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SYNERGISTIC APPROACHES IN ANTI-CERVICAL CANCER RESEARCH: MOLECULAR DOCKING AND IN VIVO ANALYSIS

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Abstract

Background/aim: Analysis compounds containing antioxidant activity of magenta plant leaves (*Peristrophe Bivalvis (L.)* Merr) in preventing oxidative stress and predicting its potential as a candidate medicinal compound for cervical anticancer. **Materials and methods**: The cervical anticancer activity of *Peristrophe Bivalvis* (L.) Merr was conducted by in silico and in vivo methods. The in silico research design was performed using molecular docking techniques, while the in vivo research design was carried out using Elisa by examining the levels of MDA, SOD, GSH and Nrf2 tissue in experimental animal models that experienced oxidative stress. **Results**: The ethanol extract of magenta leaves has antioxidant activity that can prevent oxidative stress through its activity in reducing Malondialdehyde (MDA) levels, increasing Superoxide Dismutase levels and Glutathione levels through the regulation of Nuclear Factor Erythroid Related Factor-2 (Nrf2). The molecular docking results of magenta leaf compounds showed a high affinity to target proteins, namely Nrf2/Keap1, CDK2, and EGFR. **Conclusion**: This study concluded that the ethanol extract of magenta leaves has good antioxidant activity. The molecular docking approach also shows the potential of active compounds from magenta leaves ethanol extract as cervical anticancer agents.

Keywords: Peristrophe Bivalvis, Cervical Cancer, Antioxidant.

INTRODUCTION

Cancer is a term for a disease that occurs when abnormal cells divide uncontrollably and can invade surrounding tissues (1). One of the most common cancers that cause death, especially in women is cervical cancer (2). Cervical cancer is a malignant tumor in women caused by a group of viruses known as human papillomavirus (HPV) (3). Recent studies have shown that oxidative stress is involved in cancer development as it can cause DNA damage, alteration of tumor suppressor genes, development of malignancy and resistance to treatment (4). Oxidative stress can also lead to inflammatory reactions, can cause damage to tissues, and can cause decreased levels of endogenous antioxidants such as Glutathione (GSH), Superoxide Dismutase (SOD) and increased levels of Malondialdehyde (MDA) (5). In cervical cancer, cervical epithelial tissue is exposed to oxidative stress that drives the development of persistent chronic virus infections. Cervical epithelial tissue has an antioxidant system consisting of the transcription factor nuclear factor erythroid-2-related factor 2 (Nrf2). Nrf2 maintains redox balance in cells by controlling gene transcription (6). In normal conditions, Nrf2 can be suppressed by the Keap1 protein. Keap1 (Kelch-like ECH-associated protein 1) is a suppressor protein that binds to Nrf2 and drives its degradation through the ubiquitin proteasome way. The overexpression of Nrf2 in cervical cancer is significantly associated with decreased Keap1 expression.

Therefore, the Nrf2/Keap1 signaling way may be a promising therapeutic strategy in cervical cancer (4).

Some experimental evidence suggests that antioxidants have a vasoprotective role through the Nrf2-ARE-related pathway (7). Antioxidants have the ability to neutralize free radical molecules by contributing one of their own electrons so as to inhibit oxidative reactions and prevent cell damage. Oxidative reactions are reactions that can cause degenerative diseases such as cancer (8,9).

Several studies have suggested that secondary metabolites from plants are a source of natural antioxidants. One of the plants described as having promising antioxidant activity is the magenta plant (*Peristrophe bivalvis (L.)* Merr) (10). Some of the compounds contained in magenta plant leaves are essential oils, fatty oils, saponins, tannins, flavonoids, anthocyanins, and phenolics (11), while the main components found in magenta leaves extract are peristrophine, perisbivalvin, pelargonidin, and apioside (12). Water-extracted pigments from magenta leaves are known to have good free radical capture activity (13). Based on this antioxidant activity, further testing of magenta plant leaf extract as an anticancer agent is essential. The design and development of new drugs can now be done in silico using biocomputing studies to analyze interactions based on structure or known as molecular docking (14,15). Therefore, in this study, compounds that have antioxidant activity from magenta plant leaves were tested, namely peristophine, perisbivalvin, pelargonidin, and apioside. The target receptors used in this study are Nrf2/Keap1 receptors which have an important role in the generation of endogenous antioxidants, as well as CDK2 and EGFR receptors which are instrumental in cell proliferation. Various secondary metabolites in magenta leaves can have benefits as antioxidants by counteracting free radicals that can cause cancer. In this study, in vivo testing was conducted to see the effect of giving ethanol extract of magenta leaves (*Peristrophe bivalvis (L.)* Merr) in preventing oxidative stress as seen from the levels of Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione (GSH), through the regulation of nuclear factor erythroid related factor - 2 (Nrf2).

