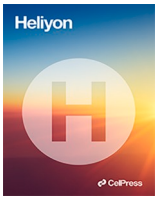
Heliyon 10 (2024) e39142

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Network pharmacology and bioinformatic integrative analysis reveals candidate gene targets and potential therapeutic of East Kalimantan propolis against hepatocellular carcinoma

Paula Mariana Kustiawan [a,1], Khalish Arsy Al Khairy Siregar [a,b,1], Muhammad Miftah Jauhar [b], Donny Ramadhan [c], Etik Mardliyati [d], Putri Hawa Syaifie [b,*]

[a] Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Samarinda, East Kalimantan, 75124, Indonesia
[b] Center of Excellence Life Sciences, Nano Center Indonesia, South Tangerang, 15314, Indonesia
[c] Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Bogor, Indonesia
[d] Research Center for Vaccine and Drug, National Research and Innovation Agency (BRIN), Bogor, 16911, Indonesia

ARTICLE INFO

Keywords:
East Kalimantan propolis
Hepatocellular carcinoma
Network pharmacology
Molecular docking
And molecular dynamics

ABSTRACT

Introduction: Hepatocellular Carcinoma (HCC) is commonly treated with surgery, liver transplantation, and chemotherapy, but recurrence and metastasis remain challenges. Natural complementary therapies like propolis, known for its hepatoprotective properties, are gaining interest due to limited efficacy and toxicity of conventional chemotherapy. This study aims to identify core targets for HCC, assess the therapeutic potential of East Kalimantan propolis (EKP) from stingless bees, and analyze the molecular interactions.

Methods: EKP compounds were analyzed using target prediction tools related to HCC, alongside clinical data from the Gene Expression Omnibus (GEO) database, to identify overlapping genes with clinical relevance. The selected genes were then subjected to protein-protein interaction (PPI), GO and KEGG enrichment, immunohistochemical comparison and survival analysis to identify potential core targets and related pathways for HCC therapy. Furthermore, molecular docking and dynamics were conducted to verify the molecular interactions and stability of EKP compounds with targets.

Results: 108 genes have been selected as HCC potential targets, which mostly associated with MicroRNAs in cancer, chemical carcinogenesis, and viral carcinogenesis pathways. These targets were obtained by overlapping genes from GEO clinical databases and target predictors. PPI network analysis revealed 4 main targets of propolis in HCC. Furthermore, differential expression genes, survival analysis, and Immunohistochemical analysis from databases suggested that AKR1C3 and MAPK1 promote HCC progression and shorten survival rate of HCC patients. Molecular docking and dynamic studies confirmed strong binding affinity and stability of Baicalein, Chrysin, Quercetin, and Myricetin with receptor targets within simulation time.

Conclusions: This study provides insight into the mechanism of action of EKP on HCC and identifies AKR1C3 and MAPK1 as candidate target treatments for future drug development.

* Corresponding author.
E-mail address: putri@nano.or.id (P.H. Syaifie).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e39142
Received 25 June 2024; Received in revised form 1 October 2024; Accepted 8 October 2024
Available online 19 October 2024

1. Introduction

Propolis is one of the promising natural ingredients with widely reported studies regarding its efficacy on hepatoprotective role. It is strongly correlated to complex composition of active molecules in propolis. There are more than 500 bioactive compounds identified in various types of propolis [1–3]. It is mostly composed of polyphenol, including phenolic acids, terpenoids, and flavonoids. Based on our previous studies, propolis extract from Trigona bees in East Kalimantan, Indonesia contains high flavonoids, polyphenols, and has antioxidant, anti-inflammatory, antibacterial and cytotoxic activity against various cancer cells, (including HepG2), specifically in inducing apoptosis mechanism. The biological activity of propolis is in accordance with the mechanism of natural compounds that works as hepatoprotectors [4–9].

Interestingly, common compounds found in propolis have anti-hepatocellular carcinoma activity. Chrysin and quercetin as flavonoids, p-coumaric acid as benzoic acid derivatives, kaempferol as flavanonols, as well as myricetin, genkwanin and baicalein have been reported as common substances found in propolis [10–12]. Chrysin could effectively inhibit the growth of HCC cells by in vivo and in vitro study [13]. Myricetin can affect down regulation of YAP expression that leads to inhibition of HCC cell proliferation and induce cell apoptosis [14]. While, quercetin affect down regulation of JAK2/STAT3 signaling pathway in HCC growth both in vitro and in vivo [15]. Kaempferol, p-coumaric acid, genkwanin, baicalein, and caffeic acid also reported to have potential anti HCC cancer and hepatoprotective effects in various pathway based on previous reports [16–19].

Among all the cancer types, HCC ranks third globally in cancer-related mortality and the most common primary liver cancer [20]. Among all action treatment for HCC patients, liver transplantation supplemented with chemotherapy drugs was the common [21,22]. However, postoperative recurrence and advanced metastasis pose challenges in HCC treatment [22].

Conventional chemotherapy drugs that currently available also exhibit low selectivity and serious toxic effects, with drug efficiency rarely exceeding 25 % [23]. In addition, the application of the 'one drug, one target' treatment strategy often fails to capture the dynamic balance of human biological systems, increasing inefficiency and triggering other health problems [24]. As a result, additional research into novel therapeutic strategies is required for the prevention of liver cancer. One such strategy is the use of natural components as a complementary therapy [25]. Based on our previous studies, East Kalimantan propolis is a promising natural ingredient to combat HCC. As far as we know, there is no study on exploring the benefits of EKP as complement therapy in HCC. The mechanism underlying propolis as an anti-HCC agent needs to be investigated due to its diversity of active compounds and its potential

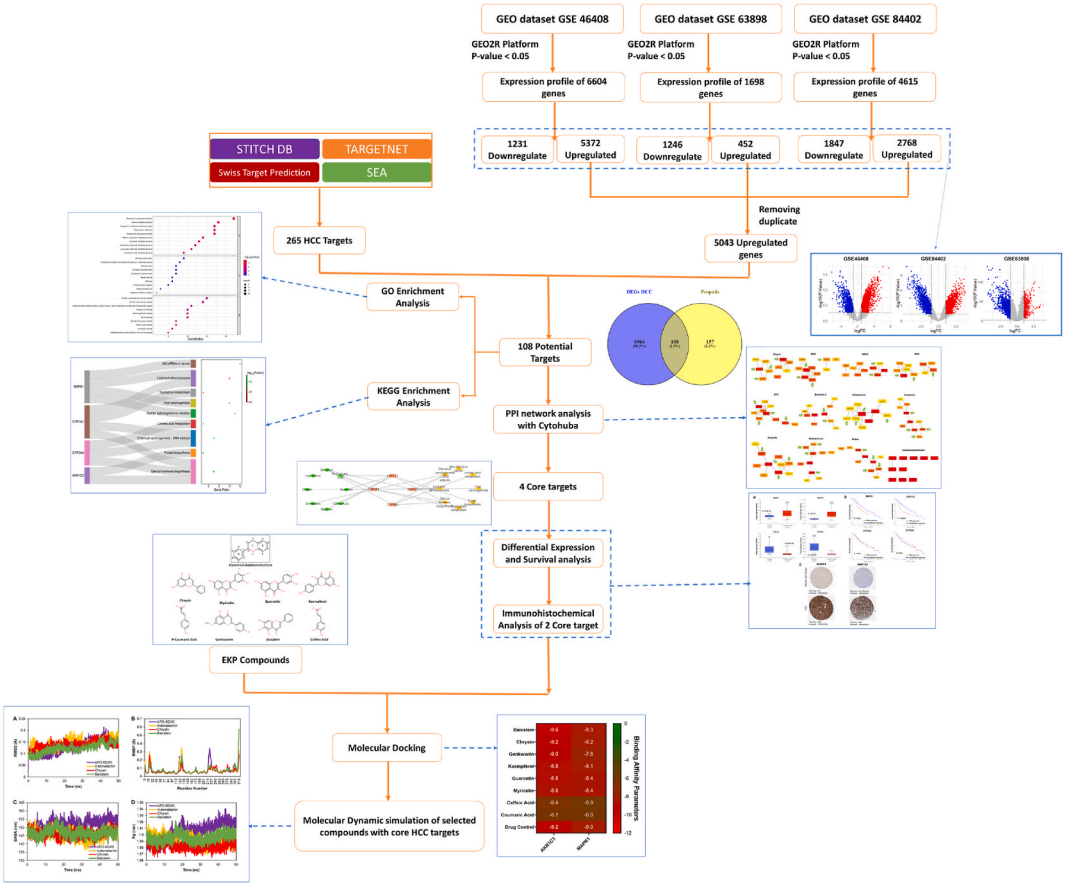


Fig. 1. Flow Chart used in this study.

to act synergistically on various target, raising questions about its mechanism of action.

Network pharmacology, one of current trend on systems-biology based methodology in in silico study [26]. This approach thoroughly explains the complex interactions between various components and targets [27]. New insight into important regulatory and gene therapy targets can be obtained from the network and sub-networks mapped [28]. Thus, we can understand and create complex networks by constructing and visualizing 'drugs-target-disease' interaction networks [29,30]. The use of network pharmacology is quite significant and comprehensive strategy for predicting the underlying mechanism, disease target, pathway and drug interactions.

This study aims to uncovering the potential molecular mechanisms and pathways of EKP in HCC, by using data from the Gene Expression Omnibus (GEO) database to identify differential expressed genes in HCC [31]. The selected genes were then subjected to additional investigation using PPI analysis, GO and KEGG enrichment analysis to identify possible targets for HCC therapy [32]. This investigation combines molecular docking and molecular dynamics analysis was employed to verify molecular interaction and stability of the drug with the target gene [33]. As far as we know, this is the first study in uncovering of the possible molecular mechanism and potential targets for EKP of *Homotrigona apicalis* bee for anti-HCC effects by in silico approach. The findings of this study could serve as a basis for future experimental investigations and advance our understanding of the molecular mechanisms underlying East Kalimantan propolis's activity. The workflow of this research is shown in Fig. 1.

2. Methods

2.1. EKP Extraction

Raw propolis was collected in Lempake District, Samarinda, East Kalimantan, Indonesia from stingless bee (*Homotrigona apicalis*). Voucher specimens of bee were deposited at Faculty of Pharmacy, East Kalimantan Muhammadiyah University (Indonesia). Raw propolis were dried and stored at −20 °C. Dry propolis smashed into powder and placed in a maceration vessel. Ethanol solution in concentration of 70 % was added into maceration vessel in ratio of 1:10 with propolis. Then, it was left at room temperature for 72 h. After seven days, the solution was filtered and residue extract was repeated for the maceration process until getting clear solution. Then, all extract solution was combined and filtered through Whatman Filter paper. EKP yielded by evaporating the solution over waterbath shaker at temperature of 60 °C.

2.2. Structural preparation of EKP compounds

In our prior study, we have been reported eleven compounds identified in EKP by using UPLC-MS/MS with Multiple Reaction Monitoring (MRM) approach [34]. There were eleven references compounds of phenolics used in analysis. However, only eight compounds confirmed. For structural preparation, the 3D structures of eight compounds were access through https://pubchem.ncbi.nlm.nih.gov/and saved in.sdf file. Then the structure was prepared using Autodock 4.2 by merging non-polar hydrogen molecules, adding polar hydrogen molecules and applied Gasteiger charges. Table 1 showed eight EKP compounds along with CIDs.

2.3. Determination of HCC targets by gene expression profiling

In this study three microarray expression profile data on HCC (GSE46408, GSE84402, and GSE63898) were obtained from GEO database http://www.ncbi.nlm.nih.gov/geo/. showed a total of normal and affected patients on HCC that used in this study. GEO2R [35] platform was used to analyze this data set and visualized in volcano plots using the R program (ggplot2) [36]. The statistical analysis threshold was determined as an adjusted *p-value* <0.05. Table 2 Proteins with log2 FC greater than or equal to 1 were considered up-regulated and proteins with log2 FC less than or equal to −1 are considered to be downregulated in HCC [37].

2.4. Target prediction of EKP compounds in treating HCC

The Canonical SMILES of eight propolis compounds used to identify potential disease targets using various bioinformatics tools, they were STITCH (http://www.stitch.embl.de/) [46], SwissTargetPrediction (http://www.swisstargetprediction.ch/) [47], SEA (https://www.sea.bkslab.org/) [48], and TargetNet (http://www.targetnet.scbdd.com/) [49]. While, the UniProt Database (http://www.uniprot.org/id-mapping) used to align target information and protein terminology to ensure consistency and standardization

Table 1
Eight compounds of EKP.

Compounds	Molecular Formula	Molecular weight (g/mol)	CID
Chrysin	$C_{15}H_{10}O_4$	254.24	5281607
Myricetin	$C_{15}H_{10}O_8$	318.23	5281672
Quercetin	$C_{15}H_{10}O_7$	302.23	5280343
Kaempferol	$C_{15}H_{10}O_6$	286.24	5280863
P-Coumaric Acid	$C_9H_8O_3$	164.16	637542
Genkwanin	$C_{16}H_{12}O_5$	284.26	5281617
Baicalein	$C_{15}H_{10}O5$	270.24	5281605
Caffeic Acid	$C_9H_8O_4$	180.16	689043

Table 2
Three sets of gene expression profiles for HCC analyzed in this study.

Dataset	Sample Size (Normal vs Affected)	Sequencing Platform	Locations	References
GSE46408	12 (6/6)	GPL4133 Agilent-014850 Whole Human Genome Microarray 4 × 44K G4112F	Taiwan	[38]
GSE84402	28 (14/14)	GPL570 [(HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array]	China	[39]
GSE63898	396 (168/228)	GPL13667 [HG-U219] Affymetrix Human Genome U219 Array	United State America	[40–45]

of protein names [50]. Subsequently, the disease targets were combined and removed duplicates. Finally, a Venn diagram, Venny 2.1 (https://www.bioinfogp.cnb.csic.es/tools/venny/), was used to identify disease targets that overlapped with DEG of HCC [51].

