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Original Article

EXPLORING THE ANTIBACTERIAL POTENTIAL OF *C. GIGANTEA* IN THE GEOTHERMAL AREA OF IE JUE: A BIOINFORMATICS APPROACH

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ABSTRACT

Objective: The Aceh geothermal region offers an enticing possibility for research into prospective contributions to the medical field, particularly in dentistry. *C. gigantea*, a hardy plant that can withstand high temperatures, grows in this area.

Methods: This plant contains a large number of secondary metabolites that are known to be useful in herbal medicine. However, there has not been much research done in this field of oral health; thus finding the plant's potential will require a cheap detecting tool. One method for this investigation is molecular docking, which is a part of bioinformatics. Molecular docking is a method used to examine how drugs interact with proteins, such as the proteins from the halitosis-related bacteria *Porphyromonas gingivalis* (*P. gingivalis*).

Results: In this study, computational technologies will be used to find antibacterial properties. In particular, the antibacterial effects of chemical compounds from Biduri leaves (*C. gigantea*) discovered in the Ie Jue geothermal zone are examined. The study uses molecular docking to compute the interactions of the five Biduri metabolites: lupenyl acetate, α-amyrin, phytol, β-amyrenyl acetate, and methyl ester with the Kgp protein in P. gingivalis.

Conclusion: The data show a range of binding affinities, with α-amyrin showing the most promising results with a binding affinity of -9.7 kcal/mol for Kgp protein. According to this study, P. gingivalis, the bacteria that cause halitosis, may be susceptible to the antibacterial effects of *C. gigantea* leaves.

Keywords: Bioinformatics, Docking, *C. gigantea*, Antibacterial, *Porphyromonas gingivalis*

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INTRODUCTION

Nearly half of the world's geothermal energy resources are concentrated on the island of Sumatra, which is home to 81 geothermal energy of Indonesia [1]. One of these resources is Mountain Seulawah Agam in Aceh, which includes Ie Jue as one of its manifestation zones [2, 3]. In contrast to other areas, geothermal zones typically have greater temperatures and pH levels, lending development to unique characteristics and various plant life. *Calotropis gigantea* grows nicely in this geothermal zone [3].

The Asclepiadaceae family includes *Calotropis gigantea,* commonly referred to as Biduri or Bak Rubek in Aceh. This plant's many parts, particularly the leaves, are frequently utilized as herbal medicines [1]. Flavonoids, alkaloids, saponins, triterpenoids, tannins, polyphenols, calcium oxalate, and other compounds are among those that exist in biduri leaves [4–6]. According to Sharma (2017), *Calotropis gigantea* mouthwash individually showed a statistically significant reduction in the number of *S. mutans* in saliva [7]. Similar results from Kumar (2010) showed that *C. gigantea* is an effective natural source of anti-Candida compounds [8]. Additionally, a study by Sharma (2017) showed how effectively *C. gigantea* leaves extract worked to prevent *S. mutans* from developing in the mouth [9].

More than 750 different bacterial species present in the oral cavity, some of which trigger oral diseases such as halitosis [10]. The unpleasant odor of poor breath is caused by volatile sulfur compounds (VSCs), which are formed by bacterial enzyme activities involving sulfur-containing amino acids [11]. *Porphyromonas gingivalis* are important mediators in this process. For example, *P. gingivalis* causes halitosis through gingipain enzymes and outer membrane proteins. These bacteria use particular enzymatic processes to carry out their functions. For instance, the enzyme L-methionine-adeaminogmercaptomethane-lyase (METase) turns L-cysteine into ammonia and methyl mercaptan, while L-cysteine-desulfhydrase transforms L-cysteine into pyruvate, ammonia, and hydrogen sulfide [12]. Researchers are looking at possible interactions between bacterial proteins and ligands or chemicals obtained from *C. gigantea* in light of the bacteria's capacity to produce VSCs and the potential for *C. gigantea* leaves to suppress bacterial development.

