

Research Article

Antibacterial Mechanisms of Constituents from Galla chinensis Revealed by Experimental and Virtual Screening-Based Studies

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Many traditional Chinese medicines (TCMs) have been confirmed to have antibacterial activities. However, very few substances have been found to be active against Gram-negative bacteria. This study aimed to identify antimicrobial activity substances against Gram-negative bacteria from fourteen TCMs. Fourteen TCMs with antibacterial potential were chosen for quantitative extraction and antibacterial activity assay, and the plant with the highest activity against *Escherichia coli* was selected to construct the component-target network. The following virtual screening and enzyme inhibition experiments were performed to analyse the antibacterial mechanisms of the compounds from Galla chinensis. The chemical constituents of Galla chinensis were identified by chemical fingerprinting. 1, 2, 3, 4, 6-Penta-O-galloyl- β -D-glucose (PGG) from Galla chinensis exhibited significant inhibition activity against adenylyl transferase (ATase) of *E. coli* and antibacterial activity against *E. coli*. Meanwhile, PGG was identified in the Galla chinensis ethanol extract as the abundant ingredient with a high content of 1.95% (w/w). PGG enriched in Galla chinensis is a promising natural antibiotic with the mode of action inhibiting ATase activity. To our knowledge, this is the first study attributing the antibacterial activity of PGG to its affinity with ATase.

1. Introduction

Antibiotics are one of the most significant medical advances, and their discovery had a great effect on the treatment of human diseases. However, widespread excessive dispensing and inappropriate use of antibiotics have led to the creation of resistant strains [1]. Antimicrobial resistance in bacterial pathogens has been a major cause of high morbidity and mortality [2]. Therefore, new antibacterial agents are crucially needed to overcome resistant bacteria. Many secondary metabolites of plants with the potential of inhibiting drug-resistant bacteria have been found and their pharmacological effects have been gradually explored to seek ingredients with strong antibacterial activity [3–5].

The overall therapeutic effect of TCMs in clinical antiinfective treatments has been witnessed in its long-term clinical application [6]. Many Chinese medicines have been confirmed to have antibacterial activities [6–8]. However, compared with Gram-positive bacteria, Gramnegative ones are more resistant to antibiotics due to their impermeable barriers and cell membranes. In order to explore active natural ingredients with a significant activity against Gram-negative bacteria, 14 TCMs used to treat diseases associated with bacterial infections, such as diarrhoea, dysentery, and skin infections, were selected for minimal inhibitory concentration (MIC) and total antibacterial activity (TAA) screening (Table 1).

Galla chinensis, a traditional Chinese medicine, is a gall formed mainly by the aphid *Melaphis chinensis* (Bell) Baker

	TABLE 1: Ethn	ic traditional uses of 14 medicinal plants.	
Scientific name	Part	Application	References
Galla chinensis	Fruit	Treat intestinal deficiency and diarrhea; folliculitis; and tinea pedis	[9–11]
Coptis chinensis Franch.	Root	Diarrhea; dysentery; and pyogenic infections of ear canal	[12, 13]
Elsholtzia ciliata (Thunb.) Hyl.	Leaf, flower, root	Diarrhea; beriberi; antibacterial; anti-inflammatory; and relieving fever	[14, 15]
Origanum vulgare L.	Leaf, flower, root	Diarrhea; bronchitis; pruritus; and flu	[16-18]
Maesa japonica (Thunb.) Moritzi. ex Zoll.	Leaf, root	Dysentery	[19]
Houpoea officinalis Rehder & E.H.Wilson	Bark	Cholera	[20-22]
Paulownia fortunei (Seem.) Hemsl.	Bark, leaf, flower, root	Parotitis; bacteriological diarrhea; enteritis; conjunctivitis; dysentery; and tonsillitis	[23-25]
Zanthoxylum nitidum (Roxb.) DC.	Root	Rheumatic arthralgia; inflammatory diseases	[26, 27]
Syringa oblata Lindl.	Flower	Bacterial dysenteries; enteritis; upper respiratory tract infection; and tonsillitis	[28, 29]
llex cornuta Lindl. & Paxton	Leaf	Diarrhea	[30]
Pteris multifida Poir.	Leaf, root	Dysentery; hepatitis; enteritis; diarrhea; typhoid; and cholecystitis	[19, 31, 32]
Rheum officinale Baill.	Root	Diarrhea and hepatitis	[33 - 35]
Cassia fistula L.	Fruit	Diarrhea; skin infections; nasal infection; flu; oral ulcers; and leprosy	[36 - 38]
Platycladus orientalis (L.) Franco.	Leaf	Dermatitis; diarrhea; cystitis; psoriasis; mumps; and bacterial dysentery	[39, 40]

