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Best regards

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The manuscript submitted to the Tropical Journal of Natural Product Research https://www.scopus.com/sourceid/21100933230 SCOPUS by the corresponding author is undergoing the peer-review process.

Title: Analysis of Potential Poly(ADP-Ribose) Polymerase 2 (PARP2) Inhibitor in Nyale Worm (*Eunice sp.*) Extract for Ovarian Cancer: An In Silico Approach

Journal: Tropical Journal of Natural Product Research www.tjnpr.org

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Manuscript No: TJNPR FB304ARN

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A. MANUSCRIPT

Journal	**Tropical Journal of Natural Product Research**
Manuscript Number	FB304AR
Type of paper	Research paper
Title of paper	Analysis of Potential Poly (ADP-Ribose) Polymerase 2 (PARP2) Inhibitor in Nyale Worm (*Eunice sp.*) Extract for Ovarian Cancer: An In Silico Approach
Name of Authors	

B. REVIEWER'S SPECIFIC COMMENTS PER SECTION OF MANUSCRIPT

Abstract	OK
Introduction	OK. See manuscript for a few corrections
Methodology	A lot of corrections to be effected
Results	There are many omissions and corrections to be made to this section.
Discussion	Not fully discussed. See corrections in the manuscript
Conclusion	Just repetition of results
References	Did not follow journal style
Figures, Tables	Tables are ok. Figures should properly titled and corrected.

C. REVIEWER'S GENERAL COMMENTS AND REMARKS

Comments may be continued onto another sheet if necessary.

The research conception is good. However, there are major revisions that the authors should effect to make the manuscript readable.

There are obvious omissions in the materials and methodology. Collection, authentication and preparation of the study sample were omitted. The make, model and instrumental condition as well as the chromatogram of the GC-MS were not stated. Some of the molecular docking software and their versions were not stated. There are a lot of gaps in the discussion, and authors many not much acquainted with molecular docking studies. They did not show clear understanding and the theory of Lipinski rule of five. Some of the ligands interactions mentioned in the discussion were not shown in the figures referred. The conclusion should be written to reflect the findings and goals of the research, not mere repetition of the results. The references and figures should be corrected.

D. REVIEWER'S RECOMMENDATION

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Accept with minor revisions (editor will check),specific comments to the editor below	
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Analysis of Potential Poly (ADP-Ribose) Polymerase 2 (PARP2) Inhibitor in Nyale Worm (*Eunice sp.*) Extract for Ovarian Cancer: An In Silico Approach

Abstract

Cancer is an umbrella term for a large group of diseases that are characterized by abnormal cell growth and adjacent tissue or organ invasion. One of the most common cancers in Indonesia is ovarian cancer. Recently, PARP enzyme inhibitor use as a therapy for cancer, including ovarian cancer, has become more common. Apart from the standard PARP inhibitor drug, natural resources are also found to have high potential for cancer therapy. Marine biotas are known for their capability to produce biomolecules which can inhibit the cell mitosis of their rivals or predators. One of the marine biotas that are commonly consumed in Lombok Island is Nyale worm. This research aimed to analyze the potential PARP, particularly PARP2, inhibitor compounds in Nyale worm extract for ovarian cancer by using molecular docking with *in silico* approach. Compounds identification was conducted by using gas chromatography-mass spectrometry (GC-MS) and molecular docking was done with PyRx v.0.8 software. There were three potential PARP2 inhibitor compounds, tricyclo[10.2.1.02,11]pentadeca-4,8-diene, tricyclo[8.6.0.02,9]hexadeca-3,15-diene, and linoleic acid. The binding affinity energy of these three compounds were lower compared with that of the native ligand 3-aminobenzamide. The lower value of the energy means greater molecular binding stability and PARP2 inhibition mechanism.

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Key words: DNA repair, Nyale worm, ovarian cancer, PARP, PARP2 inhibitor

Introduction

Cancer is an umbrella term for a large group of diseases that are characterized by abnormal cell growth and adjacent tissue or organ invasion (1). One of the most common cancers in Indonesia is ovarian cancer. As of 2018, there were 14.896 new cases of ovarian cancer making it the tenth disease with the most new cases in Indonesia according to Globocan data (2). With a total of 9.581 deaths, ovarian cancer is also the seventh cancer with the highest number of deaths (3).

Anticancer therapy targeting Poly(ADP-ribose) polymerase (PARP) enzyme was originally proposed by Mendel. PARP enzyme detects the DNA single-strand break (SSB) and causes DNA repair in cancer cells through base exicional repair (BER) mechanism (4). PARP uses NAD^+ that is transferred to the glutamate, aspartate, and lysine residues acceptor to catalyze ADP-ribose for auto-modification. This facilitates DNA repair through the formation of chromatine structures by replacing the histone and signaling the DNA repair complex protein. There are 17 enzymes of the PARP superfamily in humans, including PARP1 and PARP2 (5, 6). Recently, PARP enzyme inhibitor use as a therapy for cancer, including ovarian cancer, has become more common (6-8). An orally-administered PARP inhibitor standard drug, 3-aminobenzamide, is effective in enhancing the damage of the cancer cell DNA (6, 9).

Apart from the standard drug, natural resources are also found to have high potential for cancer therapy. Marine biotas are known for their capability to produce biomolecules which can inhibit the cell mitosis of their rivals or predators (10, 11). A marine worm, *Hermione hystrix*, is reported to have antimitotic-cytotoxic activity towards sea urchin *Paracentrotus lividus* embrio (12). Several other marine biotas such as sponges, mollusks, and cyanobacteria are also reported to have anticancer compounds (13).

Lombok Island is rich in marine biota. One of the renowned marine biotas found in Kuta Mandalika beach, a famous tourism destination in Central Lombok, is Nyale worm. It is

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commonly consumed by the local community. Nyale worm (*Eunice sp.*) is a member of Polychaeta class that includes three other species, *Lysidice sp.*, *Neanthes*, and *Aphrodite* (14).

The anticancer properties of Nyale worm have not been widely researched. Therefore, this research aimed to analyze the potential PARP2 inhibitor compounds in Nyale worm extract for ovarian cancer by using molecular docking with *in silico* approach. The compounds were compared with a standard drug for inhibition target mechanism against PARP2 enzyme.

2. Materials and Methods

2.1 Chemistry

Quantitative analysis with gas chromatography-mass spectrometry (GC-MS) was conducted to identify ~~every specific~~ the bioactive compounds ~~contained~~ present in Nyale worm extract. The amount of compounds contained in the extract was shown by the number of peaks in the GC-MS chromatogra~~mphy~~. Meanwhile, the name of each compound was interpreted from the GC-MS spectral data.

2.2 Protein/Macromolecule

PARP2 (GDP: 3KCZ) structure was obtained from rscb.org in the Protein Data Bank (PDB) format. PARP2 structure consisted of two chains, chain A and chain B. Each chain contained inhibitor ligand 3-aminobenzamide. PARP2 PDB structure was prepared using PyMOL ~~application~~software (state the version).

2.3 Ligand and drug screening

Twenty compounds were identified by GC-MS in the Nyale worm extract ~~were identified by GC-MS and~~ The compounds identity were confirm through chemical ~~n~~ searched using PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The bioavailability of the compounds was assessed according to Lipinski's Rule of Five using SwissADME (http://www.swissadme.ch/). Assessment of human intestinal absorption (HIA) was conducted

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with the use of PreADMET predictor (https://preadmet.webservice.bmdrc.org/). Ligands were prepared using Avogadro software (15).

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2.4 Molecular docking

Molecular docking of the twenty compounds in Nyale worm extract to the PARP2 protein was done with PyRx v.0.8 software (16). The molecule binding target area was X: 19.5762, Y: 2.9482, Z: 20.3313 and Dimension (A) X: 11.3241, Y: 8.1201, Z: 10.2827. This was the binding site of 3-aminobenzamide, a widely-used PARP2 inhibitor standard drug. The active binding site on PARP2 was observed in Computed Atlas of Surface Topography of Proteins (CASTp) (sts.bioe.uic.edu/castp/index.html?3kcz) (17). The result of the protein interaction and ligand binding residue identification was visualized with PyMOL and Discovery Studio R17.

3. Result and Discussion

3.1 Result

3.1PARP2 inhibitor mechanism for cancer cell

PARP2 working mechanism in Figure 3 shows that DNA repair is a potential target to kill ovarian cancer cell (18). The SSB is often found in proliferating cells. The PARP2 inhibitor affects BER, preventing the DNA repair to occur. The SSB then turns into double-strand break (DSB) leading to inhibition of cell proliferation~~which harms the cell~~. It may also affects the cell recombinant if the homologous recombination deficiency (HRD) is present. This condition renders the DSB irreparable, inducing cell apoptosis (19).

Based on the PARP2 molecule structure shown in Figure 2, PARP2 was found to have NAD^+ cofactor (denoted by arrow). NAD^+ has a pivotal role in DNA repair process. NAD^+ breaks down into nicotinamide and ADP-ribose to form poly(ADP-ribose) (PAR) which

binds to the DNA repair protein acceptor (20). Previous studies reported that inhibiting NAD^+ significantly hampered the DNA repair by PARP2, leading to cell apoptosis (21, 22).

