Pharmacogn J. 2023; 15(2): 393-398
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Analysis of the Metabolite Compound of the Ethanol Extract of *Chrysanthemum cinerariifolium* Stem and Activity for inhibition of Oral Squamous Cell Carcinoma (OSCC) *in silico* study

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ABSTRACT

Background: Oral cancer is a deadly disease that is expected to increase yearly. Current cancer treatment methods have side effects. *C. cinerariifolium* plants have potential as anticancer agents. **Objective**: To evaluate the anti-OSCC properties of the ethanol extract of *C. cinerariifolium* stems *via* an *in-silico* study. **Materials and Methods:** Analysis of active compounds in ethanol extracts of *C. cinerariifolium* stems using TLC and UPLC-QToF-MS/MS metabolic profiling. The data were analysed statistically using principal component analysis (PCA). *In silico* of *C. cinerariifolium* compounds *on protein* (PI3K and Cyclin D) from OSCC. **Results**: TLC procedures utilizing UV light with λ 366 nm after spraying with H_2SO_4 revealed multiple-colored spots, indicating that H_2SO_4 is a specific spray detector for terpenoid and carotene. Metabolic profiling in ethanol extract of *C. cinerariifolium* stem included Pronethalol (3.96%), 1-(4-Methoxyphenyl)-N-(1 naphthylmethyl) methanamine (7.34%), Orphenadrine (24.27%), Pentazocine (5.09%), 4-(Dodecyloxy) aniline (6.30%), Linoleamide (4.95%), and Pheophorbide A (8.05%). Orphenadrine had the highest percentage. Based on the Lipinski rule of five, pronethalol has the potential to be used as a drug-like therapy for OSCC. The anticancer activity profile is predicted by PASS online with a likely range of 0.065 to 0.385. An *in-silico* study showed that the strongest binding affinity is pronethalol to Cyclin D1 and pheophorbide A to the PI3K protein. **Conclusion**: The active metabolite of the ethanolic extract of *C. cinerariifolium* stem exhibits potency against oral squamous cell carcinoma *via* the downregulation of the cell cycle (cyclin D1) and P13K, especially pronethalol.

Key words: Cyclin D1, *Chrysanthemum cinerariifolium*, OSCC, P13K.

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History

- Submission Date: 19-01-2023;
- Review completed: 05-03-2023;
- Accepted Date: 07-03-2023.

DOI : 10.5530/pj.2023.15.61

Article Available online

http://www.phcogj.com/v15/i6

INTRODUCTION

Oral cancer is a deadly disease that causes the death of approximately 128,000 people worldwide.[1] Cases of oral cancer in Indonesia are approximately 1.5% of all new cancer cases, and 1.1% of cases cause death.[2] In 2012, 5,329 cases of oral cancer were documented in Indonesia; this number is estimated to rise by 21.5% in 2020.[3] Oral squamous cell carcinoma (OSCC) is a malignant neoplasm of the oral cavity followed by verrucous carcinoma, undifferentiated carcinoma, and salivary adenocarcinoma. More than 90% of cancers in the oral cavity are oral squamous cell carcinoma (OSCC).[4]

Cancer is characterized by excessive cell proliferation, angiogenesis, and evasion of apoptosis. Cancer formation is connected with cyclins and cyclin-dependent kinases (CDKs), which regulate the eukaryotic mitotic cycle.[1] Cyclin D1 commences the cancer cell cycle and pathogenesis; its high expression can stimulate tumor development.[5] Phosphatidylinositol-3 kinase (PI3K)-Akt-mTOR controls the progression of the cancer cell cycle through the G0-G1-S phase and reprograms the metabolism of cancer cells.[6] These cancer cell proteins (cyclin D1 and P13K) are the current targets of cancer therapy development.

Standard treatments for early-stage oral cancer include radiotherapy or surgery as first-line therapy, whereas advanced malignancies are treated with surgery, chemoradiotherapy, combination therapy, prophylaxis, and complementary medicines.[7] However, there is a side effect to this therapeutic strategy, which is the slow development of cancer cell resistance to therapy. Thus, a novel cancer therapy method is needed.[8]

By simulating the interaction between active metabolites and their target proteins, the active constituents of medicinal plants can serve as modulators of the major cellular signaling pathways involved in cancer activation.[9] Phytochemicals in herbs also have the benefits of less toxicity, minimal side effects, and bolstering patients' immune systems.[1] Ninety percent of the 121 cancer treatments were derived from herbs. Between 1981 and 2002, 48 of the 65 newly developed cancer therapies contained natural components.[8]

C. cinerariifolium plants are abundant in Indonesia, and their anticancer potential has been demonstrated.[10] According to Mutiah *et al.*[11], *C. cinerariifolium* leaf extract has anticancer activity on T47D breast cancer cells by inhibiting the cell cycle and inducing apoptosis. By establishing complex bonds through binding to ER-α (alpha estrogen receptor), attaching to the estrogen response element (ERE), and activating the corepressor protein NcoR, the flavonoid chemicals present in the *Chrysanthemum* plant can inhibit the replication and proliferation of cancer cells. Until now, there has

Cite this article: Listiyana A, Rachmawati YL, Susianti H, Nurdiana, Sujuti H, et al.. Analysis of the Metabolite Compound of the Ethanol Extract of *Chrysanthemum cinerariifolium* Stem and Activity for inhibition of Oral Squamous Cell Carcinoma (OSCC) *in silico* study. Pharmacogn J. 2023;15(2): 393-398.

been no scientific analysis of the active ingredients of *C. cinerariifolium* stems. Therefore, this study aims to explore and analyse the active metabolites of the ethanol extract of *C. cinerariifolium* stems against OSCC.

