Published in final edited form as:
J Ethnopharmacol. 2010 April 21; 128(3): 662–671. doi:10.1016/j.jep.2010.02.013.

Composition, Standardization and Chemical Profiling of *Banisteriopsis caapi*, a Plant for the Treatment of Neurodegenerative Disorders Relevant to Parkinson's Disease†

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Abstract

Ethnopharmacological relevance—*Banisteriopsis caapi*, a woody vine from the Amazonian basin, is popularly known as an ingredient of a sacred drink ayahuasca, widely used throughout the Amazon as a medicinal tea for healing and spiritual exploration. The usefulness of *B. caapi* has been established for alleviating symptoms of neurological disorders including Parkinson's disease.

Aim of the study—Primary objective of this study was to develop the process for preparing standardized extracts of *B. caapi* to achieve high potency for inhibition of human monoamine oxidases (MAO) and antioxidant properties. The aqueous extracts prepared from different parts of the plant collected from different geographical locations and seasons were analyzed by HPLC for principal bioactive markers. The extracts were simultaneously tested *in vitro* for inhibition of human MAOs and antioxidant activity for analysis of correlation between phytochemical composition of the extracts and bioactivities.

Materials and methods—Reversed-phase HPLC with photodiode array detection was employed to profile the alkaloidal and non-alkaloidal components of the aqueous extract of *B. caapi*. The *B. caapi* extracts and standardized compositions were tested *in vitro* for inhibition of recombinant preparations of human MAO-A and MAO-B. *In vitro* cell-based assays were employed for evaluation of antioxidant property and mammalian cell cytotoxicity of these preparations.

Results—Among the different aerial parts, leaves, stems/large branches and stem bark of *B. caapi*, HPLC analysis revealed that most of the dominant chemical and bioactive markers (**1**, **2**, **5**,

†Part II. For part I, see Samoylenko et al., (2010).

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7-**9**) were present in high concentrations in dried bark of large branch. A library of HPLC chromatograms has also been generated as a tool for fingerprinting and authentication of the studied *B. caapi* species. The correlation between potency of MAO inhibition and antioxidant activity with the content of the main active constituents of the aqueous *B. caapi* extracts and standardized compositions was established. Phytochemical analysis of regular/ commercial *B. caapi* dried stems, obtained from different sources, showed a similar qualitative HPLC profile, but relatively low content of dominant markers **1**, **2**, **7**, and **9**, which led to decreased MAO inhibitory and antioxidant potency compared to *B. caapi* Da Vine.

Conclusion—The ethnopharmacological use of bark of matured stem/large branch of *B. caapi* as well as whole matured stem is supported by the results obtained in this investigation. Among various constituents of *B. caapi*, harmine (**7**), harmaline (**6**) and tetrahydroharmine (**5**) are responsible for MAO-A inhibition, while two major proanthocyanidines, epicatechin (**8**) and procyanidine B2 (**9**) produce antioxidant effects. The compounds **1**-**9** can serve as reliable markers for identification and standardization of *B.caapi* aerial parts, collected in different seasons and/or from different geographical regions.

Keywords

Banisteriopsis caapi (Spruce ex Griseb.); *β*-carboline alkaloids; Proanthocyanidines; HPLC; Monoamine oxidase inhibitors; Antioxidants; Parkinson's disease

1. Introduction

Banisteriopsis (Family: Malpighiaceae) is a tropical South American genus with 92 species distributed mainly in Brazil, Bolivia, Colombia, Ecuador, and Peru (Mabberley, 1997; Schultes, 1970). *B. caapi* (Spruce ex Griseb.) Morton is an ingredient of a popular sacred and psychoactive drink ayahuasca, also known as Caapi, Pinde, Natema or Yaje. It is widely employed for prophecy, divination, and as a sacrament in the northern part of South America (Schultes and Raffauf, 1992). Traditionally, this drink is prepared by boiling the stems of *B. caapi* and adjuvant plant, either *Psychotria viridis* (chacruna) or *Diplopterys cabrerana* (oco yagé) (Schultes, 1970; Schultes and Raffauf, 1992; Schultes and Siri von Reis 1995). It should be noted that the identities of different *Banisteriopsis* species are incompletely known due to the paucity of fertile collections and lack of detailed taxonomic study. There are at least thirty different varieties of *B. caapi* that natives of Amazon have knowledge of and have different uses (Schultes and Hofmann, 1992). Earlier chemical investigation have reported the presence of *β*-carboline alkaloids harmine, harmaline and tetrahydroharmine (THH) as the principal monoamino oxidase (MAO) inhibitors, together with harmol, harmine N-oxide, harmic acid methyl ester, harmalinic acid, harmic amide, acetylnorharmine, and ketotetrahydronorharmine, from *B. caapi* (Hochstein and Paradies, 1957; Hashimoto and Kawanishi, 1975; *ibid* 1976). In addition, pyrrolidines (Kawanishi et al., 1982) shihunine and (*S*)-(+)- dihydroshihunine, and terpenoids (Aquino et al., 1991) stigmasterol, *β*-sitosterol, ursolic acid, oleanolic acid and nerolidol were also reported. The alkaloid content of *B. caapi* was determined previously by GC/MS (Rivier and Lindgren, 1972; Pires et al., 2009), LC/MS (Kawanishi et al., 1998) and HPLC (Serrano-Duenas et al., 2001), suggesting the content of harmine is highest among *β*-carbolines, followed by THH and harmaline.

Recent investigations at our laboratories on *B. caapi* Da Vine (Samoylenko et al., 2010), a cultivar propagated by cuttings (Miller, 1986) and collected from Oahu, Hawaii, have yielded two new tetrahydro-*β*-carboline-derived alkaloidal glycosides namely, banistenoside A (**1**) and banistenoside B (**2**) as major markers, a new natural THH analog tetrahydronorharmine (THNH) (**3**), and four known MAO inhibitors *β*-carbolines harmol (**4**), tetrahydroharmine (THH) (**5**), harmaline (**6**) and harmine (**7**). In addition, two potent antioxidant flavan-3-ols (−)-

epicatechin (**8**) (Sun et al., 2006) and its dimer (−)-procyanidin B2 (**9**) (Balde et al., 1991; Khan et al., 1997), were isolated for the first time from the genus *Banisteriopsis*. In this paper, we report the quantification of markers in *B. caapi* Da Vine, performed by RP-HPLC in different parts of the plant collected in different seasons and/ or different geographical regions, as well as regular/ commercial sample of *B. caapi*. Based on *β*-carboline alkaloids (**5**-**7**) and proanthocyanidines (**8** and **9**) compositions of the standardized aqueous extracts of Da Vine a few pharmaceutical compositions were prepared and evaluated for inhibition of MAO-A and antioxidant properties.

