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Invitro Activity and Docking Approach *In Silico* Leaf Extract *Syzygium polyanthum* (Wight) Walp. as a *Salmonella typhi* Inhibitor

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Abstract

Indonesian people had long used plants as traditional medicine. In addition to maintaining health, traditional medicine was in great demand and prevents disease. We often encounter *syzygium polyanthum* leaves as a complement in tamarind and pepes, which were believed to act as a preservative. This study aimed to determine qualitatively bay leaf extract as an antimicrobial, *Salmonella typhi*. Methanol extraction of *syzygium polyantum* leaves would be partitioned with hexane and ethyl acetate solvents. The anti-microbial potential was shown in the Maximum Inhibitory Concentration (MIC) test, the identification of the compound using GC-MS and its potential as antibacterial was shown by docking the molecule using docking *in silico* technique against 5L3J protein. The MIC results were shown that the hexane partition at 100 ppm had 22.09 mm inhibition and the ethyl acetate partition at 80 ppm, had 18.15 mm inhibition. The GC-MS' testing resulted of syzygium polianthum leaf extract showed the presence of alpha-pinene, alpha-cubenene, 1H-Cyclopropa [a] naphthalene, nerolidol, humulene epoxide II, phytol, 9,12,15-Octadecatrien-1-ol, Squalene, Alpha-tocopherol, Beta-sitosterol, azulene, valence, Azulene and beta-Panasinen. However, the lowest binding affinity value was obtained from the cytosterol compound of −9.45 kcal/mol in ethyl acetate and Valencene solutions of −6.93 kcal/mol in n-hexane solvent. While the positive control-Chloramphenicol was −36.93 kcal/mol. This means that the *syzygium polyanthum* leaf extract could be used as an alternative medicine for typhoid.

Keywords: *Syzygium polyanthum* (Wight) Walp., Leaf extract, MIC test, GC-MS, Docking *in silico*

Introduction

For a long time, traditional medicine had been known throughout the world, including Indonesia, which had various kinds of plants. Indonesian people had long used traditional medicine in the form of plants or natural ingredients. Traditional medicine was in great demand to maintain health and prevent disease because it had several advantages over synthetic drugs [1]. Traditional medicine used a lot of ingredients from plants which were then mixed traditionally and the uses were obtained based on experience.

The progress of science and technology which is getting faster and more sophisticated was not able to change the role of traditional medicine. So far, we did not realize that some kitchen ingredients for cooking and ornamental plants in the yard could be used and had the efficacy of treating diseases [2,3].

Infectious disease was one of the problems in the health sector that continued to develop from time to time. Infection is a disease that could be transmitted from 1 person to another or from animals to humans. Infection was caused by various microorganisms such as bacteria, viruses, fungi and protozoa [4]. Various anti-infective drugs such as antibiotics were 1 group that has been selected. The emergence of resistance had caused one particular group of antibiotics to no longer be used in therapy, on the other hand, the high price of antibiotics had made the economically weak community unable to afford them, so the use of various plants in the treatment of infectious diseases could be an option for Indonesians [5].

The chemical content of *Syzygium polyanthum* leaves was 0.05 % essential oil (citral and eugenol), tannins and flavonoids. *Syzygium polyanthum* leaves oil consisted of simple phenol, phenolic acid,

sequisterfenoid and lactone [3]. The content of essential oils, tannins, and flavonoids produced antimicrobial activity. Based on the above background, research was conducted on the efficacy of *Syzygium polyanthum* leaves as an antimicrobial against several pathogenic bacteria that could infect the human body, namely *Salmonella typhi*.

Materials and methods

This research was conducted in the Basic Chemistry Laboratory of the Untirta Faculty of Engineering, the Chemical Computing Laboratory of the Untirta Engineering Faculty, and the Microbiology Laboratory of PT Samco Farma. GC-MS analysis was carried out at the DKI Jakarta Regional Health Laboratory. The research took place from May 2019 to January 2020. The research design was described schematically in **Figure 1**. This research includes qualitative testing of flavonoids, antibacterial testing, GCMS testing and bioinformatics studies through docking *in silico*.

