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Review Article

Noni Fruit (*Morinda citrifolia* L.) Extraction and Phytochemical Analyses: A Mini Review

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ABSTRACT

Noni fruit (*Morinda citrifolia* L.) contains many beneficial bioactive ingredients and is used in traditional medicines and health supplements in tropical and subtropical countries. However, the fruit rots easily, so it must be rapidly processed to isolate bioactive ingredients with antioxidant, antimicrobial, anti-inflammatory, and anti-cancer effects. While many different noni fruit extraction methods are available in the literature, the objective of this mini-review was to briefly assess these methods and ensure appropriate method selection for the isolation of optimal bioactive ingredients.

Keywords: Antioxidants, bioactive, extractions methods, *Morinda citrifolia* L., noni fruit

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INTRODUCTION

De Almeida Lopes et al. (2018) used the Hawaiian term "noni" to describe the fruit of the plant species *Morinda citrifolia* Linn (from the Rubiaceae family), which is native to Australia and Southeast Asia. Noni habitats exist in tropical and subtropical climate zones such as Polynesia, India, the Caribbean, Central America, and Northern South America. Noni is taxonomically classified as Eukaryota (domain), Plantae

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(kingdom), Spermatophyta (phylum), Angiospermae (subphylum), Dicotyledonae (class), Gentianales (order), Rubiaceae (family), and *M. citrifolia* (species) (Rojas-Sandoval, 2017). *Morinda citrifolia* has many regional names: "Indian mulberry", "nuna", or "ach" in India, "noni" in Malaysia, "nhau" in Southeast Asia, "painkiller bush" in the Caribbean, and "cheese fruit" in Australia. There are one cultivar, *M. citrifolia* cultivar *Potteri*, and two well-known variants of *M. citrifolia*: *M. citrifolia* var. *citrifolia* and *M. citrifolia* var. *bracteate* (Figure 1) (University of Hawaii at Manoa, 2006). *Morinda citrifolia* var. *citrifolia* is the most widely used variety and offers commercial and health benefits.

Morinda citrifolia has broad, elliptical leaves that are 5–17 cm long and 10–40 cm wide. It grows to a height of 3–10 m. The tiny, tubular white flowers are grouped on flower stalks. The petiole leaves a ring-like mark on the stem. Noni fruits are oval, fleshy, and have an embossed-like look. They are formed as syncarps by united carpels, measuring 3–10 cm long and 3–6 cm wide. Fruit ripens from green to yellow, appearing somewhat wrinkled and translucent; they nearly become white when fully ripe. Fruits weigh approximately 100–300 g, and each axillary gemma typically produces only one fruit. When ripe, the pulp has a jelly-like texture, is pale yellow or colorless, and is watery and bitter. Ripe fruits have a strong butyric acid-like odor. Small reddish-brown spheres cover the skin, containing up to 200 seeds/fruit and 3–10 mm long. The seeds of noni are slimy with a dense seed coat and contain a lot of lignin, proteins (9–15%), dissolved sugars (5%), and lipids (43–50%). Linoleic acid is the most abundant lipid fraction (10–68%) in noni seeds, and concentrations change during germination, reducing up to 38% of the total content.

Cárdenas-Coronel et al. (2016) investigated changes in harvested noni fruit quality and composition at five different phases of development, ranging from dark green to transparent gray (Table 1). Acidity, accumulated dissolved solids, and reduced pH accompanied the ripening of the fruit. Fruit-softening profiles were divided into three: early (no significant softening),

(a) (b) (c)

Figure 1. *Morinda citrifolia* plants: (a) *Morinda citrifolia* var. *citrifolia*, (b) *Morinda citrifolia* var. *bracteata*, and (c) *Morinda citrifolia* cv. "Potteri" (University of Hawaii at Manoa, 2006)

Table 1
Skin color at different noni fruit ripening stages (Cárdenas-Coronel et al., 2016)

Ripeness stage	Subjective color and firmness (hardness in Newtons)	Fruit appearance	L*	C*	h°
1	Dark green, very hard (235±15 N)		62.2±2.2^{bc}	38.9±1.1^{ab}	102.5±0.8^{a}
2	Partially green-yellowfish, very hard (226±15 N)		65.2±1.7^{b}	42.3±1.1^{a}	99.8±1.2^{a}
3	Pale yellow, very hard (202±14 N)		80.4±2.1^{a}	35.1±2.6^{b}	95.5±1.3^{b}
4	Light yellow, moderately hard (165±12 N)		81.1±1.7^{a}	30.7±1.7^{c}	89.2±2.2^{c}
5	Translucent grayish, very soft (6±2N)		58.5±4.3^{c}	18.0±3.7^{d}	92.7±2.4^{b}

Note. Mean values ± standard errors; The significant changes are denoted by different superscripted lowercase letters in the L*, C*, and h° columns (n = 3, p<0.05); L* = Level of light or dark; C* = Chroma; h° = Hue angle

intermediate (significant softening), and final (dramatic softening). In the early stages, extensive depolymerization of water-soluble pectin and a significant increase in pectinase activity did not correspond to a slight decrease in firmness. Intermediate stages were associated with increased pectinase and hemicellulose activity. The final stages saw the most significant reductions in alcohol-insoluble solid yields, uronic acid, and neutral sugar composition. Also, pectinase activity increased, and hemicellulose fractions were depolymerized. Thus, noni-fruit ripening occurred in line with pectinase and hemicellulose activity, which promoted the differential disassembly of cell wall polymers. Changes in soluble fruit solids are shown in Table 2.

