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In silico investigation of *Panax ginseng* lead compounds against COVID-19 associated platelet activation and thromboembolism

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ABSTRACT

Hypercoagulability is frequently observed in patients with severe coronavirus disease-2019 (COVID-19). Platelets are a favorable target for effectively treating hypercoagulability in COVID-19 patients as platelet hyperactivity has also been observed. It is difficult to develop a treatment for COVID-19 that will be effective against all variants and the use of antivirals may not be fully effective against COVID-19 as activated platelets have been detected in patients with COVID-19. Therefore, patients with less severe side effects often turn toward natural remedies. Numerous phytochemicals are being investigated for their potential to treat a variety of illnesses, including cancer and bacterial and viral infections. Natural products have been used to alleviate COVID-19 symptoms. *Panax ginseng* has potential for managing cardiovascular diseases and could be a treatment for COVID-19 by targeting the coagulation cascade and platelet activation. Using molecular docking, we analyzed the interactions of bioactive chemicals in *P. ginseng* with important proteins and receptors involved in platelet activation. Furthermore, the SwissADME online tool was used to calculate the pharmacokinetics and drug-likeness properties of the lead compounds of *P. ginseng*. Dianthramine, deoxyharringtonine, and suchilactone were determined to have favorable pharmacokinetic profiles.

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1. Introduction

Coronavirus 2019 (COVID-19) is a global pandemic that has been associated with a large number of fatalities. Although antiviral drugs, such as remdesivir, have been approved by the United States Food and Drug Administration (FDA) to treat COVID-19 [1], none have shown mortality benefits [2]. Based on a systematic review by Polak et al (2020), 45% of COVID-19 patients were found to have capillary congestion, 39% had microthrombi, and 26% had alveolar fibrin deposits in pulmonary samples [3]. COVID-19 is widely understood to manifest as a pulmonary disease. However, studies

have revealed various mechanisms of infection with SARS-CoV-2, involving various extrapulmonary mechanisms that can cause deep vein thrombosis, myocardial ischemia, acute kidney injury, and even diarrhea [4]. Additionally, patients with COVID-19 have been reported to have high levels of D-dimer, increased prothrombin times, and low platelet counts [5]. The low platelet counts may have been caused by the aggregation of platelets or the formation of aggregates with other immune cells, such as neutrophils, monocytes, and lymphocytes. In summary, the use of antivirals may not be fully effective against COVID-19 as activated platelets have been detected in patients with COVID-19 [6–8], which suggests their role in the progression of COVID-19.

It is difficult to develop a treatment that will be effective against all variants of SARS-CoV-2 [9]. Patients with less severe side effects often turn to natural remedies, such as the Lianhua Qingwen remedy [10] or natural products, like ginger [11]. Natural products are easily available and are relatively less toxic than many drugs. Therefore, natural products are often desired to ease the side effects of COVID-19. *Panax ginseng* Meyer has been widely known for its various health benefits. It is also well-known for its anti-platelet

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activity [12]. We have recently summarized the potential benefits of *P. ginseng* against cardiovascular disease and COVID-19 because it targets the coagulation cascade and platelet activation [13]. In this study, we aimed to further validate this benefit via molecular docking to elucidate the potential treatment effects of *P. ginseng* against platelet activation.

2. Materials and methods

2.1. Molecular docking of bioactive compounds from *P. ginseng*

Compounds of *P. ginseng* were obtained from TCMSP (<https://tcmsp-e.com>). *P. ginseng* yields active compounds with an oral bioavailability ≥ 30 and a drug-likeness ≥ 0.18 (Table 1). A “drug-like” level of 0.18 is utilized as a criterion for selecting “drug-like” compounds in traditional Chinese herbs [14]. The ligand file in Structure Data File format was converted to MOL format using Openbabel software. Molecular docking analysis was performed using P-selecting, GPIIb α , and CD40L structure data from the protein data bank (PDB) [15] with IDs 1g1s, 1p9a, and 1aly, respectively. The co-crystallized structures were prepared using UCSF Chimera (Chimera, Version 1.12, RBVI, San Francisco, CA, USA) and iGEM-DOCK (Version 2.1; NCTU, Hsinchu City, Taiwan). Flexible docking was performed with iGEMDOCK using an accurate docking mode. The best docked poses were further analyzed, and 3D structure

images were prepared using UCSF Chimera. The platelet receptors that were targeted are P-selecting, GPIIb α , and CD40L as they were suggested to be related to COVID-19-related hypercoagulation and platelet hyperactivation [16–18].

