



Short communication

New cathepsin V inhibitor from stems of *Bowdichia virgilioides*

Taynara L. Silva ^a, João B. Fernandes ^a, M. Fátima das G.F. da Silva ^a,
Helder N. Consolaro ^b, Lorena R.F. de Sousa ^{a,b}, Paulo C. Vieira ^{a,c,*}

^a Departamento de Química, Universidade Federal de São Carlos, São Carlos, SP, Brazil

^b Unidade Acadêmica Especial de Química, Universidade Federal de Goiás – Regional Catalão, Catalão, GO, Brazil

^c Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil



ARTICLE INFO

Article history:

Received 20 December 2018

Accepted 6 April 2019

Available online 2 June 2019

Keywords:

Cathepsin
Cysteine peptidases
Inhibitors
Cerrado biome

ABSTRACT

Bioassay-guided fractionation of *Bowdichia virgilioides* Kunth, Fabaceae, extracts has led to the isolation of cathepsin V inhibitors. The investigation of the hexane and ethyl acetate extracts allowed the characterization of eleven compounds: lupeol, lupenone, β -sitosterol and stigmaterol in mixture, *trans p*-coumaric acid ester derivative, syringaresinol, bowdenol, 8-methoxycoumestrol, 3,4-hydroxy-7-methoxyisoflavone, 7,3'-dihydroxy-4'-methoxyisoflavone, and 5,4'-dihydroxy-7'-methoxyisoflavone. Structures of compounds were established by 1D and 2D NMR, and MS experiments. Among the isolated compounds, *trans p*-coumaric acid ester derivative and 8-methoxycoumestrol showed significant inhibition on cathepsin V, which is up to now unexplored.

© 2019 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Lysosomal cysteine peptidases are enzymes from papain family (C1) found in different organisms, these enzymes have as primary function protein degradation. Human cathepsin V (CatV, EC 3.4.22.43) is specifically expressed in thymus and testis and its functions are linked to oncology, type 1 diabetes, auto-immune and neurological diseases (Turk et al., 2012). Several secondary metabolites from plants have been described as Cat V inhibitors, such as acridone alkaloids (Severino et al., 2011) chalcones and flavones (Alvim et al., 2010). Although inhibitors of cathepsins have been discovered, many issues restrict these drug-like compounds to advance into clinical usage (Li et al., 2016). In the search for novel potent, selective and reversible inhibitors of cathepsins, we have selected *Bowdichia virgilioides*, a plant with ethnopharmacological relevance to be investigated.

Bowdichia virgilioides Kunth, Fabaceae, is a Brazilian medicinal plant from Cerrado biome also known as sucupira-preta. The tea of seeds of *B. virgilioides* is used in folk medicine for the treatment of rheumatism, arthritis and skin diseases and its bark is used against ulcers and diabetes (Barbosa-Filho et al., 2004; Thomazzi et al., 2010). The aqueous extracts of *B. virgilioides* have showed previously biological activities such as, antinociceptive activity in stem

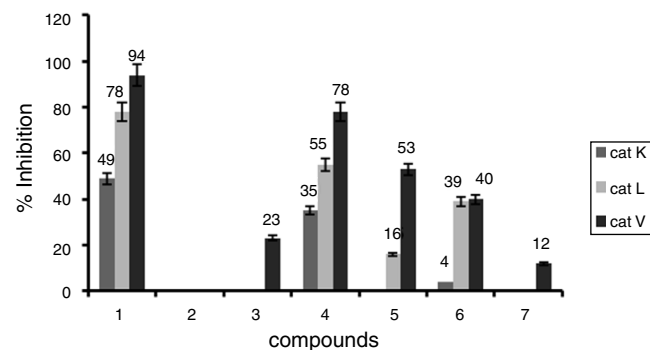


Fig. 1. Inhibitory activity of cathepsins by isolated compounds from *Bowdichia virgilioides* (1–7).

