



Revealing *Pogostemon cablin* (Blanco) Benth. and *Murraya koenigii* (L.) Spreng. action targets on AKT1 as papillary thyroid cancer therapy based on bioinformatics approach

[Revelación de las dianas de acción de *Pogostemon cablin* (Blanco) Benth. y *Murraya koenigii* (L.) Spreng. sobre AKT1 como terapia del cáncer papilar de tiroides basada en un enfoque bioinformático]

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Abstract

Context: Thyroid cancer (TC) accounts for approximately 3% of all malignant cancers, with increasing morbidity and mortality rates globally. In 2023, the United States reported 43,720 new TC cases and 2,120 TC-related deaths. Histologically, TC is classified into many types, with papillary thyroid cancer (PTC) being the most common. Current treatments, such as surgical thyroidectomy with adjuvant chemotherapy and radiotherapy, often face challenges such as tumor recurrence, metastasis, drug resistance, and toxicity. This necessitates exploring new therapeutic approaches, such as natural ingredients for complementary therapy. *Pogostemon cablin* (PC) and *Murraya koenigii* (MK) have shown anticancer properties in various cancers.

Aims: To investigate the targets and pathways of PC and MK in treating PTC based on network pharmacology, molecular docking, and molecular dynamics.

Methods: This study involved identifying common targets using bioinformatics tools, including network pharmacology, molecular docking, and molecular dynamics, to find primary targets and promote compounds in PC and MK combination.

Results: Findings identified 242 common targets, with AKT1 as a primary target, highlighting potential pathways and genes for new drug development and PTC mechanism investigation. These results suggest the PC and MK combination is a promising therapy for PTC.

Conclusions: This study underscores the potential of PC and MK combination therapy for PTC. These findings suggest that PC and MK show promising effects on the novel biomarker candidate AKT1 and its related pathways, including the cancer pathway, which is critical for metabolic pathways in PTC. In addition, this study identified that PC-MK compounds such as clonasterol, noruns-12-ene, and koenimbine were effective for PTC therapy based on molecular docking and molecular dynamics evaluation.

Keywords: AKT1; *in silico*; *Murraya koenigii*; network pharmacology; *Pogostemon cablin*; PTC therapy.

Resumen

Contexto: El cáncer de tiroides (CT) representa aproximadamente el 3% de todos los cánceres malignos, con tasas de morbilidad y mortalidad en aumento a nivel mundial. En 2023, Estados Unidos reportó 43.720 nuevos casos de CT y 2.120 muertes relacionadas con el CT. Histológicamente, el CT se clasifica en varios tipos, siendo el cáncer papilar de tiroides (CPT) el más común. Los tratamientos actuales, como la tiroidectomía quirúrgica con quimioterapia y radioterapia adyuvantes, a menudo enfrentan desafíos como la recurrencia tumoral, la metástasis, la farmacoresistencia y la toxicidad. Esto requiere explorar nuevos enfoques terapéuticos, como ingredientes naturales para la terapia complementaria. *Pogostemon cablin* (PC) y *Murraya koenigii* (MK) han demostrado propiedades anticancerígenas en diversos tipos de cáncer.

Objetivos: Investigar las dianas y vías de PC y MK en el tratamiento del CPT, basándose en la farmacología de redes, el acoplamiento molecular y la dinámica molecular.

Métodos: Este estudio implicó la identificación de dianas comunes mediante herramientas bioinformáticas, como la farmacología de redes, el acoplamiento molecular y la dinámica molecular, para encontrar dianas primarias y promover compuestos en la combinación de PC y MK.

Resultados: Los hallazgos identificaron 242 dianas comunes, con AKT1 como diana primaria, lo que destaca posibles vías y genes para el desarrollo de nuevos fármacos y la investigación del mecanismo del CPT. Estos resultados sugieren que la combinación de PC y MK es una terapia prometedora para el CPT.

Conclusiones: Este estudio subraya el potencial de la terapia combinada de PC y MK para el CPT. Estos hallazgos sugieren que PC y MK muestran efectos prometedores sobre el nuevo biomarcador candidato AKT1 y sus vías relacionadas, incluyendo la vía del cáncer, que es crucial para las vías metabólicas en el CPT. Además, este estudio identificó la eficacia de compuestos de PC-MK como clonasterol, noruns-12-eno y koenimbina en el tratamiento de PTC, basándose en la evaluación del acoplamiento molecular y la dinámica molecular.

Palabras Clave: AKT1; farmacología de redes; *in silico*; *Murraya koenigii*; *Pogostemon cablin*; tratamiento de CPT.

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Abbreviations: 3D: Three-dimensional; AKT: AKT serine/threonine kinase 1; ATC: Anaplastic thyroid cancer; BP: Biological processes; BW: Body weight; CC: Cellular components; c-Myc: c-Myc-binding protein; DNA: Deoxyribonucleic acid; EMT: Epithelial-to-mesenchymal transition; FTC: Follicular thyroid cancer; GC-MS: Gas chromatography-mass spectrometry; GO: Gene ontology; GST: Glutathione-S-transferase; KEGG: Kyoto encyclopedia of genes and genomes; LAC: Local average connectivity; LC-MS: Liquid chromatography-mass spectrometry; LDH: Lactate dehydrogenase; MD: Molecular dynamics; MF: Molecular function; MK: *Murraya koenigii*; MMPBSA: Molecular mechanical energy combined with Poisson-Boltzmann; MTC: Medullary thyroid cancer; NF- κ B: Nuclear factor- κ B; PBS: Poisson-Boltzmann; PC: *Pogostemon cablin*; PDB: Protein data bank; PLIP: Protein-ligand interaction profiler; PPI: Protein-protein interaction; PTC: Papillary thyroid cancer; QSAR: Quantitative structure-activity relationship; RCSB: Research collaboratory for structural bioinformatics; RMSD: Root-mean-square deviation; RMSF: Root-mean-square fluctuation; SMILES: Simplified molecular input line entry system; TC: Thyroid cancer.

INTRODUCTION

Thyroid cancer (TC) typically begins with the formation of thyroid nodules and is a malignant tumor affecting the endocrine system (Du et al., 2018; Prete et al., 2020). Clinical statistical data indicate that TC accounts for approximately 3% of all malignant cancers (Siegel et al., 2019; Trigo et al., 2014). Alarming, over the past few decades, the morbidity and mortality rates of TC cases have increased significantly worldwide. In 2023 alone, around 43,720 new TC cases were diagnosed in the United States, with 2,120 deaths attributed to TC (Siegel et al., 2023; Zaballos and Santisteban, 2017). From a histological perspective, TC can be classified into several subtypes: papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), medullary thyroid cancer (MTC), and undifferentiated anaplastic thyroid cancer (ATC). PTC is the most prevalent type, accounting for approximately 80% of all TC cases. It is also one of the seven most common malignancies among women and children (Abdullah et al., 2019; Gonzalez-Gonzalez et al., 2011; Hajeer et al., 2018; Ito and Miyauchi, 2020; Kim et al., 2018; Lubitz and Sosa, 2016). Conversely, ATC, though rare, is the most aggressive subtype, comprising only 1%-2% of TC cases but contributing to over 50% of TC-related deaths (Are and Shaha, 2006; Sandulache et al., 2012).

Current therapeutic strategies for TC include surgical thyroidectomy combined with adjuvant chemotherapy and radiotherapy. However, these treatments are often suboptimal due to high rates of tumor recurrence, distant metastases, drug resistance, and severe side effects (Erol et al., 2014; Saini et al., 2018; Wagle et al., 2014). In light of these limitations, the exploration of novel therapeutic strategies is crucial. A promising approach lies in precision medicine, which leverages multi-target pharmacological mechanisms to achieve better outcomes (Casas et al., 2019). Among such strategies, the use of natural products as complementary therapies has garnered attention due to their low toxicity, high efficacy, multi-target inhibition, and cost-effectiveness (Salehi et al., 2019; Sari et al., 2020; 2022; Tuli et al., 2021). *Pogostemon cablin* (Blanco) Benth. (PC), family *Lamiaceae*, and *Murraya koenigii* (L.) Spreng. (MK), family *Rutaceae*, are traditional herbal medicines with a long history of use in

Southeast Asia. Both plants exhibit significant health-promoting activities, including anticancer properties against various types of cancer. PC has been shown to inhibit HeLa cell proliferation, suppress differentiation, and promote apoptosis in human colorectal cancer cells (HCT116 and SW480) (Jeong et al., 2013). It also downregulates c-Myc, inhibits histone deacetylase enzyme activity, and activates the nuclear factor- κ B (NF- κ B) pathway (Jeong et al., 2013).

Similarly, MK exhibits a wide range of anticancer properties. Its extract has shown preventive and curative effects against tumor growth, effectively inducing apoptosis in tumor cells (Aisyah et al., 2021). The hydro-methanol extract of MK leaves, rich in polyphenols, inhibits 26S proteasome activity and induces apoptosis in breast cancer cells (MCF-7 and MDA-MB-231) (Noolu et al., 2013). Additionally, MK demonstrates anticancer activity against human colon adenocarcinoma (Caco-2) and liver cancer cells (HepG2) by increasing lactate dehydrogenase (LDH) and glutathione-S-transferase (GST) activity, along with DNA fragmentation (Patterson and Verghese, 2015). Girinimbine, a bioactive compound in MK, induces apoptosis and arrests the cell cycle in hepatocellular carcinoma (HepG2) cells (Syam et al., 2011). Despite these promising findings, the mechanisms underlying the combined action of PC and MK, particularly their effects on papillary thyroid cancer (PTC), remain unexplored. This study aims to investigate the therapeutic potential of PC and MK in PTC through a bioinformatics approach, focusing on their targets on AKT1, a key regulator in cancer progression. By elucidating these mechanisms, the study seeks to provide new insights into PTC tumorigenesis and contribute to developing more effective integrative therapies for TC patients.

On the other hand, MK is reported to have various anticancer activities, where MK extract at a dose of 300 mg/kg BW has preventive and curative effects and has the potential to be used as an antitumor, which effectively induces tumor cell apoptosis (Aisyah et al., 2021). In addition, hydro-methanol extract of MK leaves, which is rich in polyphenols, mediates inhibition of 26S proteasome proteolytic activity and induces apoptosis of MCF-7 and MDA-MB-231 breast cancer cells through decreasing cell

viability, growth kinetics, and S phase cell cycle arrest (Noolu et al., 2013). MK extract also showed anti-cancer activity against human colon adenocarcinoma Caco-2 and human liver cells HepG2 by increasing the activity of lactate dehydrogenase (LDH) and glutathione-S-transferase (GST) and DNA fragmentation. Histone-related and thus may help prevent colon cancer (Patterson and Verghese, 2015). Girinimbine also inhibits growth and induces apoptosis (intrinsic) in human hepatocellular carcinoma (HepG2) cells by decreasing cell viability, increasing DNA fragmentation, and G0/G1 phase cell cycle arrest (Syam et al., 2011). However, the detailed mechanisms involved in combining PC-MK in PTC have not been discussed.

