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3D-PHARMACOPHORE MODELING AND MOLECULAR DOCKING TO STUDY THE POTENTIAL ANTI-CANCER AGENT FROM *Ficus septica* Burm. L.

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ABSTRACT

In vitro testing showed that awar awar (*Ficus septica* Burm. L) leaf had an anticancer activity. Ethanol extract from awar-awar leaves could selectively inhibit cancer cell growth with IC^{50} values, there were MCF7 breast cancer cells (48 µg/ml), HeLa cervical cancer cells (122.4 µg/mL), and WiDR cancer cells (75.9 µg/mL). However, the active compounds that play a role in inhibiting the three cancer cells are not yet found. Therefore, this research carried out to find out the active compound using in silico. 3D-pharmacophore modeling and Molecular docking were developed for finding out the potential compound that could be acted as an anti-cancer agent. Screening pharmacophore was performed using LigandScout® 4.4 software for searching the matching pharmacophore features against chemical structure databases. Docking was performed using Autodock Tools® and visualized using Discovery Studio Visualizer® software to see the ligand interaction with the active binding site at the receptor and continue with ADMET properties to evaluating the Pharmacodynamic activities of the Hit compounds. Among 17 types of compounds tested, 11 compounds showed anticancer activity and genistin was found promising and showed potential inhibitory characteristics as an anticancer compared to other active compounds of awar-awar leaves. This study suggests that these compound could be used as a lead compound for anticancer agents.

***Keywords**: Anticancer, Awar-awar leaves, Molecular docking, Pharmacophore screening*

INTRODUCTION

Cancer is one of the leading causes of death in the world. In 2018, approximately 9.6 million deaths were caused by cancer (WHO, 2019). Of the many cases of death caused by this cancer, commonly used cancer treatments such as surgery, chemotherapy, and radiation therapy emerged (National Cancer Institute, 2021). The treatment aims to destroy cancer cells. However, these methods are not optimal and even give side effects.

Currently, various studies have been carried out to develop new drugs based on

natural ingredients that are alternatives in cancer treatment, one of which is awar-awar (*Ficus septica* Burm. L). Based on research conducted by Ika Rahmawati et al., Showed that in vitro ethanol extract from awar-awar leaves can selectively inhibit cancer cell growth with IC_{50} values, namely MCF7 breast cancer cells (48 µg/ml, HeLa cervical cancer cells (122.4 µg/mL) and WiDR cancer cells (75.9 µg / mL) (Sutedjo et al., 2016), (Anonim, 2008).

The results of in vitro studies that have been carried out are proven that awar-awar leaves had anticancer activity. However, the active compounds that play a role in inhibiting the three cancer cells are not certain. Therefore, further research carried out in silico to search potential compounds that can be used as lead compound. Molecular docking and 3D-pharmacophore modeling were established to search for the potential compound as an anti-cancer agent from this plant.

The biological activity of a compound can be explored with a computational approach by looking at the affinity of small molecular ligands to macromolecular receptors. It can be explored using the in silico method and compared with experimental methods. The molecular docking approach can describe the interactions that occur between small molecules (ligands) and proteins at the atomic level (Agarwal & Mehrotra, 2016), (Meng et al., 2011).

Pharmacophores are important features that are part of a molecule that allows ligand molecules to interact with specific target receptors. This pharmacophore is responsible for generating or inhibit biological activity (Meng et al., 2011). Apart from describing the chemical features of a molecule, the 3D pharmacophore model also determines the 3D geometry of the features of a bioactive compound. Ligand-based (LB) pharmacophore modeling involves a known set of active molecules without any information about the macromolecular target. 3D models can be used to search for bioactive molecules using virtual filtering methods or to provide chemical drug decision support during hit expansion and lead optimization (Güner & Bowen, 2014).

The introduction showed that in vitro testing has been carried out on awar-awar leaf compounds (*Ficus septica* Burm. L) and showed the activity of anticancer breast, anticancer cervical and anticancer colorectal. However, the active compounds that play a role in inhibiting the three cancer cells are not known for certain. Therefore,

further research will be carried out in silico. Pharmacophore screening and molecular docking of awar-awar leaf compounds will be conducted against cancer receptors, namely ER-α (breast cancer and cervical cancer), ER-β (breast cancer), MAP3K7/TAK1 (cervical cancer), VEGFR2-kinase and Leukotriene A4 Hydrolase (colorectal cancer). As well as prediction of drug-likeness according to Lipinski's Rule of Five through the Chem Draw Professional 15.0® application, pharmacokinetic properties (absorption and distribution profiles) through the pre-ADMET site and toxicity through the Toxtree® application.

