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RESEARCH ARTICLE

Potential of MurA Enzyme and GBAP in Fsr Quorum Sensing System as Antibacterial Drugs Target: *In vitro* and *In silico* Study of Antibacterial Compounds from *Myrmecodia pendans*

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Abstract: ***Background*:** Increasing the resistance issue has become the reason for the development of new antibacterial in crucial condition. Many ways are tracked to determine the most effective antibacterial agent. Some proteins that are a key role in bacteria metabolism are targeted, including MurA in cell wall biosynthesis and gelatinase biosynthesis-activating pheromone (GBAP) in Fsr Quorum Sensing (QS) system.

Objective: The objective of this research is the analysis of compounds **1**-**4** from *M. pendans* as antibacterial and anti-QS activity trough protein inhibition by in silico study; focus on the structure-activity relationships, to appraise their role as an antibacterial and anti-QS agent in the molecular level.

***Methods*:** Both activities of *M. pendans* compounds (**1**-**4**) were analyzed by *in silico*, compared to Fosfomycin, Ambuic acid, Quercetin, and Taxifolin as a standard. Chemical structures of *M. pendans* compounds were converted using an online program molview. The compounds were docked to MurA, GBAP, gelatinase and serine protease using Autodock Vina in Pyrx 0.8 followed PYMOL to visualization and proteis.plus program to analyze of the complex.

***Results*:** All compounds from *M. pendans* bound on MurA, GBAP, gelatinase and serine protease except compound **2.** This biflavonoid did not attach to MurA and serine protease yet is the favorable ligand for GBAP and gelatinase with the binding affinity of -6.9 and -9.4 Kcal/mol respectively. Meanwhile, for MurA and serine protease, compound **4** is the highest of bonding energy with values of -8.7 and -6.4 Kcal/mol before quercetin (MurA, -8.9 Kcal/mol) and taxifolin (serine protease, -6.6 Kcal/mol).

Conclusion: Based on the data, biflavonoid acts better as anti-QS than an inhibitor of MurA enzyme while the others can be acted into both of them either the therapeutic agent of anti-QS or antibacterial agent of MurA inhibitor.

Keyzords: Quorum sensing, *in silico,* GBAP, Gelatinase, Serine protease, MurA, Flavonoid, *M. pendans.*

1. INTRODUCTION

As infectious diseases and bacteria resistance issues have become one of the greatest threats to global health, the process of investigating new drugs is a critical point that must be done [1]. Many mechanisms to treatment growth bacteria (Gram-negative and Gram-positive) have been reported, such as disrupt the cell membrane, DNA and RNA synthesis inhibition, protein synthesis inhibition, and metabolism inhibition [2]. One of those mechanisms can be based to design or discover new antibacterial agents.

Peptidoglycan that responsible to provide mechanical resistance to the internal osmotic pressure and determining cell shape is an important component of the bacterial cell wall.

The first committed step of peptidoglycan biosynthesis has worked an enzyme called UDP-*N*-acetylglucosamine-1-carboxy vinyl transferase (MurA) [3]. This enzyme consists of a transfer of an enol pyruvate moiety from phosphoenol-pyruvate (PEP) to the C3 position of UDP-*N*-acetylglucosamine [4]. By blocking the MurA of action, it generates to

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terminate the biosynthesis of peptidoglycan that causes cell membrane disruption so that bacteria cannot survive. This mechanism is fascinating in targeting new drugs [5].

On the other hand, another approach has been known that the quorum sensing (QS) system is a regulatory system of unicellular microorganism that responsible for cell-cell communication and controls the gene expression that can be interrupted at one or more stages [6]. It consequently may enable them to suppress their virulence factors and pathogenicity. In *E. faecalis,* gelatinase and serine protease, are virulence factors contributing to biofilm formation encoded gelE-sprE whose expression is controlled by *fsr* QS system [7-9]. The fsr locus is consisted of four genes that are fsrA, fsrB, fsrC, and fsrD [10, 11]. Moreover, fsrD encoded FsrD that is a precursor of gelatinase biosynthesis-activating pheromone (GBAP), then it is derived by proteolytic activity of the FsrB. FsrB protein modifies it to become GBAP and exports it out of the cell [11]. GBAP is a cyclic peptide which acts as an autoinducing peptide. The structure of GBAP is an 11-amino-acid-residue cyclic peptide with a lactone linkage between a hydroxyl group of the third serine residue and the α-carboxyl group at the C-terminal methionine residue. When GBAP accumulating reaches approximately 1 nM, it can stimulate a two-component signal transduction cascade of FsrC-FsrA. GBAP binds to FsrC and triggers FsrA through phosphorylation. Phosphorylated FsrA binds to promoters of fsrBCD and gelE-sprE [12]. Moreover, GBAP presence also makes the FsrB protein-membrane more stable [13]. Through blocking one of QS stages such as GBAP inhibition, cell-cell communication in bacteria must be ruined and finally, bacteria will be died automatically [14]. Based on the literature, targeting anti-QS is an effective strategy to develop antibacterial agents.

