Majalah Obat Tradisional (Trad. Med. J.), September-December 2024
Vol. 29(3), p 240-248
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 22-08-2023
Revised : 04-12-2023
Accepted : 09-07-2024

Ursolic Acid and Polydatin in Melinjo Seeds Inhibit AKT1 and GAPDH Protein and HTB-179 Cells Migration

Rifki Febriansah[1]*, Irna Julita[1]
[1] School of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Bantul, Yogyakarta, Indonesia

ABSTRACT

The incidence of lung cancer in Indonesia by 2020 has reached 34.783 cases. Melinjo contains ursolic acid and polydatin, which can suppress cell proliferation and induce cell apoptosis, respectively. This study aims to determine the Melinjo seed ethyl acetate fraction (MSEAF) ability to inhibit lung cancer proliferation and migration towards HTB-179 cells using *in vitro* and *in silico* methods. Melinjo seed powder was macerated using 70% ethanol and fractionated with ethyl acetate. The fraction obtained was then analyzed using HPLC to detect the active compounds. The compounds obtained were further analyzed using bioinformatics to determine the target proteins. The docking method was performed between ursolic acid and polydatin compounds with each target protein to determine the binding affinity. The *in vitro* test was done using the MTT cytotoxicity assay and scratch wound healing assay methods. The results showed that MSEAF contains ursolic acid and polydatin with retention times of 12,475 minutes and 16,564 minutes, respectively. Ursolic acid protein targets were TP53 and AKT1 with docking scores of -6,3 kcal/mol and -7,4 kcal/mol, while polydatin target proteins were GAPDH and VEGFA with docking scores of -8,8 kcal/mol and -5,5 kcal/mol. The results of the MTT assay showed an IC_{50} value of 35,539 g/mL, and MSEAF inhibited the migration of HTB-179 cells by slowing the migration rate. This study suggested that the MSEAF contained ursolic acid and polydatin, which exhibited the ability to prevent the growth and migration of HTB-179 lung cancer, supported by the prediction of their ability to bind to TP53 and AKT1 proteins.
Keywords: bioinformatics; cell migration; cytotoxic; docking; *Gnetum gnemon* L.

INTRODUCTION

In 2020, the incidence of lung cancer in Indonesia is ranked third after breast cancer and cervical cancer. The number of new cases of lung cancer reached 34.783**,** with a percentage of 8,8%. Lung cancer is also the leading cause of cancer death in Indonesia, with a portion of 13,2% (World Health Organization, 2020). Cancer treatment is usually done by surgery, radiotherapy, radiation therapy, and/or chemotherapy. Cancer treatment has not been selective in tackling cancer due to non-specific drug targets and resistance of cancer cells. It can cause damage to normal cells and severe side effects in cancer patients (Mutiah et al., 2018).

Based on this condition, it is necessary to develop research on cancer treatment, which is relatively safer than chemotherapy. The goal of the research on cancer treatment is to increase cancer cell sensitivity and reduce the side effects caused by chemotherapy agents. Exploration of compounds from natural ingredients that have the potential to inhibit cancer growth and minimize side effects is carried out on plants. One of the plants that is rich with natural and beneficial ingredients that can be developed as an anticancer agent in lung cancer is melinjo (*Gnetum gnemon*).

In the research of Dutta et al. (2018), ursolic acid compounds were found in the leaves of *Gnetum gnemon* L. Previous study by Wang et al. (2020), ursolic acid exhibited the ability to inhibit cell proliferation of eight lung cancer cell lines with IC50 at the range of 20 μM to 30 μM. In addition to that, according to Munadhil (2016), there were trans-piceid compounds in the ethyl acetate extract of melinjo seeds. Trans-piceid/polydatin is known for various therapeutic activities such as anticancer, antioxidant, anti-inflammation, and many more (Shah et al., 2022). In a study by Zou et al. (2018)., Polydatin showed concentration-dependent cell growth inhibition properties towards lung cancer cell lines (A549 and H1299) at 25, 50, and 100 μM. The same research stated the cell migration inhibitory activity of polydatin towards the cells at the dose of 50 μM.

This research began with extracting the powder of melinjo seeds and then identifying ursolic acid and polydatin compounds using the High-Performance Liquid Chromatography (HPLC) method. The in silico tests were performed using STITCH and STRING bioinformatics and molecular

***Corresponding author : Rifki Febriansah**
Email : rifki.febriansah@umy.ac.id

DOI: 10.22146/mot.88227 | *Majalah Obat Tradisional, 29(3), 2024*
This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

docking methods. The research continued with an in vitro test using the MTT assay to test the toxic activity of the ethyl acetate fraction of melinjo seeds against HTB-179 lung cancer cells and to see the ability to inhibit cell migration using the scratch wound healing assay method. This study aims to provide preliminary data which can be resourceful for further similar research in the development of herbal compounds as chemotherapy or chemopreventive agents.

