Int. J. Electrochem. Sci., 13 **(2018)** 8983 – 8994, doi: 10.20964/2018.09.390

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Short Communication

Assessment of Noni (*Morinda citrifolia* L.*)* Product Authenticity by Solid State Voltammetry

Douglas Vieira Thomaz[1], *Renê Oliveira Couto*[2], *Aline de Oliveira Roberth*[1], *Leandra Almeida Ribeiro Oliveira*[1], *Karla Carneiro de Siqueira Leite*[1], *Maria Teresa de Freitas Bara*[1], *Paulo César Ghedini*[3], *Marize Campos Valadares Bozinis*[1], *German Sans Lobón*[1], *Eric de Souza Gil*[1], *Fabio Bahls Machado*[1,*]

[1] Faculdade de Farmácia, Universidade Federal de Goiás, Goiânia - GO, 74605-170, Brazil.
[2] Universidade Federal de São João del-Rei, Curso de Farmácia, Campus Centro-Oeste / Dona Lindu (CCO), 35501-296, Divinópolis - MG, Brazil.
[3] Instituto de Ciências Biológicas, Universidade Federal de Goiás, 74001-970 Goiânia – GO, Brazil.
[*]E-mail: famafarm@yahoo.com.br

Received: 5 April 2018 / *Accepted:* 15 May 2018 / *Published:* 5 August 2018

Noni (*Morinda citrifolia* L.) is a native fruit from Southeast Asia and Australia, widespread in tropical countries. Diverse pharmacological activities have been attributed to its raw material and products, which contain appreciable phenolic content. This paper reports the use of electrochemical methods to evaluate Noni products authenticity. Therefore, standard pharmacognostic and chromatographic techniques (TLC-UV and HPLC-PDA) were employed in order to compare the results of electroanalytical assays. An authenticity pattern for the studied samples was achieved. We also have demonstrated that the specificity and selectivity of a novel solid state differential pulse voltammetry method was comparable to both TLC-UV and HPLC-PDA results for main chemical markers detection i.e., scopoletin and rutin. Henceforth, our electroanalytical method can be a useful strategy for a quick and cheap authenticity assessment of Noni foodstuff.

Keywords: chromatography, electrochemistry, electrodes, herbal medicine, *Morinda citrifolia*.

1. INTRODUCTION

The discovery of the pharmacological properties of Noni (*Morinda citrifolia* L.) fruits at the end of the last century turned this vegetal variety in a trendy functional food. Industrial use of Noni rose due to its highly nutrient rich constitution, as well as vast antioxidant potential hence polyphenolic abundance. These attributes imbue Noni as a therapeutically relevant vegetal species, whose applications range from anti-inflammatory to anticancer [1-2].

Although Noni does indeed possess a plethora of uses in medicine, its regulatory status is still controversial around the world. Since handcraft medicinal products are widespread in popular markets, there is a clear lack of control regarding production and commerce of Noni herbal drugs, moreover their authenticity and quality control are still an issue [2-3].

In order to ensure suitable identification of vegetal samples such as those of Noni herbal medicines, authenticity assays are performed mostly by highly sensible chromatographic techniques such as High Performance Liquid Chromatography (HPLC) [4]. These techniques are however time and reagent consuming, demanding moreover high technical level and budget [5].

Due to such drawbacks, phytochemical and Thin Layer Chromatographic (TLC) methods emerged as potential alternatives for Noni products assessment [6]. Nevertheless, these methods still employ toxic solvents and reagents, as well as being somewhat inefficient towards authenticity determination [7-8].

Natural products are generally rich in structurally complex polyphenolic compounds, which possess innate electroactivity. Understanding the redox behavior of such chemicals aids the development of simpler and promptly executable electroanalytical methods for authenticity assessment.

Amongst these methods, voltammetry is a useful and low cost tool for routine quality control assessment, and mid-assay speed can be further enhanced by solid state analysis. Solid state voltammetrical assessment is based in the confection of a sample-containing electrode, which allows quick and reproducible execution, without the solvent expenditure of liquid state assessment[9].

Due to the importance of analyzing Noni-based products employed as food and in folk medicine, the aim of this work was to employ solid state voltammetry as a tool to assess their authenticity. Henceforth, standard pharmacognostic and chromatographic (TLC-UV and HPLC-PDA) methods were used to compare results.

