BUKTI KORESPONDENSI
JURNAL NASIONAL TERAKREDITASI PERINGKAT 2

Judul artikel	:	In Silico Study of Compounds Identified in *Curcuma aeruginosa* Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer
Jurnal	:	Journal of Food and Pharmaceutical Sciences (J. Food Pharm. Sci)
Edisi	:	Tahun 2025, Vol 13 No 2, hal 92-99
Penerbit	:	Integrated Research and Testing Laboratory, Universitas Gadjah Mada (LPPT-UGM)
Penulis	:	Ririn Suharsanti, Muhammad Ryan Radix Rahardhian, Lia Kusmita

Tabel Tahapan Publikasi Artikel

No	Tahapan Publikasi	Tanggal
1	Submission of manuscript	16 Mei 2025
2	Editor Decission	21 Mei 2025
3	Manuscript revision request	21 Mei 2025
4	Submit revised manuscript	22 Mei 2025
5	Acceptance Notification	2 Juni 2025
6	Payment Request	2 Juni 2025
7	Official payment receipt	12 Juni 2025
8	Copyediting Revision Request	18 Juni 2025
9	Send to Production	18 Juni 2025
10	Revised copyediting of Manuscript	19 Juni 2025
11	Article published	30 Juni 2025

1. Submission of Manuscript

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In Silico Study of Compounds Identified in Curcuma aeruginosa Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer

Ririn Suharsanti, Muhammad Ryan Radix Rahardhian, Lia Kusmita

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ID	File	Date	Type
97952-1	ririnsuharsanti, JFPS_Ririn Suharsanti_16 Mei.docx	May 16, 2025	Article Text
97954-1	ririnsuharsanti, Hasil Turnitin_Ririn Suharsanti.pdf	May 16, 2025	Other
98042-1	astridesmayanti92, 21589-Blind.docx	May 17, 2025	Article Text

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Ririn Suharsanti:

Thank you for submitting the manuscript, "In Silico Study of Compounds Identified in Curcuma aeruginosa Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer" to Journal of Food and Pharmaceutical Sciences. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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Username: ririnsuharsanti

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Prof. Dr. Abdul Rohman, M.Si., Apt

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Journal of Food and Pharmaceutical Sciences

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Our decision is: Revisions Required

Astri Desmayanti
Universitas Gadjah Mada
desmayantiastri@gmail.com

Reviewer A:
Recommendation: Revisions Required

Ttitle describes the content of papper properly and clearly

Good

Revelance of data and conclusion

Good

RECOMENDATION

Accepted with minor revision

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RECOMENDATION

Accepted with minor revision

Additional Comment

article revised according to feedback

Reviewer B:
Recommendation: Accept Submission

Ttitle describes the content of papper properly and clearly

Good

Revelance of data and conclusion

Fair

RECOMENDATION

Accepted without revision

Additional Comment

over all the manuscript was good

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We have reached a decision regarding your submission to Journal of Food and Pharmaceutical Sciences, "In Silico Study of Compounds Identified in Curcuma aeruginosa Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer".

Our decision is: Revisions Required

Astri Desmayanti
Universitas Gadjah Mada
desmayantiastri@gmail.com

Reviewer A:
Recommendation: Revisions Required

Ttitle describes the content of papper properly and clearly

Good

Revelance of data and conclusion

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RECOMENDATION

Accepted with minor revision

Additional Comment

article revised according to feedback

Reviewer B:
Recommendation: Accept Submission

Ttitle describes the content of papper properly and clearly

Good

Revelance of data and conclusion

Fair

RECOMENDATION

Accepted without revision

Additional Comment

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Ririn Suharsanti, Muhammad Ryan Radix Rahardhian, Lia Kusmita

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Round 1

Round 1 Status
Submission accepted.

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[JFPS] Editor Decision	2025-05-21 02:11 PM
[JFPS] Editor Decision	2025-06-02 06:57 PM
[JFPS] Editor Decision	2025-06-17 06:46 PM

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4. Submit revised manuscript

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[JFPS] Editor Decision

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Ririn Suharsanti, Muhammad Ryan Radix Rahardhian, Lia Kusmita:

We have reached a decision regarding your submission to Journal of Food and Pharmaceutical Sciences, "In Silico Study of Compounds Identified in Curcuma aeruginosa Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer".

Our decision is to: Accept Submission

Astri Desmayanti
Universitas Gadjah Mada
desmayantiastri@gmail.com

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2025-06-02 06:57 PM

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We have reached a decision regarding your submission to Journal of Food and Pharmaceutical Sciences, "In Silico Study of Compounds Identified in Curcuma aeruginosa Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer".

Our decision is to: Accept Submission

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desmayantiastri@gmail.com

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Participants

Astri Desmayanti (astridesmayanti92)

Ririn Suharsanti (ririnsuharsanti)

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Journal of Food and Pharmaceutical Sciences

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Participants

Astri Desmayanti (astridesmayanti92)

Ririn Suharsanti (ririnsuharsanti)

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Thank you for the proofread file that has been provided. All parts of the article are correct except for the writing of the name "Curcuma aeruginosa" in the abstract which should be written in italics. We attach our correction file and have marked it with yellow highlights thank you Ririn Suharsanti ririnsuharsanti, confirm_21589-Article Text-101234-1-18-20250617.pdf	ririnsuharsanti 2025-06-18 10:25 AM

11. Article Published Vol 13 issue 2 (30 Juni 2025) on Website

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In Silico Study of Compounds Identified in Curcuma aeruginosa Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer

Ririn Suharsanti
Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang; Central Java, 50192, Indonesia

Muhammad Ryan Radix Rahardhian
Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang; Central Java, 50192, Indonesia
https://orcid.org/0000-0001-9543-6023

Lia Kusmita
Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang; Central Java, 50192, Indonesia
https://orcid.org/0000-0001-9543-6023

DOI: https://doi.org/10.22146/jfps.21589

Journal of Food and Pharmaceutical Sciences — Volume 13 - No. 2 | April - Juni 2025

New Submission

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Submission of manuscript

Journal of Food and Pharmaceutical Sciences

1 *Original Article*

2 In Silico Study of Compounds Identified in *Curcuma* 3 *aeruginosa* Roxb Rhizome as BRAF V600E Inhibitors 4 in Melanoma Cancer

5 **Ririn Suharsanti*), Muhammad Ryan Radix Rahardhian, Lia Kusmita**

6 Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang; Central Java, 50192 , Indonesia
7 * Corresponding author: ririnsuharsanti@stifar.ac.id ; Tel.: +6282136923586

8 Received: date; Accepted: date; Published: date

9

10 **Abstract:** *Curcuma aeruginosa* Roxb rhizome contains secondary metabolite compounds and plays a role in
11 various activities such as antioxidant, antibacterial, anthelmintic, antiandrogenic, antinociceptive, and
12 anticancer. Anticancer activity that has been reported in *Curcuma aeruginosa Roxb* rhizome is limited to breast
13 and cervical cancer. The purpose of this study was to explore the potential of *Curcuma aeruginosa* Roxb rhizome
14 in melanoma cancer through the mechanism of inhibiting the BRAF V600E. The 96% ethanol extract of *Curcuma*
15 *aeruginosa* Roxb rhizome was separated to produce n-hexane (HF), ethyl acetate (EAF), and ethanol (EF)
16 fractions. The GC-MS results showed that there were 31 compounds from the three fractions. The docking
17 validation process was carried out on the native ligand N-(3-{[5-(4-chlorophenyl) -1H-pyrrolo [2,3b]pyridin3yl]
18 carbonyl}2,4difluorophenyl) propane-1-sulfonamide. All compounds were prepared as ligands for molecular
19 docking with the BRAF V600E receptor (PDB ID: 3OG7). Docking validation on native ligand showed RMSD
20 1.03Å. The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-
21 Cyclohexyl-2-propen-1-one (-6,68 kcal/mol); Cyclooctenone (-6,23 kcal/mol); and vemuravnib is still better as
22 K+ (-11.11 kcal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as
23 vemuravenib at GLN A:530, CYS A:532; ASP A:594. These results indicate that further structural development
24 is needed for better activity.

25 **Keywords:** *Curcuma aeruginosa*; GC-MS ; In Silico, BRAF V600E inhibitor, Vemuravenib
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27 1. INTRODUCTION

28 Over the past 50 years, melanoma incidence has steadily climbed globally. Melanoma is more
29 prevalent in lower latitudes and among white-skinned individuals. Melanoma is the most common
30 cancer in teenagers and young adults, but it is often more prevalent in the elderly population. In 2020,
31 melanoma of the skin is expected to account for 1.7% of all cancer diagnoses worldwide, with an
32 estimated 325,000 new cases [1]. Vemurafenib and dabravenib are BRAF mutation-inhibiting
33 chemotherapy drugs to treat melanoma [2], [3]. Approximately 50% of cutaneous melanoma patients
34 have active BRAF V600 mutations, so selective inhibitors were developed. Vemurafenib is responsive
35 in 50% of patients with BRAF V600 mutations and is longer progression-free than dacarbazine
36 (DTIC). In previous research, the reticuline compound in soursop leaves was proven to have the
37 potential to treat cancer through the BRAF V600E inhibitor mechanism in silico [4]. In addition, in
38 silico evaluation of several 4-(quinolin-2-yl)pyrimidin-2-amine derivatives as potent V600E-BRAF
39 inhibitors was carried out [5]. There are several active compounds as BRAF V 300E inhibitors, which
40 provide an opportunity for other natural ingredients to have the same activity.

41 Traditionally, the *C. aeruginose* rhizome has been used medicinally to treat stomach ache,
42 obesity and rheumatism, asthma and cough, scurvy and mental disorders [6]. Essential oil content
43 has been identified from the results of the distillation of *C. aeruginosa* Roxb. rhizomes such as

44 curzerenone (24.6%), 1,scineole (ll.O%), camphor (10.6%), zedoarol (6.3%), isocurcumenol (5.8%),
45 curcumenol (5.6%) and filranogermenone (5.5%) [7]. Other identified compounds include champor
46 (29,39%) dan germacrone (21,21%) [8], monoterpen (21,47%) berupa β-pinen dan 1,8 cineol [9], 1,8-
47 cineol (22.65%) dan germacrone (17.70%) [10], tropolene (18,1%) dan eucalyptol (17,9%) [11], β-pinene
48 (21.9%), neocurdione (16.1%) and curcumol (15.2%) [12]. Meanwhile, the compounds that were
49 successfully separated from the black turmeric extract using chromatography include germacrone,
50 zederone, dehydrocurdione, curcumenol, zedoarondiol dan isocurcumenol [13]; dehydrocurdione,
51 curcumenol, dan germacrone [14]; Pyrocurzerenone, Dehydrochromolaenin, Curzeone,
52 Linderazulene, Curzerenone, 8, 12 - Epoxy - 1 (10), 4(15), 7, 11 -germacratetraen-6-one [15]; aeruginon
53 and curcumenone [16]; dan flavon [17]. *C aeruginosa* isolates that have quite potential in various
54 activities are germacrone as antiandrogenic [13], hair growth promoter [14], antinociceptive [18], and
55 anticancer [16]. Anticancer activity that has been reported in *C. aeruginosa* Roxb rhizome is limited to
56 breast cancer (MCF-7 and T-47D) and cervical cancer (Ca Ski and HeLa S3) [19], [20]. There have been
57 no reports of *C. aeruni*osa being tested for BRAF V600E inhibitory activity as an anti-melanoma cancer
58 in silico so that it is worthy of being processed.

59 **2. MATERIALS AND METHODS**

60 *2.1. Chemical*

61 Ethanol, methanol, n-hexane, and ethyl acetate as solvents from Smart Lab, Indonesia. All reagents
62 used for the research were of analytical grade.

63 *2.2. Plant Collection*

64 *C. aeruginosa* Roxb dried rhizome from the Center for Research and Development of Traditional
65 Medicinal Plants and Medicines Tawangmangu, Central Java, harvested in February 2000.

66 *2.3. Instrumentation*

67 GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The
68 columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene
69 glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm.

70 *2.4. Software and Hardware*

71 The Protein Databank (PDB, www.rcsb.org) provided PDB ID: 3OG7 for download [4], [21]. The
72 natural chemical's 3D structure files were obtained from PubChem
73 (www.pubchem.ncbi.nlm.nih.gov). Ligands made with chemdraw 3D 15.0 for molecular docking.
74 AutoDock Tool 1.5.6 Sep_17_14 employed the molecular docking procedure for in-silico screening,
75 and Biovia Discovery Studio V21.1.0.2.20298 was used to view the results. Using a Lenovo laptop
76 running Windows 10 with a Core i3 CPU, 4 GB of RAM, 64-bit operating system, and an x-64
77 processor, pharmacokinetics and toxicity prediction are performed. The online SMILES Translator
78 (https://cactus.nci.nih.gov) was used to translate the compound into SMILES format. To forecast
79 pharmacokinetics and chemical toxicity, the SMILES-formatted molecule was processed using the
80 pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) [22].

81 *2.5. Extraction and Fractination*

82 One kg of the powdered material was macerated for three days at a ratio of 1:5 using 70% ethanol.
83 Ethanol extract (EE) was obtained by combining the filtrates and drying them out using a revolving
84 vacuum evaporator set at 60 °C and 100 rpm. Then, using solvents ranging from non-polar (n-
85 hexane), semi-polar (ethyl acetate), and polar (ethanol), the ethanol extract (EE) was separated by
86 sequential fractination to provide n-hexane (FH), ethyl acetate (EAF), and ethanol (EF). By turning
87 the vacuum evaporator at 60 °C and 100 rpm, respectively, fractions were concentrated [23], [24].

88 *2.6. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis*

89 GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The
90 columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene
91 glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm. The mobile phase used is helium
92 and was adjusted to a column velocity flow of 0.74 mL/min. Other GC-MS conditions are ion-source
93 temperature, 250 °C; interface temperature, 300 °C; pressure, 42,3 kPa; and 1 µl injector in split mode
94 with a split ratio of 153.0 with injection temperature of 300 °C. The temperature was raised to 320 °C
95 at the rate of 10 °C/min and held for 5 min. The total elution was 24 min.

96 *2.7. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents*

97 Protein and ligand preparation is the initial stage of molecular docking. AutoDock Tools-1.5.6 was
98 used to carry out 3D interaction, docking, and binding investigations. The Protein Data Bank
99 provided the target proteins for download (PDB ID: 3OG7) [4], [21]. The Biovia Discovery Studio
100 visualizer program is used to extract native ligands and water molecules from 3D structures to create
101 protein files (.pdb). Chemdraw 3D 15.0 was used to produce the test ligand file (.sdf), which was
102 retrieved from PubChem, for molecular docking. The ligand contributes charge and torsion after the
103 receptor adds charge prior to molecular docking. The grid box's dimensions and coordinates were
104 established. X: 2.643, Y: -2.28, Z: -19.403, and spacing are the grid box coordinates, and the grid box
105 size is 44 × 40 × 40Å, spacing 0.375. Molecular docking parameters include interacting amino acid
106 residues and binding affinity (kcal/mol). Interaction 2D and 3D between ligand and protein were
107 visualized using Biovia Discover Studio visualizer. The following chemical properties were predicted
108 and explained: polar surface activity (PSA), hydrogen bond acceptors (HBA), hydrogen bond donors
109 (HBD), the number of atom-to-atom bonds that can rotate (Torson), the logarithm of the coefficient
110 octanol/water partition (Log P), and molecular weight (MW). These were conducted utilizing
111 Lipinski's rule of five, a set of guidelines that aids in distinguishing between molecules that resemble
112 drugs and those that do not, using the pkCMS web tool application [5], [21], [25]. This approach might
113 forecast the higher probability of success or failure because of drug penetration and absorption.
114 Following the 3D drawing of the chemical structure using Chemdraw 3D 15.0 and its saving in a
115 particular format (.pdb), the online SMILES Translator (https://cactus.nci.nih.gov) was used to
116 convert it to SMILES format. The pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) was used
117 to process the SMILES formatted compound in order to forecast chemical toxicity and
118 pharmacokinetics [22].

119 **3. RESULTS AND DISCUSSION**

120 *3.1. Fraction Compounds*

121 According to the GC-MS data, HF was primarily composed of sesquiterpenes (63.1%) and diterpenes
122 (5.26%), with 31.58% of it being unknown. substances. The EF was made up of sesquiterpenes
123 (68.42%) and others (31.58%), whereas the EAF was made up of sesquiterpenes (42.86%), diterpenes
124 (21.43%), steroids (7.14%), and others (28.57%). Saturated fatty acids were present when IF was
125 identified. Curcumenol and epicurzerenone were the primary constituents of HF and EF, whereas
126 curcumenol and 2,4-Dispironorbornylcyclobuta-1,3-dione (ketene dimers) were the primary
127 constituents of EAF. All compounds detected from the *C. aeruginosa* fraction are shown in Figure 1.

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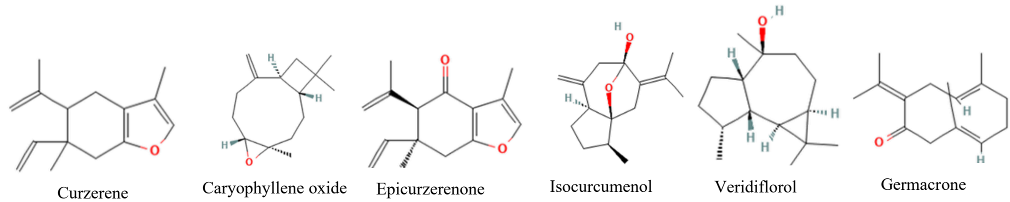

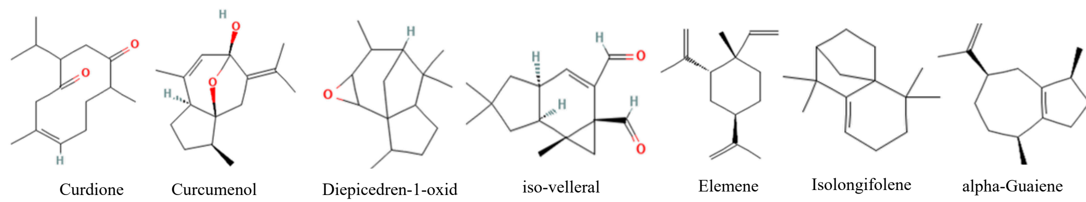

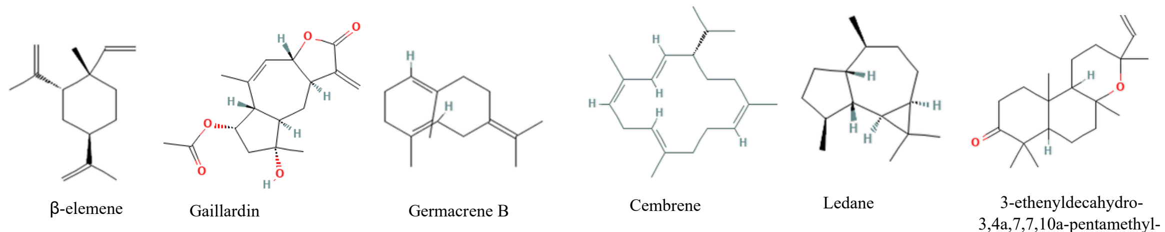

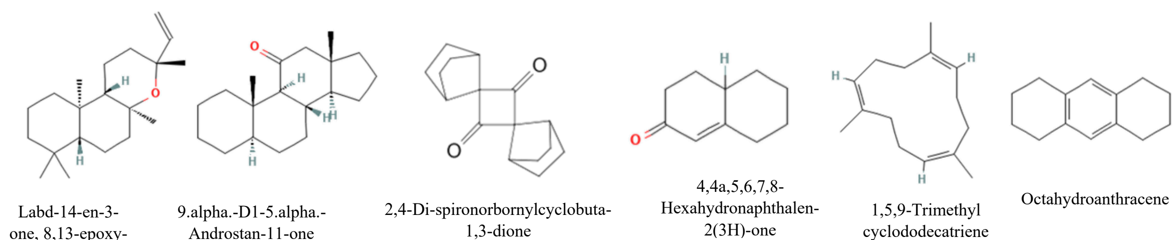

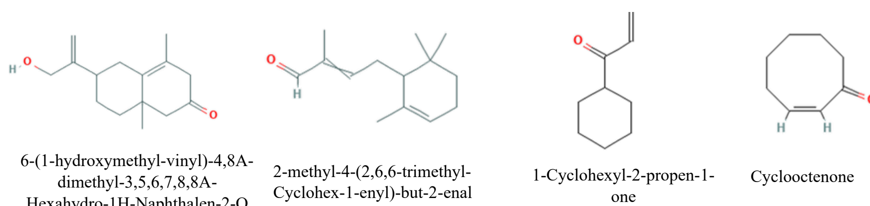

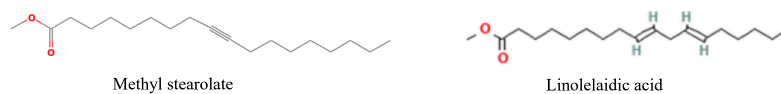

Figure 1. Compound Name and Structure Identification of *C. aeruginosa* Fractions Using GC-MS

3.1. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Redocking, or confirming the docking technique between the receptor and the native ligand, is the first step in molecular docking. RMSD 1.03A is the outcome of the validation procedure. Since the RMSD value is ≤ 2A the docking procedure can be deemed acceptable, and the RMSD obtained satisfies the validation acceptance criterion [22]. To show the stance before and after docking, native ligands are shown in two distinct colors. Native ligan in the redocking process with the BRAF V600E resistor is shown in figure 2. Vemuravenib, a commercial medication, was utilized as a control ligand against the BRAF V600E receptor (PDB ID: 3OG7), while all substances discovered by GC-MS (figure 1) were used as test ligands. ligand preparation is the initial stage of molecular docking.

