Evaluation of antioxidant, anti-inflammatory effects and molecular docking of Smiglabrone B derived from *Grewia bulot*

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Abstract

Grewia bulot is an endemic species of Vietnam's highland regions with largely unexplored bioactive and medical values. In this study, the bioactivities such as antioxidant and anti-inflammatory effects of smiglabrone B (SGB), a phenolic compound extracted from G. bulot, were investigated. The antioxidant property of SGB was determined via its free radical scavenging ability (DDPH and ABTS assays) as well as its inhibitory effect against lipid peroxidation. Moreover, the anti-inflammatory potential of SGB was proven via molecular docking test and its inhibitory effect against nitric oxide (NO) release.

The results revealed a promising antioxidant capacity of SGB with IC_{50} varying from 7.72 to 38.65 µg/mL. Besides that, the high binding affinity between SGB and inducible nitric oxide synthase or cyclooxygenase 2, two enzymes related to inflammatory response, implied the anti-inflammatory potential of SGB in silico. The data from the in vitro study indicated that SGB possessed a moderate effect in inhibiting NO release with the IC_{50} value > 100 µg/mL. These findings support the therapeutic promise of SGB in mitigating oxidative stress and inflammatory diseases.

Keywords: *Grewia bulot*, Siglabrone B, Molecular docking, Antioxidant, Anti-inflammatory, Natural products.

Introduction

Grewia bulot, also known as "Bù lốt" in Vietnamese, is an evergreen woody plant of the *Malvaceae* family, which was first recorded in 1943[13]. It is an endemic species of Vietnam indicating the wet tropical biome of Highland Floodplain forest[4]. Its height can reach 15 m, and immature twigs are covered with whitish trichomes[9]. The leaf blades have lanceolate shapes with a length of about 13-25 cm and a width of approximately 4-6 cm, the leaf base is obtuse and rounded (Figure 1).

The abaxial surfaces of leaves are covered by dense, star-shaped trichomes. The flower has oval petals, with over 15 stamens, orbicular anthers, and a nectary disc surrounding a ring of hair-like structures[5]. It has densely hairy drupes with a sour taste. In Vietnam, it is primarily distributed in highland and mountainous regions such as Quang Tri province, Hai Van pass, An Khe pass, and Di Linh district (Lam Dong province)[9]. Local people in these regions have traditionally utilized *G. bulot* for different purposes such as consuming its fruit and using its wood. Several species belonging to the *Grewia* genus have been widely used in traditional medicine and possess many bioactive compounds such as flavonoids, isoflavonoids, polyphenols, alkaloids, sterols, and terpenoids[6]. Despite that, the study regarding *G. bulot* medicinal values is still limited.

Recently, there are some studies reporting bioactivities of the extracts and essential oils extracted from *G. bulot* leaves. In the previous study, Pham et al[11] proposed that the phytochemical composition of the essential oil from *G. bulot* leaves contained 78 compounds, and exerted a potent antioxidant and anti-cancer effect whereas the anti-inflammatory capacity was not observed. Furthermore, all the extracts (using methanol, hexan, dichloromethane, ethyl acetate, and water solvent) from *G. bulot* leaves also exhibited anti-cancer effect against liver, oral, and breast cancer cell lines (HepG2, KB, and MCF-7 cells) with a variety of efficacy as well as a promising free radical scavenging capacity via DDPH assay[12].

Some bioactive compounds from the *G. bulot* have also been isolated such as quercetin, β-sitosterol, and β-amyrin[19]. Of these, β-amyrin exhibited a mild cytotoxicity against liver, breast, and lung cancer cells (HepG2, MCF-7, and SK-LU-1 cells)[19]. Moreover, all ten compounds derived from *G. bulot* including lupeol, daucosterol, inugalatolipid A, 3-(E)-coumaroyltaraxerol, taraxerol, 3-(Z)-coumaroyltaraxerol, biphenyl-3,3',4,4'-tetrol, trans-tiliroside, (3S,5R,6S,7E,9R)-7-megastigmene-3,6,9-triol, and smiglabrone B, also showed inhibitory effect against cancer cell proliferation (HepG2, SK-LU-1, KB, MCF-7 cells) with various efficacy[10].

