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# Flavonoids from *Mimosa tenuiflora* Green Propolis: Chemotaxonomy, Antioxidant, Anti-Inflammation, Anti-Bacteria, Mosquito Larvicidal Activity, and *In Silico* Approach

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Phytochemical study of green propolis alcohol extract of Brazilian Caatinga *Mimosa tenuiflora* (Willd.) Poir resulted in the separation of six flavonoids, including quercetin 3-methyl ether (**1**) and 3,3′-*O*-dimethylquercetin (**2**), and four flavanones eriodictyol 5-*O*-methyl ether (**3**), 5,4′-dihydroxy-6,7-dimethoxy-flavanone (**4**), licoflavanone (**5**), and macarangaflavanone B (**6**). All these metabolites were first isolated from green propolis and the genus *Mimosa*. Metabolites **3**–**4** also established chemotaxonomic significance since they had never been observed in the bean family Fabaceae. Flavones **1**–**2** showed strong antioxidative activity against DPPH (1,1-diphenyl-2-picryl hydrazyl) radicals with $IC_{50}$ values of 12.40 and 16.23 μg/mL, respectively. All six isolates showed anti-inflammatory effects against NO (nitric oxide) production in LPS (lipopolysaccharide)-stimulated Raw 264.7 cells, in which the activity of flavone **1** was better than the standard dexamethasone ($IC_{50}$ 13.35 μg/mL). Flavonoids **1**–**3** also strongly controlled the growth of the Gram-positive bacteria *Bacillus subtilis* ATCC 6051, and *Staphylococcus aureus* ATCC 29213 with MIC values of 32–64 μg/mL. Six isolated flavonoids exhibited strong or moderate mosquito larvicidal activity against *Aedes aegypti* and *Ae. albopictus* fourth instar larvae with the 24 h $LC_{50}$ values of 29.62–55.89 μg/mL. By *in silico* approach, compound **1** was a non-toxic agent with an $LD_{50}$ value of 5000 mg/kg.

## Introduction

Propolis is the general term for the resinous material that honeybees gather from a variety of plant sources. It is also sometimes referred to as "bee glue".[1] Bees gather, process, and use propolis, a highly adhesive and resinous material, to smooth out the interior walls of their honeycombs, seal openings, and deter intruders.[2] The resin is collected by honeybees (*Apis mellifera* L.) from leaf buds and tree bark fissures. Salivary enzymes are added after this resin is masticated, and the partially digested substance is then combined with beeswax and used in the hive. Propolis is not a pollen, nor should it be confused with "bee bread" or "royal jelly," which are entirely separate bee products, even though it may include some pollens.[1]

Propolis was reported to contain 10% volatile compounds, 50–55% resins (flavonoids, phenolic acids and, their esters), 30–40% beeswax, 5–10% pollens, and other substances.[3] The plant source dictates the chemical makeup of propolis, which is influenced by various factors like season, climate, bee species, and collection techniques. Currently, propolis has been found to include over 300 different components.[3] Propolis has been used for medicinal purposes since ancient times, at least 300 BC.[2] It is a common and local remedy in many parts of the world. Propolis was used by the Egyptians, Greeks, and Romans for its general medicinal properties as well as for the treatment of specific skin ailments.[3] Propolis has long been known for its ability to reduce inflammation and treat wounds and ulcers.[2,3] Its pharmaceutical application is still used today in treatments and personal care products.

*Mimosa tenuiflora* (Willd.) Poir., often known as "jurema-preta", is a shrubby tree belonging to the Fabaceae family.[4] It is naturally found across the Brazilian North-Eastern region of Caatinga, including beekeeping operations. The green tint of Caatinga propolis is evidenced by the salivary enzymes that

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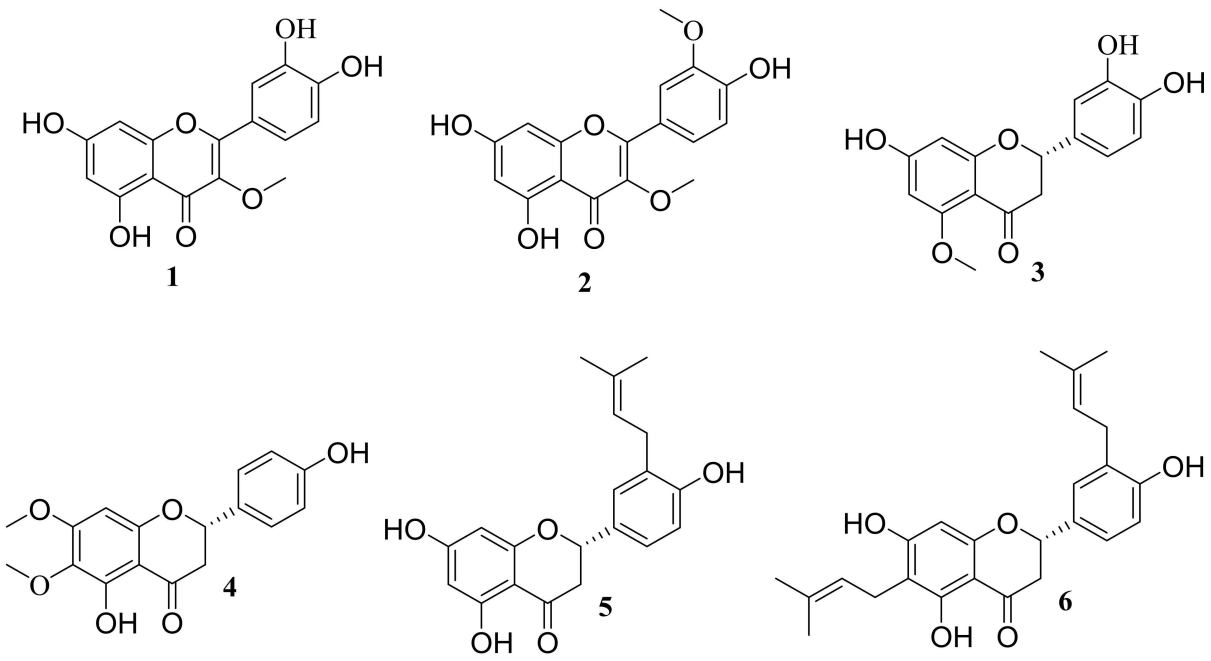


**Figure 1.** Flavonoids from *Mimosa tenuiflora* green propolis.

bees utilize to break up shoot apices and mix them with wax to make propolis.[4] It was documented that chemical constituents from *M. tenuiflora* green propolis are appropriate for immune response.[5] In this study, we aim to report the chromatographic separation of flavonoid derivatives from Northeast-Brazil Caatinga *M. tenuiflora* green propolis, and their role in chemotaxonomy. Their pharmacological values in antioxidative, anti-inflammatory, anti-bacterial, and mosquito larvicidal studies have also been considered. The obtained results are further aided by an *in silico* approach.

