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Chemical analysis and evaluation of antioxidant, antiacetylcholinesterase activity and *Artemia salina* lethality by *Asemeia ovata* (Polygalaceae)

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Abstract

The *Asemeia* genus is composed of 28 American species, some of which are found in Bahia semi-arid region. The objective of this study is to describe the chemical and biological profile of *Asemeia ovata*. The extracts were subjected to HPLC-DAD and tests for determining the total flavonoid (aluminum chloride) and phenolic content (Folin Ciocalteau). For the evaluation of antioxidant, antiacetylcholinesterase activity and lethality, the scavenging activity of DPPH techniques, adaptation of Ellman method and *Artemia salina* method were applied respectively. The chemical profile showed that the extracts are rich in phenolic compounds. The antioxidant activity evaluation showed results that varied from 5.46 mg/mL to 13.21 mg/mL. Evaluation of antiacetylcholinesterase activity did not show positive results (8.68% - 15.35). The extracts showed lethality (162.08 mg/mL-71.91 mg/mL). The results suggest *A. ovata* as a promising plant for the realization of new chemical and biological studies leading to the isolation of the bioactive compounds.

Keywords: *acetylcholinesterase; biological activities; chromatography; DPPH; fingerprint.*

Resumo

(Análise química e avaliação da atividade antioxidante, antiacetilcolinesterásica e letalidade de *Artemia salina* por *Asemeia ovata* (Polygalaceae)). O gênero *Asemeia* é composto de 28 espécies, algumas das quais são encontradas no semiárido baiano. O objetivo deste estudo é descrever o perfil químico e biológico da espécie *Asemeia ovata*. Os extratos foram submetidos a CLAE-DAD e testes para determinação do teor de flavonoides totais (cloreto de alumínio) e fenólicos (Folin Ciocalteau). Para a avaliação da atividade antioxidante, antiacetilcolinesterase e letalidade, foram aplicadas as técnicas de DPPH, adaptação do método de Ellman e método de *Artemia salina*, respectivamente. O perfil químico mostrou que os extratos são ricos em compostos fenólicos. A avaliação da atividade antioxidante mostrou resultados que variaram de 5,46 mg/mL a 13,21 mg/mL. A avaliação da atividade antiacetilcolinesterase não apresentou resultados positivos (8,68%-15,35%). Os extratos apresentaram letalidade (162,08 mg/mL-71,91 mg/mL). Os resultados sugerem *A. ovata* como uma planta promissora para a realização de novos estudos químicos e biológicos que levem ao isolamento dos compostos bioativos.

Palavras-Chave: *acetilcolinesterase; atividade biológica; cromatografia; DPPH; fingerprint*

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Introduction

Currently, the family Polygalaceae Hoffmanns & Link comprises 27 genera and about 1,300 species (PASTORE; SILVEIRA, 2016) which are widely distributed throughout the world (MARQUES; PEIXOTO, 2007; FURNESS; STAFFORD, 1995). The genus *Asemeia* Raf. *ex* Small has neotropical distribution, and comprises 28 species, most of them with seasonal occurrence in savannas or less frequently in boards or inside forest vegetation (PASTORE; ABBOTT, 2012).

Studies in Polygalaceae demonstrate the presence of several compounds including coumarins, xanthones, flavonoids and steroids (MEOTTI et al., 2006, LAPA et al., 2006). In addition, another study also quantified the methyl salicylate in different genera and species of this family, with a possible contributor to the antinociceptive activity exhibited by some species (ROCHA et al., 2012). Besides, members of this family are also known to contain chemical compounds which exhibit analgesic activity, expectorant, sedative, antifungal, and others (LAPA, 2006). However, in general, members of Polygalaceae have medicinal potential which was not deeply explored and considering its diversity and ordinary occurrence in Brazilian vegetation, it makes Polygalaceae ideal for bioprospective projects and comparative studies among species or genera (AGUIAR; ARANHA FILHO, 2008).

Although the known potential relevance of this family, only a few chemical and biological studies are available and none of them include *Asemeia ovata* (Poir.) J.F.B.Pastore & J.R.Abott. *Asemeia ovata* is a widely-distributed species, occurring from Central America to Mato Grosso do Sul States in Brazil. Despite the wide distribution, this species is more frequent in areas of Brazilian semiarid with sandy soils.

