

Research Article

Effect of Prefermentation Treatment on the Bioactive Compounds of Noni (*Morinda citrifolia* L.) Juice

Dhailly Araba Arkorful ¹, Francis Appiah ², John Owusu ¹, Ebenezer Nartey ³, and Isaac Darban ⁴

¹Department of Food and Postharvest Technology, Koforidua Technical University, PO Box 981, Koforidua, Ghana

²Department of Horticulture, Kwame Nkrumah University of Science and Technology, PMB, Kumasi, Ghana

³Department of Hospitality Management, Koforidua Technical University, PO Box 981, Koforidua, Ghana

⁴Department of Medical Laboratory Science, Koforidua Technical University, PO Box 981, Koforidua, Ghana

Correspondence should be addressed to Dhailly Araba Arkorful; da.arkorful@ktu.edu.gh

Received 12 June 2024; Accepted 7 October 2024

Academic Editor: Sunil Pareek

Copyright © 2024 Dhailly Araba Arkorful et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Morinda citrifolia L. is used as an alternative medicine in different parts of the world for treating various diseases including diabetes and heart diseases. As a functional food with a short shelf life, processing into different forms increases the shelf life while maintaining its biologically active metabolite and reducing pathogens; therefore, processing technology like natural fermentation must be well managed. The effect of prefermentation processing of noni juice extract on total flavonoid content (TFC), total phenolic content (TPC), total antioxidant activity (TAA), and antimicrobial activity for *Escherichia coli* and *Staphylococcus aureus* using minimum inhibitory concentration (MIC) was analyzed after subjecting noni fruits to 1%, 2%, and 3% of ascorbic acid and brine treatments and natural fermentation for 40 days, with the aluminum chloride calorimetric method at 420 nm for TFC, Folin–Ciocalteu reagent (FCR) at 650 nm for TPC, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay at 517 nm for TAA, where TFC ranged between 71.7 and 190.0 QE/mL, TPC between 62.6 and 63.7 mg GAE/mL, and TAA between 16.8% and 82.0%. Also, the pretreated noni extracts showed significant antimicrobial activity as compared to the untreated noni extract. In all, the pretreatments employed improved the bioactive compounds analyzed, but with variations.

Keywords: ascorbic acid; DPPH; *E. coli*; fermentation; *Morinda citrifolia* L.; noni; *S. aureus*; total antioxidant activity

1. Introduction

The consumption of fruits and vegetables is considered to be very important because they are attributed to enhancing the physiological properties of consumers due to their bioactivity [1–3]. Major bioactive compounds found in fruits and vegetables include vitamins, minerals, antioxidants, and phenolic compounds which constitute flavonoids, tannins, and phenolic acids, among others of which noni fruit is not exempted [4–6]. According to Lim et al. [7], Fitzgerald [8], and Ian and Aon [9], foods considered to possess exceptional nutrient density (superfoods) include ginseng, moringa, blueberries, ginger, and noni (*Morinda citrifolia* L.).

Morinda citrifolia L. is reported to have a wide range of health-promoting properties due to its numerous nutrients,

such as xeromine and ursolic acid, among others, known to boost immunity and inhibit the growth of cancerous tumors, respectively [10–12]. It is also reported to contain at least 200 phytochemicals such as phenolic compounds, flavonoids, antioxidants, and organic acids, among others [13, 14].

Consequently, *Morinda citrifolia* and its derived products are receiving attention [15] for both consumption and research. It has been used in complementary and alternative medicine for the treatment of diabetes, high blood pressure, menstrual cramps, heart disease, indigestion, and tuberculosis, among others, for over five decades [16–19].

The noni fruit is a functional food with a short shelf life; hence, it is processed (mostly into juice) as early as possible to prevent postharvest losses [20, 21]. The commonest

method used for expressing noni juice is the traditional natural fermentation/aging process, that is, deliberate fermentation without added starter culture method for juice extraction where the juice seeps/drips out of the noni pulp [22]. Other forms of extraction include enzyme- and microbe-assisted methods, flash evaporation, which is used in the production of noni juice concentrate, cooking or boiling, and evaporative techniques leading to dehydration and canning [21, 23–27]. During the traditional homemade extraction process, ripe fruits are kept in sealed airtight containers over a period between 10 days and 8 weeks for the juice to seep out [22, 28–30]. The processing techniques and storage can lead to chemical reactions that can be detrimental and adversely affect the medicinal component and health benefits of the juice extracted [25, 31].

Ezhilarasan, Ahmed, and Raj [32] reported that different aging times had different effects on phytochemical compounds found in noni, with the highest peaks occurring at 45-day-aged products. Different studies have reported on the methanolic, bisulphites, and metabisulphites among noni extract and their effect on the product [33, 34]. This study looks at the use of on-sulphite and nonthermal formulations, that is, ascorbic acid and sodium chloride, which are relatively inexpensive for the pretreatment of noni fruits before aging or fermentation.

Although processing improves the digestibility of food and increases microbial safety, the nutritive value of food is dependent on the selected processing method or technology [35]. Bioactive compounds present in food commodities are known to be affected by practices with the possibility of altering their phytochemical content [36, 37]. It is, therefore, important to adequately manage and use appropriate processing technology to prevent vital damage to the nutritive and quality attributes and an outbreak of pathogenic organisms leading to foodborne illnesses [38].

Against this background, there is a need to compare methods/technologies to maintain biologically active metabolites such as flavonoids, phenolic compounds, antioxidants, and antimicrobial activity available in the food and prevent its damage and losses. This study, therefore, was aimed at investigating the effect of brine and ascorbic acid treatment on quality characteristics such as total flavonoid content (TFC), total phenolic content (TPC), total antioxidant activity (TAA), and minimum inhibitory concentration (MIC) of noni juice extract. This study focuses on the noni as a functional food or medicinal plant whose therapeutic properties have been proven in its traditional use by the indigenous population. Hence, the effect of prefermentation treatments on some major bioactive compounds that could affect the potency and overall therapeutic use of the noni extract is studied.

2. Materials and Methods

2.1. Fruit Handling and Fermentation. Fresh noni samples were obtained from Bunso Arboretum located in the Eastern Region of Ghana.

The fruits were handpicked at stage four maturity (pale—yellow-white) by comparing their skin colour to a

noni fruit skin colour maturity chart by Motshakeri and Ghazali [29] and Rethinam and Sivaraman [39]. The collected fruits were placed in a paper box and then carried to the chemistry lab of the Food/Postharvest Technology Department at Koforidua Technical University.