## Results and Discussion

### Phytochemical Isolation and Chemotaxonomy

Phytochemical research of the alcoholic extract of *M. tenuiflora* green propolis led to the isolation of six compounds **1**–**6** (Figure 1). Their NMR spectroscopic data (Figures S1–S12) match well with previous reports, including two flavones quercetin 3-methyl ether (**1**) and 3,3′-*O*-dimethylquercetin (**2**), and four flavanones eriodictyol 5-*O*-methyl ether (**3**), 5,4′-dihydroxy-6,7-dimethoxyflavanone (**4**), licoflavanone (**5**), and macarangaflavanone B (**6**).[6–11] From previous studies, propolis from Asian and European regions contained a significant concentration of flavonoids, while Brazilian propolis was only accompanied by terpenoids and prenylated coumaric acids.[12] Our current result can be seen as new evidence for the presence of flavonoids in Brazilian propolis. All these isolates were found in *M. tenuiflora* plant and *M. tenuiflora* green propolis for the first time. So far, compounds **1**–**6** have never been detected in the genus *Mimosa* before. Significantly, compounds **3**–**4** were first separated from plants of the family Fabaceae. Hence, *M. tenuiflora* and its propolis products can be distinguished from other *Mimosa* plants using these separated components.

### DPPH Antioxidative Activity

The isolated flavonoids **1**–**6** have been subjected to an antioxidative assay in a model of DPPH radical scavenging (Table 1). At the highest concentration of 50 μg/mL, the SP (scavenging percentage) values of compounds **1**–**2** were more than 70%, whereas the values for compounds **3**–**6** were insignificant (SP $<$ 10%). Therefore, two flavones **1**–**2** possessed strong activity with the $IC_{50}$ values of 12.40 and 16.23 μg/mL, respectively, when ascorbic acid was used as a positive control ($IC_{50}$ 11.38 μg/mL). The isolated flavanones **3**–**6** did not show activity ($IC_{50}$ $>$ 256 μg/mL). Flavones derived from *M. tenuiflora* green propolis seem better than analogous flavanones in antioxidative treatments. It also suggests that 3′-hydroxylation is better than 3′-methoxylation when compared between **1** and **2**. In accordance with previous reports, Brazilian green propolis

**Table 1.** DPPH radical scavenging activity of the studied compounds.

| No | SP at 50 μg/mL | $IC_{50}$ (μg/mL) |
|---|---|---|
| **1** | 74.61 ± 0.76 | 12.40 |
| **2** | 71.17 ± 0.16 | 16.23 |
| **3** | 9.28 ± 0.58 | > 256 |
| **4** | 4.22 ± 0.25 | > 256 |
| **5** | 7.46 ± 0.18 | > 256 |
| **6** | 1.07 ± 0.32 | > 256 |
| Ascorbic acid | 84.42 ± 0.43 | 11.38 |

was appropriate for radical scavenging. For instance, the ethanolic extract of *Baccharis dracunculifolia* green propolis, which was collected from Bambui-Brazil, showed the $IC_{50}$ value of 9.5 μg/mL against DPPH radicals.[13] The glycolic extract of Sao Paulo-Brazil *B. dracunculifolia* green propolis showed remarkable activities against DPPH and superoxide anion radicals, and decreased $H_2O_2$ generation and $Fe^{2+}$-induced ROS (reactive oxygen species) production in isolated mitochondria.[14]

### Anti-Inflammatory Activity

Flavonoid derivatives **1**–**6** were tested for anti-inflammatory activity against NO production in LPS-stimulated Raw 264.7 cells. As shown in Table 2, compounds **1**–**3** and **5**–**6** showed activity, whereas compound **4** was inactive ($IC_{50} > 128$ μg/mL). At a concentration exceeding 64 μg/mL, both compounds **1**–**3** and **5**–**6** exhibited inhibitory percentages of more than 80%. Significantly, quercetin 3-methyl ether (**1**) with the $IC_{50}$ value of $12.66 \pm 0.34$ μg/mL is better than that of the positive control dexamethasone ($IC_{50}$ $13.35 \pm 1.52$ μg/mL). In the meantime, the $IC_{50}$ value of the remaining flavonoids run in the order of **2** ($IC_{50}$ $27.11 \pm 0.24$ μg/mL) > **3** ($IC_{50}$ $28.63 \pm 0.23$ μg/mL) > **6** ($IC_{50}$ $35.67 \pm 1.21$ μg/mL) > **5** ($IC_{50}$ $54.54 \pm 0.98$ μg/mL). Compared between **1** and **2**, 3′-hydroxylation seems better than 3′-methoxylation. Flavanone **6** is better than its derivative **5** due to 6-prenylation. Accumulating evidence indicated that the crude extracts and chemical constituents from green propolis are promising agents to treat inflammatory syndromes. The ethanol extract of Minas Gerais-Brazil green propolis, on the one hand, inhibited *in vitro* NO production in LPS-stimulated Raw 264.7 cells with the $IC_{50}$ value of 41.60 μg/mL, on the other hand, reduced the number of neutrophils *in vivo* in carrageenan-induced carrageenan with the $IC_{50}$ values of 0.72 mg/kg, i.p., or 4.17 mg/kg, o.p.[15] The anti-inflammatory effect of green propolis hydroalcoholic extract at 5–20 μg/mL against the expression of pro-inflammatory cytokines was deduced from the action of flavonoids and phenolics.[16] The ethanol extract of Brazilian green propolis exerted the $IC_{50}$ value of 200 μg/mL to decreas the luciferase activity in TNF-α (tumor necrosis factor)-stimulated HEK 293 cells transfected with NF-κB (nuclear factor κB)-luciferase reporter gene.[17] The phenolic compounds of propolis (10–100 μg/mL) have inhibitory effects on inflammatory cytokines IL-6 (interleukin-6) and TNF-α.[18] The anti-inflammatory activity of Brazilian green propolis was enhanced when encapsulated with γ-cyclodextrin.[19] Yuan et al. (2020) concluded that Chinese green propolis and Brazilian green propolis have distinct chemome, but their antioxidative and anti-inflammatory effects are similar.[20]

### Anti-Bacterial Activity

The isolated flavonoids **1**–**6** have been further studied to address their antibacterial potentials. Generally, the tested compounds **1**–**3** showed good antibacterial activity, compounds **4**–**5** were inactive, and compound **6** exhibited moderate potential (Table 3). In detail, flavonoids **1**–**3** showed strong antibacterial activity against the Gram-positive bacteria *B. subtilis* and *S. aureus* with the MIC values of 32–64 μg/mL, and moderately controlled the other Gram-positive bacterium *C. sporogens* with the MIC values of 128–256 μg/mL. In the meantime, flavanone **6** only showed activity against *S. aureus* and *C. sporogens* with the MIC values of 128 and 256 μg/mL, respectively. Regarding the Gram-negative bacteria, flavonoids