MATERIALS AND METHODS

Plant determination

C. cinerariifolium plants were obtained from Punten Village, Batu City, East Java, and determined at UPT Materia Medica Batu, East Java, Indonesia by number: 074/153/102.20-A/2-22.

Sample preparation

The stems are cut from *C. cinerariifolium* plants. Furthermore, the stems were washed, sun-dried, and sorted. The stem was ground into a powder.

Water content analysis

Using a moisture content analyser, the water content was analysed. The percentage of the water content measurement is displayed on the device's display.

Extraction of *C. cinerariifolium* stem

C. cinerariifolium stem powder was placed into an Erlenmeyer flask, and 96% ethanol was added at a ratio of 1:20. The mixture was extracted using UAE (Ultrasonication Assisted Extraction) for 2 minutes with three replications. In addition, the filtrate was evaporated using a rotary evaporator at 50°C to obtain a crude extract and then concentrated using an oven at 40°C until the extract's consistency became concentrated.[10]

Thin layer chromatography (TLC)

In identifying compounds with a TLC silica gel plate, 60 F254 was utilized as a stationary phase by optimizing the mobile phase components n-hexane and ethyl acetate (8: 2). While using H_2SO_4 10% will change the color's appearance, using the Thin Layer Chromatography (TLC) Visualizer, distinguish compound stains. After dripping the extract onto the stationary phase, it was eluted. When the elution process approached the stationary phase's upper limit, it was halted. In addition, the formation of spot stains on the TLC plate was observed using TLC-Visualizer in multiple rays, namely, white light, ultraviolet (UV) light with λ 254 nm, and UV light with λ 366 nm, which were then compared between TLC plates before and after H_2SO_4 spraying.[11,12]

Metabolite profiling analysis

Metabolite determination from *C. cinerariifolium* stem extract utilizing the UPLC-QToF MS/MS instrument with three replicates. The obtained data were in the form of chromatograms processed with Masslynk version 4.1 software. Data analysis was based on the peak area and m/z spectrum of each peak, followed by later detection with www.chemspider.com, https://pubchem.ncbi.nih.gov/, and https://massbank.eu database.

In a 10 ml volumetric flask, dissolve 10 mg of *C. cinerariifolium* stem ethanol extract in methanol and add 5 µl of microsyringe. The liquid used was a mixture of (A) Water (HPLC grade)/formic acid (Merck, Darmstadt, Germany) 99.9/0.1 [v/v]; (B) Acetonitrile (Merck, Darmstadt, Germany)/formic acid 99.9/0.1 [v/v] and the gradient elution system. The data are in the form of peak area and m/z spectra for each detected peak and were analyzed using the databases www.chemspider.com, https://pubchem.ncbi.nih.gov/, and https://massbank.eu.

Statistical analysis

The data profile was then analysed statistically by principal component analysis (PCA) using Minitab version 17.0. The identification data from the extract component were categorized according to the sample's origin. Principal component analysis (PCA) was applied to the area percentages to generate the loading plot and score plot. Minitab 17 was used to perform PCA (Minitab Inc., Pennsylvania, USA).

In silico study

The 3D structure of the compound was taken from the PubChem database integrated with NCBI (https://pubchem.ncbi.nlm.nih.gov/), while the 3D structure of the protein (PI3K and Cyclin D) was taken from the Protein Data Bank database (https://www.rcsb. org/). The active compounds with PubChem ID are 1-(4-methoxyphenyl)-N-(1-naphthylmethyl) methanamine (101900082); orphenadrine (45040161); pentazocine (441278); 4-(dodecyloxy) aniline (3498633); linoleamide (6435901); pheophorbide A (253193); and pronethalol (4930). Proteins as controls with PubChem ID are Linoed Acid for Cyclin D control (445639) and Acetylshikonin for PI3K control (479501). The 3D structures of Cyclin D1 and PI3K were downloaded from the database. The Protein Data Bank (PDB) ID for Cyclin D1 (2 W9F) and PI3K (4l23).

