Iran. J. Biotechnol. April 2024;22(2): e3718 DOI: 10.30498/ijb.2024.398939.3718

Research Article

Docking-Based Virtual Screening Method for Selecting Natural Compounds with Synergistic Inhibitory Effects Against Cancer Signalling Pathways Using a Multi-Target Approach

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Received: 2023/10/29 ; **Accepted:** 2024/03/09

Background: The complexity of the molecular mechanisms in cancer has led to the ineffectiveness of treatments for many years. To date, the one-target-one-drug approach has shown disappointing consequences for completely eradicating cancer. In comparison, a multi-targeting approach has presented improved outcomes, which may result from the synergistic inhibition of various molecular mechanisms simultaneously. In this regard, medicinal plants contain a wide range of natural compounds that could inhibit multiple targets simultaneously.

Objectives: This study aims to introduce a methodology for identifying medicinal plants that contain effective natural compounds with the most possible synergistic effects to inhibit cancer survival and proliferation in a multi-targeted manner.

Materials and Methods: To select targets, the signaling pathways involved in cancer development were defined from the KEGG database, and the protein-protein interactions (PPIs) of genes within these pathways were investigated using the STRING software. Then 14 proteins with the highest degree were identified as targets. Using the NPASS database, natural compounds were initially filtered based on their IC_{50} against 50 cancer cell lines. Finally, a total of 1,107 natural compounds were docked to the 14 selected targets involved in cancer and 5 targets involved in general drug side effects.

Results: The targets with the highest protein interactions, as identified by PPI analysis on cancer signaling pathways, were selected as hub proteins. Natural compounds with IC_{50} less than 20000 nM against cancer cell lines were then docked to these selected targets using the NPASS database. Natural compounds with low binding energy to the selected targets were identified as potential synergistic inhibitors of cancer progression if used together. Additionally, plants reported with the widest range of identified natural compounds were introduced as potential sources of synergistic effects against cancer development.

Conclusions: We have proposed a methodology for pre-screening the natural compounds database to identify potential compounds with a high likelihood of producing a synergistic response against multiple molecular mechanisms in cancer. However, further validation methods are necessary to confirm their effectiveness.

Keywords: Cancer, Docking, Multi target, Natural compounds, Screening

1. Background

Cancer is one of the major public health problems worldwide and the second leading cause of death globally (1). The high mortality rate of cancer has made drug development a crucial area of focus in the fight against the disease. For many decades, the one-target-one-ligand approach has been used to create highly selective drugs that minimize unwanted off-target effects by targeting and blocking or deactivating specific molecular processes (2). Despite this, several studies have revealed disappointing outcomes in the treatment of complex diseases like cancer when using the one-target-one-ligand approach (3). This is due to multiple drug resistance mechanisms associated with cancer, including **a)** mutational and gene expression heterogeneity of different subpopulations in each tumor, which can reduce the same responsiveness of the tumor cells to a single target drug (4). **b)** Mutations in the target of administered drugs: These mutations can reduce the binding affinity of the drug to the mutated target. An example is the BRAF V600E mutant gene which has been shown in lung adenocarcinoma and makes BRAF resistant to the designed inhibitor, Vemurafenib (5). Another mechanism is the continued activation of downstream proteins of the blocked target in a signaling pathway, several studies have demonstrated the resistance of cancer cells to EGFR inhibitors due to PIK3CA mutations, which is a protein downstream of the signaling pathway, and could cause activation of the signaling pathway despite the blocked target in the upstream of the pathway leading to cancer growth (6). **c)** Cancer cells evolve alternative and parallel signaling pathways to compensate for the blocked survival pathway (7). The data illustrates the importance of using multi-target strategies to inhibit multiple molecular mechanisms in cancer progression. Nowadays, multi-drug therapy is being proposed as an alternative approach for the treatment of complex diseases (8).

Evidence shows that the prescription of multiple drugs to inhibit multiple targets can enhance therapeutic efficiency and reduce the chance of resistance in the illness, resulting in the suppression of more types of resistance mechanisms (9,10). However, it is worth clarifying how to identify the best drug combination with the most efficient concentration of each constituent to maximize the success rate of treatment with the least side effects.

Various parts of many plants have been demonstrated to have anti-cancer properties for many types of cancer so far. Therefore, the natural compound content of these plants is supposed to be an effective source for cancer treatment (11). In this regard, the anti-cancer activity of several natural compounds has been verified by *in vitro*, *in vivo*, and in silico studies. Thanks to advances in bioinformatics tools and molecular biology knowledge, herbal medicine has been identified as a potential multi-target treatment due to the wide range of interactions between their phytochemicals and different target proteins (12). The wide range of natural compounds found in each plant species can provide multiple targeting potencies of medicinal plants, which can exert synergistic therapeutic effects (12). The synergistic effect arises when each individual factor increases the effect of the other factors. This leads to the observation of a total effect that is greater than what was predicted (13). Many experimental studies confirm the additive effects of the phytochemical composition in plants and fruits for cancer treatment, compared to the individual effects of a single compound (14, 15). For example, the combination of apple extract and 3-β-D-glucoside synergistically reduced EC_{50} of MCF-7 cell proliferation to 2 and 4-fold lower than apple extracts and 3-β-D-glucoside alone, respectively (16). In addition, a high-throughput screening of natural compounds has shown that the combination of curcumin and urosolic acid could reduce tumor volume more significantly than each compound individually (17).