The noni fruit juice processing industry is very dependent on the quality of the noni fruit and its juice, which means it depends on fruit production on the land. Research by Prakash et al. (2022) revealed the effects of chemical treatment on the fruiting capacity of noni, fruit yield, and antioxidant properties of the fruit. Noni plants bearing fruit are sprayed with chemicals on their leaves, namely boric acid (BA), gibberellic acid (GA_3), sucrose solution, and water as a control. Fruiting, fruit yield, and fruit growth rate increased significantly in plants treated with BA, GA_3, and sucrose compared with the control treatment. The treatment did not affect the antioxidant capacity, total soluble solids (TSS) of fruit, and total phenol content (TPC). Treatment of 20% sucrose and BA (100 and 200 ppm) showed the highest fruit yield. Table 3 shows the impact of chemical treatment on noni fruit.

Extraction method reviews can inform researchers of the best noni fruit extraction methods, e.g., reviewing method weaknesses and strengths for optimal bioactive ingredient isolation.

NONI FRUIT

Phytochemical Content

Noni fruit physicochemical properties were characterized by Carrillo-López and Yahia (2011) consisting of 90% water content, 3.72 of acidity, 9.87% of dry material, 8°Brix of total dissolved solids, 2.5% protein, 0.15% lipid, 8.27 g/L of fructose, 11.97 g/L of glucose, 155 mg/100 g of vitamin C, 3,900

Table 2
Alcohol-insoluble solid (AIS) yield and uronic acid (UA) content changes in ais, cellulose compared with firmness (N), and non-cellulosic neutral sugars (NS) during noni fruit ripening (Cárdenas-Coronel et al., 2016)

Ripeness (stage)	Firmness (N)	AIS yield (g/100 g FW)	UA (g/100 g FW)	NS (g/100 g FW)	Cellulose (g/100 g FW)
1	235.75 ± 15.61 ^{a}	5.38 ± 0.80 ^{a}	1.52 ± 0.06 ^{a}	1.60 ± 0.17 ^{a}	0.65 ± 0.07 ^{a}
2	226.58 ± 15.47 ^{a}	4.78 ± 0.99 ^{a}	1.49 ± 0.09 ^{ab}	1.50 ± 0.15 ^{a}	0.65 ± 0.04 ^{a}
3	202.66 ± 14.19 ^{a}	4.88 ± 0.69 ^{a}	1.36 ± 0.07 ^{ab}	1.46 ± 0.15 ^{a}	0.50 ± 0.05 ^{a}
4	165.08 ± 12.50 ^{b}	4.23 ± 0.36 ^{a}	1.23 ± 0.05 ^{b}	1.39 ± 0.21 ^{a}	0.50 ± 0.10 ^{a}
5	6.40 ± 3.12 ^{c}	2.33 ± 0.68 ^{b}	0.42 ± 0.03 ^{c}	0.85 ± 0.14 ^{b}	0.49 ± 0.07 ^{a}

Note. Mean values ± standard errors; The significant differences for different letters (n = 3; p<0.05) in the same columns

Table 3

Number of fruit, growth attributes, fruit yield, antioxidant capacity (AEAC), and total phenol content (TPC) in noni plants treated with different chemicals (Prakash et al., 2022)

Chemical treatment	Fruit Numbers per plant	Fruit growth rate (mm/day)	Mature fruit weight (g)	Fruit total soluble solids (TSS) (°Brix)	AEAC (mg/100 g FW)	TPC (mg/100 g FW)
Control (Water)	5.30 ± 0.30^{d}	0.33 ± 0.04^{c}	215.30 ± 8.70^{b}	7.43 ± 0.14^{a}	506.80 ± 14.10^{b}	150.20 ± 1.90^{d}
Sucrose 20%	12.60 ± 0.60^{bc}	0.46 ± 0.05^{b}	287.50 ± 6.70^{a}	7.70 ± 0.13^{a}	313.50 ± 11.20^{b}	125.80 ± 1.60^{abc}
Sucrose 5%	10.60 ± 0.50^{ab}	0.44 ± 0.05^{ab}	281.40 ± 13.90^{a}	7.62 ± 0.11^{a}	359.70 ± 13.80^{a}	133.40 ± 3.50^{bc}
BA 100 ppm	11.00 ± 0.80^{ac}	0.41 ± 0.04^{ab}	265.10 ± 13.50^{a}	7.80 ± 0.09^{a}	330.00 ± 10.90^{a}	119.20 ± 2.00^{a}
BA 200 ppm	11.10 ± 0.90^{ac}	0.43 ± 0.04^{ab}	260.90 ± 7.20^{a}	7.70 ± 0.11^{a}	319.80 ± 8.80^{a}	122.10 ± 2.80^{ab}
GA_3 20 ppm	8.60 ± 0.40^{a}	0.40 ± 0.04^{a}	257.70 ± 8.70^{ab}	7.43 ± 0.12^{a}	347.20 ± 11.30^{a}	136.40 ± 3.10^{c}
GA_3 40 ppm	9.90 ± 0.50^{a}	0.42 ± 0.05^{ab}	271.40 ± 13.20^{a}	7.40 ± 0.12^{a}	346.90 ± 11.30^{a}	132.00 ± 3.50^{bc}

