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Original Study

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Potential of natural antioxidant compound in Cymbopogon nardus as anti-cancer drug via HSP-70 inhibitor: A bioinformatics approach

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Abstract: Citronella grass (*Cymbopogon nardus)* is a plant containing many metabolite compounds which prevent and treat various diseases, one of which is cancer. Antioxidant compounds found in citronella have been shown to improve the immune system by increasing cytokines. The activity of changing homeostasis generates free radicals. Free radicals causing protein damage so that Heat Shock Protein-70 (HSP70) is overexpressed. HSP70 has a role as a chaperon. Mutations in the anti-apoptotic protein HSP70 are one of the causes of cancer. This current research aims to determine the potential of compounds present in the citronella plant stem as anti-cancer through inhibition of HSP-70. The method was a bioinformatics approach, namely the in-silico method which provided a simulation of binding protein ligands to HSP-70 as inhibitor mechanism. The results of this study indicated that there was a potential for citronella compounds, namely spathulenol binding to HSP-70. Spathulenol compounds interact with Hsp70 via the positions Thr204, Gly12, Gly203, Thr14, Lys71, Asp10, Val369, Asp199, Val337, Gly338, Asp366, Gly339, Pro365, Glys201, & Glys202 with Van der Waals bonds and hydrogen bonds on Thr13. In the complex, there was one unfavorable bond formed on the O atom of the query ligand. From the results above, it can be concluded that the Spathulenol compound is predicted to act as an inhibitor of Hsp70 protein activity because it inhibits the binding site of the native ligand on Hsp70. The stability of the binding interaction produced by Spathulenol allows a response to Hsp70 inhibitor activity. By inhibiting the activity of Hsp70 inhibitors, it is possible to inhibit the formation and proliferation of cancer cells.

Keyword: *Cymbopogon nardus; HSP70; cancer cells*

INTRODUCTION

Cancer is a complex disease that involves many types of biological interactions at various scales, physical, temporal and biological[1]. Cancer develops as a function of various biological interactions and events both in the domains between individual genes and proteins, and at the cellular and physiological levels between functionally diverse somatic cells[2]. At the genetic level, the genetic code interacts synergistically to achieve homeostasis. The genetic code is expressed into hundreds to thousands of proteins in cells[3].

Heat Shock Protein-70 (HSP-70) is a protein which plays a role in maintaining cell homeostasis and is also a molecular chaperone that maintains

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stability of protein levels, interactions between proteins and inhibit protein aggregation[4]. The presence of cancer is the result of an unbalanced biochemical reaction in the cells. In cancer patients, HSP70 is overexpressed so that HSP is used as an important prognostic factor in malignant diseases such as cancer[5]. Overexpression of HSP70 can inhibit apoptosis and prevent caspase activation in various cellular models through various cellular stressors[6], including accumulation of misfolded proteins, reactive oxygen species (ROS) or DNA damage[7].

Various ways of drug agents are used as anti-cancer in various fields, both in the pharmaceutical field and in the use of natural ingredients from plants. Research on traditional medicine has begun to develop, one of which is research on the citronella plant (Cymbopogon nardus L. Rendle.). Citronella grass is efficacious as a traditional medicine because it contains active compounds such as saponins, flavonoids, polyphenols, alkaloids, and essential oils (Rofi, 2021). These compounds function as antiprotozoal, anti-inflammatory, antimicrobial, antibacterial, anti-diabetic, anticholinesterase, molluscicide, and antifungal. Citronella grass is also easily cultivated and accessed by many people so it is flexible to be used as medicine[11]. Several studies have shown that compounds present in citronella have the potential to improve the immune system as protection from Sars Cov-1912. The geraniol compound of citronella essential oil also has the potential for anti-cancer chemoprevention, namely breast cancer MCF-713.

The potential of citronella compounds as anti-cancer through the inhibition of HSP70 can be seen through a mechanical model-based bioinformatics approach that explains the biochemical process using the in-silico method[14]. In silico is used to describe experiments performed with the aid of a computer. The in-silico test can be used to determine the interaction between a compound and the target molecule, one of which is the receptor[15]. Based on the information above, a bioinformatics technology is needed that shows the biochemical reaction of HSP70 as a target molecule with compounds from citronella.

MATERIAL AND METHOD

The PuChem database (https://pubchem.ncbi.nlm.nih.gov/) was used in this study for the preparation of chemical compounds samples from the results of GCMS analysis of citronella grass stems. Thirty-four compounds were successfully obtained from the database with PubChem Compound ID (CID) information. consisting of CID 7503, CID 460, CID 10329, CID 332, CID 7041, CID 1715136, CID 1146, CID 6432308, CID 92138, CID 92231, CID 95997 CID 12301996, CID 226486, CID 518516, CID 3084311, CID 521216, CID 5974, CID 592628, CID 9983, CID 26397, CID 527256, CID 75303, CID 594234, CID 554084, CID 12366, CID 565584, CID 606866, CID 548034, CID 543959, CID 15256789, CID 5363269, CID 7678, CID 123409, & CID 8791, other information was 3D structure with file structure data format (sdf). The process of energy minimization and conversion of sdf files to protein databank format (pdb) was carried out on all samples of compounds through OpeBabel 2.4.1 software. Minimization of energy in the compound aims to increase the flexibility of the molecule through positive bond energy. The 3D structure of the target protein, HSP70 (RCSB ID: 4IO8), was obtained from the RCSB PDB database (https://www.rcsb.org/), protein sterilization was carried out using PyMol 2.5 version software by removing water molecules and contaminant ligands [16,17].