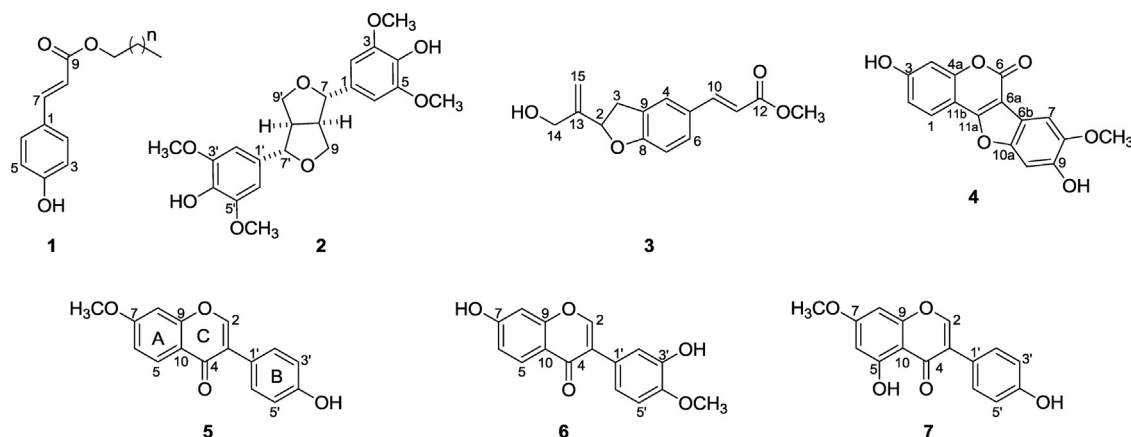
bark (Silva et al., 2010), analgesic and anti-inflammatory activities in leaves and bark (Thomazzi et al., 2010).

In this work, the phytochemical investigation of stems of *B. virgilioides* was conducted through the screening of fractions with inhibitory activity on cathepsins K, L and V. Triterpenes, coumaric acids, furofuran lignans, benzofuran derivative, coumestrol and isoflavones were isolated from hexane and ethyl acetate extracts and identified by 1D and 2D NMR spectra and MS (Fig. 1).

The chemical investigation of the hexane extract of stems guided by enzyme assays lead to *trans p*-coumaric acid ester derivative (1) which showed to be inhibitor of cat L and V, and from EtOAc extract was obtained the active compound 8-methoxycoumestrol (4).

* Corresponding author.

E-mail: pcvieira@fcrp.usp.br (P.C. Vieira).



Materials and methods

General materials

The NMR experiments (1D and 2D) were acquired on a Bruker DRX-400 NMR spectrometer (^1H : 400 MHz; ^{13}C : 100 MHz) using TMS as internal reference and the deuterated solvents (CDCl_3 , $(\text{CD}_3)_2\text{CO}$, CD_3OD). These solvents have been obtained from Merck. MS spectrum was acquired on API2000 LC/MS/MS system with triple quadrupole mass spectrometer (MDS-Sciex/Applied Biosystems). HPLC equipment was a model SPD-M10A, Photodiode Array (PDA) detectors (Shimadzu), software Class-VP and a Shimadzu GC-17A. Chromatographic procedures were carried out using HPLC, (columns: phenyl, 5 μ and 10 μ ; phenyl-hexyl, 5 μ and 10 μ), silica gel 60 (Merck, 230–400 mesh) and Sephadex LH-20 (Amersham Pharmacia Biotech AB). Solvents used were from Vetec. Thin-layer chromatography (TLC) on pre-coated aluminum silica 60 F₂₅₄ (Merck) was used to visualize compounds by UV_{254/366} and reaction with sulfuric vanillin solution.

Plant material

Bowdichia virgilioides Kunth, Fabaceae, stems were collected in December 2010 in the cerrado at Federal District-Gama/DF (DF 480, lot 01, SMA), Brazil. The plant material was identified by Dr. Helder Nagai Consolaro and deposited at the EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) Herbarium of the Genetic Resources and Biotechnology (CENARGEN) (voucher n° EG676).