Note. Mean values ± standard errors

There were significant differences in:

the number of fruit: Analysis of variance (ANOVA) with F, df (6, 49) = 15.08, $p<0.0001$

the fruit growth rate: ANOVA with F, df (6, 63) = 9.951, $p\leq0.0001$

the fruit weight: ANOVA with F, df (6,91) = 4.891, $p = 0.0002$

TSS on fruit was not significantly different: ANOVA with F, df (6, 77) = 2.012, $p = 0.0740$

There is a significant difference in:

TPC: ANOVA with F, df (6, 63) = 13.83, $p<0.0001$

AEAC: ANOVA with F, df (6, 63) = 31.86, ($p<0.0001$

Different letters indicate significant differences based on Tukey's test ($p<0.05$)

BA = Boric acid; GA_3 = Gibberellic acid

mg/L of potassium, 214 mg/L of sodium, 14 mg/L of magnesium, and 28 mg/L calcium. The most important dry matter components were dissolved solids, dietary fiber, and protein. Aspartic acid, glutamic acid, and isoleucine were the major amino acids. 8.4% of the dry matter was minerals, particularly potassium, sulfur, calcium, and phosphorus. Ascorbic acid, 24–158 mg/100 g of dry matter, and provitamin A are the vitamins in noni. From fruit and other noni plant parts, more than 150 phytochemicals have been identified and primarily included phenolic compounds, organic acids, and alkaloids. Phenolic compounds included anthraquinones (damnacanthal, morindone, and morindin), aucubin, asperuloside, and scopoletin. The primary organic acids were caproic acid and caprylic acid. The phytochemical composition of ripe *M citrifolia* L. fruit (no seeds) is shown in Table 4.

Fifty-one phenolic compounds were identified using techniques from gas chromatography and mass spectrometry. The ripe fruit contained many carboxylic acids, especially octanoic acid (about 70% of the extracts) and hexanoic acid (about 8% of the total amount of extracts), alcohol (3-methyl-3-buten-1-ol), esters (methyl octanoate and methyl decanoate), ketones (2-heptanone), and lactones ((E)-6-dodeceno-Y-lactone). Decanoic acid, octanoic acid, and 2 E-nonenal concentrations decreased during fruit ripening, while the concentrations of several esters (methyl hexanoate, ethyl octanoate, methyl octanoate, and methyl 4 E-decenoate) increased. 3-methyl-3-buten-1-il hexanoate, unsaturated esters, and 3-methyl-3-buten-1-il octanoate were also significantly decreased during ripening (Carrillo-López & Yahia, 2011). Singh and Singh (2013) examined bioactive components in *M. citrifolia* L. fruit, leaves, and seeds, and the presence of polyphenols, tannins, flavonoids, carotenoids, ascorbic acid, nitrate, oxalic acid, and phytate has been shown. However, when water extraction was used, free radical scavenging activity (RSA) significantly inhibited 1,1-diphenyl-2-picrylhydrazyl (DPPH) ($p < 0.05$) when compared with ethanol and acetone extraction methods. The highest Ly-Tyr and Leu-enkephalin levels were found in fruit. Strong correlations ($R^2 = 0.774$; $p = 0.021$) existed between antioxidant and carotenoid activities. The bioavailability of *M. citrifolia* and its free radical scavenging activity supported its numerous health benefits. Phytochemical *M. citrifolia* fruit components and antioxidant activity are shown in Tables 5 and 6, respectively.

Table 4
The phytochemical of Morinda citrifolia *L. ripe fruit (with no seeds) (de Almeida Lopes et al., 2018)*

Physicochemical parameters	Content
Water content (%)	90.00–91.00
Total carbohydrates (%)	5.27–9.60
Dissolved solids (°Brix)	9.00–9.20
Protein (%)	2.36–2.50
Fat (%)	0.04–0.30
Total fiber (%)	1.00
Ash content (%)	0.66–1.34
Sodium (mg/100 g)	19.76
Potassium (mg/100 g)	3900–5012
Titratable acidity (g/100 g)	3.20–6.82
pH	3.54–4.00

Table 5
Phytochemical and anti-nutrient components in Morinda citrofolia *fruit (Singh & Singh, 2013)*

Fruit	Phytochemical (mg/100 g)					Anti-nutrient (mg/100 g)			
	Phenol	Flavonoid	Tannin	Carotenoid (µg/100 g)	Vitamin C	Nitrate	Phytate	Oxalate	Saponin
Raw	213.4 ± 3.7	266.6 ± 4.0	524.8 ± 3.3	600.0 ± 8.3	139.2 ± 6.0	17.3 ± 2.1	369.6 ± 5	29.2 ± 1.5	236.0 ± 3.2
Ripe	250.7 ± 7.2	190.0 ± 2.7	525.3 ± 2.6	515.7 ± 5.2	149.5 ± 6.9	22.0 ± 4.6	471.8 ± 4.8	37.3 ± 2.1	234.8 ± 1.5