The main problem in the study of therapy and drug development is effective and safe if used by humans. So, we have to find a lead compound and treatment method that no result in the side effect. One of the solutions is using the natural product as a source of the active compound and utilize the weakness pathogenesis and virulence bacteria system [15, 16]. Flavonoid is a secondary metabolite that has been reported as an anti-QS agent in *P. aeruginosa* [17] by mode action binding to LuxR-type receptor protein [18]. It supposes that secondary metabolite of *M. pendans* potent be QS inhibitor while derivate peptide compounds [19] and other compounds such as siamycin [20], avellanin C [21], cyclodepsipeptides [22], arthroamide [23] have anti QS activity in pathogenic gram-positive bacteria. Furthermore, some research reported quercetin [18] and naringenin [24] inhibited the QS system of negative gram bacteria *via* binding with LuxI-type AHL synthases and/or LuxR-type AHL receptor proteins while derivative benzoic acid attenuates QS activity *via* alteration of metabolic pathways involved in QS-dependent processes [18]. Meanwhile, phenolic compounds are suggested as MurA inhibitor because having ether linkage like Fosfomycin [25] has been known as an inhibitor of MurA enzyme [26].

Myrmecodia pendans called Sarang Semut is one of the herbal plants that is widely used in West Papua. This plant is the potential to be developed in modern herbal medicines because they can grow well as epiphytic plants, therefore the exploitation will not endanger the environment [27]. Our previous research found that terpenoid and phenolic compounds of *M. pendans* inhibit the growth of *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, and *P. gingivalis* ATCC 33277 [28]. Recently, two antibacterial biflavonoids from *M. pendans* inhibit MurA enzyme work with IC_{50} of 21.7 and 151.3 ppm [29], but it is still no clear what mechanism occurs in the inhibition process. Consequently, the present study was designed to predict the molecular mechanism of action of the antibacterial and anti-QS compound from *M. pendans* using *in silico* techniques.

Through *in silico* studies, it is supposed to give information about a prediction of the drug ability of antibacterial and anti-QS compound from *M. pendans* and prediction of the potential target proteins to which these compounds bind. Furthermore, it is hoped to be a guide for discovering novel antipathogenic agents.

2. MATERIALS AND METHODS

2.1. Materials and Chemicals: *In vitro* Assay

The tested organism is *Enterococcus faecalis* ATCC 29212 (purchased from fisher scientific). Other reagent used included Brain Heart Infusion broth (Oxoid, CM1135), Muller Hinton agar (Oxoid, CM0337), Fosfomycin and chlorhexidine as positive control purchased from Merck C. Ltd., Paper disc 6 mm (Sigma-Aldrich, Z741310), aquabidest (Ikapharmindo Putramas), microplate 96 well (Iwaki, 3820 024), filter tips (Biologix, code 22-0010, 22-0200, and 22-1000), parafilm (Sigma-Aldrich P7688-1EA) and *E. coli* MurA assay kit [Profoldin, No. Cat MURA500KE].

Material Brain Heart Infusion broth (Oxoid, CM1135), Muller Hinton agar (Oxoid, CM0337), paper disc 6 mm (Sigma-Aldrich, Z741310), aquabidest (Ikapharmindo Putramas), microplate 96 well (Iwaki, 3820 024), filter tips (Biologix, ode 22-0010, 22-0200, and 22-1000), parafilm (Sigma-Aldrich P7688-1EA). Material Brain Heart Infusion broth (Oxoid, CM1135), Muller Hinton agar (Oxoid, CM0337), paper disc 6 mm (Sigma-Aldrich, Z741310), aquabidest (Ikapharmindo Putramas), microplate 96 well (Iwaki, 3820 024), filter tips (Biologix, code 22-0010, 22-0200, and 22-1000), parafilm (Sigma-Aldrich P7688-1EA).

2.2. Materials: *In silico* Assay

There are four target proteins include MurA, gelatinase biosynthesis-activating pheromone (GBAP) gelatinase, and serine protease and that are used in this research. All of these 3-dimensional structures were retrieved from UniProt knowledgebase (http://www.uniprot.org/) MurA Q831A8, GBAP G8ADP0, gelatinase Q833V7, and serine proteinase A1YGV8, while ligands were three flavonoids and two phenolic compounds. The compounds **1**-**4** were isolated from *M. pendans* is obtained from previous research [28, 29]. They are **1**) butein or 2',3,4,4'-Tetrahydroxychalcone, **2**) biflavonoid, **3**) 3''-methoxy-epicatechin-3-*O*-epicatechin, and **4**) dibenzo-p-dioxin-2,8-dicarboxylic acid. The three ligands as positive control were **Q** or Quercetin (CID 5280343), **T** or Taxifolin (CID 439533), **F** or Fosfomycin (CID 446987), and **AA** or ambuic acid (CID 11152290) that retrieved from

PubChem compound database (https://www.ncbi.nlm.nih.gov/pccompound).

2.3. Instruments

Laminar airflow, incubator (Memmert, IN55), anaerobic jar (Oxoid, AG0025A), autoclave, microplate reader (Biochrom EZ read 400, 80-4001-40), micropippet (Eppendorf, 3120000062 and 3120000054), colony counter (Schuett-Biotec, 3081502).

