

Original Article

 Simultaneous liquid chromatography- tandem mass spectrometry method to quantify epicatechin and procyanidin B2 in rat plasma after oral administration of *Trichilia catigua* (catuaba) extract and its application to a pharmacokinetic study

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ABSTRACT

Trichilia catigua A. Juss., Meliaceae, known as catuaba in Brazil, is traditionally used for the treatment of stress, sexual impotence and memory deficits. To our knowledge, there is no analytical method described in literature for simultaneous quantification of catuaba extract marker substances in biological matrices. The aim of this study was to develop and validate a bioanalytical method by LC-MS/MS to quantify epicatechin and procyanidin B2 in rat plasma after administration of standardized extract of *T. catigua*. Chromatographic separation was achieved with a C18 column, methanol and 0.1% aqueous formic acid at a flow rate of 0.25 ml/min. Detection was performed using electrospray ionization in negative mode. The lower limits of quantification were 5 ng/ml and 12.5 ng/ml for procyanidin B2 and epicatechin, respectively. Intra- and inter-day assays variability were less than 15%. The extraction recovery was 104% for epicatechin and 74% for procyanidin B2 using one-step liquid-liquid extraction with ethyl acetate. Epicatechin and PB2 were detected in plasma up to 300 min after oral administration of 400 mg/kg of standardized extract of *T. catigua* in rats. This rapid and sensitive method for the analysis of the epicatechin and procyanidin B2 in rat plasma can be applied to pharmacokinetic studies.

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Introduction

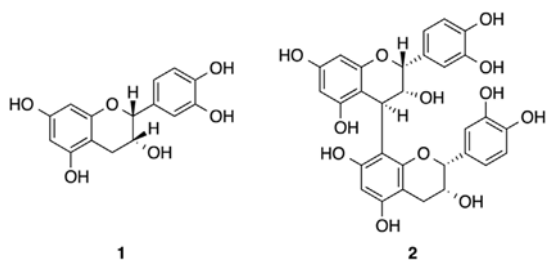
Trichilia catigua A. Juss., Meliaceae, popularly known as catuaba, is part of the rich Brazilian flora and has been traditionally used as a tonic to treat fatigue, stress, impotence and memory deficit (Pizzolatti et al., 2002). Distinct extracts from catuaba barks have been evaluated regarding the biological activity by *in vitro* and *in vivo* models. The extracts were found to: induce and prolong a sustained relaxation of cavernous bodies (Antunes et al., 2001); reverse ventricular fibrillation (Pontieri et al., 2007); induce histological and functional protection in a pre-clinical model of

cerebral ischemia (Truitt et al., 2015); generate antiviral activity for herpesvirus and poliovirus in a culture of HEp-2 cells (Espada et al., 2015); produce anti-inflammatory activity by the inhibition of the phospholipase A2 (PLA2) (Barbosa et al., 2004), produce antidepressant (Campos et al., 2005; Chassot et al., 2011; Taciany Bonassoli et al., 2012), antioxidant (Resende et al., 2011; Tang et al., 2007), bacteriostatic (Pizzolatti et al., 2002) and trypanocidal activity (Pizzolatti et al., 2003).

The chemical composition of the acetate fraction of the *T. catigua* extract has been described as: epicatechin (1), procyanidin B2 [epicatechin-(4β→8)-epicatechin] (2) cinchonain IIa and IIb, cinchonain Ia, Ib, and catechin, as well other condensed tannins (Longhini et al., 2013). This particular extract is referred in this paper as “standardized extract of *T. catigua* (SETc)” and has been described as responsible for multiple activities: antioxidant (Lonn

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et al., 2012), neuroprotective in cerebral ischemia models (Truitt et al., 2015), preventive of neuronal cell death (Truitt et al., 2015) and antidepressant (Tacyan Bonassoli et al., 2012).



Extracts of different plant species also contain **1** and **2** as active constituents. Both **1** and **2** also present activity when used isolated. Compound **1** has been related to the neuroprotective effects and to the prevention of neuronal cell death *in vitro* (Matsuoka et al., 1995; Schroeter et al., 2000), and **2** to antioxidant, anti-tumor and growth promoting activity (Lopes et al., 2009; Sakano et al., 2005).

Despite the several SETc pharmacological and biological studies, no information on its pharmacokinetic behavior has been described up to now. The extract active constitutes exposure over time, as well as the relationship of the exposure with the activity, are crucial to evaluate the viability of this extract to become a true active pharmaceutical ingredient (API).

