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Dynamic Bioactivity of Ceylon Gooseberry *(Dovyalis hebecarpa)* Fruit across Maturity Stages: Phytochemicals, Antioxidants and Anti-inflammatory Potential

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Abstract

The under-utilized fruit plant, Ceylon gooseberry *(Dovyalis hebecarpa)* has significant medicinal and commercial potential that is yet to be explored. This study aimed to address an existing research gap by comparing the levels of phenols, flavonoids, antioxidant and anti-inflammatory properties of Ceylon gooseberry fruit at three maturity stages; near-ripe, ripe and over-ripe. Freeze-dried fruit samples were extracted using 80% methanol through a 72-hour maceration process. Total contents of phenols and flavonoids were measured respectively by Folin-Ciocalteu and Aluminum chloride methods. The antioxidant activities of the extracts were evaluated using the DPPH and ABTS assays, with ascorbic acid as the positive control. The anti-inflammatory activity was determined by the HRBC and protein denaturation assays, with ibuprofen as the positive control. Among the three extracts, the highest TPC was demonstrated by the near-ripe stage, as 2.0138 ± 0.7143 mg GAE/g. The maximum TFC value was also depicted by the near-ripe extract as 0.2104 ± 0.0049 mg QE/g. From the DPPH assay, the most potent IC_{50} value was obtained as 0.3085 mg/mL from the near-ripe extract, while the most potent IC_{50} value from the ABTS assay, 0.1293 mg/mL was also recorded by the near-ripe extract. The near-ripe extract exhibited the lowest IC_{50} value in the HRBC assay, measuring 0.0547 mg/mL. In the protein denaturation assay, the minimum IC_{50} value of 0.0965 mg/mL was observed for the near-ripe extract. The study indicates that Ceylon gooseberry fruit possesses an impressive phytochemical content, antioxidant and anti-inflammatory properties with optimal benefits when consumed at the near-ripe stage.

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1.0 Introduction

Sri Lanka is regarded as one of the biodiversity hotspots in the world (Abeysuriya, Bulugahapitiya and Jayatissa, 2021; Perera *et al.*, 2022). It has been blessed with a wide range of fruits, although several still remain under-utilized. In Sri Lanka, these fruits are mostly only sold at village markets (Perera *et al.*, 2022). There is a dearth of studies on under- utilized crops in Sri Lanka (Bandula and Nath, 2020). The selected test plant, named *Dovyalis hebecarpa* or Ceylon gooseberry or Ketambilla is one out of the over sixty species of under-utilized fruit plants available in Sri Lanka, and it is a fruit plant that is native to Sri Lanka and South India (Gunathilake and Ranaweera, 2016; Perera *et al.*, 2022). The fruit is a deep purple exotic berry. The Ceylon gooseberry has an international market where it is sold at a high

cost, due to the extreme colour, appealing taste and charming appearance. The fruit is marketed to be consumed as fresh fruit or to be used in the production of juices and jams. It is known that Ceylon gooseberry fruit intake will ensure protection from harmful free radicals to the human body, through the plant bioactive compounds available. Plants are a reservoir of numerous phytochemicals, including phenols, flavonoids, alkaloids, tannins etc.The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. Phenolic compounds (PCs) are secondary products of bioactive molecules, which are mostly natural, produced in plants mainly in plant tissues. These are also antioxidant, antimicrobial, anti-inflammatory and antiproliferative and are useful in different industries. PCs have a role to play in pigmentation of plants, astringency and UV protection as well as parasites and insect protection. They are found in a variety of sources such as fruits and vegetables (Albuquerque *et al.*, 2021).

To date, more than 4,500 different flavonoids have been identified (Harborne and Williams, 2000). They are a larger category of phenolic compounds. Flavonoids have been discovered in a lot of plant tissues, including plant cell vacuoles. Flavonoids are available as monomers, dimers and oligomers. In plants, some flavonoids may serve a protective function for the plant from UV radiation. Flavonoids comprise several plant metabolites such as flavanones, flavones, flavonols, isoflavonoids, aurines, chalcones, catechins and anthocyanins (Tiwari and Rana, 2015). Flavonoids are also known as Vitamin P in plants (Rebaya *et al.*, 2015).

