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# Thin Layer Chromatography Methods for Rapid Identity Testing of *Morinda citrifolia* L. (Noni) Fruit and Leaf

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**Abstract:** *Morinda citrifolia* L., commonly known as noni, is a growing global commodity. As such, there is a need for rapid and inexpensive identity tests of noni fruit and leaf products. Thin Layer Chromatography (TLC) methods were developed for the identification of deacetylasperulosidic acid in noni fruit and leaf products. TLC methods were also developed for the identification of scopoletin in noni fruit products and rutin in noni leaf products. TLC results were supported by High Performance Liquid Chromatography (HPLC) analyses. The concentrations of marker compounds detected by HPLC indicate that these TLC methods have good sensitivity and utility in the identification of noni fruit and leaf ingredients from a range of global sources, as well as noni based commercial products. These methods do not require expensive instrumentation or specialized laboratories, and are readily transferable to laboratories operating under a variety of circumstances.

**Key words:** Identity testing, *Morinda citrifolia*, thin layer chromatography

## INTRODUCTION

*Morinda citrifolia* Linnaeus, noni, fruit juice and powder are growing global commodities. In recent years, noni fruit puree and juice comprised 22 to 46% of total agricultural exports from French Polynesia to the United States (Service du Commerce Exterieur de Polynesie Frances, 2008a). In 2005 alone, more than 6,000 metric tons of noni fruit puree was produced (Service du Commerce Exterieur de Polynesie Frances, 2008b). Since 2004, noni has been Samoa's major agricultural export, with a peak annual export of approximately 1.5 million liters of juice and 167 metric tons of dried fruit (Rogers *et al*., 2010). In 2003, Noni fruit juice was approved by the European Union as a novel food ingredient to be used in pasteurized fruit beverages (European Commission, 2003). That approval was based on the assessment of noni fruit juice from French Polynesia (Scientific Committee on Food, 2002). Since that approval, additional sources of noni fruit juice have been approved for sale within the EU under the substantial equivalence application procedure. More than 40 substantial equivalence approvals within the EU have been granted. These sources are from a variety of nations which include French Polyensia, Fiji, Dominican Republic, Panama, Costa Rica, Samoa, U.S.A. (Hawaii), Tonga, Vanuatu, Cook Islands, Palau, Solomon Islands, and Nauru (European Commission, 2010a). Additionally, noni leaves were approved in 2008 as a novel food within the EU, for the purpose of making infusions (European Commission, 2008). Very recently, noni fruit puree and noni fruit juice concentrate received approval as novel food ingredients to be used in a variety of food product categories (European Commission, 2010b).

The increasing production and sources of noni fruit and leaves necessitates standardization regarding identity testing. Analyses of commercial noni products have indicated some quality differences, as well as possible misidentification of noni fruit raw materials (West *et al*., 2006; European Food Safety Authority, 2008; Deng *et al*., 2010a). Methods have been published that might be useful for identity testing, but these require expensive instrumentation and are costly to perform. There is a need for simple, acurate and cost effective methods for authenticating noni fruit and leaf materials. This is especially true when considering the sources of noni, which are often developing countries. Many producers do not have the means to conduct testing which requires investment in costly equipment. Additionally, rapid testing needs to be available to governmental inspectors around the world to confirm the identity of noni fruit and leaves that are imported into their respective nations. To address the need for low cost, yet acurate, analytical methods for identifying authentic noni fruit and leaf raw material and commercial products, Thin Layer Chromatography (TLC) methods were developed. TLC is a widely used analytical method for quality control in food production and agriculture. It is used to detect a wide range of plant constituents (Sherma, 2000).

The accuracies of the current TLC methods were confirmed by High Performance Liquid Chromatography (HPLC). The newly developed TLC methods can be

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performed in unspecialized laboratories and do not require any expensive analytical instrumentation. Chemical reference standards may be employed in the assay, or certified reference plant materials may be used. The use of certified noni reference material makes this method readily accessible to most quality control departments and governmental food inspection agencies.

## MATERIALS AND METHODS

Chemical marker standards, scopoletin, rutin, and deacetylasperulosidic acid (DAA), were isolated directly from the freeze-dried noni fruit and leaf powder. Identity and purity (>98%) were confirmed by NMR, MS, and HPLC (Deng *et al*., 2007, 2010a, b). The marker compounds were dissolved in methanol (MeOH) to a concentration of 1 mg/mL.

Eight raw noni fruit samples (F1-F8) were collected from different locations including French Polynesia (Tahiti, Moorea, and Motu Fareone), Tonga, Dominican Republic, Okinawa, Thailand, and Hawaii. The fruit samples were stored below 0ºC before use. Fruits were thawed, mashed and extracted (2 g) twice with 125 mL methanol (MeOH), aided by sonication for 30 min. The solvent was removed under vacuum in a rotary evaporator. The MeOH extracts were then redissolved in 10 mL of MeOH.