To our knowledge, the analytical methods described in the literature solely provide information regarding the quantification of tannins using high performance liquid chromatography (HPLC) in chemical matrices (Beltrame et al., 2006; Longhini et al., 2013). No methodology for simultaneous quantification of **1** and **2** in plasma has been described. The aim of this study was to develop and validate an analytical method to quantify **1** and **2** in rat plasma following the administration of SETc and conduct a pharmacokinetic analysis with both constituents.

Material and methods

Chemical, reagents and equipment

Methanol and formic acid were HPLC grade purchased from Tedia (Brazil), while ethyl acetate was analytical grade purchased from Synth (Brazil). Ultra-purified water was produced by a Milli-Q equipment (Millipore Corp Burlington, MA). Epicatechin (**1**) and quercetin (internal standard - IS) were obtained from Sigma-Aldrich (Brazil).

Procyanidin B2 (**2**) was isolated from *Trichilia catigua* and the SETc used in pharmacokinetic study was obtained as described by Longhini et al. (2013) from *T. catigua* as well and kindly provided by Dr. João Carlos Palazzo de Mello (Pharmaceutical Biology Laboratory – Palafto). The bark of *T. catigua* was acquired from Caetité, Bahia, Brazil and a voucher specimen was identified by Dr. Cássia Mônica Sakuragui, Universidade Federal do Rio de Janeiro, RJ, Brazil, and deposited at the Herbarium of Universidade Estadual de Maringá (HUEM#19.434), Maringá, PR, Brazil.

Quantifications were performed on a HPLC system Waters Alliance e2965 tandem mass spectrometer triple quadrupole Quattro Premier XE using an electrospray ionization (ESI) source. Data acquisition, instrument controls and post run analysis were performed using MassLynx version 4.1 (Waters Technology, Milford, MA, USA).

Experimental conditions for LC–MS/MS

Chromatography was performed with an ACE3 C₁₈-300 column (50 mm × 2.1 mm i.d.) with 3 μm of particle size (ACE, Aberdeen,

UK), in line with a guard column ACE C₁₈-300 (Aberdeen, UK), at a temperature of 36 °C. The mobile phase was composed of 0.1% aqueous formic acid and methanol (50:50, v/v) at a flow rate of 0.25 ml/min. Temperature of autosampler was kept at 4 °C. Injection volume was 10 μl. The full analytical run time was 4 min, a flow switching was used and just MS data from 0.5 to 3 min was recorded. Detection was performed using electrospray ionization in the negative mode. Direct infusion of **1**, **2** and quercetin was used to identify the deprotonate molecule [M–H][–] and after a collision energy optimization, the best fragments were chosen, allowing the determination of the multiple reactions monitoring (MRM). Ion transition *m/z* of 288.5 > 244.7 for **1**, *m/z* 576.5 > 423.6 for **2** and *m/z* 300.5 > 150.5 for IS were used for the quantification of the compounds. Desolvation and cone gas flow were 600 and 35 l/h, respectively at 320 °C. The temperature of the ion source was maintained at 120 °C. Capillary voltage was set to 4.0 kV. The collision energy for both **1** and **2** was 15 kV, and for the IS it was 25 kV. Cone voltages of 30 kV were applied for all analytes. Collision-induced dissociation was performed using argon in the collision cell.

Standard curve and quality control samples

Stock solutions of **1** and **2** were prepared in methanol at the concentration of 1 mg/ml. The IS stock solution of 100 ng/ml of quercetin was prepared in mobile phase. All solutions were prepared daily. Intermediate dilutions of **1** and **2** combined were prepared by dilution in methanol:water (50:50 v/v). To prepare the calibration standards and quality controls, different aliquots (the volume would depend on the desired final concentration) of the stock solutions of **1** and **2** were diluted with 20 μl of IS solution in rat plasma to a final volume of 100 μl. In order to prevent analyte degradation by plasma enzymes, 20 μl of acetic acid (50 mmol/l) was also included (Jabor et al., 2010). The volumetric flasks were placed on a vortex for 1 min prior to a one-step liquid–liquid extraction. Liquid–liquid extraction was performed by the addition of 500 μl of ethyl acetate, stirred for 1 min in vortex and then centrifuged for 15 min at 11,200 × *g* and 4 °C. In another microtube the acetate fraction (400 μl) was collected and evaporated to dryness at 38 °C. The residue was reconstituted in 100 μl of mobile phase for injection onto the LC–MS/MS system.

Method validation

The bioanalytical method was validated according to the US Food and Drug Administration guideline (FDA, 2013). The following parameters were included in the validation: selectivity, lower limit of quantification (LLOQ), stability, standard curve range, accuracy, precision, recovery and matrix effect.

