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Research Article

Phytochemical Screening of Balinese Traditional Herbal as Anticancer Candidates Using GC-MS and Molecular Docking Analysis

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Abstract. Indonesia's biodiversity holds great potential for drug development, including through the use of traditional medicinal plants such as tapak liman (Elephantopus scaber L.), suruhan (Peperomia pellucida L. Kunth), and temu ireng (Curcuma aeruginosa Roxb.). These plants have been used in traditional medicine and are known to contain bioactive compounds with potential anticancer properties. Tapak liman contains compounds capable of inducing apoptosis in cancer cells. Suruhan is known to have cytotoxic and immunomodulatory effects, while temu ireng contains compounds with antioxidant and anticancer activity. This study aims to identify and quantify the bioactive compounds in these three plants using Gas Chromatography-Mass Spectrometry (GC-MS) and to explore their therapeutic potential in cancer treatment. The results of GC-MS analysis successfully identified six main compounds that have potential as anticancer agents, namely Phytol, Caryophyllene, Apiol, Germacrone, Germacrene B, and one additional compound. Temu ireng contains Germacrone, Germacrene B, and Caryophyllene, while suruhan contains Caryophyllene, Apiol, and Phytol. Meanwhile, tapak liman shows the presence of bioactive compounds that support cytotoxic activity. The focus of this study is on the interaction of bioactive compounds with the Bcl-2 protein, which plays a role in inhibiting apoptosis in cancer cells. Additionally, molecular analysis revealed that Germacrone, Germacrene B, Caryophyllene, and Apiol can also bind to the p53 protein, known as a guardian of the genome and a trigger for apoptosis. These findings suggest that the three plants have high potential as natural sources of anticancer agents. Further in vitro and in vivo studies are recommended to test the biological efficacy of these compounds in the context of cancer therapy, thereby bridging traditional knowledge with modern scientific approaches in the development of natural-based medications.

Keywords: Bioactive, Cancer, Suruhan, Tapak liman, Temu ireng.

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1. Introduction

Cancer is a disease in which cells lose control of their cell cycle and die as a result of the production of carcinogens that deactivate tumor suppressor genes.[1] The accumulation of genetic and epigenetic mutations in the body may result in the transformation of cells that are resistant to growth-inhibiting signals, proliferate uncontrollably, and escape immunological responses, leading to cancer.[2] Cancer is currently the leading cause of illness and mortality worldwide, with a record 19.9 million new cases and 10 million deaths per year. According to the World Health Organization (WHO) and International Agency for Research on Cancer (IARC) in the 2022 Global Burden of Cancer Study, the global case count is predicted to rise up to 30.2 million by

2040.[3] The impact of cancer extends beyond the individual; it affects families and society as a whole, compromising emotional well-being, financial stability, and increasing the strain on public healthcare resources. Therefore, identifying effective and timely treatments for cancer is essential. Cancer treatment may involve a combination of surgery, chemotherapy, radiation, and targeted therapy; although most targeted cancer therapies using monoclonal antibodies or small molecules are still undergoing clinical trials.

Natural based product from herbal plants has begun to be recognized as promising anti-cancer agents because of their varied chemical structures and biological activities. This herbal plants are rich in bioactive compounds including flavonoids, alkaloids, terpenoids, and polyphenols that may possessed numerous anticancer effects.[4] These compounds are known to promote apoptosis, hinder cell proliferation and cell cycle by disrupting microtubule formation and target topoisomerases, inhibit angiogenesis, modulate crucial signaling pathways, modulate tumor microenvironment, counteract drug resistance, and stimulate immune responses. Herbal based anti-cancer treatments provide distinct therapeutic benefits, particularly through selective toxicity towards cancer cells, which helps minimize the side effects commonly associated with traditional chemotherapy.