Table 6
Morinda citrifolia *fruit extracts antioxidant activity and half maximal inhibitory concentration (IC_{50}) values (Singh & Singh, 2013)*

Fruit	Antioxidant activity (%)			IC_{50} value (µg/ml)		
	Methanol extraction	Acetone extraction	Aqueous extraction	Methanol extraction	Acetone extraction	Aqueous extraction
Raw	58.6 ± 0.9	63.9 ± 0.7	75.1 ± 0.6	75.5 ± 8.3	122.1 ± 5.8	219.9 ± 5.3
Ripe	74.4 ± 7.3	51.2 ± 0.2	72.8 ± 0.5	213.9 ± 64.3	10.2 ± 1.9	199.7 ± 4.4

Lohani et al. (2019) reported that noni fruit contained phytochemical and polysaccharide components with different biological activities. The main chemical classes were betalain, indole, glucosinolates, organosulfides, sulfides, mono or polyphenolic compounds, terpenes (isoprenoids and terpenoids), and organic acids. Bioactive functions of noni fruit components include anti-inflammatory bioactive components (such as scopoletin, quercetin, and ursolic acid), anti-dyslipidemia and anti-diabetes mellitus bioactive components (for example, deacetylasperulosic acid, oleuropein, saponins, and rutin), neuroprotective bioactive components (rutin and scopoletin), and anti-cancer bioactive components (fatty acid glycosides, iridoids, anthroquinones, and polysaccharides).

The ingredients of noni fruit include vitamins (provitamin A, ascorbic acid), phenols (scopoletin, damnacanthal), organic acids (caprylic acid, caproic acid), minerals, and amino acids (aspartic acid). *In vitro* studies using animals have shown that noni exhibits cardiovascular effects, including anti-cancer, antibacterial, anti-inflammatory, antioxidant, and analgesic properties (Chan-Blanco et al., 2006).

In accordance with the Direktorat Produksi dan Distribusi Kefarmasian (2017), noni fruit contains a minimum of 0.02% scopoletin, while thick noni fruit extracts contain a minimum of 0.38% scopoletin and no less than 10.1% (when the solvent is ethanol). Noni fruit simplicia has a minimum water-soluble extract content of 21.3% and a minimum ethanol-soluble extract of 9.8%. Riyanto and Rohman (2007) successfully isolated scopoletin from the chloroform fraction of a noni fruit methanol extract. Scopoletin exhibited a

DPPH RSA of a half maximal inhibitory concentration (IC_{50}) value of 348.79 µg/ml. The main constituent of *M. citrifolia* L., which contributes to its antioxidant, hepatoprotective, anti-inflammatory, and immunomodulatory properties, was proposed as scopoletin. Scopotoletin has been proposed as a biochemical marker to identify and control noni fruit quality and its derivatives (Tasfiyati et al., 2022). The phytochemical characteristics of noni juice are shown (Dussossoy et al., 2011) (Table 7).

Yang et al. (2007) reported that fresh noni fruit juice free-RSA for DPPH was 140 mg ascorbic acid equivalent (AAE)/100 ml, and total phenol was 210 mg gallic acid equivalent (GAE)/100 ml. Over 90% of free RSA was lost due to fruit fermentation for 3 months, while 20% was lost by fruit dehydration at 50°C. A free-RSA reduction of >90% was achieved when fresh noni juice was stored for 3 months at 24°C. Free-RSA decreased 10 to 55% during noni powder or noni juice storage at 4 and -18°C, respectively, for 3 months. Free-RSA reductions were much greater when noni juice or puree was treated with heat or dehydration than with total phenol reduction. Thus, noni powder or frozen fresh noni juice processing is preferable to juice fermentation to maintain substantial antioxidant properties/levels in noni fruit products.

Table 7
Phytochemical characteristics of noni juice (Dussossoy et al., 2011)

Characteristics	Content
pH	3.40 ± 0.10
Dry weight[a]	7.37 ± 0.06
Dissolved solids[b]	5.80 ± 0.00
Titratable acidity[c]	1.76 ± 0.01
Fructose[d]	2.44 ± 0.02
Glucose[d]	2.07 ± 0.01
Total polyphenols[e]	47.60 ± 2.00
Total vitamin C[f]	97.10 ± 2.30
Dehydroascorbate acid[f]	26.00 ± 0.80
Ascorbate acid[f]	71.10 ± 1.40

Note. Mean values ± standard errors of three experiments; [a] = %/fresh weight; [b] = Degree Brix; [c] = g citric acid/100 g fresh weight; [d] = g/100 g fresh weight; [e] = mg gallic acid equivalent/100 g fresh weight; [f] = mg/100 g fresh weight

Guo et al. (2020) investigated noni fruit and fermented juice antioxidant activity in an *in vitro* study. When compared with fermented juice, fresh fruit had a higher antioxidant capacity. However, fermented juice exerted more preventive effects on alcohol-induced acute liver injury, based on liver function and structural integrity measurements. Thus, noni juice exhibited hepatoprotective effects.