Flavonoids are produced from phenylalanine. They are pigments of plants that correspond to mesmerizing colours that are exhibited in flowers all over the world. Plants that contain a variety of secondary metabolites, such as phenolics, flavonoids, and carotenoids exhibit antioxidant activity because of their chemical structures and redox properties (Chakraborty *et al.*, 2015). Antioxidants are referred as "any substance that when present at relatively low concentrations, compared with those of the oxidizable substrate, significantly delays or inhibits oxidation of that substrate" (Halliwell *et al.*, 1995). Sri Lankans relied on indigenous medicine and a complete balanced diet as the provider of anti-oxidant compounds ever since civilization. The process of oxidation is inhibited by antioxidants even when available at very low concentrations. They have a multifaceted activity in the body. The plant derived antioxidants aid in the transformation of free radicals to low reactivity or non-radical compounds (Yadav, Kumari, Yadav, J P Mishra, *et al.*, 2016). Our body has a protective system against reactive oxygen species, where antioxidants play an important and indispensable character (Ou *et al.*, 2002). The secret behind enjoying a maximum health and wellbeing is our first line of protection which is fulfilled by the antioxidants (Yadav, Kumari, Yadav, J.P. Mishra, *et al.*, 2016). Several studies set witness to the fact that a diet with plenty of antioxidants provides a plus impact on the health in long term (Sin, Liu and Lam, 2013).

Beyond their antioxidant roles, phenolic compounds and flavonoids possess notable anti-inflammatory properties, which arise from their ability to modulate inflammatory pathways and mediators. These compounds inhibit key enzymes involved in inflammation, such as cyclooxygenase (COX), lipoxygenase (LOX), and phospholipase A2, thereby reducing the production of pro-inflammatory prostaglandins, leukotrienes, and arachidonic acid derivatives (Al-Khayri *et al.*, 2022). Flavonoids, in particular, exert anti-inflammatory effects by suppressing the activation of nuclear factor-kappa B (NF-κB), a transcription factor that regulates the expression of genes encoding cytokines like tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), as well as inducible nitric oxide synthase (iNOS) and COX-2 (Liu *et al.*, 2023). Additionally, phenolics scavenge reactive oxygen and nitrogen species that amplify inflammatory responses, stabilize cell membranes, and inhibit histamine release from mast cells, collectively mitigating acute and chronic inflammation (Lopez-Corona *et al.*, 2022). In fruits, these bioactive molecules contribute to anti-inflammatory potential by interfering with oxidative stress signalling and downregulating pro-inflammatory pathways, making them promising agents for preventing inflammation-related disorders such as arthritis, cardiovascular diseases, and autoimmune conditions. Literature reports studies conducted to investigate the presence of a few different phytochemicals, such as polyphenols, anthocyanins etc., in Ceylon gooseberry fruits. In one of the studies, the extraction of polyphenols in Ceylon gooseberry pulp was optimized, and phenolic acids and flavonoids with high concentration and high antioxidant capacities were detected. The possibility of developing the fruit into a good source of bioactive compounds to be used in functional foods was pointed out (Bochi *et al.*, 2014). Ceylon gooseberry was characterized to have anthocyanins, especially cyanidin derivatives with high antioxidant activity, having been determined by DPPH and Oxygen Radical Absorbance Capacity (ORAC) assays.

In the study, the mutual possibility of fruit use as a natural food colorant and health-enhancing component was underlined (Bochi *et al.*, 2015). This paper evaluated the physicochemical and bioactive compounds, as well as polyphenols and anthocyanins in the fruits of Ceylon gooseberry, with good antioxidant and antimicrobial properties being identified. It was proposed that the fruit can be used pharmaceutically and in the food industry (Rodrigues *et al.*, 2022). This study aims to address the absence of a comparative study on phytochemical and antioxidant capacity of three different maturity stages of the whole fruit of Ceylon gooseberry.

This analytical, comparative investigation into the phytochemical content and antioxidant capacity of Ceylon gooseberry fruits at three maturity stages (near-ripe, ripe and over-ripe) would fill a research gap in determining the level of bioactive compound content in the fruits at each maturity stage so that at the most appropriate stages, the fruit could be harvested with the maximum available level of bioactive compounds and therefore, be better used in functional foods and nutraceuticals.

2.0 Materials and Methods

2.1 Collection of samples

The Ceylon gooseberry plant was authenticated by the Bandaranaike Memorial Ayurvedic Research Institute, and a voucher specimen (Accession No: 3427) was deposited for future reference. Collected fruit samples were pooled and subsequently sorted into three maturity stages Figure 1 based on physical appearance, texture, and firmness. Observable ripening characteristics were used to define each stage. The Near-ripe stage was characterized by a firm texture and predominantly green-orange epicarp. The Ripe stage exhibited mixed orange-purple pigmentation with moderate softness. The Over-ripe stage displayed a uniform deep purple colour and visibly softened pulp. For each maturity stage, 60 healthy, disease-free fruits were selected and divided into three independent biological replicates (n = 3).

