Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Network Pharmacology Analysis with Molecular Docking of Phytochemicals of *Panax ginseng* against Osteosarcoma

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Submitted: 09-Nov-2021 Revised: 19-Nov-2021 Accepted: 24-Dec-2021 Published: 28-Mar-2022

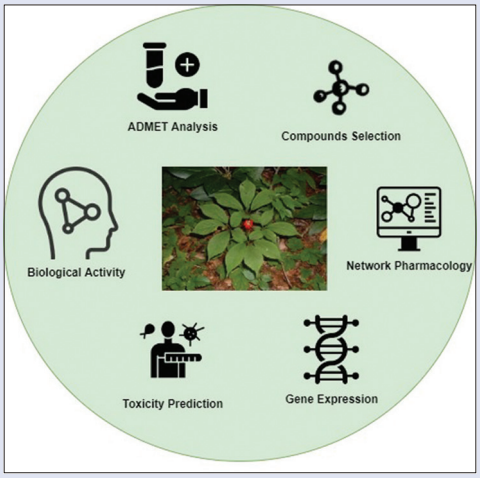
ABSTRACT

Background: *Panax ginseng* is a perennial medicinal herb also known as Korean ginseng or Insam (인삼), commonly found in Korean Peninsula. These herbs are used to treat different types of diseases. Recent studies have shown that phytochemicals found in *P. ginseng* harbor anticancer activity against various cancers. However, the biological and molecular mechanisms of these phytochemicals are still unknown in osteosarcoma (OS). **Materials and Methods:** This study utilized the network pharmacology method comprised target prediction, gene enrichment and ontology, KEGG pathway analysis, and gene expression studies. The obtained results were used to predict the interaction of phytochemicals with the human CDKL3 domain implicated in OS using molecular docking. Toxicity and pharmacokinetic elements of these phytochemicals were also identified. **Results:** Results showed that Fumarine and Inermin are bioactive phytochemicals that have a multimodal effect on multiple targets and pathways involved in the progression of OS. These compounds were able to regulate the expression of genes and interacted with human CDKL3. These compounds have good pharmacokinetic and toxicological characteristics. However, they exert a high risk of hepatotoxicity. **Conclusion:** The present study provided a predicted mechanism of action of bioactive phytochemicals of *P. ginseng* in the inhibition of OS.

Key words: Fumarine, inermin, molecular docking, network pharmacology, osteosarcoma, *Panax ginseng*, pharmacokinetics, toxicity

SUMMARY

- The phytochemicals contained in the P. *ginseng* has been explored in the treatment of osteosarcoma (OS) using Network Pharmacology method
- Drug likeness and pharmacokinetic screening identified six potential medicinal compounds such as Fumarine, Inermin, Frutinone A, Celabenzine, Nepetin and Suchilactone
- Fumarine and Inermin showed moderate anti-OS activity identified by Network Pharmacological analysis
- Molecular docking validation was reconfirmed that Fumarine and Inermin are potential candidate for the treatment of OS.



Abbreviations used: ADMET: absorption, distribution, metabolism, excretion and toxicity, BATMAN-TCM: Bioinformatics Analysis Tool for Molecular mechanism of Traditional Chinese Medicine, BBB: Blood Brain Barrier, CDKL3:Cyclin dependent kinase like 3, KEGG: Kyoto Encyclopedia of Genes and Genomes, OS: Osteosarcoma, PASS: Prediction of activity spectra for biologically active substances, P. ginseng: Panax ginseng, TCMSP: Traditional Chinese Medicine System Pharmacology Database, TTD: Therapeutic Target Database.