**Table 2.** The NO inhibitory activity of the studied compounds.

| Concentration (μg/mL) | Inhibitory percentage (%) | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **5** | **6** | Dexamethasone |
| 128 | $90.55 \pm 2.99$ | $98.28 \pm 2.41$ | $95.36 \pm 3.43$ | $96.22 \pm 1.64$ | $86.94 \pm 2.92$ | $87.42 \pm 1.20$ |
| 64 | $94.16 \pm 3.10$ | $87.63 \pm 1.68$ | $80.04 \pm 2.14$ | $93.13 \pm 3.41$ | $85.57 \pm 3.41$ | $54.47 \pm 1.81$ |
| 32 | $78.69 \pm 3.85$ | $54.12 \pm 1.70$ | $57.73 \pm 1.43$ | $15.64 \pm 1.27$ | $31.44 \pm 1.27$ | $40.55 \pm 1.24$ |
| 16 | $59.28 \pm 2.05$ | $9.79 \pm 0.27$ | $4.64 \pm 0.19$ | $5.84 \pm 0.48$ | $5.67 \pm 0.34$ | $32.97 \pm 1.31$ |
| $IC_{50}$ | $12.66 \pm 0.34$ | $27.11 \pm 0.24$ | $28.63 \pm 0.23$ | $54.54 \pm 0.98$ | $35.67 \pm 1.21$ | $13.35 \pm 1.52$ |

**Table 3.** Antimicrobial activity of the studied compounds.

| Microbial strains | | MIC (μg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | Streptomycin | Tetracycline |
| Gram (+) | *B. subtilis* | 32 | 32 | 32 | >512 | >512 | >512 | 4 | |
| | *S. aureus* | 64 | 32 | 32 | >512 | >512 | 128 | 8 | |
| | *C. sporogens* | 256 | 128 | 128 | >512 | >512 | 256 | 8 | |
| Gram (−) | *E. coli* | 128 | 32 | 32 | >512 | >512 | 256 | | 4 |
| | *P. aeruginosa* | 256 | 128 | 64 | >512 | >512 | >512 | | 4 |

**1**–**3** possessed the MIC value of 32–256 μg/mL against the bacteria *E. coli* and *P. aeruginosa*, while flavonoid 6 only inhibited *P. aeruginosa* with the MIC value of 256 μg/mL. In contrast to antioxidative activity, 3′-methoxylated flavone **2** showed better antibacterial activity than 3′-hydroxylated flavone **1**. Flavanone **6** is superior to flavanone **5** in the antibacterial model due to prenylation at carbon C-6. The four isolated flavonoids of Taiwanese green propolis, propolins C, D, F, and G, exhibited MIC values of 2.5–20 μg/mL against bacteria *B. subtilis* and *S. aureus*.[21] Brazilian green propolis ethanolic extract monitored the growth of *S. aureus* with the $MIC_{90}$ value of 246.3 μg/mL.[22] Hence, the current evidence confirms the value of green propolis constituents in antibacterial treatments.

### Mosquito Larvicidal Assay

In the next assay, we continue to use the isolated flavonoids **1**–**6** to assess their larvicidal activity against mosquitoes *Ae. aegypti* and *Ae. albopictus* for 24 and 48 h treatments. The isolates having $LC_{50} < 50$ μg/mL were considered strong active agents, but moderate activity with 50 μg/mL $< LC_{50} <$ 100 μg/mL, and weak activity with 100 μg/mL $< LC_{50} <$ 750 μg/mL, as well as those products in the range of $LC_{50} > 750$ μg/mL will be inactive.[23] Regarding *Ae. aegypti* larvae, the 24 h $LC_{50}$ values of $< 50$ μg/mL were assigned to compounds **1**–**5**, whereas compound **6** was accompanied by the $LC_{50}$ value of 55.89 μg/mL (Table 4). In the meantime, the 24 h $LC_{90}$ values of $< 50$ μg/mL belong to compounds **4**–**5**. For 48 treatments, compound **5** had the best $LC_{50}$ value of 21.11 μg/mL and the best $LC_{90}$ value of 27.21 μg/mL, in contrast to those of compound **6**. It suggests

**Table 4.** Mosquito larvicidal activity of the studied compounds.

| No | $LC_{50}$ (95% confidence levels) | $LC_{90}$ (95% confidence levels) | $\chi^2$ | *p* |
|---|---|---|---|---|
| 24 h treatment (*Ae. aegypti*) | | | | |
| **1** | 39.62 (36.70–42.61) | 59.76 (54.54–67.54) | 1.2550 | 0.740 |
| **2** | 32.91 (30.37–35.70) | 54.44 (48.78–63.02) | 2.8302 | 0.419 |
| **3** | 34.24 (31.70–37.11) | 50.57 (46.63–55.86) | 4.0967 | 0.251 |
| **4** | 33.67 (30.92–37.51) | 40.69 (36.69–47.63) | 0.0013 | 1.000 |
| **5** | 34.38 (31.76–37.81) | 42.37 (38.77–47.90) | 2.6561 | 0.448 |
| **6** | 55.89 (52.99–60.44) | 73.15 (65.97–89.74) | 0.1684 | 0.983 |
| Permethrin | 0.0094 (0.0082–0.0107) | 0.0211 (0.0185–0.0249) | 57.6 | 0.000 |
| 48 h treatment (*Ae. aegypti*) | | | | |
| **1** | 30.21 (28.37–33.23) | 38.37 (34.54–46.48) | 0.0333 | 0.998 |
| **2** | 27.37 (26.00–29.45) | 36.03 (32.56–44.12) | 4.4114 | 0.220 |
| **3** | 28.82 (26.84–31.35) | 39.74 (36.31–45.00) | 8.9877 | 0.029 |
| **4** | 32.09 (29.81–35.58) | 39.65 (35.73–47.19) | 0.0065 | 1.000 |
| **5** | 21.11 (19.81–22.32) | 27.21 (25.76–29.16) | 0.6002 | 0.896 |
| **6** | 42.98 (39.83–45.61) | 57.84 (53.99–64.10) | 0.2392 | 0.971 |
| 24 h treatment (*Ae. albopictus*) | | | | |
| **1** | 39.85 (36.62–42.57) | 51.74 (48.42–56.34) | 0.0518 | 0.997 |
| **2** | 34.87 (32.12–37.87) | 58.93 (52.72–68.31) | 2.2221 | 0.528 |
| **3** | 27.52 (25.25–30.02) | 50.16 (44.41–58.97) | 5.4132 | 0.144 |
| **4** | 37.52 (34.35–40.43) | 47.18 (43.77–51.62) | 0.0110 | 1.000 |
| **5** | 29.62 (27.61–32.29) | 39.88 (36.41–45.33) | 1.7203 | 0.632 |
| **6** | 49.75 (47.10–52.49) | 66.31 (60.72–79.22) | 0.9755 | 0.807 |
| 48 h treatment (*Ae. albopictus*) | | | | |
| **1** | 32.77 (30.31–36.41) | 40.10 (36.15–47.39) | 0.0033 | 1.000 |
| **2** | 27.18 (25.67–29.07) | 37.41 (33.88–44.32) | 6.8123 | 0.078 |
| **3** | 25.47 (23.64–27.74) | 36.87 (33.65–41.55) | 4.2840 | 0.232 |
| **4** | 33.78 (31.43–36.54) | 44.24 (40.45–50.00) | 0.0467 | 0.997 |
| **5** | 23.53 (22.53–24.89) | 31.11 (29.11–34.20) | 1.0332 | 0.793 |
| **6** | 44.22 (41.13–46.86) | 60.17 (55.95–67.40) | 0.3880 | 0.943 |

$LC_{50}$: 50% Lethal concentration, $LC_{90}$: 90% Lethal concentration.

that reducing 6-prenyl group would help to increase mosquito larvicidal activity against *Ae. aegypti* larvae. It is also found that 3′-methylated flavone **2** is always better than 3′-hydroxylated flavone **1** against these larvae for both 24 h and 48 h treatments.

