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Anti-oxidative, anti-hyperglycemic and anti-obesity properties of selected edible leafy plants of Sri Lanka

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ABSTRACT

Five selected edible leafy plants of Sri Lanka were studied *in vitro* to assess their anti-oxidative, anti-hyperglycemic and anti-obesity properties. Powdered samples of the leafy plants, namely Le-kola pala (LE), Kora kaha (KK), Koppa (KO), Stevia (ST) and Yaki naran (YK) were extracted with hexane, ethyl acetate (EtOAc) and methanol (MeOH) sequentially. The extracts were evaluated for total phenolic content, antioxidant potential and *in vitro* alpha amylase, alpha-glucosidase and lipase inhibitory activities using relevant assays. The highest alpha-glucosidase inhibitory activity was displayed by YK followed by KO. The highest alpha-amylase inhibitory activity was displayed by LE followed by YK. Among all plant extracts, only LE showed a moderate lipase inhibitory activity. These extracts displayed good antioxidant potential, which correlated well with the enzyme inhibitory activities. This study concluded that the extracts of selected edible leafy plants are a potent source of bioactive compounds that claim various pharmacological properties and can be used as safer alternative remedy for managing diabetes.

1. Introduction

Sri Lanka is a tropical Island nation having about 4143 plant species belonging to 1522 genera and 214 families. About 1025 of these are endemic to the country, whilst the rest of the species have been brought from various parts of the world at different times (Herat, 2007). Plants are a great source of food, medicines, firewood, construction materials etc. Since time immemorial, they are known to provide various phyto-therapeutics to humankind. The traditional medical system called Ayurveda in Sri Lanka dates back to several thousand years and uses medicinal plant preparations in the form of infusions, decoctions, concoctions and ointments to treat or cure diseases. Different parts of plants such as stem bark, flowers, fruits, leaves and roots are employed for various treatments. The polyherbal formulation called paspanguwa, for instance, is used to have five ingredients, namely 'Pathpadagam' (*Hedyotis corymbosa*), 'Veniwalgata' (*Coscinium fenestratum*), Ginger (*Zingiber officinale*), 'Katuwalbatu' (*Solanum xanthocarpum*), and Coriander (*Coriandrum sativum*) to treat illnesses, including fever, cough, cold, headache and overall body aches (Soyza *et al.*, 2017). Plants known as 'Kapukinissa' (*Abelmoschus moschatus* Medik), 'Hathavariya' (*Asparagus falcatus* L.) and 'Katukarandu' (*Barleria prionitis* L.) are used for treating kidney diseases as they possess diuretic effects (Amarasiri *et al.*, 2020). *Argyreia populifolia* Choisy, *Garcinia cambogia* (Gaertn.) Desr., *Hibiscus furcatus* Willd, *Mollugo cerviana* (L.) Ser., *Nyctanthes arbor-tristis* (L.) Gaertn., *Ophiorrhiza mungos* L. and *Pothos scandens* L. are some of the other plants used for treating inflammation-related diseases such as osteoarthritis, rheumatoid arthritis, inflammatory bowel disease, gout, asthma, cardiovascular disease, cancer, and central nervous system-related diseases like depression and Parkinson's disease (Napagoda *et al.*, 2020).

One of the greatest challenges of our time is to confront global prevalence of chronic and non-communicable diseases such as diabetes, obesity, cancers and cardiovascular diseases. Among the non-communicable diseases, diabetes becomes the major health burden worldwide as an estimated 25% of global population is suffering from the disease and its complications (Salehi et al., 2019). People are mostly dependent on synthetic drugs as therapeutics to control these diseases. Use of these synthetic drugs as cure for the diseases is associated with several disadvantages and limitations, which include high cost, drug resistance, adverse side effects etc. Hence, scientific community throughout the world look into plants as safer alternatives for currently available synthetic drugs in treatment of these ailments (Salehi et al., 2019). Occurrence of phyto-constituents such as polyphenols, flavonoids, alkaloids, carotenoids, terpenoids, saponins and glycosides in plants is believed to be responsible for the beneficial biological activities against these diseases.

Generally, consumption of several leafy plants is said to be remarkably effective in controlling diabetes and obesity (Sekhon-Loodu & Rupasinghe, 2019). Edible leafy plants are generally considered as free

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from adverse side effects and rich in phyto-nutrients, which may exert anti-diabetic effect. Hence, there is a pressing need for searching safer phyto-therapeutics for diabetes and obesity. According to traditional medical scriptures, leaves of Kiri anguna (*Tylophora pauciflora),* Thebu (*Costus speciosus*-Thebu), Curry plant *(Murraya koenigii)*, Kowakka (*Coccinia grandis* (L.) J. Voigt), Adhathoda (*Adhathoda vasica*), Mukunuwenna (*Alternanthera sessilis*), Ranawara (*Cassia auriculata*), Gotukola (*Cantella asiatica*) and many other leafy plants are identified as effective in controlling diabetes (Ediriweera & Rathnasooriya, 2009). Aside these, there are some other plants which are lesser known, but believed to have anti-diabetic potential according to traditional knowledge. Among them, Le-kola pala (*Premna procumbens* Moon), Koppa (*Polyscias scutellaria* Fosberg), Stevia *(Stevia rebaudiana* Bertoni),), Yaki naran (*Atlantia ceylanica*), and Kora kaha (*Memecylon umbellatum*) are just a few to mention here. As there is scarcity of scientific evidences for the claimed medicinal properties and phyto-chemical distribution of these plants, it would be worthwhile to assess their anti-hyperglycemic, anti-oxidative and anti-obesity properties through sequential extraction process using hexane, ethyl acetate and methanol as solvents.

2. Materials and methods

2.1. Plant materials

Five leafy plants, namely Le-kola pala (LE), Kora kaha (KK), Koppa (KO), Stevia (ST) and Yaki naran (YK) were evaluated in this study. All plant samples were collected from North Central and Central Provinces of Sri Lanka. Plants (LE, KK, KO, ST and YK) were cross-checked by a botanist and the voucher specimens were deposited in the Popham's Arboretum of NIFS located in Dambulla, Sri Lanka. The collected leave samples were washed with running tap water and dried at 55 ˚C in a forced air drying oven (Biobase, model - BOV-V230F, China) for 8–10 h. The dried leaves were ground into powder form and the powdered leaves were kept under storage at 4 ˚C for further analysis.

2.2. Reagents

Enzymes namely, porcine pancreatic alpha-amylase, alpha-glucosidase (from *Saccharomyces cerevisiae*) and porcine pancreatic lipase were purchased from Sigma-Aldrich. All the other chemicals used in the study were analytical grade unless otherwise specified.

2.3. Preparation of crude extracts

Two hundred grams powdered leaves of individual plant type (LE, KK, KO, ST and YK) was sequentially extracted with hexane, EtOAc and MeOH using ultra-sonication (Rocker ultrasonic cleaner, model-Soner 206H) for 30 min and the extraction was repeated three times for each solvent type. The extracts were concentrated under reduced pressure using a rotor-evaporator (Heidolph, Laborota 4000) under reduced pressure. Finally, the resulted crude extracts were vacuum dried (Vacuum oven, Heraeus instrument, Germany) for 3–4 h. The crude extracts were stored at −18 ˚C until further analysis.

