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Research Article

Integrating The Network Pharmacology and Molecular Docking Confirmed with In Vitro Toxicity to Reveal Potential Mechanism of Non–Polar Fraction of *Cyperus rotundus* Linn as Anti-Cancer Candidate

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Abstract
Cyperus rotundus Linn is a plant that is historically used in traditional medicine with anti-cancer potential. Despite the evidence of *C. rotundus* anti-cancer effect on various human carcinoma cell lines, its pharmacological mechanism remains unclear, particularly its non-polar fraction. This study was employed to provide mechanistic insight regarding the anti-cancer properties of *C. rotundus* non-polar fraction by integrating in silico and in vitro approach. The network pharmacology study was used to observe the molecular targets of n-hexane fraction of *C. rotundus*, confirmed by molecular docking simulation using Autodock 4.2. The in vitro toxicity using BSLT method was used to strengthen the in silico result. The network pharmacology investigation revealed several core targets including PI3K, MAPK1, mTOR, RAF1, and NF-κB in the potential anti-cancer mechanism of *C. rotundus*. The molecular docking study illustrated that compound 3 (Isopetasol) and compound 9 (alpha-cyperone) as the most promising compound in n-hexane fraction of C. rotundus, with free binding energies consistently less than -7 kcal/mol in all targets. The in vitro BSLT signified the in silico results, highlighting the highest toxicity of fraction 3 exhibited among others. Integrating the network pharmacology and molecular docking simulation along with in vitro toxicity have provided evidence of the anti-cancer potential of n-hexane fraction of *C. rotundus*. Specific compounds and the molecular targets responsible for its anti-cancer properties have been identified, warranting further investigations.

Keywords: network pharmacology, molecular docking, anti-cancer, *Cyperus rotundus*, LC-MS

1. INTRODUCTION

Cancer is one of the leading causes of mortality across the globe, with the prevalence in 2020 was approximately 19.3 million of new case along with 10 million of death [1]. Even though the current cancer management technologies provide a promising outcome, they still have significant limitations. Patients often face challenges such as insufficient efficacy, lack of targeted action, and prohibitive costs [2]. Moreover, cancer chemotherapy (i.e. doxorubicin, cisplatin, sorafenib) as a whole grapples with two critical issues: the emergence of drug-resistant cancer cells and the occurrence of severe side effects. These toxic reactions can precipitate a cascade of health complications, ranging from hepatotoxicity [3] and cardiotoxicity [4] to nephrotoxicity [5].

Despite advancements in conventional treatments, the search for more effective and less toxic anticancer agents continues, with natural products emerging as a promising source [6]. Several compounds derived from natural products have been extensively used clinically for the management of cancer, including vincristine and vinblastine from *C. roseus* [7], taxol from *T. brevifolia* [8], and epipodophyllotoxin from *P. peltatum* [9]. However, these compounds have been reported to exhibits tremendous side effects including liver toxicity and steastosis [10][11] and cardiovascular toxicity [12]. *Cyperus rotundus* Linn, commonly known as nutgrass, is a perennial plant widely distributed in tropical and subtropical regions. *C. rotundus* has long been used in traditional community to treat broad range of health problem and scientifically demonstrated plentiful biological activities, including anti-inflammation [13], antidiabetic [14], and antimicrobial activities [15]. Current reports have also showed the anti-cancer properties of *C. rotundus* extracts [16].

Mannareddy et al (2017) reported the cytotoxic effect of *C. rotundus* extracts in several cancer cells (i.e. HeLa, MCF-7, and HepG2) [17]. Other similar

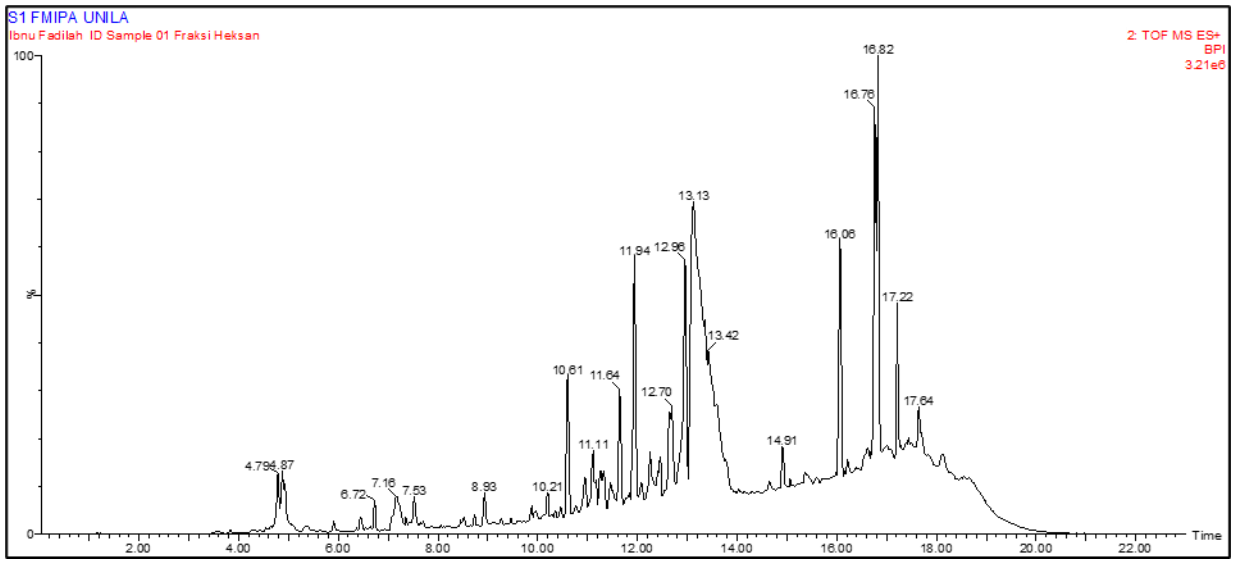

Figure 1. LC chromatogram of the n-hexane fraction of *C. rotundus*.

studies also demonstrated cytotoxic activity of *C. rotundus*, highlighting the non-polar extract and fraction as the most prominent portion [16][18]. However, the mechanistic insight in which this anti-cancer effect of the non-polar fraction of *C. rotundus* is fully unelucidated. The extensive research only focused on crude extracts or isolated compounds which limit the comprehensive understanding of molecular interactions between phytocompounds and the molecular targets, along with their synergistic effects [19][20]. One of the promising strategies in efficiently predicting and analyzing the potential mechanism of action of natural product is integrating the network pharmacology and molecular docking [21][22]. Integrating these approaches can be a complementary alternative to reduce time-consuming and resource intensive of conventional experimental methods.

This study was purposed to provide mechanistic insight of anti-cancer properties of *C. rotundus* non-polar fractions, addressing the conventional experimental limitations by integrating the in silico and in vitro studies. A more comprehensive understanding regarding the pathways and molecular targets involved in anti-cancer activity of *C. rotundus* will be provided by combining the network pharmacology, molecular docking and experimental toxicity assay.

This study aims to address these limitations by combining in silico approaches with in vitro validation to elucidate the potential anticancer mechanisms of the non-polar fraction of *C. rotundus*. By integrating network pharmacology and molecular docking analyses with experimental toxicity assays, we seek to provide a more comprehensive understanding of the molecular targets and pathways involved in the anticancer activity of *C. rotundus*. This study will be significantly contributed for the growing body of knowledge on natural product-based anticancer agents and will be demonstrating the integration of computational and experimental approaches in drug discovery. The findings from this study may pave the way for the development of novel, targeted anticancer therapies derived from *C. rotundus* and other medicinal plants.

2. MATERIALS AND METHODS

2.1. Materials

Chemicals and reagents utilized in this work were in analytical grade purchased from Merck, Germany as follows: aquadest, n-hexane, chloroform, methanol, glacial acetic acid, concentrated sulfuric acid (H_2SO_4), $FeCl_3$, chloroform, silica gel TLC plate GF254, silica gel 60 (0.063 – 0.200 mm), and cerium sulfate. The in silico study was employed using some software including PyRx 0.8, Cytoscape 3.10.1, and Biovia Discovery Studio Visualizer.

2.2. Methods

2.2.1. Sample Collection and Preparation

Fresh *C. rotundus* were collected from Tanggamus, Lampung-Indonesia. The samples were then botanically authenticated in Laboratory of Herbarium, Department of Biology, Faculty of Natural Science, Universitas Lampung. The samples were sorted, washed, and dried in an oven at 50 °C for 24 h. The oven-dried samples were further sorted and took the rhizome followed by pulverization and sieving.