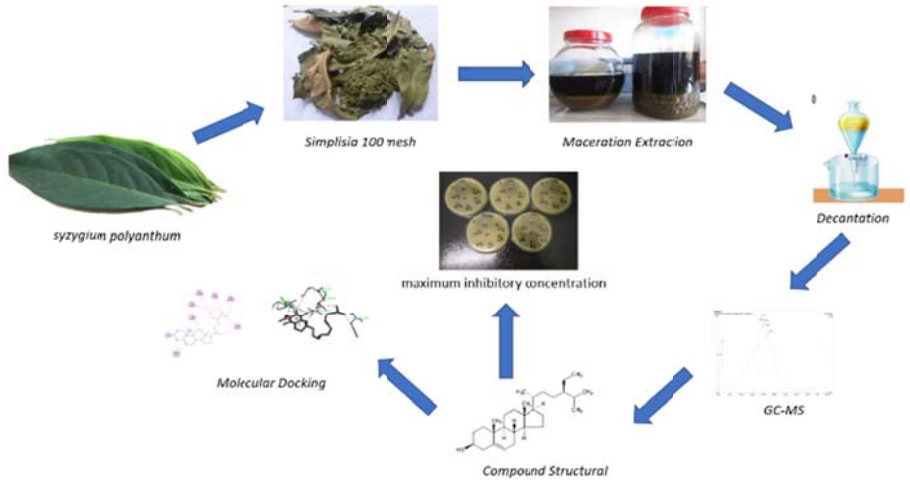


Figure 1 Research schematic.

Simplicia preparation [6]

Fresh *Syzygium polyanthum* leaves were sorted and washed then dried in the air underneat the sun light to minimize the amount of water contained in the leaves. The leaves were crushed using a mixer to form a dry powder with a size of 50 - 100 mesh and then weighed. The water conthent should be under 10 %.

Syzygium polyanthum leaf extract preparation [6]

Syzygium polyanthum simplisia was macerated with ethanol 96 % solvent for 3 days. Every day, the solvent was replaced with a new solvent. The filtrate was separated from the waste, then partitioned using n-hexane and ethyl acetate. Then, the filtrate was separated into 2, let stand for a few minutes, and the top was taken. Furthermore, the filtrate was concentrated using a rotary evaporator until the solvent content residue of 10 - 15 %. The concentrated filtrate was proceeded to dry in a vacuum oven for 18 h at a temperature of 40 ± 5 °C. The result was a thick extract formed. This thick extract was thought to be a *Syzygium polyanthum* crude extract.

Agar diffusion method [8]

Inoculum was prepared by dissolving 3 to 5 colonies of *Salmonella typhi* ATCC bacteria from 24 h agar culture in sterile saline. Turbidity was adjusted until 0.5 Mc Farl was obtained. Muller Hinton Agar (20 mL) was poured into each of the 90 mm Petri dishes. The bacterial suspension (100 µL) was evenly spread on the MH media using a Pasteur pipette and allowed to dry. Using a sterile borer, the well with a diameter of 6 mm was swabbed into the agar. 60 µL, each of the test solution, and negative control (60 µL salina) and positive control (chloramphenicol) were added carefully into the well designated on the agar surface containing bacteria. The plates were stored for 30 min and then incubated at 37 ± 1 °C for 24 h. The plates were checked for growth and the zone of resistance measured using a ruler. The endpoint of inhibition is where growth begins.

GC-MS method [9]

The instruments used were the Agilent Technologies 7890-Gas Chromatograph with Auto Sampler and 5975 Mass Selective Detector as well as the Chemstation data system. With electron impact 70 eV ionization mode. HP Ultra 2 capillary column Length 30 m, ID 0.20 mm, film thickness 0.11 mm. The temperature in the oven, namely the initial temperature at 80 ℃, hold for 0 min, increase at 3 ℃/min to 150 ℃ and finally increase 20 ℃/min to 280 ℃. Injection port temperature, Ion Source Temperature, Interface Temperature, Quadrupole Temperature are 250, 230, 280 and 140 ℃, respectively. Helium is a carrier gas. 1.2 mL/min that constant flow column mode. Injection volume 1 mL.