MATERIALS AND METHODS

Materials

Melinjo seeds (*Gnetum gnemon* L.), Ethanol 70% (General Labora/grade pro analysis), ethyl acetate (General Labora/technical grade), aquadest (General Labora/technical grade), Fungizone 0.5%, Penicillin-streptomycin 1 % v/v (Gibco), Fetal Bovine Serum (FBS) 10% v/v (Gibco), PBS, MTT 5 mg/mL in culture media, HTB-179 cells (UMY Culture Lab), Dimethyl Sulfoxide (DMSO), Trypsin 0.25%, Sodium Dodecyl Sulfate (SDS) stopper reagent in 10% HCL (Merck), Dulbecco's Modified Eagle Medium (DMEM), ligand structure and protein (PDB file).

Methods

Extraction and fractionation

Melinjo seed powder was extracted by maceration method using 70% ethanol. A total of 2.500 grams of powder dissolved in a ratio of 1:10 is put into the macerator. The powder was soaked for five days and re-macerated for two days. The extract obtained was then filtered using flannel cloth, and this procedure was repeated three times with the same solvent. The macerate was concentrated with a rotary evaporator to obtain an ethanolic extract of melinjo seeds. The ethanol extract of melinjo seeds was fractionated using a separating funnel with the liquid-liquid method using ethyl acetate and ethanol as a solvent with a 1:1 ratio. The extract and solvent were put into a separating funnel, shaken for 15 minutes, and rested until two different layers of ethyl acetate and ethanol formed. The top layer, an ethyl acetate layer, was concentrated with a rotary evaporator at 60°C and then placed in a water bath to obtain a thick ethyl acetate fraction of melinjo seeds.

High-Performance Liquid Chromatography (HPLC)

The system used in the study was conducted according to the previous literature. Identifying ursolic acid followed previous study conducted by Taralkar and Chattopadhyay (2012). The sample of the ethyl acetate fraction of melinjo seeds used for the identification was weighed as much as 5 mg, dissolved in methanol to 100 ml, filtered with millipore, and degassed for 15 minutes with an ultrasonicator. The stationary phase used to identify ursolic acid was a C-18 column with acetonitrile: methanol (80:20) used as the mobile phase. Elution was carried out isocratic by injecting a 20 µl sample and detected at a wavelength of 210 nm with a flow rate of 1 mL/min. HPLC system for polydatin detection followed the methods of Baran et al. (2019), in which the sample was weighed as much as 5 mg and dissolved in 1 ml of mobile phase phosphate buffer pH 2.4: acetonitrile then filtered and degassed for 15 minutes with the same procedure as the sample for ursolic acid detection. 20µl of the sample detected under 307nm wavelength. The stationary phase was column C-18 with a mobile phase of phosphate buffer pH 2.4: acetonitrile (95:5, 90:10, 80:20, 75:25, 70:30, 0:100) using gradient elution. From this procedure, the retention time (Rt) can be observed through chromatograms, and the retention time from the experiment was then compared to the reference literature of each HPLC system. The similar retention times were assumed to represent the similar compounds between the literature and the analyzed sample.

Cytotoxic Test with MTT Assay Method

The cytotoxic test of the melinjo seeds ethyl acetate fraction (MSEAF) on HTB-179 lung cancer cells was carried out by inserting 100 µl of cells with a density of $5x10^3$ into a 96-well plate and an empty well to be used as a control, then incubated for 24 hours at 37°C to cells can adhere and adapt to the bottom of the well. After that, the culture media was washed using PBS and added to the test sample, namely the ethyl acetate fraction of melinjo seeds from each concentration series, and replicated three times. Then 100 µl of complete media was added to the well-containing cells and then incubated for 24 hours. At the end of incubation, 0.5 mg/mL MTT culture medium was prepared. Then, the culture medium containing the sample was removed and washed with PBS and 100 µl of 0,5 mg/mL MTT into each well, incubated at 37°C for 4 hours, and then viewed under an inverted microscope with purple formazan crystals formed, indicating that the living cells reacted with MTT. After 4 hours, the media with MTT was discarded and washed. After that, a 10% SDS stopper solution was given in 0.1 N HCl to dissolve the formazan crystals. The plate was shaken on a shaker for 10 minutes and read on an ELISA reader with a wavelength of 595 nm. The absorbance data obtained from the ELISA reader was analyzed using linear regression to predict the

cell concentration, and the formula can calculate the IC_{50} value:

$$\% \text{ of viable cells} = \frac{treatment\ absorbance - media\ control\ absorbance}{cell\ control\ absorbance - media\ control\ absorbance} 100\%$$

Scratch Wound Healing Assay Test

Cells grown on 24-well plates of 7,5 x 10^4 were incubated for 24 hours in complete media. Next, a scratch is made on the bottom surface of the well using the yellow tip. The old media was discarded and washed with PBS until the released cells were removed. In the last wash, pictures of each group were taken using an inverted microscope. Cells were then given media and 500 l of ethyl acetate extract of melinjo seeds and observed at certain hours after treatment with the same microscope magnification. The migration rate among the treatments was then compared.

***In Silico* Bioinformatics Test with STITCH and STRING**

Direct protein targets of ursolic acid and polydatin were obtained from STITCH. Then, the indirect protein target was searched using the STRING database with a minimum interaction score of 0,7 and a maximum number of interactors of 20. Then, look for genes that regulate lung cancer using PubMed with the keyword "Non-Small Cell Lung Cancer". Create a Venn diagram between all target proteins and genes that regulate lung cancer using the Venni 2.1 website. Intersecting proteins are considered targets of ursolic acid and polydatin in lung cancer, which will then be visualized using a Cytoscape, and the target protein with the highest degree score is selected.

