ORIGINAL ARTICLE

Potential target and mechanism exploration from α-mangostin against triple-negative breast cancer: An *in silico* study

Rafly Mochamad Rivaldo, Paulus Chandra

Undergraduate Medical Study Program, Faculty of Medicine, Universitas Padjadjaran, Sumedang, West Java, Indonesia

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ABSTRACT

Triple-negative breast cancer (TNBC) is one of the most common types of serious breast cancer. Due to the absence of therapeutic hormone receptors, TNBC treatment generally involves chemotherapy which results in various side effects and resistance development. Herbal compounds, including α-mangostin, have shown potential anticancer effects against TNBC. However, rigorous screening is needed to uncover its mechanisms and characteristics. The aim of this study was to understand the molecular mechanism of α-mangostin against TNBC and its possible limitations. The study design used is an *in silico* study. The study involved database mining and compound characteristic analysis. Network pharmacology and molecular docking were also done to explore potential target and molecular mechanisms against TNBC. There was no statistical analysis conducted as this study relies on predefined algorithms and simulation models. Instead, a parameter threshold was used for each analysis to ensure its reliability. Prediction of Activity Spectra for Substances prediction and Gene Ontology–Kyoto Encyclopedia of Genes and Genomes enrichment predicted potential anticancer effects of α-mangostin through the regulation of enzyme activity and apoptotic pathway. Compound property predictions showed α-mangostin to have promising drug-likeness with sufficient bioavailability and low biodegradability. However, α-mangostin still has some potential limitations in water solubility and toxicity risks. Through network pharmacology, 75 potential target proteins of α-mangostin in TNBC cases were found. The top three most significant of which (AKT1, CTNNB1, and HSPAA91) were proven to bind with α-mangostin through molecular docking. Study results suggested α-mangostin to have a promising anticancer and chemopreventive activity with great drug-likeness and pharmacokinetic properties. It was revealed that α-mangostin can bind to key components in TNBC-related pathways, including AKT1, CTNNB1, and HSP90AA1 proteins. However, further experimental studies may be needed to verify its effectiveness as well as possible solubility and toxicity limitations.

Key words: *In silico*, molecular docking, pharmacokinetic, pharmacologic network, triple-negative breast cancer, α-mangostin

Address for correspondence:
Mr. Paulus Chandra,
Faculty of Medicine, Universitas Padjadjaran, Sumedang, 45363, West Java, Indonesia.
E-mail: 21pauluschandra1203@gmail.com

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INTRODUCTION

Triple-negative breast cancer (TNBC), characterized by the lack of progesterone receptor, estrogen receptor, and human epidermal growth factor receptor-2, accounts for approximately 10%–20% of breast cancer cases.[1] It has been a serious global challenge due to endocrine therapy unavailability, aggressive progression, high metastasis ratio, and poor prognosis.[1]

Therapies that are commonly carried out include chemotherapy which in large quantities can be toxic to other body cells.[2] This is further complicated by chemotherapy resistance resulting in a recurrence ratio and mortality rate of up to 50% and 37%, respectively.[3] These highlight the need for novel therapeutic options against TNBC.

One potential compound is α-mangostin, a xanthone derivative compound contained in mangosteen fruit, which has been shown to have anticancer effects.[4,5] However, the underlying mechanisms and compound characteristics have not been fully understood, which can be a significant limitation in its development as a novel drug. Therefore, this research aimed to comprehensively explore the molecular mechanisms of α-mangostin against TNBC and its potential limitations through network pharmacology and molecular docking.

MATERIALS AND METHODS

Ligand selection and Prediction of Activity Spectra for Substances prediction

The two-dimensional (2D) structure of α-mangostin (CID: 5281650) was collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov), and Prediction of Activity Spectra for Substances (PASS) software (https://way2drug.com/passonline/predict.php) was used to determine α-mangostin biological activities. The results were considered significant if the value of Pa (probability "to be active") > Pi (probability "to be inactive") value and Pa value > 0.7.[6]

Drug-likeness and pharmacokinetics

The drug-likeness of α-mangostin was analyzed using the SwissADME tool (https://www.swissadme.ch/). Furthermore, the admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) was used to assess and predict the pharmacokinetic properties of α-mangostin.

