Chempublish Journal
Journal Homepage: online-journal.unja.ac.id/chp
Department of Chemistry
Faculty of Science and Technology
Universitas Jambi
ISSN (Online): ISSN 2503-4588
No. 23/E/KPT/2019

Article

Anticaries Potential of Temu Kunci-Serai Ethyl Acetate Extract Combination: In Vitro and Molecular Studies Approach

Muhammad Priyadi[1], Rizki Rachmad Saputra[2*]

[1]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Palangka Raya, Palangka Raya 7311, Central Kalimantan, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Palangka Raya, Palangka Raya 7311, Central Kalimantan, Indonesia

Abstract

Dental caries is one of the problems in dental disorders suffered by many people. There have been many ways of handling such as using temu kunci and lemongrass plants. This study aims to determine the antibacterial activity of caries in vitro and predict the mechanism of action of the bacteria that cause caries *Streptococcus mutans*. Temu kunci and lemongrass were extracted using ethyl acetate solvent. antibacterial tests against *Streptococcus mutans* were carried out using diffusion tests with a combination treatment of temu kunci: serai extracts 5% b/v: 5% b/v, 5% b/v: 10% b/v, and 10% b/v: 5% b/v. The positive control used amoxicillin and DMSO as a negative control. Potency as an anticaries drug of drived compound from Temu Kunci and Serai were evaluated by molecular docking using glucosyltransferase (3AIC). The results showed that the combination of temu kunci and serai with concentration 10% b/v: 5% b/v has potential as anticaries against *Streptococcus mutans*. Molecular studies depicted that Panduratin A, Isopanduratin, and 1,3-O-di-p-coumaroylglycerol have great activity toward 3AIC, respectively. Especially for Panduratin A and Isopanduratin, those compound depicted great and similar binding affinity (-8.4 kcal mol^{-1}) that lower than Acarbose as native ligand (-8.3 kcal mol^{-1}). Furthermore, those compound binding similarity ilustrated activity mechanism similarly with native ligand toward receptor. Additionally, the profiling drug-target interaction suggested Temu Kunci's derived compounds have great potential as anticaries treatment.

Keywords: t*emu kunci, serai, molecular docking, Streptococcus mutans, 3AIC*

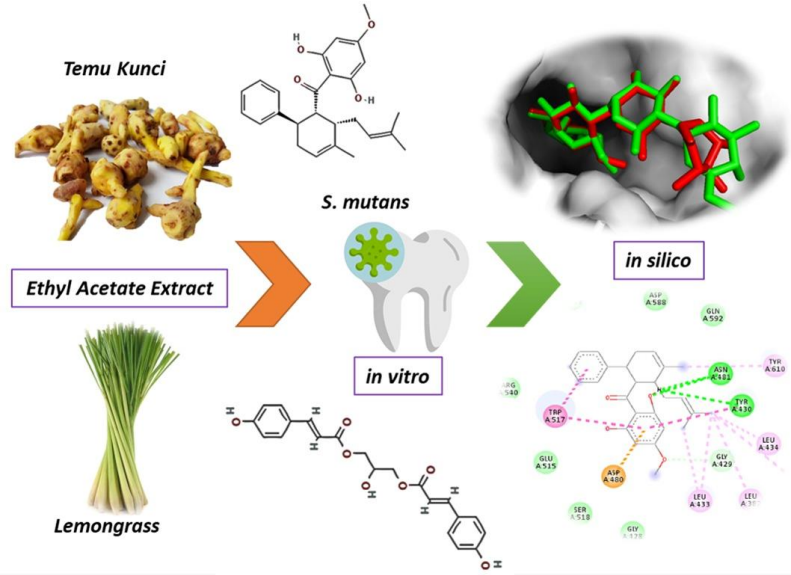
Introduction

Dental and oral problems are experienced by every member of society, such as dental caries. Dental caries disease is a chronic dental disorder condition characterized by plaque so tooth decay occurs due to bacterial activity, lifestyle, and food. Bacteria that can cause dental caries such as *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus sp.*, *Staphylococcus sp.*, *Fusobacterium sp.*, and *Corynebacterium sp.* [1]. *Streptococcus mutans* is the most dominant bacterial species causing dental caries so it is widely studied [2]. Around 530 million children globally who experience dental caries [3].

* Corresponding author
Email addresses: rizkirachmads@mipa.upr.ac.id

DOI: 10.22437/chp.v7i1.26098
Received 15 June 2023; **Accepted** 25 July 2023; **Available online** 30 July 2023

Graphical Abstract



The results of RISKESDAS 2018 show that the proportion of dental and oral problems in Indonesia is 57.6% [4]. The issue of dental caries is very important to be handled through prevention and treatment. Medical therapies that can be carried out in dental caries management include limiting caries-causing bacterial infections, reducing risk factors, remineralization, and periodic dental care [5,6]. In bacterial infection of dental caries, the use of antibiotics is at risk of resistance so other therapeutic approaches such as herbal medicines can be taken [1]. Materials that have the potential to be developed in the treatment of dental caries are temu kunci (*Boesenbergia rotunda*) and serai or lemongrass (*Cymbopogon citratus*).

Traditionally, temu kunci rhizome can be used as a vegetable, spice, and even treatment for several diseases. Some potential pharmacological activities of temu kunci compounds such as antifungal, antibacterial, anti-inflammatory to anticancer [7]. Serai essential oil contains compounds that can be used in food, cosmetics, and medicine [8,9]. Temu kunci and serai on their way can be used as antibacterial *Streptococcus mutans* [10–12]. Previous combination research has been conducted between temu kunci and serai which has the potential as an anticaries [13,14]. Ethyl acetate was used as a solvent according to research conducted by Elfahmi et al.[15] and Priyadi et al[16]. It is known that ethyl acetate extracts of temu kunci and serai contain compounds of alkaloid, terpenoid, flavonoid, phenol and quinone groups. Ethyl acetate can attract polar and non-polar compounds. There has been no specific research on the class of compounds in extracts extracted using ethyl acetate solvent so that it can be developed such as combination therapy between temu kunci and serai.