***In Silico* Molecular Docking Test**

According to PDB ID, the target protein structure was downloaded via the Protein Data Bank (PDB). The preparation of protein and ligand files was conducted using a DS visualizer to remove polar hydrogens, other ligands/macromolecules, and other atoms until pure ligand/protein structure was obtained. Then, the structure was changed into a pdbqt extension after further preparation to charges for both protein and ligand using Autodock tools. Docking was done by AutodockVina through the command prompt, and the results of docking in the form of affinity and RMSD values were obtained. The molecular docking was first conducted between the protein targets and their native ligands for validation. Polydatin and ursolic acid 3D structures were obtained from PubChem in SDF format, then prepared and changed into pdbqt format as the native ligand before being docked to each protein target. Several conformations will be obtained, and the best docking conformation can be analyzed towards the affinity and RMSD value where a good stability affinity was expressed by the lowest score value on a molecule at an RMSD value < 2Å. The best conformation is then visualized with the DS Visualizer application to see the position of the ligand and protein and a three-dimensional picture of their interactions.

RESULTS

Extraction and Fractionation

Maceration was carried out by dissolving 2.500 grams of dry powder of melinjo seeds into 70% ethanol in a ratio of 1:10 to obtain 20.500 mL of extract, which evaporated and fractioned with liquid-liquid partition. The result of fractionation is 36,218 grams of thick extract with a yield of 3,96%.

High-Performance Liquid Chromatography (HPLC)

A study by Taralkar & Chattopadhyay (2012), acetonitrile: methanol (80:20) as the mobile phase with isocratic elution and C-18 as the stationary phase used to identify ursolic acid. This study was used as a literature standard for analyzing ursolic acid compounds with a retention time of 12,36 minutes. The MSEAF was analyzed under the same HPLC conditions, and a peak at 12,475 minutes, suspected to be ursolic acid, was obtained.

Meanwhile, in the identification of polydatin, research by Baran et al., 2019 was used as a literature standard using phosphate buffer pH 2.4 (A): acetonitrile (B) as the mobile phase with a stationary phase of C-18. The gradient composition was (A: B), 95:5 at 0 min, 90:10 at 5 min, 80:20 at 10 min, 75:25 at 20 min, 70:30 at 30 min and 0:100 at 40 min. Based on this source, the retention time of polydatin was 16,5 minutes.

Cytotoxic Test with MTT Assay Method

ELISA reader absorbance results were used to calculate the percentage of cell viability and IC_{50} values through linear regression. The linear regression equation from MSEAF y = -70,703x + 159,64 was used to calculate the IC_{50} value and was found to be 35,539 g/mL, which was included in the toxic category. A concentration of 250 g/mL resulted in 3,21% cell viability, which means that MSEAF at a concentration of 250 g/mL could kill HTB-179 cells as much as 96,79%. Meanwhile, in HTB-179 cells treated with Carboplatin, the regression equation y= -61,273x + 193,16 with IC_{50} value = 216,985 g/mL was categorized as

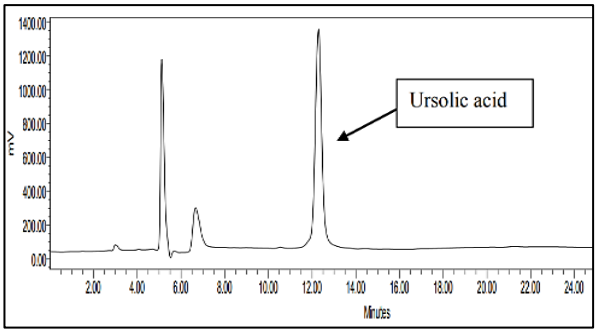


Figure 1. Standard chromatogram of ursolic acid (Taralkar & Chattopadhyay, 2012)

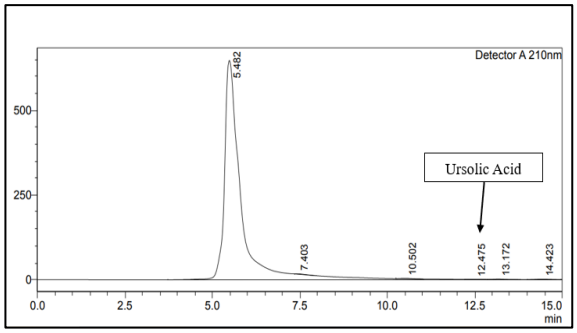


Figure 2. MSEAF chromatogram on ursolic acid analysis

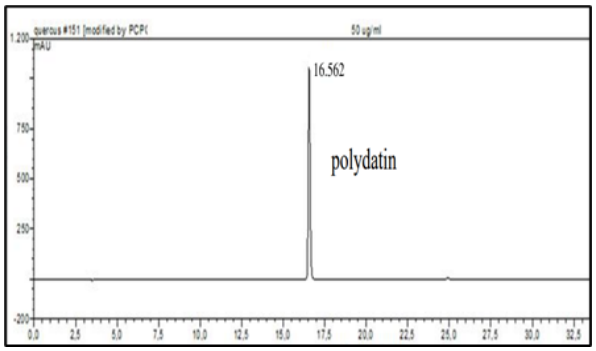


Figure 3. Standard chromatogram of polydatin (Baran et al., 2019)

