Reversed-phase-liquid chromatography method for separation and quantification of gallic acid from hydroalcoholic extracts of *Qualea grandiflora* and *Qualea parviflora*

Mariana L. de Mesquita[1,2,3], Waleska F. Leão[2,3], Magda R. A. Ferreira[2], José E. de Paula[4], Laila S. Espindola[1], Luiz A. L. Soares[2,3]

[1]Laboratory of Pharmacognosy, University of Brasília – UnB, Brasília-DF, [2]Post Graduate Programme in Therapeutic Innovation, Federal University of Pernambuco – UFPE, Recife-PE, [3]Pharmacognosy Laboratory, Federal University of Pernambuco – UFPE, Recife-PE, [4]Plant Anatomy Laboratory, University of Brasília – UnB; Brasília-DF, Brazil

Submitted: 26-06-2013 Revised: 17-08-2013 Published: 24-09-2015

ABSTRACT

Background: *Qualea parviflora* and *Qualea grandiflora* (Vochysiaceae), commonly known in Brazil as "pau-terra" and "pau-terrinha," respectively, have been widely used in the treatment of ulcer and gastritis. These therapeutic effects are attributed to various compounds present in the plants, including phenolic compounds such as gallic acid, due to their important antioxidant activity. **Objective:** The aim of the present study was to validate a high performance liquid chromatography with diode array detection (HPLC-DAD) method for the quantitative determination of gallic acid in the stem bark of *Q. parviflora* and *Q. grandiflora* hydroalcoholic extracts. **Materials and Methods:** The chromatography analysis was successfully achieved on a Dionex column, Acclaim® 120 (250 mm × 4.60 mm, 5 µm) with a gradient elution of water and methanol at a flow rate of 0.8 mL/min and ultraviolet detection at 280 nm. **Results:** The validation data, including linearity, precision, specificity, accuracy and robustness of this method demonstrated good reliability and sensitivity. **Conclusion**: The method is able to quantify gallic acid in the stem bark of both species. What is more, the chromatographic peaks showed good resolution and there are also the advantages of easy sample preparation and a short time between each injection.

Key words: Gallic acid, high-performance liquid chromatography with diode array detection, *Qualea grandiflora, Qualea parviflora*, validation

Access this article online

Website: www.phcog.com

DOI: 10.4103/0973-1296.166062

Quick Response Code:

INTRODUCTION

Plants provide a potential source of important molecules.[1,2] The Brazilian Cerrado biome is the second largest ecological area after Amazonia, with approximately 2 million Km², and 44% is comprised of endemic flora.[3] Species found in the Cerrado biome have been used by local traditional communities to treat various diseases,[4,5] but despite this there are very few studies that highlight the quality control of those plants.

Address for correspondence:
Dr. Luiz A. L. Soares, Pharmacognosy Laboratory, Department of Pharmaceutical Sciences, Federal University of Pernambuco – UFPE.
Prof. Arthur de Sá, s/n, Cidade Universitária, 50740-521 Recife-PE, Brazil.
E-mail: lals@pq.cnpq.br

This study focuses on *Qualea parviflora* (pau-terra, pau-ferro, pau-de-tucano) and *Qualea grandiflora* (cinzeiro, boizinho, pau-terrinha), Brazilian Cerrado trees of the Vochysiaceae family with a large distribution in Brazil.[5] The genera *Qualea* sp. (leaves and bark) are widely used in folk medicine[6] to treat diarrhea with blood, intestinal colic, amebiasis,[7] skin diseases and inflammatory processes,[8,9] but specially ulcers and gastritis.[7,10,11]

According to recent pre-clinical pharmacological studies, the methanolic extract from *Q. parviflora* bark has gastroprotective, antidiarrheal, antispasmodic, and intestinal anti-inflammatory effects, and is an effective anti-ulcerogenic and antimicrobial treatment with no detectable acute toxic effects.[12,13] The oral administration of the hydroalcoholic extract of the bark of *Q. grandiflora* was investigated for its ability to prevent and heal lesions in the gastric mucosa as it shows an important antiulcer activity.[14]

The chemical composition of *Qualea* genus plants mainly includes triterpenes, flavonoids and phenolic compounds such as pyrogallic and catequinic tannins and phenolic acids like ellagic acid derivatives and gallic acid.[6,14]

Gallic acid is a phenolic compound that is a secondary metabolite in a variety of plant roots and stems. Several reports indicate that it has anti-cancer, anti-inflammatory, cardioprotective, antioxidant, anti-diabetic and anti-ulcer properties.[15-18]

