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Evaluation of antioxidant, anti-inflammatory and anti-diabetic properties of noni fruit (*Morinda citrifolia* L.) and its simulated gastrointestinal digesta fractions

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ABSTRACT

Noni (*Morinda citrifolia* L.) has been used for years in traditional medicine. As noni fruit is not utilized by the food industry in Sri Lanka, this study focused on determining the bioactives and functional properties of ripe fruit extract (methanolic and water) and simulated gastrointestinal digesta fractions. Methanol (80%) showed higher extractability of bioactives than water. Moreover, antioxidant and anti-inflammatory properties were higher, and anti-diabetic properties were lower in methanolic extracts than those in water extracts. The total phenolics (TPC) of 198.6 ± 2.48 µmol gallic acid equivalent/g FW, and ascorbic acid, anthocyanin, and β-carotene contents of 53.01 ± 1.47, 57.33 ± 1.01, and 0.27 ± 0.04 µg/g FW, respectively were evident in the fresh fruit. DPPH and ABTS radicals scavenging percentages of the fresh fruit were 97.09 and 98.98, respectively. The total antioxidant capacity of the fresh fruit was 33.94 mg AAE/g FW. Singlet oxygen and Nitric oxide scavenging abilities of the fresh fruit were above 90%. Percentages of heat-induced hemolysis, protein denaturation inhibition, and proteinase inhibitory activities of the fresh fruit at 2 µg/mL were 37.14, 42.32, and 5.23, respectively. Furthermore, alpha-amylase and alpha-glucosidase inhibitory activities of the fresh fruit at 2 µg/mL were 13.7 and 17.0%, respectively. Bioactives and antioxidant, anti-diabetic, and anti-inflammatory activities of dialysable fractions were significantly lower ($p < 0.05$) than their originals. Positive correlations between TPC and antioxidant activities, and anti-inflammatory and antioxidant activities were evident. This study revealed the therapeutic benefits and appealing sensory attributes that should be developed when producing Noni fruit-incorporated products.

Keywords: Antioxidant; Anti-diabetic and anti-inflammatory activities; Bio-accessibility; In vitro digestion; Noni fruit

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1. Introduction

Noni (*Morinda citrifolia L.*) or ‘ Ahu’ plant is grown in many tropical and subtropical areas of the world and is regarded as a sustainable crop with potential for commercial use (Chan-Blanco et al., 2006). Citrifolia is the most grown variety, which is reported to possess the greatest health and economic importance among other varieties/cultivars (Abou Assi et al., 2017). Although there are seasonal patterns in flowering and fruit-bearing, the fruits are harvested throughout the year (Chan-Blanco et al., 2006). In Sri Lanka, noni or ‘*Ahu*’ fruits have been used for years by practitioners of traditional medicine. Numerous studies have shown that noni fruit has a wide range of therapeutic effects, as reported based on clear scientific evidence of *in vitro* and *in vivo* studies, or clinical trials. Phytochemicals of noni fruits are reported to possess antimicrobial, antiseptic, antifungal, antioxidant, anti-inflammatory, anti-arthritic, anti-cancer, antidiabetic, antiemetic, anti-viral, anti-parasitic, anti-tuberculosis, and anti-inflammatory activities (Abou Assi et al., 2017). Moreover, wound healing, memory enhancing, anxiolytic and sedative, analgesic, gastric ulcer healing, gout and hyperuricemia healing, anti-psoriasis healing,

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immunity enhancing, osteoporotic and otoscopic enhancing, anthelminthic, analgesic, hypotensive, LDL oxidation preventive and immune enhancing effects are also reported (Abou Assi et al., 2017).

No adverse effects of noni fruits have been observed in extensive toxicological studies, including tissue culture work, animal experiments and human clinical trials (West et al., 2006). Moreover, noni fruit was reported to be acceptable for human consumption, based on official safety evaluations done by the European Union (European Commission, 2002). Therefore, noni juice has been approved as safe by regulatory bodies of many countries and has become one of the popular fruit juices in the global market as a wellness drink. Although the juice prepared from the ripe fruits is the most predominant noni product, both the leaves and the fruits are sold in tablet, tea, and juice forms (Kulathunga & Ldam, 2017; Su et al., 2005; Abou Assi et al., 2017). The market annual sales worth of US $ 1.3 billion had been reported for noni products (Abou Assi et al., 2017). Furthermore, its use in the United States has become more widespread as reflected by the commercial availability of processed noni products in health food stores, chain grocery stores specialized in natural foods and on the Internet (Kulathunga & Ldam, 2017).

Many scientific publications have indicated presence of compounds possessing a variety of nutritional and functional properties in the fruit. However, the complete physiochemical composition of the fruit is not available and current knowledge of these constituents is not satisfactory (Inada et al., 2017). Research on noni mostly focusses on its potential as a new source of bioactives (Jahurul et al., 2021). Although nearly 200 phytochemicals have been identified and isolated from various parts of noni plant, elucidation of complete phytochemical composition, and isolation and identification of bioactives and their activities are not fully addressed (Abou Assi et al., 2017).

Even though noni fruit is reported to be a promising source of dietary antioxidants and to possess anti-inflammatory and anti-diabetic activities, literature on the effect of digestion on the bioaccessibility and dialysability of major phytochemicals is not available. Moreover, bioactives in food are mostly chemically extracted and quantified, extent of their bioavailability is uncertain, as the type and amount of bioactives absorbed into our body possibly depends on the effect of digestion and dialysability on such constituents (Gunathilake et al., 2018a). Therefore, studies on the bioavailability, dialysability, and metabolic fate of bioactives in noni fruits are important for elucidating their potential as a raw material or as an ingredients for adding value to food products (Tagliazucchi et al., 2010).