MATERIALS AND METHODS

This study is experimental research within silico and in vivo research designs. The in-silico research design was conducted using molecular docking techniques, while the in vivo one was carried out using Elisa by examining the levels of MDA, SOD, GSH) and Nrf2 tissue in experimental animal models suffering from oxidative stress.

Materials

The materials used in the in silico research are target proteins obtained from the Protein Data Bank (PDB) website (https://www.rcsb.org) using a specific PDB code, as well as two-dimensional and three-dimensional structures of the ligands used, drawn using the MarvinJS online website (https://marvinjs-demo.chemaxon.com). For the in vivo study, the materials used include magenta leaves obtained from Banjar Semebaung, Bitera Village, Gianyar Bali with an altitude of 0-425m above sea level and with dark green leaf characteristics, male Wistar rats from the Pharmacology Laboratory of Udayana University Bali, and standard rat feed. The materials used were pro-analysis quality such as methanol and CMC-Na and Whatman No. 4 filter paper. The assay kits were MDA, Nrf2, SOD, GSH, 96 well ELISA kit, made by Bioassay Technology Laboratory.

Instrument. The instruments used in the in silico research include HP laptop devices with specifications of Intel(R) Core (TM) i3-10110U CPU @ 2.10GHz - 2.59 GHz, 8.00 GB RAM (7.83 GB usable), with Intel(R) UHD graphics card equipped with AutoDock Tools version 1. 5.7, Autodock4, Open Babel, PyMOL for education under the license of I Wayan Surya Rahadi, Discovery Studio Visualizer v16.1.0.15350, as well as online MarvinJS website, Protein Data Bank (PDB), and ADMETlab 2.0. In addition, the equipment used in the in vivo study were Digital Scale analytical balance (Ohaus, USA), oven (Memmert), cabinet dryer, thermometer, blender (Phillips), 40 mesh sieve, rotary vacuum evaporator (Buchii), water bath, centrifugator (MSE Micro Centaur), Pyrex glassware, and ELISA reader Stat Fax 4700.

Methods

In Silico Testing. The MarvinJS website was used to obtain files in .sdf format. The .sdf files were converted into .pdb files using the Open Babel software. Target protein preparation was accomplished by downloading from the Protein Data Bank (RCSB PDB). Furthermore, the removal of unnecessary chains, the removal of water molecules, and the addition of hydrogen were performed using Autodock Tools. In this study, the receptors used were Nrf2 receptor with identity 4XMB, Keap1 with identity 6TYM, CDK2 with identity 3TI1, and EGFR with identity 4I23.

The acceptance of the docking program is done by re-docking the original ligand against the target protein. The method can be said to be valid if it has a Root Mean Square Deviation (RMSD) value smaller than 2.0 Å. The molecular docking process was performed by docking the test compound against the target protein using a procedure that has been validated with AutoDock Tools software version 1.5.7.

The physicochemical parameters and ADMET profiles (absorption, distribution, metabolism, excretion and toxicity) of peristophine, perisbivalvin, pelargonidin, and apioside compounds were predicted by the Lipinski rule of five using ADMETlab 2.0 website. Data analysis of the docking results was expressed by the binding energy (ΔGbinding) value and the interaction between the compound and the target protein's amino acid residues. The visualization of docking results was performed using Discovery Studio Visualizer software.

In Vivo Testing. In this study, the in vivo testing used a randomized posttest only control group design. The samples used in this study consisted of 27 males white Wistar rats aged 8-9 weeks and weighing 100-200 g which were then divided into 3 groups, namely intervention group 1 (the control group), intervention group 2 (administration of 125 mg/KgBB ethanol extract) and intervention group 3 (administration of 250 mg/KgBB ethanol extract).

This study used the quantitative sandwich enzyme immunoassay (ELISA) technique consisting of the examination of the Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Glutathione (GSH) levels and the examination of Nrf2 levels using Rat Nrf2 ELISA Kit 96, made by Bioassay Technology Laboratory.