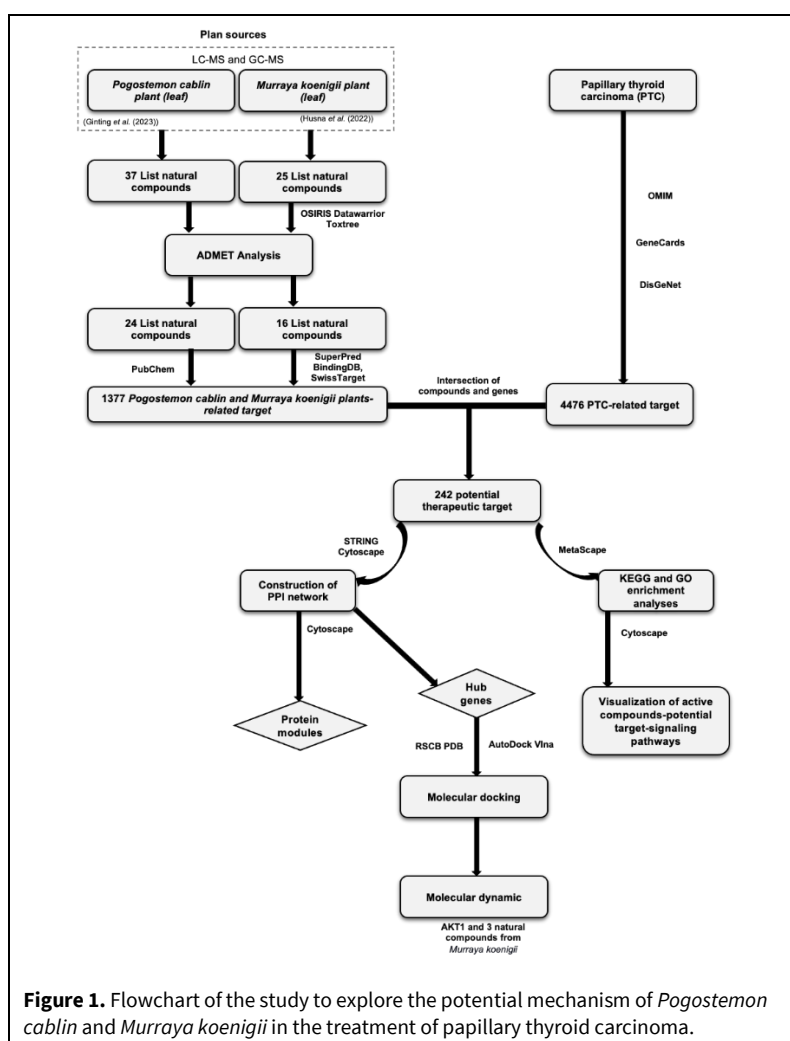
Looking at the potential of PC-MK in the treatment of various cancers and to better understand the mechanism of action and potential synergistic therapeutic targets of the combination of PC and MK, this study aims to reveal the potential molecular mechanisms and pathways of the combination of PC-MK in PTC. This study identified 242 common targets of the

AKT1 gene, which is the meeting point between PC, with AKT1 being the primary target. In addition, this study also highlights the available pathways, and the genes involved in them, which can provide a theoretical basis for developing new drugs and investigating PTC mechanisms. These findings show its potential as a promising PTC therapy.

MATERIAL AND METHODS

Workflow of the study

This study was designed to identify the potential molecular mechanisms and pathways of the PC-MK compound combination in PTC through a bioinformatics approach. This analysis includes the evaluation of signaling pathways, biological processes, and key target genes suspected of playing a role in the mechanism of action of PC-MK on PTC. This approach is expected to obtain a deeper understanding of the effect of PC-MK compounds on PTC. The research workflow is shown in Fig. 1.



Screening compounds of *Pogostemon cablin* and *Murraya koenigii*

The list of identified compounds in *Pogostemon cablin* and *Murraya koenigii* was obtained from Ginting et al. (2023) and Husna et al. (2022), which used gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) analysis for compound identification. Furthermore, the three-dimensional (3D) structures of the identified compounds were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) (Kim et al., 2023). Lipinski Rule of Five analysis was performed to screen the compounds based on suitable and safe physicochemicals and oral bioavailability using OSIRIS DataWarrior (<https://openmolecules.org/datawarrior/>) and Tox-tree (<https://toxtree.sourceforge.net/>) (Lipinski, 2004; Patlewicz et al., 2008; Sander et al., 2015), where the analysis considered five criteria: 1) preliminary screening (mutagenic, tumorigenic, irritant, reproductive effects), 2) structural alert for genotoxic carcinogenicity, 3) structural alert for non-genotoxic carcinogenicity, 4) mutagen potential of *S. typhimurium* TA100 based on QSAR (quantitative structure-activity relationship) and 5) carcinogen potential based on QSAR. Furthermore, structures showing mutagenic potential, tumorigenic, reproductive-toxic, or irritant properties were carefully excluded.

Potential target genes and identification of PTC-related targets finding

Furthermore, the pre-filtered PC and MK compounds were further analyzed to identify potential targets based on the Canonical SMILES of each compound using various webserver SuperPred (<https://prediction.charite.de/>), BindingDB (<https://www.bindingdb.org/>), SwissTargetPrediction (<http://www.swisstargetprediction.ch/>) (Daina et al., 2019; Liu et al., 2007; Nickel et al., 2014). In addition, OMIM (<https://www.omim.org/>), GeneCards (<https://www.genecards.org/>), and DisGeNet (<https://www.disgenet.org/>) web servers were used to find genes associated with PTC (Piñero et al., 2019; Stelzer et al., 2016). Finally, Venn diagrams were used to identify PTC common targets.

Identification of main targets of PC-MK for PTC

Based on Common Target PC-MK integration results on PTC from the previous stage, the STRING webserver (<https://string-db.org/>) was used to generate a protein-protein interaction (PPI) network (Szklarczyk et al., 2023). Where an interaction score >0.700 (high confidence) filters the network formed within it. Furthermore, the PPI network that had been

formed was further analyzed and visualized using Cytoscape software version 3.10 (Shannon et al., 2003). This study used the CytoNCA plug-in to identify essential gene targets in the network (Tang et al., 2015). Using this plug-in, we calculate various network centrality measures, including Betweenness, Closeness, Degree, Eigenvector, LAC, and use the median value of each score to filter the target three times (Hu et al., 2023).

Functional annotation

In exploring gene ontology (GO), including molecular function (MF), cellular components (CC), biological processes (BP), and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis, the latest study from Mariana Kustiawan et al. (2024) was applied, using the DAVID web server (<http://david.ncifcrf.gov>) and the SRplot webserver (<https://www.bioinformatics.com.cn/en>) were used to help visualize the results of GO and KEGG bar plots (Dennis et al., 2003).

Molecular docking

AutoDock Vina (version 1.2.5) was used to perform molecular docking and calculate the binding affinity score, where we carried out the simulation three times to get a convincing average binding affinity result (Trott and Olson, 2009). The AKT1 protein structure (PDB ID: 3O96) was sourced from the RCSB PDB (<https://www.rcsb.org/>) (Burley et al., 2021; Wu et al., 2010). The 44 compounds of PC-MK, along with the native ligand of the protein, AKT Inhibitor VIII 1-(1-(4-(7-phenyl-1H-imidazo[4,5-g]quinoxalin-6-yl)benzyl)piperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-one, also known as IQO. Their structures were refined using Discovery Studio (BIOVIA, San Diego, USA) (BIOVIA, 2019), which facilitated the removal of water molecules and ligands and the addition of polar hydrogens to prepare the target protein for docking. Finally, the three compounds with the highest binding affinity results, as well as the native ligand resulting from molecular docking on AKT1, will be analyzed using the protein-ligand interaction profiler (PLIP) web server (<https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index>) to see the interactions formed during the molecular docking simulation (Adasme et al., 2021; Salentin et al., 2015; Siregar et al., 2024).

Molecular dynamics

Molecular dynamics simulations were conducted for 50 ns using the AMBER14 forcefield (Krieger and Vriend, 2015; Maier et al., 2015) under physiological conditions as follows: pH 7.4, 0.9 NaCl concentration,

0.997 g/mL of water density, 1 bar pressure, and 310°K temperature with a cubic cell shape. All simulations were executed in YASARA version 21 (Krieger and Vriend, 2015), with 2000-time steps and a specified simulation box size. The simulation trajectories were subsequently analyzed using YASARA macros, specifically md_analyze.mcr and md_analyzeres.mcr. Structural dynamics were characterized by the root-mean-square deviation (RMSD) of atomic positions and the root-mean-square fluctuation (RMSF) of each residue position, measured in angstroms (Å). The calculation of the stability value of bond energy or molecular mechanical energy combined with Poisson-Boltzmann (MMPBSA) was carried out using the Poisson-Boltzmann (PBS) method in the macro md_analyzebindingenergy.mcr from YASARA (Meylani et al., 2023; Miller et al., 2012).

RESULTS

Screening compounds and identification of PTC-related targets

A total of 242 common targets for PC-MK in PTC were obtained from the intersection of protein searches using SMILES of 24 PC and 16 MK compounds that had been filtered through ADMET screening and gene target searches using SwissTargetPrediction, BindingDB, and SuperPred webserver. In addition, PTC-related protein searches were also conducted using the keyword "Papillary Thyroid Carcinoma" on various web servers (Fig. 2).

Identifying core targets of PC-MK for PTC

A total of 242 common targets of PC-MK in PTC were analyzed using protein-protein interaction (PPI) network modules via STRING, with a combined score threshold set at >0.700 (high confidence), resulting in 1225 edges and 189 nodes (Fig. 2). Four core proteins were obtained based on filtering results three times using CytoNCA, and six algorithms were used (AKT1, IL6, SRC, and STAT3) (Fig. 3). Finally, these four proteins were analyzed further to see their level of importance in the available enrichment analysis data.

