Inhibitory Activity and Docking Studies of Cathepsin V for Isoflavanoids from *Dalbergia miscolobium* Benth

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**Abstract**

Plant extracts from *Dalbergia* genus have demonstrated a wide range of biological activities including, analgesic, antidiabetic, anti-inflammatory, and antimicrobial. In this work, the chemical study of the extracts from the leaves and branches of *Dalbergia miscolobium* led to the isolation and identification of five isoflavonoids: prunetin, di-O-methyldaidzein, 8-O-methylretusin, duartina and sativan employing nuclear magnetic resonance data. The inhibition activity of these isoflavonoids was screened against cathepsin V at a concentration of 100 µM. Duartin and sativan showed remarkable activity against cathepsin V displaying 89% and 88% inhibition values, respectively. Also, docking simulations to predict the binding mode of isoflavonoids into this protein were performed and results showed that the duartin is nicely bound to the cathepsin V and stabilized by two hydrogen bonds. The isoflavans duartin and sativan showed a significant inhibition percentage of cathepsin V, which can be considered as targets into cathepsin V inhibitors investigation and further chemical study of *Dalbergia* species may afford novels isoflavonoids cathepsin inhibitors.

**Keywords:** Natural products; prunetin; di-O-methyldaidzein; 8-O-methylretusin; Duartina; sativan; biological activity.

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The Fabaceae family includes approximately 20,000 species distributed in 630 genera. It is the second most important family in nutritional and economic aspects, behind only the family of cereals. The genus *Dalbergia* is distributed mainly in tropical and subtropical regions of Africa, America, and Asia. In those
continents, approximately 250 Dalbergia species have been cataloged. In Brazil, the genus is found throughout different ecosystems showing 21 endemisms.

Various plant extracts from Dalbergia have demonstrated a wide range of biological activities such as analgesic, antidiabetic, anti-inflammatory, anti-anxiety, antidepressant, antileptogenic, antimicrobial, antioxidant, antifungal, among others. Isoflavones are typical in Fabaceae and were evaluated by molecular docking as cathepsin-L like inhibitors. Cathepsins are lysosomal cysteine peptidases implicated in many pathological conditions. These enzymes have the primary function of degrading proteins, non-selectively, inside of the lysosome and are found in various organs and tissues. Specifically, cathepsin-V (cat-V) is expressed in thymus, testis and corneal epithelium and its biological role has been associated with cancer progression, atherosclerosis, type 1 diabetes, auto-immune and neurological diseases.

In this report, for the first time, Dalbergia miscolobium Benth. was explored to obtain isoflavones with inhibitory activity against cat-V. We present the isolation and characterization of five isoflavones. Cat-V inhibition tests were performed, and to explain a remarkable inhibitory activity, docking studies were performed to predict the binding mode in this protein.

2. Experimental

2.1. Apparatus, materials, and chemicals

NMR spectra were recorded on a Bruker DRX-400 or a Varian 400 MHz (1H: 400 MHz; 13C: 100 MHz) instrument using CDCl3 (TMS as an internal reference) and CD3OD as solvents (Sigma Aldrich). The column chromatography was performed using silica gel 60 (70-230 mesh), flash silica gel (230-400 mesh), and Sephadex LH-20. The high-performance liquid chromatography (HPLC) analysis were performed in an Agilent G1311C-1260 with a quaternary pump coupled to a UV-Vis detector with a diode array (DAD, model G1315D-1260) using a C-18 Phenomenex column (250 mm × 4.5 mm, 5 μm) on analytical mode and (250 mm × 9.4 mm, 5 μm) on semi-preparative mode. The GC-MS analyses were performed in a Shimadzu, model GCMS QP5000, equipped with a DB-5 capillary column (30 m × 0.25 mm). The high-resolution mass spectra were obtained in an FT-ICR-MS device from MS Bruker Daltonics, model solariX.