Finally, the aim of this study was to establish the phytochemical profile using High Pressure Liquid Chromatography with Diode-Array Detector (HPLC-DAD). In addition, it sought to evaluate the antioxidant and antiacetylcholinesterase activity *in vitro* and its lethality on the microcrustacean *Artemia salina*.

Experimental

Plant material

Asemeia ovata was gathered in the area of the State University of Feira de Santana, (Short in Portuguese UEFS) located in Feira de Santana city, state of Bahia, Brazil, in August 2013. The identification was made by comparing the gathered material with the voucher specimen (168952 HUEFS) deposited in the Herbarium of the Biology Department of State University of Feira de Santana by the taxonomic expert in the family José Floriano B. Pastore.

Preparation of extracts

All plant material gathered (whole plant) was dried, grinded into powder and subjected to maceration using methanol extraction technique with solvent exchange every 3 days, repeated 5 times. The solvent was evaporated on rotatory evaporator at 50 °C, yielding the dried crude extract (404.8 g - 13.9 %). Approximately 3g of the crude extract was separated to perform the tests, and the remainder was partitioned with organic solvents of different polarities (hexane, chloroform and ethyl acetate). The solvents were evaporated to obtain the hexane extracts (HE): 62.31 g (15,33%); chloroform (CE): 33.97 g (8,39%) and ethyl acetate (EE): 15,09 g (3,73%).

Fingerprint

The analyses were performed using a Varian chromatograph with diode array detector Varian ProStar. The chromatographic separation was performed by column LiChroCART Purospher StaR® RP18-e (250 mm x 4.6 mm i.d.) (5µm) (Merck, Darmastad, Germany) combined with an appropriate pre-column from Merck. The elution gradient used was 0.7% acetic acid solution and acetonitrile (MeCN): 0.7% acetic acid (8:2) (Table 1). The chromatographic conditions included: flow rate of 1 mL/min, injection volume of 20 µL, wavelength range 220-600 nm. The chromatograms were compared at different wavelengths, as were the number of peaks and resolution.

Table 1. Gradient elution used for fingerprint analysis

Time (min)	Acetic Acid %	Acetic Acid + MeCN %
0:00	95	5
1:00	95	5
5:00	90	10
12:00	83	17
30:00	20	80

Determination of total phenolic and flavonoid

The phenolic contents of extracts were determined by the Folin Ciocalteu colorimetric method with modifications (GEORGÉ et al., 2005). Briefly, aliquots of 10 mg of extracts samples were, respectively, dissolved in 10 ml distilled water. This solution (0.5 ml) was mixed with 2 ml of 7.5% sodium carbonate (Na_2CO_3) and 2.5 ml of Folin–Ciocalteau reagent. The reaction mixture absorbance was measured at 760 nm (BEL Photonics, Model 1105). Gallic acid standard was used to build a calibration curve and results were expressed in mg equivalent gallic acid (EGA)/100 g of extract.

The total flavonoid content was determined according to the aluminum chloride colorimetric method. Samples of the extracts were prepared in methanol solutions. The absorbance was performed at 415 nm on spectrophotometer (BEL Photonics, Model 1105) and from the results a calibration curve was built with the standard quercetin, whose equation was used to calculate the total flavonoid content. The flavonoid content was expressed in g of equivalents quercetin (EQ)/100 g extract (POTHITIRAT et al., 2009).

Evaluation of antioxidant activity

The antioxidant activity of the extracts was performed by testing the scavenging of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (MALTERUD et al., 1993). Then solutions were prepared of the crude methanolic extract, hexane, chloroform and ethyl acetate in various concentrations.

To perform the test, 3 mL aliquot of the methanol solution of DPPH (45 µg/mL) was taken and 50 µL of the sample was added. The propyl gallate was used as a positive control (5 mg/mL). The reaction was monitored by UV-VIS, with λ = 517 nm on spectrophotometer (BEL Photonics, Model 1105), the absorbance being evaluated at 0 and 15 minutes. Readings were performed in triplicate.

The scavenging activity of DPPH by the sample was calculated in percentage, from the following formula: % scavenging of DPPH = 100 $(A_{t0} - A_{t15})$ / $(A_{t0p} - A_{t15p})$, where A_{t0} = initial absorbance of the sample; A_{t20}= final absorbance of the sample; A_{t0s} = standard initial absorbance and A_{t20s} = standard of the final absorbance. A graph was plotted correlating the mean values of % of DPPH scavenged versus the concentrations tested of the *A. ovata* extract, using 95% confidence interval. Using linear regression of graph points, the straight-line equation was derived which was used to calculate the EC_{50}.

