RESEARCH ARTICLE

Proximate composition, bioactive constituents, and therapeutic potentials of pasteurized Noni juice derived from *Morinda citrifolia* (L.) growing in Sri Lanka

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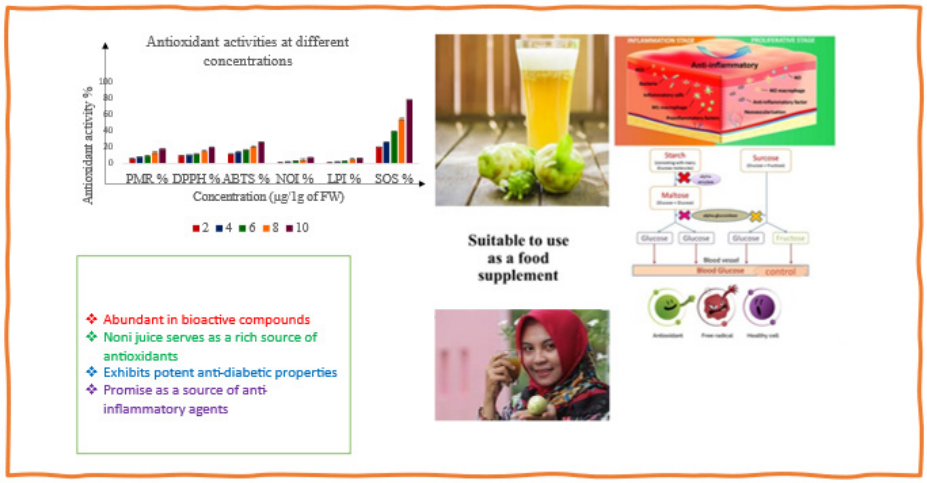

Highlights

- Pasteurized fruit juice of *Morinda citrifolia* (L.) or 'Ahu' is a globally acclaimed for wellness yet faces consumer reluctance due to myths and taste in Sri Lanka.
- Juice shows strong antioxidant properties by reducing free radicals, inhibiting lipid peroxidation, and suppressing singlet oxygen.
- It exhibits an anti-diabetic and anti-inflammatory properties.

Ceylon Journal of Science 53 (1) 2024: 87-96
DOI: http://doi.org/10.4038/cjs.v53i1.8141

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Proximate composition, bioactive constituents, and therapeutic potentials of pasteurized Noni juice derived from *Morinda citrifolia* (L.) growing in Sri Lanka

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Received: 09.05.2023; Accepted: 06.02.2024

Abstract: *Morinda citrifolia* (L.), also known as Noni or Ahu in Sri Lanka, is infrequently consumed as a dietary supplement within the country due to its association with myths and perceived toxicity. Nonetheless, in Sri Lanka, limited scientific research has been undertaken in the past to explore the therapeutic potential of noni, with public awareness primarily focused on its export-driven cultivation. The study focused on evaluating the proximate composition, bioactive constituents, and therapeutic potential of hydro-methanolic extracts from pasteurized noni fruit juice, employing an 80% methanol extraction method, filtration, and assessment through standard assays to elucidate its potential health benefits. The juice showed noteworthy antioxidant potential through DPPH scavenging activity (57.90 ± 0.40 µg/mL) and ABTS scavenging activity (42.95 ± 1.33 µg/mL), as well as high nitric oxide inhibition activity (164.56 ± 3.22 µg/mL), suggesting its anti-inflammatory properties. The juice also displayed potential as an anti-diabetic agent by inhibiting alpha-amylase (27.68 ± 0.36 µg/mL) and alpha-glucosidase (25.67 ± 1.03 µg/mL). Furthermore, it exhibited potential in protecting against certain pathological processes through heat-induced hemolysis inhibition (36.08 ± 1.08 µg/mL), protein denaturation inhibition (56.14 ± 1.11 µg/mL), and proteinase inhibitory activity (38.04 ± 0.56 µg/mL). These findings support the potential use of pasteurized fruit juice as a functional food ingredient, offering benefits to both the food industry and consumers. Further research can be directed towards the development of Noni fruit-incorporated food products that possess desirable sensory attributes to mitigate any potential aversion associated with its taste and odor profiles.

***Keywords*:** *Morinda citrifolia,* Noni fruit**,** Antioxidant, Anti-diabetic, Anti-inflammatory, Dietary supplement, Sri Lanka

INTRODUCTION

Morinda citrifolia (L.), or noni, is a perennial herb that originated in Southeast Asian countries, and its fruit has been consumed for over 2000 years in folk medicine (Ali et al.,2016). The plant thrives in various tropical environments with infertile, acidic, and alkaline soils, as well as excessively dry and wet weather conditions (Silva et al., 2016). Noni fruits were initially used by native Polynesians as a common ethnomedicine to treat various illnesses (Deng et al., 2010). Although the fruits are edible, the objectionable odor and flavor of the fruit flesh (Joshi et al., 2012) restrict their consumption in the fresh form. However, noni fruit juice is considered a wellness drink due to its therapeutic values, reported to be effective in treating many diseases, including cancer, hypertension, and inflammation (Basar et al., 2010), diabetes, cardiovascular diseases, infection, arthritis, asthma, and pain (Wang et al., 2009), as well as mental depression, diarrhea, and indigestion (Krishnakumar et al., 2015). Therefore, noni fruits have gained worldwide attention, and the demand for commercial noni fruit products in the global market has increased.