2.2. Drug-likeness analysis

The SwissADME, a free web tool, was used to evaluate the pharmacokinetics and drug-likeness (physicochemical and ADME properties) of the *P. ginseng* compounds [19]. Briefly, the Canonical SMILES for the chemical compounds were obtained from PubChem (<http://pubchem.ncbi.nlm.nih.gov/>). The resulting Canonical SMILES were used as input on the SwissADME website (<http://www.swissadme.ch/>). The output files and images were imported directly from the website. The BOILED-Egg (Brain Or Intestinal Estimated permeation predictive) model provides a rapid and straightforward evaluation for human intestinal absorption (HIA) and blood-brain barrier (BBB) permeation. The BOILED-Egg predictive model computes the lipophilicity and polarity of the chemical molecules and then outputs the WLOGP versus tPSA plot. Whereby WLOGP was the log P method developed by Wildman and Crippen for calculating lipophilicity; tPSA was the topological polar surface area for calculating polarity [19,20].

3. Results

Ginsenoside Rh2 was best docked with P-selecting and had the lowest binding energy; i.e., -108.90 kcal/mol, among the other screening compounds, followed by dianthramine and deoxyharringtonine with binding energies of -100.73 and -93.26 kcal/mol, respectively (Table 2). These compounds were predicted to interact with P-selecting mostly via van der Waals (vdW) contact with amino acid residues LYS8, ALA9, LYS112, and HIS114 (Supplementary material 1).

P-selecting has a strong affinity for specific glycoprotein counterreceptors, including P-selecting glycoprotein ligand-1 (PSGL-1). The amino acid residues ARG85 and HIS114 in human P-selecting form critical contacts with sulfates at TYR7 and TYR10 in PSGL-1 [21], whereas PSGL-1 TYR7 forms a backbone-to-backbone hydrogen bond with the amide nitrogen of P-selecting LYS112 [21]. Aside from vdW contact, the docking revealed that hydrogen bonds can be formed at LYS112 with eight compounds, including ginsenoside Rg5, kaempferol, suchilactone, girinimbine, panaxadiol, fumarine, and dianthramine. Furthermore, inermin, fumarine, fruitinone A, gomisins B, arachidonate, celabenzine, suchilactone, and dianthramine interacted with HIS114 via hydrogen bonding. Dianthramine was observed to interact with the two amino acid residues via hydrogen bonding, as shown in Fig. 1. These interactions suggest that the active compounds found in *P. ginseng* could work collectively to directly or indirectly disrupt the interaction between P-selecting and PSGL-1 (Fig. 2).

Table 1

Bioactive Compounds of *Panax ginseng* From Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCM-SP)

	Molecule Name	OB (%)	DL
1	Dianthramine	40.44	0.19
2	Arachidonate	45.57	0.20
3	Aposiopolamine	66.64	0.21
4	Kaempferol	41.88	0.24
5	Girinimbine	61.21	0.31
6	Frutinone A	65.90	0.34
7	Diop	43.59	0.39
8	Celabenzine	101.88	0.48
9	Inermin	65.83	0.53
10	Suchilactone	57.51	0.55
11	Ginsenoside Rh2	36.31	0.55
12	Chrysanthemaxanthin	38.72	0.58
13	Malkangunin	57.71	0.62
14	Beta-sitosterol	36.91	0.75
15	Alexandrin	36.91	0.75
16	Stigmasterol	43.82	0.75
17	Ginsenoside Rh4	31.11	0.77
18	Ginsenoside Rg5	39.56	0.78
19	Panaxadiol	33.08	0.79
20	Deoxyharringtonine	39.27	0.81
21	Fumarine	59.26	0.82
22	Gomisins B	31.99	0.82

OB; oral bioavailability, DL; drug-likeness.

Table 2

Interaction of the Receptors of the PDBs (1g1s, 1p9a, and 1aly) With the Active Compounds in *Panax ginseng* Extract

Binding sites	Ligands/compounds	Total energy (kcal/mol)	vdW (kcal/mol)	HBond (kcal/mol)
P-selecting (1g1s)	Ginsenoside Rh2	-108.90	-84.10	-24.80
	Dianthramine	-100.73	-68.25	-30.29
	Deoxyharringtonine	-93.26	-70.90	-22.36
GPIIb α (1p9a)	Ginsenoside Rh2	-102.34	-75.76	-26.57
	Deoxyharringtonin	-98.70	-79.70	-19.00
	Dianthramine	-93.06	-63.57	-29.74
CD40L (1aly)	Dianthramine	-116.2	-81.35	-33.19
	Ginsenoside Rh2	-112.5	-85.20	-27.26
	Suchilactone	-102.6	-78.85	-23.77

vdW; Van der Waals, HBond; hydrogen bond.

