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Mini-Research Article

Comparative Evaluation of the Anti-oxidant Potential of Selected Plants Used for Diabetes in Sri Lanka

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Abstract

Introduction: Plants are good sources of antioxidants, which have been used to treat diseases such as diabetes, arthritis, and cancer. Herbals used for diabetes have anti-oxidant potential, which could reduce complications and improve patients' health conditions. This study focused on comparing anti-oxidant activity in leaves of *Gymnema sylvestre* and *Costus pictus*, seeds of *Nigella sativa* and *Trigonella foenum graceum,* and fruit of *Momordica charantia*. Methods: The plant samples were collected from the Jaffna District and authenticated in the University of Jaffna. The extract of each plant was prepared by maceration. DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was used to evaluate the *in vitro* anti-oxidant activity of methanolic extracts of these plant extracts using ascorbic acid as standard. The folin-ciocalteu method was used to quantify the total phenolic content present in the plant extracts. One-way ANOVA was used to compare the anti-oxidant activity of extracts with standard. Pearson correlation was used to assess the correlation between anti-oxidant activity and the total phenolic content of the extracts. Results: The IC_{50} values for DPPH assay were 1246.66±10.91, 1917.89±13.86, 813.82±2.08, 767.87±2.39, and 729.81±3.28 µg/ml, and the total phenolic content were 13.23±0.23, 14.70±0.54, 18.04±0.24, 20.92±0.31, and 35.72±0.59 mg GAE/g for *T. foenum graceum, M. charantia, G. sylvestre, N. sativa, C. pictus* respectively. *C.pictus* showed the highest anti-oxidant activity, and *M. charantia* showed the least antioxidant activity among the tested plant extracts. *C. pictus* leaf extract had high total phenolic content. *T. foenum graceum* seed extract had low total phenolic content among the tested plant extracts. Anti-oxidant activity of tested plant extracts was significantly low compared to ascorbic acid ($p<0.001$). Also, anti-oxidant activities of tested plant extracts showed significant differences between them ($p<0.001$). Conclusion: *N. sativa* and *C. pictus* exhibited potential anti-oxidant activities and could be beneficial in preventing and controlling complications in patients with diabetes.

Keywords: Anti-oxidant activity, Total phenolic content, Diabetes, Herbals, Sri Lanka

Introduction

Diabetes is the most frequent endocrine illness that affects the general population. There will be 366 million patients with diabetes worldwide by 2030, up from 171 million in 2000 [1]. Diabetes has traditionally been treated using herbal remedies. Therefore, many herbal medicines are recommended for diabetes because they have no or fewer side effects [2].

Many varieties of herbs in Sri Lanka have been proven to treat ailments across many generations.

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Those herbs have been consumed to control diseases such as diabetes, arthritis, and cancer. This traditional medicinal system has been in practice for the past 3000 years and is used as the first approach for disease control [3]. Most of these plants exhibited anti-oxidant activities [4].

Free radicals have unpaired electrons and are chemically unstable and reactive species. They interact with the macromolecules (DNA, proteins, and lipids) of cells, which affects the cell structure and functionality [5]. Anti-oxidants have the potential to neutralize such free radicals. Several studies reported that oxidative stress causes the development or worsening of diabetes [3]. Oxidative stress causes the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [6]. Diabetes is diagnosed by elevated blood sugar, during which ROS production is increased [7]. It resulted in elevated oxidative damage to vital macromolecules. In diabetic patients, oxidative stress causes insulin resistance, malfunction of beta and endothelial cells, and decreased insulin secretion [8]. Patients with diabetes have an overproduction of ROS, and it results in the progression of complications [9].

Anti-oxidant therapies have potential value in controlling the detrimental effects of free radicals [10], which cause damage to body cells and results in degenerative diseases [11]. Herbals are used in treating diabetes for their hypoglycemic effects by patients. Anti-oxidant potential effects of such plants have additional effects in controlling the complications among patients with diabetes.

Plants have several medicinally active phytochemicals, including phenolic compounds, which are one of the secondary metabolites of plants. Phenolic compounds account for 45% of secondary phytochemical compounds of plants [12]. Phenolic compounds have different compounds, including flavonoids, phenolic acids, stilbenes, tannins, and coumarins [13]. These phenolic compounds showed potential anti-oxidant potentials [14]. Several studies reported that consuming fruits and vegetables enriched with polyphenols increases the anti-oxidant potential of blood or serum. It is due to increased levels poly phenols in blood [15,16]. The presence of other phytocompounds also could be responsible for anti-oxidant activity by additive and synergistic effects [17].