Considering *Ae. albopictus* larvae, six flavonoids **1**–**6** exerted the 24 h $LC_{50}$ values of $<50$ μg/mL, especially compounds **3** and **5**, which had great effects with the 24 h $LC_{50}$ values of 27.52 and 29.62 μg/mL, respectively. For the 24 h $LC_{90}$ results, only compound **5** exhibited good activity with $LC_{90}$ dose of 39.88 μg/mL. In the case of 48 h treatment, both six tested samples **1**–**6** possessed significant results with the $LC_{50}$ values of 22.53–44.22 μg/mL, and the $LC_{90}$ values of 40.10–60.17 μg/mL. In comparison, compound **2** with 3′-methoxylation is better than compound **1** with 3′-hydroxylation. Compound **5** is superior to congener **6**, reflecting that more prenylation is good for anti-inflammation and anti-bacteria, but not good for mosquito larvicidal activity. Yaseen and Ali in 2022 reported that the alcoholic extracts of Iraqi propolis were almost complete in killing *Culex pipiens molestus* 3rd instar larvae for 3 days of treatment.[24] The methanol extract of Nigerian propolis successfully inhibited *Cu. pipiens pipen* 4th instar larvae with the $LC_{50}$ values of 3.38 and 3.69 mg/L in tap and distilled water, respectively.[25] This is the first time we have provided new information, thereby exhibiting the great role of green propolis constituents in mosquito larvicidal treatments.

## *In Silico* Approach

### Docking Study

The COX-2 enzyme has demonstrated as a crucial molecular target in inflammatory treatment, while the Keap1 is considered a potential therapeutic target for diseases through oxidative stress intermediates.[26–28] Therefore, these targets were selected in this study, and docking simulations were applied to gain a better understanding of the binding modes and inhibitory potential of **1** against COX-2 (PDB ID: 5KIR) and Keap1 (PDB ID: 4L7B). Prior verification before docking was performed through the re-docking of the original crystallized ligands into the active site of the targets. The Root Mean Square Deviation (RMSD) of the crystallized ligands after docking compared to before docking was calculated. As shown in Figures S13–S14, the results indicated that the obtained values are less than 2 Å, the RMSD values for rofecoxib and 1VV in complexes with 5KIR and 4L7B are 0.44 Å and 0.72 Å, respectively, demonstrating the success of the AutoDock Vina v1.2.3 docking protocol in predicting the correct orientation of the ligands with high confidence.[29,30]

After validation, docking of compound **1** was performed, showing the binding affinities with COX-2 and Keap1 of −9.279 kcal/mol and −8.186 kcal/mol, respectively. The docked flavone **1** showed favorable pi-pi stacked, pi-alkyl, pi-sigma, and hydrogen bonding interactions with several key amino acids inside COX-2 (PDB ID: 5KIR) and Keap1 (PDB ID: 4L7B) active sites (Table 5). The docked pose of **1** in the COX-2 enzyme active site showed two hydrogen bonds between the hydrogen atom of the hydroxyl group attached to the C-5 and two residues Ser353 and Tyr355 (Figure 2). Hydroxyl group at carbon C-7 formed two hydrogen bonds with residues Phe518 and Gln192. Additionally, flavone **1** displayed two pi-sigma interactions with amino acid residues Leu352 and Val523, as well as two pi-alkyl interactions with Val523 and Leu352 (Figure 2). It is noteworthy that two pi-alkyl interactions with these residues of the known inhibitor rofecoxib have also been observed, and the key amino acids Tyr355, Gln192, and Phe518 for effective inhibition of COX-2 have been reported previously.[31]

Regarding the Keap1 receptor, flavone **1** showed several important interactions. This compound created a hydrogen bond with Ser602 with an interaction distance of 1.96 Å. Furthermore, this compound has two pi-pi stacked interactions

**Table 5.** Binding affinities and features for **1** and the co-crystallized ligands within the active site of selected targets.

| Type | Compound | Binding affinity (kcal/mol) | Ligand-protein interactions | |
|---|---|---|---|---|
| | | | Amino acid residue | Type |
| COX-2 | 1 | −9.279 | Phe518 (2.55 Å), Gln192 (2.08 Å), Tyr355 (3.04 Å), and Ser353 (2.16 Å)<br>Val349<br>Leu353, and Val523 | Conventional hydrogen bond<br>Pi-alkyl<br>Pi-sigma |
| | Rofecoxib | −9.338 | Arg513 (2.46 Å)<br>Ser353<br>Leu352, Val523, Val349, and Ala527<br>His90, and Ala527 | Conventional hydrogen bond<br>Pi-sigma<br>Pi-alkyl<br>Carbon hydrogen bond |
| Keap1 | 1 | −8.186 | Ser602 (1.96 Å)<br>Tyr572, and Tyr334<br>Gly603 | Conventional hydrogen bond<br>Pi-pi stacked<br>Carbon hydrogen bond |
| | 1VV | −11.55 | Arg415 (2.82 Å), Ser602 (2.69 Å), and Asn414 (3.39 Å)<br>Ala556, and Arg415<br>Tyr572<br>Tyr334, Ala556, and Arg415 | Conventional hydrogen bond<br>Pi-sigma<br>Pi-pi stacked, and carbon hydrogen bond<br>Pi-alkyl, and alkyl |