Pharmacological network

Network pharmacology is an approach to understanding the interactions between selected drugs and their multiple targets.[7] The target of the α-mangostin was obtained from the similarity ensemble approach (http://sea.bkslab.org/) and SwissTargetPrediction (http://www.swisstargetprediction.ch/) databases. Meanwhile, the TNBC-related targets were obtained from GeneCards (https://www.genecards.org) and DisGeNET (https://www.disgenet.org/search) for *Homo sapiens* with "triple negative breast cancer" as the keyword.

Protein–protein interaction

Protein–protein interaction (PPI) is an integral part of network pharmacology to predict the target protein function and the molecule's medicinal properties.[8] Initially, both the α-mangostin and TNBC-related targets were compared in VENNY (https://bioinfogp.cnb.csic.es/tools/venny/). Then, the String 11.0 tool (https://stringdb.org/) was used to determine the interactions between the intersecting targets obtained from the Venn diagram. The results were then visualized using Cytoscape 3.10.1 software, and proteins with a degree count >2 times the median were considered core proteins.

Enrichment analyses

Gene Ontology (GO) enrichment analysis was used to evaluate potential biological processes, cellular composition, and function of target molecules. Meanwhile, the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was utilized to explore potential biological pathways and functions related to their targets. Both enrichment analyses were performed using the DAVID tool (https://david.ncifcrf.gov/) with $P < 0.01$ as the threshold.[9]

Molecular docking

The 2D ligand structure of each core protein was obtained from the PubChem database. The amino acid sequences for the targets were downloaded from the universal protein source and their homology model was built using Modeller 10.4. Due to the absence of a native ligand, the blind docking method was used with a simulation repetition of 1000 times. The resulting lowest binding energy was selected and subsequently analyzed for their interactions.

RESULTS

Prediction of Activity Spectra for Substances prediction

Possible anticancer activities from α-mangostin were predicted using PASS prediction [Table 1]. PASS results showed α-mangostin to be apoptosis agonist and antineoplastic indicating its potential usage as an anticancer compound.

Compound property prediction

The analytical results regarding physicochemical properties and drug-likeness are shown in Table 2. According to the results, α-mangostin has a relatively light molecular weight of 410.46 Dalton (≤500) and a log*P* value of 4.14 (≤5), further confirming its lipophilicity and membrane permeability. Pharmacokinetic analyses suggested α-mangostin to have a relatively high bioavailability value of 0.55. However, the value of log S Estimated solubility (ESOL) for α-mangostin is

very low with a value of −6.35, indicating that the molecule is not water soluble. As for drug-likeness, α-mangostin followed every drug likeness rule used in this study except Muegge's rule.

The pharmacokinetic properties of α-mangostin are shown in Table 3. It was predicted to have good gastrointestinal absorption, strengthening its possibility to be administered orally and absorbed by the human intestinal mucosa. Negative biodegradation values indicate that α-mangostin has the potential to remain in the body system for a longer period of time. However, α-mangostin was indicated to have mitochondrial and respiratory toxicity risks.

Pharmacological analysis of α-mangostin against triple-negative breast cancer

A total of 4063 TNBC-related targets were obtained from GeneCards and DisGeNET databases. Meanwhile, α-mangostin was found to have 128 targets. After comparison, 76 intersecting targets were found [Figure 1].

Based on the String results, the PPI network contains 75 nodes and 892 edges [Figure 2a]. The target network diagram was then described using Cytoscape 3.10.1 and 11 nodes with a degree value >42 were found [Figure 2b]. Among them, AKT1, CTNNB1, and HSP90AA1 were found to be the three most important core targets based on degree value.

Gene Ontology function and Kyoto Encyclopedia of Genes and Genomes pathway enrichment

GO enrichment analysis was carried out on 76 targets resulting in 203 biological processes, 30 cell compositions, and 40 molecular functions. Ten results with the lowest *P* value from each group are shown in Figure 3. Results suggested α-mangostin to exert its effects mainly in cytoplasm and cytosol in which it affects enzyme binding and protein kinase molecularly as well as the biological process of protein phosphorylation and apoptotic regulation.