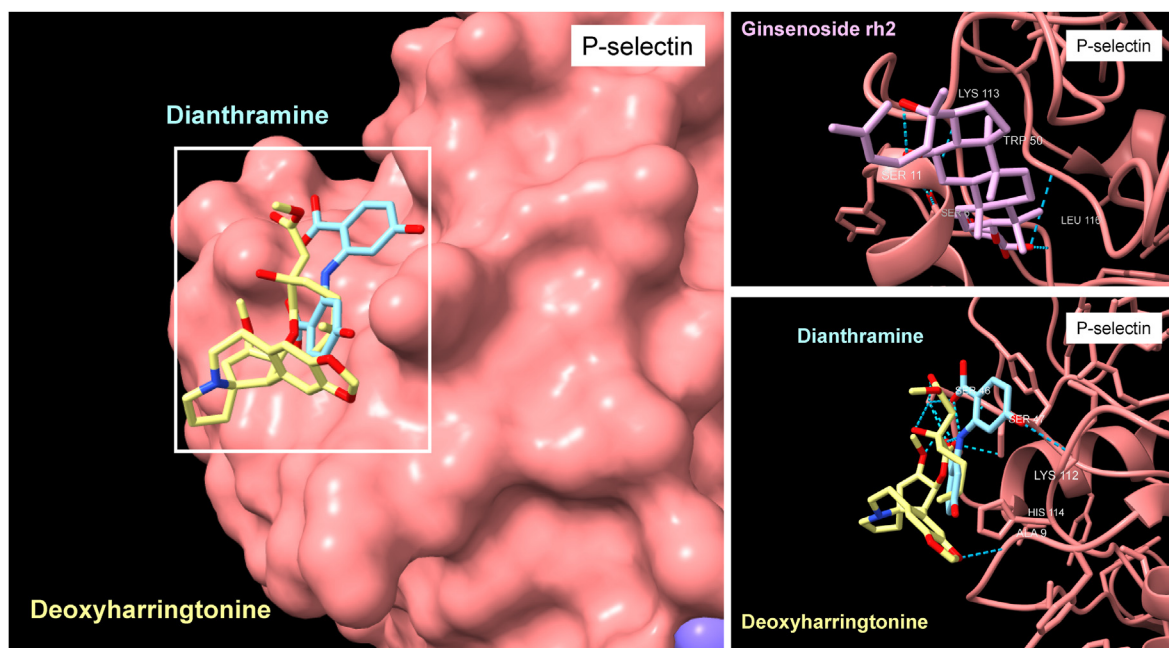


Fig. 1. Interaction between P-selectin (peach surface) and ginsenoside Rh2 (pink), dianthramine (blue), and deoxyharringtonine (yellow). Dashed lines indicate intermolecular hydrogen bonds.

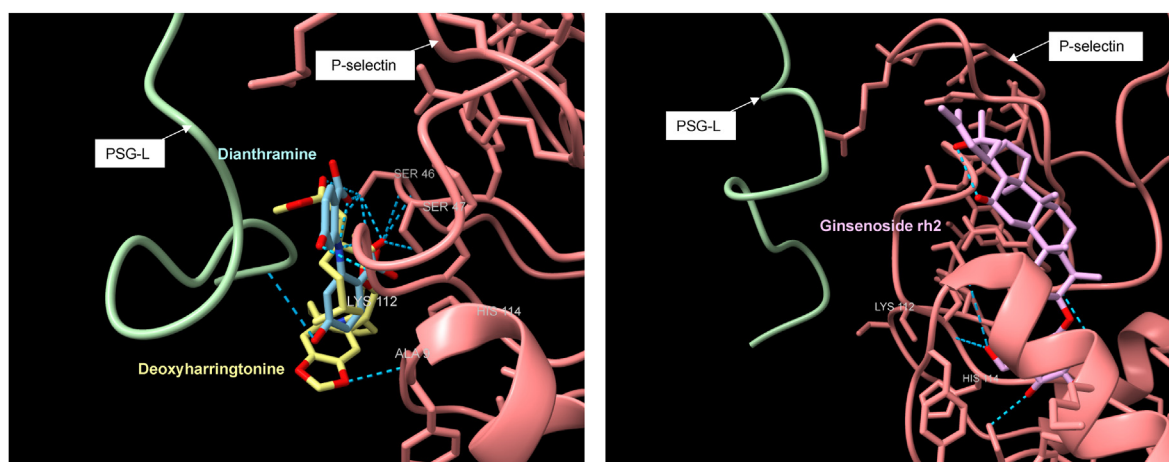


Fig. 2. Interaction between P-selectin (peach) and PSG-L (green), in the presence of docked ligands (ginsenoside Rh2, dianthramine, and deoxyharringtonine). Dashed lines indicate intermolecular hydrogen bonds.

The amino acid residues CYS20 and CYS33 are highly conserved across a large variety of species, and substitution of these amino acids appears to not be tolerated. Affected patients with biallelic Bernard-Soulier syndrome have been reported to also have cysteine amino acid substitutions in other cysteine amino acid residues in the extracellular domain of the GPIIb α protein (CYS81 and CYS225), indicating that cysteine amino acid substitutions likely affect protein structure and function [22]. The three chemical compounds with the lowest binding energy may not directly affect binding of the GPIIb α protein with vWF.

