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Preparation and evaluation of the pentavalent antimony-quercetin complex (SbV-QUE)

Preparação e avaliação do complexo antimonial pentavalente-quercetina (SbV-QUE)

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ABSTRACT

Leishmaniasis is a parasitic disease that has the first choice of treatment medications with antimony as active ingredient. These characteristics justify the search for new compounds with easy administration. In this context, there are quercetin, flavonoid leishmanicidal with chelation power, enabling complexation with metalloids, such as antimony. Thereby, this research aims to prepare and evaluate the complex antimony-quercetin (SbV) in solution and powder form. Precursor materials were quercetin anhydrous (methanolic solution) and potassium hexahydroxoantimonate (V) (aqueous solution), in 1: 1 ratio. After the formation of the complex SbV-QUE SOL (solution), it was dried by rotoevaporation, lyophilization or spray drying. For characterization of SbV-QUE SOL, spectrofluorimetry and UV-Vis spectrophotometry were utilized. In addition, for SbV-QUE powder, X-ray diffraction (XRD), energy dispersive X-Ray analysis (EDX), Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA) and fluorescence microscopy were made. In SbV-QUE SOL, an increase of fluorescence and the bathochromic shift in UV-Vis were observed, demonstrating the formation of the complex. In SbV-QUE powder, a difference of crystallinity between precursors and products was perceived, by XRD analysis. Analyzing DX results, a presence of antimony was observed in the samples. FT-IR indicated the binding site of the complex. Finally, the same thermal profile for the powdered complexes was observed, in addition to the micrographs elucidating the surface morphologies of quercetin and SbV-QUE powder. This confirms the characteristic of post-drying fluorescence. Therefore, the formation of the complex was evidenced, which is essential for further investigation of its use, aiming at leishmaniasis treatment.

Keywords: leishmaniasis; antimonial; quercetin.

RESUMO

Leishmaniose é uma doença parasitária que tem a primeira escolha de medicamentos de tratamento com antimônio como ingrediente ativo. Essas características justificam a busca por novos compostos de fácil administração. Neste contexto, há a quercetina, flavonóide leishmanicida com poder de quelação, possibilitando a complexação com metalóides, como o antimônio. Assim, esta pesquisa tem como objetivo preparar e avaliar o complexo antimônio-quercetina (SbV) em solução e em pó. Os materiais precursores foram a quercetina anidra (solução metanólica) e o hexa-hidroxoantimonato de potássio (V) (solução aquosa), na proporção de 1: 1. Após a formação do complexo SbV-QUE SOL (solução), este foi seco por rotoevaporação, liofilização ou secagem por atomização. Para caracterização do SbV-QUE SOL, utilizou-se espectrofotometria e espectrofotometria UV-Vis. Além disso, para pó SbV-QUE, difração de raios X (XRD), análise de energia dispersiva de raios X (EDX), espectroscopia de infravermelho por transformada de Fourier (FT-IR), análise termogravimétrica (TGA) e microscopia de fluorescência foram feitas. No SbV-QUE SOL, observou-se um aumento da fluorescência e o deslocamento batocrômico no UV-Vis, demonstrando a formação do complexo. No pó SbV-QUE, a diferença de cristalinidade entre precursores e produtos foi percebida pela análise de XRD. Analisando os resultados do DX, a presença de antimônio foi observada nas amostras. FT-IR indicou o local de ligação do complexo. Por fim, foi observado o mesmo perfil térmico dos complexos em pó, além das micrografias elucidando as morfologias da superfície da quercetina e do pó SbV-QUE. Isto confirma a característica da fluorescência pós-secagem. Portanto, evidenciou-se a formação do

complexo, essencial para uma investigação mais aprofundada de seu uso, visando o tratamento da leishmaniose.

Palavras-chave: leishmaniose; antimonial; quercetina.

