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RESEARCH

Antioxidant, Anti-Diabetic and Anti-Inflammatory Activities of *Passifloara foetida* grown in Sri Lanka

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ABSTRACT

Passiflora foetida (Padagedi) is identified as an underutilized fruit in Sri Lanka, used in traditional medicine for several diseases. The objective of this study was to determine the antioxidant, anti-diabetic, and anti-inflammatory properties of the leaves and fruits of *P. foetida*. Methanol (100 %) was used to prepare the plant extracts. The antioxidant activity was determined using total phenolic content (TPC), total flavonoid content (TFC), total alkaloid content (TAC), 2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging, ferric reducing antioxidant power (FRAP), and oxygen radical absorption capacity (ORAC) assays. The anti-diabetic effects of plant extracts were determined by using anti-glycation and anti-amylase inhibitory assays. Cell viability was analyzed by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay. Based on the findings, the leaf extract of *P. foetida* showed a higher TPC (21.46±1.56 mg GAE/g of extract), TFC (4.25±0.23 mg QE/g of extract), TAC (4.37±6.50 mg/g of extract), DPPH (10.91±1.29 mg TE/g of extract), $ABTS^{+}$ (105.80±18.62 mg TE/g of extract), FRAP (11.85±1.45 mg TE/g of extract), and ORAC (46.23±3.58 mg TE/g of extract) compared to its fruit extract. The anti-amylase and anti-glycation activities were high in the leaf extract of *P. foetida* (30.7% and 77.65 % respectively). The fruit extract of *P. foetida* had the highest cell viability and nitric oxide (NO) production-inhibition in RAW macrophage (264.7 cells) compared to the fruit extract. It can be concluded that *P. foetida* has good therapeutic properties, and could be a potential source for pharmaceuticals and nutraceuticals industries.

INTRODUCTION

Passiflora foetida (common name: wild passion fruits or wild watermelon and locally known as dalbatu or padagedi) is a notable medicinal plant with important phytochemicals. It is a member of the Passifloraceae family that provides therapeutic benefits against many diseases (Casierra-Posada, 2016). The genus *Passiflora* is extremely important for the development of novel medications. *Passiflora foetida* has a long history of usage in traditional medicine for conditions like hypertension, anxiety, diarrhea, issues in intestinal tract, and throat, ear infections, fever, skin diseases, and asthma.

The species originates from South America and distributed to many tropical areas such as Southeast Asia, Hawaii, and South China (Padhye et al., 1963). It is mostly available in riverbanks, dry forest floors, and wayside thickets, covering the top thorny shrubs and also grow near hamlets. *Passiflora edulis*, *P. leschenaultia*, *P. mollissima,* and *P. subpeltata* are reported as the mostly available wild species of this family.

Phytochemicals found in plants are divided into primary and secondary metabolites. Primary metabolites (proteins, carbohydrates, fats, minerals, and vitamins) are needed for the growth and development of plant parts. Secondary metabolites derived from plants are needed for the survival of the plant in its environment. The major group of phytochemicals of *P. foetida* are alkaloids, phenols, glycosides, flavonoids, and cyanogenic compounds [Dhawan et al., 2004; Fernandes et al., 2013; and Saravanan et al., 2014], including β-carbolines, harmala alkaloids, coumarins, maltol, phytosterols, and cyanogenic glycosides. Further, the species have reported to be rich in phenolic compounds, amino acid α-alanine, and organic acids, including butyric, linoleic, formic, oleic, malic, linolenic, myristic, and palmitic acids (Chen et al., 2018) as well as d-fructose, d-glucose and raffinose (Ramaiya et al., 2012).

The plant-derived compounds are thought to provide positive health effects while not being a necessary ingredient in the human diet (Akunyili, 2003). Sasikala et al. (2011) found that this plant has anti-inflammatory activity. Further, studies on *P. foetida* have shown that its extracts have a wide range of exciting therapeutic effects, including antidiarrheal, antiulcerogenic, analgesic, antidepressant, anti-inflammatory, anti-hypertensive, hepatoprotective, anticancer, antibacterial, and antinociceptive effects [Mohanasundari et al., 2007; Sasikala, 2011]. Similar to luteolin and chrysoeriol, that, have strong anti-inflammatory activities, various bioactive chemicals identified from *P. foetida*, particularly flavonoids, have demonstrated significant pharmacological activity.