Docking molecular method [6,10-12]

Protein preparation

The receptor protein is obtained from https://www.rcsb.org/structure/5L3J with code 5L3J was downloaded and saved in PDB format (*.pdb). Next to preparation using the Discovery Studio software, unused ligands or molecules such as water molecules were removed. Then the protein preparation was stored in pdb (*. Pdb) format. In AD4 protein preparation, the missing atoms were identified and repaired. The polar hydrogen atoms and Gasteiger charge were added into protein. The protein molecule was stored in pdbqt (*.pdbqt).

Ligand preparation

In this test, the ligands were compounded from the fractionation tested by GC-MS with an abundance of more than 1 % and qualification/similarity more than 90 %. The structure of the compound was confirmed via PubChem http://pubChem.ncbi.nml.nih.gov and downloaded in the SDF format (*.sdf). The next step was using Chem3D Ultra to minimize the steric energy and storing the structure in * .pdb form.

Docking molecular

The prepared receptors were opened in AutoDock software and the format was converted to PDBQT. Then the ligand was converted in its pair formation and stored as *. pdbqt. After the ligands and receivers had been prepared, a working area (grid box) was made after the positive control grid box and followed the docking process. After the docking process was completed, the bonding affinity was stored and the docking ligand was analysed for the bonding site via the Discovery Studio visualization device. The validation structure was performed in Pymol to measure the RMSD of less than 2 A.

Results and discussion

Maximum inhibitory concentration test results

The antibacterial activity test in this study used 4 treatment groups with a concentration of bay leaf extract 100, 75, 50 and 25 ppm and 2 control groups, namely positive control and negative control. The positive control was chloramphenicol 5 µg/disk. Chloramphenicol is a potent antibiotic and bactericidal compound against *Salmonella* species with a sensitivity value of 99.05 % and a resistance effect of 0.95 % [13]. Tween 20 is a nonionic surfactant that is non-toxic and can unite the non-polar (lipophilic) and polar (hydrophilic) parts of secondary metabolite compounds. The concentration of tween 20 as a negative control is usually 2 - 8 % [14].

Table 1 MIC Testing against *Salmonella typhi*.

Solvent	Concentration ppm				Positive Control*	Negative Control**
	20	50	80	100		
Ethyl Acetic Partition	9.85	12.65	14.75	13.65	35.22	6.00
n-Hexane Partition	8.65	9.70	12.35	22.10	34.85	6.00

*Kloramfenikol, 5 ppm; **Tweet 20, 5 %

The results of the antibacterial test obtained that the hexane solvent partition at a concentration of 100 ppm had a clear zone with a diameter of 22.10 mm. The more activity of the antibacterial compounds contained will cause inhibition of bacterial growth, this is indicated by a diameter of clear area around the test specimen's *Syzygium polyanthum*. The ethanol extract of *Syzygium polyanthum* leaves has an

antibacterial effect against *Salmonella sp.* Bacteria with a clear zone in the range of 21 - 27 mm [15]. The concentration of the sample have antibacterial effect is stronger if it has a diameter range between 10 - 20 mm [16]. Although its inhibitory ability against *Salmonella typhi* is still below the ability of chloramphenicol, because the sample used is still in the form of a partition which is a collection of non-pure compounds [17].

GC-MS test results

The results of the GC-MS analysis on the partition of the n-hexane solvent and the toluene solvent were taken for only a few compounds in which the % of abundance was greater than 1 %. The majority of the results were for the partition of ethyl acetate: Squalene (8 %), Beta-sytostero (4.9 %), Phytol (5.7 %), Nertolidol (3 %), 1H-cyclopropanaphthalene (2.2 %) and Alpha-tocopherol (4.6 %). At partion of n-hexane: Valence (4.9 %), alpha-pinene (4.9 %), alpha-Cubebone (3.6 %), nerorolidol (2.8 %) and Humulene epoxy II (2 %). Although the results for the 18 compounds presented in **Tables 2** and **3** are the same as with Rahim *et al.* [18].

Table 2 n-hexane partition compound in GC-MS result.