2. Materials and Methods

2.1. General experimental procedures

The HPLC system consisted of a Model 2695 Alliance Separations Module equipped with a 2996 photodiode array detector, and a computerized data station equipped with Waters Empower 2 software (Waters, Milford, MA). Separation was achieved on a Gemini C18 110Å column (Phenomenex, 150 × 4.6 mm I.D.; 5 μm particle size; Phenomenex Inc., Torrance, CA, USA) and operated at 30 °C. The column was equipped with a 2 cm LC-18 guard column (Phenomenex Inc., Torrance, CA, USA). Plant material was extracted by either Coffee Maker (Mr. Coffee®, ISX-43) or Accelerated Solvent Extractor (Dionex®, ASE-200) using H_2O as a solvent. Water extracts were freeze-dried using Freeze Dry System (Labconco®, Freezone 4.5). Samples were dried using a Savant Speed Vac Plus SC210A Concentrator. The isolated compounds were visualized by observing the TLC plates under UV light at 254 or 365 nm, followed by spraying separately with Dragendorff's and/or 1% vanillin-H_2SO_4 spray reagents.

2.2. Plant material

Fresh leaves, stems, and large branches of *B. caapi* were collected from the island of Oahu, Hawaii, in August and November 2007, and June 2008, as well as from Hilo (Big island), Hawaii, USA, in October, 2007. This plant, a cultivar known as Da Vine, is produced from cuttings of South American mother plant transplanted in Hawaii (Miller, 1986). A reference specimen was collected from Oahu, and a voucher specimen (HLA # 7835) was deposited at the Herbarium of Harold Lyon Arboretum, University of Hawaii. The collector of the voucher specimen of the plant at Lyon Arboretum was Dr. Kenneth M. Nagata (Accession # L-81.0727; collector # 2789; dated 03/13/1984). The regular *B. caapi* (mature stems) samples analyzed in this study (BC-Ex-1 – BC-Ex-4) were procured online from different commercial sources and also through NCNPR sources during 2005-2007. All samples used in this work are preserved using the standard procedures for collection, drying, grinding and packaging at the NCNPR.

2.3. Preparation of plant extracts

The detailed methods for the preparation of standardized extracts from stem and other plant parts of *B. caapi* cultivar Da Vine and regular/ commercial samples were described in our previous report (Samoylenko et al., 2010).

2.4. Compounds and reference samples

The marker compounds **1**-**9** were isolated and identified from *B. caapi* cultivar Da Vine as previously described (Samoylenko et al., 2010) (Fig. 1). The reference standards of harmol, harmine, harmaline, and (−)-epicatechin were purchased from Sigma-Aldrich (St. Louis, MO), while others were available in our laboratories.

2.5. HPLC analysis and quantification of markers

For catechin analysis, the mobile phase consisted of water (A) and acetonitrile (B), both containing 0.1% acetic acid which were applied in the following gradient elution: 92% A/ 8%

B hold for 5 min; increased to 22% B in next 35 min. The total run time was 40 min, and flow rate was 0.7 mL/min. The peaks were assigned based on their retention times and UV spectra. Detection wavelength was at 279 nm. The injection volume was 10 μL.

For alkaloid analysis, the mobile phase consisted of acetonitrile containing 0.1% acetic acid (X) and 50 mM ammonium acetate (pH = 4.2) (Y), and applied in the following gradient method: 0 – 20 min, 5% X/ 95% Y to 13% X/ 87% Y; 20–25 min, 13% X/ 87% Y to 22% X/ 78% Y; 25–30 min, 22% X/ 78% Y to 25% X/ 75% Y. Each run was followed by a 5 min wash with 100% acetonitrile and an equilibration period of 15 min. The flow rate was adjusted to 0.85 mL/min. The injection volume was 10 μL. Detection wavelengths were used at 293 nm for **1**, **2**, **3** and **5**, 320 nm for **4** and **6**, and 367 nm for **7**.

An individual stock solution of the standard compounds (**1**-**9**) was prepared at a concentration of 1.0 mg/mL in methanol or methanol/water (50:50, v/v). The calibration curve was prepared at five different concentration levels. The range of the calibration curve was 1.0–500 μg/mL for banistenoside A (**1**), banistenoside B (**2**), tetrahydronorharmine (**3**), tetrahydroharmine (**5**) and (−)-epicatechin (**8**), 5.0–500 μg/mL for (−)-procyanidine B2 (**9**), and 0.2–100 μg/mL for harmol (**4**), harmaline (**6**) and harmine (**7**), respectively, for HPLC-UV analysis.

2.6. Preparation of standardized compositions

Tetrahydroharmine (**5**) (0.3 mg), harmaline (**6**) (1.0 mg), harmine (**7**) (1.0 mg), epicatechin (**8**) (1.0 mg) and procyanidin B2 (**9**) (0.6 mg), each separately, were dissolved in appropriate amount of DMSO to make individual stock solutions with concentration 1.5 mg/mL for compounds **5** and **9**, and 1.0 mg/mL for **6**-**8**. These stock solutions were combined in appropriate calculated ratio and the total volume of each stock solution was normalized to 0.5 mL by DMSO. This gives the standardized compositions **1**-**3** with concentrations of **5**-**9** assigned in the Table 2. Calculations for composition preparation were based on simulation of natural *B. caapi* extract (5 mg) to be dissolved in 0.5 mL DMSO giving a stock solution with concentration equivalent to 10 mg/mL. *B. caapi* extract DMSO solutions (10 mg/mL, 0.5 mL) were used as the references. All prepared DMSO solutions were used as stocks for further MAO-A and antioxidant assays carried out in duplicates.

2.7. Inhibition kinetics assay using recombinant human MAO-A and MAO-B

Recombinant human monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B) were obtained from BD Biosciences (Bedford, MA), and the assay procedures used as described previously (Samoylenko et al., 2010; Parikh et al., 2002). The IC_{50} values for extracts, pure compounds and reference standards were determined at five concentrations of 1, 0.1, 0.01, 0.001 and 0.0001 μg/mL for MAO-A, whereas for MAO-B, percent inhibitions of all crude extracts were evaluated at three concentrations, 100, 10 and 1 μg/mL. All experiments were carried out in duplicate.