2.4. Determination of total poly-phenolic content

The total phenolic content (TPC) of leafy extracts of edible plants was determined by using Folin-Ciocalteu reagent, according to the methods described by Gunarathne et al. (2022) with slight modifications. Solutions of crude plant extracts were prepared by dissolving 1 mg of sample in 10 mL 3% DMSO for all three extracts. A 50 μL portion of the sample solution was mixed with 15 μL of distilled water and 105 μL of 10% Folin-Ciocalteu reagent in a 96 well micro-plate. After 3 min, 80 μL portion of 7.5% Na_2CO_3 was added and the mixture was incubated for 30 min at room temperature in the dark. The absorbance was measured at 765 nm. The results were expressed as mg of gallic acid equivalent (GAE) per gram of crude extract.

2.5. Evaluation of antioxidant activity

2.5.1. Determination of DPPH radical scavenging activity

The assay was performed according to the method described by Gunarathne et al. (2022) with some modifications. Initially, a concentration series of crude extracts ranging from 3.91 ppm to 2000 ppm were prepared by dissolving the crude extract in 3% DMSO. A 150 μL portion from each concentration was mixed with 60 μL of 0.3 mM DPPH solution in 96 well micro-plate and the mixture was incubated for 30 min in dark at room temperature. The absorbance was measured using plate reader (Synergy HTX Biotek Multimode reader, Biotek instruments, USA) at 517 nm against the control. Ascorbic acid was used as the positive control in this assay. The percentage radical scavenging activity (RSA%) was calculated using the following equation and the IC_{50} values were calculated graphically by plotting percentage of RSA% against the sample concentration of each extract.

$$RSA\% = \frac{\delta A \text{ control } - \delta A \text{sample}}{\delta A \text{ control}} \times 100$$

Where;

$$\delta A_{control} = Absorbance_{control} - Absorbance_{control\ blank},$$
$$\delta A_{sample} = Absorbance_{sample} - Absorbance_{sample\ blank}$$

2.5.2. Determination of ferric reducing antioxidant power

This assay was carried out according to the method described by Gunarathne et al. (2022). As the first step, 150 ppm sample solution was prepared by dissolving crude extracts in 3% DMSO. Then a fifty μL portion of sample solution was mixed with 200 μL FRAP reagent and incubated for 4 min at room temperature. The absorbance values were measured at 593 nm and the results were expressed as μmole of $FeSO_4$ per g of crude extract. The FRAP reagent was prepared by mixing 10 mM TPTZ solution (in 40 mM HCL), 10 mM $FeCl_3{\cdot}6H2O$ solution with 300 mM acetate buffer (pH 3.6) in the ratio of 1:1:10 and subjected to heating up to 37 ˚C just prior to use. In this study, ascorbic acid was used as the positive control.

2.5.3. Determination of $ABTS^+$ radical scavenging activity

The ABTS assay was performed according to the method describe by Marikkar et al. (2016) with some modifications. The stock solution of $ABTS^+$ radical cation was prepared by mixing equal proportions of ABTS (7.8 mM) and potassium persulfate (2.45 mM) at room temperature for 16 h in dark. As the next step, the prepared ABTS working solution was diluted with PBS (pH 7.4) to an absorbance of 0.70 ± 0.02 at 743 nm. In sample preparation, 150 ppm sample solution was prepared by mixing crude extracts in 3% DMSO. A fifty μL portion of sample solution was mixed with 150 μL portion of ABTS working solution. Thereafter, the mixture was incubated for 10 min at room temperature and the absorbance was measured at 734 nm. Ascorbic acid was used as the positive control and the results were expressed as μmole of Trolox per gram of crude extract.

2.6. Evaluation of enzyme inhibitory activity

2.6.1. Determination of α- amylase inhibitory activity

α- amylase inhibitory activity of crude extracts of selected edible leafy plants was performed according to the methods described by Nickavar et al. (2008) and Gunarathne et al. (2022) with slight modifications. As the first step, a concentration series (6000 - 42,000 ppm) of plant extracts were prepared by dissolving the crude extracts in distilled water with 5% DMSO. Then, a fifty μL of each sample solution was mixed with equal amount of α- amylase enzyme solution (20 mg/mL) in a semi centrifuge tube. After incubating the mixture for 30 min at room temperature, a 100 μL portion of 1% starch solution was added into it. Then the mixture was further incubated for another 10 min at room temperature. In the next step, 100 μL portion of DNSA (3,5-dinitrosalicylic

acid) reagent was added into the mixture and incubated for 15 min at 85 °C in a water bath. Thereafter, the final mixture was cooled down to room temperature and diluted with 900 μL of distilled water. A 200 μL portion of final mixture was transferred into the 96 wells microplate and the absorbance was measured at 540 nm. In this study, acarbose (Glucobay tablet) was used as the positive control. The percentage enzyme inhibition was calculated using the following equation and the IC_{50} values were calculated graphically by plotting percentage α- amylase inhibition against the sample concentration of each extract.

$$Percentage\ \alpha - amylase\ inhibition = \frac{\delta A_{control} - \delta A_{sample}}{\delta A_{control}} \times 100$$

Where;

$$\delta A_{control} = Absorbance_{control} - Absorbance_{control\ blank},$$
$$\delta A_{sample} = Absorbance_{sample} - Absorbance_{sample\ blank}$$

2.6.2. Determination of α-glucosidase inhibitory activity

α-glucosidase inhibitory activity of crude extracts of edible leafy plants was determined according to the method described by Gunarathne et al. (2022). Initially, a concentration series (3.91-1000 ppm) of plant extracts were prepared by dissolving the crude extracts in distilled water with 3% DMSO. Thereafter, a hundred μL of 30 mM phosphate buffer (pH 6.5) was added into 96 wells micro-plate, followed by mixing with 25 μL of sample solution. As the next step, 25 μL α-glucosidase enzyme solution (12.5 μL/ mL) was added into it and incubated for 5 min at 37 °C. After that, 50 μL portion of pNPG (*p*-nitrophenyl-α-D-glucopyranoside) solution (0.8 mg /mL) was added and followed by incubating for another 30 min at 37 °C. In the present study, acarbose (Glucobay tablet) was used as the positive control. The absorbance value was measured at 410 nm and the percentage α - glucosidase inhibitory activity was calculated by using the following equation. The IC_{50} values were calculated graphically by plotting percentage α- glucosidase inhibition against the sample concentration of each extract.

$$Percentage\ \alpha - glucosidase\ inhibition = \frac{\delta A_{control} - \delta A_{sample}}{\delta A_{control}} \times 100$$

Where;

$$\delta A_{control} = Absorbance_{control} - Absorbance_{control\ blank}$$
$$\delta A_{sample} = Absorbance_{sample} - Absorbance_{sample\ blank},$$

2.6.3. Determination of lipase inhibitory activity

Lipase inhibitory assay was conducted according to the method described by Chedda et al. (2016) with minor modifications. As the main reagent, 100 mM phosphate buffer (pH 7.4) was prepared with sodium chloride, potassium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate and Triton-X-100. In addition, *p*-NPB working solution was prepared by mixing 10 μL portion of *p*-NPB with 10 mL acetonitrile. Thereafter, a concentration series (19.55 −5000 ppm) of plant extracts was prepared by dissolving the crude extracts in distilled water with 3% DMSO. After that, a hundred μL of phosphate buffer (pH 7.4) was added into 96 wells microplate, followed by mixing with 25 μL of sample solution. Then, 50 μL enzyme solution was added and incubated for 15 min at 37 °C. After that, 25 μL portion of *P*-NPB working solution was mixed and incubated for another 30 min at 37 °C. The absorbance was measured at 400 nm. Orlistat (Orslim tablet) was used as the positive control in this study. The percentage lipase inhibitory activity was calculated by using the following equation. The IC_{50} values were calculated graphically by plotting percentage lipase inhibition against the sample concentration of each extract.