Even though many studies have been conducted in many countries on pharmacological investigations and the chemical composition of noni fruits, very few studies on those grown in Sri Lanka are reported. Moreover, the phytochemical composition varies within the same plant species, depending on soil nutrient composition, climatic season, plant development stage, natural association with other plants, methods of storage of raw materials and their processing, and extraction procedures (Kulathunga & Ldam, 2017). Therefore, this research focused on determining bioactives, as well as the antioxidant, anti-inflammatory, and anti-diabetic activities of 80% methanolic and water extracts and simulated gastrointestinal digesta fractions of ripe noni fruits. Since fresh noni fruit is rarely consumed by Sri Lankans and is not utilized as a raw material or ingredient by the food industry, the results of this study could be instrumental in popularizing the fruit grown in Sri Lanka.

2. Material and Methods

Noni fruits were locally collected from trees grown in *Katugastota*, located in the Kandy district of Sri Lanka, in July 2022. The ripe fruits selected based on visual color and shape were vacuum packaged in low-density polyethylene bags (0.05 mm in thickness) and stored at -18 ℃ until further analysis.

2.1. Preparation of fruit extracts

Methanolic extracts of the frozen fruit flesh were prepared as described previously (Kumari & Gunathilake, 2020). The ground fruit flesh (1.00 g) was mixed with 15 mL of 80% methanol/water (v/v). The mixture was soaked overnight, vortexed at high speed for 5 min, and then centrifuged (EBA 20 centrifuge, Hettich, Tuttlingen, Germany) at 2600 g for 10 min at room temperature. The obtained extracts were subjected to filtration using Whatman No. 42 filter paper (Whatman Paper Ltd, Maidstone, UK). Subsequently, the filtered extracts underwent evaporation in a rotary evaporator (HAHNVAPOR, Model HS-2005 V, HAHNSHIN Scientific, Korea) under a vacuum at a temperature of 40°C. The resulting evaporated extracts were then stored at -18 °C until analysis within a period of 1 week. Water extracts of the frozen fruit flesh were prepared as described previously (Siddiqui et al., 2021) with slight modifications, and the filtrates were stored at -18°C until further analysis.

2.2. Preparation of in-vitro gastrointestinal digesta fractions

Fresh fruit was subjected to gastrointestinal digestion following the method described by (Hettiarachchi et al., 2021) with slight modifications. Fresh fruit flesh weighing 10g was separately added to 10 mL of fresh saliva donated by a healthy adult. To simulate chewing, the mixture was pounded in a mortar and pestle for a minute. After that, they were incubated at 37°C for 10 minutes. Exact 5 mL of each fruit digestion were taken, diluted with 0.9% NaCl, and then filtered using Whatman #42 filter paper. For subsequent examination, the filtered aliquot was kept in amber-colored bottles. To render salivary amylase inactive, the pH of the mixture was raised to 2.0 using a 6.0 mol/L solution of HCl.

The sample was then treated with 50 mL of 0.9% NaCl and 4.0 mL of pepsin solution (940 mg/mL in 0.1 M HCl), all while keeping the pH at 2.0. The mixture was incubated for an hour at 37°C and 100 rpm in a LabTech shaking water bath (DAIHAN LABTECH, Korea). After the material had been digested in the stomach, a 5 mL aliquot was taken, diluted, and filtered through Whatman #42 filter papers. It was then measured and stored at -18°C for future analysis.

A piece of cellulose membrane dialysis tubing (average flat width: 33 mm, MWCO 12,000 Da) was cut to a length of 15.0 cm. To prepare it for the intestinal digestion phase, both the inner and outer surfaces of the tubing were rinsed with a 0.9% NaCl solution. Subsequently, the dialysis bag was filled with 5.5 mL of NaCl (0.9%) and 5.5 mL of $NaHCO_3$ (0.5 M). The open end of the bag was securely sealed with clips, and it was immediately immersed into the gastric digestion mixture. The bag, containing the mixture, was then placed in a shaking water bath, and incubated at 37°C and 100 rpm for 45 minutes.

Subsequently, the mixture was brought to a pH of 6.5 using $NaHCO_3$. Following this, the pancreatin-bile blend, comprising 2

mg/mL of pancreatin and 12 mg/mL of bile extract dissolved in 0.1 M $NaHCO_3$, was introduced. The entire concoction was then incubated at 37°C for an extra 2 hours. Once the digestion process concluded, the pH of the mixture was measured and found to be in the range of 7 to 7.5. The Aliquot was collected from the intestinal phase, filtered, measured, and stored for analysis, following the same procedure as before. The content present in the dialysis bag was transferred to measuring cylinders and diluted to a final volume of 14 mL using a 0.9% NaCl solution. Subsequently, the diluted mixture was filtered through Whatman #42 filter papers, measured, and stored at -18°C for further analysis.

2.3. *Determination of bioactives, antioxidant activity, and anti-diabetic and anti-inflammatory properties of the test samples (extracts and gastrointestinal digesta fractions)*

2.3.1. *Bioactives*

The total phenolic contents of the test samples were determined by the Folin - Ciocalteau's reagent method (Kumari & Gunathilake, 2020) with some modification, using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan). The absorbance of the reaction mixtures was measured at 760 nm, against a blank. Total phenolic content was calculated based on a standard curve constructed with gallic acid and expressed as µmol of gallic acid equivalents (GAE) per g on a fresh weight basis.