Temu kunci extract is known to contain several compounds in ethyl acetate extract or its fraction. Methanol extract of temu kunci with ethyl acetate fraction has shown pinosembrin, cardamonin, pinostrobin, 4-hydroxy panduratin A, and panduratin A[17]. Ethyl acetate extract contains cardamonin[18], alpinetin [15], panduratin A [19,20] and isopanduratin A [21]. Ethyl acetate fraction of serai contains potential compounds such as p-coumaric acid, caffeic acid, 1,3-O-di-p-coumaroylglycerol, and 1-O-caffeoyl-3-O-p-coumaroylglycerol[22,23]. Temu kunci has a number of compounds such as isopanduratin that have the potential to inhibit the growth of *Streptococcus mutans* [21]. The compound approach for the inhibition of *Streptococcus mutans* has one of the anticaries mechanism drug targets on the glucosyltransferase protein (3AIC). The 3AIC as a potential candidate protein target can be studied in silico through molecular

docking of compounds contained in temu kunci and serai. This is because 3AIC is the most frequent target protein for molecular testing and as important component in cell wall building and the mechanism of dental caries formation in *Streptococcus mutans*. Inhibition of 3AIC by active compounds of secondary metabolites derived from temu kunci and lemongrass can cause damage to bacterial cells, causing the death of *Streptococcus mutans* bacterial cells [24–26].

Based on the problems that occur and various background references to traditional anticaries therapy, it is encouraging to be able to develop dental caries treatment. Therefore, research is needed on the antibacterial activity of the combination of temu kunci and serai, especially on *Streptococcus mutans* bacteria both through an in vitro approach and its mechanism in silico.

Experimental Section

Materials

temu kunci rhizome, serai (lemongrass), DMSO (Merck) Nutrient Agar (Merck), Brain Heart Infuse (Merck), McFarland Media, distilled water, 70% ethanol, paper disc (Advantec), cotton swab, and Streptococcus mutans bacteria. Materials used in the in-silico test include glucansucrase/glucosyltransferase (PDB ID: 3AIC), acarbose, and amoxicillin obtained from the website database https://www.rcsb.org/. Phytochemical compound structures of temu kunci (pinostrobin, pinocembrin, isopanduratin, cardamonin, and alpinetin) and serai (p-coumaric acid, caffeic acid, 1,3-O-di-p-coumaroylglycerol, and 1-O-caffeoyl-3-O-p-coumaroylglycerol) obtained from the chemical web library database https://pubchem.ncbi.nlm.nih.gov/.

Procedure

Plant material preparation

Temu kunci and serai were obtained from UPT Materia Medica Batu, East Java in the form of powdered simplisia that had been determined (074/333A/102.7/2020). 100 g each of temu kunci and serai powder was extracted using ethyl acetate as much as 1 liter by soaking for ± 3 days. Then the solution was filtered and the filtrate was evaporated at 50°C until a thick extract was obtained. It was extracted by previous research[16].

Antibacterial testing

The antibacterial testing method is the Kirby-Bauer agar diffusion method using disc paper (6 mm) and nutrient agar as the growth medium for *Streptococcus mutans*. The test sample group consisted of a combination of temu kunci and serai extracts (5% b/v: 5% b/v, 5% b/v: 10% b/v, and 10% b/v: 5% b/v) dissolved in DMSO and amoxicillin 30 µg/mL as a positive control. The disc paper was dipped in the test sample solution for 15 minutes and placed in a petri dish containing nutrient agar media and *Streptococcus mutans*. Incubate the petri dish at 37°C for 24 hours and measure the diameter of the antibacterial inhibition zone (mm) by looking at the clear area on the test media.

Hardware and software preparation

In this study, various molecular docking tools were utilized for different purposes. Autodock Vina 4 from The Scripps Institute in the USA was employed to perform the molecular docking simulation process. PyMol was utilized to visualize the binding pocket of the receptors. For receptor preparation, binding site analysis, and interpretation of 2D interactions, BIOVIA Discovery Studio was utilized. The software was downloaded from https://discover.3ds.com. The desktop used for the study runs on Windows 10 pro and is equipped with an AMD A8-7410 APU (4 CPUs, 2.2 GHz, 4 GB RAM). OpenBabel 2.3.2 ACD/marvinSketch, specifically the freeware version 10.00, is installed on the system.

Ligand preparation

The ligand's two-dimensional structure was generated using marvinsketch and saved in the hin format using OpenBabel 2.3.2 software. However, prior to that, the geometry of the ligand was optimized using Hyperchem. The optimization result was saved in the pdb format, which can be read by Autodock Vina 4.

Macromolecule preparation

The 3D structures of glucansucrase (ID 3AIC) was obtained from the Protein Data Bank (PDB) website at www.rcsb.org.

A ligand that was originally bound to the macromolecule from its side active site was modified to convert it into a monomer macromolecule. Furthermore, the water content of the macromolecule was removed and saved in the pdb format. The native ligands for the proteins is alpha-acarbose molecule, and the resolution of the receptor proteins is 3.11 Å. To prepare the macromolecule for docking simulations, the native ligand structures and water molecules were eliminated using AutoDockTool-1.5.6. The Kollman charge calculation was applied, and polar hydrogen atoms were added. Then, BIOVIA Discovery was utilized to identify the binding sites within the macromolecules. The binding site is where the natural ligand is typically located and where biological activity is most likely to occur. All amino acids within the radius of the binding site were used for the ligand-protein molecular docking process.