Antiacetylcholinesterase Activity

A method developed by Ellman (ELLMAN et al., 1961) adapted by Rahman (RAHMAN et al., 2001) was used for the evaluation of antiacetylcholinesterase activity. The method which is based on the reaction of thiocholine with 5,5'-dithio-bis- (2-nitrobenzoic acid) to form a yellow color product. Then, they were deposited in the wells of the microplate 140 µL of phosphate buffer (pH 7.5), 20 µL of the enzyme acetylcholinesterase (0.5 U/ml) and 20 µL of the extracts to be tested (1 mg/mL). The plate was then incubated at room temperature for 15 minutes and then added to this 10 µL of 5-5'-dithiobis [2-nitrobenzoic acid] (10 mM) and 10 µL of acetylthiocholine iodide (15 mM). The absorbance was measured at 405 nm in an ELISA MultiskanTM GO 3.2. The results were compared with the commercial standard Eserine (physostigmine).

Artemia salina Lethality

In assessing the lethality, approximately 10 *naupliis* of *Artemia salina* were transferred to flasks containing artificial seawater and the extracts in five different concentrations. The tests were made in triplicate. The counting of the dead and live animals was performed after 24 h (MEYER et al., 1982; SERRANO; ORTEGA; VILLAR, 1996). LC_{50} was determined using the lethality of the *naupliis* of *A. salina* in contact with crude extracts and fractions (hexane, chloroform and ethyl acetate) of *A. ovata*.

Results e Discussion

Chemical Analysis

The methanolic extracts (ME), chloroform (CE) and ethyl acetate (EE) showed better resolution of the chromatograms at 280 nm, while the hexane extract (HE) performed at 250 nm (Figure 1). Chromatographic analysis showed variation in the profile of all extracts. The presence of several phenolic compounds, mainly phenolic acids (λmax = 220-290 nm and λmax = 330 approx.) (TERMENTZI; KEFALAS; KOKKALOU, 2008) and flavonoid compounds (λmax = 240-285 nm and λmax = 300-400 nm) (Zuanazzi 2000) in crude, chloroform and ethyl acetate extracts (Figure 2) were observed. Spectra with the characteristic of flavonoids was detected in the hexane extract, but due to the nature of the extract, it is suggested that they are methoxylated flavonoids. In a study of the *Polygala japonica* species, the fingerprint could also identify the presence of flavonoids (HONG-LAN et al., 2010).