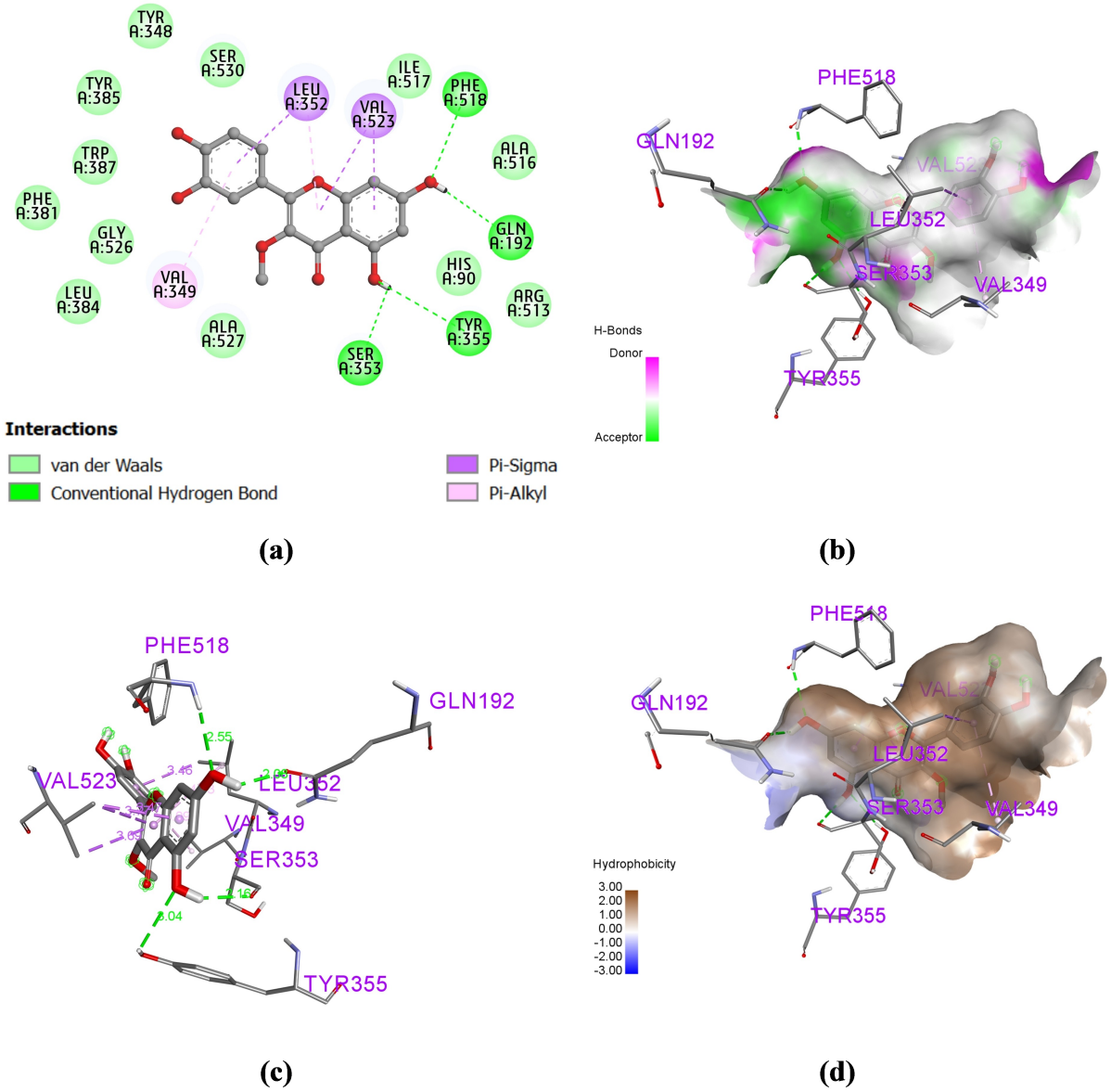


**Figure 2.** Predicted interaction mode for **1** with COX-2 enzyme. (A,B) Correspond to 2D and 3D diagrams of **1** interacting with amino acid residues. (C,D) Correspond to 3D hydrophobicity and hydrogen bonds surface map comparing substrates and COX-2 enzyme.

with amino acid residues Tyr572 and Tyr334 (Figure 3). Notably, all the important residues interacting with **1** were also observed in the complex of the well-known inhibitor 1VV with the Keap1 protein.

## ADMET Profiling

To gain a better understanding of the potentials of drug candidates, and avoid failures in subsequent experiments, the drug-likeness properties using Lipinski's rule and ADMET evaluation were taken into account.[32,33] In this study, calculations of Lipinski's proposed physicochemical parameters indicate that compound **1** does not violate any rules (molecular weight = 316 g/mol < 500 g/mol, hydrogen bond acceptor = 7 < 10, hydrogen bond donor = 4 < 5, logP = 2.31 < 5, and molar refractivity = 78.94 < 130). It also suggests that compound **1** is likely to be easily permeable, and quickly pass through biological membranes due to its molecular weight being less than 500 and efficient interaction with biological targets, as indicated by acceptable ranges for hydrogen acceptors and donors. Therefore, it shows potential in the use of compound **1** for drug development.

The pharmacokinetic parameters estimated by the pkCSM webserver are presented in Table S1.[34] Concerning absorption kinetics, the solubility of **1** in water is significant with the oral absorption capability at 76.069%. This compound has low Caco-2 permeability with a log Papp value < 0.9 cm/s. The skin permeability of **1** is considerably significant with log kp < −2.5 cm/s, indicating the potential for dermal absorption.

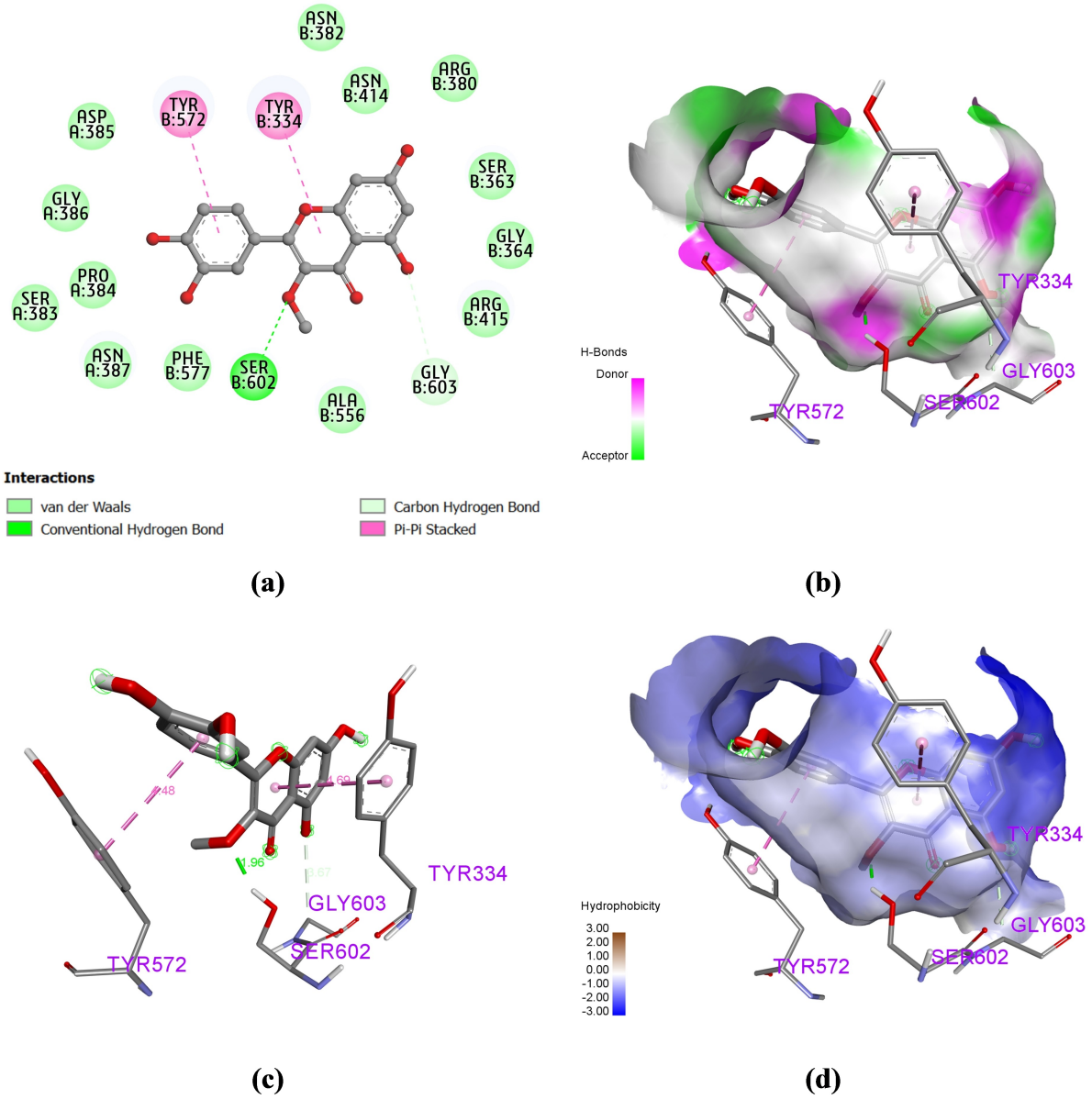


**Figure 3.** Predicted interaction mode for **1** with Keap1 protein. (A, B) Correspond to 2D and 3D diagrams of **1** interacting with amino acid residues. (C, D) Correspond 3D hydrophobicity and hydrogen bonds surface map comparing substrates and Keap1 protein.