Docking analysis

The Auto Dock tool, version 4.0, was utilized to perform molecular docking of the ligand of Temu Kunci and serai compounds to the active site of the target proteins (3AIC). The receptor was modified to include polar hydrogen bonds (H-bonds). The internal degree of freedom and torsions of the ligand were specified using the "Ligand torsions" menu option in Auto Dock. The "autogrid" option was employed to generate grid maps that represent the protein's characteristics. The ligand-receptor structure with the lowest energy, as determined by the docking simulation, was considered the best. The results were visualized using the BIOVIA Discovery Visualizer software.

Data analysis

Data obtained from the results of in vitro tests were analyzed using SPSS 25th edition with the statistical method of one-way ANOVA followed by the post hoc tuckey test at the 95% significance level and In silico tests were analyzed by comparing the results of affinity, energy, and binding between ligand-receptor.

Results and Discussions

Antibacterial testing

The results of the antibacterial test of the temu kunci:serai combination carried out by the diffusion method against *Streptococcus mutans* can be seen in Table 1. The test results show the treatment of temu kunci:serai 5%:5% w/v and 5%:10% w/v concentration has an inhibition zone diameter value of 1.28 mm and 7.23 mm with the resistant category. The treatment of temu kunci:serai 10%:5% w/v amounted to 15.23 mm with an intermediate category compared to the positive control which had an inhibition zone diameter of 26.03 mm with a susceptible category. Antibacterial activity category following CLSI 2018 reference[27].

The results of the one-way ANOVA test of 5 treatments against *Streptococcus mutans* obtained significant value = 0.000 < 0.05 so that 5 groups produced post hoc tuckey differences to determine differences between treatments.

Tabel 1. Antibacterial test against *Streptococcus mutans* and statistical analysis.

Sample	Inhibition zone (mm)	Category[27]	p-value (sig.)
Positive control (amoxicillin 30 µg)	26.03 ± 0.47	Susceptible	
Temu Kunci : Serai (5%:5% w/v)	1.28 ± 0.20	Resistant	
Temu Kunci : Serai (5%:10% w/v)	7.23 ± 1.63	Resistant	0.000
Temu Kunci : Serai (10%:5% w/v)	15.23 ± 1.24	Intermediate	
Negative control (DMSO)	-	-	
Etanol extract of Temu Kunci (5%)*	11.17 ± 0.29		
Etanol extract of Serai (5%)*	9.33 ± 0.75		

*Reference for comparing [13]

The value of tuckey analysis showed a significant difference between the positive control treatment, negative control, and the three combinations of temu kunci-serai while there was no significant difference between the negative control and temu kunci-serai due to the small inhibition zone value.

In another study, lemongrass ethyl acetate extract was found to contain terpenoids, steroids, tannins, saponins, glycosides, phenols, and flavonoids [28]. Lemongrass has the potential to be combined with various antibacterial compounds [29]. While, temu kunci has more potential antibacterial compounds and has been successfully isolated to be identified. The analysis showed that changes in the concentration ratio of the extracts could affect the antibacterial activity against *Streptococcus mutans*.

Temu kunci extract is known to increase the antibacterial activity quite strongly compared to the addition of serai extract. Therefore, the extracts can promise the potential activity of intermediate antibacterial compounds with several mechanisms of action. This can be a form of synergy between secondary metabolites adjusted to the concentration of extracts used so that it depends on the balance of which activity is stronger. synergy between extracts can improve activity performance compared to only being used singly [30]. The effect of temu kunci:lemongrass combination can be a direction and further research such as research on the combination of reuterin and catechin in inhibiting the growth of *Streptococcus mutans* [31].

Moculer studies

Molecular docking is a highly effective approach for discovering new ligands that can bind to receptors with known structures. It plays a crucial role in the development of structure-based drugs. To evaluate the interaction energies between these newly discovered ligands and the receptors, scoring functions are employed [32]. Through molecular docking, a binding energy, represented as (ΔG°), is generated. This binding energy serves as an indicator of the irreversible interaction between the ligand and the receptor. A decrease in the binding energy value signifies a more stable association between the ligand and receptor. Enhanced activity is observed when the ligand-receptor interactions become more stable [33].

This experiment involved redocking the native ligand in its original state towards the protein receptor to validate the docking method. The measured result was on Figure 1, expressed using the root mean square deviation (RMSD), which indicates the deviation from the binding position when redocking was performed using the binding pose obtained from the crystal structure. A lower RMSD value signifies a higher quality of the docking pose achieved. Ideally, a good RMSD value should be equal to or less than 2 Å. The RMSD values obtained from this experiment was 1.588 Å for the redocking of the native ligands acarbose in glucosyltransferase, (ID 3AIC). These RMSD values were calculated using the PyMOL program [34]. Figure 2 illustrates the structures of the native ligand, common drug, and derivative compounds of Temu Kunci and serai.

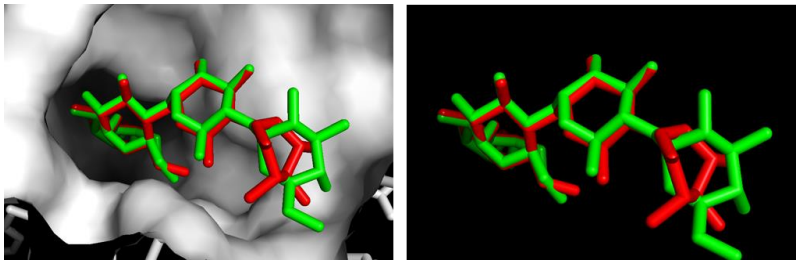
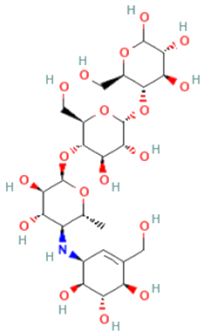
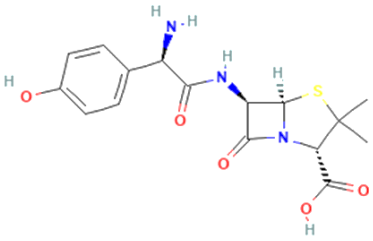


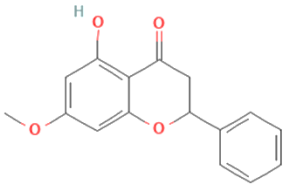
Figure 1. 3D visualisation of re-docking result. Green is origin pose of native ligand (Acarbose) inhibitor and red is re-docking result.