The binding affinity to the native ligand was -6.6 kcal/mol. This value indicated the energy needed to bind to the PARP2 receptor. The lower the value, the higher the possibility of a compound to tightly bind to the PARP2 receptor (23). The amount of energy of the binding affinity resulted from the molecular docking can be made as a reference to compare the amount of energy generated by each compound.

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3.2 Human intestinal absorption (HIA)

Percentage of HIA (% HIA) tells the absorbability of the compounds in the small intestine. Table 1 indicates that the compounds in Nyale worm extract had high absorption level (HIA > 90%). This means that the compounds ~~are more absorbable~~have good oral absorption profile and can reach the ovarian cancer cell receptor if ~~the extract is~~ administered orally. Thus, oral administration can increase the efficacy of the compounds (24).

3.3 Lipinski's Rule of Five

Assessment according to Lipinski's Rule of Five parameter before docking can ~~i~~ensure the ability of the compound to reach the appropriate receptor binding site (25). As shown in Table 1, the molecular docking could be proceeded for all compounds since two violations of Lipinski's Rule of Five were not found (26).

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3.4 Molecular docking

There were three potential PARP2 inhibitor compounds, tricyclo[10.2.1.$0^{2,11}$]pentadeca-4,8-diene, tricyclo[8.6.0.$0^{2,9}$]hexadeca-3,15-diene, and linoleic acid (Table 1). Molecular docking can predict the amount of energy generated among two or more interacting or binding molecules (27). The binding affinity energy of the three compounds were lower compared with that of the native ligand 3-aminobenzamide. Figure 2 shows the visualization of PARP2 where the four compounds bound to the same

active site. The lower value of the energy resulting ~~ed~~ from docking (kcal/mol) means greater molecular binding stability and PARP2 inhibition mechanism (28).

Interaction between PARP2 and 3-aminobenzamide shown in Table 2 explains its ~~the~~ affinity for PARP2 inhibitor. The side chain residue of TYR473 formed pi-alkyl bond with the imidazole ring. The bond between GLU558 and nitrogen atom at the end of the imidazole chain formed two hydrogen bonds. The backbone of TRP427 and HIS428 bound to the nitrogen atom, also forming the hydrogen bonds. The backbone residue of GLY429 and SER470 formed hydrophobic bonds. TYR462 caused an interaction with the cyclic amine substituent (proline) in the benzamidine ring to the backbone of GLY429. The residue of LYS469, TYR462, ALA464, PHE463 formed hydrophobic bonds as well.

The low binding affinity of tricyclo[8.6.0.0.0^{2,9}]hexadeca-3,15-diene results from interaction ~~could be caused by the binding~~ of TYR473 residue ~~to~~ with the cyclooctane ring of the inhibitor ligand. This residue functioned as a bridge for the bond between TYR462 and inhibitor. LYS469 and ALA464 also bound to the cyclooctane ring, forming pi-alkyl bond. TYR462 residue had a key role in the binding to the side chain of cyclooctane ring and in determining the binding of the inhibitor compound. The side chain residue of SER470, GLY429, PHE463, HIS428, GLN332, and TYR455 helped with the binding to the PARP2 receptor and yielded the hydrophobic bonds.

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~~From~~ Similarly, the interaction between the inhibitor compound tricyclo[10.2.1.0^{2,11}]pentadeca-4,8-diene and the PARP2 receptor, ~~it was located~~indicates that TYR473 bound to the cyclodecane ring, forming two pi-alkyl bonds. Likewise, residue TYR462 formed pi-alkyl bonds (with what?) causing low and stable binding affinity energy.

Interaction between GLY429 and oxygen atom from the linoleic acid created hydrogen bond. The bond also created from the interaction between residue SER470 and hydrogen atom, contributing the amount of energy generated (29). TYR473, LYS469, and and

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ALA464 bound to the linoleic chain to form pi-alkyl bonds. Linoleic acid, also known as omega-6, is reported to have anticancer properties (30). Study by Zhang stated that linoleic acid could deliver a significant improvement in ~~the~~ breast cancer treatment (31). Another study ~~informed~~ reported that conjugated linoleic acid ~~was~~ has antiproliferative activity and is able to activate the cell death pathway (32).

Residues TYR473 and TYR462 ~~had important contribution~~were actively involved in the interaction with the three inhibitors from the Nyale worm extract. The result of the interactions ~~can be made as a reference regarding the similarity~~ of the inhibitor compounds from the Nyale worm extract compares favourably with the commercially used native ligand with respect to the ~~of~~similarity of the binding domains. ~~among the inhibitor compounds contained in the extract and the commercially used native ligand. Eventually, The result of this research was able to analyze the potential inhibitor compounds based on their bonds with PARP2.~~

4. Conclusion

Nyale worm is a commonly consumed marine biota in Lombok Island. This research analyzed the potentials of PARP2 inhibitor compounds in Nyale worm extract for ovarian cancer by using molecular docking with in silico approach. There were three potential PARP2 inhibitor compounds, tricyclo[10.2.1.$0^{2,11}$]pentadeca-4,8-diene, tricyclo[8.6.0.$0^{2,9}$]hexadeca-3,15-diene, and linoleic acid. The binding affinity energy of these three compounds were lower compared with that of the native ligand 3-aminobenzamide. The lower value of the energy means greater molecular binding stability and PARP2 inhibition mechanism.

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5. Conflict of interest

There is no conflict of interest.

6. Acknowledgement

This research was funded by Yayasan Pesantren Al-Azhar.

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Table and figure

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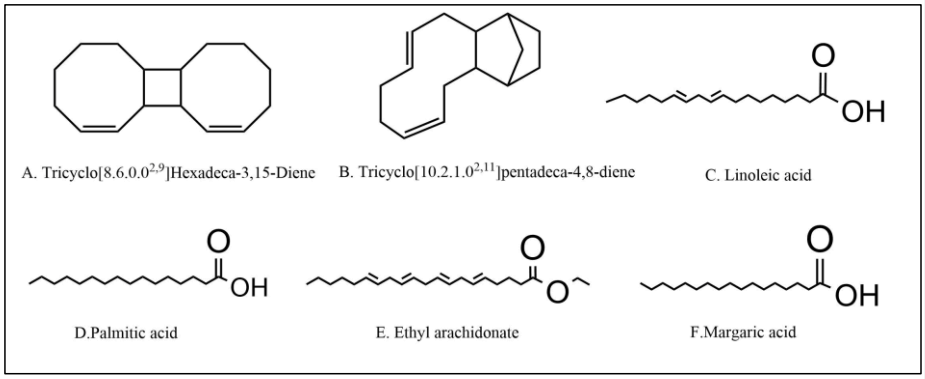

Figure 1 Some the ~~C~~compounds ~~contained~~ identified in Nyale worm (*Eunice sp.*)

Table 1. Identification of the potential PARP2 inhibitor compounds contained in Nyale worm extract based on their bioavailability and HIA.

No	Compound Name	Molecular Formula	Da	H-donor	H-acceptor	LogP	HIA (%)	Binding Affinity (kcal/mol)
1	Tricyclo[8.6.0.0^{2,9}]hexadeca-3,15-diene	$C_{16}H_{24}$	202.34	0	0	4.02	100	-8.8
2	3-Aminobenzamide (native ligand)	$C_7H_8N_2O$	136.15	2	1	0.32	90.98	-6,6
3	Margaric acid	$C_{17}H_{34}O_2$	270,45	1	2	5,57	98,40	-6,2
4	9-Octadecenal	$C_{18}H_{34}O$	266,46	0	1	5,94	100	-6,1
5	Myristic acid	$C_{14}H_{28}O_2$	228,37	2	1	4,45	978.483	-5,9
6	Pentadecylic acid	$C_{15}H_{30}O_2$	242,40	1	2	4,84	98,11	-5,9
7	Stearic acid	$C_{18}H_{36}O_2$	284,48	1	2	5,93	98,44	-6,2
8	Linoleic acid	$C_{18}H_{32}O_2$	280,45	1	2	5,45	98,37	-6,7
9	Palmitic acid	$C_{16}H_{32}O_2$	256,42	1	2	5,20	98,29	-6.1
10	Methyl myristate	$C_{15}H_{30}O_2$	242,40	2	0	4,81	100	-5,8
11	Ethyl arachidonate	$C_{22}H_{36}O_2$	332,52	0	2	6,42	100	-5,9
12	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.46	1	2	5,71	98.43	-6.6

13	Benzene, 1,2-dimethyl-	$C_6H_4(CH_3)_2$	106.17	0	0	2.83	100	-5.6
14	Hexadecanoic acid	$C_{18}H_{36}O_2$	284,48	0	2	5,79	100	-6.0
15	Tricyclo[10.2.1.0^{2,11}]pentadeca-4,8-diene	$C_{15}H_{22}$	202.34	0	0	4.02	100	-8.4
16	Methyl palmitate	$C_{17}H_{34}O_2$	270.45	0	2	5.54	100	-5.9
17	Ethyl myristate	$C_{16}H_{32}O_2$	256, 42	0	2	5,17	100	-6.0
18	Ethyl palmitate	$C_{18}H_{36}O_2$	284.48	2	0	5.90	100	-5.9
19	Methyl stearate	$C_{19}H_{38}O_2$	298.50	0	2	6.24	100	-6,3
20	Ethyl stearate	$C_{20}H_{40}O_2$	312,53	0	2	6,71	100	-5,9
21	Dythol	$C_{27}H_{46}O$	386,65	1	1	6,67	100	-3.1