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DOI: 10.4103/pm.pm_518_21

Access this article online
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INTRODUCTION

Osteosarcoma (OS) is a primary skeletal tumor in which aberration in the bone-forming mesenchymal cells causing the formation of an immature osteoid matrix.[1] This type of tumor has a high malignancy rate and usually originates in soft tissues. An approximate incidence of OS reported around ~4 million cases per year in adults, whereas ~5 million cases per year in children (0–19 years).[2] The disease etiology is still elusive yet; however, some studies indicated that exposure to radiation initiates OS formation reported in 2% of cases. However, it has been largely attributed to germline mutation in p53 protein.[3]

OS is characterized as surface and central bone tumors classified by the World Health Organization system of histological classification.[3] Almost 90% of OS cases are diagnosed as central tumors. The musculoskeletal tumor society has devised a staging system (I-III) to observe and characterize the tumor progression.[4] Stage I and II are low-grade tumors, whereas Stage III are highly malignant and vulnerable to metastasis.[4]

Cite this article as: Shah FH, Kim SJ. Network pharmacology analysis with molecular docking of phytochemicals of *Panax ginseng* against osteosarcoma. Phcog Mag 2022;18:175-82.

The therapy for OS is comprised of surgery, in which amputation of the affected limb is performed combined with post-therapy chemotherapy. However, in high-grade tumors, the treatment fails to avert the proliferation and expansion of OS.[5] Another arising issue with this tumor is the development of resistance to chemotherapy which makes it more challenging to avert the recurrence and metastasis.[6-8] That is due to the involvement of multiple protein factors facilitating such processes.[8] So far, no targeted treatments have been discovered for this disease to prolong the survival rate. The discovery of new therapeutic molecules able to perform multiple biological activities, including anticancer with anti-metastatic properties is of great concern to be able to deter cancer growth and development. Network pharmacology is defined as an integration of system biology, network analysis, and *in silico* drug discovery methods to determine the multiple pharmacological activities of a compound against various diseases.[9,10] This method combined with medicinal phytochemicals can be beneficial to discover therapeutic with biological activity against multiple targets in the studied disease.

In our study, we used the network pharmacology method to explore the phytochemicals of *Panax ginseng* against OS. *P. ginseng* is an ethnobotanical plant of the Korean Peninsula and China that belongs to the family of Araliaceae, Genus: Panax L, and Species: *P. ginseng* Meyer.[11] Traditionally, the dried form of the plant, especially the roots, has been used to treat several diseases, including cancer and inflammation.[12] Recently, these plants have been utilized to extract various bioactive compounds whose biological activity is yet to be determined. The present study obtained all these compounds contained in *P. ginseng* and explored the activity against OS using network pharmacology combined with *in silico* interaction studies.

MATERIALS AND METHODS

Selection of bioactive compounds in *Panax ginseng*

The current study utilized *P. ginseng* to analyze its therapeutic effect on OS. We have accessed the traditional Chinese medicine (TCM) system pharmacology database,[13] to acquire bioactive compounds from this plant. In the search box, *P. ginseng* was searched as a keyword, and compounds information related to this plant was downloaded in the excel file. The compounds were selected based on drug-likeness (DL), oral bioavailability (OB), toxicity class, and Lipinski's rule of five.

Screening of target genes

Gene cards database was used to procure gene targets involved in the progression of OS. Bioinformatics Analysis Tool for Molecular mechANism (BATMAN) TCM,[14] and DIGEP-Pred,[15] webservers were accessed to predict the effect of phytochemicals on the target's genes. The canonical SMILES of these compounds were used to retrieve the prediction results of the compound's induced effect on genes.

Molecular docking

The compound's interaction was evaluated against human CDKL3 kinase is an important molecular target of OS.[16] This analysis was facilitated by IGEMDOCK, and the compounds were targeted toward the 38R active site of CDKL3. The amino acids residues present in the 38R active site are VAL18, VAL10, LYS33, PHE79, GLU80, ILE82, THR85, GLU129, LEU132, and CYS142. The interaction of compounds with these residues was considered, and interactions other these residues were discarded.

Profiling of toxic characteristics

Toxic characteristics such as acute toxicity dose, organ-specific damage, and adverse effects prediction were predicted with GUSAR,[17] ROSC-Pred,[18] and Adver-Pred database.[19] These characteristics were determined by providing the canonical SMILES of compounds.