$$Percentage\ lipase\ inhibition = \frac{\delta A_{control} - \delta A_{sample}}{\delta A_{control}} \times 100$$

Where;

$$\delta A_{control} = Absorbance_{control} - Absorbance_{control\ blank}$$
$$\delta A_{sample} = Absorbance_{sample} - Absorbance_{sample\ blank},$$

2.7. Phyto-chemical profiling by LC-MS

Phyto-chemical profiling was conducted by following the LC-MS analysis as reported by Gunarathne et al. (2022) with some modifications. A five g of each leafy plant powder was extracted with 25 mL portion of 70% aqueous methanol (HPLC grade methanol, >99.99% in ultrapure water) using ultra-sonication for 30 min. After that, the extract was subjected to a filtration via a syringe filter (25 mm, 0.45 um). Then, the filtrate was taken to the LC-MS analysis. The system used in the LC-MS analysis was an ultra-high pressure liquid chromatograph (UHPLC) (UltiMate™ 3000, Thermo Scientific, Germany) equipped with a set of equipment, including auto sampler (ACC-3000), quaternary pump (LPG-3400SD) and diode array detector (DAD - 3000), which was able to take signals at 224 nm, 254 nm, 280 nm and 360 nm wavelengths. In order to obtain fragment ion *m/z*, an iron trap mass spectrometer (LCQ FLEET, Thermo Scientific, USA) equipped with electrospray ionization (ESI) source operating in full scan, auto MS^n mode was fitted to the system. From the sample, a 10 μL portion was injected to the system equipped with an Ascentis RP – Amide column (5 μm) (Supelco Analytical 15 cm x 4.6 mm, Merck, Germany). In there, the mobile phase was a mixture of methanol (A) and acidic water comprising 0.01% formic acid (B). The solvents were flowed to the system at a flow rate of 0.400 mL/min in a gradient elution. Where the gradient process was arranged as; 90% of solvent B from 0 to 5 min, 90–2% of solvent B from 5 to 65 min, 2% of solvent B from 65 to 70 min, 2–90% of solvent B from 70 to 75 min, 90% of solvent B from 75 to 80 min. The MS spectra was taken in negative iron mode and the parameters were adjusted as; sheath N_2 gas flow rate, 36 arbitrary units; aux N_2 gas flow rate, 9 arbitrary units; heat temperature, 350 ºC; spray voltage, 4.50 kV; capillary voltage, −40.00 V; capillary temperature, 320 ºC; tube lens, −95.00 V. The mass chromatogram was obtained from 110 to 1500 *m/z*. In this study, ten phenolic compounds were used as authentic standards while calibrating the same setting source parameters for qualitative detection and quantitative analysis.

2.8. Statistical analysis

All measurements in this study were obtained by analysis in triplicate ($n = 3$). The results were presented as mean ± standard deviation (SD). Data were analyzed statistically by one-way ANOVA using Minitab 17 software package. When the F values were significant, mean differences were compared using Tukey's test at the 5% level of probability. The correlation between bioactivities and the strength of the relationship were evaluated by Pearson's linear correlation analysis at the 5% level of probability.

3. Results

3.1. Total poly-phenolic content (TPC)

The TPC of the leaf extracts of the plants are presented in the Table 1. Among the crude extracts, the highest TPCs were found for MeOH extracts while the lowest TPCs were observed for hexane extracts, but with the exception of KO. Among the MeOH extracts, the highest TPC was recorded for LE (150.72 ± 0.58 mg of GAE/ g of extract), while the lowest TPC was recorded for KO (13.54 ± 0.48 mg of GAE/ g of extract). TPC of MeOH extracts were aligned as LE> YK> ST> KK> KO in descending order, showing significant ($p<0.05$) differences among all samples. Among the EtOAc extracts, the highest TPC was observed for YK (36.98 ± 4.68 mg of GAE/ g of extract), whereas the lowest TPC was

Table 1
Total phenolic content (yield within parenthesis) of different crude extracts of edible leafy plants[1].

Leafy Plant Type	TPC (mg of GAE/ g of extract)		
	Hexane (Yield%, w/w)	EtOAc (Yield%, w/w)	MeOH (Yield%, w/w)
LE	$4.72^{a} \pm 0.00$ (0.56)	$17.67^{b} \pm 1.26$ (0.72)	$150.72^{e} \pm 0.58$ (1.34)
KK	$3.06^{a} \pm 0.00$ (1.19)	$9.26^{a} \pm 2.90$ (0.54)	$43.59^{b} \pm 1.17$ (0.98)
KO	$30.36^{c} \pm 1.17$ (1.52)	$20.43^{b} \pm 2.98$ (0.87)	$13.54^{a} \pm 0.48$ (2.42)
ST	$21.53^{b} \pm 1.91$ (1.04)	$23.46^{b} \pm 1.72$ (2.09)	$68.41^{c} \pm 1.65$ (4.05)
YK	$40.01^{d} \pm 2.53$ (0.88)	$36.98^{c} \pm 4.68$ (0.74)	$84.13^{d} \pm 2.34$ (1.28)

[1] Each value in the table represents the mean ± standard deviation. Means within each column sharing different superscripts are significantly (α=0.05) different at 95% confident. Abbreviations: LE, Le-kola pala; KK, Kora kaha; KO, Koppa; ST, Stevia; YK Yaki naran.

Table 2
Distribution of selected phenolic compounds present in MeOH extracts of edible leafy plants[1].

Phenolic compound	Content (mg / 100 g of leaf powder)				
	LE	KK	KO	ST	YK
Caffeic acid	$51.00^{e} \pm 0.01$	$3.40^{c} \pm 0.00$	$0.24^{a} \pm 0.00$	$27.96^{d} \pm 0.01$	$1.69^{b} \pm 0.00$
Caffein	$1.97^{c} \pm 0.00$	$3.44^{d} \pm 0.01$	$0.18^{a} \pm 0.00$	$10.60^{e} \pm 0.01$	$1.20^{b} \pm 0.00$
Catechin	$3.37^{d} \pm 0.00$	$0.88^{b} \pm 0.00$	$0.60^{a} \pm 0.00$	$16.35^{e} \pm 0.01$	$1.64^{c} \pm 0.01$
Chlorogenic acid	$7.14^{d} \pm 0.01$	$2.53^{b} \pm 0.00$	$4.48^{c} \pm 0.01$	$0.42^{a} \pm 0.00$	$10.10^{e} \pm 0.01$
Ferulic acid	$0.90^{c} \pm 0.00$	$0.67^{b} \pm 0.00$	$0.31^{a} \pm 0.00$	$0.94^{d} \pm 0.00$	$1.04^{e} \pm 0.00$
Gallic acid	ND	ND	ND	ND	ND
p-coumaric acid	$29.96^{e} \pm 0.01$	$1.45^{d} \pm 0.00$	$0.37^{b} \pm 0.00$	$0.23^{a} \pm 0.00$	$0.46^{c} \pm 0.00$
Rutin	$519.12^{e} \pm 0.01$	$79.26^{c} \pm 0.00$	$12.25^{a} \pm 0.01$	$34.61^{b} \pm 0.01$	$183.82^{d} \pm 0.01$
Sinapic	$11.66^{d} \pm 0.00$	$0.20^{a} \pm 0.00$	$0.74^{c} \pm 0.01$	$0.67^{b} \pm 0.00$	$13.81^{e} \pm 0.01$
Vanillin	$3.88^{e} \pm 0.00$	$1.29^{d} \pm 0.01$	$0.47^{b} \pm 0.00$	$0.49^{c} \pm 0.00$	$0.38^{a} \pm 0.00$