Sina et al. (2021) compared the phytochemical content of noni fruit juice obtained by squeezing noni fruit and fruit juice that had been fermented for two months. Both treatments showed the presence of flavonoids, alkaloids, catechic tannin, saponosids, gallic tannin, reducing compounds, *o*-heteroside, and leuco-anthocyanin. The antiradical activity of fresh fruit juice (41.67%) was higher than that of fermented fruit juice (21.28%), even compared to ascorbic acid (37%). The best concentration required to inhibit 50% of DPPH radicals is fresh fruit (0.024 mg/ml), followed by ascorbic acid (0.027 mg/ml) and fermented fruit (0.047 mg/ml).

Another study reported that noni fruit extract powder, generated by spray drying, showed 28.36% DPPH inhibitory activity. Thus, fruit extract powder could be used as a food additive or raw material in foods (Krishnaiah et al., 2012). Yang et al. (2010) investigated the antioxidant levels, ascorbic acid, and total phenolic content of noni juice and noni powder stored at 24°C. After two weeks of storage, noni juice exposed to light had lost 32% of total phenolic content, 89% of ascorbic acid, and 46–65% of antioxidant capacity, or approximately 8, 22, and 9–15% more when compared with juice that not exposed to light. After four weeks, 97% of ascorbic acid had been lost in the light-exposed and non-light-exposed juices. The properties of antioxidants in light and non-exposed juices had not changed significantly at 12 weeks. After 12 weeks, noni powder exposed to light had lost 21% total phenol, 17% ascorbic acid, and 23–36% antioxidant capacity or approximately 13, 4, and 7–19% more when compared with powder not exposed to light. Thus, superior powder antioxidant properties in brown packaging were maintained over and above the properties seen in transparent packaging. The degradation of antioxidant properties of noni juice has been reduced effectively for 2 weeks because of protection from light, whereas it took 3 months for noni powder.

Ruhomally et al. (2016) reported that in ripe noni fruit, there was 76.24 ± 1.13 mg/100 g ascorbic acid content, 748.40 ± 8.85 µg GAE/g fresh weight (FW) of TPC in ripe fruit extracts, while 770.34 ± 2.27 µg GAE/g FW of TPC in raw fruit extract. Both ripe and unripe noni fruit extracts strongly inhibit nitric oxide, hydroxyl radicals, and superoxide. Iron reduction capacity ranged from 11.26 ± 0.33 to 11.90 ± 0.20 mM Fe^{2+}/g FW, while the IC_{50} values for iron (II) chelating activity of ripe and unripe fruit extract were 0.50 ± 0.01 and 1.74 ± 0.01 g FW/ml, respectively.

Noni fruit contains many bioactive substances, including polyphenols and flavonoids, which provide antioxidant benefits for human health. Thus, optimal bioactive ingredient extraction is required to collect and purify these ingredients.

Fruit Extraction

Solid-liquid extraction is a heterogeneous process that involves transferring solutes from a solid to a liquid. Complex solute combinations, such as extractable plant chemicals, can be extracted at different degrees depending on plant location (outer surface, pores, or vacuoles). The following five steps are involved in solvent transfer processes: (1) solvent from the liquid solvent to the surface of the extracted particles, (2) solvent diffusion into the solid matrix, (3) component dissolution, (4) solvent transfer through the solid matrix, and (5) solvent transfer from the solid's outer surface to the liquid solvent (Conde et al., 2010).

According to Seader et al. (2011), leaching, also known as solid-liquid (or liquid-solid) extraction, requires a liquid solvent to separate soluble components (solute or leachate) from a solid material.

The solute diffuses from the solid into the solvent around it. The desired result can be either an extracted solid fraction, an insoluble solid, or both. Leaching can be conducted using batch, semi-continuous, or continuous processes. Leaching stage outputs comprise a liquid-free solid material called "overflow" and a wet solid called "underflow". Leaching is followed by washing to reduce solute content in the liquid phase of the underflow. These processes generate a final underflow, the extracted solid moistened with an almost pure solvent, and a final overflow, or an extract containing part of the solvent and most of the solute.

Because solute concentrations in solids vary during extraction processes, the rates at which extracts are obtained are not linear with respect to time. It causes unstable or non-stationary conditions. During interaction times between particles containing solids and the extraction solvent, several phenomenological events occur (Veggi et al., 2013):

1. Solvent penetration into the solid matrix
2. Component dissolution and/or breakdown
3. Solute transport out of the solid matrix and extracted solute migration from the outer solid surface into the solution.