Previous studies have only focused on the anti-cancer effect of bioactive compounds extracted from *G. bulot*, but the information regarding other bioactivities (such as antioxidant and anti-inflammatory effects) of these compounds is still lacking. Among them, smiglabrone B (SGB) is a phenolic compound which is only reported from the well-known traditional remedy-tufuling or glabrous greenbrier (*Smilax glabra*). The antioxidant and anti-inflammatory effects of SGB from *G. bulot* have not been investigated yet.

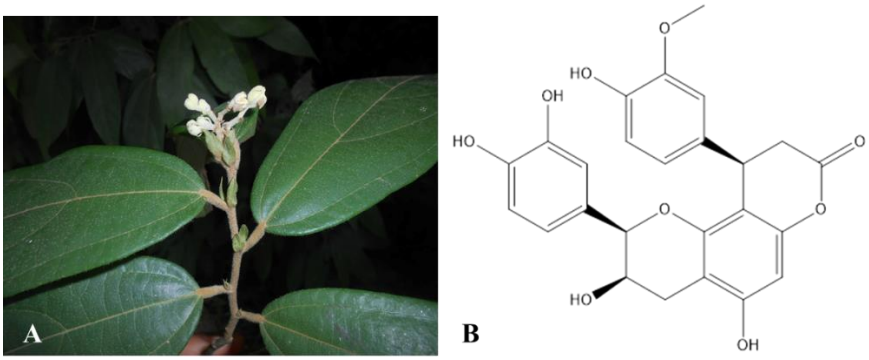


Figure 1: The sample of *Grewia bulot* (A) and its bioactive compound, smiglabrone B (B)

Therefore, we conducted this study to predict the anti-inflammatory effect of this compound *in silico* as well as to investigate antioxidant and anti-inflammatory effects of this compound *in vitro*.

Material and Methods

Reagents and Chemicals: All reagents and chemicals used in this study were at analytical grade or suitable for cell culture. Trolox, thiobarbituric acid, sodium nitrite,1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), lipopolysaccharide (LPS), and dexamethasone were obtained from Sigma Aldrich. Ascorbic acid and tricloacetic acid were manufactured by the Fisher company. Dulbecco's Modified Eagle's Media (DMEM) and FBS were purchased from Cytiva company. Smiglabrone B (SGB) derived from *G. bulot* was acquired from Mr. Pham Viet Ty, Faculty of Chemistry, University of Education, Hue University, Vietnam (Figure 1)[10].

DPPH assay: The antioxidant capacity of the compound was evaluated via DPPH, ABTS free radical scavenging assays, and its inhibitory effect on lipid peroxidation. The DPPH assay is a spectrophotometric method for detecting the percentage of DPPH free radical scavenged by the compound (smiglabrone B). Briefly, the compound was dissolved in methanol, and subsequently diluted in deionized water to obtain the appropriate concentration, while the reference (ascorbic acid) was diluted in deionized water.

100 µL of the sample (the compound or reference) with a variety of concentrations was diluted with 100 µL of DPPH solution (final concentrations 100, 20, 8, 0.4 µg/mL). Deionized water (100 µL) was combined with the same volume of DDPH solution (0,25 mM in methanol) for the control. The mixture was kept at a dark room with a regulated temperature (25 ± 2°C) for 0.5 hour. The absorbance of the mixture was read at the wavelength of 517 nm, and the percentage of DPPH scavenged by the sample was determined using the following equation:

$$SC\ (\%) = (1 - A_{sa}/A_{co}) \times 100\ (\%)$$

where A_{sa} was the absorbance of the sample, and A_{co} was the absorbance of the control[17].

