Int. J. Electrochem. Sci., 11 **(2016)** 9519 – 9528, doi: 10.20964/2016.11.31

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Electrochemical Behavior of Crude Extract of *Brosimum gaudchaudii* and Its Major Bioactives, Psoralen and Bergapten

Morgana Fernandez Alecrim[1], *Germán Sanz Lobón*,[1,2], *Rúbia D'Arc Machado*[1], *Edilson Ribeiro de Oliveira Júnior*[1], *Ruiter Lima Morais*[1], *Kênnia Rocha Rezende*[1], *Boniek Vaz Gontijo*[2], *Wallans Torres Pio dos Santos*[3], *Eric de Souza Gi1*[1*]

[1]Faculdade de Farmácia and [2]Instituto de Química, Universidade Federal de Goiás, Rua 240 com 5a avenida, Setor Universitário, Goiânia, Goiás, Brasil
[3]Departamento de Farmácia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Campus JK, 39100-000 Diamantina, MG, Brasil
[*]E-mail: ericsgil@ufg.br

Received: 14 July 2016 / *Accepted:* 2 September 2016 / *Published:* 10 October 2016

Roots from *Brosimum gaudchaudii* Trécul (Moraceae), popularly known as Mama-Cadela, is a traditional medicinal shrub from Brazilian "Cerrado" widely used in the photochemotherapy of vitiligo. Their main actives are the prenylated coumarins, Psoralen (Ps) and bergapten (Bp), which under UV exposure generate active compounds that form adducts with DNA. Though such reactions may involve electron transfer reactions, the redox behavior of BGT and their major isolated furanocoumarins, remain unknown. Therefore the aim of this study was to evaluate the electrochemical behavior of Ps, Bp and crude extracts of BGT by means of voltammetry. The cyclic voltammograms performed for Bp and Ps, confirmed the electroactivity only for Bp, which presented a anodic peak potential at E_{p1a} = 1.2 V. Similar behavior was observed for the BGT crude extract. In turn, from the differential pulse voltammograms (DPV), it was observed two small anodic peaks, 1a and 2a at lower peak potentials, which are consistent with hydroquinone and catechol like compounds. Also, it was observed that the main anodic peak occurred close to the Bp peak potential, 1a, whereas no peak was observed for Ps. Therefore, from voltammetric assays, it could be inferred that Bp is present in BGT crude extracts, which were corroborated by HPLC coupled to mass spectrometry. An cheaper and simpler electroanalytical procedure was proposed in order to estimate the amount of Bp in BGT, which despite the low repeatability presented comparable recovery compared to HPLC-PDA.

Keywords: Bergapten, Psoralen, Vitiligo, Psoriasis, *Mama-cadela*

1. INTRODUCTION

Psoriasis and vitiligo are two common skin diseases, both of autoimmune etiology, which have worldwide incidence of c.a. 2.5 % and 0.8 % respectively [1]. Psoriasis is caused by hyperproliferation and abnormal differentiation of epidermal cells, that may affect the skin, mucous membranes and eventually the joints [2,3]. Yet the vitiligo is mainly a consequence of an autoimmune attack of melanocytes, leading to a progressive loss of pigmentation, forming hypochromic spots on the skin, which are more obvious in sun-exposed areas [3,4]. The exact pathogenesis of both diseases is unknown, but it is widely accepted that the genetic predisposition is started by environmental stressing factors [4].

The impact of the related symptoms of both diseases reaches a psychosocial level, meanwhile the currently available treatments are only limited on the improvement of life quality of patients. Moreover, the handling of these disorders involves a combination therapies, in which the severity and extension of the lesions, as well as, the age of patience dictates the therapeutic procedures.

The care of vitiligo employs the use of steroids or immunomodulators of topical use, which are the most suitable for recent and less extensive injuries. For the treatment of psoriasis symptoms, the use of topical medications based on anthralin, vitamin D3 and steroids into the affected area is the first option [1-5].

The photochemotherapy, also called PUVA therapy, in which topical or systemic use of Psoralens are combined to ultraviolet A (UVA), have been used for both dermatitis [4-6].