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on the plant leaves *Rhus chinensis* Mill., *Rhus potaninii* Maxim. and *Rhus punjabensis* var. sinica (Diels) Rehder & E.H.Wilson. Galla chinensis has been used extensively in Chinese traditional medicine for over 1400 years. It was first recorded in "Ben Cao Shi yi" as a stop diarrhoea Chinese medicine. The "Compendium of Materia Medica" also described its benefit in the treatment of diarrhoea, vomiting, and sores. Modern pharmacological studies have shown that Galla chinensis has a variety of pharmacological actions, including anticaries, antibacterial, antiviral, anticancer, antioxidant, anti-inflammatory, and neuroprotective properties [9, 41].

Based on the antibacterial activity, the TCM with the strongest activity against Gram-negative bacteria represented by *E. coli* was selected to systematically explore antibacterial active ingredients and target proteins, molecular docking, and inhibition of target protein activity.

2. Materials and Methods

2.1. Plant Material and Traditional Uses. Galla chinensis, Coptis chinensis Franch., Elsholtzia ciliata (Thunb.) Hyl., Origanum vulgare L., Maesa japonica (Thunb.) Moritzi. ex Zoll., Houpoea officinalis Rehder & E.H.Wilson, Paulownia fortunei (Seem.) Hemsl., Zanthoxylum nitidum (Roxb.) DC., Syringa oblata Lindl., Ilex cornuta Lindl. & Paxton, Pteris multifida Poir., Rheum officinale Baill., Cassia fistula L., and Platycladus orientalis (L.) Franco. were commercially obtained and then identified by one of the authors (H. F. Chen). Voucher specimens (No. SCBG/NPL/2019051-No. SCBG/NPL/2019064) were preserved at the Laboratory of Natural Products Chemistry and Biology, South China Botanical Garden, Chinese Academy of Sciences. These samples to be tested were naturally sun-dried and ground, and 100 g of each sample was weighed for experimentation. Standard substance PGG was purchased from Med Chem Express.

2.2. Bacterial Strains and Culture Conditions. Staphylococcus aureus ATCC6538 and E. coli ATCC 8739 were obtained commercially from Guangdong Institute of Microbiology (Guangzhou, China). The bacterial solution stored at -4° C was thawed at room temperature and 100 μ L of the bacterial solution was taken in 5 mL of sterilised hydrolysed casein peptone medium and incubated overnight in a 37°C incubator.

2.3. Extraction. 100 g of pulverized sample was immersed in 95% ethanol for 48 h. The immersion was repeated 3 times in the ratio of herbs to ethanol 1:3 (v/v). The filtrations were mixed and concentrated under reduced pressure to obtain the ethanol extract and weighed.

2.4. Chemical Fingerprint of G. chinensis Extract. Chemical fingerprinting of the Galla chinensis ethanol extract was performed on a Thermo Ultimate 3000 instrument, coupled with a Thermo Orbitrap Elite spectrometer, with an ACQUITY UPLC HSS T3 column (Waters, 100×2.1 mm, $1.8 \,\mu$ m). Ethanol extract was analysed by UPLC-TQ-MS/MS (ultraperformance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry) using an automated full-dependent MS/MS scan with a gradient program of MeCN-H₂O (0.1% formic-acid) (0–25 min, 5–95%, 25–30 min, 95%, 30–35 min, 5%; 0.2 mL/min; MS scan 100–1200 Da) [42].