Commercial noni fruit juice products (J1-J4), originating from Tahiti, Dominican Republic, Hawaii, and Costa Rica, produced by different manufacturers, were purchased at local markets or via the internet. Prior to analysis, all samples were filtered through a 0.45 μm nylon membrane filter and then purified by Solid-Phase Extraction (SPE) with Waters OASISS® extraction cartridges. SPE cartridges were first equilibrated with water, followed by methanol. The samples were then loaded onto the cartridge and washed with 5% MeOH, followed by 100% MeOH. The MeOH eluate was retained for TLC analysis.

Four different noni fruit powder capsule products were also purchase. The producers of the capsules are located in French Polynesia (C1), Hainan, South China Sea (C2), Hawaii (C3), and Indonesia (C4). One gram of the capsule contents was extracted with 5 mL MeOH, aided by sonication for 10 min. The MeOH extracts were filtered, and the solvent removed by evaporation under vacuum at 50ºC. The extract was then redissolved in 1 mL of MeOH.

Noni leaves were collected from French Polynesia (L1), Tonga (L2), Panama (L3), and Saipan, Northern Mariana Islands (L4). These samples were air-dried. Two grams of each sample were extracted with 100 mL of ethanol (EtOH) in a sonicator for 30 min. The extractants were filtered and the solvent removed by evaporation in a rotary evaporator under vacuum at 45ºC. The EtOH extracts were redissolved with 5 mL of MeOH. Voucher specimens of all samples are deposited in our laboratory.

Analysis of the deacetylasperulosidic acid content of the samples was performed by high performance liquid chromatography (HPLC), according to a previously reported method (Deng, 2010). Extracted solid samples and juices were dissolved in solvents, mixed thoroughly and then filtered through a 0.2 μm PTFE filter for HPLC analysis. Deacetylasperulosidic acid (DAA) was accurately weighed and then dissolved in an appropriate volume of MeOH to produce corresponding standard solutions ranging from 0.00174 to 1.74 mg/mL. Calibration curves of the standards were plotted after linear regression of the peak areas versus concentrations. Chromatographic separation was performed on a Waters 2690 separations module coupled with 996 PDA detectors, equipped with a C18 column. Elution was accomplished with two mobile phases, MeCN, and 0.1% formic acid in $H_2O$ (v/v), with a flow rate of 0.8 mL/min. A linear gradient of 100% aqueous formic acid (0.1%) for 0-5 min, followed by 70% aqueous formic acid and 30% MeCN for 40 min, was used. The PDA detector was monitored in the range of 210-400 nm. The injection volume was 10 μL for each of the sample solutions. The column temperature was maintained at 25ºC.

Analysis of scopoletin and rutin content of the samples was also performed by HPLC, according to a previously reported method (Deng *et al*., 2010a). Chemical standards of scopoletin and rutin 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 were prepared by diluting the stock solutions with MeOH at different concentrations. All stock and working solutions were maintained at 0ºC in a refrigerator. Samples were extracted and dissolved in MeOH. Chromatographic separation was performed on a Waters 2690 separations module coupled with 996 a photodiode array (PDA) detector, and equipped with a C18 column. The mobile phase system was 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 quantified at 365 nm.

Table 1 summarizes the mobile phases and visualization methods used in the identification of each chemical marker. Sample solutions (5 μL each) were spotted onto silica gel 60 F254 TLC plates (Merck, Darmstadt, Germany), and developed with the three mobile phases. Visualization of the compounds was performed with either UV lamps or coloring spray reagent.

Table 1: TLC method parameters for chemical markers of noni fruit and leaves

| Chemical marker | Mobile phase | Visualization method | Spot characteristic |
|---|---|---|---|
| Deacetylasperulosidic acid | dichloromethane: methanol: water (13: 6: 1, v:v:v) | spray reagent: 2% anisaldehyde, 10% sulfuric acid in EtOH | blue colored spot |
| Scopoletin | dichloromethane: methanol (19: 1, v:v) | UV lamp, 365 nm | fluorescent light blue spot |
| Rutin | ethylacetate: formic acid: water (7: 1.5: 1.7, v:v:v) | UV lamp, 254 nm | dark spot |

This study was conducted during 2010 at the laboratories of Tahitian Noni International, American Fork, Utah, and U.S.A.