Nitric oxide (NO) is a key result of inflammation, and the nitric oxide synthases (NOS), which contain inducible NOS (iNOS), endothelial NOS, and neuronal NOS, regulate its synthesis. Interestingl, iNOS is extensively expressed in macrophages, and in some inflammatory and autoimmune illnesses, activating it impact on organ death (Murakami and Ohigashi, 2007). The water extracts and crude extracts of *P. foetida* leaves and fruits have a wide range of health benefits, including antibacterial, antioxidant, and anti-inflammatory activities and can be used to treat symptoms like hysteria, insomnia, headaches, and asthma (Mohansundari, 2007). The *in vitro*, LPS-stimulated RAW 264.7 cells produced less NO when exposed to certain flavonoids, notably flavones like luteolin. The anti-inflammatory and immune regulatory properties of flavonoids may be influenced by the inhibition of NO generation (Nguyena et al., 2015).

The objective of the present study was to determine the phytochemical composition, antioxidant, anti-diabetic, and anti-inflammatory properties of leaves, and fruits of *P. foetida* grown in Sri Lanka with the aim of evaluating the potential for using as a source for natural products.

METHODOLOGY

Collection and preparation of plant materials

The ripened fruits and matured leaves of *P. foetida* were collected from the areas of

Laggala, Pallepola and Dambulla (Matale District, Central Province, Sri Lanka). The plant authentication was done by the Industrial Technology Institute (ITI), Sri Lanka, and voucher specimens (leaves WF 08-1 and fruits WF 08-2) were deposited at the Herbal Technology Section (HTS) at the ITI. The collected leaf and fruit samples of *P. foetida* were cleaned with running tap water followed by distilled water. The fruit pericarp and leaves were dried separately using Heat Pump Dryer (MY Meiya Electric Equipment) at 40 ℃ for 20 h and ground to obtain a fine powder. The powder was vacuum packed and stored at -20℃ for further analyses.

Chemical reagents

Methanol, hexane, ethyl acetate, ethanol, dimethyl sulfoxide (DMSO), gallic acid, Folin-Ciocalteu (FC) reagent, sodium carbonate, 1.2% aluminium chloride, quercetin, monosodium dihydrogen orthophosphate, disodium monohydrogen orthophosphate , sodium chloride, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt ($ABTS^+$), potassium persulphate, sodium acetate tetrahydrate, Glacial acetic acid, ferric chloride, sodium fluorescein, 2,4,6 tripyridyl-s-triazine (TPTZ), 6-hydroxy-2-5-7-8- tetramethylchroman-2carboxylic acid (Trolox), potassium persulphate, and 2, 2′-azobis (2-amidinopropane) dihydrochloride (AAPH) were used for *in vitro* biochemical assays. All chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich, USA.

Preparation of plant extracts

Ten grams of each of dried leaf and fruit samples of *P. foetida* was extracted separately using 100 % methanol in a shaking water bath at 45 ℃, at 60 rpm for 48 h and the excess methanol was evaporated by a rotary evaporator. A known amount of the sample was dissolved in 20% DMSO and phosphate buffer to have final concentration of 100 mg/ml for *in vitro* biochemical analyses. All sample vials were covered with aluminium foil to protect from light.

Estimation of Total Polyphenol Content (TPC)

The total polyphenol content (TPC) of leaf and fruit extracts of *P. foetida* was determined by the Folin-Ciocalteu colorimetric method, described by Singleton and Lamuela-ravents, (1999) with minor modifications using 96 well microplates. Briefly, 110 µL of FC reagent was added to 20 µL of sample and vortexed. Subsequently, 70 µL of 10 % sodium carbonate solution was added and incubated at room temperature for 30 min, and absorbance was measured at 765 nm using a 96-well microplate reader (Spectra Max Plus 384, Molecular Devices, USA). This procedure was repeated three times. Gallic acid was used as the reference standard at different concentrations (16-1000 µg/ml), and the results were presented as milligrams of Gallic acid equivalent per gram of extract (mg GAE/g of extract).