2.5. Antibacterial Activity Assay. The MICs of the ethanol extract against the two bacteria were determined utilizing 96-well plate serial dilution assays [43]. The bacterial culture medium after overnight incubation was formulated to an appropriate bacterial suspension concentration by ultraviolet spectrophotometry. Generally, the optical density (OD) value at the wavelength of 600 nm was reduced to 0.065–0.075, which was equivalent to a bacterial suspension concentration of S. aureus and E. coli (10⁵-10⁶ CFU/mL). The 14 test samples diluted with dimethyl sulfoxide (DMSO) to a certain concentration, PGG, and control substances (vancomycin hydrochloride and kanamycin sulfate) were diluted with DMSO to 100 mg/mL, 0.1 mg/mL, 1 mg/mL respectively. Then, 20 µL of each was seeded into the first row of the 96-well plate, with 2 parallel wells for each. Next, $180\,\mu\text{L}$ of the resazurin-bacterial liquid mixture was loaded into the first row, while $100 \,\mu\text{L}$ of the resazurin-bacterial liquid mixture was loaded into the other rows. After blending the first row, $100 \,\mu$ L of its solution was transferred to the second row to mix evenly, and the operations of the remaining rows were the same as above. By this method, certain gradient concentrations were made by 2-fold dilution; E. coli was observed after culturing in a constant temperature incubator at 37°C for 6 hours, and S.aureus was observed after culturing for 8 hours. The MIC value was determined based on the colour change and turbidity of the solution in the well of the plate. The experiment was independently repeated 3 times.

2.6. Molecular Docking Studies

2.6.1. Selection and Preparation of Ligands. The known 49 chemical components of Galla chinensis were retrieved from the previous references [44] and TCMSP (traditional Chinese medicine systems pharmacology database) database (https://tcmspw.com/tcmsp.php). 2D, 3D SDF files, and Canonical SMILES data for these compounds were obtained from the PubChem (https://pubchem.ncbi.nlm.nih.gov/), TCMSP (https://tcmspw.com/tcmsp.php), ChemSrc (https://www. chemsrc.com/) databases, or generated via ChemOffice software.

2.6.2. Target Prediction of 49 Chemical Components of Galla chinensis. The structural files of 49 ligands prepared above were imported into the Pharm Mapper database (https://lilab-ecust.cn/pharmmapper/index.html) to obtain the potential protein target files. The scores from the 49 predicted target files were ranked from high to low, and then, the top

30 PDB IDs were imported into the UniProt database (https://www.uniprot.org/uploadlists/). The protein names were converted into standard gene names, and the relevant files of the predicted proteins belonging to the *E. coli* species (ECOLI) were downloaded.

2.6.3. Construction of Galla chinensis Component-Target Networks. Based on the 49 chemical components of Galla chinensis and the genes corresponding to the proteins belonging to the *E. coli* species as predicted above, the component-target network was constructed via Cytoscape3.7.2 [45].

2.6.4. Molecular Docking. The protein structures (PDB IDs: 2NYA, 2OPX, 1NLM, 2GFP, 2ZM5, 2IMO, 2NRO, 2WDV, 1V4A, and 3CA8) were downloaded from the PDB database (https://www.rcsb.org/). Molecular docking analysis was performed using Schrödinger suit. Compounds were prepared using LigPrep and docked to the active site using Schrödinger Maestro's Glide module. A three-dimensional image of molecular modelling results was achieved using PyMOL, and a two-dimensional interaction diagram was achieved using Maestro.

2.6.5. 1V4A Protein Construction. E. coli expression vector PET-28a-1V4A-His was constructed by Nco I/Xho I Enzymatic escharotomy. All the clones were constructed by PCR-based strategy, and protein expression and purification were performed according to previous methods [46]. Briefly, the exogenous DNA fragment was constructed and subcloned into the PET-28a expression vector. The protein was expressed and purified from *E. coli* BL21 (DE3) under natural circumstances.

2.6.6. PGG Inhibits 1V4A Protein Activity Assay. To assess inhibition of 1V4A enzymatic activity by PGG, the deadenylylation of glutamine synthetase (GS)-AMP was monitored as the formation of γ -glutamylhydroxamate utilizing the γ -glutamyl transferase activity of GS as previously described with slight modifications [46]. The γ -glutamyl transferase activity was measured using a GS activity assay kit (Solarbio) following the manufacturer's protocol.

Briefly, the assay began by combining $100 \ \mu$ L solutions of $30 \ \mu$ M 1V4A protein in BSA (1 mg/mL) containing 10% PEG 6000 with $100 \ \mu$ L of 1V4A reaction mixture (2x). This reaction mixture included varying concentrations of PGG dissolved in DMSO, Hepes-HCl (100 mM, pH 7.6), BSA (2 mg/mL), potassium phosphate (50 mM), MgCl₂ (10 mM), ATP (2 mM, pH 7.6), as well as 2-oxoglutarate (40 mM), and GS-AMP (100 nM). 0.1% DMSO served as the negative control.

