applied sciences

MDPI

Article

An Analysis of Commercial Noni Juice: LC-MS/MS Phenolic Profiles and Cytotoxic Activity

Joel B. Johnson *,†, Janice S. Mani † and Mani Naiker

School of Health, Medical & Applied Sciences, Central Queensland University, Bruce Hwy, North Rockhampton, QLD 4701, Australia

* Correspondence: joel.johnson@cqumail.com

† These authors contributed equally to this work.

Abstract: Fermented juice from the noni tree (*Morinda citrifolia*) is a traditional medicinal product used by South Pacific Islanders to treat a wide range of ailments, including cancer, inflammation and obesity, as well as improving overall wellbeing. Many of its bioactive properties have been suggested to arise from the high antioxidant capacity and phenolic content found in the juice. However, there have been limited investigations into the phenolic profiles of noni juice produced locally in the Pacific. This study aimed to investigate the chemical composition and bioactive properties of noni juice. The first phase of this study used liquid chromatography with tandem mass spectrometry (LC-MS/MS) to characterise the phenolic composition of five brands of commercial noni juice produced in the South Pacific region. A total of 21 phenolic compounds were putatively identified, with the most abundant generally being rutin, 4-hydroxybenzoic acid and gentisic acid. Vastly differing phenolic profiles were found between the noni juice brands. Significant differences were also found in their antioxidant capacities and total phenolic contents. Of the three major phenolic compounds identified, gentisic acid showed the highest antioxidant activity (640% higher than Trolox). Additionally, the noni juice showed no significant anti-acetylcholinesterase activity and no to moderate cytotoxicity against two cancer cell lines (HeLa and HT29). These results indicate that the phytochemical profiles—and hence, the expected bioactive properties—are likely to vary significantly between different noni juice brands. Furthermore, the anti-cancer activity of non-concentrated noni juice appears to be relatively low.

Keywords: *Morinda citrifolia*; total phenolic content; targeted profiling

Citation: Johnson, J.B.; Mani, J.S.; Naiker, M. An Analysis of Commercial Noni Juice: LC-MS/MS Phenolic Profiles and Cytotoxic Activity. *Appl. Sci.* **2022**, *12*, 13034. https://doi.org/10.3390/app122413034

Academic Editor: Małgorzata Muzolf-Panek

Received: 18 October 2022
Accepted: 16 December 2022
Published: 19 December 2022

1. Introduction

Noni juice, made from fermenting the fresh fruit juice of the noni tree (*Morinda citrifolia* L.), is a traditional folk medicine and tonic used by Pacific Island populations for the treatment of a broad range of health conditions and diseases [1–3]. Its major purported health benefits include improving cardiovascular health, reducing inflammation, providing anti-obesity and analgesic effects and protecting against tobacco-induced DNA damage [4–6]. Moderate anti-cancer effects have also been reported [2,7,8]. Noni juice may also improve immune health [9] and provide a feeling of overall wellbeing [10].

Many of these health benefits have been attributed to the exceptionally high antioxidant activity found in noni juice—much higher than that of any other fruit juice [4]. However, the mechanism of action for most of its biological properties remains understudied [6]. Despite the popular interest in noni juice, there have been relatively few studies investigating the typical chemical composition of this matrix in detail. Complicating this further is the fact that different brands of noni juice may have different proportions of ingredients and thus different compositions; this may lead to varying biological effects [4]. A study by Wang et al. [11] found promising anti-cancer activity against breast cancer induced in female rats, while several other reports of in vitro and in vivo activity have also been reported [12].

In terms of its chemical properties, Yang et al. [13] found a high level of free radical scavenging activity and total phenolic content in fresh noni juice from Guam. Additionally, the phenolic content and antioxidant capacity were found to decrease over time as the samples were stored. Similarly, Bramorski et al. [14] found high antioxidant capacity and total phenolic content in noni juice sourced from a Brazilian distributor. Recently, Wang et al. [15] studied the changes in volatile compounds and the total phenolic and flavonoid content during the fermentation process of noni juice, finding that the flavonoid content increased with increasing fermentation time, while the total phenolic content decreased slightly.

Barraza-Elenes et al. [16] used LC-MS to identify and quantify individual phenolic compounds present in noni bagasse from Mexico, and fresh juice pressed from the fruit. In a similar study, Yan et al. [17] used LC-MS to quantify the major phenolic compounds present in eight samples of noni juice, most of which were produced in China. However, there is limited information on the phenolic profiles of commercial noni juice produced in the Pacific Island region. Consequently, the aim of this study was to characterise the antioxidant capacity, phenolic contents and cytotoxic activity of commercial noni juice samples produced in this region.

2. Materials and Methods

2.1. Sample Collection and Preparation

Five commercial brands of noni juice (Figure 1) were procured in May 2021 from local suppliers in Rockhampton (Qld, Australia) and online. The samples were purchased in sealed bottles (stored and shipped at room temperature by the manufacturer), which were then stored in a refrigerator (2 °C) upon receipt at the laboratory. A brief chemical characterisation of these samples has already been published [7]. The details and label descriptions are provided in Table 1, while Table 2 gives the nutritional values of each brand.

Figure 1. The five samples of commercial noni juice.

Table 1. Label descriptions of the noni juice samples included in this study.

Brand (Abbreviation)	Country of Origin	Label Description	Typical Shelf Life
Tahitian Organic Noni (TON)	Tahiti	Never reconstituted—always fresh. No preservatives, colouring agents, or sugars.	3.5 years
Cook Island Noni juice (CIN)	Cook Island	100% *Morinda citrifolia* fruit extract. Non-Pasteurised	3 years
Dynamic Health Noni (DHN)	Tahiti	Contains no added sugar, artificial colour or preservatives. Due to the pure nature ingredients in this product, taste, colour and consistency may vary.	2 years
Fijian Noni (FN)	Fiji	Made from pure fruits (wild collection). No additives added. Pasteurized for optimum quality.	2 years
Life Health Noni (LHN)	New Zealand	100% noni fruit juice.	4.5 years

Table 2. Nutritional panel information for the commercial noni juice samples. Values are provided per 100 mL of juice.

Parameter	TON	CIN	DHN	FN	LHN
Energy (KJ)	70	ND	70	88	104
Protein (g)	ND	ND	ND	0.8	0.4
Fat (g)	ND	ND	ND	<0.1	0.1
Saturated Fat (g)	ND	ND	ND	<0.1	<0.1
Carbohydrates (total) (g)	<3.3	ND	0	3.1	5.50
Sugars (g)	<3.3	ND	ND	2.5	3.10
Sodium (mg)	ND		ND	8	16
Potassium (mg)	163.33	ND	100	220	ND
Calcium (mg)	ND	ND	ND	10	ND
Magnesium (mg)	ND	ND	ND	10	ND
Vitamin C (mg)	16.67	ND	ND	ND	ND
Iron	6.67	ND	ND	ND	ND

ND = no data.