MATERIALS AND METHODS

Materials

Hardware: Asus X441U series netbook with specifications: Windows 10 Home, Intel® CoreTM i3 CPU 7020U 2,3 GHz processor, 4GB DDR4 RAM.

Software: Windows 10 Home Operating System 64-bit LigandScout® 4.4 (Free Trial 30 Days), AutoDock Tools®, ChemDraw® Ultra 12.0, Chem3D® Pro 12.0, Notepad++®, Discovery Studio Visualizer®, Toxtree®, PubChem site, Protein Data Bank, Binding Database, DUD-E, and Pre-ADMET.

Method

1. Ligand-Protein preparation and Pharmacophore modeling

1.1. Receptor Preparation

The macromolecules used in this study were ER-α (code: 3ERT, resolution: 1.9 Å), ER-β (code: 1QKM, resolution: 1.8 Å), MAP3K7/TAK1 (code 5V5N, resolution: 2.01 Å), LTA4H (code: 3U9W, resolution 1.25 Å), and VEGFR -2 kinases receptors (code: 3C7Q resolution: 2,1 Å) obtained from X-ray crystallographic methods which were downloaded from the Protein Data Bank with the site http://www.rsbc.org/pdb/. The identity of these macromolecules which are in the format .pdb.

1.2. Ligan Preparation

The ligands used were tamoxifen, genistein, takinib, BIBF 1120, and bestatin as a comparison and test compounds from awar-awar leaf (*Ficus septica* Burm. L.) which were downloaded through the site http://PubChem.nbi.nlh.nih.gov with the .sdf format and then converted with the Chemdraw® program to become a compound with .mol format.

1.3. Ligand-Based Pharmacophore Model

By using the LigandScout® 4.3 program and making 3D pharmacophores based on ligands with 20 databases of active compounds and 100 databases of decoy compounds that are

downloaded through the site http://dude.docking.org in the .sdf format then converted and saved in the .ldb format.

1.4. Validation of Pharmacophore Screening

Virtual screening aims at the maximum enrichment of active compounds on the hit list. Therefore, the method is usually validated by assessing the accuracy of discrimination between active and decoy compounds. A good pharmacophore model will be able to identify the most known active compounds as little as possible. The analysis used to evaluate the results of validation is the AUC100 value, this parameter is used validly if the value is AUC100≥70% (Agarwal & Mehrotra, 2016).

1.5. Pharmacophore Feature Matching Screening

In medicinal chemistry research, drug database screening is a very crucial Bioinformatics technique for the drug discovery process. This research using LigandScout for matching the pharmacophore features with the drug databases available drugs. Results analysis can be done by looking at the value of the 'Pharmacophore Fit Score' which is displayed in the bottom table and by displaying the ROC chart.

2. Ligand-Protein preparation and Docking Protein Preparation

2.1. Receptor Preparation

Downloaded complex proteins (ER-α (code PDB: 3ERT), ER-β (code PDB: 1QKM), MAP3K7/TAK1 (code PDB: 5V5N), VEGFR-2 kinase (code PDB: 3C7Q), and LTA4H (code PDB: 3U9W)) are separated between macromolecule and ligand used Discovery Studio Visualizer®, and then hydrogen atoms were added and in the last stage of preparation, restrained minimization was performed

2.2. Ligan Preparation

Ligands or compounds were redrawn using the ChemDraw® Ultra 12.0 program and the energy minimization using the Chem3D® Pro 12.0 program and then saved in the .pdb format. After preparation, the physicochemical properties of the compounds were determined based on Lipinski's Rule of Five.

2.3. Validation

Method validation was done to find out whether the program for molecular docking is according to the requirements or not. Validation of the molecular docking method was done by re-docking between the default ligands from the target receptor using Autodock Tools® software. The analysis used to evaluate the results of validation is the RMSD value, the binding site found and the parameters used are considered valid if the RMSD value is ≤ 2Å.

2.4. Grid Preparation

The grid of the selected target structure was prepared by using Autodock Tools®.

2.5. Docking

After all the docking settings are complete then running can be done using Autogrid4 and Autodock4. the resulting parameters are (ΔG and Cluster) and compare the results obtained from one another. Determination of ligand conformation from docking results is done by selecting the ligand conformation which has the lowest bond energy (best position). The position and orientation of the ligand on macromolecules, as well as the amino acids bound to the ligand, are visualized using Discovery Studio Visualizer® software to see the ligand interaction with the active binding site at the receptor.