[1S] Each value in the table represents the mean ± SD of three replicates. The means that do not share a similar superscription letter within the rows are significantly different at 95% confident (α=0.05) Abbreviations: LE, Le-kola pala; KK, Korakaha; KO, Koppa; ST, Stevia; YK, Yaki naran; ND, Not detected.

recorded for KK. No significant (p>0.05) difference was observed among the EtOAc extracts of LE, KO and ST. In the case of hexane extracts, TPC were aligned as YK> KO> ST> LE> KK in descending order. A significantly (p<0.05) higher TPC was recoded for YK (40.01 ± 2.53 mg of GAE/ g of extract) when compared to those of other plant extracts. In these extracts, the lowest TPC was observed for KK (3.06 ± 0.00 mg of GAE/ g of extract), while a non-significant (p>0.05) difference was observed between KK and LE.

3.2. Phenolic profiles of edible leafy plants

The distribution of selected phenolics in MeOH extract of edible leafy plants is presented in Table 2. Out of the ten authentic phenolic standards, nine were detected in all plant extracts. Among the phyto-chemicals, rutin was the most abundant phenolic compound in all leafy plant types. The highest rutin content was observed for LE (519.12 ± 0.01 mg/ 100 g of leaf powder) while the lowest content was displayed by KO (12.25 ± 0.01 mg/ 100 g of leaf powder). The proportions of rutin were significantly (p < 0.05) different among these plant extracts and the values can be aligned in the ascending order of KO < ST < KK < YK < LE. According to the results provided in the Table 2, caffeic acid content of leafy plant types ranged from 51.00 ± 0.01 mg to 0.24 ± 0.00 mg / 100 g of leaf powder, where the highest and the lowest contents were observed in LE and KO, respectively. The amounts of caffeic acid in the plant extracts showed significant (p < 0.05) differences among all five leafy plant types and the values were aligned in the order of KO < YK < KK < ST < LE. As shown in Table 2, p-coumaric acid content varied from 29.96 ± 0.01 mg to 0.23 ± 0.00 mg / 100 g of leaf powder, where the highest and the lowest contents of p-coumaric acid was displayed by LE and ST, respectively. Significant (p < 0.05) differences were observed with regards to p-coumaric acid content among all five leafy plant types and the values can be arranged in the order of ST < KO < YK < KK < LE. The data presented in Table 2 shows the catechin contents of the five edible leafy plants, where the highest and the lowest catechin contents were observed in ST (16.35 ± 0.01 mg/ 100 g of leaf powder) and KO (3.37 ± 0.00 mg/ 100 g of leaf powder), respectively. With regards to catechin content, significant (p < 0.05) differences were observed among the five leafy plant types and the values were aligned in the ascending order of KO < KK < YK < LE < ST. According to Table 2, YK displayed the highest sinapic acid content with a value of 13.81 ± 0.01 mg/ 100 g of leaf powder, while the lowest content was observed for KK (0.20 ± 0.00 mg/ 100 g of leaf powder). Among the five leafy plants, a significant (p<0.05) difference was noticed with regard to sinapic acid content and the values were aligned in the order of KK < ST < KO < LE < YK. When considering the caffeine content of these edible leafy plants, all five plant types exhibited a significant (p< 0.05) difference. According to results, the highest and the lowest caffeine contents were displayed by the ST (10.60 ± 0.01 mg/ 100 g of leaf powder) and KO (0.18 ± 0.00 mg/ 100 g of leaf powder), respectively and the values were aligned in the ascending order of KO < YK < LE < KK < ST. According to Table 2, the highest and the lowest vanillin contents were observed for LE (3.88 ± 0.00 mg/ 100 g of leaf powder) and YK (0.38 ± 0.00 mg/ 100 g of leaf powder), respectively. Among the different plant extracts, there were significant (p < 0.05) differences with regards to vanillin contents and the values were in the order of YK < KO < ST < KK < LE. When considering ferulic acid content of MeOH extract of different plants, it was found to be low in concentrations relative to other phenolic compounds. The highest and the lowest ferulic acid contents were recorded for YK (1.04 ± 0.00 mg/ 100 g of leaf powder) and KO (0.31 ± 0.00 mg/ 100 g of leaf powder), respectively. The ferulic acid content were aligned in the ascending order of KO < KK < LE < ST < YK and the values were significantly (p < 0.05) different from each other.

Table 3
Antioxidant activities of hexane, EtOAc and MeOH crude extracts of edible leafy plants[1].

Assay	Plant Type	Type of Extract		
		Hexane	EtOAc	MeOH
DPPH radical scavenging activity (IC_{50} value/ ppm)	LE	WK	486.33^{b} ±29.83	383.55^{a} ± 28.29
	KK	ND	ND	1676.82^{c} ± 60.73
	KO	777.15 ± 41.99	539.89^{c} ± 16.77	>2000
	ST	WK	547.42^{c} ± 31.27	1696.43^{c} ± 78.79
	YK	1988.48 ± 89.12	158.29^{a} ± 7.18	1498.51^{b} ± 43.81
Ferric reducing antioxidant power (μmole of $FeSO_4$ / g of crude extract)	LE	10.33^{a} ± 0.76	295.66^{d} ± 23.61	2774.33^{e} ± 138.20
	KK	ND	151.46^{b} ± 10.00	299.59^{b} ± 39.80
	KO	ND	90.50^{a} ± 9.08	67.70^{a} ± 8.57
	ST	84.53^{b} ± 7.84	240.03^{c} ± 21.09	567.52^{c} ± 7.49
	YK	496.24^{c} ± 40.17	335.29^{d} ± 30.97	1796.67^{d} ± 29.69
$ABTS^{+}$ radical scavenging activity (μmole of trolox / g of crude extract)	LE	ND	62.08 ± 5.368	1601.29^{e} ± 20.77
	KK	ND	ND	491.54^{c} ± 17.64
	KO	ND	ND	22.73^{a} ± 3.57
	ST	ND	ND	269.60^{b} ± 6.18
	YK	80.54 ± 5.52	135.86 ± 9.03	671.42^{d} ± 23.71

[1] Each value in the table represents mean of three replicates ± standard deviation. Means that do not share a similar superscription letter in the same column are significantly different at 95% confident (α=0.05). Abbreviations: LE, Le-kola pala; KK, Kora kaha; KO, Koppa; ST, Stevia; YK, Yaki naran; ND, Not detected; WK, Weak activity.