2.8. Cell Based Assay for Antioxidant Activity

The effect of samples on the generation of intracellular reactive oxygen species (ROS) in myelomonocytic HL-60 cells was determined by the DCFH-DA (2′,7′-dichlorofluorescin diacetate) method (i.e., six concentrations of 62.50, 31.25, 15.63, 7.82, 3.91 and 1.95 μg/mL were used), as described previously (Samoylenko et al., 2010; Rosenkranz et al., 1992; Scudiero et al., 1988).

3. Results and Discussion

3.1. HPLC Analyses and Quantification

The qualitative and quantitative analyses of isolated markers **1**-**9** were carried out on a reversed phase C-18 column by HPLC according to published methods for *β*-carboline alkaloids (Abourashed et al., 2003) (using harmine, harmaline, harmol and harmane as standards) and green tea catechins (Khokhar et al., 1997) (using catechin and epicatechin as standards). These markers (**1**-**9**) were consistently assigned and quantified in fresh and dried samples of young and mature stem, bark, debarked stem, large branch and leaves (Table 1). *B. caapi* aqueous extracts demonstrated 10 dominating signals in the HPLC chromatograms for alkaloids, while 2 major peaks were identified in the chromatogram of catechins analysis. For the alkaloid analysis, compounds **1, 2, 4** and **5** (banistenoside A, banistenoside B, THNH, THH) were detectable at 293 nm, whereas a wavelength of 320 nm was more suitable for sensitive monitoring of **3** and **7** (harmol and harmine), and 367 nm for **6** (harmaline). Peaks 1, 2 and 4 (R*t* 17.74, 18.91, 22.66 min) were isolated and identified for markers **1**, **2** and **4**, respectively, together with known biomarkers **3** and **5**-**7** as peaks 3, 5, 6 and 7 (R*t* 21.21, 24.88, 28.38, 29.08 min) in the HPLC (Figure 2). Three additional peaks A, B and C at R*t* 16.92, 20.84 and 19.50 were also detected and identified (not isolated) as THH analogs, according to UV spectra (Fig. 4). In the analysis of catechins, epicatechin (**8**) and procyanidin B2 (**9**), with R*t* 22.53 and 20.25 min, were detectable at 279 nm (Figure 3). Two additional peaks at R*t* ca 7.0 and 14.0 were also detected but not identified. Initial screening experiment showed that an acidic mobile phase containing acetonitrile (0.1% HOAc) and 50 mM ammonium acetate at a pH 4.2 enhances peak shape and separation of *β*-carbolines, with a flow rate of 0.85 mL/min, while the two catechins were conveniently eluted using acetonitrile (0.1% HOAc) and Water (0.1% HOAc) with a flow rate of 0.7 mL/min. In both the cases, reversed phase column (Gemini C18, 5 μm, 150 × 4.6 mm) was used and peak purity was confirmed by studying the photodiode array (PDA) data of all peaks of interest; no indication for gross impurities could be found for majority of the markers. All samples (extracts and standards) were injected in triplicate, indicated the precision of the method.

The isolated markers (**1**-**9**) were employed for chemical profiling of various extracts of *B. caapi* Da Vine plant parts, collected in different seasons from two different Islands of Hawaii; thereby markers **1**-**9** were consistently assigned and quantified from aqueous extracts prepared either by maceration or ASE extractor (Table 1). Compounds **1, 2, 4**, **8** and **9** were not reported previously as markers from the genus *Banisteriopsis*, but being main markers, especially **8** and **9** are indicators of potent antioxidant value of the material, were quantified. The content of all markers was significantly higher in the dried sample compared to the corresponding fresh material. Using ASE extraction method, the leaves and young stems afforded higher yields of extracts with higher markers content, while maceration appears to be more efficient for matured plants. The contents of dominant *β*-carboline derived markers were significantly higher in dried bark of matured stem/ large branch [banistenoside A (**1**; 0.31, 0.71%), banistenoside A (**2**; 0.81, 1.93%), harmine (**7**; 0.28, 0.67%), THH (**5**; 0.04, 0.1%), and epicatechin (**8**; 0.67, 0.43%) and procyanidin B2 (**9**; 0.49, 0.16%)], followed by dried matured stem/ large branch [**1** (0.21, 0.27%), **2** (0.38, 0.57%), **5** (0.15, 0.21%), **7** (0.23, 0.35%), **8** (0.06, 0.17%) and **9** (0.12, 0.62%)]. The bioactive marker harmaline (**6**) was present in small amounts only (0.02-0.05%), compared to harmine (**7**), while THNH (**4**) and harmol (**3**) were present in these samples as minor markers. The content of the two major markers (**1** and **2**) was comparable to the total amount of all markers (**3**-**9**) in the large branch. The active alkaloid **7** was more predominant in most of the stem extracts, followed by **5** and **6**. Incidentally, harmine (**7**) was reported as the major biomarker in various *B. caapi*, followed by **5** and **6 (**Schultes and Raffauf, 1992;Rivier and Lindgren, 1972;Callaway et al., 2005), which was in agreement with our observation. The content of *β*-carbolines in fresh or dried leaves were much less than the other plant parts.

Analysis of regular/ commercial *B. caapi* stem extracts revealed consistently assignable and quantifiable peaks for **1**-**9**, however, the content of the major markers **1** (0.15, 0.17%), **2** (0.07, 0.15%), **7** (0.04, 0.13%) and **9** (0.03, 0.38%) were found to be relatively less in BCEx-1 and BCEx-4 (Table 1), compared to corresponding dried matured stem/ large branch of Da Vine; while epicatechin (**8**) was the only marker present in higher amounts in BCEx-4 (0.25% vs. 0.06-0.17%). No significant variations were found for the same plant parts collected in different season and/or different locations.