Utilizing bioinformatic assays like molecular docking is one of the most time-and cost-efficient high-throughput approaches [13, 14]. A computer research method called molecular docking integrates the disciplines of biology and chemistry to forecast interactions between various molecules, including ligands and proteins. These interactions can provide information that makes it less difficult to find drug targets, side effects, and drug resistance [13-16]. The aim of this study is to explore the interactions between ligands from *C. gigantea* in the Ie Jue, geothermal zone of mount Seulawah Agam and the proteins from the halitosis-causing bacteria *P. gingivalis*. It is predicted that the results of these interactions will be used as a first standard for selecting *C. gigantea* compounds with potential antibacterial activities.

MATERIALS AND METHODS

Methods

This type of research was conducted with *In silico* by analyzing *C. gigantea* 's leaf compounds from Ie Jue geothermal area as antibacterial against the causes of halitosis like P. gingivalis based on bioinformatics in molecular docking study. Selected target proteins from P. gingivalis bacteria is Kgp. This protein is downloaded via the Protein website Data Bank (https://www.rcsb.org/). Whereas a compound that will act as a ligand is a compound resulting from GC-MS ethanol extract *C. gigantea* leaves that have been analyzed based on Lipinski's rule and downloaded as 3D ligand via Pubchem (https://pubchem.ncbi.nlm.nih.gov/). Compound proteins and ligands were prepared using Chimera Software and grid box settings using

AutodockVina. The results of the scoring are 9 (nine) conformation results were visualized with Pymol and Discovery Studio.

RESULTS AND DISCUSSION

Based on GC-MS analysis results (in the other research), 16 metabolite compounds were found contained in the ethanol extract of *C. gigantea* leaves with different area percent compounds. In this study, 5 out of 16 metabolite compounds contained in the extract are selected because these five compounds are the main components contained in the ethanol extract of *C. gigantea* leaves. Afterward, the selected ligands were subjected to an evaluation based on four fundamental parameters associated with molecular characteristics and the way drugs behave within the human body. These four parameters, outlined in table 1, include the Hydrogen Bond Donors (HBD), Hydrogen Bond Acceptors (HBA), Molecular Weight (MW), and lipophilicity (LogP). Based on parameters the following results were obtained.

Table 1: Lipinski analysis results of metabolite compounds in Biduri leaf from Ie Jue geothermal area

No	Metabolite compounds	Compound formula	HBD	HBA	MW	Log P
1	Lupenyl acetate	C32H52O2	0	2	468.75 g/mol	7.08
2	α-amyrin	C30H50O	1	1	426.72g/mol	6.92
3	Phytol	C20H40O	1	1	296.53 g/mol	5.25
4	β-amyrenil acetate	C32H52O2	0	2	468.75 g/mol	7.08
5	Methyl ester	C31H48O3	0	3	468.71 g/mol	5.92

HBD-Hydrogen Bond Donors, HBA-Hydrogen Bond Acceptors

The results of molecular interaction in this study include both the binding affinity value (expressed in Kcal/mol) and the way in which the ligands and proteins interact (table 2). In this table, it is evident that α-amyrin exhibited the lowest binding affinity values, both interacting with proteins from *P. gingivalis* (Kgp). The protein Kgp has the best value when it interacted with α-amyrin compounds with binding affinity values -9.7 kcal/mol and -8.7 kcal/mol.

Table 2: Scoring function for binding affinity of protein and ligand interactions

No	Ligand	PubChem ID	Binding affinity
			Kgp
1	Lupenyl acetate	92157	-8.9
2	α-amyrin	73170	-9.7
3	Phytol	5280435	-4.5
4	β-amyrenil acetate	73145	-9.3
5	Methyl ester	5319706	-8.7

The interaction between α-amyrin and the other compounds with Kgp protein can be observed in fig. 1. The compound α-amyrin binding to Kgp targeted receptors with pi-alkyl interactions (PHE533 and TYR407) and alkyl interactions (LEU546 and HIS412). However, the visualization results indicate that α-amyrin has no hydrogen binding interaction with both receptors. The visual representation highlights a clear interaction occurring between the proteins of bacteria and the ligand originating from *C. gigantea*.