3.3. Evaluation of antioxidant activity

3.3.1. DPPH radical scavenging activity

The IC_{50} values for DPPH radical scavenging activity of edible leafy plants are given in Table 3. According to the results, radical scavenging activity of EtOAc extracts of most of the leafy plant types were generally higher when compared to those of MeOH and hexane extracts, except for LE and KK. Among MeOH extracts, the lowest and the highest IC_{50} values were observed for LE (383.55 ± 28.29 ppm) and KO (>2000 ppm), respectively. The IC_{50} values of MeOH extracts were aligned as LE< YK< KK< ST< KO in ascending order. Nevertheless, no significant ($p > 0.05$) difference was noticed between the MeOH extracts of KK and ST. When considering the EtOAc extracts, the lowest IC_{50} value was displayed by YK (158.29 ± 7.18 ppm) and the value was significantly ($p < 0.05$) lower than those of EtOAc extracts of the rest of plant types. Further, no significant ($p > 0.05$) difference was seen between the IC_{50} values of EtOAc extracts of KO and ST. In the case of hexane extracts, KO and YK showed IC_{50} values of 777.15 ± 41.99 ppm and 1988.48 ± 89.12 ppm, respectively. Meanwhile, the hexane extracts of LE and ST showed only a weak antioxidant potential in terms of DPPH radical scavenging activity. In the case of KK, only the MeOH extract displayed the radical scavenging activity. Among all crude extracts, the strongest anti-oxidative property in terms of DPPH radical scavenging activity was displayed by EtOAc extract of YK. When compared to crude plant extracts, ascorbic acid exhibited a strong % RSA with a IC_{50} value of 8.39 ± 0.05 ppm.

3.3.2. Ferric reducing antioxidant power

The data presented in Table 3 shows the results obtained for FRAP assay. In this study, the highest FRAP values were observed for all the MeOH extracts and the lowest values were displayed by the hexane extract except for YK. When considering the MeOH extracts, the extracts were significantly ($p<0.05$) different from each other in terms of FRAP values and the values can be aligned in the ascending order of KO< KK< ST< YK< LE. The highest FRAP value was recorded for LE (2774.33 ± 138.20 μmole of $FeSO_4$ / g of crude extract), while the lowest value was displayed by the KO (67.70 ± 8.57 μmole of $FeSO_4$ / g). Among the EtOAc extracts, the highest FRAP value was observed for YK (335.29 ± 30.97 μmole of $FeSO_4$ / g). LE also showed a higher FRAP value, but the value was not significantly ($p> 0.05$) different from that of YK. Although FRAP values of EtAOc extracts can be aligned in the ascending order of KO < KK < ST < LE < YK, no significant ($p > 0.05$) difference was observed between EtOAc extracts of LE and ST and EtOAc extract of KK and KO. Among the hexane extracts, the highest FRAP value was observed for YK (496.24 ± 40.17 μmole of $FeSO_4$ / g), while no reducing power was displayed by KK and KO. Among all crude extracts, the highest antioxidative property in terms of ferric reducing power was displayed by MeOH extract of LE. The reducing power of each solvent extract of this study was significantly ($p< 0.05$) lower than that of ascorbic acid (10,316.26 ± 19.20 μmole of $FeSO_4$ / g).

3.3.3. $ABTS^{+}$ radical scavenging activity

The data presented in Table 3 shows the results of ABTS assay. The $ABTS^{+}$ radical scavenging values were observed only for the MeOH extracts in most of the plant types. Among MeOH extracts, the highest radical scavenging activity was observed for LE (1601.29 ± 20.77 μmole of trolox/ g) while the lowest value was observed for KO (22.73 ± 3.57 μmole of trolox/ g). The values of MeOH extracts can be aligned as LE< YK< KK< ST< KO in the ascending order. Among EtOAc extracts, radical scavenging activity was shown by YK (135.86 ± 9.03 μmole of trolox/ g) and LE (62.08 ± 5.368 μmole of trolox/ g). In the case of hexane extracts, radical scavenging activity was observed only for YK (80.54 ± 5.52 μmole of trolox/ g). Among all crude extracts, the highest antioxidative ability in terms of ABTS radical scavenging activity was exerted by MeOH extract of LE.When compared to crude plant extracts, ascorbic acid (control) displayed a strong radical scavenging activity with a value of 1783.17 ± 2.62 μmole of trolox/ g.

3.4. Enzyme inhibitory activity

3.4.1. α- amylase inhibitory activity

IC_{50} values of the α-amylase inhibitory activity of the edible leafy plants are given in Table 4. Among all crude extracts, only MeOH extracts displayed strong inhibitory activity against α-amylase. On the other hand, only LE, ST and YK from EtOAc extracts showed inhibitory

Table 4
Enzyme inhibitory activities of hexane, EtOAc and MeOH crude extracts of edible leafy plants[1].

Assay	Plant Type	Type of Extract		
		Hexane	EtOAc	MeOH
α- Amylase inhibitory activity (IC_{50} value/ppm)	LE	ND	2980.30 ^{a} ± 23.54	2083.36^{a} ± 74.41
	KK	ND	ND	4040.49^{d} ± 19.29
	KO	ND	ND	3419.14^{c} ± 45.44
	ST	ND	4434.77^{b} ± 86.91	5438.49^{e} ± 85.16
	YK	ND	4387.32^{b} ± 63.51	2330.52^{b} ± 48.11
α- Glucosidase inhibitory activity (IC_{50} value/ppm)	LE	37.58 ^{d} ± 1.05	30.97^{a} ± 0.45	42.7^{b} ± 2.12
	KK	56.51^{e} ± 1.26	151.50^{c} ± 11.84	ND
	KO	31.72^{c} ± 0.13	425.98^{d} ± 14.13	18.08^{a} ± 0.27
	ST	21.79^{b} ± 0.81	130.85^{c} ± 10.32	225.57^{d} ± 8.85
	YK	7.71^{a} ± 0.40	85.24^{b} ± 2.27	125.14^{c} ± 5.41
Lipase inhibitory activity (IC_{50} value/ppm)	LE	>1000	>2000	864.00 ± 73.15
	KK	>1000	>2000	>1000
	KO	>1000	1369.43 ± 72.38	>1000
	ST	>1000	>2000	>1000
	YK	>1000	>2000	>1000

[1] Each value in the table represents mean of three replicates ± standard deviation. Means that do not share a similar superscription letter in the same column are significantly different at 95% confident (α=0.05). Abbreviations: LE, Le-kola pala; KK, Kora kaha; KO, Koppa; ST, Stevia; YK, Yaki naran; ND, Not detected; WK, Weak activity.

activity. However, all the hexane extracts did not show any inhibitory activity except KK. Among the MeOH extracts, the highest and the lowest inhibitory activities were shown by LE (2083.36 ± 74.41 ppm) and ST (5438.49 ± 85.16 ppm). The IC_{50} values for α-amylase inhibitory activity of MeOH extracts were aligned in the order of LE< YK< KO< KK< ST and the values were significantly (p<0.05) different each other. Similarly, among EtOAc extracts, significantly (p<0.05) higher inhibitory activity was observed for LE (2980.30 ± 23.54 ppm), but no significant (p>0.05) difference was observed between YK and ST. Among all crude extracts, MeOH extract of LE displayed the highest anti-hyperglycemic property in terms of α-amylase nhibitory potential. When compared to all crude plant extracts, acarbose (control) was found to show the strongest α-amylase inhibitory activity with a IC_{50} value of 0.18 ± 0.01 ppm.