No	Compound	Empiric Structure	Molecular Weight (mol/gr)	Retention Time	% Peak
1	Valence	$C_{15}H_{24}$	204	8.072	4.921
2	Alpha-Panasinen	$C_{15}H_{24}$	204	34.848	1.515
3	Nerolidol	$C_{15}H_{26}$	206	36.417	2.845
4	Humulene Epoxide II	$C_{15}H_{24}O$	220	38.772	2.060
5	Alpha- Cubebene	$C_{15}H_{24}$	204	28.330	3.633
6	Azulene	$C_{15}H_{24}$	204	33.538	1.255
7	Alpha-pinene	$C_{10}H_{24}$	174	8.072	4.921

Source: Test Resulted of GC-MS from n-hexane partition's *syzygium polyanthum leaves*

Table 3 Ethyl acetate partition compound in GC-MS result.

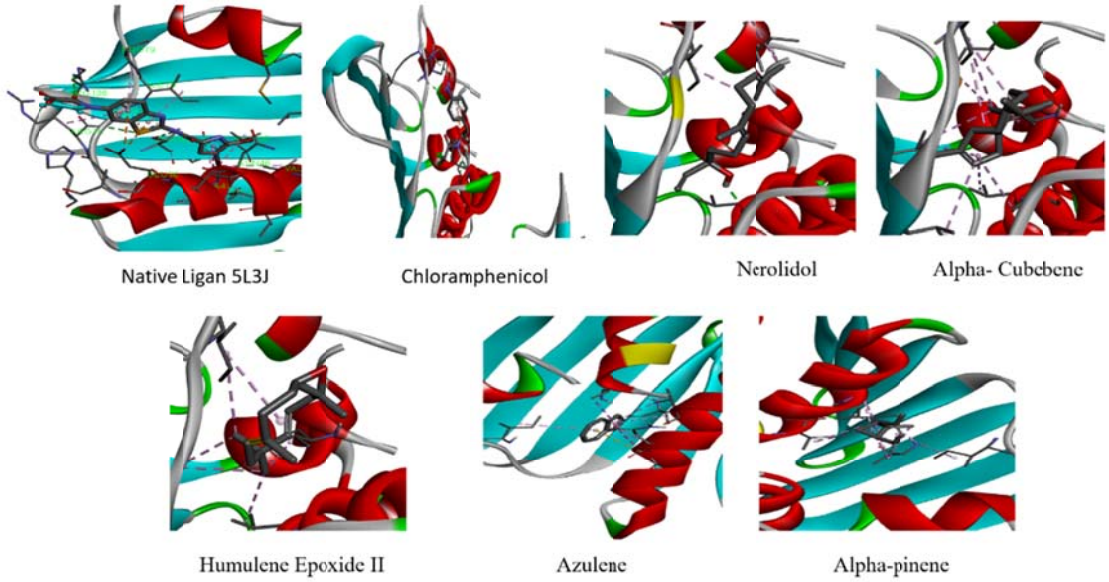
No	Compound	Empiric Structure	Molecular Weight (mol/gr)	Retention Tine	% Peak
1	Alpha-pinene	C10H16	136.24	8.076	1.068
2	Alpha-cubenene	C15H24	204.35	28333	1.690
3	1H-Cyclopropa[a]naphthalene	C15H24	204	33894	2.226
4	Nerolidol	$C_{15}H_{26}O$	222	36423	3.085
5	Humulene epoxide II	$C_{15}H_{24}O$	220	38782	1.557
6	Phytol	$C_{20}H_{40}O$	296	56151	5.715
7	9,12,15-Octadecatrien-1-ol	$C_{18}H_{32}O$	264	57341	1.037
8	Squalene	$C_{30}H_{62}$	422	75455	8.345
9	Alpha-tocopherol	$C_{29}H_{50}O_2$	430	83081	4.660
10	Beta-sitosterol	$C_{29}H_{50}O$	414	88038	4.959
11	Neophytadiene	C20H38	278	46723	1.347

Source: Test Resulted of GC-MS from ethyl acetate partition's *syzygium polyanthum leaves*