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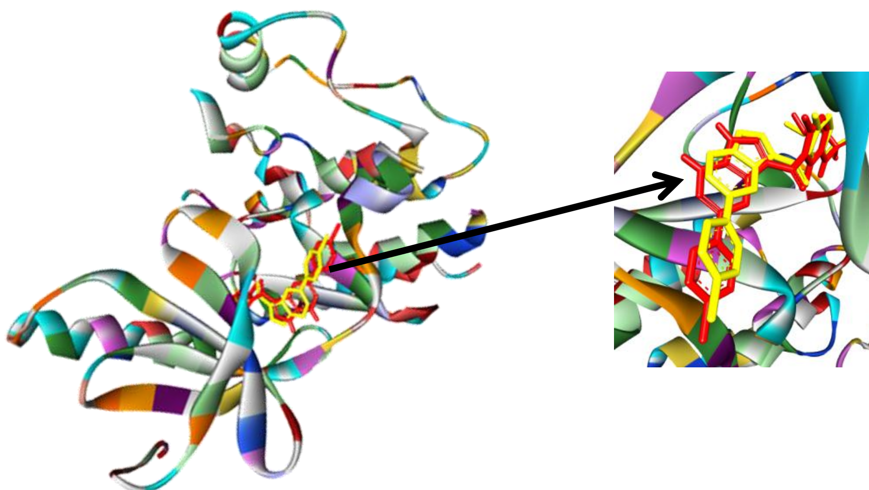

178 **Figure 2.** 3D diagram for the interaction of native ligand with BRAF-V600E receptor (yellow =before
179 and red = after redocking)

180 The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-
181 Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still
182 better as K+ (-10.593,68kal/mol). The smaller binding affinity value, the affinity between the receptor
183 and ligand was higher and the vice versa, the greater the binding affinity value, the affinity between
184 receptors and the ligands is getting lower [5], [22]. All three compounds do not bind to key amino
185 acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594 [22]. The
186 amino acid residues that were shown to interact with ligands displays in table 1 and illustrates the
187 interaction between ligands and the BRAF-V600E receptor using 2D visualization displays in figure
188 2.

189 **Table 1.** Molecular interactions present in the selected complex ligands and BRAF V600E receptor
190 and the amino acids involved.

Complex	Binding Energy (kcal/mol)	Inhibition Constant/ Ki (µM)	Amino acid residues
1-Cyclohexyl-2-propen-1-one	-6,68	12,63	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one	-6,89	8,96	Arg 575, Asp 576, Leu 577, Lys 578, Ser 616, Trp 619, Met 620, Ala 621, Val 626, Tyr 633, Ser 637, Asp 638, Ala 641
Cyclooctenone	-6,23	27,21	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
Vemuravenib	-11.11	7,42	Ile-463, Val-471, Ala- 481, Lys-483, Leu-505, Leu-514, Phe-516, Ile-527, Thr-529, **Gln-530**, Trp-531, **Cys-532**, Ser-535, Ser-536, His-539, Phe-583, **Asp-594**, Asp-593, Phe-595, Gly-596

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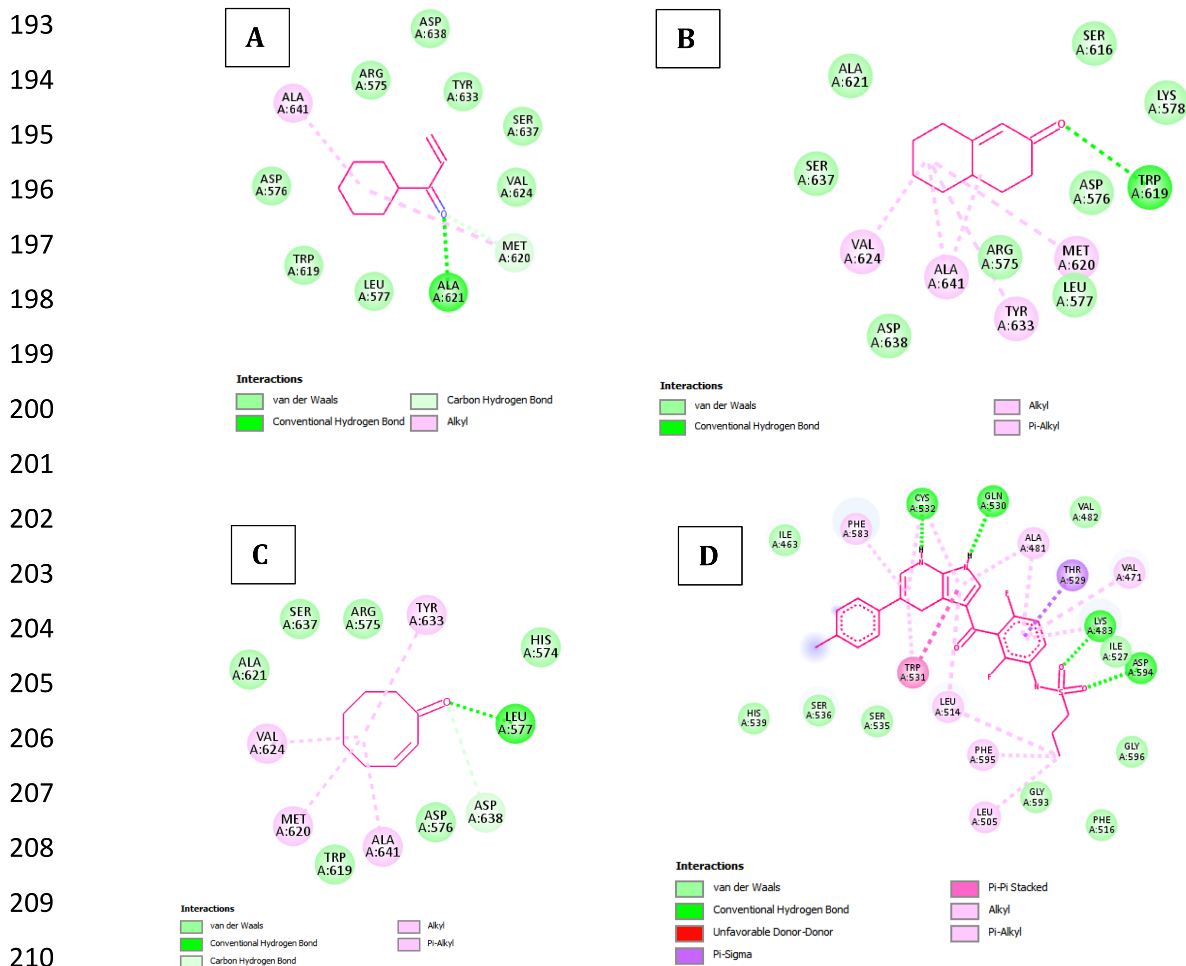

Figure 3. 2D Visualization of Complex Interactions Between Ligands 1-Cyclohexyl-2-propen-1-one **(A)**, 4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one **(B)**, Cyclooctenone **(C)**, and Vemuravenib **(D)** with BRAF V600E receptor

The likelihood that a molecule has the same or superior activity than BRAF V600E increases with the number of amino acid similarities between the reference chemical and crucial amino acid. GLN A:530, CYS A:532, ASP A:594, and THR A:529 are important amino acids linked to the BRAF V600E receptor. The reticuline compound has the same hydrogen-bonded amino acids (GLN 530 and ASP 549) as the reference compound Vemurafenib/key amino acids [22]. Conversely, compounds that had better binding scores than vemurafe-nib and a decent MolDock score (≥– 158.139) and Rerank score (≥– 118.607) were recognized as possible hits [5].

Out of all the compounds, the three identified compounds found by GC-MS were found to have the smallest binding affinities. The pkCMS online tool was used to further investigate these compound's pharmacokinetic and toxicity characteristics (ADMET). The Lipinski test uses passive diffusion to ascertain whether a substance in cell membranes is hydrophobic or hydrophilic. According to Lipinski's guidelines, a ligand must have a molecular weight of less than 500 Da and a LogP value of less than 5. molar refractivity between 40 and 130, donor hydrogen bonds < 5, and acceptor hydrogen bonds < 10. Cell membranes are more readily penetrated by ligands with molecular weights less than 500 Da than by those with molecular weights greater than 500 Da. The polarity of the ligand in fat, oil, and non-polar solvents is correlated with the logP value. Ligands that are widely dispersed throughout the body and have a log P value greater than 5 will interact more readily via the lipid bilayer layer of cell membranes. As a result, the ligand becomes more hazardous and its sensitivity to binding to the target molecule decreases. Because they are more broadly distributed throughout the body and are kept in

233 lipid membranes for longer, excessively hydrophobic compounds typically have a high level of toxicity.
234 The ligand is hydrophobic and has a tendency to dissolve in water when the log P value is less. Since
235 the ligand cannot cross the lipid bilayer membrane, its Log P value cannot be negative. The biological
236 activity of a ligand or medicine is correlated with the amount of hydrogen bonds in the donor and
237 acceptor. The amount of energy needed for absorption increases with the strength of the hydrogen bond
238 [25]. Table 2 displays the outcomes of the molecular docking studies, which showed that the three
239 compounds satisfied Lipinski's guidelines.

240 **Table 2.** Ligand's Lipinski Rules of Five

Complex	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar surface activity (PSA)
1-Cyclohexyl-2-propen-1-one	138.21	2.3218	0	1	62.125
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	150.221	2.4659	0	1	67.484
Cyclooctenone	124.183	2.0758	0	1	55.760

241

242 When evaluating the pharmacokinetics of novel pharmacological compounds, ADMET estimates are
243 essential. If a compound's anticipated value is more than 0.09, it has significant Caco2 permeability.
244 Human colorectal cancer epithelial cells are known as Caco-2 cells. To estimate oral drug absorption,
245 Caco-2 cell monolayers are frequently employed as an in vitro model of the human intestinal mucosa.
246 The volume needed for a drug to be uniformly distributed and produce the same concentration as in
247 blood plasma is known as the Steady State Volume of Distribution (VDss). Excretion in log (ml/min/kg)
248 is predicted by total clearance (CLtot). Hepatic clearance (liver metabolism and biliary clearance) and
249 renal clearance (renal excretion) are the two primary parts of drug clearance. AMES toxicity is
250 frequently used to evaluate a compound's capacity to cause mutagenesis using bacteria. Positive
251 findings suggest that the substance is mutagenic and carcinogenic [22]. If a compound's predictive value
252 is greater than 0.90, it is deemed to have high CaCO-2 permeability [26]. It has good permeability
253 because the test results showed a value greater than 0.90. When the volume of distribution (VDss) is
254 less than 0.71 L/kg (log VDss<-0.15), it is deemed low; when it is greater than 2.81 L/kg (log VDss>0.45),
255 it is deemed excessive [27]. In table 3, all compounds are in the range volume distribution requirements
256 so that it can be predicted that all these compounds can be distributed evenly to provide the same
257 concentration as in blood plasma. Based on table 3, all ligands have good pharmacokinetic parameter.

258 **Table 3.** Pharmacokinetics (ADMET) Parameters of Ligands

Complex	Absorption Caco2 permeability	Distribution VDss (human)	Metabolism (CYP2D6 substrate)	Excretion (Total Clearance)	AMES toxicity	Hepato toxicity
1-Cyclohexyl-2-propen-1-one	1.085	0.148	No	0.221	No	No
4,4a,5,6,7,8-Hexahydrona	1.501	0.344	No	0.112	No	No

phthalen-2(3H)-one						
Cyclooctenone	1.487	0.136	No	0.213	No	No

4. CONCLUSION

The three best components of *C. aeruginosa* fraction with the smallest binding affinity and meet the pharmacokinetic requirements are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still better as K+ (-10.593,68kal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. All three compounds have good pharmacokinetic parameter. These results indicate that further structural development is needed for better activity.

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Conflicts of interest: The authors declare no conflict of interest

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In Silico Study of Compounds Identified in Curcuma aeruginosa Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer

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In Silico Study of Compounds Identified in *Curcuma aeruginosa* Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer

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Abstract: *Curcuma aeruginosa* Roxb rhizome contains secondary metabolite compounds and plays a role in various activities such as antioxidant, antibacterial, anthelmintic, antiandrogenic, antinociceptive, and anticancer. Anticancer activity that has been reported in *Curcuma aeruginosa Roxb* rhizome is limited to breast and cervical cancer. The purpose of this study was to explore the potential of *Curcuma aeruginosa* Roxb rhizome in melanoma cancer through the mechanism of inhibiting the BRAF V600E. The 96% ethanol extract of *Curcuma aeruginosa* Roxb rhizome was separated to produce n-hexane (HF), ethyl acetate (EAF), and ethanol (EF) fractions. The GC-MS results showed that there were 31 compounds from the three fractions. The docking validation process was carried out on the native ligand N-(3-{[5-(4-chlorophenyl) -1H-pyrrolo [2,3b]pyridin3yl] carbonyl}2,4difluorophenyl) propane-1-sulfonamide. All compounds were prepared as ligands for molecular docking with the BRAF V600E receptor (PDB ID: 3OG7). Docking validation on native ligand showed RMSD 1.03Å. The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68 kcal/mol); Cyclooctenone (-6,23 kcal/mol); and vemuravnib is still better as K+ (-11.11 kcal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. These results indicate that further structural development is needed for better activity.

Keywords: *Curcuma aeruginosa*; GC-MS ; In Silico, BRAF V600E inhibitor, Vemuravenib

1. INTRODUCTION

Over the past 50 years, melanoma incidence has steadily climbed globally. Melanoma is more prevalent in lower latitudes and among white-skinned individuals. Melanoma is the most common cancer in teenagers and young adults, but it is often more prevalent in the elderly population. In 2020, melanoma of the skin is expected to account for 1.7% of all cancer diagnoses worldwide, with an estimated 325,000 new cases [1]. Vemurafenib and dabravenib are BRAF mutation-inhibiting chemotherapy drugs to treat melanoma [2], [3]. Approximately 50% of cutaneous melanoma patients have active BRAF V600 mutations, so selective inhibitors were developed. Vemurafenib is responsive in 50% of patients with BRAF V600 mutations and is longer progression-free than dacarbazine (DTIC). In previous research, the reticuline compound in soursop leaves was proven to have the potential to treat cancer through the BRAF V600E inhibitor mechanism in silico [4]. In addition, in silico evaluation of several 4-(quinolin-2-yl)pyrimidin-2-amine derivatives as potent V600E-BRAF inhibitors was carried out [5]. There are several active compounds as BRAF V 300E inhibitors, which provide an opportunity for other natural ingredients to have the same activity.

Traditionally, the *C. aeruginose* rhizome has been used medicinally to treat stomach ache, obesity and rheumatism, asthma and cough, scurvy and mental disorders [6]. Essential oil content has been identified from the results of the distillation of *C. aeruginosa* Roxb. rhizomes such as

curzerenone (24.6%), 1,scineole (ll.O%), camphor (10.6%), zedoarol (6.3%), isocurcumenol (5.8%), curcumenol (5.6%) and filranogermenone (5.5%) [7]. Other identified compounds include champor (29,39%) dan germacrone (21,21%) [8], monoterpen (21,47%) berupa β-pinen dan 1,8 cineol [9], 1,8-cineol (22.65%) dan germacrone (17.70%) [10], tropolene (18,1%) dan eucalyptol (17,9%) [11], β-pinene (21.9%), neocurdione (16.1%) and curcumol (15.2%) [12]. Meanwhile, the compounds that were successfully separated from the black turmeric extract using chromatography include germacrone, zederone, dehydrocurdione, curcumenol, zedoarondiol dan isocurcumenol [13]; dehydrocurdione, curcumenol, dan germacrone [14]; Pyrocurzerenone, Dehydrochromolaenin, Curzeone, Linderazulene, Curzerenone, 8, 12 - Epoxy - 1 (10), 4(15), 7, 11 -germacratetraen-6-one [15]; aeruginon and curcumenone [16]; dan flavon [17]. *C aeruginosa* isolates that have quite potential in various activities are germacrone as antiandrogenic [13], hair growth promoter [14], antinociceptive [18], and anticancer [16]. Anticancer activity that has been reported in *C. aeruginosa* Roxb rhizome is limited to breast cancer (MCF-7 and T-47D) and cervical cancer (Ca Ski and HeLa S3) [19], [20]. There have been no reports of *C. aeruni*osa being tested for BRAF V600E inhibitory activity as an anti-melanoma cancer in silico so that it is worthy of being processed.

2. MATERIALS AND METHODS

2.1. Chemical

Ethanol, methanol, n-hexane, and ethyl acetate as solvents from Smart Lab, Indonesia. All reagents used for the research were of analytical grade.

2.2. Plant Collection

C. aeruginosa Roxb dried rhizome from the Center for Research and Development of Traditional Medicinal Plants and Medicines Tawangmangu, Central Java, harvested in February 2000.

2.3. Instrumentation

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm.

2.4. Software and Hardware

The Protein Databank (PDB, www.rcsb.org) provided PDB ID: 3OG7 for download [4], [21]. The natural chemical's 3D structure files were obtained from PubChem (www.pubchem.ncbi.nlm.nih.gov). Ligands made with chemdraw 3D 15.0 for molecular docking. AutoDock Tool 1.5.6 Sep_17_14 employed the molecular docking procedure for in-silico screening, and Biovia Discovery Studio V21.1.0.2.20298 was used to view the results. Using a Lenovo laptop running Windows 10 with a Core i3 CPU, 4 GB of RAM, 64-bit operating system, and an x-64 processor, pharmacokinetics and toxicity prediction are performed. The online SMILES Translator (https://cactus.nci.nih.gov) was used to translate the compound into SMILES format. To forecast pharmacokinetics and chemical toxicity, the SMILES-formatted molecule was processed using the pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) [22].

2.5. Extraction and Fractination

One kg of the powdered material was macerated for three days at a ratio of 1:5 using 70% ethanol. Ethanol extract (EE) was obtained by combining the filtrates and drying them out using a revolving vacuum evaporator set at 60 °C and 100 rpm. Then, using solvents ranging from non-polar (n-hexane), semi-polar (ethyl acetate), and polar (ethanol), the ethanol extract (EE) was separated by sequential fractination to provide n-hexane (FH), ethyl acetate (EAF), and ethanol (EF). By turning the vacuum evaporator at 60 °C and 100 rpm, respectively, fractions were concentrated [23], [24].

2.6. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm. The mobile phase used is helium and was adjusted to a column velocity flow of 0.74 mL/min. Other GC-MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 42.3 kPa; and 1 μl injector in split mode with a split ratio of 153.0 with injection temperature of 300 °C. The temperature was raised to 320 °C at the rate of 10 °C/min and held for 5 min. The total elution was 24 min.