After incubating at 30°C for 30 min in a water bath, an aliquot of $10\,\mu$ L was withdrawn from the 1V4A reaction mixture and added to the test tubes for the GS activity assay (100 μ l reaction system). Another 10 μ L was taken for the blank tubes (100 μ L reaction system) [46]. Following the instructions of the GS activity assay kit (Solarbio), reagents II

and III were added sequentially to the test tubes, while reagents I and III were added sequentially to the control tubes. Both sets were mixed thoroughly and incubated at 30°C for an additional 30 min. The reaction was stopped by adding the stop reagents. The quantity of γ -glutamylhydroxamate produced was determined by measuring the absorbance at 540 nm: $\Delta A_{540} = A_{\text{test 540}} - A_{\text{blank 540}}$.

2.6.7. Statistical Analysis. Experiments were replicated 3 times and the mean was calculated. TAA was calculated with the formulate (mL/g) = the extraction yield ×1000/MIC value (mg/mL) [43]. The TAA value indicates how much water or solvent can be added to 1 g of the extract to still inhibit pathogen growth. The half-maximal inhibitory concentration (IC₅₀) of PGG was calculated using the log (inhibitor) vs. response – Variable slope (four parameters) equation in Prism V8 (GraphPad).

3. Results

3.1. Galla chinensis Exhibited the Greatest Antimicrobial Activity against E. coli. First, we assessed the extraction yields of 14 TCMs. As shown in Table 2, the highest extraction yield was observed in Galla chinensis (32.7%), followed by Cassia fistula L (27.27%) and Rheum officinale Baill (22.66%).

Furthermore, we assessed the antibacterial activity of 14 TCMs. In Table 2, the ethanol extracts of Galla chinensis, *Paulownia fortunei* (Seem.) Hemsl., and *Coptis chinensis* Franch. displayed strong activities against *E. coli* with MIC values in a range of 0.625–1 mg/mL, and their TAA values were 327 mL/g, 185.6 mL/g, and 167 mL/g, respectively. Meanwhile, the ethanol extracts of *Coptis chinensis* Franch., *Houpoea officinalis* Rehder & E.H.Wilson, and *Paulownia fortunei* (Seem.) Hemsl. exhibited significant activities against *S. aureus* with MIC values in a range of 0.06–0.08 mg/mL, and their TAA values were 2672 mL/g, 1820 mL/g, and 1484.8 mL/g, respectively.

By comparing the antibacterial activity, the activities of 14 TCMs against Gram-positive bacteria (*S. aureus*) were determined to be markedly stronger than their activities against Gram-negative bacteria (*E. coli*), which was consistent with the previous introduction. Strikingly, Galla chinensis exhibited the greatest antimicrobial activity against *E. coli*.

3.2. Construction of Galla chinensis Component-Target Networks. As displayed in Figure 1 and Supplementary materials Table 1, 49 Galla chinensis chemical components were obtained from TCMSP and PubChem databases including 11 tannins: WBZ1-WBZ11, 4 phenolic acids: WBZ12-WBZ15; 17 amino acids: WBZ16-WBZ32; 8 fatty acids: WBZ33-WBZ40; and 9 other types of compounds: WBZ41-WBZ49. Among them, tannins (also referred to as Gallo tannic acid or tannic acid), the main component of Galla chinensis, accounted for about 50–70% of Galla chinensis [47–49].

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TOM	Extraction note $(0/)$	S. au	reus	E. coli		
ICMS	Extraction rate (%)	MIC (mg/mL)	/mL) TAA (mL/g) MIC (mg/mL)		TAA (mL/g)	
Galla chinensis	32.7	1	327	1	327	
Coptis chinensis Franch.	16.7	0.06	2672	1	167	
Elsholtzia ciliata (Thunb.) Hyl.	18.75	1.25	150	10	18.75	
Origanum vulgare L.	9.2	3.13	29.44	12.5	7.36	
Maesa japonica (Thunb.) Moritzi. ex Zoll.	14.2	>50	<2.84	5	28.4	
Houpoea officinalis Rehder & E.H.Wilson	11.38	0.06	1820	1	113.8	
Paulownia fortunei (Seem.) Hemsl.	11.6	0.08	1484.8	0.625	185.6	
Zanthoxylum nitidum (Roxb.) DC.	14.6	10	14.6	5	29.2	
Syringa oblata Lindl.	20.64	1.25	165.12	1.25	165.12	
Ilex cornuta Lindl. & Paxton	10.27	2	51.35	2	51.35	
Pteris multifida Poir.	5.73	>10	<5.73	5	11.46	
Rheum officinale Baill.	22.66	5	45.32	5	45.32	
Cassia fistula L.	27.27	2.5	109.08	5	54.54	
Platycladus orientalis (L.) Franco.	21.75	2.5	87	10	21.75	

TABLE 2: MIC and TAA of the ethanol extracts of 14 TCMs.