The ability of inhibitor activity by query compounds from citronella grass stems on HSP70 in this study was predicted through molecular docking simulations. Molecular docking is used to measure the specific activity ability of the ligand and the pattern of molecular interactions through the value of binding affinity[18]. This study used PyRx 0.9.9 version software to identify the ability of inhibitor activity on Hsp70 & TNFR1 and activator on R by spathulenol compounds from citronella grass stems through molecular docking simulation with grid position Center (Å) X: 11,444 Y: -3.991 Z: 15,181 Dimension (Å) X: 36,592 Y: 38.011 Z:

35,415, Center (Å) X:6.891 Y: 30,858 Z:11,548 Dimension (Å) X:34,254 Y:29,278 Z:29,846, & Center (Å) X: 19,813 Y: 8.863 Z: 38,209 Dimension (Å) X: 39,358 Y: 37,212 Z: 53,400. Visualization of the 3D structure of the protein-ligand complex was carried out using PyMol 2.5 version software, the structure was displayed with cartoons, transparent surfaces, and selection coloring[19].

Identification of molecular interactions of compounds from citronella grass stems with all target proteins in this study were identified through the Discovery Studio 2016 version of the software. Types of chemical bond interactions such as Van der Waals, hydrogen, hydrophobic, electrostatic, and pi are present in molecular complexes. The interaction formed is a weak bond that plays a role in triggering the activity of the target protein[20].

The validation of the docking results was carried out through molecular dynamic (MD) simulation which aimed to identify the flexibility of ligand binding in the protein domain. The flexibility of the bond was shown by the RMSF plot on the CABS-flex 2.0ver server (http://biocomp.chem.uw.edu.pl/CABSflex2). Parameters used for MD simulation are RNG seed, temperature, side-chain, C-alpha, rigidity, & trajectory. A stable binding interaction on the ligand-protein complex should have an RMSF value <4Å[21].

The spatulenol compound from citronella grass stem extract with the strongest binding activity on Hsp70 was tested with the SwissADME server (http://www.swissadme.ch/) for the prediction of adsorption and distribution via Canonical SMILE. The prediction aims to identify physicochemical properties, bioavailability score, and prediction as a drug-like molecule using various methods such as Lipinski, Ghose, Egan, and Muegge[22]. Validation of inhibitory properties on specific compounds was further identified through the Molinspiration Chemoinformatics server (https://www.molinspiration.com/cgi-bin/properties), predictions with positive results as inhibitors on query compounds were shown through more positive values on probability scores[23].

RESULTS AND DISCUSSION

The simulation of the interaction of the ligand binding with the target specific domain in this study was carried out using the blind docking method. Blind docking aims to identify the ability of ligand binding activity on a specific domain by ignoring the functional position on the protein[24]. The most negative binding affinity for the ligand is used as a determinant of the ability of the ligand to affect the activity of the target protein. Binding affinity is the Gibbs energy formed when ligands and proteins interact and work according to the laws of Thermodynamics[25,26]. A negative value of binding affinity determines the binding activity, a more negative value indicates the ligand bond with the strongest bond. If the binding affinity value of the candidate compound is more negative, it is predicted to have activity on the target protein which refers to the response of inhibitors and activators[27,28].

The docking simulation in this study aimed to determine the inhibitory activity of compounds from citronella grass stem extract on Hsp70 via grid docking (Center (Å) X: 11,444 Y: -3.991 Z: 15,181 Dimension (Å) X: 36,592 Y: 38,011 Z: 35,415). The compounds from the citronella grass stem extract with the most negative binding affinity values for each target protein were Spathulenol (-7.9 kcal/mol), 10-epi-.gamma.-eudesmol (-8.3 kcal/mol), and Torreyol (-6.3) (Table 1). The compound with the most negative binding affinity value was predicted to trigger stronger activity on Hsp70 than other compounds. Visualization of the Spathulenol_Hsp70 molecular complex was carried out through rigid and transparent surfaces, cartoons, sticks structures with colored selection (Figure 1).

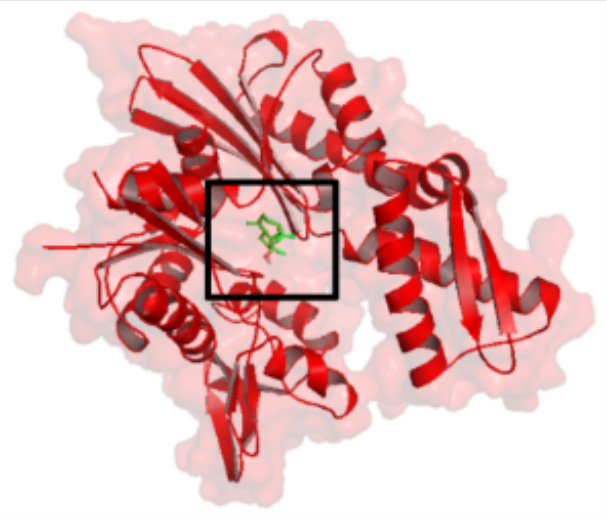

Figure 1. Structural visualization from the docking simulation Spathulenol_Hsp70.