Table 2 Compounds interaction

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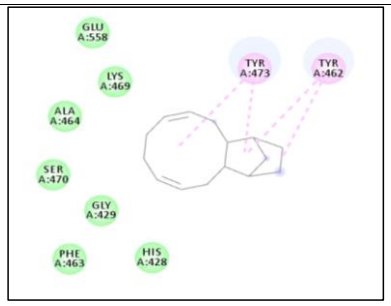
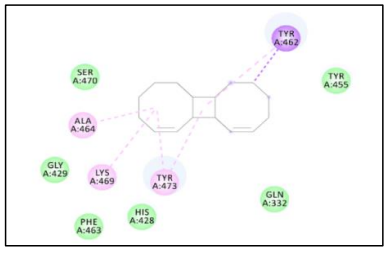
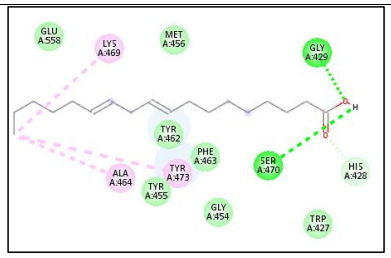
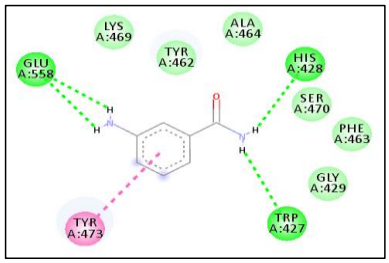
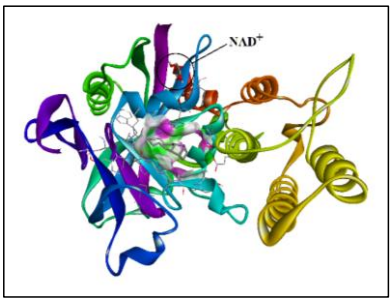
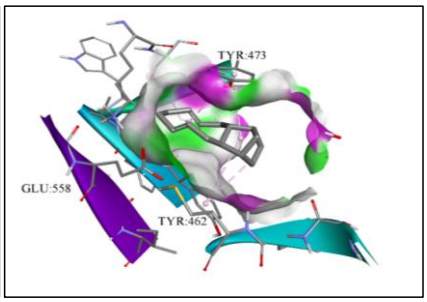
No.	Compound Name	Molecular Visualization	Interaction
1.	Tricyclo[10.2.1.0^{2,11}] pentadeca-4,8-diene		TYR473, TYR462, GLU558, LYS 469, ALA 464, SER 470, GLY 429 , PHE 463, HIS 428
2	Tricyclo[8.6.0.0^{2,9}]hexadeca-3,15-diene		TYR462, TYR473, LYS469, ALA464, TYR455, GLN332, HIS428, PHE463, GLY429, SER470

3	Linoleic acid		ALA464, TYR473, GLY429, HIS470,MET429, SER470, TRP427, GLY454, PHE463, TYR462, TYR455,
4	3-Aminobenzamide		TYR473, GLU558, HIS428, TRP427, LYS469, TYR462, ALA464, SER470,

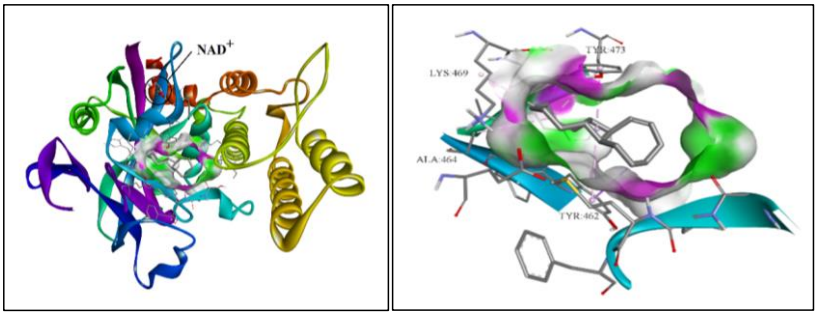
Protein Visualisation	Active Site Visualisation

A.Tricyclo[10.2.1.0^{2,11}]pentadeca-4,8-diene

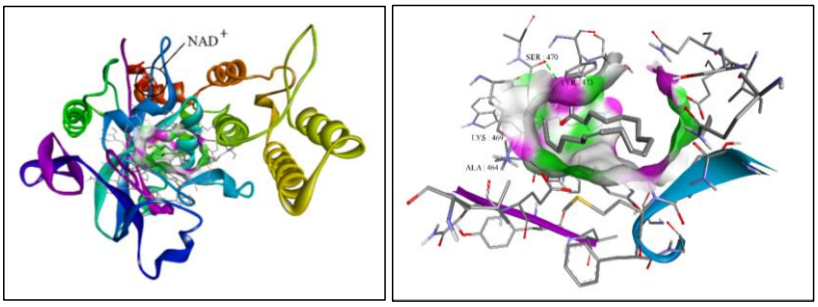




B. Tricyclo[8.6.0.0^{2,9}]hexadeca-3,15-diene



C. Linoleic acid



D. 3-Aminobenzamide

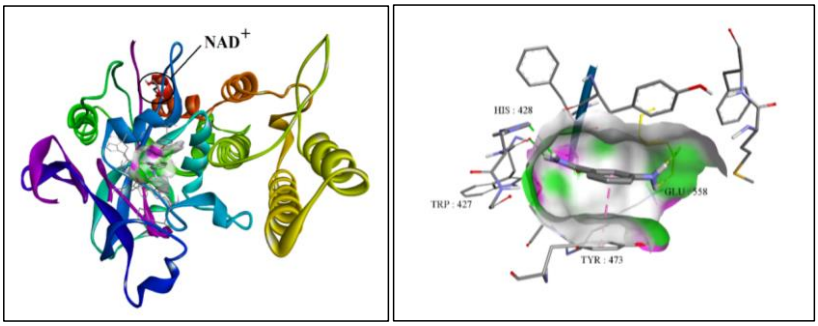


Figure2. Visualization of the inhibitor compounds against PARP2

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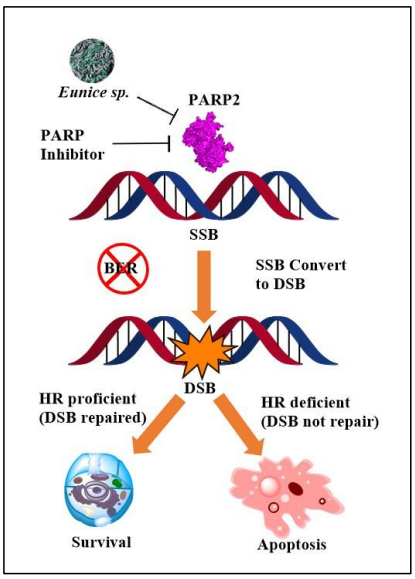

Figure 3. PARP2 inhibitor working mechanism

Trop J Nat Prod Res, June 2022; 6(6):xxxx ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Analysis of Potential Poly (ADP-Ribose) Polymerase 2 (PARP2) Inhibitor in Nyale Worm (*Eunice sp.*) Extract for Ovarian Cancer: An *In Silico* Approach

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ARTICLE INFO

Article history:
Received 16 May 2022
Revised 17 June 2022
Accepted 28 June 2022
Published online xxxxxxxxx

ABSTRACT

Cancer is an umbrella term for a large group of diseases that are characterized by abnormal cell growth and adjacent tissue or organ invasion. One of the most common cancers in Indonesia is ovarian cancer. Recently, PARP enzyme inhibitor used as a therapy for cancer, including ovarian cancer, has become more common. Apart from the standard PARP inhibitor drug, natural resources are also found to have high potential for cancer therapy. Marine biotas are known for their capability to produce biomolecules which can inhibit the cell mitosis of their rivals or predators. One of the marine biotas that are commonly consumed in Lombok Island is Nyale worm. This research aimed to analyze the potential PARP, particularly PARP2, inhibitor compounds in Nyale worm extract for ovarian cancer by using molecular docking with *in silico* approach. Compounds identification was conducted by using gas chromatography-mass spectrometry (GC-MS) and molecular docking was done with PyRx v.0.8 software. There were three potential PARP2 inhibitor compounds, tricyclo[10.2.1.02,11]pentadeca-4,8-diene, tricyclo[8.6.0.02,9]hexadeca-3,15-diene, and linoleic acid. The binding affinity energy of these three compounds were lower compared with that of the native ligand 3-aminobenzamide. The lower value of the energy means greater molecular binding stability and PARP2 inhibition mechanism.

Keywords: DNA repair, Nyale worm, Ovarian cancer, PARP, PARP2 inhibitor.