Functional enrichment analysis

To elucidate the biological mechanism of PC-MK against PTC, we conducted GO enrichment analysis on 242 common targets of PC-MK for PTC. We illustrated the top 10 signaling pathways in a bar plot in Fig. 4A, sorted by p-values from smallest to largest (supplementary Table S1). The GO analysis was categorized into three main groups: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). The top 10 GO terms for each category are visualized in a bar plot (Fig. 4B and supplementary Table S2). The length and color of the bars represent the enrichment gene count and p-values, respectively, with darker colors indicating smaller p-values and longer bars representing a higher number of enriched therapeutic genes.

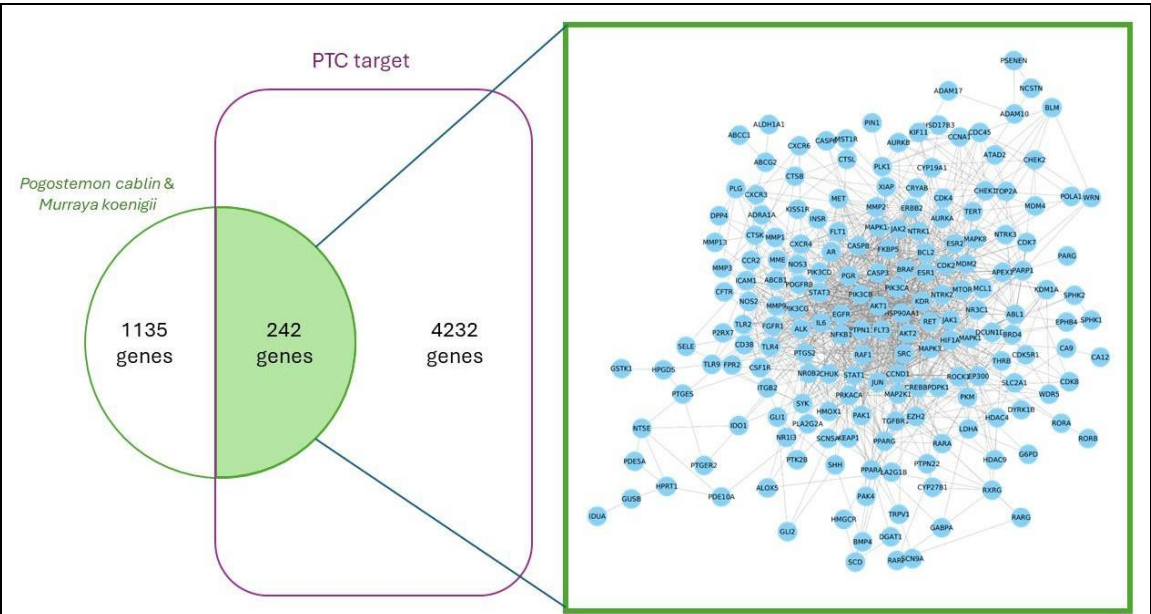
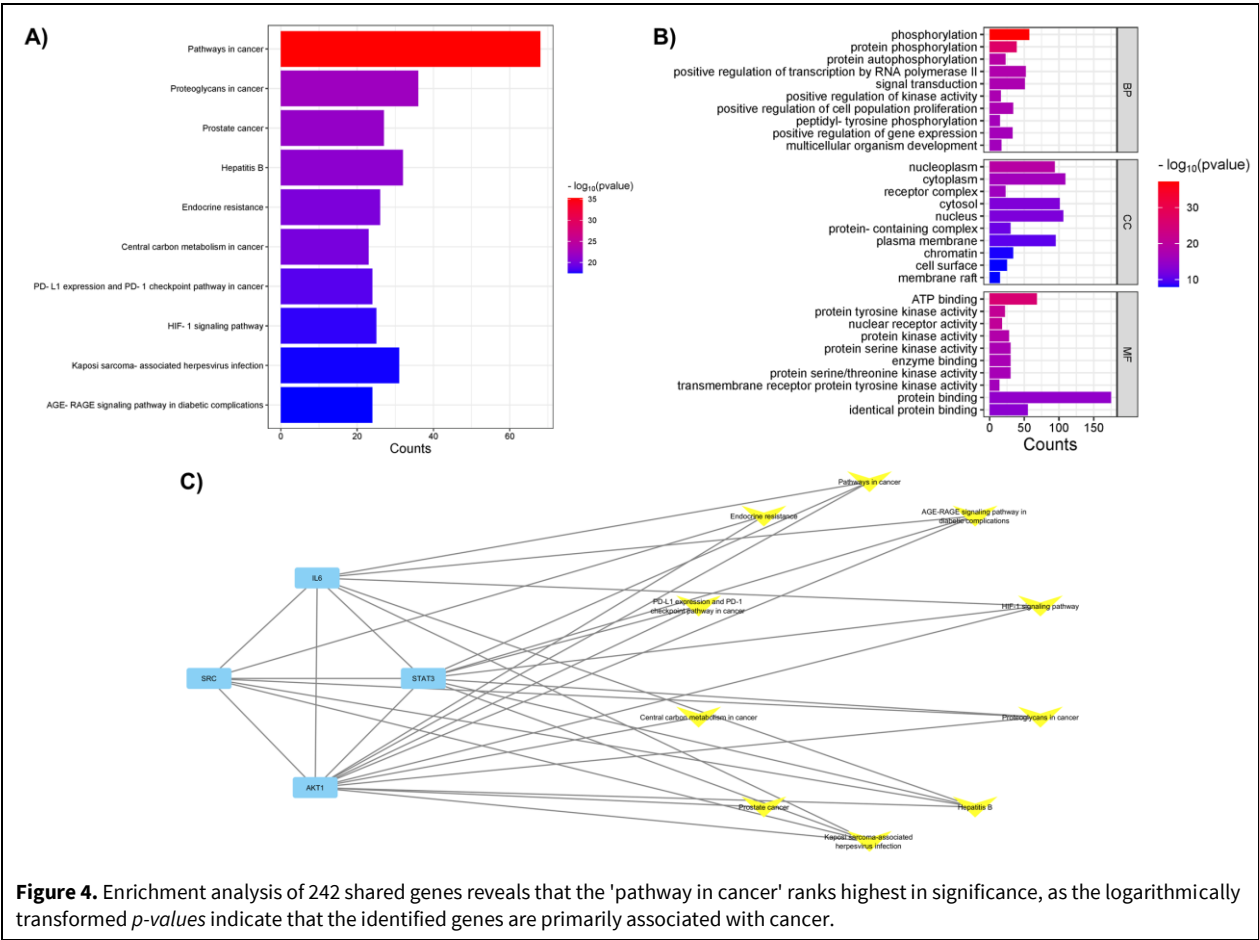
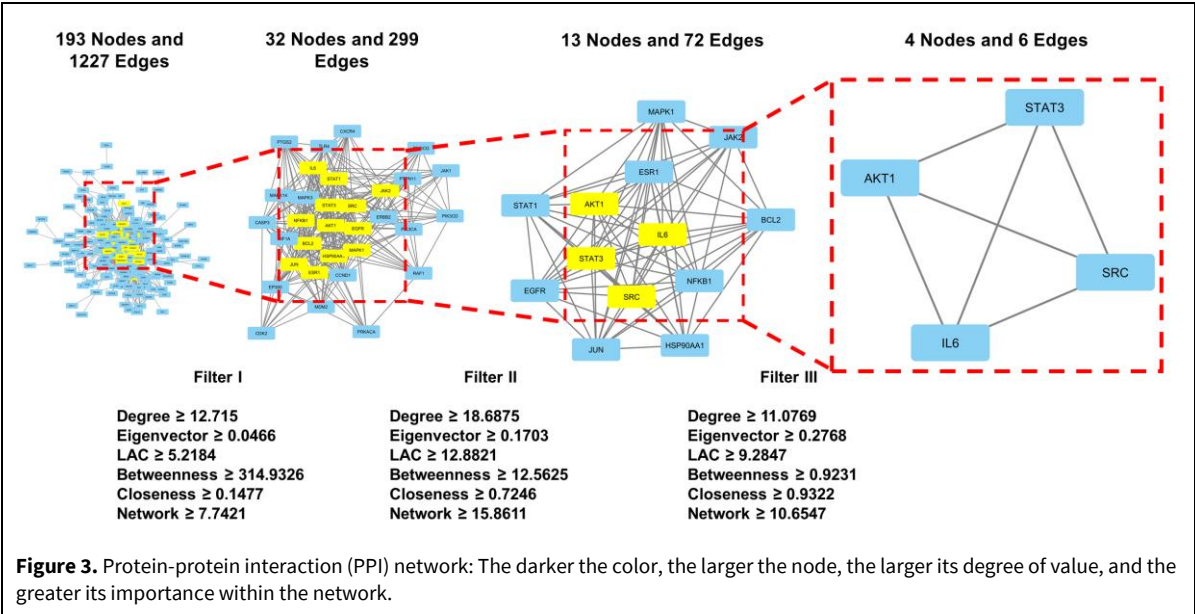


Figure 2. Common target Pogostemon cablin and Murayya koenigii and PTC-related target.



Furthermore, potential therapeutic targets were identified through KEGG pathway enrichment analysis. Among the four key genes identified via the protein-protein interaction (PPI) network, AKT1 emerged as a critical hub gene, appearing across all KEGG pathways analyzed (Fig. 4C), particularly within the cancer-related pathways. As a result, the AKT1 gene was selected for further investigation through molecular docking. These findings can potentially guide future investigations and identify promising treatment targets.

Molecular docking

In this study, although AKT Inhibitor VIII showed strong binding affinity to AKT1 by showing a docking score (-14.2 Kcal/Mol), we still chose three compounds that showed strong binding affinity to AKT1 even though they were not stronger than AKT Inhibitor VIII. Where the compounds clionasterol, noruns-12-ene, and koenimbine have docking scores of -10.9, -10.8, and -10.5 Kcal/Mol, respectively. Even though it is not stronger compared to AKT Inhibitor VIII, based on the docking results show that this compound also has potential as a therapeutic candidate that is close to an AKT1 inhibitor, and those three compounds could bind in the same location as AKT Inhibitor VIII, which potentially has a similar function as its inhibitor (Fig. 5). Further analysis, analysis of significant interactions with active residues forming hydrogen and hydrophobic bonds of the three compounds and AKT Inhibitor VIII is presented in Table 1 and Fig. 6. Finally, the four ligands will be analyzed further to see their stability in molecular dynamics.