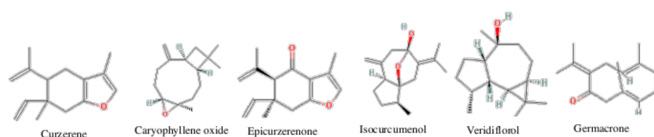
2.7. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

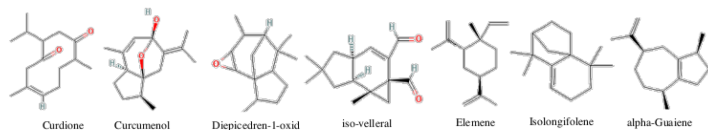
Protein and ligand preparation is the initial stage of molecular docking. AutoDock Tools-1.5.6 was used to carry out 3D interaction, docking, and binding investigations. The Protein Data Bank provided the target proteins for download (PDB ID: 3OG7) [4], [21]. The Biovia Discovery Studio visualizer program is used to extract native ligands and water molecules from 3D structures to create protein files (.pdb). Chemdraw 3D 15.0 was used to produce the test ligand file (.sdf), which was retrieved from PubChem, for molecular docking. The ligand contributes charge and torsion after the receptor adds charge prior to molecular docking. The grid box's dimensions and coordinates were established. X: 2.643, Y: -2.28, Z: -19.403, and spacing are the grid box coordinates, and the grid box size is 44 × 40 × 40Å, spacing 0.375. Molecular docking parameters include interacting amino acid residues and binding affinity (kcal/mol). Interaction 2D and 3D between ligand and protein were visualized using Biovia Discover Studio visualizer. The following chemical properties were predicted and explained: polar surface activity (PSA), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), the number of atom-to-atom bonds that can rotate (Torson), the logarithm of the coefficient octanol/water partition (Log P), and molecular weight (MW). These were conducted utilizing Lipinski's rule of five, a set of guidelines that aids in distinguishing between molecules that resemble drugs and those that do not, using the pkCMS web tool application [5], [21], [25]. This approach might forecast the higher probability of success or failure because of drug penetration and absorption. Following the 3D drawing of the chemical structure using Chemdraw 3D 15.0 and its saving in a particular format (.pdb), the online SMILES Translator (https://cactus.nci.nih.gov) was used to convert it to SMILES format. The pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) was used to process the SMILES formatted compound in order to forecast chemical toxicity and pharmacokinetics [22].

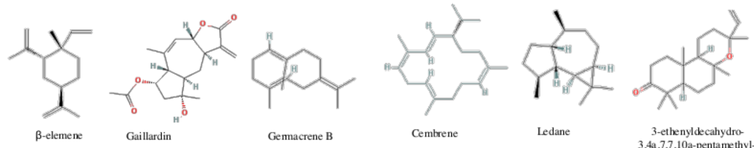
3. RESULTS AND DISCUSSION

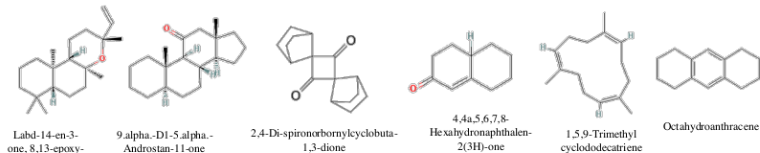
3.1. Fraction Compounds

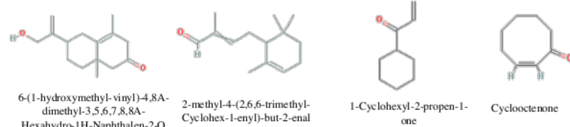
According to the GC-MS data, HF was primarily composed of sesquiterpenes (63.1%) and diterpenes (5.26%), with 31.58% of it being unknown. substances. The EF was made up of sesquiterpenes (68.42%) and others (31.58%), whereas the EAF was made up of sesquiterpenes (42.86%), diterpenes (21.43%), steroids (7.14%), and others (28.57%). Saturated fatty acids were present when IF was identified. Curcumenol and epicurzerenone were the primary constituents of HF and EF, whereas curcumenol and 2,4-Dispironorbornylcyclobuta-1,3-dione (ketene dimers) were the primary constituents of EAF. All compounds detected from the *C. aeruginosa* fraction are shown in Figure 1.











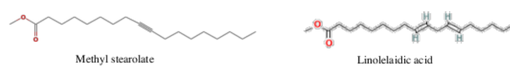


Figure 1. Compound Name and Structure Identification of *C. aeruginosa* Fractions Using GC-MS

3.1. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Redocking, or confirming the docking technique between the receptor and the native ligand, is the first step in molecular docking. RMSD 1.03A is the outcome of the validation procedure. Since the RMSD value is ≤ 2A the docking procedure can be deemed acceptable, and the RMSD obtained satisfies the validation acceptance criterion [22]. To show the stance before and after docking, native ligands are shown in two distinct colors. Native ligan in the redocking process with the BRAF V600E resistor is shown in figure 2. Vemuravenib, a commercial medication, was utilized as a control ligand against the BRAF V600E receptor (PDB ID: 3OG7), while all substances discovered by GC-MS (figure 1) were used as test ligands. ligand preparation is the initial stage of molecular docking.

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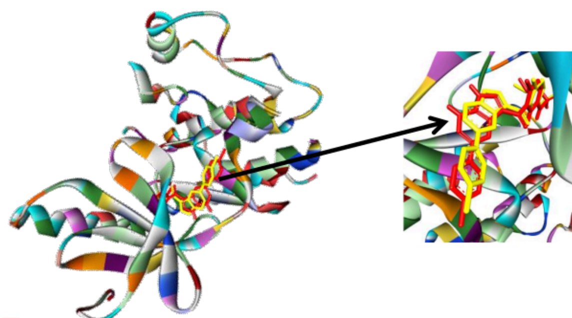

178 **Figure 2.** 3D diagram for the interaction of native ligand with BRAF-V600E receptor (yellow =before
179 and red = after redocking)

180 The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-
181 Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still
182 better as K+ (-10.593,68kal/mol). The smaller binding affinity value, the affinity between the receptor
183 and ligand was higher and the vice versa, the greater the binding affinity value, the affinity between
184 receptors and the ligands is getting lower [5], [22]. All three compounds do not bind to key amino
185 acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594 [22]. The
186 amino acid residues that were shown to interact with ligands displays in table 1 and illustrates the
187 interaction between ligands and the BRAF-V600E receptor using 2D visualization displays in figure
188 2.

189 **Table 1.** Molecular interactions present in the selected complex ligands and BRAF V600E receptor
190 and the amino acids involved.

Complex	Binding Energy (kcal/mol)	Inhibition Constant/ Ki (μM)	Amino acid residues
1-Cyclohexyl-2-propen-1-one	-6,68	12,63	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one	-6,89	8,96	Arg 575, Asp 576, Leu 577, Lys 578, Ser 616, Trp 619, Met 620, Ala 621, Val 626, Tyr 633, Ser 637, Asp 638, Ala 641
Cyclooctenone	-6,23	27,21	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
Vemuravenib	-11.11	7,42	Ile-463, Val-471, Ala- 481, Lys-483, Leu-505, Leu-514, Phe-516, Ile-527, Thr-529, **Gln-530**, Trp-531, **Cys-532**, Ser-535, Ser-536, His-539, Phe-583, **Asp-594**, Asp-593, Phe-595, Gly-596

191 ,
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Figure 3. 2D Visualization of Complex Interactions Between Ligands 1-Cyclohexyl-2-propen-1-one **(A)**, 4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one **(B)**, Cyclooctenone **(C)**, and Vemuravenib **(D)** with BRAF V600E receptor

The likelihood that a molecule has the same or superior activity than BRAF V600E increases with the number of amino acid similarities between the reference chemical and crucial amino acid. GLN A:530, CYS A:532, ASP A:594, and THR A:529 are important amino acids linked to the BRAF V600E receptor. The reticuline compound has the same hydrogen-bonded amino acids (GLN 530 and ASP 549) as the reference compound Vemurafenib/key amino acids [22]. Conversely, compounds that had better binding scores than vemurafe-nib and a decent MolDock score (≥− 158.139) and Rerank score (≥−118.607) were recognized as possible hits [5].

Out of all the compounds, the three identified compounds found by GC-MS were found to have the smallest binding affinities. The pkCMS online tool was used to further investigate these compound's pharmacokinetic and toxicity characteristics (ADMET). The Lipinski test uses passive diffusion to ascertain whether a substance in cell membranes is hydrophobic or hydrophilic. According to Lipinski's guidelines, a ligand must have a molecular weight of less than 500 Da and a LogP value of less than 5. molar refractivity between 40 and 130, donor hydrogen bonds < 5, and acceptor hydrogen bonds < 10. Cell membranes are more readily penetrated by ligands with molecular weights less than 500 Da than by those with molecular weights greater than 500 Da. The polarity of the ligand in fat, oil, and non-polar solvents is correlated with the logP value. Ligands that are widely dispersed throughout the body and have a log P value greater than 5 will interact more readily via the lipid bilayer layer of cell membranes. As a result, the ligand becomes more hazardous and its sensitivity to binding to the target molecule decreases. Because they are more broadly distributed throughout the body and are kept in

233 lipid membranes for longer, excessively hydrophobic compounds typically have a high level of toxicity.
234 The ligand is hydrophobic and has a tendency to dissolve in water when the log P value is less. Since
235 the ligand cannot cross the lipid bilayer membrane, its Log P value cannot be negative. The biological
236 activity of a ligand or medicine is correlated with the amount of hydrogen bonds in the donor and
237 acceptor. The amount of energy needed for absorption increases with the strength of the hydrogen bond
238 [25]. Table 2 displays the outcomes of the molecular docking studies, which showed that the three
239 compounds satisfied Lipinski's guidelines.

240 **Table 2.** Ligand's Lipinski Rules of Five

Complex	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar surface activity (PSA)
1-Cyclohexyl-2-propen-1-one	138.21	2.3218	0	1	62.125
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	150.221	2.4659	0	1	67.484
Cyclooctenone	124.183	2.0758	0	1	55.760

241

242 When evaluating the pharmacokinetics of novel pharmacological compounds, ADMET estimates are
243 essential. If a compound's anticipated value is more than 0.09, it has significant Caco2 permeability.
244 Human colorectal cancer epithelial cells are known as Caco-2 cells. To estimate oral drug absorption,
245 Caco-2 cell monolayers are frequently employed as an in vitro model of the human intestinal mucosa.
246 The volume needed for a drug to be uniformly distributed and produce the same concentration as in
247 blood plasma is known as the Steady State Volume of Distribution (VDss). Excretion in log (ml/min/kg)
248 is predicted by total clearance (CLtot). Hepatic clearance (liver metabolism and biliary clearance) and
249 renal clearance (renal excretion) are the two primary parts of drug clearance. AMES toxicity is
250 frequently used to evaluate a compound's capacity to cause mutagenesis using bacteria. Positive
251 findings suggest that the substance is mutagenic and carcinogenic [22]. If a compound's predictive value
252 is greater than 0.90, it is deemed to have high CaCO-2 permeability [26]. It has good permeability
253 because the test results showed a value greater than 0.90. When the volume of distribution (VDss) is
254 less than 0.71 L/kg (log VDss<-0.15), it is deemed low; when it is greater than 2.81 L/kg (log VDss>0.45),
255 it is deemed excessive [27]. In table 3, all compounds are in the range volume distribution requirements
256 so that it can be predicted that all these compounds can be distributed evenly to provide the same
257 concentration as in blood plasma. Based on table 3, all ligands have good pharmacokinetic parameter.

258 **Table 3.** Pharmacokinetics (ADMET) Parameters of Ligands

Complex	Absorption Caco2 permeability	Distribution VDss (human)	Metabolism (CYP2D6 substrate)	Excretion (Total Clearance)	AMES toxicity	Hepato toxicity
1-Cyclohexyl-2-propen-1-one	1.085	0.148	No	0.221	No	No
4,4a,5,6,7,8-Hexahydrona	1.501	0.344	No	0.112	No	No

phthalen-2(3H)-one						
Cyclooctenone	1.487	0.136	No	0.213	No	No

259

260 **4. CONCLUSION**

261 The three best components of *C. aeruginosa* fraction with the smallest binding affinity and meet the
262 pharmacokinetic requirements are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-
263 Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still
264 better as K+ (-10.593,68kal/mol). All three compounds do not bind to key amino acid residues of BRAF
265 V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. All three compounds have good
266 pharmacokinetic parameter. These results indicate that further structural development is needed for
267 better activity.

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In Silico Study of Compounds Identified in Curcuma aeruginosa Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer

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Original Article

In Silico Study of Compounds Identified in *Curcuma aeruginosa* Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer

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Abstract: *Curcuma aeruginosa* Roxb rhizome contains secondary metabolite compounds and plays a role in various activities such as antioxidant, antibacterial, anthelmintic, antiandrogenic, antinociceptive, and anticancer. Anticancer activity that has been reported in *Curcuma aeruginosa Roxb* rhizome is limited to breast and cervical cancer. The purpose of this study was to explore the potential of *Curcuma aeruginosa* Roxb rhizome in melanoma cancer through the mechanism of inhibiting the BRAF V600E. The 96% ethanol extract of *Curcuma aeruginosa* Roxb rhizome was separated to produce n-hexane (HF), ethyl acetate (EAF), and ethanol (EF) fractions. The GC-MS results showed that there were 31 compounds from the three fractions. The docking validation process was carried out on the native ligand N-(3-{[5-(4-chlorophenyl) -1H-pyrrolo [2,3b]pyridin3yl] carbonyl}2,4difluorophenyl) propane-1-sulfonamide. All compounds were prepared as ligands for molecular docking with the BRAF V600E receptor (PDB ID: 3OG7). Docking validation on native ligand showed RMSD 1.03Å. The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68 kcal/mol); Cyclooctenone (-6,23 kcal/mol); and vemuravnib is still better as K+ (-11.11 kcal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. These results indicate that further structural development is needed for better activity.

Keywords: *Curcuma aeruginosa*; GC-MS ; In Silico, BRAF V600E inhibitor, Vemuravenib

1. INTRODUCTION

Over the past 50 years, melanoma incidence has steadily climbed globally. Melanoma is more prevalent in lower latitudes and among white-skinned individuals. Melanoma is the most common cancer in teenagers and young adults, but it is often more prevalent in the elderly population. In 2020, melanoma of the skin is expected to account for 1.7% of all cancer diagnoses worldwide, with an estimated 325,000 new cases [1]. Vemurafenib and dabravenib are BRAF mutation-inhibiting chemotherapy drugs to treat melanoma [2], [3]. Approximately 50% of cutaneous melanoma patients have active BRAF V600 mutations, so selective inhibitors were developed. Vemurafenib is responsive in 50% of patients with BRAF V600 mutations and is longer progression-free than dacarbazine (DTIC). In previous research, the reticuline compound in soursop leaves was proven to have the potential to treat cancer through the BRAF V600E inhibitor mechanism in silico [4]. In addition, in silico evaluation of several 4-(quinolin-2-yl)pyrimidin-2-amine derivatives as potent V600E-BRAF inhibitors was carried out [5]. There are several active compounds as BRAF V 300E inhibitors, which provide an opportunity for other natural ingredients to have the same activity.

Traditionally, the *C. aeruginose* rhizome has been used medicinally to treat stomach ache, obesity and rheumatism, asthma and cough, scurvy and mental disorders [6]. Essential oil content has been identified from the results of the distillation of *C. aeruginosa* Roxb. rhizomes such as curzerenone (24.6%), 1,scineole (ll.O%), camphor (10.6%), zedoarol (6.3%), isocurcumenol (5.8%), curcumenol (5.6%) and filranogermenone (5.5%) [7]. Other identified compounds include champor (29,39%) dan germacrone (21,21%) [8], monoterpen (21,47%) berupa β-pinen dan 1,8 cineol [9], 1,8-

cineol (22.65%) dan germacrone (17.70%) [10], tropolene (18,1%) dan eucalyptol (17,9%) [11], β-pinene (21.9%), neocurdione (16.1%) and curcumol (15.2%) [12]. Meanwhile, the compounds that were successfully separated from the black turmeric extract using chromatography include germacrone, zederone, dehydrocurdione, curcumenol, zedoarondiol dan isocurcumenol [13]; dehydrocurdione, curcumenol, dan germacrone [14]; Pyrocurzerenone, Dehydrochromolaenin, Curzeone, Linderazulene, Curzerenone, 8, 12 - Epoxy - 1 (10), 4(15), 7, 11 -germacratetraen-6-one [15]; aeruginon and curcumenone [16]; dan flavon [17]. *C aeruginosa* isolates that have quite potential in various activities are germacrone as antiandrogenic [13], hair growth promoter [14], antinociceptive [18], and anticancer [16]. Anticancer activity that has been reported in *C. aeruginosa* Roxb rhizome is limited to breast cancer (MCF-7 and T-47D) and cervical cancer (Ca Ski and HeLa S3) [19], [20]. There have been no reports of *C. aeruni*osa being tested for BRAF V600E inhibitory activity as an anti-melanoma cancer in silico so that it is worthy of being processed.

2. MATERIALS AND METHODS

2.1. Chemical

Ethanol, methanol, n-hexane, and ethyl acetate as solvents from Smart Lab, Indonesia. All reagents used for the research were of analytical grade.

2.2. Plant Collection

C. aeruginosa Roxb dried rhizome from the Center for Research and Development of Traditional Medicinal Plants and Medicines Tawangmangu, Central Java, harvested in February 2000.

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2.3. Instrumentation

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm.

2.4. Software and Hardware

The Protein Databank (PDB, www.rcsb.org) provided PDB ID: 3OG7 for download [4], [21]. The natural chemical's 3D structure files were obtained from PubChem (www.pubchem.ncbi.nlm.nih.gov). Ligands made with chemdraw 3D 15.0 for molecular docking. AutoDock Tool 1.5.6 Sep_17_14 employed the molecular docking procedure for in-silico screening, and Biovia Discovery Studio V21.1.0.2.20298 was used to view the results. Using a Lenovo laptop running Windows 10 with a Core i3 CPU, 4 GB of RAM, 64-bit operating system, and an x-64 processor, pharmacokinetics and toxicity prediction are performed. The online SMILES Translator (https://cactus.nci.nih.gov) was used to translate the compound into SMILES format. To forecast pharmacokinetics and chemical toxicity, the SMILES-formatted molecule was processed using the pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) [22].

2.5. Extraction and Fractination

One kg of the powdered material was macerated for three days at a ratio of 1:5 using 70% ethanol. Ethanol extract (EE) was obtained by combining the filtrates and drying them out using a revolving vacuum evaporator set at 60 °C and 100 rpm. Then, using solvents ranging from non-polar (n-hexane), semi-polar (ethyl acetate), and polar (ethanol), the ethanol extract (EE) was separated by sequential fractination to provide n-hexane (FH), ethyl acetate (EAF), and ethanol (EF). By turning the vacuum evaporator at 60 °C and 100 rpm, respectively, fractions were concentrated [23], [24].

2.6. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene

glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm. The mobile phase used is helium and was adjusted to a column velocity flow of 0.74 mL/min. Other GC-MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 42,3 kPa; and 1 µl injector in split mode with a split ratio of 153.0 with injection temperature of 300 °C. The temperature was raised to 320 °C at the rate of 10 °C/min and held for 5 min. The total elution was 24 min.

2.7. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Protein and ligand preparation is the initial stage of molecular docking. AutoDock Tools-1.5.6 was used to carry out 3D interaction, docking, and binding investigations. The Protein Data Bank provided the target proteins for download (PDB ID: 3OG7) [4], [21]. The Biovia Discovery Studio visualizer program is used to extract native ligands and water molecules from 3D structures to create protein files (.pdb). Chemdraw 3D 15.0 was used to produce the test ligand file (.sdf), which was retrieved from PubChem, for molecular docking. The ligand contributes charge and torsion after the receptor adds charge prior to molecular docking. The grid box's dimensions and coordinates were established. X: 2.643, Y: -2.28, Z: -19.403, and spacing are the grid box coordinates, and the grid box size is 44 × 40 × 40Å, spacing 0.375. Molecular docking parameters include interacting amino acid residues and binding affinity (kcal/mol). Interaction 2D and 3D between ligand and protein were visualized using Biovia Discover Studio visualizer. The following chemical properties were predicted and explained: polar surface activity (PSA), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), the number of atom-to-atom bonds that can rotate (Torson), the logarithm of the coefficient octanol/water partition (Log P), and molecular weight (MW). These were conducted utilizing Lipinski's rule of five, a set of guidelines that aids in distinguishing between molecules that resemble drugs and those that do not, using the pkCMS web tool application [5], [21], [25]. This approach might forecast the higher probability of success or failure because of drug penetration and absorption. Following the 3D drawing of the chemical structure using Chemdraw 3D 15.0 and its saving in a particular format (.pdb), the online SMILES Translator (https://cactus.nci.nih.gov) was used to convert it to SMILES format. The pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) was used to process the SMILES formatted compound in order to forecast chemical toxicity and pharmacokinetics [22].

3. RESULTS AND DISCUSSION

3.1. Fraction Compounds

According to the GC-MS data, HF was primarily composed of sesquiterpenes (63.1%) and diterpenes (5.26%), with 31.58% of it being unknown. substances. The EF was made up of sesquiterpenes (68.42%) and others (31.58%), whereas the EAF was made up of sesquiterpenes (42.86%), diterpenes (21.43%), steroids (7.14%), and others (28.57%). Saturated fatty acids were present when IF was identified. Curcumenol and epicurzerenone were the primary constituents of HF and EF, whereas curcumenol and 2,4-Dispironorbornylcyclobuta-1,3-dione (ketene dimers) were the primary constituents of EAF. All compounds detected from the *C. aeruginosa* fraction are shown in Figure 1.