Introduction

Cancer is an umbrella term for a large group of diseases that are characterized by abnormal cell growth and adjacent tissue or organ invasion.[1] One of the most common cancers in Indonesia is ovarian cancer. As of 2018, there were 14,896 new cases of ovarian cancer making it the tenth disease with the most new cases in Indonesia according to Globocan data.[2] With a total of 9,581 deaths, ovarian cancer is also the seventh cancer with the highest number of deaths.[3]
Anticancer therapy targeting Poly(ADP-ribose) polymerase (PARP) enzyme was originally proposed by Mendel. PARP enzyme detects the DNA single-strand break (SSB) and causes DNA repair in cancer cells through base exicional repair (BER) mechanism.[4] PARP uses NAD^+ that is transferred to the glutamate, aspartate, and lysine residues acceptor to catalyze ADP-ribose for auto-modification. This facilitates DNA repair through the formation of chromatin structures by replacing the histone and signaling the DNA repair complex protein. There are 17 enzymes of the PARP superfamily in humans, including PARP1 and PARP2.[5,6] Recently, PARP enzyme inhibitor use as a therapy for cancer, including ovarian cancer, has become more common.[6,7,8] An orally-administered PARP inhibitor standard drug, 3-aminobenzamide, is effective in enhancing the damage of the cancer cell DNA.[6, 9]

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Citation: Arjita IPD, Rozikin R, Adnyana IGA, Saputra IPBA, Zoraya SI. Analysis of Potential Poly (ADP-Ribose) Polymerase 2 (PARP2) Inhibitor in Nyale Worm (*Eunice sp.*) Extract for Ovarian Cancer: An In Silico Approach. Trop J Nat Prod Res. 2022; 6(6):xxxx

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.
Apart from the standard drug, natural resources are also found to have high potential for cancer therapy.

Marine biotas are known for their capability to produce biomolecules which can inhibit the cell mitosis of their rivals or predators.[10, 11] A marine worm, *Hermione hystrix*, is reported to have antimitotic-cytotoxic activity towards sea urchin *Paracentrotus lividus* embrio.[12] Several other marine biotas such as sponges, mollusks, and cyanobacteria are also reported to have anticancer compounds.[13]
Lombok Island is rich in marine biota. One of the renowned marine biotas found in Kuta Mandalika beach, a famous tourism destination in Central Lombok, is Nyale worm. It is commonly consumed by the local community. Nyale worm (*Eunice sp.*) from *Eunicidae* family is a member of Polychaeta class that includes three other species, *Lysidice sp*., *Neanthes*, and *Aphrodite*.[14]
The anticancer properties of Nyale worm have not been widely researched. Therefore, this research aimed to analyze the potential PARP2 inhibitor compounds in Nyale worm extract for ovarian cancer by using molecular docking with *in silico* approach. The compounds were compared with a standard drug for inhibition target mechanism against PARP2 enzyme.

Materials and Methods

Sample collection and extraction
Nyale worms were collected from the coastal waters of Kuta Mandalika,Central Lombok. Dried samples were ground in a mortar and macerated in 250 mL ethanol 96% for 24 hours and n-hexane (99%) for 8 h. The residue was extracted three times with ethanol until it was colorless for ethanol extraction. Evaporator at 68°C was used for the solvent (n-hexane) removal.

Chemistry

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Quantitative analysis with gas chromatography-mass spectrometry (GC-MS) Shimadzu 2010 was conducted to identify the bioactive compounds present in Nyale worm extract.

Protein/Macromolecule
PARP2 (GDP: 3KCZ) structure was obtained from rscb.org in the Protein Data Bank (PDB) format. PARP2 structure consisted of two chains, chain A and chain B. Each chain contained inhibitor ligand 3-aminobenzamide. PARP2 PDB structure was prepared using PyMOL 2.5.2.

Ligand and drug screening
Twenty compounds were identified by GC-MS in the Nyale worm extract The compounds identity were confirm through chemical searched using PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The bioavailability of the compounds was assessed according to Lipinski's Rule of Five using SwissADME (http://www.swissadme.ch/). Assessment of human intestinal absorption (HIA) was conducted with the use of PreADMET predictor (https://preadmet.webservice.bmdrc.org/). Ligands were prepared using Avogadro 1.2.[15]

Molecular docking
Molecular docking of the twenty compounds in Nyale worm extract to the PARP2 protein was done with PyRx v.0.8 software.[16] The molecule binding target area was X: 19.5762, Y: 2.9482, Z: 20.3313 and Dimension (A) X: 11.3241, Y: 8.1201, Z: 10.2827. This was the binding site of 3-aminobenzamide, a widely-used PARP2 inhibitor standard drug. The active binding site on PARP2 was observed in Computed Atlas of Surface Topography of Proteins (CASTp) (sts.bioe.uic.edu/castp/index.html?3kcz).[17] The result of the protein interaction and ligand binding residue identification was visualized with PyMOL 2.5.2 and Discovery Studio R17.

Results and Discussion

PARP2 inhibitor mechanism for cancer cell
PARP2 working mechanism in Figure 3 shows that DNA repair is a potential target to kill ovarian cancer cell.[18] The SSB is often found in proliferating cells. The PARP2 inhibitor affects BER, preventing the DNA repair to occur. The SSB then turns into double-strand break (DSB) leading to inhibition of cell proliferation. It may also affects the cell recombinant if the homologous recombination deficiency (HRD) is present. This condition renders the DSB irreparable, inducing cell apoptosis.[19]
PARP2 was found to have NAD^+ cofactor (denoted by arrow). NAD^+ has a pivotal role in DNA repair process. NAD^+ breaks down into nicotinamide and ADP-ribose to form poly(ADP-ribose) (PAR) which binds to the DNA repair protein acceptor.[20] Previous studies reported that inhibiting NAD^+ significantly hampered the DNA repair by PARP2, leading to cell apoptosis.[21,22]
The binding affinity of 3-Aminobenzamide was -6.6 kcal/mol. This value indicated the energy needed to bind to the PARP2 receptor. The lower the value, the higher the possibility of a compound to tightly bind to the PARP2 receptor.[23]

Human intestinal absorption (HIA)
Percentage of HIA (% HIA) tells the absorbability of the compounds in the small intestine. Table 1 indicates that the compounds in Nyale worm extract had high absorption level (HIA > 90%). This means that the compounds have good oral absorption profile and can reach the ovarian cancer cell receptor if administered orally. Thus, oral administration can increase the efficacy of the compounds.[24]

Lipinski's rule of five
Assessment according to Lipinski's Rule of Five parameter before docking can ensure the ability of the compound to reach the appropriate receptor binding site.[25,26]

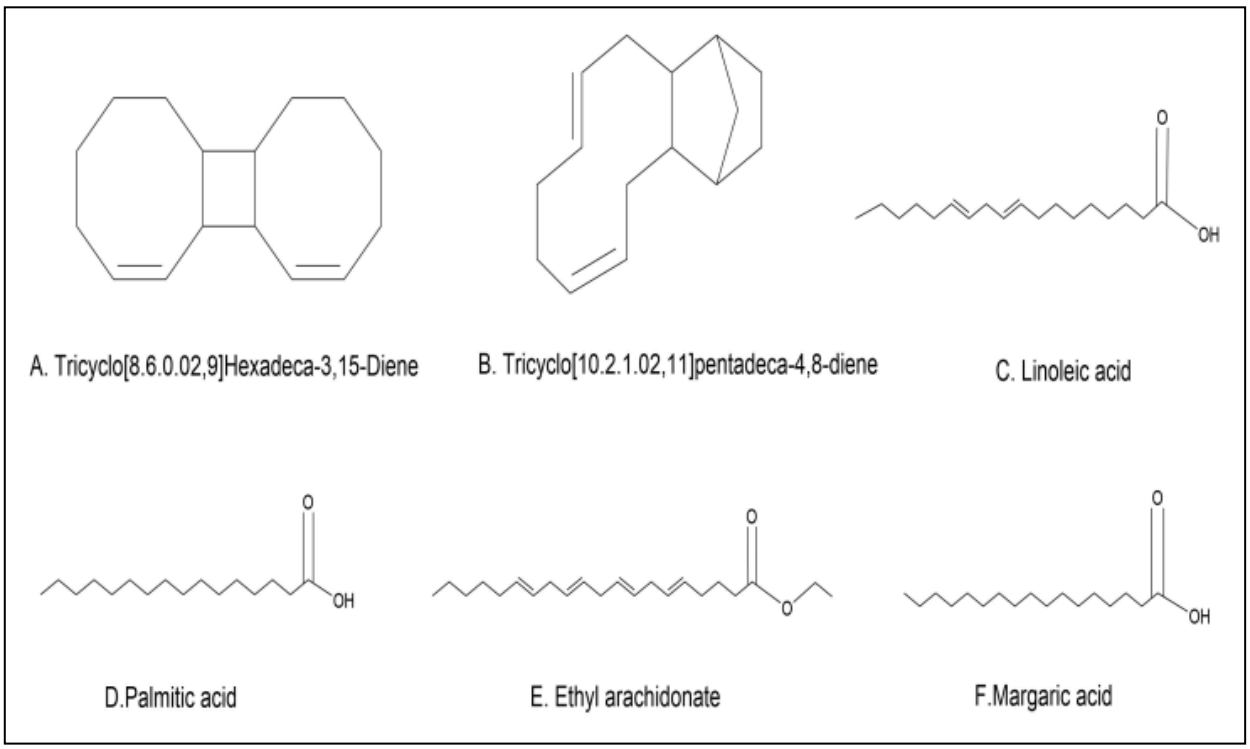

Figure 1: Some the compounds identified in Nyale worm (*Eunice sp.*)