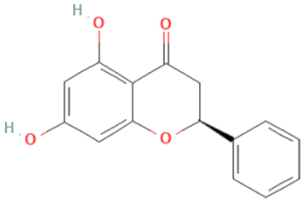
a. Acarbose (native ligand)



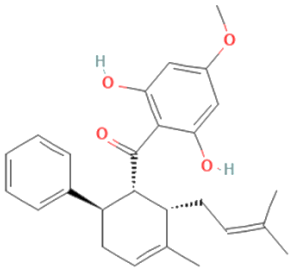
b. Amoxicillin (common Drug)



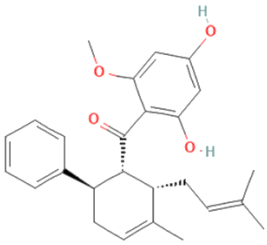
c. Pinostrobin



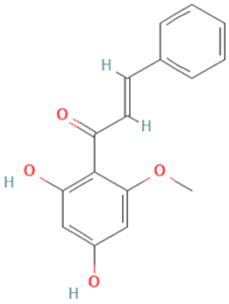
d. Pinocembrin



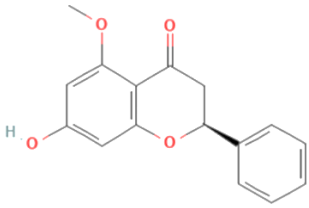
e. Panduratin A



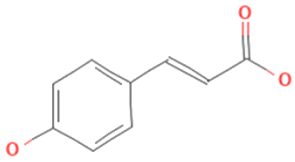
f. Isopanduratin



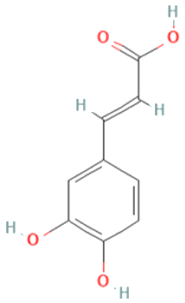
g. Cardamonin



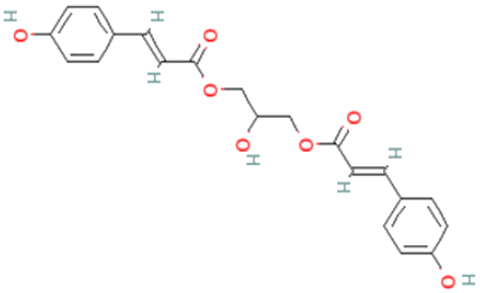
h. Alpinetin



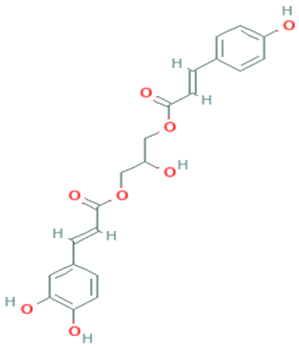
i. P-coumaric acid



j. Caffeic Acid



k. 1,3-O-di-p-coumaroylglycerol



l. 1-O-caffeoyl-3-O-p-coumaroylglycerol

Figure 2. (a) Native ligand, (b) Common Drug, and (c-f) Temu Kunci's compound and (g-l) Serai's compound as a ligand candidate.

In order to determine the binding energies between each ligand and the 3AIC receptor protein, molecular docking was employed, and the outcomes are presented in Table 2 and Table 3. The results of the molecular docking analysis for the derivative compounds of Temu Kunci and serai were obtained.

Based on the results obtained from redocking the native ligands acarbose and amoxicillin as common drug, binding energy values of -8.3 kcal mol^{-1} and -8.2 kcal mol^{-1} were determined, respectively. Docking studies indicated that in the docking process on 3AIC (as shown in Table 2), approximately two chemical compounds derived from Temu Kunci and one chemical compound derived from Serai exhibited lower binding energy compared to the control group (Acarbose and Amoxcillin). These compounds included Panduratin A with a binding energy of -8.4 kcal/mol, Isopanduratin with -8.4 kcal/mol, 1,3-O-di-p-coumaroylglycerol with -8.5 kcal/mol (refer to Table 2). Those compound exhibited lower binding energy than the control drug and were capable of binding to target protein. A lower binding affinity value suggests that the ligand can interact more easily with macromolecules. This characteristic enhances the effectiveness of the ligand in combating or treating certain illnesses. It is important to note that if the ligand is blocked, the healing process cannot occur. The ligand interacts with the active site of the macromolecule, and this interaction involves specific residues (amino acids) of the macromolecule. When a bond is formed, the resulting interaction can elicit a response from the body. The nature of the interaction between the ligand and receptor determines the specific responses generated [33].

Tabel 2. Binding Energy Rank of compound derive from Temu Kunci.

No	Compound	Binding Energy (kcal/mol)
1	Panduratin A	-8.4
2	Isopanduratin	-8.4
3	**Acarbose (NL)**	**-8.3**
4	**Amoxicillin (CD)**	**-8.2**
5	Alpinetin	-8.0
6	Pinocembrin	-7.8
7	Pinostrobin	-7.5
8	Cardamonin	-7.3

Tabel 3. Binding Energy Rank of compound derive from Serai.

No	Compound	Binding Energy (kcal/mol)
1	1,3-O-di-p-coumaroylglycerol	-8.5
2	**Acarbose (NL)**	**-8.3**
3	**Amoxicillin (CD)**	**-8.2**
4	caffeic acid	-7.1
5	1-O-caffeoyl-3-O-p-coumaroylglycerol	-7.0
6	p-coumaric acid	-6.1

Under normal circumstances, control compounds function by inhibiting the biological activity of the target protein through the formation of molecular complexes when they bind to it. Acarbose is a drug employed as an inhibitor of 3AIC. It operates by binding to the active site of the target protein, utilizing the interaction of hydrophobic bonds, effectively obstructing the activation [35]. The docking results reveal that the ligands produce various binding energy scores when interacting with the target protein, ultimately influencing the biological activity of the protein. Ligands with lower binding energy compared to the controls exert a more significant impact on the target protein [33].