Data obtained in laboratory studies were analyzed using One Way Anova (normal and homogeneous distributed data) and continued Post Hoc test (LSD) between groups.

RESULTS

Magenta Leaves Antioxidant Activity Test

When testing the antioxidant activity of magenta leaves plants in vivo, the control group (P0) obtained MDA levels of 4.555 nmol/ml, the intervention group of 125 mg/kgBB magenta leaves ethanol extract (P1) obtained levels of 2.733 nmol/ml, and the intervention group administering 250 mg/kgBB magenta leaves ethanol extract (P2) obtained levels of 2 nmol/ml. The lowest MDA levels were obtained in the intervention group of 250 mg/kgBB magenta leaves ethanol extract (P2).

The results of tissue Nrf2 levels obtained in this study were 5.026 ng/ml in the control group (P0), 9.501 ng/ml in the intervention group given 125 mg/kgBB magenta leaves ethanol extract (P1), and 14.472 ng/ml in the intervention group given 250 mg/kgBB magenta leaves ethanol extract (P2). The highest tissue Nrf2 levels were obtained in the intervention group given 250 mg/kgBB magenta leaves ethanol extract (P2).

The results of SOD levels obtained in this study were 1.693 ng/ml in the control group (P0), 2.63 ng/ml in the intervention group given 125 mg/kgBB magenta leaves ethanol extract (P1), and 3.813 ng/ml in the intervention group given 250 mg/kgBB magenta leaves ethanol extract (P2). The highest SOD levels were obtained in the intervention group given 250 mg/kgBB magenta leaves ethanol extract (P2).

The results of GSH levels obtained in this study were 3.535 ng/ml in the control group (P0), 5.995 ng/ml in the intervention group given 125 mg/kgBB magenta leaves ethanol extract (P1), and 7.548 ng/ml in the intervention group given 250 mg/kgBB magenta leaves ethanol extract (P2). The highest GSH levels were obtained in the intervention group given 250 mg/kgBB magenta leaves ethanol extract (P2).

The results of Malondialdehyde (MDA), tissue Nrf2, Superoxide Dismutase (SOD), and Glutathione (GSH) levels are completely presented in the table below.

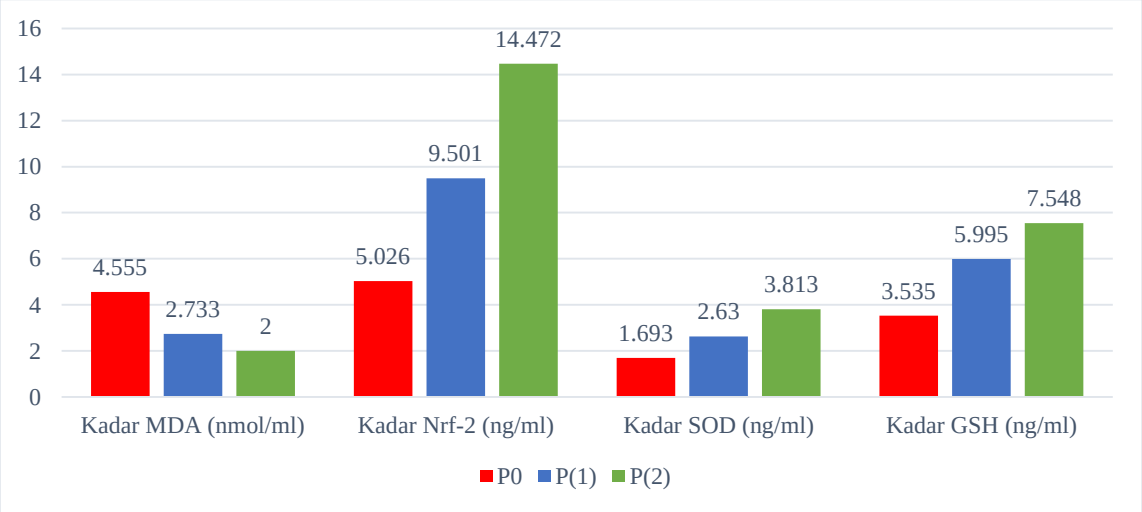

Figure 1: Average levels of MDA, tissue Nrf2, SOD, and GSH in each group in the blood of Wistar rats