1 INTRODUCTION

Leishmaniasis is a disease with high rate mortality and widespread distribution and an anthropozoonosis, whose etiological agent is a protozoan of the genus leishmanial that is transmitted by infected hematophagous phlebotomine flies (NEVES et al, 2016). In the treatment of this infection, the use of chemotherapy is necessary and the first choice drug used is based on antimony, a semimetal with main oxidation states the forms +3 and +5. Currently, the composition of the drugs uses the pentavalent form, because of a lesser toxicity than the trivalent one (VIEIRA, 2008). However, treatments with antimony still present some difficulties, such as the parenteral administration route, which associated to a long treatment period, causes many patients to quit, as well as their high toxicity to essential organs such as liver, kidneys and spleen (BRAZIL, 2006).

Therefore, it is legitimate the search for more natural medications, with less toxicity and more efficiency. The quercetin is a flavonoid with recognized leishmanicidal activity against the amastigote stage of the Leishmania (*Leishmania*) *donovani parasite* (TASDEMIR et al., 2006; VILA-NOVA et al., 2012), L. (L.) *amazonensis* (MUZITANO et al., 2006) and for the amastigote and promastigote forms of L. (L.) *infantum chagasi*, with better results than second-line drugs such as amphotericin B and pentamidine (VILA-NOVA et al., 2012). Due to its electron-donor groups, the quercetin has chelating properties that allows it to complex with antimony. This complex has been investigated, but there are few studies that describe its therapeutic activity, most of them are used in water decontamination and flavonoid determination (VISWANATHAN et al., 2000; ROJAS et al., 2013).

Consequently, this study aimed to synthesize the pentavalent-quercetin antimonial complex (SbV-QUE) in solution and powder form, as well as to characterize them in order to develop a compound to be used in leishmaniasis treatment.

2 METHODOLOGY

2.1 MATERIAL

Potassium hexahydroxoantimonate (V) (Sigma-Aldritch, USA) and quercetin anhydrous (Sigma-Aldritch, USA) were the precursor materials used for formation of the

complex quercetin-antimony (V). The solvents used to solubilize the reagents, respectively, were ultrapure water (MiliQ, Merck, Germany) and methanol (Dinâmica, Brazil).

2.2 SYNTHESIS OF THE ANTIMONY-QUERCETIN COMPLEX IN SOLUTION (SbV-QUE)

50 mL of potassium hexahydroxoantimonate (V) (7.60 mM) aqueous solution were mixed directly with 50 mL of quercetin methanol solution (0.66 mM), without mechanical shaking. The formation of the complex occurred instantly with the mixture of solutions (adapted from VISWANATHAN; SRIRAM; YOGEESWARAN, 2000).

2.3 CHARACTERIZATION OF THE SbV-QUE COMPLEX (SOLUTION)

For fluorimetric determination, a molecular spectrofluorimetry (Shimadzu, RF-5301PC spectrofluorometer, Tokyo, Japan) with excitation at 370 nm was used. The UV-Vis spectrophotometry results were obtained by using Micronal AJX-6100PC spectrophotometer, SP, Brazil.

2.4 DRYING PROCESS AND CHARACTERIZATION OF THE SbV-QUE COMPLEX (POWDER)

Three different processes using the following techniques dried the SbV-QUE complex: rotoevaporation (SbV-QUE ROTA), lyophilization (SbV-QUE LIO) and spray drying (SbV-QUE SD). The IKA® RV10 rotary evaporator at 80ºC when the aim was the total drying of the solution and, at 60ºC, when only the methanol was removed (preliminary process used for lyophilization and spray drying).

The solution was placed in a freezer for 48h prior to the drying procedure, using Terroni® LD1500 lyophilizer (São Carlos, Brazil). Lastly, the Büchi B-290 mini-spray dryer (New Castle, USA) was utilized with an inlet temperature of 200ºC and outlet temperature varying between 75ºC and 85ºC, 33% pump rate and 85% aspirator rate. All procedures were performed and all products were stored away from light.

