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Antioxidant capacity, total phenols, and ascorbic acid content of noni *(Morinda citrifolia)* fruits and leaves at various stages of maturity

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Abstract—Traditional healers and modern manufacturers both recommend noni *(Morinda citrifolia)* fruits and leaves for good health. We determined antioxidant capacity, total phenols, and ascorbic-acid content of noni fruits and leaves in various stages of maturity. Submature white hard noni fruits had ORAC values of 1880 μmol Trolox, VCEAC of 188-199 mg, total phenols of 285 mg gallic acid, and ascorbic acid of 224 mg per 100 g. Their antioxidant capacity was 1.2–2.2 times, their total phenols 1.5 times, and their ascorbic acid content 7.0 times those of immature green noni fruits, and were 1.1–1.5, 1.3, and 1.3 times greater than those of ripe fruits. Young, medium, and old noni leaves exhibited ORAC values of 671–731 μmol Trolox, VCEAC of 7.2-35.2 mg, and total phenols of 14.6–16.0 mg gallic acid per gram dry weight. Mature noni fruit and noni leaves are therefore good sources of dietary antioxidants.

Introduction

Noni *(Morinda citrifolia L.)* is among the traditional Polynesian medicinal plants used as remedies in the Pacific with a long history (Nelson & Elevitch 2006). Traditional islander healers use the noni leaves, fruits, roots, barks, stems, and flowers (Pawlus & Kinghorn 2007). Pacific traditional healers commonly use noni leaves and green immature fruit; modern Hawaiian home healers commonly use ripe noni fruits (McClatchey 2002). Modern noni manufacturers produce processed noni juice from ripe noni fruits as dietary supplements and produce herbal tea from noni leaves (Nelson et al. 2006) .

Both noni leaves and fruits are used to treat diseases in many countries and regions (McClatchey 2002, Pawlus & Kinghorn 2007), but the leaves are most frequently used for external treatments and the fruits for internal treatments (Pawlus & Kinghorn 2007). For example, noni fruits are used as emmenagogues, blood purifiers, antiemetic agents, and tonic supplements and for digestive disorders, tuberculosis, urinary-tract ailments, stimulation of appetite and central nervous system, hypertension, diabetes, and depression, whereas the leaves are often used as a poultice for rheumatic or swelling joints, fish stings, broken bones and sprains, wounds and ulcers, and boils and burns (Pawlus & Kinghorn 2007).

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In *in vitro* and *in vivo* (animal) studies, noni fruits and their juices or extracts have been shown to scavenge free radicals, inhibit LDL oxidation, stimulate the immune system, and exhibit anticancer and anti-inflammatory properties (Kamiya et al. 2004, Akihisa et al. 2007, Yang et al. 2007, Palu et al. 2008). In *in vitro* and *in vivo* (animal) studies, noni leave have been shown to scavenge free radicals and inhibit LDL oxidation and UVB-induced activator protein-1 (Sang et al. 2003, Zin et al. 2006, Takashima et al. 2007).

The differences of antioxidant characteristics of noni fruits and leaves at various stages of maturity have not been identified. Investigating antioxidant characteristics of noni fruits and leaves at different stages of maturity may help understanding the difference in use of noni fruits and leaves between traditional healers and modern noni manufacturers. The information may also be useful for noni manufacturers to select right stages of noni fruits and leaves for processing products to benefit consumers. Our objective of this study was to determine antioxidant capacity, total phenol content, and ascorbic acid content of noni fruits and leaves at various stages of maturity.

Materials and Methods

Materials

Sodium fluorescein, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, sodium carbonate monosodium phosphate monohydrate, disodium phosphate heptahydrate, glacial acetic acid, metaphosphoric acid, ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 2,6-dichloroindophenol sodium salt hydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteau reagent, gallic acid, potassium metabisulfite, potassium persulfate, and citric acid were purchased from Spectrum Chemicals (Gardena, CA, USA). The chemicals 2,2-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and 2,3'-azino-bis(3-ethylbenthiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Wako Pure Chemical Industries (Chuo-Ku, Osaka, Japan).