Target prediction of 49 chemical components from Galla chinensis against *E. coli* was carried out using a pharmacophore matching platform, Pharm Mapper server. A total of 63 putative *E. coli* targets were predicted for 49 chemical components (Supplementary materials Table 2).

Furthermore, the component-target network was constructed via Cytoscape software based above 49 chemical components from Galla chinensis and 63 predicted *E. coli* targets. As shown in Figure 2, multiple components could act on the same target, for instance, 1,2,4,6-tetra-O-galloyl- β -D-glucose (TGG, WBZ3) and 1,2,3,4,6-penta-O-galloyl- β -D-glucose (PGG, WBZ4) both acted on the same potential target glnE. Meanwhile, one component could bind to multiple targets; for instance, PGG acted on five potential target proteins including glnE, dnaX, astB, elbB, and nudC.

3.3. Docking and Visualizing of Galla chinensis Chemical Components toward Target Protein of E. coli. To better understand the plausible antimicrobial activity mechanism of Galla chinensis, the top ten score target proteins of *E. coli* (PDB IDs: 2NYA, 2OPX, 1NLM, 2GFP, 2ZM5, 2IMO, 2NRO, 2WDV, 1V4A, and 3CA8) were chosen to further dock with 49 chemical components via Schrödinger suit [50]. The new top ten docking results were listed in Table 3. The results revealed that PGG (WBZ4) and 1v4a-TGG (WBZ3) both exhibited promising binding interactions for 1V4A with docking score below -7.7. 1V4A represents the crystal structure of the N-terminal domain of *E. coli* adenylyl transferase (ATase) that catalyses the deadenylylation and subsequent activation of GS, a key enzyme in response to signals of nitrogen and carbon status [51].

To further reveal the outstanding affinities of PGG and TGG binding to target protein 1V4A, detailed docking and visualizing were performed. The interactions between TGG and 1V4A are shown in Figures 3(a) and 3(b), the hydroxyl of PGG engaged in eight direct hydrogen-bonding interactions with GLU306, GLU327, ARG324, GLN331, GLU254, and ASP223 of target protein 1V4A. In Figures 3(c) and 3(d), the hydroxyl of PGG engaged in ten direct hydrogen-bonding interactions with GLU231, GLU327, GLU306, TRP253,

ASP173, and ARG225 of target protein 1V4A. Therefore, we hypothesized that TGG and PGG might exert antibacterial effects by inhibiting the activity of the 1V4A.

3.4. PGG Inhibits the Activity of the 1V4A. 1V4A represents the N-terminal domain of the E. coli ATase, which is accountable for the deadenylylation process catalyzing GS-AMP to active GS. To evaluate the inhibition of 1V4A enzymatic activity by PGG, initially, the 1V4A protein from E. coli was synthesized and purified using established procedures [46]. Subsequently, the deadenylylation of GS-AMP was closely monitored as the formation of y-glutamylhydroxamate utilizing the γ -glutamyl transferase activity of GS as previously described with slight modifications [46]. The y-glutamyl transferase activity was determined via utilizing a commercial GS activity assay kit to monitor the formation of y-glutamyl isohydroxamic acid. In this work, we determined that PGG exhibited inhibition of ATase-1V4A activity with IC_{50} value of 37.01 µg/mL (Figure 3(e)). Although PGG is present in both the ATase-1V4A activity assay and the GS activity assay reaction systems, the concentration of PGG in the GS activity assay reaction system is 1/10 of that in the ATase-1V4A activity assay reaction system [46]. To rule out the inhibitory effect of PGG on GS activity, we tested the impact of the maximum concentration of PGG ($10 \,\mu g/mL$) on GS activity. The results indicate that $10 \,\mu\text{g/mL}$ PGG does not significantly inhibit GS activity, suggesting that PGG directly inhibits ATase-1V4A activity.

