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BIOACTIVE PROPERTIES OF THREE SRI LANKAN MEDICINAL PLANTS AND THEIR CHEMICAL PROFILING

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Summary

In this study methanol extracts of *P. emblica* (Pe), *C. auriculata* (Ca) and *H. indicus* (Hi) were screened for inhibitory activities of enzymes α-amylase and α-glucosidase. Hexane and ethyl acetate fractions of Pe displayed α-amylase and α-glucosidase enzyme inhibitory activity whereas, only the ethyl acetate fractions of Ca and Hi showed good inhibitory activity for both enzymes. In addition, water extracts of these three plants showed a significant inhibition for α-amylase and α-glucosidase enzymes. Results of qualitative phytochemical analysis for methanolic crude extracts revealed the presence of alkaloids, flavonoids, tannins and terpenoids in all three plants whereas saponins and steroids were found only in Ca and Hi. Moreover, all organic extracts of the three plants exhibited good antioxidant activity and hexane extract of Pe showed good inhibitory activity against pathogenic Gram +ve and –ve bacteria. The GCMS profile of the hexane fractions of Pe identified the presence of α-amyrin, β-amyrin, sitosterol and stigmasterol metabolites which may be responsible for the observed high bioactivity.

Keywords: Bioactivity, Medicinal plants, Enzyme activity

Introduction

Diabetes mellitus is one of the major non-communicable diseases currently affecting mankind. Currently, around 2.8% of the world's population suffer from this and it is estimated to reach 5.4% by 2025 (Patel *et al.* 2012). Diabetes mellitus type II has become the most prevalent form of diabetes due to the complications linked with the disease. Effective control of blood glucose level is vital to improve the quality of life of the patients. Currently available anti-diabetic drugs are reported to cause several side effects specially dysfunction of kidneys. Hence, recently there is increased interest on natural anti-diabetic agents of low toxicity (Lee & Jeon 2013).

Throughout decades, medicinal plants have played an important role in the field of health care (Srivastava *et al.* 2011). The, herbal medicine play a vital role in improving the health and quality of life (Ye *et al.* 2010; Grover *et al.* 2002). Moreover, herbal

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supplements can be used as an adjuvant or as favourable alternative therapy for diabetic patients.

Sri Lanka being a tropical country possesses a high diversity especially in plants. The plants have been found to be a good source of phytochemicals carrying diverse biological activities. Currently, only few studies have been accompanied on the active compounds from commonly consumed Sri Lankan medicinal plants. Importantly, there should be more studies to find the natural compounds from plants exhibiting recognizable biological activities for developments of drug leads.

The present study assessed in vitro biological activity with special reference to anti-diabetic activity. The selected medicinal plants are *Aegle marmelos* (beli- flowers), *Aegle marmelos* (beli-fruits), *Aerva lanata* (polpala), *Cassia auriculata* (ranawara), *Coscinium fenestratum* (venival), *Hamides musindicus* (iramusu), *Phyllanthus emblica* (nelli), *Scoparia dulcis* (walkottamalli), *Sida rhombifolia* (babila), and *Tinospora cordifolia* (rasakinda) which are extensively used in Sri Lankan Ayurvedic medicine. First-of-all ten plant extracts were screened for its anti-diabetic activity and among them the plants with high anti-diabetic activity were subjected to further analysis.

Methodology

The selected medicinal plants were purchased from an ayurvedic store in 2017 and were identified and authenticated by the Herbarium unit of the Peradeniya Botanical Gardens, Peradeniya, Kandy, Sri Lanka. The plant samples were washed with running tap water and air dried under shade for one week. The dried sample was visually screened for any fungal contamination. Next the dried plant samples were ground into powder (100 g) and sonicated with methanol (400 ml) for 30 minutes. Solutions were filtered using cotton wool and filtrates were collected. The powders were re-extracted three times. The filtrates were evaporated to dryness using a rotary evaporator at 40^{O}C. All crude extracts were screened for their α-amylase and α-glucosidase inhibitory activity. Crude extracts with good enzyme inhibitory activity were further partitioned using hexane, dichloromethane and ethyl acetate. Each fraction was collected separately and evaporated. Each fraction was further assessed for α-amlylase, and α- glcosidase enzyme inhibition activity, antioxidant activity (against ABTS radicals) and antimicrobial activity.

The α-amylase inhibitory activity of the plant crude extracts was assessed by the glucose oxidase method (GOD) described by Visvanathan *et al.* 2016 and IC50 were calculated. The α-glucosidase inhibitory effect of plant crude extracts was assessed by the method described by Ye *et al.* (2010).

The antimicrobial activity of the plant extracts were assessed using the method described by Napagoda *et al.* 2018 with slight modifications. The minimum inhibitory concentrations (MIC) of the plant extracts were calculated. Staphylococcus aureus (G +ve), Escherichia coli (G –ve) and Pseudomonas aeruginosa (G –ve) were used for the assay. The antimicrobial activity, as well as the minimum inhibitory concentrations (MIC) of plant extracts was determined by the nutrient broth method in 96-well microtitre plates as described by Bussman *et al,* (2010) and Napagoda *et al.,* (2018) with slight modifications.

The ABTS radical scavenging activity of the plant extracts were determined by the method described by Shalaby & Shanab (2013) with some modifications. Trolox was used as the standard reference solution.

Methanolic crude extracts of antidiabetic active plants were tested for alkaloids, flavonoids, tannins, saponnins, terpenoids and steroids (Gul *et al.* 2017; Baba & Malik 2015; Kumar *et al.* 2013; Ramamurthy and Sathiyadevi 2017; Vaghasiya, Dave and Chanda 2011).