3.4.2. α- glucosidase inhibitory activity

The IC_{50} values for α- glucosidase inhibitory activity of selected edible leafy plants are given in Table 4. All crude extracts of leafy plants exerted inhibitory activity against α- glucosidase except MeOH extract of KK. The higher inhibitory potentials (lower IC_{50} values) were exhibited by most of the hexane extracts, except KO and LE. Among hexane extracts, significant (p<0.05) difference was observed for IC_{50} values of inhibitory activity against α- glucosidase enzyme, whereas the highest and the lowest activities were observed for YK (IC_{50} = 7.71 ± 0.40 ppm) and KK (IC_{50} = 56.51 ± 1.26 ppm), respectively. The IC_{50} values of hexane extracts of leafy plants followed the ascending order of YK< ST< KO< LE< KK. Among EtOAc extracts, LE exhibited the strongest inhibitory activity against α- glucosidase with a IC_{50} value of 30.97 ± 0.45 ppm, while the lowest activity was exerted by KO. Among the EtOAc extracts, the IC_{50} values followed the order of LE< YK< ST< KK< KO and no significant (p>0.05) difference was observed between KK and ST. Among MeOH extracts, KO displayed the strongest inhibitory activity (IC_{50} = 18.08 ± 0.27 ppm), while KK (IC_{50} >2000 ppm) exhibited the weakest inhibitory activity. IC_{50} values of MeOH extracts of leafy plants followed the order of KO< LE< YK< ST< KK. Among all crude extracts, hexane extract of YK displayed the highest anti-hyperglycemic property in terms of α- glucosidase inhibitory activity. When compared to all crude plant extracts, acrabose exhibited a weak α-glucosidase inhibitory activity with a IC_{50} value of 386.00 ± 16.03 ppm.

3.4.3. Lipase inhibitory activity

The data given in Table 4 shows the IC_{50} values for lipase inhibitory activity displayed by the leaf extracts. Among the MeOH extracts, LE showed inhibitory activity against lipase with a IC_{50} value of 864.00 ± 73.15 ppm. At 1000 ppm concentration other plants namely, ST, KK, YK and KO showed percentage inhibition of 44.40 ± 2.25%, 41.04 ± 2.58%, 38.35 ± 2.92% and 30.15 ± 2.71%, respectively. It can be suggested that the IC_{50} value of these extracts could be higher than 1000 ppm. Among EtOAc extracts, the highest lipase inhibitory activity was exhibited by KO (55.46 ± 4.88% at 2000 ppm concentration) with a IC_{50} value of 1369.43 ± 72.38 ppm and rest of the other EtOAc extracts showed relatively lower activity. According to these results, the IC_{50} value of all the other extracts could be higher than 2000 ppm. In addition, the results obtained suggested that the IC_{50} values of hexane extracts could be higher than 1000 ppm. Moreover, at the 125 ppm concentration, ST showed the highest inhibition against lipase (45.14 ± 3.15%) while KK (33.76 ± 1.09%), KO (30.34 ± 0.91%), YK (29.47 ± 1.15%) and LE (28.31 ± 0.77%) showed lower inhibitory activity. Among all crude extracts, MeOH extract of LE had the highest anti-obesity property in terms of anti-lipase activity. When compared to all crude extracts, orlistat (control) showed significantly (p< 0.05) higher lipase inhibition with a IC_{50} value of 63.74 ± 1.70 ppm.

3.5. Correlation analysis of bioactivities

Pearson's correlation coefficients (r) for bioactivities of MeOH, EtOAc and hexane extracts are presented in Table 5. Referring to the r values obtained for MeOH extract, TPC showed strong significant (p<0.05) positive correlation with FRAP value (r = +0.951), $ABTS^{+}$

Table 5
Pearson's linear correlation coefficients (r) within TPC, DPPH, FRAP, ABTS, inhibition of α-amylase, inhibition of α-glucosidase and inhibition of lipase of MeOH, EtOAc and hexane extracts of edible leafy plants.

Extract		TPC	DPPH	FRAP	ABTS	α-Amylase	α-Glucosidase
	TPC						
	DPPH	+0.607	–				
	FRAP	+0.951	+0.544	–			
MeOH	ABTS	+0.946	+0.574	+0.929	–		
	α-Amylase	+0.381*	- 0.105*	+0.458*	+0.416*	–	
	α-Glucosidase	+0.156*	+0.194*	+0.046*	−0.167*	−0.185*	–
	Lipase	+0.837	+0.638	+0.678	+0.779	+0.114*	+0.198*
	TPC	–					
	DPPH	+0.618	–				
	FRAP	+0.585	+0.409*	–			
EtOAc	ABTS	+0.73	+0.326*	+0.794	–		
	α-Amylase	+0.639	+0.782	+0.858	+0.645		
	α-Glucosidase	−0.098*	+0.006*	−0.814	−0.518	−0.602	
	Lipase	+0.177*	+0.199*	−0.489*	−0.083*	−0.325*	+0.833
	TPC	–					
	DPPH	+0.806	–				
	FRAP	+0.724	+0.327*	–			
Hexane	ABTS	+0.699	+0.322*	+0.976	–		
	α-Amylase	†	†	†	†	–	
	α-Glucosidase	−0.858	−0.716	−0.794	−0.716	†	
	Lipase	+0.107*	+0.219*	+0.177*	+0.033*	†	−0.555

* No significant correlation ($p>0.05$),.
† Not detected. Abbreviations: TPC, total phenolic content; DPPH, DPPH radical scavenging activity; FRAP, ferric reducing antioxidant power; ABTS, $ABTS^+$ radical scavenging activity.

radical scavenging activity (r = +0.946) and lipase inhibitory activity (r = +0.837). In addition, strong significant ($p<0.05$) positive correlation was observed for DPPH radical scavenging activity (at the 2000 ppm concentration) with TPC (r = +0.607), FRAP (r = +0.544), ABTS (r = +0.574), and lipase inhibitory activity (r = +0.638). Furthermore, FRAP value displayed strong significant ($p<0.05$) positive correlation with $ABTS^+$ radical scavenging activity (r = +0.929) and lipase inhibitory activity (r = +0.678). $ABTS^+$ radical scavenging activity of MeOH extract also showed strong significant ($p<0.05$) correlation with lipase inhibitory activity. However, α-amylase and α-glucosidase inhibitory activities did not show significant ($p>0.05$) correlation with TPC and other bioactivities of MeOH extract.

In the case of EtOAc extracts, strong significant ($p<0.05$) positive correlation was observed within TPC and DPPH radical scavenging activity (at 2000 ppm concentration) (r = +0.618), FRAP value (r = +0.585), $ABTS^+$ radical scavenging activity (r = +0.730) and inhibition of α-amylase (r = +0.639). Unlike the MeOH extracts, both α-amylase (at 7000 ppm concentration) and α-glucosidase inhibitory activities (IC_{50} value) showed significantly ($p<0.05$) strong correlation with most of the bioactivities, where inhibition of α-amylase showed strong correlation with TPC (r= +0.639), DPPH (r= +0.782), FRAP (r= +0.858), $ABTS^+$ (r= +0.645) and inhibition of α-glucosidase (r=−0.602). On the other hand, inhibition of α- glucosidase exhibited strong positive correlation with inhibition of lipase (r = +0.833) and strong negative correlation with inhibition of α-amylase (r=−0.602), FRAP (r = −0.814) and $ABTS^+$(r = −0.518). In addition, FRAP value showed significantly ($p<0.05$) stronger positive correlation with $ABTS^+$ (r = +0.794).