Indonesia has extraordinary biodiversity, including traditional medicinal plants that have long been used to treat various diseases, such as tapak liman (*Elephantopus scaber L.*), suruhan (Peperomia pellucida L. Kunth), and temu ireng (*Curcuma aeruginosa* Roxb.), which has shown great potentials as sources of bioactive compounds with pharmacological activity; including anticancer properties. Tapak liman comes from the Asteraceae family that grows endemically in tropical regions and has been used in traditional medicine to treat fever, infections, liver disorders, and wounds. Phytochemical studies have identified that this plant contains various active compounds such as flavonoids, sesquiterpene lactones, triterpenoids, and phenolic compounds that exhibit antioxidant, anti-inflammatory, cytotoxic, and hepatoprotective activities.[5,6] One of the compounds found in tapak liman, deoxyelephantopin, has been shown in vitro to induce apoptosis in cancer cells through regulation of the NF-ϰB pathway and activation of caspase. Suruhan, also known as Chinese betel, is a tropical herbaceous plant from the Piperaceae family commonly used to treat urinary tract infections, headaches, hypertension, and skin diseases. Suruhan contains flavonoids, alkaloids, sesquiterpenoids such as dillapiole and α-farnesene, and other bioactive compounds with antioxidant, antimicrobial, and anticancer effects.[7,8] Components such as peperochromen-A and β-caryophyllene in suruhan are reported to contribute to cytotoxic and immunomodulatory effects, particularly in the case of cancer. Temu ireng is a member of the Zingiberaceae family that has long been used in traditional medicine to treat digestive disorders, menstrual issues, and as a postpartum tonic. The rhizomes of this plant contain flavonoids, saponins, triterpenoids, alkaloids, and curcuminoids that acts as antioxidants and anticancer agents.[9,10] Recent research shows that fermenting temu ireng into kombucha produces bioactive compounds such as zedoarondiol and 3,5-di-tert-butyl-4-hydroxybenzoic acid, which, through in silico approaches, have been shown to inhibit the activity of ERα and PR proteins in cancer cells.[9] Additionally, temu ireng has the potential to improve physiological quality through hepatoprotective effects and enhanced biological performance.[10]

These three plants are thought to have a stronger effect on cancer cell proliferation than their individual use. Flavonoids and sesquiterpene lactones from tapak liman induce apoptosis, while flavonoids and alkaloids from suruhan suppress cell proliferation and oxidative stress, and phenolic compounds and triterpenoids from temu ireng support the inhibition of cancer signaling pathways and the recovery of normal cells. Therefore, these three plants are highly promising candidates for development as locally sourced herbal phytopharmaceuticals. The purpose of this study on the characterization of Balinese traditional herbal plants, specifically through the analysis of extracts from tapak liman, suruhan, and temu ireng using Gas Chromatography-Mass Spectrometry (GC-MS), is to identify and quantify bioactive compounds contained in these plants that may serve as potential anticancer agents. By analyzing these extracts, we aims to identify the specific chemical constituents of the plants that may contribute to anticancer properties and to assess the potential therapeutic benefits of these compounds in cancer treatment by exploring the biochemical activity between the compunds and the protein using molecular docking, Overall, this study will try to bridge traditional medicinal knowledge with modern

scientific approaches to enhance the understanding of potential natural anticancer therapies.

2. Materials And Methods

Sample Collection and Extraction

Samples consisting of the leaves and stems of tapak liman and suruhan, and the roots of temu ireng were collected in Gianyar and Tabanan. To achieve a consistent dry mass, the samples were dried at temperatures between 50 and 60°C. The samples were then powdered using a blender and macerated with 96% ethanol in a 3:1 ratio (sample powder to ethanol) in a sealed aluminum foil container. The maceration method was employed for extraction, where the materials were steeped in 96% ethanol for three days (3 x 24 hours). The extract was subsequently filtered using filter paper to obtain the filtrate, which was then evaporated in the dark using an evaporator to yield a precipitate.

Secondary Metabolites Characteristics (GC-MS test)

GC-MS analysis of the extract of suruhan (*Peperomia pellucida (L.) Kunth*), temu ireng (*Curcuma aeruginosa*), tapak liman *(Elephantopus scaber L.*) were performed using the equipment GC-MS Agilent 7890B and MSD 5977 A. The injector was operated at 250 °C and the oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min. The GC-MS was injected with a 1 µL sample. The identification of components was based on previous research libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library attached to the GC-MS instrument and the results obtained have been tabulated.