2.5. GO and KEGG enrichment analysis on potential target genes in treating HCC

To find significantly enriched biological processes and signaling pathways in the Common Target of EKP in HCC, enrichment analysis was carried out. First, org.Hs.eg.db was used to convert Gene symbols to Ensembl IDs [52]. Next, the R package clusterProfiler was used to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Ontology (GO) analysis using the "enrichGO" and "enrichKEGG" functions, respectively, utilizing the KEGG database (https://www.kegg.jp/) [53,54]. A significance threshold of p-value<0.05 and q-value<0.05 was used to select for GO-enriched categories, and the degree of enrichment was expressed as the log10 transformed p-value. Significant categories were those having a p-value and q-value of less than 0.05. For both the KEGG analysis and the three GO enrichment analysis modules—biological processes (BP), cellular components (CC), and molecular functions (MF)—the top 10 enriched findings were displayed [55].

2.6. Determination of main HCC targets by PPI network construction

To select the main disease targets, a PPI network analysis was carried out using the STRING version 11.5 (https://string-db.org) [56]. Interactions that had the greatest confidence with a combined score more than 0.900 (strictest confidence filter) were saved and imported into Cytoscape (version 3.7.2) [57]. The cytoHubba plugin was utilized to investigate protein relationships using protein ranking methods, which include local and global based methods (degree, edge percolation component (EPC), maximum environmental component (MNC), maximum neighborhood component density (DMNC), and maximum clique centrality (MCC), congestion, eccentricity, closeness, radiality, betweenness and stress, and clustering coefficient) in order to conduct a more thorough analysis of the primary disease targets that are crucial to HCC [58].

2.7. Evaluation of main HCC targets of EKP

After obtaining proteins that have important interactions, Gene Expression Evaluation and Survival Analysis were carried out using the UALCAN webserver (https://ualcan.path.uab.edu/) [59]. This webserver results an analysis of Gene Expression and Survival analysis based on cancer OMICS data. This data used as reference in selecting potential disease targets by comparing the protein expression levels in HCC and normal samples, as well as the overall survival rates of HCC patients. Subsequently, selected main disease targets were subjected to immunohistochemical observations from the Human Protein Atlas (HPA) database (http://www.proteinatlas.org/) [60]. This database provided valuable information on protein expression levels of the hub genes in cancerous specimens compared to normal specimens [61]. The HPA database consists of over 10 million pictures illustrating human protein expression patterns in various tissues and cells [62]. The results of this evaluation will determine the HCC main targets by EKP which would be verified in the Molecular Docking and Dynamics Simulation studies.

2.8. Molecular docking of EKP compounds with main targets of HCC

Based on previous evaluations, the AKR1C3 and MAPK1 have been selected as potential therapeutic targets and their binding

Table 3
Grid settings for specific docking.

Proteins	PDB ID	Grid Position					
		Center			Dimensions		
		x	***y***	***z***	***x***	***y***	***z***
AKR1C3	6GXK	−1.36	28.513	18.989	40	40	40
MAPK1	1TVO	6.23	−3.419	17.175	40	40	40

interaction with propolis compounds were continuously verified using Molecular Docking. The protein structures of AKR1C3 and MAPK1 were obtained from RCSB Protein Data Bank (http://www.rcsb.org/) [63–66]. While, the structures of eight EKP compounds and references drug (Indomethacin, AKR1C3 Inhibitor and Selumetinib,MAPK1 Inhibitor) were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [67]. Then, protein and ligand (compounds) was prepared by using the BIOVIA Discovery Studio Visualizer software and the Open Babel tool integrated into the PyRx software version 0.9.9. The docking procedure was carried out using AutoDock Vina software integrated into PyRx [68,69]. Grid coordinates were assigned to the active site of each protein, as shown in Table 3. BIOVIA Discovery Studio Visualizer and PyMOL software visualized the docking results in three dimensions [70,71]. Finally, the top two compounds with the highest binding affinity (lowest binding energy) on the binding with AKR1C3 and MAPK1, will be analyzed using the PLIP (Protein-Ligand Interaction Profiler), https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index webserver to see the interactions formed during molecular docking simulation [72,73]. The formula for binding free energy in Autodock was used an estimated free energy of binding [74]:

$$\Delta G\left(\frac{kcal}{mol}\right) = G\,bound\left(\frac{kcal}{mol}\right) - G\,unbound\left(\frac{kcal}{mol}\right)$$

Note: ΔG = Gibbs energy/Free binding energy G bound = Final intermolecular energy + Final torsional energy + Torsional free energy G unbound = unbound system's energy.

2.9. Molecular dynamics of selected EKP compounds and main HCC targets

Molecular dynamics simulation was used to confirm the most significant binding affinity data found by molecular docking studies [75]. The GROMACS program and the CHARMM36 force field for every complex, including chemicals and drug control, were used to perform the molecular dynamics simulation. The simulation was carried out using the TIP3P water model, and the necessary quantity of sodium and chloride ions were added to create a 0.15 NaCl solution [76]. The system was subjected to periodic boundary conditions (PBCs) in all spatial directions. The study employed LINCS algorithms to facilitate the analysis while imposing constraints on all hydrogen bonds. To reduce the impact of van der Waals and short-range electrostatic interactions, a distance cutoff of 1.2 nm was employed. The calculation of the long-range electrostatic forces was done using the Particle Mesh Ewald algorithm (PME). The system's energy was reduced by utilizing the steepest descent algorithm. Subsequently, the system was permitted to attain an equilibrium state via the NVT ensemble employing the V-Rescale thermostat at a temperature of 310 K. This was followed by achieving equilibrium through the NPT ensemble utilizing the Parrinello-Rahman barostat at a pressure of 1 atm. The individual chemicals were subjected to a simulation lasting 50 ns, while the complex system underwent a simulation lasting 10 ns [77,78]. During the MD simulations, root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), and Radius of gyration (Rg) analysis were used to measure stability of the docked complexes using following equation:

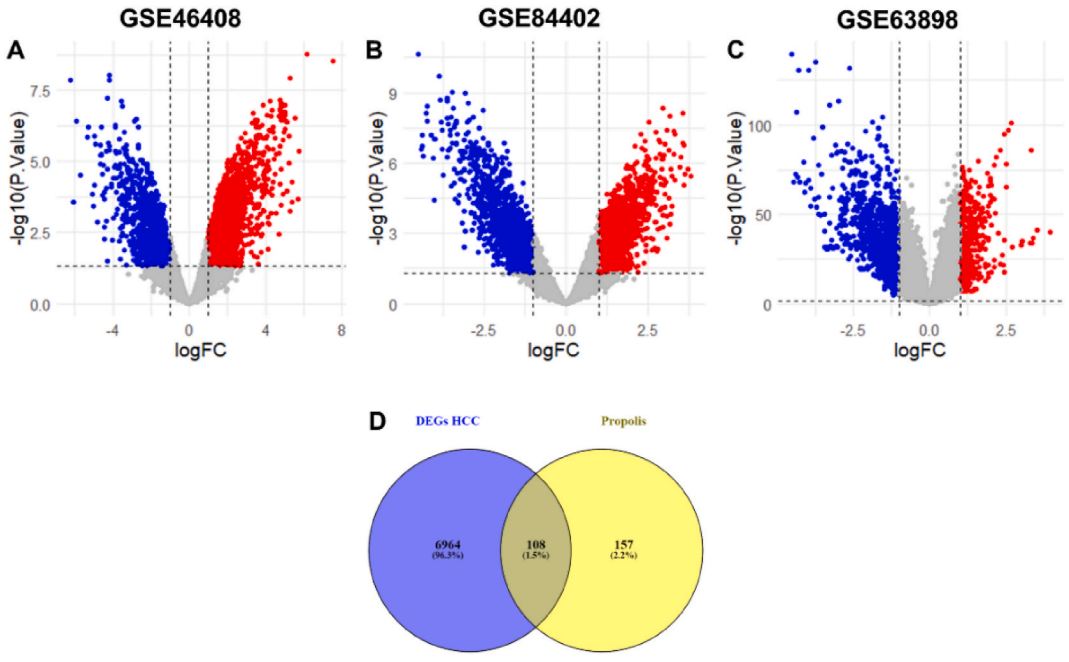


Fig. 2. Volcano plot of the distribution genes in patients with HCC in DEGs (A) GSE46408, (B) GSE84402, (C) GSE63898, Red depict high expression genes and blue depict lower expression genes in patients with HCC, (D) intersection between DEG HCC (GSE46408, GSE84402, and GSE63898) and potential targets from EKP compounds.

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(x_i^m - x_i^1\right)^2 + \left(y_i^m - y_i^1\right)^2 + \left(z_i^m - z_i^1\right)^2} \quad (1)$$

$$RMSF = \sqrt{\frac{1}{T}\sum_{i=1}^{T}(x_i - \bar{x})^2} \quad (2)$$

$$Rg = \sqrt{\frac{1}{N}\sum_{i=1}^{N}|r(i) - r_{center}|^2} \quad (3)$$

Where N represent as the number of protein atoms in formula (1) and formula (3), xm, ym, zm is the initial coordinates and x1, y1, z1 is the trajectory coordinates at frame t (specified time) in formula (1), T represent as trajectory frame numbers and $\bar{x}$ is the time averaged position in formula (2), r(i) demonstrate the coordinates of the atom i and rcenter is the center of mass [79].

3. Results

3.1. Target prediction of EKP compounds in treating HCC

By using gene expression profiling from GEO databases, a total of 5043 upregulated genes in HCC-affected groups were collected. These gene were obtained from three microarray expression profile data (GSE46408, GSE84402, and GSE63898), in which these gene were categorized as up-regulated and down-regulated based on logFC values in filtering process. These data sets were visualized in Fig. 2A, B, and C, as well as in Table 4. Moreover, 265 potential targets from an input of eight canonical smiles of EKP compounds in target prediction tools (SwissTargetPrediction, SEA, STITCH and TargetNet) were obtained. After overlapping the 5043 upregulated genes in HCC with 265 potential targets by EKP compounds, a total of 108 potential targets of EKP in HCC were obtained (Fig. 2D).

3.2. Result of GO and KEGG enrichment analysis

108 potential targets of HCC were used to perform GO and KEGG enrichment analysis in R package cluster Profiler. Fig. 3 displays the top ten enriched GO terms for each category. From the analysis of biological processes, the majority of therapeutic targets are rich in Long-chain fatty acid metabolic process, Steroid metabolic process, Unsaturated fatty acid metabolic process, Olefinic compound metabolic process, Icosanoid metabolic process, Response to xenobiotic stimulus, etc. The top ten enriched GO terms in each category are shown in Table 5. From the perspective of biological processes, the therapeutic targets are mainly enriched in Long-chain fatty acid metabolic process, Steroid metabolic process, Unsaturated fatty acid metabolic process, Olefinic compound metabolic process, Icosanoid metabolic process, Response to xenobiotic stimulus, etc. For cellular components, Protein kinase complex, Chromosomal region, Mitotic spindle, Midbody, Protein kinase complex, etc. For molecular functions, including Protein serine kinase activity, monooxygenase activity, Steroid hydroxylase activity, *etc*.

KEGG pathway analysis of the differentially expressed genes uncover numerous significantly enriched pathways, including MicroRNAs in cancer, chemical carcinogenesis - DNA adducts, Linoleic acid metabolism, Nitrogen metabolism, Human cytomegalovirus infection, viral carcinogenesis, Tryptophan metabolism, Lipid and atherosclerosis, *etc*. (Fig. 4). The top ten enriched pathways which are known to have crucial roles in signaling pathways linked to advancement of HCC, are displayed in Fig. 6 and Table 6. In general, the result of GO and KEGG enrichment analyses shed light on the biological processes and pathways which dysregulated in the conditions under study and may offer prospective therapeutic targets for further investigation.

3.3. Protein Protein interaction analysis of potential HCC core targets

A total of 108 potential targets of EKP in HCC were subjected to PPI network analysis using STRING with the use of highest confidence parameter of combined score >0.900 (the strictest confidence filter) and Cytoscape software integrated with the *cytoHubba* plugin. In results, 21 targets were obtained. Among the 21 proteins, CYP3A4, AKR1C3, CYP1A1, and MAPK1 were at the top with their occurrence in ten different methods. In addition, the CDK1 protein was present in eight methods, CXCL12 and RXRA in seven methods, CYP1A2 in five methods, CYP17A1, CYP2C19, HSD17B2, PIK3R1, and ESR1 in four methods CYP2C8, COMT, NR0B2, NR1I2 proteins

Table 4
Datasets of HCC from the GEO database, comprising sequencing platforms and groups (affected vs control).

Dataset	Sequencing Platform	Control	Affected	Total Analysis Gene	
				Upregulated	Downregulated
GSE46408	GPL4133	6	6	5372	1232
GSE63898	GPL570	168	228	452	1246
GSE84402	GPL14951	14	14	2768	1847

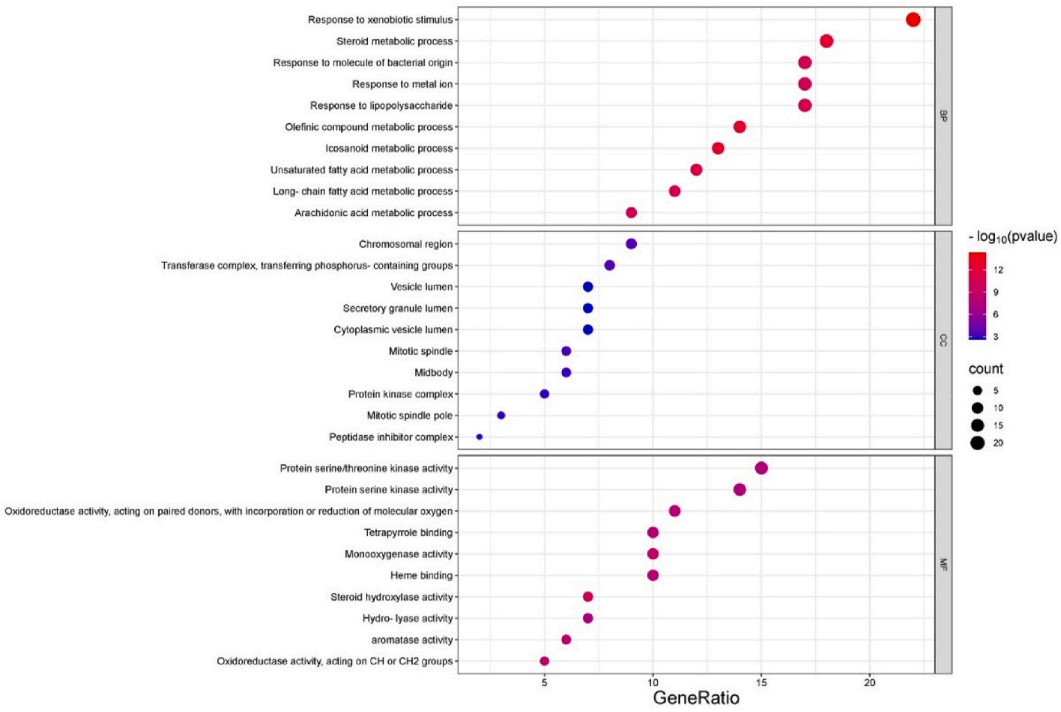


Fig. 3. The results of GO enrichment analysis pertaining to biological processes, cellular components, and molecular functions.

in three methods, lastly, DNM1, NEK2, CYP2C9, and ALDH2 were only present in two methods. In addition, graphical representation of the occurrence of filtered protein in various cytoHubba methods is shown in Fig. 5 and Table 7. The final results discovered four main targets, they are CYP3A4, AKR1C3, CYP1A1, and MAPK1.