The protein-ligand molecular complexes were formed by specific types of weak bonds that play a role in triggering the activation of specific biological responses such as inhibitory activities. The activity of the ligand as a protein activator was shown by binding to the cofactor region29. Hydrogen bonds and Van der Walls play a role in the stability of drug molecules to trigger the inhibitory activity of the target protein30. The unfavourable bond interactions formed in the molecular complex must be below three in order for the complex to remain stable31. Spathulenol compounds interacted with Hsp70 via the positions Thr204, Gly12, Gly203, Thr14, Lys71, Asp10, Val369, Asp199, Val337, Gly338, Asp366, Gly339, Pro365, Glys201, & Glys202 with Van der Waals bonds and hydrogen bonds on Thr13. In the complex, there is one unfavourable bond formed on the O atom of the query ligand (Figure 2).

Hsp70 activation was carried out by acetylation at the active site with lysine residues by the native ligand HOPX to produce a biological response to Hsp7032. Spathulenol compound was predicted to act as an inhibitor of Hsp70 protein activity because it inhibits the binding site of the native ligand on Hsp70.

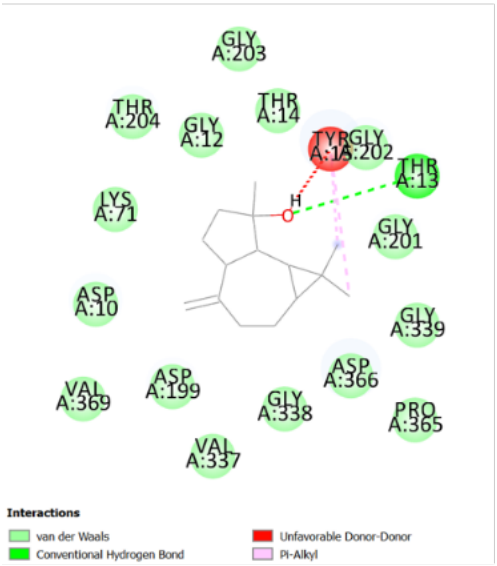

Figure 2. The ligand-protein interaction Spathulenol_Hsp70.

Table 1. The binding affinity from molecular docking simulation

No	Compound	PubChem CID	Binding Affinity (kcal/mol)
			Hsp70
1	Benzyl chloride	7503	-4.3
2	Phenol, 2-methoxy- (CAS)	460	-5.2
3	2,3-Dihydro-Benzofuran	10329	-5.2
4	2-Methoxy-4-vinylphenol	332	-5.5
5	Phenol, 2,6-dimethoxy- (CAS)	7041	-5.3
6	Phenol, 2-methoxy-4-(1-propenyl)-, (E)- (CAS)	1715136	-6.9
7	Methanamine, N,N-dimethyl- (CAS)	1146	-2.3
8	Naphthalene, 1,2,3,4,4A,5,6,8A-Octahydro-7-	6432308	-7.0
9	Elemol	92138	-6.4
10	Spathulenol	92231	-7.9
11	3',5'-Dimethoxyacetophenone	95997	-6.1
12	endo-1-bourbonanol	12301996	-5.7
13	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	226486	-6.2
14	10-epi-.gamma.-eudesmol	518516	-7.6
15	Torreyol	3084311	-7.1
16	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-	521216	-5.9
17	1-Hexadecanaminium, N,N,N-trimethyl-, bromide	2681	-5.5
18	1-Cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)	592628	-6.1
19	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- (CAS)	9983	-6.1
20	Heptadecanoic acid, ethyl ester (CAS)	26397	-5.4
21	Rosifoliol	527256	-7.7
22	1-Naphthalenamine, 4-bromo- (CAS)	75303	-6.3
23	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-	594234	-7.3
24	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)	554084	-6.3
25	Hexadecanoic acid, ethyl ester (CAS)	12366	-5.5
26	Longifolenaldehyde	565584	-7.1
27	4-(2,6,6-Trimethyl-cyclohex-1-enyl)-butyric acid	606866	-7.0
28	2-Methyl-5-(2,6,6-trimethyl-cyclohex-1-enyl)-pentane-2,3-diol	548034	-7.2
29	2H-Benzocyclohepten-2-one, decahydro-9a-methyl-, trans- (CAS)	543959	-7.1
30	1-Allyl-3-methylcyclohex-2-enol	15256789	-5.6
31	Ethyl Oleate	5363269	-5.6
32	1-Phenyl-2-propanone	7678	-5.3
33	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS)	123409	-5.4
34	Dodecanoic acid, phenylmethyl ester (CAS)	8791	-5.8

MD simulation was carried out to validate the docking results in this study. MD aimed to identify the stability of the protein-ligand molecular complex with reference to the RMSF33 value. The results of the MD analysis showed the stability of the RMSF in the complex produced by the compound Spathulenol_Hsp70 (Figure 3). The following is a link to the results of the molecular dynamic simulation from this study (http://212.87.3.12/CABSflex2/job/eb5d17d97cae2c3/) for Spathulenol_Hsp70. The stability of the binding interaction produced by Spathulenol allowed a response to Hsp70 inhibitor activity.