Molecular Docking:

The AutoDock 4.2.6 software was downloaded from the official Scripps Research Institute website (http://autodock.scripps.edu/) to utilize the docking method. The processed protein molecule was imported into the AutoDock 4.2.6 workspace. After adding polar hydrogen atoms, the Kollman and Gasteiger charges of the protein were calculated. The protein was then saved in PDBQT format and used as the target. The grid centers for each protein were selected to align with their active sites. The optimal protein–ligand conformations were determined based on their maximum binding affinities using AutoDock 4.2.6. Interactions between the ligands and proteins were visualized using the Discovery Studio client 2021, and three-dimensional protein–ligand configurations were analyzed to investigate the binding mechanisms.[11]

3. Results and Discussion

The GC-MS results for the extracts of temu ireng (*Curcuma aeruginosa*), suruhan (*Peperomia pellucida (L.) Kunth*) and tapak liman (*Elephantopus scaber L.*) exhibited a GC-MS peak quality of over 50%. The ethanolic extract of temu ireng (*Curcuma aeruginosa*) contained 10 secondary metabolites (Table 1), with (E)-3,13-Tetradecadien-2-one showing the highest peak area at 52.37%, followed by Epicurzerenone (4.17%), Germacrone (2.65%), and Germacrene B (0.18%). The majority of the chemicals present are sesquiterpenes, followed closely by terpenoids and ketones. When comparing these groups, sesquiterpenes are typically nonpolar, whereas terpenoids and ketones can exhibit more polar characteristics. Utilizing 96% ethanol as a solvent is effective for extracting both polar and nonpolar molecules, making it a versatile choice in chemical extractions. Ethanol is an effective solvent for extracting natural products due to its neutrality and broad solubility range. It can dissolve various molecules, including hydrophilic substances that mix well with water and lipophilic compounds such as lipids. Its antibacterial properties reduce the risk of contamination and help preserve the quality of the extracts. From a technical standpoint, ethanol is both affordable and safe; it evaporates quickly after extraction, which simplifies concentration processes. These qualities make ethanol a popular choice for extracting bioactive compounds, particularly those with significant antioxidant effects.

Table 1. Chemical constituents (secondary metabolites) found in ethanolic temu ireng extract (*Curcuma aeruginosa*)

Constituents	Type of secondary metabolites	Retentention Time (RT) (Minutes)	Molecular formula	Molecular weight (g/mol)	Peak area	Boiling point
(1R)-Camphor	Monoterpenoids	4.631	$C_{10}H_{16}O$	152.23	1.06	204 °C
Isoborneol	Terpenoids	4.720	$C_{10}H_{18}O$	154.25	0.41	214 °C
Alpha-terpineol	Terpenoids	4.950	$C_{10}H_{1O}$	154.25	0.41	219 °C
Caryophyllene	Sesquiterpenes	6.635	$C15H_{24}$	204.36	0.23	264 °C
Germacrene B	Sesquiterpenes	7.517	$C15H_{24}$	204.3511	0.18	288 °C
Epicurzerenone	Sesquiterpenes	7.756	$C_{15}H_{18}O_2$	230.30	4.17	321 °C
Spathulenol	Sesquiterpenes	7.906	$C_{15}H_{24}O$	220.35	1.71	296 °C
Alpha-selinene	Sesquiterpenes	8.084	$C_{15}H_{24}$	204.35	1.19	135 °C
Germacrone	Sesquiterpenes	8.331	$C_{15}H_{22}O$	218.33	2.65	330.3 °C
(E)-3,13-Tetradecadien-2-one	Ketone	15.005	$C_{14}H_{26}O$	210.36	52.37	290.4 °C