Noni fruits are harvested year-round, although there are seasonal variations in flowering and fruit production (Chan-Blanco et al., 2006). The fruit of the noni plant typically matures within 9 to 12 months after planting. When unripe, the fruit is dark green, and when ripe, it has a lumpy, green to yellowish-white appearance with polygonal sections on its outer surface (Mani et al., 2021). Noni is widely utilized as an alternative and complementary therapy due to its potent antioxidant activity and established health benefits. It has a long history of medicinal use in Southeast Asia, Polynesia, Australia, and particularly in the Hawaiian Islands where it is the second most significant medicinal plant. In the United States, noni's popularity has grown, as evidenced by the commercial availability of processed products in health food stores, natural food specialty chains, and online. Noni is commonly sold in tablet, tea, and juice forms, with the juice being the most prevalent option (Su et al., 2005; Carrillo-López and Yahia, 2011; Abou Assi et al., 2017).

It appears that noni juice is as safe as other common fruit juices. No adverse effects of noni fruits have been observed in extensive toxicological studies, including tissue culture work, animal experiments, and human clinical trials (Samarasinghe et al., 2023b; West et al., 2006). Noni fruit was reported to be acceptable for human consumption, based on official safety evaluations done by the European

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Union (European Commission, 2002). The absence of convincing evidence for a causal relationship between the occurrence of acute hepatitis and the consumption of noni juice is reported (European Food Safety Authority, 2006). Chemical analysis and genotoxicity tests revealed that noni juice did not show any genotoxic potential (Samarasinghe et al., 2023b), and genotoxic anthraquinones were not found in the juice (Westendorf et al., 2007). With an increase in widespread use in general and potential use, particularly among pregnant women, a prenatal developmental toxicity test conducted to further evaluate the safety of noni juice revealed no toxicity effect of the fruit on the developing embryo/fetus (Brett et al., 2008).

The physiochemical screening of the fruit juice showed the presence of anthraquinones, saponins, and scopoletin (Satwadhar et al., 2011). Bioactive screening of the fermented fruit juice reported the presence of superior vitamin content (C, B_1, B_2, B_3, and B_{12}), alkaloids, anthraquinones, antioxidants, essential oils, flavonoids, saponins, scopoletin, and sugars (Nandhasri et al., 2005). Numerous studies have shown that noni has a wide range of therapeutic effects, as reported based on clear scientific evidence from in vitro and in vivo studies, or clinical trials. The phytochemical components of noni demonstrate a broad spectrum of activities, encompassing antimicrobial, antiseptic, antifungal, antioxidant, anti-inflammatory, anti-arthritic, anti-cancer, antidiabetic, antiemetic, anti-viral, anti-parasitic, and anti-tuberculosis effects (Abou et al., 2017; Senthilkumar et al., 2016). Moreover, wound healing, memory enhancing, anxiolytic and sedative, analgesic, gastric ulcer healing, gout and hyperuricemia healing, anti-psoriasis healing, immunity enhancing, osteoporotic and otoscopic enhancing, anthelminthic, analgesic, hypotensive, LDL oxidation preventive and immune enhancing effects are also reported (Abou et al., 2017; Senthilkumar et al., 2016).

Even though phytochemical composition and pharmacological examinations of noni fruits have been conducted worldwide (Kulathunga & Arawwawala, 2017), very limited research has been carried out in Sri Lanka (Samarasinghe et al., 2023a). Moreover, the phytochemical makeup of a given plant species is affected by geographical conditions and the methods of processing the fresh fruit and extracting bioactive compounds (Menković et al., 2000). Thus, in this research, we studied the proximate composition, bioactive compounds, antioxidant, anti-inflammatory, and anti-diabetic activities of pasteurized noni fruit juice prepared from noni fruits growing in Sri Lanka.

MATERIALS AND METHODOLOGY

Plant material

Ripe fruits were obtained from trees grown in the *Katugasthota* area of the Kandy district, Sri Lanka. The fruits, selected based on color and shape, were vacuum-packaged in polyethylene bags, and stored at -18°C until further analysis.

Chemicals and reagents

The reagents used for the study included DNP (2,4-dinitrophenylhydrazine), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), trichloroacetic acid, thiobarbituric acid (TBA), Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromano-2-carboxylic acid (Trolox), acarbose, yeast α-glucosidase from *Saccharomyces cerevisiae*, porcine pancreatic α-amylase, egg yolk homogenate, sodium dodecyl sulfate, sodium potassium tartrate, sodium phosphate, disodium orthophosphate, bovine albumin, casein, trypsin, and dinitro salicylic acid color reagent. All other common chemicals utilized in this research were of analytical grade, and they were purchased from Sigma-Aldrich, St. Louis, MO, through Analytical Instrument Pvt Ltd., Colombo, Sri Lanka.

Preparation of pasteurized noni fruit juice extracts

The ripened fruit flesh was squeezed through a muslin cloth, and the resultant juice was pasteurized at 90°C for 1 min (Skrede et al., 2000). Methanolic extracts of the pasteurized juice were prepared as described by (Kumari & Gunathilake, 2020), and 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, as described by (Samarasinghe et al., 2023c).