2.Objectives

Generally, in silico virtual screening of natural compounds plays a crucial role in biotechnology by efficiently screening large libraries of natural product databases. It enables the rapid exploration of chemical space, leading to the discovery of bioactive compounds with potential applications in drug development, agriculture, and industry. This approach aids in the development of targeted therapies by selectively identifying compounds that can inhibit disease-related pathways, particularly in areas such as cancer treatment. By optimizing compound selection, virtual screening saves time and resources, bridging traditional knowledge with computational techniques and fostering sustainable and nature-inspired solutions. In summary, in silico virtual screening of natural compounds accelerates the discovery of bioactive molecules, fueling innovation in biotechnology.

Specifically, in this study, we aim to provide a methodology for identifying medicinal plants containing effective natural compounds with the most possible synergistic effects to inhibit cancer survival and proliferation in a multi-target manner. To achieve this, many computational tools have enabled us to explore thousands of natural compounds in order to find selective potent inhibitors for desired multiple targets. In our study, we have focused on cancer signaling pathways to identify multiple targets. This approach has previously been confirmed as a potential alternative way for cancer treatment (18).

3. Materials and Methods

3.1. Target Identification

The associated signaling pathways in cancer were obtained from the KEGG database (KEGG:hsa05200) (19). The pathways included several canonical signaling pathways involved in the proliferation, survival, and malignancy of cancer cells. Each signaling pathway was completed using information from the individual pathway in the KEGG database. In each pathway, some genes exerted an induction effect for proliferation, survival, and inhibition of apoptosis, while others had an inhibitory outcome for cancer progress mechanisms. Since the aim was to implement an antagonist effect of natural compounds to inhibit cancer progression, only the inducing genes in each pathway were selected as targets. Therefore, based on the information in each pathway, the suppressor genes were deleted.

Using the online database STRING v11.5 (20), protein-protein interactions of all signaling pathways genes were obtained and the results were analysed using Cytoscape software v3.9 (21). The hub proteins were defined using the degree calculation of each node. Finally, proteins with the highest degree and defined structures in the PDB database (www.pdb.org) were selected as targets.Transcription factors were not selected due to their reported undrugability (22).

3.2. Receptor Preparation

Since our goal is to use inhibitor binding sites of each target for local docking, the complex structure of sleced targets with previous inhibitors was retrieved from the RCSB Protein Data Bank (www.pdb.org) with the *.pdb format. The complexed ligands, water molecules, and cofactors were removed, and the structures were minimized energetically, using MGLTools v1.5.7 (http://mgltools.scripps.edu/), also hydrogen atoms were added. Before removing ligands, we identified specific amino acid residues that had interacted with the ligands and selected them to be included in the grid box region.

3.3. Ligand Library Selection

Ligands used for this study were filtered out from natural compounds in the NPASS (v1.0) database (http://bidd.group/NPASS/). NPASS database contains 35032 natural compounds isolated from 25041 organisms with reported activity records on more than 5000 targets. Our aim is to create a library of the most potent anti-cancer natural compounds. To achieve this, natural compounds with an IC_{50} of less than 20,000 nm against 50 cancer cell lines, which were reported more than two times, were isolated. The structures of the selected compounds were downloaded in SMILES format and converted to a single list of SDF files for virtual screening. Finally, all structures were converted to *.pdbqt format and energetically minimized in the PyRx 0.8 virtual screening tool (23).

3.4. Docking

For the purpose of binding energy calculation between targets and the created library, we implemented molecular docking to predict the binding affinity of the compound's library against selected targets. The PDB structures of selected targets were converted to pdbqt in PyRx software. The grid box is positioned within the protein region that had previously served as a binding site for other compounds. Therefore, we adjusted the grid box to cover the binding site of the removed ligand and its surrounding residues. Local docking of the created library containing 1107 natural compounds to 14 target proteins was performed using AutoDock Vina in PyRx with the exhaustiveness equal to 8 $kCal.moL^{-1}$.

3.5. Off target - Side Effect Analysis

We aimed to reduce the possible off-target interactions of our natural compounds library with unwanted proteins which could lead to side effects. Based on the literature, five proteins that have the potential to interact with drugs off-target were selected as the potential proteins for off-target drug interactions, as they are mainly involved in metabolic pathways (24). After the PDB structures of these proteins were downloaded, water and cofactors were deleted, and they were converted to *. pdbqt format in PyRx software.

The residues surrounding the previous inhibitors in their PDB structures were selected to adjust the grid box in local docking. The created natural compounds library was then docked to all 5 proteins using AutoDock Vina in PyRx.

3.6. Plant Identification

Based on the off-target docking results, compounds with an average energy binding of less than -7 for all five targets were removed from the created natural compounds library. Subsequently, the compounds with an energy binding lower than -8 for each of the 14 targets were isolated from the remaining compounds. Eventually, the plants containing each selected compound were identified from the NPASS database, and the plants that contained the most selected compounds were introduced. The overall workflow of this study is shown in **Figure 1**.