2.2.2. Extraction

3 kg sun-dried powder of *C. rotundus* rhizome were macerated using 6 L of a technical grade methanol 96% in a glass chamber for 24 hours at room temperature with occasionally stirred. The extract was then filtered using a Whatman filter paper No. 1, and the menstruum was collected. The residues were re-macerated same as the previous steps, and the collected menstruum was evaporated using a vacuum rotary evaporator (Buchi, Germany). The yield of extract was then calculated using the following formula 1 as reported by Setiawansyah et al (2024) [23].

$$\text{Yield (\%)} = \frac{Crude\ extract\ (g)}{Dry\ powdered\ sample\ (g)} x\ 100\% \quad \textbf{(1)}$$

2.2.3. Fractionation and Sub-fractionation

The crude extract underwent fractionation through liquid-liquid extraction using solvents of varying polarity: n-hexane, chloroform, and methanol. Initially, 30 g of crude extract was

Table 1. LC-MS result of the n-hexane fraction of *C. rotundus*.

Peak	RT (min)	$[M^+H]^+$ (m/z)	Formula	Compound name	Similarity (%)
1	4.79	146.10	$C_{10}H_{12}N$	Not identified	-
2	4.87	146.11	$C_{10}H_{12}N$	6-methylazecine	67.77
3	6.72	235.17	$C_{15}H_{23}O_2$	Isopetasol	81.72
4	7.16	160.11	$C_{11}H_{14}N$	3-ethyl-1,2-dihydroquinoline	58.39
5	7.53	146.10	$C_{10}H_{12}N$	2-ethyl-benzeneacetonitrile	73.12
6	8.93	146.10	$C_{10}H_{12}N$	Not identified	-
7	10.21	115.06	C_9H_{10}	1-ethynyl-4-(λ2-methyl) benzene	80.65
8	10.61	105.03	C_7H_5O	2-formylbenzene-1-ylium	85.71
9	11.11	146.10	$C_{10}H_{12}N$	2,3,4-trimethylbenzonitrile	80.00
10	11.64	217.16	$C_{15}H_{21}O$	1,2-dehydro-α-cyperone	76.47
11	11.94	318.30	$C_{18}H_{40}NO_3$	phytosphingoshine	61.90
12	12.25	219.18	$C_{15}H_{23}O$	α-cyperone	80.00
13	12.70	146.10	$C_{10}H_{12}N$	Not identified	-
14	12.96	146.10	$C_{10}H_{12}N$	Not identified	-
15	13.13	217.16	$C_{12}H_{25}OS$	S-ethyl-3,7- dimethyloctanethioate	37.09
16	13.42	234.19	$C_{15}H_{24}NO$	3-(3-methyl-2-oxo-6-(prop-1-en-2- yl) cycloheptyl)butanenitrile	39.05
17	14.91	146.10	$C_{10}H_{12}N$	Not identified	-
18	16.06	146.10	$C_{10}H_{12}N$	2-(4-ethylphenyl) acetonitrile	71.26
19	16.76	146.10	$C_{10}H_{12}N$	Not identified	-
20	16.82	146.10	$C_{10}H_{12}N$	Not identified	-
21	17.22	219.18	$C_{15}H_{23}O$	Not identified	-
22	17.64	309.28	$C_{20}H_{36}O_2$	1,1,4a,6-tetramethyl-5-(3-methylpent-4-en-1-yl)-ecahydronaphthalene-2,6-diol	63.29

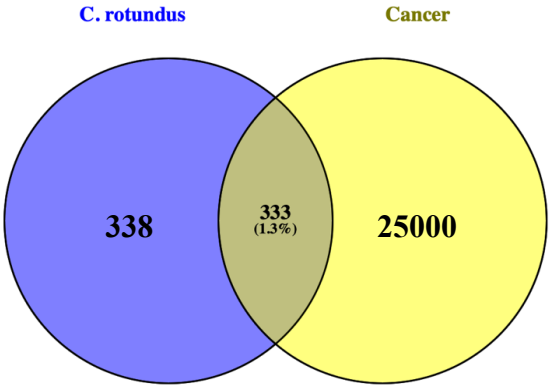

Figure 2. Venn diagram of cross-matching genes from n-hexane fraction of *C. rotundus* with cancer-related genes.

dissolved in 50 mL of methanol. This solution was then combined with 50 mL of n-hexane in a separation funnel, allowing the non-polar compounds to migrate into the n-hexane layer, which was then isolated and collected. Subsequently, the remaining methanol layer was mixed with 50 mL of chloroform in a separation funnel, extracting compounds of intermediate polarity. This entire separation process was repeated three times for each solvent, ensuring thorough extraction.

A classical column chromatography was used to further fractionate the compounds from *C. rotundus* fraction. An isocratic elution was performed on the n-hexane fraction using silica gel 60 (0.063 – 0.200 mm) as the stationary phase and n-hexane – ethyl acetate (15:1), n-hexane – ethyl acetate (7:3), and 100% methanol as the mobile phase with the ratio of sample – stationary phase was 1:30. The sub-fractions were then subsequently collected into vials and monitored using TLC, derivatized with cerium sulfate, and visualized in UV light of 254 nm.

2.2.4. Thin Layer Chromatography Analysis

Sample preparation involved dissolving 10 mg of the samples in 1 mL of methanol, followed by a 5-minute centrifugation. The resulting test solution (2 µL) was applied as a 5 mm band on aluminum silica gel 60 GF254 TLC plates (6 x 10 cm). Chromatographic separation was achieved through linear ascending development in a twin-trough chamber under saturated conditions, utilizing an n-hexane-ethyl acetate (15:1) solvent system. Post-development, the plate underwent derivatization using various reagents including cerium sulfate, Dragendorff, ninhydrin, and vanillin sulfate. Subsequent visualization was performed under visible light, as well as UV light at wavelengths of 254 nm and 366 nm, to comprehensively analyze the separated components.

2.2.5. Network Pharmacology Study

2.2.5.1. Target Identification

The chemical structure of compound identified by LC-MS, represented by SMILES notation, was retrieved from PubChem. This data was then input into Super-PRED, focusing on Homo sapiens mode, to predict the potential targets. The STRING database was utilized to standardize protein IDs and remove duplicates. Targets associated with cancer were gathered from the GeneCards database. Finally, the VENNY 2.1 tool was employed to identify both unique and shared targets between phytocompound from n-hexane fraction and cancer.

2.2.5.2. Protein-Protein Interaction (PPI) Construction

The proteins identified in the earlier stage were analyzed using the STRING databases with multiple protein features. The analysis parameters were set as follows: organism - Homo sapiens, network type - full STRING network, confidence score - high (0.400), and false discovery rate (FDR) stringency - medium (5%). The resulting PPI data was exported in TSV format via the "explore" option. This data was then imported into Cytoscape 3.10.1 software for visualization and construction

of the PPI network.

2.2.5.3. Network Construction and Topological Analysis

The protein-protein interaction (PPI) data in TSV format was processed using Cytoscape 3.10.1 software for topological analysis. The software's network analyzer tool was used to perform a network topology parameter analysis. In the resulting network, nodes represent targets, pathways, and compounds, while edges illustrate the interactions between these elements. The significance of a node is determined by its degree of connectivity - nodes with more direct connections to others are considered more influential in the network.

2.2.5.4. Gene Ontology and KEGG Enrichment Analysis

The ShinyGO 0.80 tool was employed to analyze the functional roles of the identified targets. This analysis encompassed Gene Ontology (GO) categories including biological processes, cellular components, and molecular functions, as well as KEGG pathways. The analysis was conducted with a false discovery rate (FDR) threshold of 0.05. The results of this analysis were then visualized using bar plot maps to illustrate the significant pathways and processes associated with the intersecting targets.