Pharmacological evidences indicate a correlation between the presence of gallic acid in plants and its antiulcer activity.[19,20] The mechanism of action in the treatment and prevention of the gastric ulcer has been studied, and it seems that its antioxidant properties, immunomodulator proteins and inhibition of mitochondrial apoptosis are related to this pharmacological activity.[19] Therefore, gallic acid was proposed as a phytochemical marker for *Qualea*, owing to its biological properties and presence in *Qualea* species.[6,21]

There are several reports regarding the use of HPLC to quantify gallic acid in different herbal matrices such as juice and beverages;[22,23] commercial teas;[24] and traditional species,[25,26] however, few of them reported validated methods to identify and quantify this phytochemical marker in Cerrado plants.[27,28]

This study is incorporated into a research group program that aims for the valorization of the Brazilian Cerrado biome plants, the biome being well known as a biodiversity hotspot.[29,30] The first phytopharmaceutical from this biome was approved in 2009 by the Brazilian Health Surveillance Agency. The product is an ointment containing the dry extract from *Stryphnodendron adstringens* barks, whose chemical makers are total tannins. In spite of the relevance of the two species of *Qualea* (*Q. parviflora* and *Q. grandiflora*) for the traditional treatment of several gastrointestinal disorders, the lack of specifications for their quality control remains a key challenge for the development of new herbal-based medicines from the Cerrado bioma.

Thus, in the present study, a simple and fast high performance liquid chromatography with diode array detection (HPLC-DAD) method was developed and validated for the qualitative and quantitative determination of gallic acid in hydroalcoholic extracts from stem barks of *Q. parviflora* and *Q. grandiflora*. The quantification of the marker will provide the scientific basis for the quality standardization to support the technological and clinical developments of phytopharmaceuticals from this Cerrado species.

MATERIALS AND METHODS

Materials

The methanol employed in the experiments was HPLC grade (J. T. Baker, Phillipsburg, NJ, USA) and was filtered through a 0.45 μm membrane filter prior to use. Ultrapure water was obtained by passing redistilled water through a Direct-Q UV3 system, Millipore (Billerica, MA, USA).

Plant material

Q. grandiflora and *Q. parviflora* (Vochysiaceae) were collected in the Cerrado biome, around Brasília, Distrito Federal, Brazil, in 2012. The species were identified by botanist Prof. José Elias de Paula. The voucher herbarium specimens (*Q. grandiflora*-UB 3746; *Q. parviflora*, UB 3742) were deposited in the University of Brasília (UB/UnB) Herbarium.

Preparation of plant extracts

Dried and powdered stem barks of *Q. parviflora* and *Q. grandiflora* were submitted to successive exhaustive extractions with a hydroalcoholic solution through a maceration process. The crude extracts of *Qualea parviflora* hydroalcoholic (QP) and *Qualea grandiflora* hydroalcoholic (QG), respectively, were obtained after the complete evaporation of the solvents under reduced pressure at 40°C as previously described.[31] The extracts were stored at −20°C.

Instrumentation and chromatographic conditions

High-performance liquid chromatography analyzes were conducted on an Ultimate 3000, Thermo Scientific® system (Texas, USA) equipped with a degasser, a DAD, and an automatic injector with a 20 μL loop. HPLC data acquisition was accomplished with Chromeleon software. Validation of the method was performed using a Dionex column, Acclaim® 120 (particle diameter 5 μm, 250 × 4.60 mm) equipped with a pre-column of octadecylsilanized silica (5 μm). The mobile phase consisted of water (+0.05% trifluoroacetic acid [TFA]) as solvent A and methanol (+0.05% TFA) as solvent B, with the linear gradient as follows: 0 min 15% B; 12 min 40% B; 14 min 74% B; 16 min 15% B; 18 min 15% B. The total analysis time was 18 min. The solvent flow was kept constant at 0.8 mL/min during the whole analysis. Ultraviolet (UV) spectra were recorded between 190 and 400 nm, and the detection was accomplished at 280 nm.

Validation of the method

Validation of the analytical method was performed according to the validation guide for analytical and bioanalytical methods published by National Agency

of Sanitary Vigilance-RE nº 899-2003,[32] and the ICH guideline specifications.[33]

Specificity

The specificity was determined by analysis of chromatograms of gallic acid and the solutions of QP and QG extracts. The DAD detector in association with the software resource provided by the workstation and co-elution of the standard and the extracts were employed to confirm the purity across the peak at a retention time corresponding to that of gallic acid.