3.4. Compounds-target-pathway (C-T-P) network construction of HCC in treatment with EKP

Compound-target-pathway (C-T-P) network was constructed to visualize four selected main HCC targets which connected to eight EKP compounds and their related pathway in KEGG analysis results. Fig. 6 depicted the main targets that related on chemical carcinogenesis, MicroRNAs in cancer, steroid hormone biosynthesis, lipid and atherosclerosis, tryptophan metabolism and linoleic acid metabolism. Based on C-T-P network, all propolis compounds connected the main targets in treating HCC (Fig. 6). These interaction of EKP compounds with the main targets will be validated through molecular docking and molecular dynamic simulations. However, evaluation of these targets based on their gene expression levels and survival rates in HCC patients were firstly evaluated to identify the specific targets.

3.5. Evaluation of main disease targets of EKP in treating HCC

Our analysis suggests that AKR1C3 and MAPK1 are promising candidate of HCC targets by EKP. This finding was supported by the mRNA expression of AKR1C3 and MAPK1 that significantly upregulated in HCC tissue compared with normal (Fig. 7A). In addition, the correlation of overexpressed targets in HCC tissue was confirmed through the results of the Kaplan-Meier survival curve. The results showed that high expression of AKR1C3 and MAPK1 significantly impact on low survival rate and poor prognosis of HCC patients (Fig. 7B). Furthermore, additional observations of AKR1C3 and MAPK1 in HCC and normal tissue in immunohistochemical expression were shown in Fig. 7C. This finding revealed that MAPK1 and AKR1C3 may promote carcinogenesis progression in liver hepatocellular carcinoma.

3.6. Molecular docking of EKP compounds with MAPK1 and AKR1C3 as core targets in treating HCC

In this study, molecular docking protocol was firstly validated by re-docking crystallized native ligand of protein structures and measure the RMSD. Molecular docking was performed using the crystal structures of AKR1C3 and MAPK1 (PDB ID: 6GXK and 1TVO). The results of RMSD native ligand in validation step were below 2 Å for both AKR1C3 and MAPK1. Subsequently, eight compounds of propolis were docked using the same protocol. Based on the binding free energy value in the molecular docking results with the major targets, the best propolis compounds were identified. The energy released during bond formation and contact between a protein and a ligand (small molecules) is known as binding free energy, or binding affinity (ΔG). The more negative values indicating the more stable

Table 5
The top ten results of GO enrichment analysis.

	ID	Description	*p-value*	*p*.adjust	Qvalue
BP	GO:0009410	Response to xenobiotic stimulus	4.87E-15	1.57E-11	1.02E-11
BP	GO:0006690	Icosanoid metabolic process	2.81E-13	2.48E-10	1.61E-10
BP	GO:0120254	Olefinic compound metabolic process	3.03E-13	2.48E-10	1.61E-10
BP	GO:0008202	Steroid metabolic process	3.08E-13	2.48E-10	1.61E-10
BP	GO:0033559	Unsaturated fatty acid metabolic process	2.10E-12	1.35E-09	8.77E-10
BP	GO:0032496	Response to lipopolysaccharide	1.06E-11	5.71E-09	3.70E-09
BP	GO:0002237	Response to molecule of bacterial origin	2.70E-11	1.01E-08	6.54E-09
BP	GO:0019369	Arachidonic acid metabolic process	2.70E-11	1.01E-08	6.54E-09
BP	GO:0010038	Response to metal ion	2.81E-11	1.01E-08	6.54E-09
BP	GO:0001676	Long-chain fatty acid metabolic process	3.21E-11	1.03E-08	6.71E-09
CC	GO:0061695	Transferase complex, transferring phosphorus-containing groups	3.02E-04	3.81E-02	3.29E-02
CC	GO:0098687	Chromosomal region	3.17E-04	3.81E-02	3.29E-02
CC	GO:0072686	Mitotic spindle	4.98E-04	3.98E-02	3.44E-02
CC	GO:0030496	Midbody	9.33E-04	4.83E-02	4.17E-02
CC	GO:1902911	Protein kinase complex	1.16E-03	4.83E-02	4.17E-02
CC	GO:0097431	Mitotic spindle pole	1.21E-03	4.83E-02	4.17E-02
CC	GO:1904090	Peptidase inhibitor complex	1.62E-03	4.94E-02	4.26E-02
CC	GO:0034774	Secretory granule lumen	2.10E-03	4.94E-02	4.26E-02
CC	GO:0060205	Cytoplasmic vesicle lumen	2.21E-03	4.94E-02	4.26E-02
CC	GO:0031983	Vesicle lumen	2.29E-03	4.94E-02	4.26E-02
MF	GO:0008395	Steroid hydroxylase activity	6.36E-11	2.64E-08	2.02E-08
MF	GO:0004497	Monooxygenase activity	4.83E-10	1.01E-07	7.68E-08
MF	GO:0016725	Oxidoreductase activity, acting on CH or CH2 groups	2.87E-09	3.99E-07	3.05E-07
MF	GO:0070330	aromatase activity	5.81E-09	5.52E-07	4.22E-07
MF	GO:0016705	Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	7.48E-09	5.52E-07	4.22E-07
MF	GO:0020037	Heme binding	7.96E-09	5.52E-07	4.22E-07
MF	GO:0046906	Tetrapyrrole binding	1.57E-08	9.33E-07	7.13E-07
MF	GO:0004674	Protein serine/threonine kinase activity	2.47E-08	1.29E-06	9.83E-07
MF	GO:0106310	Protein serine kinase activity	2.81E-08	1.30E-06	9.93E-07
MF	GO:0016836	Hydro-lyase activity	7.52E-08	3.13E-06	2.39E-06

and strong interaction of complex. This result of the molecular docking represented in kcal/mol. Therefore, ligands with the stronger binding affinity had more negative binding free energy in kcal/mol. Thus, the compounds with lower binding energy than references drug control was selected to molecular dynamics simulation in order to validate the findings of new drug candidates [80].

In this study, the docking results of the AKR1C3 showed that baicalein and chrysin had lowest binding energy with the targets in −9.6 and −9.2 kcal/mol, respectively. While, AKR1C3 reference drug (Indomethacin) showed similar binding energy to chrysin (−9.2 kcal/mol). Docking results of MAPK1 showed that quercetin and myricetin had lower binding energy (−8.4 kcal/mol) than its

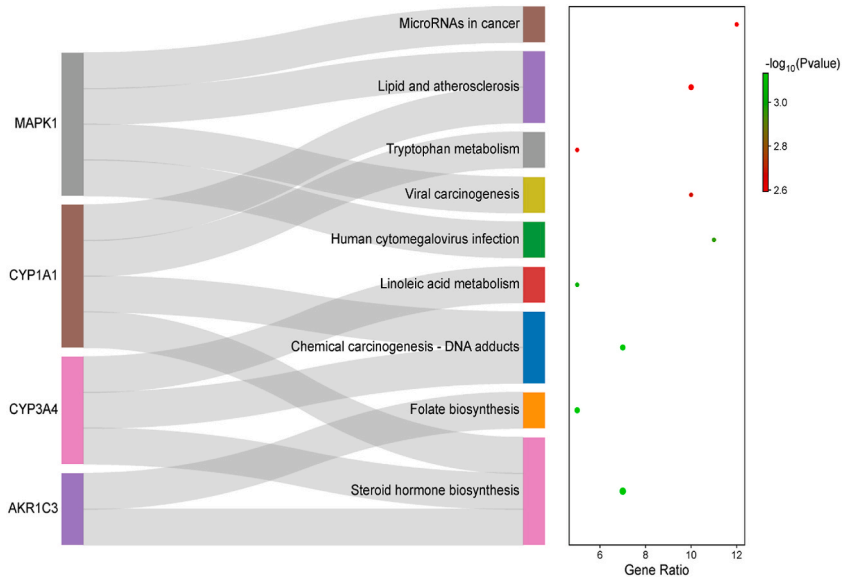


Fig. 4. Sankey diagram for Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway analysis.

Table 6
The top ten results of KEGG enrichment analysis.

ID	Description	Genes
hsa00140	Steroid hormone biosynthesis	AKR1C3, HSD17B2, CYP17A1, CYP3A4, CYP1A2, CYP1A1, COMT
hsa00790	Folate biosynthesis	AKR1C3, QDPR, AKR1B10, ALPL, AKR1B1
hsa05204	Chemical carcinogenesis - DNA adducts	PTGS2, CYP3A4, CYP2C9, CYP2C8, CYP2C19, CYP1A2, CYP1A1
hsa00591	Linoleic acid metabolism	CYP3A4, CYP2C9, CYP2C8, CYP2C19, CYP1A2
hsa00910	Nitrogen metabolism	CA12, CA5A, CA2, CA5B
hsa05163	Human cytomegalovirus infection	CXCL12, PTK2, PTGS2, PTGER4, PTGER2, MAPK1, PIK3R1, RHOA, CREB1, CDK6, CDK4
hsa05203	Viral carcinogenesis	CDK1, HDAC4, SYK, MAPK1, PIK3R1, RHOA, HDAC2, CREB1, CDK6, CDK4
hsa00380	Tryptophan metabolism	CAT, MAOB, ALDH2, CYP1A2, CYP1A1
hsa05417	Lipid and atherosclerosis	RXRA, PTK2, MAPK1, PIK3R1, MMP9, MMP1, RHOA, CYP2C9, CYP2C8, CYP1A1
hsa05206	MicroRNAs in cancer	CDC25B, HDAC4, PTGS2, MAPK1, PLAU, PIK3R1, MMP9, MCL1, RHOA, HDAC2, DNMT1, CDK6

reference drug, Selumetinib (−7.8 kcal/mol). This result supported that baicalein, chrysin, quercetin, and myricetin show better binding affinity than the reference drug (Fig. 8). Interaction analysis of selected propolis compounds with AKR1C3 and MAPK1 depicted in Table 4. The presence of hydrogen and hydrophobic bonds give significant effect on lower binding energy and the stability of interactions. Several active residues of proteins were assessed for their ability to form hydrogen and hydrophobic bonds with propolis compounds, supported by the close distance of interaction (Table 8). Furthermore, baicalein, chrysin, quercetin, and myricetin will be subjected to molecular dynamics to validated their stability and docking results.

Molecular analysis of interaction between top ligands and binding site of MAPK1 shown in Fig. 9. This finding revealed that all ligand binds to the same key residues in active site of protein. Lys54, Gln71, Gln105, and Met108 form strong hydrogen bonds with ligands. While, Ile31, Val39, Ala52, and Lys54 form strong hydrophobic contact with ligands. Previous study by Sizhen Gu and team (2020), also found that the most active ligand of MAPK1 was bind with lysine residue of Lys54, alanine residue of Ala52 and methionine residue of Met108 [81]. Selumetinib used as reference compound for MAPK1. The molecular docking result revealed that both quercetin and myricetin have more stronger binding to MAPK1 than selumetinib based on the molecular interaction and binding score. Therefore, both quercetin and myricetin have potential activity to act on MAPK1. The similar result was also demonstrated in the molecular docking of AKR1C3 with propolis compounds. Fig. 10 showed that all ligands bind to the same binding site of protein. Tyr55, Lys84 and Asn167 evaluated as active residues which form hydrogen bonds with ligands. While, Val228, Tyr216, and Phe306 form hydrophobic bonds with ligands. Both ligand baicalein and chrysin have comparable binding score with indometachin as reference substance. Tyr55 has reported as main binding point amino acids of AKR1C3 which is part of catalytic tetrad with Lys84, moreover, the most active compounds that targeting AKR1C3 reportes to form hydrophobic bond with Tyr216 in Gabriele Möller and team research [82]. Therefore, both ligands of baicalein and chrysin have potential activity on targeting AKR1C3.

3.7. Molecular dynamics

Molecular Dynamics studies were performed to understand the binding stability of the ligand-complexes. First, molecular dynamic

Fig. 5. PPI network analysis with Cytohuba plugin for selecting main targets of HCC by propolis.

Table 7
List of the targets included in at least two methods from twelve different methods of the cytoHubba plugin Cytoscape.