ABTS assay: The ABTS assay was implemented following Nguyen et al[8] procedure with some slight changes in details. Briefly, the ABST solution was prepared by mixing ABTS (7 mM) and $K_2S_2O_8$ (2.45 mM). Mixture was allowed to stand in a dark room at room temperature. The ABTS solution was diluted in acetate buffer to obtain the working solution. Then, the working ABTS solution (1900 µL) was incorporated with 100 µL of the sample (the compound ascorbic acid) to obtain appropriate concentrations (100, 20, 8, 0.4 µg/mL) or 100 µL of deionized water (control). The mixture was kept in a dark room at a regulated temperature (25 ± 2°C) for 15 min, the absorbance of the mixture was read at the wavelength of 734 nm[20].

The percentage of ABTS scavenged by the sample was determined using the following equation:

$$SC\ (\%) = (1 - A_{sa}/A_{co}) \times 100\ (\%)$$

where A_{sa} was the absorbance of the sample, and A_{co} was the absorbance of the control. The half maximal scavenging concentration of the sample (SC_{50}) is a measure to determine the concentration of the compound at which 50% of DPPH or ABTS free radical is scavenged, and SC_{50} is calculated.

Inhibition of lipid peroxidation: The assessment of lipid peroxidation was conducted by measuring malondialdehyde (MDA) content, the by-product of lipid peroxidation. The thiobarbituric acid reactive substances (TBARS) method was employed to determine MDA level in samples according to Dinh et al[3] protocol with some slight modifications[3]. Livers were collected from healthy C57BL/6J mice (35-40 g) and were homogenized with 10 volumes of PBS solution (pH 7.4) on ice to collect the homogenate. A volume of liver homogenate (1 mL) was incorporated with 0,1 mL of sample (the compound or the reference-trolox) with a variety of concentrations 0.8 mL PBS, and 0.1 mL Fenton reagent, with the final concentrations of 100, 20, 8, 0.4 µg/mL.

The control mixture without the sample was also prepared. The reaction mixture was incubated at 37°C for 0.5 hour before adding a volume of 10% trichloroacetic acid (1 mL). Following the centrifugation at 12,000 rpm for 5 min, the supernatant was obtained and incorporated with 1 mL 0.8% thiobarbituric acid, the mixture was subsequently boiled at

100°C for 0.25 hour. The mixture was cooled to room temperature and the absorbance of the wavelength at 532 nm was recorded. The percentage of lipid peroxidation inhibition by the sample was determined using the following equation:

$$I (\%) = (1 - A_{sa}/A_{co}) \times 100 (\%)$$

where A_{sa} was the absorbance of the sample, and A_{co} was the absorbance of the control. The half maximal inhibitory concentration of the sample (IC_{50}) represents the concentration of the compound required to inhibit 50% of lipid peroxidation, and IC_{50} is calculated. The study was approved by the Animal Research Ethics Committee of Ton Duc Thang University (TDTU-AEC) (Approval letter no. 5/HDTVDD).

Docking study: The anti-inflammatory effect of the compound was predicted via calculation of the binding affinity between the compounds with two key enzymes associated with the inflammatory reaction, named as cyclooxygenase 2 (COX-2), and inducible nitric oxide synthase (iNOS). The 3D structure of dexamethasone (reference medicine, positive control) was obtained from the Pubchem database whereas SMILES (Simplified Molecular Input Line Entry System) of the compound (smiglabrone B) was acquired from the NEI-MPDB database and its 3D structure was subsequently reconstructed by smiles2pdb online tool developed by the NovoPro Bioscience Inc. (retrieved from www.novoprolabs.com/tools/smiles2pdb).

The 3D structures of mouse COX-2 and iNOS were obtained from the Protein Data Bank database with the following PDB IDs: 8ET0 (COX-2); 1QW4 (iNOS). Then, the PyMOL-3.1.3 software was employed to dehydrate, to remove co-crystallized ligands, and to prepare the protein sequence of the target proteins. The protein structures were subsequently treated by the AutoDock Tools-1.5.6 software for adding appropriate charges and linkages, as well as setting up the grid box parameters. The molecular interactions between dexamethasone or smiglabrone B versus the two target proteins (COX-2, iNOS) were simulated by Auto Dock Vina 1.1.2 software. The best 2D images of docking results were visualized by the Discovery Studio Visualizer software.