2. EXPERIMENTAL

2.1. Samples and Reagents

The following samples were purchased in the Central Market of Goiânia-GO, Brazil: Ten samples of fresh Noni fruits (FF), two samples of dried fruit (DF), one liquid extract (LE) and three different samples of commercially available Noni capsules (C1, C2 and C3) formulated with crude Noni dried extract.

Dried extract powder (DE) of pharmaceutical grade was purchased from Shaanxi Jihae Phytochem, China. Considering that Noni is also marketed in handmade formulations, a sample herein called liquid extract “Auriana” (LA) was obtained from a local farmer.

LA sample was prepared from Noni fruits previously cleaned and decontaminated followed by compression in sterilized jars. The jars were kept to environmental temperature (± 25°C) and the resulting liquid was then removed as final product.

The Chemical Reference Substance (CRS) of scopoletin and rutin were purchased from Extrasynthese® (Z.I Lyon Nord, France) and Sigma-Aldrich® Co. (St. Louis, Missouri, USA), respectively. Ultrapure water was obtained from Milli-Q® system (Millipore, São Paulo, SP, Brazil) and used to prepare the solutions. All the other solvents and reagents used were of analytical grade.

2.2. Extract preparation and Botanical Analysis

A Radwag® XA110 (Poland) analytical balance was used to measure 5g of each dried sample and 10g of fresh fruits. The extraction of major compounds was carried out in an ultrasonic bath for 15 minutes, Ultra Cleaner® USC-4800 (Unique, Barueri, SP, Brazil). All extractions were performed with 10 mL analytic grade ethanol under sonication for 15 min. The extracts were thereafter dried and stored at 4^{o}C. A macroscopic analysis was performed in order to assess the morphological features of fresh and dried Noni fruit samples (FF and DF) [10-11].

2.3. Pharmacognostic Identification

Pharmacognostic identification of coumarins, flavonoids, anthraquinones and alkaloids were carried out according to official compendia [12].

2.4. Choromatographic Analysis

2.4.1. TLC-UV

TLC-UV analyses were performed in order to detect the presence of the major compounds, namely scopoletin and rutin in all samples. Liquid extracts were then filtered by using Millex® filter (Millipore, São Paulo, SP, Brazil). The liquid samples were analyzed without previous dilution. All samples were kept in Eppendorf® vials till further analysis.

Scopoletin and rutin standard solutions were prepared in order to reach 0.2 mg/mL and 1 mg/mL final concentration. Silica gel F254 plates from Silicycle® (Ville de Québec, QC, Canada), 10 x 20 cm, were used for chromatographic analysis. Herein, 5 µL of each sample was applied using a micropipette Tedia PET (Tedia do Brasil, Rio de Janeiro, RJ, Brazil).

Chromatographic separation was performed on mobile phase comprising pure dichloromethane for scopletin and ethyl acetate:formic acid:water mixture (v/v/v, 7:1.5:1.7) for rutin and other flavonoids. The development of scopoletin assay was achieved in UV light at 365 nm [6]. Flavonoids were revealed by spraying NP-PEG reagent and then in UV light at 365 nm, whereas rutin was selectively identified when a yellow spot was seen [13].

2.4.2. HPLC-PDA

The HPLC-PDA assays were performed in a Waters® (Barueri, SP, Brazil) High-Performance Liquid Chromatograph equipped with quaternary pump, separation module e2695, and Photodiod

Array® (PDA 2998) detector coupled to the software Empower® for data acquisition. The preparation of samples was achieved by mixing the dried extracts in methanol (1 g/mL), following by 20 minutes of sonication and filtering through Millex® filter. The mobile phase was composed of a mixture of acetonitrile: methanol: ultrapure water acidified with acetic acid 2% (10:17:73, v/v/v) [4]. The injection volume was 10 µL. The chromatographic separation was carried out in an isocratic mode with a flow-rate of 1.0 mL/min into a reverse-phase column, Zorbax XDB® C-18 (25 cm x 4,6 mm, 5 µm), purchased from Agilent (Santa Clara, CA, USA), placed into a thermostat controlled oven compartment with a temperature of 30°C. The elution time was fixed at 20 min and detection was at 350 nm.

2.5. Standards

The standards solutions of scopoletin and rutin were prepared to reach the final concentration of 64 and 200 µg/mL, respectively. The quantification of scopoletin and rutin was performed by undertaking the area of their corresponding peaks.

