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

LC-MS Based Metabolite Profiling Leaves Extract of *Pluchea indica* With Antioxidant Activity

Afidatul Muadifah[1,*], Dara Pranidya Tilarso[2], Momodou Salieu Sowe[3], Indra Lasmana Tarigan[4], Khoirul Ngibad[5], Sonia Devi Yuliantari[6]

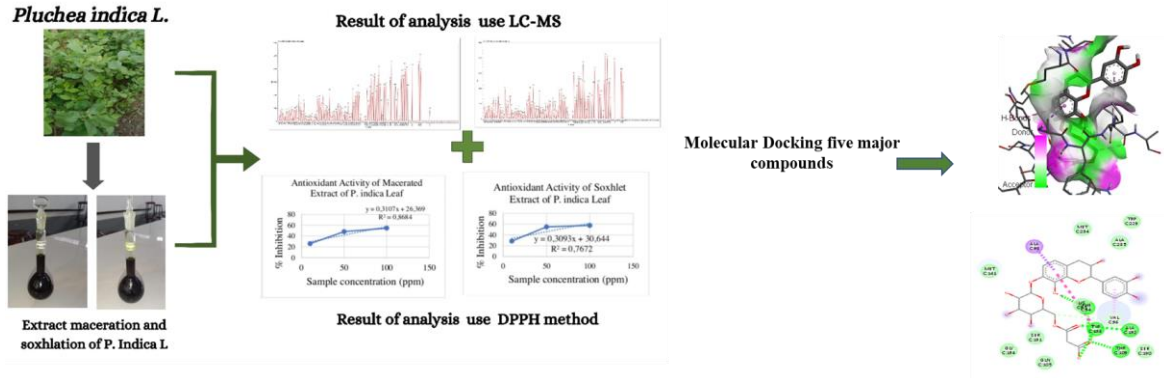
[1,2,6]*Department of Pharmacy, STIKES Karya Putra Bangsa, Tulungagung, Indonesia*
[3]*Departement of Chemistry, University of The Gambia, Gambia*
[4]*Department of Chemistry, Faculty of Science and Technology, Universitas Jambi, Jambi, Indonesia*
[5]*Department of D3 Health Analyst, Maarif Hasyim Latif University, Sidoarjo, Indonesia*

Abstract

Pluchea indica, a plant known for producing secondary metabolites such as flavonoids, alkaloids, tannins, saponins, terpenoids, and phenols, exhibits notable antioxidant activity. This study aimed to compare the antioxidant compound profiles of *P. indica* leaves using LC-MS analysis and evaluate their antioxidant activities through DPPH assays. Additionally, molecular docking was conducted to predict the binding interactions between NADPH oxidase (receptor) and selected small-molecule ligands (compounds from *P. indica*). Leaf extracts of *P. indica* obtained through maceration and Soxhlet extraction were analyzed via LC-MS to identify their chemical composition. Antioxidant activity was assessed using the DPPH assay at three concentration levels: 10 ppm, 50 ppm, and 100 ppm, with absorbance measured at 515 nm using a UV-vis spectrophotometer. Furthermore, molecular docking simulations were performed for the top five compounds against the NADPH oxidase receptor (PDB ID: 4Z3D) to determine their antioxidant potential. The LC-MS analysis of Soxhlet-extracted *P. indica* leaves identified 112 compounds, with the top five being Kaempferol 3-glucosyl-(1→2)-[glucosyl-(1→3)-rhamnoside], Quercetin 3-glucosyl-(1→2)-rhamnoside-7-glucoside, Luteolin-7-O-(6''-malonylglucoside), 5,7,4'-trihydroxy-6,3'-dimethoxyisoflavone 7-O-(6''-glucosylglucoside), and 5,7,4'-trihydroxy-6-methoxyisoflavone 4'-O-(6''-glucosylglucoside). Antioxidant activity assays revealed IC_{50} values of 76.76 ppm and 62.58 ppm for maceration and Soxhlet extracts, respectively. Molecular docking results highlighted 5,7,4'-trihydroxy-6-methoxyisoflavone 4'-O-(6''-glucosylglucoside) as having the best binding affinity, with a free energy value of -5.58 kcal/mol, an inhibition constant of 51.14 µM, and five hydrogen bonds involving GLN 105, PHE 94, VAL 96, SER 139, and ALA 235.

Keywords: *: Antioxidant, LC-MS, Molecular Docking, Pluchea indica*

Graphical Abstract



* Corresponding author
Email addresses: afidatul.muadifah@stikes-kartrasa.ac.id

DOI: https://doi.org/10.22437/chp.v8i2.36869
Received October 09th 2024; **Accepted** December 20th 2024; **Available online** December 31st 2024

Introduction

In the modern era, supported by the development of technology and science, changes in people's lifestyles have a negative impact on health. Lifestyle changes can lead to a decline in people's quality of life due to a decrease in the production of antioxidant compounds in the body, which are compounds used to neutralize the presence of free radicals, thus triggering oxidative stress [1]. Oxidative stress is a condition where the number of free radicals is not balanced with the number of antioxidants in the body. Oxidative stress can trigger the aging process and the onset of degenerative diseases, one of which is cancer [2]. One of the causes of cancer is the presence of free radicals that attack human body cells; these free radicals are the leading cause of cell damage so that they can trigger cancer [3]. The existence of oxidative stress causes the human body to lack an excess supply of antioxidants, there by requiring the use of exogenous antioxidants [4]. Exogenous antioxidants are obtained from foods that enter the body and play a crucial role in fighting excess free radicals in the body [5].

One plant that contains bioactive compounds that act as antioxidants is *Pluchea indica (P. indica)*, which grows wild. The antioxidant content in the leaves of *P. indica* can inhibit free radical reactions by binding free radicals and damage to cells [6]. Research antioxidant of *P. indica* leaves in maceration extract using 96% ethanol is classified as very strong, which produces an IC_{50} value of 37.25 ppm [7]. Antioxidant activity in *P. indica* leaves extracted by Soxhletation with a % inhibition value of 51.681 for saline *P. indica* and 88.377 for non-saline *P. indica* [8]. Based on the relevance of the research, it can be concluded that *P. indica* leaves can be extracted using cold and hot methods. The extraction carried out is by maceration and Soxhletation. Maceration and Soxhletation are methods that have differences in the temperature used during the extraction process. Maceration and Soxhletation both experience the soaking process, but for Soxhletation, the soaking process occurs after the condensation process [9]. The research aims to determine the antioxidant effect of compounds in *P. indica* leaves extracted by cold and hot extraction identified by Liquid Chromatography-Mass Spectrometry (LC-MS). We investigated the effect of maceration and Soxhletation methods on the compound content of *P. indica* leaves. The maceration and soxhlet extracts of *P. indica* leaves were then tested using the DPPH method to identify potential antioxidant compounds. Afterthan, molecular docking: compounds in maceration and soxhletation results of *P. indica* leaves towards NADPH oxisade receptors (pdb id:4z3d) as antioxidant