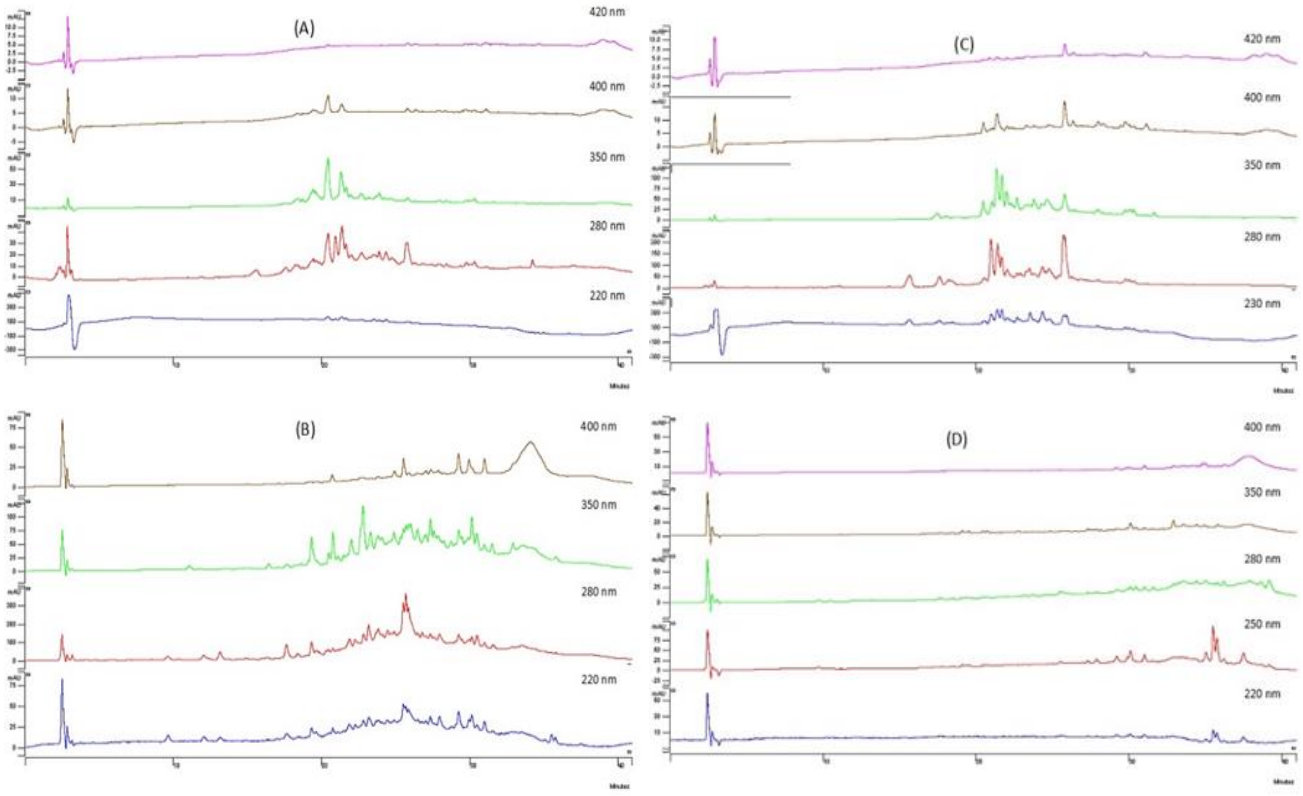

Figure 1. Comparison of the chromatograms at various wavelengths of (A) EBAO, (B) ECAO, (C) EAAO and (D) EHAO.
Fonte: own authorship

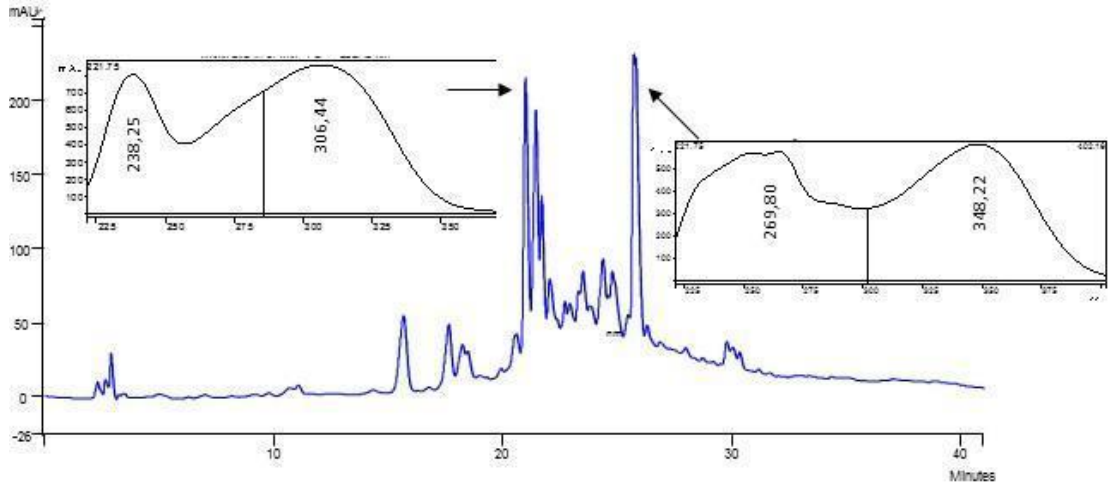

Figure 2. Chromatogram and UV spectra characteristic of *Asemeia ovata.* Fonte: own authorship

The total phenolic content obtained by the Folin Ciocalteu method for ME was 12.37 g/100 g. Values were obtained for CE, HE and EE 5.82 g/100 g, 1.69 g/100 g and 2.95 g/100 g, respectively. In the quantification of total flavonoids, the values for ME, CE and EE were obtained. However, the HE did not present sufficient levels to be detected by the used technique. Thus, 2.34 g/100 g for ME, 1.39 g/100 g in CE and 0.93 g/100 g for EE was obtained (Table 2).

