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Short communication

# A quantitative comparison of phytochemical components in global noni fruits and their commercial products

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ABSTRACT

The fruits of noni (*Morinda citrifolia* L.) have been used as a medicinal food for centuries in a wide range of tropical regions, and are increasingly attracting more attention worldwide. Due to the increase of commercial noni fruit products in the global market, an extensive phytochemical comparison of noni fruits and their juice products seems imperative to understand their internal quality. To this end, we developed an HPLC method, established phytochemical fingerprints, and quantitatively compared the characteristic components in 7 noni fruits and 13 commercial fruit juices originating from the Caribbean, Central America, the Central and South Pacific, and Asia. The results showed that scopoletin, rutin, quercetin, and 5,15-dimethylmorindol were detected in all the samples, although at varying concentrations. Together, these components could be used as a reference for identification and authentication of raw noni fruits and their commercial products. Meanwhile, the variation in phytochemical content in noni fruits and juices may be attributed to the diversity of geographical environments (soil, sunlight, temperature, precipitation, etc.) and post-growth factors (harvesting, storage, transportation, manufacturing processes, formulation, etc.). Further, the variation may also suggest different toxicological and pharmacological profiles. As such, scientific data of efficacy and safety conducted on one noni fruit or juice may not be applicable to all others, including those from the same origins.



## 1. Introduction

Noni (*Morinda citrifolia* L., Rubiaceae) is a small evergreen shrub or tree growing in tropical and subtropical areas worldwide. Originally native to Southeastern Asia, the noni plant was spread to Australia, Hawaii, French Polynesia Islands, and other tropical areas through possible water-dispersal of buoyant seeds, or by being transported by early migrants or voyagers (Degener, 1929; Setchell, 1924). Among many other trivial names are *Indian Mulberry*, H*ai Ba Ji*, *Nono* or *Nonu*, *Cheese Fruit*, and *Nhau*. As a popular ethnomedicine among indigenous Polynesians, noni fruits were traditionally used for the improvement of various health problems, such as cancer, infection, arthritis, diabetes, asthma, and pain (Wang et al., 2002).

Modern scientific research has shown that noni fruits possess antioxidant, anti-inflammatory, liver-protective, and immunomodulatory effects (Deng et al., 2007; Liu, Ma, Gao, & Jiang, 2008; Palu et al., 2008; Pawlus & Kinghorn, 2007; Su et al., 2005; Wang, Anderson, Nowicki, & Jensen, 2008). So far, over 100 secondary metabolites have been identified in noni fruits. The structures of these are classified as flavonoids, lignans, iridoids, coumarins, anthraquinones, polysaccharides, terpenoids, sterol, and fatty acids. (Deng et al., 2007; Pawlus & Kinghorn, 2007). Pharmacologically synergetic effects among the components in noni fruits may account for its diversified health benefits.

Commercialisation of noni fruits as a medicinal food and dietary supplement has tremendously facilitated its availability worldwide, boosted its use, and brought its benefits to more people. Since the first commercial noni fruit product, Tahitian Noni® juice, was launched in 1996, countless noni products have emerged in the global market. The quality of commercial noni fruit products may vary significantly, attributing to different geographical conditions (soil, sunlight, precipitation, and air) and post-growth factors (harvesting, storage, transportation, manufacturing processes, etc.). As such, there are concerns regarding the consistency of phytochemical profiles of products from different areas. Are noni fruit commercial products marketed worldwide equally efficacious and safe? To address these concerns, we developed an analytical HPLC method, established phytochemical fingerprints, and conducted an extensive quantitative comparison of characteristic components in noni fruits obtained from seven major areas of noni cultivation. Additionally, 13 commercial noni fruit juices acquired from the worldwide market were also analysed by the same methods. The commercial noni products investigated in this study represent major global suppliers of noni fruits, including Japan (Okinawa),

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Southern China (Hainan), Thailand, Indonesia, Hawaii, Dominican Republic, El Salvador, Costa Rica, Tonga, French Polynesia and Tahiti.

## 2. Experimental

### 2.1. Chemicals and standards

Methanol (MeOH), water ($H_2O$), and dichloromethane ($CH_2Cl_2$) of HPLC grade were purchased from Fisher Scientific Co. (Fair Lawn, NJ, USA). HPLC grade acetonitrile (MeCN) and analytical grade trifluoroacetic acid (TFA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Chemical standards of scopoletin (**1**), quercetin-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (rutin, **2**), and quercetin (**3**) were isolated in our laboratory from noni fruit and leaves from Tahiti. The purities (>99%) and structures were determined by HPLC, MS, and NMR (Deng et al., 2007). 5,15-Dimethylmorindol (5,15-DMM, **4**) was kindly donated by Dr. Kohei Kamiya, Kobe, Japan. The standards were accurately weighed and then dissolved in an appropriate volume of MeOH/MeCN to produce corresponding stock standard solutions. Working standard solutions for calibration curves and the determination of limits of quantitation (LOQs) were prepared by diluting the stock solutions with MeOH at different concentrations. All stock and working solutions were maintained at 0 °C in a refrigerator.

### 2.2. Instrumentation and chromatographic conditions

Chromatographic separation was performed on a Waters 2690 separations module coupled with 996 a photodiode array (PDA) detector, and equipped with an Atlantis C18 column (4.6 mm × 250 mm; 5 μm, Waters Corporation, Milford, MA, USA). The pump was connected to a mobile phase system composed of three solvents: A; MeCN, B; MeOH, and C; 0.1 TFA% in $H_2O$ (v/v). The mobile phase was programmed consecutively in linear gradients as follows: 0 min, 10% A, 10% B, and 80% C; 15 min, 20% A, 20% B, and 60% C; 26 min, 40% A, 40% B, and 20% C; 28–39 min, 50% A, 50% B, and 0% C; and 40–45 min, 10% A, 10% B, and 80% C. The elution was run at a flow rate of 1.0 mL/min. The UV spectra were monitored in the range of 210 and 450 nm, and 365 and 410 nm were selected for the quantitative analysis of **1**–**4**. The injection volume was 50 μL for each of the sample solutions. The column temperature was maintained at 25 °C. Data collection and integration were performed using Waters Millennium software version 32.