3. EXPERIMENTAL

3.1. Antibacterial Assay: *In Vitro* Study

To determine antibacterial activity, compound **1**-**4** were tested by disc diffusion method according to CLSI protocols [30, 31]. All compounds were prepared at concentrations 1000 and 5000 ppm that diluted in methanol-water (1:1, v/v). Those solutions were impregnated into a disc (diameter 6 mm) then placed on the agar that has been added bacteria solution (0.1 mL, 0.5 Mc Farland) before. The inhibition zone of each compound was measured after incubation for 24 h at 37°C. Furthermore, Minimum inhibition concentration (MIC) and Minimum bactericidal concentration (MBC) value of all compounds were determined. This assay using microdilution methods in microplate 96-well following to CLSI protocols. Each compound solution was performed in 2%. Those solutions (0.1 mL) were added into well until obtained twelve concentrations of a compound by serial two-fold dilution. Then, cultured bacteria 0.5 Mc Farland were loaded into well. After incubation for 24 h at 37°C, the absorbance of the sample was measured using a microplate reader at 620 nm. Every solution in the well was spread on the agar followed incubation for 24 h to determine MBC value.

3.2. Inhibitory Enzyme MurA Assay

This testing was conducted to the manual procedure of the *E. coli* MurA assay kit [Profoldin, No. Cat MURA500KE]. Each sample (0.6 µL) prepared in various concentrations in the range 0.039 to 5 mM by serial two-fold dilution was loaded into well. Then, the premix solution (26.4 µL) and 3 µL of the 10x enzyme-substrate were added into the sample followed incubation at 37°C for 60 min. This mixture is hereinafter referred to as the reaction mixture. Next, 45 µL of the Dye MPA3000 was added into the 30 µL of the reaction mixture. This mixture was incubated for 5 min and the light absorbance was measured at 650 nm.

3.3. *In silico* Characterization of the *Myrmecodia Pendans* Compounds 1-4

The characteristics of compounds **1**-**4** were confirmed using two online programs. The chemical structures of the four *M. pendans* compounds were converted using an online program molview (http://molview.org) in the MOL file. The 3-D structure of the MOL file for all compounds was retrieved from the PubChem Compound database. Those MOL files were used to convert the chemical structure into 3D using OPEN BABEL 2.4.1 program, in PDB format [32]. While the 3-D structure model of MurA GBAP, gelatinase, and serine protease was built using the SWISS-MODEL server (https://swissmodel.expasy.org/) in PDB format [33].

3.4. Molecular Docking between MurA / GBAP/ Gelatinase/ Serine Protease with *M. pendans* Compounds 1-4

Automated docking studies were performed using Autodock Vina in open source software PyRx 0.8 [34, 35]. Four target proteins (MurA, GBAP, gelE, and sprE) were loaded and become as macromolecule. All compounds **1-4** from *M. pendans* as ligands were subject for binding to each protein target; the ligands were free for blind docking. The docking process was started by selecting macromolecule and the four ligands. Moreover, step by step was followed as manual instruction until calculating the bonding energy of macromolecule-ligand appeared. The selected conformations were conformation with the lowest binding energy which had a bonding energy score that less than 1.0Å in positional root-mean-square deviation (RMSD) [36].

3.5. Complex MurA/GBAP/Gelatinase/Serine Protease - *M. pendans* Compounds 1-4 Visualization and Analysis

The final step is to analyze of docking results using PYMOL and online program proteins.plus [37]. Docking poses and molecular interaction of each protein-ligand complex can be visualized by PYMOL. To show which residues bind to a ligand, proteins.plus program analyze the protein-ligand complex file and then the picture of molecular interactions come out in 2-D structure. For the best visualization, those molecular interactions were showed in the 3D molecular picture. The docking poses of each protein-ligand complex was compared to the 3-D structure of a protein that bound ligands on catalytic sites of each protein (Binding site of Fosfomycin for MurA, ambuic acid for GBAP). It is supposed to evaluate the similarity of ligation pose of the compound to another compound that bound ligands on that sites.

4. RESULTS

The result of the antibacterial activity of compounds 1-4 against *E. faecalis* ATCC 29212 are represented inhibition zone, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) value. Meanwhile, the inhibitory activity of the MurA enzyme was showed by the IC_{50} value. All data have been reported on the previous research [28, 29]. Based on the data, Fosfomycin is the highest of antibacterial activity but compound 2 is the most favorable on the inhibition of MurA.

5. DISCUSSION

5.1. Bioavailability and Antibacterial Activity Prediction of M. pendans Compounds 1-4 Through Molecular Interaction with MurA, GBAP, Gelatinase, and Serine Protease

Automated Docking *M. pendans* compounds on MurA, GBAP, gelatinase and serine protease showed that those all compounds could bind to them except compound **2** with

MurA and serine protease (Table **1**). It deduces all compound will be distributed and presence in the body because the binding to those proteins affect the distribution or elimination of compound [38]. In gelatinase binding, all compounds have bonding energy lower than -5 Kcal.mol^{-1}), nevertheless their binding affinity of serine protease is the worst of the other target proteins. Furthermore, the complex of ligand **2**-serine protease has the lowest binding affinity (16.7 Kcal.mol^{-1}) indicated that compound **2** was not bound to serine protease anymore. Even though compound **2** does not fit for serine protease inhibitor, it has the highest of bonding energy on gelatinase (-9.4 Kcal.mol^{-1}) and bonding on GBAP (-6.9 Kcal.mol^{-1}). At two other proteins, compound **4** is the most favorable with bonding energy of MurA and serine protease of -8.7 and -6.4 Kcal.mol^{-1}, respectively.