## RESULTS AND DISCUSSION

Results of the HPLC analyses indicate a wide range in the concentrations of marker compounds among the various sources of noni fruit and leaves, and resulting commercial products. The analyses confirmed the presence of deacetylasperulosidic acid in all samples, as well as scopoletin in all noni fruit based samples. Rutin was detected in all leaf samples. The DAA content of the MeOH extract of raw noni fruit samples was 13.8-42.9 mg/g, while scopoletin was present in the range of 0.7-6.9 mg/g. Noni fruit powder capsules contained 7.2-11.8 mg DAA/g and 0.1-0.4 mg scopoletin/g. DAA and scopoletin occurred in commercial noni juice products at 0.2-1.7 mg/mL and 3.7-21.2 µg/mL, respectively. Dried noni leaf samples contained DAA and rutin in the range of 0.9-3.1 mg/g and 0.4-3.6 mg/g, respectively.

In the scopoletin analyses, fluorescent light blue spots were visible to the unaided eye on the developed TLC plates, when viewed under long wave UV light, 365 nm. The scopoletin standard produced the most intensely fluorescent light blue spot. Every noni fruit, juice, and capsule also produced the same spot with a retention factor (Rf) of 0.5, but with differing intensities (Fig. 1 and 2). Scopoletin was also detected by this TLC method at the lowest concentration in any sample, which was 3.7 µg/mL found in sample J3 (Fig. 1). At this low concentration, a faintly fluorescent light blue spot was still visible.

Rutin was visible on the developed TLC plate under short wave UV light, 254 nm, with an Rf of 0.5. At this wavelength, the silica gel will fluoresce green, whereas the rutin absorbs the light and appears as a dark spot. Under the conditions of this analysis, rutin was visible in all noni leaf samples (Fig. 3). The lowest concentration visualized in these analyses, as confirmed by the HPLC analyses, was 0.4 mg/g.

Upon heating, visualization of the DAA standard was accomplished with the 2% anisaldehyde spray reagent. A blue color developed, according to results with similar iridoid glycosides (Harborne, 1998). Blue color development, upon treatment with the anisaldehyde reagent and heating, also occurred with all samples, regardless of origin or form, with an Rf of 0.3

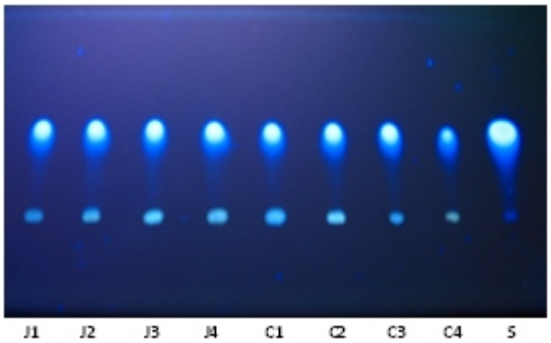


Fig. 1: Scopoletin analysis of commercial noni juice and noni capsules by TLC. C1-C4: commercial noni capsules from different manufacturers; J1-4: commercial noni fruit juices from different manufacturers. S: scopoletin

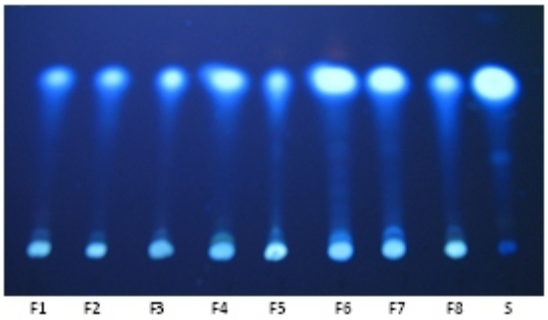


Fig. 2: Scopoletin analysis of noni fruit by TLC. F1-F8: noni fruit samples from different regions. S: scopoletin

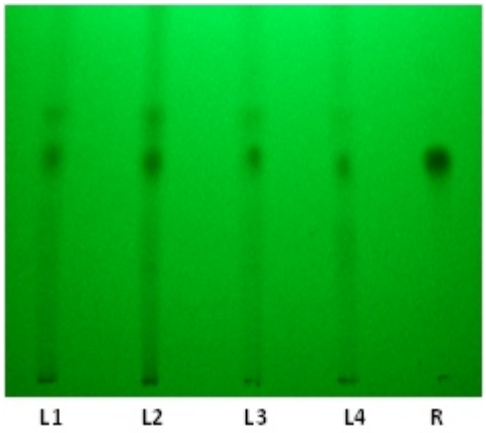


Fig. 3: Rutin analysis of noni leaf by TLC. L1-L4: noni leaf samples from different regions. R: rutin