The ethanolic extract of temu ireng was found to contain ten secondary metabolites, with (E)-3,13-Tetradecadien-2-one being the most abundant at 52.37%**.** This compound's significant presence suggests it may play a crucial role in the extract's biological activities. The ethanolic extract of temu ireng was found to contain ten secondary metabolites, with (E)-3,13-Tetradecadien-2-one being the most abundant at 52.37%**.** This compound's significant presence suggests it may play a crucial role in the extract's biological activities. Other metabolites such as Epicurzerenone (4.17%), Germacrone (2.65%), and Germacrene B (0.18%) also contribute to the overall bioactivity of temu ireng**.** Each of these compounds may possess unique properties that could synergistically enhance the extract's therapeutic effects. The presence of specific secondary metabolites in temu ireng raises the possibility of anticancer properties. Compounds like (E)-3,13-Tetradecadien-2-one have been studied for their potential cytotoxic effects on cancer cells. Research indicates that certain terpenoids and ketones can induce apoptosis in cancer cells, inhibit tumor growth, and possess anti-inflammatory properties, which could further support their role in cancer prevention.

Some secondary metabolites were also found in the ethanolic extract of suruhan (*Peperomia pellucida (L.) Kunth*), which contains eight constituents. Among these, 9,12,15-Octadecatrienoate acid exhibited the highest peak area at 28.25%, followed by Phytol (16.63%), Apiol (10.50%), Bicyclogermacrene (3.19%), and the smallest being 4-methyl-1H-pyrrole-2-carbaldehyde (1.04%) (Table 2).

Table 2. Chemical constituents (secondary metabolites) found in ethanolic suruhan extract (*Peperomia pellucida (L.) Kunth*)

Constituents	Type of secondary metabolites	Retentention Time (RT) (Minutes)	Molecular formula	Molecular weight (g/mol)	Peak area	Boiling point
Caryophyllene-oxide	Sesquiterpenes	6.635	$C_{15}H_{24}O$	220.3505	6.55	245.3 °C
Pentadecane	Aromatic hydrocarbon	6.999	$C_{15}H_{32}$	212.41	6.09	270.6 °C
Bicyclogermacrene	Sesquiterpenes	7.122	$C_{15}H_{24}$	204.3511	3.19	267.8 °C
Apiol	Benzene	7.840	$C_{12}H1_4O_4$	222.24	10.50	285 °C
Phytadiene	Terpenoids	9.168	$C_{20}H_{38}$	278.5	3.25	349 °C
Phytol	Diterpenes	10.424	$C_{20}H_{40}O$	296.5	16.63	204°C
9,12,15 – Octadecatrioenoate acid	Methylester, Polyunsaturated fatty acid	10.791	$C_{18}H_{30}O_2$	278.43	28.25	443.4°C
4-methyl-1H-pyrrole-2-carbaldehyde	Aromatic compound	14.845	C_6H_7NO	109.13	1.04	226 °C

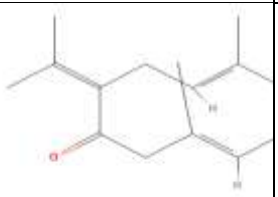
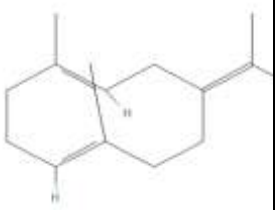
Tapak liman (*Elephantopus scaber L.*) contains certain metabolites, similar to the two extracts mentioned above. According to Table 3, the GC-MS results showed that 2,6-Octadien-1-one, 3,7-dimethyl-1-phenyl- (E) had a peak area of 48.36%, while phytol (2.66%) was also identified in the extracts of tapak liman, as well as in those of *Peperomia pellucida (L.) Kunth (Table 3).*

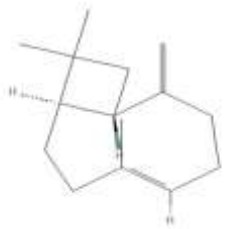
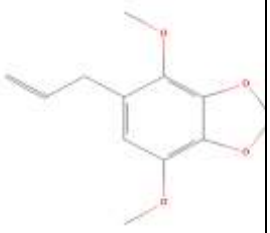
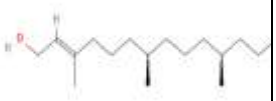
Table 3. Chemical constituents (secondary metabolites) found in ethanolic tapak liman extract (*Elephantopus scaber L.*)