Extraction and isolation

The stems were kept in an oven at 40 °C until completely dried. Afterwards, the stems were converted into a powder (854 g). The powder was extracted with ethanol in a macerating process, after solvent filtration and evaporation in a rotavap under reduced pressure afforded the ethanolic extract (69.6 g). Ethanolic extract was resuspended in $\text{MeOH:H}_2\text{O}$ (1:3) solvents at room temperature. Liquid-liquid partition of ethanolic extract (30.2 g) was performed generating hexane, EtOAc and hydroalcoholic extracts. The EtOAc extract (BvSEa) and hexane extract (BvSH) showed significant inhibition of cathepsins L and V. BvSH (1.3 g) was fractionated using silica gel 60 (SiO_2) column chromatography (CC) (230–400 mesh, 16.0 $\times$ 7 cm, hexane/EtOAc/MeOH, gradient), affording four fractions (H1 until H4).

Fractions H2 and H3 showed inhibitory activity of cathepsins (K, L and V) and were chromatographed over conditions (H2: SiO_2 , 230–400 mesh, 28.0 $\times$ 4 cm, 7.5:2.5 hexane/EtOAc, isocratic) and (H3: SiO_2 , 230–400 mesh, 25.0 $\times$ 2 cm, 9.7:0.3 hexane/EtOAc, isocratic), respectively. From CC of H2 (0.7 g) was obtained lupeol

(256 mg) and lupenone (14.5 mg). From CC of H3 (0.4 g) was obtained β -sitosterol and stigmastrol in mixture (15.3 mg), and *trans p*-coumaric acid ester derivative (1) (0.8 mg).

BvSEa (11.6 g) was chromatographed (SiO_2 , 230–400 mesh, 20 $\times$ 5 cm, 9:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$ isocratic) yielding ten fractions (A1 until A10). TLC was used for analytical analysis and enzymatic assay of fractions was carried out. The fractions with more than 70% of inhibition (A2, A4 and A6) were fractionated again.

HPLC of A2 (11.5 mg) was performed (phenyl-hexyl, 10 μ , 25 $\times$ 0.46 cm, 4.7:5.3 $\text{MeOH/H}_2\text{O}$ isocratic) and the compound 5,4'-dihydroxy-7'-methoxyisoflavone (7) (0.9 mg) has been identified in the first fraction obtained from HPLC. Fraction four derivative of HPLC was subjected to CC (SiO_2 , 230–400 mesh, 30 $\times$ 2 cm, 3.0:2.0 hexane/acetone, isocratic) giving seven fractions. Among the seven, fraction 4 was fractionated (phenyl, 5 μ , 25 $\times$ 0.46 cm, 7:3 $\text{MeOH/H}_2\text{O}$ isocratic) leading to 4-hydroxy-7-methoxyisoflavone (5) (0.5 mg), and CC of fraction 6 (Sephadex® LH-20, 53 $\times$ 1.5 cm, MeOH, isocratic) led to syringaresinol (2) (0.3 mg).

The fraction A4 (104 mg) derived from BvSEa was fractionated using CC (SiO_2 , 230–400 mesh, 36 $\times$ 1.5 cm, 9.8:0.2 $\text{CH}_2\text{Cl}_2/\text{MeOH}$, isocratic) and the first and sixth fraction obtained of this CC was further purified by HPLC (phenyl-hexyl, 10 μ , 25 $\times$ 0.46 cm, 4.7:5.3 $\text{EtOH/H}_2\text{O}$ isocratic) affording 8-methoxycoumestrol (4) (0.5 mg) and 7,3'-dihydroxy-4'-methoxyisoflavone (6) (1 mg), respectively.