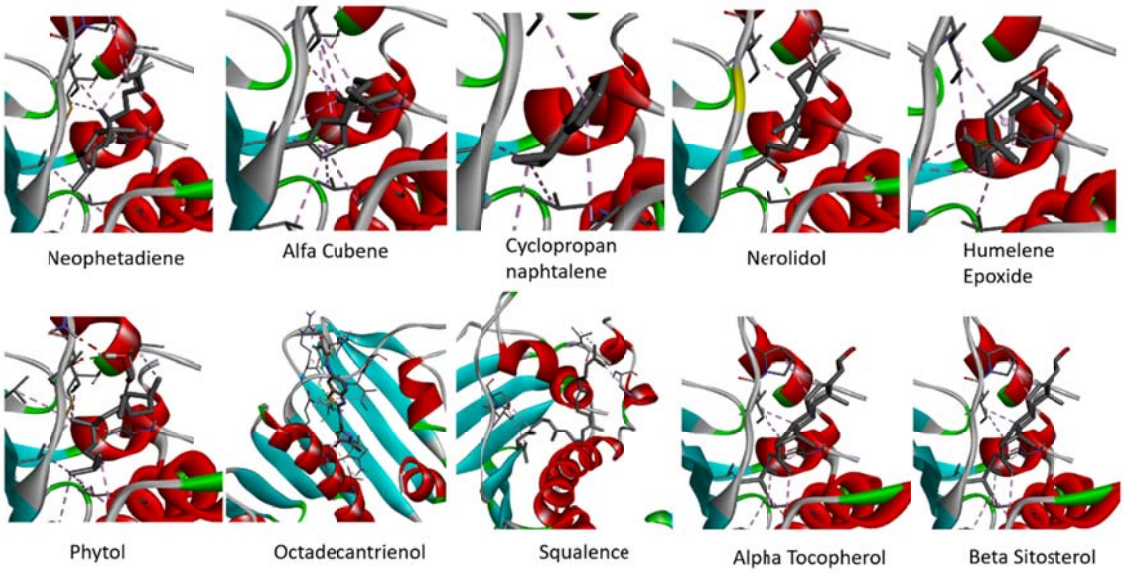
Docking *in silico* result

The interaction that occurs between the compounds resulting from the partitioning of n-hexane and toluene with the receptor is indicated by the binding affinity value, the lower the binding affinity value,

the stronger the interaction between the ligand and the receptor. Test results were compared with positive check antibiotics, chloramphenicol [19]. The docking method is known as valid if the RMSD value is less than 2 Å [20]. The lower the RMSD value, the better the expected position of the ligand, because the closer the original conformation to [21]. Meanwhile, the lower the affinity value of the bond, the lower the energy required for the bond. In addition, the compounds have varied as ligands have been divided into 2, namely the variation in the ethyl acetate solvent and the hexane solvent.



(A)



(B)

Figure 3 (A) Hydrogen bondings n-hexane partition ligands - 5LJ3 receptors, and (B) Hydrogen bondings ethyl acetate partition ligands - 5LJ3 receptors.

The results of the validation of the comparative ligands had a Binding Affinity value of −6.93 kcal/mol, an RMSD value of 0 Å.

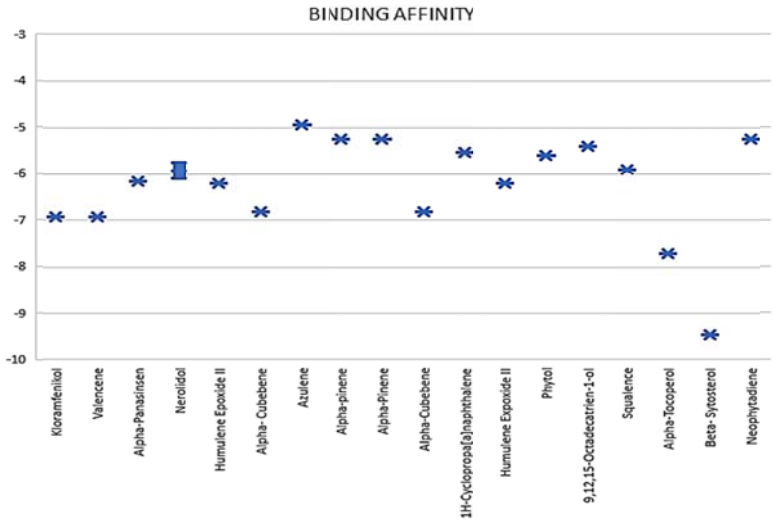


Figure 4 Binding affinity value ligand - receptor 5L3J.