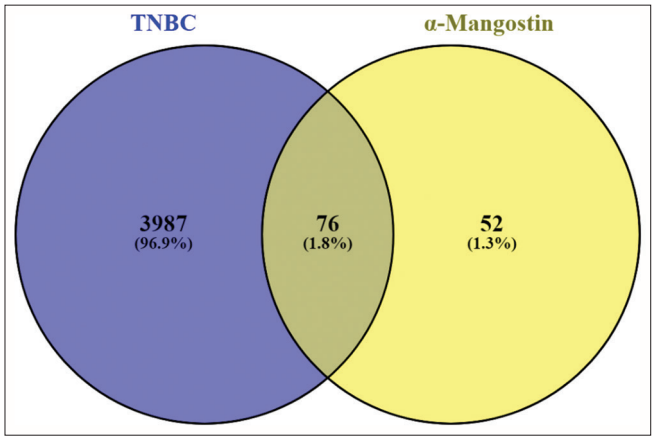

Figure 1: Number of α-mangostin and triple-negative breast cancer-associated targets. TNBC: Triple-negative breast cancer

KEGG pathway enrichment analysis results showed that 76 targets were involved in 138 signaling pathways. The 15 results with the lowest *P* values are shown in Figure 4. KEGG results showed that α-mangostin was involved in the cancer signaling pathway (hsa05200), PI3K-Akt signaling pathway (hsa04151), HIF-1 signaling pathway (hsa04066), and cell senescence (hsa04218).

Table 1: Prediction of α-mangostin activity

Pa	Pi	Activity
0.95	0.004	Membrane integrity agonist
0.875	0.005	Apoptosis agonist
0.865	0.003	UGT1A9 substrate
0.858	0.01	Chlordecone reductase inhibitor
0.855	0.003	Lipid peroxidase inhibitor
0.854	0.018	CDP-glycerol glycerophosphotransferase inhibitor
0.831	0.008	Antineoplastic
0.824	0.003	Monophenol monooxygenase inhibitor
0.817	0.002	CYP1A1 inhibitor
0.799	0.002	NOS_2 expression inhibitor

UGT1A9: Uridine diphosphate glucuronosyltransferase family 1 member A9, CDP-glycerol: Cytidine diphosphate glycerol, CYP1A1: Cytochrome P450, family 1, subfamily A, polypeptide 1, NOS_2: Nitric oxide synthase 2

Table 2: AdmetSar physicochemical properties and drug-likeness prediction of α-mangostin

Molecular formula	C24H26O6
Molecular weight	410.46 g/mol
H-bond acceptor	6
H-bond donor	3
Molar refractivity	119.99
Topological polar surface area	100.13 Å^2
Log $P_{o/w}$ (iLOGP)	4.14
Log S (ESOL)	−6.35
Drug-likeness	
Lipinski	Yes
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	No
Bioavailability score	0.55

ESOL: Estimated solubility, iLOGP: Implicit Log P

Table 3: SwissADME pharmacokinetic property prediction of α-mangostin

Pharmacokinetic	
Properties	**Results**
Gastrointestinal absorption	+
Biodegradation	−
Ames mutagenesis	−
Hepatotoxicity	−
Nephrotoxicity	−
Mitochondria toxicity	+
Respiratory toxicity	+

ADME: Absorption, Distribution, Metabolism, and Excretion, Positive (+): Predicted to have the corresponding characteristic, Negative (-): Predicted to not have the corresponding characteristic