The most promissing psoralens in PUVA therapy are the prenylated furocoumarins, 5-methoxypsoralen (bergapten, Bp) and 7H-furo[3,2-g]chromen-7-one (psoralen or psoralene, Ps), Figure 1. These furanocoumarins are obtained mainly from the roots of *Brosimumgaudichaudii Trécul* (BGT), popularly known as “Mama-Cadela”, a medicinal bush of Brazilian "Cerrado" [7,8].

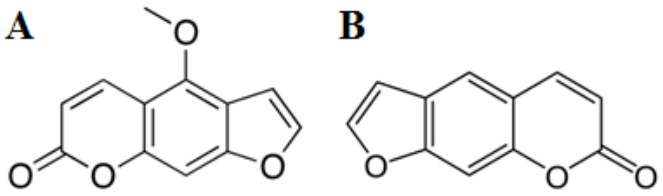

Figure 1. Chemical structure of the furanocoumarins, Bergapten (A) and Psoralen (B).

The UV radiation activates Ps and Bp, generating reactive compounds that modify the DNA of the epidermis cells thus reducing the skin renewal cycle and inflammatory signaling factors, therefore improving the clinical condition of Psoriasis [3-6]. In vitiligo the interaction of these substances with DNA, induces the production of melanocytes, hence promoting the skin pigmentation [8].

Considering that the action of these compounds is associated with photo stability and photo induction of reactive species, which may invariably involve electron transfer processes, the electrochemical characterization of these furanocoumarins is a relevant task [8,9].

Furthermore, the redox behavior of BGT crude extract or its main furanocoumarins is unknown. Since, the electrochemical characterization highlights the required potential for anodic and

cathodic processes, it allow inferring about the pro or anti-oxidant role of chemicals. On the other hand, the Faradaic peak current can express the kinetics of electron transfers and the concentration of electroactive species [9-12], which owing to the simplicity and low consume of reagents may offer interesting alternatives for qualitative and quantitative analysis of such phytopharmaceuticals.

Therefore the aim of this study was to evaluate the electrochemical behavior of Ps, Bp and also the crude extracts of *Brosimum gaudchaudii Trécul* (BGT) by voltammetric techniques. Also, this study can be extended to the development of analytical methodologies for purposes of quantification and identification of these phytochemicals in pharmaceuticals and natural products. The data herein obtained were corroborated by HPLC coupled to MS and PDA detectors.

2. EXPERIMENTAL

2.1 Reagents and Samples

The furanocoumarins, Ps and Bp, both of analytical standard grade (purity > 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and acetonitrile HPLC grade were from J.T. Baker (Center Valley, PA, USA). Other reagents were of analytical grade from Vetec (Duque de Caxias, RJ, Brasil) and Proquímios (Duque de Caxias, RJ, Brasil). Ultrapure water was obtained from an Elga PureLab Option-Q®7/15 water purification system (Elga Berkefeld GmbH, Celle, Germany).

Root bark extract of *Brosimum gaudichaudii* Trécul (BGT), supplied by the company Sauad Farmacêutica®, were solubilized in methanol 50% (v/v), sonicated in an ultrasound for 10 min and filtered through syringe filter (Nylon 0.45 μm). The extract was prepared with material-to-solvent ratio of 6.6:1.0 $mg.mL^{-1}$.

Stock solutions were prepared in ethanol-deionised water (50:50, v/v) and stored at 5 ºC. Solutions of different concentrations of furanocoumarins were prepared by dilution of the appropriate quantity in supporting electrolyte.

The Phosphate Buffer (PBS) and all other supporting electrolyte solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 0.1 μS cm^{-1})**.**

2.2 Electrochemical Measurements

Voltammetric experiments were carried out using a μAutolab running with GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands. Measurements were carried out using a three-electrode system in a 1.0 mL one-compartment electrochemical cell (Cypress System Inc., USA). Carbon Paste (CP) electrode was the working electrode, Pt wire the counter electrode and the Ag/AgCl (3 $mol.L^{-1}$ KCl) reference electrode.