4. Results

4.1. Target Identification

The cancer signaling pathways were retrieved from the KEGG database, and they were composed of 325 individual genes. Based on detailed information on each specific pathway, 58 genes from the signaling pathways were added to complete the list of genes involved in cancer signaling pathways, and 46 genes were removed due to their inhibitory effects on cancer progression.

Protein-protein interaction data for proteins involved in cancer signaling pathways is detailed in **Table S1.** Degree calculations of the nodes demonstrated a range from 3 to 232, with 45 proteins having a degree higher than 100. Eventually, after applying four factors, including high degree, inside the cell activity, not being a transcription factor, and PDB structure existence, 14 proteins, including AKT1, EGFR, CTNNB1, SRC, MAPK1, CCND1, PIK3CA, STAT3, RHOA, JAK2, MMP9, NFKB1, IKBKB, and BRAF, were selected as targets (**Table 1**). It has also been considered that the selected targets belong to different canonical signaling pathways to inhibit all possible molecular mechanisms in cancer.

The crystal structures of selected targets with PDB-ID: 3mvh, 6duk, 7afw, 2bdj, 5nhj, 2w9z, 3hhm, 6nuq, 1cc0, 6bbv, 1gkc, 1svc, 4kik, and 3tv6 were used for further analysis. For each structure, the amino acid residues surrounding the active site that had previously interacted with another inhibitor were selected as the local region for docking.

Table 1. Selected targets based on calculated PPI degree and their signaling pathways involve in cancer.

Selected targets	PDB code	PPI Degree	Molecular mechanism in cancer
AKT1	3mvh	232	PI3K signaling pathway
EGFR	6duk	216	ERK , PI3K signaling pathway
CTNNB1	7afw	173	WNT signaling pathway
SRC	2bdj	166	Multiple signaling pathway
MAPK1	5nhj	161	ERK signaling pathway
CCND1	2w9z	157	Cell cycle
PIK3CA	3hhm	152	PI3K signaling pathway
STAT3	6nuq	150	JAK-STATE signaling pathway
RHOA	1cc0	127	Invasion
JAK2	6bbv	110	JAK-STATE signaling pathway
MMP9	1gkc	105	Invasion
NFKB1	1svc	83	NF-kappa signaling pathway
IKBKB	4kik	52	NF-kappa signaling pathway
BRAF	3tv6	45	ERK signaling pathway