Genes	Occurrences	Present In (Methods)
CYP1A1	10	MCC, MNC, Degree, EPC, BottleNeck, EcCentricity, Closeness, Radiality, Betwenness, Stress
AKR1C3	10	MCC, MNC, Degree, EPC, BottleNeck, EcCentricity, Closeness, Radiality, Betwenness, Stress
MAPK1	10	Degree, Closeness, MNC, EPC, Betwenness, BottleNeck, Radiality, Stress, MCC, EcCentricity
CYP3A4	10	MNC, Degree, EPC, Closeness, Stress, Radiality, Betwenness, MCC, EcCentricity, DMNC
CDK1	8	Degree, BottleNeck, Betwenness, Radiality, Closeness, Stress, DMNC, MCC
CXCL12	7	MNC, MCC, Degree, EPC, Closeness, Radiality, Betwenness
RXRA	7	EPC, BottleNeck, Radiality, Betwenness, Stress, Closeness, EcCentricity
CYP1A2	5	EPC, MNC, Degree, MCC, Stress
CYP17A1	4	MCC, DMNC, EPC, Radiality
CYP2C19	4	MNC, MCC, EPC, Degree
HSD17B2	4	DMNC, MCC, EcCentricity, ClusteringConff
PIK3R1	4	MNC, Degree, Stress, Closeness
ESR1	4	BottleNeck, Closeness, Stress, Degree
CYP2C8	3	EcCentricity, MNC, EPC
COMT	3	BottleNeck, Betwenness, Stress
NR0B2	3	EcCentricity, BottleNeck, Radiality
NR1I2	3	Radiality, Closeness, EcCentricity
DNM1	2	DMNC, ClusteringConff
NEK2	2	DMNC, ClusteringConff
CYP2C9	2	MNC, EcCentricity
ALDH2	2	Betwenness, BottleNeck

results revealed that AKR1C3 protein alone have average RMSD of ~0.125 Å after 50 ns trajectories. The RMSD fluctuation appears to not change too much after it binds with Baicalein, Chrysin, and Indomethacin to form complexes; the average RMSD for each complex with AKR1C3 is approximately ~0.123 Å, ~0.141 Å, and ~0.147 Å. Fig. 11A showed that In apo-6GXK, means AKR1C3 alone, it is form a stable systems in the beginning until 12 ns, then the fluctuation continues to increase during the simulation up to 0.214 Å, then stabilizes again until the end of the simulation. While, Baicalein-AKR1C3 complex have a stable RMSD movement for 10 ns and fluctuated until final simulation of 50 ns. Furthermore, the Chrysin-AKR1C3 complex reached a stable state at the beginning of the simulation to 30 ns, and no significant fluctuations occurred until final. However, it is different from the Indomethacin complex during the simulation. Various fluctuations indicate that Indomethacin has a higher degree of flexibility than other complexes (Fig. 11A).

Then the RMSD result on apo-1TVO, means MAPK1 alone has an average RMSD of ~0.232 Å. While, Quercetin, Myricetin, and

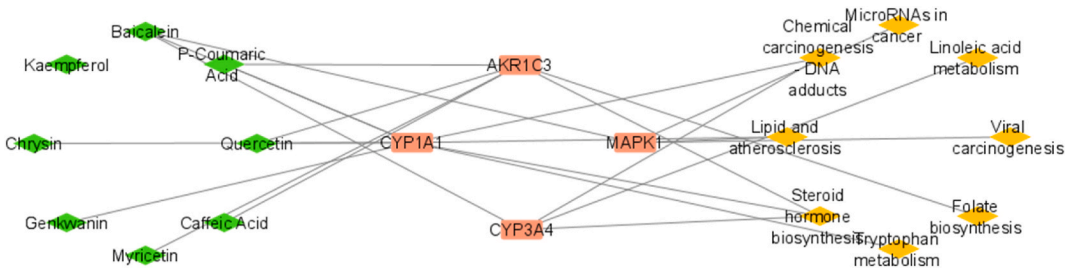


Fig. 6. Network construction of East Kalimantan propolis compounds-Target-Pathway (C-T-P), green diamond depict propolis compound, red rectangle depict main target, and orange diamond depict related pathways.

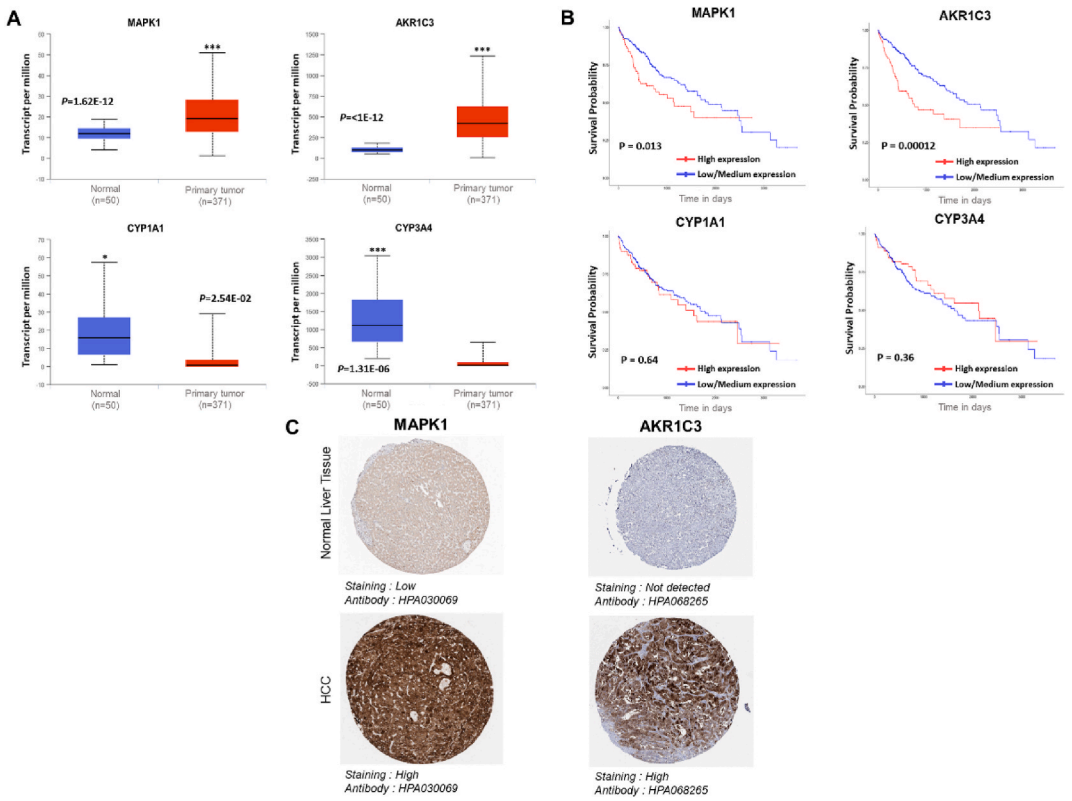


Fig. 7. Differential expression of four core genes in normal tissues and HCC Analysis expression in HCC and adjacent normal tissue (A), survival analysis based on UALCAN database (B) and Immunohistochemical images of MAPK1 and AKR1C3 (C). *P < 0.05, and ***P < 0.001.

Selumetinib complexes with MAPK1 has an average RMSD of ~0.213 Å, ~0.187 Å, and ~0.231 Å, respectively (Fig. 12A). MAPK1 was stable state at the beginning to 15 ns, after which the fluctuations continued to increase gradually until the end of the simulation. The Quercetin and Myricetin complex with MAPK1 were consistently stable from the beginning to the end of the simulation. Selumetinib was showed there is various fluctuations occurred until the end of the simulation. Finally, the results of observing the RMSD profiles of both proteins are approximately below 2 Å, indicating that each complex is in a stable state (Fig. 12A).

Furthermore, the solvent-accessible surface area was analyzed to understand changes in protein surface area. AKR1C3 had a higher SASA Profile with an average of ~152 nm compared to the Indomethacin complex, which -had a lower average SASA value of ~145 nm (Fig. 11C). On the other hand, MAPK1 has a lower SASA profile with an average of ~29 nm, while Quercetin's SASA profile was higher with an average of ~183 nm. This defines the lower SASA profile value as indicating tighter folding and reduced solvent accessibility (Fig. 12C).

Finally, to understand the structural stability of the protein-ligand complex, we determined the compactness of the protein structure by calculating Rg. Based on the Rg plot, it shows that the structural dynamics of AKR1C3, Baicalein, Chrysin, and Indomethacin complexes are relatively stable throughout the simulation time, with average Rg values of ~1.90 nm, ~1.89 nm, ~1. 88 nm and ~1.89 nm, respectively (Fig. 11D). Then, the structural dynamics of MAPK1, Quercetin, Myricetin, and Selumetinib complexes are

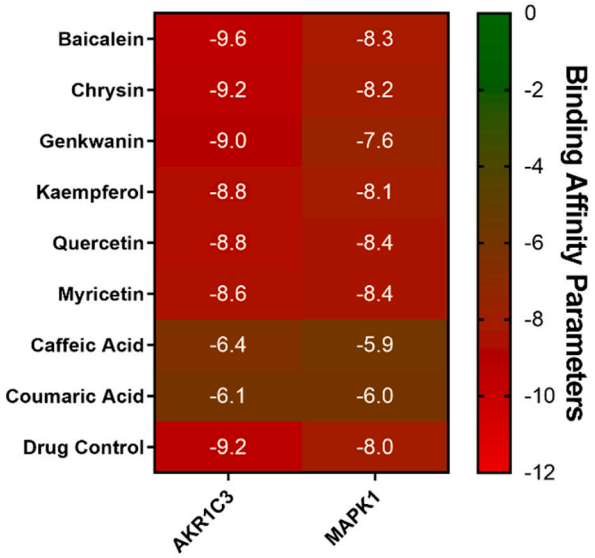


Fig. 8. Heatmap docking results.

Table 8
Best Results Docking and Drug Control with Protein-ligand interaction in detail.

Protein	Ligand	Binding Affinity (Kcal/Mol)	Interaction			
			Hydrogen Bond	Distance (Å)	Hydrophobic Interaction	Distance (Å)
AKR1C3	Baicalein	−9.6	Tyr55	22.6	Tyr216	3.79
			Lys84	2.84	Trp227	3.45
			His117	3.03	Val228	3.66
			Gln190	2.28	Tyr305	3.59
					Phe305	3.53
	Chrysin	−9.2	Tyr55	2.40	Val228	3.56
			Lys84	3.42	Phe306	3.66
			Ser166	3.13		
			Asn167	2.01		
	Indomethacin (Reference)	−9.2	Asn167	2.12	Tyr216	3.67
			Gln222	3.30	Phe306	3.35
MAPK1	Quercetin	−8.4	Ala52	2.57	Ile31	3.59
			Lys54	2.47	Val39	3.92
			Gln105	3.74	Ala52	3.74
			Met108	2.14	Lys54	3.51
			Asp111	3.02	Leu156	3.59
			Lys114	2.70		
	Myricetin	−8.4	Lys54	2.24	Ile31	3.96
			Glu71	3.06	Val39	3.85
			Ile103	2.49	Ala52	3.74
			Gln105	2.03		
			Met108	3.35		
			Thr110	2.77		
	Selumetinib (Reference)	−7.8	Lys54	3.56	Ile31	3.76
			Glu71	2.13	Val39	3.65
					Ala52	3.99
					Lys54	3.71

also relatively stable throughout the simulation time, with average Rg values of ~2.17 nm, ~2.19 nm, ~2.18 nm, and ~2.19 nm, respectively (Fig. 12D).

4. Discussion

HCC is the sixth most frequently diagnosed cancer and accounts for approximately 70–85 % of global cases, posing a significant health problem [83,84]. The primary treatment of early-stage HCC patients is commonly involved surgical resection and liver transplantation, often combined with chemotherapy. However, the recurrence patients after surgery, often experiences an advance

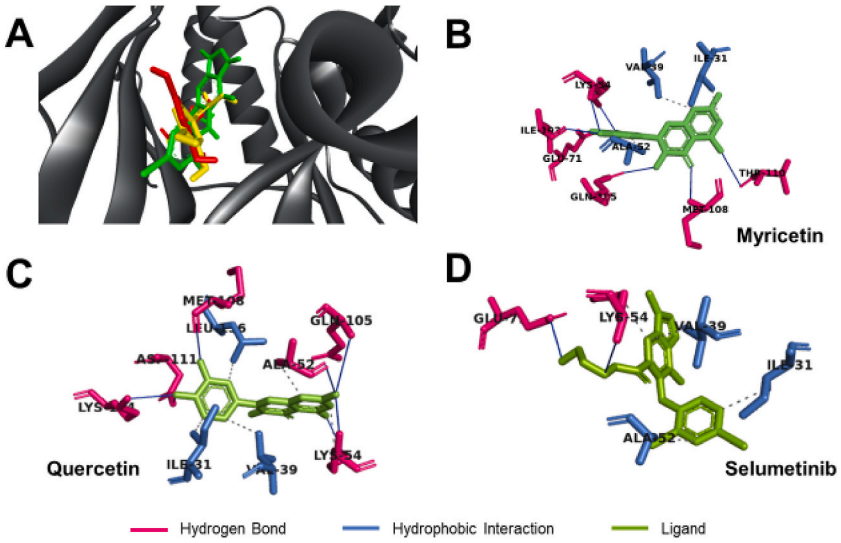


Fig. 9. (A) Superposition of Myricetin (red), Quercetin(yellow), and Selumetinib (green) nside the binding cavity of MAPK1. Amino acid interaction of MAPK1 with (B) Myricetin, (C) Quercetin, and (D) Selumetinib.

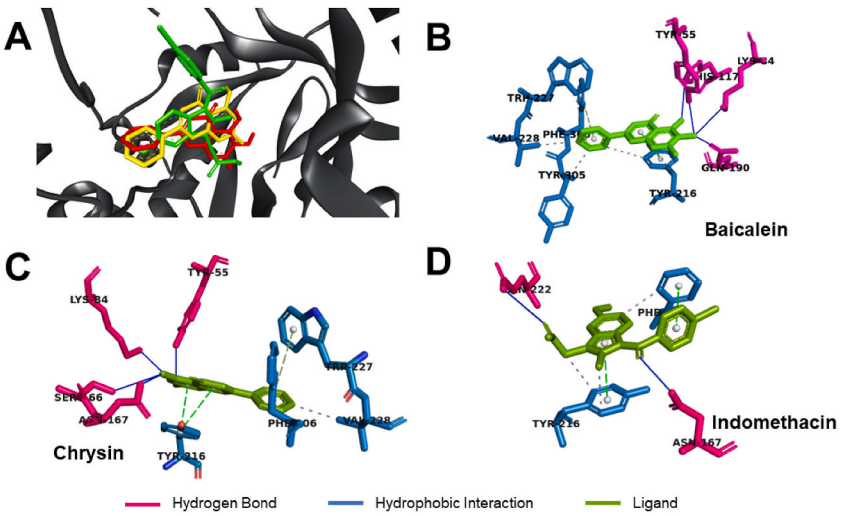


Fig. 10. (A) Superposition of Baicalein (red), Chrysin (yellow), and Indomethacin (green) nside the binding cavity of AKR1C3. Amino acid interaction of AKR1C3 with (B) Baicalein, (C) Chrysin, and (D) Indomethacin.

metastasis [85]. Following surgical resection, the recurrence rate of HCC can rise to 70 % after two and five years, especially in cases with the symptoms of AFP (alpha fetoprotein) levels that exceeding 2000 ng/dL and micrometastasis invasion from primer tumor to the main portal trunk. This condition significantly reduced the survival rate of patients to less than 50 % [86]. In recent medication, laparoscopic liver resection has emerged as an effective and a minimally invasive method for HCC resection. However, this treatment is more expensive than open surgery [87]. Therefore, natural complementary treatment may play a crucial role in new strategies for postoperative HCC treatment. Based on our previous study, East Kalimantan propolis exhibits significant bioactivity, containing rich antioxidant, polyphenol and flavonoids content as essential components for HCC treatments. Moreover, our previous reports indicate that east Kalimantan propolis have cytotoxic activity on human liver cancer cell line (HepG2 cell) [4,5].