The CP was prepared by mixing rigorously, 100 mg of dried graphite with 20 mg of mineral oil (nujol®). The obtained paste was inset in support electrode, consisting in a teflon tube overpassed by

metallic conductor with a cavity (*d* = 1.5 mm and depth of 0.5 mm). The CPE was polished in paper and conditioned by CV scans from 0 to 1 V, scan rate of 100 $mV.s^{-1}$ in 0.1 M PBS, pH 7.0 or until a steady state baseline voltammograms were obtained before each electrochemical experiment. Following this procedure ensured very reproducible experimental results. Other carbon electrodes, *i.e.* glassy carbon (GC) and Boron Doped Diamond (BDD) electrodes (both of *d*= 1.5 mm) were also employed.

The pH measurements were carried out with a Quimis pHmeter Q400A5 with an Quimis combined glass electrode QA338-ECD model. All experiments were done at room temperature (25 ± 1 ºC) and microvolumes were measured using EP-10 and EP-100 Plus Microliter Pippettes (Eppendorf Co, Enfield, Connecticut/USA).

The experimental conditions for differential pulse (DP) voltammetry were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 10 $mV\ s^{-1}$. For square wave (SW) voltammetry were: pulse of 50 mV, frequency of 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate of 100 $mV\ s^{-1}$ were used. The Cyclic Voltammetry (CV) was scanned from 0 to 1.3 V at 100 $mV.s^{-1}$.

2.3 Chromatographic analysis of BGT in HPLC-PDA

Chromatographic separation was performed on ACE 5 C18 column (150 mm x 4.0 mm, 5 µm) adjusted at 50 °C. Elution was carried out with 0.1% phosphoric acid: acetonitrile (68:32, v/v) at flow rate of 1.0 mL min^{-1} and sample volume of 10 µL. Run time analysis was set at 13 min and detector wavelength at 245 nm. Mobile phase was filtered through 0.22µm PTFE membrane filter (Millipore, Bedford, MA, USA) and degassed in an ultrasonic bath (USC-2800, Unique, Brazil) prior to use. The HPLC system consisted of a Thermo Accela series 600 autosampler including a quaternary pump (model 1250), column oven and degasser, as well as PDA detector (80 Hz). Data acquisition were performed using ChemStation software.

Stock solutions (SSs) for Ps and Bp stock solutions (SSs, 1.0 $mg.mL^{-1}$) were prepared in acetonitrile, and further diluted in Ringer solution (USP 33) to yield intermediate solutions. Both were used to set up quality control samples and calibration curve at three (20.0; 50.0 and 100.0 µg/mL) and, six concentration levels (0.1; 1,0; 2.5; 5.0; 7.5 e 10.0 $µg.\ ml^{-1}$), respectively.

Selectivity was evaluated by blank samples Ringer solution using three different reagent sources were screened for interferent peaks on retention time of Ps and Bp. Peak purity analysis was also assessed by comparing upslope, apex and downslope UV/VIS spectral similarity for all compounds. Accuracy and intra- and inter-day precision were evaluated by analyzing triplicate quality control (QC) samples at low, mid and high (0.5; 4.5; 8.5 µg/mL) Ps and Bp concentrations on two different days. The lower limit of quantification (LLOQ) was determined by injecting replicate solutions (n=6) of known Ps and Bp samples. Both intra- and inter-day precision were assessed by calculating average, standard deviation, and relative standard deviation of the replicate measurements. Sample precision and accuracy was lower than $\pm$ 5% standard deviation from average and nominal concentration, respectively.

The psoralens content (% w/w) in BGT extract were determined by HPLC/DAD by comparison with analytical standard, which was used to make a calibration curve.

2.4 Mass Spectrometry Analysis

The mass spectrometric analysis was carried out in microTOF III (Brucker Daltonics, Bremen, Germany) equipped with commercial source of ions ESI (Brucker Daltonics, Bremen, Germany). The assays were performed in positive mode (ESI+) by direct injection (3 μL.min^{-1}) of samples, which were pretreated by extraction in 1 mM formic acid methanol solution. Other experimental conditions included the use of nitrogen nebulizer gas at 200^{o}C and 1bar leading to a drying gas flux of 3.5 L.min^{-1}; capillary voltage of -4 kV; end plate offset of -500 V; skimmer of 35 V e collision voltage of - 1.5 V. The spectra's were acquired from two microscans that were processed by using a Data Analysis software (Brucker Daltonics, Bremen, Germany).