Constituents	Type of secondary metabolites	Retentention Time (RT) (Minutes)	Molecular formula	Molecular weight (g/mol)	Peak area	Boiling point
Hexadecanoic acid	Polyunsaturated fatty acid	9.548	$C_{16}H_{32}O_2$	256.42	2.81	351°C
Phytol	Diterpenoid	10.421	$C_{20}H_{40}O$	296.5	2.66	204 ^{0}°C
Ethyl linoleate	Ethyl ester	10.736	$C_{20}H_{36}O_2$	308.5	8.33	224 °C
Naphthalene	Aromatic compounds	14.521	$C_{10}H_8$	128.1705	7.58	218 °C
2,6-Octadien-1-one, 3,7-dimethyl-1--phenyl-, (E)-	Aromatic ketone	14.916	$C_{16}H_{20}O$	228.33	48.36	302 °C

This study has been identified six chemicals with potential anticancer properties based on GC-MS data from three plant extracts: temu ireng, suruhan, and tapak liman. These chemicals are Phytol, Caryophyllene, Apiol, Germacrone, and Germacrene B. Several compounds identified through GC-MS analysis have been mapped according to previous research with their potential anticancer activity (see Table 4). Germacrone**,** Germacrene B**,** and Caryophyllene were detected in temu ireng**.** Additionally, Caryophyllene was also present in suruhan**,** along with Apiol and Phytol**.** Furthermore, in tapak liman**,** Phytol**,** known for its anticancer properties, was also identified. Other studies have reported similar secondary metabolites, such as Germacrone and Caryophyllene, in different plant species. For instance, research on other members of the Zingiberaceae family often highlights the presence of Germacrone due to its bioactive properties. Previous studies have documented the anticancer potential of Phytol and Caryophyllene. Phytol has been linked to apoptosis in cancer cells, while Caryophyllene has shown promise in reducing tumor growth in various tumor models. Research shows that these six compounds can target proteins involved in cancer cell growth and induce apoptosis. They also affect cellular signaling pathways related to the growth and spread of cancer cells. For example, phytol can reduce Akt activation, which often supports cell growth and survival in cancer, by inhibiting the activity of phosphoinositide 3-kinase (PI3K) (Table 4).

Table 4. Potency of secondary metabolites found in ethanol extract of temu ireng (*Curcuma aeruginosa*), suruhan (*Peperomia pellucida (L.) Kunth*), and tapak liman (*Elephantopus scaber L.*)

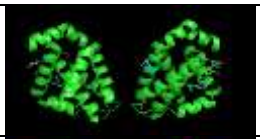
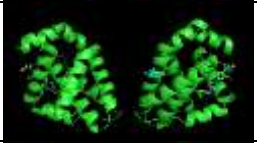
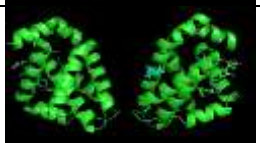
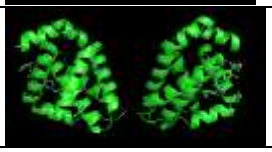
Constituents	Chemical Structure	Anticancer potential
Germacrone		Germacone demonstrated a strong cytotoxic effect on BGC823 cells, decreasing cell growth by inducing G2/M phase cell cycle arrest and promoting cell death. It also showed that germacrone modulated cell cycle-associated protein production and mitochondria-mediated apoptosis.[13]
Germacrene B		In vitro cytotoxic studies revealed that the essential oil Xylopia laevigata extracts were cytotoxic to all tumor cell lines examined. In the in vivo anticancer investigation, tumor growth inhibition rates were 37.3-42.5 percent, with germacrene B extracted 3.22-7.31% from Xylopia laevigata extracts as the major components.[14]