Previous molecular docking simulation using ginsenoside Rg1 and GPIIb α showed that ginsenoside Rg1 forms a stable structure with GPIIb α by creating hydrogen bonds with the LEU214, LYS189, CYS211, GLU212, TYR215, ARG17, and THR266 residues of the GPIIb α protein [23]. According to the author, this binding pattern implied that ginsenoside Rg1 inhibits GPIIb α activity by occupying the

amino acid N-terminal of GPIIb α . In our study, the binding of ginsenoside Rh2, deoxyharringtonine, and dianthramine produced binding energies of -102.3 , -98.7 , and -93.1 kcal/mol, respectively (Table 2). The three compounds primarily interacted with common residues on the GPIIb α protein; i.e., ASN134, GLU135, ASN157, ASN158, ASN159, LEU160, GLU181, ASN182, and SER183 (Supplementary material 2). These binding sites, however, did not correspond to the functional amino acids involved in the interaction between GPIIb α and von Willebrand factor (vWF). The functional amino acids that were identified as part of this interaction were between positions 227 and 242 of platelet GPIIb α 's beta-switch loop [24]. These results indicated the three chemical compounds may have no direct effect on the GPIIb α protein's binding to vWF (Fig. 3). As a result, the interaction between GPIIb α -vWF and the docked compounds were not illustrated.

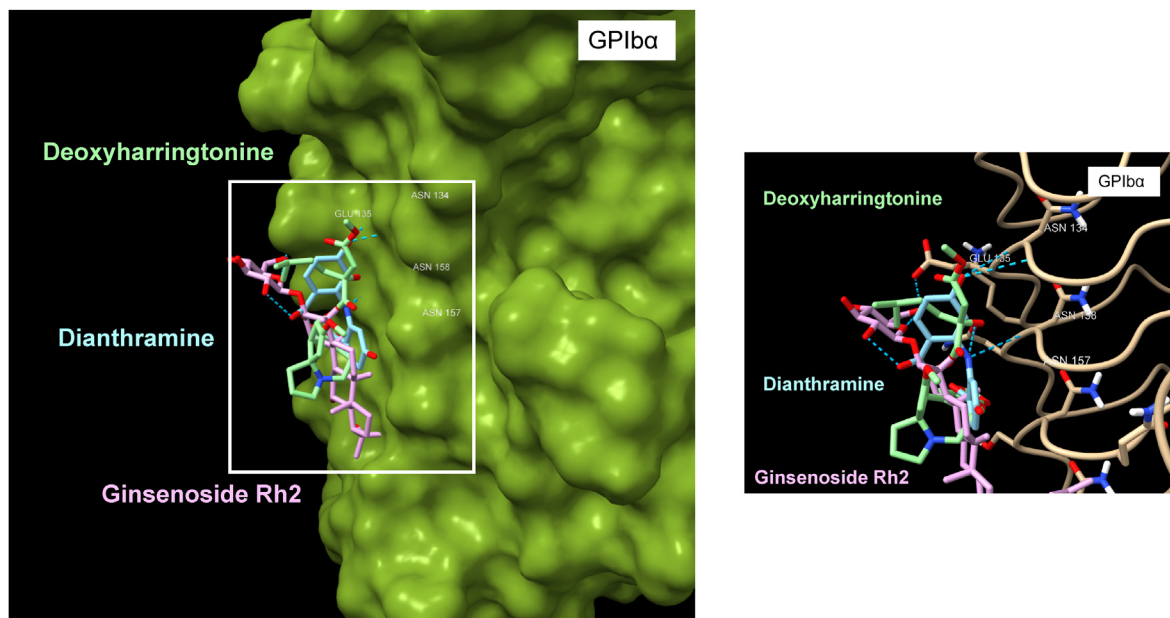


Fig. 3. Interaction and binding of ginsenoside Rh2 (pink), deoxyharringtonine (green), and dianthramine (blue) to GPIIb/IIIa (green surface). Dashed lines indicate intermolecular hydrogen bonds.

CD40L is expressed by activated platelets. CD40L's primary receptor is CD40, which is expressed constitutively on B cells, macrophages, dendritic cells, neutrophils, endothelial cells, T-cells, and platelets [25–27]. LYS143, GLY144, and TYR145 have been identified as CD40-CD40L interaction “hot spots” in CD40L. CD40L mutants LYS143ALA and TYR145ALA bind significantly less to CD40 [28]. Based on structural modeling of the CD40-CD40L complex, extended mutagenesis experiments contributed to the discovery of additional residues in CD40L (TYR146, ARG203, and GLN220) and CD40 (GLU74, and GLU117) that contribute to CD40-CD40L interactions [29].

