variety of bioactive properties, including antioxidant, anticancer, antibacterial, antidiabetic, and hepatoprotective properties[10-13].

Phytochemicals are naturally occurring compounds in various parts of the plants which can protect the liver by providing medicinal value or nutrients. These phytochemical compositions of the different medicinal plants mainly depend on the region where those are cultivated, the climate of that particular region, and the method and period of collection[14]. Therefore, the present study was to comparatively evaluate the qualitative and quantitative analysis of phytochemical constituents of leaves of above mentioned four medicinal plants.

MATERIALS AND METHODS

Collection of plant materials

The selected fresh leaves of four different medicinal plants (**Table I** and **Figures 1-4**) were collected from Jaffna District from September to October 2020. These plants were botanically authenticated in the National Herbarium Centre, Department of National Botanic Gardens, Peradeniya, Sri Lanka.

Table I. Medicinal plants used for the study

Plant Botanical name	Family	Common name		
		Sinhala	Tamil	English
Murraya koenigii (L.) Spreng.	Rutaceae	*Karapincha*	*Kariveppillai*	Curry leaf
Enicostemma axillare (Lam) A. Raynal	Gentianaceae	Nahi, Maja-Makka booti	*Vallaruku*	Indian white head
Gymnema sylvestre R. Br.	Apocynaceae	*Masbedda*	*Kurincha*	Cow plant
Tinospora cordifolia (Wild) Hook.f.	Menispermaceae	*Raskinda*	*Seenthil*	Moonseed

Figure 1. *Murraya koenigii*

Figure 2. *Tinospora cordifolia*

Figure 3. *Enicosrtemma axillare*

Figure 4. *Gymnema sylvestre*

Preparation of plant materials

The collected fresh leaves were washed with tap water for several times to remove the soil and dust particles, and those were air-dried systematically at room temperature for three weeks to avoid direct loss of phytoconstituents from sunlight. The shade-dried plant leaves were ground using the pulverizer and sieved up to 80 meshes. It was then homogenized to a fine powder and kept in air-tight containers separately for further analysis at room temperature (31±3°C).

Preparation of plant extracts

The leaf powder of each medicinal plant was extracted with ethanol using the cold extraction technique. A total of 50 g of powdered materials of each plant's leaves were separately weighed and placed in 500 ml of culture bottles. 150 mL of 100% absolute ethanol (1 : 3) was added to it and mixed well. The lid of each bottle was covered with parafilm. The solution was kept for five days with occasional shaking using a shaker at 150 rpm for 15 minutes every morning and evening. After that, those were filtered through Whatman No.1 filter paper. The part of filtered content was concentrated using a rotatory evaporator (Buchi), and another part was kept in the refrigerator at 4°C for further use. The analysis was done for three replicates of each medicinal plant leaf.

Qualitative analysis of phytochemicals[15-20]

The preliminary phytochemical screening of the ethanol extracts of each medicinal plant leaves powder was carried out using recommended laboratory procedures to detect the presence of different phytochemicals such as alkaloids, flavonoids, tannins, steroids, glycosides, phenols, terpenoids, saponins, coumarins, anthraquinones and quinines.

Phytochemical screening for flavonoids (alkaline reagent test)

Each 2 mL of filtered sample was mixed with a few drops of 20% NaOH. The formation of intense yellow color was detected. Then, a few drops of 70% diluted hydrochloric acid were added, and the yellow color disappeared. The formation and disappearance of the yellow color indicate the presence of flavonoids.

Phytochemical screening for phenols (ferric chloride test)

Each 2 mL of filtered sample was mixed with 2 mL of 5% aqueous $FeCl_3$.The formation of the blue color points out the occurrence of phenols.

Phytochemical screening for tannins (ferric chloride test)

Each 2 mL of filtered sample was added with 10% of alcoholic $FeCl_3$. The formation of the black/brownish blue directs the occurrence of tannins.