Nigella sativa [18], *T. foenum graceum* [19], *M. charantia* [20], *G. Sylvestre* [21], *and C. pictus* [22] exhibited different pharmacological activities including anti-diabetic and anti-oxidant activities. These plants are easily found in Sri Lanka, and people use them to treat diabetes in various parts of Sri Lanka. This study compares the total phenolic content and anti-oxidant potential of these plants.

Methods

This study was carried out to determine the total phenolic content and anti-oxidant potential of selected herbals that were used for the treatment of diabetes in Sri Lanka.

Plant materials

Fresh plant parts and seeds of plants stated in Table 1 were collected from the Jaffna District. Plant materials were authenticated in the Department of Botany, University of Jaffna.

Chemicals

Methanol, Desiccant (silica gel), Folin-Ciocalteu, Sodium carbonate, Gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, Dil. Hydrochloric acid, Wagner's reagent, 10% Lead acetate, Ferric chloride solution, Glacial acetic acid, Fehling's solution, Benedicts reagent, 0.2% Ninhydrin, Chloroform and Concentrated sulphuric acid.

Instruments

Electrical grinder, Rotary evaporator, Mechanical

shaker and UV Spectrophotometer (Model - Jenway 6305).

Preparation of plant extracts

Plants were collected and prepared for extraction according to previous studies [23,24]. Plant parts were dried under shade and powdered using a grinder. Dried powder of plant parts was extracted according to a previous study [25]. The maceration method was used for extraction. Fifty grams of pulverized plant parts were soaked individually with 200 mL methanol at room temperature for 24 - 48 hours. A mechanical shaker was used to ensure continuous mixing. The mixtures were filtered using a vacuum filter through Whatman filter paper. Filtrates were collected and then transferred to a rotary evaporator to get the crude extract by evaporating the solvent at 40 - 50 ºC. Then, the extracts were refrigerated at 2 - 4 ºC till further use.

Evaluation of antioxidant activity by DPPH scavenging assay

The free radical scavenging ability of plant extracts was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) [27]. Ten milligrams of DPPH reagent were dissolved in 250 mL of methanol to produce 0.004% (w/v) of DPPH solution, which was then kept at room temperature and in the dark. Control was made by 3 ml of DPPH and 2 mL of methanol. A UV spectrophotometer (Model – Jenway 6305) was used to determine the absorbance of the control at 517 nm against blank (methanol). Each procedure was replicated three times.

30 mg of plant extracts were accurately weighed and dissolved in 30 mL of methanol. Stock solution (1 mg/ml) was diluted with methanol to get 62.5, 125, 250, 500, and 1000 µg/mL concentrations. Then, 3 mL of freshly made DPPH solution (0.004% (w/v)) was added into 2 mL of various concentrations of plant extracts. Then, the resultant solutions were left in the dark for half an hour. A UV spectrophotometer was used to determine the absorbance of the mixtures at 517 nm against methanol as blank. This procedure was replicated three times. From the absorbance measurements of control, ascorbic acid and plant extracts, the Percentage of Inhibition was calculated using the following equation.

$$\% \text{ DPPH radical scavenging activity} = \frac{(Abs_{Control} - Abs_{sample}) \times 100}{(Abs_{Control})}$$

where,

$Abs_{control}$ - absorbance of DPPH + methanol

Abs_{sample} - absorbance of DPPH+Extract/Standard

The standard curve of ascorbic acid and respective percentage inhibition Vs concentration curve of plant extracts were plotted. IC_{50} (inhibitory concentration at 50%) was determined for ascorbic acid and the plant extracts from the graph. IC_{50} of a sample is the amount of extract required to react with 50% of the DPPH radicals.

Table 1: Details of selected plants

Plants	Family	Common names	Plant parts
Momordica charantia	Cucurbitaceae	Bitter gourd/Bitter melon	Fruit
Nigella sativa	Ranunculaceae	Black seed	Seed
Trigonella foenum graceum	Fabaceae	Fenugreek	Seed
Gymnema sylvestre	Asclepiadaceae	Periploca of the woods/ Gurmar	Leaves
Costus pictus	Costaceae	Insulin plant/Spiral ginger	Leaves

A stock solution of ascorbic acid was prepared by dissolving 30 mg of ascorbic acid into 30 mL of methanol. Different concentrations of ascorbic acid solution (3.13, 6.25, 12.50, 25.00, 50.00, 100.00, 200.00 µg/mL) were made using the stock solution. Then, 3 mL of freshly made DPPH solutions (0.004% (w/v)) were added into 2 mL of various concentrations of ascorbic acid solutions. Then, the resultant solutions were kept in the dark for 30 minutes. A UV spectrophotometer was used to determine the absorbance of the mixtures at 517 nm against methanol as blank.