The primary emphasis of the interaction study lies in examining hydrogen bonding interactions. Hydrogen bonds play a crucial role in determining the alignment of a ligand with a receptor, facilitating specific recognition of the ligand, and promoting interaction between the ligand and receptor [36]. By evaluating the distance between interactions, it becomes possible to predict the strength of hydrogen bonds. Additionally, apart from hydrogen bonding, hydrophobic interactions also exert a significant influence on the outcomes of binding energy. Even when the hydrophobic connection is weaker than the hydrogen bond, the presence of hydrophobic molecules suggests that ligands may still exhibit inhibitory effects (as shown in Table 4).

When a ligand binds to a protein, it forms complex molecules characterized by molecular interactions in the form of chemical bonds. These chemical bonds, which encompass weak interactions such as hydrophobic and hydrogen bonds, occur between specific atoms in the ligand and amino acid residues in the target protein [36]. To identify potential ligands as alternative inhibitors, the Discovery Studio Visualizer is employed, considering the type of chemical bond interaction and the position of amino acid residues in the target protein. According to crystallographic evidence, the inhibitor acarbose does, however, contain important residues including Glu515, Ala478, Tyr430, Asp959, Leu333, Gln960, Asp477, and Asp588 [24].

Tabel 4. Data of binding energy, binding interaction, and binding similarities of Temu Kunci and Serai derivative compounds toward 3AIC compared to Native Ligand and Common Drug.

Macromelecules (receptors)	Compounds	Binding affinity	Binding site		Binding similarity
		(kcal mol^{-1})	Hydrogen bonding	Hydrophobic interaction	
3AIC	Acarbose (Native Ligand)	-8.3	***Asp909, Tyr430, Asp477, Gln592, Gly429***	***Thr 426, Trp517, Asn481, Phe907, Leu382, Asn914,Tyr916, Asp588, Ala478, Glu515, Leu433, Tyr610, Asp480, Ser518, Gly428***	***100.00%***
	Amoxicillin (Common Drug)	-8.2	***Asn481***	***Leu382***, *Leu908,* ***Phe907***,***Gln592***, *Asn862, His587,* ***Asn914***, ***Asp909***, *Leu434,* ***Asp588***, ***Tyr916***, ***Asp477,*** *Gln960,* ***Ala478***, ***Glu515***, ***Tyr430***, *Asp517,* ***Leu433***	***65.00%***

Macromelecules (receptors)	Compounds	Binding affinity	Binding site		Binding similarity
		(kcal mol^{-1})	Hydrogen bonding	Hydrophobic interaction	
	Panduratin A	-8.4	***Asn481, Tyr430***	Ser589, **Asp588**, **Gln592**, **Tyr610**, Leu434, **Phe907, Gly429, Leu382**, **Leu433, Gly428, Asp480, Ser518, Glu515, Trp517,** Arg540	***70%***
	Isopanduratin	-8.4	***Asp909***	*Asn864,* ***Leu433****,* ***Tyr916****,* ***Phe907****,* ***Asn481****,* ***Gln592****, Leu908,* ***Leu382****,* ***Asp480****,* ***Tyr430****,* ***Tyr610****,* ***Trp517****, Ser589,* ***Asp477****, Leu434,* ***Ala478, Glu515, Asp588,*** *His587,* ***Asn914***	***80.00%***
	1,3-O-di-p-coumaroylglycerol	-8.5	*Gly716*	*Ser835, Ala833, Ala850, Ala849, Val832, Leu718, Lys651, Arg831, Val830, Val691, Ser853, Arg687, Asp852, Arg712, Ser692, Gly693, Ala717, Lys715, Asn848, Ala834*	***0.00%***

The docking parameter utilized in this experiment might also be validated by the analogies between amino acid and acarbose interactions. With respect to other ligand complexes, the comparison of the affinity character between 1,3-O-di-p-coumaroylglycerol and 3AIC in this study showed that the interaction was blocked at 3AIC in a different active site region. Comparing 3AIC's binding sides to chemical medications (native ligands and common pharmaceuticals), distinct binding sides may play a role in suppressing function in various consequences [37].

Based on Figure 3, hydrogen and hydrophobic bonds are the main components of the molecular interactions found in this study. In drug design strategies, hydrogen bonding which happens when H atoms connect with N, O, or F atoms serves as a metric reflecting the ligand's tendency to affect the biological response of target proteins. In addition to hydrogen bonds, hydrophobic bonds are important for the formation of molecular complexes, which are important for the interaction of drug molecules and result in observable modifications in the biological response of the protein target [38]. The compounds and control ligands formed molecular complexes in this study, and the existence of hydrogen and hydrophobic contacts suggests that these interactions affected the target protein's biological response. Specifically, the study identified three compounds, Panduratin A, Isopanduratin, and , 1,3-O-di-p-coumaroylglycerol with the lowest binding energy. These compounds effectively act as inhibitors, suppressing the biological activity of the target protein. This is supported by research data that Panduratin A can inhibit the growth of *Streptococcus mutans* [20]. This also applies to Isopanduratin as anticaries [21]. While 1,3-O-di-p-coumaroylglycerol likely has a different target protein pathway and mechanism of action compared to other compounds.