2.2.6. Molecular Docking Study

2.2.6.1. Preparation of Ligands and Macromolecules

All thirteen bioactive compound obtained from the n-hexane fraction were subjected for molecular docking study. The two-dimensional structure of all ligands was sketch using ChemDraw Ultra 12.0 and converted into three-dimensional structure using Chem 3D Pro. The ligand structure was then optimized through MM2 energy minimization and then saved in PDB format. On the other hand, the macromolecule used in this work PI3K, mTOR, NF -KB, MAPK1, and RAF1 as the result of the network pharmacology analysis. The x-ray crystallographic structure of the target macromolecules was retrieved from the Protein Data Bank (PDB) database with PDB IDs of 1E7U (PI3K), 4JT5 (mTOR), 4DN5 (NF-KB), 4QP4

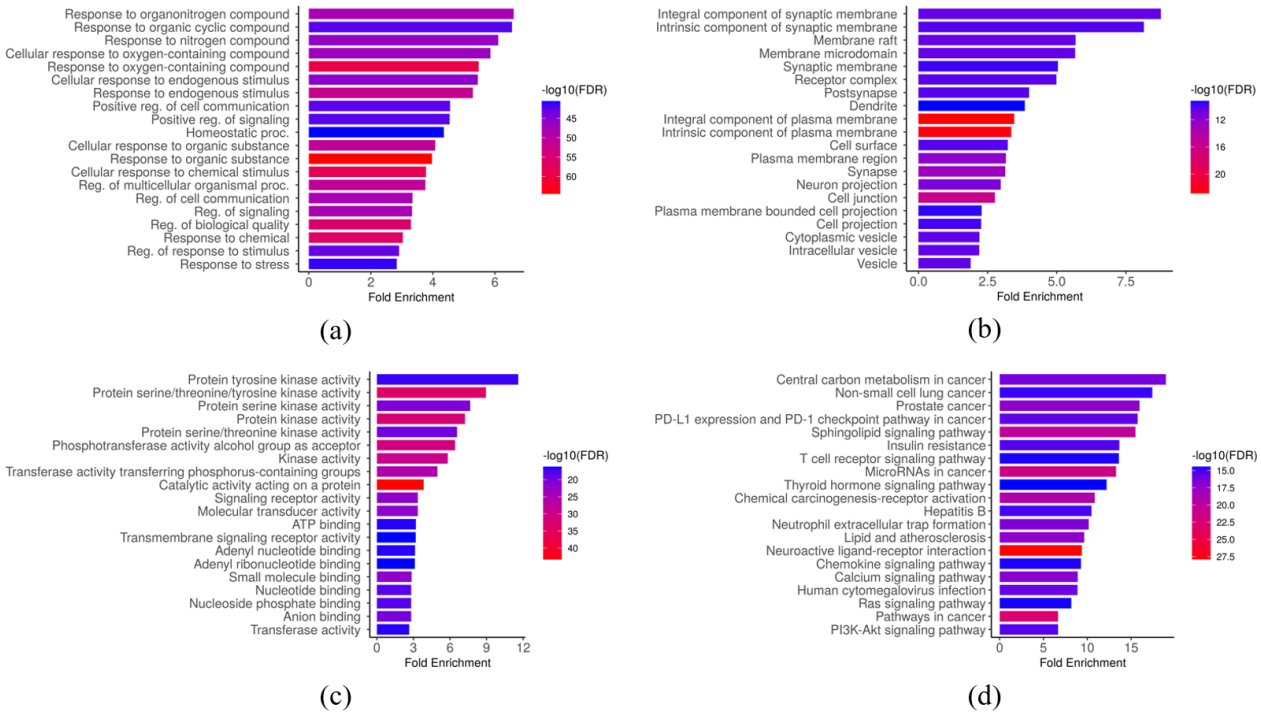

Figure 3. Enrichment analysis of potential from common target of n-hexane fraction of *C. rotundus* and cancer. (a) GO Biological Process; (b) GO Cellular Component; (c) GO Molecular Function; (d) KEGG Pathways.

Table 2. Cancer-related pathways from KEGG analysis of n-hexane fraction of *C. rotundus* genes.

KEGG Pathways	Number of genes	Fold Enrichment	Genes
Central carbon metabolism in cancer	18	18.98	MTOR, GCK, GLS, HIF1A, IDH1, KIT, NTRK1, NTRK3, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PIK3CD, PIK3R1, MAPK1, MAP2K2, RAF1, SLC2A1
Non-small cell lung cancer	17	17.43	CDK4, ALK, GRB2, JAK3, PDPK1, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PRKCA, PRKCB, MAPK1, MAP2K2, RAF1, RXRA, RXRG, STAT3
Prostate cancer	21	15.98	CHUK, MTOR, GRB2, GSTP1, HSP90AB1, AR, MMP3, MMP9, NFKB1, PDGFRA, PDGFRB, PDPK1, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PLAT, MAPK1, MAP2K2, RAF1, CCNE1
PD-L1 expression and PD-1 checkpoint pathway in cancer	19	15.76	CHUK, ALK, MTOR, HIF1A, JAK2, NFKB1, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PRKCQ, MAPK1, MAPK11, MAP2K2, PTPN11, RAF1, STAT3, TLR4, ZAP70
MicroRNAs in cancer	29	13.29	PAK4, EZH2, SIRT1, MTOR, GLS, GRB2, HDAC1, HDAC2, ITGB3, MMP9, NFKB1, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PRKCA, PRKCB, MAPK1, MAP2K2, PTGS2, RAF1, ST14, STAT3, CCNE1, RPS6KA5, HDAC4, CDC25B
Chemical carcinogenesis-receptor activation	29	10.87	CHRNB2, CHRNB4, CYP3A4, EPHX1, ESR2, MTOR, RPS6KA6, GRB2, HSP90AB1, AR, JAK2, NFKB1, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PPARA, PRKCA, PRKCB, MAPK1, MAP2K2, RAF1, BCL6, RXRA, RXRG, STAT3, KLF5, CACNA1B, IKBKE
Pathways in cancer	48	6.68	CDK4, CHUK, ESR2, ALK, MTOR, GRB2, GSTP1, HDAC1, HDAC2, HIF1A, HSP90AB1, AR, ITGB1, JAK2, JAK3, KIT, MMP9, NFE2L2, NFKB1, NOS2, NTRK1, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PIK3CD, PIK3R1, EGLN1, PPARD, PRKCA, PRKCB, MAPK1, MAPK9, MAP2K2, PTGER1, PTGER2, PTGS2, RAF1, RXRA, RXRG, SLC2A1, STAT3, TERT, CXCR4, CASP8, CCNE1, RPS6KA5, KEAP1
PI3K-Akt signaling pathway	32	6.67	CDK4, CHUK, FLT1, MTOR, GRB2, NR4A1, HSP90AB1, ITGB1, ITGB3, JAK2, JAK3, KDR, KIT, NFKB1, NOS3, NTRK1, PDGFRA, PDGFRB, PDPK1, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PRKAA1, PRKCA, MAPK1, MAP2K2, RAF1, RXRA, TLR4, CCNE1

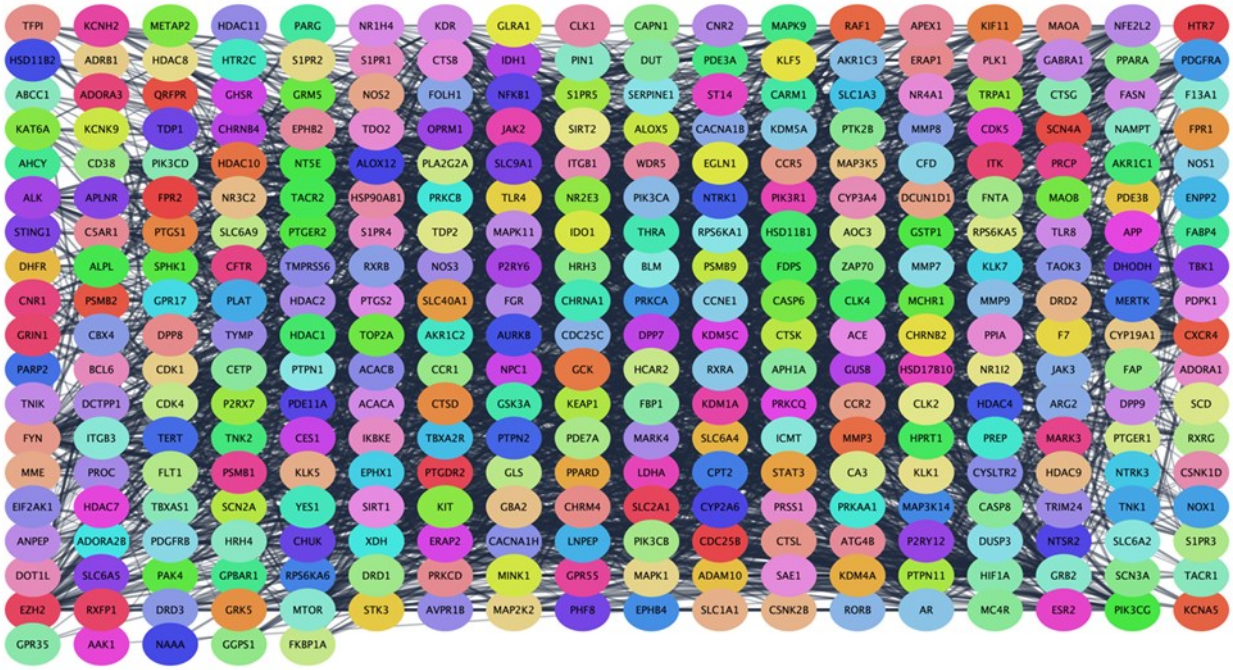

Figure 4. The PPI illustration from all target genes.