PASS and absorption, distribution, metabolism, excretion, and toxicity prediction

Other biological activities and pharmacokinetic attributes absorption, distribution, metabolism, excretion, and toxicity (ADMET) of compounds present in *P. ginseng* were also determined to explain their possible mechanism of action and safety attributes in the human body. These attributes were predicted by ADMET SAR 2.0,[20] and PASS online.[21]

RESULTS

Compounds selection in *Panax ginseng*

TCM database identified 215 medicinal compounds in *P. ginseng*. The information of these compounds was downloaded and screened for DL, oral BO, toxicity class, and Lipinski's rule of five. Parameters set for obtaining bioactive compounds were: DL >0.3, OB >20%, toxicity class >Class 4, and Lipinski's: no violations. Results of DL and OB were already indicated by TCMSP, but toxicity class and Lipinski's evaluation were facilitated by ProTox-II,[22] and SwissADME.[23] After refinement, six compounds [Table 1] qualified the criteria, which were further used for gene expression, target prediction, biological pathway governed by these targets, and enrichment and ontological analysis along with drug-target network association.

Network pharmacology analysis

Identification numbers of these compounds were procured from the PubChem database and added to the dialog box of BATMAN-TCM. The score cut off was kept at 15 for target prediction, whereas for target analyses, the adjusted $P \leq 0.05$. In Table 2, Fumarine (4970) influenced 35 genes, whereas Inermin on 14 genes. Nepetin, Celabenzine, Frutinone A, and Suchilactone had no potential effect on any target [Table 2]. Gene enrichment analysis of these genes showed that seven enriched KEGG pathways (RAS signaling pathway, Rap1 signaling pathway, PI3K-AKT signaling pathway, hematopoietic cell lineage, purine metabolism, cytokine-cytokine, and neuroactive ligand-receptor interaction,

Table 1: Physiochemical nature of medicinal compounds present in *Panax Ginseng*

Plant	Compounds	PubChem ID	Molecular Weight	Oral bioavailability (%)	Druglikeness score	Toxicity Class	Lipinski's rule of five
Panax Ginseng	Fumarine	4970	353.4	59.26	0.82	Class 4	Passed: No violations
	Inermin	91,510	284.2	65.83	0.53	Class 4	
	Frutinone A	441,965	264.2	65.90	0.34	Class 4	
	Celabenzine	442,847	379.5	101.88	0.48	Class 4	
	Nepetin	5,317,284	316.2	26.75	0.30	Class 5	
	Suchilactone	132,350,840	368.4	57.51	0.55	Class 4	

respectively) [Table 3]. Significantly enriched therapeutic target database (TTD) diseases regulated by these compounds were analgesics, chronic obstructive pulmonary disease, asthma, OS, ischemia, nausea, and vomiting, cough, dyspnea, B-lineage malignancies, allergic rhinitis, multiple organ failure, acute myelogenous leukemia, chronic urticaria, sustained ventricular tachycardia, chronic myeloproliferative disease, phyllodes tumors, macular edema, and other, as mentioned in Table 4. Gene ontological studies revealed that these two compounds have a role in initiating molecular functions such as signal transducer activity, kinase activity, and ion binding, as summarized in Table 5. Biological processes include circulatory system process, cellular protein modification process, lipid metabolic process, response to stress, signal transduction, cell-cell signaling, cell proliferation, and differentiation and locomotion, homeostatic process, anatomical structure development, and neurological system process. The compound target pathway/disease network obtained from BATMAN-TCM is illustrated in Figure 1. Gene expression induced by these compounds was explored with DIGEP-Pred. It was observed that Fumarine upregulates the *CASP2* gene, whereas Inermin downregulated *PCOLCE2* and *STK39* and upregulated *NOTCH1* and *GAS6* genes [Table 6].