Inhibitory effect against nitric oxide production: The anti-inflammatory effects of the compound or reference (dexamethasone) were further investigated via the inhibitory effects on LPS-stimulated nitric oxide (NO) production model[1]. Concisely, mouse macrophages (RAW 264.7 cell line) were sub-cultured into 96 well plate format with the complete media (DMEM + 10% FBS) containing 100 U of penicillin/mL and 100 µg of streptomycin/mL at a humidified chamber (37°C, 5% CO_2) for 1 day, before replacing with the free FBS media containing the compound or reference with different concentrations (final concentrations ranging 100, 20, 8, 0.4 µg of smiglabrone B/mL or 39.25, 7.85, 1.57, 0.31 µg of dexamethasone/mL). The negative control without the compound or reference was also prepared. Following 2 hours of media replacement, LPS (1 µg/mL) was added to the media to stimulate nitric oxide release for 1 day. A 100 µL aliquot of cell culture supernatant was incorporated with 100 µL of the Griess reagent, then the mixture was allowed to react at 25 ± 2°C for 10 min. The absorbance of the mixture at the wavelength of 540 nm was recorded, and the NO content was plotted from a standard curve of sodium nitrite. The percentage of NO inhibition by the sample was determined using the following equation:

$$I (\%) = 100 - (NO_{sa}/NO_{co}) \times 100 (\%)$$

where NO_{sa} was the NO level released in the sample-treated well, and NO_{co} was the NO level released in the control well[18]. The half maximal inhibitory concentration of the sample (IC_{50}) represents the concentration of the compound required to achieve 50% NO inhibition, and IC_{50} is calculated from table curve 2Dv4.

Cell viability: Following the LPS treatment, macrophages in wells were treated with culture media containing MTT solution (ratio 9:1) at 37°C for 240 min to measure the percentage of cell viability. The formazan crystals were subsequently solubilized by DMSO, and the absorbance of the samples was read at the wavelength of 540 nm[16]. The percentage of cell viability was determined using the following equation:

$$I (\%) = A_{sa}/A_{co} \times 100 (\%)$$

where A_{sa} was the absorbance of the sample treated wells, and A_{co} was the absorbance of the control wells[7].

Statistical analysis: The data from triplicate experimental measurements were recorded and reported as mean ± standard deviation. Statistical significance across different samples was evaluated using One-way ANOVA analysis followed by Tukey's post-hoc test (GraphPad Prism 8 software), with a predefined threshold of 5% of comparison.

Results and Discussion

Antioxidant effect of SGB: The DPPH free radical scavenging ability of SGB is presented in table 1. In brief, the compound exerted a strong scavenging activity in a dose-dependent manner within testing doses of 0.4 – 100 µg/mL ($p < 0.05$). At the low concentration (0.8 µg/mL), SGB did not exhibit the DDPH scavenging activity while the higher concentration SGB (20 µg/mL) exerted a remarkable antioxidant activity (32.59 ± 0.99 µg/mL, $p < 0.0001$). In parallel, the reference compound (ascorbic acid) also effectively scavenged the DPPH free radicals, which validated the tested results. Furthermore, the IC_{50} value of DPPH free radical scavenging ability of SGB was 5.36 times lower than that of the positive control (ascorbic acid) (38.65 ± 1.36 µg/mL versus 7.21 ± 0.53 µg/mL respectively, $p < 0.0001$). The results imply a potent antioxidant activity of SGB in terms of DPPH scavenging activity.