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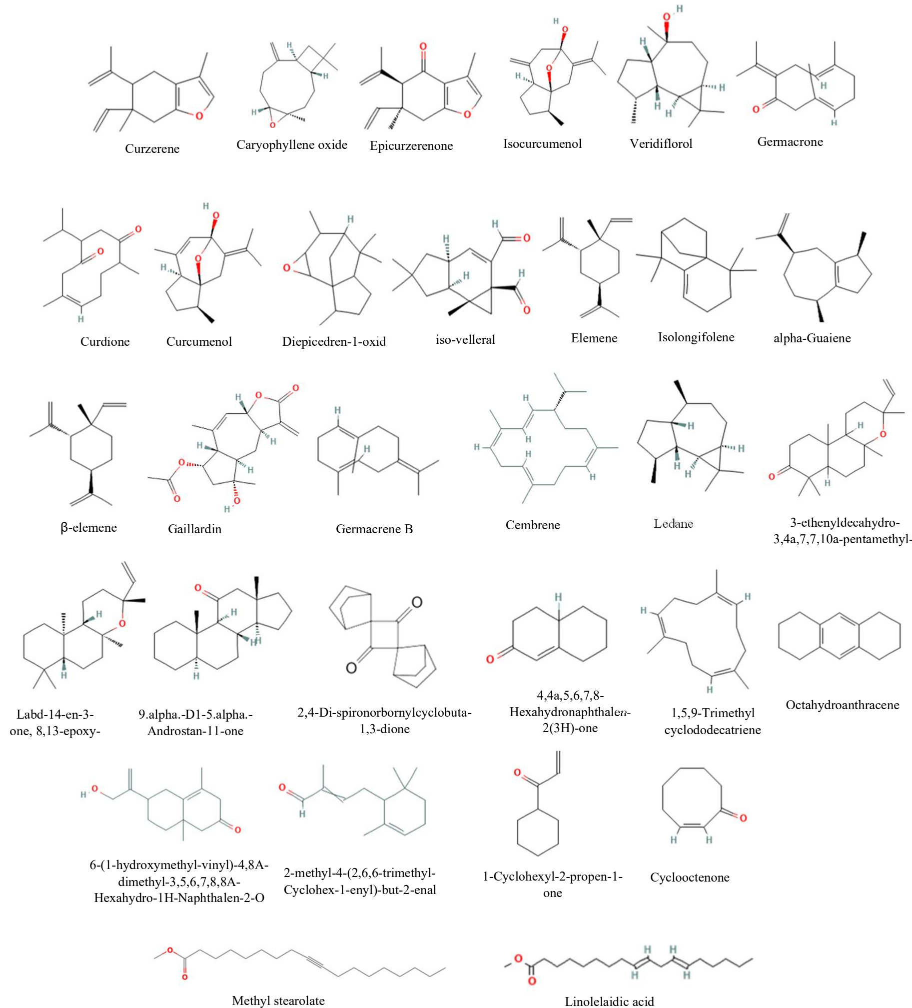

Figure 1. Compound Name and Structure Identification of *C. aeruginosa* Fractions Using GC-MS

3.1. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Redocking, or confirming the docking technique between the receptor and the native ligand, is the first step in molecular docking. RMSD 1.03A is the outcome of the validation procedure. Since the RMSD value is ≤ 2A the docking procedure can be deemed acceptable, and the RMSD obtained satisfies the validation acceptance criterion [22]. To show the stance before and after docking, native ligands are shown in two distinct colors. Native ligan in the redocking process with the BRAF V600E resistor is shown in Figure 2. Vemuravenib, a commercial medication, was utilized as a control ligand against the BRAF V600E receptor (PDB ID: 3OG7), while all substances discovered by GC-MS (Figure 1) were used as test ligands. ligand preparation is the initial stage of molecular docking.

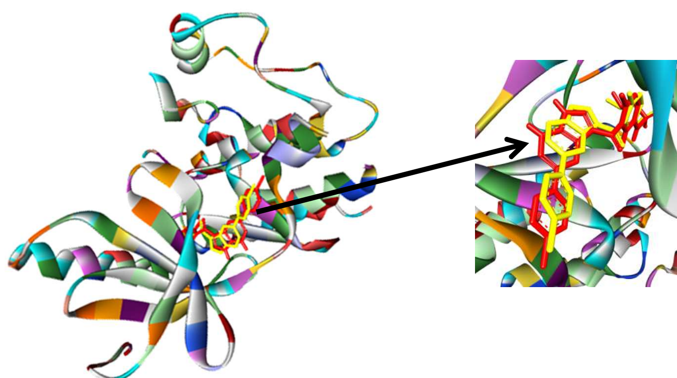

Figure 2. 3D diagram for the interaction of native ligand with BRAF-V600E receptor (yellow =before and red = after redocking)

The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still better as K+ (-10.593,68kal/mol). The smaller binding affinity value, the affinity between the receptor and ligand was higher and the vice versa, the greater the binding affinity value, the affinity between receptors and the ligands is getting lower [5], [22]. All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594 [22]. The amino acid residues that were shown to interact with ligands displays in Table 1 and illustrates the interaction between ligands and the BRAF-V600E receptor using 2D visualization displays in Figure 2.

Table 1. Molecular interactions present in the selected complex ligands and BRAF V600E receptor and the amino acids involved.

Complex	Binding Energy (kcal/mol)	Inhibition Constant/ Ki (μM)	Amino acid residues
1-Cyclohexyl-2-propen-1-one	-6,68	12,63	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one	-6,89	8,96	Arg 575, Asp 576, Leu 577, Lys 578, Ser 616, Trp 619, Met 620, Ala 621, Val 626, Tyr 633, Ser 637, Asp 638, Ala 641
Cyclooctenone	-6,23	27,21	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
Vemuravenib	-11.11	7,42	Ile-463, Val-471, Ala- 481, Lys-483, Leu-505, Leu-514, Phe-516, Ile-527, Thr-529, **Gln-530**, Trp-531, **Cys-532**, Ser-535, Ser-536, His-539, Phe-583, **Asp-594**, Asp-593, Phe-595, Gly-596

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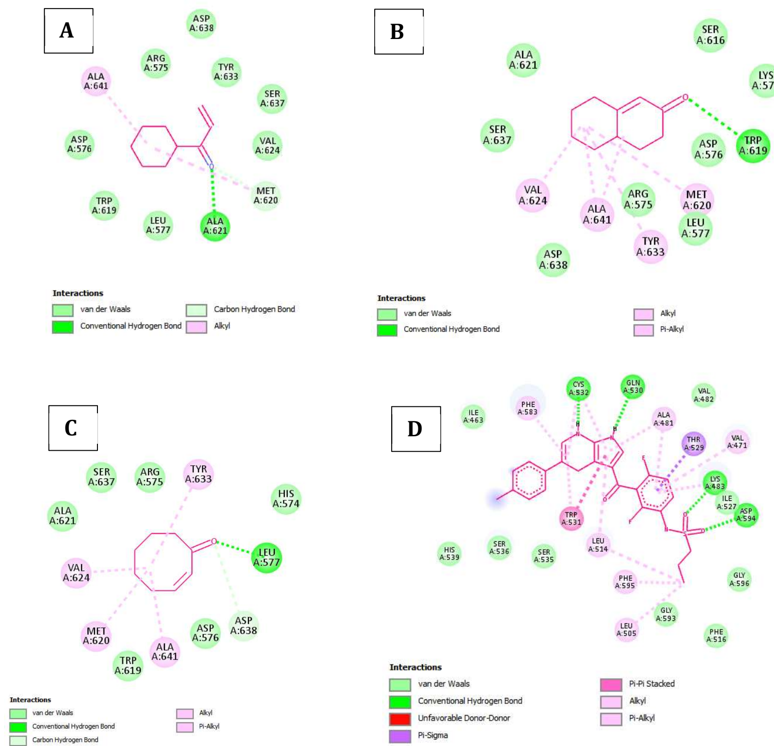

Figure 3. 2D Visualization of Complex Interactions Between Ligands 1-Cyclohexyl-2-propen-1-one **(A)**, 4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one **(B)**, Cyclooctenone (**C**), and Vemuravenib **(D)** with BRAF V600E receptor

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The likelihood that a molecule has the same or superior activity than BRAF V600E increases with the number of amino acid similarities between the reference chemical and crucial amino acid. GLN A:530, CYS A:532, ASP A:594, and THR A:529 are important amino acids linked to the BRAF V600E receptor. The reticuline compound has the same hydrogen-bonded amino acids (GLN 530 and ASP 549) as the reference compound Vemurafenib/key amino acids [22]. Conversely, compounds that had better binding scores than vemurafe-nib and a decent MolDock score (≥− 158.139) and Rerank score (≥−118.607) were recognized as possible hits [5].

Out of all the compounds, the three identified compounds found by GC-MS were found to have the smallest binding affinities. The pkCMS online tool was used to further investigate these compound's pharmacokinetic and toxicity characteristics (ADMET). The Lipinski test uses passive diffusion to ascertain whether a substance in cell membranes is hydrophobic or hydrophilic. According to Lipinski's guidelines, a ligand must have a molecular weight of less than 500 Da and a LogP value of less than 5. molar refractivity between 40 and 130, donor hydrogen bonds < 5, and acceptor hydrogen bonds < 10. Cell membranes are more readily penetrated by ligands with molecular weights less than 500 Da than by those with molecular weights greater than 500 Da. The polarity of the ligand in fat, oil, and non-polar solvents is correlated with the logP value. Ligands that are widely dispersed throughout the body and have a log P value greater than 5 will interact more readily via the lipid bilayer layer of cell membranes. As a result, the ligand becomes more hazardous and its sensitivity to binding to the target molecule decreases. Because they are more broadly distributed throughout the body and are kept in

lipid membranes for longer, excessively hydrophobic compounds typically have a high level of toxicity. The ligand is hydrophobic and has a tendency to dissolve in water when the log P value is less. Since the ligand cannot cross the lipid bilayer membrane, its Log P value cannot be negative. The biological activity of a ligand or medicine is correlated with the amount of hydrogen bonds in the donor and acceptor. The amount of energy needed for absorption increases with the strength of the hydrogen bond [25]. Table 2 displays the outcomes of the molecular docking studies, which showed that the three compounds satisfied Lipinski's guidelines.

Table 2. Ligand's Lipinski Rules of Five

Complex	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar surface activity (PSA)
1-Cyclohexyl-2-propen-1-one	138.21	2.3218	0	1	62.125
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	150.221	2.4659	0	1	67.484
Cyclooctenone	124.183	2.0758	0	1	55.760

When evaluating the pharmacokinetics of novel pharmacological compounds, ADMET estimates are essential. If a compound's anticipated value is more than 0.09, it has significant Caco2 permeability. Human colorectal cancer epithelial cells are known as Caco-2 cells. To estimate oral drug absorption, Caco-2 cell monolayers are frequently employed as an in vitro model of the human intestinal mucosa. The volume needed for a drug to be uniformly distributed and produce the same concentration as in blood plasma is known as the Steady State Volume of Distribution (VDss). Excretion in log (ml/min/kg) is predicted by total clearance (CLtot). Hepatic clearance (liver metabolism and biliary clearance) and renal clearance (renal excretion) are the two primary parts of drug clearance. AMES toxicity is frequently used to evaluate a compound's capacity to cause mutagenesis using bacteria. Positive findings suggest that the substance is mutagenic and carcinogenic [22]. If a compound's predictive value is greater than 0.90, it is deemed to have high CaCO-2 permeability [26]. It has good permeability because the test results showed a value greater than 0.90. When the volume of distribution (VDss) is less than 0.71 L/kg (log VDss<-0.15), it is deemed low; when it is greater than 2.81 L/kg (log VDss>0.45), it is deemed excessive [27]. In Table 3, all compounds are in the range volume distribution requirements so that it can be predicted that all these compounds can be distributed evenly to provide the same concentration as in blood plasma. Based on Table 3, all ligands have good pharmacokinetic parameter.

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Table 3. Pharmacokinetics (ADMET) Parameters of Ligands

Complex	Absorption Caco2 permeability	Distribution VDss (human)	Metabolism (CYP2D6 substrate)	Excretion (Total Clearance)	AMES toxicity	Hepato toxicity
1-Cyclohexyl-2-propen-1-one	1.085	0.148	No	0.221	No	No
4,4a,5,6,7,8-Hexahydrona	1.501	0.344	No	0.112	No	No

phthalen-2(3H)-one						
Cyclooctenone	1.487	0.136	No	0.213	No	No

4. CONCLUSION

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The three best components of *C. aeruginosa* fraction with the smallest binding affinity and meet the pharmacokinetic requirements are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still better as K+ (-10.593,68kal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. All three compounds have good pharmacokinetic parameter. These results indicate that further structural development is needed for better activity.

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Conflicts of interest: The authors declare no conflict of interest

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Original Article

In Silico Study of Compounds Identified in *Curcuma aeruginosa* Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer

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Abstract: *Curcuma aeruginosa* Roxb rhizome contains secondary metabolite compounds and plays a role in various activities such as antioxidant, antibacterial, anthelmintic, antiandrogenic, antinociceptive, and anticancer. Anticancer activity that has been reported in *Curcuma aeruginosa Roxb* rhizome is limited to breast and cervical cancer. The purpose of this study was to explore the potential of *Curcuma aeruginosa* Roxb rhizome in melanoma cancer through the mechanism of inhibiting the BRAF V600E. The 96% ethanol extract of *Curcuma aeruginosa* Roxb rhizome was separated to produce n-hexane (HF), ethyl acetate (EAF), and ethanol (EF) fractions. The GC-MS results showed that there were 31 compounds from the three fractions. The docking validation process was carried out on the native ligand N-(3-{[5-(4-chlorophenyl) -1H-pyrrolo [2,3b]pyridin3yl] carbonyl}2,4difluorophenyl) propane-1-sulfonamide. All compounds were prepared as ligands for molecular docking with the BRAF V600E receptor (PDB ID: 3OG7). Docking validation on native ligand showed RMSD 1.03Å. The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68 kcal/mol); Cyclooctenone (-6,23 kcal/mol); and vemuravnib is still better as K+ (-11.11 kcal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. These results indicate that further structural development is needed for better activity.

Keywords: *Curcuma aeruginosa*; GC-MS ; In Silico, BRAF V600E inhibitor, Vemuravenib

1. INTRODUCTION

Over the past 50 years, melanoma incidence has steadily climbed globally. Melanoma is more prevalent in lower latitudes and among white-skinned individuals. Melanoma is the most common cancer in teenagers and young adults, but it is often more prevalent in the elderly population. In 2020, melanoma of the skin is expected to account for 1.7% of all cancer diagnoses worldwide, with an estimated 325,000 new cases [1]. Vemurafenib and dabravenib are BRAF mutation-inhibiting chemotherapy drugs to treat melanoma [2], [3]. Approximately 50% of cutaneous melanoma patients have active BRAF V600 mutations, so selective inhibitors were developed. Vemurafenib is responsive in 50% of patients with BRAF V600 mutations and is longer progression-free than dacarbazine (DTIC). In previous research, the reticuline compound in soursop leaves was proven to have the potential to treat cancer through the BRAF V600E inhibitor mechanism in silico [4]. In addition, in silico evaluation of several 4-(quinolin-2-yl)pyrimidin-2-amine derivatives as potent V600E-BRAF inhibitors was carried out [5]. There are several active compounds as BRAF V 300E inhibitors, which provide an opportunity for other natural ingredients to have the same activity.

Traditionally, the *C. aeruginose* rhizome has been used medicinally to treat stomach ache, obesity and rheumatism, asthma and cough, scurvy and mental disorders [6]. Essential oil content has been identified from the results of the distillation of *C. aeruginosa* Roxb. rhizomes such as curzerenone (24.6%), 1,scineole (ll.O%), camphor (10.6%), zedoarol (6.3%), isocurcumenol (5.8%), curcumenol (5.6%) and filranogermenone (5.5%) [7]. Other identified compounds include champor (29,39%) dan germacrone (21,21%) [8], monoterpen (21,47%) berupa β-pinen dan 1,8 cineol [9], 1,8-

cineol (22.65%) dan germacrone (17.70%) [10], tropolene (18,1%) dan eucalyptol (17,9%) [11], β-pinene (21.9%), neocurdione (16.1%) and curcumol (15.2%) [12]. Meanwhile, the compounds that were successfully separated from the black turmeric extract using chromatography include germacrone, zederone, dehydrocurdione, curcumenol, zedoarondiol dan isocurcumenol [13]; dehydrocurdione, curcumenol, dan germacrone [14]; Pyrocurzerenone, Dehydrochromolaenin, Curzeone, Linderazulene, Curzerenone, 8, 12 - Epoxy - 1 (10), 4(15), 7, 11 -germacratetraen-6-one [15]; aeruginon and curcumenone [16]; dan flavon [17]. *C aeruginosa* isolates that have quite potential in various activities are germacrone as antiandrogenic [13], hair growth promoter [14], antinociceptive [18], and anticancer [16]. Anticancer activity that has been reported in *C. aeruginosa* Roxb rhizome is limited to breast cancer (MCF-7 and T-47D) and cervical cancer (Ca Ski and HeLa S3) [19], [20]. There have been no reports of *C. aeruni*osa being tested for BRAF V600E inhibitory activity as an anti-melanoma cancer in silico so that it is worthy of being processed.

2. MATERIALS AND METHODS

2.1. Chemical

Ethanol, methanol, n-hexane, and ethyl acetate as solvents from Smart Lab, Indonesia. All reagents used for the research were of analytical grade.

2.2. Plant Collection

C. aeruginosa Roxb dried rhizome from the Center for Research and Development of Traditional Medicinal Plants and Medicines Tawangmangu, Central Java, harvested in February 2020.

2.3. Instrumentation

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm.

2.4. Software and Hardware

The Protein Databank (PDB, www.rcsb.org) provided PDB ID: 3OG7 for download [4], [21]. The natural chemical's 3D structure files were obtained from PubChem (www.pubchem.ncbi.nlm.nih.gov). Ligands made with chemdraw 3D 15.0 for molecular docking. AutoDock Tool 1.5.6 Sep_17_14 employed the molecular docking procedure for in-silico screening, and Biovia Discovery Studio V21.1.0.2.20298 was used to view the results. Using a Lenovo laptop running Windows 10 with a Core i3 CPU, 4 GB of RAM, 64-bit operating system, and an x-64 processor, pharmacokinetics and toxicity prediction are performed. The online SMILES Translator (https://cactus.nci.nih.gov) was used to translate the compound into SMILES format. To forecast pharmacokinetics and chemical toxicity, the SMILES-formatted molecule was processed using the pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) [22].

2.5. Extraction and Fractination

One kg of the powdered material was macerated for three days at a ratio of 1:5 using 70% ethanol. Ethanol extract (EE) was obtained by combining the filtrates and drying them out using a revolving vacuum evaporator set at 60 °C and 100 rpm. Then, using solvents ranging from non-polar (n-hexane), semi-polar (ethyl acetate), and polar (ethanol), the ethanol extract (EE) was separated by sequential fractination to provide n-hexane (FH), ethyl acetate (EAF), and ethanol (EF). By turning the vacuum evaporator at 60 °C and 100 rpm, respectively, fractions were concentrated [23], [24].

2.6. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene

glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm. The mobile phase used is helium and was adjusted to a column velocity flow of 0.74 mL/min. Other GC-MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 42,3 kPa; and 1 µl injector in split mode with a split ratio of 153.0 with injection temperature of 300 °C. The temperature was raised to 320 °C at the rate of 10 °C/min and held for 5 min. The total elution was 24 min.

2.7. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Protein and ligand preparation is the initial stage of molecular docking. AutoDock Tools-1.5.6 was used to carry out 3D interaction, docking, and binding investigations. The Protein Data Bank provided the target proteins for download (PDB ID: 3OG7) [4], [21]. The Biovia Discovery Studio visualizer program is used to extract native ligands and water molecules from 3D structures to create protein files (.pdb). Chemdraw 3D 15.0 was used to produce the test ligand file (.sdf), which was retrieved from PubChem, for molecular docking. The ligand contributes charge and torsion after the receptor adds charge prior to molecular docking. The grid box's dimensions and coordinates were established. X: 2.643, Y: -2.28, Z: -19.403, and spacing are the grid box coordinates, and the grid box size is 44 × 40 × 40Å, spacing 0.375. Molecular docking parameters include interacting amino acid residues and binding affinity (kcal/mol). Interaction 2D and 3D between ligand and protein were visualized using Biovia Discover Studio visualizer. The following chemical properties were predicted and explained: polar surface activity (PSA), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), the number of atom-to-atom bonds that can rotate (Torson), the logarithm of the coefficient octanol/water partition (Log P), and molecular weight (MW). These were conducted utilizing Lipinski's rule of five, a set of guidelines that aids in distinguishing between molecules that resemble drugs and those that do not, using the pkCMS web tool application [5], [21], [25]. This approach might forecast the higher probability of success or failure because of drug penetration and absorption. Following the 3D drawing of the chemical structure using Chemdraw 3D 15.0 and its saving in a particular format (.pdb), the online SMILES Translator (https://cactus.nci.nih.gov) was used to convert it to SMILES format. The pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) was used to process the SMILES formatted compound in order to forecast chemical toxicity and pharmacokinetics [22].