(MAPK1), and 1GUA (RAF1), respectively. The macromolecule structure was then processed into Biovia Discover Studio Visualizer to remove ligand, water, heteroatoms, and unnecessary chain followed by polar hydrogen addition. The structure was then saved into PDB format.

2.2.6.2. Molecular Docking

Molecular docking was implemented using PyRx 0.8 software. The prepared macromolecule was input in PyRx and converted into pdbqt format, while the ligands was input, processed, and converted into pdbqt format using OpenBabel platform in PyRx. A global docking was employed to assess the binding affinity between ligands and macromolecule using Autodock Vina platform with the exhaustiveness of 106. The docking score was then saved into CSV format and presented as free binding energy (kcal/mol).

2.2.6.3. Molecular Interaction Visualization

Interaction of ligands with essential amino acid residues of the target macromolecule was observed using Biovia Discovery Studio Visualizer by attaching the docked pose of ligands to the active site of target macromolecule. The type of interactions and the distance was observed and presented in 2D and 3D image files.

2.2.7. In Vitro Toxicity Assessment

The toxicity of sub-fractions isolated from the n-hexane fraction of *C. rotundus* was tested on *Artemia salina* using the BSLT methods [24]. The artificial sea water which previously added with various sub-fraction concentrations, including 100 ppm, 1000 ppm, and 10,000 ppm, was used as media. Each media-containing sub-fractions was carefully filled with ten Artemia salina followed by 24 hours incubation. The number of living *Artemia salina* was considered as the survival rate.

3. RESULTS AND DISCUSSIONS

3.1. Extraction and Fractionation

A 300 g of crude extract (10% of *C. rotundus* dry weight) was yielded from the extraction process which meet the criteria of the Indonesian Herbal Pharmacopoeia (FHI). FHI states that extract yield should not be higher or equal to 10% as it represents the extraction process efficiency. The higher the yield, the more effective the extraction process is considered to be. This result is in line with the work reported by Utami et al. (2023), where the maceration method provided an extract yield of *C. rotundus* ranging from 6 to 13%, depending on the ecological environment in which the sample was grown [16].

The liquid-liquid extraction was employed for the initial fractionation of the crude *C. rotundus* crude extract. Three fractions were obtained with different polarities (i.e. n-hexane, chloroform, methanol). The highest yield was obtained from the methanol fractions followed by chloroform and n-hexane. It highlights that *C. rotundus* were mainly constituted by polar compounds. In this work, only n-hexane fraction underwent sub-fractionation using column chromatography, yielding 12 sub-fractions. The TLC profiling revealed a complex composition of each fraction. Most of them, especially compounds with aromatic rings or conjugated unsaturated bonds, interact differently with UV radiation which exhibits fluorescence. The aromatic compounds or highly conjugated systems typically provide a purple or vibrant blue fluorescence. On the other hand, green glow is considered as the signal of polyenes or similar conjugated structures. Some compounds may quench the fluorescence of the TLC plate, appearing as dark spots against the bright background. Further insight emerges through specialized visualization reagents. In example, cerium sulfate, this reagent detects several class compounds including alcohols, phenols, and other oxidizable compound with the positive indication appeared as yellow, orange, or brown spots. The Dragendorff reagent, it is used to detect alkaloid groups with its orange to reddish-brown marks as a positive marker. Ninhydrin reacts with primary and secondary amines—such as amino acids and peptides—producing vivid purple or blue spots. Meanwhile, vanillin sulfate brings terpenoids and phenolic compounds to light with an array of pink, red, or purple hues. Further characterization using cerium sulfate, Dragendorff, ninhydrin, and vanillin sulfate reagent for visualization produced a variety of colored spots, suggesting the presence of multiple classes of organic compounds including alkaloids and terpenoids, particularly mono and sesquiterpenes. This color-based differentiation provided valuable insights into the diverse chemical nature of the constituents present in each fraction,

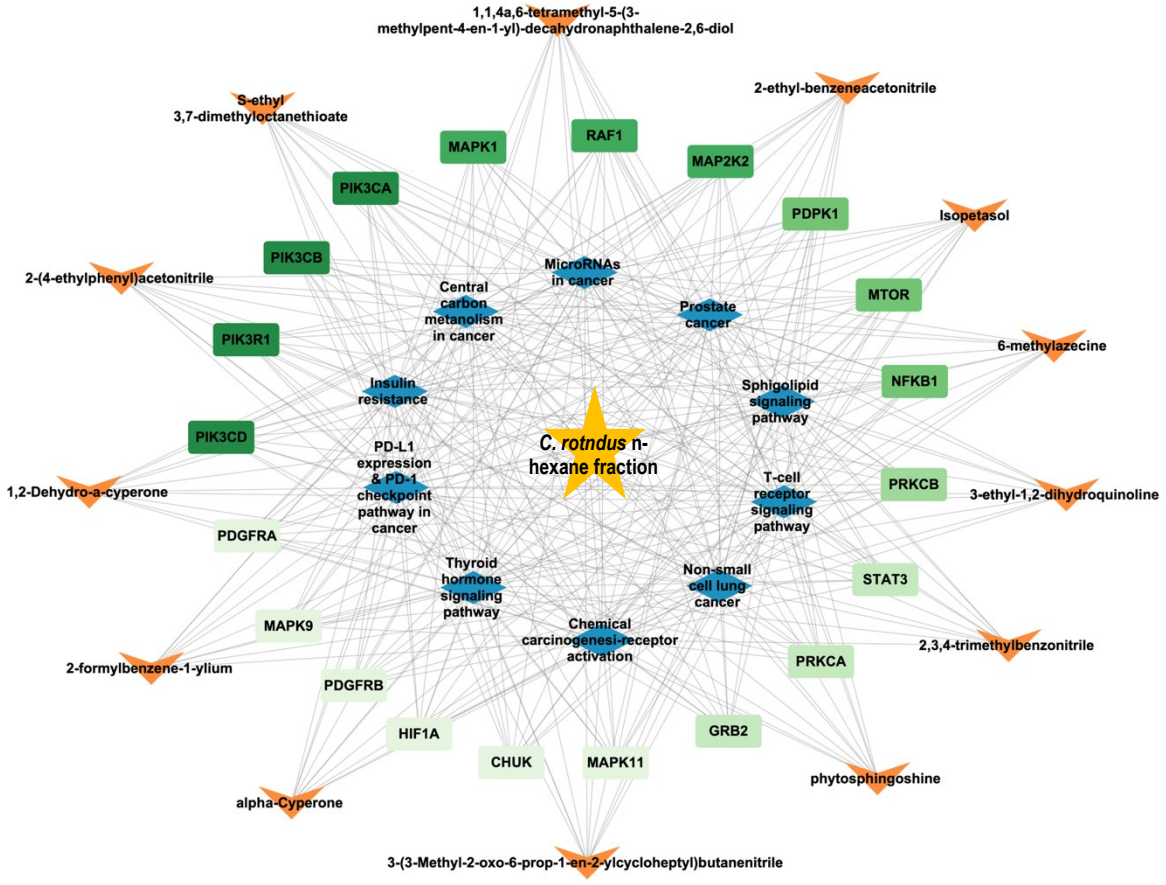

Figure 5. The network diagram of compound – genes – diseases. Soft green nodes indicate lower degree values and dark green nodes signify higher degree values.

highlighting the complexity of the isolated components from the original sample.

3.2. Liquid Chromatography – Mass Spectrometry Analysis

The LC-MS analysis of the n-hexane fraction from *Cyperus rotundus* reveals a complex mixture of compounds, as evidenced by 22 peaks observed in the chromatogram (Figure 1). Several prominent peaks (peaks 8, 10 – 14, and 17 – 19) can be observed in the chromatogram, suggesting the presence of major compounds in the n-hexane fraction. The varying intensities of the peaks indicate different concentrations or of the compounds present.

Liquid Chromatography-Mass Spectrometry (LC -MS) is often selected for bioactive compound analysis over Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) for several reasons: Nature of the Compounds, LC-MS Suitable for a wide range of bioactive compounds, including polar, non-volatile, and thermally labile compounds. Many bioactive compounds, such as alkaloids, flavonoids, and terpenoids, fall into this category. GC-MS is primarily used for volatile and thermally stable compounds. It is less effective for compounds that decompose or are non-volatile under the conditions required for GC. LC-HRMS while it provides higher resolution and accuracy, it is more specialized and may not be necessary for all bioactive compound analyses, especially if LC-MS provides sufficient detail for the current study.

To further interpret the chromatogram data, we analyzed each peak using Sirius software. Thirteen compounds successfully identified among all 22 peaks observed based on the molecular weight as detailed in Table 1. Several major peaks were not identified including peaks 13, 14, and 17 – 19. Two marker compounds from Cyperaceae family were also observed in the n-hexane fraction, including α-cyperone and 1,2-dehydro-α-cyperone.