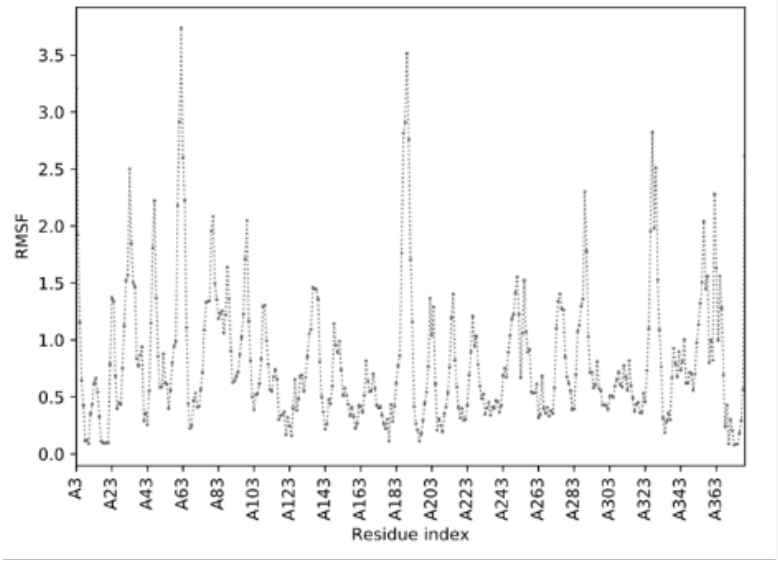

Figure 3. RMSF and residue index from MD simulation. (A) Spathulenol_Hsp70 (B) 10-epi.gamma.-eudesmol_AR (C) Torreyol_TNFR1.

Drug-like molecule analysis was used to determine the similarity of a candidate compound with a drug molecule. Similarity referred to the physicochemical properties through specific parameters such as molecular mass, high lipophilicity, hydrogen donor & acceptor bonds, and molar refractivity34. Drug-like molecule test can be done using Lipinski, Ghose, Veber, Egan, Muege, and bioavailability methods. These methods explain that candidate compounds are predicted to have similarities if they meet at least two rules and the bioavailability score of a drug candidate must be > 0.17 in order to trigger increased circulation drug molecule35. Physicochemical properties and solubility are important for early prediction of the absorption of drug candidate molecules, then prediction of bioactivity is carried out by referring to positive results as inhibitors on the query compound indicated by a more positive value on the probability score[36]. The results of the bioactivity analysis showed that Spathulenol compounds had bioactivity properties as inhibitors, the candidate compounds were drug-like and soluble (Table 2).

Heat shock protein 70 (Hsp70), one of the major stress-inducible members of the 70 kDa stress protein family (HSP70) whose expression is mainly regulated by Heat Shock Factor 1 (HSF-1) consists of at least 8 homologous members, exerts tumorigenic functions by sustaining proliferative cell signalling, increasing invasive and metastatic activity and migration and by preventing apoptotic signalling [37,38]. Hsp70 is frequently constitutively overexpressed in the cytosol and present on the plasma membrane of many different tumour types[39,40]. To promote cancer cell survival, tumorigenicity and anti-apoptotic activities such as interfering with the apoptosis signal regulating kinase 1 (ASK1) and the co-chaperone CHIP[41], blocking BAX translocation to the mitochondria[42] or by interfering with lysosomal membranes and thereby inhibiting their permeabilization[43]. Apart from its intracellular localization, Hsp70 could be transported to and anchored on the plasma membrane of tumor, but not normal cells, via tumor-specific lipid vesicular transport which was not completely unravelled[44]. Membrane Hsp70-positive tumors had been shown to actively release Hsp70 in exosomes[45] that could fuse with the plasma membrane. Since normal cells did not present Hsp70 on their cell surfaces, mHsp70 served as a tumor-specific targeting structure for in vivo imaging[46].

Table 2. Bioactivity analysis result of Spathulenol

Compounds	Activity Prediction	Physicochemical Properties	Water Solubility	Druglikeness
Spathulenol	GPCR ligand: -0.42 Ion channel modulator: -0.28 Kinase inhibitor: -0.68 Protease inhibitor: -0.36 Enzyme inhibitor: 0.06 Probable: Inhibitor	Formula: $C_{15}H_{24}O$ Weight: 220.35 g/mol Num. heavy atoms: 16 Num. arom. heavy atoms: 0 Fraction Csp3: 0.87 Num. rotatable bonds: 0 Num. H-bond acceptors: 1 Num. H-bond donors: 1 Molar Refractivity: 68.34 TPSA: 20.23 Å²	Log S (ESOL): -3.17 Class: Soluble Log S (Ali): -3.20 Class: Soluble Log S (SILICOS-IT): -2.96 Class: Soluble	Lipinski: Yes Ghose: Yes Veber: Yes Egan: Yes Muegge: No Bioavailability: 0.55 Probable: Drug-like Molecule

CONCLUSION

Compounds from citronella grass stem extract consisting of Spathulenol have the potential to inhibit the activity of Hsp70 protein. The candidate compound is proven by strong bonds through a more negative binding affinity that can form several types of weak bond interactions. The molecular complex is stable and has bioactivity as an inhibitor due to the drug-like nature of the molecule so that it is predicted to be a candidate as an anti-cancer drug.