The total flavonoid contents of the test samples were determined according to a spectrophotometric method described by Kumari and Gunathilake (2020) with slight modifications, using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan). The absorbance of the reaction mixtures was measured at 510 nm, against a blank. Total flavonoid content was calculated based on a standard curve constructed with rutin and expressed as µmol of rutin equivalents (RE) per g on a fresh weight basis.

The total anthocyanin contents of the test samples were determined according to a spectrophotometric pH differential method described by Kumari and Gunathilake (2020), using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan). The absorbance of the reaction mixtures was measured at 510 nm and 700 nm, against a blank, and the total anthocyanin content was expressed as cyanidin-3-glucoside equivalents (mg/mL).

β-carotene and lycopene contents of the test samples were determined according to a spectrophotometric method described by Kumari and Gunathilake (2020) with slight modifications, using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan). The absorbance of the reaction mixtures was measured at 453, 505, 645, and 663 nm, and β-carotene and lycopene contents were calculated as follows:

$$\beta - \text{carotene (mg/100 mL)} = 0.216A663 - 1.22A645 - 0.304A505 + 0.452A453 \quad (1)$$

$$\text{Lycopene (mg/100 mL)} = -0.0458A663 + 0.204A645 + 0.372A505 - 0.0806A453 \quad (2)$$

The ascorbic acid contents of the test samples were determined according to a spectrophotometric method described by Kumari and Gunathilake (2020). The absorbance of the reaction mixtures was measured at 520 nm, using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan).

2.3.2. *Antioxidant activity*

The total antioxidant capacity of the test samples was determined according to a spectrophotometric method described by Gunathilake and Ranaweera (2016) with slight modifications, based on the reduction of Mo (VI) to Mo (V) by the sample analyte. The absorbance of the reaction mixtures within the concentration range of 2-20 µg/1g of FW was measured at 695 nm using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan) against a blank. Total antioxidant capacity was calculated based on a standard curve constructed with ascorbic acid and expressed as mg of ascorbic acid equivalent per g of fresh fruit.

ABTS free radical scavenging activity of the test samples was determined according to a spectrophotometric method described by Kumari and Gunathilake (2020) with slight modifications. The absorbance of the reaction mixtures within the concentration range of 2-20 µg/1g of FW was measured at 734 nm using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan) against a blank. The scavenging activity was expressed as trolox equivalent per g fresh weight.

Lipid peroxidation inhibition activity of the test samples was measured, using the thiobarbituric acid-reactive species (TBARS) assay with some modifications, as described by Kumari and Gunathilake (2020). The absorbance of the reaction mixtures within the concentration range of 2-20 µg/1g of FW was measured at 532 nm, against a blank, using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan).

Nitric oxide inhibitory activity of the samples was measured according to a spectrophotometric method described by Kumari and Gunathilake (2020) with some modifications. The absorbance of the reaction mixtures within the concentration range of 2-20 µg/1g of FW was measured at 540 nm, against a blank, using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan). The inhibition activity was expressed as mg of ascorbic acid equivalent per 1 g on a fresh weight basis.

The capacity of the test samples to scavenge the 'stable' 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical was measured according to the method of (Hettiarachchi et al., 2021) with slight modifications, using Trolox as the reference standard. The absorbance of the reaction mixtures within the concentration range of 2-20 µg/1g of FW was measured at 517 nm, against a blank, using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan).

The singlet O_2 scavenging activity of the test samples was measured according to a spectrophotometric method described by Kumari and Gunathilake (2020). *N, N*dimethyl-*p*-nitrosoaniline, and histidine were used as selective scavengers and an acceptor of singlet O_2, respectively. The bleaching of *N,N*-dimethyl-p-nitrosoaniline was spectrophotometrically monitored at 440 nm for the fruit extracts within the concentration range of 2-20 µg/1g of FW. The singlet O2 scavenging activity was expressed as milligrams of gallic acid equivalent per 1 gram on a fresh weight basis.

Table 1. Content of bioactive compounds in fresh noni fruit extracts (methanolic and water) and different sample fractions of in-vitro digestive phases.

Sample fraction	TPC (µmol GAE /1g of FW)	TFC (µmol RE/1g of FW)	Anthocyanin as (µg/1g of FW)	Ascorbic (µg/1g of FW)	Lycopene (µg/1g of FW)	β-Carotene as (µg/1g of FW)
ME fruit	198.60 ± 2.48^{a}	9.19 ± 0.48^{a}	57.33 ± 1.01^{a}	53.01 ± 1.47^{a}	0.24 ± 0.06^{a}	0.27 ± 0.04^{a}
WE Fruit	139.02 ± 1.54^{b}	6.62 ± 0.38^{b}	71.67 ± 2.60^{b}	64.14 ± 2.08^{b}	0.10 ± 0.02^{b}	0.11 ± 0.01^{b}
Oral	111.22 ± 2.44^{c}	5.49 ± 0.20^{c}	40.85 ± 1.80^{c}	38.49 ± 1.48^{c}	0.08 ± 0.01^{c}	0.09 ± 0.01^{c}
Gastric	77.85 ± 1.60^{d}	4.01 ± 0.16^{d}	11.44 ± 0.68^{d}	15.39 ± 1.20^{d}	0.05 ± 0.00^{d}	0.05 ± 0.00^{d}
Intestine	19.46 ± 0.80^{e}	1.04 ± 0.08^{e}	8.70 ± 0.23^{e}	10.78 ± 1.00^{e}	0.04 ± 0.00^{d}	0.04 ± 0.00^{d}
Dialysed	5.84 ± 0.24^{f}	0.35 ± 0.02^{f}	2.09 ± 0.06^{f}	2.16 ± 0.14^{f}	0.01 ± 0.00^{e}	0.01 ± 0.00^{e}

Values are presented as mean ± *SD*, *n* = 3. Values in each column having the same letter are not significantly different ($p < 0.05$). All the values are determined per 1 gram of fresh weight (FW) of fruit.