The fruits were kept for 2 days to allow them to fully ripen (Stage 5 maturity). The fruits were washed and allowed to dry at room temperature. They were then weighed and subjected to two different treatments (brine and ascorbic acid) at three different concentrations (w/v). The fully ripe fruit had soft skin; hence, it was dipped for 60 s [4041].

Brine treatment was done using a salt solution. The brine solution (common salt dissolved in distilled water) was prepared at 1%, 2%, and 3% concentrations. The selected noni fruits were dipped in the solutions for 60 s. Ascorbic acid treatment was done by dissolving ascorbic acid in distilled water to obtain solutions of concentrations of 1%, 2%, and 3%. The selected fruits were dipped in the solutions for 60 s.

2.2. Extraction of Noni Juice. The traditional natural fermentation/aging, that is, deliberate fermentation without added starter culture method, was employed for juice extraction where the juice seeps/drips out of the noni pulp. After the various treatments, seven samples were obtained, including a controlled sample that did not undergo any type of prefermentation treatment. The fruits were then placed in sterile glass jars according to their specific treatments and then labelled and stored in the laboratory at 25°C–27°C for 40 days. After 40 days, the content of each glass jar was separated using a fine nylon cloth to extract the juice from the pulp and seeds. The juice obtained was bottled and used for the various analyses.

2.3. Analysis of Samples

2.3.1. Determination of pH. pH for all treated and untreated samples was analyzed with the Hanna HI991002 pH instrument using the AOAC 981 (2012) method. Fresh noni fruits were crushed, then the juice was squeezed out and the pH was measured and recorded before treatment and fermentation began. The pH of the noni juice extracted after treatment and fermentation was also measured and recorded.

2.3.2. Determination of the Total Soluble Solids (TSSs). The TSSs for all samples were analyzed with a Hanna HI96801 refractometer (digital) using the AOAC 932.12.2012 method. The TSS of the fresh noni sample was determined and recorded before treatment and fermentation. The TSS of the noni extract after treatment and fermentation was also measured and recorded.

2.3.3. TFC Analysis. The TFC of the noni extracts was determined using the aluminum chloride calorimetric procedure according to Tacouri, Ramful-Baboolall, and Puchooa [42] with modifications. Quercetin was used as a standard in the determination. A stock solution of the noni extract was prepared in methanol from which serial dilutions were carried out. Aluminum chloride (10%) (0.5 μ L) was measured and transferred into the test tube, then topped up with 0.5 μ L of 1 M potassium acetone solution and 1.4 mL

distilled water, and then the resulting solution was incubated for 30 min. Absorbance was measured at 420 nm with a spectrophotometer. A standard curve was prepared using different concentrations of quercetin standard (50–250 µg/mL), and the TFC determined was expressed in quercetin equivalent (QE)/mL.

2.3.4. Total Phenolic Compound Analysis. The TPC was estimated according to Singleton, Orthofer, and Lamuela-Raventos [43] with slight modifications. Noni juice extract (0.2 mL) was measured into a test tube and then oxidized with 1.25 mL of Folin–Ciocalteu reagent, and a 7.5% sodium carbonate solution (1.25 mL) was measured and added to the test tube to neutralize the reaction. The test tube was incubated for 30 min at 25°C to complete the reaction. Then, absorbance was measured at 650 nm using a spectrophotometer against a reagent blank. A standard curve was prepared using different concentrations ranging from 20 to 140 µg/mL of standard gallic acid. The total phenolic compounds determined in the noni extract were expressed in gallic acid equivalent (GAE)/mL.

2.3.5. TAA Analysis. The TAA was estimated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, according to the AOAC [44] calorimetric method with slight modifications. A stock solution of the fruit extract was prepared in methanol from a serial dilution carried out to obtain 50, 100, 150, 200, 250, and 500 µg/mL. One milliliter (1 mL) of the methanolic extracted sample was measured in a test tube. Diluted solutions (1 mL) of DPPH solution were added into the test tube and then incubated for 30 min at room temperature in a dark room to complete the reaction. The absorbance of the resulting solution was read at 517 nm using a spectrophotometer against the blank. The percentage inhibition of DPPH oxidation was calculated according to the following formula:

$$\text{DPPH}_{\text{Scavenging effect (\%)}} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} * 100$$

where A_{control} and A_{sample} are the absorbances of the control and sample, respectively.

2.3.6. Analysis of Antimicrobial Sensitivity. Microbiological analysis of the stock solution (noni) was done using routine standards protocols employed by Cheesbrough [45]. The noni extracts, both treated and untreated samples, were examined for *Escherichia coli* and *Staphylococcus aureus* loads to establish that the noni stocks were good for MIC analyses. Minimal inhibitory concentration defines the lowest concentration of an antimicrobial agent (noni extract for this study) in vitro that can inhibit the visible growth of microorganisms and also the levels of susceptibility or resistance of specific bacterial strains to apply antibiotics (noni extract) [46, 47]. Two microorganisms were used in the study, *E. coli* and *S. aureus* strains, which were clinical isolates from the Eastern Regional Hospital, Koforidua.

2.3.6.1. Dilutions of Noni Extractions. Five sterile test tubes were labelled for the fifth dilution. Then, 5 mL of sterile saline was pipetted into each of the sterile test tubes. Five milliliters of the noni extract (1%–3% ascorbic acid, 1%–3% brine, and control) was pipetted into the first tube, and subsequent dilutions were made to the fifth dilution to obtain different concentrations.

2.3.6.2. Preparation of Organism Suspension From a Broth Culture (10^5 – 10^6). Five microliters of an overnight broth culture was pipetted into 5 mL of peptone water to obtain 10^5 – 10^6 organisms per milliliter of the suspension.

2.3.6.3. Preparation of Agar Plates With Different Concentrations of Noni Extract. Two milliliters of diluted extract for each sample was dispensed into each sterile petri dish. Then, 18 mL of sterile Muller–Hinton agar was cooled and maintained at 50°C–55°C in a water bath was poured into each petri dish and swirled for even distribution of the extract. The plates were then allowed to cool to room temperature and inverted.