Molecular dynamics

MD simulations were performed for the four complexes, clionasterol-AKT1, koenimbine-AKT1, 27-norurs-12-ene-AKT1, and AKT1 inhibitor VIII-AKT1, to provide additional validation of the specific binding and stability of their interactions. The RMSD value in the backbone structure shows that the entire complex tends to fluctuate and is not constant for 50 ns, producing an average RMSD below <4 Å (Fig. 7A). On the other hand, in Fig. 7B, RMSD Movements in each complex produce varying average RMSD, where the compounds Clionasterol and AKT1 Inhibitor VIII produce average RMSD below <2 Å of (1.77 Å and 1.34 Å), and in compound 27-norurs-12-ene

yielded an average RMSD of 2.29 Å. In contrast, in koenimbine, there was a constant fluctuation in ligand movement during the simulation, resulting in an average RMSD of 5.34 Å. On the other hand, the average RMSD value of ligand during the simulation produces an average RMSD below <2 Å, whereas the compounds koenimbine and 27-norurs-12-ene produce an average RMSD of 0.98 Å and 0.76 Å, respectively. The results are close to the average RMSD of AKT1 Inhibitor VIII, which is 0.66 Å, where in Fig. 7C, it can be seen that during the simulation, there were no significant fluctuations except for the clionasterol compound. It maintained its stability until the end of the simulation with an average RMSD of 1.77 Å.

The RMSF results only showed small differences between clionasterol, koenimbine, 27-norurs-12-ene, and AKT1 Inhibitor VIII (Fig. 7D). The RMSF value shows that the binding of clionasterol, 27-norurs-12-ene, and koenimbine to AKT1 does not affect the stability of each residue, especially the active site residues of AKT1 that are formed (Table 1). Furthermore, koenimbine and clionasterol formed more hydrogen bonds than noruns-12-ene (Fig. 7E). Therefore, all ligands can bind with great affinity to AKT1 to act as its inhibitor. Finally, MM-PBSA analysis was carried out to see the binding energy produced, where the compounds clionasterol and noruns-12-ene had a stronger binding energy compared to AKT1 Inhibitor VIII (-26.79 ± 46.32 kJ/mol), forming each at -35.11 ± 35.65 kJ/mol and -39.57 ± 38.61 kJ/mol. On the other hand, the koenimbine compound has the lowest energy compared to other compounds, namely -8.18 ± 32.13 kJ/mol. So, our investigation of these three compounds shows that clionasterol and noruns-12-ene have potential inhibitory activity against AKT1 (Fig. 7F).

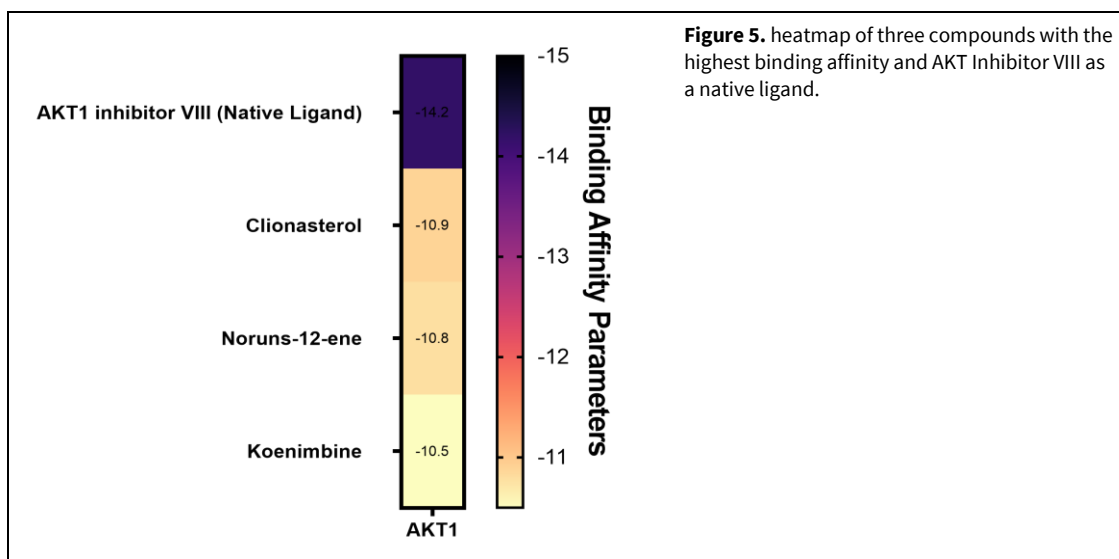


Figure 5. heatmap of three compounds with the highest binding affinity and AKT Inhibitor VIII as a native ligand.

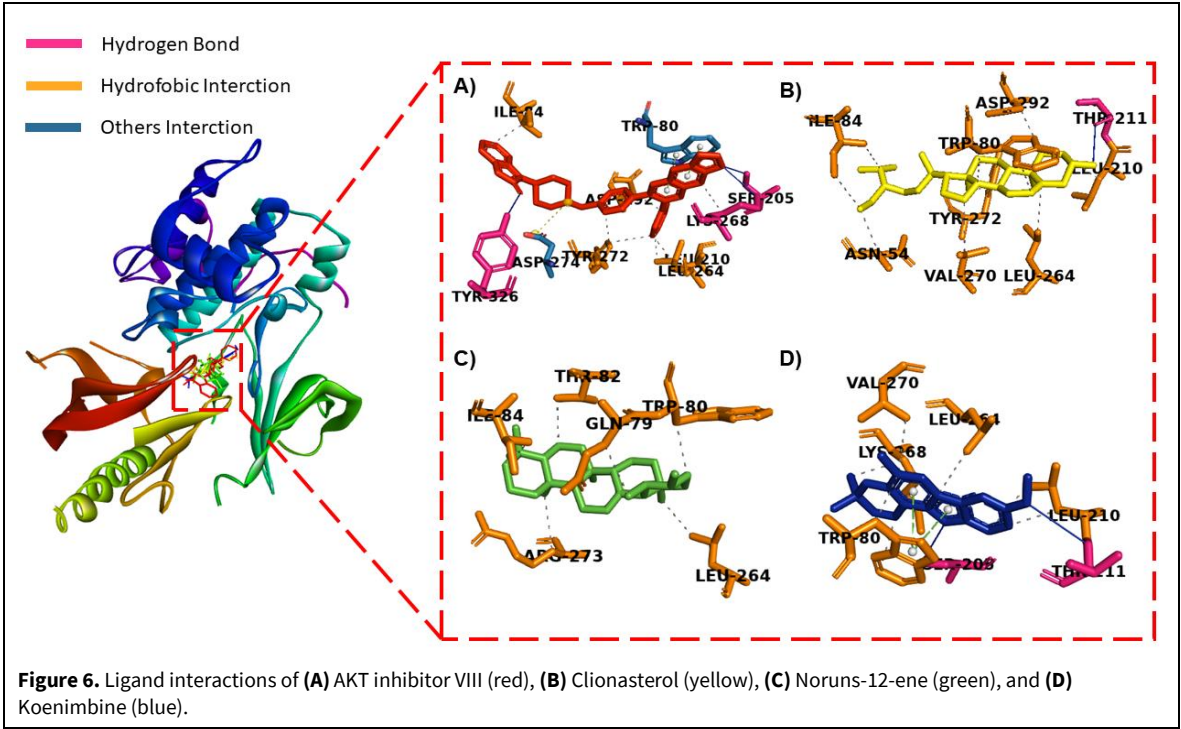
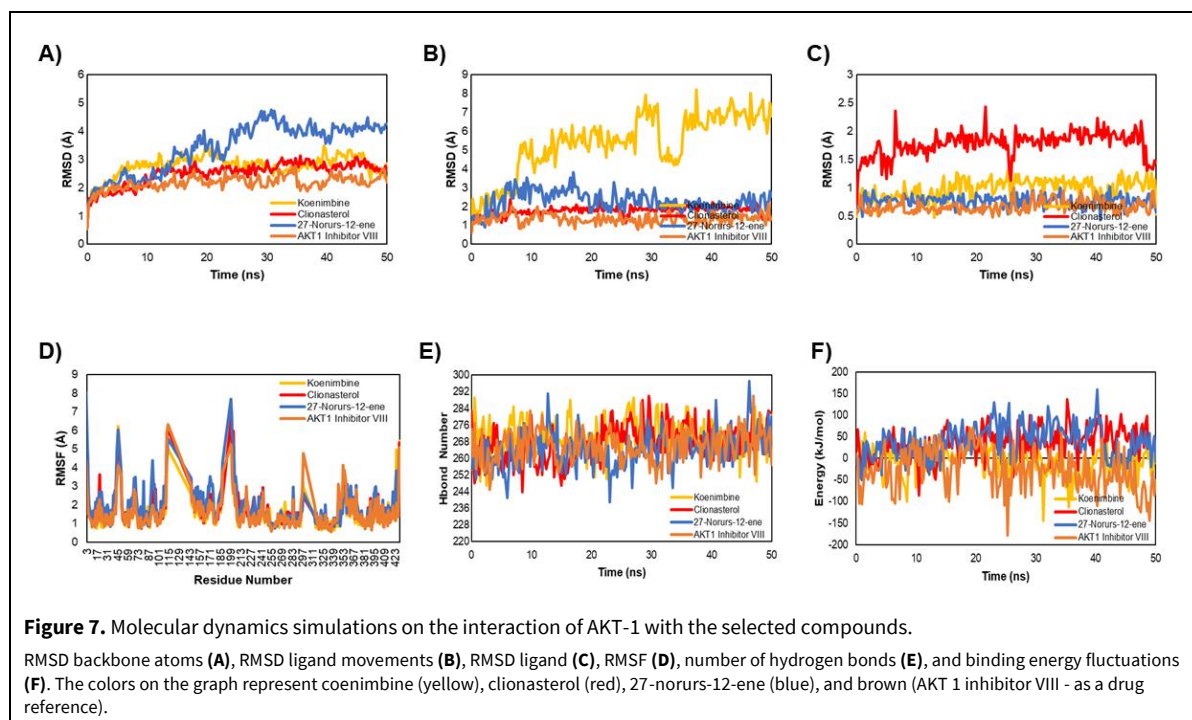


Table 1. The result of molecular docking AKT1 with three best compounds and AKT1 inhibitor as native ligand.