Table 2. Bio-accessibility and dialysability of polyphenols, flavonoids, anthocyanin, carotenoids, and ascorbic acid from fresh fruit, dried fruit, fruit juice, and dried seeds.

Bioactive compound	Bio accessibility (%)	Bioavailability (%)
Total phenolics*	14.00 ± 0.06	30.00 ± 0.02
Total flavonoids**	15.75 ± 0.01	33.97 ± 0.012
Ascorbic acid***	16.80 ± 0.10	20.00 ± 0.00
Monomeric anthocyanin***	12.13 ± 0.11	24.01 ± 0.03
β-carotene***	38.53 ± 0.23	19.05 ± 0.00
Lycopene***	38.54 ± 0.54	18.92 ± 0.04

All data are mean ± *SD* (*n*–3). *µmol gallic acid equivalent per 1 g fresh weight, **µmol rutin equivalent per 1 g fresh weight, ***µg per 1 g fresh weight.

Table 3. Antioxidant, anti-inflammatory and diabetic activities of fresh noni fruit extracts (methanolic and water) and different sample fractions of in-vitro digestion phases.

Bioactivity	Methanolic extract	Aqueous extract	Oral phase aliquot	Gastric phase aliquot	Gastro-intestinal phase aliquot	Dialysis phase aliquot
Total Antioxidant Capacity (mg AAE per g FW)	33.94 ± 0.02^{a}	28.57 ± 0.09^{b}	25.54 ± 0.76^{c}	18.78 ± 0.09^{d}	8.04 ± 0.17^{e}	2.87 ± 0.67^{f}
ABTS Radical Scavenging Activity (%)	98.98 ± 0.01^{a}	92.87 ± 0.06^{b}	88.90 ± 1.09^{c}	70.56 ± 0.45^{d}	21.09 ± 0.25^{e}	4.78 ± 0.48^{f}
DPPH Radical Scavenging Activity (%)	97.09 ± 0.03^{a}	90.78 ± 1.23^{b}	87.54 ± 0.43^{c}	68.90 ± 1.67^{d}	18.07 ± 0.53^{e}	4.34 ± 0.19^{f}
Lipid Peroxidation Inhibition Activity (%)	93.09 ± 1.67^{a}	85.90 ± 1.28^{b}	80.89 ± 0.67^{c}	62.90 ± 0.05^{d}	16.45 ± 0.39^{e}	3.98 ± 0.01^{f}
Nitric Oxide Inhibition Activity (%)	90.98 ± 1.09^{a}	84.67 ± 1.05^{b}	80.99 ± 0.43^{c}	63.09 ± 0.03^{d}	17.54 ± 0.21^{e}	4.89 ± 0.09^{f}
Singlet Oxygen Scavenging Activity (%)	99.96 ± 0.05^{a}	94.56 ± 0.76^{b}	91.78 ± 0.72^{c}	73.09 ± 0.29^{d}	23.98 ± 0.54^{e}	3.67 ± 0.05^{f}
Heat-Induced Hemolysis Inhibition (%)	99.60 ± 0.20^{a}	93.75 ± 2.78^{b}	92.90 ± 0.98c	89.06 ± 1.56^{d}	73.09 ± 1.23^{e}	33.09 ± 0.78^{f}
Protein Denaturation Inhibition (%)	99.86 ± 0.07^{a}	97.90 ± 0.98^{b}	96.09 ± 0.23^{c}	89.00 ± 0.23^{d}	80.40 ± 2.89^{e}	70.23 ± 0.09^{f}
Proteinase Inhibitory Activity (%)	99.04 ± 0.02^{a}	39.67 ± 2.89^{b}	34.30 ± 1.56^{c}	31.78 ± 0.78^{d}	13.49 ± 0.97^{e}	6.70 ± 0.98^{f}
Alpha-Amylase Inhibitory Activity (%)	93.06±1.34^{c}	94.67±1.23^{a}	93.47±0.98^{b}	65.64±1.23^{d}	56.36±0.34^{e}	51.55±0.53^{f}
Alpha-Glucosidase Inhibitory Activity (%)	94.09±2.89^{b}	98.90±1.02^{a}	92.09±1.34^{c}	85.09±1.67^{d}	70.89±3.56^{e}	60.75±3.09^{f}

Values are presented as mean ± *SD*, *n* = 3. Values in each row having the same letter are not significantly different ($p < 0.05$).

2.3.3. *Anti-diabetic properties*

The inhibition of porcine α-amylase activity in the test samples within the concentration range of 2-20 µg/1g of FW was determined using a spectrophotometric method described by Poovitha and Paranis (2016) with slight modifications, and acarbose was used as a positive control. The absorbance of the reaction mixtures was measured at 540 nm using a UV/Visible spectrophotometer (Thermo Scientific 201, USA).

α-glucosidase activity in the test samples within the concentration range of 2-20 µg/1g of FW was determined according to a spectrophotometric method described by Kim et al. (2004) with slight modifications, using acarbose as a positive control. The absorbance of the reaction mixtures was measured at 450 nm, using a UV/Visible spectrophotometer (Thermo Scientific 201, USA).

2.3.4. *Anti-inflammatory properties*

Whole human blood collected from a healthy human subject was used to prepare a 10 % erythrocyte suspension (v/v), according to a method suggested by Gunathilake et al. (2018c). Inhibition of heat-induced hemolysis of blood cell suspension by the test samples within the concentration range of 2-20 µg/1g of FW was carried out according to a spectrophotometric method described by Gunathilake et al. (2018c) with slight modifications. The absorbance of the reaction mixtures was measured at 540 nm, using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan).