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AUTHOR'S CONTRIBUTION STATEMENT

RS and PAIS prepared the samples, designed and executed the protocol and also wrote the manuscript. LM and NFA reviewed the manuscript. NFA reviewed and supervised the manuscript.

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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HASIL PENILAIAN SEJAWAT SEBIDANG ATAU *PEER REVIEW*
KARYA ILMIAH : JURNAL ILMIAH

Judul Jurnal Ilmiah (Artikel) : *"Potential of natural antioxidant compound in Cymbopogon nardus as anti-cancer drug via HSP-70 inhibitor : A bioinformatics approach"*

Jumlah Penulis : 4 orang

Status Pengusul : ~~Penulis Utama~~/Penulis ke-2/~~Penulis korespondensi~~

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LEMBAR
HASIL PENILAIAN SEJAWAT SEBIDANG ATAU *PEER REVIEW*
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Potential of natural antioxidant

by Turnitin LLC

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Original Study

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Potential of natural antioxidant compound in Cymbopogon nardus as anti-cancer drug via HSP-70 inhibitor: A bioinformatics approach

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Abstract: Citronella grass (*Cymbopogon nardus)* is a plant containing many metabolite compounds which prevent and treat various diseases, one of which is cancer. Antioxidant compounds found in citronella have been shown to improve the immune system by increasing cytokines. The activity of changing homeostasis generates free radicals. Free radicals causing protein damage so that Heat Shock Protein-70 (HSP70) is overexpressed. HSP70 has a role as a chaperon. Mutations in the anti-apoptotic protein HSP70 are one of the causes of cancer. This current research aims to determine the potential of compounds present in the citronella plant stem as anti-cancer through inhibition of HSP-70. The method was a bioinformatics approach, namely the in-silico method which provided a simulation of binding protein ligands to HSP-70 as inhibitor mechanism. The results of this study indicated that there was a potential for citronella compounds, namely spathulenol binding to HSP-70. Spathulenol compounds interact with Hsp70 via the positions Thr204, Gly12, Gly203, Thr14, Lys71, Asp10, Val369, Asp199, Val337, Gly338, Asp366, Gly339, Pro365, Glys201, & Glys202 with Van der Waals bonds and hydrogen bonds on Thr13. In the complex, there was one unfavorable bond formed on the O atom of the query ligand. From the results above, it can be concluded that the Spathulenol compound is predicted to act as an inhibitor of Hsp70 protein activity because it inhibits the binding site of the native ligand on Hsp70. The stability of the binding interaction produced by Spathulenol allows a response to Hsp70 inhibitor activity. By inhibiting the activity of Hsp70 inhibitors, it is possible to inhibit the formation and proliferation of cancer cells.

Keyword: *Cymbopogon nardus; HSP70; cancer cells*

INTRODUCTION

Cancer is a complex disease that involves many types of biological interactions at various scales, physical, temporal and biological[1]. Cancer develops as a function of various biological interactions and events both in the domains between individual genes and proteins, and at the cellular and physiological levels between functionally diverse somatic cells[2]. At the genetic level, the genetic code interacts synergistically to achieve homeostasis. The genetic code is expressed into hundreds to thousands of proteins in cells[3].

Heat Shock Protein-70 (HSP-70) is a protein which plays a role in maintaining cell homeostasis and is also a molecular chaperone that maintains

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stability of protein levels, interactions between proteins and inhibit protein aggregation[4]. The presence of cancer is the result of an unbalanced biochemical reaction in the cells. In cancer patients, HSP70 is overexpressed so that HSP is used as an important prognostic factor in malignant diseases such as cancer[5]. Overexpression of HSP70 can inhibit apoptosis and prevent caspase activation in various cellular models through various cellular stressors[6], including accumulation of misfolded proteins, reactive oxygen species (ROS) or DNA damage[7].

Various ways of drug agents are used as anti-cancer in various fields, both in the pharmaceutical field and in the use of natural ingredients from plants. Research on traditional medicine has begun to develop, one of which is research on the citronella plant (Cymbopogon nardus L. Rendle.). Citronella grass is efficacious as a traditional medicine because it contains active compounds such as saponins, flavonoids, polyphenols, alkaloids, and essential oils (Rofi, 2021). These compounds function as antiprotozoal, anti-inflammatory, antimicrobial, antibacterial, anti-diabetic, anticholinesterase, molluscicide, and antifungal. Citronella grass is also easily cultivated and accessed by many people so it is flexible to be used as medicine[11]. Several studies have shown that compounds present in citronella have the potential to improve the immune system as protection from Sars Cov-1912. The geraniol compound of citronella essential oil also has the potential for anti-cancer chemoprevention, namely breast cancer MCF-713.

The potential of citronella compounds as anti-cancer through the inhibition of HSP70 can be seen through a mechanical model-based bioinformatics approach that explains the biochemical process using the in-silico method[14]. In silico is used to describe experiments performed with the aid of a computer. The in-silico test can be used to determine the interaction between a compound and the target molecule, one of which is the receptor[15]. Based on the information above, a bioinformatics technology is needed that shows the biochemical reaction of HSP70 as a target molecule with compounds from citronella.