Proximate composition analysis

Determination of moisture content

The pasteurized juice sample (5–6)g was accurately weighed into a previously weighed moisture can and placed in an oven (G209A - Combined Laboratory Oven & Incubator) at 105°C. The sample was transferred into a desiccator in 30-minute intervals, allowed to cool down and weighed. Drying and weighing was repeated until two consecutive weights were the same, and the moisture content was calculated (Rybak-Chmielewska, 2003; Jaafar et al., 2009; Ogoloma et al., 2013) as follows,

$$\text{Moisture content (by mass \%)} = \frac{W_1 - W_2}{W_1 - W_0} \times 100\%$$

Where,

W_0 - Weight of the empty moisture can (g)

W_1 - Weight of the empty moisture can and sample before drying (g)

W_2 - Weight of the empty moisture can and sample after drying (g)

Determination of crude protein content

To determine the percentage of crude protein in the pasteurized juice sample, (0.4-1.0)g of the sample was weighed into a micro-Kjeldahl tube, along with 2 catalyst tablets and 20 mL of concentrated sulfuric acid. The sample was then digested at 400°C for 1 hour until it became colorless or pale straw. After cooling, 35 mL of distilled water was added to the tube, and it was distilled using a Kjeldahl distillation unit to trap the released ammonia.

The ammonia was subsequently titrated against 0.1 N hydrochloric acid solutions, and the percentage of nitrogen in the sample was calculated. To determine the percentage of crude protein, the percentage of nitrogen was multiplied by 6.25(Rybak-Chmielewska, 2003; Jaafar et al., 2009; Ogoloma et al., 2013).

Determination of crude fat content

To determine the crude fat content, the pasteurized juice sample was initially dried at 105°C to eliminate moisture and then weighed (5-6 g) in a thimble. Fat extraction was carried out using diethyl ether in an intermittent Soxhlet extractor for 10-15 hours, with a round-bottomed flask attached to the apparatus. The extracted diethyl ether was subsequently evaporated on a rotavapour, and the flask's weight was measured. The crude fat content was calculated and expressed per 100 g of fresh matter (Rybak-Chmielewska, 2003; Jaafar et al., 2009; Ogoloma et al., 2013).

Determination of ash content

The pasteurized juice sample, dried at 105°C to remove moisture, was accurately weighed (5 – 6 g) into a previously weighed crucible and slowly burned over a flame until the sample charred. The crucible, containing the charred sample, was then heated in a muffle furnace (FM 28, YAMATO Science Co. Ltd., Tokyo, Japan) at 550°C for 8 hours until white or light grey ash was obtained. Subsequently, the crucible was cooled in a desiccator, weighed, and the ash content was calculated and expressed per 100 g of fresh matter (Rybak-Chmielewska, 2003; Jaafar et al., 2009; Ogoloma et al., 2013).

Determination of crude fiber content

To determine the crude fiber content, a 2 g residue remaining after crude fat extraction was dried and transferred into a 400 mL beaker. Next, 50 mL of 5% sulfuric acid was added, and distilled water was added to the 200 mL mark. The solution was then boiled for 30 minutes, filtered, and washed with distilled water to remove acids. The residue was subsequently transferred to the same beaker with hot water, and 50 mL of 5% sodium hydroxide was added. After boiling for 30 minutes, the mixture was filtered and washed with distilled water to remove the bases. The residue underwent additional washing with hot water, 1% hydrochloric acid, and 95% ethanol before being transferred to an evaporating dish. After drying and weighing the dish, the dry residue ignited and weighed again. The crude fiber content was then calculated and expressed per 100 g of fresh matter (Rybak-Chmielewska, 2003; Jaafar et al., 2009; Ogoloma et al., 2013).

Bioactive components determination

Total phenolic content

The total phenolic content of the extract was determined at 760 nm using Folin-Ciocalteau's reagent method, as described by Hasperué et al. (2016), with some modifications. A UV/Visible spectrophotometer (Optima, SP-3000, Tokyo, Japan) was employed. The sample's absorbance was measured at 760 nm in triplicate, and the results were compared to a blank. A standard curve created with gallic acid was used for calibration, and the total phenolic content was expressed as μmol of gallic acid equivalents (GAE) per gram of fresh weight.

Total flavonoid content

The spectrophotometric method outlined by Zhishen et al. (1999), with slight modifications, was employed to estimate the total flavonoid content of the extract. A standard curve was generated using rutin, and the total flavonoid content of each collected sample was reported as μmol of rutin equivalent (RE) per gram of sample fresh weight.

Total anthocyanin content

The spectrophotometric pH differential method, as described by Janarny et al. (2022), was employed to estimate the total anthocyanin content of the extract. The concentrations of monomeric anthocyanins were reported as cyanidin-3-glucoside equivalents (mg/mL).

β-carotene and lycopene content

The method outlined by Janarny et al. (2022) was employed with slight modifications to estimate the β-carotene and lycopene contents of the pasteurized noni fruit extract. The absorbance was measured at 453, 505, 645, and 663 nm, and the contents of β-carotene and lycopene were calculated using the following equations.

B - carotene (mg⁄100 mL) = 0. 216A663 −1. 22A645 −0.304A505 +0. 452A453

Lycopene (mg⁄100 mL) = −0.0458A663 +0.204A645 +0. 372A505 −0.0806A453

Ascorbic acid content

The spectrophotometric method described by Khan et al. (2006) was employed to estimate the ascorbic acid content of the fruit extract. Specifically, 200 μL of the extract was mixed with 300 μL of 4% trichloroacetic acid. The DNP reagent, prepared according to Bell et al. (1981), was used. The absorbance of the sample was measured using a UV/Visible spectrophotometer at 520 nm.