2.2. Reagents

The majority of chemicals and reagents were purchased from Sigma-Aldrich Australia (Castle Hill, NSW, Australia). Most reagents used for the anticancer testing bioassays were also purchased from Sigma-Aldrich, including Dulbecco's Modified Eagle's Medium—high glucose (DMEM), Dulbecco's Phosphate Buffered Saline (PBS), L-Glutamine solution, Penicillin-Streptomycin solution and Trypsin-EDTA solution. CellTiter 96 Aqueous One Solution Reagent (MTS) and Foetal Bovine Serum (FBS) were obtained from Promega and Interpath, respectively. The methanol for use as an LC-MS grade solvent was procured from Chem-Supply (Gillman, South Australia). All bioassay reagents were frozen at −20 °C, except DMEM and PBS solutions, which were kept in the dark at 4 °C until required for use.

2.3. HPLC Analysis of Ascorbic Acid

To analyse the ascorbic acid content of the noni juice samples, we used a high-performance liquid chromatography (HPLC) method previously published by our laboratory [18]. Prior to analysis, the juice samples were 0.45 µm syringe filtered, with no dilution required. The ascorbic acid content was measured using an Agilent 1100 HPLC system with an external ascorbic acid standard calibration. Results were expressed in mg L^{-1} of the undiluted juice.

2.4. Analysis of Total Phenolic Content and Antioxidant Capacity

The Folin–Ciocalteu method was used to quantify the total phenolic content (TPC) of the juice samples, as described previously [19,20]. Similarly, their antioxidant capacity was assessed using the ferric reducing antioxidant power (FRAP) assay [19,20]. The TPC and FRAP results were expressed in equivalents of gallic acid and Trolox, respectively. The results were calculated as mg L^{-1} of the original (undiluted) juice.

2.5. LC-MS/MS Analysis of Phenolic Compounds

Targeted phenolic compounds were analysed in the noni juice samples through liquid chromatography tandem mass spectrometry (LC-MS/MS). The instrument used comprised a Nexera X2 chromatography system coupled with a Shimadzu LCMS-8040 system.

The analytical method has previously been published [21]. It used a Raptor biphenyl column (100 mm × 2.1 mm, 2.7 µm), 5 µL injection volume, 40 °C column temperature and 0.6 mL/min. The mobile phase comprised water (phase A) and methanol (phase B), each containing 5 mM ammonium formate and 0.1% formic acid. Gradient details are available in the published method [21]. The eluent was directly routed to the electrospray ionisation (ESI) module.

2.6. MS Instrument Settings

Targeted tandem mass spectrometry using ESI was performed on the eluting compounds. As detailed in Table 3, both positive and negative ionisation modes were used, depending on the ionisation characteristics of each analyte. Again, ESI conditions have been previously published [21] and targeted the specified polyphenol analytes, following precursor and product ion masses previously published in the literature [22,23].

Based on previous method development and optimisation, two ion transitions were targeted for most compounds (Table 3). The first transition was used for quantitation of the compound, while the second transition was used for the purposes of identity confirmation. Thirty phenolics, flavonoids, anthocyanins and related compounds were included in the targeted phenolic analysis method (Table 3). The limits of quantification (LOQs) were estimated by injecting a series of standards at increasing dilutions. The reproducibility was measured by comparing the peak areas of triplicate injections of standards at one concentration (~10 mg L^{-1}). The calibration curves for each of the standards are shown in the Supplementary Materials.

2.7. Acetylcholinesterase Inhibitory Activity

One previous study has suggested that noni fruit extracts may have the ability to inhibit the activity of the enzyme acetylcholinesterase (AChE), which could be useful for combating Alzheimer's disease and dementia [24]. To investigate the noni juice extracts for potential AChE inhibitory activity, an in vitro enzyme inhibition assay was conducted. The method followed Zheng et al. [25], with 40 µL of noni juice combined in a 96-well plate with 160 µL of 0.2 M phosphate buffer (at pH 7.7), 80 µL of 1 mM DTNB and 10 µL of 2 U/mL acetylcholinesterase solution. After 5 min incubation at room temperature, 15 µL of 8 mM acetylthiocholine iodide was added to each well. After another 5 min incubation at room temperature, the absorbance was measured at 405 nm using a microplate reader (Bio-Rad iMark). The inhibitory value of the extracts was calculated by comparison to the absorbance values of wells containing no added inhibitor (the positive control) and no added AChE (the negative control).

Table 3. Analytical parameters of the LC-MS/MS method.

No.	Analyte	Class	RT (min) [a]	Ionisation Mode	Precursor Ion (*m*/*z*)	MS^2 (Collision Energy) [b]	LOQ [c] (mg L^{-1})	Reproducibility (% CV) [d]
1	Gallic acid	Hydroxybenzoic acid	0.75	Neg	169.1	125.0 (17), 79.1 (24)	0.05	2.8
2	Protocatechuic acid	Hydroxybenzoic acid	1.42	Neg	153.1	108.9 (15), 108.0 (26)	0.1	3.5
3	Gentisic acid	Hydroxybenzoic acid	1.67	Neg	153.1	109.0 (15), 81.1 (21)	<0.1	3.4
4	Neochlorogenic acid	Hydroxycinnamic acid	2.56	Neg	353.2	191.1 (20), 179.1 (20)	0.09	5.3
5	4-hydroxybenzoic acid	Hydroxybenzoic acid	2.59	Neg	137.1	92.9 (16), 65.1 (29)	0.5	1.2
6	(+)-Catechin	Flavanol	3.87	Pos	291.1	139.1 (−15), 123.1 (−15)	0.1	3.6
7	Caffeic acid	Hydroxycinnamic acid	4.21	Neg	179.1	135.0 (16), 134.1 (24)	<0.1	3.9
8	Chlorogenic acid	Hydroxycinnamic acid	4.48	Neg	353.2	191.1 (16)	<0.01	5.1
9	Cyanidin 3-glucoside	Anthocyanin	5.28	Pos	449.1	287.1 (−21)	0.1	3.8
10	Salicylic acid	Hydroxybenzoic acid	5.53	Neg	137.1	93.0 (17), 65.0 (27)	0.01	3.8
11	Vanillic acid	Hydroxybenzoic acid	5.56	Neg	167.1	108.1 (20), 152.1 (16)	0.1	4.4
12	Syringic acid	Hydroxybenzoic acid	5.63	Neg	197.1	182.2 (15), 122.9 (22)	1	2.0
13	*p*-Coumaric acid	Hydroxycinnamic acid	5.73	Neg	163.1	119.0 (16), 93.0 (32)	0.1	2.6
14	Malvidin 3-glucoside	Anthocyanin	6.24	Pos	493.1	331.1 (−22), 315.1 (−51)	<0.01	1.8
15	Ferulic acid	Hydroxycinnamic acid	6.71	Neg	193.1	133.9 (19), 178.0 (15)	0.5	3.8
16	Vitexin	Flavone	6.87	Neg	431.1	311.0 (24), 283.1 (35)	<0.1	3.1
17	Rutin	Flavonol	7.03	Neg	609.2	300.0 (41), 271.1 (61)	<0.009	1.9
18	Sinapic acid	Hydroxycinnamic acid	7.12	Neg	223.1	208.2 (14), 164.1 (17)	<1	1.9
19	Quercetin 3-glucoside	Flavonol	7.14	Neg	463.1	300.0 (29), 151.2 (36)	0.01	1.9
20	Ellagic acid	Polyphenol	7.25	Neg	301.0	283.7 (27), 184.9 (30)	0.5	1.8
21	Myricetin	Flavonol	7.47	Neg	317.1	151.0 (24), 179.2 (19)	0.01	2.4
22	Resveratrol	Stilbene	7.50	Pos	229.1	135.2 (−14), 211.2 (−11)	1	2.5
23	Pelargonidin	Anthocyanin	7.92	Pos	271.0	121.1 (−34), 197.1 (−29)	ND	ND
24	Delphinidin	Anthocyanin	8.41	Pos	303.0	229 (−33), 173.1 (−34)	0.16	3.8
25	Quercetin	Flavonol	8.41	Neg	301.1	151.0 (21), 179.0 (19)	<0.01	1.7
26	Luteolin	Flavone	8.72	Neg	285.1	175.0 (27), 199.0 (25)	0.01	2.1
27	Cyanidin	Anthocyanin	9.19	Pos	287.1	121.1 (−35), 137.2 (−34)	0.09	2.6
28	Kaempferol	Flavonol	9.19	Neg	285.1	187.2 (30), 239.1 (27)	<0.1	1.7
29	Naringenin	Flavanone	9.22	Neg	271.1	151.0 (18), 119.0 (26)	<0.01	1.2
30	Apigenin	Flavonoid	9.46	Neg	269.1	117.0 (35)	0.003	3.8