3.5. Antibacterial Activity of PGG. As indicated in Table 4, PGG exhibited significant antibacterial activity against the Gram-negative bacteria (*E. coli*) with an MIC value of $250 \,\mu$ g/mL. Vancomycin hydrochloride and kanamycin sulfate were employed as positive controls for the antibacterial activity.

3.6. Identification of the Chemical Constituents of Galla chinensis. The chemical fingerprint of ethanol extract (Figure 4) was provided to understand the chemical

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FIGURE 1: Structures of 49 chemical components from Galla chinensis.

composition by UPLC-TQ-MS/MS. 18 compounds were identified including 3 tannins: PGG (WBZ4), epi-gallocatechin gallate (WBZ8), benzoic acid-3,4-dihydroxy-

5-[(3,4,5-trihydroxybenzoyl)oxy]-5-ethoxycarbonyl-2,3dihydroxyphenylester(WBZ10); 4 phenolic acids: gallic acid (WBZ12), protocatechuic acid (WBZ13), 2-hydroxy-6-



FIGURE 2: The component-target network (pink regular octagon refers to Galla chinensis, yellow regular hexagon refers to 49 components from Galla chinensis, and blue diamond refers to potential target genes).

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Protein-chemical component	Docking score	Glide score	Glide model
1v4a-1,2, 4,6-tetra-O-galloyl-β-D-glucose (TGG, WBZ3)	-8.536	-8.754	-118.370
1v4a-1,2,3,4,6-penta-O-galloyl-β-D-glucose (PGG, WBZ4)	-7.855	-8.107	-129.318
2gfp-2-hydroxy-6-pentadecyl benzoic acid (WBZ14)	-6.698	-6.76	-44.914
2nro-epigallocatechin gallate (WBZ8)	-6.632	-6.71	-77.118
2nya-linolenic acid (WBZ35)	-6.029	-6.033	-131.219
3ca8-benzoic acid (WBZ10)	-5.42	-5.416	-88.027
20px-Ginkgol (WBZ46)	-4.461	-4.462	-41.862
1nlm- linoleic acid (WBZ34)	-2.026	-2.029	-46.247
2zm5- linolenic acid (WBZ35)	-2.02	-2.024	-47.054
2imo- linolenic acid (WBZ35)	-0.179	-0.183	-25.215
1ghh- linolenic acid (WBZ35)	0.592	0.988	-21.427

pentadecylbenzoic acid (WBZ14), and 4-hydroxy-3methoxybenzoic acid (WBZ15); 3 amino acids: histidine(WBZ30), tyrosine (WBZ28), and l-isoleucine (WBZ26); 5 fatty acids: linoleic acid (WBZ34), linolenic acid (WBZ35), lauric acid (WBZ37), palmitic acid (WBZ39), and oleic acid(WBZ33); and 3 other types of compounds: methyl gallate (WBZ41), ellagic acid(WBZ43), and epigallocatechin (WBZ45).



FIGURE 3: (a). 2D interaction of TGG with target protein (PDB ID: 1V4A). (b) 3D-box of TGG with target protein 1V4A. (c) 2D interaction of PGG with target protein (PDB ID: 1V4A). (d) 3D-box of PGG with target protein 1V4A. (e) The inhibitory activities of PGG against 1V4A.



FIGURE 4: Chemical fingerprint of the Galla chinensis ethanol extract. Blue numbers represent identified compounds. The positions of peaks corresponding to compounds are indicated by orange arrows and numbers (retention time).

The content of PGG in ethanol extract was found to be 1.95% (w/w) with PGG standard sample as control, which demonstrated that Galla chinensis contains a high content of PGG.

4. Discussion

The overuse and misuse of antibiotics have led to antibiotic resistance, resulting in significant morbidity and mortality. Gram-negative bacteria present a more substantial challenge in the medical field compared to Gram-positive bacteria due to their increased resistance to traditional antibiotics. Unlike Gram-positive bacteria, Gram-negative bacteria possess an outer membrane composed of phospholipids and glycolipids, serving as a permeability barrier that makes them less susceptible to treatment. Consequently, very few substances have been identified as effective against Gram-negative bacteria. To address the escalating incidence of Gram-negative infections, it is imperative to explore new targets and drugs specifically designed to combat these types of infections.

