MOLEKUL
eISSN: 2503-0310

Articles
https://doi.org/10.20884/1.jm.2025.20.1.8673

Bioactive Compounds of *n*-Hexane Fraction of *Syzygium samarangense* Stem Bark and Molecular Docking Study as Anticancer Agent

Ahmad Misbakhus Sururi, Tukiran*, Irene Cornelia Constanty

Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Negeri Surabaya, Surabaya, East Java, Indonesia

*Corresponding author email: tukiran@unesa.ac.id

Received June 09, 2023; **Accepted** December 04, 2024; **Available online** March 20, 2025

ABSTRACT. *Syzygium samarangense* or jambu Semarang is one of the typical Indonesian plants whose stem bark is rarely used. Research reports on stem bark provide the potential for development in the medical field, especially in cancer. The main objectives of this study were to identify its bioactive content and describe its potential bioactivity as an EGFR inhibitor for anticancer candidates *in silico*. The stages of the research was started with extraction (methanol), then fractionation (*n*-hexane) to obtain *n*-hexane fraction. Then, identification of the chemical content of the fractions was carried out using LC-MS. In order to know the content of anticancer potency of the identified compounds was analyzed by molecular docking. It can be reported that there are totally 35 compounds in the fraction. Based on molecular docking analysis, There were 4 of 35 compounds in the fraction, which had anticancer potency namely: isoengeletin (i); epibetulinic acid (ii); myricitrin (iii), and stigmasterol-3-*O*-β-D-glucoside (iv). Through PASS prediction data, it is known that the four compounds have the potency to be antineoplastic and anticarcinogenic with moderate to high activity categories. Further studies, such as *in vitro* and *in vivo*, are needed to validate its bioactivity as an anticancer.

Keywords: anticancer, bioactivity, LC-MS, molecular docking, *Syzygium samarangense*

INTRODUCTION

Syzygium samarangense is one of the typical plants in Indonesia. This plant is often used in the medical field as antioxidant (Metasari et al., 2020) and antifungal (Tukiran et al., 2021), but the use of the stem bark has not still optimized yet. Research related to the plant is still limited to be fruits and leaves, but its stem bark is rarely studied. However, according to some reports, this plant is rich in secondary metabolites including flavonoids, phenolics, steroids, and terpenoids. These secondary metabolites are beneficial, especially in medicine for example as an antioxidant, anticancer, and anti-inflammatory (Tarigan et al., 2022).

Cancer is one of the most urgent problems in the world. Cancer is a condition in which cells grow abnormally in the body (WHO, 2022). All cancers occurred due to abnormalities in the DNA sequence. Mutations affect the cell's DNA sequence to change from the normal type to the cancer type. This somatic mutation alters the function of a critical gene, conferring a growth advantage on the cell and resulting in the emergence of an expanded clone (Chae et al., 2018). Identification of mutated genes has been an important goal of cancer research since the emergence of recombination DNA technology (Hsiehchen et al., 2020). The mechanism of cancer cell growth can result in the over expression of a receptor protein, one of which is the EGFR receptor (Inamura et al., 2010; Patel, 2014).

EGFR receptor or epidermal growth factor receptor is a protein found on the surface of many cells in the body (Patel, 2014). The receptor plays a vital role in cell growth and division and is involved in developing and progressing many types of cancer. Over expression of it is commonly found in many solid tumors and has been associated with poor prognosis and resistance to conventional chemotherapy (Inamura et al., 2010). EGFR is a trans membrane receptor protein that regulates cellular processes such as proliferation, differentiation, and survival (Koveitypour et al., 2019). Dysregulation of the receptor signaling pathway has been implicated in the development and progression several types of cancer, including non-small cell lung cancer (NSCLC), colorectal cancer, head and neck cancers, and breast cancer (Thakur, 2019). Targeted therapy directed against EGFR has emerged as a promising treatment strategy for cancer patients (Wee & Wang, 2017). One of the treatments to overcome excessive EGFR expression is the administration of synthetic drugs such as icotinib. However, the use of this drug has side effects, with the fatal consequence being interstitial

lung disease (ILD) (Pan et al., 2014; Zhang et al., 2014).

There have been no reports regarding the potency of the plant stem bark especially as an EGFR inhibitor, so this research is essential to be conducted. This study aimed to identify the bioactive contents from the *n*-hexane fraction of the methanol extract of the plant stem bark and describe their potentials as an EGFR inhibitor *in silico* using molecular docking and their bioactivity approaches as an anti-neoplastic and anti-carcinogenic through PASS-Online.

EXPERIMENTAL SECTION

Materials and Equipments

S. samarangense stem bark, methanol p.a. (MERCK), *n*-hexane solvent (MERCK), and filter paper (Whatmann 42 MERCK). Tools used in this study are LC-MS (Shimadzu LCMS-8040) instrugment, vacuum rotary evaporator (Buchi Rotavapor® R-300), analytical balance (OHAUS), vacuum pump, grinder, Beaker glass (PYREX), Laptop (AMD Ryzen 7 processor, 16GB RAM, 512GB SSD). Software used in this study are PyMOL, PyRx 0.8, and Discovery Studio.