[a] RT = retention time (mins); [b] MRM fragments for the related molecular ions. Note that the collision energy refers to related collision energies of each fragment ion. [c] Limit of quantification [d] CV = coefficient of variation, ND = no data.

2.8. Preparation of Crude Crystals for Cytotoxic Testing

To prepare the noni juice samples for the cytotoxic assays, approximately 30 mL of each juice sample was vacuum filtered using 0.45 µm Advantec filter paper and frozen at −80 °C overnight. The samples were then freeze dried using an FTS System-Flexi dry MP freeze drier (operating at −55 °C, 400–500 mTorr for 72 h). Finally, the crystals were weighed and stored in the fridge covered in aluminium foil.

2.9. Chemical Analysis of the Crude Crystals

Basic chemical analysis was performed on the crude crystals, namely analysis of their TPC and FRAP, following the methods described in Section 2.4.

2.10. Cytotoxic Assays

HeLa (human cervical carcinoma) and HT29 (human colorectal carcinoma) cell lines were obtained from the University of Adelaide and cultured in DMEM, supplemented with 10% FBS, 1% L-Glutamine solution and 1% Penicillin-Streptomycin solution. The cells were maintained in a T-25 flask at 37 °C, 5% CO_2 in a humidified condition until reaching approximately 80% confluency.

The anticancer potential of the crude noni crystals was assessed in the HeLa and HT29 cell lines using the MTS assay previously used by our laboratory [7,26]. Briefly, at 80% cell confluency, the old media was aspirated out and the flask was washed twice with PBS. The cells were then dislodged from the flask using Trypsin. The cell suspension was transferred to a 10 mL centrifuge tube from which a 50 µL aliquot was taken and counted using trypan blue. The cell suspension was then diluted to obtain a final concentration of 5×10^4 cells/mL, which was inoculated into 96 well plate (100 µL cells/well) in triplicate. The plate was incubated for a period of 24 h at 37 °C with 5% CO_2 prior to the addition of 100 µL of juice extracts diluted appropriately in DMEM media. Subsequently, it was incubated again for a period of 48 h at 37 °C with 5% CO_2, after which 150 µL of content from the wells was removed and 10 µL of MTS reagent was added. The absorbance reading at 490 and 630 nm was recorded using a 96-well BIO-RAD iMark plate reader after being incubated for a further 1 h at 37 °C with 5% CO_2.

Cisplatin (a commercial anticancer drug) was used as a positive control; cells exposed to DMEM only were used as the negative control. This bioassay was repeated three times for reproducibility. The percentage cell viability was calculated from the proportion of the absorbance of the test sample to the absorbance of the negative control.

2.11. Data Analysis

For LC-MS/MS work, the data were gathered in the native LabSolutions software (Shimadzu, Kyoto, Japan) and exported for subsequent analysis. All statistical testing was conducted in R Studio, running R 4.0.5 [27]. Where applicable, results are presented as mean ± 1 standard deviation. Statistical testing was performed using a one-way ANOVA with the brand as the independent variable, with a value of $p < 0.05$ taken to be statistically significant. Post-hoc Tukey testing at $\alpha = 0.05$ was used to determine the brands that were statistically different from one another.

3. Results and Discussion

3.1. Phytochemical Composition and Ascorbic Acid Content

Table 4 summarises the general phytochemical composition of the five noni juice brands, as measured using benchtop spectrophotometric methods. The Fijian noni juice showed both the highest TPC (1761 mg GAE L^{-1}) and antioxidant capacity (817 mg TE L^{-1}). The range of TPCs found here (1106–1761 mg GAE L^{-1}) were slightly higher than the TPC of commercial noni juice reported by Bramorski et al. [14] (919 mg GAE L^{-1}), but a little lower than the 2100 mg GAE L^{-1} reported by Yang et al. [13] in freshly produced noni juice from Guam. The same authors found that TPC values reduced significantly with storage [13], thus appearing to explain why the TPC values found in the present commercial

samples were lower than those typically found in fresh juice. However, it is important to note that the ripeness of the noni fruits used to manufacture the juice can also influence their TPC and antioxidant capacity [28].

Table 4. Phytochemical composition and ascorbic acid content of the noni juice samples (n = 2 replicates for each sample). Different superscript letters in the same row indicate significantly different results according to *post hoc* Tukey testing at $\alpha = 0.05$.

Analyte	FN	LHN	DHN	TON	CIN
TPC (mg GAE L^{-1})	1761 ± 64 ^{a}	1391 ± 47 ^{b}	1106 ± 6 ^{c}	1642 ± 12 ^{a}	1345 ± 58 ^{b}
FRAP (mg TE L^{-1})	817 ± 29 ^{a}	468 ± 34 ^{b}	311 ± 2 ^{c}	496 ± 10 ^{b}	196 ± 2 ^{d}
Ascorbic acid (mg L^{-1})	20.1 ± 2.9 ^{a}	15.2 ± 0.1 ^{ab}	12.2 ± 0.7 ^{b}	19.7 ± 0.3 ^{a}	16.1 ± 0.1 ^{ab}
Crude crystals					
TPC (mg GAE 100 g^{-1})	5060 ± 23	4786 ± 521	5393 ± 298	4980 ± 391	4962 ± 81
FRAP (mg TE 100 g^{-1})	4235 ± 118 ^{a}	873 ± 88 ^{c}	3244 ± 566 ^{c}	4312 ± 23 ^{a}	6389 ± 49 ^{b}

Abbreviations: FN = Fijian Noni; LHN = Life Health Noni; DHN = Dynamic Health Noni; TON = Tahitian Organic Noni; CIN = Cook Island Noni.