Based on the binding site of complex (Fig. **1**), all compounds **1-4** bind on the same pocket of three proteins (MurA, gelatinase, and serine protease). Compounds **1-4**, **AA**, **Q,** and **T** were suggested as a competitive inhibitor that can substitute each other. It is very useful to solve the resistance issue that currently fears in the medicinal field. However, in the GABP bonding, compound **2** and ambuic acid attach to a different position from the other compounds. The molecular structure of GABP that is more simple and smaller than other proteins gives many possibilities of ligand binding in various positions.

Tabel 1. Antibacterial activity of compounds 1-4 from *M. pendans* (*in vitro* study).

Sample	Inhibition Zone (mm) at Concentration (µg/mL)		Concentration (µg/mL)		
	1000	5000	MIC	MBC	IC_{50}
Compound **1**	7.45	8.25	-	-	-
Compound **2**	8.15	8.62	156	625	21.7
Compound **3**	8.05	8.55	625	1250	151.3
Compound **4**	8.05	8.15	2500	10000	-
Fosfomycin	8.4	22.7	62.5	None	99.7

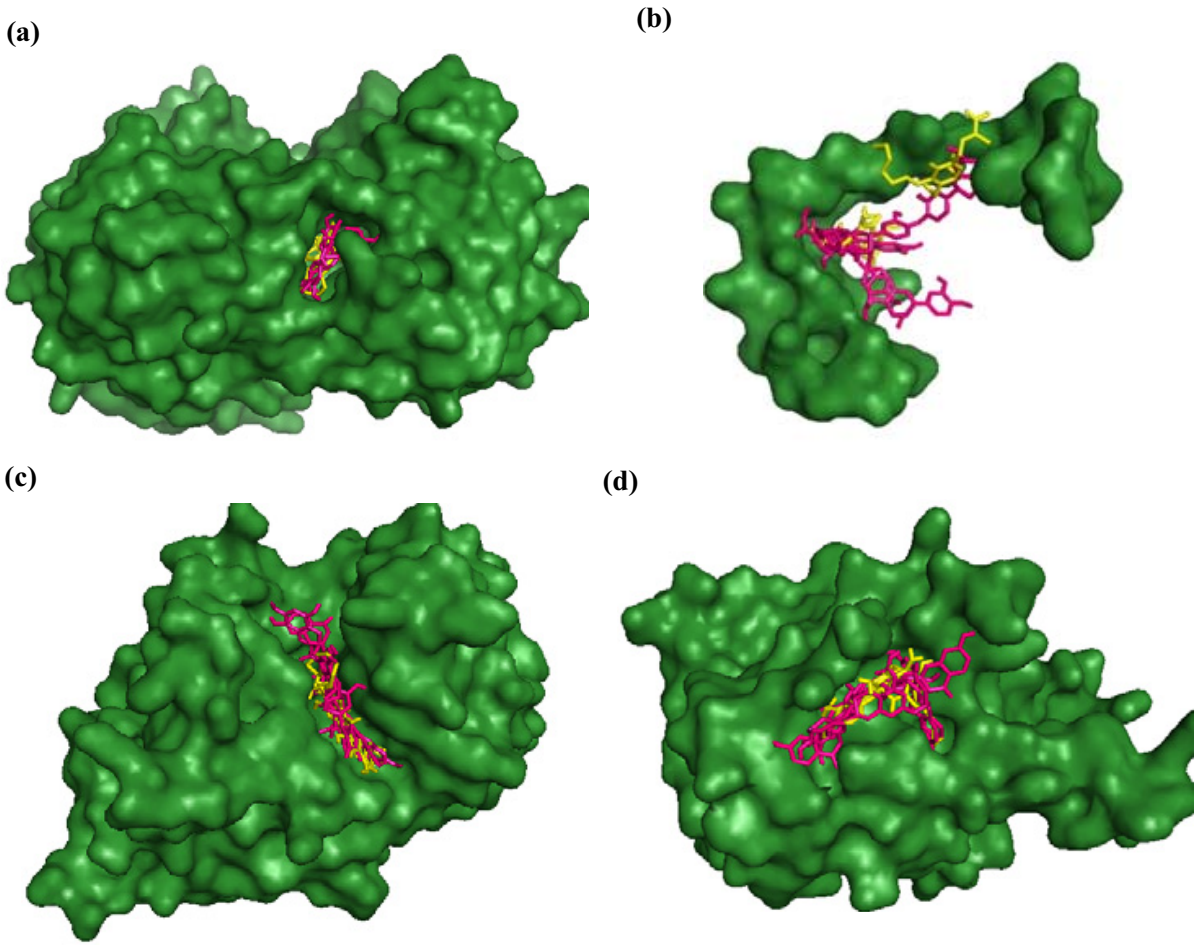

Fig. (1). The binding site of compounds **1-4** (magenta line); **AA**, **F**, **Q**, and **T** (yellow line) of MurA (**a**), GBAP (**b**), gelatinase (**c**), and serine protease (**d**). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 2. Prediction of antibacterial activity of phenolics and flavonoids from *M. pendans.*