Table 1
The free radical scavenging ability of smiglabrone B was determined via DPPH assay

Concentration (µg/mL)	% DPPH free radical scavenging	
	Smiglabrone B	**Ascorbic acid**
100	92.97 ± 1.68^{d}	91.30 ± 1.95^{d}
20	32.59 ± 0.99^{c}	77.07 ± 1.09^{c}
4	3.67 ± 0.32^{b}	35.77 ± 1.04^{b}
0.8	-2.14 ± 0.21^{a}	11.91 ± 1.16^{a}
SC_{50}	**38.65 ± 1.36******	**7.21 ± 0.53**

The superscript letters "a, b, c, d" denotes statistical differences in DPPH scavenging ability among the testing doses of the compound (Smiglabrone B) or positive control (ascorbic acid) (p<0.05). **** p<0.0001 represents the significant difference between IC_{50} values of Smiglabrone B and control.

Table 2
The free radical scavenging ability of smiglabrone B determined via ABTS assay

Concentration (µg/mL)	% ABTS free radical scavenging	
	Smiglabrone B	**Ascorbic acid**
100	90.83 ± 1.17^{d}	90.29 ± 2.13^{d}
20	80.82 ± 1.43^{c}	71.62 ± 1.05^{c}
4	33.35 ± 1.26^{b}	34.22 ± 0.91^{b}
0.8	6.99 ± 0.45^{a}	14.21 ± 0.52^{a}
SC_{50}	**7.72 ± 0.44^{ns}**	**7.83 ± 0.51**

The superscript letters "a, b, c, d" denotes statistical differences in ABTS scavenging ability among the testing doses of the compound (Smiglabrone B) or positive control (ascorbic acid) (p<0.05). The term "ns" represents no significant difference between IC_{50} values of Smiglabrone B and control.

Moreover, SGB exerted a strong antioxidant activity in terms of ABTS free radical scavenging ability (Table 2). As shown in table 2, the ABTS scavenging activity of SGB initiated at a low concentration of 0.8 µg/mL (6.99 ± 0.45%) and steadily increased at higher doses until reaching the maximal antioxidant activity at the highest testing dose of 100 µg/mL (90.83 ± 1.17%). Noticeably, the ABTS scavenging activity of SGB (IC_{50}: 7.72 ± 0.44 µg/mL) was comparable with the antioxidant activity of ascorbic acid, positive control (IC_{50}: 7.83 ± 0.51 µg/mL, p > 0.05). The results are robust and indicate SGB as a strong antioxidant as effective as ascorbic acid.

Phenolic compounds are well-known and promising antioxidants from nature with several applications in human life. According to Zeb[22], phenolic compounds can operate their antioxidant properties via several mechanisms including donation of a hydrogen atom (HAT), single electron transfer (SET), transition metal chelation, and sequential proton loss electron transfer. In this study, DPPH and ABTS free radical scavenging assay mostly focused on the action of SGB in neutralizing the free radicals, especially in HAT and SET mechanisms. Moreover, the ABTS scavenging ability of SGB appears to be higher than its DPPH scavenging capacity, which may be attributed to the polar nature of the compound.

The DPPH assay mainly deals with hydrophobic antioxidants whereas the ABTS free radical scavenging test can work well with both hydrophilic and lipophilic antioxidants, as suggested by Rumpf et al[14]. As noted, the SGB was extracted from the ethyl acetate extract and was eluted by a 2 methanol: 3 water solution in the previous study[10]. That implies SGB is a moderate polar compound. Therefore, the ABTS free radical scavenging activity of SGB is more effective than its DPPH free radical scavenging activity. Besides, the study has reported the antioxidant activity of SGB which suggests the further application of this compound in preventing oxidative stress-related diseases or implications, such as lipid peroxidation and inflammatory diseases.

Inhibitory effect of SGB against lipid peroxidation: The excessive production of free radicals or reactive oxygen species can react with unsaturated fatty acids in cell membranes, in turn leading to lipid peroxidation, a condition in which lipids are deteriorated into lipid peroxides and other oxidative adducts. Unregulated lipid peroxidation is closely related to several diseases such as atherosclerosis, neurodegenerative disorders, inflammatory diseases, and cancers. [23] Therefore, the evaluation of lipid peroxidation inhibition capacity is commonly conducted in the field of natural product research. The inhibitory effect of SGB against lipid peroxidation was evaluated via TBARS approach and the results are shown in table 3.