There was a positive correlation between the TPC and antioxidant capacity; however, this correlation was not significant ($r_3 = 0.81$; $p > 0.05$). However, previous research has reported a correlation between TPC and DPPH antioxidant capacity in this matrix [13].

In the crude crystals, the highest TPC was seen in DHN, although there was no significant variation between the different brands. The crystals from CIN also showed a very high FRAP (6389 mg TE 100 g^{-1}), although this brand had the lowest FRAP for the neat juice. In contrast to the results for the juice, the TPC and FRAP of the crude crystal extracts were not strongly correlated ($r_4 = 0.18$).

There was a lower level of variation in the ascorbic acid content of the different noni juice brands (Table 4). The FN and TON noni juice brands showed the highest ascorbic acid contents (20.1 and 19.7 mg L^{-1}, respectively), while DHN had the lowest ascorbic acid (12.2 mg L^{-1}). The ascorbic acid content was only reported on the label of one of the noni juice bottles (TON—16.67 mg L^{-1}), which was slightly lower than the experimentally determined value.

The U.S. Department of Agriculture (USDA) FoodData central has reported a vitamin C content of 30 mg/100 mL in the "Pure Noni" brand of Noni juice sourced from Florida [29], which was significantly higher than the values quantified in this study. In a review, the ascorbic acid content in Noni fruit on a wet weight basis was reported to be in the range of 53.2–76.2 mg/100 g (approximately 5–8 mg L^{-1}) [30]. Previous research has indicated that illumination during storage often affects the chemical properties and components of noni juice, including vitamin C. Although minimal exposure to light would have occurred during shipping or storage at the laboratory, the storage conditions post-manufacture are unknown for these samples.

3.2. AChE Inhibitory Activity

As shown in Table 5, all of the noni juice brands showed relatively low activity against AChE, ranging from 9.6% inhibition for DHN to 30.2% inhibition for CIN. This indicates that, in contrast the results found by Jeyabalan et al. [24], commercial samples of noni juice are unlikely to provide significant AChE inhibitory activity, particularly not at the level required to alleviate neurological conditions such as Alzeimer's disease or dementia.

Table 5. Acetylcholinesterase (AChE) inhibitory activity of the neat noni juice samples. Values are given as the percentage of total inhibition.

Noni Juice	% Inhibition
FN	26.4%
LHN	18.4%
DHN	9.6%
TON	20.3%
CIN	30.2%

Abbreviations: FN = Fijian Noni; LHN = Life Health Noni; DHN = Dynamic Health Noni; TON = Tahitian Organic Noni; CIN = Cook Island Noni.

Given the low inhibitory values, dose–response experiments were not conducted on the samples.

3.3. Quantitative Phenolic Profiling by LC-MS

Targeted LC-MS profiling of the noni juice samples revealed the presence of 21 phenolic compounds putatively identified across all five noni juice brands. Most of these compounds (14) were common to all samples, while several were only found in one or two samples (e.g., neochlorogenic acid, cyanidin 3-glucoside, sinapic acid, ellagic acid, kaempferol). Figure 2 shows the chromatogram traces for one of the noni juice samples overlaid with the combined standards solution, at a wavelength of 254 nm.

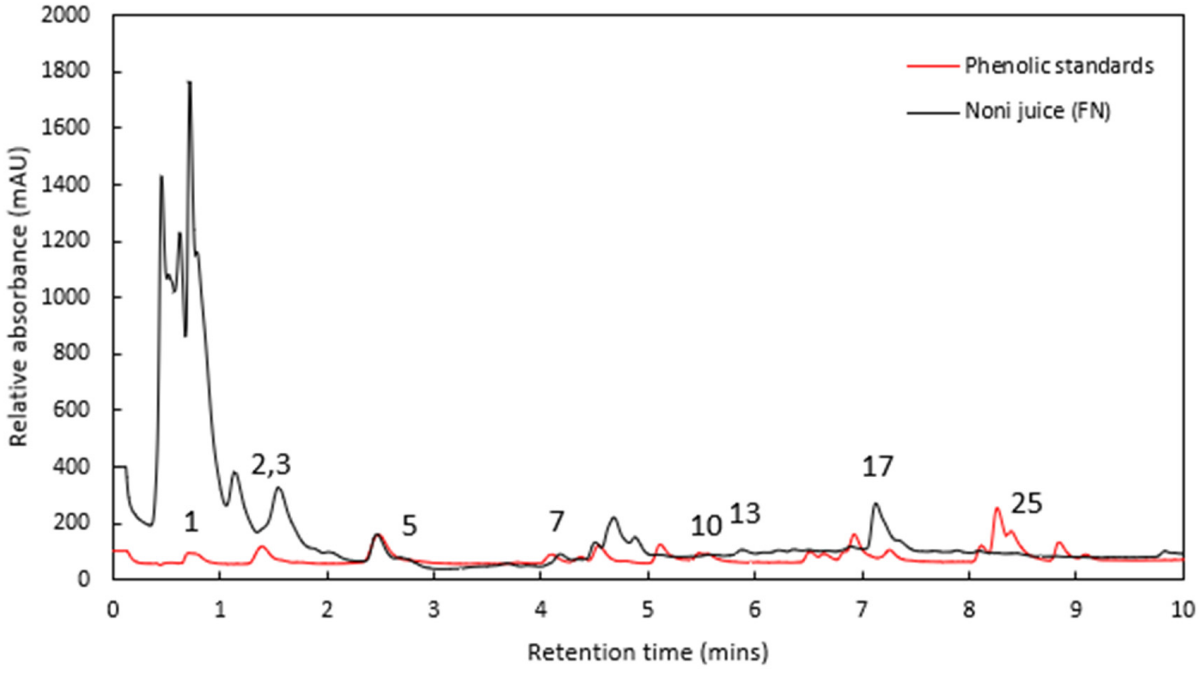

Figure 2. LC-MS chromatogram at 254 nm showing the location of selected phenolic peaks identified in one of the noni juice samples (FN = Fijian Noni). The numbered peaks correspond to the compound numbers in Table 3.

The phenolic profiles varied markedly between the various brands. The most abundant compound in three of the brands (TON, DHN and LHN) was rutin (6.8–41.2 mg L^{-1}). In contrast, the CIN brand showed virtually no rutin (0.09 mg L^{-1}). Similar differences were observed between brands for most of the detected phenolic compounds (Table 6). This agrees with the results of Yan et al. [17], who observed a high level of variation in seven phenolic constituents between eight brands of noni juice produced in China, Taiwan and Fiji. Similarly, Kim et al. [31] observed that the processing methods had a significant impact on the total phenolic content and bioactivity of noni juice samples. Consequently, it is likely

that this high level of variation in composition between brands can be attributed to the different starting materials and processes used in their manufacture.