The effect of the test samples on 1% bovine albumin protein denaturation was tested according to a spectrophotometric method described by Gunathilake et al. (2018c). The absorbance of the reaction mixtures within the concentration range of 2-20 µg/1g of FW was measured at 660 nm, using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan).

The inhibitory activity of the test samples on trypsin was measured according to a spectrophotometric method suggested by Gunathilake et al. (2018c). The absorbance of the reaction mixtures within the concentration range of 2-20 µg/1g of FW was measured at 210 nm, using a UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan).

2.4. *Data analysis*

All the experiments were carried out in triplicate, and the data were subjected to one-way analysis of variance (ANOVA), using SAS® 9.4 software (Cary, USA) to determine the difference among treatments at 0.05 significance level. When there were significant differences ($p > 0.05$), multiple mean comparisons were carried out, using the least significant difference method. All the data were expressed as mean ± standard deviation.

3. Results and Discussion

3.1. *Bio-active assays*

Bioactive compounds found in fruits offer health benefits beyond basic nutrition by addressing metabolic disorders, pro-inflammatory states, and oxidative stress, while also impacting calorie intake, and they possess the ability to influence metabolic processes and exhibit beneficial qualities such as antioxidant effects, receptor function inhibition, enzyme induction or inhibition, and gene expression modulation (Gunathilake et al., 2018b; Gunathilake & Ranaweera, 2016; Halim et al., 2019).

To assess the impact of gastrointestinal digestion on bioactive compounds, cost-effective and reproducible in vitro digestive models based on human physiology were developed (Payne et al., 2012). These models were utilized to examine structural changes, digestibility, and release of food contents under simulated gastrointestinal conditions, utilizing an in vitro static digestion model (Hettiarachchi et al., 2021). The impact of gastrointestinal digestion on total phenolics is shown in Table 1. Amounts of phenolics detected from fresh noni fruits of methanolic extracts were significantly higher ($p < 0.05$) than water extracts and digestion after the oral, gastric, and intestinal phases were significantly lower ($p < 0.05$) than those observed for water extracts and methanolic extracts of raw samples. The content of dialyzable phenolics was also significantly lower than the phenolic content present in the gastric digesta and the intestinal digesta.

Table 1 shows the effect of gastrointestinal digestion on the flavonoid content of noni fruits. The amount of flavonoids detected from the fruits of methanolic extracts were significantly higher ($p < 0.05$) than water extracts. The pattern of freeing the total flavonoid content was comparable to total phenolics. Contents of total flavonoids detected after the gastric and intestinal phases were significantly lower compared to the contents determined by methanolic extracts of fruit samples. The content of dialyzable total flavonoid was also significantly lower compared to the content of total flavonoid available in the gastric and intestinal digesta.

The influence of gastrointestinal digestion on the β-carotene and lycopene content of noni fruits is shown in Table 1. The patterns of freeing the β-carotene and lycopene were comparable to flavonoids and total phenolics. Amounts of β-carotene and lycopene detected in methanolic extracts of the fruit were significantly higher ($p < 0.05$) than in water extracts. The content of β-carotene and lycopene measured upon the gastric and intestinal phases were significantly lower ($p < 0.05$) than those observed for methanolic and water extracts of noni fruit. The content of dialyzable β-carotene and lycopene was also significantly lower compared to the same in the gastric and intestinal digesta.

The influence of gastrointestinal digestion on the ascorbic and anthocyanin content in fresh noni fruits is shown in Table 1. The pattern of freeing the ascorbic and anthocyanin were comparable to flavonoids, total phenolics, β-carotene and lycopene in the digestion phases. Amounts of ascorbic and anthocyanin in the methanolic extracts of the fruits were significantly lower ($p < 0.05$) than in water extracts. Content of ascorbic and anthocyanin measured upon the gastric and intestinal phases were significantly lower compared to the contents in methanolic and water extracts of the fruits. The content of dialyzable ascorbic and anthocyanin were also significantly lower compared to ascorbic and anthocyanin contents present in the gastric and intestinal digesta.

3.2. *Bio-accessibility and bioavailability of bio-actives*

Bioavailability refers to the absorption rate and extent of a substance for cellular metabolism, where gastrointestinal digestion, epithelial cell absorption, metabolism, tissue distribution, and bioactivity collectively influence its availability (Santos et al., 2019), while bioaccessibility refers to the quantity or percentage of a compound liberated from the food matrix in the gastrointestinal

tract for absorption, taking into account compound stability and release from food matrices (Carbonell-Capella et al., 2014). It is observed that the bioavailability of bioactive compounds which are represented by the dialysis fractions, are lower than their bioaccessible bioactive compounds except for β-carotene and lycopene as shown in Table 2.

Aliquots collected from the oral, gastric, and gastrointestinal digestion phases contain phytochemical compounds released from the food matrix, which are available for absorption, while the dialysis phase aliquot represents the bioavailable portion that is absorbed through the intestinal epithelium and enters circulation, highlighting the importance of bioaccessibility for antioxidants to exert their bioactivity (Gunathilake, 2018b; Kumari & Gunathilake, 2020; Tagliazucchi et al., 2010). The bioavailability and bioaccessibility of bioactive compounds are influenced by their diverse chemical structures, while antinutritional factors can decrease bioavailability and hinder the action of digestive enzymes (Septembre-Malaterre et al., 2018), with the stability of bioactive compounds being affected by the digestive process, ultimately impacting their bioavailability and potential health benefits, as the digestive process also plays a role in determining the potential health effects of bioactive compounds and nutrients (Kumari & Gunathilake, 2020).