3. RESULTS AND DISCUSSIONS

3.1 Electrochemical Behavior of BGT, Bp and Ps

The figure 2 presents the cyclic voltamograms obtained for Bp, Ps and BGT extract, in which no defined peak was observed for Ps. Yet, in Bp and BGT were observed an anodic peak, 1a, at high peak potential, E_{p1} = 1.2 V (pH = 7.0).

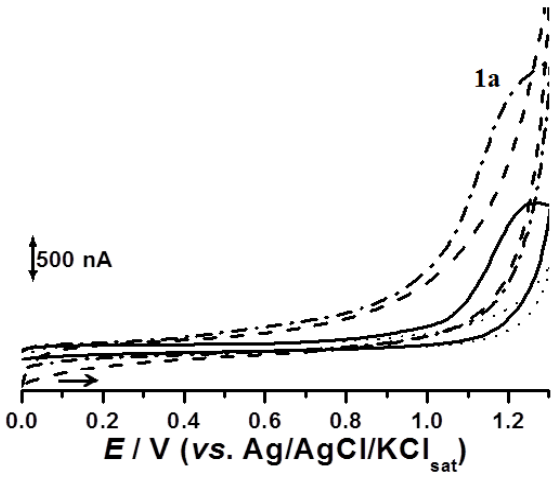

Figure 2. Cyclic voltammograms obtained for 0.1% BGT (—), 2 μM Bp (- . -) and 1 μM Ps (- - -) at CP in pH 7.0 0.1 M PBS.

Therefore, owing to this very positive peak potential, it can be inferred that the samples herein investigated presents low electron donor ability.

In order, to evaluate the presence of minor compounds with higher antioxidant potential, the more sensitive DPV was also performed (Figure 3). The baseline corrected DP voltammograms evidenced the anodic peak 1a in Bp (Figure 3A), whereas in BGT besides the high potential anodic peak, 3a, was also possible to identify two other very less intense anodic peaks, 1a and 2a, at E_{p1a} = 0.15 V and E_{p1a} = 0.45 V, respectively (Figure 3B, *inset*).

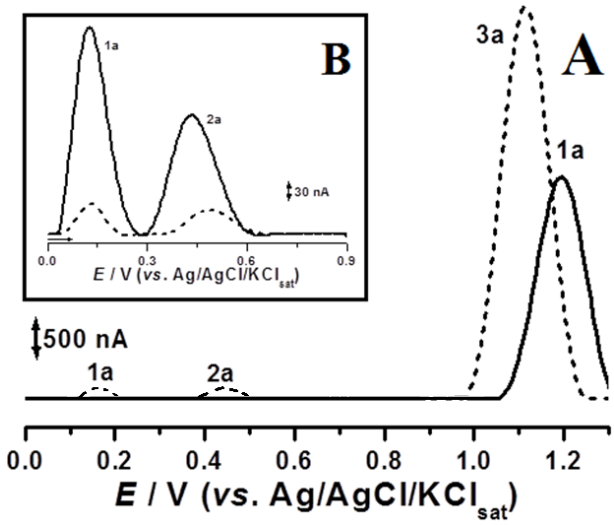

Figure 3. A) Baseline corrected DP voltammograms obtained for 1 μM Bp (—) and 0.05% BGT (---). B) Sucessive Baseline corrected DP voltammogrmas for BGT, 1st scan (—) and 2nd scan (- - -) all at CP in pH 7.0 0.1 M PBS.

Such lower anodic potential are consistent with hydroquinone and catechol like electroactive species with recognized antioxidant activity and reversible behavior.

In order to check the reversibility of redox processes, 1a and 2a, in BGT sample, as well as, the irreversibility of the high positive anodic peaks, the SWV was also performed (Figure 4).