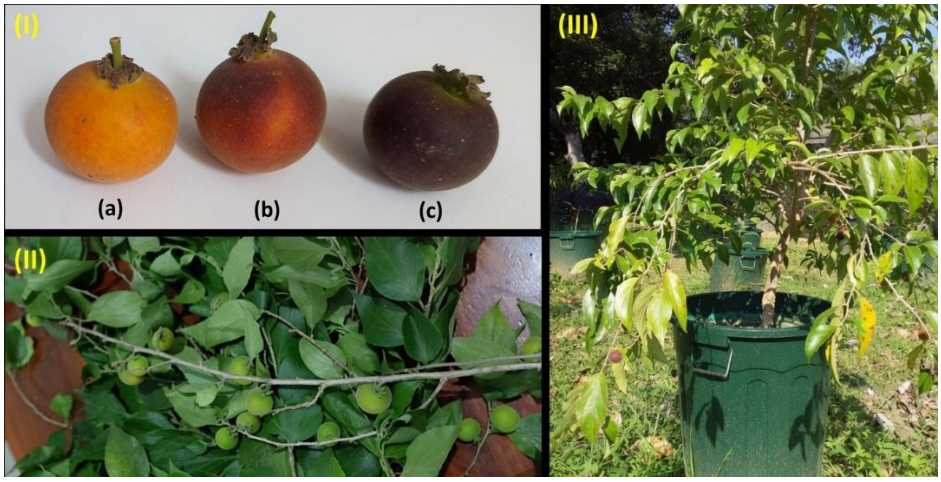

Figure 1- Ceylon gooseberry (*Dovyalis hebecarpa*); (I) -Three maturity stages of fruits (a- Near-ripe, b- Ripe, c- Over-ripe), (II) - Branches bearing fruits, (III) - The whole plant

2.2 Sample preparation

The whole fruits were washed, cleaned, and cut into small pieces. The samples were stored overnight in a -20 ºC freezer before freeze-drying for complete water removal. The freeze-dried fruit samples were powdered using a mechanical grinder, sieved and homogenized. A mass of 10 g from fruit samples in each maturity stage was separately extracted in methanol (80%) by maceration for 72 hours while shaking continuously at 120 rpm. Samples were weighed using an analytical balance (±0.001 g) accuracy. The extracts were filtered using Whatman filter paper No.1 (125 mm). The methanolic extract filtrates were evaporated under reduced pressure using a rotary evaporator at 40 °C to obtain a concentrated extract (Sasikala and Sundaraganapathy, 2017). The remaining methanol was evaporated by nitrogen purging and left open for an adequate time in the fume hood for further evaporation of the rest of the methanol.

2.3 Total phenol content

The total phenol content was assessed using the Folin-Ciocalteu method with a slight modification to the procedure of (Safdar *et al.*, 2016). A mass of 10 mg crude extract was dissolved in 1 mL of methanol and vortexed to ensure complete dissolution. From this stock, 100 µL of the sample was added to another eppendorf tube. To this, 400 µL of 7.5% Sodium carbonate and 500 µL of 10% Folin reagent were added. The samples were incubated in dark conditions for 15 minutes at room temperature. From this 200 µL was added to the well in a 96 well plate. Gallic acid was used as the standard. Absorbance was measured at 765 nm using a microplate reader. The assay was triplicated to enhance accuracy.

2.4 Total flavonoid content

The total flavonoid content was determined using the Aluminum chloride method, with a modification to the procedure of (Atanassova and Georgieva, 2011). A 2 mg portion of crude extract was dissolved in 1 mL of methanol by vortexing to ensure complete dissolution. From this solution, 500 µL was transferred to a separate eppendorf tube. To this, 500 µL of 2% Aluminium chloride solution was added. The samples were then incubated in darkness at room temperature for 1 hour. Subsequently, 200 µL of the solution was transferred to wells in a 96-well plate. Quercetin was used as the standard. Absorbance was measured at 405 nm using a microplate reader. The assay was performed in triplicate to improve accuracy.