Table 2. Content of metabolic classes valued at *Asemeia ovata*

Extract	Total flavonoids (g QE/100g)	Total phenols (g AGE/100g)
Crude	2.34	2.95
Hexane	-	1.69
Chloroform	1.39	5.82
Acetate	0.93	12.37

The phenolic and total flavonoid content showed superior results to those found with other species of the family. The crude extract of *P. sabulosa*, for example, contains about 0.00164 g/g of gallic acid of the phenolic content in dry extract, while the hexane extract and the ethyl acetate had approximately 0.00143 and 0.00160 g of gallic acid/g of dry extract, respectively (MENDES, 2008).

Antioxidant, antiacetylcholinesterase activity and *Artemia salina* lethality

The EC_{50} was found to ME of 9.46 mg/L to CE was 9.16 mg/mL, to HE was 13.21 mg/mL, and EE was 5.46 mg/mL (Table 3). A study of the hydro-alcoholic extract of *P. paniculata* showed that the antioxidant activity in EC_{50} is 61.2 mg/mL (LAPA et al., 2007). It is observed that *A. ovata*, in turn, performs better antioxidant activity.

The Antiacetylcholinesterase activity test showed percentage inhibition (% I) for ME 8.68, and 15.35 to EE. HE and CE showed no activity compared to standard Eserine. Therefore, due to lower results in the evaluation test, when compared with standard Eserine, the species under study did not show significant potential.

The lethality bioassay indicated LC_{50} equal to 162.08 mg/mL for ME, and equal to 93.25 g/mL, 90.77 mg/mL and 71.91 mg/mL for HE, CE and EE, respectively. The results of the brine shrimp lethality bioassay suggests that all extracts showed toxicity (MAYER, 1982 cited RIBEIRO et al., 2014), it considers that exhibit toxic extracts LC_{50} less than 1000 µg/mL, making them potential providers of a cytotoxic agent.

Table 3. Details of the biological activities evaluated in *Asemeia ovata*

Extract	Antioxidant Activity (CE_{50}-mg/mL)	Anticholinesterasic Activity (%I)	Lethality (CL_{50}-µg/mL)
Crude	5.46	8.68	162.08
Hexane	13.21	-	93.25
Chloroform	9.16	-	90.77
Acetate	9.46	15.35	71.91

Study with *Asemeia extraaxilaris* presented LC_{50} for crude ethanol extract of 242.23 g/mL, the fractions hexane, chloroform and ethyl acetate presented, 289.17 µg/mL, 248.22 µg/mL and 232.37 µg/mL, respectively (SILVA, 2014).

Conclusion

The results of this study showed that *Asemeia ovata* species presents a variety of compounds especially phenolic compounds, mainly phenolic acids and flavonoids. Therefore, this species has potential antioxidant activity, especially when compared to other species of the family. The lethality of extracts in the *Artemia salina* makes this a source of interest for future studies both for evidence of toxicity, providing potential cytotoxic agents, and even potential for related activities such as antimicrobial potential, parasiticide, trypanocidal and antimalarial.

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Referências

AGUIAR, A. C. A.; ARANHA FILHO, J. L. M. A família Polygalaceae na planície litorânea de Picinguaba, Ubatuba, São Paulo, Brasil. **Rev Bras Biocienc**,, v. 6, p. 321-328, 2008.

ELLMAN, G. L. et al. A new and rapid colorimetric determination of acetylcholinesterase activity. **Bio-chem Pharmacol.,** v. 7, p. 88-95, 1961.

FURNESS, S. H.; STAFFORD, P. J. The Northwest European Pollen Flora, 55 Polygalaceae. **Rev Palaeobot Palynol.,** v. 88, p. 61-82, 1995.

GEORGÉ S. et al. Rapid determination of polyphenois and vitamin C in plant-derived products. **J. Agric. Food Chem.,** v. 53, p. 1370-1373, 2005.

HONG-LAN W. et al. Chemical fingerprinting by HPLC-DAD-ELSD and principal component analysis of *Polygala japonica* from different locations in China. **Chin J Nat Med**, v. 8, p. 343-348, 2010.

LAPA, F. R. et al. Gastroprotective activity of the hydroalcoholic extract obtained from *Polygala paniculat*a L. in rats. **J Pharm Pharmacol**., v. 59, p. 1413-1419, 2007.

LAPA, F. R. et al Antinociceptive properties of the hydroalcoholic extract and the flavonoid rutin obtained from *Polygala paniculata* L. in mice. **Basic Clin Pharmacol Toxicol**., v. 104, n. 4, p. 206-315, 2006.