The deionized water was purified using a Millipore Milli-Q Plus system (Bedford, MA, USA). Hydrochloric acid (HCl) from Vetec® (Rio de Janeiro, RJ, Brazil), sodium hydroxide (NaOH) from Synth® (Diadema, SP, Brazil), acetonitrile, n-hexane, ethanol, ethyl acetate and methanol from JT Baker® (Mexico City, MX, Mexico). All commercially available chemicals and reagents were purchased from Aldrich Chemical Co. and Sigma and used without further purification.

2.2. Plant Material

Leaves and branches from Dalbergia miscolobium were collected in São Carlos city (campus of the Federal University of São Carlos - UFSCar, São Paulo, Brazil) in May 2011. The plant was identified by a botanist, Maria Inês Salgueiro Lima. A voucher specimen (8371) has been deposited in the herbarium of the Botanical Department – UFSCar.

2.3. Extraction and isolation

Dried leaves (192 g) and branches (684 g) of D. miscolobium were crushed and extracted with ethanol in an Ika Ultra Turrax for 5 min at 20000 rpm at room temperature (thrice with 200 g dried weight each). The liquid ethanol extracts were combined, filtered, concentrated under reduced pressure and resuspended in a mixture of ethanol:ultra-pure water (1:3, v/v) followed by fractionation through a liquid-liquid partition with n-hexane for leaves (HL) and branches (HB) followed by ethyl acetate only for branches (EB).

HL (5.0 g) was subjected to column chromatography (2.5 cm × 16 cm) with silica gel 60 in an elution gradient starting with n-hexane:ethyl acetate (4:1, v/v), followed by n-hexane:ethyl acetate (1:1, v/v), ethyl acetate (100%), and then methanol (100%), which allowed the collection of seven fractions after TLC analysis (named sequentially from HL1 to HL7). The HL2 fraction (227.5 mg) was subjected into a column using flash silica gel (2.0 cm × 18.0 cm) starting with n-hexane:ethyl acetate (9:1, v/v), followed
by ethyl acetate (100%) and methanol (100%), yielding nine subfractions after TLC analysis.

The complete phytochemical study also allowed the identification of the steroids, such as triterpenes α-amyrin (9), β-amyrin (10), lupeol (11) and the diterpene phytol (12) were provided in a mixture (37.5 mg) from the subfraction HL2.3. From subfraction HL2.5, sitosterol (6), campesterol (7), and stigmasterol (8) were also obtained in a mixture (5.7 mg). The structures of these substances are presented in Figure 1. The HL4 fraction (43.1 mg) was subjected to a column chromatography using flash silica gel (1.5 cm × 16.0 cm), starting with n-hexane:ethyl acetate (3:1, v/v) followed by ethyl acetate (100%) and then methanol (100%). Eight subfractions were obtained after analysis by TLC. The subfraction HL4.4 (6.7 mg) was subjected to chromatographic separation in semi-preparative HPLC, leading to the isolation of 8-O-methylretusin (3, 1.4 mg). Fraction EB5 (619.0 mg) was thrice purified through exclusion chromatography with Sephadex LH-20 (2.0 cm × 125.0 cm) as the stationary phase and isocratic elution with methanol (100%), which yielded 6.2 mg of duartin (4) and 1.1 mg of sativan (5). The latter was obtained after purification by semi-preparative HPLC.

For all HPLC analysis, the elution system started with water:methanol (9:1, v/v) to 100% methanol at 40 min, returning to the initial proportion at 46 min, and maintaining it for 50 min. A flow rate of 4 mL min⁻¹ was used.

2.4. NMR data

The structures of all isolated isoflavonoids were established using NMR data, which are presented below:

2.4.1. Prunetin (5,4'-dihydroxy-7-methoxyisoflavone) (1): ¹H NMR (400 MHz, CD₃OD): δH 8.12 (s, H-2), 7.38 (d, J = 8.8, H'-6' and H'-2'), 6.84 (d, J = 8.8, H'-5' and H'-3'), 6.55 (d, J = 2.4, H-8), 6.37 (d, J = 2.4, H-6), 3.89 (s, 7-OCH₃).