2.3.6.4. Inoculation of Organism (*E. coli* and *S. aureus*). After the plate was set, 1–2 mL of the bacterial culture was inoculated at the center of each plate. The plate was allowed to dry for 15–20 min. The dried plate was incubated in an incubator at 37°C for 18–24 h. Dilutions were done according to the extent of sensitivity; that is, when a plate showed no growth for a bacterium, the concentration of the noni extract was reduced through dilutions and plated again until the MIC was achieved.

2.4. Statistical Analysis. A completely randomized design was the basis for the analysis. A total (N) of seven (six treatment samples and one control sample) was replicated for the experiment. Data were analyzed using one-way analysis of variance (ANOVA) to establish significant differences between samples with the help of GraphPad Prism and Microsoft Excel. Tukey's multiple comparison test was used to separate treatment means at 1% ($p < 0.01$) and 5% ($p < 0.05$) significant levels.

3. Results

The pH value of the untreated noni sample reduced from 3.78 to 3.15 after 40 days, and the TSSs also reduced continuously from 8.0° to 7.04° Brix after 40 days of fermentation. The pH value of the brine-treated noni juice increased from 3.02 ± 0.188 to 3.12 ± 0.047 as the brine concentration increased. However, the pH of the ascorbic acid-treated noni extract was reduced continuously as the concentration was increased (Table 1). The TSS of both brine and ascorbic acid-treated samples decreased gradually as the concentrations increased. Noni fruit is known to contain organic acids, which are affected by fermentation, therefore affecting the pH and TSS of the juice [21, 48]. The nature (acidity and alkalinity) of the nonsulphites used in the extraction of the noni juice may have also influenced the pH and TSS.

TABLE 1: pH and total soluble solids (TSSs) of samples after 40 days.

Sample	TSS (° Brix)	pH
Brine 1%	8.93 ± 0.058	3.02 ± 0.188
Brine 2%	8.30 ± 1.015	3.04 ± 0.025
Brine 3%	7.87 ± 0.551	3.05 ± 0.186
Ascorbic acid 1%	7.67 ± 0.289	3.12 ± 0.047
Ascorbic acid 2%	7.57 ± 0.551	3.10 ± 0.036
Ascorbic acid 3%	7.03 ± 1.922	3.08 ± 0.056
Control	7.04 ± 1.002	3.15 ± 0.034

3.1. *TFC*. It is observed that there was a general rise in the TFC for brine treatments, as displayed in Figure 1, than the control. This result supports Alam et al. [49], where diverse dosages of salt concentrations increased TFC in treated purslane than in untreated purslane. It is also noted that the TFC decreased as the concentration of the brine treatments increased. The ascorbic acid treatment showed an increasing TFC with increasing treatment concentration. Overall, brine treatments recorded higher TFC values, with 1% brine recording the highest TFC (190.0 m QE/mL) compared with the other treatments. All treatments, except 1% ascorbic acid, recorded significantly ($p < 0.05$) higher TFC compared with the control.

3.2. *Total Phenolic Compounds*. The results, as shown in Figure 2, depict a slight increase at the initial concentrations of 1% and 2% but decreased as the concentration was increased to 3% in TPC for brine treatments. Brine-treated samples produced TPC higher than the control, and this agrees with the results in a similar study where increased brine concentration treatment led to increased levels of total polyphenol content of purslane [49]. Though there was a rise in the TPC in the brine samples, only 2% brine treatment (63.5 mg GAE/mL) recorded a significant difference at $p < 0.05$. The ascorbic acid treatment followed a different trend, with the least concentration (1%) recording the least TPC (62.6 mg GAE/mL), but the TPC increased with increased concentrations. The 2% and 3% ascorbic-treated samples were significantly higher than the control sample at $p < 0.01$.

3.3. *TAA*. Results from analysis of the in vitro antioxidant activity of the noni juice extracted, using the DPPH free radical scavenging assay calorimetric method, show that the scavenging activity of brine treatment decreases as the concentration of the treatments increases, as shown in Figure 3. It was observed that the least concentration (1%) of the brine solutions caused a mild salinity stress which led to 77.3% scavenging activity. The brine treatments significantly increased the TAA for 1% and 2% samples, but even though there was a rise in TAA for the 3% brine sample, it was statistically insignificant at $p < 0.05$ with the control sample.

The scavenging activity of brine treatments decreased as the concentration of the treatments increased. This might be

attributed to the induced salinity stress caused by the noni tissues triggering the production of reactive oxygen species (ROS), as asserted by Munne-Bosch and Penuelas [50]. The scavenging activity of the ascorbic acid increased as the concentration of the treatment increased, and these findings are in agreement with Jebelli Javan et al. [51] that high antioxidant capacity is obtained when foods are subjected to citric acid dipping treatment. The observed increase in TAA by ascorbic acid treatment may be due to ascorbic acid being a nonenzymatic radical scavenger [52], therefore contributing to the high scavenging activity recorded and also accounting for the increased activity as the concentration increases.

The control sample showed a scavenging activity of 16.8%. However, all the samples treated showed a higher scavenging activity than the control (untreated sample). Overall, 3% ascorbic acid showed superior scavenging activity of 82.0%.

3.4. *Antimicrobial Activity of Noni Juice*. Tables 2 and 3 show the results of antimicrobial sensitivity (resistant, sensitive, or intermediate) of the noni juice extract against *E. coli* and *S. aureus*.

Table 2 shows the results for the antimicrobial sensitivity against *Escherichia coli*. One percent (1%) ascorbic acid-treated noni extract exhibited the least inhibitory property at the highest concentration of 0.50 µg/mL, while 3% ascorbic acid-treated noni extract exhibited the highest inhibitory property at the least concentration of 0.125 µg/mL. Also, 1% brined noni extract exhibited the least inhibitory effect at the highest concentration of 0.60 µg/mL, while 3% brined noni extract exhibited the highest inhibitory effect at the least concentration of 0.25 µg/mL. The noni extract without prefermentation treatment (control) exhibited the highest inhibitory effect against *E. coli* at the lowest concentration of 0.125 µg/mL.

Table 3 shows the results for the antimicrobial sensitivity against *S. aureus*. One percent (1%) ascorbic acid noni extract exhibited the least inhibitory property at the highest concentration of 0.250 µg/mL, while 3% ascorbic acid noni extract exhibited the highest inhibitory property at the least concentration of 0.063 µg/mL. For 1% brined noni extract, the sample exhibited the least inhibitory effect at the highest concentration of 0.90 µg/mL, while the 3% brined noni extract sample exhibited the highest inhibitory effect at the least concentration of 0.60 µg/mL. The noni extract without prefermentation treatment (control) exhibited an inhibitory effect at the least concentration of 0.25 µg/mL. In all, 3% ascorbic acid noni extract exhibited the highest inhibitory effect at the lowest concentration of 0.063 µg/mL, while 1% brined noni extract exhibited the least inhibitory effect at the highest concentration of 0.90 µg/mL.