Ligand	Binding Affinity of Ligand-protein Complex (Kcal.mol^{-1})			
	MurA	GBAP	Gelatinase	Serine Protease
1	-8.1	-5.0	-7.6	-6.1
2	+2.3	-6.9	-9.4	+16.7
3	-6.7	-5.7	-8.4	-5.1
4	-8.7	-5.0	-8.4	-6.4
Ambuic acid	-7.8	-4.7	-6.9	-7.0
Fosfomycin	-4.6	-3.0	-5.0	-3.9
Quercetin	-8.6	-5.2	-8.2	-6.6
Taxifolin	-8.9	-5.2	-7,8	-6.4

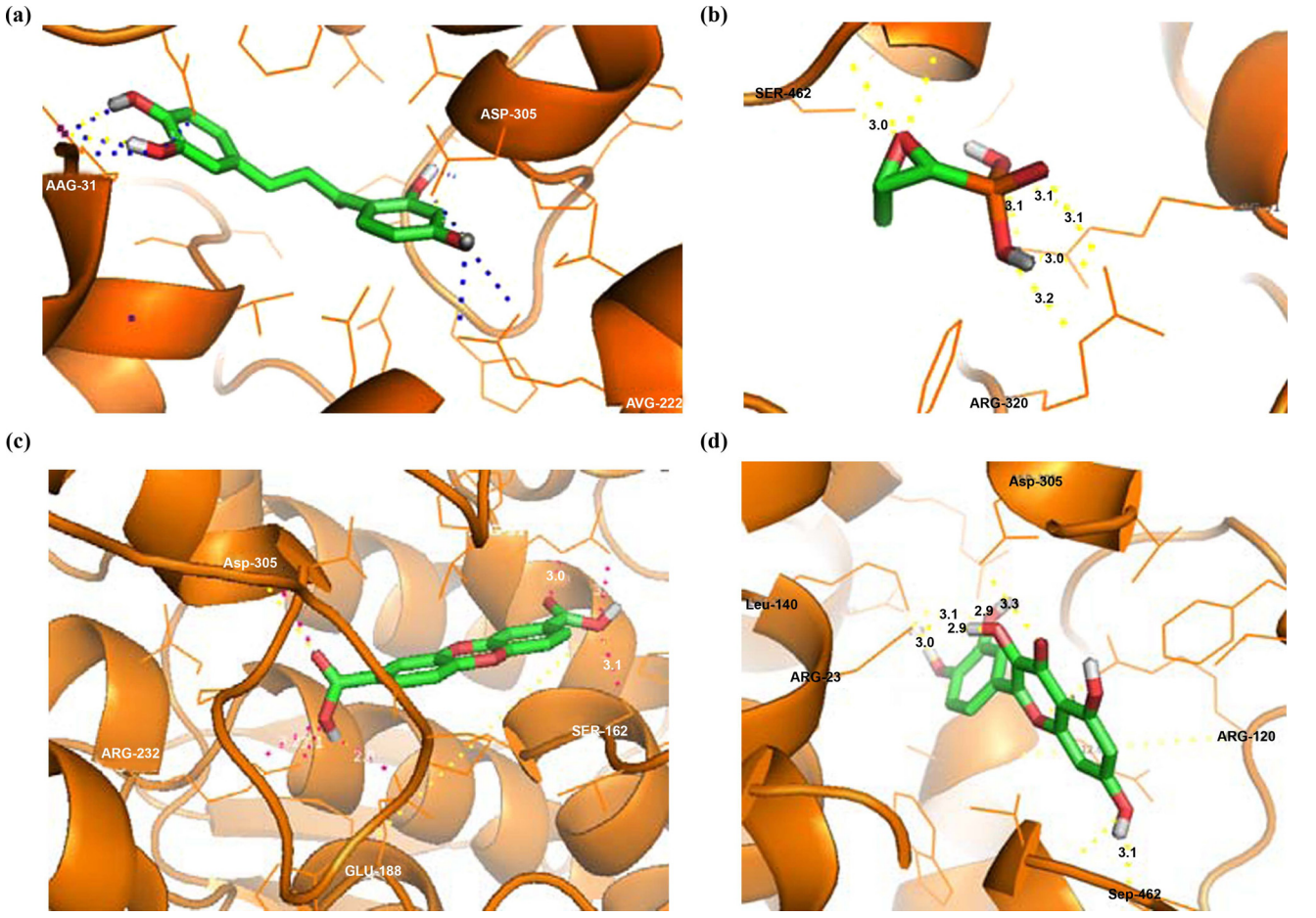

Fig. (2). Binding site on MurA for: compound **1** (**a**) and Fosfomycin (**b**). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