Furthermore, the anticancer activity of the compound was predicted by PASS Online (http://www.way2drug.com/passonline/predict.php). All compounds were continued for drug-likeness testing by the Lipinski rule of five, which has good permeability and good oral bioavailability (molecular weight <500 g/mol, logP value <5, number of hydrogen bond donors <5, and number of hydrogen bond acceptors <10) through SwissADME (http://www.swissadme.ch/)

Docking analysis was conducted using the autodockvina program on the PyRx software with the parameters RMSD maximum 2, Binding poses maximum 10, and 3 replications. A kcal/mol unit score represents bond affinity. Using Discovery Studio version 21.1.1, a 3D view of the ligand binding area on the target protein and residues, the distance, and the type of bond in the amino acid components obtained was obtained from the docking results. Cyclin D and PI3K proteins were subjected to docking analysis. The dimensions of the Cyclin D1 protein grid is X=70,558A; Y=64,058A; Z=82,242A; Volume 371.72A3; surface 311.82A2; with the grid coordinate center at X= 6.501A; Y= 5.665A; Z= 29,289 A. The dimensions of the PI3K protein grid are X=88,528A; Y=112.139A; Z=101,368A; Volume 100.63A4; surface 60.537A3; with the center of the grid coordinates at X= 18,043A; Y= 34,091A; Z= 30,205A.

RESULTS

Water content analysis

The moisture content of *C. cinerariifolium* stem powder was 3.24%.

Compound screening by TLC method-visualizer

The TLC results obtained before and after spraying H_2SO_4 stains are described in Figure 1. After being sprayed with a stain and illuminated with white light (A), purple spots can be observed on the TLC plate. On the TLC plate illuminated with UV light with λ 254 nm before spraying, brown spots can be observed (B). Before and after spraying, a difference is visible on the TLC plate under UV light with λ 366. After being sprayed, the TLC plate displayed numerous stains of various hues. Before being sprayed, the TLC plate with UV light at λ 366 revealed a red stain (C), but after being sprayed with H_2SO_4, there were spots of multiple colors, including red, pink, yellow, and orange (D).

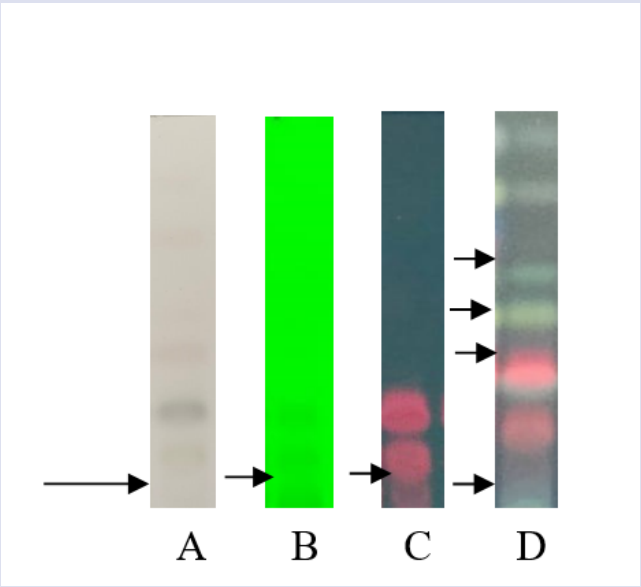

Figure 1: The appearance of TLC stains. A. TLC plate with white light after spraying with H2SO4, B. TLC plate under UV light of λ 254 nm before spraying with H_2SO_4, C. TLC plate under UV light of λ 366 nm before spraying with H_2SO_4, D. TLC plate on white light after spraying with H2SO4 (colors shown with arrow).

Metabolic profiling of the *C. cinerariifolium* stem

The UPLC-QToF-MS/MS instrument produced chromatogram-like results. The polarity of compounds that appear first on the peak chromatogram decreases as they move to the next peak. At this point, the procedure was repeated until a chromatogram with a constant peak was acquired. Several compounds have been identified in the stem extract of *C. cinerariifolium* based on detection using a database with high percentages, including pronethalol (3.96%), 1-(4-methoxyphenyl)-N-(1-naphthylmethyl) methanamine (7.34%), orphenadrine (24.27%), pentazocine (5.09%), 4-(dodecyloxy) aniline (6.30%), linoleamide (4.95%) and pheophorbide A (8.05%). The *C. cinerariifolium* stem extract contained the highest concentration of orphenadrine, which was detected at 11,827 minutes.

Prediction of the anticancer activity of *C. cinerariifolium* stems by PASS online

Anticancer activity is predicted based on the role of compounds in several cancer cases, including cancer-associated disorder treatment, breast cancer-resistant protein inhibitors, prostate cancer treatment, and cancer procoagulant inhibitors.

Anticancer activity is predicted for the identified compounds with a probability ranging from 0.098 to 0.385. Most compounds that passed the Lipinski rule of five drug-likeness tests are 1-(4-methoxyphenyl)-N-(1-naphthylmethyl) methanamine, orphenadrine, pentazocine, and pronathelol. 4-(Dodecyloxy) aniline, whereas linoleamide and pheophorbide A did not meet those requirements.

The potential of anticancer compounds such as Cyclin D1 and PI3K inhibitors

The target compound interacted with the target proteins Cyclin D1 and PI3K. Docking analysis showed that all compounds from the *C. cinerariifolium* stem extract that were analyzed showed their potential as inhibitors of Cyclin D1 and PI3K by binding several amino acids to the two target proteins. Interaction between anticancer compounds on Cyclin D1. The 3D view of the compound shows the same binding region of the active ingredient on the Cyclin D1 protein, as seen in Figure 2. The same active site residues include Lys72, Unk1, Ala187, Cys68, and Leu65. *In silico* analysis described that pronethalol has the highest binding affinity to Cyclin D1 due to its lower binding energy (-7.4 kcal/mol) compared to all other compounds and the control Linoed Acid (-4.7 kcal/mol). Pronethalol showed conventional hydrogen bonds, Pi-anion, Pi-sulfur, and Pi-alkyl interactions with Unk1 residues.