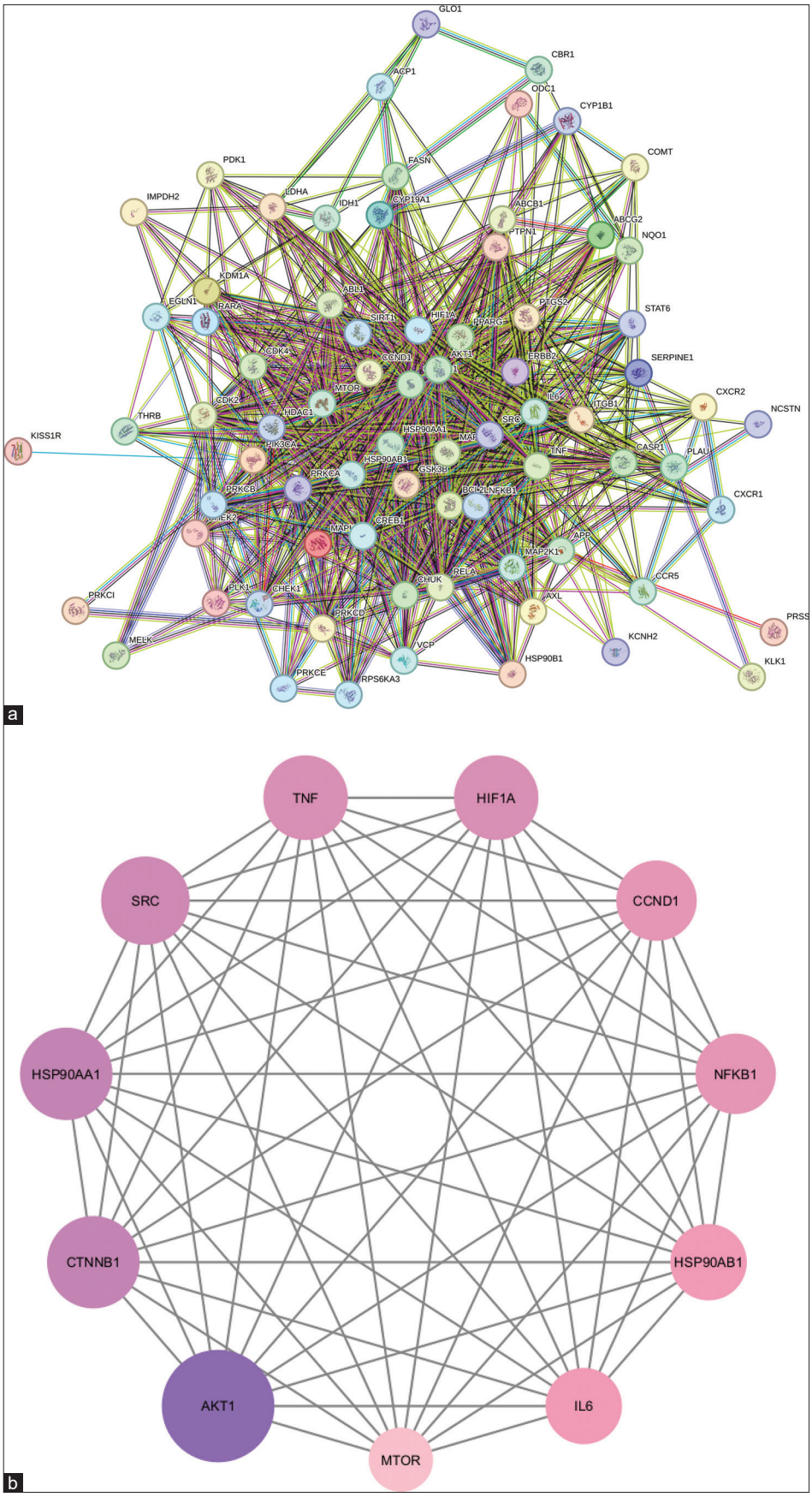

Figure 2: Protein–protein interaction network. (a) Result String, (b) 11 core targets. Circles indicate proteins. Lines represent interactions. Larger and darker nodes correspond to a higher degree value