Sample Preparation, Extraction, and Fractionation

The stem bark of *S. samarangense* is dried and ground to obtain a dried powder sample. The sample was macerated using methanol (1:4 w/v) for 1x24 hours with three repetitions. The extract was thickened with a vacuum rotary evaporator to obtain a viscous extract. The concentrated extract was dissolved with a small amount of methanol in a separating funnel and then added by an *n*-hexane solvent with a ratio of methanol and hexane which is approximately 1:3. This mixture produces two layers where the top layer is the *n*-hexane phase. The *n*-hexane phase was then concentrated using a vacuum rotary evaporator to obtain *n*-hexane fraction.

LC-MS Analysis

Determination of the compounds in *n*-hexane fraction of *S. samarangense* stem bark was conducted using the LC-MS instrument. A total of 1 µL of the sample was injected into the LC instrument using a Shim FC-ODS column (2 mm x 150 mm, particle size 3 µm) at 35 °C. Separation was performed at a 1 mL/min flow rate with an isocratic model. The ion spray needle voltage is 3.5 kV, and the capillary temperature is 400 °C. Ionization was carried out using ESI. Compounds were identified using the NIST and FSTP-NUS data libraries from LC-MS.

Druglikeness Lipinski Assay

The identified compounds were then selected using Lipinski's five rules. The drug similarity test with Lipinski's five rules was then carried out by the web server scfbio-iitd.res.in/software/drugdesign/lipinski.jsp. Compounds that can achieve at least 3 rules of the Lipinski are compounds with potential as drugs and will be simulated using molecular docking (Jayaram et al., 2012; Lipinski, 2004).

Molecular Docking Assay

Protein Preparation

The EGFR protein (PDB ID: 6VH4) obtained through the rcsb.org web server was prepared using Discovery Studio to determine its default ligand's active site and clean the protein from water, ligands, and unnecessary molecules. Proteins are stored in .pdb format (protein databank) and inputted as macromolecules in PyRx.

Ligand Preparation

Compounds that comply with Lipinski's five rules were obtained from the web server pubchem.ncbi.nlm.nih.gov/, which was minimized using OpenBabel on PyRx software and inputted as a ligand on PyRx.

Docking and Visualization

Molecular docking simulation was carried out using the Vina Wizard on PyRx 0.8 to determine the value of binding affinity and conformation of the compounds (Trott & Olson, 2010). The control compound used in this study was icotinib. The docking results were then visualized to obtain the type of data and interaction positions formed using PyMOL and Discovery Studio.

PASS-Online Prediction

PASS-Online predictions can determine the probability of a compound having a certain bioactivity. The data obtained are data on active opportunities (Pa) and inactive opportunities (Pi) (Lagunin et al., 2000; Parasuraman, 2011).

RESULTS AND DISCUSSION

Identification Bioactive Compounds of *n*-Hexane Fraction of *S. samarangense* Stem Bark

S. samarangense is one of the plants which can grow well in Indonesia. This plant is often used especially in the medical field. However, reports regarding to especially the stem bark of the plant are still rare. Based on LC-MS analysis, it can be reported that there is 35 compounds in the *n*-hexane fration. The dominant compound with the highest concentration in the fraction was cycloartenyl stearate, with a retention time of 6.93 minutes with a composition proportion of 6.93%. The chromatogram of LC-MS results can be seen in **Figure 1**, and the compounds identified can be seen in **Table 1**.

In the *n*-hexane fraction/extract, glucoside derivatives that are also found can occur due to various factors such as amphiphilic properties and solubility shifts caused by the steric effects of glucoside derivative structures. The identified glucoside compounds included steroid glucosides and terpenoid glucosides, where the steroid moiety separates into the *n*-hexane solvent while the glucoside moiety is more attracted to methanol as the initial solvent. However, due to the steric effects of the more non-polar groups, solubility shifts occur, causing the glucoside compounds to be attracted to the *n*-hexane fraction.

It has been widely reported that stigmasterol-3-*O*-β-D-glucoside or β-sitosterol-3-*O*-β-D-glucoside which is one of the steroid saponins are found in the *n*-hexane extract of a plant such as *Chorisia crispiflora* leaves (Samar et al. 2013). Several plants containing saponins in the *n*-hexane extract are *Cassia fistula* leaves (Sujatha & Asokan, 2018), *Spondias tuberosa* leaves (Anacardiaceae) (da Costa Cordeiro et al., 2018), and stem Red Dragon fruit extract (*Hylocereus polyrhizus*) (Sari et al., 2021). Also, glucoside compounds had been identified from *n*-hexane extract of *S. setigera* leaf extracts (Ushie et al., 2022). Then, sesquiterpene glucosides, specifically eudesmane glycosides is also presented in the *n*-hexane extract of *Carthamus lanatus* (San Feliciano et al., 1990), sitosteryl (60'-hentriacontanoyl)-β-D-galactopyranoside in *n*-hexane fraction from the methanol extract of *Cyperus rotundus*, glycoside derivatives from the *n*-hexane extract of *Carica papaya* leaves (Simarmata et al., 2023), etc. Certainly, these reports had ensured the data that *n*-hexane fraction of polar extracts e.g. methanol can attract glucoside derivatives such as steroids and terpenoids due to the steric effects of their structures.