Selectivity

Chromatograms of blank plasma from six rats were analyzed to check selectivity. The presence of exogenous or endogenous interferences in the matrix was evaluated.

Standard curve and LLOQ

All samples were spiked with both EPI and PB2. Seven concentrations of the analytes (5, 12.5, 20, 25, 50, 10 and 150 ng/ml), spiked with 20 ng/ml of IS and diluted in rat plasma were evaluated at three consecutive days. The ratio of areas (RA) of the **1** and **2** vs. IS were calculated. Linear regression was evaluated using the theoretical concentrations against the **1** and **2** vs. IS RAs. To determine the LLOQ, concentrations lower than 5 ng/ml for **2** and 12.5 ng/ml for

1 were assayed five times. According to FDA guideline, the LLOQ is the lowest concentration with variability lower than 20%, that the method can quantify.

Precision and accuracy

Accuracy, within- and between-day precision were evaluated using three different concentrations of **1** and **2** in rat plasma (20, 50 and 100 ng/ml). For within-day precision, five replicates of each concentration were evaluated on the same day and, for between-day, results of three consecutive days were considered. Variations of <15% between results were considered acceptable. The results of precision were expressed as relative standard deviation (RSD%) and relative error (RE%) was used to express the accuracy results.

Matrix effect and recovery

The experiments were assessed using the comparison conditions described by Lachi-Silva et al. (2015) using three different **1** and **2** concentrations (20, 50, 100 ng/ml).

Stability

The stability of samples was evaluated during short and long-term, in addition to the freeze and thaw stability assessment.

Analyses were carried out at three different concentration levels (20, 50 and 100 ng/ml) with three replicates. The samples were frozen at -80°C and thawed at room temperature ($20 \pm 5^{\circ}\text{C}$). This process was repeated for three freeze and thaw cycles, and then each sample was submitted to the liquid-extraction as described above and analyzed by LC–MS/MS. The short-time stability was evaluated in two different experiments: (i) samples were stored in the autosampler (4°C) for 6 h; (ii) samples were kept in a freezer (-20°C) for 24 h. Next, the samples were prepared and analyzed as described previously. The chromatographic results were compared against chromatographic results from freshly prepared samples. The long-term stability of **1** and **2** in rat plasma was assessed by keeping samples stored for two months at -80°C . In all stability studies **1** and **2** were considered stable if the deviation from the nominal concentration was in the range of 85–115%.

Application of the method in a pharmacokinetic study

All animal experiments were approved and in accordance with the guidelines of the Animal Ethics Committee of the State University of Maringa (protocol CEUA/UEM – 8525010616). Male albino Wistar rats ($n = 5$) with medium weigh of 0.180 kg were supplied by the animal facilities of State University of Maringa, Paraná, Brazil. The animals were housed at controlled temperature ($22 \pm 2^{\circ}\text{C}$),