4. Discussion

The TFC and TPC recorded in this study confirm the assertion of Chen et al.'s [53] results where an abundance of flavonoids and polyphenols were found in ripe noni fruit. There was general protection for the bioactive compounds

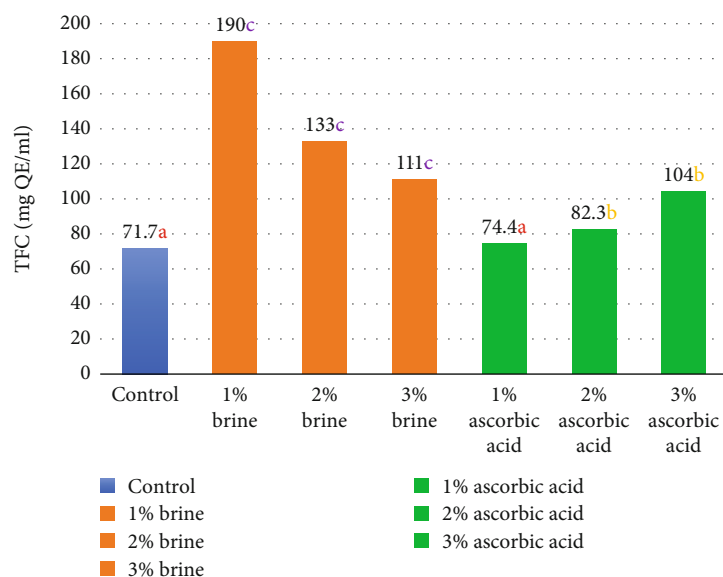


FIGURE 1: Total flavonoid content (mg quercetin equivalent/mL). a: No significant difference between the control and sample at a 95% confidence level ($p > 0.05$). b: Statistically significant difference between the control and sample at 95% confidence level ($p < 0.05$). c: Statistically significant difference between the control and sample at 99% confidence level ($p < 0.01$).

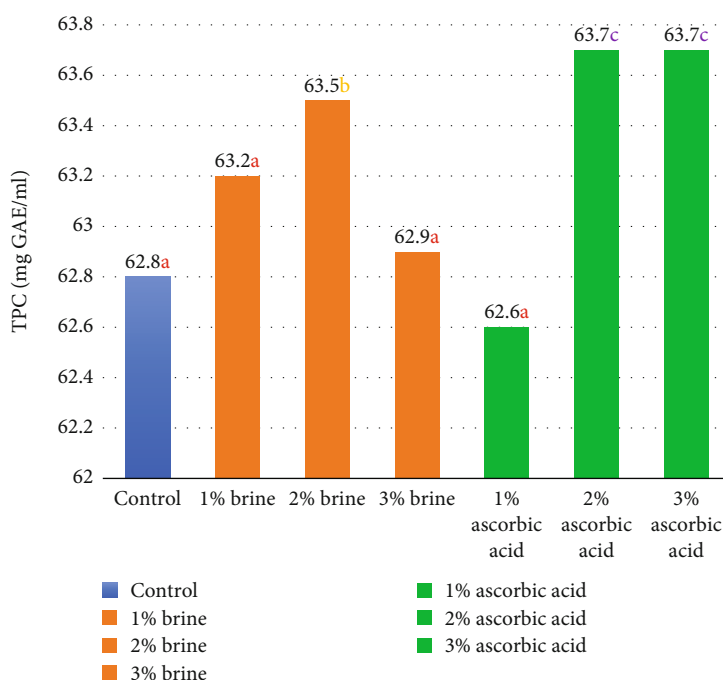


FIGURE 2: Total phenolic compounds (mg gallic acid equivalent/mL). a: No significant difference between the control and sample at a 95% confidence level ($p > 0.05$). b: Statistically significant difference between the control and sample at 95% confidence level ($p < 0.05$). c: Statistically significant difference between the control and sample at a 99% confidence level ($p < 0.01$).

using the nonsulphite pretreatment techniques employed in this study as compared with heat treatments from other works that reported the loss of some bioactive compounds due to thermal degradation and heat-induced reactions [54].

Generally, increasing brine concentration resulted in reduced bioactivity concerning TFC, total phenolic compound, and TAA. However, increasing the concentration of ascorbic acid had a reciprocal effect. The different values

obtained at varying brine concentrations for all parameters show that salt stress at diverse salinity levels results in different outcomes of bioactive compounds in noni juice. This shows that salinity stress affects functional foods, as it supports recent works by Sarker, Islam, and Oba [55], which posit that augmentation of TFC, TPC, and TAC in *Amaranthus tricolor* leaves occurs when subjected to salinity stress. A similar report was made by Petropoulos et al.

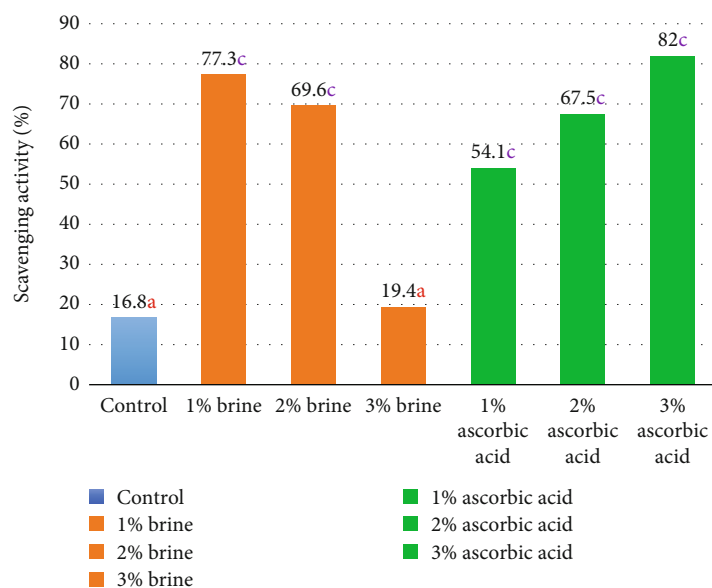


FIGURE 3: Total antioxidant activity in scavenging percentage. a: No significant difference between the control and sample at a 95% confidence level ($p > 0.05$). c: Statistically significant difference between the control and sample at a 99% confidence level ($p < 0.01$).