### 2.3. Sample collection

The raw noni fruit samples (NF1–NF7) were collected from different areas, including Tahiti and Moorea of French Polynesia, Tonga, Dominica Republic, Okinawa, Thailand, and Hawaii. The fruit samples were stored below 0 °C before use. The commercial noni products, including 13 noni fruit juices (NFJ 1–13) were obtained from global markets. These products were made from raw noni fruits originated from the following locations: Tahiti, El Salvador, Hawaii, Dominican Republic, Costa Rica, China, and Indonesia. All of these products were produced by different manufacturers. Voucher specimens are deposited in the Research and Development Laboratory of Tahitian Noni International Inc., Utah, USA.

### 2.4. Sample preparation

*Noni fruits:* the fruits NF1–NF7 were defrosted and mashed. Two g of each mashed fruit was extracted with MeOH twice (125 mL, 30 min each) using a sonicator. The MeOH extract was dried under vacuum in a rotary evaporator. The dried MeOH extracts were redissolved with 10 mL of MeOH, respectively, for HPLC analysis.

*Commercial noni juice products:* for the HPLC analysis of analytes **1**–**3**, 1 mL of noni fruit juice was mixed with 1 mL of MeOH, vortexed for 1 min, and prepared into a concentration of 0.5 mL/mL solution. For 5,15-DMM (**4**) analysis, 100 mL of juice was partitioned with 100 mL of $CH_2Cl_2$ three times to obtain $CH_2Cl_2$ extract. The extract was concentrated to dryness in a rotary evaporator under reduced pressure at 45 °C. The dried extract was dissolved with 5 mL of MeOH, for HPLC experiments. Each fruit juice sample was prepared in this manner.

All samples were filtered through a nylon microfilter (0.45 μm pore size) before HPLC experiments. The injection volume was 50 μL each of the sample solutions.

### 2.5. Method validation

For calibration curves, working solutions of reference compounds **1**–**4** were prepared by diluting the stock solutions with MeOH at five concentrations in the range of 0.02–94.4 μg/mL. The calibration curves were plotted after linear regression of the peak areas *versus* concentrations. The result showed an acceptable linearity with correlation coefficient higher than 0.999 within the range of concentrations investigated. The working solutions of **1**–**4** for LOQ determinations were prepared by diluting them sequentially. The LOQs of **1**–**4**, defined as signal/noise ratio of 10, were determined to be 0.14, 0.085, 0.11, and 3.3 ng, respectively. $CH_2Cl_2$ was used for extraction of anthraquinone 5,15-DMM referencing a previous study with good recoveries (Deng, West, Jensen, Basar, & Westendorf, 2009). All analyses were performed in triplicates, and the variations were evaluated by the standard deviation (SD) in the HPLC experiments. Identification of target compounds **1**–**4** was made by comparing the HPLC retention times and UV absorptions of target peaks with those of the reference compounds **1**–**4**

**Table 2**
Phytochemical analysis of the methanolic extracts of global noni fruits (mg/g).

| Samples | Scopoletin | Rutin | Quercetin | 5,15-DMM |
|---|---|---|---|---|
| NF1 | 0.66 ± 0.019 | 1.26 ± 0.065 | 0.040 ± 0.0065 | 0.014 ± 0.0045 |
| NF2 | 0.76 ± 0.025 | 1.44 ± 0.14 | 0.036 ± 0.0050 | 0.0055 ± 0.00070 |
| NF3 | 1.18 ± 0.040 | 2.75 ± 0.15 | 0.20 ± 0.055 | 0.0048 ± 0.00045 |
| NF4 | 0.064 ± 0.0055 | 0.053 ± 0.013 | 0.015 ± 0.0045 | 0.018 ± 0.0035 |
| NF5 | 6.87 ± 0.085 | 0.57 ± 0.071 | 0.080 ± 0.0085 | 0.26 ± 0.035 |
| NF6 | 3.59 ± 0.16 | 2.21 ± 0.085 | 0.028 ± 0.0065 | 0.014 ± 0.0045 |
| NF7 | 0.70 ± 0.065 | 1.16 ± 0.070 | 0.086 ± 0.0086 | 0.0043 ± 0.00060 |

**Table 1**
Analytical parameters of reference compounds used in the HPLC experiments.

| Reference | Linearity range (μg/mL) | Calibration equation[a] | LOQ (ng) | Correlation coefficient | Retention time (min) | UV (nm) |
|---|---|---|---|---|---|---|
| 1 | 0.19–94.4 | $y = 8.614 \times 10^7 x + 32447.32$ | 0.14 | 0.9998 | 13.96 | 365 |
| 2 | 0.32–64.0 | $y = 7.194 \times 10^7 x + 1395.66$ | 0.085 | 0.9995 | 16.21 | 365 |
| 3 | 0.042–42.0 | $y = 1.874 \times 10^8 x - 24892.34$ | 0.11 | 0.9999 | 23.01 | 365 |
| 4 | 0.02–10.0 | $y = 9.077 \times 10^7 x + 359.08$ | 3.30 | 0.9992 | 29.42 | 410 |

[a] $x$ is the concentration in mg/mL and $y$ is the peak area at designated UV wavelength.