Calibration curves of the standard

An external standard method was utilized to construct the analytical curve for gallic acid measured at a wavelength of 280 nm. A stock solution of the standard was prepared by weighing 1.0 mg of gallic acid (purity 99%) and dissolving it in 50 mL of water to give a final concentration of 0.02 mg/mL. Calibration curves of the five extract solutions, with concentrations of 0.9, 1.0, 1.1, 1.2, and 1.3 mg/mL of QP and 0.2, 0.21, 0.22, 0.23, and 0.24 mg/mL of QG, were prepared with appropriate dilution of the stock solution (2 mg/mL). Each of the five solutions was injected into the HPLC in triplicate, and the regression equation determined.

Repeatability and intermediate precision

Repeatability was estimated by assaying solutions of QP and QG at 1 mg/mL; each analysis being carried out in sextuplicate by the same operator on the same day. The chromatograms were registered and peak heights were measured. Intermediate precision was determined by analyzing, in triplicate, solutions of QP and QG at the same concentration employed in the repeatability test, on two consecutive days and by two different operators. Precision and repeatability were confirmed by the relative standard deviation (RSD) value of the intra and interday assays. The RSD was calculated by the standard deviation over the measured amount multiplied by 100.

Accuracy

The accuracy was determined by the recovery test, and was examined by adding to the main solution of the extracts at 1 mg/mL three solutions of gallic acid, at concentrations of 1.0, 1.1, and 1.2 μg/mL for QP and 2.1, 2.2 and 2.3 μg/mL for QG. Three replicate analyzes were performed.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated on the basis of the data of the regression of the analytical curve of gallic acid for QP and QG. The equations below were used to do the calculations, as required by Anvisa and recognized by ICH.[32,33]

$$LD = 3.3 \times \left(\frac{S}{I}\right) \qquad LQ = 10 \times \left(\frac{S}{I}\right)$$

S = Standard deviation for the intercept with axis Y e I = slope of the curve.

Robustness

Analyses were carried out with different flow rates of the mobile phase (0.79 and 0.81 mL/min), with different pH values of the mobile phase ranging from 2 to 4 for solvent A and from 4 to 6 for solvent B, and with different column temperatures (24 and 27°C), in order to estimate the robustness of the method.

Statistical analysis

The individual data were grouped following each experiment. The mean with the respective deviation was used as a measurement of the central tendency and dispersion (RSD). The data were submitted to statistical analysis using Excel® software.

RESULTS AND DISCUSSION

The specificity of the analytical method was confirmed using the DAD workstation software resource for the standard analyte and for each of the assayed extracts,[32] as well as by the same retention time values for the sample and the standard (8 min). The presence of gallic acid was confirmed by the retention time and UV–Vis spectra obtained from authentic samples, at λmax 271.5 nm [Figure 1].

Regarding the peak of gallic acid in both samples, the data revealed that the phenolic acid showed λmax at 271.4 nm and 270 nm for extracts from QP and QG, respectively.

From the analytical curve constructed with gallic acid as standard, the response of the UV detector at 270 nm was found to be linear in the range of 0.9-1.3 mg/mL for QP and 0.2-0.24 mg/mL for QG. The regression analysis providing the linear equation was y = 1.2451x + 0.1144 with a determination coefficient (R^2) of 0.9996 for QP; and the linear model y = 2.3602x + 0.4942 with a determination coefficient (R^2) of 0.9996 for QG.

The assay of accuracy was performed to evaluate the matrix interference. The recoveries of gallic acid from extract solutions were 106.09, 105.93, and 106.19% for QP and 105.02, 104.58 and 103.69% for QG in respect to each of the three concentrations analyzed. The results are within the acceptable limit required for the Brazilian Health Surveillance Agency (80–120%),[32] and demonstrated low interference of the extracts in the recovery of gallic acid.

Precision was evaluated through the determination of the repeatability and intermediary precision. In terms of repeatability the RSD values obtained were 1.04% for QP and 3.61% for QG. According to the RSD values obtained from peak retention times and areas during the tests, it could be interpreted that the system showed a satisfactory response with RSD <5%, as recommended by the literature.[32,33]

The assay of intermediary precision was performed in order to determine the accumulation of the random errors between different operators and days. The RSD values obtained from both peak areas and retention times of gallic acid were lower than 5%, demonstrating that there were no statistical differences between operators and days of experiments. The method showed high reproducibility and thus demonstrated low interference of the sample preparation variables such as days and operators [Table 1].