Noni Fruits and Leaves

Immature green and submature white hard noni fruits were picked from wild noni trees on Guam. The soft ripe fruits were obtained by ripening white hard noni fruits at 26–28°C and 70–75% humidity for 2 or 3 days. All noni fruits of green, white hard, and ripe soft noni fruits were grouped as three replications. In each replication, fruits were divided into two sub-groups. Each sub-group was consisted of 5 fruits of green, white hard, or ripe soft fruits. The green, white hard, and soft ripe fruits were pureed with a Samson GB-9001 multipurpose juice extractor (Greenbison, Cypress, CA, USA). The noni juice was then centrifuged at 8000 rpm in a Beckman-Coulter Allegra X-22R Centrifuge (Kansas City, MO, USA) for 10 min. The supernatant was used immediately as fruit extract or stored at –18°C for later assays.

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Young, medium, and old noni leaves were obtained up from wild noni trees on Guam. All young, medium, or old noni leaves were grouped as three replications. In each replication, the leaves were divided into two sub-groups. Each sub-group was consisted of 15-40 leaves of young, medium, or old leaves. Noni leaves were cleaned, freeze-dried in a Lock 6 freeze dry system (Lab Conco Lyph, Kansas City, Missouri, USA) at –40°C for 2 days, and ground into powders. For each assay, 1 g of powder was extracted with 20 ml of hot water at 90°C for 20 min. The mixture was centrifuged at 8000 rpm in the Beckman centrifuge for 10 min. The supernatant was collected, and the pellet was extracted three times by the same procedure. The combined supernatants were used fresh or stored at –18°C for later assays.

ORAC Assay

The antioxidant capacity of noni fruits and leaves was determined by the oxygen-radical absorbance-capacity (ORAC) assay (Huang et al. 2002). In the assay, a solution of 40 mM AAPH in 75 mM phosphate buffer (pH 7.4) was prepared daily as peroxyl-radical generator. A solution of 1.17 mM sodium fluorescein in 75 mM phosphate buffer (pH 7.4) was prepared as the redox-sensitive fluorescent indicator and stored at 5°C for analysis. In analysis, 3 µl of sodium fluorescein stock solution was diluted with 30 ml of 75-mM phosphate buffer (pH 7.4). The microplate wells were filled with 120 µl of diluted sodium fluorescein solution and 20 µl of noni fruit or leaf extract, or with blank (75-mM phosphate buffer pH 7.4), or with standard Trolox solution. After the solutions equilibrated in the Synergy™ HT Mulit-Detection microplate reader (BioTek Instruments, Winooski, VT, USA.) at 37°C for 15 min, 60 µl of 40 mM AAPH solution was added to the wells to initiate the reactions. The fluorescence of each well was then measured kinetically with an excitation wavelength at 485 nm and an emission wavelength at 528 nm. The antioxidant capacity of noni fruits or leaves was calculated and expressed in ORAC units as µmol Trolox/100 g fresh weight (fw) of fruits or g dry weight (dw) of leaves.

ABTS Assay

The antioxidant capacity of noni fruits and leaves was also assayed by the radical-scavenging of the free radical ABTS (Re et al. 1999). In the assay, the free-radical cations ($ABTS^{\bullet+}$) were generated by mixing of 2.5 ml of 7 mM ABTS diammonium salt with 0.5 ml of 15 mM potassium persulfate at 20°C. After being stored in the dark for 24 h, the $ABTS^{\bullet+}$ solution was diluted with distilled water to yield an absorbance of 0.700 at 734 nm. Two ml of the diluted $ABTS^{\bullet+}$ solution was mixed with 20 µl of diluted noni fruit or leaf extract. After 60 min at 20°C, the absorbance of the mixture was measured at 734 nm with a Varian Cary 50 UV-Vis Spectrophotometer (Varian, Walnut Creek, CA, USA). Antioxidant capacity of noni fruit or leaf extract was calculated by the formula inhibition (%) = $((A_{t=0} - A_{t=60})/A_{t=0}) \times 100$, where $A_{t=0}$ was the absorbance at the time the sample was added and $A_{t=60}$ was the absorbance after 60 min. The antioxidant capacity of noni fruits or leaves was expressed as vitamin C equivalent antioxidant capacity (VCEAC), described by (Kim, Lee, Lee, & Lee, 2002), in mg/100 g fw of fruits or g dw of leaves.