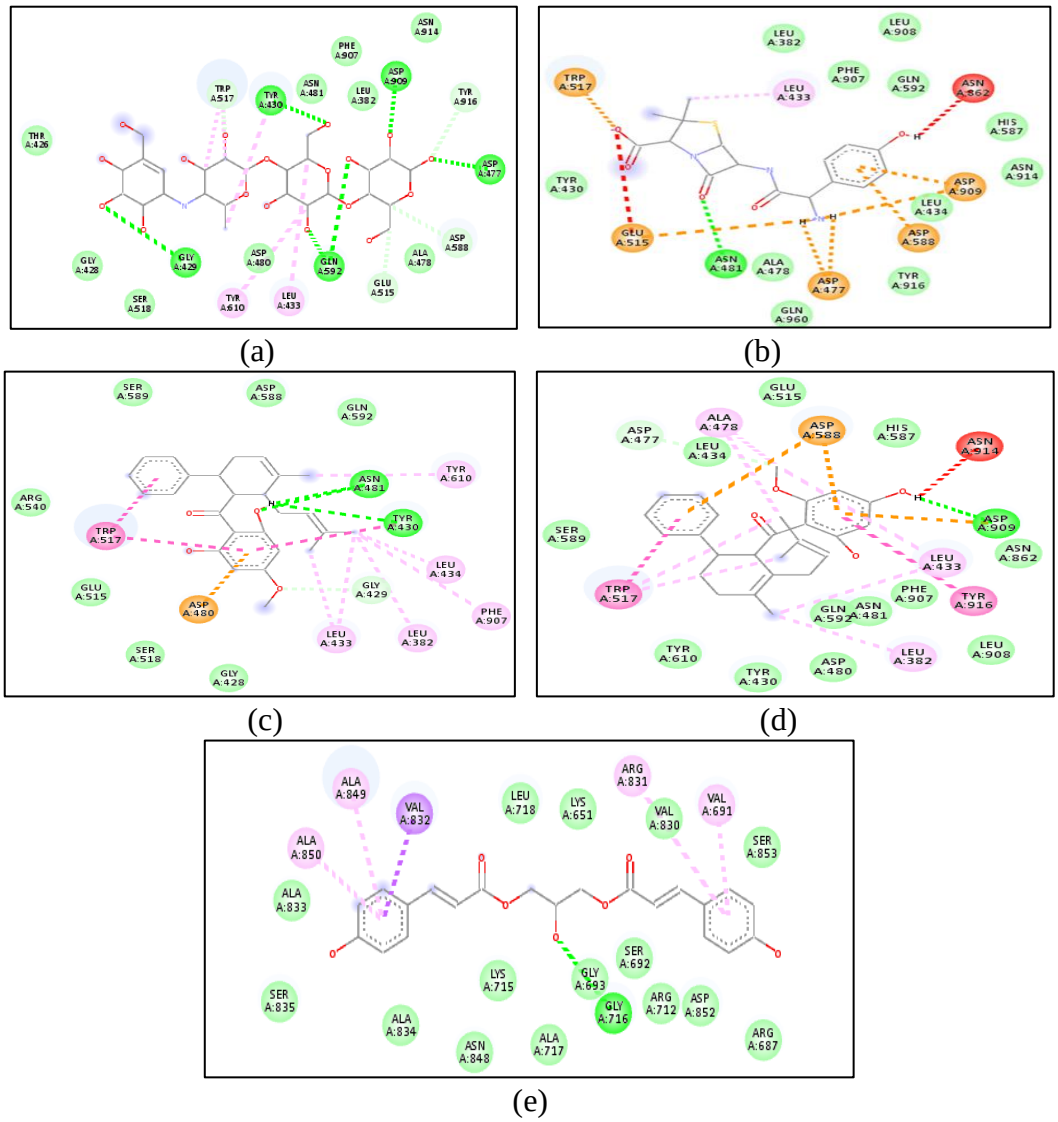


Figure 3. 2D-ilustration between 3AIC and ligand. (a) Native ligand/acarbose, (b) Amoxicillin, (c) Panduratin A, (d) Isopanduratin, and (e) 1,3-O-di-p-coumaroylglycerol.

The use of certain solvents is influenced by previous research and the identity of the target compound so that the use of ethyl acetate is an additional new information that there are still many potential compounds that can be developed from temu kunci and serai.

In vitro tests are seen as pharmacological evidence and molecular studies as reinforcement in supporting the mechanism of action of antibacterials on *Streptococcus mutans*, especially on specific target proteins such as 3AIC. Therefore, this study can confirm as well as act as preliminary research to be able to target potential compounds isolated so that they can be developed as anticaries. This research shows that the integration between in vitro and molecular studies can accelerate the discovery of alternative anticaries therapies.

Conclusions

The results showed that the combination of temu kunci and serai has potential as anticaries against *Streptococcus mutans*. Moreover, docking studies depicted descent of docking value (ΔG˚) toward Glucosiltransferase (3AIC) signaling as anti-caries receptor. Like Acarbose's ability to operate as an anti-caries native ligand, panduratin, isopanduratin, exhibited anti-caries activity both

of binding affinity and similarity. Otherwise, 1,3-O-di-p-coumaroylglycerol toward 3AIC is higher than acarbose toward 3AIC on binding affinity, but totally different on binding similarity.