2.6. Electroanalytical method

Noni samples exhibit strong adsorptive behavior (data not shown). Therefore, the use of glassy carbon electrode should be avoided, whereas carbon paste is the best alternative. Furthermore, in order to get higher faradaic current signals, differential pulse voltammetry was performed in solid state. Thus, carbon paste electrodes were modified with sample extracts in the following way: 250 µL of each extract were mixed to 70 mg of graphite powder Merck® and left to dry at room temperature for one day. Thereafter, 20 mg of Nujol® (mineral oil) was added and mixed for 5 minutes in order to obtain homogeneous paste. The employed reference and counter electrodes were respectively $Ag/AgCl/KCl_{sat}$ and platinum wire, whereas the cell contained 10 mL capacity.

2.6.1. Differential Pulse Voltammetry (DPV)

DPV was performed in Autolab® potentiostat, type III (Netherlands), coupled to GPES 4.9® software. Voltammetric assays were performed in 0.1 M phosphate buffer pH 7.0. In order to condition the paste, a conditioning time of 15 minutes was used before each run. Scan range from -0.25 to 1.25 V, scan rate of 10 mV s^{-1}, pulse width of 0.5 s and pulse amplitude of 50 mV. All experiments were performed in room temperature, and the voltammograms were treated in Origin® 8 software.

3. RESULTS AND DISCUSSION

3.1. Botanical Analysis

The weight of the ten sampled Noni fruits ranged from 88 up to 180 g. The average weight was of 167.4 g, the average length was of 10.8 cm and the average width was of 5.9 cm. According to

literature, Noni fruit has an oval shape, which is also characterized by a lumpy surface covered with polygonal sections comprising polygonal shapes of 8 to 16 cm in diameter. The mesocarp inward possesses a juicy and soft texture that is odorless when green, but becomes strongly foul as it matures. Their size and weight generally range from 50 to 300 g, whereas the color ranges from green to white green [10-11].

3.2. Pharmacognostic Identification

The search for anthraquinones and alkaloids presented negative results for all samples. On the other hand, the coumarins, presented positive results for FF, DF, LA and also for all capsules, C1, C2 and C3. Furthermore, DE and LE were negative to coumarins assay.

In the performed experiments, the presence of flavonoids was confirmed by oxalo-boric acid, concentrated sulfuric acid, alkali hydroxides, aluminum chloride and ferric chloride reactions, and was negative for all samples by Shinoda reaction.

3.2.1 TLC-UV

A summary of the results obtained in the TLC-UV analysis of all sample is presented in Table 1, which displays the values of retention factors (Rf) for both scopoletin and rutin.

Table 1. Summary of TLC-UV analysis for several samples of Noni foodstuff

Samples	Rf scopoletin (SD)	Rf rutin (SD)
FF	0.133 (0.02)	0.51 (0.08)
DF	0.134 (0.01)	0.53 (0.08)
DE	-	-
LE	-	0.56 (0.05)*
LA	0.136 (0.01)	0.55 (0.08)*
C1	0.135 (0.02)	0.57 (0.03)
C2	0.137 (0.02)	0.54 (0.08)
C3	-	0.55 (0.04)*
scopoletin CRS	0.135 (0.02)	-
rutin CRS	-	0.56 (0.08)

*Non conclusive spot pattern as revealed by NP-PEG reagent at 365 nm; CRS, Chemical Reference Substance; Rf, retention factor; SD, Standard Deviation; FF, fresh fruit; DF, dried fruit; DE, dried extract; LE, liquid extract; LA, liquid extract “Auriana”; C1-3, capsules.

The presence of fluorescent blue spots at 365 nm and a Rf of 0.135 ± 0.02 was an indicative for scopoletin. Since this pattern was observed for FF, DF, LA, C1 and C2, it can be inferred that these sample may be really obtained from Noni fruits [12-13]. Meanwhile, the absence of fluorescent blue spots in samples DE, LE and C3 suggests non authenticity of these products (data not shown).

The presence of the flavonoid rutin was related to the presence of intense yellow spots at Rf of 0.56 ± 0.08 by spraying the NP-PEG reagent on the plate and observation under 365 nm [13], which have been observed only for sample FF, C1 and C2 (data not shown). In the case of samples LA, LE and C3, the spot patterns were somewhat different, thus assay was not conclusive for polyphenolic acids or flavonoids. Yet, the absence of any spots in DE sample suggests that the dried extract may have lost such polyphenols during their industrial production (6 months).