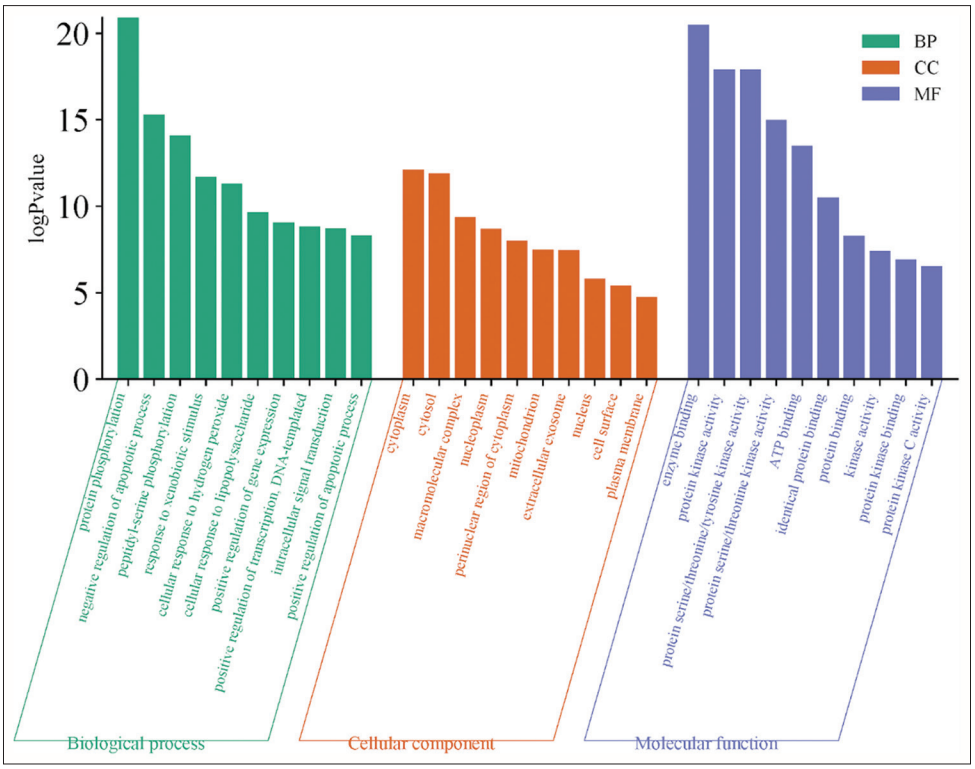

Figure 3: Top 10 results from Gene Ontology enrichment analysis. The length of each band represents the logP value of each entry

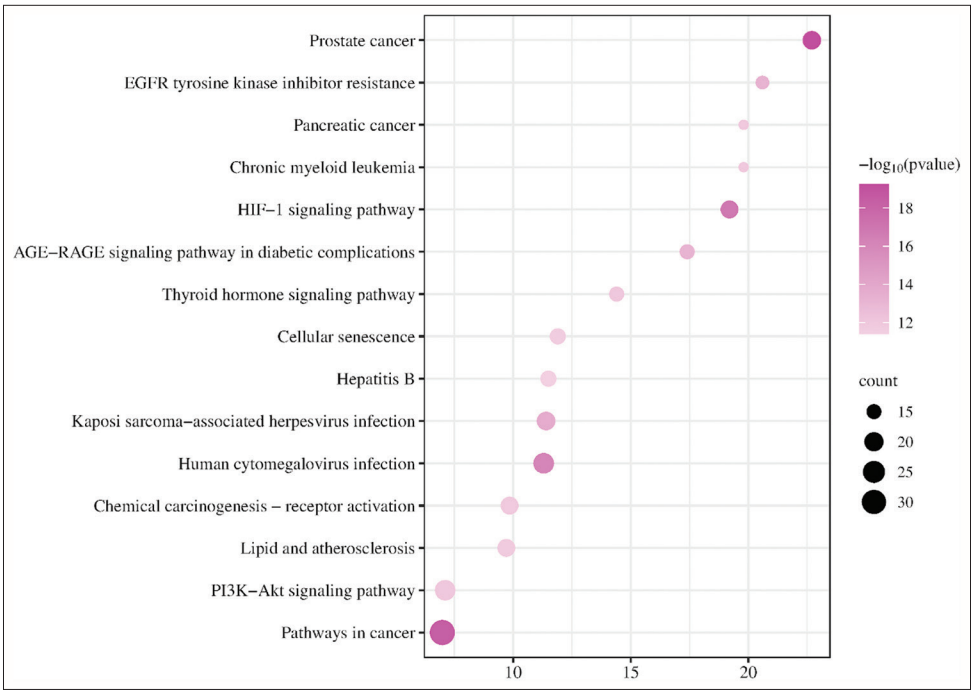

Figure 4: Top 15 results of Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis. Colors represent −log10 differences (*P* values) and bubble sizes represent counts

Molecular docking verification

To further verify α-mangostin intracellular mechanism, molecular docking was carried out with three core targets with the highest degree, namely AKT1 (P31749), CTNNB1 (P35222), and HSP90AA1 (P07900). The binding interactions [Figure 5] and binding energies [Table 4] were collected and analyzed. The binding energies of α-mangostin for AKT1, CTNNB1, and HSP90AA1 were lower than ≤−5.0 kcal/mol, which indicates that ligand binding is likely to occur.[10] These binding energies also correlate with the