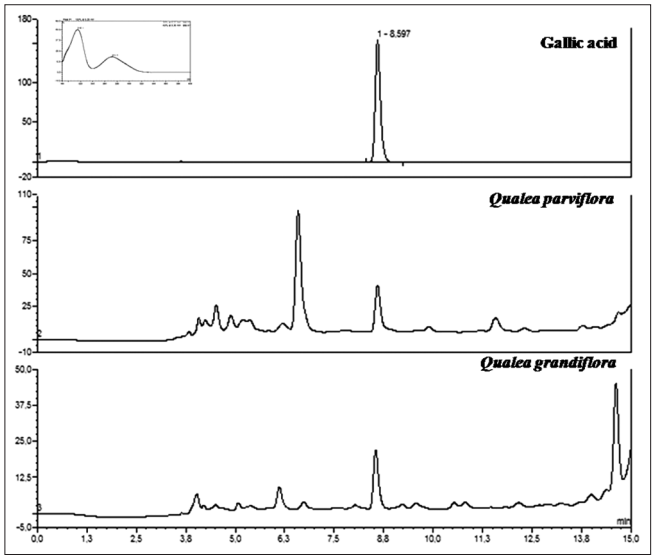

Figure 1: High performance liquid chromatography with diode array detection chromatograms and on-line ultraviolet (UV) spectrum of the standard of gallic acid, *Qualea parviflora* hydroalcoholic extract and *Qualea grandiflora* hydroalcoholic extract; chromatographic conditions: Dionex, Acclaim® 120 (particle diameter 5 mm, 250 × 4.60 mm); gradient of 5% trifluoroacetic acid in water (solvent A) and methanol (solvent B), 0–10 min 0–15% B; to 12 min 40% B; to 14 min 74% B; to 16 min 15% B; to 18 min 15% B at a flow rate of 1.0 mL/min; UV detection between 190 and 400 nm

The LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantified. The LOD values were found to be 0.1339 and 0.0288 mg/mL for QP and QG mg/mL, respectively. The LOQ was defined as the lowest concentration that can be determined with acceptable accuracy and precision at a signal-to-noise ratio of 10. LOQ values were 0.4464 and 0.0960 mg/mL, respectively.

The test of robustness showed that the method was insensitive to small variations in the flow rate, pH of the mobile phase and temperature of the oven, as demonstrated in Table 2. No significant variations, as shown by the RSDs, were found between analyzes conducted using different batches of chromatographic columns, showing that the method is robust.

CONCLUSION

Until today, the antiulcerogenic effects of *Q. grandiflora* and *Q. parviflora* were not attributed to any specific active compound. However, several biological investigations have shown that gallic acid displays important antioxidant properties related to antiulcerogenic activity. The validation assay of the proposed LC-method demonstrated its suitability for separation and quantification of gallic acid in crude hydroalcoholic extracts from stem barks of both

Table 1: Precision data of *Q. Parviflora* and *Q. Grandiflora* assessed in triplicate on two consecutive days and by two different operators

	QP		QG	
	Day 1	Day 2	Day 1	Day 2
Operator 1	0.105±0.0008 (0.76)	0.108±0.0025 (2.27)	0.203±0.0020 (0.98)	0.205±0.0012 (0.57)
Operator 2	0.108±0.0010 (0.96)	0.107±0.0030 (2.78)	0.200±0.0050 (2.52)	0.200±0.0013 (0.66)

QP: *Q. Parviflora*; QG: *Q. Grandiflora*

Table 2: Robustness of the method for *Q. parviflora* and *Q. grandiflora*

	Flow (mL/min)		Temperature (oC)		pH	
	0.79	0.81	24	27	2/4	4/6
QP	0.106±0.0035 (3.28)	0.106±0.0004 (0.34)	0.101±0.0017 (1.66)	0.102±0.0037 (3.67)	0.105±0.0006 (0.59)	0.106±0.0004 (0.40)
RSD (%)	0.69		0		0.67	
QG	0.201±0.0088 (4.37)	0.213±0.0010 (0.46)	0.206±0.0053 (2.56)	0.209±0.0016 (0.75)	0.212±0.0039 (1.82)	0.209±0.0037 (1.78)
RSD (%)	4.10		1.02		1.01	

QP: *Q. Parviflora*; QG: *Q. Grandiflora*; RSD: Relative standard deviation

Qualea species. No significant variations in peak areas or retention times were detected throughout the method evaluation. Thus, it can be concluded that the HPLC-DAD method is simple, rapid and precise and can be used to qualify and standardize phytotherapeutic agents containing these Brazilian traditional herbals.