LAPA, F. R. **Avaliação da atividade antinociceptiva, antiinflamatória e protetora gástrica do extrato hidroalcoólico bruto da *Polygala paniculata* L**. Universidade Federal do Paraná, Paraná, Brasil, 2006.

MALTERUD, K.E. et al. Antioxidant and radical scavenging effects of anthraquinones and anthrones. **Pharmacology**. v. 47, p. 77-85, 1993.

MARQUES, M. C. M.; PEIXOTO, A. L. Taxonomic study of *Polygala subgenus* Ligustrina (Chodat) Paiva (Polygalaceae). **Rodriguésia**, v. 58, n. 1, p. 95-146, 2007.

MATOS, F. J. A. **Introdução a fitoquímica experimental**. Fortaleza: Edições UFC, 1997.

MENDES, B. G. **Polygala sabulosa A. W. Bennett: obtenção de estirilpironas e cumarinas, preparo de análogos e ensaios de atividades biológicas**. Centro de Ciências Físicas e Matemáticas, Universidade Federal de Santa Catarina, Santa Catarina, Brasil, 2008.

MEOTTI, F. C. et al. Antinociceptive properties of coumarins, steroid and dihydrostyryl-2-pyrones from *Polygala sabulosa* (Polygalaceae) in mice. **J Pharm Pharmacol**., v. 58, n. 1, p. 107-12, 2006.

MEYER, B. N. et al. Brine shrimp: a convenient general bioassay for active plant constituents. **J Med Plants Res**., v. 45, p. 31-34, 1982.

PASTORE, J. F.B.; ABBOTT, J. R. Taxonomic notes and new combinations for *Asemeia* (Polygalaceae). **Kew bull.,** v. 67, p. 801-813, 2012.

PASTORE, J. F. B.; SILVEIRA, J. B. Flora das cangas da Serra dos Carajás, Pará, Brasil: Polygalaceae**. Rodriguésia**, v. 67, n. 5, p. 1451-1458, 2016.

POTHITIRAT, W. et al. Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. **Fitoterapia**, v. 80, p. 442–447, 2009.

RAHMAN, A. et al. Acetyl and butyrylcholinesterase-inhibiting triterpenoid alkaloids from *Buxus papillosa*. **Phytochemistry**, v. 58, p. 963-968, 2001.

RIBEIRO, A. R. C. et al. Study of anthelmintic activity of the ethanol extract of *Jatropha mollissima* (Pohl) Baill. (Euphorbiaceae) in *Haemonchus contortus* in sheep in semi-arid Paraiba. **Pesqui. Vet. Bras.**, v. 34, p. 1051-1055, 2014.

ROCHA, J. L. C. et al. Quantification of methyl salicylate in four kinds Polygalaceae, by HPLC-DAD. **Quim Nova.**, v. 35, n. 11, p. 2263-2266, 2012.

SERRANO, C.; ORTEGA, T.; VILLLAR, A. Biological activity of traditional medicines from Spain and Guatemala *Artemia salina* bioassay: a revision. **Phytother Res.,** v. 10, p. 118-120, 1996.

SILVA, C. B. ***Asemeia extraaxillaris* (Chodat) J.F.B. Pastore e J.R. Abbott (Polygalaceae) e *Microlobius foetidus* (subsp. *Paraguensis* (Benth.)M. Sousa et G. Andrade) (Fabaceae - Mimosoideae): Contribuição ao estudo fitoquímico e investigação das atividades biológicas (alelopática,.antiploriferativa, antineoplásica, antimicrobiana, antioxidante, tóxica e larvicida).** Setor de Ciências da Saúde, Universidade Federal do Paraná, Paraná, Brasil, 2014.

TERMENTZI, A.; KEFALAS, P.; KOKKALOU, E. LC–DAD–MS (ESI+) analysis of the phenolic content of Sorbus domestica fruits in relation to their maturity stage. **Food Chemistry**, v. 106, p. 1234-1245, 2008.

ZUANAZZI, J. A. S. Flavonoides. In: Simões CMO, Sebenkel EP, Gosmann G., Mello JCP, Mentz LA, Petrovick PR (Org.). **Farmacognosia: da planta ao medicamento**. 2 ed. Porto Alegre/Florianópolis: Ed. Universidade/UFGRS/ Ed. Da UFSC, p. 489-515, 2000.