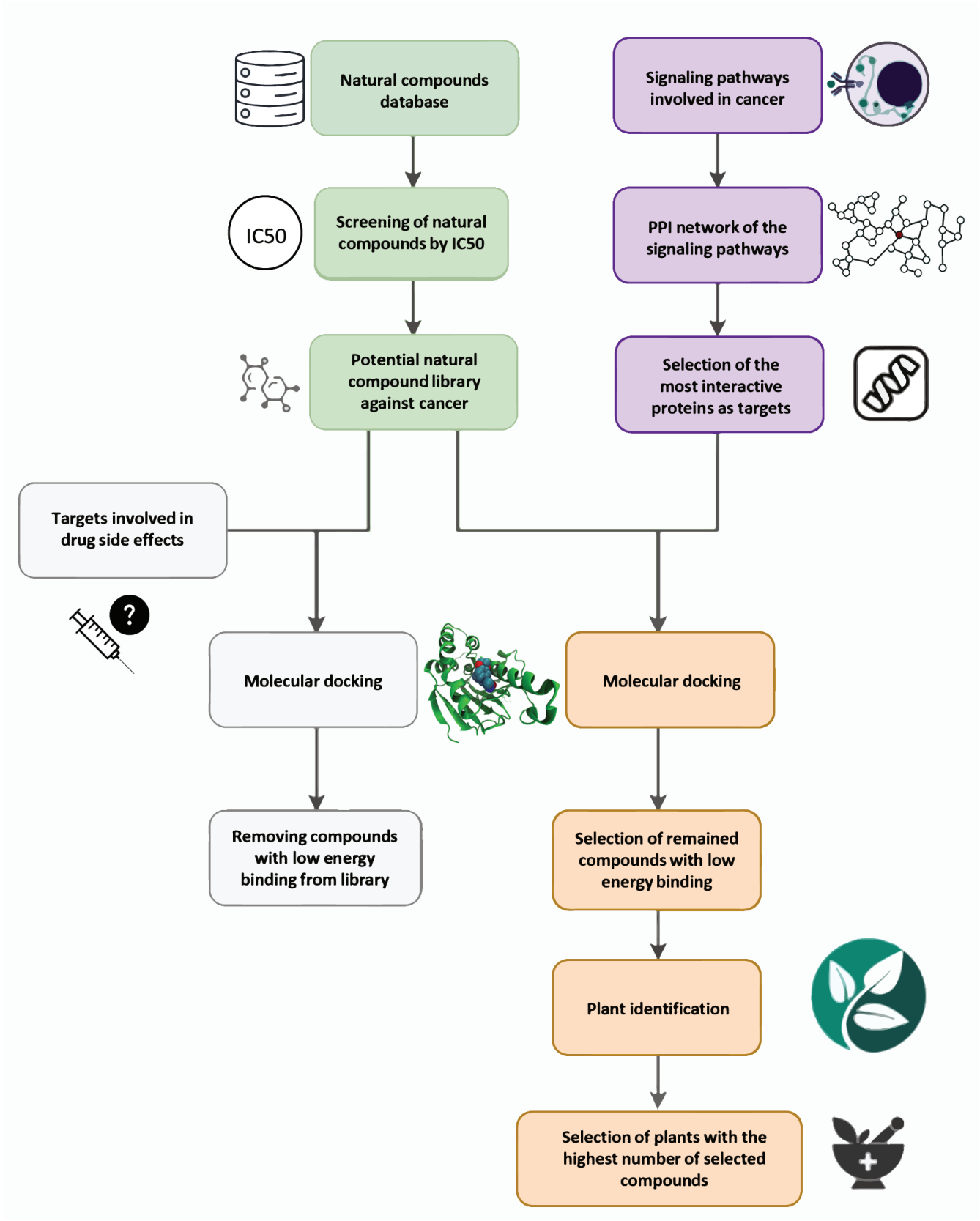

Figure 1. A pipeline workflow diagram describing the steps involved in selecting anti-cancer plants with compounds that have synergistic therapeutic effects.

4.2. Virtual Screening

A total of 1,107 out of 35,032 natural compounds from the NPASS database were used for docking-based virtual screening.

The compounds were selected based on their IC_{50} values (<20,000 nM) in the 50 tested cancer cell lines. The selected cancer cell lines for IC_{50} investigation of natural compounds are shown in **Table S2.** These compounds were previously reported as potent anticancer agents in experimental results (**Table S3** lists these compounds). **Table S4** reports the molecular docking results of the created natural compounds library to 14 target proteins. The average binding energy of the compound library for the 14 targets ranges from -15.34 to 80.18 $kCal.moL^{-1}$.

4.3. Off Target-Side Effect Analysis and Compound Selection

The molecular docking results of the proteins involved in drug-side effect with the library of selected natural compounds revealed that 283, 338, 266, 407, and 397 compounds had an energy binding lower than -7 $kCal.moL^{-1}$ and RMSD=0 for proteins Alcohol dehydrogenase, HGPRT, Carbonic anhydrase, Phenylalanine hydroxylase, and Pyruvate kinase, respectively **(Table S5**). To prevent side effects and unwanted interactions, a total of 794 compounds with an average energy binding lower than -7 $kCal.moL^{-1}$ were removed from the library. After removing compounds with low energy binding to proteins involved in the side effect, the remaining ligands with a score lower than -8 $kCal.moL^{-1}$ and zero RMSD for all 14 individual targets were selected. A total of 166 compounds were selected based on their docking score of less than -8 $kCal.moL^{-1}$ for at least one target. **Table S6** displays the compounds with energy binding less than -8 $kCal.moL^{-1}$ for each target and shows that some selected compounds can inhibit more than one target, which is an advantage that brings us closer to the goal of multi-targets.

4.4. Plant Identification

Table S7 displays the scientific names of the selected plants, the natural compounds each plant contains from the selected compounds in the previous section, and the targets expected to be inhibited by each compound based on docking results. These plants were selected because they contain the greatest number of our selected compounds. The corresponding selected compounds with their docking energy are listed in **Table 2**.

5. Discussion

Multi-target approach in the treatment of complex diseases, such as cancer, has been investigated in many studies (25). Given that numerous molecular mechanisms are involved in tumor development, multi-target approaches have the potential to suppress cancer in a broader range, leading to a higher possibility of successful treatment (26). In addition to the molecular mechanisms implicated in cancer, signaling pathways have upstream induction effects in the regulation of cancer mechanisms, such as growth, invasion, survival, and drug resistance. Therefore, we focused on finding multiple targets in the signaling pathways.

The inhibition of targets with high levels of interaction with other proteins could induce systematic inhibitory effects on all associated mechanisms, subsequently leading to a highly potent anti-cancer outcome. Hence, we prioritized the final selection based on the protein-protein interaction degree obtained from the STRING database; however, high degree proteins such as KRAS and MYC, which have been considered undruggable due to their structures, were not selected. Eleven out of the 14 selected targets play critical roles in the main signaling pathways, including PI3K, ERK, WNT, and NF-kappa, while the remaining selected targets, including CCND1, RHOA, and MMP9 are key regulators of invasion and cell division (**Table 1**)

As demonstrated in the KEGG signaling pathway, ERK signaling pathway is associated with multiple receptors like EGFR which are overexpressed in cancer (27). Therefore, targeting of downstream signaling pathways also could suppress their function in cancer. With the aim of virtual screening, we are able to filter out the vast majority of natural compounds and identify the most promising ones for further investigation in wet labs. This can significantly reduce the cost and time required to discover potential drugs. Since each type of cancer is involved in different molecular mechanisms (28), In our initial screening, we filtered out natural compounds based on their reported IC_{50} on 50 different cancer cell lines in the NPASS database. Therefore, we created a library with the most promising natural compounds for their potential against cancer cells.