3.3. Network Pharmacology Analysis

The network pharmacology study was initially done by mining the gene target of all compounds and cross-matching with the cancer-related genes obtained from GeneCards. Gene mining obtained 338 genes for all compound from n-hexane fraction and 25000 genes for cancer. Cross-matching these genes identified 333 (1.3%) common targets in close and direct associated with *C. rotundus* derived compounds and cancer as depicted in Figure 2.

These 333 common genes undergo GO functional annotation and KEGG pathways analysis. As visualized in Figure 3, compounds from n-hexane fraction of *C. rotundus* were mainly influence the response to organonitrogen compound in biological process with the function is related to integral component of synaptic membrane at the cellular component. Furthermore, compounds from n-hexane fraction of *C. rotundus* targets in the molecular function were protein tyrosine kinase and protein serine/threonine/tyrosine kinase activity. Total of eight KEGG signaling pathways relevant to cancer were identified using a significance threshold of $P < 0.05$. To assess the robustness of these findings, False Discovery Rate (FDR) values were calculated, providing a measure of pathway enrichment significance. Subsequent analysis, incorporating both the enrichment score and gene count from the bar plot, highlighted the central carbon metabolism in cancer as the most significantly enriched among the intersecting target genes. This pathway exhibited the lowest FDR value (High fold enrichment) as presented in Table 2, underscoring its potential importance in the molecular mechanisms linking the identified genes to cancer. The prominence of this pathway suggests it may be a key focus for understanding the disease process and potential therapeutic interventions.

Protein interactions were analyzed using the STRING database, based on protein IDs. To elucidate the network structure, these interaction data were visualized and subjected to topological analysis using Cytoscape 3.10.1 software. The resulting network is illustrated in Figure 4, where gene targets are depicted as circular nodes. The color intensity of each node reflects its degree of connectivity within the network, providing a visual representation of each gene's relative importance. Ten nodes, demonstrate particularly high connectivity: PIK3CA, PIK3CB, PIK3CD, PIK3R1, MAPK1, MAP2K2, RAF1, MTOR, NFKB1, and PDPK1. This high degree of interaction suggests these genes may play pivotal

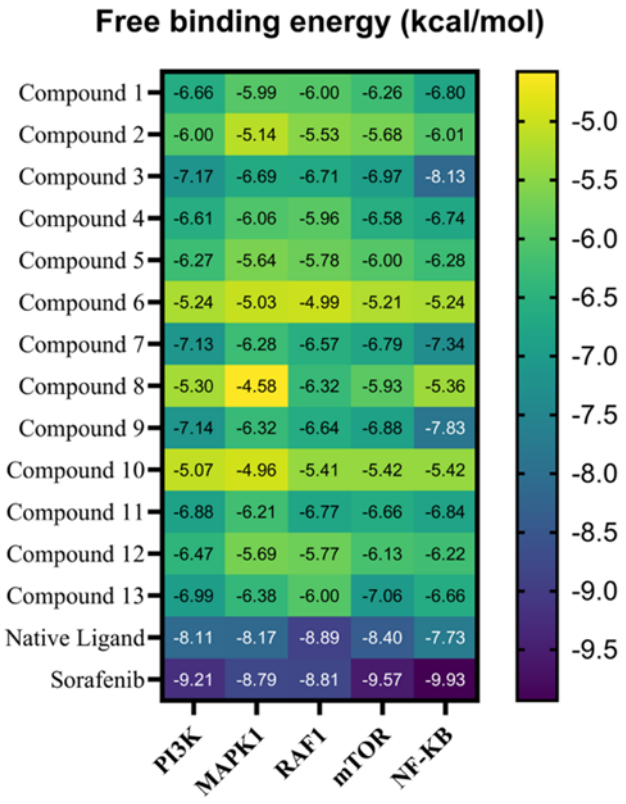

Figure 6. Molecular docking score of thirteen bioactive compounds from n-hexane fraction of *C. rotundus* rhizome.

roles in the biological processes under investigation.

The construction of a network visualization was employed to illustrate the relationship between compound from n-hexane fractions, the core targets, and the associated disease. The network diagram (depicted in Figure 5) provides a comprehensive overview of the relationship of compound, core targets, and associated diseases, which facilitates a better view understanding of potential therapeutic implication of the n-hexane fraction of *C. rotundus*.

The network pharmacology analysis of the n-hexane fractions of *C. rotundus* has brought out interesting observations regarding its potential anti-tumor properties. This analysis was able to detect central carbon metabolism in cancer as an important KEGG pathway with its core target genes being PI3K, MAPK1, mTOR, RAF1 and NF-κB. These results advocate development of a combinatorial strategy for cancer treatment while pointing at the interdependence of metabolism and cancer signaling networks. Central carbon metabolism is very important in the initiation and progression of cancers since cancer cells are known to possess diverse metabolic changes to enable faster and better cell growth [25]. The given core target genes are part of the whole signaling pathways which control this metabolic reprogramming in cancer cells and thus are part of a broad network of interrelationships which influence the activity of cancer cells.

The central component of this network is Phosphatidylinositol-3-kinase (PI3K), which is a prominent member of the PI3K/AKT/mTOR pathway. This pathway is often pathologically activated in a range of cancers in an effort to help cells survive, proliferate and reprogram metabolism. The potential inhibition of PI3K by compounds from *C. rotundus* could disrupt this critical signaling axis, offering a promising avenue for cancer therapy [26]. Metastatic progression is closely associated with the activation of many signaling pathways, and one of them includes PI3K signaling. There is intermediary between PI3K signaling pathway and MTOR. As a master regulator of cell growth and metabolism, MTOR is

often overactive in cancers. Possible alterations to mTOR signaling activity that may be present in Cyperus rotundus tonics, could modulate protein synthesis and metabolism in tumor cells [27]. Another crucial component of this signaling network is MAPK1 (Mitogen-Activated Protein Kinase 1), also known as ERK2. MAPK1 is known to be a central player in the MAPK/ERK pathway, whose main functions are cell proliferation, differentiation and survival regulation. Its hyperactivation which is seen in a number of cancers leads to excessive cell proliferation and survival. Therefore, the possible targeting of MAPK1 by *C. rotundus* compounds offers another mode of inhibition of proliferation in cancer cells [28]. The MAPK pathway works in concert with RAF1, a key player in the RAS/RAF/MEK/ERK signaling cascade. This pathway's significance cannot be overstated, particularly in the context of cancer biology, where its dysregulation often leads to unchecked cell proliferation and enhanced survival of cancer cells. Our findings regarding RAF1 as a core target are particularly intriguing. They suggest that compounds derived from *C. rotundus* might interact with this signaling cascade in meaningful ways. This interaction could potentially mirror the effects of known RAF inhibitors, a class of drugs that have shown considerable promise in clinical settings for certain types of cancer [29]. Lastly, the identification of NF-κB as a core target adds another layer of complexity to the potential anticancer effects of *Cyperus rotundus*. NF-κB, a transcription factor regulating genes involved in inflammation, immunity, and cell survival, is often constitutively active in cancer. It promotes tumor growth, angiogenesis, and metastasis, while also playing a role in metabolic reprogramming of cancer cells. The potential modulation of NF-κB activity by *Cyperus rotundus* compounds could therefore impact multiple aspects of cancer biology [30]. The identification of these interconnected core targets suggests that compounds from the n-hexane fractions of *Cyperus rotundus* may exert their anticancer effects through multiple, synergistic mechanisms. By potentially modulating these keys signaling pathways simultaneously, these compounds could influence cancer cell metabolism, proliferation, survival, and inflammation in a comprehensive manner.

3.4. Molecular Docking Study

The docking simulation of thirteen bioactive compounds exhibit different free binding energy to all target macromolecules. Figure 6 presents a heatmap of free binding energies (in kcal/mol) for various compounds, including a native ligand and Sorafenib, against five protein targets: PI3K, MAPK1, RAF1, mTOR, and NF-KB. The free binding energy indicates the strength of interaction between a compound and a protein target, with more negative values suggesting stronger binding [20][22][31]. Among the tested compounds, compound **3** (Isopetasol) and compound **9** (alpha-Cyperone) show the most promising binding profiles, with energies often below -7 kcal/mol across multiple targets. Additionally, compounds **3** and **9** even exhibit more negative free binding energy than that of the native ligand on NF-KB, suggesting they have excellent affinity on NF-KB than the native ligand have. On the other hand, compound **6** (2-formylbenzene-1-ylium) and compound **10** (S-ethyl 3,7-dimethyloctanethioate) consistently show the weakest binding across all targets, suggesting they may be less effective as potential inhibitors.