2.5 DPPH radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was performed according to a modification of the procedure (Deng, Cheng and Yang, 2011). The DPPH solution was prepared to obtain a 1 mg/ml concentration in the well. The mixture was vortexed for 15 seconds and then left to stand at room temperature for 30 minutes in the dark. Ascorbic acid was used as the standard/positive control. The stock solution for the assay was made by dissolving 1.25 mg of dried crude extract in 1 mL of methanol. A concentration gradient of the extracts (0.03125, 0.0625, 0.125, 0.25, 0.5, 1.0 mg/mL) was assessed. In a 96-well plate, 160 µL of the sample was added to each well containing 40 µL of DPPH. As the control 160 µL of methanol and 40 µL of DPPH was added. As the blank 200 µL of methanol was added. The samples were incubated in the dark at room temperature for 15 minutes. Absorbance was read at 517 nm using a microplate reader. The assay was repeated three times.

2.6 ABTS radical scavenging assay

The ABTS radical scavenging assay was conducted with a modification to the protocol of (Widowati *et al.*, 2017). ABTS solution was prepared to get a 1 mg/ml concentration in the well. Ascorbic acid served as the positive control. For the assay, a stock solution was prepared by dissolving 1 mg of dried crude extract in 1 mL of methanol. Various concentrations of the extracts (ranging from 0.03125 to 1.0 mg/mL) were tested. In a 96-well plate, each well received 20 µL of sample and 180 µL of ABTS solution. Methanol (20 µL) and ABTS solution (180 µL) were used as the control, and methanol (200 µL) only was used as the blank. The samples were incubated in darkness at room temperature for 10-15 minutes. Absorbance was measured at 734 nm using a microplate reader. This assay was repeated thrice.

Percentage inhibition was calculated using the following equation for both radical scavenging assays.

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

IC_{50} values of fruit extracts in DPPH and ABTS assays were calculated by GraphPad Prism software.

2.7 HRBC membrane stabilization assay

A modified Human Red Blood Cell membrane stabilization (HRBC) assay from (Yesmin *et al.*, 2020) was conducted to evaluate potential anti-inflammatory activity through heat-induced haemolysis, where the erythrocyte membrane served as an analogue to the lysosomal membrane, and stabilization against haemolysis indicated possible therapeutic effects. Blood (2 mL) was collected from a healthy human volunteer into an EDTA-coated tube to prevent clotting, with the volunteer having avoided NSAIDs, other medications, or alcohol for at least two weeks prior to ensure no interference with membrane stability; informed consent and institutional ethical approval were obtained beforehand (ERC, FOM, KDU under RP/2025/07). The blood sample was divided equally into two clean centrifuge tubes (1 mL each), centrifuged at 3000 rpm for 10 minutes at room temperature, and the supernatant (plasma) was carefully removed and discarded from each tube.

Sterile saline (500 µL) was added to each tube, mixed gently to resuspend the RBC pellet, centrifuged again at 3000 rpm for 10 minutes, and the supernatant was discarded; this saline washing step was repeated three times to eliminate plasma proteins and contaminants. The washed RBC pellets from both tubes were combined to yield approximately 1 mL of packed RBCs, which was transferred to a clean Falcon tube, and 9 mL of sterile saline was added to create a 1:10 (v/v) RBC dilution, mixed gently by inversion, and stored at 4°C if not used immediately but within the same day. The test sample (0.0012 g) was weighed and transferred to a clean centrifuge tube, dissolved in 200 µL of DMSO with thorough mixing using a stirrer or vortex until fully solubilized, and 800 µL of sterile saline was added to reach a total volume of 1 mL, forming a stock solution. A two-fold serial dilution was prepared from this stock using saline as the diluent to generate a range of concentrations, including positive controls, ibuprofen, and negative controls. For each concentration, 500 µL of the diluted sample was transferred to a clean centrifuge tube, 100 µL of the 1:10 RBC suspension was added and mixed gently, and triplicate samples were prepared for statistical reliability. The tubes were incubated in a water bath at 56°C for 30 minutes to induce heat-mediated haemolysis, removed and cooled to room temperature (approximately 25°C) for 10-15 minutes, and centrifuged at 3000 rpm for 10 minutes to pellet intact RBCs and debris. The supernatant (200 µL) from each tube was transferred to the wells of a 96-well microplate in triplicate, with a blank for baseline correction, and absorbance was measured at 560 nm using a microplate reader to quantify released haemoglobin. Percentage membrane stabilization was calculated as,

$$\% \text{ Membrane stabilization} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Dose-response curves were plotted for multiple concentrations, IC_{50} values were derived by GraphPad Prism software. Aseptic techniques and biohazard precautions were maintained throughout, with biological waste disposed of per institutional guidelines. The assay was conducted in triplicate.