Estimation of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined by aluminium chloride colorimetric method with slight modification using a 96 well microplate (Chandrasekara & Shahidi, 2010). A 100 µL aliquot of the sample was added to 100 µL of 2 % aluminium chloride solution. Subsequently, the mixture was vortexed and incubated at room temperature for 10 min, and absorbance was measured at 415 nm. Methanol was used as a blank. Quercetin was used as a reference standard. The standard graph for quercetin was plotted (concentration vs. absorbance), using five different concentrations (16-1000 µg/ml) with three replicates and the results were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g of extract).

Estimation of Total Alkaloid Content (TAC)

The total alkaloid content was determined using the Bromocresol Green reagent (Mathew George, 2017). Atropine was used as a standard, and chloroform was the primary solvent. The absorbance was measured at 470 nm.

Determination of antioxidant activities of leaf and fruit extracts of *P. foetida*

Antioxidant activities of leaf and fruit extract of *P. foetida* were determined with four different methods: DPPH radical scavenging assay (Blois, 1958), $ABTS^{+}$ radical scavenging activity experiment (Pellegrini et al., 1999), FRAP assay (Benzie and Strain, 1996) and ORAC assay (Tai-Hua Mu and Hong-Nan Sun, 2018). Trolox was used as the reference standard for all these assays. Absorbance was measured using a 96-well microplate reader (Spectra Max Plus 384, Molecular Devices, USA). The ORAC assay was performed using fluorescent microplate reader (SPECTRA max-Gemini EM, Molecular Devices Inc., USA). The results of DPPH, FRAP, and ORAC were expressed as milligrams of trolox equivalent per gram of extract (mg TE/g of extract). The IC_{50} value in DPPH assay was determined by plotting the concentration vs. inhibition percentage.

Determination of anti-diabetic properties of leaf and fruit extracts of *P. foetida*

The anti-diabetic effects of plant extracts were determined by using anti-glycation and anti-amylase inhibitory assays.

Determination of anti-amylase activity of leaf and fruit extracts

In vitro α-amylase inhibition assay was performed by the method described by Oyenihi et al. (2015). The dinitrosalicylic (DNS) method was used to measure the amount of maltose released under the assay conditions. A 40 µL of 1% (w/v) starch solution, 50 µL of the dried leaf or fruit sample, 800 µL of 100 mM sodium acetate buffer solution were mixed well and pre-incubated at 40 °C in a water bath with shaking for 10 min. The mixture was then incubated for an additional 15 min at 40 °C in a shaking water bath for 10 min. 50 µL of α-amylase enzyme (5 g/mL, from porcine pancreas) was added to the mixture and incubated for 15 min at 40 °C and 500 µL DNS reagent was added and boiled for another 5 min. Then the mixture was allowed for cooling in an ice bath. The control and sample negative control were prepared without sample, without enzyme, respectively. The absorbance was read at 540 nm using a 96-well microplate reader. Acarbose was used as a reference standard to plot the standard graph, concentration vs. percentage inhibition. Five different concentrations (ranging from 16 to 1000 µg/ml) of the standard solution were used with three replicates. The anti-amylase activity (percentage of inhibition) was calculated using the Equation 1:

$$Percentage\ of\ inhibition\ (\%) = \frac{[A_C - (A_S - A_b)]}{A_C} \times 100 \quad \text{.....Equation 1}$$

Where,
As = Absorbance of sample at concentration N; Ab = is the absorbance produces by the sample blank; and Ac = absorbance of control (without sample).