Ligand	Binding affinity (Kcal/Mol)	Position of chemical bonds			
		Hydrogen bond	Distance (Å)	Hydrophobic interction	Distance (Å)
AKT1 Inhibitor VIII (native ligand)	-14.2	Ser205	3.02	Ile84	3.97
		Lys268	2.95	Leu210	3.50
		Tyr326	3.69	Leu264	3.47
				Lys268	3.65
				Tyr272	3.39
				Asp292	3.58
Clionasterol	-10.9	Thr211	2.42	Asn54	3.65
				Trp80	3.68
				Ile84	3.72
				Leu210	3.89
				Leu264	3.78
				Val270	3.89
				Tyr272	3.57
				Asp292	3.89
Noruns-12-ene	-10.8	-	-	Gln79	3.70
				Trp80	3.29
				Thr82	3.43
				Ile84	3.36
				Leu264	3.68
				Arg273	3.87
Koenimbine	-10.5	Ser205	2.99	Trp80	3.55
		Thr211	3.56	Leu210	3.19
				Leu264	3.90
				Lys268	3.51
				Val270	3.89

Bold letter means catalytic residues of AKT1. *Native ligand.



DISCUSSION

In this study, we employed a network pharmacology and bioinformatics analysis approach to investigate the effects of PC-MK on PTC. A total of 242 common targets for PC-MK targets in PTC were obtained from the intersection results based on protein search outcomes using SMILES from 44 PC-MK compounds. The search utilized the SuperPred, BindingDB, and SwissTargetPrediction web servers and OMIM, GeneCards, and DisGeNet web servers to find genes associated with PTC. Furthermore, the results of our PPI analysis found four genes (AKT1, IL6, SRC, and STAT3) to be associated with PTC, and we specifically focused on AKT1 as a key biomarker candidate for a more comprehensive investigation based on PPI analysis and support for its role in various pathways. The research of Yang et al. (2013) supports this by revealing that decreased AKT1 expression can significantly inhibit cell proliferation, induce apoptosis, and inhibit thyroid cancer cell migration (Yang et al., 2013). Moreover, AKT1 activation triggers thyroid cancer progression, specifically through PI3K signaling, which plays a central role in cancer cell proliferation, antiapoptosis, and motility. Increased AKT activity is associated with advanced tumor stage and the development of several different malignancies (Kim, 2005). In thyroid cancer, an association between increased AKT activity and tumor size and invasion has been demonstrated in FTC and PTC, except in tumors with BRAF-activating mutations (Shin et al., 2004; Vasko et al., 2004). PI3K signaling has been shown to play a role in cell proliferation, survival, invasion, and

motility in PTC, FTC, and anaplastic thyroid cancer cell lines *in vitro*. Activation of AKT and other PI3K-regulated proteins, including p70S6 kinase and mammalian target of rapamycin, play important roles in thyroid cell proliferation and cancer progression (Miyakawa et al., 2003; Motti et al., 2005; Suh et al., 2003). As patients with more aggressive cancers account for most deaths from thyroid cancer, there is interest in capitalizing on this role to develop new therapies (Kim, 2005). Some recent mechanistic observations from other malignancies strongly suggest that a more detailed understanding of the AKT mechanism in thyroid cancer progression is needed before taking this step.

While it is unlikely that there is only one single pathway responsible for tumor progression, PI3K effectors, including AKT, appear to play a critical role in this process in thyroid cancer. Pharmacological and molecular inhibition of PI3K and AKT isoforms have been shown to reduce proliferation and motility in several human thyroid cancer cell lines *in vitro*. PI3K and, more specifically, AKT inhibitors reduce thyroid cancer cell cycle progression at the G2/M phase transition. They can induce apoptosis despite the overall resistance of poorly differentiated thyroid cells to apoptosis in general (Braga-Basaria et al., 2004). In addition, RET/PTC-induced cell motility and expression of osteopontin, a key regulator of epithelial-to-mesenchymal transition (EMT), are mediated by PI3K and AKT (Guarino et al., 2005). Recently, peroxisomal proliferator-activated receptor- γ agonists' ability to reverse EMT partially depends on the upregulation of

PTEN expression followed by the inhibition of AKT activity (Aiello et al., 2006). *In vivo* studies in TR β pv/pv mice that develop metastatic FTC-like thyroid cancer have shown increased AKT activation in both primary and metastatic tumor tissues (Kim et al., 2005). In addition, primary cultures of TR β pv/pv primary thyroid cancer displayed AKT-dependent cell motility in Boyden chambers, consistent with a role for AKT in metastasis in this model (Shinohara et al., 2007).

Molecular docking and molecular dynamics verification to assess the potential of the PC-MK combination for PTC therapy using the identified biomarker candidates were performed. The three best compounds from the molecular docking results with the highest binding affinity were selected: Clonasterol, noruns-12-ene, and koenimbine. These compounds exhibited strong binding affinity to the AKT1 gene, though not as high as AKT1 inhibitor VIII, indicating close integration with the gene through bond formation (Fig. 6 and Table 1). In addition, to explain the stability of protein-ligand interactions and the binding stability of the compounds clonasterol, noruns-12-ene, and koenimbine with the AKT1 structure, we carried out MD simulations and binding energy stability analysis in 50 ns using YASARA software. As a result, the compounds clonasterol and noruns-12-ene are promising compounds for PTC therapy based on RMSD, RMSF, and energy stability analysis, which shows that these compounds have strong bond energy stability compared to AKT Inhibitor VIII itself (Fig. 7).

Until we know, this is the first study to explore the potential PTC therapeutic activity of a combination of PC-MK compounds with the underlying critical pathways and targets associated with PTC. The results show that the combination of PC-MK compounds can potentially treat PTC. However, this research has limitations because the bioinformatics approach depends on the accuracy and completeness of the database used to obtain target genes. Despite these limitations, this study offers valuable insights into PC-MK compounds' potential targets and mechanisms. This suggests its influence on PTC through multiple pathways, especially those involved in cancer regulation, including proliferation, differentiation, apoptosis, and metabolic pathways.

CONCLUSION

This study underscores the potential of PC-MK combination therapy for PTC. The findings demonstrate that the PC-MK combination exhibits promising effects on the novel biomarker candidate AKT1 and its associated pathways, including the pathway in cancer, which is crucial for the metabolic pathway in

PTC. Additionally, the study identified that PC-MK compounds such as clonasterol, noruns-12-ene, and koenimbine are effective for PTC therapy based on molecular docking and molecular dynamics evaluations. However, they are not superior to the native ligand as a natural inhibitor of AKT but have the potential to bind in a similar location as its inhibitor. This suggests that the PC-MK compound combination could be used as a complementary treatment for PTC and has a potential activity similar to an AKT inhibitor. Nonetheless, further research is necessary to validate these findings and investigate the underlying mechanisms. Overall, these results have significant implications for developing new therapeutic strategies for PTC.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

- Abdullah MI, Junit SM, Ng, KL, Jayapalan JJ, Karikalan B, Hashim OH (2019) Papillary thyroid cancer: Genetic alterations and molecular biomarker investigations. *Int J Med Sci* 16(3): 450–460. <https://doi.org/10.7150/ijms.29935>
- Adasme MF, Linnemann KL, Bolz SN, Kaiser F, Salentin S, Haupt VJ, Schroeder M (2021) PLIP 2021: Expanding the scope of the protein-ligand interaction profiler to DNA and RNA. *Nucleic Acids Res* 49(W1): W530–W534. <https://doi.org/10.1093/nar/gkab294>
- Aiello A, Pandini G, Frasca F, Conte E, Murabito A, Sacco A, Genua M, Vigneri R, Belfiore A (2006) Peroxisomal proliferator-activated receptor- γ agonists induce partial reversion of epithelial-mesenchymal transition in anaplastic thyroid cancer cells. *Endocrinology* 147(9): 4463–4475. <https://doi.org/10.1210/en.2005-1610>
- Aisyah S, Handharyani E, Bermawie N, Setiyono A (2021) Effects of ethanol extract of curry leaves (*Murraya koenigii*) on HER2 and caspase-3 expression in rat model mammary carcinoma. *Vet World* 1988–1994. <https://doi.org/10.14202/vetworld.2021.1988-1994>
- Are C, Shaha AR (2006) Anaplastic thyroid carcinoma: biology, pathogenesis, prognostic factors, and treatment approaches.