2.8 Protein denaturation assay

A modified egg albumin protein denaturation assay from (Gunathilake, Ranaweera and Rupasinghe, 2018) was performed to assess potential anti-inflammatory activity by evaluating the inhibition of heat-induced denaturation, where prevention of protein unfolding and aggregation indicated therapeutic potential analogous to stabilizing lysosomal enzymes during inflammation. The test sample (2.6 mg) was weighed and transferred to an Eppendorf tube. 200 µL of dimethyl sulfoxide (DMSO) was added and vortexed thoroughly until solubilized, and 800 µL of sterile saline was added to achieve a total volume of 1 mL, forming a stock solution that was vortexed again. The stock solution was centrifuged at 3000 rpm for 10 minutes to remove any insoluble particles. A two-fold serial dilution series was prepared by transferring 500 µL of the stock or previous dilution to a new tube and adding 500 µL of saline, mixing well after each step to generate a range of concentrations, including positive controls such as ibuprofen and negative controls for comparison. From each tube in the dilution series, 250 µL of the sample was transferred to a new Eppendorf tube, 50 µL of fresh egg albumin was added, and 350 µL of saline was incorporated to reach a total volume of 650 µL, followed by gentle vortexing to ensure homogeneity. The mixtures were incubated in a water bath at 37°C for 15 minutes to allow interaction, then transferred to a water bath at 70°C for 5 minutes to induce thermal denaturation. After heating, the tubes were centrifuged at 3000 rpm for 10 minutes to pellet denatured proteins. The supernatant (200 µL) from each tube was carefully transferred to the wells of a 96-well microplate in triplicate, with a blank included for baseline correction, and absorbance was measured at 660 nm using a microplate reader to quantify turbidity or remaining soluble protein. Dose-response curves were plotted for multiple concentrations and IC_{50} values were determined. The assay was performed in triplicate.

Percentage denaturation inhibition was calculated as,

$$\% \text{ Denaturation inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

2.9 Statistical analysis

The data was subjected to an Analysis of Variance (ANOVA) at 95 % significance level. Mean separation was conducted using the Tukey test. All assays were performed in triplicate to ensure accuracy and reproducibility.

3.0 Results and discussion

3.1 Phenol and flavonoid contents

The contents of the two phytochemicals phenols and flavonoids in the three maturity stages of Ceylon gooseberry whole fruit is graphically represented in Figure 2. TPC values were expressed as mg/GAE g with reference to the Gallic acid standard curve ($y = 0.306x + 0.048$, $R^2 = 0.9726$) and TFC values were expressed as mg/QE g with reference to the Quercetin standard curve ($y = 0.7597x + 0.0305$, $R^2 = 0.9729$). The TPC values obtained were near-ripe (2.0138 ± 0.7143^a), ripe (1.8653 ± 0.3706^b), and over-ripe (1.7044 ± 0.5644^c) mg GAE/g. The TFC values reported were near-ripe (0.2104 ± 0.0049^a), ripe (0.1944 ± 0.0022^b), and over-ripe (0.1792 ± 0.0101^c) mg QE/g. Data are expressed as mean ± standard deviation. Error bars represent the standard deviation. The results of Folin-Ciocalteu method reported the highest TPC value from near-ripe whole fruit extract of Ceylon gooseberry. The results of Aluminium chloride method reported the highest TFC value also from near-ripe whole fruit extracts. As the fruit ages past the near-ripe stage, these results point to a steady decrease in phenolic and flavonoid levels, most likely as a result of enzymatic breakdown, polymerization, or usage in ripening processes including pigmentation and softening (Mahmood *et al.*, 2012; Oszmiański *et al.*, 2018). The TPC values in near-ripe Ceylon gooseberries are similar to those found in unripe strawberries (roughly 2.5-3.0 mg GAE/g) and blueberries (1.8-2.2 mg GAE/g at early maturity) when compared to other berries. According to oxidative breakdown, the phenolic content of blueberries also peaks in less ripe stages before decreasing with full ripeness (Mahmood *et al.*, 2012; Guofang *et al.*, 2019). This pattern suggests that Ceylon gooseberry deposits protective secondary metabolites early in the fruit development as a strategy against environmental stresses after which they decline as the fruit concentrates on seed distribution and appetibility (N'Dri *et al.*, 2010). The increased flavonoid concentration in near-ripe fruit is consistent with the results on raspberries, where TFC reduces by 20-30% between green and red to red-ripe fruits, highlighting the importance of flavonoids in protecting against UV radiation and pathogen threats in immature stages (Staveckienė *et al.*, 2023). From the statistical analysis performed using Minitab 22, the total phenol contents at the three maturity stages; near-ripe, ripe and over-ripe were significantly different $F(2, 6) = 22.29$, $p = 0.002$ at significance level $\alpha = 0.05$ in the ANOVA test and Tukey pairwise mean separation. The ANOVA and mean separation (Tukey) conducted for the total flavonoid contents at the three maturity stages; near-ripe, ripe and over-ripe were significantly different $F(2, 6) = 67.47$, $p < 0.001$ at significance level $\alpha = 0.05$.