3.2.2. HPLC-PDA

The retention time (Rt) of the peaks related to the standards, scopoletin (S) and rutin (R), as well as their corresponding UV spectra peaks (S1, S2, S3 and R1, R2) were used for qualitative purposes. It can be observed in Figure 1 that the Rt values obtained for scopoletin and rutin, were respectively of 9.78 and 12.41 minutes. The extracted UV spectra of scopoletin, exhibited three main peaks, S1, S2 and S3, at 229.9, 296.2 and 344.0 nm respectively. For rutin, we have observed two maximum absorption peaks, R1 and R2, at 255.9 and 354.8 nm respectively (Figure 1).

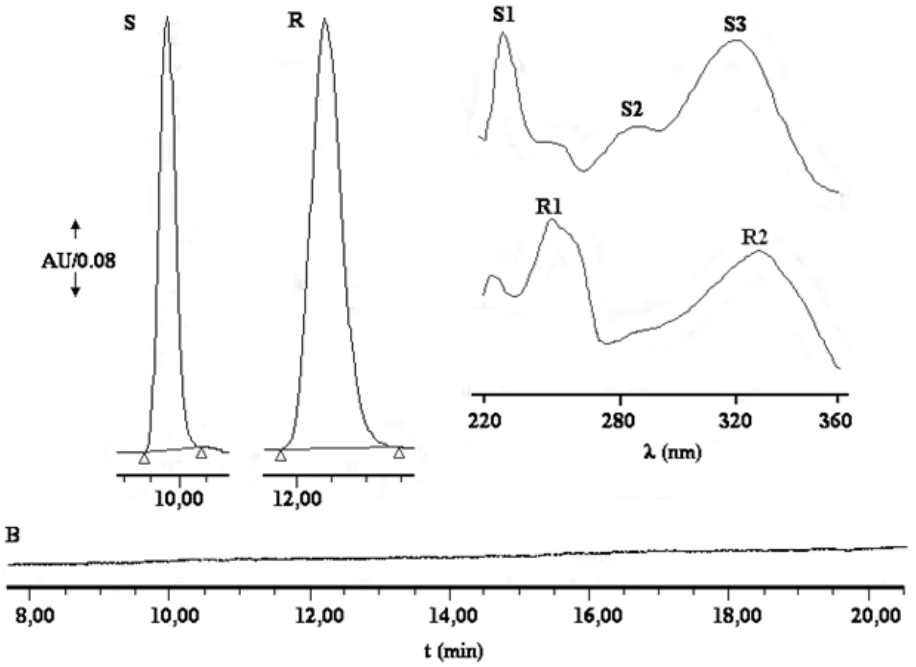

Figure 1. HPL-Chromatograms and respective UV (220 to 360 nm) spectra obtained from blank (B), scopoletin (S) and rutin (R). Experimental conditions as described in section HPLC-PDA.

These data, as well as the HPLC-PDA fingerprints of FF sample were undertaken to compare the authenticity of “Noni made” products. Figure 2 presents the chromatograms related to samples LA, DF and FF.