Regarding its interaction with the crucial P-glycoprotein cell, it is identified as a substrate for this transporter, which may influence its absorption and distribution in the body. Furthermore, compound **1** was determined not to be an inhibitor of P-glycoproteins I and II, potentially impacting their bioavailability and systemic distribution. The ability to distribute through various physiological barriers and permeate biological membranes of **1** has been also studied (Table S1). Compound **1** showed a stable volume of distribution (VDss) ($-0.15 < \log$ VDss $< 0.45$). The observed fraction unbound for **1** is 0.067, indicating a low likelihood of efficient cell membrane diffusion due to its low non-binding state. Another aspect of **1** is its poor ability to cross the blood-brain barrier (BBB), evidenced by a log BB value $< 1$ and an inability to penetrate the central nervous system (CNS), as demonstrated by log PS $< -3$, suggesting it may have no potential impact on the nervous system.

The prediction of metabolic processes was carried out by assessing the interactions of isolated compounds with the cytochrome P450 isoenzymes, and examining their role as substrates or inhibitors. Compound **1** was mostly found not to exhibit any inhibitory effect on P450, except for CYP1A2 inhibition, indicating the potential for metabolic pathway alterations through CYP1A2 intermediates.

In terms of dynamic excretion, **1** was not to be a substrate for the organic cation transporter 2 (OCT2) in the kidney. This compound has a total clearance score of 0.457 log mL/min/kg, indicating efficient elimination from the body. Toxicity assessments are outlined in Table S1. As a result of the ProTox II web server shown in Figure S15, compound **1** was classified into

category 5 with an obtained $LD_{50}$ value of 5000 mg/kg, thereby indicating its non-toxic property.[35] The predicted oral rat acute toxicity ($LD_{50}$) for **1** is 2.529 mol/kg. Furthermore, regarding oral rat chronic toxicity, the toxicity value for **1** is 2.256 log mg/kg bw/day. Additionally, determining that **1** was not an hERG I and II inhibitor contributes to cardiovascular safety, no hepatotoxicity, no skin sensitization, and non-AMES toxicity, which are positive findings with significance for future drug development. The potential ecological impact of **1** was also examined using the environmental toxicity models of *Tetrahymena pyriformis* and minnow (Table S1). The predicted toxic doses for *T. pyriformis* and minnow are 0.3 log μg/L and 2.374 log mM, respectively.

## Conclusions

For the first time, the current research describes the phytochemical performance of alcohol extract of Brazilian *M. tenuiflora* green propolis, in which flavonoids are the principal compounds. The obtained result is opposite to various reports that flavonoids were not present in Brazilian propolis. Among these isolates, some compounds can be used as chemotaxonomic agents since they have not yet been observed in either *Mimosa* plants or their propolis. Flavones derived *M. tenuiflora* green propolis are likely appropriate for antioxidant and antibacterial treatments. Meanwhile, the flavonoids of Brazilian green propolis themselves displayed potential for anti-inflammation and mosquito resistance. By docking approach, it reflects that the immune response of green propolis can be explained by the interactions between the isolated flavones and amino residues of proteins.

## Experimental Section

### General Experimental Procedure

NMR spectral data (400 MHz for $^{1}H$ and 100 MHz for $^{13}C$ NMR) were carried out on a Bruker AVANCE in methanol-$d_4$. Silica gel (40–63 μm; Thermo Fisher, USA) and Sephadex LH-20 (25–100 μm; GE Healthcare, Sweden) were used to perform column chromatography (CC). Thin-layer chromatography (TLC) was performed using pre-coated silica gel 60 $F_{254}$ (Merck, Germany). Sulfuric acid 5% in ethanol was used for visualizing TLC plates.

### Plant Material

*M. tenuiflora* green propolis was gathered from Remanso, Brazil in June 2021. The Latin name was recommended by the taxonomist Milton Groppo, Department of Biology, University of Sao Paulo. The voucher specimen SPFR-15118 was deposited at Biology Department of the University of São Paulo, Ribeirao Preto, Sao Paulo, Brazil.

### Extraction and Isolation

The dried powders of green propolis (150 g) were immersed in 90% ethanol (4×2 L x 4 h) using a Soxhlet apparatus. A crude extract (38.50 g) was obtained by evaporating the combined extracts under a decreased pressure at 50.0 °C. It was then subjected to a normal silica gel CC [*n*-hexane-acetone (1:0 to 0:1, v/v)] to yield thirteen fractions (fr.s) BGP1-BPG13. The fr. BGP4 (5.51 g) was fractionated using a normal silica gel CC [chloroform-acetone (15:1 and 9:1, v/v)], to give four fr.s BGP41-BGP44. The fr. BGP42 (1.1 g) was chromatographed over Sephadex LH-20 column [100% methanol], to produce compound **1** (35.0 mg). The fr. BGP 43 (0.9 g) was separated by preparative HPLC-UV [Phenomenex reverse phase column, 4 μm, 250×10 mm, $\lambda_{max}$ 281 and 335 nm], and eluted with acetonitrile-water (6:1, v/v, 0.1% acetic acid) to give compound **2** (19.3 mg). The fr BGP44 (2.2 g) was separated over normal silica gel CC [chloroform-ethyl acetate (3:1, v/v)], to afford compound **4** (2.5 mg).

The fr. BGP6 (2.17 g) was subjected to Sephadex LH-20 column [100% methanol], to furnish three fr.s BGP61-BGP63. The fr. BGP63 (0.8 g) was then chromatographed over Sephadex LH-20 [100% methanol], to give compound **3** (9.6 mg). The fr. BGP8 (5.15 g) was fractionated into four fr.s BGP81-BGP84, by using normal silica gel CC [chloroform-ethyl acetate (30:1 to 1:1, v/v)]. Two remaining compounds **5** (4.5 mg) and **6** (9.4 mg) were obtained from the corresponding fr.s fractions BGP83 (1.56 g) and BGP84 (1.21 g), using the same condition used for the separation of compound **4**.

### Antioxidative Assay

The antioxidative effect of flavonoids **1**–**6** was evaluated using a well-known model of DPPH radical scavenging.[36] In brief, DPPH (0.2 mM) was diluted in ethanol. 200 μL of the DPPH solution was combined with 1.3 μL of various concentrations of compounds **1**–**6** in DMSO (50–3.125 μg/mL). The mixture was then introduced to a 96-well microplate (MHB, Merck) at room temperature for 30 min. Then, optical density (OD) was determined at 517 nm using a Multiskan™ FC Microplate photometer (ThermoFisher).