Phytochemical screening for alkaloids (Dragendroff''s test)

Each 2 mL of filtered sample was dissolved individually in dilute hydrochloric acid and filtered. The filtrate was treated with Dragendroff''s reagent (solution of potassium bismuth iodide). The formation of a red precipitate indicates the presence of alkaloids.

Phytochemical screening for terpenoids (chloroform test)

Each 2 mL of filtered sample was added with 0.5 mL chloroform with 0.5 mL of acetic anhydride and a few drops of concentrated sulfuric acid. The formation of reddish-brown precipitate directs the presence of terpenoids.

Phytochemical screening for anthraquinones

Each 2 mL of filtered sample was added with potassium hydroxide. The blood red colour shows the presence of anthraquinones.

Phytochemical screening for saponin (foam test/frothing test)

Each 2 mL of filtered sample was added with 4 mL of distilled water. It will be mixed well and shaken vigorously. If foam will be produced continues for ten minutes, it designates the presence of saponins.

Phytochemical screening for quinones

Each 1 mL of filtered sample was added with 1 mL of sodium hydroxide. The formation of blue, green, or red colors shows the presence of quinones.

Phytochemical screening for coumarins

Each 1 mL of 1% filtered sample was added with 3-4 drops of 1% KOH in absolute ethanol. The formation of yellow color directs the occurrence of coumarins.

Phytochemical screening for glycosides (Keller-Kiliani test)

Each 2 mL of filtered sample was added with 0.5 mL glacial acetic acid, three drops of 1% aqueous $FeCl_3$ solution, and 0.5 mL H_2SO_4 concentrated. A brown ring formed between the layers, which showed the entity of cardiac steroidal glycosides.

Phytochemical screening for steroids

It was carried out by Salkowski's test. About 2 mL of sample was mixed with 2 mL of chloroform. Then, 2 mL of concentrated H_2SO_4 was added to it. If steroids are present, the chloroform layer will appear red, and the acid layer will show greenish-yellow fluorescence.

Quantitative analysis of phytochemicals

Quantitative analysis for total phenolic content (Folin-Ciocalteu colorimetric method)

About 20 µL of each filter was added to the test tube using a micropipette. 1.58 µL was added to each above test tube. 100 µL of Folin-Ciocalteu reagent was added to each test tube. They were mixed well using a magnetic stirrer and allowed for eight minutes after stirring. 300 µL of a sodium carbonate solution was added to each stirred solution. They were heated in a water bath at 40°C for 30 minutes. They were permitted to cool. They were again stirred well. The Absorption of each

sample was measured using a spectrophotometer at 765 nm wavelengths. A curve chart for each solution was prepared by using absorbance and concentration. The three replicates were prepared for each sample. Using the standard curve, the total phenolic content was determined and expressed in mg gallic acid equivalent (mg GAE) per g of dry matter using the following linear equation based on the calibration curve (**Figure 5**)[21].

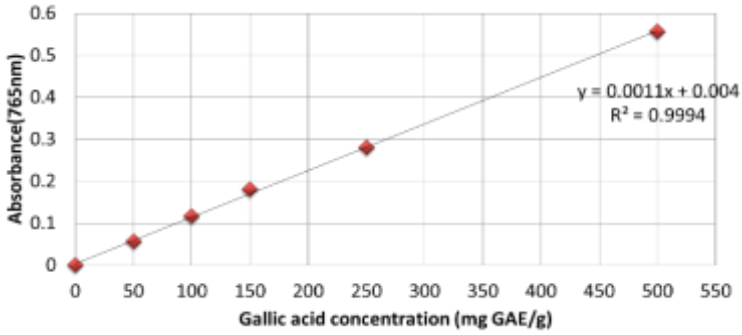

Figure 5. Standard curve for total phenolic content

Quantitative analysis for total flavonoid content (aluminum colorimetric method)

Each 0.25 mL filtered sample was added with 4.5 mL of distilled water. 0.3 mL of 5% $NaNO_2$ solution was added and allowed for 5 minutes. 0.3 mL of 10% of $AlCl_3$ was mixed and incubated for 5 minutes. 2 mL of 1N NaOH was added, and the entire volume was made to 10 mL with distilled water and mixed well. The absorbance of each sample was measured at 510 nm using a spectrophotometer. Blank was prepared using the above reagents and distilled water instead of sample. A curve chart for each solution was prepared by using absorbance and concentration. The three replicates were prepared for each sample. The flavonoid content was calculated as mg catechin equivalent (mg CAE) per gram of dry matter using the calibration curve and the following linear equation (**Figure 6**)[22].

