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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.

Fig. 1. Location of origins of noni raw fruits and commercial noni juice samples (the base map was adopted from http://english.freemap.jp/).

(Table 1). The typical HPLC chromatograms of a mixed standard solution of reference compounds **1**–**4**, noni fruits, and commercial noni fruit juices are displayed in Fig. 2.

3. Results and discussion

Noni fruits have been used as a medicinal food or dietary supplement for centuries, and are of increasing interest to consumers. At present, hundreds of commercial noni fruit products are marketed worldwide (West, Tolson, Vest, Jensen, & Lundell, 2006). The raw noni fruits of these products originate from a wide range of geographical regions, from Eastern to Western hemispheres, from coasts to mountains. Fig. 1 summarises the major origins of noni, globally, and also represents the geographical range of noni evaluated in our experiments. Among these, only a few have been scientifically evaluated by researchers and health authorities for efficacy and safety (European Commission., 2003). As such, there are concerns about authenticity and quality of commercial noni fruit products. In our investigation, we used four different compounds as references. Scopoletin and 5,15-dimethylmorindol are characteristic components in noni fruits, while rutin and quercetin are bioactive flavonoids. Together, the four compounds may provide a unique profile for phytochemical analysis of noni fruits.

3.1. Noni fruits

Our study extensively investigated the characteristics of noni fruits collected from major noni suppliers worldwide. These areas include Pacific Islands of Tahiti and Moorea of French Polynesia, Tonga, and Hawaii, as well as other countries and regions, including the Dominican Republic (Caribbean), Okinawa of Japan (Asia), and Thailand (Southeast Asia) (Fig. 1).

In our study, an analytical HPLC method was developed for establishing phytochemical fingerprints of different noni fruits. Further, a qualitative comparison of characteristic components, including scopoletin, rutin, quercetin, and 5,15-DMM was performed for these samples. A good separation of analytes **1**–**4** was obtained by using a reverse-phase C_{18} column and eluted with a linear gradient of triplet solvent system (MeOH–MeCN–H_2O). Fig. 2 (NF1–NF7) shows the typical HPLC fingerprint profiles of these samples under experimental conditions. The experimental results indicated that the noni fruits collected globally exhibit different chemical profiles: scopoletin, rutin, quercetin, and 5,15-DMM contents ranging at 0.064–6.87, 0.053–2.75, 0.015–0.20, and 0.0043–0.26 mg/g in the methanolic extracts of noni fruits, respectively (Table 2). The variation of these characteristic constituents in noni fruits from one location to the other may be caused by significant impacts from geographical environmental factors, such as soils, sunlight, temperature, moisten, air, etc. The experimental results demonstrate that noni fruits collected from different geographical locations are not identical in their phytochemical components, although analytes **1**–**4** were detected in each sample.

3.2. Noni fruit juice products

In addition to the noni fruit raw materials analysed, 13 samples of commercial noni products were obtained from global markets and analysed with the analytical method developed. These noni juice products were made from noni fruits originating from the regions of the Caribbean, Central America, the Central and South Pacific, and Asia, specifically including Tahiti, El Salvador, Dominican Republic, Costa Rica, Hawaii, China, and Indonesia (Fig. 1). A typical HPLC fingerprint of these samples is shown in Fig. 2 (NFJ1). The comparison of phytochemical fingerprints indicated that all of these 13 commercial noni juices contain characteristic components of scopoletin, rutin, quercetin, and 5,15-DMM, as observed in all noni frutis. Further quantitative analysis showed that the contents of those components vary significantly, in the range of 0.88–34.01, 1.11–69.70, 0.14–8.33, and 0.0019–0.19 μg/mL, respectively (Table 3). Interestingly, even noni juice products from within the same regions had wide variations in **1**–**4** content, as shown in samples NFJ1–NFJ4, NFJ5–NFJ7, and NFJ8–NFJ9. The results indicated that some post-growth factors, other than environmental may also contribute to the difference of phytochemical contents in commercial noni fruit juices. These factors may include harvesting, storage, transportation, manufacturing processes, formulation, etc. We have noticed that juice processing is a very critical step for exclusion of potentially genotoxic anthraquinones. During this process, seed and skin of ripe fruit containing more anthraquinones could be effectively removed. Additionally, the "eyes" on the noni fruits contain approximately 100 times of anthraquinones than fruit puree (unpublished data). A proper process may help to separate these "eyes" from puree, and prevent anthraquinone inclusion in the products. Artificial additives or other ingredients in some commercial noni juices may also account for difference in their phytochemical profiles.