Antioxidant assays

Total antioxidant capacity

To estimate the total antioxidant capacity of the test sample, the method for reducing Mo VI to Mo V, as explained by Prieto et al. (1999), was adopted with slight modifications. A volume of three milliliters of a reagent solution comprising 4 mM Ammonium molybdate, 0.6 mM H_2SO_4, and 28 mM Na_3PO_4 was introduced to 0.1 ml of the sample. Subsequently, the reaction mixture underwent incubation for a duration of 90 minutes at 95°C and was subsequently cooled to room temperature. The final absorbance of the mixture was determined at 695 nm using a UV/Visible spectrophotometer against a blank. The standard curve was established employing ascorbic acid.

ABTS scavenging activity.

The ABTS free radical scavenging activity was assessed by combining 100 μL of the test sample with 2.9 mL of an ABTS solution and measuring absorbance at 734 nm. The spectrophotometric method, as described by Payum et al. (2013), with slight modifications, was used. The standard

curve was established using ascorbic acid. Absorbance values were adjusted for radical decay by utilizing blank solutions in the experimental procedure.

Lipid peroxidation inhibition activity

The inhibition of lipid peroxidation of the pasteurized noni fruit extract was assessed using the Thiobarbituric acid-reactive species (TBARS) assay, modified and explained by Ohkawa et al. (1979). The control, prepared without the addition of the sample extract, had its absorbance (C) measured at λ max = 532 nm. The standard curve was established using Butylated hydroxy toluene.

FRAP (Ferric Reducing Antioxidant Power) Assay

The antioxidant capacity of pasteurized noni fruit extract was measured using the FRAP assay, following the methodology outlined by Gunathilake and Rupasinghe (2014) with slight modifications. A standard curve was constructed with ascorbic acid.

Determination of 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The capacity of the prepared extracts to scavenge the 'stable' free radical DPPH was tested according to the method of Rahman et al. (2015) with slight modifications. This involved mixing a freshly prepared methanolic solution of DPPH (0.1 mM, 3.6 mL) in methanol with 0.4 mL of the test sample, with Trolox used as the reference standard. The absorbance of the resulting solution was read spectrophotometrically using a UV/Visible spectrometer (SP-3000, OPTIMA INC, Japan) at 517 nm.

Singlet oxygen ($^1O_2$) scavenging activity.

The spectrophotometric method by Chakraborty & Tripathy (1992) was adopted with slight modifications following Wang & Jiao's (2000) approach to determine the production of singlet oxygen ($^1O_2$) by sodium hypochlorite and H_2O_2. *N, N*-dimethyl-*p*-nitrosoaniline was used as a selective scavenger of singlet O_2, and histidine served as a selective acceptor. The bleaching of *N, N*-dimethyl-*p*-nitrosoaniline was monitored spectrophotometrically at 440 nm. The singlet oxygen scavenging activity of the sample was compared with the standard curve constructed using gallic acid.

Examining anti-diabetic properties

***In vitro* α- amylase inhibitory assay**

The inhibition of porcine α-amylase activity by pasteurized noni fruit extract was assessed through color development with dinitro salicylic acid at 540 nm using a UV/Visible spectrophotometer (Thermo Scientific 201, United States). The method followed the procedure described by Poovitha & Parani (2016) with slight modifications, and Acarbose was used as a positive control at varying concentrations.

α-Glucosidase inhibitory assay

In vitro α-glucosidase activity was evaluated using the spectrophotometric method by Kim et al. (2004) with slight modifications to estimate the α-glucosidase (maltase) inhibitory activity of pasteurized noni fruit extract, ex. Yeast (Sisco Research Laboratories Pvt. Ltd.). Absorbance was measured at 405 nm using a UV/Visible spectrophotometer (Thermo Scientific 201, United States), with Acarbose used as a positive control at varying concentrations.

Examining anti-Inflammatory properties

Nitric oxide inhibition activity

This assay aimed to determine the nitric oxide inhibitory activity of the test sample using the spectrophotometric method described by Samad et al. (2014) and outlined in Kumari & Gunathilake (2020) with some modifications. The pasteurized noni fruit juice was combined with sodium nitroprusside (100 mM, 1.5 mL) in a phosphate buffer with a pH of 7.2 and incubated for one hour at 30°C. Griess reagent was prepared by mixing equal volumes of sulfanilamide (1%) in phosphoric acid (2%) and naphthyl ethylenediamine dihydrochloride (0.1%) in water. Subsequently, 5 ml of this Griess reagent was added to each reaction mixture. The absorbance was then measured at λmax = 540 nm.

The inhibition percentage was calculated by determining the difference in absorbance between the sample mixture and the control. To establish the standard curve, various concentrations of ascorbic acid were employed.

Membrane lysis assays

Heat-induced hemolysis

A 10% (v/v) erythrocyte suspension was prepared from whole human blood collected from a healthy subject following the method outlined by Rashid et al. (2011), with modifications as per Gunathilake et al. (2018). The inhibition of heat-induced hemolysis by noni fruit extracts (methanolic and water) and in-vitro digested fractions was conducted using the spectrophotometric method described by Umapathy et al. (2010), with adjustments made by Gunathilake et al. (2018). The absorbance of the supernatant was measured at 540 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan).

Effect on Protein Denaturation

The test was conducted to determine the effect of pasteurized noni fruit extract and in-vitro digested aliquot fractions on 1% bovine albumin protein denaturation. This was done using the spectrophotometric method outlined by Juvekar et al. (2009), with some modifications as per Gunathilake et al. (2018), and the absorbance was measured at 660 nm with a UV/VIS spectrometer.