For the products characterization in powder form: powder X-ray diffraction (XRD), using a Shimadzu XRD-6000 (Kyoto, Japan); Energy Dispersive X-Ray Analysis (EDX) using a Shimadzu EDX-7000/8000 (Kyoto, Japan); Fourier transform infrared spectroscopy (FT-IR) through a Thermo Scientific Smart OMNI-Sampler Nicolet iS10 FT-IR Spectrometer (Massachussetts, USA); Thermogravimetric Analysis (TGA) using a Shimadzu, model DTG-

60 (Kyoto, Japan); and Fluorescence microscopy through a ZEISS Axionvision microscope (Oberkochen, Germany) were utilized.

The XRD used CuKα with 30 Kv voltage and 30 mA current, a Ni filter and data collected in a 2θ range between 3-40 degrees. FTIR data on the functional groups of the material used had obtained in a range of 500-4000 cm^{-1} using a KBr (1: 100) insert. The EDX analysis was made under vacuum, considering the carbon balance. Moreover, TGA was analyzed at a temperature range of 100°C to 800 °C, with a heating rate of 10 °C/min and compared to losses of materials under the same conditions. For fluorescence microscopy, Alexa filter (red) at a wavelength of 546 nm was used.

3 RESULTS AND DISCUSSION

3.1 ANTIMONY-QUERCETIN COMPLEX - SOLUTION

After mixing aqueous solution of potassium hexahydroxoantimonate (V), which is a source of SbV, and anhydrous quercetin solution in a proportion 1:1, an instantaneous change of color from yellow to orange with a pH approximately to 7 was observed, with quercetin in its neutral form.

By the fluorimeter analysis (Figure 1a), it was possible to observe that quercetin presents low fluorescence, increasing significantly when there is complex formation, as realized by Viswanathan, Sriram e Yogeeswaran (2000), who, besides this characteristic, also noted a color change.

Through UV-Vis technique, presented in figure 1b, a bathochromic shift from 371 (free quercetin) to 379 nm in the SbV-QUE SOL complex was noticed, as described by Tong et al. (2016), in which quercetin bands were shifted from 255.67 to 259.13 nm for band II and 375 to 391nm, for band I. That happens because of the extension of conjugated system, when complexation occurs (SANNA et al. 2015). This result and the fluorimetry analysis proved the SbV-QUE complex formation.

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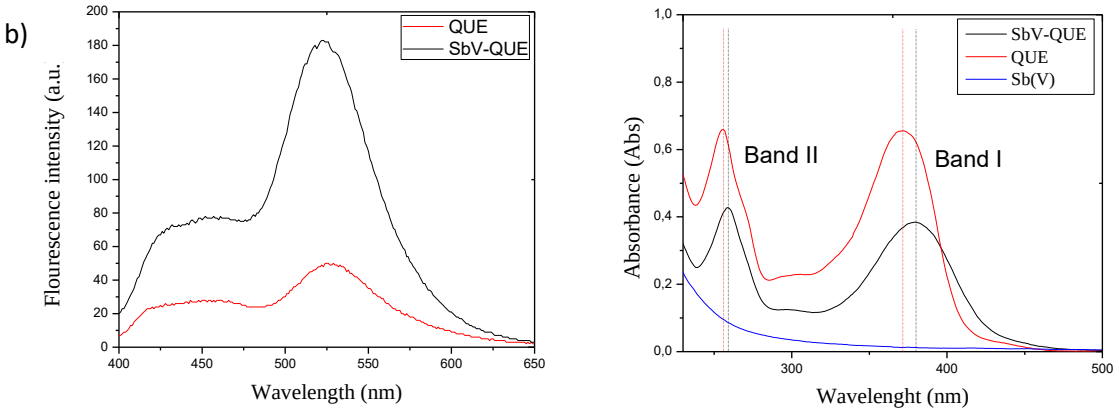
Figure 1: a) Fluorescence of the SbV-QUE complex in relation to quercetin; b) UV-Vis spectra of SbV-QUE complex, quercetin (QUE) e antimony (SbV).