Interaction between anticancer compounds on PI3K. The 3D view of the compound shows the binding sites of the same active ingredient in the P13K protein, as seen in Figure 3. Residues on the same active side, Unk1, Asn677, Phe666, Met811, and Cys838. An *in-silico* study also showed the strongest binding affinity and shared a higher number of hydrogen bonds between pheophorbide A (-9,4 kcal/mol) and PI3K compared to all compounds and the control acetylshikonin (-8 kcal/mol). Pheophorbide A showed conventional hydrogen bonds, Pi-anion, Pi-donor hydrogen bonds, Pi-sigma, and Pi-alkyl interactions with Unk1 and Gln682 residues. The bond formed in anticancer compounds to cancer proteins (Cyclin D1 and P13K) is hydrogen, hydrophobic, and electrostatic.

DISCUSSION

Water content analysis

The principle of moisture analysis is to use infrared or halogen lamps as a heat source to evaporate the water content in the simplicia powder to shorten the testing time (3 to 15 minutes).[13,14] The water content in this powder still meets SNI 01-4320-1996 requirements for herbal powder for beverages, namely, 3-5%.[15] The purpose of measuring the water content in the ethanol extract of *C. cinerariifolium* stems is to determine the stability of the extract and dosage form, as well as the amount of water remaining to facilitate the drying of the extracted sample and prevent rapid fungal growth.[16]

Compound screening by TLC method-visualizer

Thin-layer chromatography (TLC) is a physicochemical separation technique based on a liquid-based mobile phase and a solid-based stationary phase.[17] The TLC method is easy to apply, cost-effective, and widely utilized to analyses natural products. This technique is utilized for the chemical and biological testing of herbal products,[12] identifying specific secondary metabolites, and separating these metabolites' constituents.[18] The phytochemical screening of *C. cinerariifolium* stem extract using silica gel plate 60 F254 aims to accelerate the extract's dissolution due to the presence of ultrasonic waves.[19] The research results on the ethanol extract of *C. cinerariifolium* stem showed a yellow color using UV light with λ 341-389 nm after being sprayed with H_2SO_4 towards the flavonol group. In contrast, red indicates the terpenoid group. Orange refers to carotene.[18]

Polyphenols, such as flavonoids, tannins, and curcumin, have antioxidant properties. Cancers such as hepatoma (Hep-G2), cervical carcinoma (HeLa), and breast cancer are susceptible to the cytotoxic effects of flavonoids (MCF-7). Terpenoids are divided into several classes based on the number of their building blocks, including monoterpenes (e.g., carvone, geraniol, d-limonene, and perillyl alcohol), diterpenes (e.g., retinol and trans-retinoic acid), and triterpenes (e.g., bellic acid, lupeol, oleanic, and ursolic acid). Terpenoids inhibit cancer cell proliferation, growth, and invasion *via* cytotoxic effects. Carotenoids serve as anti-inflammatory and immunomodulatory agents.[20]

Metabolic profiling of the *C. cinerariifolium* stem

UPLC-QToF-MS/MS method for metabolic profiling determination. This method has the benefits of high resolution, speed, and sensitivity and is effective for identifying the structure of components of natural