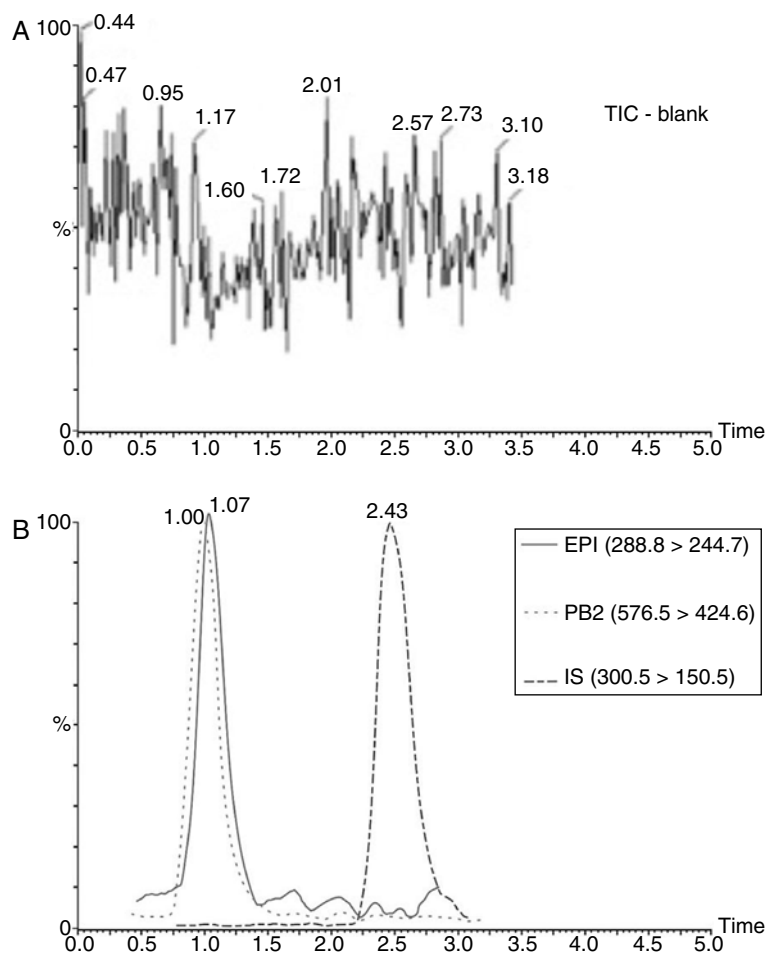
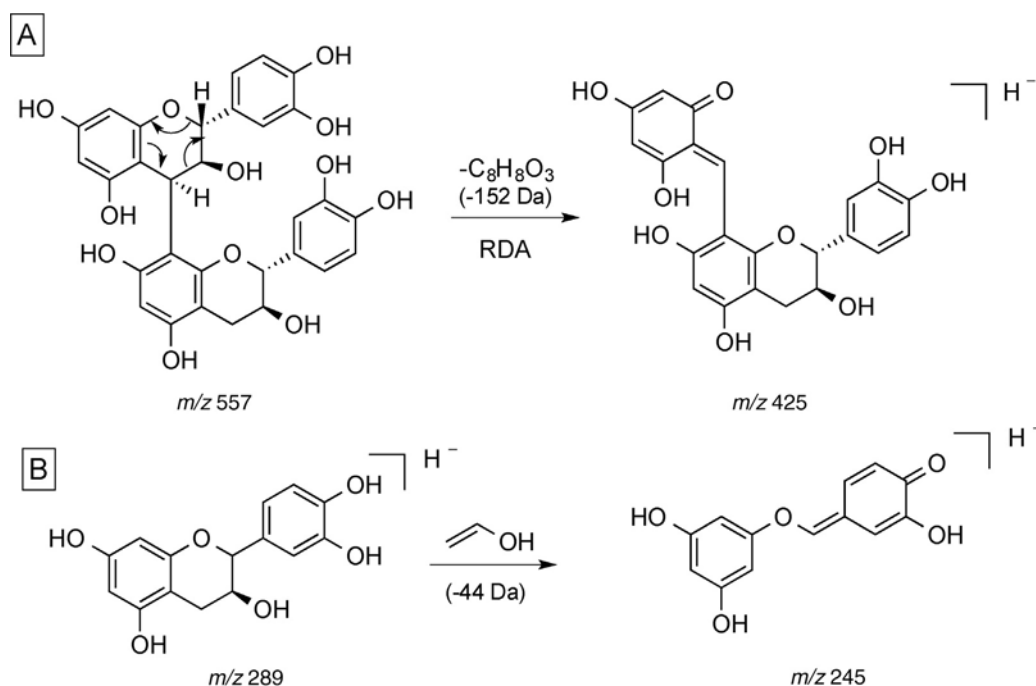


Fig. 1. Representative chromatograms obtained from: (A) blank plasma; (B) plasma spiked with 100 ng/ml of quercetin (IS), 100 ng/ml of procyanidin B2 (PB2) and 100 ng/ml of epicatechin (EPI). All the mass transitions are described in the figure legend.



Scheme 1. Fragmentation reactions of the precursor procyanidin B2 (A) and epicatechin (B) with the respective main fragments and the m/z for each form.

under 12-h light/12-h dark cycles and had food and water *ad libitum*.

A solution of SETc at 80 ± 8 mg/ml was prepared in glycerol:water (0.1:1) and administered to three animals orally (gavage) at the dose of 400 mg/kg, which corresponded to 2.67 ± 0.29 mg of **1** and 12.15 ± 0.62 mg of **2**. Samples of 300 μ l of blood, from the right tail vein, were collected in heparinized microtubes at times 5, 15, 30, 45, 60, 90, 120, 150 and 180 min after administration. Samples were immediately centrifuged at $7.5 \times g$ for 15 min at $4^\circ C$ and an aliquot of 100 μ l was transferred to another microtube. Next, 20 μ l of acetic acid (50 mmol/l) was added and the samples were kept at $-80^\circ C$ until HPLC–MS/MS analysis.

The plasma concentration data were used to plot plasma concentration (C_p) versus time (t) graphs, using Microsoft Excel (Microsoft, Seattle, WA, USA) and to calculate the non-compartmental pharmacokinetic parameters (Gibaldi and Perrier, 1982).