The results of the normality and homogeneity test analysis of Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione (GSH), and Nrf2 tissue levels obtained showed that the data were normally distributed and homogeneous variants with values ($p>0.05$). The results of the analysis using One Way Anova showed that the

distribution of ethanol extract of magenta leaves (*Peristrophe bivalvis (L.)* Merr) 125 mg/KgBB, and distribution of ethanol extract of magenta leaves (*Peristrophe bivalvis (L.)* Merr) 250 mg/KgBB. 250 mg/KgBB indicated significantly different levels of MDA, tissue Nrf2, SOD, and GSH with a value of ($p<0.05$). The Post Hoc test present a significant mean difference among the control group and the intervention group. The Post Hoc test data among the control group and the intervention group is described in Table 1.

Table 1: Difference in averaged levels of MDA, tissue Nrf2, SOD, and GSH between intervention groups

	Intervention Group	MDA level (ng/ml)		Nrf2 (ng/ml)		SOD (ng/ml)		GSH (ng/ml)	
		Average	*p*	Averaged	*p*	Averaged	*P*	Averaged	*p*
P0	P1	1,8222*	0,000	-4,4744*	0,020	-0,9367*	0,000	-0,2460*	0,000
	P2	2,5555*	0,000	-9,4455*	0,000	-2,1200*	,000	-4,0133*	0,000
P1	P0	-1,8222*	0,000	4,4744*	0,020	0,9367*	0,000	2,4600*	0,000
	P2	0,7333*	0,002	-4,9711*	0,032	-1,1833*	0,000	-1,5533*	0,01
P2	P0	-2,5555*	0,000	9,4455*	0,000	2.1200*	0,000	4,0133*	0,000
	P1	-0,7333*	0,002	4,9711*	0,032	1.1183*	0,000	1,5533*	0,01

Molecular Docking of Magenta Leaves Compounds as Cervical Anticancer Agents

Based on in vivo research, it is known that magenta leaves have antioxidant activity that can prevent oxidative stress through the adjustment of the Nuclear Factor Erythroid Related Factor-2 (Nrf2). Research have shown that there is a relationship between plant antioxidants and the reduction of chronic diseases, thus antioxidants are thought to protect against cancer development and metastasis (16). Therefore, in silico research was conducted to predict the potential of compounds from magenta plant leaves that act as cervical anticancer agents. In the in-silico research, a molecular docking method was used. This method requires the visualization of the two-dimensional and three-dimensional structures of the test compounds which are presented in Figure 2.

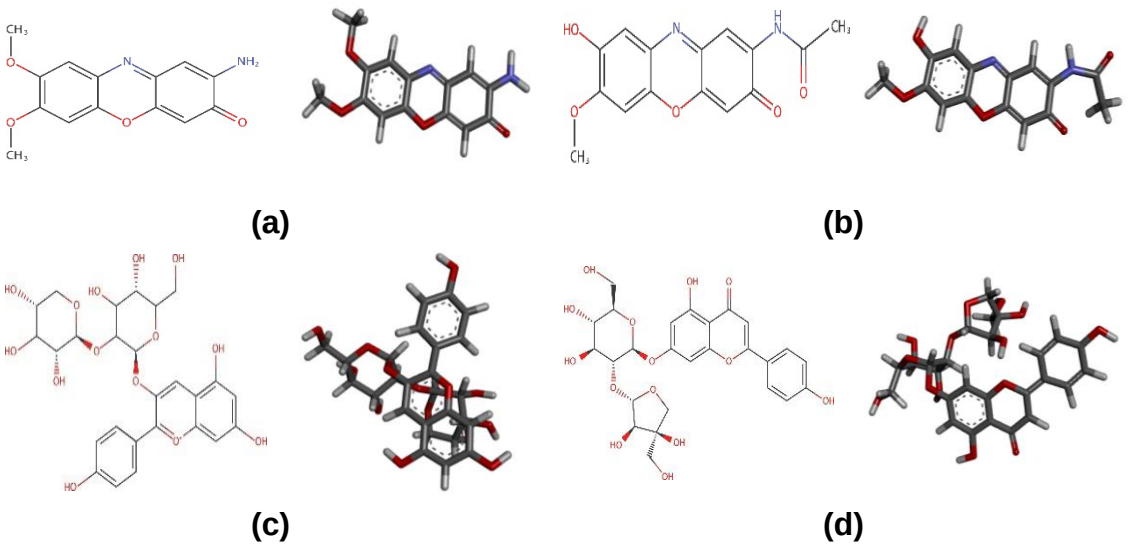

Figure 2: Structure of Test Compounds (a) Peristrophine; (b) Perisbivalvin; (c) Pelargonidin; and (d) Apioside

The acceptance of the docking program was analyzed based on the root mean square deviation (RMSD) value gained by redocked native ligands towards the target proteins.