References

1. El Sherbiny, G. M. (2014). Control of growth Streptococcus mutans isolated from saliva and dental caries. *Ournal of Current Microbiology and Applied Sciences*, *3*(10), 1–10.
2. Lemos, J. A., Palmer, S. R., Zeng, L., Wen, Z. T., Kajfasz, J. K., Freires, I. A., Abranches, J., & Brady, L. J. (2019). The Biology of Streptococcus mutans. *Microbiology Spectrum*, *7*(1). https://doi.org/10.1128/microbiolspec.GPP3-0051-2018
3. World Health Organization. (2019, June 13). *Ending childhood dental caries: WHO implementation manual*. Geneva: World Health Organization. https://apps.who.int/iris/handle/10665/330643
4. Kementerian Kesehatan RI. (2019). *Riset Kesehatan Dasar 2018*.
5. Pitts, N. B., Zero, D. T., Marsh, P. D., Ekstrand, K., Weintraub, J. A., Ramos-Gomez, F., Tagami, J., Twetman, S., Tsakos, G., & Ismail, A. (2017). Dental caries. *Nature Reviews Disease Primers*, *3*(1), 17030. https://doi.org/10.1038/nrdp.2017.30
6. Yon, M. J. Y., Gao, S. S., Chen, K. J., Duangthip, D., Lo, E. C. M., & Chu, C. H. (2019). Medical Model in Caries Management. *Dentistry Journal*, *7*(2), 37. https://doi.org/10.3390/dj7020037
7. Chahyadi, A., Hartati, R., Wirasutisna, K. R., & Elfahmi. (2014). Boesenbergia Pandurata Roxb., An Indonesian Medicinal Plant: Phytochemistry, Biological Activity, Plant Biotechnology. *Procedia Chemistry*, *13*, 13–37. https://doi.org/10.1016/j.proche.2014.12.003
8. Do, D. N., Nguyen, D. P., Phung, V.-D., Le, X.-T., Le, T. M., Do, V. M., Minh, B. Q., & Luu, X. C. (2021). Fractionating of Lemongrass (Cymbopogon citratus) Essential Oil by Vacuum Fractional Distillation. *Processes*, *9*(4), 593. https://doi.org/10.3390/pr9040593
9. Oladeji, O. S., Adelowo, F. E., Ayodele, D. T., & Odelade, K. A. (2019). Phytochemistry and pharmacological activities of Cymbopogon citratus: A review. *Scientific African*, *6*, e00137. https://doi.org/10.1016/j.sciaf.2019.e00137
10. Hwang, J.-K., Shim, J.-S., & Chung, J.-Y. (2004). Anticariogenic activity of some tropical medicinal plants against Streptococcus mutans. *Fitoterapia*, *75*(6), 596–598. https://doi.org/10.1016/j.fitote.2004.05.006
11. Taweechaisupapong, S., Singhara, S., Lertsatitthanakorn, P., & Khunkitti, W. (2010). Antimicrobial effects of Boesenbergia pandurata and Piper sarmentosum leaf extracts on planktonic cells and biofilm of oral pathogens. *Pakistan Journal of Pharmaceutical Sciences*, *23*(2), 224–231.
12. Ortega Cuadros, M., Tofiño Rivera, A. P., Merini, L. J., & Martinez Pabon, M. C. (2018). Antimicrobial activity of Cymbopogon citratus (Poaceae) essential oil on Streptococcus mutans biofilm and cytotoxic effect on keratinocytes and fibroblasts. *Revista de Biología Tropical*, *66*(4), 1519. https://doi.org/10.15517/rbt.v66i4.33140
13. Hati, A. K., Dyahariesti, N., & Yuswantina, R. (2019). Penetapan Kadar Flavonoid Dan Uji Aktivitas Antibakteri Ekstrak Sereh (Cymbopogon nardus) Dan Temu Kunci (Boesnbergia pandurata Roxb) Terhadap Bakteri Streptococcus Mutans. *Indonesian Journal of Pharmacy and Natural Product*, *2*(2), 71–78. https://doi.org/10.35473/ijpnp.v2i2.264
14. Kawengian, S. A. F., Wuisan, J., & Leman, M. A. (2017). Uji daya hambat ekstrak daun serai (Cymbopogon citratus L) terhadap pertumbuhan Streptococcus mutans. *E-GIGI*, *5*(1), 7–11. https://doi.org/10.35790/eg.5.1.2017.14736
15. Elfahmi, Aziz, S., & Sulaiman, I. (2017). Isolation of flavonoid compound from ethyl acetate extract of fingeroot (Boesenbergia Pandurata (Roxb.) Schlechter) rhizome. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *8*(4), 1192–1198.
16. Priyadi, M., Chusna, N., Isnawati, I., & Indriani, O. (2021). Profil Fitokimia Ekstrak Etil Asetat Temu Kunci (Boesenbergia rotunda L.) dan Serai (Cymbopogon citratus). *Jurnal Pharmascience*, *8*(1), 45. https://doi.org/10.20527/jps.v8i1.9725
17. Adhikari, D., Gong, D.-S., Oh, S. H., Sung, E. H., Lee, S. O., Kim, D.-W., Oak, M.-H., & Kim, H. J. (2020). Vasorelaxant Effect of Boesenbergia rotunda and Its Active Ingredients on an Isolated Coronary Artery. *Plants*, *9*(12), 1688. https://doi.org/10.3390/plants9121688
18. Atun, S., Sri, H., & Rakhmawati, A. (2018). Potential Bioactive Compounds Isolated from Boesenbergia rotunda as Antioxidant and Antimicrobial Agents. *Pharmacognosy Journal*, *10*(3), 513–518. https://doi.org/10.5530/pj.2018.3.84

19. Song, M. S., Shim, J. S., Gwon, S. H., Lee, C. W., Lee, C. W., Kim, H. S., & Hwang, J. K. (2008). Antibacterial activity of panduratin A and isopanduratin A isolated from Kaempferia pandurata Roxb. against acne-causing microorganisms. *Food Science and Biotechnology*, *17*(6), 1357–1360.

20. Rukayadi, Y., Lee, K.-H., & Hwang, J.-K. (2009). Activity of panduratin A isolated from Kaempferia pandurata Roxb. against multi-species oral biofilms in vitro. *Journal of Oral Science*, *51*(1), 87–95. https://doi.org/10.2334/josnusd.51.87

21. Hwang, J.-K., Chung, J.-Y., Baek, N.-I., & Park, J.-H. (2004). Isopanduratin A from Kaempferia pandurata as an active antibacterial agent against cariogenic Streptococcus mutans. *International Journal of Antimicrobial Agents*, *23*(4), 377–381. https://doi.org/10.1016/j.ijantimicag.2003.08.011

22. Hacke, A. C. M., Marques, J. A., Vellosa, J. C. R., Boligon, A. A., da Silva, F. D., de Souza, D., Bonini, J. S., Rocha, J. B. T., & Pereira, R. P. (2018). Ethyl acetate fraction of Cymbopogon citratus as a potential source of antioxidant compounds. *New Journal of Chemistry*, *42*(5), 3642–3652. https://doi.org/10.1039/C7NJ04352J