The compound (SGB) exhibited a strong inhibitory effect against lipid peroxidation with the highest treatment dose of 100 µg/mL (73.81 ± 2.75%). The positive correlation between the treatment doses and inhibition capacity highlighted the protective effect of SGB. As presented in table 3, the IC_{50} value of SGB was 3.47 times higher than

that of Trolox, the positive control with a statistically significant difference ($p < 0.001$), which indicated that the anti-lipid peroxidation effect of SGB is lower than that of Trolox. A previous study found that the 70% methanolic extract of *Euglena tuba* (70%), well-known antioxidant microalgae, demonstrated an excellent anti-lipid peroxidation activity[2].

Nevertheless, its potency was found to be nearly 29.95 times weaker than that of Trolox[2]. The data suggest SGB as a potent inhibitor of lipid peroxidation. In this study, the antioxidant capacity of SGB is reported not only in free radical scavenging activity in both DPPH or ABTS models but also in the context of suppression of lipid peroxidation, which highlights the antioxidant capacity of this compound (SGB).

***In silico* prediction of SGB-targeted proteins:** To further investigate the potential bioactivity of SGB, such as the anti-inflammatory effect, the molecular docking was performed to evaluate the interactions and binding affinities between SGB with inflammation-related proteins like COX-2 and iNOS (Table 4). As shown in table 4, SGB exhibited a high affinity with all targeted proteins with binding energies ranging from -7.2 to -9.3 kcal/mol (below threshold -7 kcal/mol). Of note, the binding energy of SGB with iNOS was lower than the positive control, dexamethasone, the widely prescribed glucocorticoid for treatment of inflammatory diseases (-9.3 versus -8.8 kcal/mol respectively). That suggests the potent anti-inflammatory effect of SGB.

Dexamethasone has been commonly used as the positive control to validate the data from various anti-inflammatory assays *in silico*. In this study, the binding energies of dexamethasone with COX-2 and iNOS were relatively low (-8.0 and -8.8 kcal/mol respectively), which was consistent with the prior studies. For example, the binding energy of dexamethasone with COX-2 (8ET0) was low -6.14 kcal/mol in the previous study[15]. Furthermore, dexamethasone bound to COX-2 via 2 hydrogen bonds in residues His356 and Phe580, and it formed two interactions with iNOS via hydrogen linkages (Figure 2). The data highlights strong binding affinities of dexamethasone with target proteins and the inhibitory effect against inflammatory response. In parallel, SGB also bound to COX-2 via 5 hydrogen bonds in Gln350, Gly354, Tyr355, His356, and Asn581 residues as well as an alkyl interaction in Lys358 residue.

Besides that, SGB exhibited a stable interaction with iNOS via hydrogen bonds in Arg260, Tyr341, Asp376 residues and other interactions with Gln257, Glu371, Arg375, Arg382 and Trp457 residues. Note that the binding energy of SGB with iNOS was lower as compared to that of SGB and COX-2. The data indicate the SGB possesses a potent anti-inflammatory effect, selectively targeting the iNOS pathway. To validate the results from the docking study, an inhibitory assay for nitric oxide (NO) production was further performed.

Anti-inflammatory effect of SGB at *in vitro* level: The results from this study reconfirmed the strong anti-inflammatory effect of dexamethasone with a low IC_{50} value (12.74 ±1.17 µM, equivalent to about 5 µg/mL) for inhibiting NO production (Table 5). The anti-inflammatory of dexamethasone was remarkably observed even in the low concentration of 0.8 µM (equivalent to about 0.31 µg/mL) with approximately 32.67 ± 1.05% inhibition on NO production. As shown in table 5, there was a positive correlation between the treatment doses of SGB (0.4 - 100 µg/mL) and the inhibitory percentage of NO production in wells ranging from 0.92% to 26.91%, proposing the anti-inflammatory effect of SGB.