3. RESULTS AND DISCUSSION

3.1. Fraction Compounds

According to the GC-MS data, HF was primarily composed of sesquiterpenes (63.1%) and diterpenes (5.26%), with 31.58% of it being unknown. substances. The EF was made up of sesquiterpenes (68.42%) and others (31.58%), whereas the EAF was made up of sesquiterpenes (42.86%), diterpenes (21.43%), steroids (7.14%), and others (28.57%). Saturated fatty acids were present when IF was identified. Curcumenol and epicurzerenone were the primary constituents of HF and EF, whereas curcumenol and 2,4-Dispironorbornylcyclobuta-1,3-dione (ketene dimers) were the primary constituents of EAF. All compounds detected from the *C. aeruginosa* fraction are shown in Figure 1.

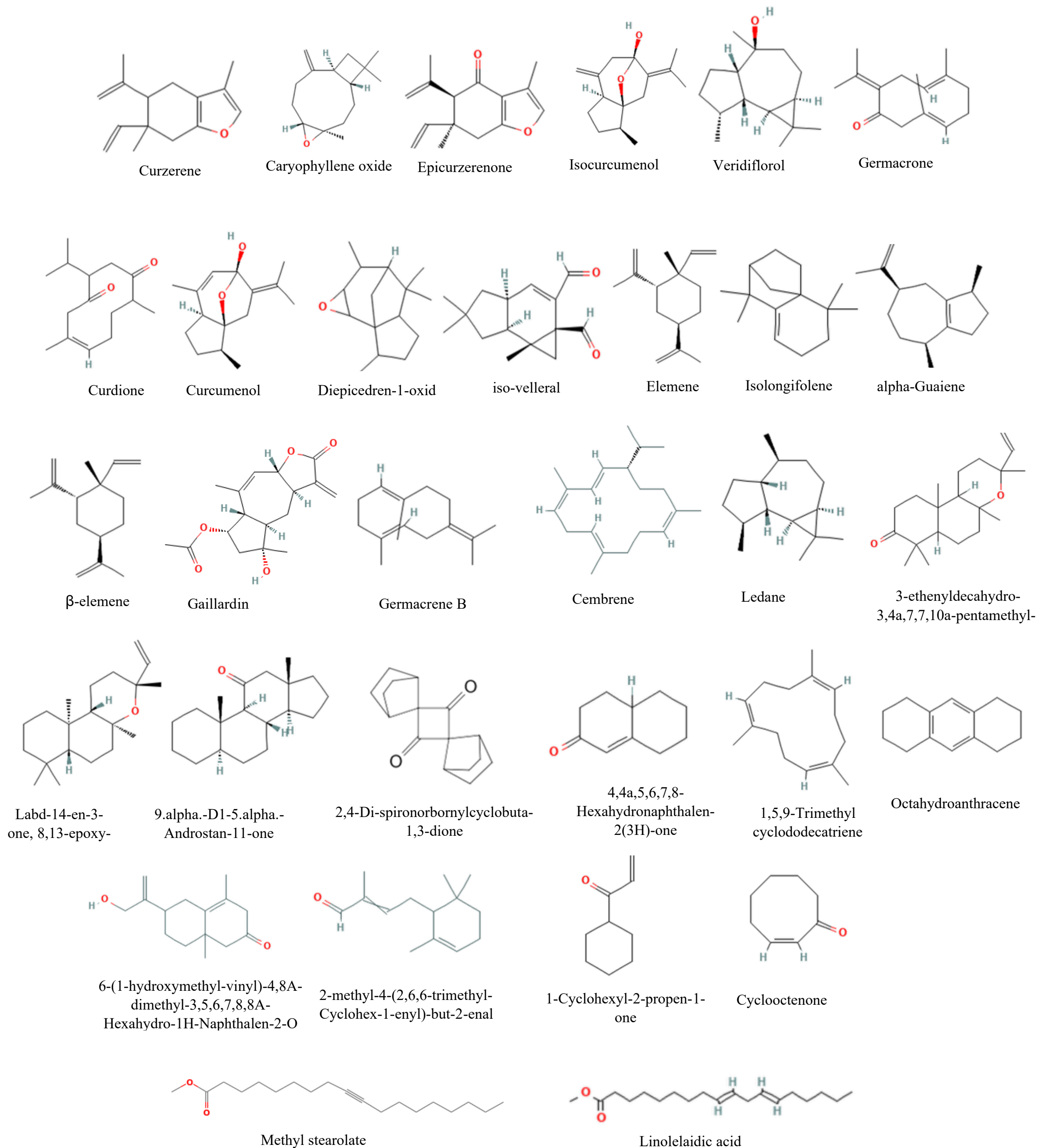

Figure 1. Compound Name and Structure Identification of *C. aeruginosa* Fractions Using GC-MS

3.1. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Redocking, or confirming the docking technique between the receptor and the native ligand, is the first step in molecular docking. RMSD 1.03A is the outcome of the validation procedure. Since the RMSD value is ≤ 2A the docking procedure can be deemed acceptable, and the RMSD obtained satisfies the validation acceptance criterion [22]. To show the stance before and after docking, native ligands are shown in two distinct colors. Native ligan in the redocking process with the BRAF V600E resistor is shown in Figure 2. Vemuravenib, a commercial medication, was utilized as a control ligand against the BRAF V600E receptor (PDB ID: 3OG7), while all substances discovered by GC-MS (Figure 1) were used as test ligands. ligand preparation is the initial stage of molecular docking.

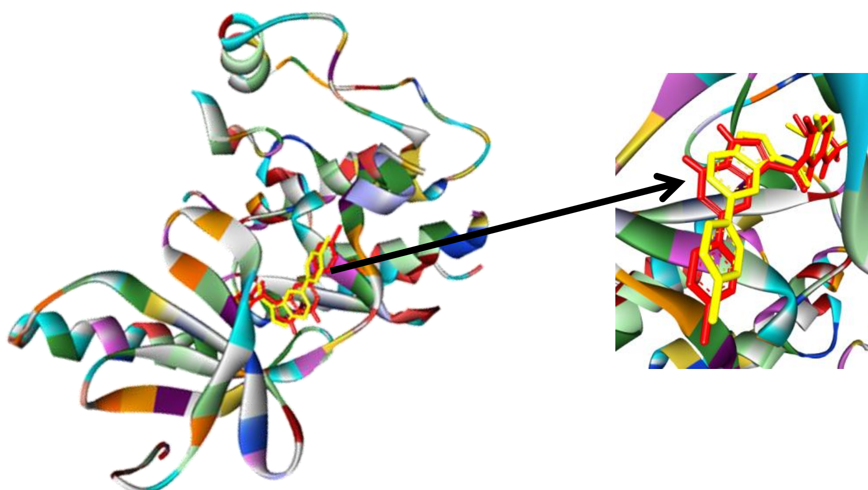

Figure 2. 3D diagram for the interaction of native ligand with BRAF-V600E receptor (yellow =before and red = after redocking)

The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still better as K+ (-10.593,68kal/mol). The smaller binding affinity value, the affinity between the receptor and ligand was higher and the vice versa, the greater the binding affinity value, the affinity between receptors and the ligands is getting lower [5], [22]. All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594 [22]. The amino acid residues that were shown to interact with ligands displays in Table 1 and illustrates the interaction between ligands and the BRAF-V600E receptor using 2D visualization displays in Figure 2.

Table 1. Molecular interactions present in the selected complex ligands and BRAF V600E receptor and the amino acids involved.

Complex	Binding Energy (kcal/mol)	Inhibition Constant/ Ki (µM)	Amino acid residues
1-Cyclohexyl-2-propen-1-one	-6,68	12,63	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one	-6,89	8,96	Arg 575, Asp 576, Leu 577, Lys 578, Ser 616, Trp 619, Met 620, Ala 621, Val 626, Tyr 633, Ser 637, Asp 638, Ala 641
Cyclooctenone	-6,23	27,21	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
Vemuravenib	-11.11	7,42	Ile-463, Val-471, Ala- 481, Lys-483, Leu-505, Leu-514, Phe-516, Ile-527, Thr-529, **Gln-530**, Trp-531, **Cys-532**, Ser-535, Ser-536, His-539, Phe-583, **Asp-594**, Asp-593, Phe-595, Gly-596

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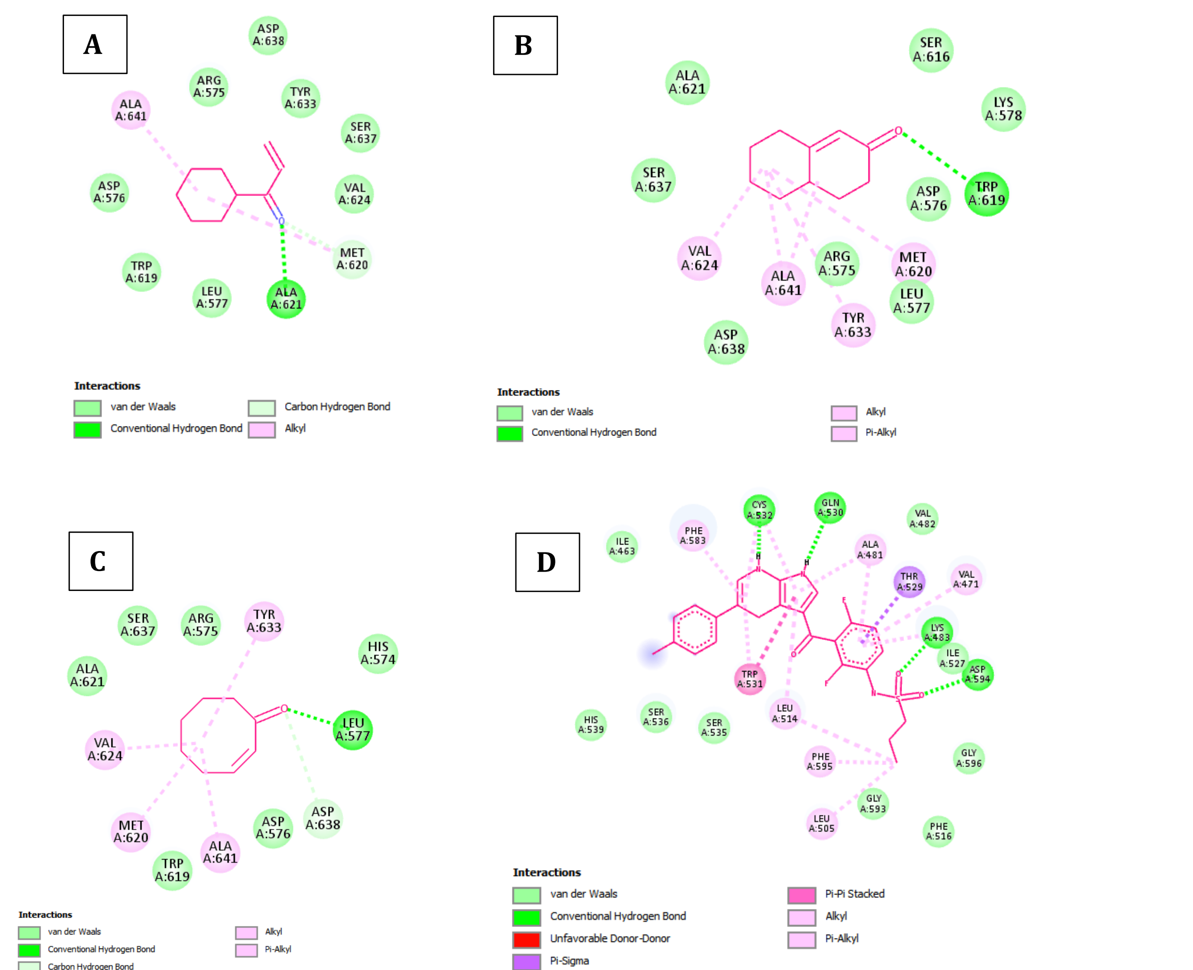

Figure 3. 2D Visualization of Complex Interactions Between Ligands 1-Cyclohexyl-2-propen-1-one **(A)**, 4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one **(B)**, Cyclooctenone **(C)**, and Vemuravenib **(D)** with BRAF V600E receptor

The likelihood that a molecule has the same or superior activity than BRAF V600E increases with the number of amino acid similarities between the reference chemical and crucial amino acid. GLN A:530, CYS A:532, ASP A:594, and THR A:529 are important amino acids linked to the BRAF V600E receptor. The reticuline compound has the same hydrogen-bonded amino acids (GLN 530 and ASP 549) as the reference compound Vemurafenib/key amino acids [22]. Conversely, compounds that had better binding scores than vemurafe-nib and a decent MolDock score (≥− 158.139) and Rerank score (≥− 118.607) were recognized as possible hits [5].

Out of all the compounds, the three identified compounds found by GC-MS were found to have the smallest binding affinities. The pkCMS online tool was used to further investigate these compound's pharmacokinetic and toxicity characteristics (ADMET). The Lipinski test uses passive diffusion to ascertain whether a substance in cell membranes is hydrophobic or hydrophilic. According to Lipinski's guidelines, a ligand must have a molecular weight of less than 500 Da and a LogP value of less than 5. molar refractivity between 40 and 130, donor hydrogen bonds < 5, and acceptor hydrogen bonds < 10. Cell membranes are more readily penetrated by ligands with molecular weights less than 500 Da than by those with molecular weights greater than 500 Da. The polarity of the ligand in fat, oil, and non-polar solvents is correlated with the logP value. Ligands that are widely dispersed throughout the body and have a log P value greater than 5 will interact more readily via the lipid bilayer layer of cell

membranes. As a result, the ligand becomes more hazardous and its sensitivity to binding to the target molecule decreases. Because they are more broadly distributed throughout the body and are kept in lipid membranes for longer, excessively hydrophobic compounds typically have a high level of toxicity. The ligand is hydrophobic and has a tendency to dissolve in water when the log P value is less. Since the ligand cannot cross the lipid bilayer membrane, its Log P value cannot be negative. The biological activity of a ligand or medicine is correlated with the amount of hydrogen bonds in the donor and acceptor. The amount of energy needed for absorption increases with the strength of the hydrogen bond [25]. Table 2 displays the outcomes of the molecular docking studies, which showed that the three compounds satisfied Lipinski's guidelines.

Table 2. Ligand's Lipinski Rules of Five

Complex	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar surface activity (PSA)
1-Cyclohexyl-2-propen-1-one	138.21	2.3218	0	1	62.125
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	150.221	2.4659	0	1	67.484
Cyclooctenone	124.183	2.0758	0	1	55.760

When evaluating the pharmacokinetics of novel pharmacological compounds, ADMET estimates are essential. If a compound's anticipated value is more than 0.09, it has significant Caco-2 permeability. Human colorectal cancer epithelial cells are known as Caco-2 cells. To estimate oral drug absorption, Caco-2 cell monolayers are frequently employed as an in vitro model of the human intestinal mucosa. The volume needed for a drug to be uniformly distributed and produce the same concentration as in blood plasma is known as the Steady State Volume of Distribution (VDss). Excretion in log (ml/min/kg) is predicted by total clearance (CLtot). Hepatic clearance (liver metabolism and biliary clearance) and renal clearance (renal excretion) are the two primary parts of drug clearance. AMES toxicity is frequently used to evaluate a compound's capacity to cause mutagenesis using bacteria. Positive findings suggest that the substance is mutagenic and carcinogenic [22]. If a compound's predictive value is greater than 0.90, it is deemed to have high Caco-2 permeability [26]. It has good permeability because the test results showed a value greater than 0.90. When the volume of distribution (VDss) is less than 0.71 L/kg (log VDss<-0.15), it is deemed low; when it is greater than 2.81 L/kg (log VDss>0.45), it is deemed excessive [27]. In Table 3, all compounds are in the range volume distribution requirements so that it can be predicted that all these compounds can be distributed evenly to provide the same concentration as in blood plasma. Based on Table 3, all ligands have good pharmacokinetic parameter.

Table 3. Pharmacokinetics (ADMET) Parameters of Ligands

Complex	Absorption Caco2 permeability	Distribution VDss (human)	Metabolism (CYP2D6 substrate)	Excretion (Total Clearance)	AMES toxicity	Hepato toxicity
1-Cyclohexyl-2-propen-1-one	1.085	0.148	No	0.221	No	No
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	1.501	0.344	No	0.112	No	No
Cyclooctenone	1.487	0.136	No	0.213	No	No

4. CONCLUSION

The best potential components exploration of *C. aeruginosa* fraction with the smallest binding affinity and meet the pharmacokinetic requirements are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still better as K+ (-10.593,68kal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. All three compounds have good pharmacokinetic parameter. These results indicate that further structural development is needed for better activity.

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Conflicts of interest: The authors declare no conflict of interest

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1 *Original Article*

2 In Silico Study of Compounds Identified in *Curcuma* 3 *aeruginosa* Roxb Rhizome as BRAF V600E Inhibitors in 4 Melanoma Cancer

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8 Received: 16 May 2025; Revised: 22 May 2025; Accepted: 02 June 2025; Published: June 2025

9 **Abstract:** Curcuma aeruginosa Roxb rhizome contains secondary metabolite compounds and plays a role in
10 various activities such as antioxidant, antibacterial, anthelmintic, antiandrogenic, antinociceptive, and
11 anticancer. Anticancer activity that has been reported in Curcuma aeruginosa Roxb rhizome is limited to breast
12 and cervical cancer. The purpose of this study was to explore the potential of Curcuma aeruginosa Roxb
13 rhizome in melanoma cancer through the mechanism of inhibiting the BRAF V600E. The 96% ethanol extract
14 of Curcuma aeruginosa Roxb rhizome was separated to produce n-hexane (HF), ethyl acetate (EAF), and
15 ethanol (EF) fractions. The GC-MS results showed that there were 31 compounds from the three fractions. The
16 docking validation process was carried out on the native ligand N-(3-{[5-(4-chlorophenyl) -1H-pyrrolo
17 [2,3b]pyridin3yl] carbonyl}2,4difluorophenyl) propane-1-sulfonamide. All compounds were prepared as
18 ligands for molecular docking with the BRAF V600E receptor (PDB ID: 3OG7). Docking validation on native
19 ligand showed RMSD 1.03Å. The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-
20 6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68 kcal/mol); Cyclooctenone (-6,23 kcal/mol); and vemuravnib
21 is still better as K+ (-11.11 kcal/mol). All three compounds do not bind to key amino acid residues of BRAF
22 V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. These results indicate that further structural
23 development is needed for better activity.

24 **Keywords:** *Curcuma aeruginosa*; GC-MS ; In Silico, BRAF V600E inhibitor, Vemuravenib
25

26 1. INTRODUCTION

27 Over the past 50 years, melanoma incidence has steadily climbed globally. Melanoma is more
28 prevalent in lower latitudes and among white-skinned individuals. Melanoma is the most common
29 cancer in teenagers and young adults, but it is often more prevalent in the elderly population. In 2020,
30 melanoma of the skin is expected to account for 1.7% of all cancer diagnoses worldwide, with an
31 estimated 325,000 new cases [1]. Vemurafenib and dabravenib are BRAF mutation-inhibiting
32 chemotherapy drugs to treat melanoma [2], [3]. Approximately 50% of cutaneous melanoma patients
33 have active BRAF V600 mutations, so selective inhibitors were developed. Vemurafenib is responsive
34 in 50% of patients with BRAF V600 mutations and is longer progression-free than dacarbazine
35 (DTIC). In previous research, the reticuline compound in soursop leaves was proven to have the
36 potential to treat cancer through the BRAF V600E inhibitor mechanism in silico [4]. In addition, in
37 silico evaluation of several 4-(quinolin-2-yl)pyrimidin-2-amine derivatives as potent V600E-BRAF
38 inhibitors was carried out [5]. There are several active compounds as BRAF V 300E inhibitors, which
39 provide an opportunity for other natural ingredients to have the same activity.