The validation results on the target proteins 4XMB, 6TYM, 3TI1, and 4I23 showed an RMSD value of 0.001 Å presented in Table 2, and the overlay results of the redocked native ligand and the crystallographic native ligand can be viewed in Figure 3.

Table 2: Method Validation Results on Target Protein

No	Makromolekul	Ligand	RMSD (Å)
1.	4XMB	*41P*	0,001
2.	6TYM	*08A*	0,001
3.	3TI1	*Sunitinib*	0,001
4.	4I23	*Dacomitinib*	0,001

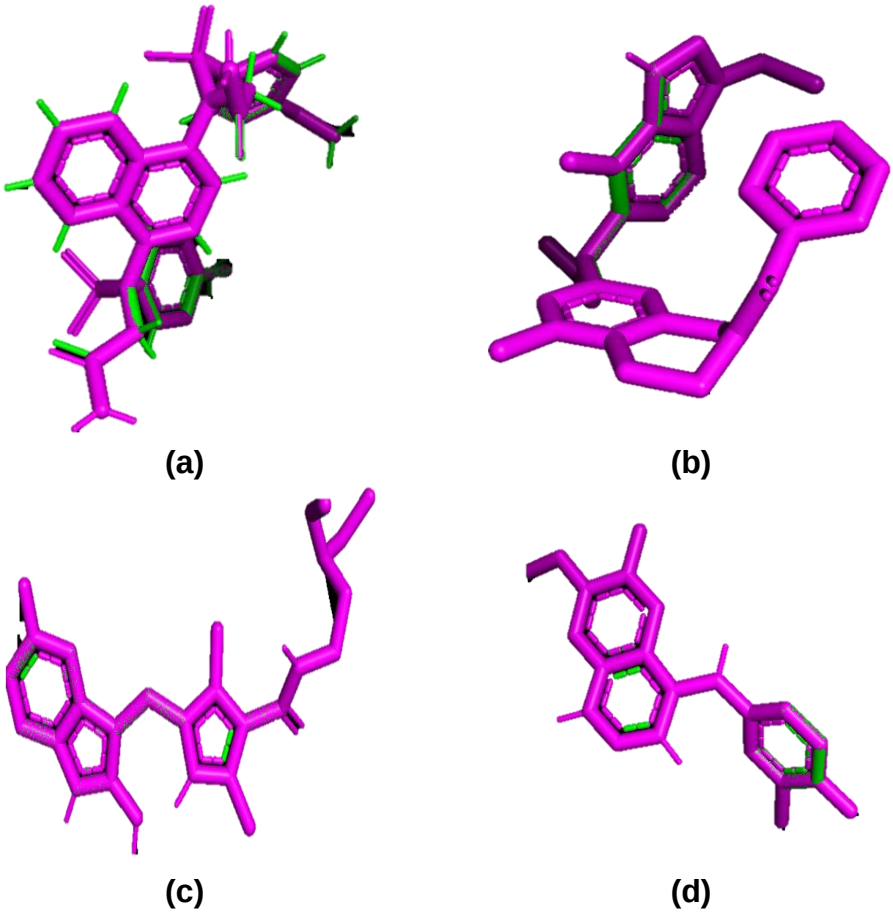

Figure 3: Overlay Results of Redocked Native Ligands (Red) and Crystallography Resulted Native Ligands (Green) (a) 4XMB; (b) 6TYM; (c) 3TI1; and (d) 4I23

The molecular docking parameters in this study were collected from the AutoDock Tools program with the parameter observed being the binding energy value. The results of binding energy values between target proteins with native ligands and test compounds are displayed in Table 3.