TABLE 2: Antimicrobial sensitivity for *E. coli*.

<i>E. coli</i>	1st Dil	1st Dil	2nd Dil	3rd Dil	4th Dil	5th Dil	6th Dil
Ascorbic acid	0.60 M	0.500 M	0.250 M	0.125 M	0.063 M	0.031 M	0.016 M
1%	—	S	I	R	R	R	—
2%	—	S	S	I	R	R	—
3%	—	S	S	S	I	R	—
Brine							
1%	S	S	R	R	R	R	—
2%	S	S	I	R	R	R	—
3%	S	S	S	I	R	R	—
Control	S	S	S	I	R	R	—

Abbreviations: Dil = dilution, I = intermediate, R = resistant, S = sensitive.

TABLE 3: Antimicrobial sensitivity for *S. aureus*.

<i>S. aureus</i>	1st Dil	1st Dil	1st Dil	1st Dil	2nd Dil	3rd Dil	4th Dil	5th Dil
Ascorbic acid	0.900 M	0.800 M	0.60 M	0.500 M	0.250 M	0.125 M	0.063 M	0.031 M
1%	—	—	—	S	S	R	R	R
2%	—	—	—	S	S	S	I	R
3%	—	—	—	S	S	S	S	R
Brine								
1%	S	I	R	R	R	R	R	R
2%	S	S	R	R	R	R	R	R
3%	S	S	S	I	R	R	R	R
Control	—	—	S	S	S	I	R	R

Abbreviations: Dil = dilution, I = intermediate, R = resistant, S = sensitive.

[56], who indicated that salinity stress changes phenolic compounds, flavonoid content, and antioxidant activity in *Cichorium spinosum*.

Also, Neffati et al. [57] found that high sodium chloride concentrations reduce TPC and TAA in coriander. These results point to responses to salinity stress by different

plants. In addition, Munne-Bosch and Penuelas [50] found that the production of radical oxygen species due to induced salinity stress and oxidative stress within the plant accounts for the declining TAA. Different concentrations of salt affect the TPC, TAA, and TFC differently. Likewise, the production of phenolic compounds was noted to increase when the salinity stress level was increased to 2% but dropped when increased to 3%. The increase in salinity could lead to stress-induced radical oxygen species production due to the oxidative stress in the plant. Because of the damaging effect of ROS, it guards itself by deactivating ROS with its nonenzymatic antioxidants [50], hence reducing TPC and TAA at high sodium chloride concentrations. Minimal salinity stress could lead to the elevation of some phenolic compounds such as salicylic acid, gallic acid, and quercetin, among others [55]. This is due to the induced stress phenylpropanoid pathway being biotic and abiotic, hastening the production of phenolic compounds [58, 59]. Furthermore, stress triggers the secretion of jasmonic and methyl jasmonic acids [60], which leads to the enzymatic activation of phenylamine ammonia-lyase (PAL), which leads to the buildup of phenolic compounds [61]. This shows that noni juice needs a minimum level of brine pretreatment to increase TPC, TFC, and TAA.

Samarasinghe, Illeperuma, and Gunathilake [33] confirmed that methanolic extract from fresh noni fruits had impressive phenolic and antioxidant content, as reported by many studies, including Thirukkumar et al. [62] and Maskat and Tan [63]. Different concentrations of ascorbic acid had a different effect on the juice. The increase in concentration increased the TPC might be because the treatment prevented enzymatic browning and also antisenescence attributes, as noted by Sedaghat and Zahedi [64]. Comparing the TFC within the ascorbic acid treatment saw an inclining activity as the concentration was increased, and it also showed a tremendous increase when compared to the control sample. This is in agreement with the work by Turmanidze et al. [31], where ascorbic acid-treated extract had increased polyphenols (flavonoids from the majority of all compounds) in frozen strawberry and raspberry fruits. It is important to note that the overall antioxidant ability is influenced by the phenolic compound constituent [65].

The antimicrobial activity of the noni juice extracts was evaluated against *E. coli* (gram-negative) and *S. aureus* (gram-positive) MIC. Noni fruit has been reported to have effectiveness against bacteria, with greater potency against gram-negative bacteria as compared to gram-positive bacteria [66] due to the differences in their cell walls. *E. coli* possess a thin peptidoglycan cell wall, while *S. aureus* has a thicker cell wall which serves as a protective barrier against external substances [67]. Overall, the ascorbic acid-treated sample exhibited the highest sensitivity against both *E. coli* and *S. aureus* with 3% ascorbic acid noni extract showing a superior antimicrobial property. This might be because ascorbic acid treatment increases the phytochemical content in the extracted juice as seen on the TPC, TFC, and TAA figures while preventing degradation of nutrients due to oxidation and salt stress, hence augmenting its microbial action [10, 68].

The brined noni extract exhibited the least sensitivity against *S. aureus*. The organism's ability to thrive in saline conditions might be the cause of the low sensitivity [69]. Three percent brined noni extract exhibited better sensitivity against the organism than 1% brined noni extract due to the difference in the concentrations. The noni extract without any prefermentation treatment (control) exhibited moderate sensitivity against both organisms. This may be due to the phytochemical content of the extracted juice giving it some antimicrobial characteristics [3, 66]. Also, the fermentation and aging process affects the amount and concentration of some major components in the extracted juice, enhancing compounds with antimicrobial properties present in the juice [32, 70]. The MIC for both organisms is in support of West et al. [71], where noni juice was treated with methanol segregated with n-butanol. It was reported that *E. coli* had a higher sensitivity than *S. aureus*.

5. Conclusion

It is observed that prefermentation treatments employed improved the quality of the noni juice extract, though some were statistically insignificant. It can be concluded that ascorbic acid treatments elevate the antioxidant activity, flavonoid, phenolic content, and antimicrobial ability of the noni juice. Also, minimum levels of salt application to the noni fruits are needed to augment the bioactive (antioxidant activity, flavonoid, and phenolic) content of the juice extracted. It is important to note that the concentration of the compounds used in the treatments affected the levels of bioactive compounds in the juice extracted, but there was a general increase in the pretreated noni juice extract than the nontreated juice extract. It is therefore recommended that during the traditional fermentation extraction method of noni juice, some form of pretreatment of the noni fruit should be done to preserve some bioactive compounds in the juice.