In this study, we employed a network pharmacology and bioinformatics analysis approach to investigate underlying mechanism of East Kalimantan propolis in treating HCC [88]. A total of 108 potential HCC core targets of EKP compounds were identified through microarray expression profile datasets (GSEs) and various target prediction tools. KEGG pathway analysis of these targets revealed that the "microRNAs in cancer" pathway was the top result, as it is associated with most of the identified genes. Previous reports stated that the gene encoding miR-122 plays an important role in liver metabolism by inhibiting tumorigenesis and contributing to the maintenance of liver homeostasis [89,90]. Additionally, according to reports, microRNA can help overcome the side effects of Sorafenib, a first-line treatment for hepatocellular carcinoma, by inhibiting its target TYRO3 through the PI3-Kinase/AKT pathway [91,92].

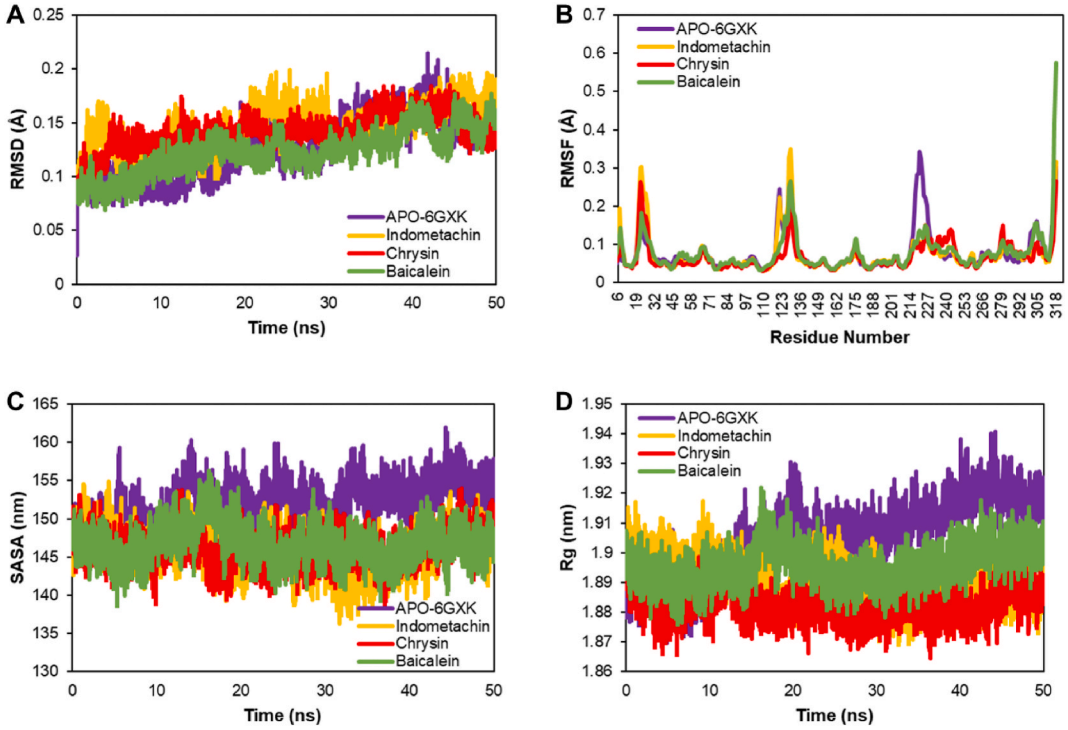


Fig. 11. Molecular dynamics simulation for AKR1C3. Analysis of (A) RMSD (Root Mean Square Deviation); (B) RMSF (Root Mean Square Fluctuations); (C) SASA (Solvent Accessible Surface Area) and (D) Rg (Radius of Gyration).

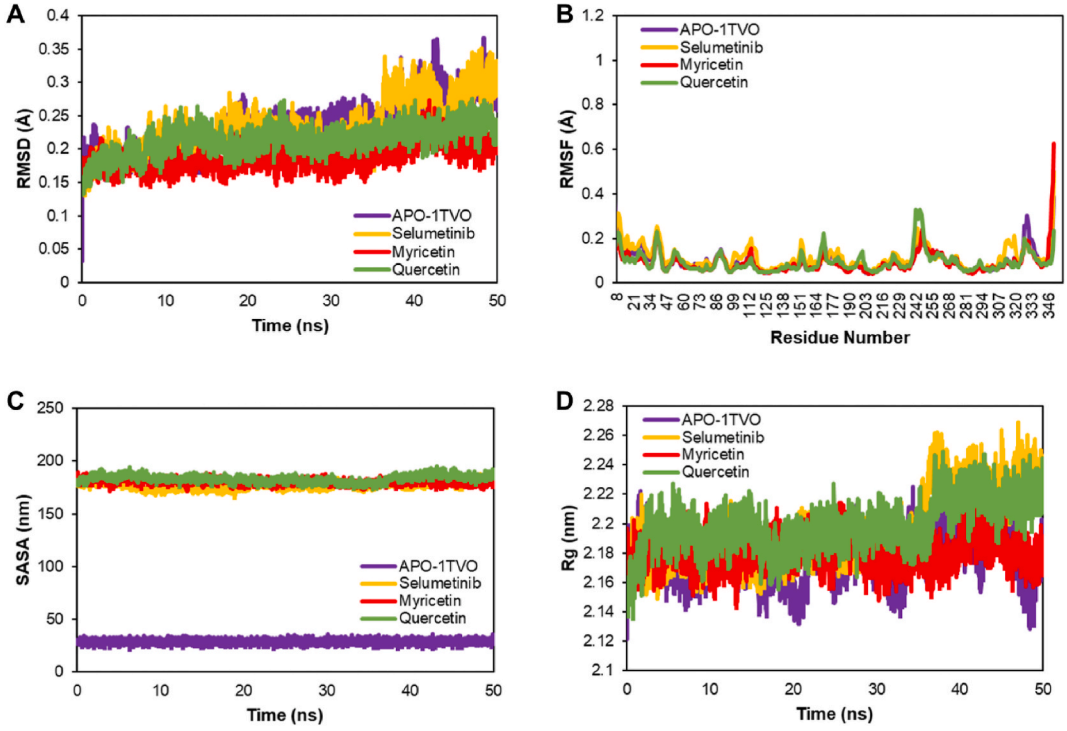


Fig. 12. Molecular dynamics simulation for MAPK1. Analysis of (A) RMSD; (B) RMSF; (C) SASA and (D) Rg.

Additionally, pathways such as lipids and atherosclerosis, viral carcinogenesis, and other HCC-related signaling pathways (as seen in Fig. 4.) were also significantly enriched in the KEGG analysis of the core targets.

Furthermore, our finding reveals the core targets mainly enriched in the biological process of long-chain fatty acid metabolic process, steroid metabolic process, unsaturated fatty acid metabolic process, olefinic compound metabolic process, icosanoid metabolic process, and response to xenobiotic stimulus. A previous study reported that the metabolic process causing HCC is the dysregulation of fatty acid metabolism caused by oncogene dysregulation, triggering metabolic reprogramming that results in carcinogenesis [93]. Other metabolic processes mention the role of alcohol catabolism in influencing lipid metabolism, causing hepatic steatosis and inhibiting fatty acid oxidation (FAO) [94]. Thus, the results of biological process enrichment are mostly centered around various metabolic processes [95].

In results, among those potential HCC targets by EKP, there are 4 main targets (CYP1A1, AKR1C3, MAPK1, and CYP3A4) as the most significance based on PPI analysis. However, only two of them, AKR1C3 and MAPK1, were chosen as core targets in HCC treatment based on their overexpression in HCC tissue and their association with poor prognosis in HCC patients. These targets affect significantly on lower survival rates of HCC patients with supported the high expressed level of targets in HCC tissues. Additionally, AKR1C3 and MAPK1 play a role in steroid hormone synthesis and microRNAs in cancer, respectively.

According to previous reports, the AKR1C3 gene was identified as a potential core targets for HCC therapy, playing a role in controlling cell growth and/or differentiation [96]. It further activated the essential signaling pathways such as MEK/ERK and androgen receptor pathways, contributing to aggressive growth and colony formation of HCC cells [97,98]. A prior study have demonstrated that inhibition of AKR1C3 using the inhibitor indometachin results in a notable reduction in tumor growth both in vitro and in vivo. This effect is linked to the suppression of AKR1C3 activity leads to reduction of PGF2α and inhibited the PGF2α receptor (PTGFR) which significantly reduces HCC growth [99]. Targeting AKR1C3-PGF2α-PTGFR pathway may also offer a new therapeutic approach for HCC treatment. Additionally, another prior study reported that AKR1C3 over expression enhances the proliferation of HCC cells and contributes to acute sorafenib resistance. Downregulation of AKR1C3 restrained cell proliferation and increased the sensitivity of liver cancer cells to sorefanib [100]. Thus, the prior study suggests that the combination of AKR1C3 inhibitor and sorafenib might improve treatment outcomes for HCC patients by targeting multiple aspects of tumor growth and drug resistance mechanisms.

Similarly, the MAPK1 gene was reported as an oncogene causing accelerated proliferation, migration, and invasion of HCC cells [101]. While no specific therapies currently target MAPK1 in HCC, studies suggest its potential as a therapeutic target for inhibiting tumor growth [102–104]. Research has shown that MAPK1 is more frequently expressed in HCC tissues than in peritumoral cirrhotic liver tissues (PCLTs), linking its expression to HCC development and progression. Increased MAPK1 expression is also associated with lower tumor differentiation, which correlates with more aggressive tumors and poorer outcomes. This makes MAPK1 a potential target for identifying poorly differentiated and aggressive HCC cases [102]. Therefore, both AKR1C3 and MAPK1 emerge as promising candidate targets for HCC treatment.

Furthermore, molecular docking and molecular dynamics were conducted to assess the potential of EKP compounds to target AKR1C3 and MAPK1. In results, baicalein, chrysin, quercetin, and myricetin exhibited stable complex interaction with AKR1C3 and MAPK1 with their RMSD values below 2 Å within simulations. these indicate that all complex reached equilibrium within 50 ns. Similarly, the RMSF, Rg and SASA results also demonstrated that baicalein, chrysin, quercetin, and myricetin remained stable throughout the simulation time. In comparison with indomethacin and selumetinib as reference drugs, the molecular behavior to AKR1C3 and MAPK1 were similar with baicalein, chrysin, quercetin, and myricetin. Indomethacin is nonsteroidal anti-inflammatory drugs that act as AKR1C3 inhibitor to combat cancer [105]. While, selumetinib is an MAPK1 inhibitor [106]. Therefore, these findings suggest that AKR1C3 and MAPK1 may be potential therapeutic targets of EKP in the treatment of HCC, particularly with baicalein, chrysin, quercetin, and myricetin.

Numerous previous studies supported our findings on EKP selected compounds (baicalein, chrysin, myricetin and quercetin) in treating HCC. Baicalein has been reported to demonstrate an anti-metastatic effect by inhibiting the invasion and metastasis of HCC tumor cell growth through the suppression of the MEK-ERK signaling pathway [107,108]. Chrysin has reported to effectively inhibit tumor progression in the treatment of HCC by inducing HCC cell ERK1/2 phosphorylation [109]. Additionally, it enhanced mouse anti-tumor immunity and increased the proportion of CD4/CD8 positive T cells in the tumor tissue of the H22 xenograft mouse model [110]. Other studies revealed that Chrysin had a protective effect on the liver, showing a decrease in ALP, AST, ALT, yGT, and LDH. Furthermore, Chrysin was able to prevent the formation of preneoplastic nodules [111]. Lastly, Quercetin and Myricetin exhibited anti-proliferative effects and activation of growth factor-mediated cell migration by inhibiting AKT signaling [112]. This suggests that these potential EKP compounds hold promise in the treatment of HCC.

As far as we know, this is the first study on revealing mechanism pathways of East Kalimantan propolis compounds in treating HCC diseases and identified potential core targets in HCC treatment. Despite, the valuable insights provided by this research, it is important to acknowledge the limitations associated with the Network Pharmacology approach. A key factor is the precision and comprehensiveness of the database that was utilized to find the target genes. The computer-based predictions provide only a preliminary exploration of the potential mechanisms of drug action, and the possible errors could be appeared as the databases not fully updated [113,114]. These findings must be validated through future pharmacological experiments and clinical trials. However, this work provides insightful information about the possible targets and processes of EKP compounds, demonstrating their effects on HCC through various pathways, particularly the MicroRNA cancer pathway, which plays significant role in regulating proliferation, differentiation, apoptosis, and metabolic pathways [115,116].

5. Conclusion

This study highlights the potential application of East Kalimantan propolis as potential supplement for therapy HCC. By using integrative bioinformatics analysis of network pharmacology with differential expression genes analysis, survival analysis and Immunohistochemical images, we examined AKR1C3 and MAPK1 as potential gene targets that may have strong correlation with severity of HCC. Overall, in network pharmacology, 108 potential targets of propolis in treating HCC related to several pathways in KEGG results, mainly MicroRNAs in cancer and chemical carcinogenesis. Survival and immunohistochemical analysis from databases showed that AKR1C3 and MAPK3 may promote carcinogenesis progression in HCC. From our molecular docking results of eight propolis compounds, quercetin and myricetin revealed as best ligands for MAPK1, while chrysin and baicalein revealed as best ligands for AKR1C3. Molecular dynamics confirm that binding stability of propolis ligand complex with targets within 50 ns simulation based on RMSD, RMSF, Rg and SASA analysis. Therefore, this study provides insight into candidate targets, pathways and therapeutic compounds of East Kalimantan Propolis in HCC. However, to verify these results and investigate the underlying mechanisms, additional investigation is necessary. Overall, these results may have important implications for new therapeutic strategies for HCC.