Total phenolic content by folin-ciocalteu method

The Folin-ciocalteu method was used to quantify the phenolic content of the methanolic extracts of plant materials [26]. The Folin-ciocalteu reagent solution was made by mixing 20 mL of the reagent with 180 mL of distilled water. Blank was prepared by mixing 5 mL of Folin ciocalteu reagent, 1 mL of methanol, and 4 mL of 7.5% sodium carbonate solution.

30 mg of each plant extract were accurately weighed and dissolved in 30 mL of methanol individually. Then, 5 mL of prepared Folin-ciocalteu reagent solution was mixed with 1 mL of each plant extract. These mixtures were let to stand at room temperature for 5 minutes. Approximately 4 mL of saturated sodium carbonate was added to these mixtures and were incubated for 30 minutes. Then, the absorbance of these plant extracts was determined at 756 nm in a spectrophotometer. This whole procedure was replicated three times. The phenolic content of plant extracts was determined as Gallic acid equivalents (GAE), or milligrams per gram of dried plant material, using the Gallic acid calibration curve.

Calibration curve of gallic acid

About 100 mg of gallic acid was dissolved in 100 mL of distilled water. Then, by serial dilution, various quantities of gallic acid solutions (3.90, 7.81, 15.62, 31.25, 62.50, 125.00, 250.00 µg/mL) were made. Then, 5 mL of Folin-ciocalteu reagent solution was mixed with 1 mL of different concentrations of gallic acid solutions. The resultant mixtures were let to stand at room temperature for 5 minutes. To these mixtures, approximately 4 mL of sodium carbonate was added, and the resultant mixtures were incubated for half an hour. Then, the absorbance of the blue colour solutions was determined at 756 nm using a UV-Spectrophotometer. This procedure was replicated three times. The calibration curve for gallic acid was constructed by plotting absorbance *vs* concentration.

Statistical analysis

The data was analysed by using SPSS software version 25. All experiments were triplicated and presented as mean with standard deviation. One-way ANOVA was used to determine the significance of anti-oxidant activities between extracts and the standard. A p-value less than 0.05 was considered statistically significant. Total phenolic content and anti-oxidant activity of plant extracts were assessed for correlation using the Pearson correlation test.

Results

Yield percentages for the methanolic extracts of *M. charantia, N. sativa, T. foenum graceum, G. sylvestre* and *C. pictus* were 11.88, 12.46, 5.08, 10.50, and 4.38% respectively.

The total phenolic content and anti-oxidant activity of plants are depicted in Table 2. According to the results, the total phenolic content of the plant was highest in *C. pictus* and lowest in *T. foenum* as tabulated in Table 2. R^2 values of calibration curves of gallic acid and ascorbic acid were found to be 0.0049 and 0.9936 respectively.

The anti-oxidant activity was estimated in terms of IC_{50} value, which is the sample concentration needed to scavenge 50% of DPPH free radical. When the IC_{50} value of a plant, its anti-oxidant

activity, the anti-oxidant activity of plant extracts was highest in *C. pictus* and lowest in *M. charantia* as presented in Table 2.

According to the one-way ANOVA analysis, the anti-oxidant potential of all plant extracts were significantly low compared to the standard ($p<0.001$). Also, there were significant differences in anti-oxidant activities between plant extracts ($p<0.001$). The Pearson correlation analysis revealed no significant correlation ($r=-0.598$, $p=0.287$) between total phenolic content and anti-oxidant activity of plant extracts.

Discussion

Anti-oxidants protect body cells from free radicals, which cause several complications and disease conditions such as diabetes, and cancer [14]. Patients with diabetes have more free radicals due to high blood sugar, and they are more susceptible to several complications. More free radicals are formed due to the auto-oxidation of blood sugar in patients with diabetes [28]. Although the body produces anti-oxidants, it cannot efficiently combat abundant free radicals produced in patients with diabetes [29]. They need anti-oxidant supplements to counteract oxidation stress. Even though several synthetic anti-oxidants are available in the market, they have several adverse effects on continuous usage [30]. Consuming natural anti-oxidants containing fruits and vegetables could effectively combat free radicals with no side effects [31].

Patients with diabetes have consumed several herbals which had demonstrated anti-diabetic activity. The anti-oxidant potential of such herbals was evaluated using the DPPH method, which is the most used method for estimating anti-oxidants and is rapid, simple, and inexpensive [32]. Methanol was used as a solvent for extraction since it is a polar solvent that can extract anti-oxidant components and other phytochemicals [33]. The maceration method was used for extraction since it can extract maximum extractable matter from plant parts [34].