Out of the 1107 natural compounds, 503 had a binding score lower than -7 $kCal.moL^{-1}$ on average for all targets, as shown in **(Table S4)**. These results indicate that our initial screening, which was based on IC_{50},

Table 2. List of selected compounds and their corresponding docking energy to each of our 14 selected targets.

Compound id	RHOA (1cc0)	MMP9 (1gkc)	NFKB1 (1svc)	SRC (2bdj)	CCND1 (2w9z)	PIK3CA (3hhm)	AKT1 (3mvh)	BRAF (3tv6)	IKBKB (4kik)	MAPK1(5nhj)	EGFR (6duk)	STAT3 (6nuq)	JAK2 (6bbv)	CTNNB1 (7afw)	Avg
NPC129372	-7.3	-7.1	-6.5	-8.1	-6.2	-5	-2.5	-9.2	-6.9	-7.8	-5.6	-6.6	-6.4	-2.5	-6.26429
NPC136816	-7.6	-7.1	-6.2	-7.1	-7.1	-5.3	-2.5	-7.3	-7.7	-8.7	-6	-5.6	-5.4	1.1	-5.89286
NPC140446	-7.1	-7.8	-6.1	-7.1	-7.7	-6.9	-5.9	-9	-7.8	-8.4	-5.8	-5.3	-6.8	-2.1	-6.7
NPC472896	-7.4	-7.2	-6.9	-7.8	-7.2	-6.5	-5.9	-8.7	-3.4	-8.4	-4.9	-6.3	-1.7	17.3	-4.64286
NPC472897	-8.1	-6.9	-7.2	-7.7	-6.7	-6.5	-2.8	-8	-1.9	-8.7	-6.1	-6.5	-5.7	2.6	-5.72857
NPC472898	-6.6	-7	-6.2	-7.3	-6.3	-5.4	1.1	-7.2	-6.4	-8.7	-6	-5.2	-3.1	7.1	-4.8
NPC472899	-6.6	-6.6	-6.1	-7.3	-6.3	-4.8	1.8	-7.5	-4.4	-8.7	-5.5	-5.2	-3.1	5.9	-4.6
NPC472900	-7.6	-7.4	-6.6	-7.7	-6.5	-6.2	3.1	-8.6	-7.1	-7.9	-4.4	-5.7	-4.5	-3.3	-5.74286
NPC472987	-7.5	-7.7	-6.6	-8	-6.7	-4.6	-2	-8.7	3.2	-8.3	0.1	-6	1.1	10.1	-3.68571
NPC116862	-7.3	-7.4	-6	-9.8	-8.3	-2.8	10.7	-10.7	0.3	-9	-3.1	-5.6	-2.4	20.8	-2.9
NPC208553	-7.5	-7.5	-5.8	-9.3	-6.7	-5.2	-1.1	-9.6	-2.7	-8.3	-4.3	-5.3	-7.5	9.1	-5.12143
NPC260194	-5.9	-6.7	-5.1	-7.7	-5.8	-7.2	-1.7	-9.2	-6.7	-7.8	-6.7	-6	-5.9	0.3	-5.86429
NPC300827	-6.2	-6.2	-5.9	-7.3	-6.9	-7.3	-4.8	-8.6	-7.8	-8.1	-2.1	-6.4	-6.9	-0.9	-6.1
NPC471623	-7.4	-5.8	-6.2	-7.9	-6.8	-8	-0.4	-11	-2	-8.8	-6	-6.1	-7.4	1.6	-5.87143
NPC472375	-6.6	-6.8	-5.8	-7.1	-7.1	-6.2	10.5	-9.4	-3.6	-7.4	-6.3	-5.7	-4.3	19.3	-3.32143
NPC472391	-7.5	-7	-6.4	-7.1	-7.3	-4.8	-0.6	-9.7	-1.5	-8.8	-2.8	-4.6	0.4	18.8	-3.49286
NPC472392	-7.7	-6.5	-6	-9.1	-7.3	-4.4	2	-9.4	-0.6	-9.3	-0.5	-5	-5.6	17.6	-3.7
NPC472393	-6.9	-6.6	-5.4	-7.7	-7	-5.5	1.5	-8.5	-1.4	-7.7	-3.9	-4.4	-4.6	0.9	-4.8
NPC67246	-7.5	-7.4	-5.7	-8.1	-6.9	-6.5	-0.3	-8.3	-4	-9.4	-6.4	-6.1	-5.1	5.3	-5.45714