ACKNOWLEDGMENTS

The authors thank CNPq (480128/2012-0, 302113/2012-6) and FACEPE (IBPG-0423-4.03/11, APQ-1296-4.03/12). The authors were also grateful to Andrew Alastair Cumming for editing this paper.

REFERENCES

1. da Costa RC, Santana DB, Araújo RM, de Paula JE, do Nascimento PC, Lopes NP, *et al.* Discovery of the rapanone and suberonone mixture as a motif for leishmanicidal and antifungal applications. Bioorg Med Chem 2014;22:135-40.
2. Carneiro Albernaz L, Deville A, Dubost L, de Paula JE, Bodo B, Grellier P, *et al.* Spiranthenones A and B, tetraprenylated phloroglucinol derivatives from the leaves of *Spiranthera odoratissima*. Planta Med 2012;78:459-64.
3. Klink CA, Machado RB. A conservação do Cerrado brasileiro. Megadiversidade 2005;1:147-55.
4. Veloso Cde C, de Oliveira MC, Oliveira Cda C, Rodrigues VG, Giusti-Paiva A, Teixeira MM, *et al.* Hydroethanolic extract of *Pyrostegia venusta* (Ker Gawl.) Miers flowers improves inflammatory and metabolic dysfunction induced by high-refined carbohydrate diet. J Ethnopharmacol 2014;151:722-8.
5. Rios MN, Pastore Junior F. Plantas da Amazônia: 450 espécies de uso geral. Universidade de Brasília; 2011. p. 3361-7.
6. Neto FC, Pilon AC, Silva DH, Da Silva Bolzani V, Castro-Gamboa I. Vochysiaceae: Secondary metabolites, ethnopharmacology and pharmacological potential. Phytochem Rev 2011;10:413-29.
7. Rodrigues VE, Carvalho, D. A . Etnobotanical Survay of Medicinal Plants in the Dominion of Meadows in the Region of the Alto Rio Grande – Minas Gerais. Ciên Agrotec 2001;25:102-23.
8. de Toledo CE, Britta EA, Ceole LF, Silva ER, de Mello JC, Dias Filho BP, *et al.* Antimicrobial and cytotoxic activities of medicinal plants of the Brazilian cerrado, using Brazilian cachaça as extractor liquid. J Ethnopharmacol 2011;133:420-5.
9. Costa ES, Hiruma-Lima CA, Lima EO, Sucupira GC, Bertolin AO, Lolis SF, *et al.* Antimicrobial activity of some medicinal plants of the Cerrado, Brazil. Phytother Res 2008;22:705-7.
10. Bonacorsi C, da Fonseca LM, Raddi MS, Kitagawa RR, Vilegas W. Comparison of Brazilian Plants Used to Treat Gastritis on the Oxidative Burst of *Helicobacter pylori*-Stimulated Neutrophil. Evid Based Complement Alternat Med 2013;2013:851621.
11. Bonacorsi C, da Fonseca LM, Raddi MS, Kitagawa RR, Sannomiya M, Vilegas W. Relative antioxidant activity of Brazilian medicinal plants for gastrointestinal diseases. J Med Plants Res 2011;5:4511-8.
12. Mazzolin LP, Kiguti LR, da Maia EO, Fernandes LT, da Rocha LR, Vilegas W, *et al.* Antidiarrheal and intestinal antiinflammatory activities of a methanolic extract of *Qualea parviflora* Mart. in experimental models. J Ethnopharmacol 2013;150:1016-23.
13. Mazzolin LP, Nasser AL, Moraes TM, Santos RC, Nishijima CM, Santos FV, *et al. Qualea parviflora* Mart.: An integrative study to validate the gastroprotective, antidiarrheal, antihemorragic and mutagenic action. J Ethnopharmacol 2010;127:508-14.
14. Hiruma-Lima CA, Santos LC, Kushima H, Pellizzon CH, Silveira GG, Vasconcelos PC, *et al. Qualea grandiflora*, a Brazilian "Cerrado" medicinal plant presents an important antiulcer activity. J Ethnopharmacol 2006;104:207-14.
15. Li D, Liu Z, Zhao W, Xi Y, Niu F. A straightforward method to determine the cytocidal and cytopathic effects of the functional groups of gallic acid. Process Biochem 2011;46:2210-14.
16. Kam A, Li KM, Razmovski-Naumovski V, Nammi S, Chan K, Li GQ. Gallic acid protects against endothelial injury by restoring the depletion of DNA methyltransferase 1 and inhibiting proteasome activities. Int J Cardiol 2014;171:231-42.
17. Bhadoriya U, Sharma P, Solanki SS. *In vitro* free radical scavenging activity of gallic acid isolated from *Caesalpinia decapetala* wood. Asian Pac J Trop Dis 2012;2:S833-6.
18. Ho HH, Chang CS, Ho WC, Liao SY, Wu CH, Wang CJ. Anti-metastasis effects of gallic acid on gastric cancer cells involves inhibition of NF-kappaB activity and downregulation of PI3K/AKT/small GTPase signals. Food Chem Toxicol 2010;48:2508-16.
19. Abdelwahab SI. Protective mechanism of gallic acid and its novel derivative against ethanol-induced gastric ulcerogenesis: Involvement of immunomodulation markers, Hsp70 and Bcl-2-associated X protein. Int Immunopharmacol 2013;16:296-305.
20. Nanjundaiah SM, Annaiah HN, Dharmesh SM. Gastroprotective Effect of Ginger Rhizome (*Zingiber officinale*) Extract: Role of Gallic Acid and Cinnamic Acid in H(+), K(+)-ATPase/*H. pylori* Inhibition and Anti-Oxidative Mechanism. Evid Based Complement Alternat Med 2011;2011:249487.
21. Sousa CM, Silva HR, Vieira GM Jr, Ayres MC, Da Costa CL, Araújo DS, *et al*. Total phenolics and antioxidant activity of five medicinal plants. Quím Nova 2007;30:351-5.
22. Vichapong J, Santaladchaiyakit Y, Burakham R, Srijaranai S. Cloud-point extraction and reversed-phase high performance liquid chromatography for analysis of phenolic compounds and their antioxidant activity in Thai local wines. J Food Sci Technol 2014;51:664-72.
23. Chen H, Zuo Y, Deng Y. Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography. J Chromatogr A 2001;913:387-95.
24. Zuo Y, Chen H, Deng Y. Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector. Talanta 2002;57:307-16.
25. Arceusz A, Wesolowski M. Quality consistency evaluation of *Melissa officinalis L*. commercial herbs by HPLC fingerprint and quantitation of selected phenolic acids. J Pharm Biomed Anal 2013;83:215-20.
26. De Souza TP, Holzschuh MH, Lionço MI, González Ortega G, Petrovick PR. Validation of a LC method for the analysis of phenolic compounds from aqueous extract of *Phyllanthus niruri* aerial parts. J Pharm Biomed Anal 2002;30:351-6.
27. Carvalho MG, Freire FD, Raffin FN, Aragão CF, Moura TF. LC determination of gallic acid in preparations derived from *Schinus terebinthifolius* Raddi. Chromatographia Suppl 2009;69:S249-53.
28. Lopes GC, Sanches AC, Toledo CE, Isler AC, Mello JC. Quantitative determination of tannins im three species of