3.2 Antioxidant activity

The IC_{50}, which stands for half-maximal inhibitory concentration, is highly utilized and provides crucial information about how effective a pharmaceutical agent is. It quantifies the amount of drug required to reduce a biological process by half, serving as a measure of the potency of an antagonist drug in pharmacological studies (Aykul and Martinez-Hackert, 2016). Similarly, the IC_{50} of the fruit samples represents the concentration at which the initial absorbance of oxidants decreases by half (Chakraborty *et al.*, 2015). The results of DPPH assay recorded low IC_{50} values as near-ripe (0.3085), ripe (0.4155), and over-ripe (0.5046) mg/mL for the whole fruit extracts, proving the potency of Ceylon gooseberry as a source of antioxidants. The standard Ascorbic acid showed an IC_{50} value of 0.1360 ± 0.0073 mg/mL.

The IC_{50} values obtained by the ABTS assay were near-ripe (0.1293), ripe (0.1864), and over-ripe (0.2770) mg/mL. The standard Ascorbic acid showed an IC_{50} value of 0.0983 ± 0.0010 mg/mL. The antioxidant activity (measured using both DPPH and ABTS radical scavenging assays) has proved consistent with the phytochemical patterns, with lower IC_{50} values demonstrating greater strength. The IC_{50} values obtained indicate the concentration that will inhibit 50 % of the radicals and indicate the high radical scavenging capacity of near-ripe fruits due to high contents of phenolics and flavonoids that either donate electrons or hydrogen atoms to quench free radicals (Bochi *et al.*, 2014; Dharmadeva *et al.*, 2018). Differences in DPPH and ABTS assays can be related to the different radical mechanisms, where DPPH may be more suited to the hydrophobic antioxidants and ABTS to the hydrophilic antioxidants, showing a complex antioxidant property of Ceylon gooseberry (Boontang and Simla, 2023).

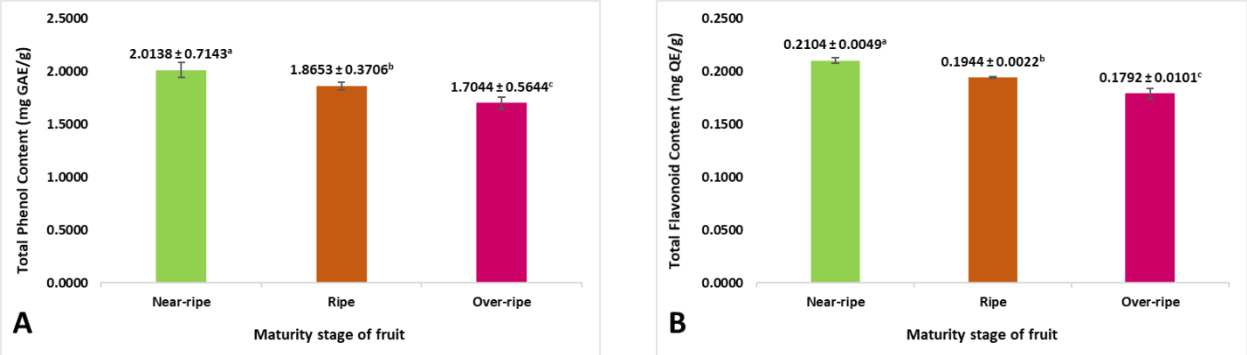

Figure 2- Graphical representation of phytochemical content in Ceylon Gooseberry whole fruit extracts; A- TPC (standard- Gallic acid y = 0.306x +0.048), B- TFC (standard- Quercetin y = 0.7597x +0.0305)