40 Traditionally, the *C. aeruginose* rhizome has been used medicinally to treat stomach ache,
41 obesity and rheumatism, asthma and cough, scurvy and mental disorders [6]. Essential oil content
42 has been identified from the results of the distillation of *C. aeruginosa* Roxb. rhizomes such as
43 curzerenone (24.6%), 1,scineole (ll.O%), camphor (10.6%), zedoarol (6.3%), isocurcumenol (5.8%),
44 curcumenol (5.6%) and filranogermenone (5.5%) [7]. Other identified compounds include champor
45 (29,39%) dan germacrone (21,21%) [8], monoterpen (21,47%) berupa β-pinen dan 1,8 cineol [9], 1,8-

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cineol (22.65%) dan germacrone (17.70%) [10], tropolene (18,1%) dan eucalyptol (17,9%) [11], β-pinene (21.9%), neocurdione (16.1%) and curcumol (15.2%) [12]. Meanwhile, the compounds that were successfully separated from the black turmeric extract using chromatography include germacrone, zederone, dehydrocurdione, curcumenol, zedoarondiol dan isocurcumenol [13]; dehydrocurdione, curcumenol, dan germacrone [14]; Pyrocurzerenone, Dehydrochromolaenin, Curzeone, Linderazulene, Curzerenone, 8, 12 - Epoxy - 1 (10), 4(15), 7, 11 -germacratetraen-6-one [15]; aeruginon and curcumenone [16]; dan flavon [17]. *C aeruginosa* isolates that have quite potential in various activities are germacrone as antiandrogenic [13], hair growth promoter [14], antinociceptive [18], and anticancer [16]. Anticancer activity that has been reported in *C. aeruginosa* Roxb rhizome is limited to breast cancer (MCF-7 and T-47D) and cervical cancer (Ca Ski and HeLa S3) [19], [20]. There have been no reports of *C. aeruni*osa being tested for BRAF V600E inhibitory activity as an anti-melanoma cancer in silico so that it is worthy of being processed.

2. MATERIALS AND METHODS

2.1. Chemical

Ethanol, methanol, n-hexane, and ethyl acetate as solvents from Smart Lab, Indonesia. All reagents used for the research were of analytical grade.

2.2. Plant Collection

C. aeruginosa Roxb dried rhizome from the Center for Research and Development of Traditional Medicinal Plants and Medicines Tawangmangu, Central Java, harvested in February 2020.

2.3. Instrumentation

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm.

2.4. Software and Hardware

The Protein Databank (PDB, www.rcsb.org) provided PDB ID: 3OG7 for download [4], [21]. The natural chemical's 3D structure files were obtained from PubChem (www.pubchem.ncbi.nlm.nih.gov). Ligands made with chemdraw 3D 15.0 for molecular docking. AutoDock Tool 1.5.6 Sep_17_14 employed the molecular docking procedure for in-silico screening, and Biovia Discovery Studio V21.1.0.2.20298 was used to view the results. Using a Lenovo laptop running Windows 10 with a Core i3 CPU, 4 GB of RAM, 64-bit operating system, and an x-64 processor, pharmacokinetics and toxicity prediction are performed. The online SMILES Translator (https://cactus.nci.nih.gov) was used to translate the compound into SMILES format. To forecast pharmacokinetics and chemical toxicity, the SMILES-formatted molecule was processed using the pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) [22].

2.5. Extraction and Fractination

One kg of the powdered material was macerated for three days at a ratio of 1:5 using 70% ethanol. Ethanol extract (EE) was obtained by combining the filtrates and drying them out using a revolving vacuum evaporator set at 60 °C and 100 rpm. Then, using solvents ranging from non-polar (n-hexane), semi-polar (ethyl acetate), and polar (ethanol), the ethanol extract (EE) was separated by sequential fractination to provide n-hexane (FH), ethyl acetate (EAF), and ethanol (EF). By turning the vacuum evaporator at 60 °C and 100 rpm, respectively, fractions were concentrated [23], [24].

2.6. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm. The mobile phase used is helium and was adjusted to a column velocity flow of 0.74 mL/min. Other GC-

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MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 42,3 kPa; and 1 µl injector in split mode with a split ratio of 153.0 with injection temperature of 300 °C. The temperature was raised to 320 °C at the rate of 10 °C/min and held for 5 min. The total elution was 24 min.

2.7. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Protein and ligand preparation is the initial stage of molecular docking. AutoDock Tools-1.5.6 was used to carry out 3D interaction, docking, and binding investigations. The Protein Data Bank provided the target proteins for download (PDB ID: 3OG7) [4], [21]. The Biovia Discovery Studio visualizer program is used to extract native ligands and water molecules from 3D structures to create protein files (.pdb). Chemdraw 3D 15.0 was used to produce the test ligand file (.sdf), which was retrieved from PubChem, for molecular docking. The ligand contributes charge and torsion after the receptor adds charge prior to molecular docking. The grid box's dimensions and coordinates were established. X: 2.643, Y: -2.28, Z: -19.403, and spacing are the grid box coordinates, and the grid box size is 44 × 40 × 40Å, spacing 0.375. Molecular docking parameters include interacting amino acid residues and binding affinity (kcal/mol). Interaction 2D and 3D between ligand and protein were visualized using Biovia Discover Studio visualizer. The following chemical properties were predicted and explained: polar surface activity (PSA), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), the number of atom-to-atom bonds that can rotate (Torson), the logarithm of the coefficient octanol/water partition (Log P), and molecular weight (MW). These were conducted utilizing Lipinski's rule of five, a set of guidelines that aids in distinguishing between molecules that resemble drugs and those that do not, using the pkCMS web tool application [5], [21], [25]. This approach might forecast the higher probability of success or failure because of drug penetration and absorption. Following the 3D drawing of the chemical structure using Chemdraw 3D 15.0 and its saving in a particular format (.pdb), the online SMILES Translator (https://cactus.nci.nih.gov) was used to convert it to SMILES format. The pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) was used to process the SMILES formatted compound in order to forecast chemical toxicity and pharmacokinetics [22].

3. RESULTS AND DISCUSSION

3.1. Fraction Compounds

According to the GC-MS data, HF was primarily composed of sesquiterpenes (63.1%) and diterpenes (5.26%), with 31.58% of it being unknown. substances. The EF was made up of sesquiterpenes (68.42%) and others (31.58%), whereas the EAF was made up of sesquiterpenes (42.86%), diterpenes (21.43%), steroids (7.14%), and others (28.57%). Saturated fatty acids were present when IF was identified. Curcumenol and epicurzerenone were the primary constituents of HF and EF, whereas curcumenol and 2,4-Dispironorbornylcyclobuta-1,3-dione (ketene dimers) were the primary constituents of EAF. All compounds detected from the *C. aeruginosa* fraction are shown in Figure 1.

3.2. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Redocking, or confirming the docking technique between the receptor and the native ligand, is the first step in molecular docking. RMSD 1.03A is the outcome of the validation procedure. Since the RMSD value is ≤ 2A the docking procedure can be deemed acceptable, and the RMSD obtained satisfies the validation acceptance criterion [22]. To show the stance before and after docking, native ligands are shown in two distinct colors. Native ligan in the redocking process with the BRAF V600E resistor is shown in Figure 2. Vemuravenib, a commercial medication, was utilized as a control ligand against the BRAF V600E receptor (PDB ID: 3OG7), while all substances discovered by GC-MS (Figure 1) were used as test ligands. ligand preparation is the initial stage of molecular docking.

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Curzerene
Caryophyllene oxide
Epicurzerenone
Isocurcumenol
Veridiflorol
Germacrone

Curdione
Curcumenol
Diepicedren-1-oxid
iso-velleral
Elemene
Isolongifolene
alpha-Guaiene

β-elemene
Gaillardin
Germacrene B
Cembrene
Ledane
3-ethenyldecahydro-3,4a,7,7,10a-pentamethyl-

Labd-14-en-3-one, 8,13-epoxy-
9.alpha.-D1-5.alpha.-Androstan-11-one
2,4-Di-spironorbornylcyclobuta-1,3-dione
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one
1,5,9-Trimethyl cyclododecatriene
Octahydroanthracene

6-(1-hydroxymethyl-vinyl)-4,8A-dimethyl-3,5,6,7,8,8A-Hexahydro-1H-Naphthalen-2-O
2-methyl-4-(2,6,6-trimethyl-Cyclohex-1-enyl)-but-2-enal
1-Cyclohexyl-2-propen-1-one
Cyclooctenone

Methyl stearolate
Linolelaidic acid

Figure 1. Compound Name and Structure Identification of *C. aeruginosa* Fractions Using GC-MS

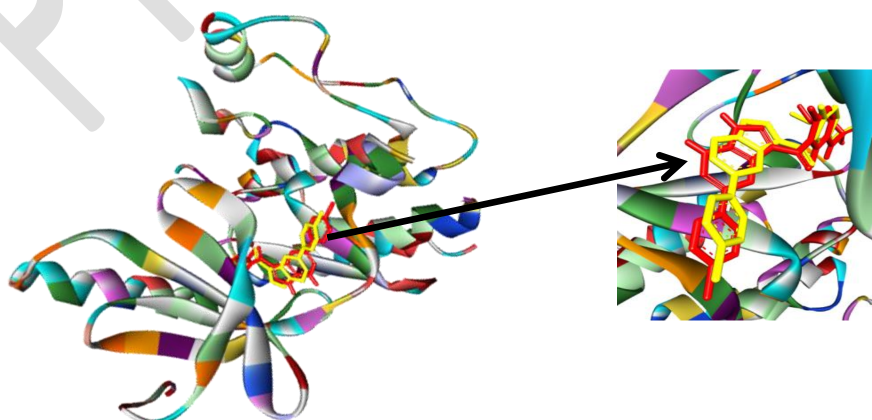

Figure 2. 3D diagram for the interaction of native ligand with BRAF-V600E receptor (yellow =before and red = after redocking)

The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still better as K+ (-10.593,68kal/mol). The smaller binding affinity value, the affinity between the receptor and ligand was higher and the vice versa, the greater the binding affinity value, the affinity between receptors and the ligands is getting lower [5], [22]. All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594 [22]. The amino acid residues that were shown to interact with ligands displays in Table 1 and illustrates the interaction between ligands and the BRAF-V600E receptor using 2D visualization displays in Figure 2.

Table 1. Molecular interactions present in the selected complex ligands and BRAF V600E receptor and the amino acids involved.

Complex	Binding Energy (kcal/mol)	Inhibition Constant/Ki (µM)	Amino acid residues
1-Cyclohexyl-2-propen-1-one	-6.68	12.63	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one	-6.89	8.96	Arg 575, Asp 576, Leu 577, Lys 578, Ser 616, Trp 619, Met 620, Ala 621, Val 626, Tyr 633, Ser 637, Asp 638, Ala 641
Cyclooctenone	-6.23	27.21	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
Vemuravenib	-11.11	7.42	Ile-463, Val-471, Ala- 481, Lys-483, Leu-505, Leu-514, Phe-516, Ile-527, Thr-529, **Gln-530**, Trp-531, **Cys-532**, Ser-535, Ser-536, His-539, Phe-583, **Asp-594**, Asp-593, Phe-595, Gly-596

,

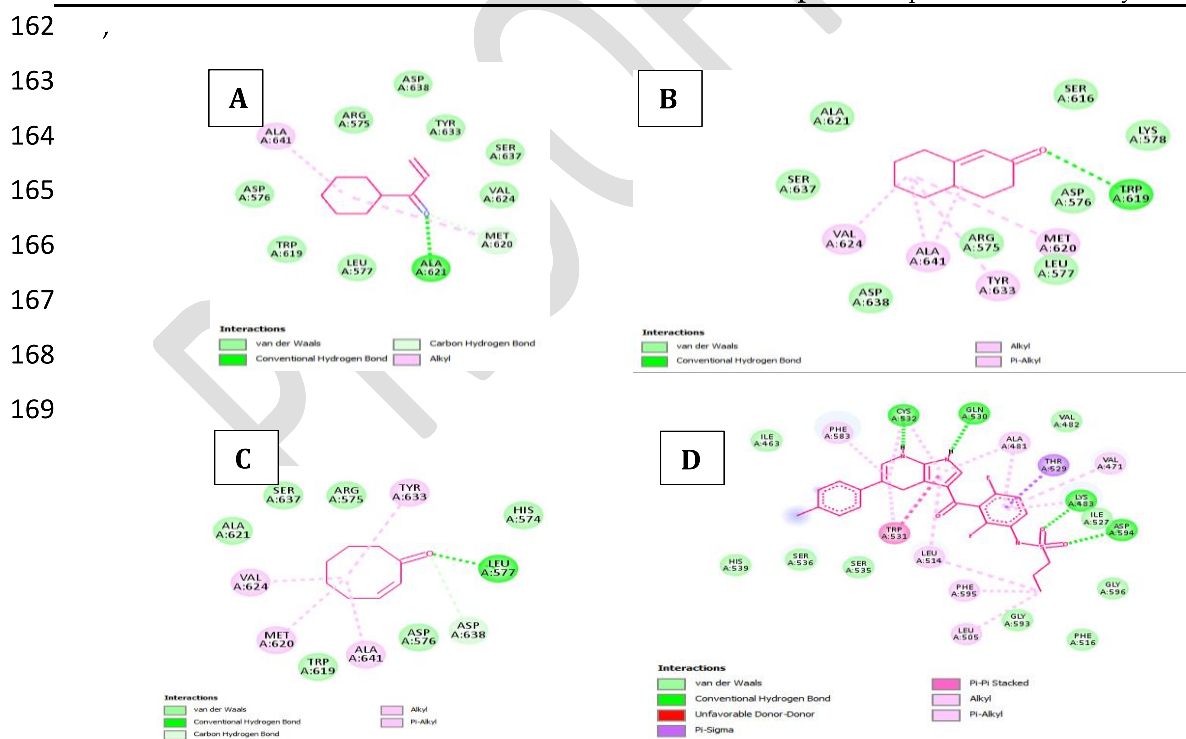

Figure 3. 2D Visualization of Complex Interactions Between Ligands 1-Cyclohexyl-2-propen-1-one **(A)**, 4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one **(B)**, Cyclooctenone **(C)**, and Vemuravenib **(D)** with BRAF V600E receptor

180 The likelihood that a molecule has the same or superior activity than BRAF V600E increases
181 with the number of amino acid similarities between the reference chemical and crucial amino acid. GLN
182 A:530, CYS A:532, ASP A:594, and THR A:529 are important amino acids linked to the BRAF V600E
183 receptor. The reticuline compound has the same hydrogen-bonded amino acids (GLN 530 and ASP 549)
184 as the reference compound Vemurafenib/key amino acids [22]. Conversely, compounds that had better
185 binding scores than vemurafe-nib and a decent MolDock score (≥− 158.139) and Rerank score (≥−
186 118.607) were recognized as possible hits [5].
187 Out of all the compounds, the three identified compounds found by GC-MS were found to
188 have the smallest binding affinities. The pkCMS online tool was used to further investigate these
189 compound's pharmacokinetic and toxicity characteristics (ADMET). The Lipinski test uses passive
190 diffusion to ascertain whether a substance in cell membranes is hydrophobic or hydrophilic. According
191 to Lipinski's guidelines, a ligand must have a molecular weight of less than 500 Da and a LogP value of
192 less than 5. molar refractivity between 40 and 130, donor hydrogen bonds < 5, and acceptor hydrogen
193 bonds < 10. Cell membranes are more readily penetrated by ligands with molecular weights less than
194 500 Da than by those with molecular weights greater than 500 Da. The polarity of the ligand in fat, oil,
195 and non-polar solvents is correlated with the logP value. Ligands that are widely dispersed throughout
196 the body and have a log P value greater than 5 will interact more readily via the lipid bilayer layer of
197 cell membranes. As a result, the ligand becomes more hazardous and its sensitivity to binding to the
198 target molecule decreases. Because they are more broadly distributed throughout the body and are kept
199 in lipid membranes for longer, excessively hydrophobic compounds typically have a high level of
200 toxicity. The ligand is hydrophobic and has a tendency to dissolve in water when the log P value is less.
201 Since the ligand cannot cross the lipid bilayer membrane, its Log P value cannot be negative. The
202 biological activity of a ligand or medicine is correlated with the amount of hydrogen bonds in the donor
203 and acceptor. The amount of energy needed for absorption increases with the strength of the hydrogen
204 bond [25]. Table 2 displays the outcomes of the molecular docking studies, which showed that the three
205 compounds satisfied Lipinski's guidelines.

206 **Table 2.** Ligand's Lipinski Rules of Five

Complex	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar surface activity (PSA)
1-Cyclohexyl-2-propen-1-one	138.21	2.3218	0	1	62.125
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	150.221	2.4659	0	1	67.484
Cyclooctenone	124.183	2.0758	0	1	55.760

207

208 When evaluating the pharmacokinetics of novel pharmacological compounds, ADMET
209 estimates are essential. If a compound's anticipated value is more than 0.09, it has significant Caco-2
210 permeability. Human colorectal cancer epithelial cells are known as Caco-2 cells. To estimate oral drug
211 absorption, Caco-2 cell monolayers are frequently employed as an in vitro model of the human
212 intestinal mucosa. The volume needed for a drug to be uniformly distributed and produce the same
213 concentration as in blood plasma is known as the Steady State Volume of Distribution (VDss). Excretion
214 in log (ml/min/kg) is predicted by total clearance (CLtot). Hepatic clearance (liver metabolism and
215 biliary clearance) and renal clearance (renal excretion) are the two primary parts of drug clearance.
216 AMES toxicity is frequently used to evaluate a compound's capacity to cause mutagenesis using
217 bacteria. Positive findings suggest that the substance is mutagenic and carcinogenic [22]. If a
218 compound's predictive value is greater than 0.90, it is deemed to have high Caco-2 permeability [26]. It

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219 has good permeability because the test results showed a value greater than 0.90. When the volume of
220 distribution (VDss) is less than 0.71 L/kg (log VDss<-0.15), it is deemed low; when it is greater than 2.81
221 L/kg (log VDss>0.45), it is deemed excessive [27]. In Table 3, all compounds are in the range volume
222 distribution requirements so that it can be predicted that all these compounds can be distributed evenly
223 to provide the same concentration as in blood plasma. Based on Table 3, all ligands have good
224 pharmacokinetic parameter.

225 **Table 3.** Pharmacokinetics (ADMET) Parameters of Ligands

Complex	Absorption Caco2 permeability	DistributionVDss (human)	Metabolism (CYP2D6 substrate)	Excretion (Total Clearance)	AMES toxicity	Hepato toxicity
1-Cyclohexyl-2-propen-1-one	1.085	0.148	No	0.221	No	No
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	1.501	0.344	No	0.112	No	No
Cyclooctenone	1.487	0.136	No	0.213	No	No

226

227 ## 4. CONCLUSION

228 The best potential components exploration of *C. aeruginosa* fraction with the smallest binding
229 affinity and meet the pharmacokinetic requirements are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-
230 one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol);
231 and vemuravnib is still better as K+ (-10.593,68kal/mol). All three compounds do not bind to key
232 amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. All
233 three compounds have good pharmacokinetic parameter. These results indicate that further
234 structural development is needed for better activity.

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Original Article

In Silico Study of Compounds Identified in *Curcuma aeruginosa* Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer

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Abstract: Curcuma aeruginosa Roxb rhizome contains secondary metabolite compounds and plays a role in various activities such as antioxidant, antibacterial, anthelmintic, antiandrogenic, antinociceptive, and anticancer. Anticancer activity that has been reported in Curcuma aeruginosa Roxb rhizome is limited to breast and cervical cancer. The purpose of this study was to explore the potential of Curcuma aeruginosa Roxb rhizome in melanoma cancer through the mechanism of inhibiting the BRAF V600E. The 96% ethanol extract of Curcuma aeruginosa Roxb rhizome was separated to produce n-hexane (HF), ethyl acetate (EAF), and ethanol (EF) fractions. The GC-MS results showed that there were 31 compounds from the three fractions. The docking validation process was carried out on the native ligand N-(3-{[5-(4-chlorophenyl) -1H-pyrrolo [2,3b]pyridin3yl] carbonyl}2,4difluorophenyl) propane-1-sulfonamide. All compounds were prepared as ligands for molecular docking with the BRAF V600E receptor (PDB ID: 3OG7). Docking validation on native ligand showed RMSD 1.03Å. The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68 kcal/mol); Cyclooctenone (-6,23 kcal/mol); and vemuravnib is still better as K+ (-11.11 kcal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. These results indicate that further structural development is needed for better activity.

Keywords: *Curcuma aeruginosa*; GC-MS ; In Silico, BRAF V600E inhibitor, Vemuravenib

1. INTRODUCTION

Over the past 50 years, melanoma incidence has steadily climbed globally. Melanoma is more prevalent in lower latitudes and among white-skinned individuals. Melanoma is the most common cancer in teenagers and young adults, but it is often more prevalent in the elderly population. In 2020, melanoma of the skin is expected to account for 1.7% of all cancer diagnoses worldwide, with an estimated 325,000 new cases [1]. Vemurafenib and dabravenib are BRAF mutation-inhibiting chemotherapy drugs to treat melanoma [2], [3]. Approximately 50% of cutaneous melanoma patients have active BRAF V600 mutations, so selective inhibitors were developed. Vemurafenib is responsive in 50% of patients with BRAF V600 mutations and is longer progression-free than dacarbazine (DTIC). In previous research, the reticuline compound in soursop leaves was proven to have the potential to treat cancer through the BRAF V600E inhibitor mechanism in silico [4]. In addition, in silico evaluation of several 4-(quinolin-2-yl)pyrimidin-2-amine derivatives as potent V600E-BRAF inhibitors was carried out [5]. There are several active compounds as BRAF V 300E inhibitors, which provide an opportunity for other natural ingredients to have the same activity.