3.2. In vitro MAO-A Inhibitory Activity of Standardized Extracts and Pharmaceutical Compositions of β-Carboline Alkaloids and Proanthocyanidines

The *in vitro* MAO-A inhibitory and antioxidant activities of the standardized extracts and pharmaceutical compositions, prepared from the active constituents identified and isolated from *B. caapi* cultivar Da Vine, were evaluated. Three MAO-A inhibitory *β*-carboline alkaloids, THH (**5**), harmaline (**6**) and harmine (**7**), and two antioxidants, epicatechin (**8**) and procyanidin B2 (**9**), were used for the preparation of the standardized compositions. Three compositions were prepared based on optimum range of marker constituents in standardized *B. caapi* aqueous extracts consisting **5** (0.29-3.48%), **6** (0.12-0.59%) **7** (1.26-3.71%), **8** (0.6-5.4%) and **9** (0.9-7.2%); composition **1** with the lowest concentration of the compounds **5-9**, composition **2** with intermediate amounts and composition **3** with the highest amounts of these compounds (Table 2).

Three pharmaceutical compositions together with the standardized extracts of *B. caapi* (Da Vine) and *B. caapi* from commercial sources were subjected to *in vitro* evaluation of MAO-A inhibitory and antioxidant activities. Compositions **2** and **3** showed most potent MAO-A inhibitory activity (IC_{50} 0.027 μg/mL and 0.024 μg/mL, respectively), which is comparable to two different *B. caapi* (Da Vine) standardized extracts (IC_{50} 0.029 μg/mL and 0.032 μg/mL, respectively) (Table 2). The amount of the two most potent MAO-A inhibitor *β*-carboline alkaloids harmaline (**6**) and harmine (**7**) of the standardized extracts of *B. caapi* (Da Vine) dried bark of large branch (**BCdBS**: 0.16% and 3.71%) and whole dried matured stem (**BCDVSBig-07-08**: 0.48% and 3.66%) was found to be similar to those of compositions **2** (0.36% and 2.49%) and **3** (0.59% and 3.71%). On the other hand, composition **1** with the lowest amounts of **6** and **7** (0.12% and 1.26%, respectively) showed MAO-A inhibitory activities (IC_{50} 0.047 μg/mL *vs.* 0.059 μg/mL) similar to commercial *B. caapi* dried matured stem extract (**BCEx-4**) with comparable concentrations of **6** and **7** (0.24% and 1.36%). The content of **6** and **7** in the **BCEx-4** was found to be lower than the two Da Vine standardized extracts used for comparison. The antioxidant activity of compositions **2** and **3** was found to be equally potent to those of *B. caapi* commercial extract due to the presence of similar amounts of active antioxidant constituents **8** and **9** (Table 2). However, *B. caapi* (Da Vine) whole dried matured stem showed weak antioxidant activity, like composition **1**, due to lower amounts of **8** and **9**. On the other hand, *B. caapi* large branch extract (**BCdBS**) was found to be more potent than compositions **2**, **3** and **BCEx-4**, although they had lower amounts of **8** and **9**, suggesting the presence of additional antioxidant constituent(s). Collectively, standardized/ pharmaceutical compositions containing principal bioactive constituents (**5**-**9**) demonstrated *in vitro* MAO-A inhibitory and antioxidant activity generally comparable to those of *B. caapi* aqueous extracts with corresponding composition of bioactive constituents.

4. Conclusion

Various standardized extracts of *B caapi* were prepared, using maceration and ASE extraction methods, to determine high potency of MAO-A inhibitory and antioxidant activities. The predominant chemical and bioactive markers were present in high concentrations in dried bark of matured stem/ large branch, followed by whole dried stem and debarked stem [i.e., with the

range of concentration of markers: **1** (0.21-0.71%), **2** (0.38-1.93%), **5** (0.04-0.21%), **7** (0.23-0.67%), **8** (0.06-0.67%) and **9** (0.12-0.49%)], while harmaline (**6**) was present in small amounts only (0.02-0.05%). The variation of MAO-A inhibitory and antioxidant activities of various standardized extracts (Table 1) and compositions (Table 2) may be attributed to their different phytochemical compositions with respect to *β*-carboline alkaloids (**5**-**7**) and proanthocyanidins (**8** and **9**), respectively. Thus, the low content of key bioactive markers (**5**, **7** and **9**) in regular sample of *B. caapi* dried stems BCEx-1 (0.1%, 0.04%, 0.03%), compared to those of dried matured stems/ large branch, stem bark and debarked stem of Da Vine, is likely to be responsible for relatively less potent MAO-A inhibitory and antioxidant activities. However, the presence of two inactive major marker glycosides, based on azepino[1,2-*a*] tetrahydro-*β*-carboline nucleus (**1** and **2**) (Samoylenko et al., 2010), warrants further biological investigation of their aglycones. Collectively, various dried stem extracts demonstrated potent MAO-A inhibitory and antioxidant activity, thereby suggesting an optimal marker composition consisting **1** (0.81-3.93%), **2** (0.50-10.72%), **5** (0.29-3.48%), **6** (0.12-0.59%) **7** (1.26-3.71%), **8** (0.6-5.4%) and **9** (0.9-7.2%) in a standardized *B. caapi* phytopharmaceutical preparation. Interestingly, pharmaceutical composition **2** and **3**, prepared using only active constituents (compounds **5**-**9**) of *B. caapi* demonstrated similar *in vitro* MAO-A inhibitory and antioxidant activity compared to those displayed by *B. caapi* stem extracts (Table 2).

Traditionally, *B. caapi* is used as an adjuvant plant with *Psychotria viridis* (chacruna) or *Diplopterys cabrerana* (oco yage) for the preparation of the scared drink Ayahausca, which is widely used throughout the Amazon as a psychoactive tea for healing and spiritual exploration. It was established that monoamine oxidase (MAO) inhibitory activity of *β*-carboline alkaloids of *B. caapi* protects the psychoactive constituent dimethyltryptamine (DMT) of *Psychotria viridis* or *Diplopterys cabrerana* from oral degradation (i. e., caused by MAO) (Leal and Elisabetsky, 1996). Therefore, the presence of different *β*-carboline alkaloids (**3**-**7**) as main chemical markers reaffirms the traditional use of *B. caapi* as an adjuvant plant for the preparation of Ayahausca.