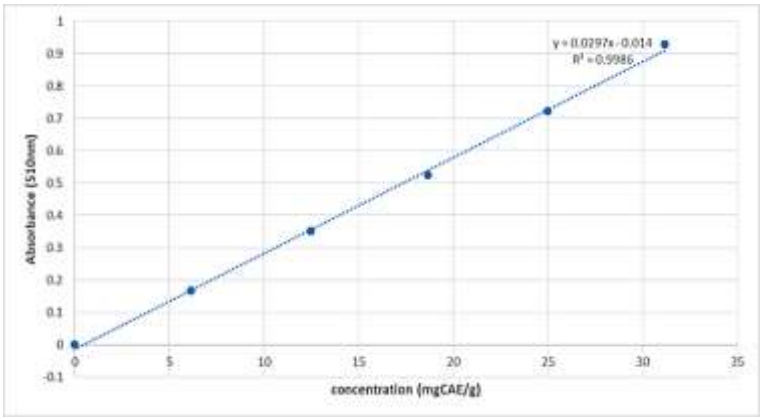

Figure 6. Standard curve for total flavonoid content

Quantitative analysis for total tannin content (Folin-Ciocalteu colorimetric method)

Each 0.5 mL of filtered sample was added with 3.75 mL of distilled water and 0.25 mL of Folin-Ciocalteu reagent, 0.5 mL of 35% sodium carbonate. The absorbance of each sample was measured at 725 nm using a spectrophotometer. The blank was prepared using the above reagents with distilled water instead of the sample. A curve chart (**Figure 7**) for each solution was prepared by using absorbance and concentration. The three replicates were prepared for each sample. The estimation of the total tannin content was carried out in three replicates. The tannin content of the samples was measured in mg/ml of tannic acid[23].

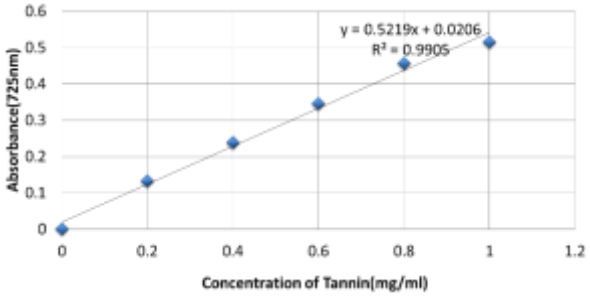

Figure 7. Standard curve for total tannin content

Quantitative analysis for total alkaloid content

About 5 g of the three samples of each powder material were balanced into a 250 mL beaker, and 200 mL of 20% of acetic acid was added and enclosed to stand for 4 hours. They were filtered, and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to each extract until the precipitous was completed. The whole solution was permitted to settle down, and the precipitate was collected by filtration through the accurately weighed filter paper. The filtrate is the alkaloid, which was dried in the oven for four hours and balanced. Total alkaloid content was measured as mg per g of air-dried material using the **Equation 1**[24].

$$\%\ Alkaloid\ content = \frac{w1-W2}{M} \times 100\% \quad \ldots [1]$$

W_1 : weight of the precipitate with the filter paper
W_2 : weight of the empty filter paper
M : weight of the sample

Statistical data analysis

The results were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple comparisons at probability value ($p \leq 0.05$) using the SAS statistical program (version 9.1.3). In each analysis, three replicates were maintained for each sample.