Table 6. Quantitative profiling of phenolic compounds in the noni juice samples using the developed LC-MS/MS method (values given as mg L-1 of the undiluted juice).

No.	Putative Identification	CIN	TON	DHN	FN	LHN
1	Gallic acid	0.28	0.62	0.33	0.41	0.18
2	Protocatechuic acid	0.30	0.93	0.67	1.74	0.95
3	Gentisic acid	0.15	0.97	0.47	3.21	5.53
4	Neochlorogenic acid	ND	ND	0.02	ND	ND
5	4-hydroxybenzoic acid	5.84	4.36	15.53	8.92	4.76
6	(+)-Catechin	ND	ND	ND	ND	ND
7	Caffeic acid	0.29	1.32	0.73	2.20	1.37
8	Chlorogenic acid	ND	0.01	0.06	ND	0.01
9	Cyanidin 3-glucoside	ND	ND	ND	0.03	0.05
10	Salicylic acid	0.26	1.15	0.53	1.84	2.11
11	Vanillic acid	0.01	0.24	0.14	0.34	0.21
12	Syringic acid	ND	ND	ND	ND	ND
13	*p*-Coumaric acid	0.23	0.71	0.55	1.23	0.97
14	Malvidin 3-glucoside	ND	ND	ND	ND	ND
15	Ferulic acid	0.10	0.29	0.29	0.94	0.51
16	Vitexin	ND	ND	ND	ND	ND
17	Rutin	0.09	41.18	28.41	2.96	6.82
18	Sinapic acid	ND	ND	ND	0.20	0.15
19	Quercetin 3-glucoside	0.01	1.42	1.27	0.55	1.70
20	Ellagic acid	ND	ND	0.05	ND	0.07
21	Myricetin	ND	ND	ND	ND	ND
22	Resveratrol	ND	ND	ND	ND	ND
23	Pelargonidin	0.06	0.18	0.06	0.16	0.14
24	Delphinidin	0.20	0.51	0.28	0.59	2.50
25	Quercetin	0.14	0.51	0.27	0.42	2.13
26	Luteolin	ND	ND	ND	ND	ND
27	Cyanidin	ND	0.04	ND	0.05	0.08
28	Kaempferol	ND	ND	ND	0.01	0.09
29	Naringenin	ND	ND	ND	ND	ND
30	Apigenin	ND	ND	ND	ND	ND
Sum of identified compounds		7.96	54.44	49.66	25.8	30.33

ND = not detected. Abbreviations: FN = Fijian Noni; LHN = Life Health Noni; DHN = Dynamic Health Noni; TON = Tahitian Organic Noni; CIN = Cook Island Noni.

All six of the phenolic compounds reported by Yan et al. [17] were also detected in this work (gentisic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid and quercetin). Additional compounds reported here include five hydroxybenzoic acids (gallic, protocatechuic, 4-hydroxybenzoic, salicylic and vanillic acid), two hydroxycinnamic acids (chlorogenic and neochlorogenic acid), one dimerised polyphenol (ellagic acid) and five flavonoids (luteolin, rutin, quercetin-3-glucoside and kaempferol).

Five anthocyanins were also detected in the noni juice samples—cyanidin, cyanidin-3-glucoside, malvidin-3-glucoside, pelargonidin and delphinidin. Although there have been limited studies reporting individual anthocyanins from *M. citrifolia* fruit, [32] did detect a moderate concentration of total anthocyanins (16 mg/100 g) in this species. Furthermore, many brands of noni juice are reported to include grape and blueberry juice, in addition to noni fruit juice [14]. Glycosides of all four anthocyanin types detected here (cyanidin, malvidin, pelargonidin and delphinidin) have been reported from grapes or blueberries [33,34]. Consequently, this may be the source of some of these anthocyanins, rather than the noni fruit itself.

3.4. Assessment of Antioxidant Activity of the Major Phenolic Compounds

Previous preliminary work has isolated the most antioxidant-active fractions of noni juice and found that these included phenol and flavonoid compounds [35]. However, the exact identity of the most antioxidant-active compounds has not been described to date. Consequently, in order to identify the major phenolic compounds contributing to the high total phenolic content and antioxidant activity of the noni juice, the TPC and FRAP assays were performed on pure standards of the most abundant phenolic compounds identified in this study—rutin, gentisic acid and 4-hydroxybenzoic acid. These were chosen due to their high concentrations (i.e., >3 mg L^{-1}) across one or more noni juice brands.

These results (Table 7) indicated that gentisic acid showed the highest total phenolic content and antioxidant activity out of the three major phenolic acids. This compound showed a total phenolic content approximately 10% higher than gallic acid and an antioxidant activity over 7 times higher than Trolox, a synthetic derivative of vitamin E. This agreed with previous research, which found that gentisic acid had the second-highest antioxidant activity out of a number of phenolic compounds tested using the DPPH assay [36].

Table 7. The slope of the TPC and FRAP calibrations for the main phenolic compounds identified in noni juice samples, relative to the respective standards (gallic acid for TPC or Trolox for FRAP).

Compound	TPC	FRAP
Gallic acid	1	-
Trolox	-	1
Rutin	0.44	0.95
Gentisic acid	1.10	7.40
4-hydroxybenzoic acid	0.75	0

Interestingly, 4-hydroxybenzoic acid showed no antioxidant activity using the FRAP assay. This concurs with theoretical modelling that suggests that 4-hydroxybenzoic acid would have the lowest antioxidant activity out of a number of 4-hydroxybenzoic acid derivatives [37].

Although the possibility of interactions or matrix effects was not considered in this study, gentisic acid showed the highest antioxidant activity, followed by rutin. Interestingly, the LHN sample contained only slightly less gentisic acid than rutin; this sample also showed the highest antioxidant capacity (Table 4). In contrast, the rutin content of the DHN brand of noni juice was over 60 times higher than the gentisic acid concentration in this sample; it contained the second-lowest antioxidant capacity. Further investigations would be required to determine any possible matrix effects on the antioxidant activity.

3.5. Cytotoxic Activity

The various Noni juice crystals showed significantly different ($p < 0.05$) activity against the HeLa cells (Figure 3). The CIN extracts showed the highest potency (cell viability of $63 \pm 1\%$), significantly different to the negative control, whereas the FN, LHN and DHN showed lower potency (76–90% cell viability). Aside from CIN, the effects of all extracts on the HeLa cells were not significantly different from that of the negative control; hence deeming them to have no or low cytotoxicity. It is possible that longer cell exposure times (>48 h) to the Noni juice extracts would have resulted in a greater reduction in cell viability. Nevertheless, these results suggest that commercial samples of noni juice do not appear to have very high cytotoxic activity against the tested cell lines.