NPC293136	-6.3	-6.8	-5.4	-7.1	-4.8	-6.9	-7.5	-7.7	-5.8	-7.2	-8.8	-5.6	-6.9	-3.2	-6.42857
NPC329615	-5	-6.8	-5.5	-7.3	-5.7	-6.5	-6.9	-7.8	-6.4	-7.1	-8.3	-5	-6.2	-3.4	-6.27857
NPC329829	-5.7	-4.9	-4.2	-6.4	-4.7	-6.4	-7.2	-8	-6.5	-7.3	-7.9	-5.1	-6.2	-3.4	-5.99286
NPC329838	-5.7	-5.4	-4.4	-6.6	-5.6	-6.6	-6.9	-7.1	-5.8	-6.4	-8.4	-4.5	-6.3	-3.3	-5.92857
NPC473478	-4.9	-6.2	-5.4	-6.3	-4.6	-6.4	-6.6	-7.3	-6	-7.1	-8	-5.5	-6.7	-3.8	-6.05714
NPC473780	-5.9	-5.7	-5.6	-6.9	-4	-6.2	-7	-7.4	-6.4	-7.1	-8.3	-4.7	-7.4	-3.7	-6.16429
NPC218510	-6.7	-6.4	-5.7	-7.3	-5.8	-6.3	-4.2	-8.4	-7	-7	-6.6	-5.6	-7.4	-5.1	-6.39286
NPC36531	-6.1	-6.5	-5.9	-7.5	-5.9	-5.9	-7.1	-8.3	-6.9	-7	-6.4	-5.6	-7.9	-4.7	-6.55
NPC471154	-6.3	-6.7	-5.4	-7.1	-6.3	-6.3	-5.5	-7.7	-6.5	-6.9	-7	-6.1	-8	-4.4	-6.44286
NPC471181	-6.2	-6.8	-6	-7.3	-5.9	-5.7	-7.2	-8.2	-7.9	-7.2	-7.3	-5.4	-8.2	-4.8	-6.72143
NPC471183	-5.9	-6.1	-5.2	-6.2	-5.7	-5.8	-3.5	-8.7	-7.6	-7.1	-7.2	-5.7	-7.3	-3.9	-6.13571
NPC476879	-7	-6.7	-6.2	-7.4	-6.6	-5.1	-3	-7.5	-5.4	-8.3	-4.3	-6.7	-3.8	1.9	-5.43571
NPC476880	-7.4	-7.6	-7.1	-8.5	-7.2	-5.2	1.4	-9.1	-4.7	-9.3	-2.2	-6.6	-0.8	12.5	-4.41429
NPC476881	-7.3	-8.2	-6.7	-8.3	-7.1	-5.6	-1.7	-10.4	-5.6	-8.4	-5.4	-6.2	-2.6	11.4	-5.15
NPC476882	-7.6	-7.1	-6.5	-7.6	-6.8	-3.8	-2.2	-9.7	-2.2	-8.3	-6.4	-5.8	-2.3	19.2	-4.07857
NPC476883	-7.1	-8	-6	-7.4	-7.1	-4.8	-4.1	-9.4	-4.9	-8.3	-3.7	-6.2	-4.6	7.4	-5.3
NPC476884	-7.6	-7.2	-6.1	-7.6	-6.5	-3.2	7.8	-8.6	-1.4	-8.6	-8.7	-4.2	-4.7	35.1	-2.25
NPC476885	-7.9	-7.4	-6.2	-7.4	-6.5	-5.2	-0.4	-8.4	-3.8	-8.5	-5.8	-6.4	-3.5	12	-4.67143
NPC476886	-7.6	-6.9	-7.4	-8.3	-6.6	-5.3	3.4	-9.2	-3.8	-8.8	-2.3	-6	-2.8	7.1	-4.60714
NPC476889	-7	-7.3	-6.4	-7.8	-7.6	-5.2	2.9	-8.3	-4.7	-8.9	-5.4	-7.6	-3.3	-2.1	-5.62143
NPC253201	-6.9	-6.5	-6.1	-7.9	-6.6	-5.5	-1.9	-7.7	-7.8	-8.1	-5.1	-5.9	-3.3	-1.8	-5.79286
NPC307781	-7.8	-7.6	-5.8	-8.4	-6.5	-6.3	-6.6	-8.8	-7	-8.9	-5.1	-6.6	-4.7	-5.4	-6.82143
NPC472668	-7.1	-6.9	-6.3	-8.1	-6.9	-4	-5.2	-8.4	-7.2	-7.8	-6.8	-6.3	-6.9	-5.6	-6.67857
NPC472669	-6.6	-7	-6.3	-7.9	-6	-6.1	2.6	-7.7	-5.8	-8	-3.9	-6.1	-6	-1.2	-5.42857
NPC472673	-7.5	-7.3	-6.1	-8.1	-6.6	-5.7	-1.3	-8.3	-6.4	-8.1	-7.4	-6.4	-4.5	-2.9	-6.18571