Among the hexane extract, α-glucosidase inhibiting activity showed significantly ($p<0.05$) stronger negative with TPC (r = −0.858), DPPH radical scavenging activity (r = −0.716), FRAP value (r = −0.794), $ABTS^+$ radical scavenging activity (r = −0.716) and inhibition of lipase (r = −0.555). In addition, TPC showed significantly ($p<0.05$) strong positive correlation with DPPH (r = +0.806), FRAP value (r = +0.724) and $ABTS^+$ radical scavenging activity (r = +0.699). On the other hand, significantly ($p<0.05$) strong positive correlation was observed between ABTS and FRAP value (r= +0.976).

4. Discussion

4.1. TPC and phenolic distribution

Polyphenols are an important sub-class of secondary metabolites, which are responsible for most of the biological properties of plants. Based on the data of this study, the TPC of the plant extracts were dependent on the type of plant as well as the extracting medium. This is in accordance with the findings reported previously by Benoite and Vigasini (2021). According to previous studies, polyphenolic compounds are found to reduce risks of vast range of diseases as they possess numerous biological properties, including antioxidant, anti-tumor, anticancer, anti-diabetic, anti-inflammatory activities and cardioprotective effect (Farha et al., 2020; Alam et al., 2022). The present study suggests that LE and YK are potent sources of polyphenolic compounds among other candidates and they are capable of reducing risks of diseases, such as diabetes, cancers, cardiovascular diseases etc.

The TPC of LE, KO and YK were scantily reported in the literature to compare the results of the present study. However, few studies conducted on KK leaves indicated the presence of phytochemicals (Krishnamurthy & Asha, 2011; Killedar et al., 2014). According to Krishnamurthy and Asha (2011), phenols were present in both hot and cold EtOH extracts of KK. In another study, Tamilvannan et al. (2019) estimated the TPC in EtOH, MeOH and EtOAc of KK. The results showed that the highest TPC was observed for MeOH extract. Several studies were performed to evaluate the effect of different factors that affect the TPC of ST leaves. Zaidan et al. (2019) reported that the TPC present in the polar extracts including aqueous, acetone and EtOH of ST were higher than TPC of hexane extract of ST. According to Garcia-Mier et al. (2021) the TPC of ST was also dependent on the type of fertilization method such as organic or conventional. Garcia-Mier et al. (2021) further observed that organic stevia had 33% more TPC than the conventional one. Halim et al. (2019) previously observed that the TPC of ST leaves was dependent on the method of drying. Other than these, various other factors such as source of origin and particle size of dried leaves could also affect the TPC of ST (Grozeva et al., 2015).

Rutin is a common phenolic compound found in most of the fruits and vegetables with promising health benefits (Farha et al., 2020). Caf-

feic acid is another plentiful phenolic compound found in a wide array of food sources including tea, coffee, spices, oils, fruits and vegetables. Caffeic acid has also been known to exert several biological properties, including anti-oxidative, anti- inflammatory, anti-cancer, and neuroprotective properties (Alam et al., 2022). Similar to caffeic acid, p-coumaric acid is also widely distributed among fruits, vegetables, cereals etc. This has been known to be responsible for a range of medicinal properties including anti-oxidative, anti-inflammatory, anti-diabetic, hepato-protective, nephron-protective, neuro-protective, anti-neoplastic and anti-microbial properties (Ferreira et al., 2018).

Catehin is the predominant flavonoid present in the tea leaves and is further classified under the category of flavonoids (Ratnani & Malik, 2022). Catechin is known to possess several medicinal properties, which include anti-oxidative, anti- carcinogenic, anti-inflammatory and anti-ultraviolet effects and normalizing of blood glucose, blood pressure and cholesterol levels (Yu et al., 2010). Sinapic acid is another group of phenolics found in a range of food sources such as rice, wheat, vegetables, citrus fruits, cereals, oil seeds, spices, and vinegar (Pandi & Manickam, 2021). According to previous studies, sinapic acid is known to possess various pharmacological properties, which include anti-oxidative, anti-cancer, anti-diabetic anti-inflammatory, hepato-protective, cardio-protective, neuro-protective, reno-protective, anxiolytic and anti-bacterial properties (Pandi & Manickam, 2021). Caffeine is a distinguished stimulant and a predominant phenolic constituent present in coffee, tea, cocoa and cola (Akomolafe et al., 2017). It is known to possess pharmacological effect as a central nervous system stimulant, cognitive enhancing agent as well as an anti-oxidant (Akomolafe et al., 2017). When compared to other phenolic compounds, vanillin and ferulic acid were present in relatively lower concentrations among the leafy plant types used in this study.Vanillin is the principal constituent found in vanilla beans. According to Anand et al. (2019), vanillin is found to possess pharmacological activities against ailments namely, Parkinson's disease, Alzheimer's disease, Huntington's disease, depression and cancers. Ferulic acid is a derivative of caffeic acid, which is widely distributed in various fruits, vegetables, coffee and beer. Ferulic acid is known to exert therapeutic effects against several disease conditions, including diabetes, cancers, cardiovascular diseases and alzheimer's diseases.

4.2. Anti-oxidative properties

Imbalance in free radical generation and elimination inside the body would result in oxidative stress, which is a harmful phenomenon that can adversely affect several cellular structures inside the body (Pizzino et al., 2017). The results of this study suggest the potent antioxidant ability of these plants to maintain the oxidative stress in the body by neutralizing free radicals produced by metabolic processes.

For comparison purpose, there is hardly any information in the literature about the antioxidant potential of LE, KO and YK in terms of radical scavenging activity. However, few studies have been reported on the effect of different factors on the antioxidant potential of ST (Rao et al., 2014). For instance, Halim et al. (2019) stated that the DPPH radical scavenging activity of ST leaves was dependant on either the effect of preservation or the method of drying of leaves. According to another study by Garcia-Mier et al. (2021), the antioxidant activity of ST leaves is also affected by fertilization method either organic or conventional. In the case of KK, relatively few studies have been performed (Tamilvannan et al., 2019). The highest antioxidant activity was observed in MeOH extract and EtOH extract of KK leaves when compared to the extracts of non-polar solvents (Anbukkarasi et al., 2014). These observations suggested that the antioxidant potential of KK could be due to polar compounds present in KK leaves. Separately, Bharathi et al. (2016) stated that the antioxidant potential of KK was due to umbelactone, a ketone group present in KK.

Based on the findings of the present study, the antioxidant activity of leafy plants in terms of FRAP values was dependant on the plant-type and the extracting solvent. While studying *in vitro* antioxidant activities of ST leaves and callus Tadhani et al. (2007) observed that the highest antioxidant activity was for aqueous extract of ST leaves (38.24 ± 0.36 mg of trolox equivalent/ g of sample) and the results were roughly similar to the FRAP value for MeOH extract of ST leaves (37.40 ± 1.58 mg of trolox equivalent/ g). The antioxidant activity by means of FRAP value is also dependent on the type of drying treatments; the fresh leaves of ST was found to show significantly (p<0.05) higher antioxidant activity than dried ST leaves (Halim et al., 2019). In addition, Gaweł-Bęben et al. (2015) reported that the highest ability to chelate $Fe^{2+}$was observed by for 96% EtOH extract of ST with a IC_{50} value of 2.08 μg flavonoids/ mL. According to another study on KK, significantly (p<0.05) higher reducing power was reported for MeOH extract with a IC_{50} value of 42.23 μg/ml (Tamilvannan et al., 2019). Other than these, there is hardly any literature on FRAP ability of LE, KO, YK and KK to compare the results of the present study.