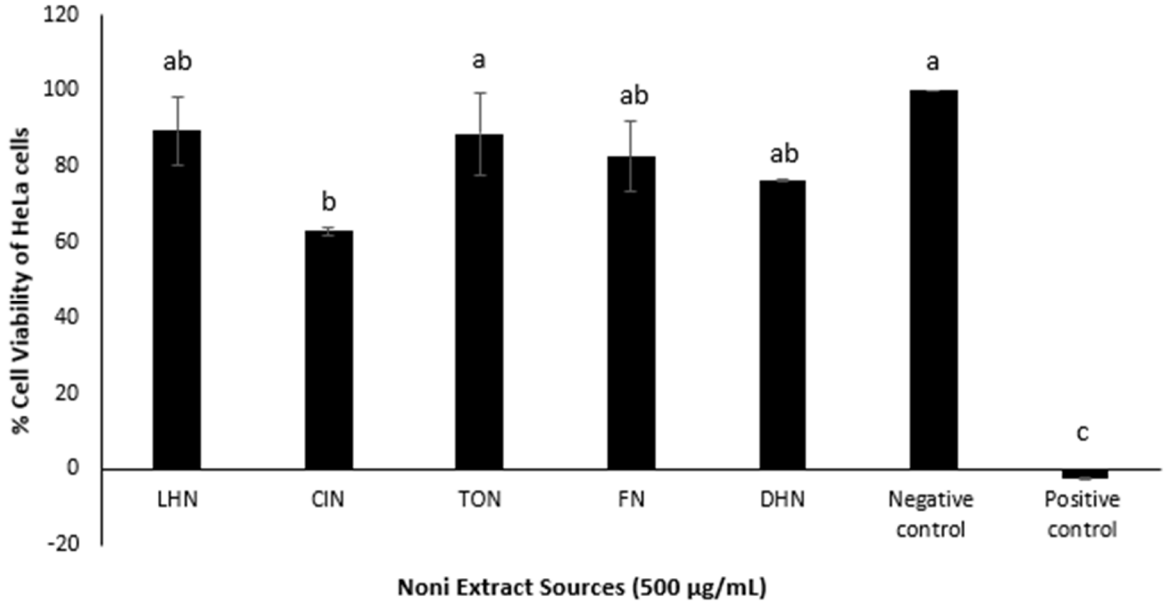

Figure 3. The percentage cell viability of HeLa cells treated with different sources of Noni juice extracts at 500 µg/mL. Different letters on the bar indicate significant difference between the different sources of Noni crude extracts ($p < 0.05$). Negative control: cells without treatment; positive control: cells treated with 10 µg/mL cisplatin (chemotherapy drug). Abbreviations: FN = Fijian Noni; LHN = Life Health Noni; DHN = Dynamic Health Noni; TON = Tahitian Organic Noni; CIN = Cook Island Noni.

Previously, Gupta et al. [38] reported that Noni juice and cisplatin either alone or in combination were able to induce apoptosis in HeLa cells through the mitochondrial pathway. Their findings also reported that cisplatin showed higher cell potency compared to Noni juice, comparative to the results of this study. However, the combination of Noni juice and cisplatin showed additive effects, suggesting that it can be used as a chemo adjuvant in the treatment of cervical cancer [38,39]. Another study suggested that in vitro, a 'concentrated component' in Noni juice and not the pure Noni juice may firstly stimulate the immune system to 'possibly' assist the body fight the cancer and then kill a small percentage (0–36%) of cancer depending on the type [2]. This may explain the low potency of the Noni juice extracts (0–30%) (Figure 3) obtained in this study. Further fractionation and isolation of Noni juice is required to identify the 'component' responsible for the cytotoxic activity.

To further investigate the cytotoxic activity, colon cancer cell lines (HT29 cells) were treated with the Noni juice extracts, with the findings shown in Figure 4. Cell viability decreased to 70–89% upon treatment with the Noni juice extracts, which was lower than the negative control (100% cell viability). This indicated that Noni extracts to some degree induced a cytotoxic effect on HT29 cell lines. There were no significant differences found between the various sources of Noni juice extracts, hence a comparison of their cytotoxicity is inconclusive.

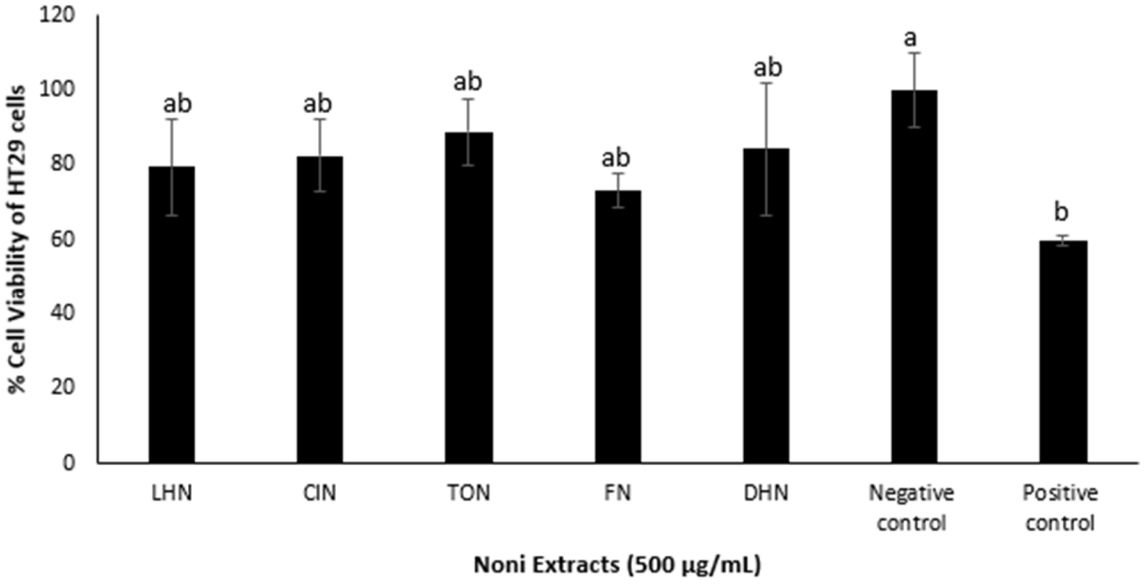

Figure 4. The percentage cell viability of HT29 cells treated with different sources Noni juice extracts at 500 µg/mL. Different letters on the bar indicate significant difference between the different sources of Noni crude extracts ($p < 0.05$). Negative control: cells without treatment; positive control: cells treated with 10 µg/mL cisplatin (chemotherapy drug). Abbreviations: FN = Fijian Noni; LHN = Life Health Noni; DHN = Dynamic Health Noni; TON = Tahitian Organic Noni; CIN = Cook Island Noni.

