Journal of Ethnopharmacology 127 (2010) 357–367

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Banisteriopsis caapi, a unique combination of MAO inhibitory and antioxidative constituents for the activities relevant to neurodegenerative disorders and Parkinson's disease

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ARTICLE INFO

Article history:
Received 5 August 2009
Received in revised form 13 October 2009
Accepted 22 October 2009
Available online 30 October 2009

Keywords:
Banisteriopsis caapi (Spruce ex Griseb.)
β-Carboline alkaloids
Proanthocyanidines
Monoamine oxidase inhibitors
Antioxidants
Parkinson's disease

ABSTRACT

Aim of the study: Parkinson's disease is a neurological disorder mostly effecting the elder population of the world. Currently there is no definitive treatment or cure for this disease. Therefore, in this study the composition and constituents of the aqueous extract of *Banisteriopsis caapi* for monoamine oxidases (MAO) inhibitory and antioxidant activities were assessed, which are relevant to the prevention of neurological disorders, including Parkinsonism.

Materials and methods: The aqueous extract of *Banisteriopsis caapi* stems was standardized and then fractionated using reversed-phase (RP) chromatography. Pure compounds were isolated either by reversed-phase (RP) chromatography or centrifugal preparative TLC, using a Chromatotron®. Structure elucidation was carried out by 1D and 2D NMR, Mass, IR and Circular Dichroism spectroscopy and chemical derivatization. Chemical profiling of the extract was carried out with RP-HPLC. The inhibitory activity of MAO-A, MAO-B, acetylcholinesterase, butyrylcholinesterase and catechol-*O*-methyl transferase enzymes, as well as antioxidant and cytotoxic activities of both *Banisteriopsis caapi* extract and isolated compounds was evaluated.

Results: An examination of the aqueous extracts of *Banisteriopsis caapi* cultivar Da Vine yielded two new alkaloidal glycosides, named banistenoside A (**1**) and banistenoside B (**2**), containing "azepino[1,2-a]tetrahydro-β-carboline" unique carbon framework. One additional new natural tetrahydronorharmine (**4**), four known β-carbolines harmol (**3**), tetrahydroharmine (**5**), harmaline (**6**) and harmine (**7**), two known proanthocyanidines (−)-epicatechin (**8**) and (−)-procyanidin B2 (**9**), and a new disaccharide β-D-fructofuranosyl-(2 → 5)-fructopyranose (**14**) together with known sacharose (**15**) and β-D-glucose (**16**) were also isolated. In addition, the acetates of **1**, **2**, **8**, **9**, **14** and **15** (compounds **10**–**13**, **17**, **18**) were also prepared. Harmaline (**6**) and harmine (**7**) showed potent *in vitro* inhibitory activity against recombinant human brain monoamine oxidase (MAO)-A and -B enzymes (IC_{50} 2.5 and 2.0 nM, and 25 and 20 μM, respectively), and (−)-epicatechin (**8**) and (−)-procyanidin B2 (**9**) showed potent antioxidant and moderate MAO-B inhibitory activities (IC_{50} < 0.13 and 0.57 μg/mL, and 65 and 35 μM). HPLC analysis revealed that most of the dominant chemical and bioactive markers (**1**, **2**, **5**, **7**–**9**) were present in high concentrations in dried bark of large branch. Analysis of regular/commercial *Banisteriopsis caapi* dried stems showed a similar qualitative HPLC pattern, but relatively low content of dominant markers **1**, **2**, **7**, and **9**, which led to decreased MAO inhibitory and antioxidant potency.

Conclusion: Collectively, these results give additional basis to the existing claim of *Banisteriopsis caapi* stem extract for the treatment of Parkinsonism, including other neurodegenerative disorders.

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doi:10.1016/j.jep.2009.10.030

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). *Banisteriopsis caapi* (Spruce ex Griseb.) Morton (Vine of the Soul) (Schultes and Raffauf, 1992) is an ingredient of the popular sacred and psychoactive drinks Ayahuasca, also known as Caapi, Pinde, Natema or Yaje, which is widely used for prophecy, divination, and as a sacrament in the northern part of South America (Schultes, 1970; Schultes and Siri von, 1995). However, to the best of our knowledge, no traditional drink prepared only from *Banisteriopsis caapi* has been consumed for such uses. Earlier chemical investigations have reported the presence of β-carboline alkaloids (β-CA) harmine, harmaline and tetrahydroharmine (THH) as the principal MAO inhibitors, together with other β-CA's, from *Banisteriopsis caapi* (Hochstein and Paradies, 1957; Hashimoto and Kawanishi, 1975, 1976; Callaway et al., 2005). In addition, two pyrrolidines, shihunine and (*S*)-(+)-dihydroshihunine (Kawanishi et al., 1982), and terpenoids (Aquino et al., 1991) were also reported. The alkaloid content of *Banisteriopsis caapi* was determined previously by GC/MS (Rivier and Lindgren, 1972), LC/MS (Kawanishi et al., 1998), and HPLC (Serrano-Dueñas et al., 2001), suggesting the content of harmine is highest among β-CA's, followed by THH and harmaline.