Table 2: Target Prediction of Fumarine and Inermin by BATMAN-TCM

Compounds	Predicted Target
Fumarine (4970)	PDGFRB, NTRK1, CSF1R, PDGFRA, KIT, ABL1, DDR1, HTR3A, CSF1, EPOR, DDR2, PDGFRL, BICD1, ZEB2, MST1R, NRP1, SORT1, SLC6A3, PDE7B, PDE4A, OPRD1, PGD, SCN5A, PDE4D, CHRNA10, PDE7A, OPRM1, HRH1, PDE4C, GRIN3A, PDE4B, DNMT1, PDE8B, SLC6A2, PDE8A
Inermin (91510)	SEC14L3, PPP2CA, PRKCA, NR1I2, ALOX5, PPP2CB, SEC14L2, DGKA, PRKCB, SEC14L4, CNR2, TYR, CNR1

Molecular docking results

We have discarded Suchilactone, Celabenzine, Nepetin, and Frutinone A from further analysis based on their inactivity in the network pharmacological analysis. Fumarine and Inermin were then used to check the interaction with CDKL3 protein, which is highly upregulated in OS cells and provides them with proliferative properties. The standard docking algorithm of IGEMDOCK was selected, which is comprised a population size of 200, generation: 70, and a number of docked solutions = 3. Site-directed docking was performed, and the results were characterized on hydrogen interaction of compounds with the active site of CDKL3 protein. IGEMDOCK analysis revealed that Fumarine [Figure 2] and Inermin [Figure 3] established hydrogen bonding with LYS33 amino acid of CSKL3 protein, indicating a similar mechanism of action [Table 7].

Table 3: Enriched genes KEGG pathway governed by Fumarine and Inermin

Compounds	KEGG pathway ID	KEGG pathway name	Adjusted *P*	Targets
Fumarine, Inermin	hsa00230	Purine Metabolism	2.64e-004	PDE4A, PDE4B, PDE4C, PDE4D, PDE7A, PDE7B, PDE8A, PDE8B (8)
	hsa04014	Ras Signaling Pathway	1.20e-002	ABL1, CSF1, CSF1R, KIT, PDGFRA, PDGFRB (6)
	hsa04015	Rap1 Signaling Pathway	1.16e-002	CNR1, CSF1, CSF1R, KIT, PDGFRA, PDGFRB (6)
	hsa04060	Cytokine-Cytokine Receptor Interaction	2.46e-002	CSF1, CSF1R, EPOR, KIT, PDGFRA, PDGFRB (6)
	hsa04080	Neuroactive Ligand-Receptor Interaction	9.17e-003	CHRNA10, CNR1, CNR2, GRIN3A, HRH1, OPRD1, OPRM1 (7)
	hsa04151	PI3K-Akt Signaling Pathway	8.34e-003	CSF1, CSF1R, EPOR, KIT, PDGFRA, PDGFRB, PPP2CA, PPP2CB (8)
	hsa04640	Hematopoietic Cell Lineage	1.20e-002	CSF1, CSF1R, EPOR, KIT (4)

Table 4: Enriched therapeutic target database Results of Fumarine and Inermin

Compounds	TTD ID	Adjusted *P*	Targets
Fumarine, Inermin	Analgesics	1.01e-003	(CHRNA10, CNR1, CNR2, NTRK1, OPRD1, OPRM1, SCN5A) 7
	Pain, unspecified	1.62e-004	(CNR1, CNR2, NTRK1, OPRD1, OPRM1) 5
	Chronic obstructive pulmonary disease	6.36e-006	(PDE4A, PDE4B, PDE4C, PDE4D) 4
	Asthma	3.59e-002	(PDE4A, PDE4B, PDE4C, PDE4D) 4
	Osteosarcoma	6.36e-006	(PDE4A, PDE4B, PDE4C, PDE4D) 4
	Ischemia	2.51e-002	(HRH1, OPRD1) 2
	Nausea and vomiting	1.97e-002	(HRH1, HTR3A) 2
	Cough	1.67e-003	(OPRD1, OPRM1) 2
	Dyspnea	4.46e-003	(OPRD1, OPRM1) 2
	B-lineage malignancies	3.72e-002	(PRKCB) 1
	Seasonal allergic rhinitis	3.72e-002	(HRH1) 1
	Acute lymphoblastic leukaemia (therapy-refractory)	3.72e-002	(HRH1) 1
	Chronic urticaria	3.72e-002	(HRH1) 1
	Sustained ventricular tachycardia	3.72e-002	(SCN5A) 1
	Chronic myeloproliferative diseases	3.72e-002	(PDGFRB) 1
	Phyllodes tumours	3.72e-002	(KIT) 1
	Macular edema	3.72e-002	(PRKCB) 1
	Malignant phyllodes tumours	3.72e-002	(KIT) 1
	Hypereosinophilic syndrome	3.72e-002	(PDGFRA) 1
	Epileptic seizures	3.72e-002	(SCN5A) 1
	Opioid-induced bowel dysfunction	3.72e-002	(OPRM1) 1
	Refractory partial epilepsy	3.72e-002	(SCN5A) 1
	Motion sickness	3.72e-002	(HRH1) 1
	Gastrointestinal stromal tumors	3.72e-002	(KIT) 1
	Myelodisplastic syndrome	3.72e-002	(CSF1) 1