Anti-glycation activity assay of leaf and fruit extracts

The *in vitro* α-glucosidase inhibitory activity of leaf and fruit extracts of *P. foetida* were carried out according to the method described by Matsuura et al. (2014) with some modifications. An 80 µL aliquot of 0.1 % Bovine Serum Albumin (BSA) solution (100 mg BSA in 10 ml with 0.2% sodium azide), and 50 mM phosphate buffer (pH 7.4) were added to 360 µL of D - glucose sample (20 % D – Glucose solution in water with 0.2 % sodium azide). The mixture was incubated at 60 °C for 40 h. A 400 µL of the reaction mix was removed and added to 60 µL of 100% (w/v) trichloroacetic acid in 1.5 mL Eppendorf tubes. It was centrifuged at 15,000 rpm at 4 °C for 6 min. The supernatant of the advanced glycation end products-BSA (AGEs-BSA) was removed and dissolved in 1 mL of phosphate buffer saline (pH 10), vortexed until the pellet dissolved completely and the fluorescence intensity was read at excitation and emission wavelengths of 370 nm and 440 nm using a 96-well fluorescence microplate reader (Spectra Max, Gemini EM, Molecular Devices, Inc., USA). The anti-glycation activity (percentage of inhibition) was computed using the Equation 2, and the IC_{50} value was derived, using rutin as the positive control.

$$Percentage\ of\ inhibition\ (\%) = \frac{[F_c - F_b] - [F_s - F_{sb}]}{[F_c - F_b]} \times 100$$. Equation 2

Where,
Fc = Florescence of control;
Fb = Florescence of BSA;
Fs = Florescence of sample; and
Fsb = Florescence of sample blank.

The Herbal Technology Section of the ITI, Sri Lanka has verified the *in vitro* bioassay methods. (ISO: 9005 certified Laboratory).

In vitro assay for Nitric Oxide (NO) inhibitory activity of the fruit extract

The Griess assay was used to measure the inhibition of NO production by the fruit extracts of nine underutilized fruits including *P. foetida*. The fruit extracts of all other selected fruits were prepared by following the same method of preparation and extraction used for *P. foetida*. The Griess assay was applied according to the modified method of Dirsch et al. (1998). Briefly, RAW 264.7 cells were stimulated to produce inflammation using recombinant mouse interferon (IFN) (BD Pharmingen, San Diego, CA, USA) and lipopolysaccharide (LPS) from Escherichia coli (Sigma). Griess reagent [(1% sulfanilamide/0.1% N-(1-naphtyl)], ethylenediamine dihydrochloride in 2.5% H_3PO_4 was mixed with an equal part of the cell-culture medium of control or extract-treated RAW 264.7 cells. The RAW 264.7 cells were treated with extract doses of 100, 10, and 1 µg/mL. In order to determine the percentage of NO inhibition, the color development corresponding to the NO level was measured at 550 nm using a microplate reader (SpectraMax Plus 384, Molecular Devices, Inc., Sunnyvale, CA, USA) using the Equation 3:

$$\text{Percentage of NO inhibition } (\%) = \frac{NO\ level\ of\ control\ cells - NO\ level\ of\ extract\ treated\ cells}{NO\ level\ of\ control} \times 100$$ .....Equation 3

Cytotoxicity assay of the fruit extract

The cell viability was determined by the MTT assay. The fruit extract of nine underutilized fruits including *P. foetida* were tested. The 3T3-L1 cells were seeded at a density of 1 × 10^4 cells/well in 96-well plates for 24 h with complete cellular attachment. The fruit extracts were diluted with medium (RPMI 1640 medium supplemented with 10% fetal bovine bovine serum (FBS), 2 mmol L^{-1} L-glutamine, 100 U mL^{-1} penicillin, and 100 mg mL^{-1} streptomycin) and added to individual wells with three replicates, followed by 48 h of incubation at 37 °C in a humidified atmosphere containing of 5% CO_2. Then, 20 µL MTT (5 mg/mL) was added to the sample medium, followed by 2 h of incubation. A 100 µL of DMSO was added to dissolve the MTT of the formazan complex formed by shaking for 10 min, after removing the liquid in the plate. The optical density was then determined at 540 nm in wavelength. In relation to the viability percentage, which was set to 100%, the proportion of viable cells was determined.

Statistical Analysis.