In general, the HeLa cell line was more susceptible to the TON, CIN and DHN, whereas FN and LHN were found to be more potent against HT29. Furthermore, although some correlation between the phytochemistry and cytotoxic activity of the five sources of Noni juice was noted in this study, comparison in terms of TPC and cytotoxic activity against the HT29 cell line was inconclusive.

4. Conclusions

This study conducted a detailed investigation of the phenolic composition of commercial noni juice from the Pacific region using targeted LC-MS/MS, as well as an investigation of their biological activity using in vitro cytotoxicity screening. A total of 21 phenolic compounds were identified across the five noni juice brands, with the most abundant compounds generally being rutin, 4-hydroxybenzoic acid and gentisic acid. However, the phenolic profiles varied drastically between brands. This indicates that the phytochemical composition of Pacific Island noni juice is not standardised and is highly dependent upon the chemistry of the source materials and/or manufacturing processes used. For example, the rutin content ranged from 0.1–41 mg L^{-1}. A similar level of variation was also observed in the total phenolic contents and antioxidant capacities, while moderate variation was seen in the cytotoxic activities. Furthermore, non-concentrated noni juice does not appear to show significant anti-cancer activity. Future work is necessary to directly link the content of individual phenolics to specific bioactive properties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app122413034/s1.

Author Contributions: Conceptualization, J.B.J.; methodology, J.B.J. and J.S.M.; software, J.B.J.; validation, J.B.J.; formal analysis, J.B.J. and J.S.M.; investigation, J.B.J. and J.S.M.; resources, J.B.J. and M.N.; data curation, J.B.J.; writing—original draft preparation, J.B.J.; writing—review and editing, J.B.J., J.S.M. and M.N.; visualization, J.B.J.; supervision, M.N.; project administration, J.B.J.; funding acquisition, M.N. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a New Staff Grant (RSH/5343) awarded by CQUniversity to one of the authors (MN). One of the authors (JJ) acknowledges support from the Australian Government in the form of a Research Training Program Scholarship.

Data Availability Statement: Data are available upon request from the corresponding author.

Acknowledgments: Thanks to Jens Altvater from Shimadzu for assistance in setting up the LC-MS method.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gupta, R.K.; Patel, A.K. Do the health claims made for *Morinda citrifolia* (Noni) harmonize with current scientific knowledge and evaluation of its biological effects. *Asian Pac. J. Cancer Prev.* **2013**, *14*, 4495–4499. [CrossRef] [PubMed]
2. Brown, A.C. Anticancer Activity of *Morinda citrifolia* (Noni) Fruit: A Review. *Phytother. Res.* **2012**, *26*, 1427–1440. [CrossRef] [PubMed]
3. Pande, M.; Naiker, M.; Mills, G.; Singh, N.; Voro, T. The Kura files: Qualitative social survey. *Pac. Health Dialog* **2005**, *12*, 85–93. [PubMed]
4. West, B.J.; Deng, S.; Isami, F.; Uwaya, A.; Jensen, C.J. The Potential Health Benefits of Noni Juice: A Review of Human Intervention Studies. *Foods* **2018**, *7*, 58. [CrossRef] [PubMed]
5. Didin Wahyu, A.; Mulya Dwi, W.; Sri, W.; Aris, S.; Hideo, T.; Muhaimin, R.I. Noni Juice (*Morinda citrifolia*) to Prevent Cancer Progression in Mice Induced DMBA and Cigarette Smoke Exposure. *Pharmacogn. J.* **2020**, *12*, 946–951. [CrossRef]
6. Brown, A.C.; Apau-Ludlum, N. Chapter 14—Noni (*Morinda citrifolia*) Fruit as a Functional Food and Dietary Supplement for an Aging Population. In *Complementary and Alternative Therapies and the Aging Population*; Watson, R.R., Ed.; Academic Press: San Diego, CA, USA, 2009; pp. 299–316. [CrossRef]
7. Mani, J.S.; Johnson, J.B.; Naiker, M. The Phytochemistry and Anticarcinogenic Activity of Noni Juice. *Eng. Proc.* **2021**, *11*, 16. [CrossRef]
8. Ma, L.-d.; Lin, G.-b.; Yang, L.-b.; Cao, J.-l.; Wang, J.; Chen, Q.-d.; Li, W.-q.; Zhong, W.-j. *Morinda citrifolia* (Noni) Juice Suppresses A549 Human Lung Cancer Cells via Inhibiting AKT/Nuclear Factor-κ B Signaling Pathway. *Chin. J. Integr. Med.* **2021**, *27*, 688–695. [CrossRef]
9. Palu, A.K.; Kim, A.H.; West, B.J.; Deng, S.; Jensen, J.; White, L. The effects of *Morinda citrifolia* L. (noni) on the immune system: Its molecular mechanisms of action. *J. Ethnopharmacol.* **2008**, *115*, 502–506. [CrossRef]
10. Westendorf, J.; Mettlich, C. The benefits of noni juice: An epidemiological evaluation in Europe. *J. Med. Food Plants* **2009**, *1*, 64–79.
11. Wang, M.-Y.; Peng, L.; Anderson, G.; Nowicki, D. Breast cancer prevention with *Morinda citrifolia* (noni) at the initiation stage. *Funct. Foods Health Dis.* **2013**, *3*, 203–222. [CrossRef]
12. Boontha, S.; Kaewjaiboon, N.; Rattanatanyapat, P.; Nanto, W.; Taolam, S.; Buranrat, B.; Pitaksuteepong, T. Cytotoxicity and cell migration suppression by noni fruit extract on Michigan Cancer Foundation-7 human breast cancer cells and development of topical microemulsions. *Pharmacogn. Mag.* **2018**, *14*, 499–506. [CrossRef]
13. Yang, J.; Paulino, R.; Janke-Stedronsky, S.; Abawi, F. Free-radical-scavenging activity and total phenols of noni (*Morinda citrifolia* L.) juice and powder in processing and storage. *Food Chem.* **2007**, *102*, 302–308. [CrossRef]
14. Bramorski, A.; Cherem, A.d.R.; Marmentini, C.P.; Torresani, J.; Mezadri, T.; Costa, A.d.A.S. Total polyphenol content and antioxidant activity of commercial Noni (*Morinda citrifolia* L.) juice and its components. *Braz. J. Pharm. Sci.* **2010**, *46*, 651–656. [CrossRef]
15. Wang, Z.; Dou, R.; Yang, R.; Cai, K.; Li, C.; Li, W. Changes in Phenols, Polysaccharides and Volatile Profiles of Noni (*Morinda citrifolia* L.) Juice during Fermentation. *Molecules* **2021**, *26*, 2604. [CrossRef] [PubMed]
16. Barraza-Elenes, C.; Camacho-Hernández, I.L.; Yahia, E.M.; Zazueta-Morales, J.J.; Aguilar-Palazuelos, E.; Heredia, J.B.; Muy-Rangel, D.; Delgado-Nieblas, C.I.; Carrillo-López, A. Analysis by UPLC–DAD–ESI-MS of Phenolic Compounds and HPLC–DAD-Based Determination of Carotenoids in Noni (*Morinda citrifolia* L.) Bagasse. *J. Agric. Food Chem.* **2019**, *67*, 7365–7377. [CrossRef] [PubMed]
17. Yan, Y.; Lu, Y.; Jiang, S.; Jiang, Y.; Tong, Y.; Zuo, L.; Yang, J.; Gong, F.; Zhang, L.; Wang, P. Quantitative determination of bioactive constituents in *Noni Juice* by High-performance liquid chromatography with electrospray ionization triple quadrupole mass spectrometry. *Pharmacogn. Mag.* **2018**, *14*, 70–75. [CrossRef]
18. Johnson, J.B.; Budd, C.; Mani, J.S.; Brown, P.; Walsh, K.B.; Naiker, M. Carotenoids, ascorbic acid and total phenolic content in the root tissue from five Australian-grown sweet potato cultivars. *N. Z. J. Crop Hortic. Sci.* **2021**, *50*, 32–47. [CrossRef]
19. Johnson, J.; Collins, T.; Skylas, D.; Quail, K.; Blanchard, C.; Naiker, M. Profiling the varietal antioxidative content and macrochemical composition in Australian faba beans (*Vicia faba* L.). *Legume Sci.* **2020**, *2*, e28. [CrossRef]
20. Johnson, J.B.; Mani, J.S.; Skylas, D.; Walsh, K.B.; Bhattarai, S.P.; Naiker, M. Phenolic profiles and nutritional quality of four new mungbean lines grown in northern Australia. *Legume Sci.* **2020**, *3*, e70. [CrossRef]
21. Johnson, J.B.; Hoyos, B.E.; Mani, J.S.; Reynolds, M.; Altvater, J.; Naiker, M. Identification of phenolics responsible for the high antioxidant activity in Burdekin plum (*Pleiogynium timoriense*) fruit. *Food Chem. Adv.* **2022**, *1*, 100081. [CrossRef]