Druglikeness Lipinski's Analysis

Druglikeness analysis is used to predict the potency of a compound as a drug candidate. Druglikeness analysis in this study used Lipinski's five rules including the following parameters: molar mass < 500 Da; log P (lipophilicity) < 5; hydrogen bond donors <5; hydrogen bond acceptors < 10; and molar refractivity 40-130 (Lipinski, 2004). Compounds can be said to have potency as drug agents if they achieve 3 parameters of the Lipinski rules. As a result, there are 22 of 35 compounds achieving at least 3 rules of the Lipinski rules and have the potency as drug agents as shown in **Table 1**.

Molecular Docking Analysis

Molecular docking analysis is one approach that can be used to determine the potency of a compound as a drug candidate by inhibiting specific target proteins (Wang et al., 2019). Docking will tether the ligand to the receptor's active site to obtain a receptor-ligand complex with a binding affinity value (Abdul-Hammed et al., 2022). As a result, there are 22 compounds that comply with the Lipinski rule docked with EGFR protein have binding affinity values ranging from -5.2 kcal/mol to -9.3 kcal/mol (presented in **Table 2**). From this, there are 4 compounds found having lower binding affinity values than the control drug (icotinib), namely isoengeletin (i); epibetulinic acid (ii); myricitrin (iii), and stigmasterol-3-*O*-β-D-glucoside (iv). The fourth compound has the lowest binding affinity compared to the other compounds. Binding affinity is the value of the stability of the complex formed between the receptor and the ligand. A stable complex has a low binding affinity value and will also has optimal activity inhibition (Awaluddin et al., 2023; Muhamed-Ahmed et al., 2022).

The results of visualizing the interaction of protein receptors and ligands (seen in **Figure 3**) showed that the ligands and receptors can form various interactions. The interactions formed are vulnerable to the stability of the complexes formed. In this study, the interactions formed are hydrogen bonds, electrostatic bonds, hydrophobic bonds, unfavorable bonds, and Van der Waals forces. A stable complex is also a complex having few unfavorable bonds (Freire, 2008). In addition, there are amino acid residues or interaction positions that are the same as the control drug compounds, as seen in **Table 3**. The similarity of the inhibition positions indicated that these compounds have similar activity to the control drug compounds (Kharisma et al., 2021; Sururi et al., 2024). As a result, isoengeletin has a binding position with 4 binding positions; myricitrin and stigmasterol-3-*O*-β-D-glucoside have similarities at 5 amino acid positions, whereas epibetulinic acid had no resemblance to the icotinib control.

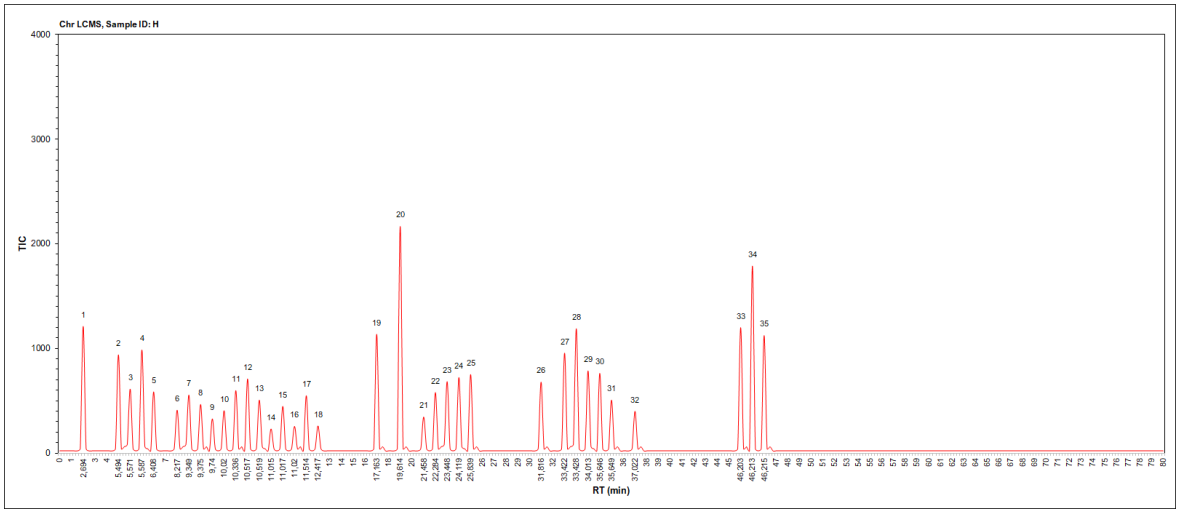

Figure 1. Chromatogram of LCMS analysis of *n*-hexane fraction of *S. samarangense* stem berk