Statistical analysis was performed using SAS statistical software. All experimental data for leaf and fruit extracts were analyzed by t-test. The mean values of each parameter for leaf and fruit were compared using Tukey multiple comparison test (p = 0.05). All the values were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Total polyphenol content (TPC), total flavonoid content (TFC), total alkaloids content (TAC) of the leaf and fruit extracts of *Passiflora foetida*

There was no significance difference between the TPC and TAC values of leaf and fruit extracts of *P. foetida* (p≥=0.05). The TPC, TFC and TAC values in the leaf extract of *P. foetida*, were numerically higher than those of the fruit extract (Table 1).

In vitro antioxidant activity of leaf and fruit extracts of *P. foetida*

There was no significant difference (p>0.05) of DPPH values between the leaf and fruits of *P. foetida*, however, the fruit extract showed a higher DPPH scavenging activity compared to that of the leaf extract (Table 3).

Table 1. Total Phenolic Content, Total Flavonoid Content, and Total Alkaloid Content of leaf and fruit extracts of *P. foetida*

Plant extract	TPC(mg GAE/g of extract)	TFC(mg QE/g of extract)	TAC(mg /g of extract)
P. foetida leaf extract	21.46±1.56[a]	4.25±0.23[a]	4.37±6.50[a]
P. foetida fruit extract	18.99±1.16[a]	2.50±0.21[b]	3.50±0.15[a]

Results presented as mean ± standard deviation, n=3. The values within each column followed by the same letter are not significantly different at p=0.05. TPC: total phenolic content; TFC: total flavonoid content; TAC: total alkaloid content

Table 2. *In vitro* antioxidant activity of leaf and fruit extracts of *Passiflora foetida*

Plant extract	DPPH (mg TE/g of extract)	FRAP(mg TE/g of extract)	$ABTS^+$(mg TE/g of extract)	ORAC(mg TE/g of extract)
P. foetida leaf extract	10.91±1.29[a]	11.85±1.45[a]	105.80±18.62[a]	46.23±3.58[a]
P. foetida fruit extract	11.13±1.83[a]	6.47±0.31[b]	127.84±14.90[b]	29.90±1.43[b]

Results are presented as mean ± standard deviation, n=3. The values within each column followed by the same letter are no significantly different at p=0.05. ORAC: oxygen radical absorbance capacity; FRAP: ferric reducing antioxidant power; ABTS: ABTS radical scavenging activity; DPPH: DPPH radical scavenging activity

Table 3. The α-amylase inhibitory activity of leaf and fruit extracts of *P. foetida*

Scientific name	Assay Concentration (µg/ml)	Inhibition percentage (%)	
		LE	FE
P. foetida	33.7	NI	NI
	67.5	NI	NI
	135	2.77± 0.02 [a]	1.45±0.71[a]
	270	7.65± 0.05[b]	12.66 ±0.06 [a]
	540	30.70±0.05 [a]	19.46±0.04 [b]

*NI= No inhibition (n=3) Results are expressed as mean ± standard deviation. The values within each raw followed by the same letter are no significantly different at p= 0.05. LE= leaf extract, FE= fruit extract. This table shows the percentage of inhibition of α-amylase at the different concentrations.

Significant differences ($p<0.05$) were observed between the $ABTS^+$, FRAP and ORAC values between the leaf and fruit extracts of *P. foetida*. The $ABTS^+$ activity was significantly ($p=\leq 0.05$) high in the leaf extract with an IC_{50} value compared to that of the fruit extract. The FRAP value was also significantly higher ($p<0.05$) in the leaf extract than that of the fruit extract. These results suggested that the antioxidant activity in leaf extracts of *P. foetida* is significantly higher than that of the fruit extract

The α-amylase inhibitory activity of leaf and fruit extracts of *Passiflora foetida*

The α-amylase inhibitory activity of the fruit extract was higher than that in the leaf extract of *P. foetida* (Table 3). The inhibitory activity was not detected values for the two lowest concentrations used in the assay for both the leaf and fruit extracts. The highest enzyme-inhibitory activity was reported in the leaf extracts at the concentration of 540 µg/ml (30.7%). At the concentration of 270 µg/ml the fruit extract showed a higher α-amylase inhibition than that of the leaf extract. These values showed a poor linear response (data not presented) and therefore, the IC_{50} was not calculated.