CRediT authorship contribution statement

Paula Mariana Kustiawan: Writing – review & editing, Supervision, Resources, Funding acquisition, Formal analysis, Data curation. **Khalish Arsy Al Khairy Siregar:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Muhammad Miftah Jauhar:** Formal analysis, Data curation. **Donny Ramadhan:** Software, Investigation. **Etik Mardliyati:** Writing – review & editing, Supervision, Software, Resources, Funding acquisition. **Putri Hawa Syaifie:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by National Innovation Research Agency (BRIN); Indonesia in funding program of Riset dan Inovasi untuk Indonesia Maju (RIIM), Contract Number: 46/IV/KS/05/2023 and 123/URK/C.6/H/2023. The authors express their gratitude to Nano Center Indonesia and Universitas Muhammadiyah Kalimantan Timur for providing research facilities.

Abbreviation

EKP : East Kalimantan Propolis
HCC : Hepatocellular Carcinoma
PPI : Protein-Protein Interaction
AKR1C3 : Aldo-keto reductase family 1 member C3
MAPK1 : Mitogen-activated protein kinase 1
GEO : Gene Expression Omnibus
RMSD : Root Mean Square Deviation
RMSF : Root Mean Square Fluctuation
GO : Gene Ontology
KEGG : Kyoto Encyclopedia of Genes and Genomes
BP : Biological Processes
CC : Cellular Components
MF : Molecular Functions
HPA : Human Protein Atlas

References

[1] S. Huang, C.-P. Zhang, K. Wang, G. Li, F.-L. Hu, Recent advances in the chemical composition of propolis, Molecules 19 (2014) 19610–19632, https://doi.org/10.3390/molecules191219610.
[2] P.M. Kustiawan, P.H. Syaifie, K.A.A.K. Siregar, D. Ibadillah, E. Mardliyati, New Insights of Propolis Nanoformulation and its Therapeutic Potential in Human Diseases, ADMET DMPK, 2024, https://doi.org/10.5599/admet.2128.
[3] P.H. Syaifie, D. Ibadillah, M.M. Jauhar, R. Reninta, S. Ningsih, D. Ramadhan, A.G. Arda, D.W.C. Ningrum, N.M.N. Kaswati, N.T. Rochman, E. Mardliyati, Phytochemical profile, antioxidant, enzyme inhibition, acute toxicity, in silico molecular docking and dynamic analysis of *Apis mellifera* propolis as antidiabetic supplement, Chem. Biodivers. 21 (2024), https://doi.org/10.1002/cbdv.202400433.

[4] P.M. Kustiawan, K. Lirdprapamongkol, T. Palaga, S. Puthong, P. Phuwapraisirisan, J. Svasti, C. Chanchao, Molecular mechanism of cardol, isolated from Trigona incisa stingless bee propolis, induced apoptosis in the SW620 human colorectal cancer cell line, BMC Pharmacol Toxicol 18 (2017) 4–13, https://doi.org/10.1186/s40360-017-0139-4.

[5] P.M. Kustiawan, S. Puthong, E.T. Arung, C. Chanchao, In vitro cytotoxicity of Indonesian stingless bee products against human cancer cell lines, Asian Pac. J. Trop. Biomed. 4 (2014) 549–556, https://doi.org/10.12980/APJTB.4.2014APJTB-2013-0039.

[6] P.M. Kustiawan, H. Okuyoshi, Y. Kuse, H. Izawa, Y. Saito, K. Tsuruma, C.C.H. Hara, M. Shimazawa, Protective effects of Indonesian propolis against light-induced retinal damage, International Journal of Toxicological and Pharmacological Research 9 (2017) 163–170.

[7] P.M. Kustiawan, A. Aziz, V.N. Yuliawan, Antioxidant and antibacterial activity of various fractions of heterotrigona itama propolis found in kutai kartanegara, Open Access Maced J Med Sci 10 (2022) 531–534, https://doi.org/10.3889/oamjms.2022.7766.

[8] M.A. Batistuta, A. Aulia, P.M. Kustiawan, Review : potensi aktivitas anti virus Dari produk alami lebah kelulut, J. Farm. Udayana (2021) 144, https://doi.org/10.24843/JFU.2021.v10.i02.p06.

[9] A. Aziz, V.N. Yuliawan, P.M. Kustiawan, Identification of secondary metabolites and antibacterial activity of non polar fraction from heterotrigona itama propolis, Journal of Fundamental and Applied Pharmaceutical Science 2 (2021) 23–33, https://doi.org/10.18196/jfaps.v2i1.12406.

[10] P. Bhargava, D. Mahanta, A. Kaul, Y. Ishida, K. Terao, R. Wadhwa, S.C. Kaul, Experimental evidence for therapeutic potentials of propolis, Nutrients 13 (2021) 2528, https://doi.org/10.3390/nu13082528.

[11] S. Altabbal, K. Athamnah, A. Rahma, A.F. Wali, A.H. Eid, R. Iratni, Y. Al Dhaheri, Propolis: a detailed insight of its anticancer molecular mechanisms, Pharmaceuticals 16 (2023) 450, https://doi.org/10.3390/ph16030450.

[12] A. Pascoal, X. Feás, T. Dias, L.G. Dias, L.M. Estevinho, The role of honey and propolis in the treatment of infected wounds, in: Microbiology for Surgical Infections, Elsevier, 2014, pp. 221–234, https://doi.org/10.1016/B978-0-12-411629-0.00013-1.

[13] W. Rong, N. Wan, X. Zheng, G. Shi, C. Jiang, K. Pan, M. Gao, Z. Yin, Z.-J. Gao, J. Zhang, Chrysin inhibits hepatocellular carcinoma progression through suppressing programmed death ligand 1 expression, Phytomedicine 95 (2022) 153867, https://doi.org/10.1016/j.phymed.2021.153867.

[14] M. Li, J. Chen, X. Yu, S. Xu, D. Li, Q. Zheng, Y. Yin, Myricetin suppresses the propagation of hepatocellular carcinoma via down-regulating expression of YAP, Cells 8 (2019) 358, https://doi.org/10.3390/cells8040358.

[15] L. Wu, J. Li, T. Liu, S. Li, J. Feng, Q. Yu, J. Zhang, J. Chen, Y. Zhou, J. Ji, K. Chen, Y. Mao, F. Wang, W. Dai, X. Fan, J. Wu, C. Guo, Quercetin shows anti-tumor effect in hepatocellular carcinoma LM3 cells by abrogating JAK2/STAT3 signaling pathway, Cancer Med. 8 (2019) 4806–4820, https://doi.org/10.1002/cam4.2388.

[16] B. Han, Y.-Q. Yu, Q.-L. Yang, C.-Y. Shen, X.-J. Wang, Kaempferol induces autophagic cell death of hepatocellular carcinoma cells via activating AMPK signaling, Oncotarget 8 (2017) 86227–86239, https://doi.org/10.18632/oncotarget.21043.

[17] M.U. Ijaz, A. Ishtiaq, N. Ehsan, M. Imran, G. Zhu, Hepatoprotective potential of genkwanin against aflatoxin B1-induced biochemical, inflammatory and histopathological toxicity in rats, The Pakistan Veterinary Journal 42 (2022) 493–498, https://doi.org/10.29261/pakvetj/2022.048.

[18] B. Bie, J. Sun, Y. Guo, J. Li, W. Jiang, J. Yang, C. Huang, Z. Li, Baicalein: a review of its anti-cancer effects and mechanisms in Hepatocellular Carcinoma, Biomed. Pharmacother. 93 (2017) 1285–1291, https://doi.org/10.1016/j.biopha.2017.07.068.

[19] W. Gu, Y. Yang, C. Zhang, Y. Zhang, L. Chen, J. Shen, G. Li, Z. Li, L. Li, Y. Li, H. Dong, Caffeic acid attenuates the angiogenic function of hepatocellular carcinoma cells via reduction in JNK-1-mediated HIF-1α stabilization in hypoxia, RSC Adv. 6 (2016) 82774–82782, https://doi.org/10.1039/C6RA07703J.

[20] P. Konyn, A. Ahmed, D. Kim, Current epidemiology in hepatocellular carcinoma, Expert Rev Gastroenterol Hepatol 15 (2021) 1295–1307, https://doi.org/10.1080/17474124.2021.1991792.

[21] C. Meneghelli, L.S.D. Joaquim, G.L.Q. Félix, A. Somensi, M. Tomazzoli, D.A. da Silva, F.V. Berti, M.B.R. Veleirinho, D. de O.S. Recouvreux, A.C. de Mattos Zeri, P.F. Dias, M. Maraschin, Southern Brazilian autumnal propolis shows anti-angiogenic activity: an in vitro and in vivo study, Microvasc. Res. 88 (2013) 1–11, https://doi.org/10.1016/j.mvr.2013.03.003.

[22] C.-Y. Liu, K.-F. Chen, P.-J. Chen, Treatment of liver cancer, Cold Spring Harb Perspect Med 5 (2015) a021535, https://doi.org/10.1101/cshperspect.a021535.

[23] M. Maluccio, A. Covey, Recent progress in understanding, diagnosing, and treating hepatocellular carcinoma, CA Cancer J Clin 62 (2012) 394–399, https://doi.org/10.3322/caac.21161.

[24] A.I. Casas, A.A. Hassan, S.J. Larsen, V. Gomez-Rangel, M. Elbatreek, P.W.M. Kleikers, E. Guney, J. Egea, M.G. López, J. Baumbach, H.H.H.W. Schmidt, From single drug targets to synergistic network pharmacology in ischemic stroke, Proc. Natl. Acad. Sci. USA 116 (2019) 7129–7136, https://doi.org/10.1073/pnas.1820799116.

[25] D. Suresh, A.N. Srinivas, A. Prashant, K.B. Harikumar, D.P. Kumar, Therapeutic options in hepatocellular carcinoma: a comprehensive review, Clin. Exp. Med. 23 (2023) 1901–1916, https://doi.org/10.1007/s10238-023-01014-3.

[26] U. Chandran, N. Mehendale, S. Patil, R. Chaguturu, B. Patwardhan, Network pharmacology, in: Innovative Approaches in Drug Discovery, Elsevier, 2017, pp. 127–164, https://doi.org/10.1016/B978-0-12-801814-9.00005-2.

[27] K. Sachdev, M.K. Gupta, A comprehensive review of feature based methods for drug target interaction prediction, J Biomed Inform 93 (2019) 103159, https://doi.org/10.1016/j.jbi.2019.103159.

[28] H. Vaghasia, S. Sakaria, J. Prajapati, M. Saraf, R.M. Rawal, Interactive bioinformatics analysis for the screening of hub genes and molecular docking of phytochemicals present in kitchen spices to inhibit CDK1 in cervical cancer, Comput. Biol. Med. 149 (2022) 105994, https://doi.org/10.1016/j.compbiomed.2022.105994.

[29] S. Li, B. Zhang, Traditional Chinese medicine network pharmacology: theory, methodology and application, Chin. J. Nat. Med. 11 (2013) 110–120, https://doi.org/10.1016/S1875-5364(13)60037-0.

[30] Z. Tanoli, Z. Alam, A. Ianevski, K. Wennerberg, M. Vähä-Koskela, T. Aittokallio, Interactive visual analysis of drug–target interaction networks using Drug Target Profiler, with applications to precision medicine and drug repurposing, Brief Bioinform (2018), https://doi.org/10.1093/bib/bby119.

[31] M.U. Kakar, M.Z. Mehboob, M. Akram, M. Shah, Y. Shakir, H.W. Ijaz, U. Aziz, Z. Ullah, S. Ahmad, S. Ali, Y. Yin, Identification of differentially expressed genes associated with the prognosis and diagnosis of hepatocellular carcinoma by integrated bioinformatics analysis, BioMed Res. Int. 2022 (2022) 1–12, https://doi.org/10.1155/2022/4237633.

[32] C. Ma, T. Xu, X. Sun, S. Zhang, S. Liu, S. Fan, C. Lei, F. Tang, C. Zhai, C. Li, J. Luo, Q. Wang, W. Wei, X. Wang, F. Cheng, Network pharmacology and bioinformatics approach reveals the therapeutic mechanism of action of baicalein in hepatocellular carcinoma, Evid. base Compl. Alternative Med. 2019 (2019) 1–15, https://doi.org/10.1155/2019/7518374.

[33] J. Sun, T. Han, T. Yang, Y. Chen, J. Huang, Interpreting the molecular mechanisms of yinchenhao decoction on hepatocellular carcinoma through absorbed components based on network pharmacology, BioMed Res. Int. 2021 (2021) 1–22, https://doi.org/10.1155/2021/6616908.

[34] P. Mariana Kustiawan, K.A.A.K. Siregar, P.H. Syaifie, F. Zein Muttaqin, D. Ibadillah, M. Miftah Jauhar, N. Djamas, E. Mardliyati, N. Taufiqu Rochman, Uncovering the anti-breast cancer activity potential of east Kalimantan propolis by in vitro and bioinformatics analysis, Heliyon 10 (2024) e33636, https://doi.org/10.1016/j.heliyon.2024.e33636.

[35] T. Barrett, S.E. Wilhite, P. Ledoux, C. Evangelista, I.F. Kim, M. Tomashevsky, K.A. Marshall, K.H. Phillippy, P.M. Sherman, M. Holko, A. Yefanov, H. Lee, N. Zhang, C.L. Robertson, N. Serova, S. Davis, A. Soboleva, NCBI GEO: archive for functional genomics data sets—update, Nucleic Acids Res. 41 (2012) D991–D995, https://doi.org/10.1093/nar/gks1193.

[36] H. Wickham, D. Navarro, T.L. Pedersen, ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag, New York, 2016.