Table 1. Compounds of *n*-hexane fraction and druglikeness lipinski's profile

Peak	Comp. (%)	Compound	Lipinski's Rule of Five Parameters					Note
			MW[a]	HA[b]	HD[c]	LP[d]	MR[e]	
1	4.70	Eugenol	164.20	2	1	2.01	49.06	Yes
2	3.63	β-Caryophyllene	204.35	0	0	4.63	68.78	Yes
3	2.36	Eugenin	206.19	4	1	0.24	55.97	Yes
4	3.81	Eugenol acetate	206.24	3	0	2.43	58.54	Yes
5	2.26	Benzyl benzoate	212.24	2	0	3.41	62.21	Yes
6	1.58	Pinocembrin	256.25	4	2	1.27	69.55	Yes
7	2.14	(−)-Strobopinin	270.28	4	2	1.52	74.51	Yes
8	1.80	8-Methylpinocembrin	270.28	4	2	1.52	74.51	Yes
9	1.26	Uvangoletin	272.30	4	2	1.91	76.47	Yes
10	1.56	Stercurensin	284.31	4	2	2.08	81.75	Yes
11	2.31	2',4'-Dihydroxy-6'-methoxy-3'-methyldihydrochalcone	286.32	4	2	2.15	81.43	Yes
12	2.74	4',6'-Dihydroxy-3',5'-dimethyl-2'-methoxychalcone	298.33	4	2	2.31	86.72	Yes
13	1.96	7-Hydroxy-5-methoxy-6,8- dimethylflavanone	298.33	4	1	2.00	83.95	Yes
14	0.90	Aurentiacin	298.33	4	1	2.31	86.22	Yes
15	1.72	2',4'-Dihydroxy-6'-methoxy3',5'-dimethylchalcone	298.33	4	2	2.31	86.72	Yes
16	0.99	(+)-6,8-Di-*C*-methylpinocembrin-5-methylether	298.33	4	1	2.00	83.95	Yes
17	2.12	Myricetin	318.24	8	6*	-1.08	80.06	Yes
18	1.00	Biflorin	354.31	9	6*	-2.45	84.12	Yes
19	4.39	β-Sitosterol	414.71	1	1	6.73*	133.23	Yes
20	8.39	Lupeol	426.72	1	1	6.92*	135.14	Yes
21	1.33	**Isoengeletin**	434.39	10	6*	-1.42	103.95	Yes
22	2.24	Betulin	414.71	1	1	6.73*	133.23	Yes
23	2.64	**Epibetulinic acid**	456.70	3	2	5.82*	136.91	Yes
24	2.79	**Myricitrin**	464.38	12*	8*	-2.32	111.02	Yes
25	2.91	Mearnsitrin	478.40	12*	7*	-2.11	115.49	Yes
26	2.62	Myricetin-3-*O*-(4"-*O*-malonyl)-α-L-rhamnopyranoside	610.52*	16*	10*	-3.62	141.98*	No
27	3.77	**Stigmasterol-3-*O*-β-D-glucoside**	574.83*	6	4	3.85	165.14*	Yes
28	4.60	β-Sitosterol-D-glucoside	576.85*	6	4	3.96	165.61*	Yes
29	3.04	Campesterol glucoside	562.82*	6	4	3.78	160.81*	Yes
30	2.96	Desmanthin 1	616.48*	16*	10*	-2.50	146.73*	No
31	1.96	Myricetin-3-(3"-galloylrhamnoside)	616.48*	16*	10*	-2.50	146.73*	No
32	1.6	Strictinin	634.45*	18*	11*	-2.42	141.85*	No
33	4.64	Sitosteryl stearate	681.17*	2	0	9.66*	219.88*	No
34	6.93	Cycloartenyl stearate	693.18*	2	0	9.82*	221.79*	No
35	4.35	Lupenyl stearate	693.18*	2	0	9.82*	221.79*	No

Note: [a]molecular weight; [b]hydrogen bond acceptor; [c]hydrogen bond donor; [d]lipophilicity; [e]molar refractivity; * = does not comply with lipinski's rule

Table 2. Binding affinity of compounds based on molecular docking analysis

No	Compound	Binding Affinity (kcal/mol)
1	Icotinib (Control Drug)	-8.9
2	eugenol	-5.2
3	β-Caryophyllene	-6.5
4	Eugenin	-6.2
5	Eugenol acetate	-5.7
6	Benzyl benzoate	-6.3
7	Pinocembrin	-7.7
8	(−)-Stropopinin	-8.4
9	8-Methylpinocembrin	-8.3
10	Uvangoletin	-6.9
11	Stercurensin	-7.2
12	2',4'-Dihydroxy-6'-methoxy-3'-methyldihydrochalcone	-7.2
13	4',6'-Dihydroxy-3',5'-dimethyl-2'-methoxychalcone	-6.8
14	7-Hydroxy-5-methoxy-6,8- dimethylflavanone	-8.4
15	Aurentiacin	-6.8
16	2',4'-Dihydroxy-6'-methoxy3',5'-dimethylchalcone	-6.8
17	(+)-6,8-Di-*C*-methylpinocembrin-5-methyl ether	-8.4
18	Myricetin	-8.3
19	Biflorin	-8.2
20	β-Sitosterol	-8.6
21	Lupeol	-8.7
22	**Isoengeletin**	**-9.1**
23	Betulin	-8.2
24	**Epibetulinic acid**	**-9.1**
25	**Myricitrin**	**-8.9**
26	Mearnsitrin	-8.8
27	**Stigmasterol-3-*O*-β-D-glucoside**	**-9.3**
29	β-Sitosterol-D-glucoside	-8.1
30	Campesterol glucoside	-8.8