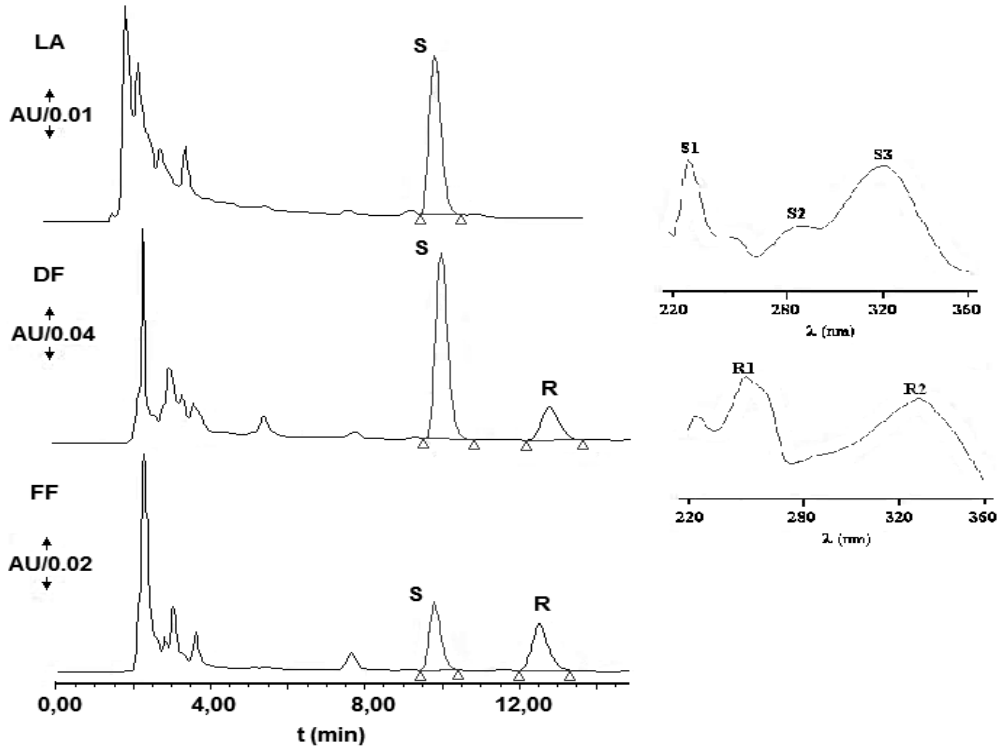

Figure 2. HPL-Chromatograms obtained for fresh fruit (FF), dried fruit (DF) and liquid extract "Auriana" (LA). Experimental conditions as described in section HPLC-PDA.

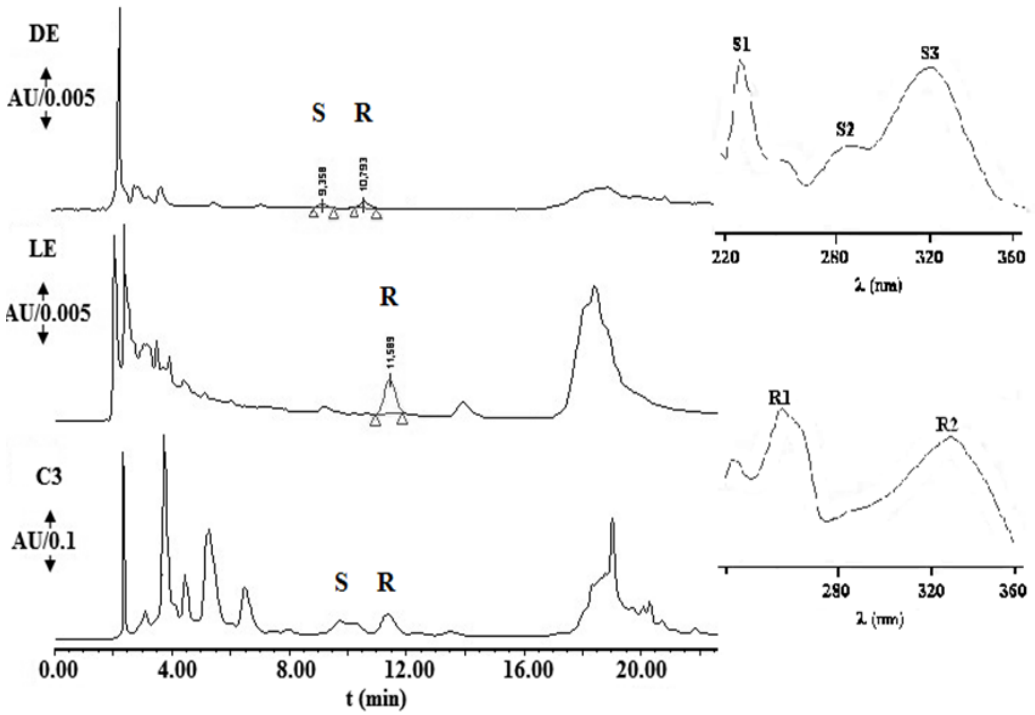

Figure 3. HPL-Chromatograms obtained for dried extract (DE), liquid extract (LE) and capsule 3 (C3). Experimental conditions as described in section HPLC-PDA.

The HPLC-PDA fingerprints of samples DF and LA were similar to those observed for FF, hence proving the authenticity of such products. Likewise, accepting the RSD of 2% [13-14], the biomarker scopoletin was found in all samples. The peak R was not observed to LA.

In turn, the finger print obtained for samples C1 and C2 (not shown), was very close to the one obtained for DF (Figure 2), hence suggesting that these solid dosage forms might be prepared with dried Noni fruit.