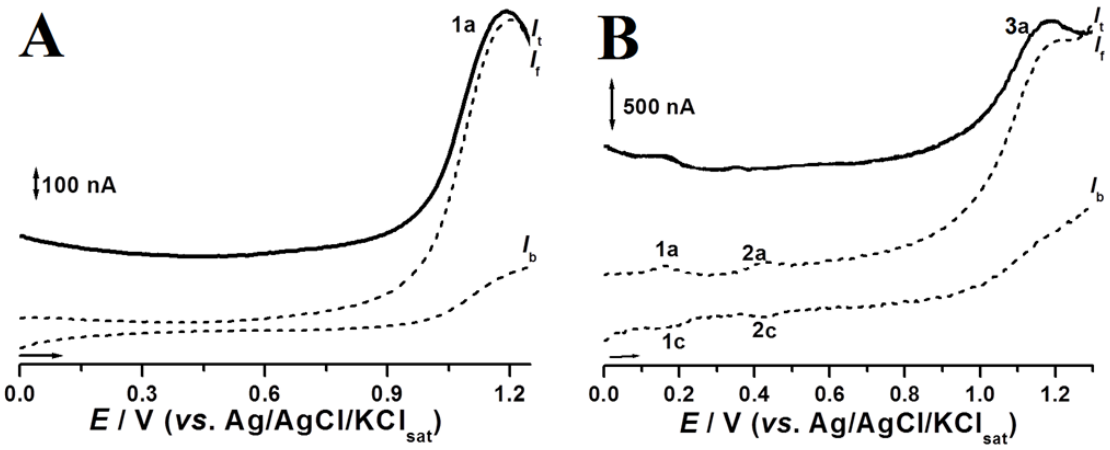

Figure 4. SW voltammograms obtained for 1μM Bp (A) and 0.05% BGT (B) at GC in pH 7.0 0.1 M PBS.

As expected no corresponding reduction signal was observed for the anodic peaks, 1a, in Bp (Figure 4A) and peak 3a in BGT extract (Figure 4B). On the other hand, the related cathodic peaks, 1c and 2c of less positive anodic peak potential, 1a and 2a, in BGT prove the reversibility of such redox processes (Figure 4B).

A strong adsorption behavior leading to electrode passivation was observed for all systems, herein investigated (Figure 3 B), including at BDD (not shown). Indeed, this fact is in agreement with literature, especially for coumarins [9-13] and has dictated the choice for the renewable carbon paste electrode.

Indeed, such polymerization reactions were well described for the coumarin, umbelliferone [13], whereas the oxidation mechanism proposed for scopoletin, another relevant coumarin, also corroborates for remarkable electrode passivation process [14].

Finally, taking into account the electroactivity and biological relevance of Bp, an electroanalytical method was proposed to quantify this compound in BGT extract.

The DPV was performed from 1 to 1.25 V for increasing Bp concentrations and a calibration graph was plotted by taking the corresponding anodic (Figure 5).