Material and Methods

Materials and Instrumentations

P. indica leaves were collected from Krandegan Hamlet, RT 002 RW 002, Kalidawir Village, Kalidawir Sub-district, Tulungagung Regency, Indonesia. The materials used were *Pluchea indica* leaves as samples, chloralhydrate reagent, 10% HCl reagent, 96% ethanol (absolute), distilled water, Mg powder, Mayer reagent, Wagner, Dragendorff, chloroform, ammonia, sulfuric acid, 2N HCl, $FeCl_3$, DPPH compound (2,2-diphenyl-2-picrylhydrazili) is a synthetic with free radical and pure vitamin C (ascorbic acid) which acts as a positive control. The main materials used in molecular docking were the five highest compounds from LCMS results stored in pdb format in 3D. The receptor structures (target proteins) of IL-10 (1LQS) and IFN-γ (3BES) are also stored in pdb format on the respective database webservers. The tools used are an LCMS (Liquid et al.) instrument (Shimadzu LCMS-800) and to determine the category of antioxidant compounds using a UV-VIS spectrophotometer instrument (N4S), which will provide data in the form of absorbance to calculate IC_{50}. Other tools are a set of Soxhletation apparatus, a set of maceration tools, a set of glass tools (Pyrex), a microscope, analytical scales (Acis), black cloth to cover the sample during the drying process, a blender, a lab spatula, aluminum foil, water bath for the concentration process, mesh 60 sieves to sift *P. indica* leaf simplicia, filter paper, and spirit burner. The tools used in molecular docking are hardware, namely Lenovo PC IdeaCentre AIO 5i 24IAH7 F0GR006RID Strorm Gray (Intel Core i7 12700H, Win11 Home, 16GB DDR4, Intel ARC

A370M 4GB GDDR6) and PyRx software, ChemDraw Ultra version 22.0, Chem 3D version 22.0, AutoDockTools, Discovery Studio Visualizer 2021 and UCSF Chimera. The webserver used is RCSB (Research Collaboratory for Structural Bioinformatics).

Methods

Sample Preparation. Samples of *Pluchea indica* leaves were picked from Krandegan Hamlet, RT 002 RW 002, Kalidawir Village, Kalidawir Sub-district, Tulungagung Regency, Indonesia. The sampling technique is purposive sampling, which takes samples based on predetermined and considered criteria, namely non-random sampling [10]. *The collected Pluchea indica leaves were subjected* to the stages of wet sorting, washing using running water, chopping, drying with sunlight covered with black cloth, and dry sorting [11]. *Dried Pluchea indica* leaves were mashed using a blender and then meshed using a 60-sieve to achieve a uniform size that is not too fine or too coarse so that the distillation liquid can penetrate easily [12].

Simplicia Characteristic Test. Macroscopic Test: Macroscopic tests were carried out through organoleptic testing to determine the shape, color, smell, and taste of the simplicia, which were then compared with the literature [13].

Drying Shrinkage Test. Drying shrinkage is the weight reduction between the fresh sample and the dried simplicia, which describes the weight reduction of the material in the drying process and illustrates the simplicia lost due to the heating process [14].

Water Content Test. Water content testing was carried out by weighing the test sample in a porcelain cup containing 1 g and heating it at 105 ^{0}C for 30 mins. After 15 mins, it was cooled, and the powder was weighed again [12].

Extraction. This study carries out two extractions: maceration and Soxhletation. The method of maceration was carried out by soaking 200 g of dry leaves of P. indica L in 100 ml of 96% ethanol for three days. The leaves were then filtered and concentrated using a water bath at 50^{o}C, obtaining a thick extract [15]. Soxhletation was carried out using a solvent of 96% ethanol, as much as 500 ml. This extraction process was carried out for 15 cycles, then filtered, and the filtrate was taken to concentrate using a water bath with a temperature of 50^{o}C to obtain a thick extract [9].

Extract Characterization Test. Ethanol Free Test: The ethanol-free test aims to determine the presence of ethanol in an extract. Positive ethanol-free results are characterized by test extracts that do not smell of esters, which are typical of ethanol [16].

Analysis of Compounds Using LC-MS. Test content compound results of extracts of the leaves of *Pluchea indica* were carried out with LC-MS by applying the method of dissolving sample-free ethanol using methanol pa with a ratio of 1:5, i.e., with 2 mg of each leaf extract dissolved in 10 ml methanol pa. Stage protein precipitation with a filter using a 0.45 cellulose acetate filler micrometer was then conducted. The sample was injected with as much as 1 microlite into the LC-MS instrument. LC (Liquid Chromatography) is connected to a spectrometer mass Quadrupole Time- of flight (QTOF) equipped with source ionization Electrospray Ionization (ESI). The mass spectrometry (MS) is a QTOF system with a positive ionization mode. The instrument's Electrospray Ionization (ESI) parameters were set at a temperature capillary 350 ^{o}C and atomizer gas 60ML/HR, source voltage 5.0V. Full scan mode from m/z 100-5000 was performed with a temperature source of 100^{o}C. UPLC column used Shimadzu Shim Pack FC-ODS (2 mm x 150mm, 3µm) with eluent 95% ethanol and regulated water on rate total flow 0.5 mL/ min.

Antioxidant Activity Test Using DPPH Method. Determining the Optimum Wavelength of DPPH Solution: DPPH solution was made with a level of 50 ppm, weighing as much as 5 mg of DPPH dissolved in 100 ml of ethanol in a volumetric flask. DPPH solution is kept at a low temperature with minimal light. Determination of DPPH absorbance aims to identify how much of the sample can be absorbed by DPPH compounds. This determination is done by taking 4 ml of DPPH solution, leaving it for 30 minutes, and measuring the absorbance. The optimum wavelength of 50 ppm DPPH stock solution was

determined with a UV-Vis spectrophotometer in the 400-800 nm range [17].

Antioxidant Activity of Pluchea indica Leaf Extract and Vitamin C. Antioxidant activity was determined from the IC50 value calculated using the DPPH method with a UV-Vis spectrophotometer. *Pluchea indica leaf* extract was made in stock solution with a concentration of 100 ppm, then diluted with three concentration series of 10 ppm, 50 ppm, and 100 ppm. 2 mL was taken from each solution and put into a test tube. Each test tube was added with 4 mL of DPPH solution. The mixture was incubated in a dark room for 30 minutes. Then, the absorbance of the solution was measured using a UV-Vis spectrophotometer [17].

Vitamin C was made into three concentration series, namely 1 ppm, 5 ppm, and 10 ppm. Testing was done by pipetting 2 ml of sample solution from various concentrations. Each concentration was added with 4 ml of 50 ppm DPPH in a closed test tube and left to rest for 30 mins, after which the absorption was measured. The absorbance results were used to calculate the percent of free radical remission and then entered into the equation obtained from the linear regression curve to obtain the IC_{50} value [18].

Determination of Percentage of Antioxidant Activity of Pluchea indica Leaf Extract and Vitamin C. The IC_{50} value is calculated based on the measured absorbance data, and the percentage of immersion between the DPPH radical and the sample solution can be calculated using the equation 1, 2, and 3 [6].

$$\% \text{ Inhibition} = (BA-SA)/BA \times 100\% \quad \ldots\ldots\ldots\ldots (1)$$

BA: Blank absorbance; SA: Sample absorbance

The calculation of antioxidant activity was entered into the equation 2.

$$y = a+bx \quad \ldots\ldots\ldots\ldots (2)$$

with the concentration (µg/mL) as the abscissa (x-axis) and the % antioxidant activity value as the ordinate (y-axis) [17]. The IC_{50} calculation formula is:

$$IC_{50} = \frac{(50-a)}{b} \quad \ldots\ldots\ldots\ldots (3)$$

Ligand Structure Preparation. Ligand preparation was carried out by converting the 2-dimensional (2D) molecular structure of the five main compounds (LCMS results) drawn using ChemDraw Ultra version 22.0 then converted into a 3-dimensional (3D) structure model using the Chem3D application version 22.0 (pdb file format). Then add hydrogen ions to the ligand using Discovery Studio 2021 software and save in PDF file format. Furthermore, optimize the ligand using the AutoDockTools program, and adjust the number of torsion bonds on the ligand and save in pdbqt file format.