3.3. *Effect of digestion on antioxidant, anti-inflammatory and antidiabetic activities*

Plant extracts have attracted a lot of attention in the food industry due to their diverse functional and sensory qualities, appealing color, and flavor. These qualities establish plant extracts as non-toxic, reasonably priced, and environmentally friendly natural additives that improve product quality, facilitate the development of functional foods, and potentially offer alternatives to harmful synthetic compounds (Plaskova & Mlcek, 2023). Furthermore, research was conducted to assess *Severinia buxifolia* for its phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities, and the findings indicate that methanol serves as the optimal solvent for extracting bioactive compounds from *S. buxifolia* branches. Methanolic extract showed promise as an antioxidant and anti-inflammatory agent for the nutraceutical and pharmaceutical industries (Truong et al., 2019). By the way, we utilized an aqueous fruit extract also in our research, which is distinct from other solvent extracts. Additionally, water plays a crucial role as a medium for the biochemical reactions within the body. The aqueous extract used in the study is a rich source of potent chemicals that are simple to add to a wide range of goods, such as pharmaceuticals and baked goods. This extract can be obtained easily, safely, affordably, and in an environmentally sustainable manner (El-Desouky, 2021).

3.3.1. *Antioxidant properties*

Antioxidants are gaining increasing attention from scientists in the food industry and medical field due to their protective effects against oxidative deterioration in food products and oxidative stress-related diseases in the body. The effective exploration of natural antioxidant sources and the development of new antioxidant compounds necessitate reliable methods for evaluating antioxidant activity. Oxidation leads to the production of various reactive oxygen species and free radicals, causing specific damage to cells (Munteanu & Apetrei, 2021). A single assay method is insufficient for assessing the antioxidant potential of endogenous compounds, and it is important to use multiple antioxidants assays due to variations in assay principles and experimental conditions among different methods (Rahman et al., 2015).

All the antioxidant activities have direct strong positive correlation with phenolic compound availability. When phenolic bio-actives amount is higher in samples, ability of antioxidant capacity is also higher. A positive correlation may not always exist between the total phenolic content and antioxidant activities. It may be due to other compounds that possess the radical scavenging abilities, phosphomolybdenum reduction ability, nitric oxide inhibition ability, singlet oxygen inhibition ability and lipid peroxidation ability also indicated as antioxidant activity. All the antioxidant activities of fresh Noni fruit detected after the oral, gastric, intestinal and dialysis phases were significantly low ($P< 0.05$) compared to those measured by methanolic and aqueous extractions of raw samples as reported in Table 3. All antioxidant activities were significantly higher ($p < 0.05$) for the methanolic extractions of raw samples compared to those measured for the aqueous extractions as mentioned in Table 3.

Previous research conducted by Gunathilake et al. (2018c) has also reported similar observations regarding the alterations in antioxidant properties of leafy vegetables during simulated in vitro gastrointestinal digestion. According to Bouayed et al. (2012), the reduction in antioxidant activity during intestinal digestion may be attributed to interactions between phenolics and various dietary components such as dietary fiber and proteins that are released from food matrices during gastrointestinal digestion, thereby affecting the solubility and availability of phenolics and, consequently, the antioxidant capacity. Fig. 1 presents the regression analysis conducted to examine the relationship between the measured antioxidant activity and the total phenolic content in fresh noni fruit extracts, revealing a correlation between the six antioxidant activities and the total phenolic content of the noni fruits. Similarly, previous studies by Gunathilake and Ranaweera (2016) on leafy vegetables and Kumari and Gunathilake (2020) on *Syzygium caryophyllatum* have also reported strong correlations between phenolic contents and antioxidant activities.

3.3.2. *Anti-inflammatory properties*

The term "inflammation" typically refers to the intricate biological reaction of vascular tissues to damaging stimuli. Additionally, inflammation is linked to pain and includes alterations to membranes, an increase in vascular permeability, and an increase in protein denaturation, among other things (Gunathilake et al., 2018). Reactive oxygen and nitrogen species are produced by activated macrophages during inflammation, and these species can cause oxidation of lipids, proteins, and nucleic acids. Reactive aldehydes produced by the oxidation of polyunsaturated fatty acids can permeate throughout the cell (Gunathilake & Ranaweera, 2016). Although certain synthetic anti-inflammatory medications, such as steroids, nonsteroidal anti-inflammatory medications (NSAIDs), and immune suppressants, are well-established for use in inflammatory illnesses, their long-term usage is constrained by the associated negative effects (Harirforoosh et al., 2013). Therefore investigation of plant based treatments are easily available, safe and cost effective for anti-inflammatory disorders (Kazemi et al., 2018).

All the anti-inflammatory activities of fresh Noni fruit detected after the oral, gastric, intestinal and dialysis phases were significantly low ($p < 0.05$) compared to those measured by

methanolic and aqueous extractions of raw samples as reported in Table 3. All anti-inflammatory activities were significantly higher ($p < 0.05$) for the methanolic extractions of raw samples compared to those measured for the aqueous extractions as shown in Table 3. According to a correlation study conducted by Gunathilake et al. (2018c) has provided evidence suggesting that the observed anti-inflammatory properties could be attributed to the presence of antioxidant bioactive compounds, including polyphenols, flavonoids, and carotenoids.