The fraction A6 (111.5 mg) from BvSEa was submitted to CC with sephadex LH20 and SiO_2 and after two chromatographic steps were obtained bowdenol (3) (1.2 mg) and 4-hydroxy-7-methoxyisoflavone (5) (0.6 mg). The CC conditions to isolate these compounds: A6 fraction (sephadex LH20, 50 $\times$ 3 cm, MeOH, isocratic); fraction 8 from column A6 was submitted to CC (SiO_2 , 230–400 mesh, 23 $\times$ 2 cm, 3:2 hexane/acetone, isocratic) leading to compound 3; fraction 10 from A6 column was purified with CC (SiO_2 , 230–400 mesh, 30 $\times$ 1.5 cm, 3:2 hexane/acetone, isocratic) leading to compound 5. The isolated compounds were characterized by 1D and 2D NMR and MS and comparison with literature data (Prakash and Prakash, 2012; Costa et al., 2010; Kort et al., 1996; Durango et al., 2002; Park et al., 2010; Melo et al., 2001; Ingham et al., 1981; Du et al., 2006; Vitor et al., 2004).

Cathepsins assay

Cathepsins V, K and L were obtained by expression in *Pichia pastoris* and detailed conditions of assay were the same as previously described (Ramalho et al., 2014; Severino et al., 2011).

Table 1¹H NMR spectroscopic data for isoflavones **5** and **7** (400 MHz, CD₃OD), and for isoflavone **6** (400 MHz, (CD₃)₂CO).

H	5	6	7
2	8.17 (s)	8.04 (s)	8.12 (s)
5	8.07 (d, 9.0)	7.97 (d, 8.8)	
6	6.95 (dd, 9.0, 2.0)	6.90 (dd, 8.8, 2.4)	6.37 (d, 2.5)
7	3.84 (s) OMe		3.88 (s) OMe
8	6.85 (d, 2.0)	6.76 (d, 2.4)	6.37 (d, 2.5)
2'	7.49 (d, 8.5)	7.15 (d, 1.9)	7.39 (d, 8.5)
3'	7.01 (d, 8.5)		6.86 (d, 9.0)
4'		3.85 (s) OMe	
5'	7.01 (d, 8.5)	6.95 (d, 8.3)	6.86 (d, 9.0)
6'	7.49 (d, 8.5)	7.05 (dd, 8.4, 1.8)	7.39 (d, 8.5)

Results and discussion

Seven compounds **1–7** were isolated from *B. virgilioides* extracts. Hexane and EtOAc extracts of stems of *B. virgilioides* were tested in a concentration of 125 µg/ml against cathepsins K, L and V. The less polar extract showed significant inhibition on Cat L and V, with 91% and 97% of inhibition, respectively. Likewise, the EtOAc extract inhibited Cat K, L and V (70, 97 and 99%, respectively).

Compounds from hexane extract were characterized as lupeol (Prakash and Prakash, 2012), lupenone (Prakash and Prakash, 2012), β-sitosterol and stigmaterol in mixture (Costa et al., 2010), and an active compound was isolated and identified from bioguided study as a derivative of *trans p*-coumaric acid (**1**) (Kort et al., 1996) showing 78% of inhibitory activity on cat L and 94% of inhibition on cat V when evaluated at the concentration 50 µg/ml (Fig. 1). It was observed by ¹H NMR experiment that compound **1** had a long carbon chain, however we could not determine it by MS.

Several chromatographic steps of EtOAc extract guided by enzymatic assay conducted to the isolation of the active compound 8-methoxycoumestrol (**4**) (Durango et al., 2002), together with other constituents, syringaresinol (**2**) (Park et al., 2010;), bowdenol (**3**) (Melo et al., 2001), 4-hydroxy-7-methoxyisoflavone (**5**) (Ingham et al., 1981), 7,3'-dihydroxy-4'-methoxyisoflavone (**6**) (Du et al., 2006), and 5,4'-dihydroxy-7'-methoxyisoflavone (**7**) (Vitor et al., 2004).

Except for compound **3** and the terpenes from hexane extract, all compounds characterized from *B. virgilioides* were not identified previously in this species. Among the compounds, syringaresinol (**2**), 8-methoxycoumestrol (**4**) and the isoflavones **5**, **6** and **7** are new in *Bowdichia* genus (Barbosa-Filho et al., 2004; Melo et al., 2001; Vellozo et al., 1999a,b; Juck et al., 2006). Experiments of MS and NMR such as ¹H NMR, HSQC, HMBC, and 1D NOE were performed for these compounds and results were compared to literature data (Prakash and Prakash, 2012; Costa et al., 2010; Kort et al., 1996;

Durango et al., 2002; Park et al., 2010; Melo et al., 2001; Ingham et al., 1981; Du et al., 2006; Vitor et al., 2004).