Inhibition of MAO by harmine and harmaline provide protection against neurodegeneration, and has a potential therapeutic value for the treatment of Parkinson's disease (Serrano-Duenas et al., 2001). In addition, oxidative stress induced by reactive oxygen species has been strongly associated with the pathogenesis of neurodegenerative disorders, including Alzheimer's diseases (Barnham et al., 2004). Collectively, these results support traditional uses of *B. caapi* stem extract in the treatment of PD (Serrano-Dueñas et al., 2001; Schwarz et al., 2003). Furthermore, this investigation suggested that the standardized extract of *B. caapi* with assigned composition of markers may be valuable for neurodegenerative diseases due to combined effect of proanthocyanidins (**8** and **9**) and *β*-carboline alkaloids (**5**-**7**); the former responsible for antioxidative, MAO-B inhibitory activities (Hou et al., 2005), and anti-neurodegenerative effects (Heo and Lee et al., 2005; Cho et al., 2008; Castillo et al., 2004), while the latter increases the release of dopamine from brain cells (Schwarz et al., 2003) and inhibiting MAO's, thus preventing its breakdown.

Acknowledgments

The authors sincerely thank Dr. Larry A. Walker, NCNPR, University of Mississippi and Professor Keith Tipton, University of Dublin, Ireland, for their valuable advise, suggestions and comments on the project; Mr. Raymond F. Baker, Harold Lyon Arboretum, University of Hawaii, for his kind supply of fresh plant materials from Oahu, HI, after collection and harvesting, Dr. Bharati Avula, NCNPR, UM, for Mass spectra. This work was supported by the National Center for Complementary and Alternative Medicine (NCCAM), National Institute of Health, Grant No. 5R21AT003409-02.

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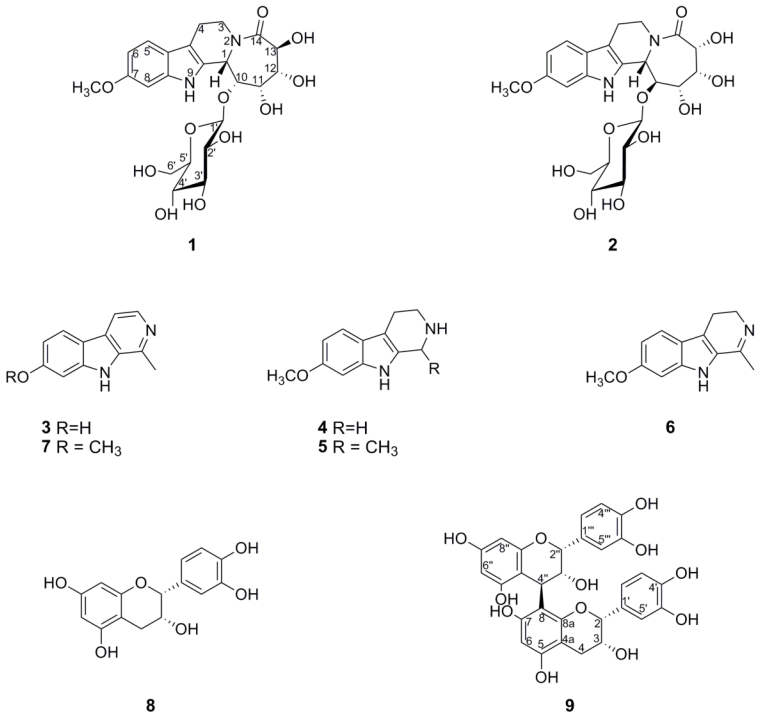
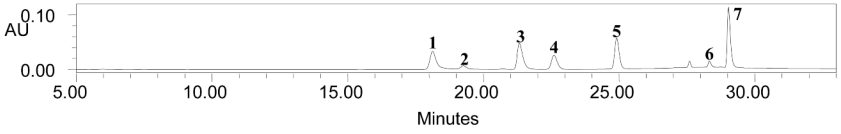


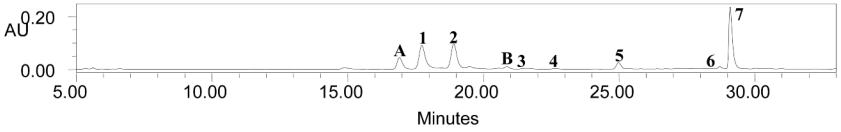
Fig. 1.

Structures of *β*-carboline alkaloids: banistenoside A (**1**), banistenoside B (**2**), harmol (**3**), tetrahydronorharmine (THNH) (**4**), tetrahydroharmine (THH) (**5**), harmaline (**6**), harmine (**7**) and proanthocyanidines: epicatechin (**8**) and procyanidin B2 (**9**) from *Banisteriopsis caapi*.

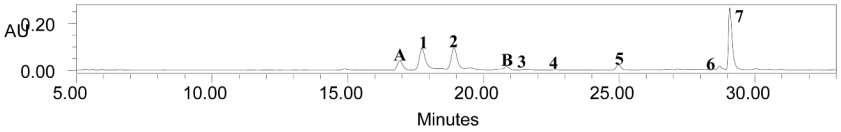
Mixture of Standards: [**1**, Banistenoside A (pk1); **2**, Banistenoside B (pk2); **3,** Harmol (pk3); **4,** THNH (pk4); **5,** THH (pk5); **6,** Harmaline (pk6) and **7,** Harmine (pk7)]



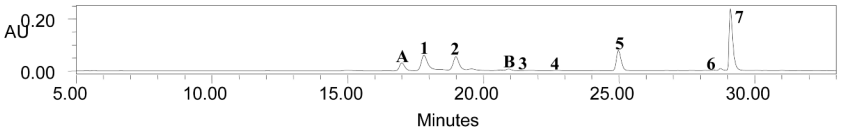
BCfBS (*B. caapi* Da Vine fresh bark of matured stem, Nov. 2007), 5.0 mg/mL



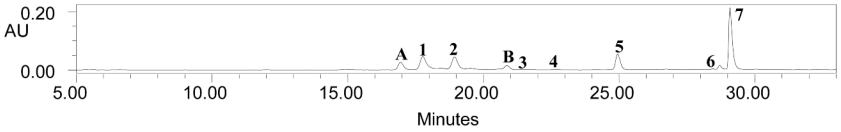
BCdBS (*B. caapi* Da Vine dried bark of matured stem, Nov. 2007), 5.0 mg/mL



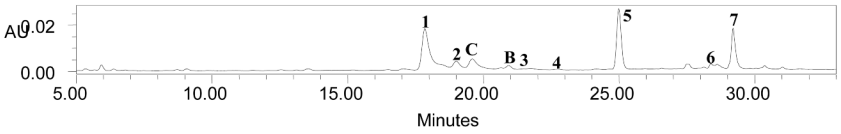
BCfDS (*B. caapi* Da Vine fresh debarked matured stem, Nov. 2007), 5.0 mg/mL