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3.2 ANTIMONY-QUERCETIN COMPLEX IN POWDER FORM

As there are no studies in literature with the evaluation of this dried complex by rotoevaporation, lyophilization and spray drying, it justifies the drying for analysis of stability and viability of its use. By XRD analysis, a difference of crystallinity between precursors and products (Figure 2) was observed. The quercetin presented well defined and with high intensity, as well as with antimony, but with low intensity. Dried SbV-QUE complexes using the three drying techniques, however, presented a similar diffractogram to characteristics of amorphous materials, suggesting a formation of a new product.

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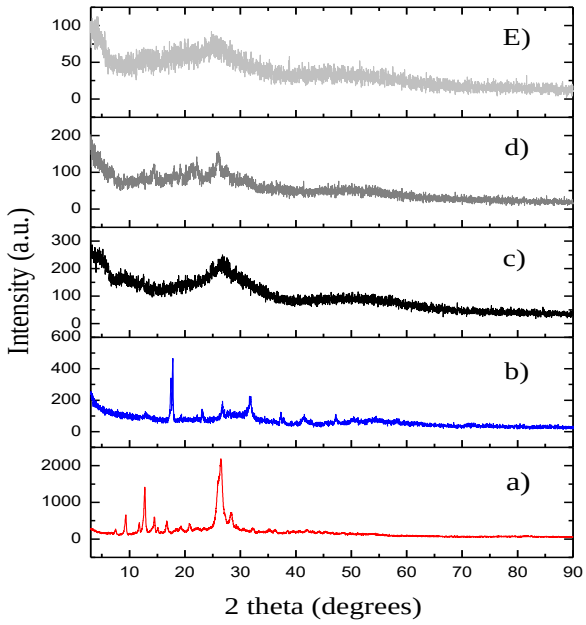
Figure 2: XRD of a) quercetin, b) antimony, c) SbV-QUE ROTA, d) SbV-QUE LIO e e) SbV-QUE SD.



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Analysing EDX results, a presence of antimony in the samples was observed, despite of drying technique. By FT-IR analysis (Table 1), we noticed that quercetin presented its main bands with 3350cm^{-1} because of the deformation of binding ⁻OH, intramolecular hydrogen bonds of free and intermolecular hydroxyl groups: *v*(C=O) with stretching in 1660cm^{-1} related to carbonyl; and band with 1318cm^{-1} related to phenol (SIMÕES et al., 2013). The pentavalent antimonial presented bands in the region from 3600 to 2250cm^{-1} corresponding to –OH, which represent adsorbed water, and the bands between 766 and 604cm^{-1} corresponding to Sb-O bond, considering the representation metal-oxygen binding (DUOMO et al., 1997).

Table 1: Comparison among different frequencies obtained by FT-IV of powdered products.

Compound	*v*(O-H)	*v*(C=O)	*V*(C=C)	*δ*(C-OH)$_{fenol}$	*v*(C-O-C)	*v*(Sb-O)
Quercetin	3438cm^{-1}	1660cm^{-1}	1512cm^{-1}	1318cm^{-1}	1260cm^{-1}	-
Sb(V)	3443cm^{-1}	-	-	-	-	766 e 604cm^{-1}
SbV-QUE ROTA	3443cm^{-1}	1653cm^{-1}	1490cm^{-1}	1318cm^{-1}	1266cm^{-1}	637cm^{-1}
SbV-QUE LIO	3441cm^{-1}	1656cm^{-1}	1491cm^{-1}	1320cm^{-1}	1265cm^{-1}	-
SbV-QUE SD	3442cm^{-1}	1654cm^{-1}	1489cm^{-1}	1320cm^{-1}	1267cm^{-1}	643cm^{-1}

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Considering Sb-QUE complex dried by rotoevaporation and spray drying, an onset of bands which are specific to Sb (V) was noticed in the spectral region between 700cm^{-1} and 600cm^{-1}, indicating not only the presence of antimony in the molecular structure, but also in the complex formation, as described by Ravichandran, Rajendran e Devapirian (2014) to Cd-O, in the cadmium-quercetin complex. For SbV-QUE LIO, the same onset was suggested, however, with a band not well defined.