In the docking result of MurA (Table **2**), compound **4** (-8.7 Kcal.mol^{-1}) is the highest of energy binding after taxifolin (-8.9 Kcal.mol^{-1}). The three highest compounds of bonding affinity, which are compound **4**, **Q** and **T**, have the same bounded residues that are Ser162A, and Asp305A (Figs. **2** and **3**). followed compound **1** and **AA** that have binding energy are -8.1 and -7.8 respectively. Both of them bind to Arg120A residue. Nevertheless, it suggests the active site of MurA is pocket which around Ser162A, Asp305A, and Arg120A. The MurA catalytic site is located in a deep cavity between the two globular domains. The surface of the two domains are Arg-120 and Pro-121 on one face and by Leu-124 on the other. Furthermore, the conformation and the bounded other residues affect the binding affinity of the ligand. Although two residues (Ser162A and Arg120A) are bounded fosfomycin called inhibitor of MurA enzyme, its binding affinity is the lowest of them before compound **2**. The Cys-115 residue that is noticed as an active site of Fosfomycin [25, 39] does not appear while Lys-22 residue as another active site exists [4] in complex quercetin, taxifolin and, ambuic acid.

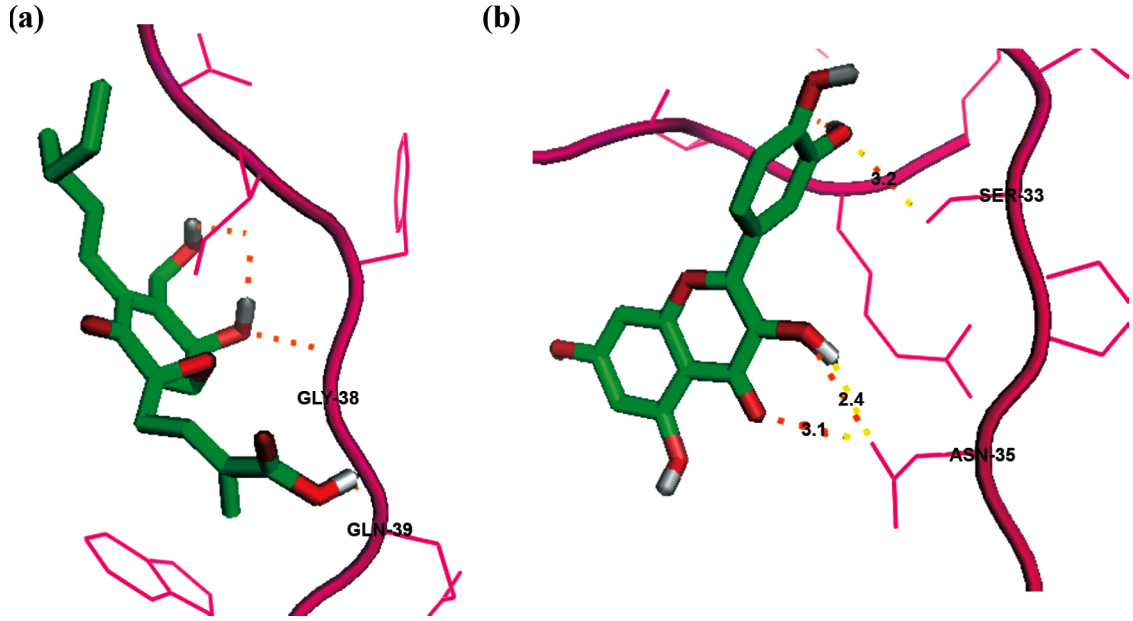

Fig. (3). Binding site on MurA for: (**a**), and Quercetin (**b**). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

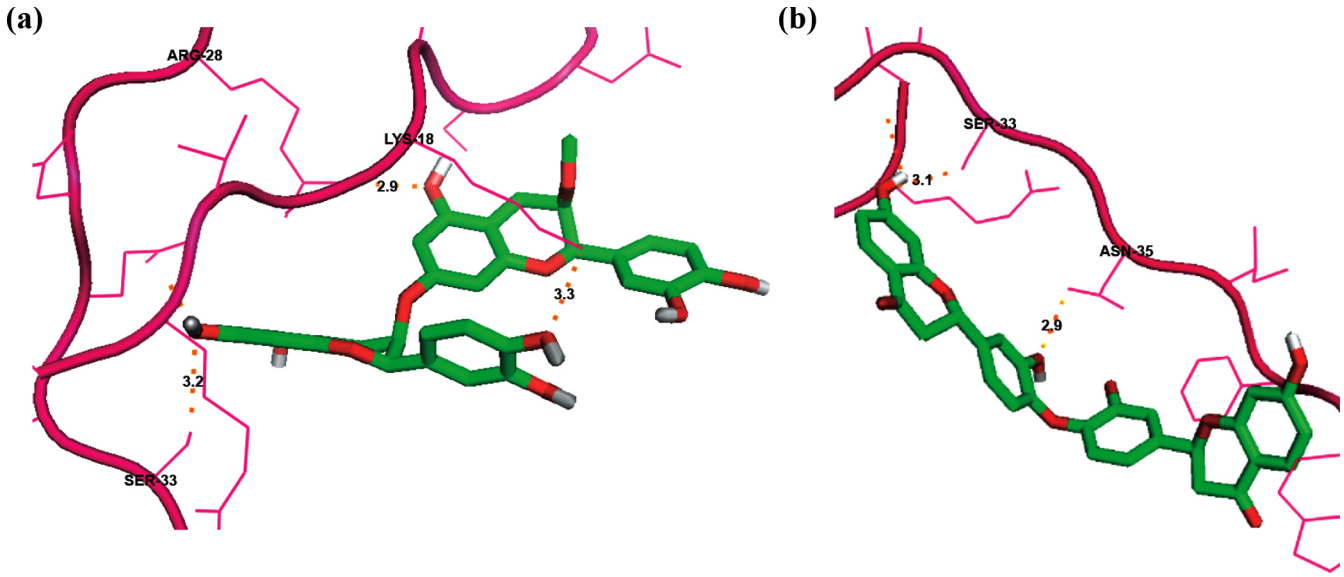

Fig. (4). The binding site on GBAP: Ambuic acid (**a**) and Quercetin (**b**), compound **2** (**c**), and **3** (**d**). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