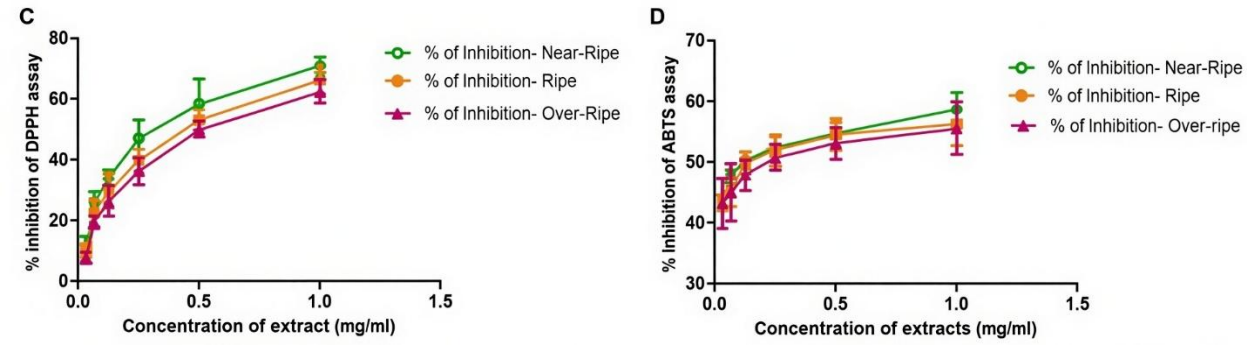

Figure 3- Graphical representation of antioxidant activity of Ceylon Gooseberry fruit extracts during assays; C- DPPH (standard IC_{50}- 0.1360±0.0073 mg/mL), D- ABTS (standard IC_{50}- 0.0983±0.0010 mg/mL)

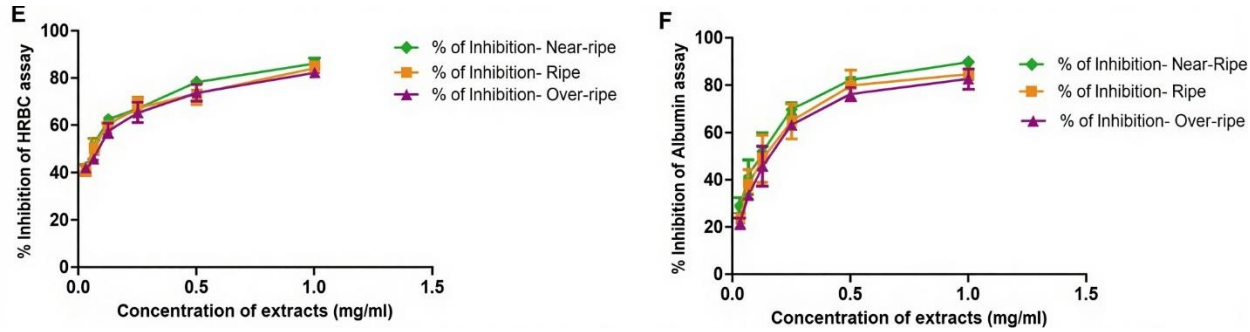

Figure 4- Graphical representation of anti-inflammatory activity of Ceylon Gooseberry fruit extracts during assays; E- HRBC (standard IC_{50}- 0.0158±0.0028 mg/mL), F- Albumin (standard IC_{50}- 0.0887±0.0008 mg/mL)

This can be attributed to the fact that the mechanism of action, the method, and the type of radical affect how antioxidants present in a plant extract reacts and the amount of antioxidant activity displayed (Abramovi *et al.*, 2017). Comparatively, unripe blackberries exhibit DPPH IC_{50} measures within 0.25-0.35 mg /mL, which decline as these berries ripen just like this case and this leads to the inference that early harvest gives antioxidant best so that patients may exploit them to combat the oxidative stress-induced illnesses (Wang and Hsin-Shan, 2000; Kuntorini *et al.*, 2022). In Ceylon gooseberry in particular, known previously published data on mature fruits find DPPH IC_{50} values at 0.4-0.5 mg/mL, which is consistent with our results on ripe and over-ripe fruits but further supports the benefits of near-ripe stages (Bochi and Milene Teixeira Barcia, Daniele Rodrigues, 2015; Da Silva *et al.*, 2022). Figure 3 shows a graphical representation of the antioxidant activity of Ceylon gooseberry during DPPH and ABTS assays. IC_{50}

values were calculated by nonlinear regression (R^2 > 0.95 for all curves).