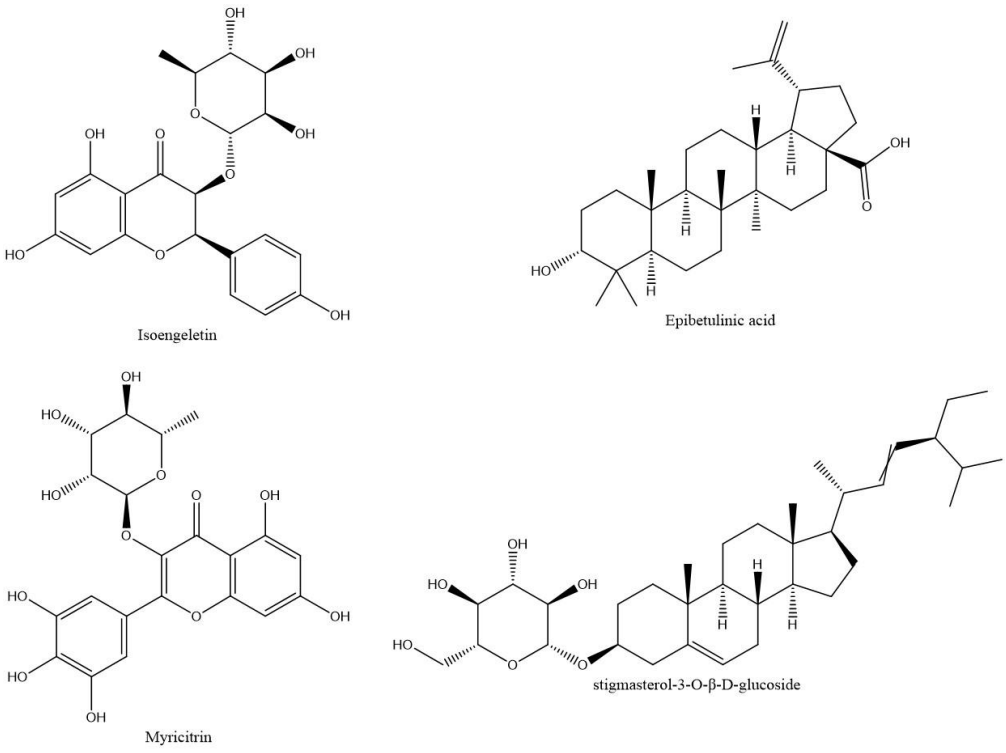

Figure 2. Structure of potential compounds

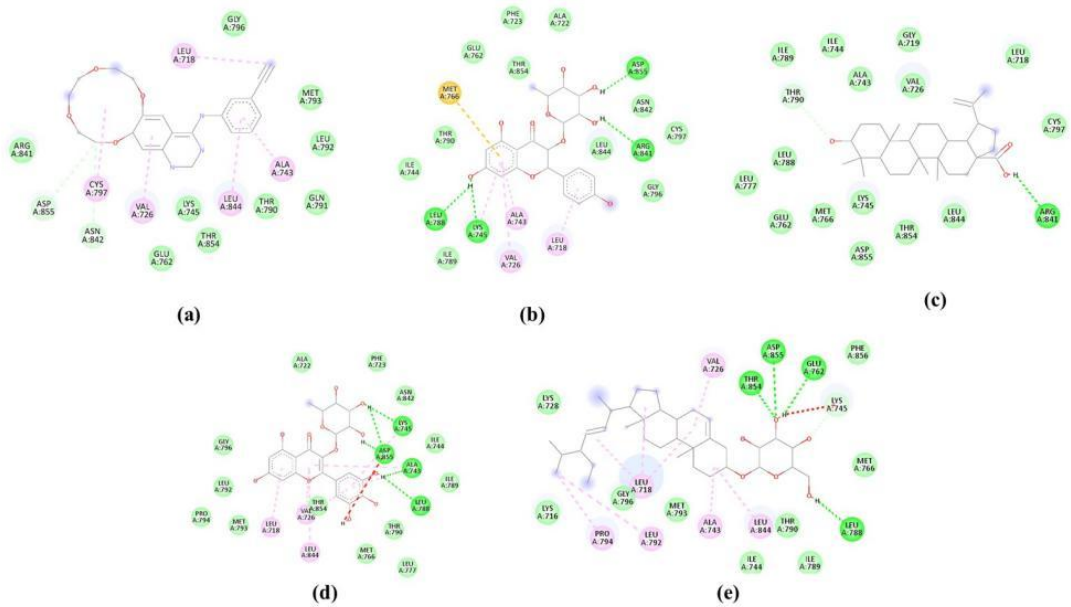

Figure 3. Visualization of potential and control compound with EGFR receptor: (**a**) Icotinib (control drug); (**b**) isoengeletin; (**c**) epibetulinic acid; (**d**) myricitrin; and (**e**) stigmasterol-3-*O*-β-D-glucoside

Table 3. Amino acid residues with similar to icotinib

Compounds	Similar Amino Acid Residue
Isoengeletin (i)	Val 726, Ala 743, Leu 718, Asp 855
Epibetulinic acid (ii)	-
Myricitrin (iii)	Leu 718, Leu 844, Val 726, Ala 743, Asp 855
Stigmasterol-3-*O*-β-D-glucoside (iv)	Leu 718, Ala 743, Leu 844, Val 726, Asp 855.