MATERIAL AND METHOD

The PuChem database (https://pubchem.ncbi.nlm.nih.gov/) was used in this study for the preparation of chemical compounds samples from the results of GCMS analysis of citronella grass stems. Thirty-four compounds were successfully obtained from the database with PubChem Compound ID (CID) information. consisting of CID 7503, CID 460, CID 10329, CID 332, CID 7041, CID 1715136, CID 1146, CID 6432308, CID 92138, CID 92231, CID 95997 CID 12301996, CID 226486, CID 518516, CID 3084311, CID 521216, CID 5974, CID 592628, CID 9983, CID 26397, CID 527256, CID 75303, CID 594234, CID 554084, CID 12366, CID 565584, CID 606866, CID 548034, CID 543959, CID 15256789, CID 5363269, CID 7678, CID 123409, & CID 8791, other information was 3D structure with file structure data format (sdf). The process of energy minimization and conversion of sdf files to protein databank format (pdb) was carried out on all samples of compounds through OpeBabel 2.4.1 software. Minimization of energy in the compound aims to increase the flexibility of the molecule through positive bond energy. The 3D structure of the target protein, HSP70 (RCSB ID: 4IO8), was obtained from the RCSB PDB database (https://www.rcsb.org/), protein sterilization was carried out using PyMol 2.5 version software by removing water molecules and contaminant ligands [16,17].

The ability of inhibitor activity by query compounds from citronella grass stems on HSP70 in this study was predicted through molecular docking simulations. Molecular docking is used to measure the specific activity ability of the ligand and the pattern of molecular interactions through the value of binding affinity[18]. This study used PyRx 0.9.9 version software to identify the ability of inhibitor activity on Hsp70 & TNFR1 and activator on R by spathulenol compounds from citronella grass stems through molecular docking simulation with grid position Center (Å) X: 11,444 Y: -3.991 Z: 15,181 Dimension (Å) X: 36,592 Y: 38.011 Z:

35,415, Center (Å) X:6.891 Y: 30,858 Z:11,548 Dimension (Å) X:34,254 Y:29,278 Z:29,846, & Center (Å) X: 19,813 Y: 8.863 Z: 38,209 Dimension (Å) X: 39,358 Y: 37,212 Z: 53,400. Visualization of the 3D structure of the protein-ligand complex was carried out using PyMol 2.5 version software, the structure was displayed with cartoons, transparent surfaces, and selection coloring[19].

Identification of molecular interactions of compounds from citronella grass stems with all target proteins in this study were identified through the Discovery Studio 2016 version of the software. Types of chemical bond interactions such as Van der Waals, hydrogen, hydrophobic, electrostatic, and pi are present in molecular complexes. The interaction formed is a weak bond that plays a role in triggering the activity of the target protein[20].

The validation of the docking results was carried out through molecular dynamic (MD) simulation which aimed to identify the flexibility of ligand binding in the protein domain. The flexibility of the bond was shown by the RMSF plot on the CABS-flex 2.0ver server (http://biocomp.chem.uw.edu.pl/CABSflex2). Parameters used for MD simulation are RNG seed, temperature, side-chain, C-alpha, rigidity, & trajectory. A stable binding interaction on the ligand-protein complex should have an RMSF value <4Å[21].

The spatulenol compound from citronella grass stem extract with the strongest binding activity on Hsp70 was tested with the SwissADME server (http://www.swissadme.ch/) for the prediction of adsorption and distribution via Canonical SMILE. The prediction aims to identify physicochemical properties, bioavailability score, and prediction as a drug-like molecule using various methods such as Lipinski, Ghose, Egan, and Muegge[22]. Validation of inhibitory properties on specific compounds was further identified through the Molinspiration Chemoinformatics server (https://www.molinspiration.com/cgi-bin/properties), predictions with positive results as inhibitors on query compounds were shown through more positive values on probability scores[23].

RESULTS AND DISCUSSION

The simulation of the interaction of the ligand binding with the target specific domain in this study was carried out using the blind docking method. Blind docking aims to identify the ability of ligand binding activity on a specific domain by ignoring the functional position on the protein[24]. The most negative binding affinity for the ligand is used as a determinant of the ability of the ligand to affect the activity of the target protein. Binding affinity is the Gibbs energy formed when ligands and proteins interact and work according to the laws of Thermodynamics[25,26]. A negative value of binding affinity determines the binding activity, a more negative value indicates the ligand bond with the strongest bond. If the binding affinity value of the candidate compound is more negative, it is predicted to have activity on the target protein which refers to the response of inhibitors and activators[27,28].

The docking simulation in this study aimed to determine the inhibitory activity of compounds from citronella grass stem extract on Hsp70 via grid docking (Center (Å) X: 11,444 Y: -3.991 Z: 15,181 Dimension (Å) X: 36,592 Y: 38,011 Z: 35,415). The compounds from the citronella grass stem extract with the most negative binding affinity values for each target protein were Spathulenol (-7.9 kcal/mol), 10-epi-.gamma.-eudesmol (-8.3 kcal/mol), and Torreyol (-6.3) (Table 1). The compound with the most negative binding affinity value was predicted to trigger stronger activity on Hsp70 than other compounds. Visualization of the Spathulenol_Hsp70 molecular complex was carried out through rigid and transparent surfaces, cartoons, sticks structures with colored selection (Figure 1).