Near Supercritical Fluid Carbon Dioxide Extraction (SF-CO_2)

Chen et al. (2009) extracted noni fruit using the near SF-CO_2 method. Fresh fruit was washed in running water, cut into pieces, dried for 5 days in an oven at 37°C, ground to a powder, and sifted through a 20 mesh. The powder moisture content was <2%. The powders were stored at -80°C in plastic bottles to limit the damage. A 50 ml stainless steel vessel has been used as the extraction chamber. Near SF-CO_2 was performed at 35 or 50°C and 1,500 or 3,500 psi pressure for 3 hr statically, hereafter a dynamic extraction for 1 hr. The vessel was filled with 10 g of sample, and the extracted analyte was accommodated in a 20 ml measuring flask containing 5 ml of absolute ethanol. An ice bath was used to enhance dynamic extraction in a 20 ml volumetric flask. The extracted samples were re-suspended in 100% ethanol, dried at ambient temperature, and kept at -80°C. Using the constant weight method, the sample weight after extraction was divided by the initial weight (10 g) to generate extraction data. Before analyzing antioxidant activity, samples were re-suspended in absolute ethanol and centrifuged for 10 min at 4°C and 8,000 x *g*. For antioxidant activity tests, supernatants were gathered and serially diluted (the final ethanol concentration was less than 1%). Extracted contents and antioxidant activities are summarized in Table 8.

Ethanol Extraction

Thoo et al. (2010) extracted dried *M. citrifolia* fruit powder to generate phenolic antioxidants using 0 to 100% (v/v) of ethanol solvents, 20 to 120 min of extraction times, and 25 to 65°C of extraction temperatures. Dried fruit powder (2 g) was extracted in

Table 8
Phenol content and antioxidant activity of noni fruit extracts (Chen et al., 2009)

Extraction conditions	Total phenolic content (mg/g)	Antioxidative activity (IC_{50})			
		DPPH radical scavenging activity (mg/L)	Hydroxyl radical scavenging activity (mg/L)	Hydrogen peroxide scavenging activity (g/L)	Ferrous ion chelating activity (g/L)
3,500 psi; 35°C (method A)	2.59 ± 0.08	157.07 ± 3.26	Undefined	Undefined	Undefined
3,500 psi; 50°C (method B)	6.14 ± 0.41[a]	373.97 ± 6.59[a]	Undefined	2.73 ± 0.65	Undefined
1,500 psi; 35°C (method C)	0.49 ± 0.004[a,b]	597.26 ± 24.43[a,b]	Undefined	1.03 ± 0.05	Undefined
1,500 psi; 50°C (method D)	14.82 ± 0.83[a,b,c]	651.81 ± 24.03[a,b,c]	Undefined	Undefined	Undefined

Note. Mean values ± standard errors; IC_{50} = Effective concentration to deliver 50% response; Undefined indicates very low values/no inhibitory activity because IC_{50} values were too large to be detected; [a] = Significant difference between this method and method A; [b] = Significant difference between this method and method B; [c] = Significant difference between this method and method C

20 ml solvent in a temperature-controlled shaker or water bath at a constant speed and specific temperature. The crude extract was refined through Whatman No. 1 filter paper, after which antioxidant compound and antioxidant capacity levels were assessed. The results demonstrated that the extraction conditions significantly impacted antioxidant capacity and phenolic compounds. With a TPC value of 919.95 mg GAE/100 g DW, a TFC of 472.73 mg CE/100 g DW, a 2,20-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) inhibition of 791.71 µmol Trolox equivalent antioxidant capacity (TEAC)/100 g DW, and a DPPH inhibition of 1928.5 µmol TEAC/100 g DW, the ideal extraction conditions were 40% ethanol for 80 min at 65°C. Depending on the extraction duration (r = 0.938) and ethanol content (r = 0.932), TPC, and DPPH showed a substantial correlation.

Ultrasonic-Assisted Extraction (UAA), Pulsed Electric Field-Assisted Extraction (PEFAE), and Hot Water Extraction (HWE)

Li et al. (2020) compared the effects of different methods of extraction on physicochemical characteristics, polysaccharide extraction yield, antiproliferative capabilities, and antioxidant activity of *M. citrifolia* L. Three extraction methods were compared: HWE, PEFAE, and UAA. Fully ripe noni fruits (brown and soft) were washed and dried at 55°C, then dried into powder and passed through a 60-mesh sieve. A Soxhlet system (75°C for 6 hr) using ethanol (90%, v/v) has been used to remove grease and impurities from the powder. The residue was dehydrated at 50°C for 48 hr after filtration. UEA generated the highest extraction yields, smallest

molecular weights, best antioxidant activity, and excellent antiproliferative abilities. Antioxidant activity values represented that UAE, HWE, and PEFAE donated hydrogens to inhibit DPPH radicals. However, UAE extracts had better ABTS radical inhibitory activity when compared with HWE and PEFAE. Also, UAE extracts had better hydroxyl radical inhibitory activity when compared with HWE and PEFAE methods.

High Hydrostatic Pressure Extraction (HHPE)

Jamaludin et al. (2020) extracted noni fruit using the HHPE method. The fruit was washed, sliced thinly, freeze-dried, ground, and sieved using a 20–50 mesh sieve. A high-pressure food processor (Frescal MFP-7000, Mitsubishi Heavy Ind., Japan) was used to perform HHPE; 1 g dry fruit powder and 30 ml extraction solvent (ethanol solution) were placed into a polyethylene bag, then placed into another polyethylene (PE) bag, and vacuum sealed. The HHPE was operated at room temperature, and water was poured into the high hydrostatic pressure (HHP) vessel as a pressure transfer medium. A hydraulic pump generated pressure and maintained the required processing time. After the pressure was released, the PE bag was removed, the extract was filtered, and the filtrate was diluted in methanol and analyzed for bioactive content.