In this study, we found that Galla chinensis exhibited the most potent anti-*E. coli* activity among the 14 Chinese herbs tested. Galla chinensis, a widely used traditional Chinese medicine, is known for its antidiarrheal, antiparasitic, and antibacterial properties. Despite having a normal MIC value, Galla chinensis demonstrated the highest extraction yields (32.7%) and the highest total antibacterial activity (TAA) value (327 mL/g) against *E. coli*. This outcome suggests that the exceptional antibacterial effectiveness of Galla chinensis

is attributed to its high content of specific antibacterial components.

Galla chinensis tannin is a type of hydrolyzable tannin that consists of a central glucose core, surrounded by several gallic acid units, and further gallic acid units can be attached. Chemical fingerprinting showed gallic acid (WBZ12) is the highest peak in Galla chinensis's ethanol extract, which means the highest content. Meanwhile, PGG (WBZ4) has been identified in the Galla chinensis ethanol extract with a high content of 1.95% (w/w).

To better understand the potential antimicrobial ingredients and action mechanism of Galla chinensis, the component-target network was constructed between 49 chemical components and 63 predicted *E. coli* targets. Docking and visualizing of Galla chinensis chemical components toward top ten target proteins showed that PGG exhibited notable docking scores for 1V4A. PGG is a naturally occurring glucose pentagallic acid ester that belongs to the hydrolysable tannins and is chemically classified as a gallotannin. Unfortunately, the highest constituent, gallic acid, might not have been screened through molecular docking due to its potentially small molecular weight. Gallic acid is commonly considered to be an effective natural antibacterial agent [52].

In our study, PGG inhibited bacterial growth in accordance with PGG's previously reported broad-spectrum activity, with MICs in the 50–500 μ g/mL range [53]. PGG's antibacterial activity has been attributed to its chelation of iron and its binding with lipopolysaccharide [54], but this study is the first to our knowledge to attribute its antibacterial activity to its affinity with ATase.

ATase, encoded by *glnE*, is a signal transduction enzyme widely distributed in bacteria, and in some bacteria is essential for cellular viability [55]. The enzyme regulates the activity of glutamine synthetase by adenylylation and deadenylylation in response to intracellular signals of nitrogen status. Docking analysis demonstrated PGG exhibited notable docking scores to ATase-1V4A of E. coli mainly due to forming ten hydrogen bonds with key residues located at the active site pocket of the enzyme (Figures 3(c), 3(d)). It is speculated that PGG may act on active sites and hinder the chelation of Mg²⁺ with the conserved nucleotidyltransferase domains, resulting in inhibition of the bimetallic mechanism of ATase and followed glutamine synthetase activity, and ultimately repressing nitrogen assimilation, protein synthesis, and life activities [51]. Therefore, ATase may be an important potential target for PGG's antibacterial activity. Previous studies have indicated that PGG also inhibits other proteins in the enzyme inhibition assay. PGG exhibited a strong inhibitory effect on H⁺, K⁺-ATPase (IC₅₀: 166 nM) and relatively weak inhibitory effects on Mg²⁺-ATPase (IC₅₀: >10 µM) [56]. Additionally, PGG was identified as a dual inhibitor of urokinase-type plasminogen activator and plasmin, with IC₅₀ values of 6.861 μ M and 149.0 μ M, respectively. These studies suggest that PGG is a compound with broad inhibitory activity against enzymes, but it exhibits some level of selectivity in its inhibition of different enzymes [57].

In present study, PGG could be considered a promising anti-*E. coli* natural product that can suppress ATase catalytic activity with an IC_{50} value of $37.01 \,\mu$ g/mL, and we believe that the structure and mechanistic insights presented here would support the development of potent Gram-negative inhibitors.

5. Conclusions

Based on the present results, Galla chinensis and its key active ingredient PGG exhibit a strong antibacterial activity against *E. coli*, and PGG exhibits a significant inhibitory activity against ATase of *E. coli*, which may become one of the important potential targets for anti-Gram-negative bacteria.

Data Availability

The data that have been used are confidential.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Supplementary Materials

The following supporting information is provided in Supplementary Materials. Supplementary information Table 1. 49 chemical compounds isolated from Galla chinensis. Supplementary information Table 2. Potential targets of 49 chemical components from Galla chinensis. (*Supplementary Materials*)

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