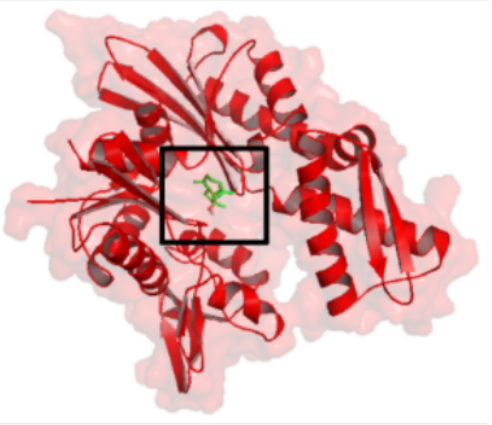

Figure 1. Structural visualization from the docking simulation Spathulenol_Hsp70.

The protein-ligand molecular complexes were formed by specific types of weak bonds that play a role in triggering the activation of specific biological responses such as inhibitory activities. The activity of the ligand as a protein activator was shown by binding to the cofactor region29. Hydrogen bonds and Van der Walls play a role in the stability of drug molecules to trigger the inhibitory activity of the target protein30. The unfavourable bond interactions formed in the molecular complex must be below three in order for the complex to remain stable31. Spathulenol compounds interacted with Hsp70 via the positions Thr204, Gly12, Gly203, Thr14, Lys71, Asp10, Val369, Asp199, Val337, Gly338, Asp366, Gly339, Pro365, Glys201, & Glys202 with Van der Waals bonds and hydrogen bonds on Thr13. In the complex, there is one unfavourable bond formed on the O atom of the query ligand (Figure 2).

Hsp70 activation was carried out by acetylation at the active site with lysine residues by the native ligand HOPX to produce a biological response to Hsp7032. Spathulenol compound was predicted to act as an inhibitor of Hsp70 protein activity because it inhibits the binding site of the native ligand on Hsp70.

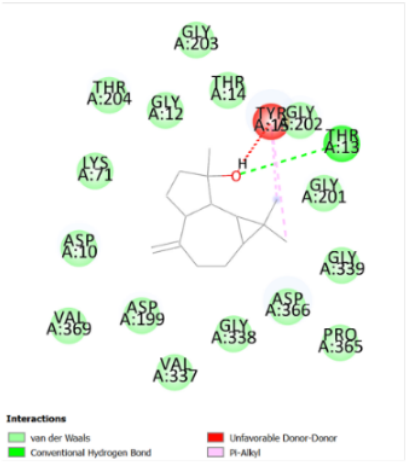

Figure 2. The ligand-protein interaction Spathulenol_Hsp70.

Table 1. The binding affinity from molecular docking simulation

No	Compound	PubChem CID	Binding Affinity (kcal/mol)
			Hsp70
1	Benzyl chloride	7503	-4.3
2	Phenol, 2-methoxy- (CAS)	460	-5.2
3	2,3-Dihydro-Benzofuran	10329	-5.2
4	2-Methoxy-4-vinylphenol	332	-5.5
5	Phenol, 2,6-dimethoxy- (CAS)	7041	-5.3
6	Phenol, 2-methoxy-4-(1-propenyl)-, (E)- (CAS)	1715136	-6.9
7	Methanamine, N,N-dimethyl- (CAS)	1146	-2.3
8	Naphthalene, 1,2,3,4,4A,5,6,8A-Octahydro-7-	6432308	-7.0
9	Elemol	92138	-6.4
10	Spathulenol	92231	-7.9
11	3',5'-Dimethoxyacetophenone	95997	-6.1
12	endo-1-bourbonanol	12301996	-5.7
13	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	226486	-6.2
14	10-epi-.gamma.-eudesmol	518516	-7.6
15	Torreyol	3084311	-7.1
16	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-	521216	-5.9
17	1-Hexadecanaminium, N,N,N-trimethyl-, bromide	2681	-5.5
18	1-Cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)	592628	-6.1
19	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- (CAS)	9983	-6.1
20	Heptadecanoic acid, ethyl ester (CAS)	26397	-5.4
21	Rosifoliol	527256	-7.7
22	1-Naphthalenamine, 4-bromo- (CAS)	75303	-6.3
23	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-	594234	-7.3
24	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)	554084	-6.3
25	Hexadecanoic acid, ethyl ester (CAS)	12366	-5.5
26	Longifolenaldehyde	565584	-7.1
27	4-(2,6,6-Trimethyl-cyclohex-1-enyl)-butyric acid	606866	-7.0
28	2-Methyl-5-(2,6,6-trimethyl-cyclohex-1-enyl)-pentane-2,3-diol	548034	-7.2
29	2H-Benzocyclohepten-2-one, decahydro-9a-methyl-, trans- (CAS)	543959	-7.1
30	1-Allyl-3-methylcyclohex-2-enol	15256789	-5.6
31	Ethyl Oleate	5363269	-5.6
32	1-Phenyl-2-propanone	7678	-5.3
33	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS)	123409	-5.4
34	Dodecanoic acid, phenylmethyl ester (CAS)	8791	-5.8

MD simulation was carried out to validate the docking results in this study. MD aimed to identify the stability of the protein-ligand molecular complex with reference to the RMSF33 value. The results of the MD analysis showed the stability of the RMSF in the complex produced by the compound Spathulenol_Hsp70 (Figure 3). The following is a link to the results of the molecular dynamic simulation from this study (http://212.87.3.12/CABSflex2/job/eb5d17d97cae2c3/) for Spathulenol_Hsp70. The stability of the binding interaction produced by Spathulenol allowed a response to Hsp70 inhibitor activity.