Variations in affinity among the compounds can potentially be explained by their differing interactions with specific amino acid residues within the macromolecular structure. In the NF-KB for example (depicted in Figure 7), the native ligand exhibits a rich tapestry of interactions, including conventional hydrogen bonds, van der Waals forces, pi-sigma interactions, and pi-alkyl interactions. This diverse interaction profile is typical of naturally evolved ligands, which often display high affinity and specificity for their targets.

The complexity of these interactions underscores the sophisticated molecular recognition mechanisms that have developed through evolutionary processes, as elucidated by Bissantz et al. (2010) in their comprehensive review of protein-ligand interactions [32]. While sorafenib presents a different yet equally complex interaction pattern. Its binding mode includes conventional hydrogen bonds, attractive charge interactions, pi-sigma and amide-pi stacked interactions, as well as some unfavorable donor-donor interactions. This

multifaceted interaction profile aligns with sorafenib's known promiscuity across multiple kinases, a characteristic that contributes to both its broad therapeutic efficacy and its side effect profile, as detailed in the seminal work by Wilhelm et al. (2006) [33]. The presence of attractive charge interactions is particularly noteworthy, likely contributing significantly to its binding affinity, while the unfavorable donor-donor interactions suggest some degree of strain in the binding pose – a common compromise in drug-target interactions where optimal geometry for all interactions is rarely achieved. In stark contrast, Compound 3 displays a markedly simpler interaction profile, primarily consisting of van der Waals interactions, alkyl and pi-alkyl interactions, and notably, an unfavorable acceptor-acceptor interaction. This simplicity is intriguing, especially given that Compound **3** is labeled as the "best compound." This apparent paradox challenges conventional wisdom that more complex interaction profiles necessarily lead to higher affinity or better drug candidates. It suggests that the quality and positioning of interactions may be more crucial than their quantity, a concept increasingly recognized in modern drug design strategies.

The simpler profile of Compound **3** raises several important points for consideration. Firstly, it may indicate a higher degree of specificity, potentially reducing off-target effects - a desirable

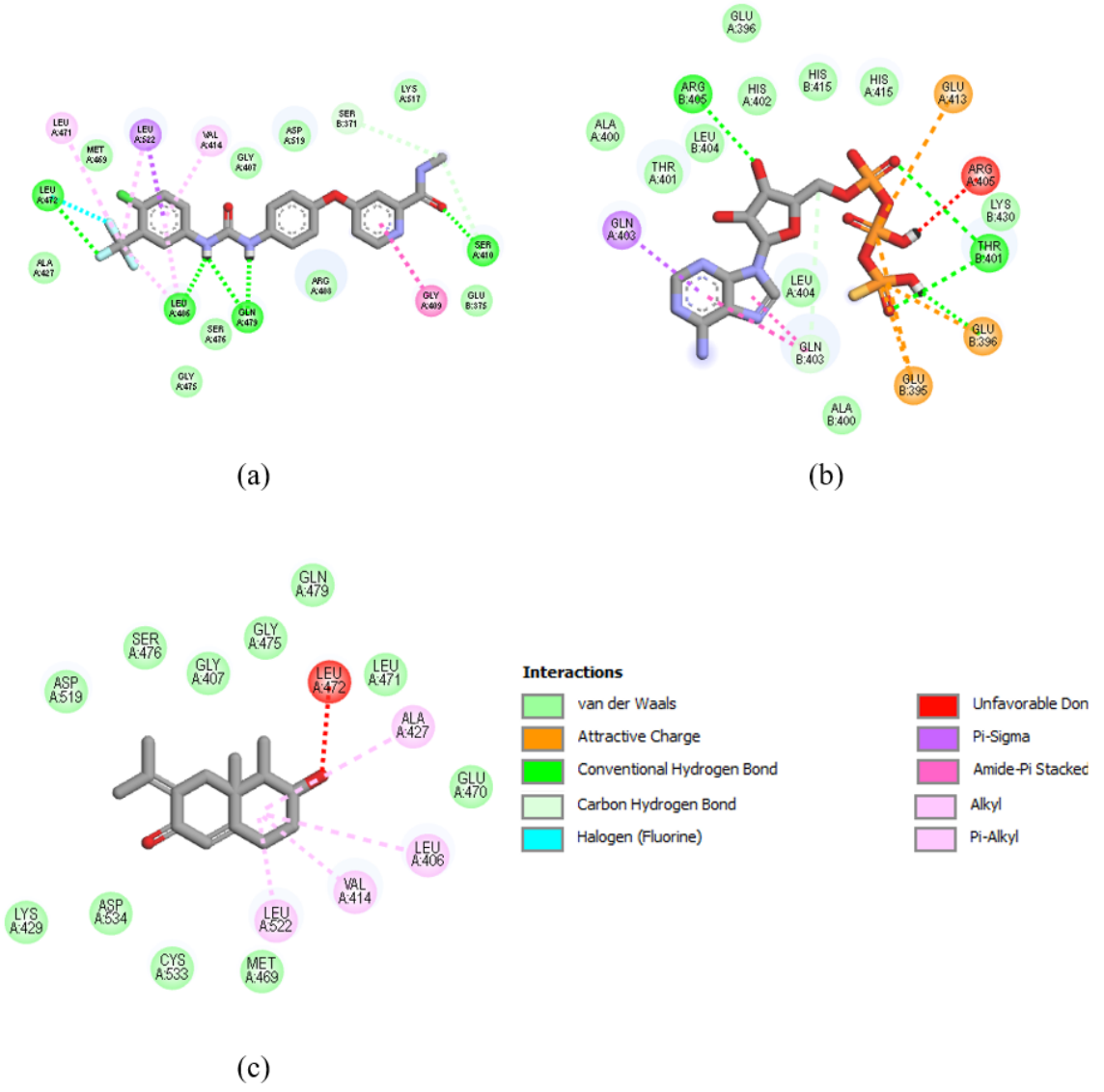

Figure 7. Two-dimensional molecular interaction of (a) native ligand, (b) sorafenib, and (c) compound 3 with amino acid residues of NF-KB.

characteristic in drug development. However, as Anastassiadis et al (2011) demonstrated in their comprehensive kinase inhibitor study, high specificity doesn't always correlate with improved therapeutic outcomes [34]. The strong alkyl and pi-alkyl interactions observed could provide substantial binding energy through hydrophobic effects, as discussed by Bissantz et al. (2010), potentially compensating for the fewer number of interactions [32]. Moreover, the reliance on fewer key interactions might make Compound **3** more susceptible to resistance mutations, a common issue with highly specific kinase inhibitors as reviewed by Barouch-Bentove et al. (2011) [35]. This underscores the delicate balance between specificity and robustness against resistance that must be navigated in drug design.

The multi-target approach illustrated in this docking study is particularly relevant in cancer research, where targeting multiple pathways simultaneously can be more effective than single-target therapies due to the complex nature of cancer signaling networks [36]. Compounds **3** and **9** emerge as the most promising candidates for further investigation. Their ability to bind strongly to multiple targets suggests they could potentially act as multi-kinase inhibitors, similar to Sorafenib but possibly with a different selectivity profile. This could be advantageous in targeting cancer cells that have developed resistance to existing therapies. The strong binding of many compounds to PI3K and NF-KB is noteworthy. The PI3K pathway is often hyperactivated in various cancers and plays a crucial role in cell survival, proliferation, and metabolism [37]. NF-KB, on the other hand, is a key regulator of inflammation and immune responses, which are increasingly recognized as important factors in cancer progression [30]. Compounds that can effectively inhibit these targets could potentially have significant anti-cancer effects. It's interesting to note that while Sorafenib shows the strongest binding overall, some compounds show comparable or even stronger binding to specific targets. For example, compounds **3** and **9** bind more strongly to NF-KB than native ligand does. This suggests that some of these compounds might have unique target profiles that could be exploited for developing new cancer therapies or overcoming resistance to existing drugs.

3.5. Toxicity Assay

The Brine Shrimp Lethality (BSL) method was utilized to evaluate the toxicity of fractions derived from nutgrass tuber extracts. The study examined mortality rates over a 24-hour period, revealing a clear positive correlation between extract concentration and shrimp mortality. Concentrations ranging from 10 to 10,000 µg/mL were tested for each fraction. The mortality endpoint of this experiment was determined through 30 seconds of controlled observation, during which the absence of shrimp movement indicated that the shrimp had perished and allowed for the observation of living shrimp in motion. Mortality percentages were meticulously calculated for each concentration across all fractions.