Results and discussion

Optimization of LC–MS/MS analysis

The chromatographic conditions for **1** and **2** analysis consisted on a mixture of methanol and 0.1% aqueous formic acid (50:50, v/v) as the mobile phase, as well as a C18 column as the stationary phase. The total ions chromatogram (TIC) presented a coelution of **1** and **2** with retention times at 1.10 and 1.01 min, respectively. However, both **1** and **2** mass chromatograms can be clearly distinguished from each other (Fig. 1). The IS presented an isolated peak at 2.47 min.

The detector parameters were initially assessed by direct infusion of EPI and PB2 standards solutions (one solution per substance)

into the electrospray source. Both substances showed better response to a negative ionization mode. EPI and PB2 exhibited the precursor ion at a m/z of 288.5 and 576.5 respectively, corresponding to the deprotonate molecule form $[M-H]^-$. After collision induced dissociation with argon, using 15 kV collision energy, the ion at m/z of 244.7 for **1** and 423.6 for **2** were the main fragment observed. The IS exhibited the precursor ion at m/z 300.5 $[M-H]^-$, and its fragmentation generated a fragment ion with m/z of 150.5. Thus, for MRM the ion pairs chosen for the **1**, **2** and IS were: m/z 288.5 \rightarrow 244.7, 576.5 \rightarrow 423.6 and 300.5 \rightarrow 150.5, respectively. Scheme 1 shows the fragmentation reaction of **2** and **1**.

Considering the heterogeneity of the plasma matrix, different solvents for sample pretreatment processes (extraction) were tested. The solvents methanol, acetonitrile, dichloromethane and ethyl acetate and their mixtures were evaluated to achieve maximal recovery of **1** and **2**. For all solvents tested, the same technique was applied, the one-step liquid–liquid extraction. The procedure was adequate to extract the analyte and clean up interferences. The best reproducibility and recovery was obtained using only ethyl acetate while other solvents did not achieve the same recoveries as obtained for ethyl acetate. After these results, ethyl acetate was defined as the solvent for recovery of **1** and **2** from plasma samples.

Method validation

Good selectivity and absence of plasma interference was observed (Fig. 1). The analytical method presented linearity ($r^2 = 0.9983$ for **1** and $r^2 = 0.9956$ for **2**). The concentration range was defined as 12.5–150 ng/ml for **1** and 5–150 ng/ml for **2** in rat plasma. The fitted linear equation was y (ng/ml) = $0.01x + 0.0496$ (ng/ml) for **1** and y (ng/ml) = $0.0041x + 0.0351$ (ng/ml) for **2**. The

Table 1
Accuracy and precision (intra and inter-day) of epicatechin (EPI) in rat plasma

Spiked concentration (ng/ml)	Mean observed concentration (ng/ml)	Accuracy (RE%)	Precision (RSD%)
<i>Intra-day (n=5)</i>			
12.5	14.31	114.46	0.75
20	20.25	101.24	0.50
50	47.93	95.86	2.76
100	100.70	100.70	1.23
<i>Inter-day (n=5x3 dias)</i>			
12.5	14.33	114.65	0.48
20	20.64	103.21	1.16
50	48.98	97.95	2.12
100	99.65	99.65	2.51

RSD% = relative standard deviation; RE% = relative error

Table 2
Accuracy and precision (intra and inter-day) of procyanidin B2 (PB2) in rat plasma

Spiked concentration (ng/ml)	Mean observed concentration (ng/ml)	Accuracy (RE%)	Precision (RSD%)
<i>Intra-day (n=5)</i>			
5	4.37	87.47	0.53
20	17.98	17.98	1.23
50	56.59	56.59	0.65
100	99.08	99.08	0.79
<i>Inter-day (n=5x3 dias)</i>			
5	4.66	93.21	1.15
20	20.67	103.33	1.57
50	53.91	107.81	1.60
100	106.23	106.23	3.34

RSD% = relative standard deviation; RE% = relative error

Table 3
Matrix effect and recovery of epicatechin and procyanidin B2 in rat plasma (n=3)

Concentration (ng/ml)	Matrix effect % (mean ± sd)	Recovery % (mean ± sd)
<i>Epicatechin</i>		
20	85.50 ± 0.62	106.44 ± 1.99
50	88.24 ± 0.56	101.37 ± 1.65
100	90.12 ± 1.18	104.80 ± 0.85
<i>Procyanidin B2</i>		
20	90.20 ± 0.45	71.70 ± 0.75
50	84.93 ± 0.55	72.24 ± 2.81
100	85.32 ± 1.08	74.68 ± 0.85

sd = standard deviation

method also showed good sensitivity: a LLOQ value of 12.5 ng/ml for **1** with precision of 0.48% and accuracy of 14.65% was verified. Meanwhile for **2**, the LLOQ value was 5 ng/ml with a precision of 1.15% and an accuracy of -6.79%.