Macromolecular Preparation. The three-dimensional macromolecules NAHDP Oxidase were downloaded from the Protein Data Bank data site https://www.rcsb.org with PDB codes used are 4Z3D. Macromolecules are separated from solvents and native ligands or non-standard residues using the UCSF Chimera application. Native ligands and unnecessary residues are removed by clicking the select feature then clicking residues and selecting all nonstandard, then selecting the actions feature, clicking atoms/bonds then clicking delete. Macromolecular (receptor) files are saved in pdb format. Next, the macromolecules were optimized using AutoDockTools by adding hydrogen ions and Kollman charges and saved in pdbqt file format.

Molecular Docking. The molecular docking process was carried out using PyRx software based on AutoDock Tools. The macromolecular structure (receptor) and ligand that have been optimized separately are stored in one folder. The molecular docking process uses a grid box and energy minimization parameters according to the validation results. The grid box parameter settings are carried out using the grid box coordinates determined based on the receptor ligand coordinates used in the docking validation process. Furthermore, docking was carried out using PyRx software with the AutoDock wizard feature. The docking data displayed is in the form of binding affinity values and amino acid residue interactions. The docking results are stored in pdb format.

Visualization and Analysis of Docking Results. The visualization process is carried out to see the interactions that occur in the docking results between the receptor and ligand. Visualization of the docking results is carried out using Discovery Studio Visualizer 2021 software.

Results and Discussions

Simplicia Physicochemical Properties

The results of the macroscopic test of *P. indica* Leaf Simplicia are dark green, have a typical smell of *P. indica*, and have a strong taste. On the other hand, we did dried shrinkage test, we found the result approximately 9% which is fit to good simplicia standard.

Furthermore, the water content of simplicia also a part of important properties, which is less than 10% to avoid fungal growth because enzymatic reactions only occur when the water content is less than 10%. The results obtained are 8.2%; it can be concluded that the drying shrinkage of *P. indica* leaf simplicia is by quality standards to minimize contamination.

The results of the ethanol-free test of maceration and Soxhletation extracts do not smell typical of ethanol, meaning that maceration and Soxhletation extracts from *P. indica* leaves do not contain ethanol.

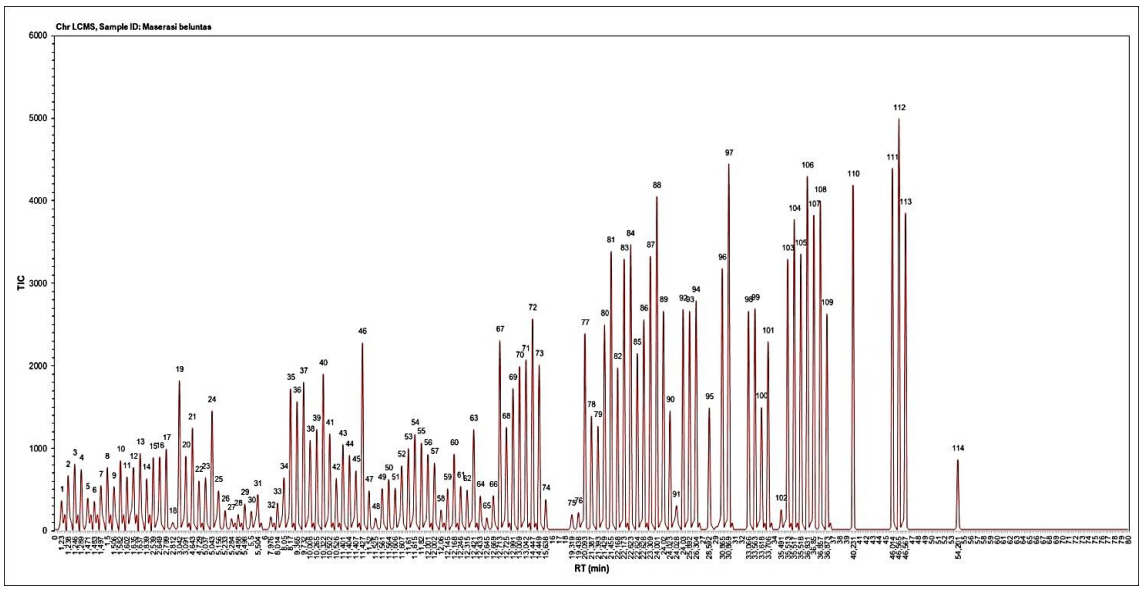


Figure 1. LC-MS Chromatogram of *P. indica extract* (maceration process) Analysis conditions: column Shimadzu Shim Pack FC-ODS (2mm x 150mm, 3µm); flow rate 0.5 mL/min; injection volume 1 µL; solvent ethanol 95%; column temperature 35 ºC

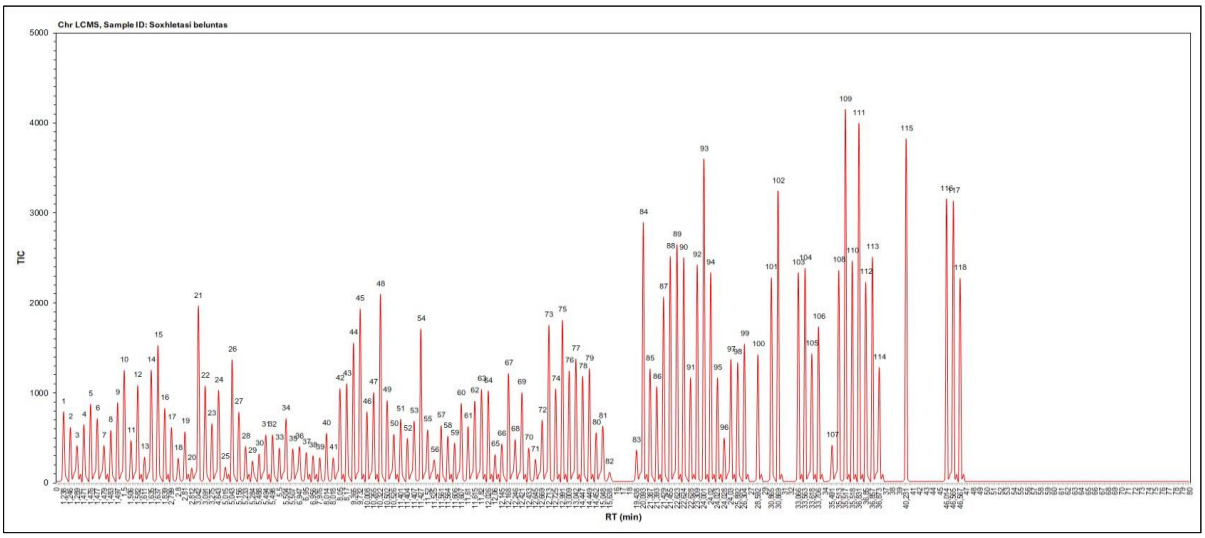
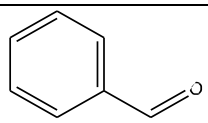
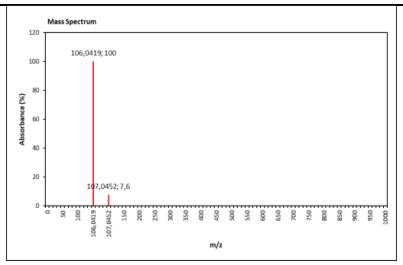
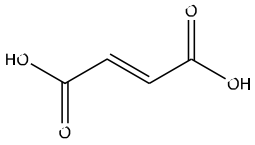
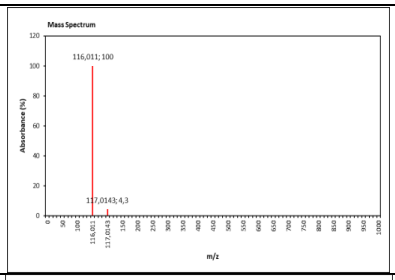
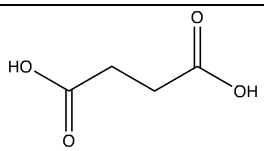
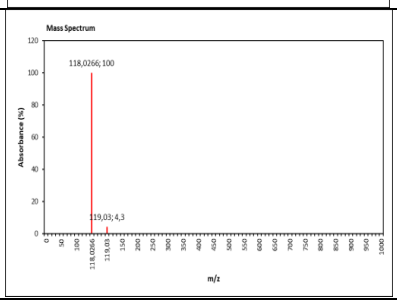