Table 3: Docking Parameters of Original Ligand and Test Compound

GDP Target Code	Ligand	*Binding Energy* (kcal/mol)	Hydrogen Bonding
4XMB (NRF2/KEAP1)	*41P**	-10,60	Ile461, Arg415, Asn414, Ser363
	Peristrophine ***	-5,12	Asn414, Ser363, Ser555
	Perisbivalvin ***	-4,90	Ile461, Gly509, Gln530
	Pelargonidin ***	-3,99	Gly509, Ile461, Tyr525
	Apioside ***	-5,30	Tyr525, Arg415, Asn414, Ser602
6TYM (NRF2/KEAP1)	*08A**	-14,94	Ser508
	Peristrophine ***	-6,79	Ser508, Ser602, Arg483
	Perisbivalvin ***	-5,02	Gln530
	Pelargonidin ***	-6,43	Arg483, Dms702, 08a703
	Apioside ***	-2,81	Ser508, Arg483
3TI1 (CDK2)	*Sunitinib***	-8,96	Leu83, Glu81, Ile10
	Peristrophine ***	-6,58	Asp86
	Perisbivalvin ***	-7,38	Leu83, Glu81, Asp86
	Pelargonidin ***	-5,62	Leu83
	Apioside ***	-4,34	Asp86, Glu12
4I23 (EGFR)	*Dacomitinib***	-6,06	-
	Peristrophine ***	-5,57	Met793, Lys745
	Perisbivalvin ***	-6,28	Glu762, Met793
	Pelargonidin ***	+26,31	Thr790, Met793
	Apioside ***	+47954,42	Tyr727

Description:

* = Native ligand

** = Drug compound

*** = Test compound

The prediction of the physicochemical developments of the test compounds was drifting out by analyzing the Lipinski rule of five which predicts the ability of the test compounds to diffuse through the cell membrane. The results of the Lipinski rule of five analysis of the four test compounds are presented in Table 4.

Table 4: Lipinski rule of five analysis of test compounds on magenta leaves

Compound Name	Molecular Weight (<500 Dalton)	*H-Bond acceptors* (<10)	*H-bond donors* (<5)	logP (<5)	*Molar Refractivity* (40-130)
Peristrophine	272	6	2	1,228	74,29
Perisbivalvin	300	7	2	1,426	79,73
Pelargonidin	565	14	9	-0,249	132.69
Apioside	564	14	8	0,769	132.56

Description:

BM. = The molecular weight (MW) must be <500.

H-acceptor bond = H-acceptor bonds are expressed in terms of the number of O with N atoms < 10

H donor bond = H donor bond expressed in O-H with N-H groups <5

LogP = Log fat/water partition coefficient should be <5

Molar Refractivity = A measure of the total polarization of moles of a substance

The toxicity parameters in this study were achieved through a pharmacokinetic prediction program, ADMETlab 2.0 website. The toxicity specification of the test compounds are in Table 5.

Table 5: Toxicity Prediction Results of Magenta Leaves Compounds

Ligand	Parameter			
	Acute Toxicity Rule	***Genotoxic***	***NonGenotoxic***	***In vitro mutagenicity***
Peristrophine	*0 Alert*	*3 Alert*	*1 Alert*	-
Perisbivalvin	*0 Alert*	*3 Alert*	*1 Alert*	- -
Pelargonidin	*0 Alert*	*0 Alert*	*0 Alert*	+
Apioside	*0 Alert*	*0 Alert*	*0 Alert*	+

Description:

- = No potential

-- = Very low potential

+ = Potential

DISCUSSION

The antioxidant activity test in this study used ethanol extract of magenta leaves given to an animal model of oxidative stress using Wistar male white rats divided into 3 groups, namely the control group (P0), the 125mg/KgBB ethanol extract intervention group (P1), and the 250mg/KgBB ethanol extract intervention group. Tests carried out include the examination of Malondialdehyde (MDA), Nrf2 tissue, Superoxide Dismutase (SOD), and Glutathione (GSH) levels systematic using quantitative sandwich enzyme immunoassay (ELISA) techniques.

Malondialdehyde (MDA) is one of the oxidation products formed from the process of lipid peroxidation in cell membranes. High MDA levels indicate an increase in oxidative stress and free radicals in the body, so the role of antioxidants is needed in excessive an increase in MDA levels (17). The administration of 125 mg/KgBB and 250 mg/KgBB ethanol extracts of magenta leaves to the oxidative stress experimental animal model is able to reduce the state of oxidative stress, resulting in lower MDA levels compared to the group without extract administration. The 250 mg/KgBB dose has the best ability as an antioxidant as it caused the MDA levels in the group given this dose to be lower than the levels of the control group.