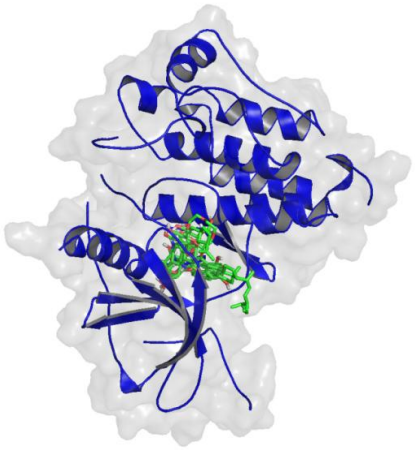

Figure 4. Surface area interaction of potential and control compounds in active site

Table 4. PASS Prediction result of potential compound as anti-neoplastic and anti-carcinogenic substances

Compounds	Anti-neoplastic	Anti-carciogenic
Isoengeletin (i)	Pa= 0,819	Pa = 0,909
	Pi= 0,010	Pi= 0,002
Epibetulinic acid (ii)	Pa= 0,925	Pa= 0,493
	Pi= 0,005	Pi=0,020
Myricitrin (iii)	Pa= 0,878	Pa= 0,955
	Pi= 0,005	Pi= 0,001
Stigmasterol-3-O-β-D-glucoside	Pa= 0,630	Pa= 0,847
	Pi= 0,039	Pi= 0,004

PASS Prediction

PASS Prediction is a web server to provide a web server that predicts more than 300 pharmacological effects and biochemical mechanisms based on the structural formula of a substance. It can be used efficiently to find new targets (mechanisms) for some ligands and vice versa to reveal new ligands for some biological targets (Abdul-Hammed et al., 2022; Sururi et al., 2022). As presented in **Table** 4, it can be shown that the four potential compounds have potency as anti-neoplastic and anti-carcinogenic, with values of Pa (active potential) and Pi (inactive potential). As reported that a Pa value > 0.7 is categoried as high, while a Pa value > 0.3 is categorized as medium (Rahmaningsih & Pujiastutik, 2019; Sururi et al., 2022). Compounds (i), (ii), and (iii) have high potency as anti-neoplastic agents, while compound iv has moderate potency as anti-neoplastic agents. Anti-neoplastic agents are used for treating cancer (commonly called anticancer drugs) (Guichard et al., 2017). In addition, compounds i, iii, and iv have high potency, while compound (iii) has moderate potency as anti-carcinogenic. Anti-carcinogenic substances can counteract carcinogenic effects or inhibit the development of cancer cells (WHO, 2003). The difference between anti-neoplastic and anti-carcinogenic is that anti-neoplastic used for cancer treatment is commonly called anticancer, while anti-carcinogenic is a drug used to counteract carcinogenic (preventive) effects.

CONCLUSIONS

Based on exposure above, it can be concluded that there are 35 compounds found in the *n*-hexane fraction of the methanol extract of *S. samarangense* stem bark. However, there are 4 compounds having potency as anticancer, namely: isoengeletin; epibetulinic acid; myricitrin; and stigmasterol-3-*O*-β-D-glucoside. Based on PASS prediction, it can be stated that the four compounds have medium-high active potential as anti-neoplastic and anti-carcinogenic substances.

ACKNOWLEDGEMENTS

Thanks to Mr. Ariesandy as the laboratory assistant who has helped in the LC-MS process.

REFERENCES

Abdul-Hammed, M., Adedotun, I. O., Olajide, M., Irabor, C. O., Afolabi, T. I., Gbadebo, I. O., Rhyman, L., & Ramasami, P. (2022). Virtual screening, ADMET profiling, PASS prediction, and bioactivity studies of potential inhibitory roles of alkaloids, phytosterols, and flavonoids against COVID-19 main protease (M(pro)). *Natural Product Research*, *36*(12), 3110–3116. https://doi.org/10.1080/14786419.2021.1935933

Awaluddin, F., Batubara, I., & Tri Wahyudi, S. (2023). Virtual Screening of Natural Compounds Against Six Protein Receptors Coded by The SARS-CoV-2 Genome. *Molekul. 18*(1), 147–158. https://doi.org/10.20884/1.jm.2023.18.1.7884.

Chae, Y. K., Anker, J. F., Bais, P., Namburi, S., Giles, F. J., & Chuang, J. H. (2018). Mutations in DNA repair genes are associated with increased neo-antigen load and activated T cell infiltration in lung adenocarcinoma. *Oncotarget*. 9(8):7949-7960. https://doi.org/10.18632/oncotarget.23742.

da Costa Cordeiro, B.M.P., Nataly Diniz de Lima Santos, Magda Rhayanny Assunção Ferreira, Larissa Cardoso Corrêa de Araújo, Alexsander Rodrigues Carvalho Junior, Alan Diego da Conceição Santos, Ana Paula de Oliveira, Alexandre Gomes da Silva, Emerson Peter da Silva Falcão, Maria Tereza dos Santos Correia, Jackson Roberto Guedes da Silva Almeida, Luís Cláudio Nascimento da Silva, Luiz Alberto Lira Soares, Thiago Henrique Napoleão, Márcia Vanusa da Silva1, & Patrícia Maria Guedes Paiva. (2018). Hexane extract from Spondias tuberosa (Anacardiaceae) leaves has antioxidant activity and is an anti-Candida agent by causing mitochondrial and lysosomal damages. *BMC Complementary and Alternative Medicine*, 18:284. https://doi.org/10.1186/s12906-018-2350-2.