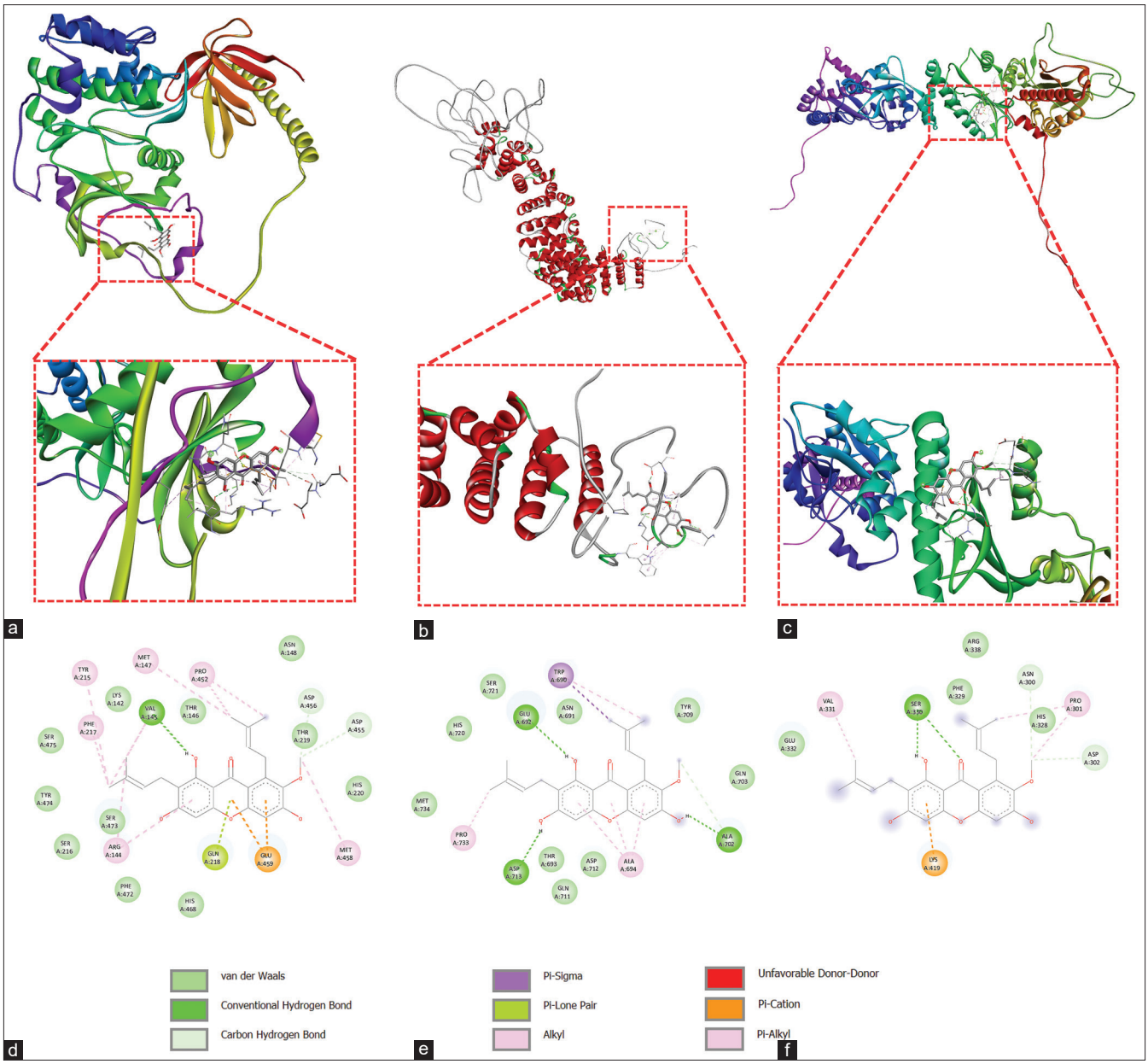

Figure 5: Docking results of the three highest core targets with α-mangostin. Crystal structures of AKT1 (a), CTNNB1 (b), and HSP90AA1 (c) bound to α-mangostin. Two-dimensional interactions between α-mangostin with AKT1 (d), CTNNB1 (e), and HSP90AA1 (f)

Table 4: Binding energy of α-mangostin to the three core proteins

Binding energy	
Protein	**Energy (kcal/mol)**
AKT1	−8.00
CTNNB1	−6.32
HSP90AA1	−5.99

AKT1: RAC-α serine/threonine-protein kinase, CTNNB1: Catenin β-1, HSP90AA1: Heat shock protein 90 α family class A member 1

number of hydrogen polar and hydrophobic bonds as α-mangostin bound to 11 amino acids in AKT1 (ARG144, VAL145, MET147, TYR215, PHE217, GLN218, PRO452, ASP455, ASP456, MET458, and GLU459), 6 amino acids in CTNNB1 (TRP690, GLU692, ALA694, ALA702, ASP713, and PRO733), and 4 amino acids in HSP90AA1 (PRO301, SER330, VAL331, and LYS419).