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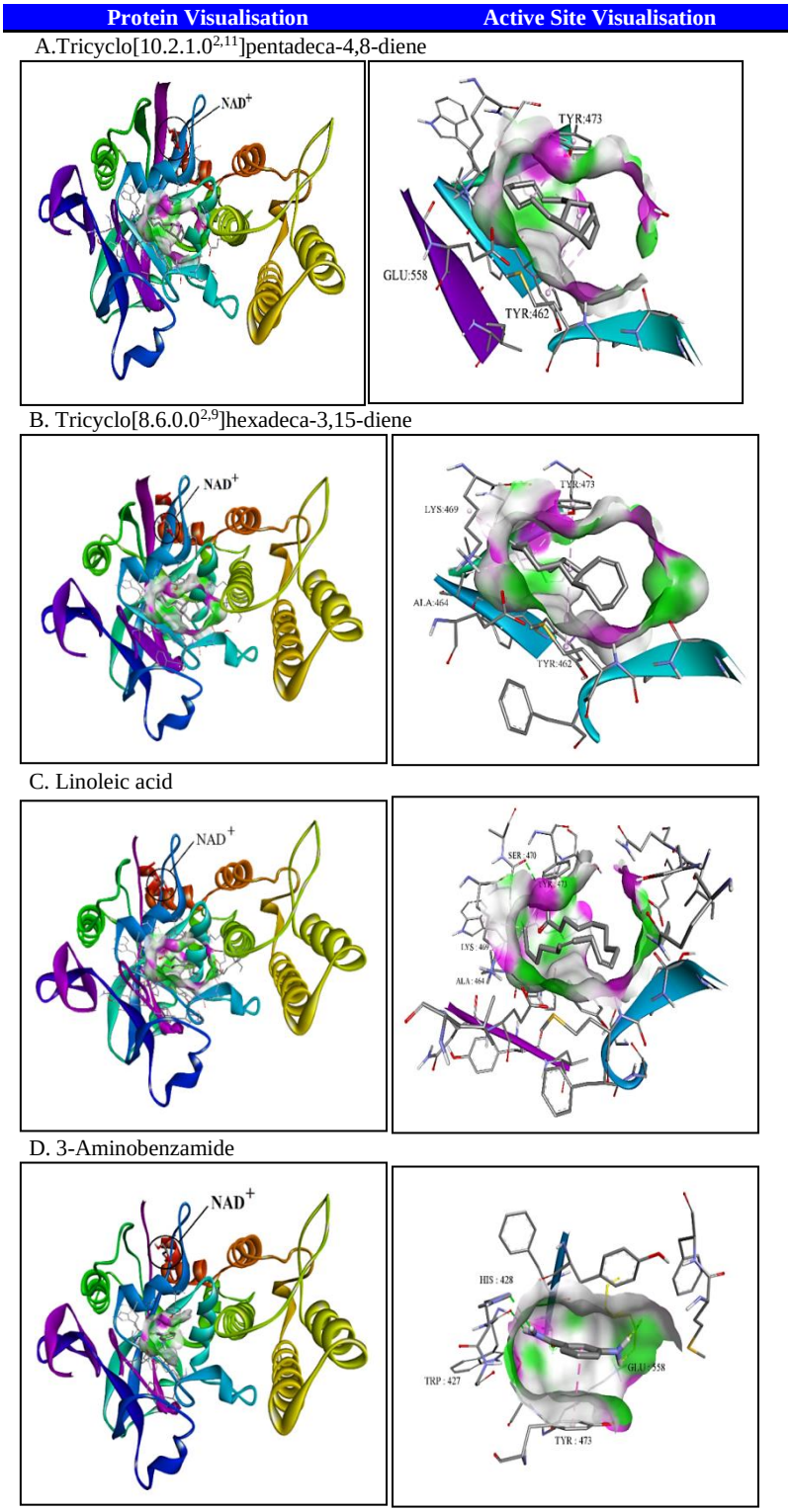

Figure 2: Visualization of the inhibitor compounds against PARP2

Molecular docking

There were three potential PARP2 inhibitor compounds (Figure 1**)**, tricyclo[10.2.1.0^{2,11}]pentadeca-4,8-diene, tricyclo[8.6.0.0^{2,9}]hexadeca-3,15-diene, and linoleic acid (Table 1). Molecular docking can predict the amount of energy generated among two or more interacting or binding molecules.[27] The binding affinity energy of the three compounds were lower compared with that of the native ligand 3-aminobenzamide. Figure 2 shows the visualization of PARP2 where the four compounds bound to the same active site. The lower value of the energy resulting from docking (kcal/mol) means greater molecular binding stability and PARP2 inhibition mechanism.[28]

Interaction between PARP2 and 3-aminobenzamide shown in Table 2 explains its affinity for PARP2 inhibitor. The side chain residue of TYR473 formed pi-alkyl bond with the imidazole ring. The bond between GLU558 and nitrogen atom at the end of the imidazole chain formed two hydrogen bonds. The backbone of TRP427 and HIS428 bound to the nitrogen atom, also forming the hydrogen bonds. The backbone residue of GLY429 and SER470 formed hydrophobic bonds. TYR462 caused an interaction with the cyclic amine substituent (proline) in the benzamidine ring to the backbone of GLY429. The residue of LYS469, TYR462, ALA464, PHE463 formed hydrophobic bonds as well.

The low binding affinity of tricyclo[8.6.0.0.0^{2,9}]hexadeca-3,15-diene results from interaction of TYR473 residue with the cyclooctane ring of the inhibitor ligand. This residue functioned as a bridge for the bond between TYR462 and inhibitor. LYS469 and ALA464 also bound to the cyclooctane ring, forming pi-alkyl bond. TYR462 residue had a key role in the binding to the side chain of cyclooctane ring and in determining the binding of the inhibitor compound.

Similarly, the interaction between the inhibitor compound tricyclo[10.2.1.0^{2,11}]pentadeca-4,8-diene and the PARP2 receptor, indicates that TYR473 bound to the cyclodecane ring, forming two pi-alkyl bonds. Likewise, residue TYR462 formed pi-alkyl bonds withtricyclo[10.2.1.0^{2,11}]pentadeca-4,8-diene causing low and stable binding affinity energy.

The interaction between the GLY 492 residue with the oxygen molecules contained in the linoleic acid causes the formation of hydrogen interactions. Hydrogen interactions are also formed at the SER470 residue that binds the H atoms contained in the linoleic acid.[29] TYR473, LYS469, and ALA464 bound to the linoleic chain to form pi-alkyl bonds. Linoleic acid, also known as omega-6, is reported to have anticancer properties.[30] Study by Zhang stated that linoleic acid could deliver a significant improvement in breast cancer treatment.[31] Another study reported that conjugated linoleic acid has antiproliferative activity and is able to activate the cell death pathway.[32]

Residues TYR473 and TYR462 were actively involved in the interaction with the three inhibitors from the Nyale worm extract. The result of the interactions of the inhibitor compounds from the Nyale worm extract compares favourably with the commercially used native ligand with respect to the similarity of the binding domains.

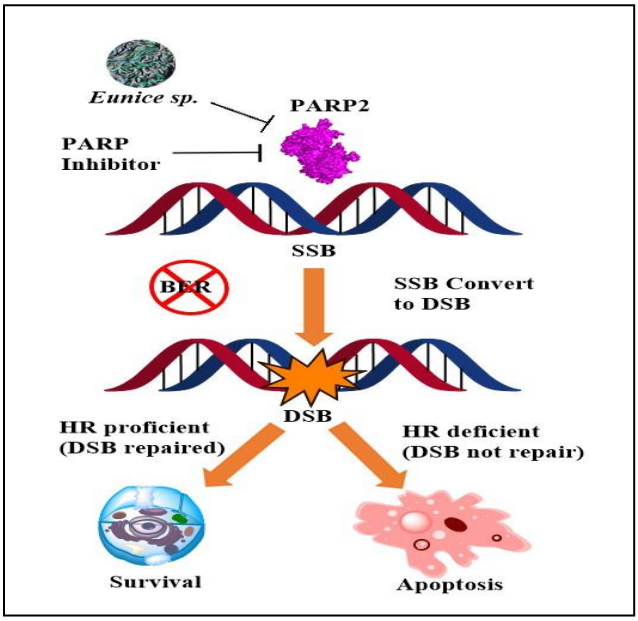

Figure 3: PARP2 inhibitor working mechanism

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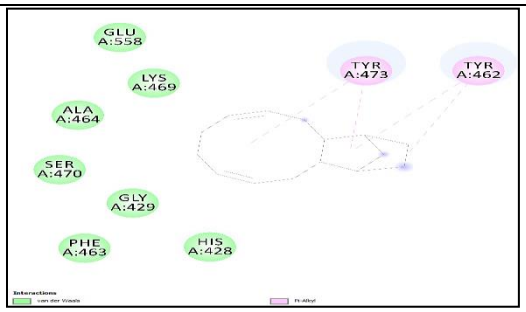
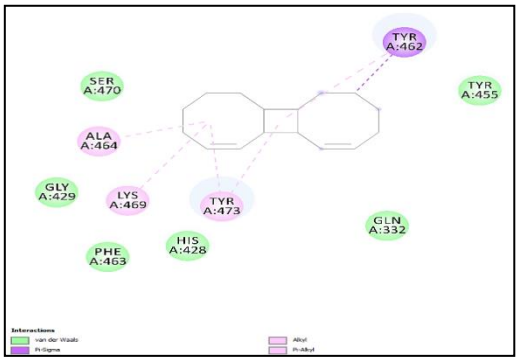
Table 1: Identification of the potential PARP2 inhibitor compounds contained in Nyale worm extract based on their bioavailability and HIA.