DPPH Assay

The antioxidant capacity of noni fruits and leaves was also determined by scavenging of DPPH free radicals (Brand-William et al. 1995). The diluted noni fruit extracts (10–60 µl) and leaf extract (10–80 µl) were added to 3 ml of solution of 0.025 g/l $DPPH^{\bullet}$ in methanol. After 40 min the absorbance of the $DPPH^{\bullet}$ solution was measured at 515 nm with the spectrophotometer. The concentrations of $DPPH^{\bullet}$ were calculated with the calibration curve $A_{(515nm)} = 31.7[DPPH^{\bullet}] - 0.0006$. The percentage of DPPH remaining after 40 min was calculated from $\% DPPH^{\bullet}_{rem} = ([DPPH^{\bullet}]_{40}/[DPPH^{\bullet}]_{0})100$, where $[DPPH^{\bullet}]_{0}$ is the initial concentration of DPPH free radicals and $[DPPH^{\bullet}]_{40}$ is the concentration of DPPH free radicals after 40 min. The amount of noni fruit or leaf extract necessary to reduce the initial $DPPH^{\bullet}$ concentration by 50% was expressed as VCEAC in mg/100 g fw of fruits or g dw of leaves.

Total Phenol Analysis

The total phenolic contents of noni fruits and leaves were measured with the Folin-Ciocalteu reagent (Slinkard & Singleton 1977). Twenty µl of diluted noni fruit or leaf extract was first mixed with 1.58 ml of distilled water; 100 µl of Folin-Ciocalteu reagent and 300 µl of saturated Na_2CO_3 (20%) were then added. After the solution was incubated at 40°C for 30 min, the absorbance of the solution was measured at 765 nm with the spectrophotometer. Total phenols of noni fruits or leaves were calculated from the standard curve and expressed as mg gallic acid (GAE) per 100 g fw of noni fruits or g dw of leaves.

Ascorbic Acid Analysis

Ascorbic-acid content of noni fruits was determined by the dichloroindophenol titrimetric methods (AOAC 1995). A 0.3-ml sample of fresh centrifuged noni fruit extract was mixed for 10 sec with 20 ml of solvent prepared with glacial acetic acid and metaphosphoric acid. The solution was then titrated with 2,6-dichloroindophenol solution until it appeared pink for 10 seconds. The ascorbic acid content of noni juice was calculated based on the ascorbic acid standard curve conducted at the same condition.

Statistical Analysis

Three replications were performed in all experiments. In each replications, 2 assays from two sub-groups were conducted for analysis. The mean of antioxidant characteristics was based on the data from 2 assays × 2 sub-groups × 3 replications. The differences among means were identified by analysis of variance and least-significant-difference tests conducted with SPSS 12.0 for Windows (SPSS, 2003). Mean differences were considered significant at the $P < 0.05$ level.

Results

White hard noni fruits exhibited antioxidant capacity and total phenols and ascorbic-acid contents significantly higher than those of immature green noni fruits,

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except for the antioxidant capacity in the ORAC assay (Table 1). The VCEAC, total phenols, and ascorbic-acid content of white hard noni fruits are 2.1, 1.3, and 7.1 times greater than those of immature green noni fruits, respectively. White hard noni fruits exhibited significantly greater antioxidant capacity in ABTS assay and ascorbic acid content than did ripe soft noni fruits. The ORAC value, VCEAC, total phenols, and ascorbic acid content of white hard noni fruits were 1.1, 1.3–1.5, 1.3, and 1.3 times greater than those of ripe soft noni fruits, respectively. White hard and ripe noni fruits did not differ significantly in antioxidant capacity in the ORAC and DPPH assay or in total phenols (Table 1). Although the medium and old noni leaves exhibited antioxidant capacity and total phenol content greater than those of young noni leaves, none of the differences was significant (Table 2).