Stryphnodendron by high performance liquid chromatography. Braz J Pharm Sci 2009;45:135-43.

29. de Assis PA, Theodoro PN, de Paula JE, Araújo AJ, Costa-Lotufo LV, Michel S, *et al*. Antifungal ether diglycosides from *Matayba guianensis* Aublet. Bioorg Med Chem Lett 2014;24:1414-6.
30. Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J. Biodiversity hotspots for conservation priorities. Nature 2000;403:853-8.
31. de Mesquita ML, de Paula JE, Pessoa C, de Moraes MO, Costa-Lotufo LV, Grougnet R, *et al*. Cytotoxic activity of Brazilian Cerrado plants used in traditional medicine against cancer cell lines. J Ethnopharmacol 2009;123:439-45.
32. ANVISA. Resolution - RE n. 899: Guide for Validation of Analytical and Bioanalytical Methods; 2003.
33. ICH-International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Q2 (R1): Validation of Analytical Procedures: Text and Methodology; 2005.

Cite this article as: de Mesquita ML, Leão WF, Ferreira MR, de Paula JE, Espindola LS, Soares LA. Reversed-phase-liquid chromatography method for separation and quantification of gallic acid from hydroalcoholic extracts of *Qualea grandiflora* and *Qualea parviflora*. Phcog Mag 2015;11:316-21.

Source of Support: Nil, **Conflict of Interest:** None declared.