The anti-glycation activity of leaf and fruit extracts of *P. foetida*

This anti-glycation activity determined for three assay concentrations of the leaf and fruit extracts of *P. foetida* is shown in Table 4. The leaf extract of *P. foetida* exhibited the highest inhibitory activity at a concentration of 200 µg/ml followed by fruit extract at the same concentration.

Determination of NO production and cell viability

The Griess assay was conducted to assess the anti-inflammatory activity using LPS-induced RAW macrophages and determined the NO production inhibitory and cytotoxicity effects (Lee et al., 2010). 100 µg/mL concentration of *P. foetida* pre-incubated with LPS for 24 h and observed the inhibitory effect of nitric oxide (NO) production level against the macrophage cell line RAW 264.7, in the fruit extracts (Figure 1). The results revealed that the fruit extract of *P. foetida* has significant NO production suppression activity compared to the control (LPS 1 µg/mL treated) and was the same as the blank treatment (without LPS treated) against the macrophage cell line compared to the other fruit extracts. Nine fruit extracts exhibited the least or no cytotoxicity similar to the untreated control cells (Figure 2). The compounds in the fruit extract of *P. foetida* did not affect the viability of RAW 264.7 macrophage cells. The results were similar to that of the study Thi Yen Nguyen et al., (2015) and Park et al., (2018). Characteristic inhibition of NO production could support the anti-inflammatory activity of the human body.

Table 4. Anti-glycation activities of the leaf and fruit extracts of *P. foetida*

Scientific name	Assay Concentration (µg/ml)	Inhibition percentage (%)	
		LE	FE
P. foetida	50	39.98±0.14^{b}	51.50±7.22^{a}
	100	65.20±1.73^{a}	63.46±1.89^{b}
	200	77.65±7.34^{a}	72.64±0.71^{b}

Results presented as mean ± standard deviation, n=3. The values within each raw followed by the same letter are significantly different at p =≤ 0.05. LE= leaf extract, FE= fruit extract. This table shows the percentage of inhibition of α-glucosidase at the different concentrations.

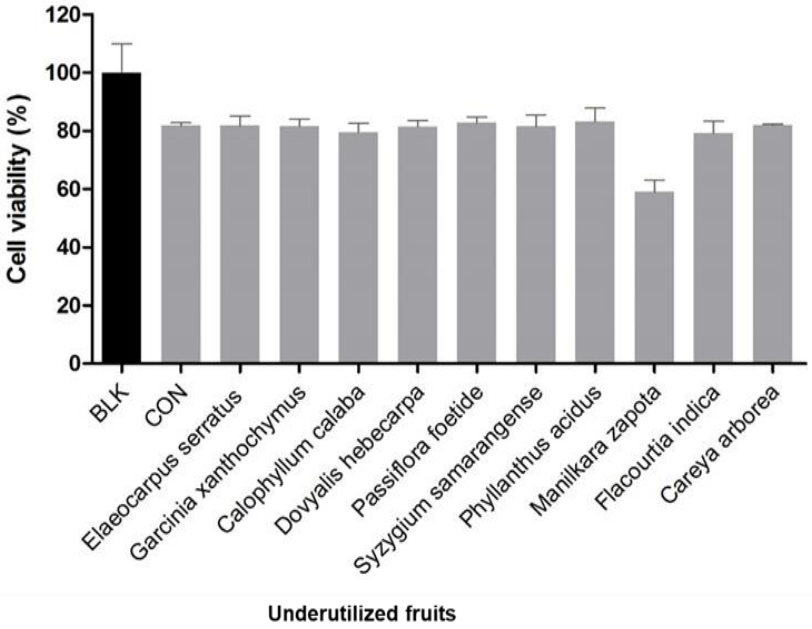

Figure 1. The inhibition Nitric Oxide (NO) production in macrophage cell line, RAW264.7 of fruit extract of underutilized fruits in Sri Lanka