23. Wang, H., Zhang, R., Zhang, K., Chen, X., & Zhang, Y. (2022). Antioxidant, Hypoglycemic and Molecular Docking Studies of Methanolic Extract, Fractions and Isolated Compounds from Aerial Parts of Cymbopogon citratus (DC.) Stapf. *Molecules*, *27*(9), 2858. https://doi.org/10.3390/molecules27092858

24. Nijampatnam, B., Zhang, H., Cai, X., Michalek, S. M., Wu, H., & Velu, S. E. (2018). Inhibition of Streptococcus mutans Biofilms by the Natural Stilbene Piceatannol Through the Inhibition of Glucosyltransferases. *ACS Omega*, *3*(7), 8378–8385. https://doi.org/10.1021/acsomega.8b00367

25. Atta, L., Khalil, R., Khan, K. M., Zehra, M., Saleem, F., Nur-e-Alam, M., & Ul-Haq, Z. (2022). Virtual Screening, Synthesis and Biological Evaluation of Streptococcus mutans Mediated Biofilm Inhibitors. *Molecules*, *27*(4), 1455. https://doi.org/10.3390/molecules27041455

26. Jauhar, M. M., Syaifie, P. H., Arda, A. G., Ramadhan, D., Nugroho, D. W., Ningsih Kaswati, N. M., Noviyanto, A., Rochman, N. T., & Mardliyati, E. (2023). Evaluation of propolis activity as sucrose-dependent and sucrose-independent Streptococcus mutans inhibitors to treat dental caries using an in silico approach. *Journal of Applied Pharmaceutical Science*, *13*(3), 071–080. https://doi.org/10.7324/JAPS.2023.45365

27. Performance Standards for Antimicrobial Susceptibility Testing, 28th ed, Pub. L. No. M100, Wayne, PA: Clinical and Laboratory Standards Institute 1 (2018).

28. sayed, H., issa, mohammady, mahmoud, magda, abdelkader, emad, & ismail, hamadi. (2022). Phytochemical Screening and Antioxidant Activity of some Egyptian Medicinal Plants. *Journal of Modern Research*, *4*(1), 14–20. https://doi.org/10.21608/jmr.2021.83376.1075

29. Tadtong, S., Watthanachaiyingcharoen, R., & Kamkaen, N. (2014). Antimicrobial Constituents and Synergism Effect of the Essential Oils from Cymbopogon citratus and Alpinia galanga. *Natural Product Communications*, *9*(2), 1934578X1400900. https://doi.org/10.1177/1934578X1400900237

30. Prince, A., Roy, S., & McDonald, D. (2022). Exploration of the Antimicrobial Synergy between Selected Natural Substances on Streptococcus mutans to Identify Candidates for the Control of Dental Caries. *Microbiology Spectrum*, *10*(3), e02357-21. https://doi.org/10.1128/spectrum.02357-21

31. Zhang, G., Tan, Y., Yu, T., Wang, S., Liu, L., & Li, C. (2021). Synergistic antibacterial effects of reuterin and catechin against Streptococcus mutans. *LWT*, *139*, 110527. https://doi.org/10.1016/j.lwt.2020.110527

32. Ali, H. I., Ashida, N., & Nagamatsu, T. (2008). Antitumor studies. Part 4: Design, synthesis, antitumor activity, and molecular docking study of novel 2-substituted 2-deoxoflavin-5-oxides, 2-deoxoalloxazine-5-oxides, and their 5-deaza analogs. *Bioorganic & Medicinal Chemistry*, *16*(2), 922–940. https://doi.org/10.1016/j.bmc.2007.10.014

33. Syarafina, Z. Y. I., Safithri, M., Bintang, M., & Kurniasih, R. (2022). In Silico Screening of Cinnamon (Cinnamomum burmannii) Bioactive Compounds as Acetylcholinesterase Inhibitors. *Jurnal Kimia Sains Dan Aplikasi*, *25*(3), 97–107. https://doi.org/10.14710/jksa.25.3.97-107

34. Prahasti, A. E., Yuanita, T., & Rahayu, R. P. (2022). Computer Aided Drug Discovery Utilization in Conservative Dentistry. *Journal of International Dental and Medical Research*, *15*(2), 899–903.

35. Ariefin, M., & Saputra, R. R. (2023). Study Natural Compound of Eleutherine americana as a SaR-CoV-2 Therapeutic Agent : In Silico Approach. *ALCHEMY:Journal of Chemistry*, *11*(1), 41–49. https://doi.org/10.18860/al.v11i1.18018

36. Arora, R., de Beauchene, I. C., Polanski, J., Laine, E., & Tchertanov, L. (2013). Raltegravir flexibility and its impact on recognition by the HIV-1 IN targets. *Journal of Molecular Recognition*, *26*(9), 383–401. https://doi.org/10.1002/jmr.2277

37. Bare, Y., Sari, D. R. T., Rachmad, Y. T., Krisnamurti, G. C., & Elizabeth, A. (2019). In Silico Insight the Prediction of Chlorogenic Acid in Coffee through Cyclooxygenase-2 (COX2) Interaction. *Biogenesis: Jurnal Ilmiah Biologi*, *7*(2), 100–105. https://doi.org/10.24252/bio.v7i2.9847

38. Kharisma, V. D., Kharisma, S. D., Ansori, A. N. M., Kurniawan, H. P., Witaningrum, A. M., Fadholly, A., & Tacharina, M. R. (2021). Antiretroviral effect simulation from black tea (Camellia sinensis) via dual inhibitors mechanism in HIV-1 and its social perspective in Indonesia. *Research Journal of Pharmacy and Technology*, *14*(1), 455–460. https://doi.org/10.5958/0974-360X.2021.00083.4