3.3.3. *Antidiabetic properties*

Breaking down of long-chain carbohydrates catalyze by alpha amylases (such as salivary and pancreatic alpha amylases) which contribute to convert starch into oligosaccharides, whereas shorter starch chains and disaccharides are broken down by alpha glucosidases (such as maltase glucoamylase and sucrase-isomaltase) to produce glucose for intestinal absorption and raising blood glucose levels as a result (DeFronzo et al., 2015). Alpha-glucosidases' and alpha-amylases' activity can be inhibited using synthetic medicines as treatment options for type-2 diabetes. Acarbose is well known as a highly effective competitive inhibitor of intestinal alpha glucosidases such as sucrase, maltase, glucoamylase, and glucanosyltransferase. Acarbose is mixed inhibitor of alpha amylase (Kim et al., 1999). But these inhibitors typically cause gastrointestinal adverse effects (Shai et al., 2010). Inhibiting the function of these enzymes in patients with type-2 diabetes may reduce hyperglycemia.

All the antidiabetic activities of fresh Noni fruit detected after the oral, gastric, intestinal, and dialysis phases were significantly low ($p < 0.05$) compared to those measured by methanolic and aqueous extractions of raw samples as reported in Table 3. Whereas, according to the results shown in Table 3, antidiabetic activities of aqueous extractions were significantly higher ($p < 0.05$) compared to methanolic extractions of raw Noni fruit.

3.4. *Correlation between total phenolics and antioxidant activities*

As shown in Fig. 1 DPPH radical scavenging activity, phosphomolybdenum reduction activity, nitric oxide inhibition activity, ABTS radical scavenging activity, lipid peroxidation inhibition activity, and singlet oxygen inhibition activity showed R^2 values of 0.9953, 0.9858, 0.9849, 0.9815, 0.9594 and 0.9562, respectively, which indicated strong positive correlation with total phenolics content. It was evident that the six antioxidant activities of noni fruits are correlated with their total phenolic content. Furthermore, carotenoids are important antioxidants, which possess the capability of protecting against photooxidative processes due to the efficient scavenging of peroxyl radicals and singlet molecular oxygen (Eggersdorfer & Wyss, 2018). However, the type of carotenoid molecules freely found in different phases of the digestion process, their relative stability at different digestive media, and their relative existence in the food matrix determine the antioxidant properties of carotenoids (Gunathilake et al., 2018b). Generally, a decrease in the contents of certain bioactives including phenolics, flavonoids, and carotenoids was observed during each digestion process and dialysis executed via a semi-permeable cellulose membrane and, therefore, significantly ($p < 0.05$) lower antioxidant activities were observed in the digestion of noni fruits as reported in Table 3.

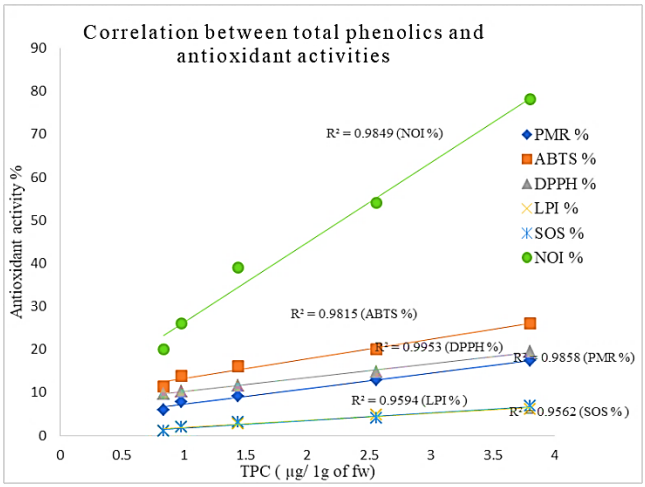

Fig. 1. Correlation between antioxidant activities and total phenolics content.

3.5. *Correlation between antioxidant and anti-inflammatory activities*

Inflammation produces ROS(Reactive oxygen species) and RNS (Reactive nitrogen species), which defend against infections but can damage tissues in excess; scavenging them with anti-inflammatory bioactive compounds is vital (Kumari & Gunathilake, 2020; Sharma et al., 2007). The hydroxyl radical (•OH) is a highly reactive and hazardous radical formed from H2O2 in the presence of metal ions. It plays a significant role in lipid peroxidation by oxidizing polyunsaturated fatty acids (PUFAs) (Gunathilake & Ranaweera, 2016). Lipid peroxidation is linked to diseases and aging, but antioxidant defenses inhibit it through prevention, scavenging, repair, and adaptive responses (Niki et al., 2005).

According to Fig. 2 nitric oxide inhibition activity showed a strong positive correlation with protein denaturation inhibition (R^2 = 0.9903), proteinase inhibition (R^2 = 0.9568), and heat-induced hemolysis inhibition (R^2 = 0.9360) respectively. According to Fig. 2 singlet oxygen inhibition activity showed a strong positive correlation with protein denaturation inhibition (R^2 = 0.9925), proteinase inhibition (R^2 = 0.9638), and heat-induced hemolysis inhibition (R^2 = 0.9613) respectively. According to Fig. 2 lipid peroxidation inhibition activity showed a strong positive correlation with heat-induced hemolysis inhibition (R^2 = 0.9949), protein denaturation inhibition (R^2 = 0.9579), and proteinase inhibition (R^2 = 0.8980), respectively.

3.6. *Correlation between antioxidant and antidiabetic activities*

Long-term diabetes mellitus is linked to various complications, including atherosclerosis, myocardial infarction, neuropathy, and nephropathy, which have traditionally been associated with chronically elevated glucose levels and resulting oxidative stress. Mechanisms contributing to increased oxidative stress in diabetes involve non-enzymatic glycosylation, auto-oxidative glycosylation, and metabolic stress.