2.4.2. Di-O-methyldaidzein (7,4'-dimethoxyisoflavone) (2): ¹H NMR (400 MHz, CD₃OD): d 8.22 (s, H-2), 8.11 (d, J = 9.6, H-5), 7.48 (d, J = 8.8, H'-6' and H'-2'), 7.08 (m, 7-OH and 8-OH overlapped), 6.98 (d, J = 9.6, H'-5' and H'-3'), 3.95 (s, 7-OCH₃), 3.83 (s, 4'-OCH₃).

2.4.3. 8-O-Methylretusin (8,4'-dimethoxy-7-hydroxyisoflavone) (3): ¹H NMR (400 MHz, CD₃OD): d 8.21 (s, H-2), 7.78 (d, J = 8.8, H-5), 7.48 (d, J = 8.8, H'-6' and H'-2'), 6.99 (d, J = 8.8, H'-5' and H'-3'), 6.94 (d, J = 8.8, H-6), 3.94 (s, 8-OCH₃), 3.83 (s, 4'-OCH₃).

2.4.4. Duartin (7,5'-dihydroxy-8,4',6'-trimethoxyisoflavon) (4): ¹H NMR (400 MHz, CD₃OD): d 6.70 (d, J = 8.6, H-3'), 6.65 (d, J = 8.6, H-5'), 6.60 (d, J = 8.6, H-2'), 6.37 (d, J = 8.6, H-6), 4.29 (ddd, J = 10.2, 3.2 and 2.0, H-2 eq), 3.97 (td, J = 10.4 and 0.8, H-2 ax), 3.40-3.47 (m, H-3), 2.91 (dd, J = 15.6 and 11.0, H-4 ax), 2.80 (dd, J = 15.6 and 4.4, H-4 eq), 3.84 (s, 6'-OCH₃), 3.83 (s, 4'-OCH₃), 3.78 (s, 8-OCH₃).
2.4.5. Sativan (7-hydroxy-2′,4′-dimethoxyisoflavan) (5): 1H NMR (400 MHz, CD3OD): δ 7.04 (d, J = 8.4, H-6′), 6.87 (d, J = 8.4, H-5′), 6.54 (d, J = 2.4, H-3′), 6.48 (dd, J = 8.6 and 2.4, H-5′), 6.31 (dd, J = 8.2, and 2.4, H-6), 6.22 (d, J = 2.4, H-8), 4.19 (dddd, J = 10.4, 3.2 and 2.0, H-2ax), 3.64 (m, H-2eq), 3.49-3.41 (m, H-3), 2.92 (dddd, J = 15.6, 10.8 and 0.8, H-4ax), 2.77 (dddd, J = 15.6, 5.2 and 1.2, H-4eq), 3.83 (s, 2′-OCH3), 3.77 (s, 4′-OCH3).

2.5. Inhibitory activity studies

Recombinant human cat-V was produced using the Pichia pastoris expression system as previously described. The molar concentration of the enzyme was determined by active site titration with E-64 following the conditions previously described. Stock solutions of the flavonoids were prepared at a concentration of 1 mM in dimethyl sulfoxide and the inhibitors were screened against the cat-V at the concentration of 100 μM. The in vitro enzyme inhibition experiments were carried out in triplicate (in 96-well black plates) as previously described. The final volume of the reaction mixture was 200 μL, kept under stirring. Each well contained 191 mL of a 100 mM sodium acetate buffer pH 5.5 containing 5 mM EDTA and 5 mM dithioerythritol (DTE), 2 mL of 1 mM Z-Phe-Arg-MCA dissolved in dimethyl sulfoxide, 5 mL of sample, and 32 nM of cat-V. The enzyme was activated for 5 min with DTE at 27 °C, and then the reaction mixture was incubated for 5 min with the sample. The reactions were started by the addition of the fluorogenic substrate 4-methylcoumaryl-7-amide (MCA) and measurements were conducted using the Molecular Devices Spectra MAX GEMINI XS (excitation 355 nm, and emission 460 nm). Control assays were performed without inhibitor (negative control) and in the presence of the irreversible inhibitor for cysteine peptidase, E-64 (positive control). The percentage of inhibition was calculated according to the equation: % inhibition = 100 × (1 – Vc / Vo), where Vc and Vo are initial velocities (enzyme activities) determinate in presence and in the absence of inhibitor, respectively.