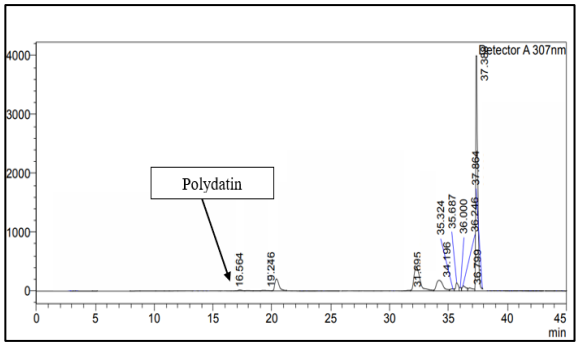


Figure 4. MSEAF chromatogram on polydatin analysis

moderately toxic. The percentage of cell viability at a concentration of 1000 g/mL was 3,49%, which means that a concentration of 1000 g/mL could kill 179 HTB cells as much as 96,51%.

Scratch Wound Healing Assay Test

The cell migration inhibitory ability of MSEAF was analyzed by using Carboplatin as a positive control and HTB-179 cultured cells without extract or drug as a negative control. The test is carried out by making scratches on the 24-well plate using a yellow tip. The doses used were IC_{50} of MSEAF 35,5 g/ml, ½ IC_{50} of MSEAF 17,75 g/ml, ¼ IC_{50} of MSEAF 8,875 g/ml, IC_{50} of Carboplatin 216,98 g/ml and ½ IC_{50} of Carboplatin 108,5 g/ml. After being treated, incubation and observations were carried out at 0, 3, 6, 18, 21, and 24 hours by taking pictures using an inverted microscope. The image that has been obtained is then analyzed to measure the scratch width using Image Raster 3.0 software, which is then used to calculate the cell migration rate. The negative control was used as the standard of comparison. The scratches in the control closed faster than the other treatments. For more details, the migration speed of each treatment was calculated. The IC_{50} MSEAF treatment showed activity that could inhibit cell migration with a lower migration rate than the control. The longer the observation time, the more the ability of MSEAF to inhibit cell movement decreases. Migration rate of control, IC_{50} MSEAF, ½ IC_{50} MSEAF, ¼ IC_{50}, IC_{50} Carboplatin, and ½ IC_{50} Carboplatin, respectively, at the 6th hour was 2,862 µm/hour; 1,152µm/hour; 2,154 µm/hour; 2,725 µm/hour; 0,864 µm/hour and 0,993 µm/hour. At the 6th hour, IC_{50} MSEAF inhibited the migration rate by 59.7% against the control, while IC50 Carboplatin inhibited the migration rate to the control by 69.8%.

***In Silico* Bioinformatics Test with STITCH and STRING**

DTPs from ursolic acid were obtained from STITCH (http://stitch.embl.de), and 10 DTPs were obtained. ITPs searched through STRING (https://string-db.org) obtained 200 ITPs, while the genes that regulate Non-Small Cell Lung Cancer (NSCLC) were 2188. Slices were searched from the data on target proteins and genes regulating NSCLC, and 91 protein targets were obtained. Visualization was carried out on 91 target proteins using a Cytoscape to see which proteins had the most potential to become protein targets by paying attention to the degree score of each protein. Based on the visualization results, the TOP 10 with the

Table I. IC_{50} Value

Type of treatment	Linear Regression Equation	IC_{50} Value	Description
FEABM	Y = -70.703x + 159.64	35,539	Toxic
Carboplatin	Y = -61.273x + 193.16	216,985	Moderately toxic

highest degree score was obtained, and two proteins were taken as targets, namely TP53 and AKT1, with degree scores of 75 and 62, respectively. Then, the same thing was done to determine the target protein from polydatin, with DTPs 10 and ITPs 200. The results of the slices obtained 73 protein targets, which were then visualized using a Cytoscape to obtain the TOP 10 proteins. Two proteins with the highest degree scores, namely GAPDH and VEGFA, with degree scores of 66 and 62, were used as target proteins. The top 10 proteins obtained from both of the compounds can be observed in table III and tamble IV.

In Silico Molecular Docking Test

The results of the test showed that the interaction of ursolic acid with TP53 and AKT1 proteins had a docking score of -6.3 kcal/mol and -7.4 kcal/mol, which were lower than Carboplatin, namely -4.5 kcal/mol and 4.7 kcal/mol. This means that the affinity energy of ursolic acid compounds is lower than Carboplatin, so the bonds formed are stronger and more stable. The same test was also carried out on polydatin compounds. The docking score obtained from the interaction of polydatin compounds with GAPDH and VEGFA proteins is -8.8 kcal/mol and -5.5 kcal/mol, respectively. The docking score of Carboplatin was higher, that is, -4.6 kcal/mol and -3.6 kcal/mol for GAPDH and VEGFA proteins.