Caryophyllene		On MCF-7, DLD-1, and L-929 cell lines, beta-caryophyllene increased paclitaxel's anticancer efficacy. These findings show that beta-caryophyllene enhances paclitaxel's transit through the membrane, hence increasing its anticancer activity.[4] β-Caryophyllene of chilli pepper exerts inhibitory activity in NSCLC cells possibly by affecting miR-659-3p-targeted SphK1.[15]
Apiol		Apiole was the most effective chemical, particularly at inhibiting the proliferation of COLO 205 colon cancer cells. AP-02's cytotoxicity was much lower in normal colon epithelial (FHC) cells than in other normal cells from the breast, lung, or liver. This chemical has been shown to suppress human colon cancer cell (COLO 205) development by inducing G0/G1 cell cycle arrest and apoptotic cell death.[16]
Phytol		In A549 cells, phytol reduced AP-1 and NF-ϰB luciferase activity in a dose-dependent manner. Phytol also significantly reduced the levels of MMP9, IL-6, VEGFA, IL-8, and NFKBIA in A549 cells, but had no effect on H69 cells. Phytol reduced A549 cell growth and migration via the PI3K-Akt signaling pathway, according to bioinformatic and immunoblotting analyses.[17]

BCL-2 and p53 proteins are two critical apoptosis regulators that have opposing roles in cell survival, particularly in cancer. BCL-2 (B-cell lymphoma 2), an anti-apoptotic protein, blocks the activation of the intrinsic apoptosis pathway by preserving the outer mitochondrial membrane and inhibiting the release of cytochrome C. Overexpression of BCL-2 has been associated to a variety of malignancies, including melanoma, lung cancer, breast cancer, and chronic lymphocytic leukemia, making it an important target for cancer treatment. In contrast, the tumor suppressor gene p53 promotes apoptosis via direct protein interactions and gene transcription mechanisms.[18,19] **Table 4** shows how likely substances are to bind to the BCL-2 receptor. The Gibbs Free Energy (ΔG) values reflect the interaction between the BCL-2 receptor protein and the active chemical ligand. Negative ΔG values indicate a stable connection, while positive values suggest instability. A lower ΔG value means a stronger bond between the ligand and the target.[20] This suggests that the ligand could act as an inhibitor or agonist, indicating an effective interaction with the target.

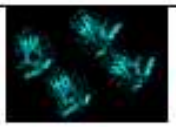
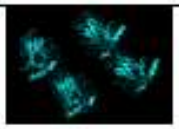
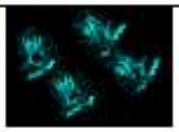
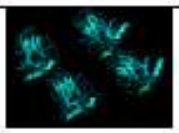
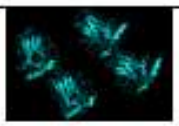
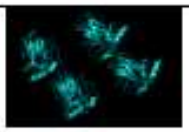
Bcl-2 is an important protein that regulates apoptosis, or programmed cell death. It controls this process by forming pairs with certain pro-apoptotic members of the Bcl-2 family.[18] Table 5 shows that Caryophyllene-oxide, Germacrone, Germacrene B, and Caryophyllene have more negative values compared to the other two chemicals, Apiol and Phytol. This study focuses on the Bcl-2 protein, which acts as an anti-apoptotic protein. This means that Bcl-2 helps prevent cell death, including in cancer cells. By reducing the levels of Bcl-2, apoptosis (programmed cell death) can be triggered, which is essential for treating cancer.