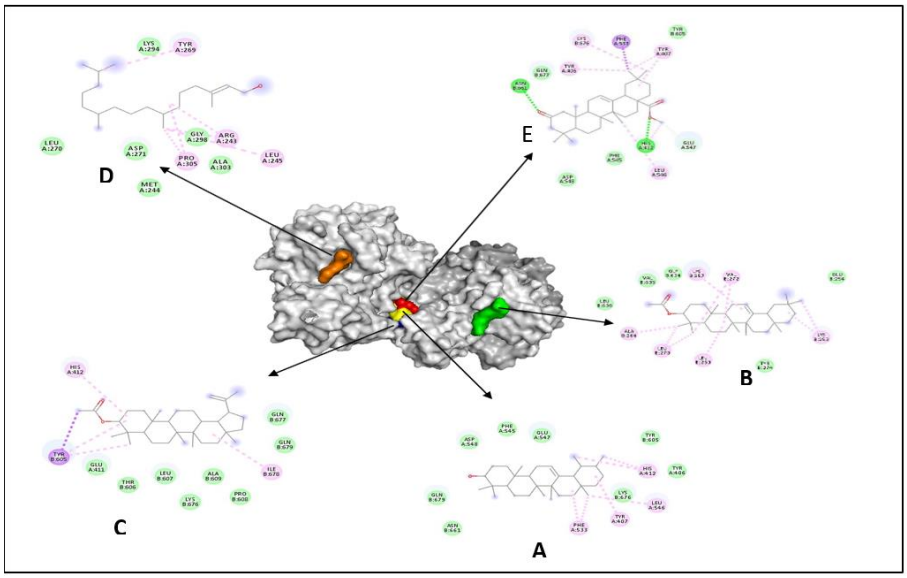

Fig. 1: Visualization of the interaction of α-amyrin with Kgp receptors and the metabolites (ligand of *C. gigantea* such as: (A) α-amyrin, (B) β-amyrenil acetate, (C) Lupenyl acetate, (D) Phytol, and (E) Methyl ester

The interaction outcomes resulting from the five compounds contained in *C. gigantea* with bacterial proteins from *P. gingivalis* can be observed in the table below. From the table, it is evident that only methyl ester exhibits hydrogen and hydrogen carbon interactions (table 3).

Table 3: Ligand interactions with Kgp bacterial protein of *P. gingivalis*

Compounds	Interaction type of Kgp (*P. gingivalis*)					
	Hidrogen	**Alkyl**	**Pi-Alkyl**	**Carbon hydrogen**	**Pi-Sigma**	**Van deer waals**
Lupenyl Acetate	-	ILE678	HIS412	-	TYR605	GLN667 GN678 PTO608 ALA609 LYS609 LYS676 LEU607 THR606 GLU411
α-amyrin	-	LEU546	PHE533 TYR407 HIS412	-	-	ASP548 PHE545 GLU547 TYR605 TYR406 LYS676 GLN679 ASN661
Phytol	-	ARG243 LEU245 PRO305	TYR269	-	-	LYS294 LEU270 ASP271 GLY298 ALA303 MET244
β-amyrenil acetate	-	LYS257 LYS253 LEU260 LEU270 ALA264 VAL272	-	-	-	GLU254 TYR274 LEU636 VAL635 GLY634
Methyl ester	ASN661 HIS412	LYS676	GLU406 TYR47	GLU547	PHE533	PHE545 ASP548 GLN677 TYR605

ILE: Isoleusin, HIS: Histidin, TYR: Tirosin, GLN: Glutamin, ALA: Alanan, LYS: Lisin, LEU: Leusin GLU: Asam glutamate, PHE: Fenilalanin, ASP: Asam aspartate, ASN: Asparagin, ARG: Arginin, PRO: Prolin, MET: Metionin, VAL: Valin, GLY: Glisin, SER: Serin, THR: Treonim