3. ADMET profiling analysis

The ADMET property analysis is extremely significant for evaluating the Pharmacodynamic activities of the Hit compounds. Tests carried out include absorption and distribution as well as toxicity tests which include mutagenic and carcinogenic properties of compounds. Tests are carried out using a special program that is carried out using a special program conducted online on the site http://preadme.bmdrc.kr/.

4. Toxicity Prediction

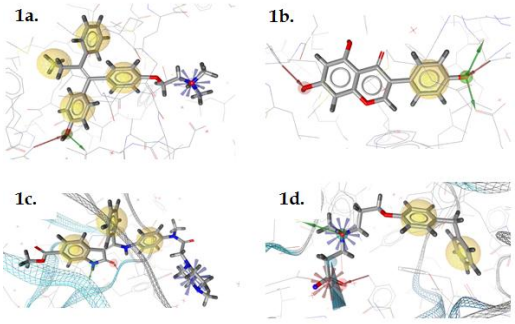
Toxicity prediction is done to predict the toxicity level of a compound in the body. Toxicity prediction was carried out using the Toxtree® program, based on the parameters of the Cramer Rules, Beighni/Bossa Rulebase, and Kroes TTC.

RESULTS AND DISCUSSION

1. Pharmacophore Feature Matching Screening

The pharmacophore virtual screening aims at the maximum enrichment of active compounds on the hit list. The first stage in pharmacophore screening is to identify the pharmacophores of the ligand and receptor complexes, by identifying the pharmacophores of the natural ligands that are attached to each of the target receptors including ER-α, ER-β, VEGFR-2 kinase, and LTA4H Figure 1. The results of this pharmacophore screening will be used as a comparative pharmacophore to look for test ligands that have the same biological activity based on similar pharmacophores. Therefore, the method is usually validated by assessing the accuracy of discrimination between 20 databases of active compounds and 100 databases of decoy compounds. The results show that of the total active compounds and decoys that are 0,97 for ER-α, 1,00 for ER-β, 0,87 for VEGFR-2 kinase, and 0,99 for

LTA4H Table. 1. This means that the method can be used because it meets the requirements, namely the value of AUC100 ≥ 70% / 0.7. AUC value > 0.7, and a hit score > 0.7 was used as a virtual screening model [18].

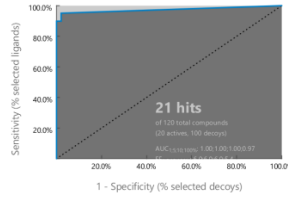
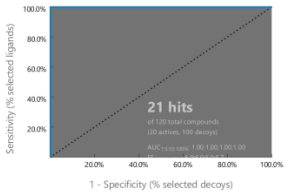
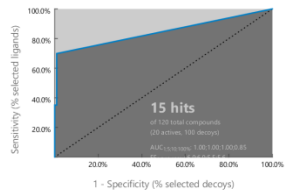
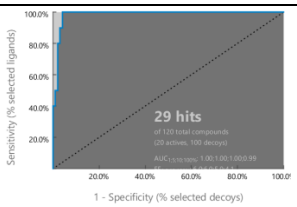


Description : red arrows : hydrogen bond acceptor (HBA), greens arrow : hydrogen bond donor (HBD) and yellow spheres : hydrophobic sites)

Figure 1. (1a) Pharmacophore screening results for estrogen receptors - alpha, (1b) Pharmacophore screening results for estrogen receptor-beta, (1c) Pharmacophore screening results for vascular endothelial growth factor receptor-2 kinase (VEGFR-2 kinase), (1d) Pharmacophore screening results for leukotriene A4 hydrolase receptors.

After testing the pharmacophores on 17 active compounds of awar-awar leaf, the results obtained 2 hit compounds against estrogen-α receptors namely genistin and 4-hydroxy-3-methoxyacetophenone with a value of pharmacophore fit score in a row of 48.82% and 47,57%.

Table 1. Validation curve (ROC curve)

Receptor	ROC Curve	AUC	GH Score
ER-α		0,97	0,90
ER-β		1,00	0,95
VEGFR-2 Kinase		0,85	0,87
LTA4H		0,99	0,70

These two compounds have similar pharmacophore features such as the native ligand (tamoxifen). And obtained 2 hit compounds against estrogen-β receptors namely beta-amyrin and kaempferitrin with a value of pharmacophore fit score in a row of 47.57% and 45,35%. These two compounds have similar pharmacophore features such as the native ligand (genistein). And obtained 2 hit compounds against VEGFR-2 kinase receptors namely genistin and 4-hydroxy-3-methoxyacetophenone with a value of

pharmacophore fit score in a row of 42.33% and 35,18%. These two compounds have similar pharmacophore features such as the native ligand (BIBF 1120), And obtained 1 hit compounds against LTA4H receptor namely genistin and 4-hydroxy-3-methoxyacetophenone with a value of pharmacophore fit score was 35,12% and have similar pharmacophore features such as the native ligand (sc57461a) Table 2.