22. Abbattista, R.; Ventura, G.; Calvano, C.D.; Cataldi, T.R.I.; Losito, I. Bioactive Compounds in Waste By-Products from Olive Oil Production: Applications and Structural Characterization by Mass Spectrometry Techniques. *Foods* **2021**, *10*, 1236. [CrossRef] [PubMed]
23. Bouhafsoun, A.; Yilmaz, M.A.; Boukeloua, A.; Temel, H.; Harche, M.K. Simultaneous quantification of phenolic acids and flavonoids in *Chamaerops humilis* L. using LC–ESI-MS/MS. *Food Sci. Technol.* **2018**, *38*, 242–247. [CrossRef]
24. Jeyabalan, S.; Subramanian, K.; Cheekala, U.M.R.; Krishnan, C. In vitro & ex vivo acetylcholinesterase inhibitory activity of *Morinda citrifolia* Linn (Noni) fruit extract. *Pharmacogn. J.* **2017**, *9*, 900–905. [CrossRef]
25. Zheng, B.; Yuan, Y.; Xiang, J.; Jin, W.; Johnson, J.B.; Li, Z.; Wang, C.; Luo, D. Green extraction of phenolic compounds from foxtail millet bran by ultrasonic-assisted deep eutectic solvent extraction: Optimization, comparison and bioactivities. *LWT* **2021**, *154*, 112740. [CrossRef]
26. Mani, J.; Johnson, J.; Hosking, H.; Walsh, K.; Neilsen, P.; Naiker, M. In vitro anticancer screening of crude plant polar extracts. In Proceedings of the 4th Edition of Global Conference on Pharmaceutics and Novel Drug Delivery Systems, Online, 6–8 September 2021.
27. R Core Team. *R: A Language and Environment for Statistical Computing*, version 4.0.2; R Foundation for Statistical Computing: Vienna, Austria, 2020.
28. Yang, S.-C.; Chen, T.-I.; Li, K.-Y.; Tsai, T.-C. Change in phenolic compound content, reductive capacity and ACE inhibitory activity in noni juice during traditional fermentation. *J. Food Drug Anal.* **2007**, *15*, 10. [CrossRef]
29. USDA. FoodData Central. Available online: https://fdc.nal.usda.gov/ (accessed on 23 November 2022).
30. Chang, S.K.; Alasalvar, C.; Shahidi, F. Superfruits: Phytochemicals, antioxidant efficacies, and health effects—A comprehensive review. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 1580–1604. [CrossRef]
31. Kim, J.M.; Jeon, Y.H.; Jeong, Y.J.; Yoon, K.Y. Comparison of bioactive composition, antioxidant activity, and nitric oxide inhibition effect of enzyme-treated and commercial noni juice. *Korean J. Food Sci. Technol.* **2020**, *52*, 75–80. [CrossRef]
32. Gadicherla, V.; Challa, S.; Basaveswara Rao, M.; Kunda, P.; Prudhvi, R. *Morinda Citrifolia* (Noni) fruit protects the exocrine pancreatic dysfunction against L-arginine induced acute pancreatitis in rats. *Pharmacogn. Mag.* **2019**, *15*, 328–334. [CrossRef]
33. Ghassempour, A.; Heydari, R.; Talebpour, Z.; Fakhari, A.R.; Rassouli, A.; Davies, N.; Aboul-Enein, H.Y. Study of New Extraction Methods for Separation of Anthocyanins from Red Grape Skins: Analysis by HPLC and LC-MS/MS. *J. Liq. Chromatogr. Relat. Technol.* **2008**, *31*, 2686–2703. [CrossRef]
34. Grace, M.H.; Xiong, J.; Esposito, D.; Ehlenfeldt, M.; Lila, M.A. Simultaneous LC-MS quantification of anthocyanins and non-anthocyanin phenolics from blueberries with widely divergent profiles and biological activities. *Food Chem.* **2019**, *277*, 336–346. [CrossRef]
35. Han-qiao, L.; Chi, C.; Lu, Z.; Jian-guo, C. Screening of antioxidant components in noni fermentation juice. *Nat. Prod. Res. Dev.* **2016**, *28*, 1479. [CrossRef]
36. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT—Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
37. Vafiadis, A.P.; Bakalbassis, E.G. A computational study of the structure-activity relationships of some p-hydroxybenzoic acid antioxidants. *J. Am. Oil Chem. Soc.* **2003**, *80*, 1217–1223. [CrossRef]
38. Gupta, R.K.; Banerjee, A.; Pathak, S.; Sharma, C.; Singh, N. Induction of mitochondrial-mediated apoptosis by *Morinda citrifolia* (Noni) in human cervical cancer cells. *Asian Pac. J. Cancer Prev.* **2013**, *14*, 237–242. [CrossRef] [PubMed]
39. Gupta, R.K.; Singh, N. *Morinda citrifolia* (Noni) alters oxidative stress marker and antioxidant activity in cervical cancer cell lines. *Asian Pac. J. Cancer Prev.* **2013**, *14*, 4603–4606. [CrossRef]