Table 1. Antioxidant capacity, total phenols, and ascorbic acid content of green (immature), white hard (mature), and ripe soft noni fruits (fresh weight).

	ORAC assay, Trolox mol/100 g	ABTS assay, VCEAC mg/100 g	DPPH assay, VCEAC mg/100 g	Total phenols, (gallic acid mg/100 g)	Ascorbic acid (mg/100 g)
Green	1610 ± 131 A	93.9 ± 3.5 C	86.8 ± 19.5 B	187.0 ± 20.6 B	32.0 ± 7.2 C
White hard	1882 ± 230 A	199.5 ± 8.2 A	188.0 ± 8.5 A	284.8 ± 25.9 A	224.3 ± 23.0 A
Ripe soft	1766 ± 157 A	133.9 ± 8.2 B	166.7 ± 3.2 A	225.3 ± 41.0 AB	173.7 ± 2.1 B

Means with different letters in the same column differed significantly ($P < 0.05$).

Table 2. Antioxidant capacity and total phenol content of noni leaves (dry weight) at various stages of maturity. Age did not affect either value significantly.

	ORAC assay, Trolox μmol/g	ABTS assay, VCEAC mg/g	DPPH assay, VCEAC mg/g	Total phenols (allic acid mg/g)
Young	671.0 ± 43.7	26.4 ± 6.8	6.80 ± 1.68	14.6 ± 4.6
Medium	688.6 ± 59.5	35.2 ± 8.5	7.24 ± 2.46	16.1 ± 2.7
Old	731.5 ± 56.8	26.3 ± 0.5	9.74 ± 3.90	15.0 ± 1.9

Discussion

Antioxidant Capacity of Noni Fruits

Mature noni fruits exhibited the same level of antioxidant capacity (ORAC value) as navel oranges and tangerines compared to the report by Wu et al. (2004). The antioxidant activity, total phenols, and ascorbic-acid contents of mature noni fruits in our study were much greater than those reported by Chan-Blanco et al. (2007). For unripe noni fruits pulp, Chan-Blanco et al. (2007) reported ORAC values of 740 μmol Trolox/100 g, phenols of 4.1 mg GAE/100 g, and ascorbic acid of 39.1 mg/100 g; their corresponding values for ripe fruit pulp were 800, 5.1, 31.6, respectively. The differences between that study and ours may be attributable to the difference in sample preparation. Potent antioxidants reported from noni fruits are neolignan, americanin A, 3,3'-bisdemethylpinoresinol, morindolin,

and isoprincepin, which inhibit copper-induced low-density lipoprotein oxidation (Kamiya et al. 2004, Su et al. 2005).

The greater antioxidant capacity of white hard and ripe soft noni fruits indicate that they provide higher dietary antioxidants than do green noni fruits. Dietary antioxidants can prevent chronic and degenerative diseases—cancer, heart disease, and neurological disorders—by mitigating oxidative stress in the human body (Morrissey & O'Brien 1998). The high dietary antioxidant content of mature noni fruits supports the practice by modern home healers of using ripe fruits as an internal remedy and by modern noni processors of promoting noni products as dietary supplements.

Effects of Noni Fruit Maturation and Ripening

Maturation from green to white hard noni fruits increased antioxidant capacity, total phenols, and ascorbic-acid content; ripening from the white hard to the ripe soft stage decreased the three values (Table 1). The decrease in ascorbic acid content was significant. Chan-Blanco et al. (2007) also observed an increase in antioxidant capacity and total phenols from unripe to ripe noni fruits and a decrease in these values after ripening, but the changes were not significant. Other fruits and vegetables also show different levels of antioxidant activity, total phenols, and ascorbic acid content at different stages of maturation and ripening. For example, ripening increases the ascorbic acid content, total phenols, and antioxidant activity of sweet cherry (Serrano et al. 2005), but it decreases antioxidant activity and total phenols of blueberry (Castrejon et al. 2008) and lowers antioxidant activity and ascorbic-acid content of *Rubus coreanus* fruit (Park et al. 2008).