DISCUSSION

Extraction and Fractionation

The process of extracting melinjo seeds (*Gnetum gnemon*. L) is conducted through the maceration method. Maceration is a simple method of extraction that is widely used and shows no significant changes in heat-sensitive compounds (Gori et al., 2021). In principle, fractionation is a process of withdrawing the active compound in an extract using two kinds of solvents that do not mix (Cahyani, 2018). This process resulted in a yield of 3.96% from 36.218 grams of fractionated thick extract.

High-Performance Liquid Chromatography (HPLC)

This qualitative test identifies the presence or absence of ursolic acid and polydatin compounds in MSEAF by comparing the Rt of the analyte with the Rt of the standard. MSEAF analysis was also carried out to identify polydatin by making the same HPLC conditions as the reference source and obtaining a retention time of 16,564 minutes, representing the probability of polydatin compound in the MSEAF. This study identified ursolic acid with the same HPLC conditions as the reference source and obtained a peak at 12.475 minutes.

Both polydatin and ursolic acid in MSEAF appeared in a small peak, representing only a small amount of those compounds. The phytochemical contents of a plant might differ depending on the species, part of the plants, and environmental factors such as light, temperature, and soil contents (Mudau et al., 2022). Thus, phytochemicals might be detected at various concentrations among studies in different literatures.

Cytotoxic Test with MTT Assay Method

This test aimed to determine the toxicity level of MSEAF against HTB-179 lung cancer cells with Carboplatin as a comparison. Carboplatin is the control compound as this platinum analogue has been the first-line therapy for NSCLC in severe stages (IIB -IV) (Griesinger et al., 2019). The parameter that is used as a benchmark is the IC_{50} value. IC_{50} showed levels capable of inhibiting cell proliferation by 50% of the population. The results of the IC_{50} MSEAF value are in the toxic category, while Carboplatin, as a comparison, is in the moderately toxic category. Based on these data values, MSEAF has a more significant IC_{50} value than Carboplatin. This means that MSEAF has good potential to be further developed as an anticancer agent.

Scratch Wound Healing Assay Test

Scratch wound healing assay test was done to obtain data related to the effect of treatment on the rate of cell migration. Cell migration is involved in many pathological processes, such as cancer metastasis, and is a significant cause of death in cancer patients due to the development of cancer metastases (Justus et al., 2014). Observations were made on migration rate of control, IC_{50} MSEAF, ½ IC_{50} MSEAF, ¼ IC_{50}, IC_{50} Carboplatin, and ½ IC_{50} Carboplatin. Compared with the concentrations of ½ IC_{50} and ¼ IC_{50} MSEAF, IC_{50} MSEAF was more capable of inhibiting the migration of HTB-179 cells. This means that the ability to inhibit

Table II. Migration Rate

Treatment	Migration Rate(µm/jam)				
	3rd Hour	6th Hour	18th Hour	21st Hour	24th hour
Control	2.655	2.856	3.550	3.158	2.763
	2.835	2.868	3.354	2.939	2.572
	2.745	**2.862**	**3.452**	**3.049**	**2.668**
IC50 MSEAF 35.5 µg/ml	0.370	0.907	2.649	2.626	2.559
	1.040	1.398	2.456	2.439	2.533
	0.705	**1.152**	**2.552**	**2.533**	**2.546**
½ IC50 MSEAF 17.75	0.897	1.996	3.694	3.411	2.984
µg/ml	1.378	2.313	3.601	3.519	3.079
	1.138	**2.154**	**3.648**	**3.465**	**3.032**
¼ IC50 MSEAF 8.875	3.127	3.106	4.024	3.581	3.133
µg/ml	1.892	2.344	3.874	3.429	3.000
	2.509	**2.725**	**3.949**	**3.505**	**3.067**
IC50 Carboplatin 216.98	0.488	0.647	0.505	0.167	0.426
µg/ml	1.162	1.082	0.471	0.437	0.193
	0.825	**0.864**	**0.488**	**0.302**	**0.310**
½ IC50 Carboplatin 108.5	1.248	1.136	0.654	0.517	0.451
µg/ml	0.682	0.731	0.382	0.307	0.292
	0.965	**0.993**	**0.518**	**0.412**	**0.371**

Table III. Top 10 ursolic acid target proteins

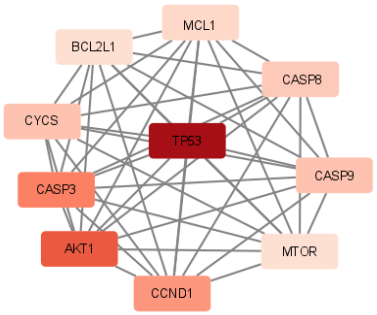
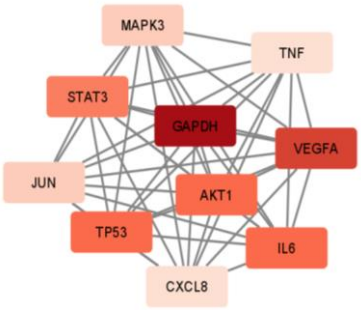
Number	Protein Symbol	Degree Score	Top 10 Visualization
1	TP53	75	
2	AKT1	62	
3	CASP3	56	
4	CCND1	53	
5	BCL2L1	50	
6	CASP9	47	
7	CYCS	47	
8	CASP8	46	
9	MCL1	44	
10	MTOR	43	