Although this study offers essential knowledge, it focused on the functional and therapeutic properties of the noni juice. Therefore, future studies should test for nutritional profile and the therapeutic effect of brine and ascorbic acid noni extracts on the human body. In the long run, targeting the integration of the findings into the development of effective medicine and food supplements.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

No funding was received for this manuscript.

References

- [1] J. Muselík, M. García-Alonso, M. P. Martín-López, M. Žemlička, and J. C. Rivas-Gonzalo, "Measurement of antioxidant activity of wine catechins, procyanidins, anthocyanins and Pyranoanthocyanins," *International Journal of Molecular Sciences*, vol. 8, no. 8, pp. 797–809, 2007.
- [2] M. Philpott, C. C. Lim, and L. Ferguson, "Dietary protection against free radicals: a case for multiple testing to establish structure-activity relationships for antioxidant potential of anthocyanic plant species," *International Journal of Molecular Sciences*, vol. 10, no. 3, pp. 1081–1103, 2009.
- [3] B. Thorat, A. Kambale, and K. Patil, "Noni fruit crop is a versatile medicinal plant," *Journal of Medicinal Plants Studies*, vol. 5, no. 5, pp. 247–249, 2018, <https://www.researchgate.net/publication/328408032>.
- [4] R. H. Liu, "Dietary bioactive compounds and their health implications," *Journal of Food Science*, vol. 78, pp. 1750–3841, 2013.
- [5] T. Mehmood, A. Afzal, F. Anwar, N. Memon, A. A. Memon, and R. Qadir, "Variation in phenolic acids and antibacterial attributes of peel extracts from ripe and unripe [*Citrus Limon* (L.) Osbeck fruit]," *Journal of Food Measurement and Characterization*, vol. 14, no. 3, pp. 1325–1332, 2020.
- [6] J. L. Slavin and B. Lloyd, "Health benefits of fruits and vegetables," *Advances in Nutrition*, vol. 3, no. 4, pp. 506–516, 2012.
- [7] S. L. Lim, N. M. Mustapha, Y. M. Goh, N. A. A. Bakar, and S. Mohamed, "Metastasis lung cancer suppression by *Morinda citrifolia* (noni) leaf compared to erlotinib via anti-inflammatory, endogenous antioxidant responses and apoptotic gene activation," *Molecular and Cellular Biochemistry*, vol. 416, no. 1–2, pp. 85–97, 2016.
- [8] M. Fitzgerald, *It's a bird! It's a plane! It's superfood! Diet cults: the surprising fallacy at the core of nutrition fads and a guide to healthy eating for the rest of us*, Pegasus Books, 2014.
- [9] Ian and Aon, "Noni fruit superfood. Noni juice benefits, nutrition and skin," 2013, July 2019, <https://www.thenaturalhealthmarket.co.uk/blogs/news/noni-fruit-super-food-html>.
- [10] R. Elkins, *The noni revolution: today's tropical wonder that can battle disease, boost energy and revitalize your health*, Woodland Publishing, Hawaii, 2002.
- [11] W. A. Whistler, *Tongan herbal medicine*, University of Hawaii Press, Hawaii, 1992.
- [12] A. Vilmara, D. Amanda, S. C. Andrea et al., "Noni (*Morinda citrifolia* L.) fruit as a new source of milk-clotting cysteine proteases," *Food Research International*, vol. 127, no. 108689, 2020.
- [13] A. C. Inada, P. Figueiredo, R. Santos-Eichler et al., "*Morinda citrifolia* Linn. (noni) and its potential in obesity-related metabolic dysfunction," *Nutrients*, vol. 9, no. 6, p. 540, 2017.
- [14] T. W. Mower, J. C. Bawden, M. C. Harmon, C. R. Stutz, and C. Wang, *U.S. Patent No. 7,749,535*, U.S. Patent and Trademark Office, Washington, DC, 2010.
- [15] O. Poterat and M. Hamburger, "*Morinda citrifolia* (Noni) fruit-phytochemistry, pharmacology, safety," *Planta Medica*, vol. 73, no. 3, pp. 191–199, 2007.
- [16] I. A. Abbott and C. Shimazu, "The geographic origin of the plants most commonly used for medicine by Hawaiians," *Journal of Ethnopharmacology*, vol. 14, no. 2–3, pp. 213–222, 1985.
- [17] O. A. Bushnell, M. Fukuda, and T. Makinodian, "The antibacterial properties of some plants found in Hawaii," *Pacific Science*, vol. 4, pp. 167–183, 1950.
- [18] M. Lohani, M. Majrashi, M. Govindarajulu et al., "Immunomodulatory actions of a Polynesian herb noni (*Morinda citrifolia*) and its clinical applications," *Complementary Therapies in Medicine*, vol. 47, article 102206, 2019.
- [19] N. Solomon, *Island noni: the tropical fruit with 101 medicinal uses, noni juice*, Woodland Publishing, Salt Lake City, Utah, 2nd edition, 1999.
- [20] A. R. Dixon, H. Mcmillen, and N. L. Etkin, "Ferment this: the transformation of noni, a traditional Polynesian medicine (*Morinda citrifolia*, Rubiaceae)," *Ecological Botany*, vol. 53, no. 1, pp. 51–68, 1999.
- [21] L. Zhang, Q. Hong, C. Yu, R. Wang, C. Li, and S. Liu, "*Acetobacter* sp. improves the undesirable odors of fermented noni (*Morinda citrifolia* L.) juice," *Food Chemistry*, vol. 401, article 134126, 2023.
- [22] Y. Chan-Blanco, F. Vaillant, A. M. Pérez, M. P. Belleville, C. Zúñiga, and P. Brat, "The ripening and aging of noni fruits (*Morinda citrifolia* L.): microbiological flora and antioxidant compounds," *Journal of the Science of Food Agriculture*, vol. 87, no. 9, pp. 1710–1716, 2007.
- [23] Y. X. Choo, L. K. Teh, and C. X. Tan, "Effects of sonication and thermal pasteurization on the nutritional, antioxidant, and microbial properties of noni juice," *Molecules*, vol. 28, no. 1, p. 313, 2023.
- [24] S. Wang, Z. Liu, S. Zhao, L. Zhang, C. Li, and S. Liu, "Effect of combined ultrasonic and enzymatic extraction technique on the quality of noni (*Morinda citrifolia* L.) juice," *Ultrasonics Sonochemistry*, vol. 92, article 106231, 2022.
- [25] J. Yang, R. Paulino, S. Janke-Stedronsky, and F. Abawi, "Free-radical-scavenging activity and total phenols of noni (*Morinda citrifolia* L.) juice and powder in processing and storage," *Food chemistry*, vol. 102, no. 1, pp. 302–308, 2007.
- [26] Y. Noor Hafizah, M. Y. Maskat, W. M. Wan Aida, and A. G. Maaruf, "Properties of canned mengkudu (*Morinda citrifolia* L.) extract during storage," *International Food Research Journal (Malaysia)*, vol. 19, no. 3, pp. 1211–1215, 2012.
- [27] H. Moniz, *Noni (Morinda citrifolia) as a pharmaceutical product* (U.S. Patent No. 5,288,491), U.S. Patent and Trademark Office, 1994.
- [28] J. Janick and R. E. Paull, "The encyclopedia of fruit & nuts," in *Experimental Agriculture*, vol. 45, p. 238, 2008.
- [29] M. Motshakeri and H. M. Ghazali, "Nutritional, phytochemical and commercial quality of noni fruit: a multi-beneficial gift from nature," *Trends in Food Science & Technology*, vol. 45, no. 1, pp. 118–129, 2015.
- [30] S. C. Nelson and C. R. Elevitch, *Noni: The Complete Guide for Consumers and Growers*, PAR, 2006.
- [31] T. Turmanidze, M. Jgenti, L. Gulua, and V. Shaiashvili, "Effect of ascorbic acid treatment on some quality parameters of frozen strawberry and raspberry fruits," *Annals of Agrarian Science*, vol. 15, no. 3, pp. 370–374, 2017.
- [32] S. Ezhilarasan, A. F. Ahmed, and S. P. Raj, *Studies on maturity indexes and quality evaluation of Noni (Morinda citrifolia) fruits*, Research Project, SRM University, 2009.
- [33] H. G. A. S. Samarasinghe, D. C. K. Illeperuma, and K. D. P. P. Gunathilake, "An assessment of the bioactive compounds and the antioxidant, anti-inflammatory, and antidiabetic potential of hydro-methanolic extracts derived from fresh