DISCUSSION

α-mangostin, a compound from the mangosteen plant, has anticancer potential against TNBC.[4,5] However, the underlying mechanisms and limitations have not been fully understood; therefore, *in silico* studies incorporating network pharmacology and molecular docking were done. PASS predictions showed that α-mangostin has agonist apoptotic activity and antineoplastic properties. These are supported by KEGG and GO enrichment results

which showed α-mangostin targets cancer and cell aging pathways, possibly through molecular functions involving enzyme binding regulations and kinase activities. These are subsequently predicted to affect various biological processes, including the disruption of protein phosphorylation and cell apoptosis.

Through database screenings and PPI network analysis, 11 core targets were obtained with AKT1, CTNNB1, and HSP90AA1 identified as the three most important targets for α-mangostin against TNBC. Molecular docking revealed α-mangostin binding with these proteins through multiple hydrogen and hydrophobic bonds. In line with these findings, Zhu *et al*. found that α-mangostin inhibits the activity of AKT1 by suppressing its phosphorylation, culminating in the reduction of cell viability.[4] AKT1 inhibitor addition has also been proven to increase progression-free and overall survival of TNBC patients.[11] This is due to AKT1 playing a key role in the PI3K/Akt/mTOR pathway, an important pathway involved in TNBC chemoresistance and survival as was reported in our KEGG enrichment analysis. Meanwhile, Yoo *et al*. found α-mangostin impacting the protein expression and pathway of β--catenin.[12] These include the Wnt/CTNNB1 pathway which affects tissue homeostasis and is hyperactive in various human cancer types.[13] Regarding HSP90AA1, its expressions are associated with poor prognosis in TNBC, but binding with α-mangostin is a novel finding that has not been analyzed before.[14]

Drug similarity and physicochemical characteristics were also analyzed with α-mangostin fulfilling the Lipinski, Ghose, Veber, and Egen's rule of five and can therefore be considered a drug-like molecule.[15] However, it does not pass Muegge's rule which determines the probability of a compound being a successful drug molecule by calculating the pharmacophore point.[16] Meanwhile, pharmacokinetic searches showed α-mangostin to be readily absorbed through the gastrointestinal tract and have good bioavailability with low biodegradability, leading to its ability to remain in the body system for a long duration.[17] These properties are crucial in maximizing α-mangostin efficacy as a potential oral administered treatment.

However, pharmacokinetic studies also revealed several potential limitations in the form of low water-solubility and mitochondrial toxicity risk. Although mitochondrial dysfunction was suggested to play a role in its anticancer effect, this risk of toxicity should be taken into consideration in its drug development to minimize unintended adverse effects.[5] In addition, α-mangostin was also predicted to have respiratory toxicity risks, but no prior study had reported on this. Despite these limitations, targeted therapy of α-mangostin will potentially be sufficient to combat limited water solubility and minimize the risk of adverse effects while maximizing its efficacy as a potential TNBC treatment.

Overall, this study has successfully elucidated the integral molecular mechanisms and protein targets of α-mangostin against TNBC, along with its possible limitations. However, further studies involving molecular dynamics or more extensive screenings for minor targets may be beneficial to complement the novel understandings found in this study. Aside from that, *in vitro* and *in vivo* studies regarding α-mangostin potential against TNBC through AKT1, CTNNB1, and HSP90AA1 were also highly encouraged to confirm their compound interactions, physiological implications, effectiveness, and safety for its intended therapeutic use.

CONCLUSION

Database predictions suggested α-mangostin to have anticancer effects, by regulating enzyme binding activities and apoptotic pathways. Predicted drug-likeness and pharmacokinetics properties showed α-mangostin to be an orally active agent for TNBC treatment. Through network pharmacology, 11 core targets of α-mangostin against TNBC were identified with AKT1, CTNNB1, and HSP90AA1 as the most integral protein targets. Subsequently, molecular docking also showed α-mangostin to bind with these three proteins through hydrogen polar and hydrophobic interactions suggesting potential implication in TNBC-related pathways which have considerable involvement from these proteins. However, further studies are still needed to verify the potential physiological implications, effectiveness, and safety.

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Conflicts of interest
There are no conflicts of interest.

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