Key findings from the BSL test as illustrated in Figure 8 highlighted fraction 3 as exceptionally potent, demonstrating high lethality across the entire concentration spectrum. This result suggests fraction 3's potential application as a biopesticide. Conversely, fraction 11 exhibited minimal inhibitory effects throughout the concentration range, indicating its non-toxic properties and potential for medicinal use.

The in vitro toxicity test based on the brine shrimp lethality assay of the n-hexane fraction of *Cyperus rotundus* has yielded intriguing results, particularly regarding fraction 3, which demonstrated strong toxicity activity. This finding aligns with in silico predictions suggesting that the n-hexane fraction can inhibit several cancer-related genes, particularly alpha-Cyperone and Isopetasol, strengthening the potential of *C. rotundus* as a source of anti-cancer compounds. Alpha-Cyperone and Isopetasol are both sesquiterpenes with similar molecular formulas. In chromatographic separations, they would likely have a higher retention time on a polar stationary phase like silica gel compared to other compounds found in *C. rotundus*. They both compounds share similar overall chemical characteristics typical of sesquiterpenes, increasing the possibility to include in the same fraction particularly in fraction 3. The use of the brine shrimp lethality test as a preliminary screening method is consistent with numerous other studies in natural product research,

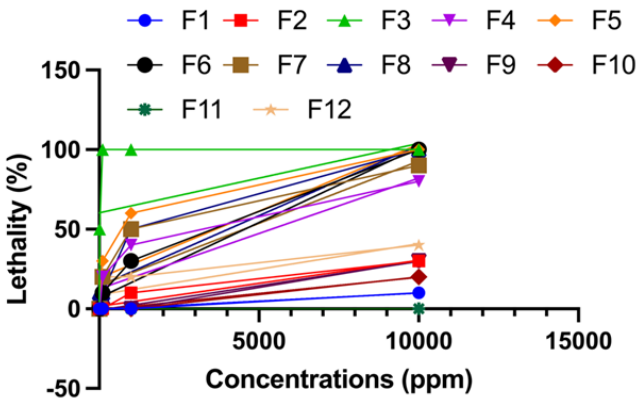

Figure 8. BSL toxicity test of the n-hexane fraction of *C. rotundus* on *Artemia salina*.

such as the work of Saleem et al (2010) on *Euphorbia serpens*, which also employed this assay to identify potential anti-cancer fractions [38]. The strong toxicity observed in the n-hexane fraction, particularly in fraction 3, is reminiscent of findings by Soumaya et al. (2013), who reported significant cytotoxic activity in the essential oil of *C. rotundus* against various cancer cell lines [39]. Their study demonstrated that the plant's components could induce apoptosis in cancer cells, supporting the potential anti-cancer properties suggested by the current brine shrimp assay results. Similarly, Susianti et al. (2024) reported the apoptotic inducing activities of *C. rotundus*, highlighting its potential in cancer treatment, which further corroborates the present findings [18].

The correlation between the in vitro results and the in silico predictions regarding cancer-related gene inhibition is particularly exciting. This synergy between computational and experimental approaches is becoming increasingly important in natural product research, as demonstrated by Atanasov et al. (2021) in their review of discovery and resupply of pharmacologically active plant-derived natural products [40]. To further validate these findings, it would be valuable to isolate and identify the specific compounds in fraction 3 responsible for the observed toxicity, possibly using techniques like those employed by Xu et al. (2008) in their phytochemical investigation of *C. rotundus* [41]. While the current results are promising, they should be viewed as a starting point for more comprehensive investigations. Future research should focus on identifying the active compounds, elucidating their mechanisms of action, and conducting in vivo studies to assess both efficacy and safety. This approach would align with the broader trends in natural product-based drug discovery, potentially leading to the development of new anti-cancer agents derived from *C. rotundus*.

4. CONCLUSIONS

Our study provides strong evidence for the anti-cancer potential of *C. rotundus* Linn and sheds light on its possible mechanisms of action. We identified key targets (PI3K, MAPK1, mTOR, RAF1, and NF-κB) involved in its anti-cancer activity. Molecular docking revealed compounds 3 and 9 as particularly promising, with strong binding to these targets, especially NF-κB. These computational findings were supported by in vitro toxicity tests, where fraction 3 showed the highest toxicity. This research lays the groundwork for understanding how C. rotundus Linn's non-polar fraction may fight cancer. We've identified specific compounds and their likely targets, opening up new paths for anti-cancer drug development from this traditional medicinal plant. Moving forward, we should focus on isolating and studying compounds 3 and 9 in more detail. While our results are promising, we acknowledge the need for further research, including tests on human cancer cells and animal models, to fully understand the effectiveness and safety of these compounds. Our integrated approach has proven valuable in natural product research and could speed up the discovery of new anti-cancer treatments from traditional medicinal plants

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Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, and F. Bray. (2021). "Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries". *CA: A Cancer Journal for Clinicians.* **71** (3): 209-249. 10.3322/caac.21660.

[2] R. Shafabakhsh, R. J. Reiter, H. Mirzaei, S. N. Teymoordash, and Z. Asemi. (2019). "Melatonin: A new inhibitor agent for cervical cancer treatment". *Journal of Cellular Physiology.* **234** (12): 21670-21682. 10.1002/jcp.28865.

[3] N. A. G. dos Santos, N. M. Martins, C. Curti, M. d. L. Pires Bianchi, and A. C. dos Santos. (2007). "Dimethylthiourea protects against mitochondrial oxidative damage induced by cisplatin in liver of rats". *Chemico-Biological Interactions.* **170** (3): 177-186. 10.1016/j.cbi.2007.07.014.

[4] P. S. Rawat, A. Jaiswal, A. Khurana, J. S. Bhatti, and U. Navik. (2021). "Doxorubicin-induced cardiotoxicity: An update on the molecular mechanism and novel therapeutic strategies for effective management". *Biomedicine & Pharmacotherapy.* **139** : 111708. 10.1016/j.biopha.2021.111708.

[5] R. P. Miller, R. K. Tadagavadi, G. Ramesh, and W. B. Reeves. (2010). "Mechanisms of Cisplatin nephrotoxicity". *Toxins (Basel).* **2** (11): 2490-518. 10.3390/toxins2112490.

[6] D. J. Newman and G. M. Cragg. (2020). "Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019". *Journal of Natural Products.* **83** (3): 770-803. 10.1021/acs.jnatprod.9b01285.

[7] M. M. Alam, M. Naeem, M. M. A. Khan, and M. Uddin. (2017). In: "Catharanthus roseus, ch. Chapter 11". 277-307. 10.1007/978-3-319-51620-2_11.

[8] V. Nikolic, I. Savic, I. Savic, L. Nikolic, M. Stankovic, and V. Marinkovic. (2011). "Paclitaxel as an anticancer agent: isolation, activity, synthesis and stability". *Open Medicine.* **6** (5): 527-536. 10.2478/s11536-011-0074-5.

[9] S. Motyka, K. Jafernik, H. Ekiert, J. Sharifi-Rad, D. Calina, B. Al-Omari, A. Szopa, and W. C. Cho. (2023). "Podophyllotoxin and its derivatives: Potential anticancer agents of natural origin in cancer chemotherapy". *Biomedicine & Pharmacotherapy.* **158** : 114145. 10.1016/j.biopha.2022.114145.

[10] M. L. Costa, J. A. Rodrigues, J. Azevedo, V. Vasconcelos, E. Eiras, and M. G. Campos. (2018). "Hepatotoxicity induced by paclitaxel interaction with turmeric in association with a microcystin from a contaminated dietary supplement". *Toxicon.* **150** : 207-211. 10.1016/j.toxicon.2018.05.022.

[11] Y. S. Kurniawan, K. Gurning, I. Iksen, and A. Bikharudin. (2024). "Fight for Cancer Diseases using Natural Compounds and Their Semisynthetic Derivatives". *Bioactivities.* 10.47352/bioactivities.2963-654X.221.

[12] E. Herradon, C. Gonzalez, A. Gonzalez, J. A. Uranga, and V. Lopez-Miranda. (2021). "Cardiovascular Toxicity Induced by Chronic Vincristine Treatment". *Frontiers in Pharmacology.* **12** : 692970. 10.3389/fphar.2021.692970.

[13] F. G. Rocha, M. M. Brandenburg, P. L. Pawloski, B. D. S. Soley, S. C. A. Costa, C. C. Meinerz, I. P. Baretta, M. F. Otuki, and D. A. Cabrini. (2020). "Preclinical study of the topical anti-inflammatory activity of Cyperus rotundus L. extract (Cyperaceae) in models of skin inflammation". *Journal of Ethnopharmacology.* **254** : 112709. 10.1016/j.jep.2020.112709.