Parkinson's disease (PD) is caused by a loss of neurons from *substantia nigra* of the brain. Once damaged, these neurons stop producing dopamine and compromise the brain's ability to control movement. It is not known what damages certain neurons in PD patients. One reason is that free radicals/toxic particles normally deactivated in the body are responsible, which can be controlled by antioxidants as adjuvant with dopamine agonist or MAO inhibitors. The usefulness of *Banisteriopsis caapi* was established for alleviating symptoms of PD (Serrano-Dueñas et al., 2001), which contains MAO inhibitor harmine as active constituent used in PD treatment (Sánchez-Ramos, 1991). A double-blind, randomized placebo-controlled trial of *Banisteriopsis caapi*, using a single dose, revealed a significant improvement in motor function of PD patients (Serrano-Dueñas et al., 2001). Tests for MAO inhibition using liver homogenate showed that *Banisteriopsis caapi* stem extract and harmine showed a concentration-dependent inhibition of MAO-A, and an increase in release of dopamine from rat striatal slices (Schwarz et al., 2003).

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 *Banisteriopsis caapi* that natives of Amazon have knowledge of and have different uses (Schultes and Hofmann, 1992). During the course of chemical and biological standardization of *Banisteriopsis caapi* under an NIH funded investigation at the NCNPR for neurological disorders relevant to Parkinsonism, an extract of *Banisteriopsis caapi* cultivar Da Vine, collected in Oahu, Hawaii, demonstrated potent *in vitro* MAO-A inhibitory and antioxidant activities. This led to the bioassay-guided isolation of two new β-carboline alkaloidal glycosides, banistenoside A (**1**) and banistenoside B (**2**), a new tetrahydronorharmine (**4**), four known β-carbolines harmol (**3**), tetrahydroharmine (**5**), harmaline (**6**) and harmine (**7**), two proanthocyanidines (−)-epicatechin (**8**) and (−)-procyanidin B2 (**9**), and a new disaccharide β-D-fructofuranosyl-(2 → 5)-fructopyranose (**14**) (Fig. 1), using regular and RP silica gel chromatography. In this paper, we report the isolation, characterization and bioactivities of isolated compounds, and HPLC analysis of *Banisteriopsis caapi* cultivar Da Vine and two regular/commercial samples of *Banisteriopsis caapi*.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were measured in $CHCl_3$ or MeOH using an AUTOPOL IV® instrument at ambient temperature; IR spectra were obtained using a Bruker Tensor 27 FTIR instrument; Circular Dichroism (CD) spectra were recorded on a Olis DCM 20 CD spectrometer. 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), using a Gemini C18 110 Å column (Phenomenex, 150 mm × 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). The NMR spectra were acquired on a Bruker Avance DRX-400 instrument at 400 MHz (^{1}H), 100 (^{13}C) in $CDCl_3$ or CD_3OD, using the residual solvent as int. standard; multiplicity determinations (DEPT) and 2D NMR spectra (COSY, HMQC, HMBC and NOESY) were obtained using standard Bruker pulse programs; HRMS were obtained by direct injection using a Bruker Bioapex-FTMS with electro-spray ionization (ESI). 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). TLC was carried out on either reversed phase silica gel (Analtech®, RP18 SiO_2, 150 μm, UV254) with MeCN–H_2O (9:1) or acetone–H_2O–NH_3·H_2O (7:3:0.1), silica gel (EMD® Chemicals Inc., SiO_2 60 F_{254}) using $CHCl_3$–MeOH (7:3) or alumina plates (EMD® Chemicals Inc., Al_2O_3 60 F_{254}) using CH_2Cl_2–MeOH (9:1) as solvent system; centrifugal preparative TLC (CPTLC) was performed by a Chromatotron® (Harrison Research Inc., model 8924), tagged with a fraction collector (SpectralChrom® CF-1) on a 1, 2 or 4 mm silica gel rotors (Analtech®, SiO_2 coated with F_{254} indicator); flash CC was carried out over reversed phase silica gel (J.T. Baker, Bakerbond® C18 prep LC SiO_2, 40 μm). Samples were dried using a Savant Speed Vac Plus SC210A Concentrator. The isolated compounds were visualized by observing under UV light at 254 or 365 nm, followed by spraying separately with Dragendorff's and/or 1% vanillin-H_2SO_4 spray reagents. The reference standards of harmol, harmine, harmaline, and (−)-epicatechin were purchased from Sigma–Aldrich (St. Louis, MO), while others were available in our laboratories.

2.2. Plant material

Fresh leaves, stems, and large branches of *Banisteriopsis caapi* cultivar Da Vine (Miller, 1986) 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. 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 *Banisteriopsis caapi* (mature stems) samples analyzed in this study (BCEx-1–BCEx-4) were procured/obtained from commercial (*via* internet) and NCNPR sources during 2005–2009. In addition, all samples used in this work are preserved using the standard procedures for collection, drying, grinding and packaging at the NCNPR.

2.3. Extraction of plant material

2.3.1. Preparation of extracts from different plant parts

The different plant parts (leaves, young and mature stems, bark and debarked stems, and large branches (diameter: 3–8 cm)) of