In all these cases, UV spectra confirmed the presence of scopoletin, as well as in samples FF, DF, C1 and C2, the presence of rutin.

As can be seen in Figure 3, the samples DE, LE and C3, respecting 2% of variation range, did not show any peak at the retention time related to scopoletin and rutin.

Although the sample DE presented small peaks at Rt of 9.358 and 10.793 min, the undertaken UV spectra (not shown) were also quite distinct of those observed for the reference standards, scopoletin and rutin (Figure 4).

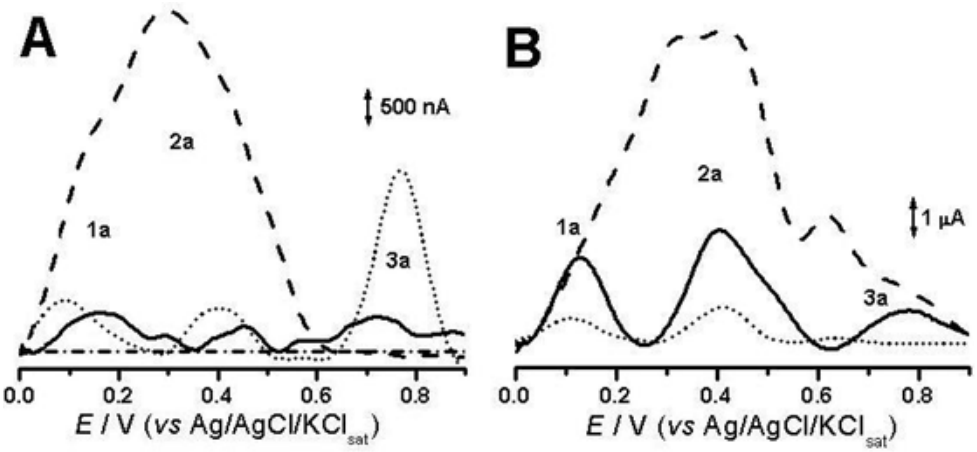

Figure 4. DP voltammograms obtained for carbon paste electrodes in the solid state analyses of dried extract (DE - - -); liquid extract (LE -·-); liquid extract "Auriana" (LA ····); fresh fruit (FF —) **(A)** and capsule 1 (C1 —); capsule 2 (C2 ····) and capsule 3 (C3 - - -) **(B)**. Experimental conditions as described in section Electroanalytical method.

Besides providing qualitative information, the performed HPLC-PDA analyses allowed the quantification of scopoletin and rutin in the studied samples (Table 2).

Table 2. Content of scopoletin and rutin calculated from HPLC-PDA peak analysis of several Noni samples

Samples	scopoletin (%)	rutin (%)
FF	8.37×10^{-4}	2.84×10^{-3}
DF	4.96×10^{-3}	4.40×10^{-3}
LA	9.0×10^{-1}	-
C1	2.02×10^{-3}	4.26×10^{-3}
C2	2.02×10^{-3}	2.73×10^{-3}

FF, fresh fruit; DF, dried fruit; DE, dried extract; LA, liquid extract "Auriana"; C1 and C2, capsules.

As expected, the contents of these chemical markers were greater in DF rather FF. Moreover, except to LA, it was observed greater contents of rutin as compared to scopoletin. On the other hand, the scopoletin content determined to LA was greater than all other samples. For both C1 and C2 samples, the amounts of scopoletin and rutin were quite small compared to DF.

3.3 Electroanalysis

Figure 4 shows the voltammograms obtained for raw materials and formulations studied at solid state voltammetry.

The authentic sample, FF (—) presents three well defined anodic peaks, 1a, 2a e 3a, at 0.17; 0.46 and 0.72 V, respectively (Fig. 4A). Thus, this profile can be used as authenticity pattern for identification purposes in quality control of Noni foodstuff. Identical profile was observed for carbon paste prepared with the dried fruit extract, DF (data not shown). LA carbon paste also presented similar profile, exhibiting three peaks, 1a, 2a e 3a, at 0.10; 0,40 e 0,77 V. Furthermore, peak amplitudes were consistent with sample phytocompound concentrations.

In turn, the comparison of DP voltammetric profiles for DE and DF (akin to FF, Figure 4A), and those obtained for C1, C2 and C3 (Figure 4B), enable the inference of the raw material employed to produce each capsule.

Complementarily, TLC spots observed in scopoletin assay were removed by scratching chromatographic paper surface and used to make modified carbon paste electrodes. The DPV profiles are presented in Figure 5.