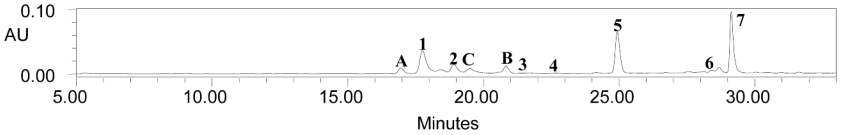
BCdDS (*B. caapi* Da Vine dried debarked matured stem, Nov. 2007), 5.0 mg/mL



BCEx-1 (*B. caapi* dried matured stem; regular/commercial), 5.0 mg/mL



BCEx-4 (*B. caapi* dried matured stem; regular/commercial), 5.0 mg/mL



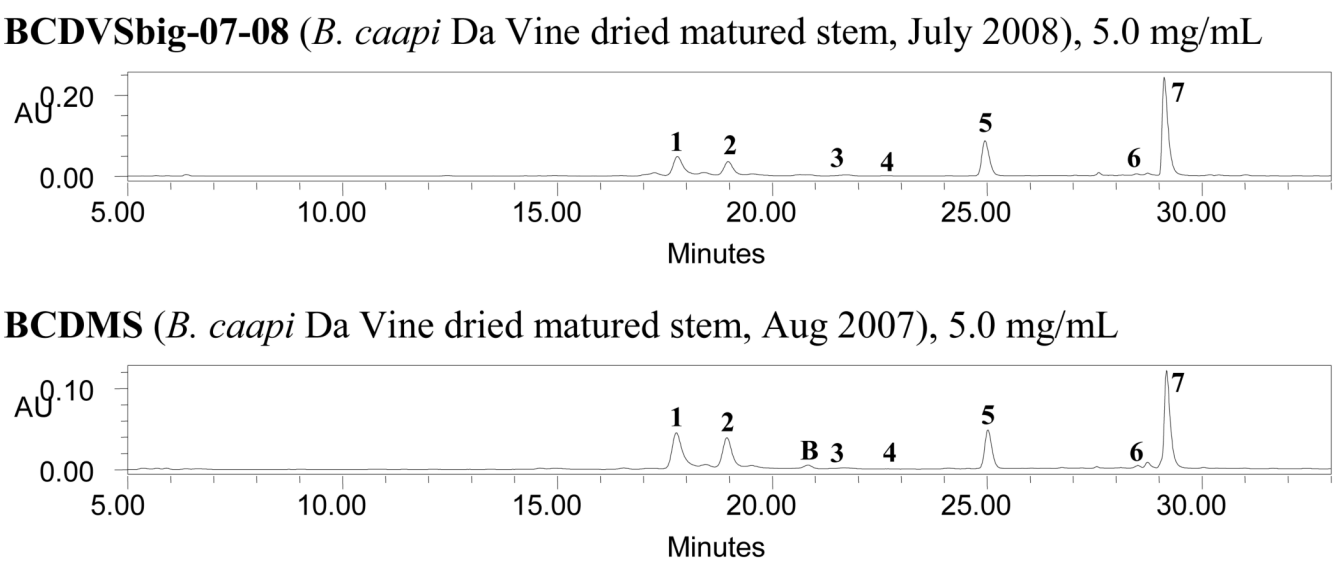
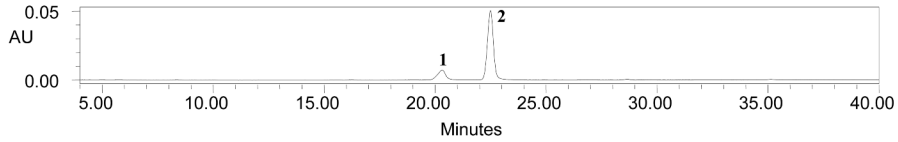
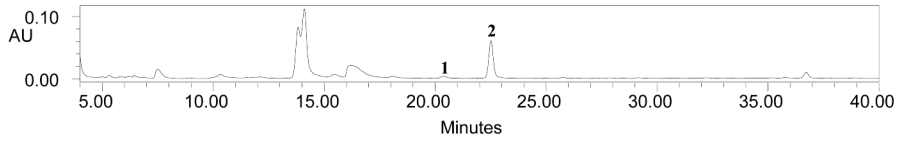


Figure 2.
HPLC Chromatograms of *B. caapi* Da Vine extracts and regular/ commercial samples at UV 293 nm for Alkaloids (Compounds **1**-**7**).

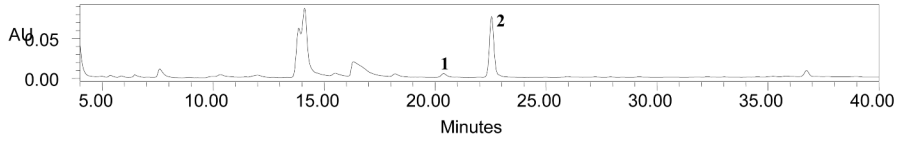
Mixture of Standards -2 (100 μg/mL) [Procyanidin B2 (**9**, pk1) and Epicatechin (**8**, pk2)]



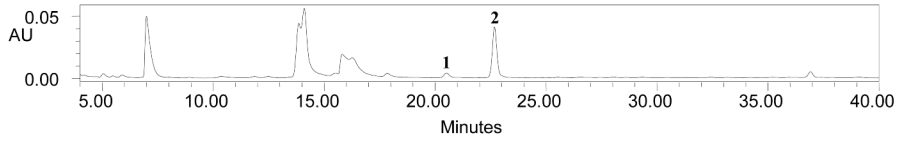
BCfBS (*B. caapi* Da Vine fresh bark of matured stem, Nov. 2007), 5.0 mg/mL



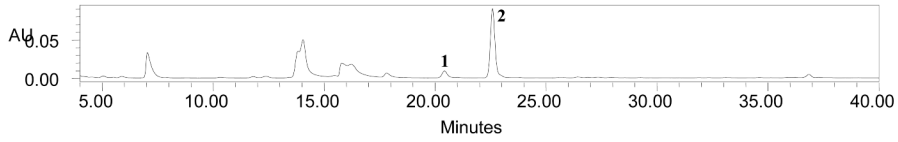
BCdBS (*B. caapi* Da Vine dried bark of matured stem, Nov. 2007), 5.0 mg/mL