[14] P. Singh, R. L. Khosa, G. Mishra, and K. K. Jha. (2015). "Antidiabetic activity of ethanolic extract of Cyperus rotundus rhizomes in streptozotocin-induced diabetic mice". *Journal of Pharmacy and Bioallied Sciences.* **7** (4): 289-92. 10.4103/0975-7406.168028.

[15] P. G. Daswani, S. Brijesh, P. Tetali, and T. J. Birdi. (2011). "Studies on the activity of Cyperus rotundus Linn. tubers against infectious diarrhea". *Indian Journal of Pharmacology.* **43** (3): 340-4. 10.4103/0253-7613.81502.

[16] N. Utami, S. Susianti, S. Bakri, B. Kurniawan, and A. Setiawansyah. (2023). "Cytotoxic activity of Cyperus rotundus L. rhizome collected from three ecological zones in Lampung-Indonesia against HeLa cervical cancer cell". *Journal of Applied Pharmaceutical Science.* 10.7324/japs.2023.113764.

[17] P. Mannarreddy, M. Denis, D. Munireddy, R. Pandurangan, K. P. Thangavelu, and K. Venkatesan. (2017). "Cytotoxic effect of Cyperus rotundus rhizome extract on human cancer cell lines". *Biomedicine & Pharmacotherapy.* **95** : 1375-1387. 10.1016/j.biopha.2017.09.051.

[18] S. Susianti, Y. Yanwirasti, E. Darwin, J. Jamsari, and A. Setiawansyah. (2024). "Diterpene alcohol fraction of Cyperus rotundus Linn essential oil regulates Bcl-2 and Bax expression inducing apoptosis on HeLa in vitro and in silico". *Journal of Applied Pharmaceutical Science.* 10.7324/japs.2024.188231.

[19] S. Kilani-Jaziri, A. Neffati, I. Limem, J. Boubaker, I. Skandrani, M. B. Sghair, I. Bouhlel, W. Bhouri, A. M. Mariotte, K. Ghedira, M. G. Dijoux Franca, and L. Chekir-Ghedira. (2009). "Relationship correlation of antioxidant and antiproliferative capacity of Cyperus rotundus products towards K562 erythroleukemia cells". *Chemico-Biological Interactions.* **181** (1): 85-94. 10.1016/j.cbi.2009.04.014.

[20] A. Setiawansyah, G. Susanti, R. Alrayan, I. Hadi, M. Ikhlas Arsul, D. Luthfiana, L. Wismayani, and N. Hidayati. (2024). "Aaptamine Enhanced Doxorubicin Activity on B-Cell Lymphoma 2 (Bcl-2): A Multi-Structural Molecular Docking Study". *Ad-Dawaa' Journal of Pharmaceutical Sciences.* 1-10. 10.24252/djps.v7i1.46796.

[21] R. Zhang, X. Zhu, H. Bai, and K. Ning. (2019). "Network Pharmacology Databases for Traditional Chinese Medicine: Review and Assessment". *Frontiers in Pharmacology.* **10**. 10.3389/fphar.2019.00123.

[22] D. Luthfiana, M. Soleha, A. Prasetiyo, W. A. Kusuma, R. Fatriani, L. Nurfadhila, N. Yunitasari, A. H. Ahkam, T. L. Wargasetia, R. Irfandi, A. N. M. Ansori, V. D. Kharisma, S. W. Naw, E. Ullah, V. Jakhmola, and R. Zainul. (2023). "Network pharmacology and molecular docking study to reveal the potential anticancer activity of Oscillatoxin D, E, and F marine cytotoxins". *Food systems.* **6** (3): 365-389. 10.21323/2618-9771-2023-6-3-365-389.

[23] A. Setiawansyah, A. T. Widiyawati, M. S. D. Sari, M. A. Reynaldi, N. Hidayati, R. Alrayan, and S. A. Nugroho. (2024). "FT-IR-based fingerprint combined with unsupervised chemometric analysis revealed particle sizes and aqueous-ethanol ratio alter the chemical composition and nutraceutical value of Daucus carota". *Natural Product Research.* 1-9. 10.1080/14786419.2024.2376351.

[24] P. J. D. de Vera, J. C. Tayone, and M. C. S. De Las Llagas. (2022). "Cyperus iria linn. Roots ethanol extract: its phytochemicals, cytotoxicity, and anti-inflammatory activity". *Journal of Taibah University for Science.* **16** (1): 854-862. 10.1080/16583655.2022.2123088.

[25] N. N. Pavlova and C. B. Thompson. (2016). "The Emerging Hallmarks of Cancer Metabolism". *Cell Metabolism.* **23** (1): 27-47. 10.1016/j.cmet.2015.12.006.

[26] B. Thibault, F. Ramos-Delgado, and J. Guillermet-Guibert. (2023). "Targeting Class I-II-III PI3Ks in Cancer Therapy: Recent Advances in Tumor Biology and Preclinical Research". *Cancers (Basel).* **15** (3). 10.3390/cancers15030784.

[27] R. A. Saxton and D. M. Sabatini. (2017). "mTOR Signaling in Growth, Metabolism, and Disease". *Cell.* **168** (6): 960-976. 10.1016/j.cell.2017.02.004.

[28] M. Burotto, V. L. Chiou, J. M. Lee, and E. C. Kohn. (2014). "The MAPK pathway across different malignancies: a new perspective". *Cancer.* **120** (22): 3446-56. 10.1002/cncr.28864.

[29] A. A. Samatar and P. I. Poulikakos. (2014). "Targeting RAS-ERK signalling in cancer: promises and challenges". *Nature Reviews Drug Discovery.* **13** (12): 928-42. 10.1038/nrd4281.

[30] K. Taniguchi and M. Karin. (2018). "NF-kappaB, inflammation, immunity and cancer: coming of age". *Nature Reviews Immunology.* **18** (5): 309-324. 10.1038/nri.2017.142.

[31] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, and A. J. Olson. (2009). "AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility". *Journal of Computational Chemistry.* **30** (16): 2785-91. 10.1002/jcc.21256.

[32] C. Bissantz, B. Kuhn, and M. Stahl. (2010). "A medicinal chemist's guide to molecular interactions". *Journal of Medicinal Chemistry.* **53** (14): 5061-84. 10.1021/jm100112j.

[33] S. Wilhelm, C. Carter, M. Lynch, T. Lowinger, J. Dumas, R. A. Smith, B. Schwartz, R. Simantov, and S. Kelley. (2006). "Discovery and development of sorafenib: a multikinase inhibitor for treating cancer". *Nature Reviews Drug Discovery.* **5** (10): 835-44. 10.1038/nrd2130.

[34] T. Anastassiadis, S. W. Deacon, K. Devarajan, H. Ma, and J. R. Peterson. (2011). "Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity". *Nature Biotechnology.* **29** (11): 1039-1045. 10.1038/nbt.2017.

[35] R. Barouch-Bentov and K. Sauer. (2011). "Mechanisms of drug resistance in

kinases". *Expert Opinion on Investigational Drugs.* **20** (2): 153-208. 10.1517/13543784.2011.546344.

[36] D. Hanahan and R. A. Weinberg. (2011). "Hallmarks of cancer: the next generation". *Cell.* **144** (5): 646-74. 10.1016/j.cell.2011.02.013.

[37] D. A. Fruman and C. Rommel. (2014). "PI3K and cancer: lessons, challenges and opportunities". *Nature Reviews Drug Discovery.* **13** (2): 140-56. 10.1038/nrd4204.

[38] M. Saleem, M. Nazir, M. S. Ali, H. Hussain, Y. S. Lee, N. Riaz, and A. Jabbar. (2010). "Antimicrobial natural products: an update on future antibioticdrug candidates". *Natural Product Reports.* **27** (2): 238-254. 10.1039/b916096e.

[39] K. J. Soumaya, M. Dhekra, C. Fadwa, G. Zied, L. Ilef, G. Kamel, and C. G. Leila. (2013). "Pharmacological, antioxidant, genotoxic studies and modulation of rat splenocyte functions by Cyperus rotundus extracts". *BMC Complementary Medicine and Therapies.* **13** : 28. 10.1186/1472-6882-13-28.

[40] A. G. Atanasov, S. B. Zotchev, V. M. Dirsch, T. International Natural Product Sciences, and C. T. Supuran. (2021). "Natural products in drug discovery: advances and opportunities". *Nature Reviews Drug Discovery.* **20** (3): 200-216. 10.1038/s41573-020-00114-z.

[41] Y. Xu, H. W. Zhang, C. Y. Yu, Y. Lu, Y. Chang, and Z. M. Zou. (2008). "Norcyperone, a novel skeleton norsesquiterpene from Cyperus rotundus L". *Molecules.* **13** (10): 2474-81. 10.3390/molecules13102474.