Previously, Garcia-Mier et al. (2021) found that fertilization methods such as organic and conventional could have influence on the antioxidant activity of ST. No significant (p>0.05) difference was observed in $ABTS^{+}$radical scavenging activity with respect to the mode of fertilization. Shailasree et al. (2013) evaluated the antioxidant activity of *Memecylon* species and reported that $ABTS^{+}$radical scavenging activity of KK with a IC_{50} value of 2.3 ± 0.22 mg/mL. Some other studies showed that MeOH, petroleum ether and chloroform extracts of KK exerted remarkable antioxidant capacity by means of $ABTS^{+}$radical scavenging activity in a dose dependent manner. Other than these, availability of the literature with regards to $ABTS^{+}$ radical scavenging activity of LE, KO and YK is scarce.

4.3. Anti-hyperglycemic properties

The findings of the present study indicated that the consumption of these leafy plants could be an effective alternative remedy to control hyperglycemia by reducing carbohydrate diagestion in the body by inhibiting α-amylase. Few studies have been conducted previously to investigate the *in vivo* anti-hyperglycemic effect of KK leaves. According to Amalraj and Ignacimuthu (1998), oral administration of EtOH extract of KK leaves tend to reduce significantly (p< 0.001) the serum glucose levels of normal and alloxan-induced diabetic mice. After evaluating the anti-diabetic and anti-obesity effect of KK leaf extracts in high-fat-diet induced obese mice, Sunil et al. (2017) reported a significant (p< 0.001) reduction in the fasting blood glucose level of obese mice after 8th week, when compared to the untreated high-fat-diet induced obese mice. Some further studies have been performed in the past to evaluate the *in vitro* α- amylase inhibitory activity of ST. According to Noreen et al. (2020), among the different fractions of ST extracted with aqueous, ethanol, methanol, chloroform, ethyl acetate, n-hexane and n-butanol as solvents, the highest α- amylase inhibitory activity was observed for aqueous fraction and the inhibitory activity of other solvent fractions was tended to follow the descending order of chloroform > ethyl acetate > n-hexane > n-butanol> ethanol > methanol. In another study, Ruiz-Ruiz et al. (2015) observed remarkable α- amylase inhibitory activity for aqueous extract of ST with a IC_{50} value of 198.40 μg/mL. In addition to these *in vitro* studies, several *in vivo* studies were conducted to evaluate anti- hyperglycemic effect of ST leaves. Hossain et al. (2011) evaluated EtOH crude extract of leaves obtained through cold extraction process and subjected to fractionation using solvents such as petroleum ether, EtOAc and chloroform. A significant (p<0.05) reduction was observed in the blood glucose levels of hyperglycemic rats by different fractions of EtOH extract of ST leaves. According to another report, medium polar leaf extract of ST was responsible for significant (p<0.05) reduction in the blood glucose level of alloxan-induced diabetic rats (Misra et al., 2011). According to Kujur et al. (2010), ST extracts of aqueous, ether and MeOH were responsible for decreasing blood glucose level in diabetic rats. This was further confirmed by Ahmad and Ahmad (2018) who suggested that the aqueous extract of ST could significantly (p<0.05) re-

duce the random blood glucose level and fasting blood glucose level of diabetic rats with reference to diabetic and non-diabetic control groups after 8th week of administration. Despite all these, there was hardly any literature on α- amylase inhibitory activity of LE, KO, YK leaves in order to compare with the results of the present study.

The results further proved that consumption of these leafy plants could be effective in managing diabetes by depleting carbohydrate digestion process by inhibiting α-glucosidase. According to Benoite and Vigasini (2021), EtOH extract of ST exhibited the α- glucosidase inhibitory activity with a IC_{50} value of 33.70±1.00 μg/mL. In contrast to this, no inhibitory activity was observed by Zaidan et al. (2019) for aqueous, MeOH, EtOH and acetone extracts of ST. Apart from these, several polyherbal formulations were also studied where ST leaves was incorporated as one the major components (Patil et al., 2012). In the case of KK, beta amyrin isolated from KK was evaluated for the α-glucosidase inhibitory potential (Sridevi et al., 2015). According to the results, at 10.0 mM dose level, beta- amyrin exerted inhibitory activity at V_{max}1.614 ± 0.1825 and K_m 3.588 ± 0.6432. However, availability of literature related to the α- glucosidase inhibitory activity of LE, KO, YK leaves has been scares to compare the results obtained in this study.

4.4. Anti-obesity properties

The findings of present study suggest that lipase inhibitory effects of these leafy plants are generally low and dependant on the plant type as well as the extracting solvent. However, the results indicate that consumption of LE could be effective in managing obesity by depleting lipid digestion process in the body. Except the study on the *in vivo* anti-hyperlipidamic potential of ST (Ahmad et al., 2018), there is hardly any literature with regard to the *in vitro* lipase inhibitory potential of these leafy plants for comparison purpose.

4.5. Significance of correlation analysis

The results of the correlation analysis suggest that the phenolic compounds present in these plant extracts have a direct influence on the ferric reducing power and the DPPH and ABTS radical scavenging activities. The results of the correlation analysis further indicated that the α-amylase inhibitory activity of EtOAc extracts and α-glucosidase inhibitory activity of hexane extracts could be due to their ferric reducing power, and the DPPH and ABTS radical scavenging activities. Apart from these, correlation analysis strongly indicated that the observed anti-lipase activity of MeOH extracts could have been caused by the phenolic constituents present in the MeOH extracts of these plants.

5. Conclusions

The five leafy plants selected in this study were found to possess anti-oxidative, anti-hyperglycemic and anti-obesity properties in varying proportions. They had the ability to play the major role as antioxidants as well as anti-hyperglycemic agents. The TPC and biological activities of plant extracts were generally dependent on the plant-type and polarity of the extracting solvent. According to UHPLC phyto-chemical profiling of the extracts of these plants, they were rich sources of phenolic compounds, with rutin as the most predominant. Among all crude extracts of plants, the highest TPC was detected in MeOH extract of LE. The highest antioxidative ability in terms of ABTS radical scavenging activity and FRAP value was exhibited by MeOH extract of LE. Among rest of the plants, the strongest anti-oxidative potential in terms of DPPH radical scavenging activity was exhibited by EtOAc extract of YK. The highest anti-hyperglycemic potential in terms of α- amylase inhibition was displayed by MeOH extract of LE. Among rest of the plants, highest anti-hyperglycemic potential in terms of α-glucosidase inhibitiory activity was displayed by hexane extract of YK. With regard to anti-obesity property, the highest anti-lipase activity was exhibited by MeOH extract of LE. The overall findings of this study suggested that these plant extracts would be useful for development of plant derived pharmaceuticals for management and control of diabetes.

Author statement

SU performed the experiment, analyzed, interpreted the data and prepared the manuscript. NM provided the concept, design of the experiment and edited the manuscript. LJ reviewed and edited the manuscript

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data Availability

Data will be made available on request.

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