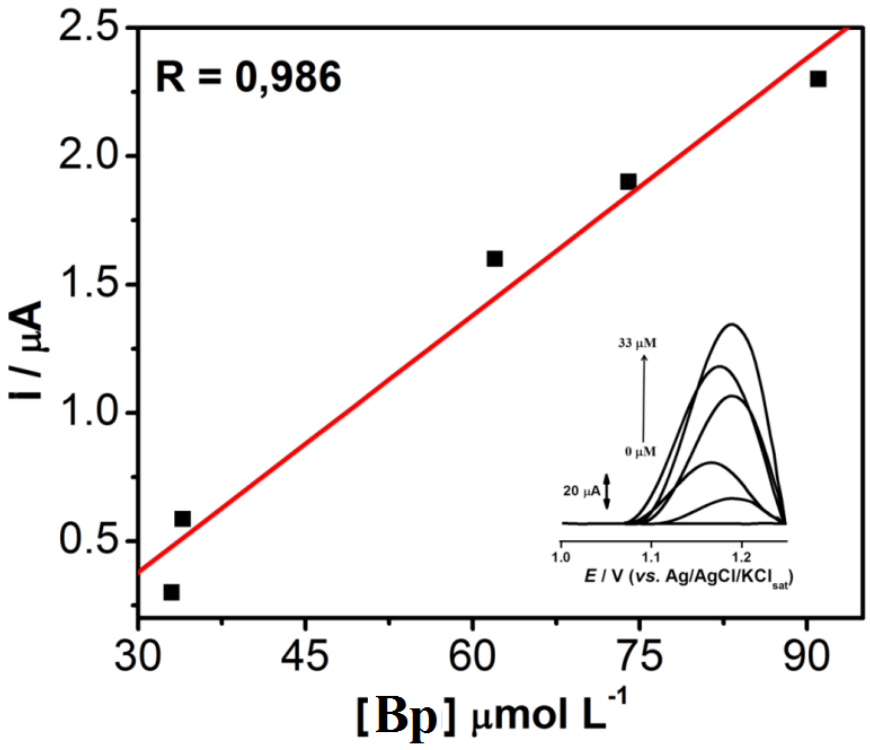

Figure 5. Calibration graph and DP voltammograms for incremental Bp concentrations (0.3 to 33 µM).

Owning to the strong adsorption behavior, an acceptable linear response, I (A) = - 6.23 . 10^{-7} + 3.339 . 10^{-8} (r = 0.986) was achieved only at low concentration range (< 33 µM), whereas the RSD for this interval were very close to the maximum value of 5%. Nevertheless, considering the simplicity,

low reagent consume, the proposed method may be still useful in order to estimate the amount of Bp in BGT extract, which was found to around 0.6%.

In a similar study a metal oxide carbon paste modified electrode was applied on the DP voltammetric quantification of Bp in commercial bergamot essential oil, in which was found c.a. 0.1 % w/w, meanwhile the related voltammograms were not presented [15]. Morevover, it is the first approach for quantitative determination of Bp in BGT crude extracts.

3.2 Identification of Bp and Ps in BGT extract by Mass Spectrometry

Figure 6 shows ESI(+) mass spectra of Bp and Ps standards. Note in Figure 6A that Bp was detected as $[M + H]^+$ of *m/z* 217.0499 and as sodium and potassium adducts $[M + Na]^+$ of *m/z* 239.0295 and $[M + K]^+$ of *m/z* 255.0022, respectively. Ps was detected in the same way as $[M + H]^+$ of *m/z* 187.0325 and as sodium adducts $[M + Na]^+$ of *m/z* 209.0145 (Figure 6B).