Previously, isoflavones, isoflavone glucoside and isoflavanones have been identified in *B. virgilioides* (Vellozo et al., 1999a,b; Juck et al., 2006). From the roots of *B. virgilioides* were isolated odoratin, afromosin, cladrastin, fujikinetin and isoflavones glucosides (Vellozo et al., 1999a,b). From the wood, isoflavones with different substitution pattern in the aromatic ring moieties were reported by Juck et al. (2006), such as, 7,8,4'-trimethoxyisoflavone and 7,3'-hydroxy-4'-methoxyisoflavone.

Compounds **5** and **7** showed a spin coupling system AA'BB' for ring B integrating for two hydrogens each, with similar chemical shifts for H-3', H-5', more shielded and for H-2', H-6' more deshielded, respectively.

The position of methoxyl group for compounds **5** and **7** was assigned by NOE experiments, which selective irradiation of the resonance frequency of H-8 (δ 6.85 and δ 6.37, compounds **5** and **7**, respectively) caused an enhancement of the signal related to OMe group at δ 3.84 and δ 3.88 (compounds **5** and **7**, respectively). In addition, when OMe was irradiated, a NOE was also observed at chemical shifts of H-8 (δ 6.85 and δ 6.37, compounds **5** and **7**, respectively).

The ring A for compounds **5** and **6** revealed the same substitution pattern characterized by the presence of an ABX coupling pattern, with two doublets related to meta coupling (H-8 and H-6) and ortho coupling (H-5 and H-6), and a doublet of doublets by coupling of H-6 to both, ortho and meta protons (H-5 and H-8, respectively) (Table 1). The methoxyl group of compound **6** in the ring B was established comparing chemical shifts as well by HMBC correlations. It was observed a long-range heteronuclear coupling of hydrogens at δ 3.85 (OMe) and δ 6.95 (H-5') with the same carbon at δ 146.19 (C-4').

The LC/MS/MS data for compound **4** showed the molecular ion [M–H][–] at 297 *m/z* providing its molecular formula as C₁₆H₉O₆.

Table 2¹H NMR spectroscopic data for 8-methoxycoumestrol (**4**) (400 MHz, (CD₃)₂CO).

C	HSQC		HMBC	1D NOE
	δ _C	δ _H	² J _{HC} ; ³ J _{HC} ; ⁴ J _{HC}	
1	123.2	7.88 (d, 8.5)		H-2
2	114.1	7.01 (dd, 8.5, 2.3)	H-4	H-1
3	155.9		H-1, H-4	
4	103.8	6.94 (d, 2.3)	H-1, H-2	
4a	161.1		H-1, H-4	
6				
6a	104.1		H-7	
6b	115.5		H-7	
7	102.5	7.44 (s)	H-10	
8-OCH ₃	147.3	3.99 (s)	H-7	H-7
9	151.1		H-7	
10	99.0	7.24 (s)	H-7	
10a				
11a				
11b	105.9		H-1, H-2, H-4	

The ^1H NMR for compound **4** showed the presence of two aromatic rings. The multiplicity of the signals as two doublets at δ 6.94 and δ 7.88, and a doublet of doublets (δ 7.88) suggested the trisubstituted ring moiety related to the coupling of H-1, H-2 and H-4. The singlets at δ 7.44 and δ 7.24 belong to hydrogens positioned *para* each other (H-7 and H-10, respectively). The singlet at δ 3.99 indicated the presence of a methoxyl group at C-8, which was determined by the selective irradiation of the resonance frequency of OMe (δ 3.99), that generated a NOE signal of H-7 (δ 7.44). The ^{13}C NMR chemical shifts were obtained from projections of HSQC and HMBC data (Table 2) suggesting the structure of compound as 8-methoxycoumestrol (Durango et al., 2002).