The current study showed that dianthramine and suchilactone were predicted to interact with CD40L at the interaction “hotspot” for CD40-CD40L at residues LYS143, GLY144, and TYR145 (Fig. 4). Particularly, dianthramine interacts with CD40L at TYR145 via a hydrogen bond with free energy of -3.50 kcal/mol (Fig. 4), while interactions with LYS143 and GLY144 via vdW contact had free energy of -0.02 and -6.42 kcal/mol, respectively (Supplementary material 3). Suchilactone interacts with CD40L through vdW contact at LYS143, GLY144, and TYR145 with a free energy of -10.26 , -3.80 , and -10.66 kcal/mol, respectively (Supplementary material 3). Ginsenoside Rh2 mainly binds to CD40L on residues that are less relevant to CD40-CD40L binding.

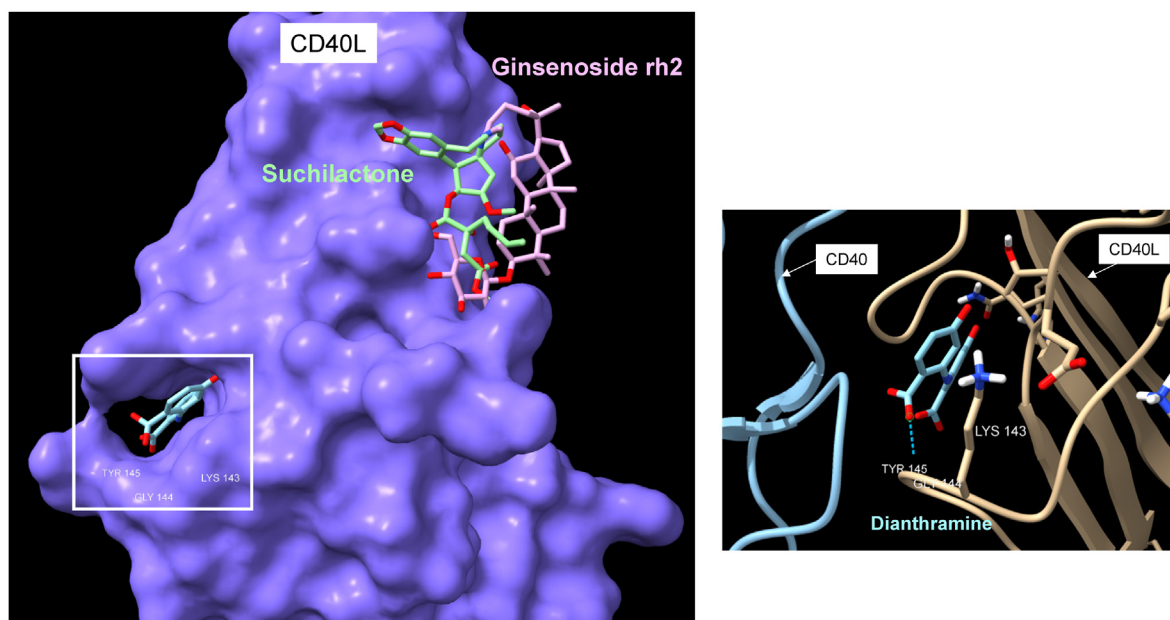


Fig. 4. The binding of dianthramine (blue), ginsenoside Rh2 (pink), and suchilactone (green) to the CD40L (purple surface). Dashed lines indicate intermolecular hydrogen bonds.