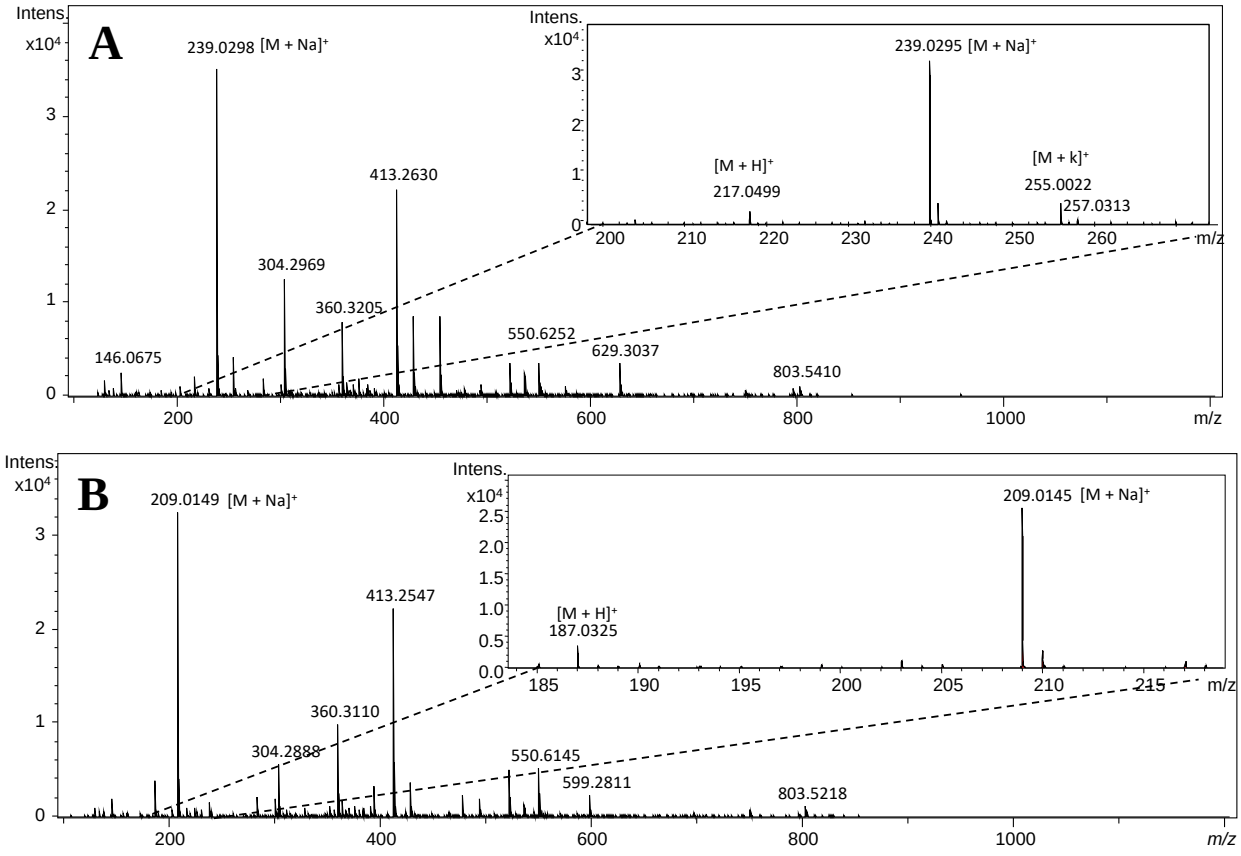

Figure 6. ESI(+) Q-TOF mass spectra of standards Bp (A) e Ps (B).

The figure 7 shows mass spectrum in the positive mode of BGT extract. Note that Ps and Bp are present in the extracts, detected as $[M + Na]^+$ of *m/z* 209.0145, 239.0295 respectively. Moreover, glucose were identified in the extract as $[M + Na]^+$ of *m/z* 203.0517.

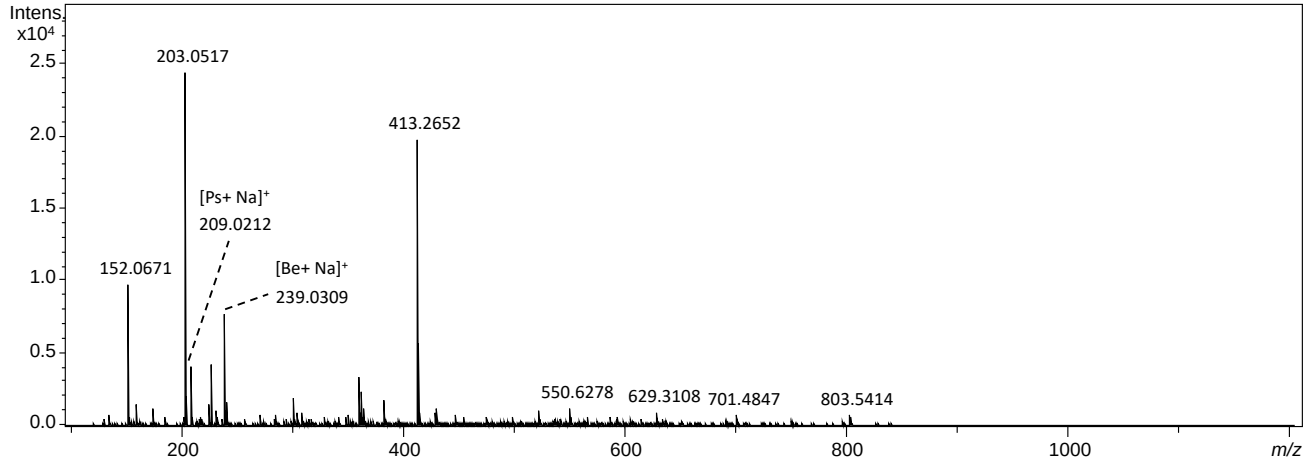

Figure 7. ESI (+) Q-TOF mass spectra of crude extract *Brosimum gaudchaudii.*

3.3 Quantification of Ps and Bp in BGT by HPLC-PDA validated method

The quantification of the BGT furanocoumarins was also performed by HPLC-PDA, and the retention time was of 5.9 min and 8.7 min, for Ps and Bp, respectively. The calibration curve meets a linear correlation coefficient (r) for Ps (y = 356483x - 14025; r = 0,999) e Bn (195961x -17251; r = 0,9997), confirming their presence. It was found to be respectively, 0.36% w/w (23.58 ± 0.17 $\mu g.mL^{-1}$) and 0.58% w/w (38.96 ± 0.03 $\mu g.mL^{-1}$) of BGT extract.