The enzyme inhibitory activity of 8-methoxycoumestrol (**4**) was determined for cathepsin V showing IC_{50} value of $17.4 \pm 1.0 \mu\text{M}$. In previous work, a fraction containing 9-methoxycoumestrol and isoflavones showed cytotoxic effects on the cell lines evaluated KB, K562 and HL60 with IC_{50} values of 17.6, 8.3 and $9.7 \mu\text{g/ml}$, respectively (Lu et al., 2009). Likewise, the EtOAc fraction from *B. virgilioides* containing similar phenolic compounds (8-methoxycoumestrol and isoflavones) showed inhibition on Cat K, L and V (70%, 97% and 99%, respectively, in concentration of $125 \mu\text{g/ml}$) (Fig. 1).

This is the first report of coumaric acid derivatives and coumestrol as cathepsins V and L inhibitors. Cytotoxicity and cathepsins V and L activities are closely related to cancer and metastasis process, suggesting that our results of inhibitory activities of Cat V and Cat L could be correlated with cytotoxic activity in tumor cell lines found previously (Lu et al., 2009; Turk et al., 2012).

Authors' contributions

TLS (PhD student) carried out phytochemical procedures and enzymatic assays. HNC contributed in collecting plant sample with identification. JBF and MFGFS contributed with critical reading of the manuscript. LRFS contributed with analysis of data and drafted the paper. PCV supervised the laboratory work contributing with chromatographic analysis, NMR and MS data and to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgment

TSL would like to thank São Paulo Research Foundation (FAPESP) for the master scholarship (Grant # 2012/11819-8). The authors would like to thank FAPESP (Grant # 2012/25299-6) and Coordination for the Improvement of Higher Education Personnel (CAPES, Finance Code 001)

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bjpp.2019.04.004](https://doi.org/10.1016/j.bjpp.2019.04.004).