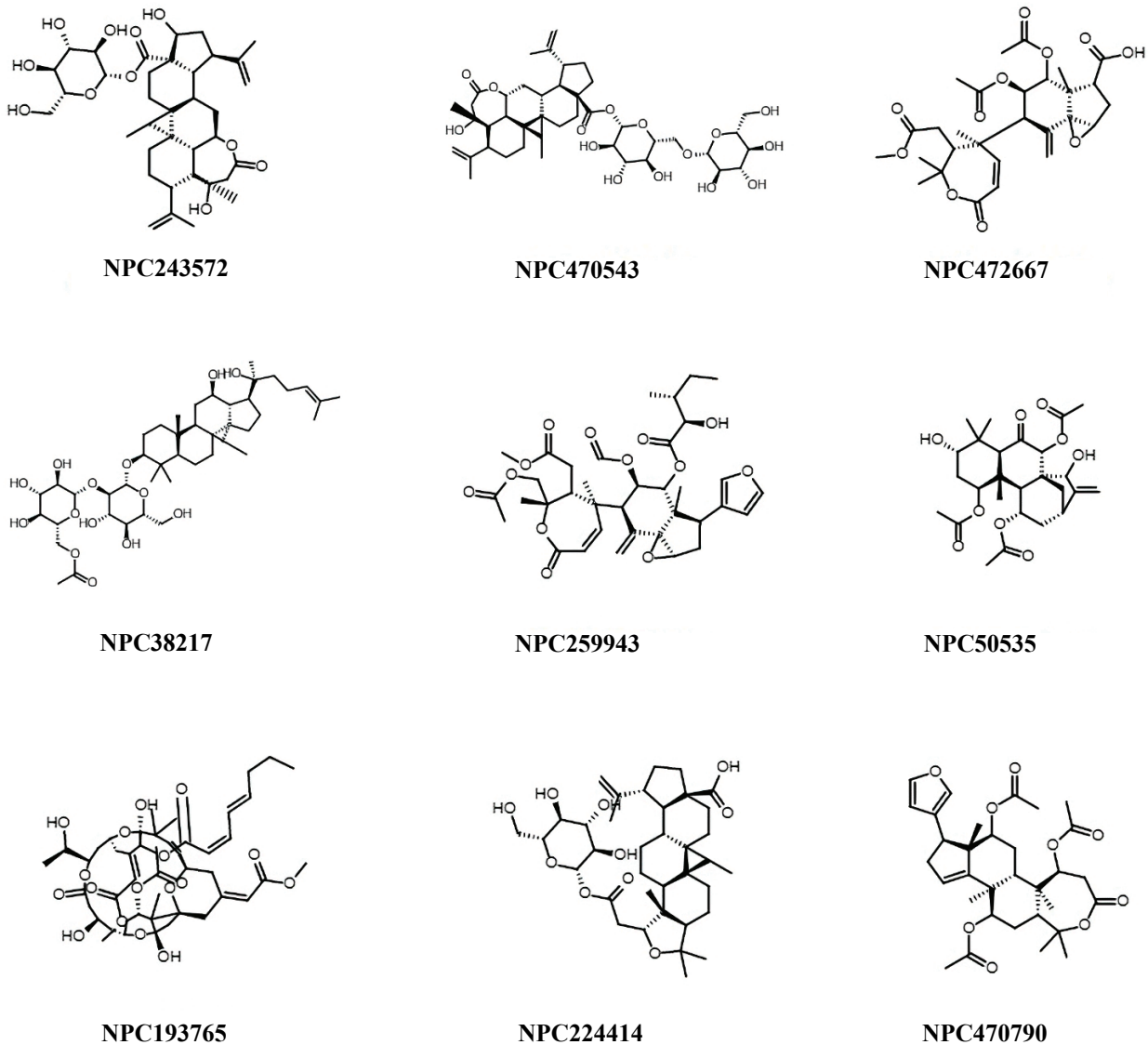

Figure 2. Structures of some natural compounds from created library with lowest binding scores in docking to the most of the targets. These compounds possess many functional groups capable of forming hydrogen bonds with different targets.

generally isolated potentially active compounds. The binding site of each protein significantly influenced the range of docking scores of the compounds (29). In the docking results of this study, the target structure 5nhj had 497 compounds with a binding score lower than -7 kCal.moL^{-1}, whereas the target structure 7afw had only 6 compounds with a binding score lower than -7 kCal. moL^{-1}. There is a scoring bias in the docking algorithm due to the protein pockets; in pockets with a larger contact area than others, the algorithm tends to give lower scores (30).

Alcohol dehydrogenase, Hypoxanthine-guanine phosphoribosyl transferase (HGPRT), Carbonic anhydrase, Phenylalanine hydroxylase, and Pyruvate kinase were selected for side effect analysis, as they are generally involved in metabolic pathways. According to studies, these proteins usually play an important role in undesirable interactions with drug compounds and can contribute to inducing side effects (24).

During our analysis, we observed that some compounds

had very low negative scores with both the main and side effect targets. As this pattern was observed across several compounds and targets, it suggests a possible bias in the docking algorithm that exaggeratedly scores these compounds. This bias could lead to false positives in molecular docking and interfere with the identification of true targets. To address this issue, it is necessary to utilize normalization computational methods for docking results (31), which is not the subject of our study. Additionally, further optimization of AutoDock Vina is necessary to improve the accuracy of docking. The structure of the compounds is another factor that may have influenced the scoring in docking. As shown in **Figure 2**, many functional groups in these compounds can form multiple hydrogen bonds, which may lead to non-specific interactions with targets, potentially explaining the extremely low docking scores. Another possible reason for this could be due to the initial selection of compounds based on the lowest IC_{50}. The compounds in the library seem to have a high level of activity, which can increase the possibility of inducing side effects. Therefore, it is recommended to remove compounds with the potential for side effects from the library as much as possible. Based on **Table S5**, many of these compounds had very low scores with proteins involved in side effects, and were therefore removed from the library. This strategy can help to reduce the probability of non-specific interactions, although the docking results do not conclusively clarify this.