Freire, E. (2008). Do enthalpy and entropy distinguish first in class from best in class? *Drug Discovery Today*, *13*(19), 869–874. https://doi.org/https://doi.org/10.1016/j.drudis.2008.07.005

Guichard, N., Guillarme, D., Bonnabry, P., & Fleury-Souverain, S. (2017). Antineoplastic drugs and their analysis: a state of the art review. *The Analyst*, *142*(13), 2273–2321. https://doi.org/10.1039/c7an00367f

Hsiehchen, D., Hsieh, A., Samstein, R. M., Lu, T., Beg, M. S., Gerber, D. E., Wang, T., Morris, L. G. T., & Zhu, H. (2020). DNA Repair Gene Mutations as Predictors of Immune Checkpoint Inhibitor Response beyond Tumor Mutation Burden. *Cell Reports Medicine*. *1*(3):100034. https://doi.org/10.1016/j.xcrm.2020.100034

Inamura, K., Ninomiya, H., Ishikawa, Y., & Matsubara, O. (2010). Is the Epidermal Growth Factor Receptor Status in Lung Cancers Reflected in Clinicopathologic Features? *Archives of Pathology & Laboratory Medicine*, *134*(1), 66–72. https://doi.org/10.5858/2008-0586-RAR1.1

Jayaram, B., Singh, T., Mukherjee, G., Mathur, A., Shekhar, S., & Shekhar, V. (2012). Sanjeevini: a freely accessible web-server for target directed lead molecule discovery. *BMC Bioinformatics*, *13*(17), 1–13. https://doi.org/10.1186/1471-

2105-13-S17-S7.

Kharisma, V., Widyananda, M., Nege, A., Naw, S., & Nugraha, A. (2021). Tea catechin as antiviral agent via apoptosis agonist and triple inhibitor mechanism against HIV-1 infection: A bioinformatics approach. *Journal of Pharmacy & Pharmacognosy Research*, *9*(4), 435–445. https://doi.org/10.56499/jppres21.1009_9.4.435

Koveitypour, Z., Panahi, F., Vakilian, M., Peymani, M., Seyed Forootan, F., Nasr Esfahani, M. H., & Ghaedi, K. (2019). Signaling pathways involved in colorectal cancer progression. *Cell & Bioscience*, *9*(1), 97. https://doi.org/10.1186/s13578-019-0361-4

Lagunin, A., Stepanchikova, A., Filimonov, D., & Poroikov, V. (2000). PASS: prediction of activity spectra for biologically active substances. *Bioinformatics*, *16*(8), 747–748. https://doi.org/ 10.1093/bioinformatics/16.8.747.

Lipinski, C. A. (2004). Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technologies*, *1*(4), 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007

Metasari, S., Elfita, E., Muharni, M., & Yohandini, H. (2020). Antioxidant compounds from the stem bark of *Syzygium samarangense* L. *Molekul*, *15*(3), 175–183. https://doi.org/10.20884/1.jm.2020.15.3.626.

MuhamedAhmed, A., Niazi, Z. R., Hanif, M., Rafey, A., Iqbal, K., Pieters, L., & Amin, A. (2022). Computational analysis and in vitro investigation on Citrus flavonoids for inflammatory, diabetic and AGEs targets. *Brazilian Journal of Pharmaceutical Sciences*, *58*. https://doi.org/10.1590/s2175-97902022e201056

Pan, H., Liu, R., Li, S., Fang, H., Wang, Z., Huang, S., & Zhou, J. (2014). Effects of icotinib on advanced non-small cell lung cancer with different EGFR phenotypes. *Cell Biochemistry and Biophysics*, *70*(1), 553–558. https://doi.org/10.1007/s12013-014-9955-y

Parasuraman, S. (2011). Prediction of activity spectra for substances. *Journal of Pharmacology & Pharmacotherapeutics*, *2*(1), 52–53. https://doi.org/10.4103/0976-500X.77119

Patel, R. (2014). EGFR Signaling and its inhibition by EGFR inhibitors in NSCLC. *International Journal of Applied Sciences and Biotechnology*, *2*(4), 375–388. https://doi.org/10.3126/ijasbt.v2i4.11263.

Rahmaningsih, S., & Pujiastutik, H. (2019). An in vitro and *in silico* evaluation of the antibacterial activity of the bioactive compounds in Majapahit (*Crescentia cujete* L.) fruit. *Veterinary World*, *12*(12), 1959–1965. https://doi.org/10.14202/vetworld.2019.1959-1965.