No	Compound Name	Molecular Formula	Da	H-donor	H-acceptor	LogP	HIA (%)	Binding Affinity (kcal/mol)
1	Tricyclo[8.6.0.0^{2,9}]hexadeca-3,15-diene	$C_{16}H_{24}$	202.34	0	0	4.02	100	-8.8
2	3-Aminobenzamide (native ligand)	$C_7H_8N_2O$	136.15	2	1	0.32	90.98	-6,6
3	Margaric acid	$C_{17}H_{34}O_2$	270,45	1	2	5,57	98,40	-6,2
4	9-Octadecenal	$C_{18}H_{34}O$	266,46	0	1	5,94	100	-6,1
5	Myristic acid	$C_{14}H_{28}O_2$	228,37	2	1	4,45	978.483	-5,9
6	Pentadecylic acid	$C_{15}H_{30}O_2$	242,40	1	2	4,84	98,11	-5,9
7	Stearic acid	$C_{18}H_{36}O_2$	284,48	1	2	5,93	98,44	-6,2
8	Linoleic acid	$C_{18}H_{32}O_2$	280,45	1	2	5,45	98,37	-6,7
9	Palmitic acid	$C_{16}H_{32}O_2$	256,42	1	2	5,20	98,29	-6.1
10	Methyl myristate	$C_{15}H_{30}O_2$	242,40	2	0	4,81	100	-5,8
11	Ethyl arachidonate	$C_{22}H_{36}O_2$	332,52	0	2	6,42	100	-5,9
12	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.46	1	2	5,71	98.43	-6.6
13	Benzene, 1,2-dimethyl-	$C_6H_4(CH_3)_2$	106.17	0	0	2.83	100	-5.6
14	Hexadecanoic acid	$C_{18}H_{36}O_2$	284,48	0	2	5,79	100	-6.0
15	Tricyclo[10.2.1.0^{2,11}]pentadeca-4,8-diene	$C_{15}H_{22}$	202.34	0	0	4.02	100	-8.4
16	Methyl palmitate	$C_{17}H_{34}O_2$	270.45	0	2	5.54	100	-5.9
17	Ethyl myristate	$C_{16}H_{32}O_2$	256, 42	0	2	5,17	100	-6.0
18	Ethyl palmitate	$C_{18}H_{36}O_2$	284.48	2	0	5.90	100	-5.9
19	Methyl stearate	$C_{19}H_{38}O_2$	298.50	0	2	6.24	100	-6,3
20	Ethyl stearate	$C_{20}H_{40}O_2$	312,53	0	2	6,71	100	-5,9
21	Dythol	$C_{27}H_{46}O$	386,65	1	1	6,67	100	-3.1

Table 2: Interaction between compounds contained in Nyale worm extract and PARP2

No.	Compound Name	Molecular Visualization	Interaction
1.	Tricyclo[10.2.1.0^{2,11}]pentadeca-4,8-diene		TYR473, TYR462, GLU558, LYS 469, ALA 464, SER 470, GLY 429, PHE 463, HIS 428
2	Tricyclo[8.6.0.0^{2,9}]hexadeca-3,15-diene		TYR462, TYR473, LYS469, ALA464, TYR455, GLN332, HIS428, PHE463, GLY429, SER470

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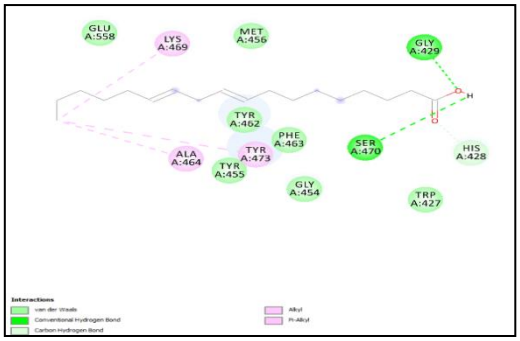
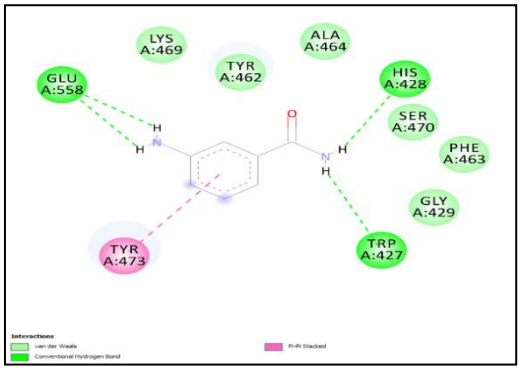
3	Linoleic acid		ALA464, TYR473, GLY429, HIS470, MET429, SER470, TRP427, GLY454, PHE463, TYR462, TYR455,
4	3-Aminobenzamide		TYR473, GLU558, HIS428, TRP427, LYS469, TYR462, ALA464, SER470,

Conclusion

This research confirms the anticancer properties in Nyale worm by analyzing the potential PARP2 inhibitor compounds in the worm extract through the use of molecular docking with *in silico* approach. Future studies in developing anticancer drug from Nyale worm extract are encouraged.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This research was funded by Yayasan Pesantren Al-Azhar.

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Tropical Journal of Natural Product Research
Official Journal of the Natural Product Research Group
Faculty of Pharmacy, University of Benin 300001, Benin City, Nigeria
Phone: +2348073184488, Email: editor.tjnpr@gmail.com; editor.tjnpr@uniben.edu; Website: www.tjnpr.org

ISSN: 2616-0684 (Print); 2616-0692 (Online), DOI: 10.26538/tjnpr, ISI IF: 0.562 (2017)
Abstracted/Indexed: Index Copernicus ICV (2017): 59.83, Open-J-Gate, EMBASE, EBSCO Host, WorldCat, AJOL, CrossRef (USA), JIF, NCBI (PubMed), CAS

SCOPUS indexed, SCImago SjR 0.13

Ref. No. 406801802217 DATE: 26th May, 2022

Metabolic and Antioxidant Department of the Faculty of Medicine, Al-Azhar Islamic University.
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Dear Dr. Arjita,

Provisional Acceptance letter for Article Manuscript Number TJNPR FB304ARN

Title: Analysis of Potential Poly(ADP-Ribose) Polymerase 2 (PARP2) Inhibitor in Nyale Worm (Eunice sp.) Extract for Ovarian Cancer: An In Silico Approach

Authors: I Putu Dedy Arjita*, Rozikin, I Gede Angga Adnyana, I Putu Bayu Agus Saputra, Sabrina Intan Zoraya

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Trop J Nat Prod Res, June 2022; 6(6):xxxx
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

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Original Research Article

Analysis of Potential Poly (ADP-Ribose) Polymerase 2 (PARP2) Inhibitor in Nyale Worm (*Eunice sp.*) Extract for Ovarian Cancer: An *In Silico* Approach

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ARTICLE INFO

Article history:
Received 16 May 2022
Revised 17 June 2022
Accepted 28 June 2022
Published online xxxxxxxxx

ABSTRACT

Cancer is an umbrella term for a large group of diseases that are characterized by abnormal cell growth and adjacent tissue or organ invasion. One of the most common cancers in Indonesia is ovarian cancer. Recently, PARP enzyme inhibitor used as a therapy for cancer, including ovarian cancer, has become more common. Apart from the standard PARP inhibitor drug, natural resources are also found to have high potential for cancer therapy. Marine biotas are known for their capability to produce biomolecules which can inhibit the cell mitosis of their rivals or predators. One of the marine biotas that are commonly consumed in Lombok Island is Nyale worm. This research aimed to analyze the potential PARP, particularly PARP2, inhibitor compounds in Nyale worm extract for ovarian cancer by using molecular docking with *in silico* approach. Compounds identification was conducted by using gas chromatography-mass spectrometry (GC-MS) and molecular docking was done with PyRx v.0.8 software. There were three potential PARP2 inhibitor compounds, tricyclo[10.2.1.02,11]pentadeca-4,8-diene, tricyclo[8.6.0.02,9]hexadeca-3,15-diene, and linoleic acid. The binding affinity energy of these three compounds were lower compared with that of the native ligand 3-aminobenzamide. The lower value of the energy means greater molecular binding stability and PARP2 inhibition mechanism.

Keywords: DNA repair, Nyale worm, Ovarian cancer, PARP, PARP2 inhibitor.

Introduction

Cancer is an umbrella term for a large group of diseases that are characterized by abnormal cell growth and adjacent tissue or organ invasion.[1] One of the most common cancers in Indonesia is ovarian cancer. As of 2018, there were 14,896 new cases of ovarian cancer making it the tenth disease with the most new cases in Indonesia according to Globocan data.[2] With a total of 9,581 deaths, ovarian cancer is also the seventh cancer with the highest number of deaths.[3]

Anticancer therapy targeting Poly(ADP-ribose) polymerase (PARP) enzyme was originally proposed by Mendel. PARP enzyme detects the DNA single-strand break (SSB) and causes DNA repair in cancer cells through base exicional repair (BER) mechanism.[4] PARP uses NAD^+ that is transferred to the glutamate, aspartate, and lysine residues acceptor to catalyze ADP-ribose for auto-modification. This facilitates DNA repair through the formation of chromatin structures by replacing the histone and signaling the DNA repair complex protein. There are 17 enzymes of the PARP superfamily in humans, including PARP1 and PARP2.[5,6] Recently, PARP enzyme inhibitor use as a therapy for cancer, including ovarian cancer, has become more common.[6,7,8] An orally-administered PARP inhibitor standard drug, 3-aminobenzamide, is effective in enhancing the damage of the cancer cell DNA.[6, 9]

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Citation: Arjita IPD, Rozikin R, Adnyana IGA, Saputra IPBA, Zoraya SI. Analysis of Potential Poly (ADP-Ribose) Polymerase 2 (PARP2) Inhibitor in Nyale Worm (*Eunice sp.*) Extract for Ovarian Cancer: An In Silico Approach. Trop J Nat Prod Res. 2022; 6(6):xxxx

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Apart from the standard drug, natural resources are also found to have high potential for cancer therapy.