3.3. Species confusion

Species confusion exists in some areas. In Malay, all *Morinda* species are called 'mengkudu', which may cause misidentification during harvesting. This may explain why catechins were only reported from local species (Zin, Hamid, Osman, Saari, & Misran, 2007). In India, eight different species in *Morinda* L. (Rubiaceae) are found, and confusion or misuse of other species with *M. citrifo-*

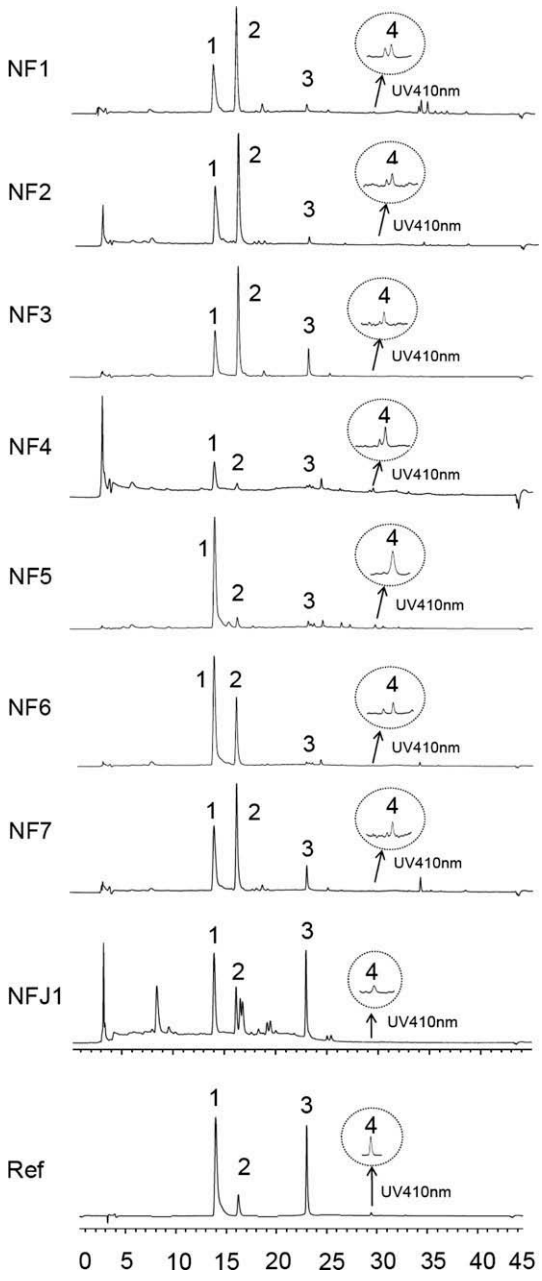

Fig. 2. Reverse-phase HPLC chromatograms of seven noni fruits (NF1–NF7) collected from different areas, a typical noni juice product (NFJ1), and mixed references (Ref). All samples were found to contain scopoletin (**1**, Rt = 13.96 min), rutin (**2**, Rt = 16.21 min), quercetin (**3**, Rt = 23.01 min), and 5,15-DMM (**4**, Rt = 29.42 min), although at a significantly wide range of concentrations. (UV 365 nm is selected for quantitation of **1**–**3**, and UV 410 nm for **4**, as shown in inserted circles which were adopted from the different HPLC chromatograms).

lia often happens (Anonymous, 1962). This is a challenge for scientific evaluation of safety and efficacy of noni fruits and their commercial products, and highlights the need for authentication and standardisation of noni fruits. The phytochemical fingerprints and quantitative analysis established in our study serve as a useful method for identifying possible adulteration of noni fruit and noni juice products.

In conclusion, our study investigated phytochemicals in noni fruits and commercial noni juice products available worldwide. Four characteristic analytes, scopoletin, rutin, quercetin, and 5,15-DMM were detected in all the noni fruits and commercial juices originating from different regions of the world, although at a significantly various range of concentration. Together, these characteristic components can be used as a reference for identification and authentication of noni fruit raw materials and commercial noni products. Meanwhile, the variation in phytochemical content in noni fruits and juices may suggest different toxicological and pharmacological properties. While there are over 300 peer-reviewed scientific publications on noni, data regarding efficacy and safety of one commercial noni product may not be applicable to any others.

Table 3
Characteristic phytochemicals in commercial noni fruit juices in worldwide market (μg/mL).

Samples	Scopoletin	Rutin	Quercetin	5,15-DMM
NFJ1	12.90 ± 0.40	23.09 ± 0.59	8.33 ± 0.17	0.045 ± 0.0075
NFJ2	1.48 ± 0.055	2.27 ± 0.12	0.48 ± 0.027	0.038 ± 0.0045
NFJ3	34.01 ± 1.05	7.80 ± 0.11	5.41 ± 0.14	0.19 ± 0.025
NFJ4	8.93 ± 0.11	16.71 ± 0.74	4.60 ± 0.17	0.023 ± 0.0035
NFJ5	20.62 ± 0.39	3.65 ± 0.12	3.69 ± 0.16	0.23 ± 0.040
NFJ6	20.11 ± 0.50	1.30 ± 0.055	1.15 ± 0.095	0.030 ± 0.0035
NFJ7	15.42 ± 0.31	1.11 ± 0.11	3.67 ± 0.18	0.19 ± 0.025
NFJ8	17.03 ± 0.29	69.70 ± 2.30	0.90 ± 0.0.021	0.11 ± 0.020
NFJ9	6.62 ± 0.12	5.01 ± 0.53	1.48 ± 0.096	0.050 ± 0.0041
NFJ10	21.19 ± 1.82	2.59 ± 0.10	1.23 ± 0.056	0.0019 ± 0.00030
NFJ11	14.58 ± 0.49	1.88 ± 0.085	0.14 ± 0.0085	0.0022 ± 0.00065
NFJ12	0.88 ± 0.075	5.57 ± 0.18	0.98 ± 0.14	0.0063 ± 0.00075
NFJ13	13.09 ± 0.91	21.29 ± 1.06	0.92 ± 0.13	0.19 ± 0.035

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