Furthermore, in the products obtained, characteristic bands were observed exclusively to quercetin in the region between 1300cm^{-1}and 900cm^{-1}, not related to the drying technique used. According to Pralhad e Rajendracumar (2004), a band at 1664cm^{-1} is attributed to carbonyl ketone stretching from aromatic ring. In this case, the band in 1660cm^{-1}, found in the pure quercetin on this study, was shifted, what suggests complex formation and indicates where possible sites of binding to the complexation are located. In other words, the carbonyl located at C-4 position has some association to the hydroxyl group located at C-3 and suggests the well-known keto-enol chelation in the final complexes.

In addition, in the complexes SbV-QUE ROTA, SbV-QUE LIO and SbV-QUE SD there is the appearance of a new band at 1490 cm^{-1}, which indicates an increase and a shift of the small band observed for quercetin, representing C=C, characteristic bond from aromatic ring (LUNA et al., 2016). According to Ficarra et al. (2002), changes in the characteristics of the precursors bands indicate the existence of a new compound with different spectroscopic bands.

The thermogravimetric analysis demonstrated that features of free quercetin decomposition (Figure 3a) have two weight losses, the first having an onset at 100°C and an end at 355.9°C, and the second with an 798.3°C. The first loss was abrupt, corresponding to 55% and indicates the decomposition of central ring of quercetin structure, or the loss of one of its two dihydroxylated rings, compatible with a solid/liquid transition (BERLIER et al., 2013). For SbV (Figure 3b), three mass losses observed. The first with an onset at 119°C and an end at 185.59°C, and with a loss of 8.60%. The second started at 185.59°C and ended at 422.90°C, with a loss of 21.24%. Finally, the third had a loss of 27.34% between 422.90°C and 815.30°C. The total loss of the compound was 57.18% and which components correspond to each loss are not found in literature, however, it is suggested that the residue of decomposition is constituted by antimony.

Considering the analysis of the complex, it was seen that there was a similar thermogravimetric profile for the three drying technique (Figure 3c-e) with three losses of mass. A first, between 100°C-318.04°C, corresponding to dehydration, with a loss of 13.07%; a second (318.04°C to 530.64°C) indicating the first decomposition of the complex with a loss of 20,915%; and the last (530.64—794.43°C) representing the oxidative decomposition of organic matter, with a loss of mass of 12.015%. With complex formation, a loss of organic matter occurred at higher temperatures than for quercetin, demonstrating a change on the profile decomposition of the compounds. A total loss of mass was 46% with a probable antimony residue (TONG et al., 2016), which was corroborated by the proportion 1:1 (quercetin:antimony) utilized in the complex synthesis.

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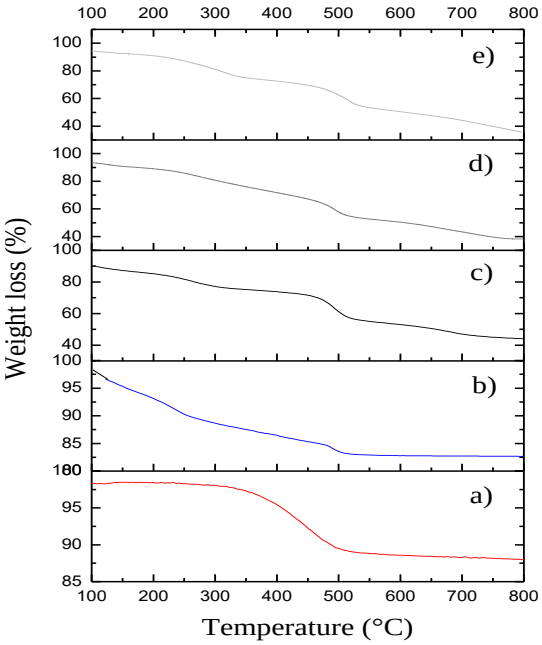
Figure 3: TGA analysis of a) quercetin, b) antimony, c) SbV-QUE ROTA, d) SbV-QUE LIO and e) SbV-QUE SD