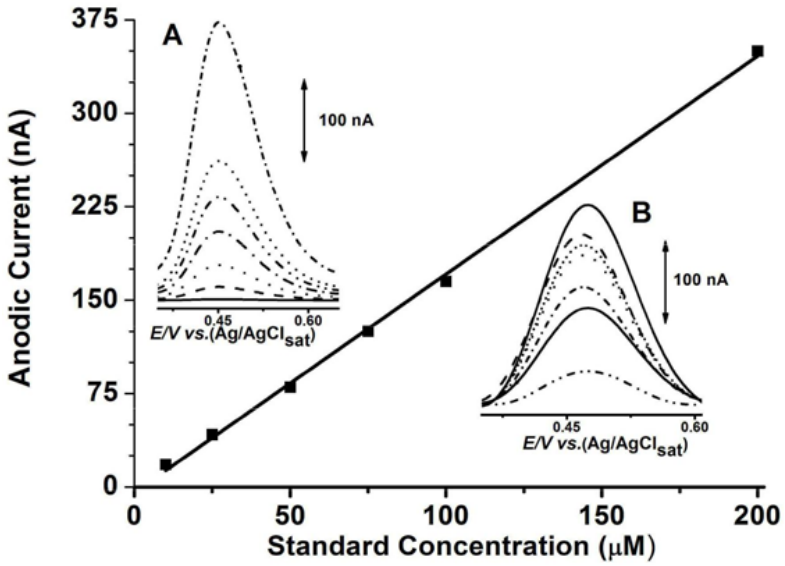

Figure 5. (**A**) DP Voltammograms shows de anodic peak of different concentrations of scopoletin standard solution obtained in 0.1 M phosphate buffer, pH 7.0 for carbon paste electrodes, which is related to calibration curve and (**B**) DP Voltammograms obtained in 0.1M phosphate buffer, pH 7.0 for carbon paste modified with scraped spots from thin-layer scopoletin plate of fresh fruit (FF ··–), dried fruit (DF ····), liquid extract "Auriana" (LA ––), capsule 1 (C1 · ·) and capsule 2 (C2 –·–), as well as the used standard (—). Pulse amplitude 50 mV, scan rate 10 $mV.s^{-1}$.

The voltammetric profile for scopoletin standard spot, showed one well defined peak, at E_{pa} = 0.49 V. Taking into account the Faraday Law and current heights observed for the positive samples, it is possible to assert the following order of concentration: LA > DF > C1 > C2 > FF, thus being in agreement with HPLC-PDA assay.

The morphological characterization and pharmacognostic screening allied to chromatographic assessment proved to be useful in the accurate identification of herbal products e.g., *Passiflora species*, which sheds light in combining different assessment tools to provide authenticity standard concerning natural products analysis[15-16].

Owing to the distinguishable features of Noni fruits, their macroscopic identification was achieved. Thus, authenticity of all Noni samples has been proven by the morphological analysis, allowing them to be used as reference for further studies towards commercial products supposedly prepared with such fruits.

The negative results for anthraquinones in the pharmacognostic identification were consistent with the fact that such phytochemicals are mostly abundant in the roots [17]. When these compounds are present in fruits, the expected concentration is very low [17-18]. On the other hand, coumarins have been found in the majority of the samples, and are considered ubiquitous in Noni fruits [19, 6].

Scopoletin is often used as biomarker to prove Noni fruit authenticity [20-21]. Moreover, Noni major compound, rutin, which is ubiquitous in plant kingdom, was also identified as non-negative parameter of authenticity [6].

HPLC-PDA analysis of all samples was also performed in order to testify the TLC assumptions about the authenticity of Noni made products. It is possible to assert that the Relative Standard Deviation (RSD) for all Rf (Table 1) was around 15%, which is acceptable for such method [22].

The absence of peak R, in LA, may be associated to the degradation of rutin during its long processing time. In fact, owing to the great electron donor character, flavonoids and other polyphenols, are susceptible to many extrinsic degradation factors, including temperature, light, oxidation and hydrolysis [23].

Nonetheless, chromatographic profiles observed by TLC-UV analysis have been confirmed by HPLC-PDA data.

The trend observed for DF, C1 and C2 samples concerning the content of scopoletin and rutin reinforce our hypothesis regarding the loss of these phenolic compounds during manufacturing steps e.g., drying and storage. These results also suggest the benefits of consuming ready prepared Noni foodstuff.