[37] A. Hermawan, H. Putri, M. Ikawati, Bioinformatic analysis reveals the molecular targets of tangeretin in overcoming the resistance of breast cancer to tamoxifen, Gene Rep 21 (2020) 100884, https://doi.org/10.1016/j.genrep.2020.100884.

[38] Y.-L. Chen, T.-H. Wang, H.-C. Hsu, R.-H. Yuan, Y.-M. Jeng, Overexpression of CTHRC1 in hepatocellular carcinoma promotes tumor invasion and predicts poor prognosis, PLoS One 8 (2013) e70324, https://doi.org/10.1371/journal.pone.0070324.

[39] H. Wang, X. Huo, X.-R. Yang, J. He, L. Cheng, N. Wang, X. Deng, H. Jin, N. Wang, C. Wang, F. Zhao, J. Fang, M. Yao, J. Fan, W. Qin, STAT3-mediated upregulation of lncRNA HOXD-AS1 as a ceRNA facilitates liver cancer metastasis by regulating SOX4, Mol. Cancer 16 (2017) 136, https://doi.org/10.1186/s12943-017-0680-1.

[40] S. Roessler, H.-L. Jia, A. Budhu, M. Forgues, Q.-H. Ye, J.-S. Lee, S.S. Thorgeirsson, Z. Sun, Z.-Y. Tang, L.-X. Qin, X.W. Wang, A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients, Cancer Res. 70 (2010) 10202–10212, https://doi.org/10.1158/0008-5472.CAN-10-2607.

[41] Y. Dong, Q. Miao, D. Li, Screening of prognostic markers for hepatocellular carcinoma patients based on multichip combined analysis, Comput. Math. Methods Med. 2022 (2022) 1–12, https://doi.org/10.1155/2022/6881600.

[42] A. Villanueva, A. Portela, S. Sayols, C. Battiston, Y. Hoshida, J. Méndez-González, S. Imbeaud, E. Letouzé, V. Hernandez-Gea, H. Cornella, R. Pinyol, M. Solé, J. Fuster, J. Zucman-Rossi, V. Mazzaferro, M. Esteller, J.M. Llovet, HEPTROMIC Consortium, DNA methylation-based prognosis and epidrivers in hepatocellular carcinoma, Hepatology 61 (2015) 1945–1956, https://doi.org/10.1002/hep.27732.

[43] B. Losic, A.J. Craig, C. Villacorta-Martin, S.N. Martins-Filho, N. Akers, X. Chen, M.E. Ahsen, J. von Felden, I. Labgaa, D. D'Avola, K. Allette, S.A. Lira, G. C. Furtado, T. Garcia-Lezana, P. Restrepo, A. Stueck, S.C. Ward, M.I. Fiel, S.P. Hiotis, G. Gunasekaran, D. Sia, E.E. Schadt, R. Sebra, M. Schwartz, J.M. Llovet, S. Thung, G. Stolovitzky, A. Villanueva, Intratumoral heterogeneity and clonal evolution in liver cancer, Nat. Commun. 11 (2020) 291, https://doi.org/10.1038/s41467-019-14050-z.

[44] A. Moeini, S. Torrecilla, V. Tovar, C. Montironi, C. Andreu-Oller, J. Peix, M. Higuera, D. Pfister, P. Ramadori, R. Pinyol, M. Solé, M. Heikenwälder, S. L. Friedman, D. Sia, J.M. Llovet, An immune gene expression signature associated with development of human hepatocellular carcinoma identifies mice that respond to chemopreventive agents, Gastroenterology 157 (2019) 1383–1397.e11, https://doi.org/10.1053/j.gastro.2019.07.028.

[45] R. Esteban-Fabró, C.E. Willoughby, M. Piqué-Gili, C. Montironi, J. Abril-Fornaguera, J. Peix, L. Torrens, A. Mesropian, U. Balaseviciute, F. Miró-Mur, V. Mazzaferro, R. Pinyol, J.M. Llovet, Cabozantinib enhances anti-PD1 activity and elicits a neutrophil-based immune response in hepatocellular carcinoma, Clin. Cancer Res. 28 (2022) 2449–2460, https://doi.org/10.1158/1078-0432.CCR-21-2517.

[46] M. Kuhn, C. von Mering, M. Campillos, L.J. Jensen, P. Bork, STITCH: interaction networks of chemicals and proteins, Nucleic Acids Res. 36 (2007) D684–D688, https://doi.org/10.1093/nar/gkm795.

[47] A. Daina, O. Michielin, V. Zoete, SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules, Nucleic Acids Res. 47 (2019) W357–W364, https://doi.org/10.1093/nar/gkz382.

[48] M.J. Keiser, B.L. Roth, B.N. Armbruster, P. Ernsberger, J.J. Irwin, B.K. Shoichet, Relating protein pharmacology by ligand chemistry, Nat. Biotechnol. 25 (2007) 197–206, https://doi.org/10.1038/nbt1284.

[49] Z.-J. Yao, J. Dong, Y.-J. Che, M.-F. Zhu, M. Wen, N.-N. Wang, S. Wang, A.-P. Lu, D.-S. Cao, TargetNet: a web service for predicting potential drug–target interaction profiling via multi-target SAR models, J. Comput. Aided Mol. Des. 30 (2016) 413–424, https://doi.org/10.1007/s10822-016-9915-2.

[50] C. Shan, X. Ji, Z. Wu, J. Zhao, Network pharmacology combined with GEO database identifying the mechanisms and molecular targets of Polygoni Cuspidati Rhizoma on Peri-implants, Sci. Rep. 12 (2022) 8227, https://doi.org/10.1038/s41598-022-12366-3.

[51] J.C. Oliveros, Venny. An Interactive Tool for Comparing Lists with Venn's Diagrams, (n.d.). https://bioinfogp.cnb.csic.es/tools/venny/index.html.

[52] M. Carlson, org.Hs.eg.db: genome wide annotation for Human. https://doi.org/10.18129/B9.bioc.org.Hs.eg.db, 2023.

[53] M. Kanehisa, M. Furumichi, M. Tanabe, Y. Sato, K. Morishima, KEGG: new perspectives on genomes, pathways, diseases and drugs, Nucleic Acids Res. 45 (2017) D353–D361, https://doi.org/10.1093/nar/gkw1092.

[54] G. Yu, L.-G. Wang, Y. Han, Q.-Y. He, clusterProfiler: an R Package for comparing biological themes among gene clusters, OMICS 16 (2012) 284–287, https://doi.org/10.1089/omi.2011.0118.

[55] H. Hu, H. Wang, X. Yang, Z. Li, W. Zhan, H. Zhu, T. Zhang, Network pharmacology analysis reveals potential targets and mechanisms of proton pump inhibitors in breast cancer with diabetes, Sci. Rep. 13 (2023) 7623, https://doi.org/10.1038/s41598-023-34524-x.

[56] J. Ma, J. Wang, L.S. Ghoraie, X. Men, B. Haibe-Kains, P. Dai, A comparative study of cluster detection algorithms in protein–protein interaction for drug target Discovery and drug repurposing, Front. Pharmacol. 10 (2019), https://doi.org/10.3389/fphar.2019.00109.

[57] M. Kohl, S. Wiese, B. Warscheid, Cytoscape: software for visualization and analysis of biological networks, 291–303, https://doi.org/10.1007/978-1-60761-987-1_18, 2011.

[58] C.-H. Chin, S.-H. Chen, H.-H. Wu, C.-W. Ho, M.-T. Ko, C.-Y. Lin, cytoHubba: identifying hub objects and sub-networks from complex interactome, BMC Syst. Biol. 8 (2014) S11, https://doi.org/10.1186/1752-0509-8-S4-S11.

[59] D.S. Chandrashekar, S.K. Karthikeyan, P.K. Korla, H. Patel, A.R. Shovon, M. Athar, G.J. Netto, Z.S. Qin, S. Kumar, U. Manne, C.J. Creighton, S. Varambally, UALCAN: an update to the integrated cancer data analysis platform, Neoplasia 25 (2022) 18–27, https://doi.org/10.1016/j.neo.2022.01.001.

[60] F. Pontén, K. Jirström, M. Uhlen, The human protein atlas—a tool for pathology, J. Pathol. 216 (2008) 387–393, https://doi.org/10.1002/path.2440.

[61] C. Dong, X. Tian, F. He, J. Zhang, X. Cui, Q. He, P. Si, Y. Shen, Integrative analysis of key candidate genes and signaling pathways in ovarian cancer by bioinformatics, J. Ovarian Res. 14 (2021) 92, https://doi.org/10.1186/s13048-021-00837-6.

[62] P.J. Thul, C. Lindskog, The human protein atlas: a spatial map of the human proteome, Protein Sci. 27 (2018) 233–244, https://doi.org/10.1002/pro.3307.

[63] M.L. Lolli, I.M. Carnovale, A.C. Pippione, W.Y. Wahlgren, D. Bonanni, E. Marini, D. Zonari, M. Gallicchio, V. Boscaro, P. Goyal, R. Friemann, B. Rolando, R. Bagnati, S. Adinolfi, S. Oliaro-Bosso, D. Boschi, Bioisosteres of indomethacin as inhibitors of aldo-keto reductase 1C3, ACS Med. Chem. Lett. 10 (2019) 437–443, https://doi.org/10.1021/acsmedchemlett.8b00484.

[64] M. Ohori, T. Kinoshita, M. Okubo, K. Sato, A. Yamazaki, H. Arakawa, S. Nishimura, N. Inamura, H. Nakajima, M. Neya, H. Miyake, T. Fujii, Identification of a selective ERK inhibitor and structural determination of the inhibitor–ERK2 complex, Biochem. Biophys. Res. Commun. 336 (2005) 357–363, https://doi.org/10.1016/j.bbrc.2005.08.082.

[65] S.K. Burley, C. Bhikadiya, C. Bi, S. Bittrich, L. Chen, G. V Crichlow, C.H. Christie, K. Dalenberg, L. Di Costanzo, J.M. Duarte, S. Dutta, Z. Feng, S. Ganesan, D. S. Goodsell, S. Ghosh, R.K. Green, V. Guranović, D. Guzenko, B.P. Hudson, C.L. Lawson, Y. Liang, R. Lowe, H. Namkoong, E. Peisach, I. Persikova, C. Randle, A. Rose, Y. Rose, A. Sali, J. Segura, M. Sekharan, C. Shao, Y.-P. Tao, M. Voigt, J.D. Westbrook, J.Y. Young, C. Zardecki, M. Zhuravleva, 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 (2021) D437–D451, https://doi.org/10.1093/nar/gkaa1038.

[66] BIOVIA, Discovery Studio Visualizer, 2019.

[67] S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B.A. Shoemaker, P.A. Thiessen, B. Yu, L. Zaslavsky, J. Zhang, E.E. Bolton, PubChem 2023 update, Nucleic Acids Res. 51 (2023) D1373–D1380, https://doi.org/10.1093/nar/gkac956.

[68] S. Dallakyan, A.J. Olson, Small-molecule library screening by docking with PyRx, in: Chemical Biology, Humana Press, New York, 2015, pp. 243–250, https://doi.org/10.1007/978-1-4939-2269-7_19.

[69] M.M. Jauhar, P.H. Syaifie, A.G. Arda, D. Ramadhan, D.W. Nugroho, N.M. Ningsih Kaswati, A. Noviyanto, N.T. Rochman, E. Mardliyati, Evaluation of propolis activity as sucrose-dependent and sucrose-independent Streptococcus mutans inhibitors to treat dental caries using an in silico approach, J Appl Pharm Sci 13 (2023), https://doi.org/10.7324/JAPS.2023.45365, 071–080.

[70] L. Schrödinger, The PyMOL Molecular Graphics System, 2023, Version 2.5.5.

[71] A.H. Harisna, R. Nurdiansyah, P.H. Syaifie, D.W. Nugroho, K.E. Saputro, Firdayani, C.D. Prakoso, N.T. Rochman, N.N. Maulana, A. Noviyanto, E. Mardliyati, In silico investigation of potential inhibitors to main protease and spike protein of SARS-CoV-2 in propolis, Biochem Biophys Rep 26 (2021) 100969, https://doi.org/10.1016/j.bbrep.2021.100969.

[72] M.F. Adasme, K.L. Linnemann, S.N. Bolz, F. Kaiser, S. Salentin, V.J. Haupt, M. Schroeder, Plip 2021: expanding the scope of the protein–ligand interaction profiler to DNA and RNA, Nucleic Acids Res. 49 (2021) W530–W534, https://doi.org/10.1093/nar/gkab294.

[73] S. Salentin, S. Schreiber, V.J. Haupt, M.F. Adasme, M. Schroeder, PLIP: fully automated protein–ligand interaction profiler, Nucleic Acids Res. 43 (2015) W443–W447, https://doi.org/10.1093/nar/gkv315.