Figure 2. LC-MS Chromatogram Result of the *P. indica* soxhlet extract (Analysis conditions: column Shimadzu Shim Pack FC-ODS (2mm x 150mm, 3µm); flow rate 0.5 mL/min; injection volume 1 µL; solvent ethanol 95%; column temperature 35 °C).

Table 1. LC-MS Chromatogram Result of the *Pluchea indica* macerate

Peak number	RT (min)	Composition (%)	Compound Result		
			Analysis	Structure	Mass Spectrum
1	1,23	0,21302	**Benzaldehyde** Chemical Formula: C_7H_6O Exact Mass: 106,0419 Molecular Weight: 106,1240 m/z: 106.0419 (100.0%), 107.0452 (7.6%)		
2	1,238	0,39034	**Fumaric acid** Chemical Formula: $C_4H_4O_4$ Exact Mass: 116,0110 Molecular Weight: 116,0720 m/z: 116.0110 (100.0%), 117.0143 (4.3%		
3	1,246	0,47112	**Succinic acid** Chemical Formula: $C_4H_6O_4$ Exact Mass: 118,0266 Molecular Weight: 118,0880 m/z: 118.0266 (100.0%), 119.0300 (4.3%		

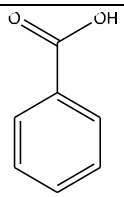
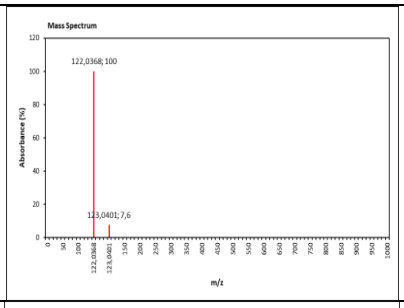
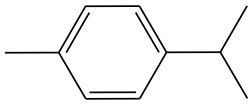
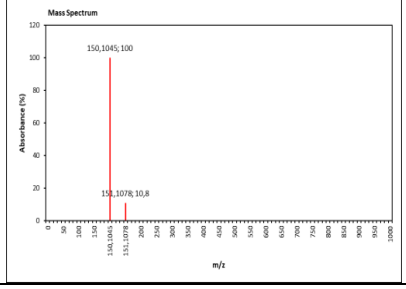
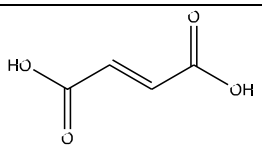
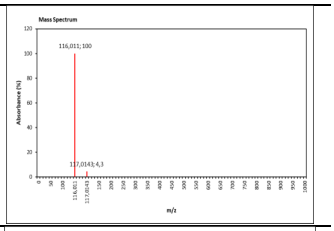
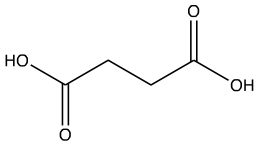
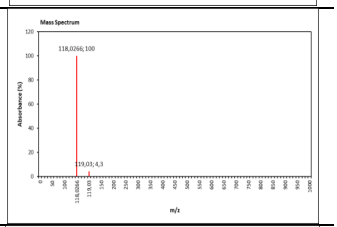
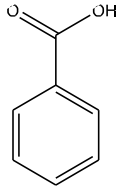
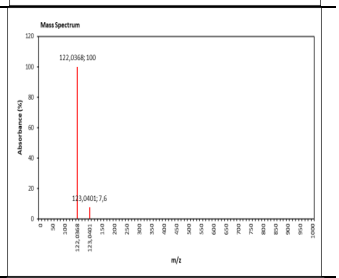
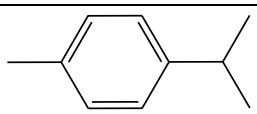
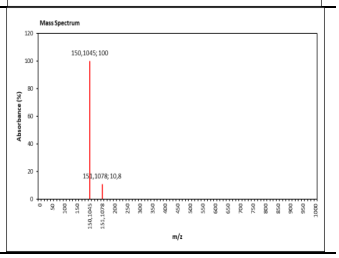
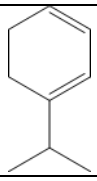
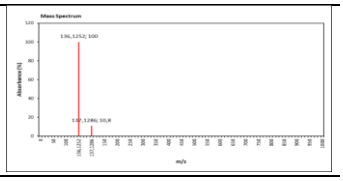
Peak number	RT (min)	Composition (%)	Compound Result		
			Analysis	Structure	Mass Spectrum
4	1,289	0,43318	**Benzoic acid** Chemical Formula: $C_7H_6O_2$ Exact Mass: 122,0368 Molecular Weight: 122,1230 m/z: 122.0368 (100.0%), 123.0401 (7.6%		
5	1,471	0,23169	**p-cymene** Chemical Formula: $C_{10}H_{14}$ Exact Mass: 134,1096 Molecular Weight: 134,2220 m/z: 134.1096 (100.0%), 135.1129 (10.8%		