Powdered crude extracts of Nelli, Ranawara and Iramusu (2 g) were stirred with diluted HCL (8ml) and then filtered. Filtrates were treated with few drops of Dragondroff's reagent. Formation of orange colour precipitate indicated the presence of alkaloids.

Methanolic plant extracts of Nelli, Ranawara and Iramusu (1ml) were mixed with diluted NaOH (1 ml). Formation of intense blue colour indicated the presence of flavonoids.

Methanolic extracts of Nelli, ranawara and Iramusu (2ml) were mixed with few drops of 1% lead acetate. Formation of yellow colour precipitate indicated the presence of tannins.

The presence of saponins was determined by Frothing test. The crude dry powders of Nelli, Iramusu and Ranawara were vigorously shaken with distilled water and was allowed to stand for 10 min and classified for saponin content as follows: No froth indicate absence of saponins and stable froth more than 1.5 cm indicated the presence of saponins.

Chloroform (2 ml) and concentrated H_2SO_4 were added with the 5ml of methanolic plant crude extracts. Formation of red colour indicated the presence of steroids.

Chloroform (2 ml) was added to 5 ml of methanolic plant extracts of Nelli, Ranawara and Iramusu and evaporated on the water bath and then boiled with 3 ml of concentrated H_2SO_4. A grey colour formed which showed the entity of terpenoids.

To analyze the volatile organic compounds is the hexane extracts, 1 mg of hexane extract residue was dissolved in 2 mL of HPLC grade hexane and filtered through a membrane filter (0.2 um PTFE filter) before analysing using GCMS (Agilent 7820A gas chromatography system coupled to Agilent 5975 series quadrupole mass spectrometer) working on EI mode, Thermo HP-5MS column (30 cm x 250 µm x 0.25 µm). 1 uL of the samples was injected. Analysis was performed at 50°C for 5 min, then (50-250°C) over 35 min using Helium as a carrier gas with a flow of 1.2 mL/min. Compounds were identified using NIST 11.0 library of mass spectra on an Agilent ChemStation software.

Results and Discussion

Enzyme inhibitory activities of tested plants are shown in Table 01. Among ten studied medicinal plants, methanolic extracts of *Phyllanthus emblica* (nelli), *Cassia auriculata* (ranawara), and *Hamidesmus indicus* (iramusu) showed highest inhibition for both α-amylase and α-glucosidase enzyme assays. Nelli showed significant inhibitory activity for α-amylase and α-glucosidase assay with an IC50 value of 21.57±0.14 µg/mL and 16.42±0.23 µg/mL respectively. Ethyl acetate fractions of all three plants exhibited highest inhibition for both enzymes. Moreover, hexane fraction of nelli demonstrated significant inhibition for α-amylase and α-glucosidase assays. Therefore, in nelli both

polar and non polar compounds could be responsible for its bioactivity while in ranawara and iramusu only polar compounds could be responsible for their bioactivity. In addition, all extracts of three plants exhibited good radical scavenging activity.

Hexane extract of nelli showed significant inhibitory activity against three pathogenic Gram +ve and Gram –ve bacteria, while dichloromethane and ethyl acetate fractions of ranawara showed moderate inhibitory activity against pathogenic bacteria while iramusu extracts didn't showed any antimicrobial activity for less than 500 ug/mL. Phytochemical analysis was carried for plants with highest inhibition for α-amylase and α-glucosidase enzyme assays. According to the phytochemical analysis, alkaloids, flavonoids, tannins and terpenoids were present in all three plant extracts whereas saponins and steroids were found only in ranawara and iramusu (Table 2). The GCMS profile of nelli hexane fraction identified the presence of secondary metabolites such as α-amyrin, β-amyrin, sitosterol and stigmasterol metabolites which may be responsible for the observed high bioactivity. Secondary metabolites are naturally occurring chemical compounds, serve as survival functions of the plant and has beneficial uses for humans as drug leads in pharmaceutical industry.

Table 01- Percentage Inhibition Values for Alpha Amylase and α-glucosidase .

Plant	Percentage Inhibition α-amylase for 2000 ppm solution (%)	Percentage Inhibition α-glucosidase for 2000 ppm solution (%)
C. fenestratum	69.52	-
T. cordifolia	71.80	-
S. rhombifolia	76.45	-
S. dulcis	**93.73**	-
A. marmelos (Flower)	**93.26**	-
P. emblica	**96.30**	**97.89**
C. auriculat	**98.10**	**98.46**
H. indicus	**91.19**	**98.99**
A. lanata	71.56	
A. marmelos (Fruit)	71.33	

Table no 02- Phytochemicals Present in *Phyllanthus emblica* (nelli), *Cassia auriculata* (ranawara), and *Hamides musindicus* (iramusu).

Plant	Alkaloids	Flavonoids	Tannins	saponins	steroids	Terpenoids
P. emblica	+	+	+	-	-	+
C.auriculat	+	+	+	+	+	+
H. indicus	+	+	+	+	+	+

Conclusions

Among the studied medicinal plants (ten), Pe, Ca and Hm demonstrated good biological activity by inhibiting both α-amylase and α- glucosidase enzyme. According to the results, both non-polar and polar compounds were responsible for the enzyme inhibitory activity of nelli while moderately and/or high polar compounds were responsible for the enzyme inhibitory activity of ranawara and iramusu. The presence of secondary metabolites α-amyrin, β-amyrin, sitosterol and stigmasterol and tocopherol could be the reason for the observed high bioactive properties of hexane fraction of nelli. Studied three plants, Nelli, Ranawara and Iramusu shows potential to be used as alternative herbal supplements to control diabetes among population after detailed research on their activities.

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