Antioxidant Capacity and Total Phenols of Noni Leaves

Noni leaves exhibited ORAC values equivalent to or higher than those of blackberry, red raspberry, and strawberry leaves (Table 2), whose ORAC values range from 205 to 728 µmol Trolox/g dry weight (Wang & Lin 2000), but the exhibited total phenol content 3.0–8.6 times lower than did those species (Wang & Lin 2000). Noni leaves had antioxidant capacity about 1.14 times and total phenols about 0.21 times those of green tea (Prior & Cao 1999). Noni-leaf extracts made with ethyl acetate or from chromatographic fractions exhibited antioxidant activity comparable to those made with butylated hydroxyl toluene (BHT) and α-tocopherol (Zin et al. 2006). Owen et al. (2007) reported total phenols in noni leaves at 2.09 mg tannic acid equivalents (TAE)/g extract. Deng et al. (2008) reported that noni leaves contain two major flavonoids—quercetin-3-O-α-1-rhamnopyranosyl-(1 → 6)-β-d-glucopyranoside and kaempferol-3-O-α-1-rhamnopyranosyl-(1→ 6)-β-d-glucopyranoside—at 0.94 and 3.71 mg/g dry weight, respectively. Although maturation affected antioxidant capacity and total phenols of blackberry, raspberry, and strawberry leaves (Wang & Lin 2000), we found no significant differences in antioxidant capacity or total phenol content among young, medium, and old noni leaves.

Effects of Antioxidant Capacity Assays

The mechanisms of assay play an important role in determining antioxidant properties of noni fruits and leaves. The significant increase in antioxidant values that we observed during maturation of noni fruits was evident in the DPPH, ABTS, FC–total phenol, and 2,6-dichloroindophenol titration assays but not in the ORAC assay. The DPPH, ABTS, and FC assays are single-electron transfer (ET) reaction–based assays, as is the 2,6-dichloroindophenol titration assay; the ORAC assay is a hydrogen-atom transfer (HAT) reaction–based assay (Huang et al. 2005). The first four assays revealed significant increases in antioxidant capacity, total phenols, or ascorbic acid content during noni fruit maturation, whereas ORAC assay trended differently in antioxidant capacity.

The VCEAC value of noni leaves assayed with $ABTS^{\bullet+}$ radicals was also observed five times greater than that assayed with $DPPH^{\bullet}$ radicals (Table 2). The reaction of antioxidants with $ABTS^{\bullet+}$ is generally much faster than those with $DPPH^{\bullet}$. The fast and slow reactions of antioxidants with $ABTS^{\bullet+}$ can achieve the steady state within 6 min, (Re et al. 1999). However, the fast, intermediate, and slow reactions of antioxidants with $DPPH^{\bullet}$ require 0-5, 5-30, and > 30 min, respectively, to achieve the steady state (Brand-William et al. 1995). The observation time in our experiments covered both fast and slow reactions in ABTS assay but only fast and intermediate reactions in DPPH assay. Therefore, the difference of noni-leaf VCEAC values was observed between the ABTS assay and the DPPH assay. In addition, the difference of VCEAC values between the two assays also indicated noni leaves may contain substantial antioxidants with slow kinetic behaviors to $DPPH^{\bullet}$ radicals.

Conclusions

Mature noni fruit had antioxidant capacity similar to that of navel oranges and tangerines; noni leaves had the same level of antioxidant capacity as green tea. Maturation increased antioxidant capacity, total phenols, and ascorbic acid content of noni fruits, and ripening beyond maturity decreased all three, but both white hard and ripe soft noni fruits had greater antioxidant capacity, total phenols, and ascorbic acid content than did immature green noni fruits. Young, medium, and old noni leaves showed similar level of antioxidant activity and total phenols. Our conclusion that mature noni fruits are good sources of dietary antioxidants supports the practices by Pacific traditional healers of using fruits as internal remedies and of noni manufacturers of processing noni products as dietary supplements.

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