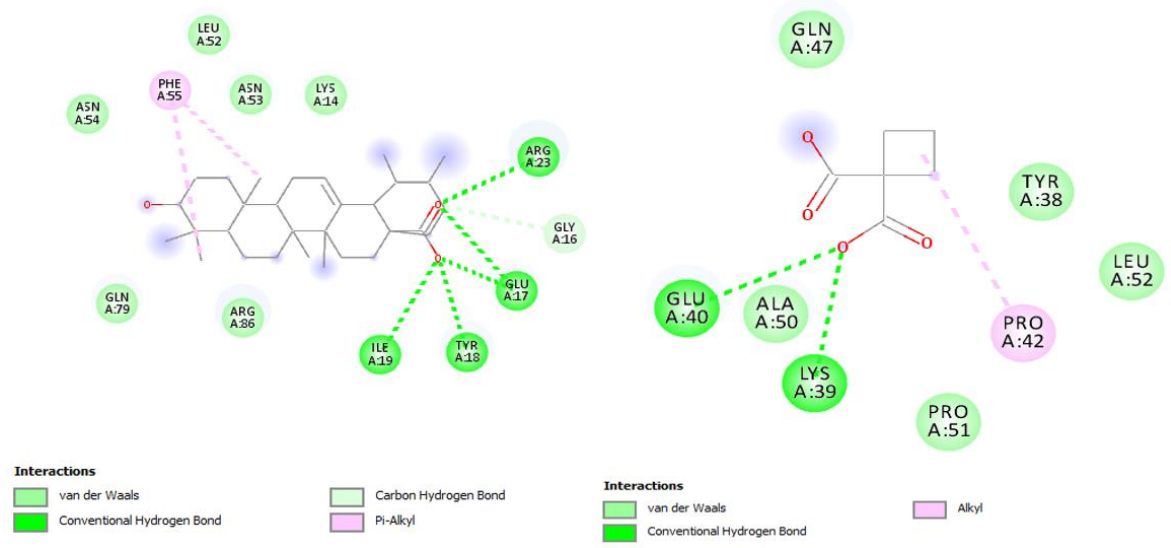
Table IV.Top 10 polydatin target proteins

Number	Protein Symbol	Degree Score	Top 10 Visualization
1	GAPDH	66	
2	VEGFA	62	
3	AKT1	59	
4	TP53	59	
5	IL6	59	
6	STAT3	58	
7	JUN	54	
8	MAPK3	54	
9	CXCL8	53	
10	TNF	53	

migration was directly proportional to the concentration of MSEAF. As a positive control, Carboplatin used a dose of IC_{50} Carboplatin 216,98 g/ml and ½ IC_{50} Carboplatin 108,5 g/ml. In the treatment group, Carboplatin could restrain the movement of HTB-179 cells until the 24th hour. The result exhibited that the migration rate is just reduced slightly over time.

Table V. Results of molecular docking ligand with protein AKT1

Compound Type	RMSD	*Docking Score*	Conformation
Asam Ursolat	1.665	- 7,4	2
Carboplatin	0.642	- 4,7	2

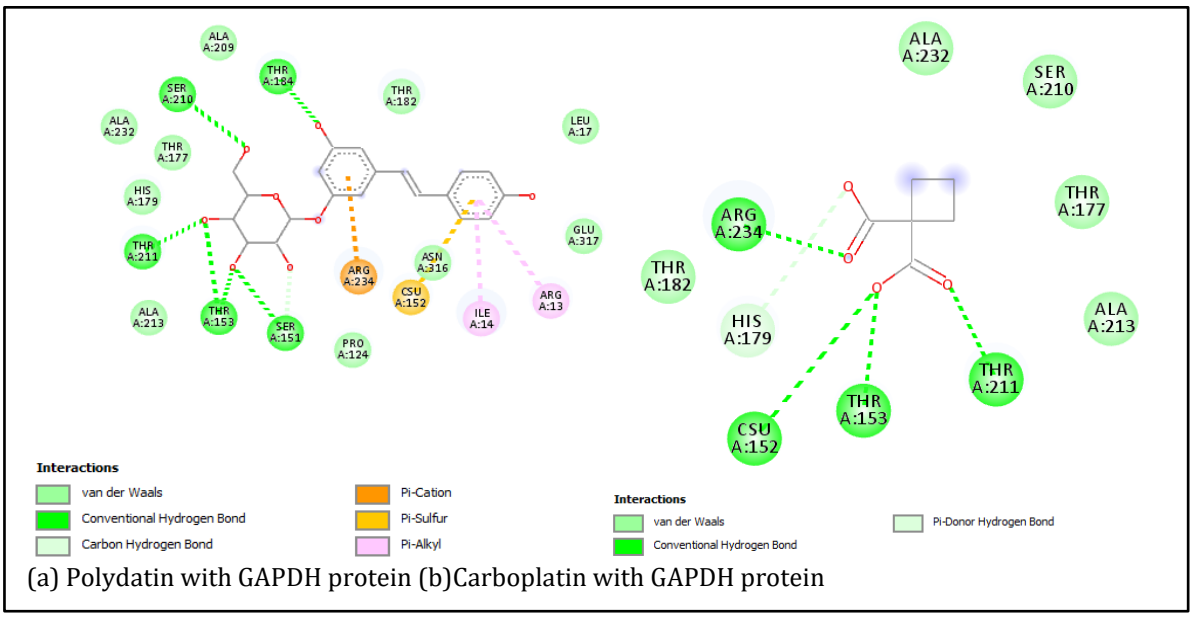


(a) Ursolic acid with AKT1 protein; (b)Carboplatin with AKT1 protein

Figure 5. 2D visualization of amino acid interactions

Table VI. Results of molecular docking ligand with protein GAPDH

Compound Type	RMSD	*Docking Score*	Conformation
Polydatin	1.913	- 8,6	2
Carboplatin	0.676	- 4,6	4