Phenolic compounds have a wide range of activities, such as antibacterial, antihyperlipidemic, anticancer, anti-oxidant, cardioprotective,

Table 2: Total phenolic content and *in vitro* anti-oxidant activity of plant extracts

Sample	Total phenolic content (mg/g GAE)	Anti-oxidant activity IC_{50} (µg/ml)	Significant level of anti-oxidant activity (p value)
Ascorbic acid	--	34.45±0.15	**<0.001**
Momordica charantia	14.70±0.54	1917.89±13.86	**<0.001**
Nigella sativa	20.92±0.31	767.87±2.39	**<0.001**
Trigonella foenum graceum	13.23±0.23	1246.66±10.91	**<0.001**
Gymnema sylvestre	18.04±0.24	813.82±2.08	**<0.001**
Costus pictus	35.72±0.59	729.81±3.28	**<0.001**

N=3 data were represented as mean with standard deviation

neuroprotective, and anti-diabetic properties. By interacting with different free radicals through the transfer of hydrogen atoms, the transfer of a single electron, the sequential proton loss electron transfer, and the chelating of the transition metals, phenolic compounds function as antioxidants [35]. The phenolic contents of tested plants varied from 35.72 to 13.23 mg/g GAE. *C. pictus* had the maximum total phenolic content, followed by *N. sativa* and *G. sylvestre,* while, *M. charantia* had the least phenolic content among tested plants. The total phenolic content obtained in this study was different from other studies done in *C. pictus* [36] *N. sativa* [37]*, G. sylvestre* [38]*, M. charantia* [39] *and T. foenum graceum* [40]. This difference in total phenolic content in different studies could be due to solvent selection, method of extraction, and method of estimation. Further, the origin of plants, growing conditions, harvesting time, storage conditions, and regional variation affect the amount of phenolic compounds present in plants [41].

The antioxidant activity of plant extracts was expressed as an IC_{50} value obtained from DPPH scavenging activity. A low IC_{50} value denotes high anti-oxidant potential [38]. All plant extracts showed significantly low anti-oxidant activity compared to standard ascorbic acid. A significant difference in anti-oxidant activities between plant extracts were observed ($p<0.001$). Plant extracts' anti-oxidant activity ranged from 729.81 to 1917.89 µg/mL. *Costus pictus* showed the highest activity, and *M. charantia* showed lowest activity among the tested plants. All plant extracts showed concentration-dependent activity. It demonstrated the presence of compounds with potential anti-oxidant activity in all tested plants. Further, *G. sylvestre* and *N. sativa* showed similar inhibition patterns at different concentrations. It could be due to the presence of approximately equal phenolic contents (total phenolic content in *G. sylvestre* and *N. sativa* were 18.04 and 20.92 mg/g GAE respectively) in both plants.

The antioxidant potential of plant extracts is mainly due to phenolic compounds [42]. However, in this study, there was no significant correlation between total phenolic content and anti-oxidant potential of plant extracts ($r = -0.598$, $p=0.287$). *C. pictus* had the highest amount of phenolic compounds, which contributed to its higher anti-oxidant potential; while, *M. charantia* had a low quantity of phenolic compounds, resulting in its lower anti-oxidant potential. Even though *N. sativa* and *G. sylvestre* had comparatively low total phenolic compounds among tested plants, they exhibit significantly high ($p=0.001$) anti-oxidant potential. It could be due to the presence of other phytocompounds that might have anti-oxidant potential, and additive and synergistic effects. Further, the polyphenolic content of plants is due to several compounds. Over 8,000 molecules have been reported under phenolic compounds [41]. Such individual compounds showed different levels of anti-oxidant potential. Although some plants have low phenolic compounds, they showed good anti-oxidant potential due to individual compounds with high anti-oxidant potentials related to their chemical structure and anti-oxidant mechanism.

Several experimental, clinical, and epidemiological studies have reported on beneficial effects of anti-oxidants in treating diabetes and its complications [43]. Consumption of anti-oxidants in the natural forms present in fruits, vegetables, and herbals could prevent and control the various complications, especially among patients with diabetes. Numerous herbals have been used to treat diabetes in Sri Lanka. Choosing herbals for diabetes with more anti-oxidant potential could be beneficial to improve the health conditions of patients with diabetes.

Conclusion and Recommendations

Among the selected plants used for diabetes in Sri Lanka, *C. pictus* leaf extract had the highest anti-oxidant activity, and *M. charantia* had the lowest

anti-oxidant activity. *C.pictus* and *N. sativa* have potential to be used effectively to prevent and control complications in patients with diabetes due to their anti-oxidant content.

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