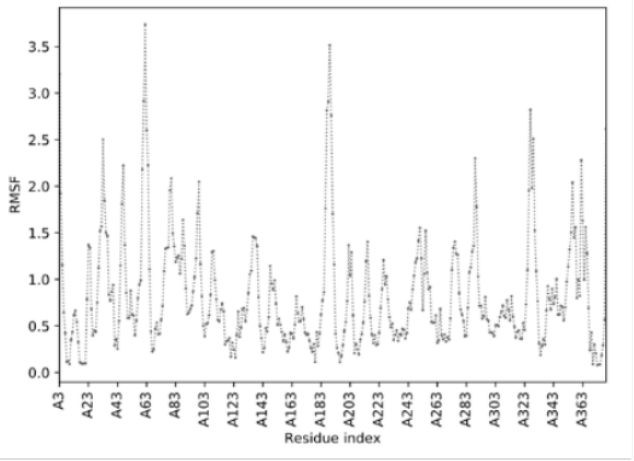

Figure 3. RMSF and residue index from MD simulation. (A) Spathulenol_Hsp70 (B) 10-epi.gamma.-eudesmol_AR (C) Torreyol_TNFR1.

Drug-like molecule analysis was used to determine the similarity of a candidate compound with a drug molecule. Similarity referred to the physicochemical properties through specific parameters such as molecular mass, high lipophilicity, hydrogen donor & acceptor bonds, and molar refractivity34. Drug-like molecule test can be done using Lipinski, Ghose, Veber, Egan, Muege, and bioavailability methods. These methods explain that candidate compounds are predicted to have similarities if they meet at least two rules and the bioavailability score of a drug candidate must be > 0.17 in order to trigger increased circulation drug molecule35. Physicochemical properties and solubility are important for early prediction of the absorption of drug candidate molecules, then prediction of bioactivity is carried out by referring to positive results as inhibitors on the query compound indicated by a more positive value on the probability score[36]. The results of the bioactivity analysis showed that Spathulenol compounds had bioactivity properties as inhibitors, the candidate compounds were drug-like and soluble (Table 2).

Heat shock protein 70 (Hsp70), one of the major stress-inducible members of the 70 kDa stress protein family (HSP70) whose expression is mainly regulated by Heat Shock Factor 1 (HSF-1) consists of at least 8 homologous members, exerts tumorigenic functions by sustaining proliferative cell signalling, increasing invasive and metastatic activity and migration and by preventing apoptotic signalling [37,38]. Hsp70 is frequently constitutively overexpressed in the cytosol and present on the plasma membrane of many different tumour types[39,40]. To promote cancer cell survival, tumorigenicity and anti-apoptotic activities such as interfering with the apoptosis signal regulating kinase 1 (ASK1) and the co-chaperone CHIP[41], blocking BAX translocation to the mitochondria[42] or by interfering with lysosomal membranes and thereby inhibiting their permeabilization[43]. Apart from its intracellular localization, Hsp70 could be transported to and anchored on the plasma membrane of tumor, but not normal cells, via tumor-specific lipid vesicular transport which was not completely unravelled[44]. Membrane Hsp70-positive tumors had been shown to actively release Hsp70 in exosomes[45] that could fuse with the plasma membrane. Since normal cells did not present Hsp70 on their cell surfaces, mHsp70 served as a tumor-specific targeting structure for in vivo imaging[46].

Table 2. Bioactivity analysis result of Spathulenol

Compounds	Activity Prediction	Physicochemical Properties	Water Solubility	Druglikeness
Spathulenol	GPCR ligand: -0.42 Ion channel modulator: -0.28 Kinase inhibitor: -0.68 Protease inhibitor: -0.36 Enzyme inhibitor: 0.06 Probable: Inhibitor	Formula: $C_{15}H_{24}O$ Weight: 220.35 g/mol Num. heavy atoms: 16 Num. arom. heavy atoms: 0 Fraction Csp3: 0.87 Num. rotatable bonds: 0 Num. H-bond acceptors: 1 Num. H-bond donors: 1 Molar Refractivity: 68.34 TPSA: 20.23 Å²	Log S (ESOL): -3.17 Class: Soluble Log S (Ali): -3.20 Class: Soluble Log S (SILICOS-IT): -2.96 Class: Soluble	Lipinski: Yes Ghose: Yes Veber: Yes Egan: Yes Muegge: No Bioavailability: 0.55 Probable: Drug-like Molecule

CONCLUSION

Compounds from citronella grass stem extract consisting of Spathulenol have the potential to inhibit the activity of Hsp70 protein. The candidate compound is proven by strong bonds through a more negative binding affinity that can form several types of weak bond interactions. The molecular complex is stable and has bioactivity as an inhibitor due to the drug-like nature of the molecule so that it is predicted to be a candidate as an anti-cancer drug.

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AUTHOR'S CONTRIBUTION STATEMENT

RS and PAIS prepared the samples, designed and executed the protocol and also wrote the manuscript. LM and NFA reviewed the manuscript. NFA reviewed and supervised the manuscript.

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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