Precision and accuracy are presented in Tables 1 and 2 for **1** and **2**, respectively. The accuracy of **1** within-day batches ($n=5$)

Table 4
Freeze and thaw, short-term and long-term stability of epicatechin and procyanidin B2 in rat plasma (n=3)

Concentration (ng/ml)	Freeze and thaw(RE%)	Short-term6h injector (RE%)	Short-term24h, -20°C (RE%)	Long-term2 months(RE%)
<i>Epicatechin</i>				
20	96.39	97.62	96.26	85.76
50	98.45	97.72	92.44	86.53
100	97.42	91.77	97.88	85.05
<i>Procyanidin B2</i>				
20	92.02	100.32	98.61	88.23
50	97.98	99.60	92.89	85.43
100	96.90	95.73	102.51	87.52

RE% = relative error

Table 5
Non-compartmental pharmacokinetic parameters of EPI and PB2 in plasma following oral administration of 400 mg/kg SETc in rats. EPI dose was 2.67 ± 0.29 mg and PB2 dose was 1.15 ± 0.62 mg. Data reported as arithmetic mean ± standard deviation (n=3)

Parameter (unit)	EPI	PB2
$t_{1/2}$ (min)	59.31 ± 20.27	110.10 ± 12.78
AUC _{0-t} (mg.min.l-1)	9.59 ± 3.58	17.80 ± 6.17
AUC _{0-∞} (mg.min.l-1)	10.83 ± 3.35	25.07 ± 6.70
Cl/F (L/min)	0.27 ± 0.12	0.064 ± 0.016
MRT (min)	130.75 ± 9.48	177.87 ± 14.77
C _{max} (mg/l)	0.054 ± 0.04	0.108 ± 0.046
T _{max} (min)	80 ± 35	53 ± 11

$t_{1/2}$ = half-life time, ASC_{0-t} = area under the curve from zero to t, ASC_{0-∞} = area under the curve from zero to infinite, Cl/F = clearance/bioavailability, MRT = mean residence time, C_{max} = maximum plasma concentration, T_{max} = maximum time

and between-day batches ($n=5 \times 3$ consecutive days) ranged from 95.86 to 101.24% and from 97.95 to 103.21%, respectively, as shown in Table 1.

The accuracy of **2** within-day batches ($n=5$) and between-day batches ($n=5 \times 3$ consecutive days) ranged from 89.92 to 113.18% and from 103.33 to 107.81%, respectively, as shown in Table 2.

During the evaluation of the matrix effect, the recoveries ranged from 85.5 to 90.2% of the initial spiked **1** and from 84.9 to 90.2% for spiked **2** (Table 3). The recoveries from plasma at concentrations of 20, 50 and 100 ng/ml were 106.44, 101.37 and 104.8%, respectively for **1**, and 71.70, 72.24 and 74.68%, respectively for **2**. For matrix effect and recovery, the validation guidelines have not yet established the acceptance values, but the consensus for matrix effect is no more than 15% of variation and for recovery is acceptable a value that presents reproducibility with precision and accuracy.

Compounds **1** and **2** were found to be stable under the studied short and long-term stability conditions (Table 4).

As far as we known, this is the first time an LC-MS/MS method was developed to quantify **1** and **2** simultaneously in a biological matrix.

Application of the method to a pharmacokinetic study

This was used to determine the preliminary noncompartmental pharmacokinetic parameters after oral administration of 400 mg/kg of SETc to rats, demonstrating its applicability to pharmacokinetic studies (Fig. 2 and Table 5).

The half-life values compared to the mean residence time (MRT) parameter indicate a high elimination rate of **1** and **2**. The clearance/bioavailability (Cl/F) ratios for **1** and **2** were 0.27 and 0.57 l/min, respectively, which corresponds to 1.35 and 2.85 l min⁻¹ kg⁻¹.