2. Drug-likeliness property analysis

A drug like features (Lipinski's rule of five) of the selected best Hits was confirmed using Drug-likeliness property analysis. A good drug contains properties such as well distribution throughout the system, absorbed in the timeline as well as shows good metabolism property (Kalyaanamoorthy & Chen, 2011). Table 3 shows selected some properties from 17 compounds at awar-awar such as Molecular Weight (MW), Hydrogen bond donor (HBD), Hydrogen bond acceptor (HBA), Predicted Aqueous Solubility (QP log S). The test results showed that all test compounds meet the requirements of Lipinski's rule of five.

Table 2. Results of Awar-awar Leaf Compound Farmacophore Screening

No	Compound	Matching Features	Fit Score
Estrogen Receptor – Alpha			
1.	Tamoxifen		-
2.	Genistin		48,82
3.	4-hydroxy-3-methoxyacepto phenone		47,05
Estrogen Receptor – Beta			
1.	Genistein		-
2.	Beta- Amyrin		47,57
3.	Kaempferitrin		45,35
Vascular Endothelial Growth Factor Receptor-2 kinase			
1.	BIBF 1120		-
2.	4-hydroxy-3-methoxyacepto phenone		42,33
3.	Genistin		35,18
Leukotriene A4 hydrolase Receptor			
1.	Bestatin		35,12
2.	4-hydroxy-3-methoxyacepto phenone		

Description : blue = aromatic ring
yellow = hydrophobic interaction
red = hydrogen bond acceptor
green = hydrogen bond donor

3. Docking against the Target

The molecular docking process is done by using AutoDock Tools. The parameters observed for the determination of the affinity of the test compounds against receptors are the value of the energy bond (ΔG) and the inhibition constant (KI). The more negative the energy bond value and the smaller the inhibition constant value shows the higher ligand affinity and also the

amino acid residue from the hydrogen bond interaction with the receptor (Agarwal & Mehrotra, 2016).

Table 3. Physicochemical properties of compounds based on the Lipinski rule of five

No.	Ligan	MW	QP log S	HBD	HBA	Meet requirement/ not meet the requirement
1	1-triacontanol	438	14,58	1	1	meet requirement
2	3,4,5-trimethoxyacetophenone	210	1,08	0	4	meet requirement
3	4-hydroxy-3-methoxyacetophenone	166	1,28	3	1	meet requirement
4	α-Amyrin	426	10,66	1	1	meet requirement
5	β-Amyrin	426	10,66	1	1	meet requirement
6	β-Stigmasterol	412	9,96	1	1	meet requirement
7	Coumarine	146	1,41	0	1	meet requirement
8	Dehydroantofine	360	0,55	0	3	meet requirement
9	Deydrotylophorine	350	0,18	0	4	meet requirement
10	Ficuseptine A	455	3,24	1	7	meet requirement
11	Genistin	432	0,91	6	10	meet requirement
12	Kaempferitrin	578	-0,15	0	4	meet requirement
13	Myristic acid	220	6,15	1	1	meet requirement
14	Palmitic acid	256	7,21	1	1	meet requirement
15	Phenanthroindolizidine	273	4,96	0	1	meet requirement
16	Stigmasterol	413	9,96	1	1	meet requirement
17	Tylophorine	393	4,21	0	5	meet requirement

Requirements: 1. BM <500 mg / mol
2. Log P <5
3. Donor Hydrogen <5
4. Hydrogen acceptor <10

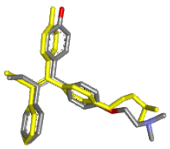
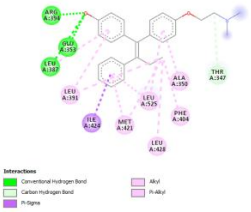
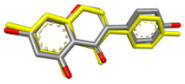
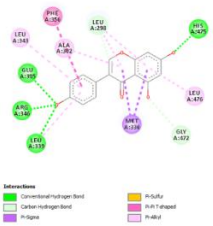
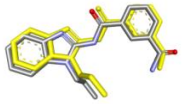
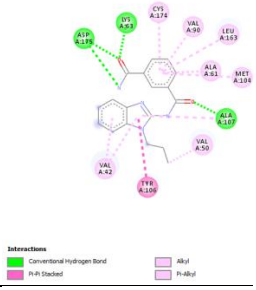
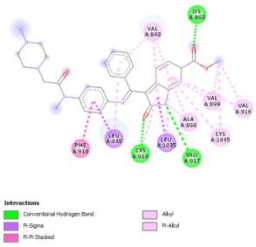
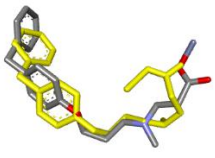
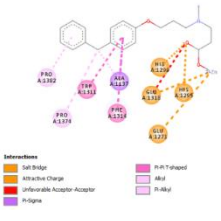
The first step is validation by redocking the standard ligand and determining the coordinates used in molecular docking and docking grid volume Table 4.