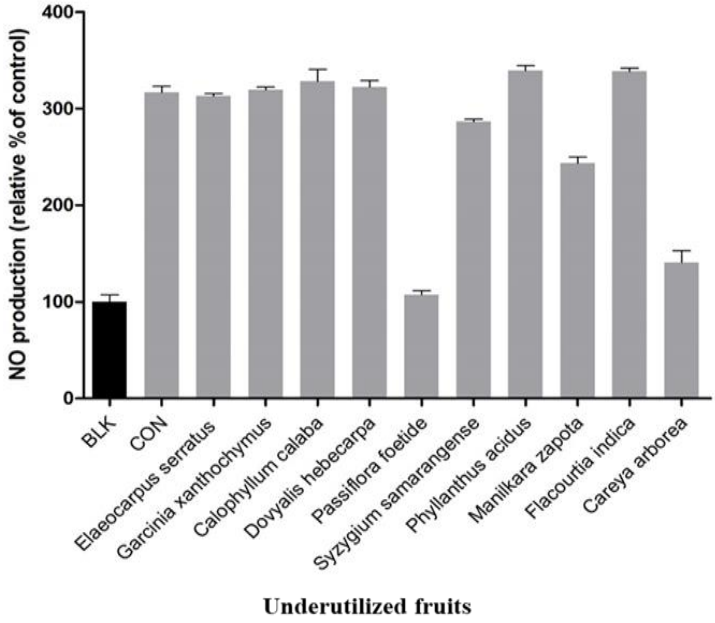

Figure 2. Effect of fruit extracts of underutilized fruits in Sri Lanka on cell viability in RAW 264.7 macrophages determined by the MTT assay

A class of polyphenolic chemicals known as flavonoids have anti-inflammatory, hydrolytic, oxidative enzyme inhibitory, and free-radical scavenging activities. Flavanoids act as scavenging or chelating substances in the body. By transferring a single electron, flavanoids can scavenge two types of free radicals, namely superoxide and hydroxyl radicals. Due to their interactions with different systems of the human body and their anti-inflammatory, hypolipidemic, hypoglycemic, and antioxidant properties, consumption of flavonoids may be helpful to human health. By acting on enzymes and pathways involved in anti-inflammatory processes, plant extracts have the ability to change how cells implicated in inflammatory processes function (Vijayakumar, 2008). The

present study revealed that the leaf extract of *P. foetida* has higher flavonoid contents (4.25±0.23 mg QE/g of extract) compared to that of the fruit extract. Previous studies have shown a variability of the TFC in the methanolic leaf extract (100%) of *P. foetida*; i.e. 10.52±0.59 mg/100 g (Chiavaroli et al., 2020), 205.59±6.57 mg quercetin equivalent/g of sample (Sisin et al., 2014), and 205.59±6.57 mg QE/g of sample (Sisin et al., 2017), which were higher than those reported in the present study.

All plants frequently contain phenolic chemicals, which have a variety of biological effects. This could have a positive impact on human health (Asir et al., 2014). Among the two extracts, the phenolic content was found to be high in leaf extract (21.46±1.56 mg GAE/g of extract). The TPC of hexane extract was 16.78±0.26 mg GAE/100 g of extract. According to Ruflno et al., (2010), values up to 100 mg GAE/100 g show a low concentration of total phenols; values between 100 and 500 mg GAE/100 g represent a moderate concentration of phenols; and values above 500 mg GAE/100g represent a high concentration of phenols. Thus, the present study reported a low amount of total phenol content; the results were below 100 mg GAE/100 g of extract. Chiavaroli et al. (2020) showed the TPC value of the methanolic extract of *P. foetida* was 24.59±0.24 mg GAE/g. According to Sisin, et al. (2014), the TPC of *P. foetida* methanolic (100%) leaf extract was 82.09±13.82 mg GAE/g. The TPC of methanolic leaf extract was 82.09±13.82 mg GAE/g (Sisin et al., 2017), which was higher than the present study.