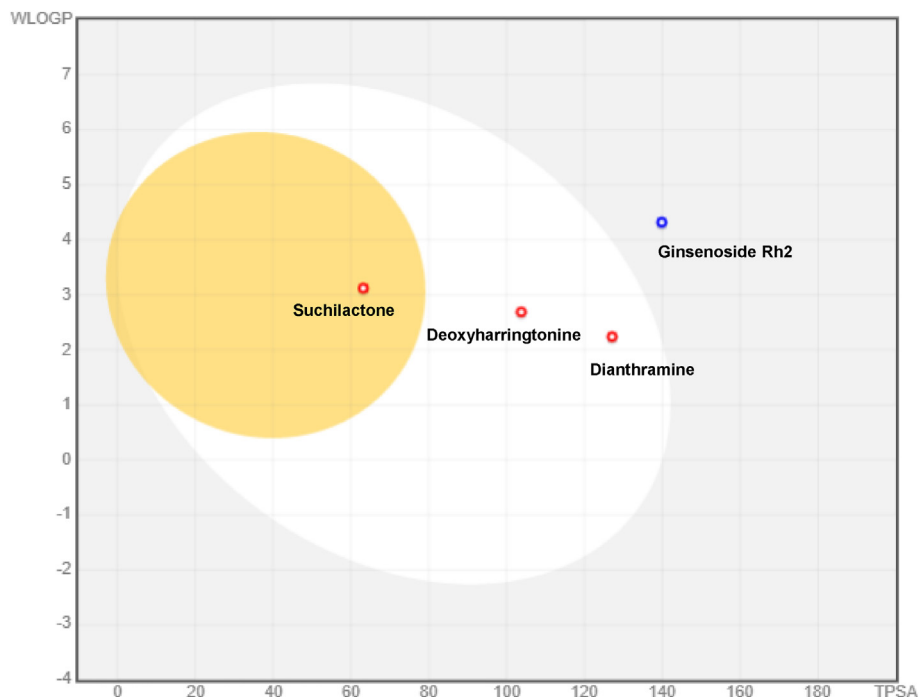


Fig. 5. BOILED-Egg analysis of the compounds. The yolk (yellow region) indicates the high probability of brain penetration; white indicates a high probability of passive absorption by the gastrointestinal tract. Molecules that are predicted to be effluated by P-glycoprotein from the central nervous system are indicated by blue whereas red molecules were predicted otherwise.

In this study, dianthramine was seen as a potent compound candidate in regard to its interactions with the key ligands and/or receptors analyzed in this study. Dianthramine interacts strongly through the formation of hydrogen bonds with the amino acid residues, which are mainly the key residues for binding sites related to activation of thrombosis and platelet activation (CD40L and P-selecting, respectively). SwissADME calculates bioavailability scores and drug-likeness differently than TCMSP. The former was used for Martin's calculation of bioavailability scores [30]. We used $OB \geq 30$ and drug-likeness ≥ 0.18 values from TCMSP as criteria to target chemical compounds in *P. ginseng* that were predicted to be suitable candidates for our molecular docking analysis. We then used SwissADME to investigate the pharmacokinetics (Fig. 5) and physicochemical properties (Fig. 6) of the selected compounds that were highly associated with platelet activation. When comparing dianthramine to ginsenoside Rh2, dianthramine was found to have a higher drug-likeness because it had no violations of the Lipinski (Pfizer) filter, Muegge (Bayer) filter, Ghose filter, Veber filter, and Egan filter [19]. Dianthramine has a bioavailability score of 0.56, but ginsenoside Rh2 has a score of only 0.17. Deoxyharringtonine and suchilactone had a bioavailability score of 0.55. Suchilactone was predicted to pass through the blood-brain barrier (BBB) passively, as shown in the yellow region (the yolk) in the BOILED-Egg diagram (Fig. 5). This could be due to its low molecular weight (68.38 g/mol). Dianthramine and deoxyharringtonine can be passively absorbed by the GI tract but not accessing the brain (in the white), while ginsenoside Rh2 was predicted to have rather low passive absorption into the GI tract (in the grey region). In a previous study, ginsenoside Rh2 was also reported to have low oral bioavailability [31]. Ginsenoside Rh2 and suchilactone were also predicted to be effluated by the central nervous system through P-glycoprotein (indicated as blue), while red molecules were predicted otherwise (Fig. 5). The bioavailability radar generated from SwissADME for ginsenoside Rh2, dianthramine, deoxyharringtonine and suchilactone was shown in Fig. 6. The pink region on the radar indicates

the optimal range for each property which includes lipophilicity, polarity, size, solubility, flexibility and saturation. Despite having optimum properties, ginsenoside Rh2 lacked drug-likeness due to its poor GI tract absorption (Fig. 6A). Deoxyharringtonine, dianthramine, and suchilactone have good drug-likeness (Fig. 6B–D). The favorable bioavailability of these compounds suggests that *P. ginseng* may be a potentially therapeutic agent against COVID-19 related coagulopathy.

4. Discussion

Platelet counts have been suggested to be an important factor in determining the severity of COVID-19. Lower platelet counts may be related to the consumption of platelets in thrombi formation and an improvement in platelet counts may indicate clinical improvement [32]. Platelets are important players in hypercoagulability and disseminated intravascular coagulation (DIC), which are both important markers in patients with COVID-19 [33–35]. The coagulopathy of COVID-19 comprises a combination of a few coagulopathies, including DIC, thrombotic microangiopathy, a cytokine storm, and the antiphospholipid syndrome; it does not meet the criteria for any of these coagulopathies [36]. Thus, to effectively treat hypercoagulability in COVID-19, platelets are a favorable target as platelet hyperactivity has been observed in COVID-19 patients [37–39]. The levels of P-selecting and soluble CD40L (sCD40L) were shown to be impacted by the severity of COVID-19, with higher levels of P-selecting and sCD40L found in ICU patients [16]. sCD40L is present in the bloodstream, where it forms multimeric complexes on cell surfaces with the membrane-anchored full-length version of CD40L, which is involved in cell adhesion [40].