Table S7 indicates that out of the 14 selected targets, 9 could be inhibited by natural compounds found in 6 selected plants, while the remaining 5 targets (CTNNB1, STAT3, NFKB1, IKBKB, and AKT1) could not be inhibited by any of these compounds. This may be attributed to differences in the drugability of the targets, as some targets yielded more promising docking results than others. In this study, plant selection was based on the highest number of potential compounds in each plant, although this selection could be in other ways, as well as they would be specifically considered to inhibit most possible targets based on docking results.

We have introduced six plants which contained 43 of our selected compounds. The anticancer activity of these natural compounds has been previously reported in the literature. Specifically, the anticancer properties of Ginsenoside have been demonstrated *in vitro* on two cancer cell lines. Subsequent investigations revealed that its anti-cancer activity is attributed to the inhibition of the PIK3/AKT signaling pathway (32). *In vitro* studies of squadiolin A and B on various cancer cell lines, including MDA-MB-231, MCF7, A549, and HepG2, confirmed their anti-cancer properties, with IC_{50} values below 5 micromolar, affirming our initial hypothesis regarding the anticancer potential of these compounds (33). Cephalomannine, another compound of interest, has demonstrated inhibitory effects on hypoxic lung cancer cells by interfering with the APEX1/HIF-1α interaction (34). In a study by Shamsee *et al*., Icterogenin was isolated from the leaves of Lantana Camera, and its impact on MCF-7 cell viability and proliferation was assessed using the MTT test. The results indicated a dose-dependent reduction in cell viability with an IC_{50} value of 180.6 $\mu g.mL^{-1}$ (35). Paclitaxel is a widely recognized anti-cancer compound known for its microtubule-stabilizing properties as anti-tumor agents. However, recent research has revealed that paclitaxel inhibits the proliferation of breast cancer cells by targeting the PI3K/AKT signaling pathway. This dual mechanism of action highlights the potential for a synergistic response by targeting different molecular pathways in cancer cells (36).

In addition, Previous studies have confirmed the potential anticancer activities of introduced six plants in various cancer cell lines. Moreover, these plants are commonly used as traditional herbal medicines to treat numerous diseases, including cancer, in indigenous cultures worldwide. Molecular techniques have confirmed the anti-cancer properties of these plants, which include promoting apoptosis and inhibiting proliferation (37-42). **Table 3** provides information on the country of origin of each plant, as well as the organs that are predominantly used for medicinal purposes.

It is worth considering that medicinal plants contain several compounds with a variety of beneficial bioactivities (43). Therefore, in addition to the effective compounds confirmed in our study, the introduced plants may possess other compounds that synergistically enhance their anticancer properties or potentially induce other side effects; Consequently, a separate study should be conducted in this regard. Moreover, it is essential to determine the concentrations of the desired compounds in the selected plants. Geographical factors and different subspecies of the plant can affect the concentration of the desired compounds in plant tissues, which could be important in the assessment of their effective doses for medicinal use (44).

Table 3. List of selected plants with their specifications.

Plant name	Plant organs mainly used for medicinal purposes	Habitat	Ref
Taxus mairei	leaves and twigs	China	(45)
Panax notoginseng	leaves, root, rhyzom, flower and berry	China	(46)
Combretum sundaicum	leaves and flowers	South America, Africa, and Southeast Asia	(38)
Annona squamosal	leaves, bark, fruit and stem	tropics and subtropics of Asia	(39)
Schisandra neglecta	stem	southwest China	(47)
Munronia henryi	Leaves, roots	tropics and subtropics of Asia	(41,48)

Moreover, as the validation for this study, since we believe the purpose of the study is systematic inhibition of cancer signaling pathways in cancer, we could not implement conventional validation methods like MD simulation and in-vitro binding affinity tests, as these methods rely on validation of each individual inhibitory interaction of compounds. To be more specific, we believe determination of systematic and synergistic inhibition of multiple compounds can be revealed accurately using systematic methods like implementing of the agents on signaling pathways of the cells with comprehensive *in vitro* assays, hence, in the current study we have introduced screened natural compounds as the potent compounds for synergistic inhibition of the proteins involved in cancer signaling pathways, the final validation of this purpose will be met with the comprehensive *in vitro* assays of pre-selected natural compounds with docking on different cancer cell lines.

Overall, our aim was to provide an in-silico method for the pre-selection and screening of natural compounds with a high chance of synergistic response in inhibiting cancer signaling pathways. We believe that multiple targeting of key proteins in cancer mechanisms at the same time could lead to this suppression response. However, further validation methods are necessary to confirm and ultimately select the introduced natural compounds/plants.

6. Conclusions

In summary, we provided a methodology to screen any natural compound database to identify a combination of potential plants/natural compounds with synergistic inhibitory effects on cancer cell mechanisms. In addition, the docking-based screened data can be used for further analysis and validation using experimental or molecular dynamics simulation methods.

Furthermore, the method used in this study could reveal the anti-cancer activity of the less studied plants if they contain compounds with reported low IC_{50} against cancer cell lines.

Acknowledgements

The computational analysis was performed at the Laboratory of Bioinformatics and Drug Design (LBD), Institute of Biochemistry and Biophysics (IBB), University of Tehran.

Disclosure statement

All of the authors declare no competing interest.

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