2.6. Theoretical section

The binding mode of duartin (4) was predicted via molecular docking technique using software package Gold Suite 5.1 (Genetic Optimization for Ligand Docking 5.1) (CCDC Software Limited), which revealed that this isoflavan 4 showed similar complementarity as well as APC-3316 in the active site of cat-V (PDB code 1FH0).

GOLD was used by a GoldScore fitness function, which is a molecular mechanism like for the calculation of binding positions of the ligand. Docking simulations were performed inside a sphere of 7 Å radius centered at the ligand in chain A, and using the pattern parameters available, a population of 100 conformers, 100,000 operations, 95 mutations and 95 crossovers. The docked lowest-energy structure has a root mean square deviation (RMSD) of 1.462Å with respect to the corresponding crystal structure of the complex.

3. Results and Discussion

3.1. Compound identification

As a part of ongoing search for bioactive compounds from Brazilian flora, Dalbergia miscolobium Benth. was submitted to chromatographic processes, which led to the isolation of five isoflavonoids prunetin (1), di-O-methylaidzein (2), 8-O-methylretusin (3), duartin (4) and sativan (5) (Figure 1).

The typical olefinic oxymethine hydrogens at C-2 of the isoflavones derivatives were observed at δH 8.13, 8.22 and 8.21 in the 1H-NMR spectrum of compounds 1, 2 and 3, respectively (Figures S2). Compounds 4 and 5 showed typical resonances for oxymethylene and benzylic protons at C-2 and C-4, respectively, which confirmed 4 and 5 as isoflavans derivatives. After 1D and 2D NMR experiment, and comparison with those of reported in the literature, these isoflavonoids were identified as prunetin (1), di-O-methylaidzein (2), 8-O-methylretusin (3), duartin (4) and sativan (5). The stereochemistry of 4 and 5 was defined by comparison of their specific rotation with those of reported in the literature. Their 1H-NMR data recovered in deuterated methanol are also reported herein.

Prunetin (1) is an important anti-inflammatory agent and is commonly found in species from the genus Prunus. Di-O-methylaidzein (2) is usually isolated from Maackia species. The compound 8-O-methylretusin (3) has already been isolated from different species of Dalbergia.
described in other *Dalbergia* species and showed antibacterial activity. The moderate antitumoral agent duartin (4) has been described in *Machaerium* species and sativan (5) is reported as an active compound against tuberculosis. Furthermore, in the course of the phytochemical procedure, the steroids sitosterol (6), campesterol (7) and stigmasterol (8) along with the terpenes derivatives α-amyрин (9), β-amyрин (10), lupeol (11) and phytol (12) were also isolated and identified according to spectroscopic data.

3.2. Inhibitory activity

Tests of the inhibitory activity against cat-V were performed for the isolated isoflavonoids. The compounds duartin (4) and sativan (5) showed remarkable results with inhibitions percentage of 89 and 88%, respectively at a concentration of 100 mM (Table 1). 8-O-methyleutrin (3) also showed a critical inhibition ability (57%).

These results express the percentage of inhibition in only one concentration tested and not determining the real potency of inhibition of the compounds. To verify the existence of interactions between the compounds and the tested enzyme, molecular docking studies were carried out to suggest the interactions that justify the inhibition percentages.

3.3. Molecular docking studies of isoflavonoids

Two domains are presented in cat-V structure, with similar size, in which one is structurally related to the R-domain of papain consisting primarily of a twisted β-sheet and the second one is related to the papain L-domain and comprises three R-helices. The catalytic site of cat-V is in a wedge-shaped cleft at the interface between the R- and L-domains and the crucial amino acids in the catalytic machinery are Cys25 and His159. The His159 has a crucial role supporting the cysteine to keep its deprotonated state and protonating the leaving amine. Finally, it is also worth noting that the indole ring of Trp177 lies above His159 and Asn175 and seems to shield these residues from the solvent.