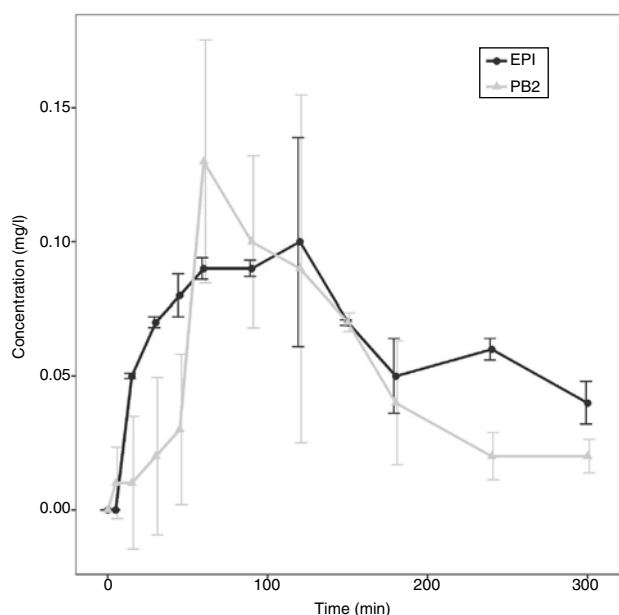


Fig. 2. Plasma pharmacokinetic profile of EPI and PB2 after oral administration of 400 mg/kg of standardized extract of *Trichilia catigua* (SETc, n = 3).

The clearance (Cl) of **1** ($1.351 \text{ min}^{-1} \text{ kg}^{-1}$) is similar to the Cl described in literature for rabbits after administration of isolated **1** ($1.31 \text{ min}^{-1} \text{ kg}^{-1}$) (Chen and Hsu, 2009). But for **2**, the Cl data described in the literature for rats after isolated and labeled **2** was 0.41 l/min (Chen and Hsu, 2009), seven times lower than the value observed in this study for **2** from SETc.

Conclusion

A rapid, efficient and selective LC–MS/MS method for simultaneous quantification of epicatechin (**1**) and procyanidin B2 (**2**) in rat plasma was developed and can be used to explore the pharmacokinetics of these compounds. The pharmacokinetic parameters described for **1** and **2** could support new studies with SETc to elucidate the pharmacokinetics and its relationship with the pharmacodynamic effect of this herbal product.

Ethical Disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Contributors

MC executed pre-clinical experiments and analytical experiments; PRG executed pre-clinical experiments; LL-S executed analytical procedures; ABB supported pharmacokinetic evaluation;

AOS, RL and JCPM produced the SETc and isolated compounds; EK was responsible for all analytical procedures, and AD was principal investigator of this work.

Conflicts of interest

The authors declare no conflicts of interest for this article.