The scavenging percentage (SP) was expressed as:

$$SP\ (\%) = [(OD_o - OD_T)/OD_o] \times 100.$$

where $OD_o$ represented the absorbance of the control reaction, and $OD_T$ was the absorbance in the presence of the tested compound or standard (ascorbic acid). Each assay was run in triplicate. The $IC_{50}$ value is referred to as a concentration that induces a half-maximal response. Table Curve 2Dv4 software was used to do a linear regression analysis of the serial SP values versus the concentrations, to determine the $IC_{50}$ value.

### Anti-Inflammatory Assay

The RAW 274.7 cells were cultured in a 96-well microplate at a density of $5\times10^4$ cells/well.[37] They were then incubated for one day at 37 °C in an incubator with 5% $CO_2$. After that, the media in each well were aspirated, and the fresh FBS-free DMEM environment was added for 3 h. Following a 2 h pre-treatment with various concentrations of the isolated flavonoids **1**–**6** (128–16 μg/mL), cells were stimulated for a further 24 h period using LPS (1 ng/mL). By measuring nitrite ($NO_2^-$), which accumulated in the culture medium, a colorimetric assay based on the Griess reagent [50 μL of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 50 μL of 0.1% (w/v) N-1-naphthylethylenediamine dihydrochloride in water] was used to determine the amount of NO generation. In brief, cell culture medium (100 μL) and Griess reagent at a rate of 1:1, v/v, were incubated at room temperature for 10 min, and then nitrite concentration was calculated by measuring the OD at 540 nm using a Multiskan™ FC Microplate photometer. Fresh culture medium was

used as blank. Dexamethasone was a positive control. Each assay was run in triplicate.

The NO inhibitory percentage was calculated as:

$$\text{Inhibition } (\%) = 100\% - (\text{concentration of } NO_{sample} / \text{concentration of } NO_{negative\ control}) \times 100.$$

The $IC_{50}$ value was determined by using Table Curve 2Dv4.

### Anti-Bacterial Assay

Three Gram-positive bacteria *Bacillus subtilis* ATCC 6051, *Staphylococcus aureus* ATCC 29213, and *Clostridium sporogenes* ATCC 15579, two Gram-negative bacteria *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027 used in this study were derived from the ATCC (American Type Culture Collection, USA). All strains have been cultured on Muller Hilton Agar (MHA, Sigma Aldrich) plates for 24 h at 37 °C. The assay was performed as described previously.[30] In brief, the isolated compounds **1**–**6** were dissolved in DMSO (5%), to get the final concentrations in the range of 512–4.0 μg/mL. A mixture of bacterial suspension (180 μL, $10^6$ CFU $ml^{-1}$ in MHA) and each tested compound (20 μL) was introduced to a 96-well microplate. This was then incubated at 37 °C, and the OD was calculated at 600 nm using a Multiskan™ FC Microplate photometer (ThermoFisher). The MIC was defined as the lowest concentration at which no growth was observed. Each assay was run in triplicate. The same manner was employed for the negative control (containing MHB, Tween, and the tested sample), and the positive controls (containing bacterial culture without the tested sample). Streptomycin and tetracycline were used as the positive controls for the Gram-positive and Gram-negative bacteria, respectively.

### Mosquito Larvicidal Assay

As described in our previous works,[23,38] the World Health Organization's approach was used to assess the larvicidal activity of flavonoids **1**–**6**. After the tested substances were dissolved in 1% stock solution of EtOH, an aliquot was taken and put in a 200 mL beaker containing 20 *Ae. aegypti* larvae in their fourth instar. The beaker was then filled with water. For the experiment, four different concentrations of the tested samples (100, 50, 25, and 12.5 μg/mL) were utilized. Permethrin, a larvicidal medication, was utilized as the positive control, and ethyl alcohol as the negative control. The mortality of larvae was noted following 24 h and 48 h of exposure to the various concentrations. The experiments were repeated in four replicates at room temperature, and the same procedure was applied to *Ae. albopictus* larvae.

The mortality rate (MR) was expressed as follows:

$$MR = (M_o - M_t)/(100 - M_t) \times 100.$$

Where $M_o$ and $M_t$ denoted the mortality of the treat and control groups, respectively. The $LC_{50}$ and $LC_{90}$ values, 95% confidence limits, and chi-square values were obtained using XLSTAT v. 2018.5 (Addinsoft, France).

### Molecular Docking and ADMET Calculations

The macromolecule structures of human cyclooxygenase-2 (COX-2) enzyme and Kelch-like ECH-associated protein 1 (Keap1) were sourced from the RCSB PDB databases with an entry ID of 5KIR and 7C5E, respectively.[39,40] The downloaded protein models were subjected to structural preparation using the AutoDockTools v1.5.6 software. This involved assigning the Kollman partial charges of the entire protein and adding the missed polar hydrogens that were absent from the coordinate file (*.pdb file). Additionally, the structure was cleared of co-crystallized water, solvent, and ion molecules to allow the chosen ligand to freely dock in the target's active site. Then, the prepared targets were transformed into files with the extension *.pdbqt.[41] For ligand preparation, the energy of compound **1** was minimized and then converted into a *.pdbqt file. Molecular docking proceeded on the AutoDock Vina 1.2.0 platform, with the exhaustiveness set at a value of 400. A grid box was established with an identified grid center, which was mostly based on information obtained from the central coordinates of the co-crystallized inhibitors within downloaded protein models, an external size of 22 Å×22 Å×22 Å and spacing of 1 Å to accommodate all essential amino acids using the grid generation tool within AutoDockTools software. After docking simulation, the best-docked pose of **1** was selected to be analyzed for protein-ligand interactions and visualized via Discovery Studio Visualizer software. The evaluation of drug-like properties and related characteristics such as absorption, distribution, metabolism, excretion, and toxicity were assessed using the pkCSM server at www.biosig.lab.uq.edu.au/pkcsm.[42]

## Supporting Information

Figures S1–S15 and Table S1.

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## Conflict of Interests

The authors declared no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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[1] G. A. Burdock, *Food Chem. Toxicol.* **1998**, *36*, 347–363.
[2] J. M. Sforcin, *J. Ethnopharmacol.* **2007**, *113*, 1–14.
[3] L. Zhu, J. Zhang, H. Yang, G. Li, H. Li, Z. Deng, B. Zhang, *Front. Nutr. Sci.* **2023**, *10*, 1066789.
[4] N. T. Son, V. P. Ribeiro, J. K. Bastos, *Biochem. Syst. Ecol.* **2022**, *104*, 104473.
[5] A. A. Sartori, N. T. Son, M. D. S. Honorio, N. Ripari, K. B. Santiago, A. M. Gomes, W. F. Zambuzzi, J. K. Bastos, J. M. Sforcin, *J. Ethnopharmacol.* **2024**, *319*, 117297.