Table 5: Enriched gene ontology results of Fumarine and Inermin

Compounds	Function	Gene ontology ID	Gene ontology term name	Adjusted *P*	Targets
Fumarine, Inermin	Molecular function	GO: 0004871	Signal Transducer Activity	8.33e-009	(CNR1, CNR2, CSF1R, DDR1, DDR2, EPOR, GRIN3A, HRH1, HTR3A, KIT, MST1R, NR1I2, NRP1, NTRK1, OPRD1, OPRM1, PDE8A, PDE8B, PDGFRA, PDGFRB, PDGFRL, PPP2CA, SORT1, ZEB2) 24
		GO: 0016301	Kinase Activity	3.31e-006	(ABL1, CSF1, CSF1R, DDR1, DDR2, DGKA, KIT, MST1R, NRP1, NTRK1, PDGFRA, PDGFRB, PDGFRL, PPP2CA, PRKCA, PRKCB, ZEB2) 17
		GO: 0043167	Ion Binding	3.32e-002	(ABL1, ALOX5, DGKA, DNMT1, KIT, NR1I2, NRP1, PDE4A, PDE4B, PDE4C, PDE4D, PDE7A, PDE7B, PDE8A, PDE8B, PPP2CA, PPP2CB, PRKCA, PRKCB, TYR, ZEB2)
	Cellular component	GO: 0005829	Cytosol	8.40e-004	(ABL1, ALOX5, BICD1, DGKA, NRP1, PDE4A, PDE4B, PDE4C, PDE4D, PDE7A, PDE7B, PDE8A, PDE8B, PGD, PPP2CA, PRKCA, PRKCB) 17
		GO: 0005886	Plasma Membrane	3.31e-006	(ALOX5, CHRNA10, CNR1, CNR2, CSF1, CSF1R, DDR1, DDR2, DGKA, EPOR, GRIN3A, HRH1, HTR3A, KIT, MST1R, NRP1, NTRK1, OPRD1, OPRM1, PDE4A, PDGFRA, PDGFRB, PPP2CA, PRKCA, PRKCB, SCN5A, SLC6A2, SLC6A3, SORT1) 29
	Biological process	GO: 0006464	Cellular Protein Modification Process	8.40e-004	(ABL1, CSF1, CSF1R, DDR1, DDR2, DGKA, DNMT1, KIT, MST1R, NRP1, NTRK1, PDGFRA, PDGFRB, PDGFRL, PPP2CA, PPP2CB, PRKCA, PRKCB, ZEB2) 17
		GO: 0006629	Lipid Metabolic Process	1.06e-002	(ALOX5, CNR1, CSF1R, HRH1, KIT, NR1I2, PDGFRA, PDGFRB, PPP2CA, SEC14L2) 10
		GO: 0006810	Transport	2.04e-003	(ABL1, BICD1, CHRNA10, CNR1, CSF1R, GRIN3A, HTR3A, NR1I2, NRP1, OPRD1, PDE4C, PDE8B, PDGFRB, PRKCA, PRKCB, SCN5A, SEC14L2, SEC14L3, SEC14L4, SLC6A2, SLC6A3, SORT1) 22
		GO: 0007165	Signal Transduction	4.63e-009	(ABL1, CNR1, CNR2, CSF1, CSF1R, DDR1, DDR2, DGKA, EPOR, GRIN3A, HRH1, HTR3A, KIT, MST1R, NR1I2, NRP1, NTRK1, OPRD1, OPRM1, PDE4A, PDE4B, PDE4C, PDE4D, PDE7A, PDE7B, PDE8A, PDE8B, PDGFRA, PDGFRB, PDGFRL, PPP2CA, PPP2CB, PRKCA, PRKCB, SORT1, ZEB2) 36