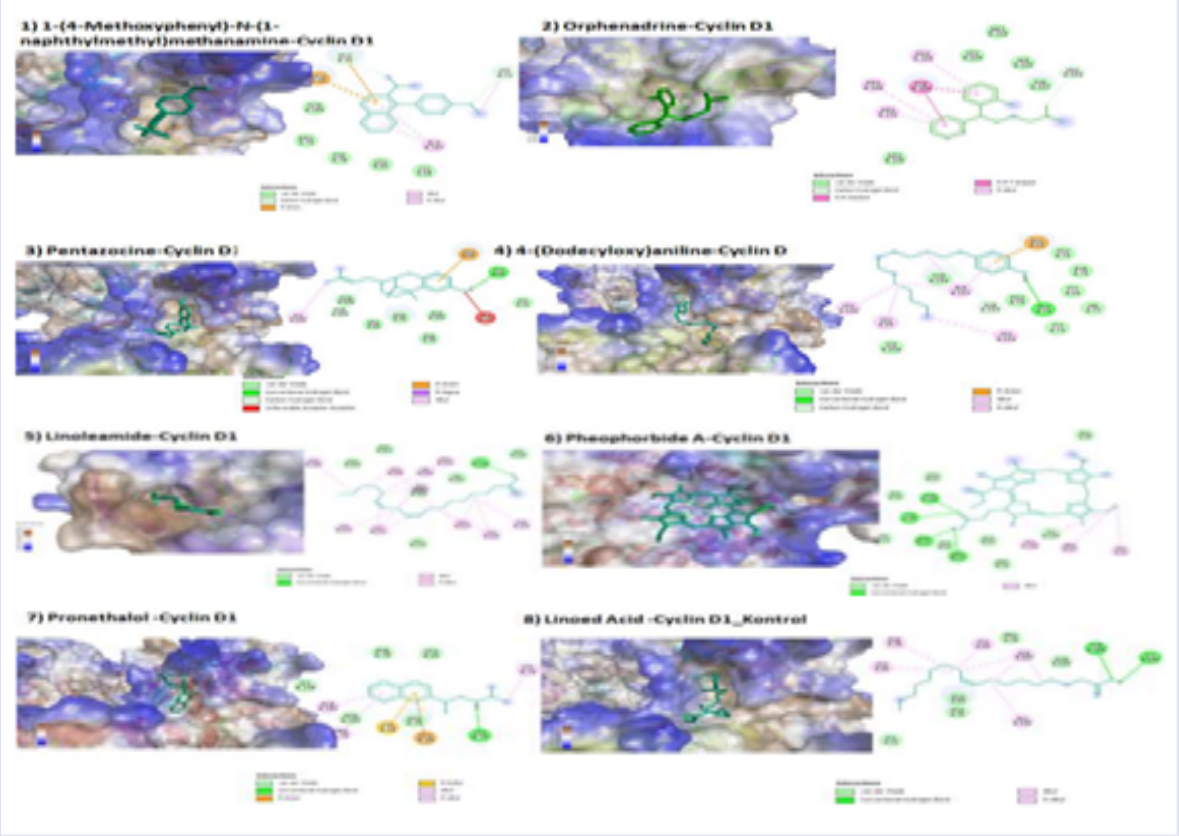

Figure 2: Interaction between anticancer compounds on Cyclin D1 protein.

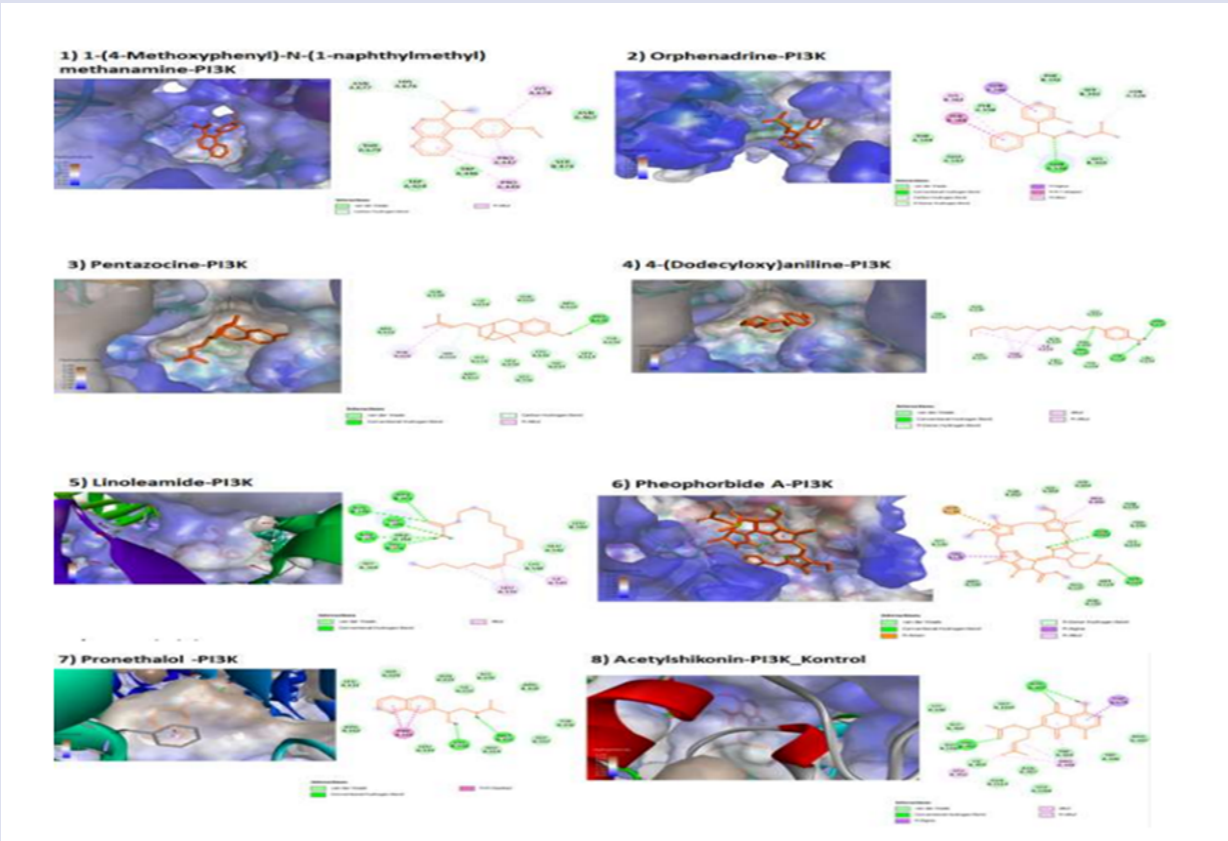

Figure 3: Interaction between anticancer compounds on the PI3K protein.

organic compounds and mixtures.[10] Metabolic profiling of ethanol extract of *C. cinerariifolium stem* is Pronethalol, 1-(4-Methoxyphenyl)-N-(1-naphthylmethyl) methanamine, Orphenadrine, Pentazocine, 4(Dodecyloxy) aniline, Linoleamide, and Pheophorbide A.