[74] A.D. Hill, P.J. Reilly, Scoring functions for AutoDock, in: Glycoinformatics, Springer, New York, 2015, pp. 467–474, https://doi.org/10.1007/978-1-4939-2343-4_27.
[75] J.-F. Cao, Y. Gong, M. Wu, L. Xiong, S. Chen, H. Huang, X. Zhou, Y. Peng, X. Shen, J. Qu, Y. Wang, X. Zhang, Molecular docking and molecular dynamics study Lianhua Qingwen granules (LHQW) treats COVID-19 by inhibiting inflammatory response and regulating cell survival, Front. Cell. Infect. Microbiol. 12 (2022), https://doi.org/10.3389/fcimb.2022.1044770.
[76] J. Lemkul, From proteins to perturbed Hamiltonians: a suite of tutorials for the GROMACS-2018 molecular simulation package [article v1.0], living J, Comput Mol Sci 1 (2019), https://doi.org/10.33011/livecoms.1.1.5068.
[77] R. Umitaibatin, A.H. Harisna, M.M. Jauhar, P.H. Syaifie, A.G. Arda, D.W. Nugroho, D. Ramadhan, E. Mardliyati, W. Shalannanda, I. Anshori, Immunoinformatics study: multi-epitope based vaccine design from SARS-CoV-2 spike glycoprotein, Vaccines (Basel) 11 (2023) 399, https://doi.org/10.3390/vaccines11020399.
[78] M.M. Jauhar, I. Anshori, M. Yosephine, P.H. Syaifie, A.H. Harisna, D.W. Nugroho, E. Mardliyati, A.G. Arda, In silico study of natural xanthones as potential inhibitors of alpha-glucosidase and alpha-amylase, Record Nat. Prod. (2022) 125–144, https://doi.org/10.25135/rnp.337.2203.2391.
[79] S. Ghahremanian, M.M. Rashidi, K. Raeisi, D. Toghraie, Molecular dynamics simulation approach for discovering potential inhibitors against SARS-CoV-2: a structural review, J. Mol. Liq. 354 (2022) 118901, https://doi.org/10.1016/j.molliq.2022.118901.
[80] M. Safithri, D. Andrianto, A. Gaisani Arda, P. Hawa Syaifie, N. Mardia Ningsih Kaswati, E. Mardliyati, D. Ramadhan, M. Miftah Jauhar, D. Wahyu Nugroho, D. Anggraini Septaningsih, L. Tria Melati, M. Hudayanti, E. Sarah, R. Alifibi Putera Irsal, N. Taufiqu Rochman, The effect of red betel (piper crocatum) water fraction as tyrosinase inhibitors: in vitro, molecular docking, and molecular dynamics studies, J. King Saud Univ. Sci. (2023) 102933, https://doi.org/10.1016/j.jksus.2023.102933.
[81] S. Gu, Y. Xue, Y. Gao, S. Shen, Y. Zhang, K. Chen, S. Xue, J. Pan, Y. Tang, H. Zhu, H. Wu, D. Dou, Mechanisms of indigo naturalis on treating ulcerative colitis explored by GEO gene chips combined with network pharmacology and molecular docking, Sci. Rep. 10 (2020) 15204, https://doi.org/10.1038/s41598-020-71030-w.
[82] G. Möller, V. Temml, A. Cala Peralta, O. Gruet, P. Richomme, D. Séraphin, G. Viault, L. Kraus, P. Huber-Cantonati, E. Schopfhauser, J. Pachmayr, J. Tokarz, D. Schuster, J.-J. Helesbeux, K.A. Dyar, Analogues of natural chalcones as efficient inhibitors of AKR1C3, Metabolites 12 (2022) 99, https://doi.org/10.3390/metabo12020099.
[83] D. Magalhães, J. dos Santos, A. Frutuoso, A. Mesquita, Human epidermal growth factor receptor 2 (HER2) expression by immunohistochemistry and its clinical significance in hepatocellular carcinoma: a single-center analysis, Cureus (2023), https://doi.org/10.7759/cureus.34724.
[84] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J Clin 68 (2018) 394–424, https://doi.org/10.3322/caac.21492.
[85] J. Balogh, D. Victor, E.H. Asham, S.G. Burroughs, M. Boktour, A. Saharia, X. Li, M. Ghobrial, H. Monsour, Hepatocellular carcinoma: a review, J. Hepatocell. Carcinoma 3 (2016) 41–53, https://doi.org/10.2147/JHC.S61146.
[86] H. Imamura, Y. Matsuyama, E. Tanaka, T. Ohkubo, K. Hasegawa, S. Miyagawa, Y. Sugawara, M. Minagawa, T. Takayama, S. Kawasaki, M. Makuuchi, Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy, J. Hepatol. 38 (2003) 200–207, https://doi.org/10.1016/S0168-8278(02)00360-4.
[87] C. Jia, H. Li, N. Wen, J. Chen, Y. Wei, B. Li, Laparoscopic liver resection: a review of current indications and surgical techniques, Hepatobiliary Surg. Nutr. 7 (2018) 277–288, https://doi.org/10.21037/hbsn.2018.03.01.
[88] Y. Yu, S. Ding, X. Xu, D. Yan, Y. Fan, B. Ruan, X. Zhang, L. Zheng, W. Jie, S. Zheng, Integrating network pharmacology and bioinformatics to explore the effects of dangshen (codonopsis pilosula) against hepatocellular carcinoma: validation based on the active compound luteolin, Drug Des Devel Ther 17 (2023) 659–673, https://doi.org/10.2147/DDDT.S386941.
[89] W.-C. Tsai, S.-D. Hsu, C.-S. Hsu, T.-C. Lai, S.-J. Chen, R. Shen, Y. Huang, H.-C. Chen, C.-H. Lee, T.-F. Tsai, M.-T. Hsu, J.-C. Wu, H.-D. Huang, M.-S. Shiao, M. Hsiao, A.-P. Tsou, MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis, J. Clin. Invest. 122 (2012) 2884–2897, https://doi.org/10.1172/JCI63455.
[90] S. Hsu, B. Wang, J. Kota, J. Yu, S. Costinean, H. Kutay, L. Yu, S. Bai, K. La Perle, R.R. Chivukula, H. Mao, M. Wei, K.R. Clark, J.R. Mendell, M.A. Caligiuri, S. T. Jacob, J.T. Mendell, K. Ghoshal, Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver, J. Clin. Invest. 122 (2012) 2871–2883, https://doi.org/10.1172/JCI63539.
[91] M.Y. Pratama, D. Pascut, M.N. Massi, C. Tiribelli, The role of microRNA in the resistance to treatment of hepatocellular carcinoma, Ann. Transl. Med. 7 (2019) 577, https://doi.org/10.21037/atm.2019.09.142, 577.
[92] T.D. Kabir, C. Ganda, R.M. Brown, D.J. Beveridge, K.L. Richardson, V. Chaturvedi, P. Candy, M. Epis, L. Wintle, F. Kalinowski, C. Kopp, L.M. Stuart, G.C. Yeoh, J. George, P.J. Leedman, A microRNA-7/growth arrest specific 6/TYRO3 axis regulates the growth and invasiveness of sorafenib-resistant cells in human hepatocellular carcinoma, Hepatology 67 (2018) 216–231, https://doi.org/10.1002/hep.29478.
[93] M. Wang, J. Han, H. Xing, H. Zhang, Z. Li, L. Liang, C. Li, S. Dai, M. Wu, F. Shen, T. Yang, Dysregulated fatty acid metabolism in hepatocellular carcinoma, Hepat Oncol 3 (2016) 241–251, https://doi.org/10.2217/hep-2016-0012.
[94] P. Ramadori, F. Cubero, C. Liedtke, C. Trautwein, Y. Nevzorova, Alcohol and hepatocellular carcinoma: adding fuel to the flame, Cancers 9 (2017) 130, https://doi.org/10.3390/cancers9100130.
[95] X. Kang, L. Bai, X. Qi, J. Wang, Screening and identification of key genes between liver hepatocellular carcinoma (LIHC) and cholangiocarcinoma (CHOL) by bioinformatic analysis, Medicine 99 (2020) e23563, https://doi.org/10.1097/MD.0000000000023563.
[96] M. Gholizadeh, S.R. Mazlooman, M. Hadizadeh, M. Drozdzik, S. Eslami, Detection of key mRNAs in liver tissue of hepatocellular carcinoma patients based on machine learning and bioinformatics analysis, MethodsX 10 (2023) 102021, https://doi.org/10.1016/j.mex.2023.102021.
[97] P. Zhu, R. Feng, X. Lu, Y. Liao, Z. Du, W. Zhai, K. Chen, Diagnostic and prognostic values of AKR1C3 and AKR1D1 in hepatocellular carcinoma, Aging 13 (2021) 4138–4156, https://doi.org/10.18632/aging.202380.
[98] X. Liu, K. Wang, L. Wang, X. Fan, Network pharmacology combined with experimental validation reveals the anti-tumor effect of duchesnea indica against hepatocellular carcinoma, J. Cancer 14 (2023) 505–518, https://doi.org/10.7150/jca.76591.
[99] K.-S. Jeng, P.-Y. Cheng, Y.-H. Lin, P.-C. Liu, P.-H. Tseng, Y.-C. Wang, C.-F. Chang, C.-M. Leu, Aldo-keto reductase family member C3 (AKR1C3) promotes hepatocellular carcinoma cell growth by producing prostaglandin F2α, Oncol. Res. 32 (2024) 163–174, https://doi.org/10.32604/or.2023.030975.
[100] J. Zheng, Z. Yang, Y. Li, L. Yang, R. Yao, Knockdown of AKR1C3 promoted sorafenib sensitivity through inhibiting the phosphorylation of AKT in hepatocellular carcinoma, Front. Oncol. 12 (2022), https://doi.org/10.3389/fonc.2022.823491.
[101] H. Wang, J. Ke, Q. Guo, K. Barnabo Nampoukime, P. Yang, K. Ma, Long non-coding RNA CRNDE promotes the proliferation, migration and invasion of hepatocellular carcinoma cells through miR-217/MAPK1 axis, J. Cell Mol. Med. 22 (2018) 5862–5876, https://doi.org/10.1111/jcmm.13856.
[102] Y. Zhang, S. Wang, D. Li, J. Zhnag, D. Gu, Y. Zhu, F. He, A systems biology-based classifier for hepatocellular carcinoma diagnosis, PLoS One 6 (2011) e22426, https://doi.org/10.1371/journal.pone.0022426.
[103] S.-M. Wang, P.-W. Yang, X.-J. Feng, Y.-W. Zhu, F.-J. Qiu, X.-D. Hu, S.-H. Zhang, Apigenin inhibits the growth of hepatocellular carcinoma cells by affecting the expression of microRNA transcriptome, Front. Oncol. 11 (2021), https://doi.org/10.3389/fonc.2021.657665.
[104] Y. Zheng, C. Zhou, X.-X. Yu, C. Wu, H.-L. Jia, X.-M. Gao, J.-M. Yang, C.-Q. Wang, Q. Luo, Y. Zhu, Y. Zhang, J.-W. Wei, Y.-Y. Sheng, Q.-Z. Dong, L.-X. Qin, Osteopontin promotes metastasis of intrahepatic cholangiocarcinoma through recruiting MAPK1 and mediating Ser675 phosphorylation of β-Catenin, Cell Death Dis. 9 (2018) 179, https://doi.org/10.1038/s41419-017-0226-x.
[105] A.L. Lovering, J.P. Ride, C.M. Bunce, J.C. Desmond, S.M. Cummings, S.A. White, Crystal structures of prostaglandin D2 11-ketoreductase (AKR1C3) in complex with the nonsteroidal anti-inflammatory drugs flufenamic acid and indomethacin, Cancer Res. 64 (2004) 1802–1810, https://doi.org/10.1158/0008-5472.CAN-03-2847.
[106] M. Hedayat, R. Jafari, N. Majidi Zolbanin, Selumetinib: a selective MEK1 inhibitor for solid tumor treatment, Clin. Exp. Med. 23 (2022) 229–244, https://doi.org/10.1007/s10238-021-00783-z.

[107] K. Chen, S. Zhang, Y. Ji, J. Li, P. An, H. Ren, R. Liang, J. Yang, Z. Li, Baicalein inhibits the invasion and metastatic capabilities of hepatocellular carcinoma cells via down-regulation of the ERK pathway, PLoS One 8 (2013) e72927, https://doi.org/10.1371/journal.pone.0072927.
[108] R.-R. Liang, S. Zhang, J.-A. Qi, Z.-D. Wang, J. Li, P.-J. Liu, C. Huang, X.-F. Le, J. Yang, Z.-F. Li, Preferential inhibition of hepatocellular carcinoma by the flavonoid Baicalein through blocking MEK-ERK signaling, Int. J. Oncol. 41 (2012) 969–978, https://doi.org/10.3892/ijo.2012.1510.
[109] C.-T. Wei, L.-C. Chen, Y.-P. Hsiang, Y.-J. Hung, P.-H. Chien, H. Pan, Chen-Yun-Ju, Chrysin-induced ERK1/2 phosphorylation enhances the sensitivity of human hepatocellular carcinoma cells to sorafenib, Anticancer Res. 39 (2019) 695–701, https://doi.org/10.21873/anticanres.13165.
[110] D. Xu, J. Jin, H. Yu, Z. Zhao, D. Ma, C. Zhang, H. Jiang, Chrysin inhibited tumor glycolysis and induced apoptosis in hepatocellular carcinoma by targeting hexokinase-2, J. Exp. Clin. Cancer Res. 36 (2017) 44, https://doi.org/10.1186/s13046-017-0514-4.
[111] M.S. Khan, H. Devaraj, N. Devaraj, Chrysin abrogates early hepatocarcinogenesis and induces apoptosis in N-nitrosodiethylamine-induced preneoplastic nodules in rats, Toxicol. Appl. Pharmacol. 251 (2011) 85–94, https://doi.org/10.1016/j.taap.2010.12.004.
[112] N. Yamada, R. Matsushima-Nishiwaki, O. Kozawa, Quercetin suppresses the migration of hepatocellular carcinoma cells stimulated by hepatocyte growth factor or transforming growth factor-α: attenuation of AKT signaling pathway, Arch. Biochem. Biophys. 682 (2020) 108296, https://doi.org/10.1016/j.abb.2020.108296.
[113] Y. Zeng, S. Xiao, L. Yang, K. Ma, H. Shang, Y. Gao, Y. Wang, F. Zhai, R. Xiang, Systematic analysis of the mechanism of Xiaochaihu decoction in hepatitis B treatment via network pharmacology and molecular docking, Comput. Biol. Med. 138 (2021) 104894, https://doi.org/10.1016/j.compbiomed.2021.104894.
[114] X. Jiang, J. Zhou, Z. Yu, X. Gu, Y. Lu, Y. Ruan, T. Wang, Exploration of Fuzheng Yugan Mixture on COVID-19 based on network pharmacology and molecular docking, Medicine 102 (2023) e32693, https://doi.org/10.1097/MD.0000000000032693.
[115] G. Guo, J. Zhou, X. Yang, J. Feng, Y. Shao, T. Jia, Q. Huang, Y. Li, Y. Zhong, P.S. Nagarkatti, M. Nagarkatti, Role of MicroRNAs induced by Chinese herbal medicines against hepatocellular carcinoma: a brief review, Integr. Cancer Ther. 17 (2018) 1059–1067, https://doi.org/10.1177/1534735418805564.
[116] Y. Zhou, F. Liu, C. Ma, Q. Cheng, Involvement of microRNAs and their potential diagnostic, therapeutic, and prognostic role in hepatocellular carcinoma, J. Clin. Lab. Anal. 36 (2022), https://doi.org/10.1002/jcla.24673.