References

- Alvim Jr., J., Severino, R.P., Marques, E.F., Martinelli, A.M., Vieira, P.C., Fernandes, J.B., Silva, M.F., das, G.F.da., Corrêa, A.G., 2010. Solution phase synthesis of a combinatorial library of chalcones and flavones as potent cathepsin V inhibitors. *J. Comb. Chem.* 12, 687–695.
- Barbosa-Filho, J.M., Almeida, J.R.G. da S., Costa, V.C. de O., Da-Cunha, E.V.L., Silva, M.S.da, Braz-Filho, R., 2004. Bowdichine, a new diaza-adamantane alkaloid from *Bowdichia virgilioides*. *J. Asian Nat. Prod. Res.* 6, 11–17.
- Costa, D.A., Chaves, M.H., Silva, W.C.S., Costa, C.L.S., 2010. Constituintes químicos, fenóis totais e atividade antioxidante de *Sterculia striata* St. Hil. et Naudin. *Acta Amazon* 40, 207–2012.
- Du, X., Bai, Y., Liang, H., Wang, Z., Zhao, Y., Zhang, Q., Huang, L., 2006. Solvent effect in ^1H NMR spectra of 3'-hydroxy-4'-methoxy isoflavonoids from *Astragalus membranaceus* var. *mongolicus*. *Magn. Reson. Chem.* 44, 708–712.
- Durango, D., Quiñones, W., Torres, F., Rosero, Y., Gil, J., Echeverri, F., 2002. Phytoalexin accumulation in colombian bean varieties and aminosugars as elicitors. *Molecules* 7, 817–832.
- Ingham, J.L., Keen, N.T., Mulheirn, L.J., Lyne, R.L., 1981. Inducibly-formed isoflavonoids from leaves of soybean. *Phytochemistry* 20, 795–798.
- Juck, D.B.F., Rezende, L.C.de, David, J.P., Queiroz, L.P.de, David, J.M., 2006. Two new isoflavonoids from *Bowdichia virgilioides*. *Nat. Prod. Res.* 20, 27–30.
- Kort, R., Vonk, H., Xu, X., Hoff, W.D., Crielard, W., Hellingwerf, K.J., 1996. Evidence for *trans-cis* isomerization of the *p*-coumaric acid chromophore as the photochemical basis of the photocycle of photoactive yellow protein. *FEBS Lett.* 382, 73–78.
- Li, Y.Y., Fang, J., Ao, G.Z., 2016. Cathepsin B and L inhibitors: a patent review (2010 – present). *Expert. Opin. Ther. Pat.* 27, 643–656.
- Lu, D., He, H., Wu, B., Yao, S., 2009. Cytotoxic effect on cancer cells and structural identification of phenols from *Spatholobi caulis* by HPLS-ESI-MSn. *Nat. Prod. Commun.* 4, 809–812.
- Melo, F.N., Navarro, V.R., Silva, M.S., Da-Cunha, da E.V.L., Barbosa-Filho, J.M., Braz-Filho, R., 2001. Bowdenol, a new 2,3-dihydrobenzofuran constituent from *Bowdichia virgilioides*. *Nat. Prod. Lett.* 15, 261–266.
- Park, J.-H., Yeon, S.-W., Cho, J.-G., Lee, D.-Y., Kim, Y.-S., Baek, N.-I., 2010. Lignans from silkworm droppings and their promotional activities on heme oxygenase-1 (ho-1). *J. Korean Soc. Appl. Biol.* 53, 734–739.
- Prakash, C.V.S., Prakash, I., 2012. Isolation and structural characterization of lupane triterpenes from *Polypodium vulgare*. *Res. J. Pharm. Sci.* 1, 23–27.
- Ramalho, S.D., Sousa, L.R.F.de, Nebo, L., Maganhi, S.H., Caracelli, I., Zukerman-Schpector, J., Lima, M.I.S., Alves, M.F.M., Silva, M.F. da G.F. da, Fernandes, J.B., Vieira, P.C., 2014. Triterpenoids as novel natural inhibitors of human cathepsin L. *Chem. Biodivers.* 11, 1354–1363.
- Severino, R.P., Guido, R.V.C., Marques, E.F., Brömmle, D., Silva, M.F. da G.F. da., Fernandes, J.B., Andricopulo, A.D., Vieira, P.C., 2011. Acridone alkaloids as potent inhibitors of cathepsin V. *Bioorg. Med. Chem.* 19, 1477–1481.
- Silva, J.P., Rodarte, R.S., Calheiros, A.S., Souza, C.Z., Amendoeira, F.C., Martins, M.A., Silva, P.M.R., Frutuoso, V.S., Barreto, E., 2010. Antinociceptive activity of aqueous extract of *Bowdichia virgilioides* in mice. *J. Med. Food* 13, 348–351.
- Thomazzi, S.M., Silva, C.B., Silveira, D.C.R., Vasconcellos, C.L.C., Lira, A.F., Cambui, E.V.F., Estevam, C.S., Antonioli, A.R., 2010. Antinociceptive and anti-inflammatory activities of *Bowdichia virgilioides* (sucupira). *J. Ethnopharmacol.* 127, 451–456.
- Turk, V., Stoka, V., Vasiljeva, O., Renko, M., Sun, T., Turk, B., Turk, D., 2012. Cysteine cathepsins: from structure, function and regulation to new frontiers. *Biochim. Biophys. Acta* 1824, 68–88.
- Veloze, L.S.M., Silva, B.P., Bernardo, da R.R., Parente, J.P., 1999a. Odoratin 7-O- β -D-glucopyranoside from *Bowdichia virgilioides*. *Phytochemistry* 52, 1473–1477.
- Veloze, L.S.M., Silva, B.P., Silva, da E.M.B., Parente, da J.P., 1999b. Constituents from the roots of *Bowdichia virgilioides*. *Fitoterapia* 70, 532–535.
- Vitor, R.F., Mota-Filipe, H., Teixeira, G., Borges, C., Rodrigues, A.L., Teixeira, A., Paulo, A., 2004. Flavonoids of an extract of *Pteropartum tridentatum* showing endothelial protection against oxidative injury. *J. Ethnopharmacol.* 93, 363–370.