The table 1 presents the quantitative results for Bp in BGT extract by means of HPLC-PDA and DPV.

Table 1. Quantitative determination of Bp in BGT extracts by HPLC-PDA and DPV.

	HPLC-PDA Mean ($\mu g.mL^{-1}$) ± SD*	DPV Mean ($\mu g.mL^{-1}$) ± SD*	Recovery
Bp / BGT	38.96 ± 0.03	41.65 ± 2.13	106.9%

*SD for six replicates

4. CONCLUSIONS

The redox behavior of the BGT furanocoumarins, Ps and Bp, was investigated at different carbon electrodes, being that Bp presented an anodic peak at very positive potential, whereas no redox peak was observed for Ps. In all cases was observed a very strong adsorptive behavior, which hampered the quantitative analysis. Nevertheless, the presence of both major markers was identified in BGT extract by mass spectrometry. Moreover, the HPLC-PDA proved that Bp and Ps content is of 0.36 and 0.58%, respectively. Thus, the anodic peak 3a in BGT may have a great contribution of Ps and Bp.

ACKNOWLEDGEMENTS
The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Projects: 481683/ 2013-5 and 471109/2013-4) as well as FAPEG. This work is a collaboration research project of members of the Rede Mineira de Química (RQ-MG) supported by FAPEMIG (Project: CEX - RED-00010-14)

References

1. S. Bakar-Dertlioğlu, H. Uçak, D. Çiçek, M. Batirem, *Turk. J. Pediatr.*, 54 (2012) 77.
2. D. Hisao, S. Masa-Aki Shibata, K. Kimihiro, O. Yoshinori, *J. Dermatol. Sci*., 33 (2003) 7.
3. L. Xiao-Ming, Z. Qiong, X. Shi-Zheng, W. Kazumasa, *Free Rad. Biol. Med.*, Tie-Chi Lei 50 (2011) 1177.
4. R. Speeckaert, M.M. Speeckaert, N. van Geel, *Autoimmun. Rev*., 14 (2015) 332.
5. S.M. Schleicher, *Clin. Podiatr. Med. Surg.*, 33 (2016) 355.
6. E. Jacomassi, I.S. Moscheta, S.R. Machado, *Acta Bot. Bras.*, 21 (2007) 575.
7. G.L. Pozetti, *Rev. Ciênc. Farm. Básica Apl*., 26 (2005) 159.
8. L. Serrano-Andrés, R. González-Luque, M. Marchán, J.J. Serrano-Pérez, *J. Photochem Photobiol*., 199 (2008) 34.
9. E.S. Gil, R.O. Couto, *Braz. J. Pharmacog*., 23 (2013) 542.
10. Escarpa A., *Chem. Record*, 12 (2012) 72.
11. N.S. Reis, S.H.P. Serrano, R. Meneghatti, E.S. Gil, *Lat. Am. J. Pharm*., 28 (2009) 949.
12. J.L.R. Martins, E.A. Costa, S.H.P. Serrano, S.C. Santos, E.S. Gil, *Int. J. Electrochem. Sci*., 10 (2015) 4552.
13. K.C.S Leite, L.M.F.C. Torres, L.F. Garcia, S.G. Rezende, J.R. Oliveira-Neto, F.M. Lopes, T.A. Garcia, R.M. Verly, W.T.P. Santos, E.S. Gil, *Int. J. Electrochem. Sci.*, 10 (2015) 5714.
14. L.H. Wang, M. Tso, *J. Pharm. Biomed. Anal.* 30 (2002) 593.
15. X.-R. Hu, J.-B. He, Y. Wang, Y.-W. Zhu, J.-J. Tian, *Electrochimica Acta* 56 (2011) 2919.