Nrf2 levels in both groups at doses of 125 mg/KgBB and 250 mg/KgBB were higher when compared to the control group. These results corroborate that the content of secondary metabolite compounds in ethanol extracts of magenta leaves, namely flavonoids, and anthocyanins are able to activate endogenous antioxidants through Nrf2 regulation. Nrf2 is a transcription factor that oversees the expression of antioxidant proteins, which help protect against oxidative damage. (18).

In normal or unstressed conditions, Nrf2 is retained in the cytoplasm through its binding with Keap1 and is tagged for degradation via ubiquitination by Cullin3, followed by breakdown by the proteasome (19). The presence of oxidative or electrophilic stress will cause modification of cysteine residual in Keap1. This causes a change in the shape of Keap1, preventing ubiquitination and enabling Nrf2 to move to the nucleus to stimulate the production of endogenous antioxidants. (20).

Superoxide dismutase (SOD) is one of the enzymes involved in cellular defense against oxidative stress generated by ROS (21). SOD catalyzes the change of superoxide anion to hydrogen peroxide. The catalase enzyme will then proceed to convert hydrogen peroxide into oxygen and water (22). In this study, the administration of 250 mg/KgBB magenta leaves ethanol extract to rats caused higher Superoxide dismutase (SOD) levels are measured in comparison to a control group, while glutathione (GSH) is crucial for protecting cells from free radicals, peroxides, and other toxic agents (23). Providing magenta leaves extract in the P1 and P2 intervention groups gave higher GSH enzyme activity compared to the control group. This indicates that giving ethanol extract of magenta leaves containing flavonoid compounds, as well as anthocyanins can counteract free radicals.

Several studies have mentioned that the application of antioxidants to patients undergoing chemotherapy can reduce the incidence of side effects and increase survival time [9]. The antioxidant activity test of magenta leaves that has been carried out provides results that the application of 125 mg/KgBB and 250 mg/KgBB of magenta leaves ethanol extract can reduce the state of oxidative stress compared to the group without extract application. Based on the antioxidant activity of magenta leaves, a study was conducted using the molecular docking method to predict the potential of compounds from magenta leaves that act as cervical anticancer agents.

Molecular docking analysis is one of the in silico methods that allows early identification of target molecules that have potential as anticancer agents from natural compounds (24,25). The acceptance of the docking program was analyzed using PyMOL software. The validation results on 4XMB, 6TYM, 3TI1, and 4I23 receptors showed an RMSD value of 0.001 Å. This value states that the docking method and parameter settings used are valid, thus the parameters can then be used for docking the test compounds (26). The molecular docking process of the test compound was performed using AutoDock Tools 1.5.7 software with a binding energy value parameter that describes the strength or energy required for the interaction between the receptor and the ligand (27). A lower or more negative binding energy value signifies a more stable interaction between the ligand and the receptor (28,29).

Based on the results of molecular docking conducted on Nrf2 receptors (4XMB) with the test compound, it is recognized that the Apioside compound has a binding energy value of -5.30 kcal/mol. Meanwhile, the results of molecular docking conducted on the Keap1 (6TYM) receptor with the test compound showed that the Peristrophine compound has a binding energy value of -6.79 kcal/mol. However, this value is still greater when compared to the native ligands of the 4XMB and 6TYM proteins, indicating that the magenta leaves compound is able to bind to these proteins but is less stable when compared to the native ligands.

The development of non-covalent Nrf2 activators focuses on blocking its interaction with its negative regulator, KEAP1. The inhibitor binds to the KEAP1 domain where the ETGE motif of Nrf2 binds, thus displacing, and activating Nrf2. (30). Studies revealed that significant interactions seem to occur at amino acid residues ARG415 and ARG483 on the binding pocket (20,30). In this study, the amino acid residue ARG415 was found in all magenta leaves compounds and the hydrogen bond type was found in the compound Apioside. In addition, the amino acid residue ARG483 was also found to have hydrogen bonds in Peristrophine and Pelargonidin compounds, indicating that the compounds from the magenta leaves are able to bind to the same

binding pocket as the original ligand, so they are predicted to be able to provide similar effects to the original ligand, namely as non-covalent activators of Nrf2.