- Ann Surg Oncol 13(4): 453–464. <https://doi.org/10.1245/ASO.2006.05.042>
- BIOVIA (2019) Discovery Studio Visualizer. Dassault Systèmes. <https://discover.3ds.com/discovery-studiovisualizer>
- Braga-Basaria M, Hardy E, Gottfried R, Burman KD, Saji M, Ringel MD (2004) 17-Allylamino-17-demethoxygeldanamycin activity against thyroid cancer cell lines correlates with heat shock protein 90 levels. *J Clin Endocrinol Metab* 89(6): 2982–2988. <https://doi.org/10.1210/jc.2003-031767>
- Burley SK, Bhikadiya C, Bi C, Bittrich S, Chen L, Crichlow GV, Christie CH, Dalenberg K, Di Costanzo L, Duarte JM, Dutta S, Feng Z, Ganesan S, Goodsell DS, Ghosh S, Green RK, Guranović V, Guzenko D, Hudson BP, Lawson CL, Liang Y, Lowe R, Namkoong H, Peisach E, Persikova I, Randle C, Rose A, Rose Y, Sali A, Segura J, Sekharan M, Shao C, Tao Y, Voigt M, Westbrook JD, Young JY, Zardecki C, Zhuravleva, M (2021) RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. *Nucleic Acids Res* 49(D1): D437–D451. <https://doi.org/10.1093/nar/gkaa1038>
- Casas AI, Hassan AA, Larsen SJ, Gomez-Rangel V, Elbatreek M, Kleikers PWM, Guney E, Egea J, López MG, Baumbach J, Schmidt HHHW (2019) From single drug targets to synergistic network pharmacology in ischemic stroke. *Proc Natl Acad Sci USA* 116(14): 7129–7136. <https://doi.org/10.1073/pnas.1820799116>
- Daina A, Michielin O, Zoete V (2019) SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res* 47(W1): W357–W364. <https://doi.org/10.1093/nar/gkz382>
- Dennis G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA (2003) DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 4(9): R60. <https://doi.org/10.1186/gb-2003-4-9-r60>
- Du L, Wang Y, Sun X, Li H, Geng X, Ge M, Zhu Y (2018) Thyroid cancer: Trends in incidence, mortality and clinical-pathological patterns in Zhejiang Province, Southeast China. *BMC Cancer* 18(1): 291. <https://doi.org/10.1186/s12885-018-4081-7>
- Erol V, Makay O, Icoz G, Kose T, Yazarbas U, Kumanlioglu K, Akyildiz M (2014) Prognostic factors of survival and recurrence pattern in differentiated thyroid cancer: A retrospective study from Western Turkey. *Endocr Regul* 48(04): 173–181. <https://doi.org/10.4149/endo.2014.04.173>
- Ginting B, Sufriadi E, Harnelly E, Isnaini N, Mulana F, Suparto I, Ilmiawati A, Ernawati, E, Muhammad S, Syakira M, Riski C (2023) Identification of volatile compounds contained in the therapeutic essential oils from *Pogostemon cablin*, *Melaleuca leucadendra*, and *Mentha piperita* and their purified fractions. *Adv Pharm Technol Res* 14(3): 208. <https://doi.org/10.4103/IAPTR.JAPTR.161.23>
- Gonzalez-Gonzalez R, Bologna-Molina R, Carreon-Burciaga RG, Gómezpalacio-Gastelum M, Molina-Frecher N, Salazar-Rodríguez S (2011) Papillary thyroid carcinoma: differential diagnosis and prognostic values of its different variants: review of the literature. *ISRN Oncol* 2011: 915925. <https://doi.org/10.5402/2011/915925>
- Guarino V, Faviana P, Salvatore G, Castellone MD, Cirafici AM, De Falco V, Celetti A, Giannini R, Basolo F, Melillo RM, Santoro M (2005) Osteopontin is overexpressed in human papillary thyroid carcinomas and enhances thyroid carcinoma cell invasiveness. *J Clin Endocrinol Metab* 90(9): 5270–5278. <https://doi.org/10.1210/jc.2005-0271>
- Hajeer MH, Awad HA, Abdullah NI, Almuhaissen GH, Abudalu LE (2018) The rising trend in papillary thyroid carcinoma. *Saudi Med J* 40(1): 1–6. <https://jppres.com>
- Med J 39(2): 147–153. <https://doi.org/10.15537/smj.2018.2.21211>
- Hu H, Wang H, Yang X, Li Z, Zhan W, Zhu H, Zhang T (2023) Network pharmacology analysis reveals potential targets and mechanisms of proton pump inhibitors in breast cancer with diabetes. *Sci Rep* 13(1): 7623. <https://doi.org/10.1038/s41598-023-34524-x>
- Husna F, Suyatna FD, Arozal W, Purwaningsih EH, Hanafi M, Razali R, Asrizal CW (2022) *Murraya koenigii* extract improving rate limiting enzymes on carbohydrate metabolism and GLUT-4 expression of hyperglycemic rats. *J Appl Pharm Sci* 12(12): 143–149. <https://doi.org/10.7324/JAPS.2022.121215>
- Ito Y, Miyauchi A (2020) Active surveillance of low-risk papillary thyroid microcarcinomas. *Gland Surg* 9(5): 1663–1673. <https://doi.org/10.21037/gs-2019-catp-03>
- Jeong JB, Choi J, Lou Z, Jiang X, Lee SH (2013) Patchouli alcohol, an essential oil of *Pogostemon cablin*, exhibits anti-tumorigenic activity in human colorectal cancer cells. *Int Immunopharmacol* 16(2): 184–190. <https://doi.org/10.1016/j.intimp.2013.04.006>
- Kim CS, Vasko VV, Kato Y, Kruhlak M, Saji M, Cheng SY, Ringel MD (2005) AKT activation promotes metastasis in a mouse model of follicular thyroid carcinoma. *Endocrinology* 146(10): 4456–4463. <https://doi.org/10.1210/en.2005-0172>
- Kim D (2005) AKT/PKB signaling mechanisms in cancer and chemoresistance. *Front Biosci* 10(1–3): 975. <https://doi.org/10.2741/1592>
- Kim K, Kim JH, Park IS, Rho YS, Kwon GH, Lee DJ (2018) The updated AJCC/TNM Staging System for Papillary Thyroid Cancer (8th Edition): From the perspective of genomic analysis. *World J Surg* 42(11): 3624–3631. <https://doi.org/10.1007/s00268-018-4662-2>
- Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J, Bolton EE (2023) PubChem 2023 update. *Nucleic Acids Res* 51(D1): D1373–D1380. <https://doi.org/10.1093/nar/gkac956>
- Krieger E, Vriend G (2015) New ways to boost molecular dynamics simulations. *J Comput Chem* 36(13): 996–1007. <https://doi.org/10.1002/jcc.23899>
- Lipinski CA (2004) Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discov Today Technol* 1(4): 337–341. <https://doi.org/10.1016/j.ddtec.2004.11.007>
- Liu T, Lin Y, Wen X, Jorissen RN, Gilson MK (2007) BindingDB: A web-accessible database of experimentally determined protein-ligand binding affinities. *Nucleic Acids Res* 35(Database): D198–D201. <https://doi.org/10.1093/nar/gkl999>
- Lubitz CC, Sosa JA (2016) The changing landscape of papillary thyroid cancer: Epidemiology, management, and the implications for patients. *Cancer* 122(24): 3754–3759. <https://doi.org/10.1002/cncr.30201>
- Maier JA, Martinez C, Kasavajhala K, Wickstrom L, Hauser KE, Simmerling C (2015) ff14SB: Improving the accuracy of protein side chain and backbone parameters from ff99SB. *J Chem Theory Comput* 11(8): 3696–3713. <https://doi.org/10.1021/acs.jctc.5b00255>
- Mariana Kustiawan P, Siregar KAAK, Syaifie PH, Zein Muttaqin F, Ibadillah D, Miftah Jauhar M, Djamas N, Mardiyati E, Taufiq Rochman N (2024) Uncovering the anti-breast cancer activity potential of east Kalimantan propolis by *in vitro* and bioinformatics analysis. *Heliyon* 10(13): e33636. <https://doi.org/10.1016/j.heliyon.2024.e33636>
- Meylani V, Rizal Putra R, Miftahussurur M, Sukardiman S, Eko Hermanto F, Abdullah A (2023) Molecular docking analysis of *Cinnamomum zeylanicum* phytochemicals against secreted aspartyl proteinase 4–6 of *Candida albicans* as anti-candidiasis

- oral. Results Chem 5: 100721. <https://doi.org/10.1016/j.rechem.2022.100721>
- Miller BR, McGee TD, Swails JM, Homeyer N, Gohlke H, Roitberg AE (2012) MMPBSA.py: An efficient program for end-state free energy calculations. J Chem Theory Comput 8(9): 3314–3321. <https://doi.org/10.1021/ct300418h>
- Miyakawa M, Tsushima T, Murakami H, Wakai K, Iozaki O, Takano K (2003) Increased expression of phosphorylated p70S6 kinase and Akt in papillary thyroid cancer tissues. Endocr J 50(1): 77–83. <https://doi.org/10.1507/endocrj.50.77>
- Motti ML, Califano D, Troncone G, De Marco C, Migliaccio I, Palmieri E, Pezzullo L, Palombini L, Fusco A, Viglietto G (2005) Complex regulation of the cyclin-dependent kinase inhibitor p27kip1 in thyroid cancer cells by the PI3K/AKT pathway. Am J Pathol 166(3): 737–749. [https://doi.org/10.1016/S0002-9440\(10\)62295-X](https://doi.org/10.1016/S0002-9440(10)62295-X)
- Nickel J, Gohlke BO, Erehman J, Banerjee P, Rong WW, Goede A, Dunkel M, Preissner R (2014) SuperPred: Update on drug classification and target prediction. Nucleic Acids Res 42(W1): W26–W31. <https://doi.org/10.1093/nar/gku477>
- Noolu B, Ajumeera R, Chauhan A, Nagalla B, Manchala R, Ismail A (2013) Murraya koenigii leaf extract inhibits proteasome activity and induces cell death in breast cancer cells. BMC Complement Altern Med 13(1): 7. <https://doi.org/10.1186/1472-6882-13-7>
- Patlewicz G, Jeliakova N, Safford RJ, Worth AP, Aleksiev B (2008) An evaluation of the implementation of the Cramer classification scheme in the Toxtree software. SAR QSAR Environ Res 19(5–6): 495–524. <https://doi.org/10.1080/10629360802083871>
- Patterson J, Verghese M (2015) Anticancer and toxic effects of curry leaf (Murraya koenigii) extracts. J Pharmacol Toxicol 10(2): 49–59. <https://doi.org/10.3923/jpt.2015.49.59>
- Piñero J, Ramírez-Anguita JM, Sañch-Pitarch J, Ronzano F, Centeno E, Sanz F, Furlong LI (2019) The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic Acids Res 48(D1): D845–D855. <https://doi.org/10.1093/nar/gkz1021>
- Prete A, Borges de Souza P, Censi S, Muzza M, Nucci N, Sponziello M (2020) Update on fundamental mechanisms of thyroid cancer. Front Endocrinol 11:102. <https://doi.org/10.3389/fendo.2020.00102>
- Saini S, Tulla K, Maker AV, Burman KD, Prabhakar BS (2018) Therapeutic advances in anaplastic thyroid cancer: A current perspective. Mol Cancer 17(1): 154. <https://doi.org/10.1186/s12943-018-0903-0>
- Salehi B, Varoni, EM, Sharifi-Rad M, Rajabi S, Zucca P, Iriti M, Sharifi-Rad J (2019) Epithelial-mesenchymal transition as a target for botanicals in cancer metastasis. Phytomedicine 55: 125–136. <https://doi.org/10.1016/j.phymed.2018.07.001>
- Salentin S, Schreiber S, Haupt VJ, Adasme MF, Schroeder M (2015) PLIP: Fully automated protein–ligand interaction profiler. Nucleic Acids Res 43(W1): W443–W447. <https://doi.org/10.1093/nar/gkv315>
- Sander T, Freyss J, von Korff M, Rufener C (2015) DataWarrior: An open-source program for chemistry aware data visualization and analysis. J Chem Inf Model 55(2): 460–473. <https://doi.org/10.1021/ci500588j>
- Sandulache VC, Skinner HD, Wang Y, Chen Y, Dodge CT, Ow TJ, Bankson JA, Myers JN, Lai SY (2012) Glycolytic inhibition alters anaplastic thyroid carcinoma tumor metabolism and improves response to conventional chemotherapy and radiation. Mol Cancer Ther 11(6): 1373–1380. <https://doi.org/10.1158/1535-7163.MCT-12-0041>
- Sari AN, Bhargava P, Dhanjal JK, Putri JF, Radhakrishnan N, Shaferin S, Ishisa Y, Terao K, Sundar D, Kaul SC, Wadhwa R (2020) Combination of withaferin A and CAPE provides superior anticancer potency: Bioinformatics and experimental evidence to their molecular targets and mechanism of action. Cancers 12(2): 1160. <https://doi.org/10.3390/cancers12051160>
- Sari AN, Dhanjal JK, Elwakeel A, Kumar V, Meidinna HN, Zhang H, Ishisa Y, Terao K, Sundar D, Kaul SC, Wadhwa R (2022) A low dose combination of withaferin A and caffeic acid phenethyl ester possesses anti-metastatic potential in vitro: Molecular targets and mechanism. Cancers 14(3): 787. <https://doi.org/10.3390/cancers14030787>
- Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 13(11): 2498–2504. <https://doi.org/10.1101/gr.1239303>
- Shin E, Hong SW, Kim SH, Yang WI (2004) Expression of down stream molecules of RET (p-ERK, p-p38 MAPK, p-JNK and p-AKT) in papillary thyroid carcinomas. Yonsei Med J: 45(2): 306. <https://doi.org/10.3349/ymj.2004.45.2.306>
- Shinohara M, Chung YJ, Saji M, Ringel MD (2007) AKT in thyroid tumorigenesis and progression. Endocrinology 148(3): 942–947. <https://doi.org/10.1210/en.2006-0937>
- Siegel RL, Miller KD, Jemal A (2019) Cancer statistics, 2019. CA Cancer J Clin 69(1): 7–34. <https://doi.org/10.3322/caac.21551>
- Siegel RL, Miller KD, Wagle NS, Jemal A (2023) Cancer statistics, 2023. CA Cancer J Clin 73(1): 17–48. <https://doi.org/10.3322/caac.21763>
- Siregar, KAAK, Syaifie PH, Jauhar MM, Arda AG, Rochman NT, Kustiawan PM, Mardiyati E (2024) Revealing curcumin therapeutic targets on SRC, PPARG, MAPK8 and HSP90 as liver cirrhosis therapy based on comprehensive bioinformatic study. J Biomol Struct Dyn 1–18. <https://doi.org/10.1080/07391102.2023.2301534>
- Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Stein TI, Nudel R, Lieder I, Mazor Y, Kaplan S, Dahary D, Warshawsky D, Guan-Golan Y, Kohn A, Rappaport N, Safran M, Lancet D (2016) The GeneCards Suite: From gene data mining to disease genome sequence analyses. Curr Protoc Bioinformatics 54(1). <https://doi.org/10.1002/cpbi.5>
- Suh JM, Song JH, Kim DW, Kim H, Chung HK, Hwang JH, Kim JM, Hwang ES, Chung J, Han JH, Cho BY, Ro HK, Shong M (2003) Regulation of the phosphatidylinositol 3-kinase, Akt/protein kinase B, FRAP/mammalian target of rapamycin, and ribosomal S6 kinase 1 signaling pathways by thyroid-stimulating hormone (TSH) and stimulating type TSH receptor antibodies in the thyroid gland. J Biol Chem 278(24): 21960–21971. <https://doi.org/10.1074/jbc.M300805200>
- Syam S, Abdul AB, Sukari MA, Mohan S, Abdelwahab SI, Wah TS (2011) The growth suppressing effects of girinimbine on Hepg2 involve induction of apoptosis and cell cycle arrest. Molecules 16(8): 7155–7170. <https://doi.org/10.3390/molecules16087155>
- Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, Gable AL, Fang T, Doncheva NT, Pyysalo S, Bork P, Jensen LJ, von Mering C (2023) The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res 51(D1): D638–D646. <https://doi.org/10.1093/nar/gkac1000>
- Tang Y, Li M, Wang J, Pan Y, Wu FX (2015) CytoNCA: A cytoscape plugin for centrality analysis and evaluation of protein interaction networks. Biosystems 127: 67–72. <https://doi.org/10.1016/j.biosystems.2014.11.005>
- Trigo JM, Capdevila J, Grande E, Grau J, Lianes P (2014) Thyroid cancer: SEOM clinical guidelines. Clin Transl Oncol 16(12): 1035–1042. <https://doi.org/10.1007/s12094-014-1224-4>