The results obtained from the docking process of ethyl acetate solvent indicated that the ligand variation which had the greatest binding affinity value was cytosterol ligand with a value of −9.45 kcal/mol which was closest to the binding ligand and had an RMSD value of 0.0 Å. This compound has several residues viz. PRO79, ILE 78, ALA 47, VAL 167, VAL 71, VAL 43 and VAL 120. Judging from the results of several other compounds that have been analyzed, Sytosterol has a smaller binding affinity and it can be stated that the compound can be used as an antibacterial agent [20].

Table 4 Amino acid residue result ligand - 5L3J.

LIGAN	AMINO ACID RESIDU																
	ARG136	GLY77	ILE78	ALA47	ASN46	VAL43	VAL167	VAL71	ILE78	VAL120	PRO'9	MET95	ILE94	LEU132	VAL97	HIS99	PRO79
Kloramfenikol				V		V	V		V	V	V	V					
1H-Cyclopropa[a]naphthalene			V	V			V										
9,12,15-Octadecatrien-1-ol	V	V		V													
Alpha- Cubebene			V	V		V	V	V	V	V		V					
Alpha-Panasinsen			V							V			V				
Alpha-Pinene			V	V		V	V	V									
Alpha-Tocoperol			V				V			V		V	V			V	
Azulene				V	V	V	V	V	V								
Beta- Sytosterol				V		V	V	V	V	V	V						
Humulene Expoxide II			V			V	V		V	V							
Neophytadiene				V		V	V	V	V	V	V	V	V				
Nerolidol			V			V			V		V		V				
Phytol	V	V		V		V	V	V	V	V		V	V	V			
Squalence			V												V	V	V
Valencene				V		V	V		V	V	V	V					

Figure 4 shows to the docking results of the variation of ligands in the hexane solvent, it can be observed that the variation of ligands that have the closest binding affinity value is Valencene with a value of −6.93 kcal/mol which has an RMSD value of 0.0 Å. In theory, the compound that binds to has an RMSD value below or equal to 2Å can be said to be valid. This compound has residual compounds such as ALE 78, ALA 47, VAL 43, VAL 167, VAL 120, PRO 79 and MET 95. The binding affinity of this compound is lower than other compounds and can be said to be used as anti-bacterial. The mechanism of *Syzygium polyanthum* compounds (alpha-pinene, alpha-cubenene, 1H-Cyclopropa [a] naphthalene, nerolidol, humulene epoxide II, phytol, 9,12,15-Octadecatrien-1-ol, Squalene, Alpha-tocopherol, Beta-sitosterol, azulene, valence and beta- Panasinen) as antibacterial work by inhibiting the action of DNA gyrase (Receptor 5l3J model). DNA gyrase is an antibacterial target for chloramphenicol antibiotics, as

the enzyme is present in prokaryotic cells and is important for bacterial growth [20]. Furthermore, DNA gyrases play a role in regulating the topology of DNA in cells. This enzyme requires ATP hydrolisis energy to negatively catalyze supercoiling. Hydrolysis of PTA is a task of sub-unit B of DNA gyrases [21]. As a result, when DNA gyrase is bound by antibiotics, DNA synthesis will be disrupted and eventually cause cell death [22].

Conclusions

The antimicrobial test of *Syzygium polyanthum* leaves showed that the highest diameter hexane was 22.09 mm at 100 ppm. GCMS results show that the presence of alpha-pinene, alpha-cubenene, 1H-Cyclopropa [a] naphthalene, nerolidol, humulene epoxide II, phytol, 9,12,15-Octadecatrien-1-ol, Squalene, Alpha-tocopherol, Beta-sitosterol , azulene, valence and beta- Panasinen. Evidence of its potential as a drug can be seen from the docking results, which show that the highest binding affinity value of sytosterol is −9.45 kcal/mol in ethyl acetate solution and the highest binding affinity value of the Valencene hexane solution with a value of −6.93 kcal/mol. Then the bay leaf extract can be used as an alternative medicine for typhoid.

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