- noni (*Morinda citrifolia* L.) fruits growing in Sri Lanka,” *Biology and Life Sciences Forum*, vol. 26, no. 1, p. 12, 2023.
- [34] E. Duncan, *Non-sulphite dips for fresh prepared produce*, Campden and Chorleywood Food Research Association, 1999.
- [35] A. Ruiz-Rodriguez, F. R. Marín, A. Ocaña, and C. Soler-Rivas, “Effect of domestic processing on bioactive compounds,” *Phytochemistry Reviews*, vol. 7, no. 2, pp. 345–384, 2008.
- [36] A. L. De la Rosa, J. O. Moreno-Escamilla, J. Rodrigo-Garcia, and E. Alvarez-Parrilla, “Phenolic compounds,” in *Postharvest Physiology and Biochemistry of Fruits and Vegetables*, pp. 253–271, 2019.
- [37] O. I. Minatel, V. C. Borges, I. M. Ferreira, G. A. H. Gomez, O. C. Chung-Yen, and P. G. Pereira Lima, “Phenolic compounds: Functional properties, impact of processing and bio-availability,” *Phenolic Compounds - Biological Activity*, vol. 8, 2017.
- [38] K. Warriner, A. Huber, A. Namvar, W. Fan, and K. Dunfield, “Chapter 4 Recent advances in the microbial safety of fresh fruits and vegetables,” *Advances in Food and Nutrition Research*, vol. 57, pp. 155–208, 2009.
- [39] P. Rethinam and K. Sivaraman, “Noni (*Morinda citrifolia* L.), the miracle fruit: A holistic review,” *International Journal of Noni Research*, vol. 2, no. 1, pp. 4–34, 2007.
- [40] N. Chammem, M. Kachouri, M. Mejri, C. Peres, A. Boudabous, and M. Hamdi, “Combined effect of alkali pretreatment and sodium chloride addition on the olive fermentation process,” *Bioresource Technology*, vol. 96, no. 11, pp. 1311–1316, 2005.
- [41] L.-Z. Deng, A. S. Mujumdar, Q. Zhang et al., “Chemical and physical pretreatments of fruits and vegetables: effects on drying characteristics and quality attributes – a comprehensive review,” *Critical Reviews in Food Science and Nutrition*, vol. 59, no. 9, pp. 1408–1432, 2017.
- [42] D. D. Tacouri, D. Ramful-Baboolall, and D. Puchooa, “*In vitro* bioactivity and phytochemical screening of selected spices used in Mauritian foods,” *Asian Pacific Journal of Tropical Disease*, vol. 3, no. 4, pp. 253–261, 2013.
- [43] V. Singleton, R. Orthofer, and R. M. Lamuela-Raventós, “Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent,” *Methods in Enzymology*, vol. 299, pp. 152–175, 1999.
- [44] AOAC, “Determination of antioxidant activity in foods and beverages by reaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH): Collaborative study, first action,” *Journal of AOAC International*, vol. 95, no. 6, pp. 1562–1569, 2012.
- [45] M. Cheesbrough, *District Laboratory Practice in Tropical Countries, Part 2*, Cambridge University Press, New York, 2nd edition, 2006.
- [46] B. Kowalska-Krochmal and R. Dudek-Wicher, “The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance,” *Pathogens*, vol. 10, no. 2, p. 165, 2021.
- [47] T. Singhal, “Antimicrobial Resistance: The Other Pandemic! Based on 9th Dr. IC Verma Excellence Award for Young Pediatricians Delivered as Oration on 19th Sept. 2021,” *Indian Journal of Pediatrics*, vol. 89, no. 6, pp. 600–606, 2022.
- [48] X. Xu, B. Wu, W. Zhao et al., “Shifts in autochthonous microbial diversity and volatile metabolites during the fermentation of chili pepper (*Capsicum frutescens* L.),” *Food Chemistry*, vol. 335, article 127512, 2021.
- [49] M. Amirul Alam, A. S. Juraimi, M. Y. Rafii, A. A. Hamid, F. Aslani, and M. Z. Alam, “Effects of salinity and salinity-induced augmented bioactive compounds in purslane (*Portulaca oleracea* L.) for possible economical use,” *Food Chemistry*, vol. 169, pp. 439–447, 2015.
- [50] S. Munné-Bosch and J. Peñuelas, “Drought induced oxidative stress in strawberry tree (*Arbutus unedo* L.) growing in Mediterranean field conditions,” *Plant Science*, vol. 166, no. 4, pp. 1105–1110, 2004.
- [51] A. Jebelli Javan, A. Nikmanesh, K. Keykhosravi et al., “Effect of citric acid dipping treatment on bioactive components and antioxidant properties of sliced button mushroom (*Agaricus bisporus*),” *Journal of Food Quality and Hazards Control*, vol. 2, pp. 20–25, 2015.
- [52] D. Huang, B. Ou, and R. L. Prior, “The chemistry behind antioxidant capacity assays,” *Journal of Agriculture and Food Chemistry*, vol. 53, no. 6, pp. 1841–1856, 2005.
- [53] X. Chen, C. Gu, K. Zhu, F. Xu, Z. Feng, and Y. Zhang, “Insight into the effects of different ripeness levels on the quality and flavor chemistry of noni fruit (*Morinda citrifolia* L.),” *Food Chemistry*, vol. 434, article 137408, 2024.
- [54] T. T. Nguyen, C. Rosselló, S. Mikhaylin, and C. Ratti, “Converting potato peel waste into bioactive extracts: reduction of pesticides by traditional and novel pretreatment technologies,” *Sustainable Food Technology*, vol. 2, no. 2, pp. 386–399, 2024.
- [55] U. Sarker, M. T. Islam, and S. Oba, “Salinity stress accelerates nutrients, dietary fiber, minerals, phytochemicals and antioxidant activity in *Amaranthus tricolor* leaves,” *PLoS One*, vol. 13, no. 11, article e0206388, 2018.
- [56] S. A. Petropoulos, E. Levizou, G. Ntatsi et al., “Salinity effect on nutritional value, chemical composition and bioactive compounds content of *Cichorium spinosum* L,” *Food Chemistry*, vol. 214, pp. 129–136, 2017.
- [57] M. Neffati, J. Sriti, G. Hamdaoui, M. E. Kchouk, and B. Marzouk, “Salinity impact on fruit yield, essential oil composition and antioxidant activities of *Coriandrum sativum* fruit extracts,” *Journal of Food Chemistry*, vol. 124, no. 1, pp. 221–225, 2011.
- [58] A. Giorgi, M. Mingozi, M. Madeo, G. Speranza, and M. Cocucci, “Effect of nitrogen starvation on the phenolic metabolism and antioxidant properties of yarrow (*Achillea colina* Becker ex Rchb.),” *Food Chemistry*, vol. 114, no. 1, pp. 204–211, 2009.
- [59] H. J. Kim, F. Chen, X. Wang, and J. H. Choi, “Effect of Methyl Jasmonate on Phenolics, Isothiocyanate, and Metabolic Enzymes in Radish Sprout (*Raphanus sativus* L.),” *Journal of Agricultural and Food Chemistry*, vol. 54, no. 19, pp. 7263–7269, 2006.
- [60] H. Pedranzani, R. Sierra-de-Grado, A. Vigliocco, O. Miersch, and G. Abdala, “Cold and water stresses produce changes in endogenous jasmonates in two populations of *Pinus pinaster* Ait,” *Plant Growth Regulation*, vol. 52, no. 2, pp. 111–116, 2007.
- [61] X. Liu, M. Dong, X. Chen, M. Jiang, X. Lv, and G. Yan, “Antioxidant activity and phenolics of an endophytic *Xylaria* sp. from *Ginkgo biloba*,” *Food Chemistry*, vol. 105, no. 2, pp. 548–554, 2007.
- [62] S. Thirukkumar, P. Vennila, S. Kanchana, and T. Uma Maheswari, “Studies on extraction of juice from noni fruits (*Morinda citrifolia* Linn.),” *Indian Journal of Natural Sciences*, vol. 7, no. 40, pp. 11988–11994, 2017.
- [63] M. Y. Maskat, “Effect of heat treatment on the physico-chemical properties of mengkudu (*Morinda citrifolia*) extract,”