Proteinase Inhibitory Activity

The test was conducted to estimate the inhibitory activity of pasteurized noni fruit extract on trypsin, following the spectrophotometric method outlined by Sakat et al. (2010) with modifications as suggested by Gunathilake et al. (2018), and measurements were taken at 210 nm using a UV/VIS spectrometer. The percentage of inhibition of proteinase activity was then calculated.

Data analysis

All samples were analyzed in triplicate, and one-way analysis of variance (ANOVA) was performed using SAS software (Cary, USA) to determine significant differences

at various levels. When significant differences were present ($p > 0.05$), multiple mean comparisons were conducted using the least significant difference (LSD) method. Data were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

The proximate composition of pasteurized noni juice from noni fruits growing in Sri Lanka, as mentioned in Table 1, revealed that the juice contains high moisture content (96.60 ± 2.76%) with low amounts of crude protein (0.01 ± 0.00%), crude fat (0.47 ± 0.02%), crude fiber (0.49 ± 0.02%), and ash (0.39 ± 0.01%) per 100 g of fresh weight. However, noni juice has a moderate amount of crude carbohydrates (2.03 ± 0.04%) per 100 g of fresh weight. These findings are consistent with previous studies on noni juice from different regions, which reported similar levels of moisture, protein, fat, fiber, and ash content (Joshi et al., 2012; Li et al*.,* 2020; Ma et al., 2013). It is worth noting that the chemical composition of noni juice may vary depending on the ripeness and harvesting time of the fruit, as well as the processing methods used to prepare the juice (Mani et al., 2021).

Table 1. Proximate composition of pasteurized Noni fruit juice.

Chemical composition (%) (per 100 g of fresh weight)	Pasteurized Noni fruit juice
Moisture	96.60 ± 2.76
Crude protein	0.01 ± 0.00
Crude fat	0.47 ± 0.02
Crude fiber	0.49 ± 0.02
Crude carbohydrate	2.03 ± 0.04
Ash	0.39 ± 0.01

Note: All data mean ± SD (n–3).

The present study analyzed the bioactive compound composition of pasteurized Noni juice. The results in Table 2 revealed the presence of several important bioactive compounds, including total phenolics, total flavonoids, ascorbic acid, monomeric anthocyanin, β-carotene, and lycopene. These findings contribute to our understanding of the potential health benefits associated with Noni juice consumption.

Table 2. Bioactive compounds in methanolic extracted pasteurized Noni fruit juice.

Bioactive compound	Pasteurized Noni Juice
Total phenolics *	165.85 ± 0.84
Total flavonoids **	6.36 ± 0.22
Ascorbic acid***	24.68 ± 0.89
Monomeric anthocyanin ***	8.91 ± 0.96
β-carotene ***	0.15 ± 0.02
Lycopene ***	0.14 ± 0.01

Note: All data 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.

Total phenolics, expressed as μmol gallic acid equivalent per 1 g fresh weight, were found to be 165.85 ± 0.84. Phenolic compounds are well-known for their antioxidant properties and have been linked to various health benefits, such as reducing the risk of chronic diseases like cardiovascular diseases and cancer (Samarasiri et al., 2019). The high content of total phenolics in Noni juice suggests its potential use as a functional beverage for promoting health. Total flavonoids, expressed as μmol rutin equivalent per 1 g fresh weight, were measured at 6.36 ± 0.22. Flavonoids are a subclass of phenolic compounds known for their antioxidant, anti-inflammatory, and anticancer activities (Aryal et al., 2019).The presence of flavonoids in Noni juice further adds to its potential health benefits. Ascorbic acid, also known as vitamin C, was found to be 24.68 ± 0.89 μg per 1 g fresh weight. Vitamin C is an essential nutrient with antioxidant properties that play a vital role in immune function, collagen synthesis, and protection against oxidative stress (Chambial et al., 2013). The presence of ascorbic acid in Noni juice suggests its potential as a natural source of vitamin C. Monomeric anthocyanin, expressed as μg per 1 g fresh weight, was measured at 8.91 ± 0.96. Anthocyanins are natural pigments responsible for the vibrant colors of fruits and vegetables and have been associated with numerous health benefits, including anti-inflammatory and antioxidant effects (Mattioli et al., 2020). The presence of monomeric anthocyanin in Noni juice suggests its potential as a source of these beneficial compounds. β-carotene, a precursor of vitamin A and a provitamin with antioxidant properties, was found to be 0.15 ± 0.02 μg per 1 g fresh weight. β-carotene is known for its role in maintaining vision, supporting immune function, and protecting against oxidative damage (Gul et al., 2015) The presence of β-carotene in Noni juice adds to its potential nutritional value. Lycopene, another carotenoid with antioxidant properties, was measured at 0.14 ± 0.01 μg per 1 g fresh weight. Lycopene has been associated with a reduced risk of certain cancers, particularly prostate cancer, and has shown potential in protecting against cardiovascular diseases (Khan et al., 2021). The presence of lycopene in Noni juice may contribute to its potential health benefits. Overall, the analysis of bioactive compounds in pasteurized Noni juice revealed significant levels of total phenolics, total flavonoids, ascorbic acid, monomeric anthocyanin, β-carotene, and lycopene. These findings support the potential health-promoting properties of Noni juice. However, further research is needed to explore the bioavailability and biological activities of these compounds in the context of human health.