Marine biotas are known for their capability to produce biomolecules which can inhibit the cell mitosis of their rivals or predators.[10, 11] A marine worm, *Hermione hystrix*, is reported to have antimitotic-cytotoxic activity towards sea urchin *Paracentrotus lividus* embrio.[12] Several other marine biotas such as sponges, mollusks, and cyanobacteria are also reported to have anticancer compounds.[13]

Lombok Island is rich in marine biota. One of the renowned marine biotas found in Kuta Mandalika beach, a famous tourism destination in Central Lombok, is Nyale worm. It is commonly consumed by the local community. Nyale worm (*Eunice sp.*) from *Eunicidae* family is a member of Polychaeta class that includes three other species, *Lysidice sp.*, *Neanthes*, and *Aphrodite.*[14]

The anticancer properties of Nyale worm have not been widely researched. Therefore, this research aimed to analyze the potential PARP2 inhibitor compounds in Nyale worm extract for ovarian cancer by using molecular docking with *in silico* approach. The compounds were compared with a standard drug for inhibition target mechanism against PARP2 enzyme.

Materials and Methods

Sample collection and extraction

Nyale worms were collected from the coastal waters of Kuta Mandalika,Central Lombok. Dried samples were ground in a mortar and macerated in 250 mL ethanol 96% for 24 hours and n-hexane (99%) for 8 h. The residue was extracted three times with ethanol until it was colorless for ethanol extraction. Evaporator at 68°C was used for the solvent (n-hexane) removal.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Chemistry
Quantitative analysis with gas chromatography-mass spectrometry (GC-MS) Shimadzu 2010 was conducted to identify the bioactive compounds present in Nyale worm extract.

Protein/Macromolecule
PARP2 (GDP: 3KCZ) structure was obtained from rscb.org in the Protein Data Bank (PDB) format. PARP2 structure consisted of two chains, chain A and chain B. Each chain contained inhibitor ligand 3-aminobenzamide. PARP2 PDB structure was prepared using PyMOL 2.5.2.

Ligand and drug screening
Twenty compounds were identified by GC-MS in the Nyale worm extract The compounds identity were confirm through chemical searched using PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The bioavailability of the compounds was assessed according to Lipinski's Rule of Five using SwissADME (http://www.swissadme.ch/). Assessment of human intestinal absorption (HIA) was conducted with the use of PreADMET predictor (https://preadmet.webservice.bmdrc.org/). Ligands were prepared using Avogadro 1.2.[15]

Molecular docking
Molecular docking of the twenty compounds in Nyale worm extract to the PARP2 protein was done with PyRx v.0.8 software.[16] The molecule binding target area was X: 19.5762, Y: 2.9482, Z: 20.3313 and Dimension (A) X: 11.3241, Y: 8.1201, Z: 10.2827. This was the binding site of 3-aminobenzamide, a widely-used PARP2 inhibitor standard drug. The active binding site on PARP2 was observed in Computed Atlas of Surface Topography of Proteins (CASTp) (sts.bioe.uic.edu/castp/index.html?3kcz).[17] The result of the protein interaction and ligand binding residue identification was visualized with PyMOL 2.5.2 and Discovery Studio R17.

Results and Discussion

PARP2 inhibitor mechanism for cancer cell
PARP2 working mechanism in Figure 3 shows that DNA repair is a potential target to kill ovarian cancer cell.[18] The SSB is often found in proliferating cells. The PARP2 inhibitor affects BER, preventing the DNA repair to occur. The SSB then turns into double-strand break (DSB) leading to inhibition of cell proliferation. It may also affects the cell recombinant if the homologous recombination deficiency (HRD) is present. This condition renders the DSB irreparable, inducing cell apoptosis.[19]
Based on the PARP2 molecule structure shown in Figure 2, PARP2 was found to have NAD^+ cofactor (denoted by arrow). NAD^+ has a pivotal role in DNA repair process. NAD^+ breaks down into nicotinamide and ADP-ribose to form poly(ADP-ribose) (PAR) which binds to the DNA repair protein acceptor.[20] Previous studies reported that inhibiting NAD^+ significantly hampered the DNA repair by PARP2, leading to cell apoptosis.[21,22]
The binding affinity of 3-Aminobenzamide was -6.6 kcal/mol. This value indicated the energy needed to bind to the PARP2 receptor. The lower the value, the higher the possibility of a compound to tightly bind to the PARP2 receptor.[23]

Human intestinal absorption (HIA)
Percentage of HIA (% HIA) tells the absorbability of the compounds in the small intestine. Table 1 indicates that the compounds in Nyale worm extract had high absorption level (HIA > 90%). This means that the compounds have good oral absorption profile and can reach the ovarian cancer cell receptor if administered orally. Thus, oral administration can increase the efficacy of the compounds.[24]

Lipinski's rule of five
Assessment according to Lipinski's Rule of Five parameter before docking can ensure the ability of the compound to reach the appropriate receptor binding site.[25,26]

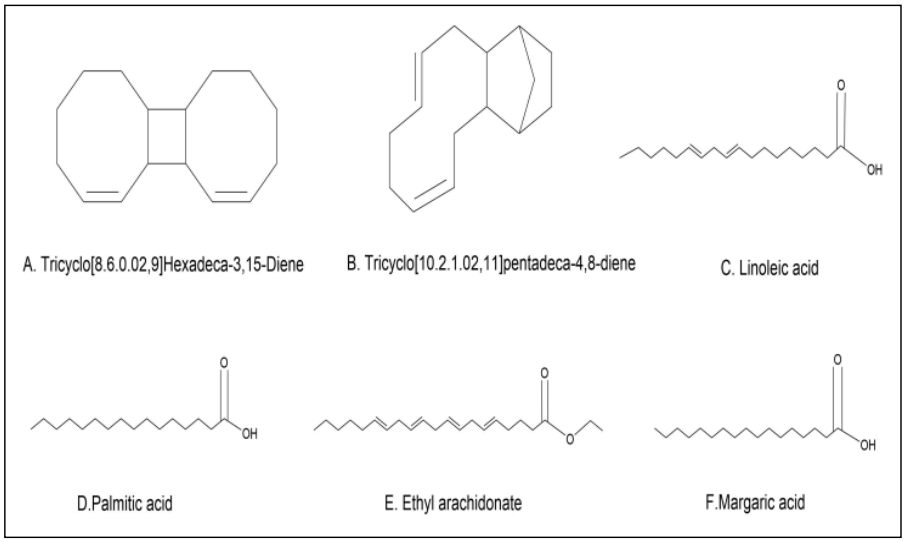

Figure 1: Some the compounds identified in Nyale worm (*Eunice sp*.)

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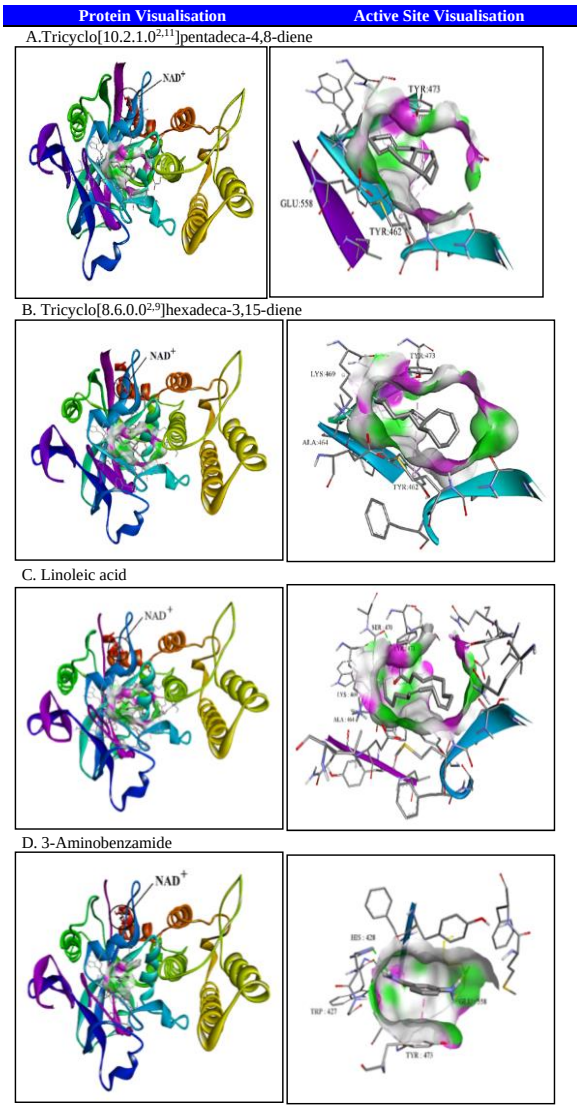