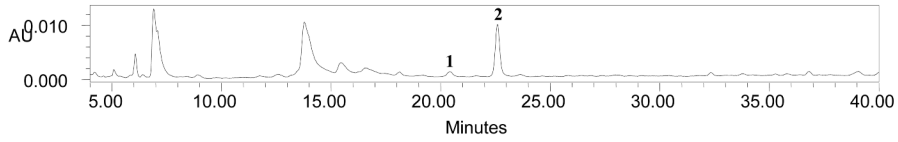
BCfDS (*B. caapi* Da Vine fresh debarked matured stem, Nov. 2007), 5.0 mg/mL



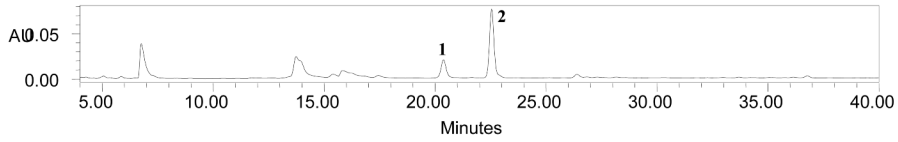
BCdDS (*B. caapi* Da Vine dried debarked matured stem, Nov. 2007), 5.0 mg/mL



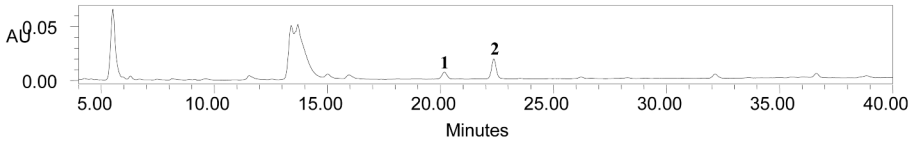
BCEx-1 (*B. caapi* dried matured stem; regular/commercial), 5.0 mg/mL



BCEx-4 (*B. caapi* dried matured stem; regular/commercial), 5.0 mg/mL



BCDVSbig-07-08 (*B. caapi* Da Vine dried matured stem, July 2008), 5.0 mg/mL



BCDMS (*B. caapi* Da Vine dried matured stem, Aug 2007), 5.0 mg/mL

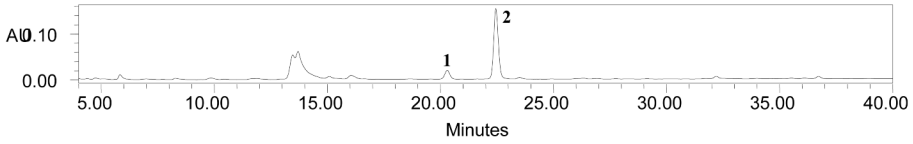
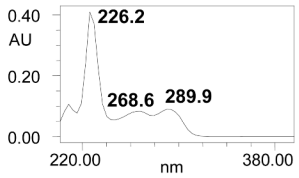


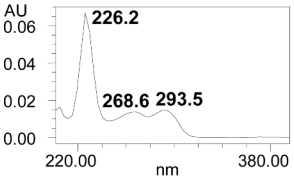
Figure 3.

HPLC Chromatograms of *B. caapi* Da Vine extracts and regular/ commercial samples at UV 279 nm for Epicatechin (**8**) and Procyanidin B2 (**9**).

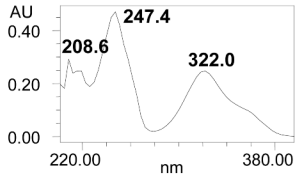
Banistenoside A (**1**): retention time = 17.74 min



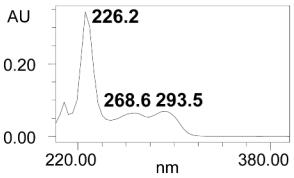
Banistenoside B (**2**): retention time = 18.91 min



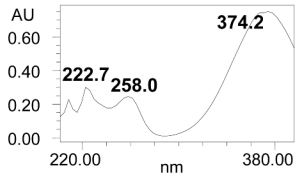
Harmol (**3**): retention time = 21.21 min



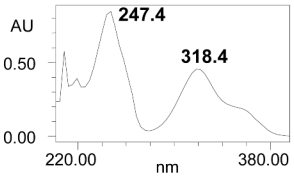
THH (**5**): retention time = 24.88 min



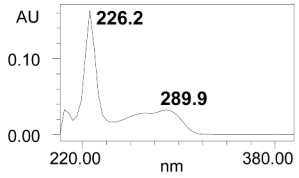
Harmaline (**6**): retention time = 28.38 min



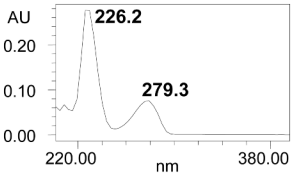
Harmine (**7**): retention time = 29.08 min



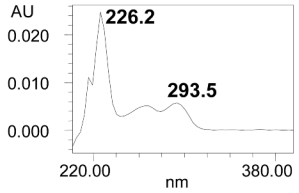
Unidentified Compound **A**: retention time = 16.92 min



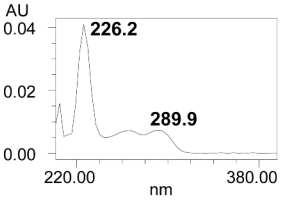
Unidentified Compound **B**: retention time = 20.84 min



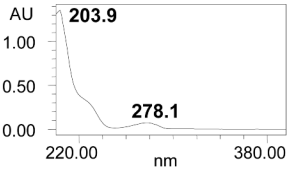
Unidentified Compound **C**: retention time = 19.50 min



THNH (**4**): retention time = 22.66 min



Epicatechin (**8**) (pk2): retention time = 22.53 min



Procyanidin B2 (**9**) (pk1): retention time = 20.25 min

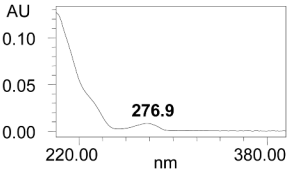


Figure 4.
UV Spectra of standards and unidentified Alkaloids.