At the ligand complex of GBAP, the binding affinity of a ligand that has flavonoid form is higher than others. Two bioflavonoids of **2** and **3** lead the binding affinity followed quercetin, taxifolin, and compound **1** with values of -5.2; -5.2 and -5.0 Kcal.mol^{-1}, respectively. Almost those flavonoids bind to Arg15A and Ser33A residues (Fig. **4**). Meanwhile, GBAP inhibitor, ambuic acid, binds different residues (Gly38A and Gln39A) and shows binding affinity that weaker than the five flavonoids above (Table **3**). With value -4/7 Kcal.mol^{-1}, placed ambuic acid is the three weakest of the ligands. However, all compounds did not bind to Trp10 residue that is the key role of the GBAP stabilization [40]. Based on the result, flavonoid is predicted to able to inhibit QS system by blocking GBAP so that it cannot bind to the FsrC and the stages QS system is disrupted.

On the gelatinase, the binding energy of the ligand complex shows the highest of the other protein. All compounds were the most suitable and the most stable of this protein, and the most favorable was compound **2** with value -9.4 Kcal.mol^{-1}. Almost compounds attach to two residues which are His332A and Tyr343A while Fosfomycin does not bind. It makes Fosfomycin ranked in the weakest ligand with the binding energy -5.0 Kcal.mol^{-1}. Nevertheless, it is supposed to His332A and Tyr343A residues that are the active site of the gelatinase.

Besides, *M. pendans* compounds except compound **2** have a binding affinity which is almost the same as ambuic

acid. Like the MurA, serine protease is not bound to compound **2**. All samples except fosfomycin and compound **2** bind the same residues including Gln44A, Ser47A, and Gly225A. It concluded that the bound pocket by them is the same. Therefore, they are the competitive inhibitor because of the same binding active site.

The result of the *in silico* study notices that *M. pendans* compound able to block some proteins that become a key role in the survival life of bacteria. They also attenuate serine protease and gelatinase known as virulence factors in *E. faecalis* [41].

5.2. Structure-activity Relationship on Antibacterial Activities of *M. pendans* Compounds 1-4

Based on the ranking of binding affinity, the binding energy of compound **1** is almost the same as quercetin and taxifolin of each protein complex. The difference range between compound **1** and **T** or **Q** was 0.2-0.8 Kcal/mol. Meanwhile, the score difference between **T** and **Q** is 0.2-0.4 Kcal/mol. It was allegedly because all three had the same structure framework that hydroxyl group in 5,7,3',4' position gives a contribution to for activity. Taxifolin and Quercetin have four hydroxyl groups, while compound **1** has only three hydroxyl groups at 7,3', and 4' positions (Fig. **5**). Consequently, it is suspected the more hydroxyl group whose compound the stronger of binding affinity [42, 43].

Discuss structure-activity relationship, compound **4** structure is different from the other *M. pendans* compounds but the activity is almost the same as compound **1**. It is in line with other research that reported a negative correlation between drug-likeness and activity of the compound. There are influencing structural properties contributing to whatever determine active or inactive a compound [44].