Figure 2: Visualization of the inhibitor compounds against PARP2

Molecular docking

There were three potential PARP2 inhibitor compounds, tricyclo[10.2.1.0$^{2.11}$]pentadeca-4,8-diene, tricyclo[8.6.0.0$^{2.9}$]hexadeca-3,15-diene, and linoleic acid (Table 1). Molecular docking can predict the amount of energy generated among two or more interacting or binding molecules.[27] The binding affinity energy of the three compounds were lower compared with that of the native ligand 3-aminobenzamide. Figure 2 shows the visualization of PARP2 where the four compounds bound to the same active site. The lower value of the energy resulting from docking (kcal/mol) means greater molecular binding stability and PARP2 inhibition mechanism.[28]

Interaction between PARP2 and 3-aminobenzamide shown in Table 2 explains its affinity for PARP2 inhibitor. The side chain residue of TYR473 formed pi-alkyl bond with the imidazole ring. The bond between GLU558 and nitrogen atom at the end of the imidazole chain formed two hydrogen bonds. The backbone of TRP427 and HIS428 bound to the nitrogen atom, also forming the hydrogen bonds. The backbone residue of GLY429 and SER470 formed hydrophobic bonds. TYR462 caused an interaction with the cyclic amine substituent (proline) in the benzamidine ring to the backbone of GLY429. The residue of LYS469, TYR462, ALA464, PHE463 formed hydrophobic bonds as well.

The low binding affinity of tricyclo[8.6.0.0.0$^{2.9}$]hexadeca-3,15-diene results from interaction of TYR473 residue with the cyclooctane ring of the inhibitor ligand. This residue functioned as a bridge for the bond between TYR462 and inhibitor. LYS469 and ALA464 also bound to the cyclooctane ring, forming pi-alkyl bond. TYR462 residue had a key role in the binding to the side chain of cyclooctane ring and in determining the binding of the inhibitor compound.

Similarly, the interaction between the inhibitor compound tricyclo[10.2.1.0$^{2.11}$]pentadeca-4,8-diene and the PARP2 receptor, indicates that TYR473 bound to the cyclodecane ring, forming two pi-alkyl bonds. Likewise, residue TYR462 formed pi-alkyl bonds withtricyclo[10.2.1.0$^{2.11}$]pentadeca-4,8-diene causing low and stable binding affinity energy.

The interaction between the GLY 492 residue with the oxygen molecules contained in the linoleic acid causes the formation of hydrogen interactions. Hydrogen interactions are also formed at the SER470 residue that binds the H atoms contained in the linoleic acid.[29] TYR473, LYS469, and ALA464 bound to the linoleic chain to form pi-alkyl bonds. Linoleic acid, also known as omega-6, is reported to have anticancer properties.[30] Study by Zhang stated that linoleic acid could deliver a significant improvement in breast cancer treatment.[31] Another study reported that conjugated linoleic acid has antiproliferative activity and is able to activate the cell death pathway.[32]

Residues TYR473 and TYR462 were actively involved in the interaction with the three inhibitors from the Nyale worm extract. The result of the interactions of the inhibitor compounds from the Nyale worm extract compares favourably with the commercially used native ligand with respect to the similarity of the binding domains.

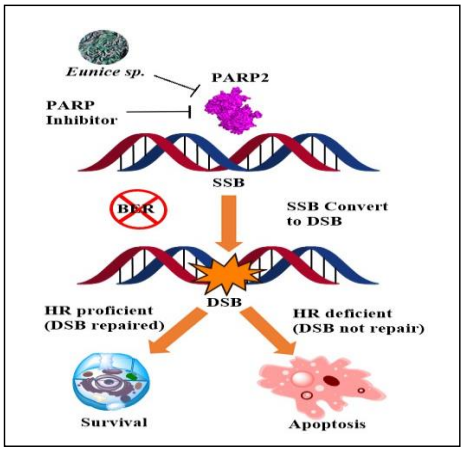

Figure 3: PARP2 inhibitor working mechanism

ISSN 2616-0684 (Print)
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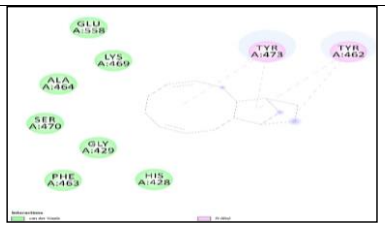
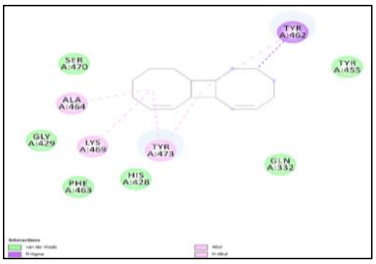
Table 1: Identification of the potential PARP2 inhibitor compounds contained in Nyale worm extract based on their bioavailability and HIA.

No	Compound Name	Molecular Formula	Da	H-donor	H-acceptor	LogP	HIA (%)	Binding Affinity (kcal/mol)
1	Tricyclo[8.6.0.0^{2,9}]hexadeca-3,15-diene	$C_{16}H_{24}$	202.34	0	0	4.02	100	-8.8
2	3-Aminobenzamide (native ligand)	$C_7H_8N_2O$	136.15	2	1	0.32	90.98	-6,6
3	Margaric acid	$C_{17}H_{34}O_2$	270,45	1	2	5,57	98,40	-6,2
4	9-Octadecenal	$C_{18}H_{34}O$	266,46	0	1	5,94	100	-6,1
5	Myristic acid	$C_{14}H_{28}O_2$	228,37	2	1	4,45	978.483	-5,9
6	Pentadecylic acid	$C_{15}H_{30}O_2$	242,40	1	2	4,84	98,11	-5,9
7	Stearic acid	$C_{18}H_{36}O_2$	284,48	1	2	5,93	98,44	-6,2
8	Linoleic acid	$C_{18}H_{32}O_2$	280,45	1	2	5,45	98,37	-6,7
9	Palmitic acid	$C_{16}H_{32}O_2$	256,42	1	2	5,20	98,29	-6.1
10	Methyl myristate	$C_{15}H_{30}O_2$	242,40	2	0	4,81	100	-5,8
11	Ethyl arachidonate	$C_{22}H_{36}O_2$	332,52	0	2	6,42	100	-5,9
12	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.46	1	2	5,71	98.43	-6.6
13	Benzene, 1,2-dimethyl-	$C_6H_4(CH_3)_2$	106.17	0	0	2.83	100	-5.6
14	Hexadecanoic acid	$C_{18}H_{36}O_2$	284,48	0	2	5,79	100	-6.0
15	Tricyclo[10.2.1.0^{2,11}]pentadeca-4,8-diene	$C_{15}H_{22}$	202.34	0	0	4.02	100	-8.4
16	Methyl palmitate	$C_{17}H_{34}O_2$	270.45	0	2	5.54	100	-5.9
17	Ethyl myristate	$C_{16}H_{32}O_2$	256, 42	0	2	5,17	100	-6.0
18	Ethyl palmitate	$C_{18}H_{36}O_2$	284.48	2	0	5.90	100	-5.9
19	Methyl stearate	$C_{19}H_{38}O_2$	298.50	0	2	6.24	100	-6,3
20	Ethyl stearate	$C_{20}H_{40}O_2$	312,53	0	2	6,71	100	-5,9
21	Dythol	$C_{27}H_{46}O$	386,65	1	1	6,67	100	-3.1

Table 2: Interaction between compounds contained in Nyale worm extract and PARP2

No.	Compound Name	Molecular Visualization	Interaction
1.	Tricyclo[10.2.1.0^{2,11}]pentadeca-4,8-diene		TYR473, TYR462, GLU558, LYS 469, ALA 464, SER 470, GLY 429, PHE 463, HIS 428
2	Tricyclo[8.6.0.0^{2,9}]hexadeca-3,15-diene		TYR462, TYR473, LYS469, ALA464, TYR455, GLN332, HIS428, PHE463, GLY429, SER470

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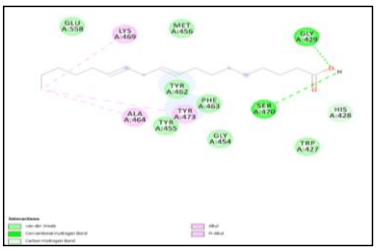
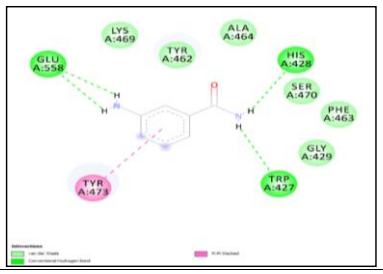
3	Linoleic acid		ALA464, TYR473, GLY429, HIS470, MET429, SER470, TRP427, GLY454, PHE463, TYR462, TYR455,
4	3-Aminobenzamide		TYR473, GLU558, HIS428, TRP427, LYS469, TYR462, ALA464, SER470,

Conclusion

This research confirms the anticancer properties in Nyale worm by analyzing the potential PARP2 inhibitor compounds in the worm extract through the use of molecular docking with *in silico* approach. Future studies in developing anticancer drug from Nyale worm extract are encouraged.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This research was funded by Yayasan Pesantren Al-Azhar.

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