3.3 Anti-inflammatory activity

The results of HRBC assay recorded low IC_{50} values as near-ripe (0.0547), ripe (0.0616) and over-ripe (0.0651) mg/mL for the whole fruit extracts, proving the potency of Ceylon gooseberry as a source of antioxidants (Figure 4). IC_{50} values were determined using nonlinear regression (R^2 > 0.95 for each curve). The standard Ibuprofen showed an IC_{50} value of 0.0158±0.0028 mg/mL. It shows an increase in membrane stabilization to heat-induced lysis, which can be likened to lysosome protection during inflammatory states (Lail *et al.*, 2021).

The IC_{50} values obtained by the protein denaturation assay were near-ripe (0.0965), ripe (0.1214) and over-ripe (0.1400) mg/mL. The standard Ibuprofen showed an IC_{50} value of 0.0887±0.0008 mg/mL. It recommends the suppression of protein unfolding that approximates that of inflammatory enzyme destabilization (Pap *et al.*, 2021). The superiority of the near-ripe fruits was further supported by anti-inflammatory potential, which was measured by HRBC membrane stabilization assay and egg albumin protein denaturation assay. Such effects could be mediated by phenolics and flavonoids that activate NF-kB pathway and inhibit enzymes as observed in other berries (Gu *et al.*, 2020). In one example, unripe raspberries are shown to be 20-40% more effective against inflammation in analogous assays than their ripe counterparts, with their 50% effect values of about 0.06-0.1 mg/mL, which is similar to our findings and indicates that the natural bioactive degradation that raspberries experience with maturity decreases their anti-chronic-inflammation efficacy (Kim and Lim, 2019; Bader *et al.*, 2022). On the whole, the results suggest that the near-ripe stage is optimal harvest time to maximize bioactivity most likely due to maximum accumulation of inherently formed defensive compounds. Thus, Ceylon gooseberry will be a promising source for functional food similar to traditional berries such as blueberries and strawberries which stand well in alleviating oxidative and inflammatory disorders (N'Dri *et al.*, 2010; Mphahlele *et al.*, 2014).

The research presents specific constraints. While the results show encouraging antioxidant and anti-inflammatory potential, they are derived solely from in vitro chemical and cell-free tests (DPPH, ABTS, HRBC, and protein denaturation), which might not completely represent physiological conditions in vivo, where bioavailability, metabolism, and absorption affect activity (Halliwell *et al.*, 1995; Abramovi *et al.*, 2017). Moreover, using 80% methanol for extraction might not effectively reflect the bioactive compounds present in typical dietary intake. The research did not include assessments of cytotoxicity or tests of cellular mechanisms to validate anti-inflammatory effects (Pap *et al.*, 2021; Liu *et al.*, 2023). Environmental and seasonal variations were also not assessed. Therefore, further in vitro cellular, in vivo, and bioavailability studies are essential before establishing conclusive health assertions.

4.0 Conclusion

It can be inferred that Ceylon gooseberry is a fruit with notable phytochemical content, antioxidant capabilities and anti-inflammatory properties. The fruit is optimal for consumption when it is near ripe, because of the comparatively high phytochemical content during that stage. Beyond the ripe and over-ripe stages, the near-ripe fruits showed the highest TPC and TFC coupled with the lowest IC_{50} values in DPPH, ABTS, HRBC, and protein denaturation assays. This under-utilized fruit, Ceylon Gooseberry *(Dovyalis hebecarpa)* is potent to be promoted for intake and developed as a natural supplement through further studies. This study highlights the fruit's potential as a natural antioxidant and anti-inflammatory agent for combating chronic diseases, suggesting that beneficial secondary metabolites peak at the near-ripe stage before declining with further ripening. More *in vivo* research, the creation of products like functional foods or supplements, and marketing in domestic and foreign markets are advised in order to capitalize on this underutilized resource. Near-ripe harvesting is emphasized for improved health benefits and sustainable use in Sri Lanka. Although the results show encouraging antioxidant and anti-inflammatory properties and imply potential uses in functional foods, these conclusions are derived entirely from *in vitro* tests. Consequently, additional *in vivo*, cell-based, and bioavailability research is necessary prior to establishing conclusive health or clinical assertions.

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Conflict of interest/Competing interest

Authors declare that they have no conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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