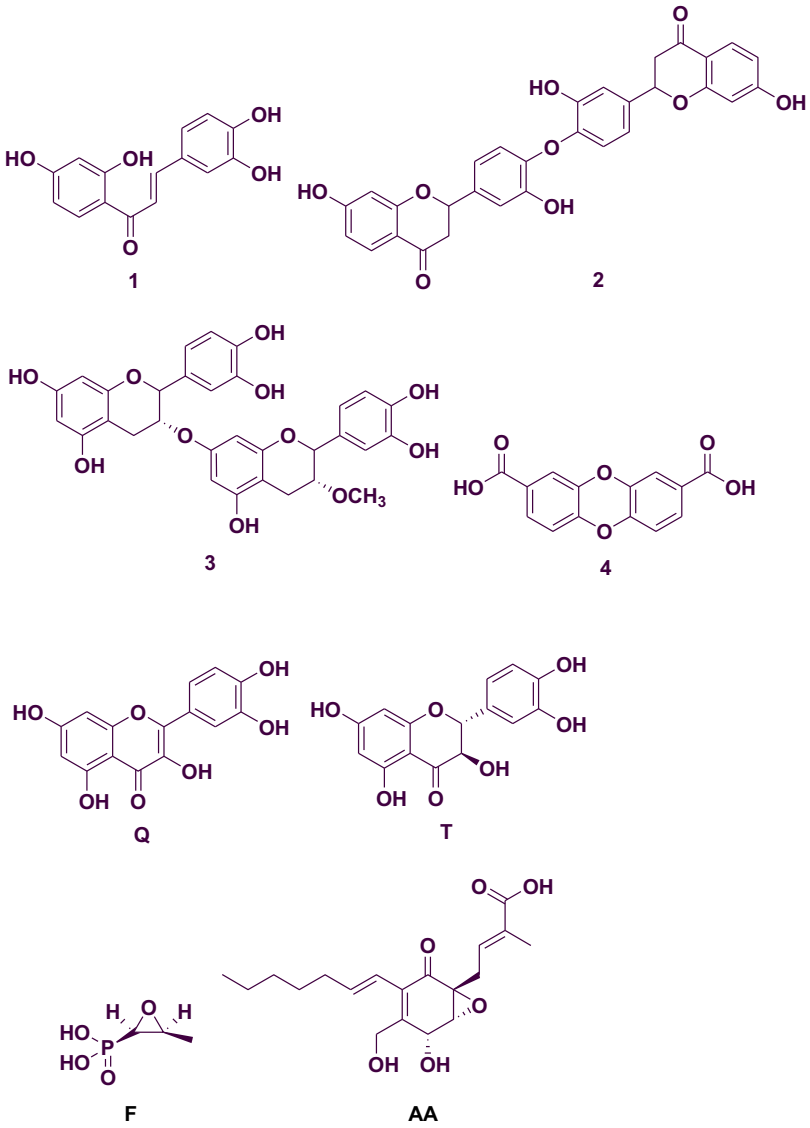

Fig. (5). Chemical structure of compounds from *M. pendans*: butein (**1**), biflavonoid (**2**), 3"-methoxy-epicatechin-3-*O* epicatechin (**3**), and dibenzo-p-dioxin-2,8-dicarboxylic acid (**4**); positive control: Quercetin (**Q**), Taxifolin (**T**), Fosfomycin (**F**), and Ambuic acid (**AA**).

Table 3. Hydrogen bond in MurA/GBAP/gelatinase/serine protease-compounds from *M. pendans*.

Ligand	Residues Binding at Ligand-protein Complex			
	MurA	**GBAP**	**Gelatinase**	**Serine protease**
1	Arg120A, Phe328A	Arg15A, Lys18A	Trp301A, Glu329A, His332A, Leu341A, Tyr343A, Asn351A	Arg46A, Ser47A, Tyr81A, Leu84A, Glu192A, Gly225A
2	Ser162A, Arg232A, Arg371A	Arg15A, Ser33A, Asn35A, Trp40A	Tyr319A, His332A, Tyr343A, Gln375A, Lys408A, Gln415A,	Tyr81A, Thr224A
3	Asn23A, Arg91A, Trp95A, Arg232A, Ala301A, Phe328A,	Arg15A, Lys18A, Ser33A	Trp301A, Glu329A, His332A, Tyr343A, His419A	Gln44A, Ser47A, Leu49A, Leu84A, Asn194A
4	Arg91A, Ser162A, Arg232A, Asp305A	NO	Asn298A, His332A, Tyr343A, Asn351A, Glu392A	Gln44A, Arg46A
Ambuic acid	Lys22A, Arg91A, Arg120A	Gly38A, Gln39A	Ala292A, Asn298A, His332A, Tyr343A, Glu352A	Gln44A, Ser47A, Tyr81A, Ser83A, Gly225A
Fosfomycin	Arg91A, Arg120A, Ser162A, Gly164A	Ser33A	Glu329A, Arg384A	Ser83A, Leu84A
Quercetin	Lys22A, Asn23A, Asp49A, Ser162A, Asp305A, Arg397A	Arg15A, Gln31A, Ser33A	Glu329A, Asn298A, His332A, Glu336A, Tyr343A	Ser47A, Ser83A, Leu84A, Gly225A
Taxifolin	Lys22A, Asn23A, Asp49A, Ser162A, Asp305A, Arg397A	Arg15A, Gln31A, Ser33A, Asn35A	Asn298A, Trp301A, Glu329A, His332A, Asn351A	Gln44A, Ser47A, Ser83A, Leu84A, Gly225A

5.3. Comparison of *In vitro* and *In silico* Assay Data

In vitro study showed that compound **2** has the highest of IC_{50} value of 21.7 µg/mL, while *in silico* study suggested it has the lowest of binding affinity of +2.3 Kcal.mol^{-1}. It is deduced that factors are contributing to the reaction. At least, we able to notice that biological entities are nonlinear systems showing '*screwed up behavior'*. Then, the second possibility, the biological system is difficult represented fully by a computer program so that possible to provide different predictions [45].

CONCLUSION

In the antibacterial agent discovery, the natural product carries fresh air to find a solution. Flavonoids and phenolic compounds from *M. pendans* have antibacterial and anti-QS activity through inhibiting MurA enzyme and GBAP. It has not been reported that *M. pendans* flavonoids are anti-QS agents. However, further studies are still needed to clarify the activity of compound through *in vitro* method so that it can be implemented clinically.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Data was retrieved from PubChem compound database (https://www.ncbi.nlm.nih.gov/pccompound).

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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