Fe (III) reduction is frequently utilized as a marker of electron-donating activity. In the reducing power assay, the Fe^{3+}/ferric cyanide complex would be reduced to the ferrous form if reductants (antioxidants) were present in the examined samples (Dorman et al., 2003). A strong reducing power was observed in the leaf extract of P. foetida used in the present study (11.85±1.45 mg TE/g of extract). Chiavaroli et al. (2020) reported that the FRAP of a methanolic extract (100 %) of *P. foetida* was 35.76±1.43 mg TE/g. Thus, the samples used in the present study had relatively lower antioxidant activities compared to values reported previously.

The DPPH and $ABTS^{+}$ are the most common methods used to determine the antioxidant activities of different plant materials. Chiavaroli et al. (2020), reported that, the $ABTS^{+}$ value of the methanolic extract (100%) of the *P. foetida* leaf to be 50.99±0.89 mg TE/100 g of extract. The higher phenolic content of *P. foetida* leaf may be responsible for the high radical scavenging activity in leaves. As they have a free OH group, phenols actively eliminate free radicals and provide defense against oxidative damage (Devaki et al., 2011). The DPPH scavenging activity of a methanolic extract of *P. foetide* (100%) was reported as 28.57±0.36 mg TE/g (Chiavaroli, et al., 2020). Furthermore, Aiane and Patil (2015) reported that DPPH and ABTS of *P. foetida* extract to be 614.405 µg/mL, 25.18 µg/mL respectively. The antioxidant activity of the leaf extracts was generally greater than that of the fruit extract (Tandoro et al., 2020), and the observation in the present study are in agreement with this. The DPPH radical scavenging activity and the FRAP of methanolic leaf extract of *P. foetida* were 1.37±1.17 mg TE/g and 0.41±0.03 mg TE/g, respectively (Sisin et al., 2017), indicating a high antioxidant activity of the samples used in the present study.

Carbohydrates in the diet are the main source of glucose, and thus, enzymes such as α-amylase and α-glucosidase that break down the carbohydrates, in human body are important to avoid a postprandial spike in blood sugar. The inhibition of α-amylase and α-glucosidase enzymes reduces carbohydrate digestion and lowers glucose absorption. This concept is used to manage the type 2 diabetes (Hullatti et al., 2015). Notably, in the present investigation, the highest anti-amylase and anti-glucosidase activities were found in the leaf extract of *P. foetida*. These results are in agreement with Paulraj et al. (2014) and Chiavaroli, et al., (2020) suggesting that, *Passiflora* species are strong inhibitors of glucosidase and amylase. The strong phenolic and flavonoid content and antioxidant activity of this fruit crop were largely responsible for this (Saravanan et al., 2014). The α- amylase inhibitors bind to the alpha bonds of polysaccharides and inhibit the breakdown of

polysaccharides into mono and disaccharides. *Passiflora foetida* showed α-amylase inhibition activity of extracts 83.85±3.63 % for at a concentration of 500 µg/ml extract (Elakya et al*.,* 2020). Interestingly, findings of the present study demonstrated low α-amylase inhibition activity (30.7% for 540 µg/ml extract) and high anti-glucosidase inhibition activity (77.65 ± 7.34% for 200 µg/ml extract) in leaf extracts, which was greater than reported previously (36.73±0.85 % for 200 µg/ml extract) by Elakya et al., (2020).

In the present study, the cytotoxicity effects of fruit extract measured by the MTT assay, and NO production inhibition activity performed using the Griess assay, reported similar results to that of Nguyen et al., (2015). The fruit extract of *P. foetida* had the highest cell viability and NO production inhibition in RAW 264.7 cells *in vitro* compared to other fruit extracts of underutilized fruits. Inhibition of NO production may lead to the anti-inflammatory activity of *P. foetida*.

CONCLUSION

In conclusion, the leaf extract of *P. foetida* shows more total phenolic, alkaloid, and flavonoid contents. Nonetheless, the leaf extract was rich in antioxidant potential and anti-diabetic properties via functioning as an inhibitor of α-amylase, α-glycosidase. The fruit extracts of *P. foetida* had a notable amount of anti-inflammatory activity, inhibiting the NO production in RAW 264.7. Hence, the results revealed that *P. foetida* has considerable levels of phytochemicals and good therapeutic properties. This should lead to further research into how such material would affect human health as potential pharmaceuticals and nutraceuticals.

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