Table 1

Quantification of markers banistenoside A (**1**), banistenoside B (**2**), harmol (**3**), THNH (**4**), THH (**5**), harmaline (**6**), harmine (**7**), epicatechin (**8**) and procyanidin B2 (**9**) of various extracts of *B. caapi*

Plant Part	Time and Place of Collection	Yield,[a] %	Compounds Quantified (Content, %)									MAO-A	Antioxidant
			1	2	3	4	5	6	7	8	9	IC_{50} (μg/mL)	IC_{50} (μg/mL)
Fresh leaves	August 2007; Oahu	0.49	0.002	0.0004	0.0004	0.000	0.004	0.0001	0.002	0.021	0.002	0.125	NT
Fresh leaves[b]		5.28	0.072	0.015	0.011	0.003	0.114	0.002	0.042	0.333	0.090	0.2	NT
Dried leaves		10.06	0.166	0.027	0.005	0.005	0.101	0.004	0.031	0.070	0.030	0.6	5.9
Fresh young stem		0.85	0.009	0.002	0.0004	0.0003	0.005	0.0003	0.001	0.002	0.002	0.2–0.67	NT
Fresh young stem[b]		11.70	0.129	0.032	0.007	0.005	0.055	0.005	0.020	0.070	0.070	0.9	NT
Dried matured stem		12.74	0.270	0.572	0.004	0.003	0.148	0.042	0.233	0.166	0.624	0.04	NT
Dried matured stem[b]		9.27	0.075	0.046	0.002	0.003	0.030	0.005	0.007	0.195	0.185	NT	NT
Fresh bark of matured stem		2.16	0.085	0.198	0.005	0.004	0.012	0.005	0.089	0.151	0.093	0.015–0.025	NT
Dried bark of matured stem		14.29	0.314	0.809	0.019	0.014	0.041	0.017	0.280	0.672	0.486	0.033	2.2
Fresh debarked young stem		2.67	0.087	0.058	0.001	0.002	0.107	0.015	0.063	0.112	0.096	0.025–0.035	NT
Dried debarked young stem		9.76	0.267	0.188	0.007	0.002	0.340	0.058	0.226	0.527	0.703	0.08–0.043	4.0
Fresh leaves	October 2007; Big Island[c]	0.60	0.005	0.001	0.001	0.0002	0.010	----	0.003	0.014	0.005	1.35	NT
Fresh young stem		0.80	0.005	0.002	0.002	0.001	0.006	----	0.002	0.011	0.002	0.58	NT
Fresh bark of large branch[d]	November 2007; Oahu	3.0	0.117	0.322	0.003	0.007	0.020	0.004	0.098	0.051	0.018	0.045	0.4
Dried bark of large branch[d]		18.1	0.711	1.926	0.018	0.040	0.103	0.029	0.672	0.434	0.163	0.03	1.9
Fresh debarked large branch[d]		5.2	0.120	0.279	0.002	0.010	0.089	0.018	0.177	0.062	0.036	0.035	3.0
Dried debarked large branch[d]		7.6	0.154	0.378	0.002	0.010	0.103	0.020	0.229	0.205	0.114	0.02	2.0
Fresh leaves	June 2008; Oahu	3.71	0.080	0.049	0.007	0.003	0.126	0.007	0.062	0.063	0.056	0.052	NT
Fresh young stem		2.50	0.027	0.041	0.005	0.001	0.016	0.004	0.040	0.008	0.010	0.16	NT
Dried young stem		8.20	0.158	0.125	0.003	0.007	0.157	0.030	0.103	0.098	0.148	0.065	NT

Plant Part	Time and Place of Collection	Yield,[a] %	Compounds Quantified (Content, %)									MAO-A	Antioxidant
			1	2	3	4	5	6	7	8	9	IC_{50} (μg/mL)	IC_{50} (μg/mL)
Fresh leaves	July 2008; Big Island	2.17	0.018	0.007	0.004	0.002	0.039	----	0.011	0.254	0.156	0.18	NT
Dried leaves		8.33	0.107	0.021	0.008	0.006	0.147	----	0.025	0.017	0.025	0.25	NT
Fresh matured stem		2.9	0.062	0.103	0.002	0.003	0.050	0.010	0.075	0.078	0.099	0.034	NT
Dried matured stem		9.56	0.210	0.382	0.004	0.008	0.207	0.046	0.350	0.057	0.115	0.028	NT
Dried matured stem (BCEx-1)	Regular/ Commercial sample	15.43	0.150	0.074	0.002	0.006	0.103	0.017	0.043	0.046	0.031	0.38	7.8
Dried matured stem (BCEx-4)		9.80	0.170	0.153	0.002	0.008	0.159	0.024	0.133	0.245	0.382	0.05	1.0

Pure compd/Standards	MAO-A	Antioxidant
	IC_{50} (nM)	IC_{50} (μg/mL)
THH (**5**)	74	NA
Harmaline (**6**)	2.5	NA
Harmine (**7**)	2.0	NA
Epicatechin (**8**)	51.7×10^3	0.16
Procyanidin B2 (**9**)	8.5×10^3	0.57
Clorgyline	1.6	NT
Deprenyl	9.0	NT
Vitamin C	NT	1.34
Doxorubicin	NT	NT

Yield of the aqueous plant extract measured by weight. Extracted with water by automated ASE-200 extractor.

[c] Plants from this collection are 3 years old.

[d] Large branches: diameter: 3-8 cm. NT = Not tested. NA = No antioxidant activity up to 31.25 μg/mL.

Table 2

In vitro MAO-A inhibitory activity of compositions **1**-**3** and *B. caapi* standardized extracts

Sample Code Name	Content of Compound in the Extract/Composition, %					Concentration of the stock DMSO solution (mg/mL)	MAO-A IC_{50} (μg/mL)	Antioxidant IC_{50} (μg/mL)
	5	**6**	**7**	**8**	**9**			
Composition **1**	0.63	0.12	1.26	0.6	0.9	10	0.047	21
Composition **2**	1.89	0.36	2.49	3.0	3.9	10	0.027	3.1
Composition **3**	3.48	0.59	3.71	5.4	7.2	10	0.024	3.8
BCdBS [a]	0.57	0.16	3.71	2.4	0.9	10	0.029	0.64
BCDVSBig-07-08 [b]	2.17	0.48	3.66	0.6	1.2	10	0.032	20
BCEx-4 [c]	1.62	0.24	1.36	2.5	3.9	10	0.059	3.8
Clorgyline	-	-	-	-	-	-	1.6 nM	-
Vitamin C	-	-	-	-	-	-	-	1.34

[a] *B. caapi* cultivar Da Vine dried bark of large branch/November 2007.

[b] *B. caapi* Da cultivar Vine dried matured stem/July 2008.

[c] *B. caapi* dried matured stem/commercial sample.