The maximal inhibitory effect (29.61 ± 2.33%) was observed in the highest treatment dose of SGB (100 µg/mL). Consequently, 50% inhibition effect was not obtained within the examined dose range of SGB with an IC_{50} value exceeding 100 µg/mL which suggested the moderate efficacy of SGB in inhibiting NO production.

The data from this study supports the above *in silico* study to reaffirm the anti-inflammatory effect of SGB. Epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate, compounds with closely related structures to SGB, have demonstrated dose-dependent inhibitory effects against NO release[21].

Table 3
The inhibitory ability of smiglabrone B on lipid peroxidation determined via TBARS assay

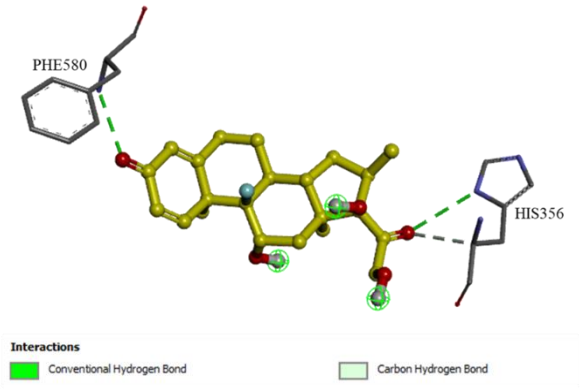
Concentration (µg/mL)	% inhibition of lipid peroxidation	
	Smiglabrone B	**Trolox**
100	73.81 ± 2.75^{d}	87.96 ± 1.48^{d}
20	52.09 ± 1.41^{c}	84.08 ± 1.02^{c}
4	15.14 ± 0.76^{b}	34.57 ± 1.07^{b}
0.8	6.34 ± 0.29^{a}	8.26 ± 0.75^{a}
IC_{50}	**25.54 ± 2.54*****	**7.36 ± 0.43**

The superscript letters "a, b, c, d" denotes statistical differences in lipid peroxidation inhibitory ability among the testing doses of the compound (Smiglabrone B) or positive control (trolox) (p<0.05). *** p<0.001 represents the significant difference between IC_{50} values of Smiglabrone B and control.

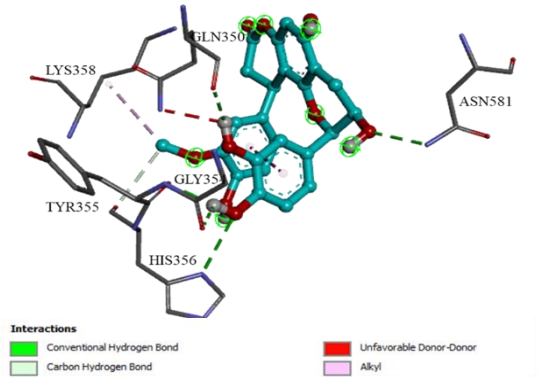
Table 4
Docking results of smiglabrone B or dexamethasone with the target proteins, such as COX-2 and iNOS.

Compounds	Binding energy (kcal/mol)	Hydrogen bonds	Residues with H-bonds	Other interactions
Mouse COX-2 (PDB ID: 8ET0)				
Dexamethasone	-8.0	2	His356, Phe580	-
Smiglabrone B	-7.2	5	Gln350, Gly354, Tyr355, His356, Asn581	Lys358
Mouse iNOS (PDB ID: 1QW4)				
Dexamethasone	-8.8	2	Cys194, Glu371	-
Smiglabrone B	-9.3	4	Arg260, Tyr341, Asp376	Gln257, Glu371, Arg375, Arg382, Trp457

Mouse COX-2 (PDB ID: 8ET0)