SARS-CoV-2 may activate endothelial cells, inducing the secretion of various inflammatory cytokines, which eventually causes inflammation of platelets and leads to their activation [41]. Platelets express a variety of receptors, including pattern recognition

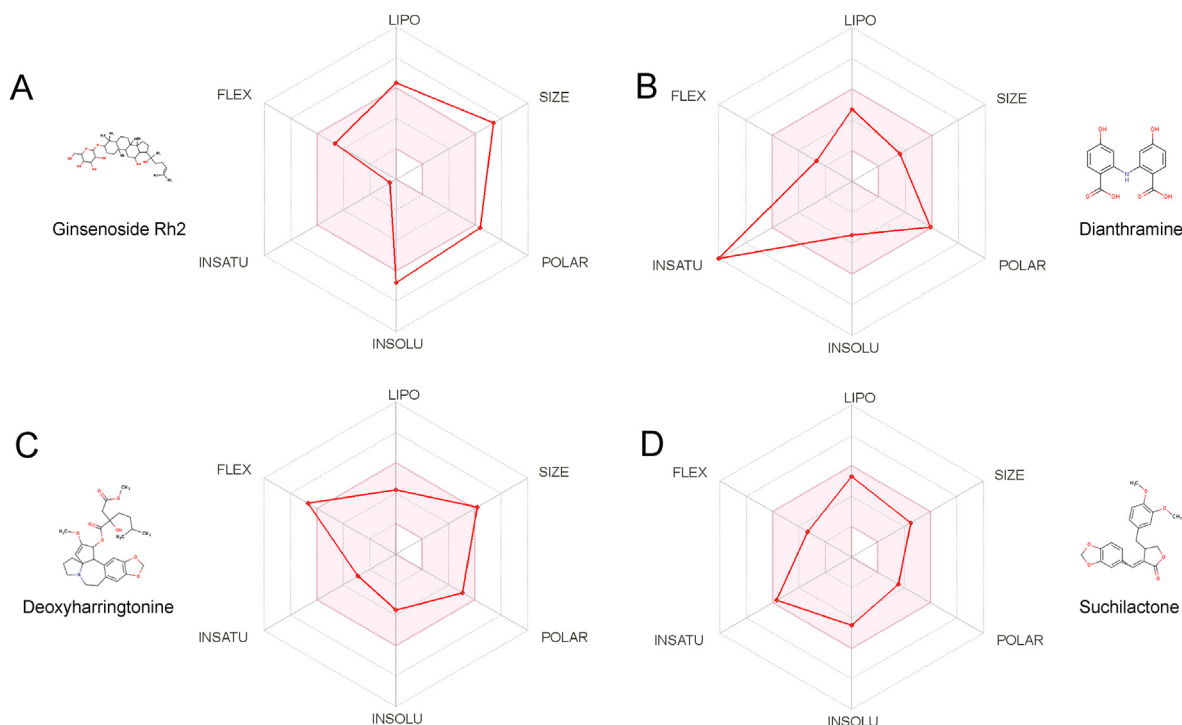


Fig. 6. Bioavailability of active compounds of *Panax ginseng*. The bioavailability radar for ginsenoside Rh2 (A), dianthramine (B), deoxyharringtonine (C), and suchilactone (D) were generated using SwissADME (<http://www.swissadme.ch/>). LIPO; lipophilicity, FLEX; flexibility, INSATU; saturation, INSOLU, solubility.

receptors and cytokine and chemokine receptors [42]. The protease-activated receptor-1 and the GPIb-IX-V complex on platelets can be activated by the coagulation cascade, which causes platelets to be activated and aggregate due to integrin α IIb β 3 [43]. When platelets are activated, CD40L translocates to the platelet surface, is cleaved, and then is shed from the platelet surface releasing sCD40L. This is known to cause inflammation, stabilize the platelet-rich thrombi, and inhibit the reendothelialization of an injured vessel in the case of thrombosis [44]. Additionally, CD40, which binds to CD40L and is an integral protein of the tumor necrosis factor receptor family (TNF-R), plays an important role in many inflammatory processes, including Crohn's disease and lupus erythematosus, and also participates in an important interaction that contributes to the progression of atherosclerosis [45]. The sCD40L secreted from activated platelets can also bind to CD40, which is found on endothelial cell surfaces. This triggers a series of inflammatory cascades and ultimately leads to atherothrombosis [46]. Patients with COVID-19 have been reported to have an elevated amount of detectable soluble CD40L (sCD40L) [47]. In a separate study, an increase in sCD40L in the plasma of patients with COVID-19 was also observed [48]. This indicated that CD40L is an important marker in COVID-19.