Table 4. Results of method validation with natural ligand redocking.

No	Receptor	RMSD	Coordinate of Grid Box	Size of grid box
1.	ER-α (3ERT)	1,162 Å	X : 30,282 Y : -1,913 Z : 24,207	40x40x40
2.	ER-β (1QKM)	0,481 Å	X : 28, 438 Y : 8,003 Z : 113,539	40x40x40
3.	MAP3K7/TAK1 (5V5N)	0,842 Å	X : -14,81 Y : -48,766 Z : -16,291	40x40x40
4.	VEGFR-2 kinase (3C7Q)	0,977 Å	X : 20,187 Y : 67,208 Z : 29,806	40x52x40
5.	Leukotrien A4 Hidrolase (3U9W)	1,180 Å	X : 29,679 Y : 1,546 Z: 1,893	30x30x30

Among 17 types of compounds from awar -awar leaf that were successfully docking, the results form docking to the ER-α receptor, the stigmasterol compound has a Gibbs free energy (ΔG) value of -11.74 kcal/mol and a KI value of 2.48 nM. From the results of docking to the ER-β receptor, phenanthroindolizidine has a Gibbs free energy (ΔG) value of -11.28 kcal/mol and a KI value of 5.43 nM. From the results of docking to VEGFR-2 kinase, two compounds were obtained, namely stigmasterol and β-stigmasterol.

Table 5. Visualization redocking.

Receptor	Overlapping the native ligand with redocking ligand	Interactions of native ligand with amino acid residues
ER-α	 Native ligand 3ERT: red-gray-blue Redocking ligand: Yellow	
ER-β	 Native ligands 1QKM: red-gray Redocking ligands: yellow	
MAP 3K7/ TAK1	 Native ligan 5V5N: red-gray-blue Redocking Ligand: yellow	
VEGF R-2 kinase	 Native ligands 3C7Q: red-gray-blue Redocking ligand: yellow	
Leukotriene A4 hydrolase.	 Native ligands 3U9W: red-gray-blue redocking ligands: yellow	

Among the two compounds that have a smaller free energy value is the stigmasterol compound with a ΔG value of -10.93 kcal/mol and a KI value of 9.67 nM. From the results of docking to the LTA4H receptor, two compounds were obtained, namely genistin and phenanthroindolizidine. Among the two compounds that have a smaller free energy value are genistin compounds with a ΔG value of -10.95 kcal/mol and a KI value of 9.38 nM Table 6.

Table 6. Results of molecular docking.

No	Compound	ΔG kkal/ mol	KI (nM)	Amino Acid Residue
Estrogen Receptor – Alfa				
1.	Tamoxifen	-11,71	2,60	**ARG 394A, GLU 353A**, LEU 387A, THR 347A
2.	1-triacontanol	-6,70	12,22 x 10^3	**GLU 353A**, LEU 387A
3.	3,4,5-trimethoxyacetophenone	-4,93	241,36 x 10^3	THR 347A, HIS 524A
4.	4-hydroxy-3-methoxyacetophenone	-5,23	147,26 x10^3	**GLU 353A, ARG 394A**, LEU 346A
5.	α-Amyrin	-7,30	4,47 x 10^3	LEU 384A, LEU 346A, MET 343A, TRP 383A
6.	β-Amyrin	-8,34	765,70	LEU 428A, LEU 391A, MET 388A, LEU 384A, TRP 383A
7.	β-Stigmasterol	-10,96	9,23	LEU 349A, PHE 404A, ALA 350A, LEU 346A, LEU 387A, LEU 391A
8.	Coumarine	-5,51	91,76 x 10^3	**ARG 394A**
9.	Dehydroantofine	-9,34	141,94	GLY 521A, ASP 351A
10.	Deydrotylophorine	-7,62	2,61 x 10^3	**GLU 353A,** LEU 346A, ASP 351A
11.	Ficuseptine A	-7,86	1,74 x 10^3	THR 347A, MET 343A, ALA 350A, ASP 351A
12.	Genistin	-7,19	5,34 x 10^3	ARG 346A, GLU 305A, LEU 387A, LEU 346A, THR 347A
13.	Kaempferitrin	-9,43	123,08	GLU 419A, LEU 346A, ASP 351A
14.	Myristic acid	-4,80	301,56 x 10^3	PHE 404A