As described by Somoza *et al.*, by analyzing the crystallographic structure of complex between cat-V and the irreversible inhibitor APC-3316...
bound in the active site, it was possible to identify the important molecular interactions, which must be considered in the investigation of duartin (4) binding mode prediction. Concerning the critical interactions observed in APC-3316/cat-V complex, three hydrogen bonds keep the APC-3316 structure tightly fitting. These interactions occur with the oxygen atom of the sulphonyl group in this inhibitor and Trp189, NH amide and Asp162, and carbonyl in amide moiety and Gly68, as illustrated in Figure 2.

Moreover, the fragments hPhe, Phe, and N-methylpiperazine in the inhibitor structure are in the S1, S2 and S3 pockets of the protease, respectively, while the sulfone phenyl group is in the S1’ region. The amino acids residues Asn64, Gly23, Gly65 and Cys63 are present in the S1 region. The specificity of the papain family members of cysteine proteases is intrinsically related to S2 region and, this is a deep slot located in the R domain. This portion is occupied with Ala133, Gly160 and Met68, while the sides with Lys155, Asn156, Leu157, Phe67, Asp158 and Gly66 amino acids. Considering the sides and bottom of the S3 portion, these regions present the residues Phe67, Arg70, Gln61, Asn64 and Gly59, and Asn60, Gly65 and Gly66, respectively. Furthermore, N-methylpiperazine fragment in the inhibitor structure is in this region. Finally, the S1’ pocket of cathepsin V, which is involved in the potency improvement but not in the selectivity of novel inhibitors, also comprehend amino acids Trp177, His159, Asp158 and Ala136 and receives the sulfone phenyl moiety of APC-3316 (Figure 2).

To better understand the potency of duartin (4), we proceeded to analyse their interactions with cat-V. The molecular docking was carried out by simulation of duartin (4) into the cat-V catalytic

Table 1. Percentage of cathepsin V inhibition for the isolated isoflavonoids from D. miscolobus

| Compound             | Cathepsin V inhibition (%) |
|----------------------|-----------------------------|
| prunetin (1)         | 38                          |
| di-O-methylaidzein (2)| 26                          |
| 8-O-methylretusin (3) | 57                          |
| duartin (4)          | 89                          |
| sativan (5)          | 88                          |

*Table 1. Percentage of cathepsin V inhibition for the isolated isoflavonoids from D. miscolobus*

![Figure 2. APC-3316/cathepsin V complex and key amino acids residues in stick. Light blue, carbon atoms; blue, nitrogen; red, oxygen; sulfur, yellow](image-url)
site. All docking runs were applied to the genetic algorithm of GOLD 5.1. The binding modes of duartin (4) in catalytic site of cat-V were depicted in Figure 3. All the amino acid residues which had interactions with duartin (4) were exhibited. In the binding mode, duartin (4) is nicely bound to the cat-V and stabilized by two remarkable hydrogen bonds. The oxygen atom of the methoxy group in duartin (4) formed a hydrogen bond with the –NH₂ moiety in Gln19 residue (2.8Å), as similarly observed to inhibitor APC-3316. Another notable hydrogen bond is performed by the oxygen atom of the methoxy group of duartin (4) and NH in indole ring present inside chain of Trp189 (3.3Å) amino acid. This interaction is equal to that carried out by APC-3316 and important for its stabilization in the cat-V active site. In this binding mode prediction, the water molecule W554 participates as a hydrogen bonding bridge between 4 and Gly23 amino acid residue which is present in the S1 region.

Furthermore, the molecular docking study results showed that the duartin (4) skeleton is closely on the structure of APC-3316 irreversible inhibitor showing a complementarity and a fill of S1 and S1’ pocket. The docking results confirmed that the hydrogen bonding interactions may contribute to the potent biological activities. This study also contributes to understanding the interesting pharmacophoric features required for the further development of isoflavone derivatives as cat-V inhibitors.