The GPIb-IX-V complex is found on megakaryocytes and platelets, the second most abundant receptor on platelets, and is a platelet receptor for the vWF, thrombin, P-selecting, coagulation factors XI and XIII, and the integrin α M β 2. This complex also contains the GPIb α , GPIb β , GPIX, and the GPV subunits GPIb α , and is also known as CD42b, which contains the binding region for the A1 domain of the vWF [49]. The vWF is a protein that participates in the formation of blood clots. When vascular injury occurs, the platelet GPIb α interacts with strand β 3 of domain A1 of the vWF, which facilitates the initial stage of platelet adhesion to the vascular subendothelium. This also activates signaling events within the platelet that lead to increased platelet activation, thrombosis, and

hemostasis [50]. In a previous study, SARS-CoV-2 was found to cause activation of platelets due to spike proteins via CD42b [18]. In patients that did not survive COVID-19 pneumonia, there was an increase in sinusoidal platelet aggregates in the hepatic microvasculature, indicated by CD42b positive staining in the liver [51]. Therefore, preventing the binding of CD42b to various coagulation factors and agonists for platelet activation may cease and prevent aggregate formation.

P-selecting (CD62P) is the largest member of the selecting family and is expressed by both platelets and endothelial cells. Soluble P-selecting is found in the plasma, which may be due to damaged platelet membranes or simple shedding [52]. Grobler et al (2020) summarized that fibrinogen, P-selecting, vWF, and D-dimers are crucial in the coagulopathies of COVID-19 patients, and they are associated with an increased risk of acute respiratory distress syndrome. Increased P-selecting is also observed with increasing severity of COVID-19, followed by the occurrence of a cytokine storm, increased D-dimer levels, and reduced levels of vWF and fibrinogen [53]. Therefore, we investigated the binding affinity of the active compounds of ginseng to GPIb α , P-selecting, and CD40L as these receptors have been reported in COVID-19 related coagulopathies, targeting platelet activation.

Natural products, such as Korean Red Ginseng, which is the steamed and dried product of *P. ginseng*, have been reported to have anti-platelet effects [12,54,55]. In this study, we sought to investigate whether the anti-platelet effect of *P. ginseng* could target COVID-19 related coagulopathies and targeting receptors that have been reported to be associated with the pathology of COVID-19. We found that various bioactive compounds of *P. ginseng* have high affinity to the receptors that were reportedly involved in the coagulopathy of COVID-19, especially dianthramine, deoxyharringtonine, and suchilactone. These minor secondary metabolites in *P. ginseng* have received little attention in the past. However, a target network analysis of ginseng bioactive

phytochemicals has been carried out on a TCM, namely the ShenZhu capsule. *P. ginseng*'s dried roots were one of the main components of the capsule. The study revealed that dianthramine was one of the bioactive compounds that has the most interactions with targets, primarily those connected related to immune and inflammatory pathways [56]. These minor secondary metabolites may play a synergistic or catalytic role in the antiplatelet effects of *P. ginseng* via multiple pathways. Validating the roles of these compounds requires additional research. From the BOILED-Egg diagram, suchilactone can pass through the BBB, while dianthramine can be passively absorbed into the GI tract. However, there have been no reported data on the toxicity of suchilactone. Thus, this has to be further validated in future studies.

The current investigation suggested that dianthramine is a potential therapeutic agent against COVID-19 induced coagulopathy by targeting the activation of platelets as it can interact with CD40L and P-selecting at their functional binding sites. Docking complexes of this natural metabolite with CD40L and P-selecting demonstrated a stable conformation, which was corroborated by the binding free energy. There are difficulties in actual *in vitro* or *ex vivo* validation of *P. ginseng* against COVID-19, however, this study shed light on the potential use of *P. ginseng* as a supplementary treatment for patients with COVID-19 with evidence of the potential binding sites via molecular docking. Nevertheless, the efficacy of dianthramine should be further validated in inhibition of platelet aggregation and against COVID-19 induced thromboembolism. In addition to iGEMDOCK analysis, future research on molecular docking analysis using a different docking software is warranted to verify inter-software outputs.

Authorship contributions

Conception and design of study: **S—C. Park, M.H. Rhee, Y. Quah, Y.Y. Lee**; acquisition of data: **Y. Quah, Y.Y. Lee**, analysis and/or interpretation of data: **Y. Quah, Y.Y. Lee**; Drafting the manuscript: **Y. Quah, Y.Y. Lee**; revising the manuscript critically for important intellectual content: **S.-J. Lee, S.D. Kim, M.H. Rhee, S—C. Park**. Approval of the version of the manuscript to be published (the names of all authors must be listed): **Y. Quah, Y.Y. Lee, S.-J. Lee, S.D. Kim, M.H. Rhee, S—C. Park**.

Declaration of competing interest

The author declares no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgr.2022.09.001>.

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