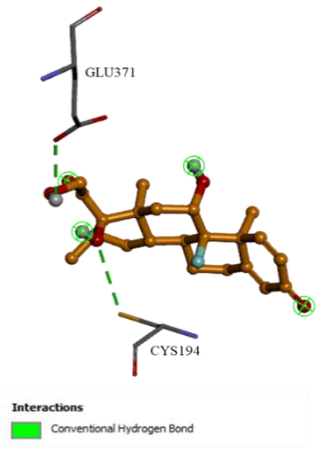


Dexamethasone

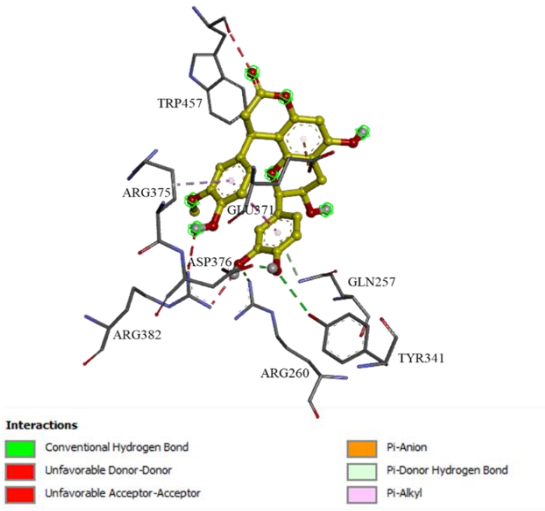


Smiglabrone B

Mouse iNOS (PDB ID: 1QW4)



Dexamethasone



Smiglabrone B

Figure 2: The best poses of 3D images demonstrating interactions between dexamethasone or smiglabrone B with cyclooxygenase -2 (COX-2) and inducible nitric oxide synthase (iNOS) proteins.

Table 5
The inhibitory ability of smiglabrone B on nitric oxide production

Concentration (µg/mL)	Smiglabrone B		Concentration (µM)	Dexamethasone	
	NO inhibition (%)	Cell viability (%)		NO inhibition (%)	Cell viability (%)
100	29.61 ± 2.33[d]	98.67 ± 1.33	100	87.59 ± 1.12[d]	91.96 ± 1.81
20	10.35 ± 0.84[c]	99.80 ± 1.80	20	55.09 ± 1.07[c]	96.49 ± 2.60
4	4.55 ± 0.43[b]		4	40.84 ± 0.89[b]	
0.8	0.92 ± 0.11[a]		0.8	32.67 ± 1.05[a]	
IC_{50}	**>100**		**IC_{50}**	**12.74 ±1.17**	

The superscript letters "a, b, c, d" denote statistical differences in inhibitory effect on nitric oxide production among the testing doses of the compound (Smiglabrone B) or positive control (dexamethasone) (p<0.05). *** p<0.001 represents the significant difference between IC_{50} values of Smiglabrone B and control.

Therefore, this study aligns with existing literature in elucidating the anti-inflammatory properties of SGB. In addition, the anti-inflammatory effect of SGB suggests that the compound is a natural product to substitute the synthetic counterparts for the treatment of inflammatory diseases and requires further modification and investigation to optimize its function as an anti-inflammatory medicine.

Conclusion

This study explored the antioxidant and anti-inflammatory effects SGB, a natural product derived from *G. bulot*. The compound demonstrated strong antioxidant properties by effectively scavenging DPPH and ABTS free radicals as well as inhibiting lipid peroxidation, with IC_{50} values ranging from 7.72 to 38.65 µg/mL. Notably, its ABTS free radical scavenging ability was found to be comparable to ascorbic acid, the reference compound. Furthermore, SGB exhibited a strong binding affinity with COX-2 and iNOS, showing binding energies between -7.2 and -9.3 kcal/mol.

Its inhibitory effect against NO production was moderate, with a peak inhibition of 29.61 ± 2.33% at 100 µg/mL. This study proposes SGB as a promising antioxidant and an alternative anti-inflammatory agent, emphasizing the need for further studies to refine its structure and to elucidate its mechanism of action before therapeutic applications against oxidative stress and inflammatory diseases.

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