Table 2. LCMS Chromatogram Result of the *P. indica* soxhlet extract

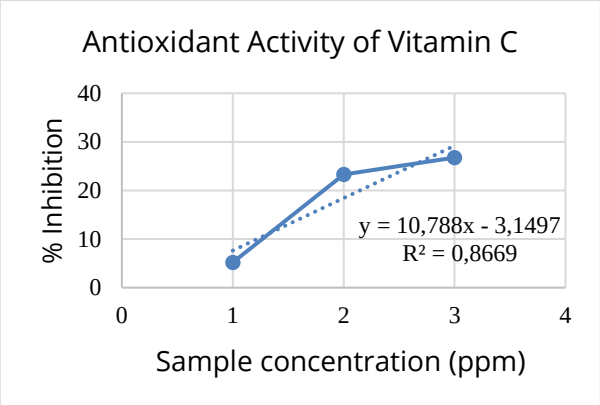
Peak number	RT (min)	Composition (%)	Compound Result		
			Analysis	Structure	Mass Spectrum
1	1,238	0,55963	**fumaric acid** Chemical Formula: $C_4H_4O_4$ Exact Mass: 116,0110 Molecular Weight: 116,0720 m/z: 116.0110 (100.0%), 117.0143 (4.3%)		
2	1,246	0,43751	**succinic acid** Chemical Formula: $C_4H_6O_4$ Exact Mass: 118,0266 Molecular Weight: 118,0880 m/z: 118.0266 (100.0%), 119.0300 (4.3%)		
3	1,289	0,29217	**benzoic acid** Chemical Formula: $C_7H_6O_2$ Exact Mass: 122,0368 Molecular Weight: 122,1230 m/z: 122.0368 (100.0%), 123.0401 (7.6%)		
4	1,471	0,45756	**p-cymene** Chemical Formula: $C_{10}H_{14}$ Exact Mass: 134,1096 Molecular Weight: 134,2220 m/z: 134.1096 (100.0%), 135.1129 (10.8%)		

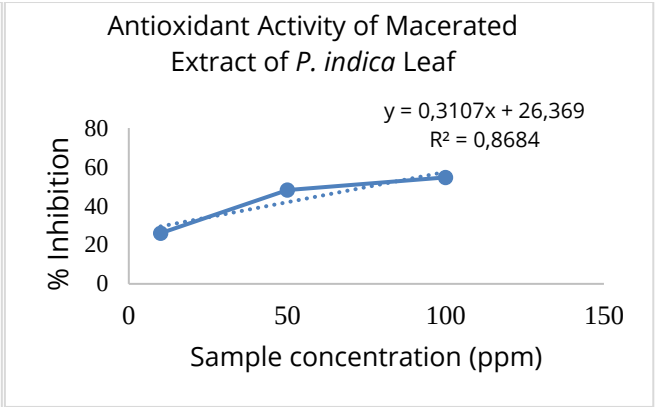
Peak number	RT (min)	Composition (%)	Compound Result		
			Analysis	Structure	Mass Spectrum
5	1,476	0,61809	**α-terpinene** Chemical Formula: $C_{10}H_{16}$ Exact Mass: 136,1252 Molecular Weight: 136,2380 m/z: 136.1252 (100.0%), 137.1286 (10.8%)		

The LC-MS results of *Pluchea indica L* extract obtained by maceration extraction method (114 compounds) and soxhletation (112 compounds) were then grouped based on the group of secondary metabolite compounds that have potential as antioxidants: Alkaloids, flavonoids, tannins, saponins, terpenoids and phenols. Furthermore, they were compared to determine the most secondary metabolite compounds (which have potential as antioxidants) between the maceration or soxhletation methods.

Table 3. Grouping of secondary metabolite compounds from LC-MS results of *P. indica*

Secondary metabolite compounds	Composition (%)	
	Maceration	Soxhletation
Flavonoid	72.4841	67.14345
Phenol	9.00625	8.95854
Terpenoids	6.11827	13.82165
Alkaloids	6.99075	5.35637
Tannins	2.2256	2.53305
Saponins	2.04458	0.2969





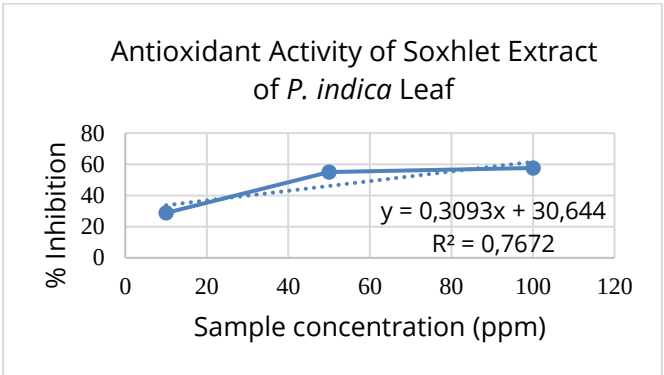


Figure 3. Linear regression of the relationship the concentration of test samples and % inhibition (Vitamin C, *Pluchea indica* leaf maceration extract, and *Pluchea indica* leaf Soxhletation extract)

The results of calculating the antioxidant activity test with DPPH of vitamin C as a positive control have an IC_{50} value of 4.927 ppm, indicating good antioxidant activity. Meanwhile, our results show that Pluchea indica leaf extract has a strong antioxidant activity with an IC_{50} value of 76.057 ppm for maceration and 62.580 ppm for Soxhletation.

Table 4. Antioxidant Activity of *Pluchea indica* extracts

Samples	Concentration (ppm)	Abs. Average	% Inhibition	IC_{50} (ppm)
Maceration extract	10	± 0.470	25,984	76.057 (Strong Antioxidant)
	50	± 0,329	48,189	
	100	± 0,288	54,646	
Soxhletation extract	10	± 0,452	28,819	62.580 (Strong Antioxidant)
	50	± 0,286	54,960	
	100	± 0,269	57,638	
Vitamin C	1	± 0,602	5,197	4.927 (Very Strong Antioxidant)
	5	± 0,487	23,307	
	10	± 0,465	26,772	

Based on this explanation, maceration and Soxhletation extractions of *P. indica* leaves have similar antioxidant content (Figure 3 and Table 4). The antioxidant activity of a sample can be determined by looking at its IC_{50} value. IC_{50} (inhibition concentration) value is a number that indicates the concentration of the sample that can reduce DPPH by 50%. A sample can be said to be a powerful antioxidant if it has an IC_{50} value < 50 ppm, a potent antioxidant if its IC_{50} value is 50 - 100 ppm, a moderate antioxidant if its IC_{50} value is 100 - 150 ppm, a weak antioxidant if its IC_{50} value is 150 - 200 ppm, and a very weak antioxidant if its IC_{50} value is > 200 ppm[24]. Both extractions produced IC_{50} values > 100 ppm, which are potent antioxidants. Extracts from the Soxhlet extraction show an IC_{50} value of 62.580 ppm, while the macerated extracts show 76.757 ppm. The greater the IC_{50} value, the smaller the antioxidant potential [18]. It is also shown by the test using LC-MS that there are many compound compositions contained in *Pluchea indica* leaf extracts that have potential as antioxidants, namely mainly flavonoids and terpenoids, which have a relatively large composition, and there are differences in composition between the two extraction methods. The maceration extract has a flavonoid composition of 72.4841% of 60 compounds, while the solution extract is 67.14345% of 57 compounds. In macerated extracts, the terpenoid group has a composition of 6.11827% of 25 compounds; in solution extracts, terpenoids are 13.82165% of 34 compounds. Flavonoids and terpenoids belong to the class of powerful antioxidants. The Soxhlet extraction has a smaller IC_{50} value than the macerated extract but is still in one activity category: potent antioxidants. The smaller the IC_{50} value, the greater the antioxidant activity and the greater the % inhibition value of a sample tested. IC_{50} value is calculated based on absorbance data that has been measured from the percentage of immersion between DPPH radicals and sample solution [19]. To ensure that the compounds in the *P. indica* extract work as antioxidants, molecular docking was carried out on the five main compounds resulting from LCMS.