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References

- Antunes, E., Gordo, W.M., de Oliveira, J.F., Teixeira, C.E., Hyslop, S., De Nucci, G., 2001. The relaxation of isolated rabbit corpus cavernosum by the herbal medicine Catuama and its constituents. *Phytother. Res.* 15, 416–421.
- Barbosa, N.R., Fischmann, L., Talib, L.L., Gattaz, W.F., 2004. Inhibition of platelet phospholipase A2 activity by catuaba extract suggests antiinflammatory properties. *Phytother. Res.* 18, 942–944.
- Beltrame, F.L., Filho, E.R., Barros, F.A.P., Cortez, D.A., Cass, Q.B., 2006. A validated higher-performance liquid chromatography method for quantification of cinchonain Ib in bark and phytopharmaceuticals of *Trichilia catigua* used as catuaba. *J. Chromatogr. A* 1119, 257–263.
- Campos, M.M., Fernandes, E.S., Ferreira, J., Santos, A.R.S., Calixto, J.B., 2005. Antidepressant-like effects of *Trichilia catigua* (catuaba) extract: evidence for dopaminergic-mediated mechanisms. *Psychopharmacology* 182, 45–53.
- Chassot, J.M., Longhini, R., Gazarini, L., Mello, J.C.P., de Oliveira, R.M.W., 2011. Pre-clinical evaluation of *Trichilia catigua* extracts on the central nervous system of mice. *J. Ethnopharmacol.* 137, 1143–1148.
- Chen, Y.-A., Hsu, K.-Y., 2009. Pharmacokinetics of (–)-epicatechin in rabbits. *Arch. Pharm. Res.* 32, 149–154.
- Espada, S.F., Faccin-Galhardi, L.C., Rincão, V.P., Bernardi, A.L.S., Lopes, N., Longhini, R., de Mello, J.C.P., Linhares, R.E., Nozawa, C., 2015. Antiviral activity of *Trichilia catigua* bark extracts for herpesvirus and poliovirus. *Curr. Pharm. Biotechnol.* 16, 724–732.
- FDA, 2013. Guidance for Industry Bioanalytical Method Validation. U.S. Department of Health and Human Services Food and Drug Administration, Rockville.
- Gibaldi, M., Perrier, D., 1982. *Pharmacokinetics*. Marcel Dekker, New York, NY, pp. 494.
- Jabor, V.A.P., Soares, D.M., Diniz, A., de Souza, G.E.P., Lopes, N.P., 2010. LC–MS–MS identification and determination of the flavone-C-glucoside vicenin-2 in rat plasma samples following intraperitoneal administration of *Lychnophora* extract. *Nat. Prod. Commun.* 5, 741–745.
- Lachi-Silva, L., Sousa, J.P.B., Montanha, M.C., Sy, S.K.B., Lopes, J.L.C., da Silva, D.B., Lopes, N.P., Diniz, A., Kimura, E., 2015. Rapid and efficient method for the quantification of lychnopholide in rat plasma by liquid chromatography-tandem mass spectrometry (LC–MS/MS) for pharmacokinetic application. *Biomed. Chromatogr.* 30, 1092–1096.
- Longhini, R., Klein, T., Bruschi, M.L., da Silva, W.V., Rodrigues, J., Lopes, N.P., de Mello, J.C.P., 2013. Development and validation studies for determination of phenylpropanoid-substituted flavan-3-ols in semipurified extract of *Trichilia catigua* by high-performance liquid chromatography with photodiode array detection. *J. Sep. Sci.* 36, 1247–1254.
- Lonni, A.A.S.G., Longhini, R., Lopes, G.C., de Mello, J.C.P., Scarmínio, I.S., 2012. Statistical mixture design selective extraction of compounds with antioxidant activity and total polyphenol content from *Trichilia catigua*. *Anal. Chim. Acta* 719, 57–60.
- Lopes, G.C., Bruschi, M.L., Mello, J.C.P., 2009. RP–LC–UV determination of proanthocyanidins in *Guazuma ulmifolia*. *Chromatographia* 69 (S2), 175–181.
- Matsuoka, Y., Hasegawa, H., Okuda, S., Muraki, T., Urano, T., Kubota, K., 1995. Ameliorative effects of tea catechins on active oxygen-related nerve cell injuries. *J. Pharmacol. Exp. Ther.* 274, 602–608.
- Pizzolatti, M.G., Koga, A.H., Grisard, E.C., Steindel, M., 2003. Trypanocidal activity of extracts from Brazilian atlantic rain forest plant species. *Phytomedicine* 10, 422–426.
- Pizzolatti, M.G., Venson, A.F., Smânia, A., Smânia, E., de, F.A., Braz-Filho, R., 2002. Two epimeric flavalignans from *Trichilia catigua* (Meliaceae) with antimicrobial activity. *Z. Naturforsch. C* 57, 483–488.
- Pontieri, V., Neto, A.S., de França Camargo, A.F., Koike, M.K., Velasco, I.T., 2007. The herbal drug Catuama reverts and prevents ventricular fibrillation in the isolated rabbit heart. *J. Electrocardiol.* 40, 534, e1–534.e8.
- Resende, F.O., Rodrigues-Filho, E., Luftmann, H., Petereit, F., Mello, J.C.P., 2011. Phenylpropanoid substituted flavan-3-ols from *Trichilia catigua* and their *in vitro* antioxidative activity. *J. Braz. Chem. Soc.* 22, 2087–2093.

- Sakano, K., Mizutani, M., Murata, M., Oikawa, S., Hiraku, Y., Kawanishi, S., 2005. Pro-cyanidin B2 has anti- and pro-oxidant effects on metal-mediated DNA damage. *Free Rad. Biol. Med.* 39, 1041–1049.
- Schroeter, H., Williams, R.J., Matin, R., Iversen, L., Rice-Evans, C.A., 2000. Phenolic antioxidants attenuate neuronal cell death following uptake of oxidized low-density lipoprotein. *Free Rad. Biol. Med.* 29, 1222–1233.
- Taciany Bonassoli, V., Micheli Chassot, J., Longhini, R., Milani, H., Mello, J.C.P., de Oliveira, R.M.W., 2012. Subchronic administration of *Trichilia catigua* ethyl-acetate fraction promotes antidepressant-like effects and increases hippocampal cell proliferation in mice. *J. Ethnopharmacol.* 143, 179–184.
- Tang, W., Hioki, H., Harada, K., Kubo, M., Fukuyama, Y., 2007. Antioxidant phenylpropanoid-substituted epicatechins from *Trichilia catigua*. *J. Nat. Prod.* 70, 2010–2013.
- Truiti, M.T., Soares, L., Longhini, R., Milani, H., Nakamura, C.V., Mello, J.C.P., de Oliveira, R.M.W., 2015. *Trichilia catigua* ethyl-acetate fraction protects against cognitive impairments and hippocampal cell death induced by bilateral common carotid occlusion in mice. *J. Ethnopharmacol.* 172, 232–237.