- Trott O, Olson AJ (2009) AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 73: 196–218. <https://doi.org/10.1002/jcc.21334>
- Tuli HS, Mittal S, Aggarwal D, Parashar G, Parashar NC, Upadhyay SK, Barwal TS, Jain A, Kaur G, Savla R, Sak K, Kumar M, Varol M, Iqbal A, Sharma AK (2021) Path of silibinin from diet to medicine: A dietary polyphenolic flavonoid having potential anti-cancer therapeutic significance. *Semin Cancer Biol* 73: 196–218. <https://doi.org/10.1016/j.semcancer.2020.09.014>
- Vasko V, Gaudart J, Allasia C, Savchenko V, Di Cristofaro J, Saji M, Ringel M, De Micco C (2004) Thyroid follicular adenomas may display features of follicular carcinoma and follicular variant of papillary carcinoma. *Eur J Endocrinol* 151(6): 779–786. <https://doi.org/10.1530/eje.0.1510779>
- Wagle N, Grabiner BC, Van Allen EM, Amin-Mansour A, Taylor-Weiner A, Rosenberg M, Gray N, Barletta JA, Guo Y, Swanson SJ, Ruan DT, Hanna GJ, Haddad RI, Getz G, Kwiatkowski DJ, Carter SL, Sabatini DM, Jänne PA, Garraway LA, Lorch JH (2014) Response and acquired resistance to everolimus in anaplastic thyroid cancer. *N Engl J Med* 371(15): 1426–1433. <https://doi.org/10.1056/NEJMoa1403352>
- Wu WI, Voegtli WC, Sturgis HL, Dizon FP, Vigers GPA, Brandhuber BJ (2010) Crystal structure of human AKT1 with an allosteric inhibitor reveals a new mode of kinase inhibition. *PLoS One* 5(9): e12913. <https://doi.org/10.1371/journal.pone.0012913>
- Yang L, He JT, Guan H, Sun YD (2013) AKT1 inhibitory DNazymes Inhibit cell proliferation and migration of thyroid cancer cells. *Asian Pac J Cancer Prev* 14(4): 2571–2575. <https://doi.org/10.7314/APJCP.2013.14.4.2571>
- Zaballos MA, Santisteban P (2017) Key signaling pathways in thyroid cancer. *J Endocrinol* 235(2): R43–R61. <https://doi.org/10.1530/JOE-17-0266>

AUTHOR CONTRIBUTION:

Contribution	Rahman FI	Sari AN	Siregar KAAK	Prakoso I	Syahraini A	Ningsih EG	Harnelly E	Zulkarnain Z
Concepts or ideas	x	x	x	x			x	x
Design	x	x	x	x	x	x		x
Definition of intellectual content					x		x	x
Literature search	x	x	x	x		x		
Experimental studies	x							
Data acquisition	x	x						
Data analysis	x	x	x					
Statistical analysis	x		x					
Manuscript preparation		x	x					
Manuscript editing		x	x					x
Manuscript review	x	x	x	x	x	x	x	x

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Supplementary data

Table S1. The top ten results of GO enrichment analysis.

Term	ID	Description	p-value	p-adjust	q-value
BP	GO:0009410	Response to xenobiotic stimulus	6.77E-29	2.83E-25	1.14E-25
BP	GO:0006979	Response to oxidative stress	6.02E-28	1.26E-24	5.08E-25
BP	GO:0034599	Cellular response to oxidative stress	1.09E-26	1.32E-23	5.33E-24
BP	GO:0048732	Gland development	1.54E-26	1.32E-23	5.33E-24
BP	GO:0062197	Cellular response to chemical stress	1.58E-26	1.32E-23	5.33E-24
BP	GO:2001233	Regulation of apoptotic signaling pathway	1.96E-26	1.37E-23	5.51E-24
BP	GO:0009411	Response to UV	3.16E-25	1.89E-22	7.61E-23
BP	GO:0009314	Response to radiation	6.33E-24	3.31E-21	1.34E-21
BP	GO:0048511	Rhythmic process	1.23E-23	5.70E-21	2.30E-21
BP	GO:0018105	Peptidyl-serine phosphorylation	2.19E-23	9.14E-21	3.69E-21
MF	GO:0004714	Transmembrane receptor protein tyrosine kinase activity	7.61E-17	3.97E-14	2.32E-14
MF	GO:0004713	Protein tyrosine kinase activity	2.51E-15	5.66E-13	3.30E-13
MF	GO:0019199	Transmembrane receptor protein kinase activity	3.26E-15	5.66E-13	3.30E-13
MF	GO:0001221	Transcription coregulator binding	3.23E-14	4.21E-12	2.46E-12
MF	GO:0042826	Histone deacetylase binding	3.73E-12	3.89E-10	2.27E-10
MF	GO:0019902	Phosphatase binding	7.01E-11	6.09E-09	3.55E-09
MF	GO:0001223	Transcription coactivator binding	8.50E-11	6.33E-09	3.69E-09
MF	GO:0140297	DNA-binding transcription factor binding	1.28E-10	8.35E-09	4.87E-09
MF	GO:0004674	Protein serine/threonine kinase activity	5.51E-10	3.19E-08	1.86E-08
MF	GO:0008395	Steroid hydroxylase activity	8.92E-10	4.65E-08	2.71E-08
CC	GO:0045121	Membrane raft	1.64E-14	2.58E-12	1.73E-12
CC	GO:0098857	Membrane microdomain	1.75E-14	2.58E-12	1.73E-12
CC	GO:0005901	Caveola	1.25E-09	1.23E-07	8.24E-08
CC	GO:0017053	Transcription repressor complex	1.27E-08	9.38E-07	6.29E-07
CC	GO:0044853	Plasma membrane raft	2.83E-08	1.67E-06	1.12E-06
CC	GO:0009925	Basal plasma membrane	2.48E-07	1.22E-05	8.17E-06
CC	GO:0016323	Basolateral plasma membrane	4.38E-07	1.85E-05	1.24E-05
CC	GO:0045178	Basal part of cell	5.44E-07	2.01E-05	1.35E-05
CC	GO:0098687	Chromosomal region	6.26E-07	2.05E-05	1.38E-05
CC	GO:0005925	Focal adhesion	1.67E-06	4.93E-05	3.31E-05

Table S2. The top ten results of KEGG enrichment analysis.

Term	ID	Description	p-value	p-adjust	q-value
KEGG	hsa05417	Lipid and atherosclerosis	2.11E-27	5.35E-25	1.88E-25
KEGG	hsa05215	Prostate cancer	5.04E-26	6.39E-24	2.25E-24
KEGG	hsa05210	Colorectal cancer	1.16E-24	9.79E-23	3.45E-23
KEGG	hsa04151	PI3K-Akt signaling pathway	3.04E-24	1.93E-22	6.80E-23
KEGG	hsa05219	Bladder cancer	1.54E-23	7.83E-22	2.76E-22
KEGG	hsa01522	Endocrine resistance	9.91E-22	4.19E-20	1.48E-20
KEGG	hsa05161	Hepatitis B	8.14E-21	2.96E-19	1.04E-19
KEGG	hsa05205	Proteoglycans in cancer	3.13E-20	9.92E-19	3.50E-19
KEGG	hsa01521	EGFR tyrosine kinase inhibitor resistance	6.29E-20	1.78E-18	6.26E-19

Table S3. Result docking AKT-1.