The effects of pressure, ethanol concentration, and time on bioactive compound extraction were individually evaluated. The ethanol concentration was varied from 30 to 70% (v/v) at 20% intervals; the extraction pressure was changed from 400 to 600 MPa at 100 MPa intervals; and extraction times varied from 5–15 min at 5 min intervals.

Extraction parameters were maintained at a moderate value if not reviewed: 50%, 500 MPa, and 10 min. Bioactive compounds that were methanol-extracted from noni fruit were: 533.4 ± 33.1 μg/g dry sample of scopoletin, 544.9 ± 21.9 μg/g dry sample of rutin, and 23.4 ± 1.0 μg/g dry sample of alizarin. The response surface methodology and the Box–Behnken design have been used to examine optimum extraction conditions. Extraction pressure and ethanol concentrations were the most statistically significant variables that produced high extraction yields. Optimal HHPE extraction conditions (>0.998 desired level) for simultaneous rutin, alizarin, and scopoletin extraction were 65% concentration of ethanol, 544 MPa, and 15 min, which generated maximum yields of 82.4% scopoletin 77.2% alizarin, and 82.2% rutin (Jamaludin et al., 2020).

High-pressure Extractor (HPE) Method

Krishnaiah et al. (2015) extracted noni fruit using the HPE method. Fresh seedless fruit were washed, cut into pieces, dried in an oven at 60°C for 2 days, then ground to powder. The powder was extracted in methanol using an HPE at specific temperature, pressure, and time values. The supernatant was separated from the powder residue by filtering through Whatman filter paper No. 4 and evaporated (at reduced pressure) to

generate a dark green viscous material. TPC = 43.18 mg GAE/10 g and DPPH radical inhibitory activity = 55.60% values were generated at 60°C, 1.5 bar pressure, and 6 hr extraction conditions.

Accelerated Solvent Extraction (ASE)

Tasfiyati et al. (2022) used ASE on noni fruit to generate scopoletin. Ripe fruit was peeled, cut into pieces, dried in an oven at 50°C for several days, ground to a powder, and filtered through an 80-mesh sieve. The powder was extracted using ASE on an automated Energized Dispersive Guided Extraction® system (CEM Corporation, USA): 1 g powder was placed in a stainless-steel sample holder with cellulose filter paper 2.5 µm. The solvent (ethanol) flowed into the sample chamber at a specified time and temperature. The extract flowed into a container through a cooling coil (at the bottom of the sample chamber). Optimum scopoletin levels were generated for 12 min at 60°C in ethanol and 1:30 (w/v) for the ratio of solid-to-solvent. After 12 min of ASE, scopoletin levels were 377.30 ± 5.27 µg/g, whereas, after a 24 hr maceration extraction process, 244.56 ± 37.31 µg/g was generated.

Solvent Variation

Chang-hong et al. (2007) extracted freeze-dried fermented noni juice using three solvents: ethyl acetate (EtOAc), 1-butanol (n-BuOH), and petroleum ether. Three phenolic antioxidant components were isolated from the EtOAc solvent: aesculetin, 3,3',4',5,7-pentahydroxyflavone (quercetin), and isoscopoletin. The EtOAc solvent extraction showed higher antioxidant activity when compared with mannitol/vitamin C, whereas petroleum ether and n-BuOH solvent extractions had less activity besides mannitol.

High-Performance Liquid Chromatography (HPLC) Extraction

A quantitative scopoletin analytical method using noni fruit water extraction (aqueous fruit extracts [AFE]) was formulated by Mahattanadul et al. (2011). Diluted fruit extracts were analyzed using the high-performance liquid chromatography with ultra-violet (HPLC-UV) spectroscopy method. A mobile phase (isocratic mixture of 0.01 M sodium acetate: acetonitrile [80:20, v/v]) was operated at 1.0 ml/min, and scopoletin content in eluents was monitored at 350 nm. A calibration curve of several scopoletin concentrations (0.05–10 µg/ml) was used to calculate AFE scopoletin concentrations. Comparing retention times (5.585 min) with a scopoletin standard (5.497 min) performed identification of scopoletin peak on AFE chromatograms. The scopoletin content was 0.85–0.87 mg in 1 g of lyophilized AFE powder.

De Moraes et al. (2019) performed HPLC to determine flavonoid and phenolic ingredients in *M. citrifolia* AFE. From chromatograms, *M. citrifolia* AFE contained (2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoic acid (6), tR 37.5 min; 2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione (5), tR 35 min; 2-(3,4-dihydroxyphenyl)-

5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl] oxan-2 yl]oxychromen-4-one (4), tR 32.5 min; (E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid (3), tR 15.1 min; (1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid (2), tR 14.9 min; and phenolic compounds (e.g., gallic acid, chlorogenic acid, caffeic acid, ellagic acid, and rosmarinic acid), flavonoids (rutin) (e.g. 3,4,5-trihydroxybenzoic acid (1), tR 5 min.

Phenolic acid and flavonoid levels in noni juice (made from fermented and pasteurized fruit) were also examined by HPLC (Lin et al., 2013). The UV spectra were recorded between 220 and 450 nm. Bioactive content is shown in Table 9.