DISCUSSION

The research was conducted with biomolecular, which is a study conducted using computer simulation with molecular docking. Molecular docking is done on *C. gigantea* leaf metabolites from the region of geothermal Ie jue with Kgp protein from *P. gingivalis* as bacteria that cause halitosis. Compounds that act as ligands are metabolite compounds from the ethanolic extract of the *C. gigantea* leaf, which has been analyzed by GC-MS by previous researchers. The Kgp protein in *P. gingivalis* is part of the gingipain structure, which is essential for the survival of bacteria and the pathological processes of bacteria [17]. Meanwhile, the protein plays a role in the formation of VSC [18].

The 3D configuration of Kgp protein was acquired as receptor molecules (macromolecules) from the official Protein Data Bank (PDB) website in. pdb format. Ligands, which were chosen based on their highest area percentages from GC-MS analysis, included lupenyl acetate, α-amyrin, phytol, β-amyrenyl acetate, and methyl ester. These five chosen compounds underwent scrutiny utilizing Christopher A. Lipinski's Rule of Five (2004) to assess their potential pharmacological and biological activities for oral drug efficacy [19]. The investigation determined that none of the selected compounds met Lipinski's criteria, particularly with respect to Log P values exceeding 5. Log P values indicate the lipophilicity of a compound, where heightened lipophilicity can influence drug solubility, selectivity, potency, and permeability. Drugs with poor solubility often result in inadequate absorption and distribution. Furthermore, compounds might tend to interact with non-target hydrophobic proteins, leading to potential toxic effects. Despite these findings, all five selected compounds met the minimal requirements of Lipinski's rule and remain suitable as ligands for this study [20].

Results of molecular docking this research is the value of binding affinity, which is the strength of the interaction between the receptor and ligands, as well as visualizations to see interactions occurring between the receptor and the ligand. Results docking of each compound has 9 conformational ratings (table 3). Every conformation has a binding affinity value different, which indicates the stability of the position ligands which, when visualized, conform selected based on the value of binding affinity lowest [21]. The results showed that the value of binding α-amyrin affinity had more potential as an antibacterial against halitosis-causing bacteria *P. gingivalis* compared to the other four compounds.

According to the results of the interaction described above, α-amyrin does not interact with either receptor in a hydrogen-binding manner, although it performs better than some other molecules that do. This is due to the fact that the interaction between a receptor and ligands depends on hydrogen bonds as well as hydrophobic binding between the receptor and ligand or dominating bonds. Alkyl, pi-alkyl, and Van der Waals interactions are the main components of-amyrin's interactions with both receptors. By creating a cohesive environment to stabilize the complex bonds created it significantly influences the stabilization of the binding structure. Hydrophobic connections are also clearly highly important in this situation. However, the software employed in this investigation was unable to visualize hydrophobic interactions formed by ligand and receptor. Therefore, additional study is required for comprehension of the interaction.

CONCLUSION

According to the study's results, *Porphyromonas gingivalis* bacterial protein receptors can bind with the five metabolites of *C. gigantea* leaves extract. With a binding affinity value of-9.7 kcal/mol on Kgp protein, the-amyrin compound molecule has the best binding affinity value when compared to each of the other compounds, according to

the docking results. Based on docking molecular studies, it may be deduced that the metabolite chemicals in the ethanol extract of *C. gigantea* leaves extract from the geothermal area of Ie Jue have the potential to be an antibacterial agent toward the bacteria *P. gingivalis*, a bacteria that causes halitosis.

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AUTHORS CONTRIBUTIONS

Diana Setya Ningsih-Conceptualization, writing, original draft preparation; Rinaldi Idroes-Conceptualization, writing, edit preparation; Khairan Khairan-Conceptualization, visualization; Subhaini Jakfar-Methodology; Iin Sundari-Supervision; Viona Diansari-Methodology; Sri Fitriani-Visualization; Afri Handayani-Software; Inas Aqifah-Software

CONFLICT OF INTERESTS

Declared none

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