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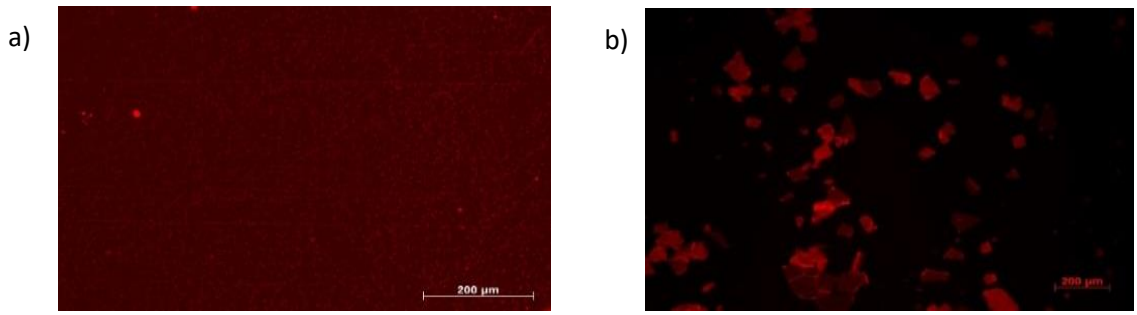
Observing fluorescence microscopy (Figure 4), it was possible to note a superficial morphology of quercetin and SbV-QUE complex, and also confirming the maintenance of its fluorescence properties, which corroborates with what was seen for the complex in solution.

Figure 4: Images of fluorescence microscopy. In: a) quercetin and b) SbV-QUE.



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4 CONCLUSION

It can be inferred that antimony-quercetin complex in solution was obtained with success, by fluorimetry and UV-Vis techniques. In addition, it was realized that, independently of which drying technique, the features of powder complex were similar. Through the XRD, a difference between crystalline planes of quercetin and potassium hexahydroxoantimonate (V) was observed, antimony source, compared to the dried complexes, with amorphous characteristics. The EDX analysis detected the presence of antimony in solid samples. FT-IR demonstrated to be essential for the indication of SbV-Que complex formation and complexation site, which occurred through the hydroxyl group of carbon at C-3 and the carbonyl at C-4. Finally, the same thermic profile for the powder complexes and the fluorescence microscopy were noted and it evidenced the morphologies of quercetin and SbV-QUE complex in powder form, confirming the maintenance of fluorescence feature after drying process. In this way, the set of techniques corroborated to evidencing the complex formation and its characterization, fundamental for the study of this product and taking in account its use for leishmaniasis treatment.

REFERENCES

BERLIER, et al. Stabilization of quercetin flavonoid in MCM-41 mesoporous silica: positive effect of surface functionalization. Journal of Colloid and Interface Science, v. 393, p. 109–118, 2013.

BORGES, K. C. Pitanga (Eugenia uniflora) desidratada por atomização e liofilização: características físico-químicas, compostos bioativos e efeito sobre a longevidade, estresse oxidativo e neurotoxicidade induzida em modelos in vivo *Caenorhabditis elegans*. Tese (Doutorado em engenharia química) - Departamento de Engenharia Química, Universidade Federal Do Rio Grande do Norte, Rio Grande do Norte, 2015.