Rank	Ligand	Docking 1	Docking 2	Docking 3	Average
Native	1-(1-(4-(7-Phenyl-1H-imidazo[4,5-g]quinoxalin-6-yl)benzyl)piperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-one	-14,21	-14,24	-14,21	-14,22
1	Clionasterol	-10,97	-10,955	-10,982	-10,969
2	27-Norurs-12-ene	-10,87	-10,867	-10,842	-10,85966667
3	Pyraquinone A	-10,787	-10,797	-10,775	-10,78633333
4	Murrayaquinone B	-10,633	-10,637	-10,649	-10,63966667
5	Koenimbine	-10,603	-10,556	-10,488	-10,549
6	Murrayamine I	-10,516	-10,496	-10,473	-10,495
7	Pyraquinone A	-9,777	-9,764	-9,762	-9,76766667
8	Siamenol	-9,648	-9,649	-9,66	-9,65233333
9	14-Diepoxy-15,17-bisnorlabdane	-9,472	-9,418	-9,431	-9,44033333
10	Alpha-Tocopherol acetate (vitamin E)	-9,524	-9,114	-9,214	-9,284
11	Aciphyllene	-8,964	-8,967	-8,965	-8,96533333
12	gamma-Gurjunene	-8,923	-8,92	-8,931	-8,92466667
13	alpha-delta-Guaiene	-8,822	-8,833	-8,822	-8,82566667
14	Ledene	-8,755	-8,814	-8,824	-8,79766667
15	alpha-Guaiene	-8,796	-8,739	-8,796	-8,777
16	Matrine	-8,759	-8,774	-8,773	-8,76866667
17	alpha-Gujene	-8,803	-8,76	-8,731	-8,76466667
18	5-p-Methoxyanilido-7-amino-8-5H-quinolone	-8,749	-8,716	-8,718	-8,72766667
19	delta-Guaiene	-8,687	-8,675	-8,699	-8,687
20	21HNaphthalenone	-8,652	-8,659	-8,657	-8,656
21	4,7-Methanoazulene	-8,497	-8,488	-8,47	-8,485
22	Globulol	-8,438	-8,446	-8,442	-8,442
23	beta-Patchoulene	-8,406	-8,415	-8,4	-8,407
24	Beta Patchoulene	-8,397	-8,375	-8,404	-8,392
25	Murralongin	-8,362	-8,365	-8,382	-8,36966667

26	beta-Selinene	-8,361	-8,365	-8,37	-8,365333333
27	Nootkatene	-8,349	-8,326	-8,341	-8,338666667
28	alpha-Selinene	-8,319	-8,327	-8,321	-8,322333333
29	13-Diepoxy-14,15-bisnorlabdane	-8,28	-8,278	-8,269	-8,275666667
30	Spathulenol	-8,196	-8,196	-8,197	-8,196333333
31	(+)-gamma-Gurjunene	-8,142	-8,137	-8,119	-8,132666667
32	Murrayone	-8,135	-8,222	-7,946	-8,101
33	Murraxonin	-8,052	-8,091	-8,1	-8,081
34	beta-Acorenol	-7,991	-7,989	-7,981	-7,987
35	alpha-Maaliene	-7,901	-7,908	-7,912	-7,907
36	cis-Z-alpha-Bisabolene epoxide	-7,758	-7,826	-7,83	-7,804666667
37	Caryophyllene oxide	-7,652	-7,594	-7,613	-7,619666667
38	Elemene	-7,597	-7,6	-7,608	-7,601666667
39	8S,13-Cedranediol	-7,437	-7,463	-7,455	-7,451666667
40	alpha-Patchoulene1	-7,43	-7,408	-7,431	-7,423
41	alpha-Patchoulene	-7,416	-7,298	-7,436	-7,383333333
42	delta-Elemene	-7,338	-7,348	-7,362	-7,349333333
43	Cycloseychellene	-7,315	-7,307	-7,329	-7,317
44	Caryophyllene epoxide	-7,307	-7,298	-7,315	-7,306666667
45	Patchouli alcohol	-7,299	-7,289	-7,297	-7,295
46	beta-Caryophyllene	-7,284	-7,29	-7,291	-7,288333333
47	Caryophyllene	-7,283	-7,283	-7,27	-7,278666667
48	Humulene	-7,208	-7,225	-7,201	-7,211333333
49	Isoaromadendrene epoxide	-7,14	-7,14	-7,133	-7,137666667
50	Pseudoselagin	-7,115	-7,11	-7,106	-7,110333333
51	Naphtalenol	-7,063	-7,07	-7,068	-7,067
52	Linolenic acid	-6,706	-7,1	-7,03	-6,945333333
53	Azulene	-6,932	-6,943	-6,93	-6,935
54	Norpatchouleneol	-6,839	-6,841	-6,833	-6,837666667
55	Seychellene	-6,821	-6,841	-6,849	-6,837
56	1R-3beta-Ethenyl-3-methyl-2alpha-1-methylethenyl-6beta-1-methylethyl-1beta-cyclohexanol	-6,573	-6,564	-6,569	-6,568666667
57	Octadecanoic acid	-6,36	-6,424	-6,308	-6,364
58	Palmitic acid	-6,172	-6,232	-6,346	-6,25
59	Benzyl methyl ether	-5,326	-5,315	-5,316	-5,319
60	E-4,4-Dimethyl-2-pentene	-4,336	-4,325	-4,328	-4,329666667
61	Pyrrolone	-3,576	-3,519	-3,566	-3,553666667

Table S4. Toxicity analysis of *Pogostemon cablin* and *Murraya koenigii*.

No.	Plant	Compound name	Pubchem ID	Initial screening	1st Alert	2nd Alert	3rd Alert	4th Alert
1	Pogostemon cablin	alpha-Guaiene	5317844	NONE	NO	NO	NO	NO
2		Azulene	9231	NONE	NO	NO	NO	NO
3		Patchouli alcohol	10955174	NONE	NO	NO	NO	NO
4		Globulol	12304985					
5		Naphtalenol	7005					
6		Spathulenol	92231					
7		delta-Guaiene	94275	NONE	NO	NO	NO	NO
8		gamma-Gurjunene	90805	NONE	NO	NO	NO	NO
9		Ledene	10910653					
10		alpha-Selinene	10856614	NONE	NO	NO	NO	NO
11		Beta Selinene	28237	NONE	NO	NO	NO	NO
12		beta-Patchoulene	101731	NONE	NO	NO	NO	NO
13		Elemene	10583	NONE	NO	NO	NO	NO
14		delta-Elemene	89316	NONE	NO	NO	NO	NO
15		alpha-Guajene	107152	NONE	NO	NO	NO	NO
16		Seychellene	519743	NONE	NO	NO	NO	NO
17		alpha-Patchoulene	521710	NONE	NO	NO	NO	NO
18		Aciphyllene	565709					
19		2(1H)Naphthalenone	612605	NONE	NO	NO	NO	NO
20		Caryophyllene oxide	1742210					
21		alpha-(delta)-Guaiene	3085917	NONE	NO	NO	NO	NO
22		BETA-CARYOPHYLLENE	5281515	NONE	NO	NO	NO	NO
23		(E)-4,4-Dimethyl-2-pentene	5326158	NONE	NO	NO	NO	NO
24		beta-Acorenol	6430766	NONE	NO	NO	NO	NO
25		Norpatchoulene	14682250	NONE	NO	NO	NO	NO
26		(+)-gamma-Gurjunene	15560285	NONE	NO	NO	NO	NO
27		Nootkatene	74427712	NONE	NO	NO	NO	NO
28		cis-(Z)-alpha-Bisabolene epoxide	91753574	NONE	YES	NO	NO	NO
29		(1R)-3beta-Ethenyl-3-methyl-2alpha-(1-methylethenyl)-6beta-(1-methylethyl)-1beta-cyclohexanol	101280480	NONE	NO	NO	NO	NO
30		alpha-Maaliene	102361380					
31		Cedran-diol	536384	NONE	NO	NO	NO	NO
32		Isoaromadendrene epoxide	534398					
1	Murraya koenigii	Mukoeinin A	5209	NONE	YES	NO	NO	NO
2		Murrayamin I	101693310	NONE	YES	NO	NO	NO

3		Murraxonin	5319960					
4		Murrayaquinone B	375141					
5		Murralongin	179620					
6		Murrayone	5319964					
7		Koenimbine	97487	NONE	YES	NO	NO	NO
8		Siamenol	477436	NONE	YES	NO	NO	NO
9		Pyrayaquinone A	13966506					
10		5-p-Methoxyanilido-7-amino-8-5H-quinolone	624340					
11		pyrrolone	14246795	NONE	YES	NO	NO	NO
12		13-Diepox-14,15-bisnorlabdane	612676	NONE	NO	NO	NO	NO
13		14-Diepox-15,17-bisnorlabdane	624203	NONE	NO	NO	NO	NO
14		Humulene	5281520	NONE	NO	NO	NO	NO
15		Benzyl methyl ether	10869	NONE	NO	NO	NO	NO
16		Caryophyllene epoxide	14350					
17		Caryophyllene	5281515	NONE	NO	NO	NO	NO
18		Clionasterol	457801	NONE	NO	NO	NO	NO
19		Palmitic acid	985					
20		Pseudoselagin	621853	NONE	NO	NO	NO	NO
21		linolenic acid	5280934	NONE	NO	NO	NO	NO
22		Matrine	91466	NONE	NO	NO	NO	NO
23		Vitamin E	14985	NONE	NO	NO	NO	NO
24		Octadecanoic acid	5281					
25		27-Norurs-12-ene	101183147	NONE	NO	NO	NO	NO

Yellow Highlight: Molecules used for molecular docking; Initial Screening: mutagenic, tumorigenic, irritant, reproductive effective using Osiris DataWarrior; 1st Alert: Structural Alert for genotoxic carcinogenicity; 2nd Alert: Structural Alert for nongenotoxic carcinogenicity; 3rd Alert: Potential S. typhimurium TA100 mutagen based on QSAR; 4th Alert: Potential carcinogen based on QSAR.