4. Conclusions

Isoflavonoids are common in Fabaceae species. The phytochemical study of D. miscolobus led to the isolation of three isoflavones and two isoflavans, a part of the known terpenes and steroids derivatives. The isoflavone 8-O-methylretusin (3) showed an important inhibition percentage of cat-V (57%), even though the best results were observed for the isoflavans duartin (89%) and sativan (88%). In silico studies, the rational understanding of the potency of isoflavan duartin (4) allowed to identify interesting hydrogen bond interactions with amino acid residues in active site of this protein. In this attempt, 8-O-methylretusin (3), duartin (4) and sativan (5) can be considered as targets into cat-V inhibitors investigation and further chemical study of Dalbergia species may afford novels isoflavonoids cathepsin inhibitors.

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Aminfar, Z.; Tohidfar, M. In silico analysis of squalene synthase in Fabaceae family using bioinformatics tools. *Journal of Genetic Engineering and Biotechnology* **2018**, *16*, 739. [CrossRef] [PubMed]

Veitch, N. C. Isoflavonoids of the leguminosae. *Natural Product Reports*** **2013**, *30*, 988. [CrossRef] [PubMed]

Nascimento, I. A.; Braz-Filho, R.; Carvalho, M. G.; Mathias, L.; Fonseca, F. A. Flavonoides e outros compostos isolados de *Mimosa artemisiana* Heringer e Paula. *Química Nova*** **2012**, *35*, 2159. [CrossRef]

Sikdar, S.; Tiwari, S.; Sapre, S.; Thakur, V. V. Simple approach for species discrimination of Fabaceae family on the basis of length variation in PCR amplified products using barcode primers. *International Journal of Current Microbiology and Applied Sciences*** **2018**, *7*, 921. [CrossRef]

Yin, X.; Huang, A.; Zhang, S.; Liu, R.; Ma, F. Identification of three *Dalbergia* species based on differences in extractive components. *Molecules*** **2018**, *23*, 1. [CrossRef] [PubMed]

Silva, T. L.; Fernandes, J. B.; Silva, M. F. G. F.; Consolario, H. N.; Sousa, L. R. F.; Vieira, P. C. New cathepsin V inhibitor from stems of *Bowdichia virgilioides*. *Brazilian Journal of Pharmacognosy*** **2019**, *29*, 491. [CrossRef]

Jing, J.; Wang, S.; Ma, J.; Yu, L.; Zhou, H. Elevated CTSL2 expression is associated with an adverse prognosis in hepatocellular carcinoma. *International Journal of Clinica and Experimental Pathology*** **2018**, *11*, 4035. [PubMed]

Severino, R. P.; Guido, R. V. C.; Marques, E. F.; Brömme, D.; Da Silva, M. F. D. G. F.; Fernandes, J. B.; Andricopulo, A. D.; Vieira, P. C. Acridone alkaloids as potent inhibitors of cathepsin V. *Biorganic and Medicinal Chemistry*** **2011**, *19*, 1477. [CrossRef] [PubMed]

Turk, V.; Stoka, V.; Vasiljeva, O.; Renko, M.; Sun, T.; Turk, B.; Turk, D. Cysteine cathepsins: from structure, function and regulation to new frontiers. *Biochimica et Biophysica Acta*** **2012**, *1842*, 68. [CrossRef] [PubMed]

Brömme, D.; Li, Z.; Barnes, M.; Mehler, E. Human cathepsin V functional expression, tissue distribution, electrostatic surface potential, enzymatic characterization, and chromosomal localization. *Biochemistry*** **1999**, *38*, 2377. [CrossRef] [PubMed]
Pharmacotherapy pathway in human nasal epithelial cells. MUC5AC expression by inactivating the TLR4/MyD88 induced inflammatory cytokine production and extracts of activities of phenolic constituents from the stem of *Maackia amurensis*. *Rev. Virtual Quim.* 2020, 12, 687. [CrossRef] [PubMed]