The present study investigated the *in vitro* antioxidant, anti-inflammatory and anti-diabetic potentials of pasteurized Noni fruit juice by evaluating its IC_{50} values for various assays which mentioned in Table 3. The results provide valuable insights into the antioxidant activity of Noni juice and its potential health benefits. Assessing the antioxidant capacity of a biological sample is a complex task that cannot be adequately captured by a single assay, as various factors, such as the compatibility with lipophilic and hydrophilic samples and potential interferences in reaction mixtures, must be considered; hence, multiple assays were employed

to evaluate the antioxidant potential of Pasteurized noni juice samples.

The total antioxidant capacity (TAC) determined through the phosphomolybdenum assay is based on either an electron transfer (ET) or hydrogen atom transfer (HAT) mechanism, leading to the reduction of molybdenum (VI) to molybdenum (V) as described by Bibi Sadeer et al. (2020). In the specific case of pasteurized Noni fruit juice, the TAC was measured to be 43.92 ± 0.33 µg/mL. Furthermore, the calculated IC_{50} value for total antioxidant capacity was compared to ascorbic acid, a well-known antioxidant, revealing an IC_{50} value of 98.49 ± 0.24 µg/ml. Bibi Sadeer et al. (2020) explained that the correlation between the phosphomolybdenum assay and other antioxidant assays remains debatable. Additionally, Prieto et al. (1999) highlighted that DPPH and ABTS•+ are capable of detecting antioxidants such as flavonoids and phenols. In contrast, the phosphomolybdenum assay generally detects antioxidants, specifically ascorbic acid, some phenolics, α-tocopherol, and carotenoids.

The current study involved the performance of two free radical scavenging antioxidant assays, namely the 2,2-Diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) assay and the Trolox equivalent antioxidant capacity or 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (TEAC or ABTS•+) assay. According to Sina et al. (2020), fresh fruit juice exhibited an IC50 of 24 µg/mL, whereas the fermented fruit juice demonstrated lower activity with an IC50 of 47 µg/mL. In our study, pasteurized noni fruit juice exhibited DPPH scavenging activity with an IC_{50} value of 57.90 ± 0.40 µg/ml. The DPPH assay measures the ability of a sample to scavenge DPPH radicals, representative of free radicals. The IC_{50} value for DPPH scavenging activity was compared to Trolox, a synthetic antioxidant compound, with an IC_{50} value of 66.11 ± 0.20 µg/mL. The comparable activity of Noni juice indicates its potential as a natural alternative to synthetic antioxidants. According to a study conducted by Selvam and Kumar (2017), Divine Noni Juice (DNJ) was tested for in vitro antioxidant activity using the ABTS method to estimate its antioxidant potency. The results indicate that Divine Noni Juice demonstrated activity in scavenging free radicals by the ABTS method, with a 50% inhibitory concentration (IC50) of 66.47 µl/mL. In comparison, the ABTS scavenging activity of pasteurized noni fruit juice was measured at 42.95 ± 1.33 µg/mL. The ABTS assay evaluates the ability of a sample to scavenge ABTS radicals and assesses its overall antioxidant potential. The IC_{50} value for ABTS scavenging activity was compared to Trolox, with an IC_{50} value of 51.17 ± 0.80 µg/mL. Noni juice exhibited similar scavenging activity, suggesting its potential as a natural antioxidant. As reported by Bibi Sadeer et al. (2020), the solubility contrast between the ABTS cationic radical, which is soluble in both organic and aqueous media, and the DPPH radical, which exclusively dissolves in organic medium, enables the ABTS assay to serve as a versatile screening tool for evaluating the antioxidant activity of both lipophilic and hydrophilic samples. Therefore, the results of these two assays showed notable differences.

The hydroxyl radical (HO•) is a potent reactive oxygen species (ROS) in biological systems, causing damage to cell membranes by reacting with polyunsaturated fatty acids. As the most harmful ROS, HO• can impair cell membranes, disrupt sugar groups, induce DNA alterations, and trigger cell apoptosis and mutations. The TBARS assay quantifies lipid peroxidation, enduring as a method to evaluate radical activity between HO• and antioxidants (Bibi Sadeer et al.,2020). Pasteurized noni fruit juice demonstrated lipid peroxidation activity with an IC_{50} value of 159.9 ± 3.21 µg/mL. Lipid peroxidation is associated with oxidative damage and various diseases. The IC_{50} value for lipid peroxidation

Table 3. IC_{50} values for antioxidant, anti-inflammatory and anti-diabetic activities of pasteurized Noni fruit juice.

In vitro bioactivity	Pasteurized fruit juice (µg/mL)	Standard (µg/mL)
Total Antioxidant Capacity	43.92 ± 0.33^{t}	98.49 ± 0.24^{q} (Ascorbic acid)
DPPH Scavenging Activity	57.90 ± 0.40^{t}	66.11 ± 0.20^{r} (Trolox)
ABTS Scavenging Activity	42.95 ± 1.33^{t}	51.17 ± 0.80^{r} (Trolox)
Lipid Peroxidation Activity	159.9 ± 3.21^{o}	60.32 ± 0.87^{r} (Butylated hydroxy toluene)
Singlet O_2 Inhibition Activity	24.87 ± 0.41^{t}	19.12 ± 0.52^{s} (Gallic acid)
Ferric Reducing Antioxidant Power Assay (FRAP assay)	81.29 ± 0.99^{q}	55.31 ± 0.28^{r} (Ascorbic acid)
Alpha-Amylase Inhibitory Activity	27.68 ± 0.36^{t}	32.61 ± 0.20^{s} (Acarbose)
Alpha-Glucosidase Inhibitory Activity	25.67 ± 1.03^{s}	44.77 ± 0.08^{r} (Acarbose)
Nitric oxide Inhibitory Activity	164.56 ± 3.22^{p}	101.47 ± 0.20^{q} (Ascorbic acid)
Heat-Induced Hemolysis Inhibitory Activity	36.08 ± 1.08	55.03 (Aspiring) {Truong *et al*., 2021}
Protein Denaturation Inhibitory Activity	56.14 ± 1.11	58.96 (Aspiring) {Truong *et al*., 2021}
Proteinase Inhibitory Activity	38.04 ± 0.56	60.89 (Aspiring) {Truong *et al*., 2021}