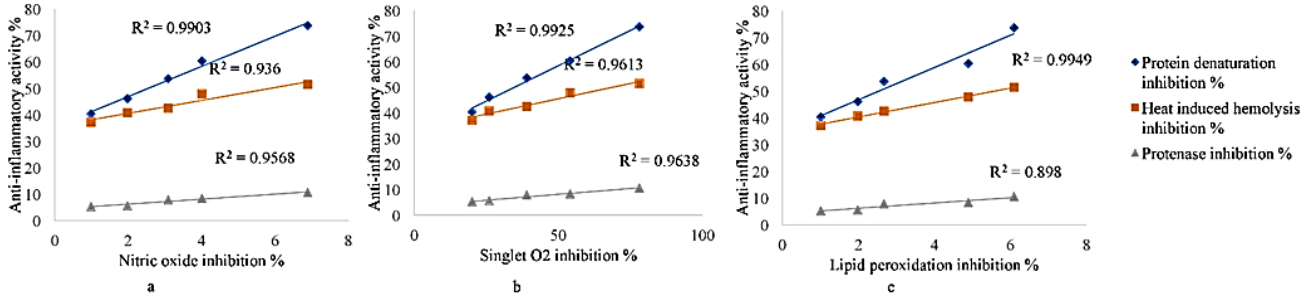

Fig. 2. a) Correlation between nitric oxide inhibition activity and anti-inflammatory activities, b) Correlation between singlet oxygen inhibition activity and anti-inflammatory activities at different concentrations, c) Correlation between singlet oxygen inhibition activity and anti-inflammatory activities at different concentrations.

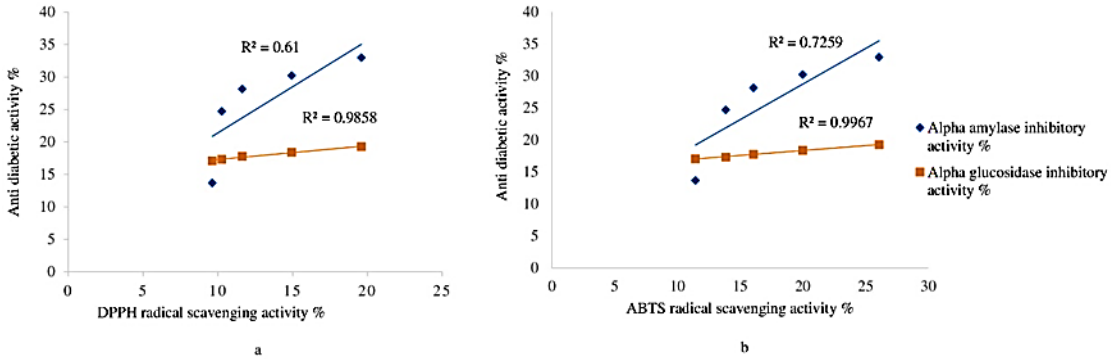

Fig. 3. a) Correlation between ABTS radical scavenging activity and anti-diabetic activities, b) Correlation between ABTS radical scavenging activity and anti-diabetic activities.

Antioxidants have been prescribed to mitigate the long-term complications of diabetes by partially reducing oxidative stress (Sabu & Kuttan, 2002, 2004).

According to Fig. 3, there was a strong positive correlation between ABTS radical scavenging activity and anti-diabetic activities. The results revealed that in Fig. 3 there was a strong positive correlation between DPPH radical scavenging ability and anti-diabetic activities. Furthermore, a previous study conducted by Nijat et al. (2021) aimed to evaluate the interrelationship between ABTS and DPPH radical scavenging activities of *Rosa rugosa* with its antidiabetic activities. This study further confirmed that the bioactive compounds responsible for the antidiabetic activities were consistent with the antioxidant activity. Moreover, the results obtained from this study indicated the impact of free radical scavenging activities on reducing diabetic mellitus. Obesity is characterized by chronic oxidative stress and inflammation, even in the absence of other risk factors, suggesting their involvement in the development of metabolic syndrome. This syndrome is associated with various illnesses, including type 2 diabetes, cardiovascular disease, arthritis, hypertension, cardiac arrest, and certain cancers. A recent study by Mba et al. (2022) utilizing *Psychotria densinervia* has provided evidence supporting this association mentioned. In a previous study conducted by Bashkin et al. (2021), the objective was to identify a dietary treatment for dysregulation of glucose homeostasis caused by chronic hyperglycemia. Screening 41 plant extracts revealed a remarkable correlation between antidiabetic and antioxidant activities, closely aligning with our research findings. Furthermore, our study further validates these findings by establishing correlations between antioxidant activity and both anti-inflammatory and anti-diabetic activities.

4. Conclusion

From the results obtained in the present study, the hydro methanolic extract of fresh Noni fruit pulp showed antioxidant activities, anti-diabetic activities, and anti-inflammatory activities. The preliminary phytochemical investigation indicates that the *Morinda citrifolia*. L. contains β- carotene, lycopene and higher in phenolic compounds, flavonoids, anthocyanin, and ascorbic content. The availability of bioactive compounds and functional properties was reduced gradually with the digestion stage of gastrointestinal digestion. Especially, there is a necessity for a complete characterization of gastrointestinal factors that could influence the bioaccessibility of bioactive compounds in *Morinda citrifolia* fruit. These plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. However, current knowledge of the bioavailability and bioaccessibility of bioactives facilitates the development of novel functional foods using *Morinda citrifolia* fruit. This research was

carried out according to an in vitro model which has some drawbacks compared to actual in vivo conditions. Therefore, further studies should be carried out to support the findings of this study, perhaps, coupled with experimental cellular and animal models to obtain more qualitatively well-correlated results with human studies.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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