Samar, S. A., Abeer, M. A. & Omayma A.E. (2013). Phytochemical Investigation and Molecular Profiling by p21 and NF-κB of *Chorisia crispiflora* Hexane Extract in Human Breast Cancer Cells *in Vitro. British Journal of Pharmaceutical Research.* 3(1): 78-89. https://www.researchgate.net/publication/256390331.

San Feliciano, A., Medarde, M., Del Rey, B., Miguel Del Corral, J. M., & Barrero, A. F. (1990). Eudesmane glycosides from *Carthamus lanatus*. *Phytochemistry*, 29(10), 3207–3211. https://doi.org/https://doi.org/10.1016/0031-9422(90)80186-K.

Sari, D.A., Sulistyarini, I. & Rahardian, M.R.R. (2021). Anti-bacterial activity of ethanol extract, *n*-hexane fraction, ethyl acetate fraction and water fraction from Dragon Fruit Stem (*Hylocereus polyrhizus*) against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Jurnal Ilmu Kesehatan,* 9(2), 2614-6703.

Sayed, H. M., Mohamed, M. H., Farag, S. F., Mohamed, G. A., & proksch, P. (2007). A new steroid glycoside and furochromones from *Cyperus rotundus* L. *Natural Product Research,* 21(4), 343–350. https://doi.org/10.1080/14786410701193056.

Simarmata, C. W. R. B., Nasution, H. M., Nasution, M. P., & Rahayu, Y. P. (2023). Phytochemical screening and isolation of steroid/triterpenoid compounds from n-hexane extract of Papaya leaves (Carrica papaya L). *Journal of Pharmaceutical and Sciences*, *6*(4 SE-Original Articles), 1819–1830. https://doi.org/10.36490/journal-jps.com.v6i4.324.

Sujatha, J. & Asokan, S. 2018. Phytochemical analysis and Antioxidant effect of hexane extract of *Cassia fistula* using FT-IR and GC-MS analysis, *International Journal of Pharmaceutics & Drug Analysis*, 6(2), 173 – 179. http://ijpda.com; ISSN: 2348-8948.

Sururi, A. M., Raihan, M., Aisa, E. R., Safitri, F. N., Constaty, I. C., & Tukiran. (2022). Anti-Inflammatory Activity of Stem Bark Dichloromethane Fraction *Syzygium samarangense* Extract as COX-2 Inhibitor: A Bioinformatics Approach. *Jurnal Kimia Riset*, *7*(2), 94–100. https://doi.org/10.20473/jkr.v7i2.39662

Sururi, A. M., Tukiran, T., Aisa, E. R., & Raihan, M. (2024). Identification of bioactive compounds and ADMET profile of stem bark of *Syzygium samarangense* and their potential as antibreast cancer and antiinflammatory. *Journal of Applied Pharmaceutical Science*, *14*(02), 273–280. https://doi.org/10.7324/JAPS.2024.143017

Tarigan, C., Pramastya, H., Insanu, M., & Fidrianny, I. (2022). *Syzygium Samarangense*: Review of

Phytochemical Compounds and Pharmacological Activities. *Biointerface Research in Applied Chemistry*, *12*(2), 2084–2107. https://doi.org/10.33263/BRIAC122.20842107

Thakur, C. (2019). *Chapter 2 - An Overview, Current Challenges of Drug Resistance, and Targeting Metastasis Associated With Lung Cancer* (P. B. T.-N.-B. T. D. D. S. for L. C. Kesharwani (ed.); pp. 21–38). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-815720-6.00002-2

Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455–461. https://doi.org/10.1002/jcc.21334

Tukiran, T., Suyatno, S., & Safitri, F. N. (2021). Identification of the Chemical Constituents of the Selected Fraction of the Dichloromethane Extract of Syzygium samarangense Stem Bark Using LC-ESI-MS and Evaluation Its Potential as Antifungal Agent. *Indonesian Journal of Chemistry*, *21*(2), 340–349.

Ushie O.A., Kendeson, A.C., Longbab, B.D., Ogofotha, P.U., Nongu, S.T. & Bako, B. 2022. Profiling Phytochemicals And Antimicrobial Activities Of Hexane And Acetone Crude Extracts Of *Sterculia Setigera* Leaf. *J. Chem. Soc. Nigeria*, 47(6), 1296 - 1302.

Wang, J., Ge, Y., & Xie, X.-Q. (2019). Development and Testing of Druglike Screening Libraries. *Journal of Chemical Information and Modeling*, *59*(1), 53–65. https://doi.org/10.1021/acs.jc/im.8b00537

Wee, P., & Wang, Z. (2017). Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways. *Cancers*, *9*(5). https://doi.org/10.3390/cancers9050052

WHO. (2003). Diet, nutrition and the prevention of chronic diseases. In *World Health Organization - Technical Report Series* (Issue 916). https://doi.org/10.1093/ajcn/60.4.644a

WHO. (2022). *World Health Organization*. Health topic

Zhang, J., Zhan, Y., Ouyang, M., Qin, Y., Zhou, C., & Chen, R. (2014). Fatal interstitial lung disease associated with icotinib. *Journal of Thoracic Disease; Vol 6, No 12 (December 31, 2014): Journal of Thoracic Disease*. https://jtd.amegroups.com/article/view/3584