No	Compound	ΔG kkal/ mol	KI (nM)	Amino Acid Residue
15.	Palmitic acid	-5,16	165,57 x 10^3	THR 347A
16.	Phenanthroindolizidine	-9,71	76,48	LEU 387A
17.	Stigmasterol	-11,74	2,48	ASP 351A
18.	Tylophorine	-8,47	622,77	ASP 351A, **GLU 353A**
Estrogen Receptor – Beta				
1.	Genistein	-10,25	30,60	GLU 305A, ARG 346A, LEU 339A, HIS 475A, GLY 472A, LEU 298A
2.	1-triacontanol	-2,93	7,15 x 10^6	-
3.	3,4,5-trimethoxyacetophenone	-6,11	33,09 x 10^3	HIS 475A
4.	4-hydroxy-3-methoxyacetophenone	-5,71	65,34 x 10^3	GLU 305A, ARG 346A, LEU 298A
5.	α-Amyrin	+20,13	-	GLY 472A
6.	β-Amyrin	+17,62	-	GLY 472A
7.	β-Stigmasterol	-10,12	37,97	MET 479A, LEU 476A, MET 295A, ILE 376A, ILE 373A
8.	Coumarine	-5,95	43,70 x 10^3	ARG 346A
9.	Dehydroantofine	-9,08	221,62	ILE 373A
10.	Deydrotylophorine	-5,55	85,16 x 10^3	-
11.	Ficuseptine A	-3,83	1,55 x 10^6	MET 336A, ILE 373A, LEU 339A, GLY 472A
12.	Genistin	+2,17	-	GLY 472A, ARG 346A, GLU 305A
13.	Kaempferitrin	+11,67	-	ILE 373A, GLY 472A, GLU 305A
14.	Myristic acid	-6,10	34,01 x 10^3	ARG 346A, GLU 305A
15.	Palmitic acid	-6,38	20,97 x 10^3	HIS 475A, GLY 472A
16.	Phenanthroindolizidine	-11,28	5,43	-
17.	Stigmasterol	-8,11	1,13 x 10^3	LEU 339A
18.	Tylophorine	-5,82	54,52 x 10^3	ILE 373A
Mitogen-Activated Protein Kinase Kinase Kinase 7				
1.	Takinib	-8,05	1,26 x 10^3	ASP 175A, LYS 63A, ALA 107A
2.	1-triacontanol	-3,76	1,75 x 10^6	GLU 108A, TRY 106A
3.	3,4,5-trimethoxyacetophenone	-4,85	278,97 x 10^3	ALA 107A
4.	4-hydroxy-3-methoxyacetophenone	-4,87	270,98 x 10^3	ALA 107A, ASP 175A
5.	α-Amyrin	-9,58	94,26	-
6.	β-Amyrin	-9,65	85,86	-
7.	β-Stigmasterol	-9,57	95,89	-
8.	Coumarine	-5,33	122,97 x 10^3	ASP 175A, LYS 63A
9.	Dehydroantofine	-8,57	522,21	LYS 63A, ARG 44A, GLU 77A
10.	Deydrotylophorine	-8,53	563,39	LYS 63A, GLU 77A, GLU 105A, GLY 110A, ASN 161A, ASP 175A
11.	Ficuseptine A	-8,59	501,44	TRY 106A, ASP 175A, PRO 160A, GLY 110A
12.	Genistin	-7,86	1,74 x 10^3	GLU 105A, ALA 107A
13.	Kaempferitrin	-6,82	10,04 x 10^3	MET 104A, ARG 44A,
14.	Myristic acid	-4,11	967,06 x 10^3	MET 104A, ASP 175A, LYS 63A
15.	Palmitic acid	-4,61	417,48 x 10^3	ALA 107A, GLU 105A
16.	Phenanthroindolizidine	-7,91	1,61 x 10^3	ALA 107A
17.	Stigmasterol	-9,68	80,50	-
18.	Tylophorine	-8,77	371,85	LYS 63A, GLU 77A, PRO 160A
Vascular Endothelial Growth Factor Receptor-2 kinase (VEGFR-2 kinase)				
1.	BIBF 1120	-10,54	18,76	LYS 868A, CYS 919A, LEU 840A, GLU 917A
2.	1-triacontanol	-2,85	8,21 x 10^6	ARG 1032A
3.	3,4,5-trimethoxyacetophenone	-4,61	415,95 x 10^3	CYS 919A, PHE 918A, LYS 920A, GLY 922A
4.	4-hydroxy-3-methoxyacetophenone	-4,30	701,96 x 10^3	GLU 917A, CYS 919A,
5.	α-Amyrin	-8,93	286,83	-
6.	β-Amyrin	-8,77	375,03	-