RESULTS AND DISCUSSION

Qualitative analysis of phytochemicals

The presence or absence of phytochemicals was evaluated using qualitative analysis of leaves from selected four medicinal plants. The results are provided in **Table II**. Saponins are found in all four plants, according to the study. Saponins contain a variety of functions, including the ability to precipitate and coagulate red blood cells, as well as the ability to bind cholesterol. It also shows foam formation in aqueous solutions and hemolytic action, and saponins have traditionally been employed as detergents and molluscicides. In addition to their industrial applications as foaming and surface-active agents, saponins have beneficial health effects against various diseases[25].

Table II. Preliminary phytochemical screening of ethanolic extracts of selected plant leaves

Phytochemicals	*M. koenigii*	*G. sylvestre*	*T. cordifolia*	*E. axillare*
Tannin (black colour)	+	+	+	+
Saponins (foam)	+	+	+	+
Flavonoid (yellow color)	-	+	+	+
Alkaloid (red precipitate)	+	+	+	+
Quinone (green or red color)	-	-	-	-
Anthraquinones (blood red color)	-	-	-	-
Glycoside (brown ring)	+	+	+	+
Terpenoids (reddish-brown precipitate)	+	-	-	+
Steroids (greenish yellow fluorescence)	+	+	+	+
Phenol (blue color)	+	+	+	+
Coumarins (yellow color)	+	+	+	+

"+" color change/precepitation observed & "-" color change/precepitation not observed

Plant steroids are vital for their cardiotonic properties and are employed in nutrition, herbal medicine, and cosmetics manufacturing. Steroids are used to stimulate bone marrow and promote growth. It promotes lean body mass and aids in preventing bone loss in older men[26]. As a result of this study, steroids were found in all four plants. Many studies have been carried out on the anti-hypoglycemic activity of terpenoids of herbal plant origin. *Murraya koenigii* and *E. axillare* show the presence of terpenoids. Flavonoids also show a wide variety of essential activities, including antihyperglycemic activity[27]. According to the study, flavonoids are present in *G. sylvestre, T. cordifolia*, and *E. axillare*.

Results show the presence of alkaloids in all selected plants. Thus, alkaloids can be concluded as one of the healers in medicinal plants, and many natural bio-resources studied may prove to be of significance in naturopathy and have properties that may be further investigated[28]. Many bioactive molecules in herbal plants may prove to be promising therapeutic tools. Previous research has proven that glycosides have a high potential in curing diabetes mellitus and many other diseases[29]. The glycosides are found in all four plants, according to the results of this study. Several natural phenolic compounds in medicinal plants provide anti-inflammatory, antioxidant, antimicrobial, and neuroprotective properties. Results show the presence of phenols in all selected plants.

According to the results, quinone & anthraquinones are absent in all selected plants. Coumarins are present in all selected plants. Coumarins have many biochemical and pharmacological properties which may be effective against diabetes and its complications, some of which are of potential therapeutic interest[30]. Tannins are thought to have various properties, including analgesic, anti-diabetic, and anti-inflammatory properties. Tannins have the potential to be an efficient kidney-relieving medication. Tannins are present in all selected plants, according to the results. These phytochemicals have a significant influence on hypoglycemic activity[31]. Therefore, they help to reduce diabetes. They contain antibacterial as well as antihyperglycemic properties. The use of natural chemical compounds from plants as antibacterial and antifungal agents is an intriguing technique for developing bioactive products and pharmaceuticals that could become practical therapeutic tools in the coming years[32].