Table 5. Binding affinity of ligand molecules with BCl2 protein

Ligand molecule	ΔG(kcal/mol)	Rmsd lb	Rmsd ub	Molecular docking
Caryophyllene oxide	-6.5	1.643	3.302	
Germacrone	-6.1	1.988	4.490	
Germacrene B	-6.1	1.486	2.767	
Caryophyllene	-6.0	1.685	2.965	
Apiol	-5.3	10.332	12.960	
Phytol	-4.1	1.921	3.687	

Note: *RMSD* root mean square deviation; *RMSD/lb* Rmsd lower bond; *RMSD/ub* Rmsd upper bond; The green structure represents the protein target, while the cyan structure represents the ligand. p53 is a protein involved in the stress response.[21-22] With a more recent review, p53 is considered a molecular center for the interactions between stressors (reactive oxygen radicals [ROS], nutritional restriction, hypoxia, telomere erosion, etc.) and cellular biological responses.[23,24] A previous study found that germacrone had strong cytotoxic effects on BGC823 cells using the MTT assay. Germacrone caused the cells to stop progressing through the G2/M phase by significantly reducing the levels of proteins like cyclin B1, cdc2, and cdc25c. Additionally, germacrone treatment activated caspase-3 and led to the cleavage of PARP in cancer cells. These results showed that germacrone inhibits cell growth by causing G2/M phase cell cycle arrest and promoting cell apoptosis. It also indicated that germacrone affects the production of cell cycle-related proteins and triggers apoptosis through mitochondrial pathways.[25] Table 6 shows that Germacrone, Germacrene B, Caryophyllene, and Apiol have negative ΔG values, suggesting their ability to bind to the p53 protein. This finding underscores the potential of these three plant extracts for in vitro testing on cancer cells, highlighting their relevance in cancer research.

Table 6. Binding affinity of ligand molecules with p53 protein

Ligand molecule	ΔG(kcal/mol)	Rmsd lb	Rmsd ub	Molecular docking
Germacrone	-5.8	1.385	5.030	
Germacrene B	-5.7	0.954	1.226	
Caryophyllene oxide	-5.2	1.630	3.523	
Caryophyllene	-5.2	1.374	2.313	
Apiol	-5.5	1.062	3.608	
Phytol	-4.5	1.444	2.874	

Note: The cyan structure represents the protein target, while the green structure represents the ligand.

Panyajai et al. (2024) found that the essential oil of black turmeric (Curcuma aeruginosa essential oil/CAEO) effectively induces apoptosis and inhibits the migration of MCF-7 and K562 cancer cells, with IC50 values of 20.18 μg/mL and 13.43 μg/mL, respectively.[26] This finding is supported by a study by Rafi et al. (2021), which discovered that nanoemulsions of black turmeric extract can effectively suppress the growth of breast cancer cells (MCF-7), with effectiveness varying based on particle size and incubation time.[27] This highlights the importance of formulation in determining the biological effectiveness of the active compounds in black turmeric. Tran et al. (2024) found that velutin, isolated from the ethyl acetate fraction, had high cytotoxic activity against HepG2, A549, and MCF-7 cancer cells, with IC50 values of 34.00 μg/mL, 30.85 μg/mL, and 41.33 μg/mL, respectively.[28] Other compounds, including dillapiole, apiol, and caryophyllene, have also been shown to inhibit the proliferation of breast and lung cancer cells (Lydia et al., 2021).[29] Although several researchs confirm P. pellucida's anticancer effects, there is variety in extraction methods, fraction kinds, and test cell types, making the conclusions inconsistent and not yet generally applied. Some research is also limited to in vitro stages, with no further testing in animal or human models; therefore, the clinical significance has not yet been proven. Regarding anticancer effectiveness, many studies have shown that E. scaber extracts can inhibit the growth of various types of cancer cells. Gong et al. (2024) found that an ethanol extract of E. scaber (EEES) can suppress the growth and spread of liver cancer cells (HepG2, Huh7, Hep3B) by inducing apoptosis and stopping the cell cycle through the PI3K/Akt pathway.

4. Conclusion

The GC-MS analysis of three extracts—temu ireng, suruhan, and tapak liman—showed many chemicals that could be developed as anticancer agents. The results from the in silico method indicate that these chemicals interact with the BCL-2 and p53 proteins, which are important in cancer development. The presence of secondary metabolites in the ethanol extracts suggests that these plants can be further studied using in vitro and in vivo methods to test their ability to inhibit cancer cell growth.

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