Assuming that scopoletin and rutin, as well as other natural phenols present innate electroactivity, voltammetry emerges as an affordable tool to be applied in the quality control of Noni and other phytopharmaceuticals.

The findings for solid state DPV were consistent with the results obtained in TLC-UV and HPLC-PDA assays. Such results ratify both the selectivity and specificity of our novel electroanalytical identification method, and reinforce its suitability along qualitative quality control of Noni products.

4. CONCLUSION

The use of different pharmacognostic and analytical techniques led to complementary results. Solid state voltammetric assessment may be an efficient strategy to determinate the authenticity of Noni products as well as scopoletin detection, hence results present undeniable similarity to those of chromatographic assays. In further investigations the experimental conditions of this innovative method will to be optimized in order to step forward into validation.

ACKNOWLEDGEMENTS
The authors gratefully acknowledge financial support from Brazilian National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Level Personnel (CAPES).

References

1. A. R. Dixon, H. McMillen, N. L. Etkin, *Economic Bot*., 53 (1999) 51.
2. R. A. Assi, Y. Darwis, I. M. Abdulbaqi, A. A. Khan, L. Vuanghao, M. H. Laghari, *Arabian J. Chem.* 10 (2017) 691.
3. Brasil, *Informe Técnico*, 25 (2007) 1.
4. S. Deng, B. J. West, C. J. Jensen, S. Basar, J. Westendorf, *Food Chem.* 116 (2009) 505.
5. E. S. Gil, Controle Físico-Químico de Qualidade de Medicamentos, *Pharmabooks*, (2011) São Paulo, Brasil.
6. B. J. West, S. Deng, *Food Sci. Technol*. 2 (2010) 298.
7. N. S. Reis, S. H. P. Serrano, R. Meneghatti, E. S. Gil, Lat. *Am. J. Pharm*. 28 (2009) 949.
8. D. Gonçalves, R. O. Couto, E. C. Conceição, N. S. Reis, E. S. Gil, *Quím. Nova*, 34 (2011) 330.
9. J. Dobes, O. Zitka, J. Sochor, B. Ruttkay-Nedecky, P. Babula, M. Beklova, J. Kynicky, J. Hubalek, B. Klejdus, R. Kizek, *Int. J. Electrochem. Sci.,* 8 (2013) 4520.
10. J. F. Morton, *Econ. Bot.,* 46 (1992) 241.
11. W. McClatchey, *Integr. Cancer*. 1 (2002) 110.
12. A. F. Costa, Farmacognosia, *Fundação Calouste Gulbenkian* (2000) Lisboa, Portugal.
13. H. Wagner and S. Bladt, Plant drug analysis: a thin layer chromatography atlas, Springer (2009) Germany.
14. Brasil, Farmacopeia Brasileira, *Agência Nacional de Vigilância Sanitária*, (2010) Brasília, Brasil.
15. L. Wosch, D. C. Imig, A. C. Cervi, B. B. Moura, J. M. Budel, C. A. M. Santos, *Rev. Bras. Farmacogn.,* 25 (2015) 328.
16. L. Wosch, K. C. Santos, D. C. Imig, C. A. M. Santos, *Rev. Bras. Farmacogn.,* 27 (2017) 40.
17. T. Anekpankul, M. Gotob, M. Sasakib, P. Pavasanta, A. Shotipruka, *Sep. Purif. Technol.,* 55 (2007) 343.
18. L. V. Lishuang, H. Chen, C. T. Ho, S. Sang, *Fitoterapia*, 82 (2011) 704.
19. O. Potterat, R. V. Felten, P. W. Dalsgaard, M. Hamburger, *J. Agric. Food Chem.,* 55 (2007) 7489.
20. Y. Ikeda, M. Wada, T. Nishigki, K. Nakamoto, *Food Chem.* 113 (2009) 1169.
21. S. Mahattanadul, W. Ridtitid, S. Nima, N. Phdoongsombut, P. Ratanasuwon, S. Kasiwong, *J. Ethnopharmacol.,* 134 (2011) 243.
22. S. R. Valentini, W. A. Sommer, G. Matioli, *Acta Sci. Health Sci.,* 26 (2004) 357.

23. M. Friedman, H. S. Jürgens, *J. Agric. Food Chem.*, 48 (2000) 2101.