Quantitative analysis of phytochemicals

The quantitative phytochemical analysis of different medicinal plant leaves is tabulated in **Table III**. Phenolic compounds are a broad and diversified class of chemicals that comprise a variety of secondary aromatic metabolites found in plants. It has been reported to have antioxidant, anti-diabetic, and antibacterial effects, among other biological activities[33]. Phenolic compounds have a wide range of pharmacological effects. Phenol's antioxidant activity mainly derives from its redox characteristics, hydrogen donors, and singlet oxygen quenchers[34]. Gallic acid has also been observed to play a synergistic role in drug-herb interactions, resulting in increased therapeutic benefit and fewer side effects. The results of this study show that the total phenolic content was significantly highest in *M. koenigii*, followed by *E. axillare*, *G. sylvestre*, and *T. cordifolia*. The antioxidant activity of ethanolic and water extracts of curry leaves is relatively high at all concentrations, but it increases as the sample concentration increases.

Table III. Quantitative analysis of phytochemicals of selected plant leaves

Plant species	Phenol (mgGAE/g)	Flavonoid (mgCAE/g)	Tannin (mg/ml)	Alkaloid (%)
M.koenigii	1960.71 ± 66.88	15.42 ± 3.50	1.223 ± 0.011	19.42 ± 0.26
E.littorale	856.84 ± 35.4	22.27 ± 0.86	1.26 ± 0.017	10.38 ± 0.31
G.sylvestre	616.92 ± 19.6	14.67 ± 1.35	1.23 ± 0.014	6.62 ± 0.25
T. cordifolia	325.61 ± 23.84	15.03 ± 1.42	1.24 ± 0.008	13.50 ± 0.33

Flavonoids are hydroxylated phenolic compounds that plants produce in response to microbial infection and have been discovered to have antibacterial properties in vitro against a wide range of pathogens. Flavonoids' antioxidative activities are attributable to various processes, including scavenging free radicals, chelation of metal ions like iron and copper, and inhibiting enzymes that generate free radicals[35]. Catechins have an anti-hyperglycemic effect, reducing blood sugar while also regulating insulin release. Catechins also have antiviral properties. The results of this study show that the total flavonoid content was highest in *E. axillare*, followed by *M. koenigii, G. sylvestre*, and *T. cordifolia*.

Tannins can inhibit the growth of many microorganisms such as fungi, yeasts, bacteria, and viruses. The results of this study show that the total tannin content was highest in E. *axillare*, followed by *M. koenigii, G. sylvestre*, and *T. cordifolia*. Tannins have antioxidant properties. They are cardio-protective, anti-inflammatory, anti-carcinogenic, and anti-mutagenic, among other things. Tannins increase glucose absorption while inhibiting adipogenesis, making them viable treatments for non-insulin-dependent diabetes mellitus (NIDDM)[36].

Plant cells are highly sophisticated chemical factories that produce secondary metabolites like alkaloids which possess significant biological properties. They exhibit good anti-microbial activity against a few bacterial pathogens causing common infections. Alkaloids are a vast and structurally diverse collection of chemicals that have been used as scaffolding for antibacterial medications like metronidazole and quinolones[37]. Alkaloids help to regulate hypoglycemic activity also[38]. The results of this study show that the total alkaloid content was highest in *M. koenigii*, followed by *G. sylvestre, T. cordifolia*, and *E. axillare*.

CONCLUSION

Medicinal plants and phytochemicals have much importance in the present scenario in developing countries where resources are limited. Regular uptake of herbal medicines containing these phytochemicals can benefit many health problems. The results of preliminary phytochemical screening using ethanolic extracts of *M. koenigii, G. sylvestre, T. cordifolia*, and *E. axillare* leaves are presented in this work. Leaves of *E. axillare* and *M. koenigii* are rich in critical specific phytochemicals and higher amounts of total phenolic and flavonoid contents than other plants. Therefore, *E. axillare* and *M. koenigii* can be used as multi-functional medicinal herbs in the traditional system of medicine and to prepare ready-to-use functional products and nutraceuticals.

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AUTHORS' CONTRIBUTION

Gowri Rajkumar: interpreted, conceived, design the analysis, supervised the experimental works and also the correction of the manuscript. **Panambara Arachchilage Harini Rangana Panambara**: performed the experiments and initially drafted the manuscript. **Vinotha Sanmugarajah**: contributed in the experimental works and assisted for manuscript writing.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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