BRAZIL. Ministério da Saúde. Manual de Vigilância e Controle da Leishmaniose Visceral. Departamento de Vigilância Epidemiológica. 1ed., Brasília: Ministério da Saúde, 2006. P. 120. Disponível em: <http://bvsms.saude.gov.br/bvs/publicacoes/manual_vigilancia_controle_leishmaniose_visceral.pdf>. Acesso em: 01 de janeiro, 2019.

DUOMO, Z et al. Polymorph control of antimony white (Sb2O3) prepared by hydrometallurgy method. Transactions of Nonferrous Metals Society of China. v.7, n.4, p.118-122, 1997.

FICARRA, R. et al. Study of flavonoids/β-cyclodextrins inclusion complexes by NMR, FT-IR, DSC, X-ray investigation. Journal of Pharmaceutical and Biomedical Analysis, v. 29, n. 6, p. 1005-1014, 2002.

LUNA, F. M. T. et al. Rapid assessment of total and polycyclic aromatic contents. Environmental Monitoring and Assessment, v. 188, p. 215, 2016.

MENEZES, J. et al. Layered double hydroxides (LDHs) as carrier of antimony aimed for improving leishmaniasis chemotherapy. Applied Clay Science, v. 91–92, p. 127–134, 2014.

MUZITANO, M.F. et al. Quercitrin: An Antileishmanial Flavonoid Glycoside From *Kalanchoe pinnata*. Planta Medica, v. 72, p. 81–83, 2006.

NEVES et al. Parasitologia Humana. 13ª Ed. Atheneu Rio, 2016.

PRALHAD, T.; RAJENDRAKUMAR, K. Study of freeze-dried quercetin–cyclodextrin binary systems by DSC, FT-IR, X-ray diffraction and SEM analysis. Journal of Pharmaceutical and Biomedical Analysis, v. 34, n. 2, p. 333-339, 2004.

ROJAS, C. et al. High sensitivity adsorptive stripping voltammetric method for antimony(III) determination in the presence of quercetin-5′-sulfonic acid. Substituent effect on sensitivity. Sensors and Actuators B: Chemical, v. 185, p. 560-567, 2013.

SANNA, D. et al. Behavior Of The Potential Antitumor VIVO Complexes Formed By Flavonoid Ligands. 2. Characterization Of Sulfonate Derivatives Of Quercetin And Morin, Interaction With The Bioligands Of The Plasma And Preliminary Biotransformation Studies. Journal of Inorganic Biochemistry, v. 153, p. 167-177, 2015.

TASDEMIR, D. et al. Antitrypanosomal And Antileishmanial Activities Of Flavonoids And Their Analogues: In Vitro, In Vivo, Structure-Activity Relationship Studies. Antimicrobial Agents and Chemotherapy, v. 50, p. 1352-1364, 2006.

TONG, L. et al. Sb(III)–Quercetin Complex:Synthesis, Characterization And Antioxidant Activity. Life Science Journal, v. 13, n. 7, p. 127-133, 2016.

VIEIRA, F. A. Estudos Sobre O Comportamento Do Antimoniato De Meglumina No Corpo Humano E Em Macacos *Rhesus.* Tese (Doutorado em Química) – Pontifícia Universidade Católica Do Rio De Janeiro - Puc-Rio, Rio de Janeiro, 2008.

VILA-NOVA, N.S. et al. Leishmanicidal and cholinesterase inhibiting acti-vities of phenolic compounds of *Dimorphandra gardneriana* and *Platymiscium loribundum*, native plants from Caatinga biome. Pesquisa Veterinária Brasileira, v. 32, n. 11, p. 1164-1168, 2012.

VISWANATHAN, P. SRIRAM, V. YOGEESWARAN, G. Sensitive spectrophotometric assay for 3-hydroxy-substituted flavonoids, based on their binding with molybdenum, antimony, or bismuth. Journal of Agricultural and Food Chemistry, v. 48, n. 7, p. 2802-2806, 2000.