- International Food Research Journal*, vol. 18, no. 3, pp. 966–970, 2011.
- [64] N. Sedaghat and Y. Zahedi, “Application of edible coating and acidic washing for extending the storage life of mushrooms (*Agaricus bisporus*),” *Food Science and Technology International*, vol. 18, no. 6, pp. 523–530, 2012.
- [65] B. De Antos, C. Sanchez-Moreno, S. De Pascual-Teresa, and M. P. Cano, “Fruit freezing principles,” in *Handbook of Fruits and Fruit Processing*, Y. H. Hui, Ed., pp. 59–80, Blackwell Publishing, Iowa, USA, 2006.
- [66] T. Su, J. Zhao, Y. Zhu et al., “Comparison of nutrient composition, phytochemicals and antioxidant activities of two large fruit cultivars of sea buckthorn in Xinjiang of China,” *Scientia Horticulturae*, vol. 324, article 112602, 2024.
- [67] Y. W. Heng, J. J. Ban, K. S. Khoo, and N. W. Sit, “Biological activities and phytochemical content of the rhizome hairs of *Cibotium barometz* (Cibotiaceae),” *Industrial Crops and Products*, vol. 153, article 112612, 2020.
- [68] L. Bouarab Chibane, P. Degraeve, H. Ferhout, J. Bouajila, and N. Oulahal, “Plant antimicrobial polyphenols as potential natural food preservatives,” *Journal of Agricultural and Food Chemistry*, vol. 99, no. 4, pp. 1457–1474, 2019.
- [69] M. A. Yusuf and T. H. A. T. A. Hamid, “Isolation and identification of bacteria in retailed smoked fish, within Bauchi metropolis,” *Journal of Pharmacy and Biological Sciences*, vol. 3, no. 1, pp. 1–5, 2012.
- [70] N. Atkinson, “Antimicrobial Substance from Flowering Plants,” *Australian Journal of Experimental Biology & Medical Science*, vol. 34, no. 1, 1956.
- [71] B. J. West, S. K. Palmer, S. X. Deng, and A. K. Palu, “Antimicrobial activity of an iridoid rich extract from *Morinda citrifolia* fruit,” *Current Research Journal of Biological Sciences*, vol. 4, no. 1, pp. 52–54, 2012.