Molecular Docking Results of NAHDP Oxidase Receptor (4Z3D).

A computational method, is used to predict the binding of a macromolecule (receptor) to a small molecule in the form of a ligand (five main compounds of LCMS). The purpose of this

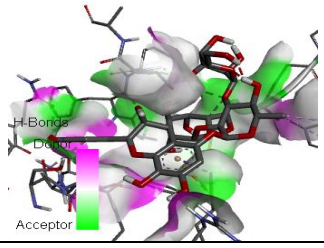
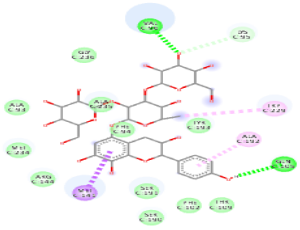
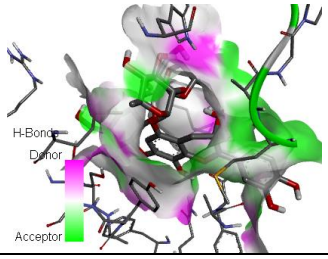
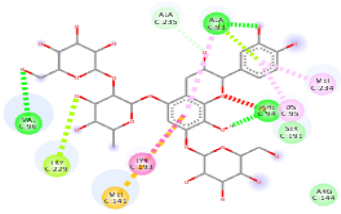
molecular docking is to determine the conformation and free energy of the bond involved in the interaction between the macromolecule (receptor) and the ligand [22]. This docking simulation helps in studying drugs or ligands or receptor/protein interactions, to obtain good geometry of the ligand-receptor complex [23]. In this research we conducted molecular docking of five major compounds; Kaempferol 3-glucosyl-(1→2)- [glucosyl-(1→3)-rhamnoside **(1),** Quercetin 3-glucosyl-(1→2)-rhamnoside-7-glucoside **(2),** Luteolin-7-O-(6''-malonylglucoside) **(3)**, 5,7,4'-trihydroxy-6,3'-dimethoxyisoflavone 7-O-(6''-glucosylglucoside) **(4),** and 5,7,4'-trihydroxy-6- methoxyisoflavone 4'-O-(6''- glucosylglucoside) **(5)** against Oxidase Receptor (NAHDP).

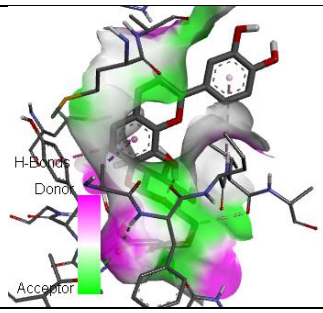
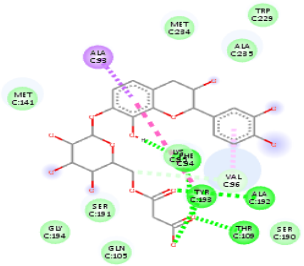
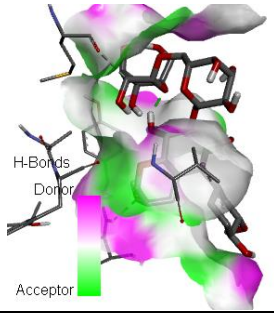
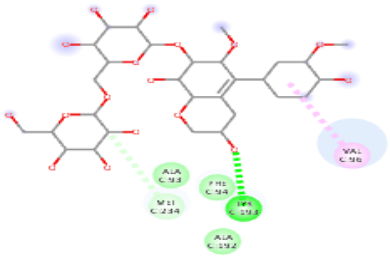
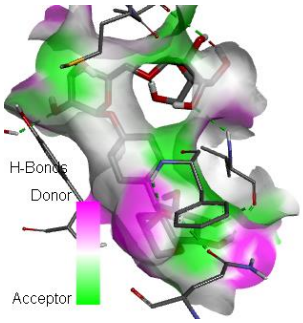
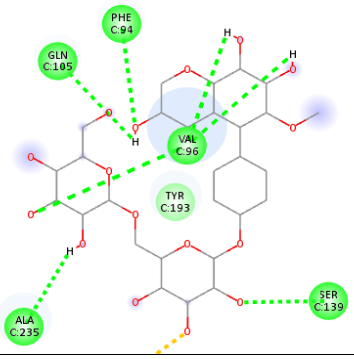
Table 5. NAHDP Oxidase Receptor (4Z3D) Docking Results

Ligans	Binding Free Energy (kkal-mol^{-1})	Inhibition Constans (μM)	Hydrogen Bonds	Hydrogen Bond Distance (Å)	Amino Acid Residues	Category	Interaction Type
(1)	-5.22	149.52	VAL 96 GLN 105 LYS 95	2.24046 2.18309 2.28311	VAL 96 LYS 95 GLN 105 TRP 229 ALA 192 MET 141 ALA 235 PHE 94 TYR 193 TYR 193 THR 109 PHE 102 SER 190 SER 191 ARG 144 MET 234 ALA 93 GLY 236	H-Bond H-Bond H-Bond Hidrofobik Hidrofobik Hidrofobik	Conv. H-Bond Carbon H-Bond Conv. H-Bond Π-alkil Π-alkil Π-sigma Van Der Waals Van Der Waals Van Der Waals Van Der Waals Van Der Waals Van Der Waals Van Der Waals Van Der Waals Van Der Waals Van Der Waals Van Der Waals Van Der Waals
(2)	-4.43	569.32	VAL 96 ALA 93 PHE 94 ALA 235	2.38135 2.56729 1.77284 3.23537	VAL 96 ALA 93 PHE 94 ALA 235 LYS 95 MET 234 TYR 193 MET 141 TRP 229 SER 191 ARG 144	H-Bond H-Bond H-Bond H-Bond Hidrofobik Hidrofobik Hidrofobik	Conv. H-Bond Conv. H-Bond Conv. H-Bond Carbon H-Bond Π-alkil Π-alkil Π- Π stacked Π-sulfur Π-Lone Pair Van Der Waals Van Der Waals
(3)	-3.03	5990	PHE 94 THR 109 ALA 192 TYR 193 VAL 96	2.68257 1.9578 2.18555 3.1745	PHE 94 THR 109 ALA 192 TYR 193 VAL 96 ALA 93	H-Bond H-Bond H-Bond H-Bond H-Bond Hidrofobik	Conv. H-Bond Conv. H-Bond Conv. H-Bond Conv. H-Bond Carbon H-Bond Π-sigma Van Der Waals

Ligans	Binding Free Energy (kkal-mol^{-1})	Inhibition Constans (μM)	Hydrogen Bonds	Hydrogen Bond Distance (Å)	Amino Acid Residues	Category	Interaction Type
					MET 234 TRP 229 ALA 235 SER 190 GLN 105 SER 191 GLY 194 MET 141		Van Der Waals Van Der Waals Van Der Waals Van Der Waals Van Der Waals Van Der Waals Van Der Waals
(4)	-5.42	107.19	TYR 193 MET 234	2.4667 3.40771	TYR 193 MET 234 VAL 96 ALA 93 PHE 94 ALA 192	H-Bond H-Bond Hidrofobik	Conv. H-Bond Carbon H-Bond Alkyl Van Der Waals Van Der Waals Van Der Waals
(5)	-5.58	51.14	GLN 105 PHE 94 VAL 96 SER 139 ALA 235	2.27997 2.41434 2.24751 2.54893 2.67562	GLN 105 PHE 94 VAL 96 SER 139 ALA 235 MET 243 TYR 193	H-Bond H-Bond H-Bond H-Bond H-Bond	Conv. H-Bond Conv. H-Bond Conv. H-Bond Conv. H-Bond Conv. H-Bond Sulfur-X Van Der Waals