Prediction of anticancer activity of the *C. cinerariifolium* stems by PASS online

The identified compounds are predicted to have anticancer activity with a probability of 0.098-0.385. If the prediction of active herbal ingredients is Pa < 0.5, it means that the substance does not show anticancer activity in experiments both *in vitro* and *in vivo*.[21] Due to the possibility that the amount of the active ingredient in the *C. cinerariifolium* stem is relatively small and is a whole compound, an extraction technique is required to extract as much of the active ingredient as possible. Interestingly, most compounds from *C. cinerariifolium* stems met the drug-likeness test criteria according to the Lipinski rule of five, including 1-(4-methoxyphenyl)-N-(1-naphthylmethyl) methanamine, orphenadrine, pentazocine, and pronathelol, so they could be candidates for anticancer drugs. Orphenadrine inhibits the formation of the active CDK-cyclin complex by inhibiting the action of cyclin-dependent kinase (CDK), which regulates the cell cycle by activating kinases.[22] Pentazocine (PTZ), a narcotic antagonist analgesic, is used to treat preoperative or postoperative cancer-related pain. In carrageenan-induced rats, PTZ also reduces oxidative stress markers such as malondyaldehyde and proinflammatory cytokines.[23] Reducing free radicals can affect the increase in the cell membrane and mitochondrial integrity for cell survival.[24]

The potential of anticancer compounds such as Cyclin D1 and PI3K inhibitors

In silico analysis revealed that pronethalol binds to Cyclin D1 with the highest affinity compared to all other compounds and the control linoed acid. Pheophorbide A has the strongest binding affinity to PI3K and the highest number of hydrogen bonds compared to all other compounds and the control acetylshikonin. According to Mutiah *et al*,[11] Bond energy represents the amount of energy required to interact with a ligand and its receptor. Lower bond energies indicate more stable bonds, leading to an increase in activity. Hydrogen, hydrophobic, and electrostatic bonds are formed between anticancer compounds and cancer proteins (Cyclin D1 and P13K) by anticancer compounds. Docking of anchor proteins by a variety of physical forces, including 1) the hydrophobic effect, 2) electrostatic force, 3) van der Waals forces, 4) hydrogen bonds, 5) ionic bonding, and 6) entropy. Hydrogen bonding and hydrophobic effects are two of the most important forces in protein interactions. The competition between ligands and proteins in extracellular media always involves water (hydrogen bonds), and bulk water can interfere with reversible biological processes. Enthalpy-entropy compensation occurs during the formation of hydrogen bonds.[25]

Protein targets such as Cyclin D1 and PI3K inhibitors play an essential role in cancer progression and serve as anticancer treatment targets.[9] Through genetic amplification or overexpression, breast cancer frequently displays elevated cyclin D1 levels. In cases of multiple myeloma, translocation of Cyclin D1 can also be detected.[26] The cell cycle involves the preparation of cells and the duplication of their genomes in daughter cells. The cell cycle comprises four successive phases: the G1 phase (during which the cell accumulates mass and metabolites required for DNA replication), the S phase (during which DNA replication occurs), the G2 phase (which is essential for accurate DNA replication), and the M phase (segregation). DNA and cell division). Most adult cells exist in a quiescent or dormant state (G0 phase). When exposed to the proper mitogenic stimuli, G0 phase cells can transition into the G1 phase.[27] Cyclin-D1 is one of the key regulators of cell proliferation that regulates the progression of the cell cycle in the nucleus and the transition of cells from the G0/G1 phase to the S phase[1] by acting as an allosteric regulator of cyclin-dependent kinase 4 (CDK) and CDK6. Cyclin D1 forms an active complex by phosphorylating and inactivating the retinoblastoma protein (RB), which promotes cell cycle progression. It then eliminates the E2F transcription factor, which regulates specific transcription genes from cell proliferation so that the degradation of cyclin D1 can be used as an intervention therapy against cancer.[5,28] Several mechanisms increase cyclin D levels, thereby increasing CDK4/6 activation: (i) increased transcription of cyclin D genes by transcription factors involved in mitogenic signaling pathways, such as Fos/Jun, Ets-2, signal transduction, and activator. of transcriptions (STATs), and nuclear factor kappa κB-light-chain-enhancer of activated B cells (NF-κB), and (ii) increased cyclin D protein translation mediated by phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt)-ribosomal S6 kinase 1 (S6K1) signaling, and (iii) stabilization and nuclear localization of cyclin D proteins, regulated by C-terminal phosphorylation of the protein.[29]