No	Compound	ΔG kkal/ mol	KI (nM)	Amino Acid Residue
7.	β-Stigmasterol	-10,89	10,42 x 10^3	-
8.	Coumarine	-5,27	137,98 x 10^3	-
9.	Dehydroantofine	-7,92	1,56 x 10^3	ASN 923A, CYS 920A
10.	Deydrotylophorine	-7,96	1,46 x 10^3	ASN 923A, ARG 1032A
11.	Ficuseptine A	-7,80	1,90 x 10^3	CYS 919A, LYS 920A, PHE 921A, ARG 1032A
12.	Genistin	-7,46	3,42 x 10^3	LYS 920A, CYS 919A, LYS 868A, PHE 1047A
13.	Kaempferitrin	-7,20	5,26 x 10^3	GLU 917A, ASN 923A,
14.	Myristic acid	-4,68	369,26 x 10^3	LYS 868A, CYS 1045A, PHE 1047A,
15.	Palmitic acid	-4,74	337,53 x 10^3	ASN 923A
16.	Phenanthroindolizidine	-7,61	2,62 x 10^3	-
17.	Stigmasterol	-10,93	9,67	CYS 919A
18.	Tylophorine	-7,47	3,35 x 10^3	CYS 919A, ARG 1032A, GLU 917A
Leukotriene A4 hydrolase Receptor				
1.	Bestatin	-10,02	45,41	PRO 1374A, ASP 1375A, GLN 1136A, TRP 1311A, TYR 1267A
2.	1-triacontanol	+2,89	-	GLY 1269A,
3.	3,4,5-trimethoxyacetophenone	-5,61		TYR 1378A, ALA 1137A, ASP 1375A
4.	4-hydroxy-3-methoxyacetophenone	-5,60	77,65 x 10^6	LEU 1365A, ASP 312A
5.	α-Amyrin	+9A, 18	-	TRP 1311A
6.	β-Amyrin	+43,66	-	TRP 1311A
7.	β-Stigmasterol	-9,20	181,34	GLU 1271A, HIS 1299A, HIS 1295A, ALA 1377A, LEU 1379A, TRP 1311A
8.	Coumarine	-6,27	25,15 x 10^6	PHE 1314A, TRP 1315A
9.	Dehydroantofine	-1,63	64,06 x 10^9	PRO 1374A, ASP 1373A,
10.	Deydrotylophorine	+3,14	-	GLN 1136A, GLY 1269A, TRP 1311A, PRO 1374A, GLU 1271A
11.	Ficuseptine A	+0,21	-	GLN 1136A, GLU 1271A, TRP 1311A, TRP1267A, PRO 1374A
12.	Genistin	-10,95	9,38	GLY 1269A, TRP 1311A, ALA 1137A
13.	Kaempferitrin	+10,37	-	GLN 1134A, MET 1270A, GLU 1271A, GLU 1296A, HIS 1295A, GLN 1136A, ASP 1375A, PRO 1374A
14.	Myristic acid	-5,68	68,89 x 10^3	TRP 1311A
15.	Palmitic acid	-7,10	6,24 x 10^6	GLN 1134A, GLN 1136A
16.	Phenanthroindolizidine	-10,21	32,71	TRY 1267A
17.	Stigmasterol	-9,99	47,20	GLU 1296A, LEU 1369A
18.	Tylophorine	-0,05	924,15 x 10^9	GLN 1136A, GLU 1271A, PRO 1374A, TRP 1311A

4. ADMET Prediction

ADMET prediction was established for 17 compounds using the admetSAR server. Protein Plasma Binding, Caco-2 probability, as well as HIA probability, indicates good value where Protein Plasma Binding represents predicted distribution based on attachment to plasma proteins, HIA (Human intestinal absorption) score is high shows better absorbance in the intestinal tract upon oral administration (Cheng et al., 2013).

Table 7. In-Silico absorption and distribution analysis using admetSAR server.