In cervical cancer, the epithelial tissue of the cervix is subjected to oxidative stress, which can lead to persistent chronic viral infections. Therefore, the mechanism of cervical cancer is related to the cell cycle expressed by HPV and causes stimulation of cell proliferation that exceeds normal limits (6,31). Cyclin-dependent kinase 2 (CDK2) and the epidermal growth factor receptor (EGFR) are receptors involved in cell proliferation (32,33). As part of the protein kinase family, CDK2 plays a role in regulating the cell division cycle in eukaryotes (34). Increased expression of CDK2 has been identified in cervical cancer cell lines. The CDK2 receptor works by forming a complex with cyclin A that dominantly controls the G1 to S phase cycle (35,36). Studies on CDK2 inhibitors also present new prospects for cancer treatment. Therefore, CDK2 inhibition is a therapeutic target designed to arrest or restore cell cycle control in irregularly dividing cells (37).

The epidermal growth factor receptor (EGFR) has been associated with the incidence of cervical cancer development (38,39). EGFR is well described regarding its involvement in several processes such as proliferation and resistance to chemotherapy (40,41). The EGFR receptor signaling pathway leads to G1/S cell cycle progression. The EGFR expression correlates with poor survival among cervical cancer patients. HPV infection was demonstrated to increase the number of EGFR receptors exposed on the cell membrane by inhibiting EGFR degradation and increasing the expression of intracellular signal transducers commonly associated with EGFR signaling (42). Thus, targeting EGFR with compounds that act as inhibitors is a promising strategy in cancer therapy (43).

Based on the docking results of the test compounds with CDK2 (3TI1) and EGFRF (4I23) receptors, the smallest binding energy value was obtained for the Perisbivalvin compound. The binding energy value of the Perisbivalvin compound (-6.28 kcal/mol) against protein 4I23 is smaller than that of the Dacomitinib compound (-6.06 kcal/mol). This suggests that the Peristrophine compound has a more stable conformation when compared to Dacomitinib.

In addition to having high potency and selectivity, the physicochemical properties of a new drug are also important to know because they are used to determine the presence of active substances in the body which will further determine its pharmacological activity (44). The Lipinski rule of five is used to determine the physicochemical properties and hydrophobic/hydrophilic character of a compound so that the ability of the compound to diffuse through cell membranes can be determined (45,46). Of the four compounds tested, it was found that the Peristrophine and Perisbivalvin compounds met the Lipinski rule of five, so it was known that the two compounds had potential as drugs that could be given orally, while the Pelargonidin and Apioside compounds do not meet the Lipinski rule of five because they violate more than one criterion (44,47).

Determining the toxicity of a compound is necessary to identify harmful effects on humans, animals, plants, or the environment. It is also one of the major steps in drug design development (48). The prediction of compound toxicity in this study refers to the Toxicophore Rules presented in Table 4. Based on Acute Toxicity Rule testing, the results obtained 0 alerts on the four test compounds. This shows that the four test

compounds are predicted not to have structures that can cause acute toxicity during oral application (49,50).

Based on Carcinogenicity testing on test compounds, the results showed that there are two magenta leaf compounds, namely Peristrophine and Perisbivalvin, which have three genotoxic structures and one non-genotoxic structure. Meanwhile, the Ames Toxicity test results revealed that there are two magenta leaves compounds, namely Pelargonidin and Apioside, which have a value of (+) which means their mutagenic potential is low, while the other two compounds, Peristrophine and Perisbivalvin, are known to have no mutagenic potential (51).

This study concluded that ethanol extract of magenta leaves has good antioxidant activity through its activity in reducing Malondialdehyde (MDA) levels, increasing Superoxide Dismutase levels and Glutathione levels through regulation of Nuclear Factor Erythroid Related Factor-2 (Nrf2). The molecular docking approach also shows the binding of active compounds from ethanol extract of magenta leaves to proteins which are molecular targets of cervical cancer. Therefore, the active compounds from ethanol extract of magenta leaves are predicted to be new candidate compounds that have activity as cervical anticancer agents.

CONCLUSION

This study concluded that the ethanol extract of magenta leaves has good antioxidant activity. The molecular docking approach also shows the potential of active compounds from magenta leaves ethanol extract as cervical anticancer agents.

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