The PI3K/mTOR pathway is a crucial cell cycle progression regulator.[30] PI3Ks play an important role in cellular processes, including proliferation, survival, differentiation, and metabolism. Regulation of the PI3K pathway in tumors results in hyperactivation of growth factor signaling, mutation or loss of PTEN, and oncogenic mutations in PIK3CA, thereby making this condition a target for PI3K inhibition-based cancer therapy.[31]

Ethanol extract from *C. cinerariifolium* stem can potentially be an oral squamous cell carcinoma inhibitory agent. According to the Lipinski rule of five, most of the compounds in C. cinerariifolium stems met the drug-likeness test criteria.[32] Sox2 is expressed in 25 cancer types. In mouse embryonal carcinoma cells, Sox2 regulates the transcription of FGF4. Sox2 is also involved in ovarian, lung, skin, brain, breast, prostate, and pancreatic cancer development, tumorigenicity, and metastasis.[33]

Pheophorbide is a chlorophyll degradation product found in green plant cells. Photodynamic therapy based on pheophorbide-A demonstrates that the generated free radicals can induce hepatocellular carcinoma cell death in humans (Hep3B). Pheophorbide An induces mitochondrial apoptosis.[34] Apoptosis is the body's attempt to prevent the metastasis of cancer cells. Loss of apoptotic control enables cancer cells to survive longer and provides more time for mutation accumulation, thereby increasing invasiveness during tumor development, stimulating angiogenesis, deregulating cell proliferation, and inhibiting differentiation. Quercetin, another active component of *C. cinerariifolium*, plays a role in activating the caspase pathway that leads to apoptosis.[35] Ethanol extract from *C. cinerariifolium* stems contains active ingredients such as flavonol, terpenoid, and carotene.

The most potent active metabolite of the ethanolic extract of *C. cinerariifolium* stem is pronethalol, which exhibits an anti-oral squamous cell carcinoma candidate *via* the downregulation of the cell cycle (cyclin D1) and P13K in an *in-silico* study. The active ingredients of ethanol extract from *C. cinerariifolium* stems have different targets in the prevention or therapy of cancer. Combining two or more compounds as a cancer treatment may be much more effective than a single compound. There is a need for *in vivo* studies employing suitable animal models to further elucidate this assumption. The combination of multiple chemotherapeutic agents may reduce the drug dose administered to patients, reducing the severity of side effects.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

Thank you to the Doctoral Study Program in Medical Science, Faculty of Medicine, Universitas Brawijaya, for their support. The author

would like to thank the BOPTN Litabdimas LP2M UIN Maulana Malik Ibrahim Malang grant from the Ministry of Religion of the Republic of Indonesia in 2021.