Table 6. Docking visualization

Compounds	Visualization Results	
	3D	**2D**
(1)		
(2)		

Compounds	Visualization Results	
	3D	2D
(3)		
(4)		
(5)		

The smaller the results of the docking process indicate that the protein ligand complex will be more stable, so that the compound is more patent [24]. Based on the statement of Azzahra et al (2021) [25], the free energy of binding (ΔG) and the inhibition constant (Ki) are parameters to determine the quality of the bond formed. The results of molecular docking can be seen from the value of the free energy of binding (ΔG) where the smaller the value of the free energy of binding, the higher the affinity between the receptor and the ligand, conversely the greater the value of the free energy of binding, the lower the affinity between the receptor and the ligand [26]. The ability of a ligand to inhibit the activity of a receptor can be analyzed through the inhibition constant (Ki) of the ligand. The smaller the inhibition constant (Ki) value, the stronger the binding affinity and the less the amount of drug needed to inhibit enzyme activity. The smaller the Ki value, the stronger the binding of the ligand to the protein [27]. The inhibition constant can be stated as strong if it has a value of ≤ 100 μM and conversely the inhibition constant is stated as weak if it has a value of ≥ 100 μM [28].

Molecular docking was carried out on five test compounds, namely Kaempferol 3-glucosyl-(1→2)- [glucosyl-(1→3)-rhamnoside, Quercetin 3-glucosyl-(1→2)- rhamnoside-7-glucoside, Luteolin-7-O-(6''- malonylglucoside), 5,7,4'-trihydroxy-6,3'-dimethoxyisoflavone 7-O-(6''-glucosylglucoside), and 5,7,4'-trihydroxy-6-methoxyisoflavone 4'-O-(6''- glucosylglucoside) which will be docked to the NADPH Oxisade receptor (PDB id: 4Z3D) which has antioxidant activity. From the docking results obtained, the best affinity was obtained for 5,7,4'-trihydroxy-6-

methoxyisoflavone 4'-O-(6''- glucosylglucoside) with a binding free energy value of -5.58 kcal/mol with an inhibition constant value of 51.14 μM and produced five hydrogen bonds GLN 105 PHE 94 VAL 96 SER 139 ALA 235. The use of the NADPH Oxidase receptor is thought to have antioxidant activity, where this molecule can work to neutralize reactive radicals (ROS and RNS) and can prevent cell damage. Carbonyl reductase w-1 in humans that depends on NADPH can contribute to the metabolism of endogenous carbonyl-containing compounds and xenobiotics. Where this enzyme works by protecting cells from cellular damage due to oxidative stress [29].

Conclusions

The results of analysis using LC-MS on macerat extracts of *Pluchea indica* leaves : 114 compounds with the five highest compounds, namely benzaldehyde, fumaric acid, succinic acid, benzoic acid, and p-cymene; and soxhleted extract: 112 compounds on with the five highest compounds, namely Kaempferol 3-glucosyl-(1→ 2)- [glucosyl-(1 → 3)-rhamnoside, Quercetin 3-glucosyl-(1 → 2)- rhamnoside-7-glucoside, Luteolin-7-O-(6''- malonylglucoside), 5,7,4'-trihydroxy-6,3'-dimethoxyisoflavone 7-O-(6''-glucosylglucoside), and 5,7,4'-trihydroxy-6-methoxyisoflavone 4'-O-(6''- glucosylglucoside). Potential antioxidant compounds in macerated and Soxhletation extracts of *Pluchea indica* leaves show strong antioxidant category through the DPPH test. The docking results obtained the best affinity at 5,7,4'-trihydroxy-6- methoxyisoflavone 4'-O-(6''- glucosylglucoside) with a binding free energy value of -5.58 kcal/mol with an inhibition constant value of 51.14 μM and produced five hydrogen bonds GLN 105 PHE 94 VAL 96 SER 139 ALA 235.

Acknowledgement

This research was supported by STIKES Karya Putra Bangsa Tulungagung in the internal research grant program for the 2024 funding year.

Author Contibutions

Conceptualization, A.M. and D.P.T.; Methodology, A.M., D.P.T., S.D.Y., I.L.T. Validation, A.M and K.N.; Formal Analysis, A.M., S.D.Y., Investigation, A.M Resources, A.M., D.P.T., and S.D.Y; Data Curation, A.M., M.S.S; Writing – Original Draft Preparation, A.M., S.D.Y; Writing – Review & Editing, A.M., I.L.T., K.N., M.S.S; Visualization, A.M.; Supervision, A,M., D.P.T and K.N; Project Administration, A.M.; Funding Acquisition, A.M., D.P.T

Conflict of Interest

There are no significant conflicts

References

[1]. Arnanda QP, Nuwarda RF. Penggunaan Radiofarmaka Teknisium-99M Dari Senyawa Glutation dan Senyawa Flavonoid Sebagai Deteksi Dini Radikal Bebas Pemicu Kanker. *Farmaka Suplemen*. 2019;14(1):1-15. https://jurnal.unpad.ac.id/farmaka/article/view/22071

[2]. Werdhawati A. Peran Antioksidan Untuk Kesehatan. *Biotek Medisiana Indones*. 2014;3(1):59-68.

[3]. Mulia K, Hasan AEZ, Suryani. Total Phenolic, Anticancer and Antioxidant Activity of Ethanol Extract of Piper retrofractum Vahl from Pamekasan and Karang Asem. *Curr Biochem*. 2016;3(2):80-90. http://biokimia.ipb.ac.id

[4]. Sianturi CY. Manfaat Lidah Buaya Sebagai Anti Penuaan Melalui Aktivitas Antioksidan. *Essence Sci Med J*. 2019;17(1):34-38.

[5]. Kusuma ASW. The Effect of Ethanol Extract of Soursop Leaves (Annona muricata L.) to Decreased Levels of Malondialdehyde. *J Major*. 2015;4(3):14-18.

[6]. Utomo Y, Chairini N, Asrori MR. Perbandingan Metode Maserasi dan Microwave-Assisted Extraction pada Daun Pluchea indica dengan Variasi Pelarut dan Uji Antioksidan. *KOVALEN J Ris Kim*. 2023;9(1):23-32. doi:10.22487/kovalen.2023.v9.i1.16155

[7]. Defitiana Wanita. Uji Aktivitas Antioksidan Ekstrak Etanol Daun Beluntas (Pluchea

indica L.) Dengan Metode DPPH (2, 2-Difenil-1-Pikrilhidrazil). *ICAJ*. 2018;2(2): 25-28.
doi: https://doi.org/10.26740/icaj.v2n2.p25-28

[8]. Dewi Susylowati, Noverita Dian Takarina, Yasman, Ikhsan Pratama, Muhammadi A. Rijal. Karakteristik Biologi dan Kandungan Antioksidan Daun Beluntas yang Hidup di Lahan Wanamina Blanakan, Subang-Jawa Barat. *SAINTEKS*. 2022;19(1): 97-107.