(a) Polydatin with GAPDH protein (b)Carboplatin with GAPDH protein

Figure 6. 2D visualization of amino acid interactions

In Silico Bioinformatics Test with STITCH and STRING

In cells, a compound can interact with proteins. Proteins that interact or become targets of compounds are called direct target proteins (DTPs), while proteins that interact with DTPs are defined as indirect target proteins (ITPs) (Hermawan et al., 2021). The degree score represents the number of interactions among the nodes (Rahman et al., 2022). The higher the degree score, the more potential to be used as a target protein. The two target proteins with the highest degree score of ursolic acid and polydatin, will be processed further in the molecular docking test.

In Silico Molecular Docking Test

This method was used to determine the interaction between the active compounds thought to be present in MSEAF, namely ursolic acid, and polydatin, with protein targets previously obtained from bioinformatics. Ursolic acid protein targets are TP53 and AKT1, while polydatin protein targets are GAPDH and VEGFA. As a comparison, the docking of Carboplatin with these target proteins was also carried out. The parameters used to assess the docking results are the RMSD value and the docking score. The RMSD value is considered good when < 2 (Soares et al., 2023). The smaller the docking score or binding affinity, the higher and more stable the bonds that form between the receptor and the compound (Flamadita et al., 2020). The results show that the affinity energy of polydatin compounds is lower than that of Carboplatin, so the bonds will be stronger and more stable. When compared further with the target protein of ursolic acid between TP53 and AKT1, AKT1 has a lower docking score, which means its binding is better than TP53. Then, for the target protein from polydatin, the GAPDH protein was chosen based on considering a lower docking value than VEGFA.

The visualization of molecular docking results of AKT1 towards ursolic acid and Carboplatin can be seen in Figure 5. The amino acids where the interaction occurs and the type of interactions can be observed. Ursolic acid and Carboplatin appeared to form several interactions towards amino acids in AKT1, such as van der Waals interactions, hydrogen bonds, and phi-alkyl bonds. Hydrogen bonds are crucial to improving the bounds between the drug and the receptor by altering solubility and other physicochemical properties (Bulusu & Desiraju, 2020). The similar interactions observed in the molecular docking result of GAPDH towards polydatin and Carboplatin. Several different interactions were exhibited in the molecular docking result of polydatin, such as phi-catio and phi-sulfur. These phi interactions are included in aromatic interactions, affecting the binding properties between a ligand and its receptor (Maulydia et al., 2022). This study found that the interactions between the analyzed compounds are better than the interactions of Carboplatin control towards the protein targets, which are also affected by the higher number of interactions that occurred in each analyzed compound configuration compared to the control.

CONCLUSION

Based on the research results, the ethyl acetate fraction of melinjo seeds was suspected to contain ursolic acid and polydatin compounds based on the HPLC method. The ethyl acetate fraction of melinjo seeds was toxic to HTB-179 cancer cells with an IC_{50} value of 35.539 g/mL. It inhibited cell migration by slowing down the migration speed of HTB-179 cells. Ursolic acid compounds have target proteins TP53 and AKT1, and polydatin target proteins GAPDH and VEGFA were obtained based on bioinformatics tests. The ursolic acid compound has a solid and stable bond to AKT1 protein with a docking score of -7.4 kcal/mol, while polydatin has a strong and stable bond to GAPDH protein with a docking score of -8.8 kcal/mol based on the molecular docking method.

ACKNOWLEDGEMENT

Thanks to the University of Muhammadiyah Yogyakarta for facilitating this research and the Ministry of Education and Culture of Indonesia for funding this research.

REFERENCES

Baran, M. Y., Şöhretoğlu, D., & Kuruüzüm Uz, A. (2019). Quantitative analysis of Polydatin in a Turkish oak: Quercus coccifera L. with HPLC-DAD. *International Journal of Secondary Metabolite*, *6*(3), 233–240. https://doi.org/10.21448/ijsm.610157

Bulusu, G., & Desiraju, G. R. (2020). Strong and weak hydrogen bonds in protein–ligand recognition. *Journal of the Indian Institute of Science*, *100*, 31-41.

Cahyani, L. . (2018). Fraksi Senyawa Antituberkulosis dari Ekstrak Larut n-Heksan Daun Jati Merah (Tectona grandis L F)., Skripsi,. *Skripsi*, 1–83.

Callil-Soares, P. H., Biasi, L. C. K., & Pessoa Filho, P. D. A. (2023). Effect of preprocessing and simulation parameters on the performance of molecular docking studies. *Journal of Molecular Modeling*, *29*(8), 251.