REFERENCES

1. Arora R. *Operculina turpethum* extract inhibits growth and proliferation by inhibiting NF-κB, COX-2 and cyclin D1 and induces apoptosis by up regulating P53 in oral cancer cells. Arch Oral Biol. 2017;80:1-9.
2. Bray F. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2018;68(6):394-424.
3. Pangaribuan C. Ekspresi P53 Dan E-Cadherin Sebagai Prediktor Prognosis Pada Karsinoma Sel Skuamosa Rongga Mulut Di Rsup Dr. Kariadi Semarang. J Kedokt Raflesia. 2019;5(1):7-17.
4. Riskayanti N, Riyanto D, Winias S. Manajemen multidisiplin Oral Squamous Cell Carcinoma (OSCC): laporan kasus. Intisari Sains Medis. 2021;12(2):621-6.
5. Montalto F, De Amicis F. Cyclin D1 in Cancer: A Molecular Connection for Cell Cycle Control, Adhesion and Invasion in Tumor and Stroma. Cells. 2020;9(12):1-15.
6. Vadlakonda L, Pasupuleti M, Pallu R. Role of PI3K-AKT-mTOR and Wnt signaling pathways in transition of G1-S phase of cell cycle in cancer cells. Front Oncol. 2013;3:85.
7. Khan T. Autophagy modulators for the treatment of oral and esophageal squamous cell carcinomas. Med Res Rev. 2020;40(3):1002-60.
8. Safarzadeh E, Shotorbani S, Baradaran B. Herbal medicine as inducers of apoptosis in cancer treatment. Adv Pharm Bull. 2014;4(1):421-7.
9. Hemamalini G, Nirmala G. In Silico Screening Of Active Component Identified In Acetone Extract Of Acacia Nilotica Leaves Against Cancer Target Protein. World J Pharm Res. 2018;7(1):1190-8.
10. Listiyana A. Anticancer Activities And Metabolite Fingerprinting of UPLC- QToF-MS/MS Method From *Chrysanthemum cinerariifolium (Trev)*. 2019;4(1):19-39.
11. Mutiah R, Inayatin A, Annisa R, Indrawijaya YY, Listiyana A. Inhibition of cell cycle and induction of apoptosis y ethanol leaves extract of *Chrysanthemum cinerariifolium (Trev.)* in T47D breast cancer cells. Indones J Pharm. 2010;31(1):1-10.
12. Urbain A, Simões-Pires C. Thin-Layer Chromatography for the Detection and Analysis of Bioactive Natural Products. Encycl Anal Chem. 2020;1-29.
13. Arezou R, Maria P, Mehrdad R. Assessment of Soil Moisture Content Measurement Methods: Conventional Laboratory Oven versus Halogen Moisture Analyzer. J Soil Water Sci. 2020;4.
14. Zambrano M, Dutta B, Mercer D, MacLean H, Touchie M. Assessment of moisture content measurement methods of dried food products in small-scale operations in developing countries: A review. Trends Food Sci Technol. 2019;88(1):484-96.
15. Anggarani MA, Ayuningsih AD, Yudianto E, Prasodi BA. The Composition of Water and Ash of Secang Wood's Simplicia and Secang Wood Herbal Drink Powder. J Phys Conf Ser. 2019;2019:1-5.
16. Komala A, Haryoto. Tests of Ash Content, Moisture Content and Dry Shrinkage of Ethanol Extracts of Capidada Leaves (*Sonneratia alba*) and Ketapang (*Terminilia cattapa*). J Nutraceuticals Herb Med. 2010;3(1):10-4.
17. Yoga IBK, Suarta W, Wrasiati L. Identifikasi Fraksi Terpisah Simplisia Daun Kacapiring Dengan Thin Layer Chromatography Identification of Separate Fractions on Kacapiring Leaf Simplisia by Thin Layer Chromatography. Media Ilm Teknol Pangan. 2019;6:105-16.
18. Yahyaoui O. Phytochemical screening and thin layer chromatography of two medicinal plants: *Adansonia digitata* (Bombacaceae) and *Acacia raddiana* (Fabaceae). J Pharmacogn Phytochem. 2017;6(1):10-5.
19. Wulandari L, Retnaningtyas Y, Mustafidah D. Development and Method Validation Densitometry Thin Layer Chromatography for Thesimultaneous Determination of Theophylline and Ephedrinehydrochloride in Tablet Dosage Form. Jkti. 2013;15(1):15-21.
20. Thoppil R, Bishayee A. Terpenoids as potential chemopreventive and therapeutic agents in liver cancer. World J Hepatol. 2011;3(9):228-49.
21. Lagunin A, Stepanchikova A, Filimonov D, Poroikov V. PASS: Prediction of activity spectra for biologically active substances. Bioinformatics. 2000;16(8):747-8.
22. Mutiah R, Marsyah A, Saputra A. Systematic Review: Phytochemical Content and Pharmacological Effects of Chrysanthemum Sp. Planar. 2021;31-41.
23. Khan Z. Physicochemical Characterizations and Pharmacokinetic Evaluation of Pentazocine Solid Lipid Nanoparticles against Inflammatory Pain Model. Pharmaceutics. 2022;14(2):409.
24. Kamran S, Sinniah A, Abdulghani MM, Alshawsh M. Therapeutic Potential of Certain Terpenoids as Anticancer Agents: A Scoping Review. Cancers (Basel). 2022;14(5):1100.
25. Li J. A Hydrophobic-Interaction-Based Mechanism Triggers Docking between the SARS-CoV-2 Spike and Angiotensin-Converting Enzyme 2. Glob Challenges. 2020;4(12):2000067.
26. Day P. Crystal structure of human CDK4 in complex with a D-type cyclin. Proc Natl Acad Sci. 2009;106(11):4166-70.
27. Qie S, Diehl JA. Cyclin D1, cancer progression, and opportunities in cancer treatment. J Mol Med. 2016;94(12):1313-26.
28. Alao J. The regulation of cyclin D1 degradation: Roles in cancer development and the potential for therapeutic invention. Mol Cancer. 2007;6:1-16.
29. Inoue K, Fry E. Aberrant Expression of Cyclin D1 in Cancer. Sign Transduct Insights. 2015;4(1):3.
30. Cai Z. Overexpressed Cyclin D1 and CDK4 proteins are responsible for the resistance to CDK4/6 inhibitor in breast cancer that can be reversed by PI3K/mTOR inhibitors. Sci China Life Sci. 2023;66(1):94-109.
31. Zhao Y. Crystal structures of PI3Kα complexed with PI103 and its derivatives: New directions for inhibitors design. ACS Med Chem Lett. 2014;5(2):138-42.
32. Qiao X. Pronethalol decreases RBPJκ to reduce Sox2 in cerebral arteriovenous malformation. Vasc Med. 2021;25(6):569-71.
33. Wuebben EL, Rizzino A. The dark side of SOX2: Cancer - A comprehensive overview. Oncotarget. 2017;8(27):44917-43.
34. Mansoori B. Photodynamic therapy for cancer: Role of natural products. Photodiagnosis Photodyn Ther. 2019;26:395-404.
35. Pfeffer CM, Singh ATK. Apoptosis: A target for anticancer therapy. Int J Mol Sci. 2018;19(2):448.

Cite this article: Listiyana A, Rachmawati YL, Susianti H, Nurdiana, Sujuti H, et al.. Analysis of the Metabolite Compound of the Ethanol Extract of *Chrysanthemum cinerariifolium* Stem and Activity for inhibition of Oral Squamous Cell Carcinoma (OSCC) *in silico* study. Pharmacogn J. 2023;15(2): 393-398.