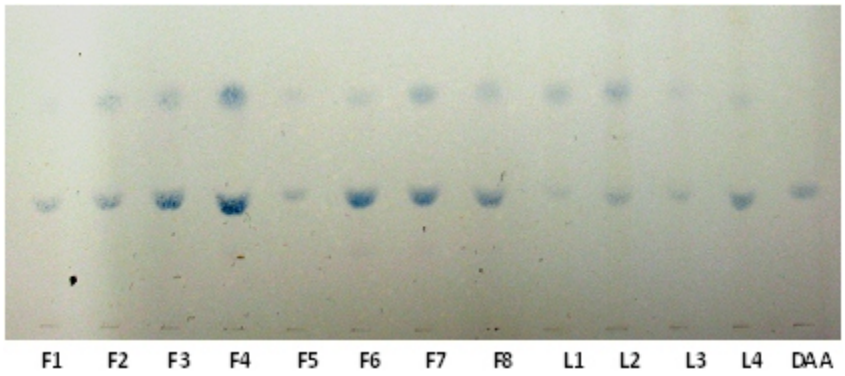


Fig. 4: Deacetylasperulosidic acid analysis of noni fruit and leaves by TLC. F1-F8: noni fruit samples from different regions. L1-L4: noni leaf samples from different regions. DAA: deacetylasperulosidic acid

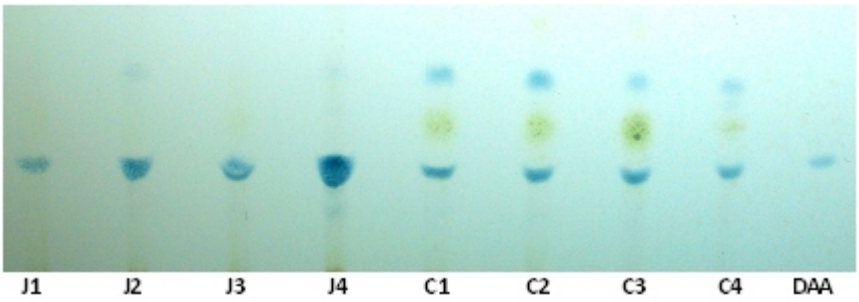


Fig. 5: Deacetylasperulosidic acid analysis of commercial noni juice and noni capsules by TLC. C1-C4: commercial noni capsules from different manufacturers; J1-4: commercial noni fruit juices from different manufacturers. DAA: deacetylasperulosidic acid

(Fig. 4 and 5). The commercial noni juice samples represent the lowest concentration ranges for DAA. Blue color development was distinctive also at the lowest concentration, 0.2 mg/mL.

Two compounds in noni fruit and two in noni leaves are useful chemical markers for identification purposes. For noni fruit, scopoletin and deacetylasperuloside were chosen as markers. Scopoletin has been found in authentic noni fruit samples from all tropical regions around the globe and has been identified in many commercial noni juice products, as well (Deng *et al*., 2010a). Iridoid glycosides are chemotaxonomic markers for plants in the Rubiaceae family (Inouye, 1988). Deacetylasperulosidic acid has been found to be the major iridoid glycoside, as well as the major phytochemical constituent, of noni fruit (Deng, 2010; Potterat *et al*., 2007; Kamiya *et al*., 2005). Deacetylasperulosidic acid is also a useful chemical marker for noni leaves (Sang *et al*., 2001). Rutin has been isolated and identified as a major flavonoid in the leaves (Deng *et al*., 2007), and, therefore, rutin was chosen as the second chemical marker for noni leaf products.

All TLC analysis results were confirmed by the result of the HPLC analyses. The sensitivity of the scopoletin TLC method is apparently very good, since as little as 3.7 µg/mL produced a fluorescent blue spot that was discernable to the unaided eye. Rutin and DAA content ranges were much higher than scopoletin, but still demonstrated good sensitivities at 0.4 mg/g and 0.2 mg/mL, respectively. Quantities lower than these are possible in some commercial products where noni ingredients are blended with others. However, detection in these products can be enhanced by concentration of the extracts produced during sample preparation.

The utility of the TLC methods for the identification of noni fruit and leaf ingredients, as well as noni based commercial products, has been demonstrated. These methods do not require expensive instrumentation or specialized laboratories. Therefore, the methods are readily transferable to analysts working under a variety of circumstances. The marker compounds utilized in these methods are characteristic of noni fruit and leaves from all regions where this plant is cultivated. As DAA is the major phytochemical in noni fruit, it is an especially useful marker for identity testing of the growing number of noni juice products being sold world-wide.

## CONCLUSION

The two iridoids, deacetylasperulosidic acid and asperulosidic acid, have been identified in noni fruit and leaf samples from different geographical regions. As such,

these two chemicals serve as useful identity markers for authentic noni fruit and leaf products. HPLC analysis confirms that the thin layer chromatography method described herein is a simple, accurate, and economic tool for performing identity testing of noni fruit and leaf products. This method can also be readily transferred to unspecialized laboratories in developing nations.

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