Dutta, P. P., Bordoloi, M., Roy, S., Narzary, B., Gogoi,

K., Bhattacharyya, D. R., Mohapatra, P. K., & Mazumder, B. (2018). Antiplasmodial activity of Gnetum gnemon Leaves and compounds isolated from them. *Natural Product Communications*, *13*(10), 1263–1265. https://doi.org/10.1177/1934578x1801301007

Flamandita, D., Lischer, K., Pratami, D. K., Aditama, R., & Sahlan, M. (2020, May). Molecular docking analysis of podophyllotoxin derivatives in Sulawesi propolis as potent inhibitors of protein kinases. In *AIP Conference Proceedings* (Vol. 2230, No. 1). AIP Publishing.

Gori, A., Boucherle, B., Rey, A., Rome, M., Fuzzati, N., & Peuchmaur, M. (2021). Development of an innovative maceration technique to optimize extraction and phase partition of natural products. *Fitoterapia*, *148*, 104798.

Griesinger, F., Korol, E. E., Kayaniyil, S., Varol, N., Ebner, T., & Goring, S. M. (2019). Efficacy and safety of first-line carboplatin-versus cisplatin-based chemotherapy for non-small cell lung cancer: a meta-analysis. Lung Cancer, 135, 196-204.

Hermawan, A., Ikawati, M., Jenie, R. I., Khumaira, A., Putri, H., Nurhayati, I. P., Angraini, S. M., & Muflikhasari, H. A. (2021). Identification of potential therapeutic target of naringenin in breast cancer stem cells inhibition by bioinformatics and in vitro studies. *Saudi Pharmaceutical Journal*, *29*(1), 12–26. https://doi.org/10.1016/j.jsps.2020.12.002

Hidayat, R., & Wulandari, P. (2021). Methods of extraction: Maceration, percolation and decoction. *Eureka Herba Indonesia*, *2*(1), 68-74.

Lezoul, N. E. H., Belkadi, M., Habibi, F., & Guillén, F. (2020). Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. *Molecules*, *25*(20), 4672.

Maulydia, N. B., Tallei, T. E., Ginting, B., Idroes, R., Illian, D. N., & Faradilla, M. (2022, January). Analysis of flavonoid compounds of Orange (Citrus sp.) peel as anti-main protease of SARS-CoV-2: A molecular docking study. In *IOP Conference Series: Earth and Environmental Science* (Vol. 951, No. 1, p. 012078). IOP Publishing.

Mudau, H. S., Mokoboki, H. K., Ravhuhali, K. E., & Mkhize, Z. (2022). Effect of soil type: Qualitative and quantitative analysis of phytochemicals in some browse species leaves found in savannah biome of South Africa. *Molecules*, *27*(5), 1462.

Munadhil, M. A. (2016). *Uji aktivitas penghambatan angiotensin converting enzyme (ACE) dan penetapan kadar fenolik total dari biji melinjo (Gnetum gnemon L.)*. Universitas Indonesia.

Mutiah, R., Suryadinata, A., & Nurani, P. S. (2018). Uji Sitotoksik Kombinasi Cisplatin dengan Ekstrak Etanol Benalu Alpukat (Dendrophthoe pentandra (L) Miq.) Pada Sel HeLa. *Majalah Kesehatan*, *5*(September), 133–143.

Rahman, M. A., Shorobi, F. M., Uddin, M. N., Saha, S., & Hossain, M. A. (2022). Quercetin attenuates viral infections by interacting with target proteins and linked genes in chemicobiological models. *In Silico Pharmacology*, *10*(1), 17.

Shah, M. A., Hamid, A., Faheem, H. I., Rasul, A., Baokbah, T. A., Haris, M., ... & Jeandet, P. (2022). Uncovering the Anticancer Potential of Polydatin: A Mechanistic Insight. *Molecules*, *27*(21), 7175.

Sukma, F. F. (2019). Identifikasi Asam Dehidroasetat dalam Produk Kosmetika dengan Menggunakan HPLC (High Performance Liquid Cromatography). *QUIMICA: Jurnal Kimia Sains dan Terapan*, *1*(2), 15-17.

Taralkar, S. V., & Chattopadhyay, S. (2012). A HPLC Method for Determination of Ursolic Acid and Betulinic Acids from their Methanolic Extracts of Vitex Negundo Linn. *Journal of Analytical & Bioanalytical Techniques*, *03*(03), 1–6. https://doi.org/10.4172/2155-9872.1000134

Wang, M., Yu, H., Wu, R., Chen, Z. Y., Hu, Q., Zhang, Y. F., ... & Zhou, G. B. (2020). Autophagy inhibition enhances the inhibitory effects of ursolic acid on lung cancer cells. *International Journal of Molecular Medicine*, *46*(5), 1816-1826.

World Health Organization. (2020). Cancer Incident in Indonesia. *International Agency for Research on Cancer*, *858*, 1–2.

Zou, J., Yang, Y., Yang, Y., & Liu, X. (2018). Polydatin suppresses proliferation and metastasis of non-small cell lung cancer cells by inhibiting NLRP3 inflammasome activation via NF-κB pathway. *Biomedicine & Pharmacotherapy*, *108*, 130-136.