Traditionally, the *C. aeruginose* rhizome has been used medicinally to treat stomach ache, obesity and rheumatism, asthma and cough, scurvy and mental disorders [6]. Essential oil content has been identified from the results of the distillation of *C. aeruginosa* Roxb. rhizomes such as curzerenone (24.6%), 1,scineole (ll.O%), camphor (10.6%), zedoarol (6.3%), isocurcumenol (5.8%), curcumenol (5.6%) and filranogermenone (5.5%) [7]. Other identified compounds include champor (29,39%) dan germacrone (21,21%) [8], monoterpen (21,47%) berupa β-pinen dan 1,8 cineol [9], 1,8-

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cineol (22.65%) dan germacrone (17.70%) [10], tropolene (18,1%) dan eucalyptol (17,9%) [11], β-pinene (21.9%), neocurdione (16.1%) and curcumol (15.2%) [12]. Meanwhile, the compounds that were successfully separated from the black turmeric extract using chromatography include germacrone, zederone, dehydrocurdione, curcumenol, zedoarondiol dan isocurcumenol [13]; dehydrocurdione, curcumenol, dan germacrone [14]; Pyrocurzerenone, Dehydrochromolaenin, Curzeone, Linderazulene, Curzerenone, 8, 12 - Epoxy - 1 (10), 4(15), 7, 11 -germacratetraen-6-one [15]; aeruginon and curcumenone [16]; dan flavon [17]. *C aeruginosa* isolates that have quite potential in various activities are germacrone as antiandrogenic [13], hair growth promoter [14], antinociceptive [18], and anticancer [16]. Anticancer activity that has been reported in *C. aeruginosa* Roxb rhizome is limited to breast cancer (MCF-7 and T-47D) and cervical cancer (Ca Ski and HeLa S3) [19], [20]. There have been no reports of *C. aeruni*osa being tested for BRAF V600E inhibitory activity as an anti-melanoma cancer in silico so that it is worthy of being processed.

2. MATERIALS AND METHODS

2.1. Chemical

Ethanol, methanol, n-hexane, and ethyl acetate as solvents from Smart Lab, Indonesia. All reagents used for the research were of analytical grade.

2.2. Plant Collection

C. aeruginosa Roxb dried rhizome from the Center for Research and Development of Traditional Medicinal Plants and Medicines Tawangmangu, Central Java, harvested in February 2020.

2.3. Instrumentation

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm.

2.4. Software and Hardware

The Protein Databank (PDB, www.rcsb.org) provided PDB ID: 3OG7 for download [4], [21]. The natural chemical's 3D structure files were obtained from PubChem (www.pubchem.ncbi.nlm.nih.gov). Ligands made with chemdraw 3D 15.0 for molecular docking. AutoDock Tool 1.5.6 Sep_17_14 employed the molecular docking procedure for in-silico screening, and Biovia Discovery Studio V21.1.0.2.20298 was used to view the results. Using a Lenovo laptop running Windows 10 with a Core i3 CPU, 4 GB of RAM, 64-bit operating system, and an x-64 processor, pharmacokinetics and toxicity prediction are performed. The online SMILES Translator (https://cactus.nci.nih.gov) was used to translate the compound into SMILES format. To forecast pharmacokinetics and chemical toxicity, the SMILES-formatted molecule was processed using the pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) [22].

2.5. Extraction and Fractination

One kg of the powdered material was macerated for three days at a ratio of 1:5 using 70% ethanol. Ethanol extract (EE) was obtained by combining the filtrates and drying them out using a revolving vacuum evaporator set at 60 °C and 100 rpm. Then, using solvents ranging from non-polar (n-hexane), semi-polar (ethyl acetate), and polar (ethanol), the ethanol extract (EE) was separated by sequential fractination to provide n-hexane (FH), ethyl acetate (EAF), and ethanol (EF). By turning the vacuum evaporator at 60 °C and 100 rpm, respectively, fractions were concentrated [23], [24].

2.6. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm. The mobile phase used is helium and was adjusted to a column velocity flow of 0.74 mL/min. Other GC-

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93 MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 42,3 kPa;
94 and 1 µl injector in split mode with a split ratio of 153.0 with injection temperature of 300 °C. The
95 temperature was raised to 320 °C at the rate of 10 °C/min and held for 5 min. The total elution was 24
96 min.

97 *2.7. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents*

98 Protein and ligand preparation is the initial stage of molecular docking. AutoDock Tools-
99 1.5.6 was used to carry out 3D interaction, docking, and binding investigations. The Protein Data
100 Bank provided the target proteins for download (PDB ID: 3OG7) [4], [21]. The Biovia Discovery
101 Studio visualizer program is used to extract native ligands and water molecules from 3D structures
102 to create protein files (.pdb). Chemdraw 3D 15.0 was used to produce the test ligand file (.sdf), which
103 was retrieved from PubChem, for molecular docking. The ligand contributes charge and torsion after
104 the receptor adds charge prior to molecular docking. The grid box's dimensions and coordinates were
105 established. X: 2.643, Y: -2.28, Z: -19.403, and spacing are the grid box coordinates, and the grid box
106 size is 44 × 40 × 40Å, spacing 0.375. Molecular docking parameters include interacting amino acid
107 residues and binding affinity (kcal/mol). Interaction 2D and 3D between ligand and protein were
108 visualized using Biovia Discover Studio visualizer. The following chemical properties were predicted
109 and explained: polar surface activity (PSA), hydrogen bond acceptors (HBA), hydrogen bond donors
110 (HBD), the number of atom-to-atom bonds that can rotate (Torson), the logarithm of the coefficient
111 octanol/water partition (Log P), and molecular weight (MW). These were conducted utilizing
112 Lipinski's rule of five, a set of guidelines that aids in distinguishing between molecules that resemble
113 drugs and those that do not, using the pkCMS web tool application [5], [21], [25]. This approach might
114 forecast the higher probability of success or failure because of drug penetration and absorption.
115 Following the 3D drawing of the chemical structure using Chemdraw 3D 15.0 and its saving in a
116 particular format (.pdb), the online SMILES Translator (https://cactus.nci.nih.gov) was used to
117 convert it to SMILES format. The pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) was used
118 to process the SMILES formatted compound in order to forecast chemical toxicity and
119 pharmacokinetics [22].

120 **3. RESULTS AND DISCUSSION**

121 *3.1. Fraction Compounds*

122 According to the GC-MS data, HF was primarily composed of sesquiterpenes (63.1%) and
123 diterpenes (5.26%), with 31.58% of it being unknown. substances. The EF was made up of
124 sesquiterpenes (68.42%) and others (31.58%), whereas the EAF was made up of sesquiterpenes
125 (42.86%), diterpenes (21.43%), steroids (7.14%), and others (28.57%). Saturated fatty acids were
126 present when IF was identified. Curcumenol and epicurzerenone were the primary constituents of
127 HF and EF, whereas curcumenol and 2,4-Dispironorbornylcyclobuta-1,3-dione (ketene dimers) were
128 the primary constituents of EAF. All compounds detected from the *C. aeruginosa* fraction are shown
129 in Figure 1.

130 *3.2. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents*

131 Redocking, or confirming the docking technique between the receptor and the native ligand, is
132 the first step in molecular docking. RMSD 1.03A is the outcome of the validation procedure. Since the
133 RMSD value is ≤ 2A the docking procedure can be deemed acceptable, and the RMSD obtained
134 satisfies the validation acceptance criterion [22]. To show the stance before and after docking, native
135 ligands are shown in two distinct colors. Native ligan in the redocking process with the BRAF V600E
136 resistor is shown in Figure 2. Vemuravenib, a commercial medication, was utilized as a control ligand
137 against the BRAF V600E receptor (PDB ID: 3OG7), while all substances discovered by GC-MS (Figure
138 1) were used as test ligands. ligand preparation is the initial stage of molecular docking.

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Curzerene | Caryophyllene oxide | Epicurzerenone | Isocurcumenol | Veridiflorol | Germacrone

Curdione | Curcumenol | Diepicedren-1-oxid | iso-velleral | Elemene | Isolongifolene | alpha-Guaiene

β-elemene | Gaillardin | Germacrene B | Cembrene | Ledane | 3-ethenyldecahydro-3,4a,7,7,10a-pentamethyl-

Labd-14-en-3-one, 8,13-epoxy- | 9.alpha.-D1-5.alpha.-Androstan-11-one | 2,4-Di-spironorbornylcyclobuta-1,3-dione | 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one | 1,5,9-Trimethyl cyclododecatriene | Octahydroanthracene

6-(1-hydroxymethyl-vinyl)-4,8A-dimethyl-3,5,6,7,8,8A-Hexahydro-1H-Naphthalen-2-O | 2-methyl-4-(2,6,6-trimethyl-Cyclohex-1-enyl)-but-2-enal | 1-Cyclohexyl-2-propen-1-one | Cyclooctenone

Methyl stearolate | Linolelaidic acid

Figure 1. Compound Name and Structure Identification of *C. aeruginosa* Fractions Using GC-MS

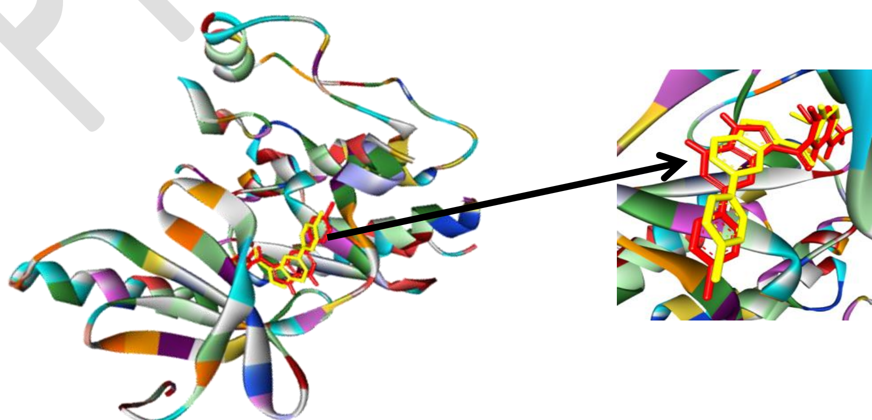

Figure 2. 3D diagram for the interaction of native ligand with BRAF-V600E receptor (yellow =before and red = after redocking)

The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still better as K+ (-10.593,68kal/mol). The smaller binding affinity value, the affinity between the receptor and ligand was higher and the vice versa, the greater the binding affinity value, the affinity between receptors and the ligands is getting lower [5], [22]. All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594 [22]. The amino acid residues that were shown to interact with ligands displays in Table 1 and illustrates the interaction between ligands and the BRAF-V600E receptor using 2D visualization displays in Figure 2.

Table 1. Molecular interactions present in the selected complex ligands and BRAF V600E receptor and the amino acids involved.

Complex	Binding Energy (kcal/mol)	Inhibition Constant/Ki (µM)	Amino acid residues
1-Cyclohexyl-2-propen-1-one	-6.68	12.63	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one	-6.89	8.96	Arg 575, Asp 576, Leu 577, Lys 578, Ser 616, Trp 619, Met 620, Ala 621, Val 626, Tyr 633, Ser 637, Asp 638, Ala 641
Cyclooctenone	-6.23	27.21	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
Vemuravenib	-11.11	7.42	Ile-463, Val-471, Ala- 481, Lys-483, Leu-505, Leu-514, Phe-516, Ile-527, Thr-529, **Gln-530**, Trp-531, **Cys-532**, Ser-535, Ser-536, His-539, Phe-583, **Asp-594**, Asp-593, Phe-595, Gly-596

,

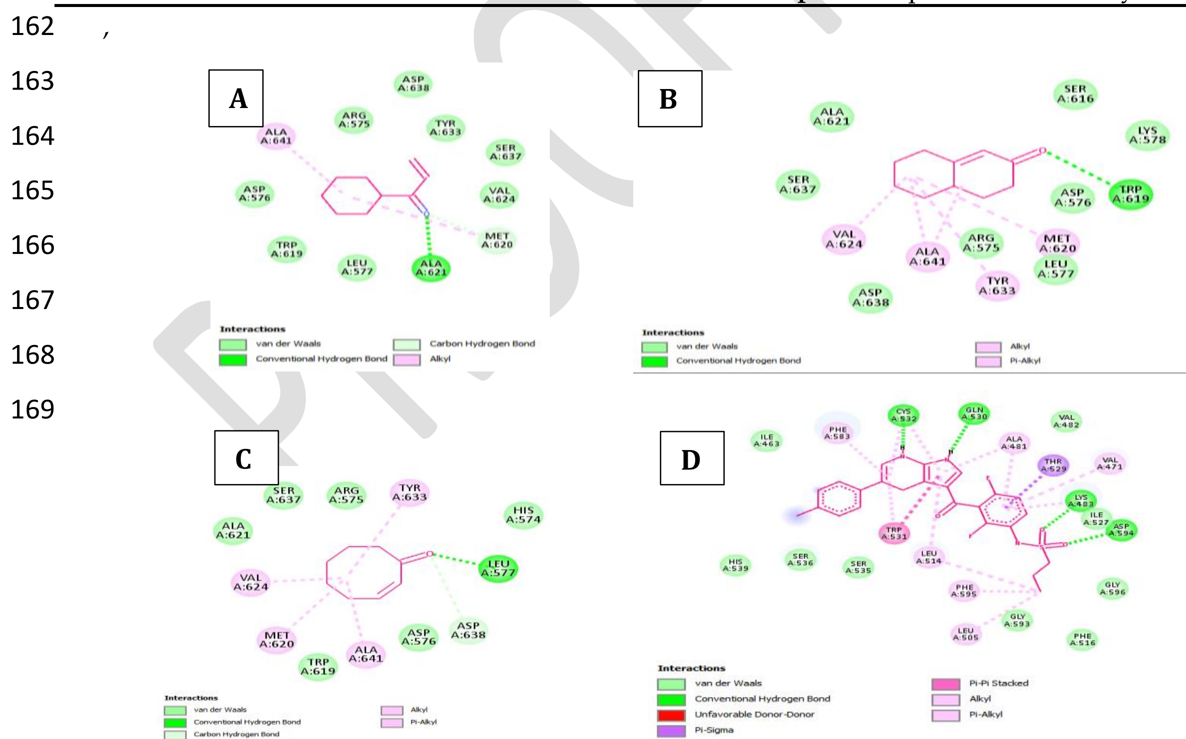

Figure 3. 2D Visualization of Complex Interactions Between Ligands 1-Cyclohexyl-2-propen-1-one **(A)**, 4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one **(B)**, Cyclooctenone **(C)**, and Vemuravenib **(D)** with BRAF V600E receptor

180 The likelihood that a molecule has the same or superior activity than BRAF V600E increases
181 with the number of amino acid similarities between the reference chemical and crucial amino acid. GLN
182 A:530, CYS A:532, ASP A:594, and THR A:529 are important amino acids linked to the BRAF V600E
183 receptor. The reticuline compound has the same hydrogen-bonded amino acids (GLN 530 and ASP 549)
184 as the reference compound Vemurafenib/key amino acids [22]. Conversely, compounds that had better
185 binding scores than vemurafe-nib and a decent MolDock score (≥− 158.139) and Rerank score (≥−
186 118.607) were recognized as possible hits [5].
187 Out of all the compounds, the three identified compounds found by GC-MS were found to
188 have the smallest binding affinities. The pkCMS online tool was used to further investigate these
189 compound's pharmacokinetic and toxicity characteristics (ADMET). The Lipinski test uses passive
190 diffusion to ascertain whether a substance in cell membranes is hydrophobic or hydrophilic. According
191 to Lipinski's guidelines, a ligand must have a molecular weight of less than 500 Da and a LogP value of
192 less than 5. molar refractivity between 40 and 130, donor hydrogen bonds < 5, and acceptor hydrogen
193 bonds < 10. Cell membranes are more readily penetrated by ligands with molecular weights less than
194 500 Da than by those with molecular weights greater than 500 Da. The polarity of the ligand in fat, oil,
195 and non-polar solvents is correlated with the logP value. Ligands that are widely dispersed throughout
196 the body and have a log P value greater than 5 will interact more readily via the lipid bilayer layer of
197 cell membranes. As a result, the ligand becomes more hazardous and its sensitivity to binding to the
198 target molecule decreases. Because they are more broadly distributed throughout the body and are kept
199 in lipid membranes for longer, excessively hydrophobic compounds typically have a high level of
200 toxicity. The ligand is hydrophobic and has a tendency to dissolve in water when the log P value is less.
201 Since the ligand cannot cross the lipid bilayer membrane, its Log P value cannot be negative. The
202 biological activity of a ligand or medicine is correlated with the amount of hydrogen bonds in the donor
203 and acceptor. The amount of energy needed for absorption increases with the strength of the hydrogen
204 bond [25]. Table 2 displays the outcomes of the molecular docking studies, which showed that the three
205 compounds satisfied Lipinski's guidelines.

206 **Table 2.** Ligand's Lipinski Rules of Five

Complex	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar surface activity (PSA)
1-Cyclohexyl-2-propen-1-one	138.21	2.3218	0	1	62.125
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	150.221	2.4659	0	1	67.484
Cyclooctenone	124.183	2.0758	0	1	55.760

207

208 When evaluating the pharmacokinetics of novel pharmacological compounds, ADMET
209 estimates are essential. If a compound's anticipated value is more than 0.09, it has significant Caco-2
210 permeability. Human colorectal cancer epithelial cells are known as Caco-2 cells. To estimate oral drug
211 absorption, Caco-2 cell monolayers are frequently employed as an in vitro model of the human
212 intestinal mucosa. The volume needed for a drug to be uniformly distributed and produce the same
213 concentration as in blood plasma is known as the Steady State Volume of Distribution (VDss). Excretion
214 in log (ml/min/kg) is predicted by total clearance (CLtot). Hepatic clearance (liver metabolism and
215 biliary clearance) and renal clearance (renal excretion) are the two primary parts of drug clearance.
216 AMES toxicity is frequently used to evaluate a compound's capacity to cause mutagenesis using
217 bacteria. Positive findings suggest that the substance is mutagenic and carcinogenic [22]. If a
218 compound's predictive value is greater than 0.90, it is deemed to have high Caco-2 permeability [26]. It

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219 has good permeability because the test results showed a value greater than 0.90. When the volume of
220 distribution (VDss) is less than 0.71 L/kg (log VDss<-0.15), it is deemed low; when it is greater than 2.81
221 L/kg (log VDss>0.45), it is deemed excessive [27]. In Table 3, all compounds are in the range volume
222 distribution requirements so that it can be predicted that all these compounds can be distributed evenly
223 to provide the same concentration as in blood plasma. Based on Table 3, all ligands have good
224 pharmacokinetic parameter.

225 **Table 3.** Pharmacokinetics (ADMET) Parameters of Ligands

Complex	Absorption Caco2 permeability	DistributionVDss (human)	Metabolism (CYP2D6 substrate)	Excretion (Total Clearance)	AMES toxicity	Hepato toxicity
1-Cyclohexyl-2-propen-1-one	1.085	0.148	No	0.221	No	No
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	1.501	0.344	No	0.112	No	No
Cyclooctenone	1.487	0.136	No	0.213	No	No

226

227 ## 4. CONCLUSION

228 The best potential components exploration of *C. aeruginosa* fraction with the smallest binding
229 affinity and meet the pharmacokinetic requirements are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-
230 one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol);
231 and vemuravnib is still better as K+ (-10.593,68kal/mol). All three compounds do not bind to key
232 amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. All
233 three compounds have good pharmacokinetic parameter. These results indicate that further
234 structural development is needed for better activity.

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239 **Conflicts of interest:** The authors declare no conflict of interest

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Dear : Ririn Suharsanti

ACCEPTANCE LETTER

Journal of Food and Pharmaceutical Research (JFPS), is pleased to inform you that the following manuscript has been accepted for publication in JFPS volume 13 issue 2 on June 2025.