Note: All data mean ± *SD* (*n*–3).

activity was compared to butylated hydroxy toluene, with an IC_{50} value of 60.32 ± 0.87 µg/mL. Although Noni juice exhibited a higher IC_{50} value, it still demonstrated notable lipid peroxidation inhibition, indicating its potential as a protective agent against oxidative damage.

Singlet oxygen, crucial in host defense by mediating the destruction of infectious agents, becomes a double-edged sword as its sustained production in chronic inflammation may inflict severe damage to tissues, contributing to ailments such as cardiovascular disease, multiple sclerosis, diabetes, cancer, and dementia, highlighting the vital significance of singlet oxygen's scavenging ability(Costa et al., 2008;Petrou et al., 2018). In terms of singlet oxygen inhibition activity, Noni fruit juice showed an IC_{50} value of 24.87 ± 0.41 µg/mL. The IC_{50} value for singlet oxygen inhibition activity was compared to gallic acid, with an IC_{50} value of 19.12 ± 0.52 µg/mL. The results suggest that singlet oxygen is being scavenged by Pasteurized noni juice in a dose-dependent manner, indicating its potential as a natural scavenger of singlet oxygen.

Furthermore, Yilmazer et al. (2016) utilized the d-ROMs test and BAP test, validated methods for evaluating oxidative stress in diverse health conditions, and found that the examined commercial noni juice demonstrated significant antioxidant activities. Nevertheless, in a study conducted by Chen et al. (2018), it was found that neurons were protected from oxidative damage induced by tert-Butyl hydroperoxide (TBHP) through the administration of noni juice extracts, specifically chloroform and aqueous fractions. The study revealed that oxidative stress, apoptosis, and cytotoxicity in SH-SY5Y cells were effectively reduced by these fractions. The mechanisms involved the upregulation of antioxidant enzymes, nuclear factor-erythroid 2 related factor 2 (Nrf2), and the inhibition of the mitochondrial pathway. The results suggest the potential use of these fractions in the prevention or therapy of neurodegeneration.

Numerous research studies have substantiated the antidiabetic potential of fermented noni juice in *vivo* and in *vitro*, revealing its efficacy in significantly reducing blood glucose levels, improving wound healing, preventing liver degeneration, inhibiting weight gain, enhancing glucose and insulin tolerance, and ameliorating various diabetic markers in animal models from diverse geographical regions, including Trinidad and Tobago, West Indies, Honolulu, HI, USA, Seoul, Korea, and Nigeria(Nerurkar et al., 2015). Nonetheless, pasteurized noni fruit juice exhibited alpha-amylase inhibitory activity with an IC_{50} value of 27.68 ± 0.36 µg/mL. Alpha-amylase is an enzyme involved in the breakdown of complex carbohydrates into simpler sugars (Shukla et al., 2015). Alpha-amylase inhibition is a significant therapeutic target in the control of the postprandial rise in blood glucose in diabetes patients(Zinjarde et al., 2011). The IC_{50} value for alpha-amylase inhibition was compared to acarbose, a known alpha-amylase inhibitor, with an IC_{50} value of 32.61 ± 0.20 µg/mL. Noni juice demonstrated comparable inhibitory activity, suggesting its potential as a natural alternative for managing carbohydrate digestion and postprandial blood sugar levels. The inhibitory activity of Noni fruit juice against alpha-glucosidase, another key enzyme involved in carbohydrate digestion, was determined with an IC_{50} value of 25.67 ± 1.03 µg/ml. The enzyme alpha glucosidase catalyzes the release of α-glucose from the non-reducing end of the substrate. The rise in blood sugar that occurs after a meal high in carbohydrates is slowed by inhibiting this enzyme(Kumar et al., 2011). The IC_{50} value for alpha-glucosidase inhibition was compared to acarbose, with an IC_{50} value of 44.77 ± 0.08 µg/mL. Moreover, Zhang et al. (2021) demonstrated the potent inhibitory activity against α-glucosidase of two novel noni alkaloids isolated from Noni Juice, extracted with ethyl acetate, with IC_{50} values of 413.7 and 364.4 µM, respectively. Hence, pasteurized noni juice helps to slow down the breakdown of complex carbohydrates into glucose, thereby reducing the postprandial increase in blood sugar levels.