The minor element of noni juice was analyzed using high-performance liquid chromatography coupled with diode-array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-MS^n) (Dussossoy et al., 2011). Nine elements were quantified, and twelve were identified. Several phenolic compounds were determined: phenolic acids (vanillic acid), coumarins (scopoletin and esculetin), vanillin and iridoids (asperulosidic acid and deacetylasperulosidic acid), and flavonoids (rutin, kaempferol rutinoside, quercetin, isoquercitrin, and quercetin derivatives). Asperulosidic acid (71.6 mg equivalent of loganic acid/100 g FW) and deacetylasperulosidic acid (159.1 mg equivalent of loganic acid/100 g FW) were the major elements in noni juice. The main polyphenol compounds were scopoletin 1.33 mg/100 g FW and rutin 4.63 mg/100 g FW. Phenolic and iridoid compound quantities are shown in Table 10.

Table 9
Flavonoid and phenolic acid content in noni fruit juice (Lin et al., 2013)

Component	Content (mg/100 ml juice)
Gallic acid	1.79 ± 0.01
Gentisic acid	19.16 ± 0.75
Chlorogenic acid	10.49 ± 0.01
p-hydroxybenzoic acid	14.12 ± 0.42
Caffeic acid	5.42 ± 0.02
Epicatechin	2.94 ± 0.03
Ferulic acid	3.36 ± 0.01
p-anisic acid	5.07 ± 0.03
Naringin	3.39 ± 0.02
Hesperidin	3.85 ± 0.02
Phenolic acid content	59.41 ± 1.22
Flavonoid content	10.61 ± 0.04
Total	69.59 ± 1.26

Table 10
Noni juice components (Dussossoy et al., 2011)

Component	Amount (mg/100 g FW)
Quercetin	0.29 ± 0.01[a]
Quercetin derivative	0.46 ± 0.02[a]
Scopoletin	1.32 ± 0.02[a]
Rutin	4.63 ± 0.04[a]
Esculetin	0.20 ± 0.01[a]
Isoquercitrin	tr[b]
Desacetylasperulosidic acid	159.1 ± 8.1[a]
Asperulosidic acid	71.6 ± 4.1[a]
Vanillic acid	0.26 ± 0.00[a]
Vanillin	0.35 ± 0.01[a]
Kaempferol derivative	tr[b]
Protocatechuic acid	tr[b]

Note. [a]Mean values ± standard errors of three independent assessments
[b]tr = Trace amounts (<0.1 mg/100 g fresh weight)

Wu et al. (2019) reported that the compounds of fresh noni fruit that related to antioxidant activity were D-tagatose, ethylsuberenol, serotinose, (R)-3, 7-dihydroxy-2, 4-dimethoxyisoflavan, ancistrotectorine, 3-hydroxy-5Z-octenyl acetate, garcimangosone C, (−)-epigallocatechin, eugenitin, musanolone C, archange-slippery, and *O*-isopentenylhalfordinol. The component that significantly increased after harvesting were DL-malic acid, palmitic acid, isooctyl acetate, marmelolactone A, isorhamnetin 3-(6-malonylglucoside), ethylparaben, ethyl (4Z)-4,7-octadienoate, coumarin, geranylacetone, (Z)-alpha-irone, cedrelanol, carotol, norecasantalol, 5-dodecyldihydro-2(3H)-furanone, (2E,4E)-2,4-dodecadienal, and BRXanthone A. These molecules may account for the antibacterial properties of noni fruit and their benefits toward the immune system and inflammation.

Extraction processes break down protective cells to release bioactive ingredients so they can be separated and purified. Physical (e.g., pressure, temperature, or electromagnetic waves), chemical extraction techniques (e.g., solvents that bind to bioactive ingredients), or a combination thereof have been successfully applied to noni fruit. Such extraction methods must minimize the impact on bioactive ingredient activity, e.g., polyphenols and flavonoids, which have multiple health benefits. Extraction methods should be selected based on equipment availability, extraction time, optimal extraction content, and environmental safety (e.g., waste produced).

A variety of extraction methods have been applied to noni fruit to obtain the optimum amount of bioactive substances. Various operating conditions, such as temperatures below 70°C, continue to be considered by conventional extraction methods that are used up to high pressure. It is intended to ensure that the extracted bioactive materials, for example, polyphenols and flavonoids with antioxidant functions, are not damaged. Solvent selection leads to solvents that are safe for the extracted material and the environment, for example, ethanol, which is polar and suitable for polyphenols and flavonoid extraction. With additional treatments, such as pressure, the use of solvents is gradually reduced.

The use of advanced equipment encourages the provision of funding, which is a major weakness in today's extraction process. However, this weakness may be overcome by maximizing the extraction results and making full use of them to minimize the loss of bioactive materials in noni fruit.

CONCLUSION

Noni fruit contains phytochemical components that have multiple health benefits. Several extraction techniques are available that can generate optimally bioactive extracts. The separation technique for noni fruit was solid-liquid extraction or leaching. Polyphenols and flavonoids, which are highly beneficial for health, are bioactive from noni fruit.

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