[9]. Maulida Rosita J, Taufiqurrahman I. Perbedaan Total Flavonoid Antara Metode Maserasi Dengan Sokletasi Pada Ekstrak Daun Binjai (Mangifera caesia) (Studi pendahuluan terhadap proses pembuatan sediaan obat penyembuhan luka). *J Kedokt Gigi*. 2017;1(1):100-104.

[10]. Lenaini I. Teknik Pengambilan Sampel Purposive dan Snowball Sampling. *Hist J Kajian, Penelit Pengemb Pendidik Sej*. 2021;6(1):33-39. http://journal.ummat.ac.id/index.php/historis

[11]. Wijaya A, Noviana. Penetapan kadar air simplisia daun kemangi (Ocimum. *J Ris Kefarmasian Indones*. 2022;4(2):185-195.

[12]. Eka Siswanto Syamsul, Olanda Anugerah RS. Penetapan Rendemen Ekstrak Daun Jambu Mawar Determination Of Mawar Jambu Leaf Extract (*Syzygium jambos* L . Alston) Based On Variation Of Ethanol Concentration With The Maseration Method. *J Ris Kefarmasian Indones*. 2020;2(3):147-157. https://jurnalfarmasi.or.id/index.php/jrki/article/view/98/75

[13]. Marpaung MP, Septiyani A. Penentuan Parameter Spesifik dan Nonspesifik Ekstrak Kental Etanol Batang Akar Kuning (Fibraurea chloroleuca Miers). *Penentuan Param … J Pharmacopolium*. 2020;3(2):58-67.

[14]. Fauziah T, Nurmayni, Putri R, Pidia S, Sari S. *Hipertensi Si Pembunuh Senyap "Yuk Kenali Pencegahan Dan Penangananya."*; 2021.

[15]. Agus Faizal I, Tri Kumala Swandari M. Metode Perbandingan Maserasi Dan Soxhletasi Ekstrak Daun Sirih Merah (*Piper crocatum* Ruiz & Pav) Terhadap Efektivitas Bakteri *Staphylococcus epidermidis*. *J Ilmu Kefarmasian*. 2023;4(1):64-72.

[16]. Tivani I, Amananti W, Rima Putri A. Uji AKtivitas Antibakteri Handwash Ekstak Daun Turi (Sesbania grandiflora L) Terhadap Staphylococus aureus. *J Ilm Manutung*. 2021;7(1):86-91.

[17]. Tatiana WS, Ria S, Tahir M, Heluth AC, Widiastuti H. Uji Aktivitas Antioksidan dengan Metode DPPH dan Uji Sitotoksik Terhadap Sel Kanker Payudara T47D pada Ekstrak Daun Kemangi. *J Farmasetis*. 2020;9(1):51-64.

[18]. Chandra B, Sari RP, Misfadhila S, Azizah Z, Asra R. Skrining Fitokimia dan Aktivitas Antioksidan Ekstrak Metanol Daun Kemangi (*Ocimum tenuiflorum* L.) dengan Metode Dpph (1,1-difenil-2-pikrilhidrazil). *J Pharm Sci*. 2019;2(2):1-8. doi:10.36490/journal-jps.com.v2i2.20

[19]. Huda C, Putri AE, Sari DW. Uji Aktivitas Antibakteri Fraksi dari Maserat Zibethinus folium terhadap Escherichia coli. *J SainHealth*. 2019;3(1):7. doi:10.51804/jsh.v3i1.333.7-14

[20]. Nasution PA, Batubara R, Surjanto. Tingkat Kekuatan Antioksidan dan Kesukaan Masyarakat Terhadap Teh Daun Gaharu (Aquilaria malaccensis Lamk) Berdasarkan Pohon Induksi dan Non-Induksi. *Peronema - For Sci Journal*. 2015;4(1):10-18.

[21]. Azalia. D, Rachmawati. I, Zahira. S, et al. Uji kualitatif senyawa aktif flavonoid dan terpenoid pada beberapa jenis tumbuhan Fabaceae dan Apocynaceae di kawasan TNGPP Bodogol. *BIOMA J Biol Makassar*. 2023;8(1):32-33. https://journal.unhas.ac.id/index.php/bioma

[22]. Frimayanti, N., Lukman, A., Nathania, L., Program, S., Farmasi, F., Farmasi, S., Tingi, I., & Farmasi, R. Studi molecular docking senyawa 1,5-benzothiazepine sebagai inhibitor dengue DEN-2 NS2B/NS3 serine protease. *Chempublish Journal*, 2021; *6*(1), 54–62. https://doi.org/10.22437/chp.v6i1.12980

[23]. Setiawan, H., & Irawan, M. I. Kajian Pendekatan Penempatan Ligan Pada Protein Menggunakan Algoritma Genetika. *Jurnal Sains Dan Seni ITS*, 2017; *6*(2), 2–6. https://doi.org/10.12962/j23373520.v6i2.25468

[24]. Akbar,N. A., S. Amin, W. T. W. *Studi In Silico Senyawa Yang Terkandung Dalam Tanaman Daun Sirih Merah (Piper crocatum RUITZ & PAV) Sebagai Kandidat Anti Sars CoV-2*. 2022; *2*, 378–391.

[25]. Azzahra, R. W., Murdaya, N., Al Shofwan, A. A., Ramadan, E., & Utami, S. D. Molecular Docking Senyawa Ekstrak Etanol Daun Kenikir (*Cosmos caudatus*) Sebagai Inhibitor Il-6 Dalam Respon Inflamasi. *Jurnal Farmasi Udayana*, 2021; *10*(2), 138. https://doi.org/10.24843/jfu.2021.v10.i02.p05

[26]. Rachmania, R. A., Hariyanti, H., & Susilawati, D. Studi Molecular Docking Senyawa Isoflavonoid Dada Ayam (Erythrina variegata) terhadap Reseptor Plasminogen sebagai Agen Trombolitik pada Penyakit Infark Miokard. *Jurnal Jamu Indonesia*, 2022; *7*(1), 12–19.

[27]. Puspita, P., Liliyani, N., & Ambarsari, R. In Silico Analysis of Active Compounds of Avocado Fruit (*Persea americana Mill*.) as Tyrosinase Enzyme Inhibitors. *Current Biochem*,2022; *9*(2), 73–87.

[28]. Putri, R. G., Safithri, M., Husnawati, H., & Kurniasih, R. Penambatan Molekuler Senyawa Aktif Sirih Merah (Piper crocatum) pada Butirilkolinesterase sebagai Kandidat Antialzheimer. *ALCHEMY Jurnal Penelitian Kimia*, 2023; *19*(1), 68. https://doi.org/10.20961/alchemy.19.1.59676.68-85

[29]. Andranai, Y., Y. Mulyani., Iskandar., S. Megantara dan J. Levita. "Molecular Docking Study, Antioxidant Activity, Proximate Content, And Total Phenol Of Lemna perpusilla torr. Grown In Sumedang, West Java, Indonesia". *Rasayan J. Chem.* 2022*;* 15(2): 1182-1189.