Excessive production of inflammatory mediators, notably nitric oxide (NO), has been associated with inflammatory diseases (Sharma et al., 2007; Suluvoy & Berlin Grace, 2017), and numerous natural compounds have been identified as inhibitors of NO synthase, exhibiting potential anti-inflammatory properties (Pepe et al.,2017). In a previous study conducted by Lee et al. (2020), the anti-inflammatory properties of noni fruit juice were investigated. Five compounds, namely asperulosidic acid, rutin, nonioside A, (2E,4E,7Z)-deca-2,4,7-trienoate-2-O-β-d-glucopyranosyl-β-d-glucopyranoside, and tricetin, were isolated and analyzed using NMR spectroscopy and LC/MS for structural elucidation. In an anti-inflammatory assay, these compounds exhibited inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated macrophages. Mechanistic studies revealed downregulation of IKKα/β, I-κBα, NF-κB p65, nitric oxide synthase, and cyclooxygenase-2, suggesting their involvement in the observed anti-inflammatory effects. Additionally, previous research by Manavalan et al. (2021) investigated Nrf2 activators in *Morinda citrifolia* L. fruit juices, revealing that ripe Noni juice fermented with Lactobacillus plantarum at 22°C activated Nrf2 and inhibited NF-κB, suggesting its potential therapeutic application in oxidative stress and inflammation. In the current investigation, pasteurized Noni fruit juice exhibited noteworthy activity in inhibiting nitric oxide, as evidenced by an IC_{50} value of 164.56 ± 3.22 µg/mL. This IC_{50} value was compared to that of ascorbic acid, which demonstrated an IC_{50} value of 101.47 ± 0.20 µg/mL in nitric oxide inhibition.

Inflammation involves cellular infiltration, where leukocytes release enzymes causing tissue damage. Cell membrane damage increases susceptibility to further harm. Red blood cell membrane inhibition could offer insights into inflammation. Stabilizing cell membranes can minimize tissue damage, making substances protecting against harm crucial in inhibiting inflammation progression (Gunathilake et al., 2018). In this study, three membrane lysis assays, including the heat-induced hemolysis inhibition assay, protein denaturation inhibition assay, and proteinase inhibitory activity assay, were employed to evaluate membrane stabilization in the inflammatory reaction. In the heat-induced hemolysis inhibition assay,

Noni fruit juice exhibited an IC_{50} value of 36.08 ± 1.08 µg/mL. The inhibitory activity of Noni fruit juice was further compared to aspirin, which demonstrated an IC_{50} value of 55.03 µg/mL (Truong et al., 2021). Noni fruit juice exhibited comparable inhibitory activity to aspirin, suggesting its potential in preventing heat-induced hemolysis. For the protein denaturation inhibition assay, Noni fruit juice showed an IC_{50} value of 56.14 ± 1.11 µg/mL. The inhibitory activity of Noni fruit juice was compared to aspirin, which exhibited an IC_{50} value of 58.96 µg/mL (Truong et al., 2021). Noni fruit juice demonstrated similar inhibitory activity to aspirin, indicating its potential in preventing protein denaturation. In the proteinase inhibitory activity assay, Noni fruit juice exhibited an IC_{50} value of 38.04 ± 0.56 µg/mL. The inhibitory activity of Noni fruit juice was compared to aspirin, which demonstrated an IC_{50} value of 60.89 µg/mL (Truong et al., 2021). Noni fruit juice exhibited comparable inhibitory activity to aspirin, indicating its potential as a proteinase inhibitor.

Additionally, a prior investigation by Yilmazer et al. (2016) utilized enzyme-linked immunosorbent assay (ELISA) to measure plasma concentrations of endothelin-1 and leptin, established inflammation markers, reflecting their increased levels in inflammation processes, with endothelin-1 playing a crucial role in inflammation across various human diseases, emphasizing the anti-inflammatory properties observed in the investigated commercial noni juice. Moreover, it was suggested by Dussossoy et al. (2011), Kokturk et al. (2013), and Motshakeri and Ghazali (2015) that the anti-inflammatory activity of noni fruit may have resulted from the presence of flavonoids, including quercetin, isoquercitrin, and rutin, coumarins such as scopoletin and esculetin, and the triterpenoid ursolic acid, which are most likely present in commercial noni juice products. As is well known, these compounds have been demonstrated to exhibit anti-inflammatory activity through the nitric oxide and prostaglandins E2 pathways.

CONCLUSION

In conclusion, this investigation has elucidated the substantial presence of diverse bioactive compounds, encompassing total phenolics, total flavonoids, ascorbic acid, monomeric anthocyanin, β-carotene, and lycopene, in pasteurized Noni fruit juice. The juice has exhibited pronounced antioxidant properties through the mitigation of free radicals, inhibition of lipid peroxidation, and singlet oxygen suppression. Moreover, it has manifested inhibitory effects on alpha-amylase and alpha-glucosidase, suggesting promising anti-diabetic potential, while also demonstrating inhibitory effects on nitric oxide, heat-induced hemolysis, protein denaturation, and proteinase enzymes, underscoring its anti-inflammatory potential. Further investigations are essential to elucidate the molecular mechanisms and assess bioactivities in vivo, utilizing animal models and human trials. The results emphasize the therapeutic potential of Noni fruit juice, offering crucial insights for researchers and healthcare practitioners, and indicating the commercial viability of noni juice production in Sri Lanka.

ACKNOWLEDGMENTS

The authors sincerely thank Prof. Siril Wijesundara, Former Director General at the Department of National Botanic Gardens, and Dr. R.H.G. Ranil from the Department of Crop Science, Faculty of Agriculture, University of Peradeniya for their invaluable assistance in identifying the '*Ahu*' plant. Additionally, gratitude is extended to the staff members of the Department of Food Science and Technology at the University of Peradeniya and Wayamba University of Sri Lanka for their unwavering support during the analysis and testing process.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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