[6] S. S. Hong, Y. H. Choi, H. J. Suh, M. J. Kang, J. H. Shin, O. O. Kwon, J. S. Oh, *J. Appl. Biol. Chem.* **2015**, *58*, 189–194.
[7] E. Alday, D. Valencia, A. Garibay-Escobar, Z. Domínguez-Esquivel, A. L. Piccinelli, L. Rastrelli, J. Monribot-Villanueva, J. A. Guerrero-Analco, R. E. Robles-Zepeda, J. Hernandez, C. Velazquez, *Sci. Nat.* **2019**, *106*, 25.
[8] M. C. Li, Z. Yao, Y. Takaishi, S. A. Tanga, H. Q. Duan, *Chem. Biodiversity* **2011**, *8*, 1112–1120.
[9] C. Fernandez, B. M. Fraga, M. G. Hernandez, *J. Nat. Prod.* **1988**, *51*, 591–593.
[10] T. S. Tjahjadarie, R. D. Saputri, M. Tanjung, *AIP Conf. Proc.* **2016**, *1718*, 060007.
[11] B. A. Schutz, A. D. Wright, T. Rali, O. Sticher, *Phytochemistry* **1995**, *40*, 1273–1277.
[12] S. El-Guendouz, B. Lyoussi, M. G. Miguel, *Chem. Biodiversity* **2019**, *16*, e1900094.
[13] W. M. Pazin, L. D. Mata Monaco, A. E. E. Soares, F. G. Miguel, *J. Apic. Res.* **2017**, *56*, 40–49.
[14] N. S. S. Guimarães, J. C. Mello, J. S. Paiva, P. C. P. Bueno, A. A. Berretta, R. J. Torquato, I. L. Nantes, T. Rodrigues, *Food Chem. Toxicol.* **2012**, *50*, 1091–1097.
[15] N. Paulino, C. Teixeira, R. Martins, A. Scremin, V. M. Dirsch, A. M. Vollmar, S. R. L. Abreu, S. L. D. Castro, M. C. Marcucci, *Plant. Med.* **2006**, *72*, 899–906.
[16] F. F. D. Santos, R. P. Morais-Urano, W. R. Cunha, S. G. D. Almeida, P. S. D. Santos R Cavallari, H. A. Manuquian, H. D. A. Pereira, R. Furtado, M. F. C. Santos, M. L. A. Silva, *J. Food Biochem.* **2022**, *46*, e14350.
[17] N. Paulino, C. Teixera, R. Martins, A. Scremin, V. M. Dirsch, A. M. Vollmar, S. R. L. Abreu, S. L. D. Castro, M. C. Marcucci, *Plant. Med.* **2006**, *72*, 899–906.
[18] L. G. Zamarrenho, M. H. F. D. Lima, J. I. Hori, J. A. Lima, S. R. Ambrosio, J. K. Bastos, D. D. Jong, A. A. Berretta, *Appl. Sci.* **2023**, *13*, 6247.
[19] G. Rimbach, A. Fisher, A. Schloesser, G. Jerz, N. Ikuta, Y. Ishida, R. Matsuzawa, S. Matsugo, P. Huebbe, K. Terao, *Int. J. Mol. Sci.* **2017**, *18*, 1141.
[20] M. Yuan, X. J. Yuan, M. Pineda, Z. Y. Liang, J. He, S. W. Sun, T. L. Pan, K. P. Li, *Food Funct.* **2020**, *11*, 268–2379.
[21] Y. W. Chen, S. R. Ye, C. Ting, Y. H. Yu, *J. Food Drug Anal.* **2018**, *26*, 761–768.
[22] R. S. Veiga, S. De Mendonça, P. B. Mendes, N. Paulino, M. J. Mimica, A. A. Lagareiro Netto, I. S. Lira, B. G. C. López, V. Negrão, M. C. Marcucci, *J. Appl. Microbiol.* **2017**, *122*, 911–920.
[23] L. T. Huong, N. T. Som, L. N. Sam, P. N. Minh, N. D. Luyen, N. H. Hung, D. N. Dai, *Z. Naturforsch. C* **2023**, *78*, 337–344.
[24] A. T. Yaseen, Y. I. M. Ali, *IOP Conf. Ser.: Earth Environ. Sci.* **2022**, *1060*, 012091.
[25] K. A. Adeniyi, I. K. Olayemi, I. A. Ukubuiwe, I. M. Salihu, Y. Garba, *Niger. J. Pure Appl. Sci.* **2016**, *8*, 53–58.
[26] Z. Ju, M. Li, J. Xu, D. C. Howell, Z. Li, F. E. Chen, *Acta Pharm. Sin. B* **2022**, *12*, 2790–2807.
[27] V. Sihvola, A. L. Levonen, *Arch. Biochem. Biophys.* **2017**, *617*, 94–100.
[28] J. W. Kaspar, S. K. Niture, A. K. Jaiswal, *Free Radical Biol. Med.* **2009**, *47*, 1304–1309.
[29] N. T. Tra, N. X. Ha, N. V. Tuyen, N. T. Thuy Linh, N. T. Thu Ha, B. T. Cham, L. T. Yen, N. The Son, *Nat. Prod. Commun.* **2023**, *18*, 1–12.
[30] T. V. Pham, N. X. Ha, N. D. Luyen, T. H. Xuan, T. Le Quoc, N. H. Hung, S. N. The, *Chem. Biodiversity* **2023**, *20*, e202300652.
[31] J. V. Ryn, G. Trummlitz, M. Pairet, *Curr. Med. Chem.* **2000**, *7*, 1145–1161.
[32] C. A. Lipinski, *Drug Discovery Today Technol.* **2004**, *1*, 337–341.
[33] L. L. Ferreira, A. D. Andricopulo, *Drug Discov. Today* **2019**, *24*, 1157–1165.
[34] D. E. Pires, T. L. Blundell, D. B. Ascher, *J. Med. Chem.* **2015**, *58*, 4066–4072.
[35] P. Banerjee, A. O. Eckert, A. K. Schrey, R. Preissner, *Nucl. Acids Res.* **2018**, *46(W1)*, W257–W263.
[36] N. T. T. Ha, P. V. Cuong, N. T. Tra, L. T. T. Anh, B. T. Cham, N. T. Son, *Vietnam J. Sci. Technol.* **2020**, *58*, 411–418.
[37] N. D. Luyen, L. M. Huong, N. T. T. Ha, N. T. Tra, L. T. Tu Anh, N. V. Tuyen, K. Posta, N. T. Son, H. P. The, *Chem. Biodiversity* **2023**, *20*, e202200456.
[38] P. T. Thuy, T. T. Hieu, D. X. Duc, H. V. Trung, N. H. Hung, W. N. Setzer, T. D. Thang, N. T. Son, *J. Mol. Struct.* **2023**, *1283*, 135264.
[39] B. J. Orlando, M. G. Malkowski, *Acta Crystallogr. Sect. F* **2016**, *72*, 772–776.
[40] S. Unni, P. Deshmukh, G. Krishnappa, P. Kommu, B. Padmanabhan, *FEBS J.* **2021**, *288*, 1599–1613.
[41] T. Viet Pham, L. H. Cuong, T. T. Hong Ha, N. D. Luyen, N. X. Ha, T. Xuan Hoang, N. T. Hao, D. H. Gioi, T. T. T. Thuy, N. T. Son, *Chem. Biodiversity* **2023**, *22*, e202301192.
[42] D. E. Pires, T. L. Blundell, D. B. Ascher, *J. Med. Chem.* **2015**, *58*, 4066–4072.

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