25 Carvalho, M. G.; Braz-Filho, R. Novas técnicas de RMN e os deslocamentos químicos dos átomos de $^1$H e $^{13}$C da isoflavana duartina. *Química Nova* 2003, 26, 335. [CrossRef]

26 Hu, H.; Li, H. Prunetin inhibits lipopolysaccharide-induced inflammatory cytokine production and MUC5AC expression by inactivating the TLRA/MyD88 pathway in human nasal epithelial cells. *Biomedicine and Pharmacotherapy* 2018, 106, 1469. [CrossRef] [PubMed]

27 Yang, G.; Ham, I.; Choi, H. Y. Anti-inflammatory effect of prunetin via the suppression of NF-κB pathway. *Food Chemical Toxicology* 2013, 58, 124. [CrossRef] [PubMed]

28 Promchai, T.; Janhom, P.; Maneerat, W.; Rattanajak, R.; Kamchongwongpaisan, S.; Pyne, S. G.; Limtharakul, T. Antibacterial and cytotoxic activities of phenolic constituents from the stem extracts of *Spaltholus parviflorus*. *Natural Product Research* 2018, 0, 1. [CrossRef] [PubMed]

29 Songsiang, U.; Wanich, S.; Pitchuanchom, S.; Netsopa, S.; Uanporn, K.; Yenjai, C. Bioactive constituents from the stems of *Dalbergia parviflora*. *Fitoterapia* 2009, 80, 427. [CrossRef] [PubMed]

30 Aye, M. M.; Aung, H. T.; Sein, M. M.; Armijos, C. A review on the phytochemistry, medicinal properties and pharmacological activities of 15 selected myanmar medicinal plants. *Molecules* 2019, 24. [CrossRef] [PubMed]

31 Hasan, N.; Osman, H.; Mohamad, S.; Chong, W. K.; Awang, K.; Zahariuluddin, A. S. M. The chemical components of *Sesbania grandiflora* root and their antituberculosis activity. *Pharmaceuticals* 2012, 5, 882. [CrossRef] [PubMed]

32 Santos, J. C.; Oliveira, C. A. N.; Varella, L.; Matos, A. P.; Terezan, A. P.; Leite, A. C.; Fernandes, J. B.; Vieira, P. C.; Silva, M. F. G. F.; Pirani, J. R. Constituìntes químicos e atividade antimicrobiana dos extratos de *Dilodendron bipinnatum* (sapindaceae). *Química Nova* 2010, 33, 2080. [CrossRef]

33 Zanon, R. B.; Pereira, D. F.; Boschetti, T. K.; Santos, M.; Athayde, M. L. Phytoconstituents isolated from dichlormethane fraction of *Vernonia tweediana* Baker leaves. *Brazilian Journal of Pharmacognosy* 2008, 18, 226. [CrossRef]

34 Olea, R. S. G.; Roque, N. F. Análise de misturas de triterpenos por RMN de $^{13}$C. *Química Nova* 1990, 13, 278. [Link]

35 Somboza, J. R.; Zhan, H.; Bowman, K. K.; Yu, L.; Mortara, K. D.; Palmer, J. T.; Clark, J. M.; McGrath, M. E. Crystal structure of human cathepsin V. *Biochemistry* 2000, 39, 12543. [CrossRef] [PubMed]

36 Cstorer, A.; Ménard, R. Catalytic mechanism in papain family of cysteine peptidases. *Methods in Enzymology* 1994, 244, 486. [CrossRef] [PubMed]

37 Watanabe, A.; Yoshimura, T.; Mikami, B.; Hayashi, H.; Kagamiyama, H.; Esaki, N. Reaction mechanism of alanine racemase from *Bacillus stearothermophilus*: X-ray crystallographic studies of the enzyme bound with N-($S'$-phosphopyridoxyl)alanine. *Journal of Biological Chemistry* 2002, 277, 19166. [CrossRef] [PubMed]