No	Compound	Absorbtion		Distribution
		CaCo-2 (nm. Sec-1)	HIA (%)	PBB (%)
1.	1-triacontanol	51,34	100,00	100,00
2.	3,4,5-trimethoxyacetophenone	53,72	97,51	81,41
3.	4-hydroxy-3-methoxyacetophenone	18,06	93,54	73,51
4.	α-Amyrin	45,28	100,00	100,00
5.	β-Amyrin	46,75	100,00	100,00
6.	β-Stigmasterol	52,34	100,00	100,00
7.	Coumarine	32,12	100,00	43,40
8.	Dehydroantofine	49,21	96,87	44,63
9.	Deydrotylophorine	57,57	98,35	49,96
10.	Ficuseptine A	41,73	97,35	45,92
11.	Genistin	8,20	42,26	39,71
12.	Kaempferitrin	5,18	13,77	42,73
13.	Myristic acid	24,07	97,85	100,00
14.	Palmitic acid	26,07	98,30	100,00
15.	Phenanthroindolizidine	34,48	100,00	83,18
16.	Stigmasterol	52,34	100,00	100,00
17.	Tylophorine	57,47	98,11	74,48

Classification::
- In Vitro CaCo-2 cell permeability (nm. Sec^{-1}): >70 higher permeability; 4-70 medium permeability; <4 low permeability
- % Human Intestinal Absorption (%HIA): 70-100% well absorbed, 20-70% moderately absorbed; 0-20% poorly absorbed
- % Plasma Protein Binding: >90% strongly bound; <90% weakly bound

Based on the prediction results, it shows that 2 (two) compounds have a poor absorption profile in the intestines, namely genistin and kaempferitrin. All compounds have the ability of permeability in intermediate $CaCO_2$ cells. Meanwhile, the distribution profile based on attachment to plasma proteins shows that the compounds 1-triacontanol, amyrin, β-amyrin, β-stigmasterol, myristic acid, palmitic acid, and stigmasterol are strongly bound to plasma proteins so that they are predicted to have poorly distributed abilities in the body Table 7.

After predicting the pharmacokinetic properties including the absorption and distribution profiles, the toxicity prediction of the test compounds was carried out. In silico toxicity was carried out to see the carcinogenicity and mutagenicity properties of the test compound, because chemical carcinogenesis is of increasing importance in drug discovery for its serious effect on human health (Yang et al., 2018). This experiment using the toxtree® application to predict the level of toxicity of a compound in the body. The parameters used in this test are Cramer Rules, Benigni/Bossa Rulebase, and Kroes TTC decision tree. The Cramer Rules test results showed that 8 test compounds were in class III which showed high toxicity and 7 compounds showed low levels of toxicity. For carcinogen and mutagenic testing, Benigni/Bossa Rulebase was used and showed negative results for all test compounds. And the Kroes TTC test shows that all test compounds have safe exposure limits Table 8.

Table 8. In-Silico toxicity analysis of active compounds from Awar-awar leaves (*Ficus septica* Burm. L)

No	Compound	Cramer rules	Benigni/bosa rulebase	Kroes TTC decision tree
1.	1-triacontanol	1	8,9	1
2.	3,4,5-trimethoxyacetophenone	1	8,9	1
3.	4-hydroxy-3-methoxyacetophenone	1	8,9	1
4.	α-Amyrin	1	8,9	1
5.	β-Amyrin	1	8,9	1
6.	β-Stigmasterol	2	8,9	1
7.	Coumarine	3	8,9	1
8.	Dehydroantofine	3	8,9	1
9.	Deydrotylophorine	3	8,9	1
10.	Ficuseptine A	3	8,9	1
11.	Genistin	3	8,9	1
12.	Kaempferitrin	3	8,9	1
13.	Myristic acid	1	8,9	1
14.	Palmitic acid	1	8,9	1
15.	Phenanthroindolizidine	3	8,9	1
16.	Stigmasterol	2	8,9	1
17.	Tylophorine	3	8,9	1

Classification:
- Cramer rules:
  1. Substances with sample chemical structures and fix which efficient modes of metabolism exist, suggesting a low order of oral toxicity
  2. Substances which possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III
  3. Substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups
- Benigni/bose rulebase:
  8. Negative for genotoxic carcinogenicity
  9. Negative for non-genotoxic carcinogenicity
- Kroes TTC decision tree:
  1. Substance would not be expected to be a safety concern
  2. Substance has safety issue but can be ignored
  3. Substance requiring specific toxicity data in determining their safety risk

CONCLUSION

A step by step computational pipeline was used to find out the potential anticancer agents from *Ficus septica* Burm. L. Molecular docking and 3D-pharmacophore modeling had accomplished to Study the potential compounds for Anti-Cancer Agent of ER-α, ER-β, VEGFR-2 kinase, and LTA4H Receptor from this plant. There were total of 17 types of compounds tested and has been found several potential compounds that can be used as lead compounds and genistin were found promising and showed potential inhibitory characteristics compared to others compound as an anticancer.

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