Manuscript Title : In Silico Study of Compounds Identified in *Curcuma aeruginosa* Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer
Authors : Ririn Suharsanti, Muhammad Ryan Radix Rahardhian, and Lia Kusmita

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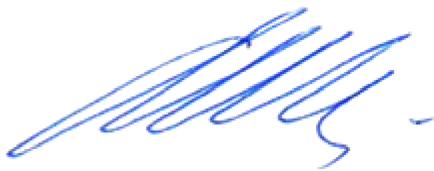

Prof. Dr. Abdul Rohman

Chief Editor

Journal of Food and Pharmaceutical Sciences

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Original Article

In Silico Study of Compounds Identified in *Curcuma aeruginosa* Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer

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Abstract: *Curcuma aeruginosa* Roxb rhizome contains secondary metabolite compounds and plays a role in various activities such as antioxidant, antibacterial, anthelmintic, antiandrogenic, antinociceptive, and anticancer. Anticancer activity that has been reported in *Curcuma aeruginosa* Roxb rhizome is limited to breast and cervical cancer. The purpose of this study was to explore the potential of *Curcuma aeruginosa* Roxb rhizome in melanoma cancer through the mechanism of inhibiting the BRAF V600E. The 96% ethanol extract of *Curcuma aeruginosa* Roxb rhizome was separated to produce n-hexane (HF), ethyl acetate (EAF), and ethanol (EF) fractions. The GC-MS results showed that there were 31 compounds from the three fractions. The docking validation process was carried out on the native ligand N-(3-{[5-(4-chlorophenyl) -1H-pyrrolo [2,3b]pyridin3yl] carbonyl}2,4difluorophenyl) propane-1-sulfonamide. All compounds were prepared as ligands for molecular docking with the BRAF V600E receptor (PDB ID: 3OG7). Docking validation on native ligand showed RMSD 1.03Å. The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68 kcal/mol); Cyclooctenone (-6,23 kcal/mol); and vemuravnib is still better as K+ (-11.11 kcal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. These results indicate that further structural development is needed for better activity.

Keywords: *Curcuma aeruginosa*; GC-MS ; In Silico, BRAF V600E inhibitor, Vemuravenib

1. INTRODUCTION

Over the past 50 years, melanoma incidence has steadily climbed globally. Melanoma is more prevalent in lower latitudes and among white-skinned individuals. Melanoma is the most common cancer in teenagers and young adults, but it is often more prevalent in the elderly population. In 2020, melanoma of the skin is expected to account for 1.7% of all cancer diagnoses worldwide, with an estimated 325,000 new cases [1]. Vemurafenib and dabravenib are BRAF mutation-inhibiting chemotherapy drugs to treat melanoma [2], [3]. Approximately 50% of cutaneous melanoma patients have active BRAF V600 mutations, so selective inhibitors were developed. Vemurafenib is responsive in 50% of patients with BRAF V600 mutations and is longer progression-free than dacarbazine (DTIC). In previous research, the reticuline compound in soursop leaves was proven to have the potential to treat cancer through the BRAF V600E inhibitor mechanism in silico [4]. In addition, in silico evaluation of several 4-(quinolin-2-yl)pyrimidin-2-amine derivatives as potent V600E-BRAF inhibitors was carried out [5]. There are several active compounds as BRAF V 300E inhibitors, which provide an opportunity for other natural ingredients to have the same activity.

Traditionally, the *C. aeruginose* rhizome has been used medicinally to treat stomach ache, obesity and rheumatism, asthma and cough, scurvy and mental disorders [6]. Essential oil content has been identified from the results of the distillation of *C. aeruginosa* Roxb. rhizomes such as curzerenone (24.6%), 1,scineole (ll.O%), camphor (10.6%), zedoarol (6.3%), isocurcumenol (5.8%), curcumenol (5.6%) and filranogermenone (5.5%) [7]. Other identified compounds include champor (29,39%) dan germacrone (21,21%) [8], monoterpen (21,47%) berupa β-pinen dan 1,8 cineol [9], 1,8-

cineol (22.65%) dan germacrone (17.70%) [10], tropolene (18,1%) dan eucalyptol (17,9%) [11], β-pinene (21.9%), neocurdione (16.1%) and curcumol (15.2%) [12]. Meanwhile, the compounds that were successfully separated from the black turmeric extract using chromatography include germacrone, zederone, dehydrocurdione, curcumenol, zedoarondiol dan isocurcumenol [13]; dehydrocurdione, curcumenol, dan germacrone [14]; Pyrocurzerenone, Dehydrochromolaenin, Curzeone, Linderazulene, Curzerenone, 8, 12 - Epoxy - 1 (10), 4(15), 7, 11 -germacratetraen-6-one [15]; aeruginon and curcumenone [16]; dan flavon [17]. *C aeruginosa* isolates that have quite potential in various activities are germacrone as antiandrogenic [13], hair growth promoter [14], antinociceptive [18], and anticancer [16]. Anticancer activity that has been reported in *C. aeruginosa* Roxb rhizome is limited to breast cancer (MCF-7 and T-47D) and cervical cancer (Ca Ski and HeLa S3) [19], [20]. There have been no reports of *C. aeruni*osa being tested for BRAF V600E inhibitory activity as an anti-melanoma cancer in silico so that it is worthy of being processed.

2. MATERIALS AND METHODS

2.1. Chemical

Ethanol, methanol, n-hexane, and ethyl acetate as solvents from Smart Lab, Indonesia. All reagents used for the research were of analytical grade.

2.2. Plant Collection

C. aeruginosa Roxb dried rhizome from the Center for Research and Development of Traditional Medicinal Plants and Medicines Tawangmangu, Central Java, harvested in February 2020.

2.3. Instrumentation

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm.

2.4. Software and Hardware

The Protein Databank (PDB, www.rcsb.org) provided PDB ID: 3OG7 for download [4], [21]. The natural chemical's 3D structure files were obtained from PubChem (www.pubchem.ncbi.nlm.nih.gov). Ligands made with chemdraw 3D 15.0 for molecular docking. AutoDock Tool 1.5.6 Sep_17_14 employed the molecular docking procedure for in-silico screening, and Biovia Discovery Studio V21.1.0.2.20298 was used to view the results. Using a Lenovo laptop running Windows 10 with a Core i3 CPU, 4 GB of RAM, 64-bit operating system, and an x-64 processor, pharmacokinetics and toxicity prediction are performed. The online SMILES Translator (https://cactus.nci.nih.gov) was used to translate the compound into SMILES format. To forecast pharmacokinetics and chemical toxicity, the SMILES-formatted molecule was processed using the pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) [22].

2.5. Extraction and Fractionation

One kg of the powdered material was macerated for three days at a ratio of 1:5 using 70% ethanol. Ethanol extract (EE) was obtained by combining the filtrates and drying them out using a revolving vacuum evaporator set at 60 °C and 100 rpm. Then, using solvents ranging from non-polar (n-hexane), semi-polar (ethyl acetate), and polar (ethanol), the ethanol extract (EE) was separated by sequential fractination to provide n-hexane (FH), ethyl acetate (EAF), and ethanol (EF). By turning the vacuum evaporator at 60 °C and 100 rpm, respectively, fractions were concentrated [23], [24].

2.6. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm. The mobile phase used is helium and was adjusted to a column velocity flow of 0.74 mL/min. Other GC-

MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 42,3 kPa; and 1 μl injector in split mode with a split ratio of 153.0 with injection temperature of 300 °C. The temperature was raised to 320 °C at the rate of 10 °C/min and held for 5 min. The total elution was 24 min.

2.7. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Protein and ligand preparation is the initial stage of molecular docking. AutoDock Tools-1.5.6 was used to carry out 3D interaction, docking, and binding investigations. The Protein Data Bank provided the target proteins for download (PDB ID: 3OG7) [4], [21]. The Biovia Discovery Studio visualizer program is used to extract native ligands and water molecules from 3D structures to create protein files (.pdb). Chemdraw 3D 15.0 was used to produce the test ligand file (.sdf), which was retrieved from PubChem, for molecular docking. The ligand contributes charge and torsion after the receptor adds charge prior to molecular docking. The grid box's dimensions and coordinates were established. X: 2.643, Y: -2.28, Z: -19.403, and spacing are the grid box coordinates, and the grid box size is 44 × 40 × 40Å, spacing 0.375. Molecular docking parameters include interacting amino acid residues and binding affinity (kcal/mol). Interaction 2D and 3D between ligand and protein were visualized using Biovia Discover Studio visualizer. The following chemical properties were predicted and explained: polar surface activity (PSA), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), the number of atom-to-atom bonds that can rotate (Torson), the logarithm of the coefficient octanol/water partition (Log P), and molecular weight (MW). These were conducted utilizing Lipinski's rule of five, a set of guidelines that aids in distinguishing between molecules that resemble drugs and those that do not, using the pkCMS web tool application [5], [21], [25]. This approach might forecast the higher probability of success or failure because of drug penetration and absorption. Following the 3D drawing of the chemical structure using Chemdraw 3D 15.0 and its saving in a particular format (.pdb), the online SMILES Translator (https://cactus.nci.nih.gov) was used to convert it to SMILES format. The pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) was used to process the SMILES formatted compound in order to forecast chemical toxicity and pharmacokinetics [22].

3. RESULTS AND DISCUSSION

3.1. Fraction Compounds

According to the GC-MS data, HF was primarily composed of sesquiterpenes (63.1%) and diterpenes (5.26%), with 31.58% of it being unknown. substances. The EF was made up of sesquiterpenes (68.42%) and others (31.58%), whereas the EAF was made up of sesquiterpenes (42.86%), diterpenes (21.43%), steroids (7.14%), and others (28.57%). Saturated fatty acids were present when IF was identified. Curcumenol and epicurzerenone were the primary constituents of HF and EF, whereas curcumenol and 2,4-Dispironorbornylcyclobuta-1,3-dione (ketene dimers) were the primary constituents of EAF. All compounds detected from the *C. aeruginosa* fraction are shown in Figure 1.

3.2. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Redocking, or confirming the docking technique between the receptor and the native ligand, is the first step in molecular docking. RMSD 1.03A is the outcome of the validation procedure. Since the RMSD value is ≤ 2A the docking procedure can be deemed acceptable, and the RMSD obtained satisfies the validation acceptance criterion [22]. To show the stance before and after docking, native ligands are shown in two distinct colors. Native ligan in the redocking process with the BRAF V600E resistor is shown in Figure 2. Vemuravenib, a commercial medication, was utilized as a control ligand against the BRAF V600E receptor (PDB ID: 3OG7), while all substances discovered by GC-MS (Figure 1) were used as test ligands. ligand preparation is the initial stage of molecular docking.

Curzerene, Caryophyllene oxide, Epicurzerenone, Isocurcumenol, Veridiflorol, Germacrone

Curdione, Curcumenol, Diepicedren-1-oxid, iso-velleral, Elemene, Isolongifolene, alpha-Guaiene

β-elemene, Gaillardin, Germacrene B, Cembrene, Ledane, 3-ethenyldecahydro-3,4a,7,7,10a-pentamethyl-

Labd-14-en-3-one, 8,13-epoxy-, 9.alpha.-D1-5.alpha.-Androstan-11-one, 2,4-Di-spironorbornylcyclobuta-1,3-dione, 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one, 1,5,9-Trimethyl cyclododecatriene, Octahydroanthracene

6-(1-hydroxymethyl-vinyl)-4,8A-dimethyl-3,5,6,7,8,8A-Hexahydro-1H-Naphthalen-2-O, 2-methyl-4-(2,6,6-trimethyl-Cyclohex-1-enyl)-but-2-enal, 1-Cyclohexyl-2-propen-1-one, Cyclooctenone

Methyl stearolate, Linolelaidic acid

Figure 1. Compound Name and Structure Identification of *C. aeruginosa* Fractions Using GC-MS

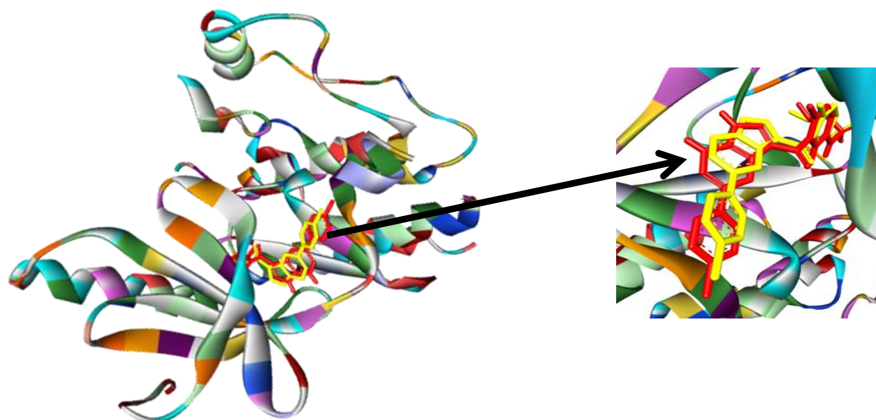

Figure 2. 3D diagram for the interaction of native ligand with BRAF-V600E receptor (yellow =before and red = after redocking)

The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still better as K+ (-10.593,68kal/mol). The smaller binding affinity value, the affinity between the receptor and ligand was higher and the vice versa, the greater the binding affinity value, the affinity between receptors and the ligands is getting lower [5], [22]. All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594 [22]. The amino acid residues that were shown to interact with ligands displays in Table 1 and illustrates the interaction between ligands and the BRAF-V600E receptor using 2D visualization displays in Figure 2.

Table 1. Molecular interactions present in the selected complex ligands and BRAF V600E receptor and the amino acids involved.

Complex	Binding Energy (kcal/mol)	Inhibition Constant/Ki (µM)	Amino acid residues
1-Cyclohexyl-2-propen-1-one	-6.68	12.63	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one	-6.89	8.96	Arg 575, Asp 576, Leu 577, Lys 578, Ser 616, Trp 619, Met 620, Ala 621, Val 626, Tyr 633, Ser 637, Asp 638, Ala 641
Cyclooctenone	-6.23	27.21	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
Vemuravenib	-11.11	7.42	Ile-463, Val-471, Ala- 481, Lys-483, Leu-505, Leu-514, Phe-516, Ile-527, Thr-529, **Gln-530**, Trp-531, **Cys-532**, Ser-535, Ser-536, His-539, Phe-583, **Asp-594**, Asp-593, Phe-595, Gly-596

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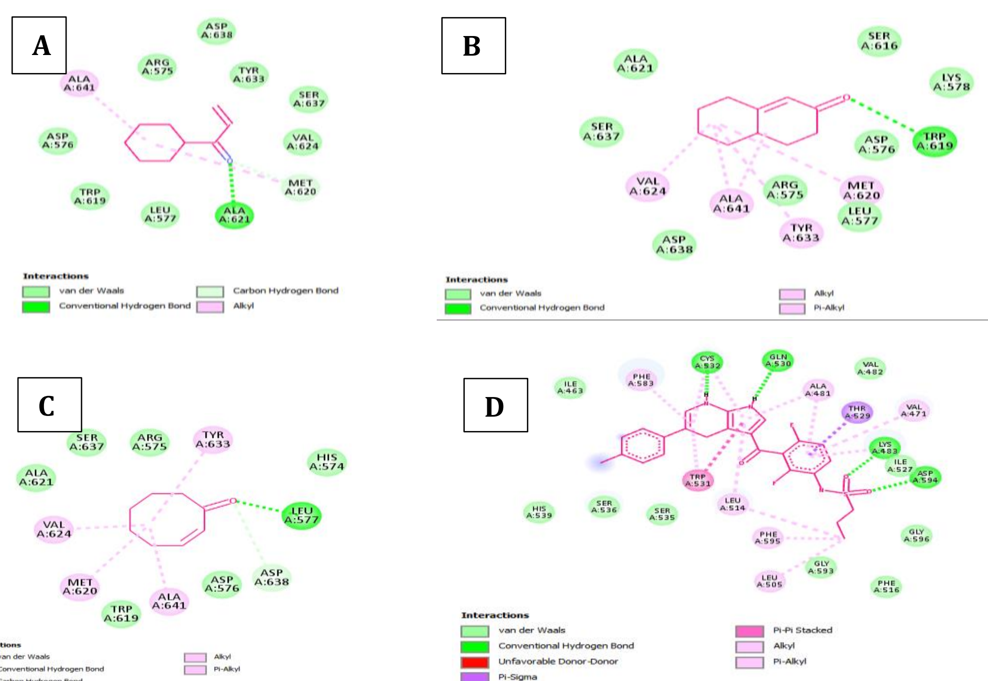

Figure 3. 2D Visualization of Complex Interactions Between Ligands 1-Cyclohexyl-2-propen-1-one **(A)**, 4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one **(B)**, Cyclooctenone **(C)**, and Vemuravenib **(D)** with BRAF V600E receptor

The likelihood that a molecule has the same or superior activity than BRAF V600E increases with the number of amino acid similarities between the reference chemical and crucial amino acid. GLN A:530, CYS A:532, ASP A:594, and THR A:529 are important amino acids linked to the BRAF V600E receptor. The reticuline compound has the same hydrogen-bonded amino acids (GLN 530 and ASP 549) as the reference compound Vemurafenib/key amino acids [22]. Conversely, compounds that had better binding scores than vemurafe-nib and a decent MolDock score (≥− 158.139) and Rerank score (≥− 118.607) were recognized as possible hits [5].

Out of all the compounds, the three identified compounds found by GC-MS were found to have the smallest binding affinities. The pkCMS online tool was used to further investigate these compound's pharmacokinetic and toxicity characteristics (ADMET). The Lipinski test uses passive diffusion to ascertain whether a substance in cell membranes is hydrophobic or hydrophilic. According to Lipinski's guidelines, a ligand must have a molecular weight of less than 500 Da and a LogP value of less than 5. molar refractivity between 40 and 130, donor hydrogen bonds < 5, and acceptor hydrogen bonds < 10. Cell membranes are more readily penetrated by ligands with molecular weights less than 500 Da than by those with molecular weights greater than 500 Da. The polarity of the ligand in fat, oil, and non-polar solvents is correlated with the logP value. Ligands that are widely dispersed throughout the body and have a log P value greater than 5 will interact more readily via the lipid bilayer layer of cell membranes. As a result, the ligand becomes more hazardous and its sensitivity to binding to the target molecule decreases. Because they are more broadly distributed throughout the body and are kept in lipid membranes for longer, excessively hydrophobic compounds typically have a high level of toxicity. The ligand is hydrophobic and has a tendency to dissolve in water when the log P value is less. Since the ligand cannot cross the lipid bilayer membrane, its Log P value cannot be negative. The biological activity of a ligand or medicine is correlated with the amount of hydrogen bonds in the donor and acceptor. The amount of energy needed for absorption increases with the strength of the hydrogen bond [25]. Table 2 displays the outcomes of the molecular docking studies, which showed that the three compounds satisfied Lipinski's guidelines.

Table 2. Ligand's Lipinski Rules of Five

Complex	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar surface activity (PSA)
1-Cyclohexyl-2-propen-1-one	138.21	2.3218	0	1	62.125
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	150.221	2.4659	0	1	67.484
Cyclooctenone	124.183	2.0758	0	1	55.760

When evaluating the pharmacokinetics of novel pharmacological compounds, ADMET estimates are essential. If a compound's anticipated value is more than 0.09, it has significant Caco-2 permeability. Human colorectal cancer epithelial cells are known as Caco-2 cells. To estimate oral drug absorption, Caco-2 cell monolayers are frequently employed as an in vitro model of the human intestinal mucosa. The volume needed for a drug to be uniformly distributed and produce the same concentration as in blood plasma is known as the Steady State Volume of Distribution (VDss). Excretion in log (ml/min/kg) is predicted by total clearance (CLtot). Hepatic clearance (liver metabolism and biliary clearance) and renal clearance (renal excretion) are the two primary parts of drug clearance. AMES toxicity is frequently used to evaluate a compound's capacity to cause mutagenesis using bacteria. Positive findings suggest that the substance is mutagenic and carcinogenic [22]. If a compound's predictive value is greater than 0.90, it is deemed to have high Caco-2 permeability [26]. It

has good permeability because the test results showed a value greater than 0.90. When the volume of distribution (VDss) is less than 0.71 L/kg (log VDss<-0.15), it is deemed low; when it is greater than 2.81 L/kg (log VDss>0.45), it is deemed excessive [27]. In Table 3, all compounds are in the range volume distribution requirements so that it can be predicted that all these compounds can be distributed evenly to provide the same concentration as in blood plasma. Based on Table 3, all ligands have good pharmacokinetic parameter.

Table 3. Pharmacokinetics (ADMET) Parameters of Ligands

Complex	Absorption Caco2 permeability	DistributionVDss (human)	Metabolism (CYP2D6 substrate)	Excretion (Total Clearance)	AMES toxicity	Hepato toxicity
1-Cyclohexyl-2-propen-1-one	1.085	0.148	No	0.221	No	No
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	1.501	0.344	No	0.112	No	No
Cyclooctenone	1.487	0.136	No	0.213	No	No

4. CONCLUSION

The best potential components exploration of *C. aeruginosa* fraction with the smallest binding affinity and meet the pharmacokinetic requirements are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still better as K+ (-10.593,68kal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. All three compounds have good pharmacokinetic parameter. These results indicate that further structural development is needed for better activity.

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