		GO: 0007267	Cell-Cell Signaling	2.01e-004	(CHRNA10, CNR1, CNR2, HRH1, HTR3A, NRP1, PDE4C, PDE7B, PDE8B, PRKCA, PRKCB, SLC6A2, SLC6A3) 13
		GO: 0008283	Cell Proliferation	1.65e-003	(CHRNA10, CSF1, CSF1R, DDR1, DDR2, KIT, MST1R, NRP1, OPRM1, PDGFRA, PDGFRB, TYR, ZEB2) 13
	Catabolic Process	GO: 0030154	Cell Differentiation	2.39e-002	(ABL1, CNR1, CSF1, CSF1R, DDR2, GRIN3A, KIT, NRP1, NTRK1, PDE4D, PDGFRA, PDGFRB, PPP2CA, SORT1, ZEB2) 15
		GO: 0040011	Locomotion	1.22e-002	(ABL1, CSF1, CSF1R, DDR2, KIT, NRP1, PDGFRA, PDGFRB, PRKCA, ZEB2) 10
		O: 0048870	Cell Motility	9.07e-004	(ABL1, CSF1, CSF1R, DDR2, KIT, NRP1, PDGFRA, PDGFRB, PRKCA, ZEB2) 10s
		GO: 0042592	Homeostatic Process	2.39e-002	(CHRNA10, CNR1, CNR2, CSF1, CSF1R, EPOR, PDGFRA, PDGFRB, SCN5A) 9
		GO: 0048856	Anatomical Structure Development	8.09e-003	(ABL1, BICD1, CHRNA10, CNR1, CSF1, CSF1R, DDR1, DDR2, EPOR, GRIN3A, KIT, NRP1, NTRK1, PDE4D, PDGFRA, PDGFRB, PPP2CA, SLC6A3, SORT1, TYR, ZEB2) 21
		GO: 0050877	Neurological System Process	6.57e-004	(ALOX5, CHRNA10, CNR1, CNR2, GRIN3A, HRH1, HTR3A, OPRD1, OPRM1, PDE7B, PRKCA, PRKCB, SLC6A2, SLC6A3, TYR) 15

Table 6: Gene expression induced by Fumarine and Inermin

Compounds	Gene expression pattern	Genes	Genes name	Biological function
Fumarine	Upregulation	CASP2	Caspase 2	Mediate Cell apoptosis in osteosarcoma cells
Inermin	Downregulation	PCOLCE2	Procollagen C-Endopeptidase Enhancer 2	Migration, Invasion and Metastasis of Osteosarcoma
	Downregulation	STK39	Serine/Threonine Kinase 39	Overexpression of STK39 is associated with high tumor proliferation and invasion
	Upregulation	NOTCH1	Notch homolog 1	Sensitizes the OS cells to Cisplatin
	Upregulation	ADAMTS9	ADAM Metallopeptidase with Thrombospondin Type 1 Motif 9	Sensitizes tumor cells to paclitaxel and inhibits growth
	Upregulation	GAS6	Growth Arrest Specific 6	Prevents Tumor cell migration and Invasion and induces apoptosis

Toxicity profiling results

Fumarine has acute toxicity of 135,100 mg/kg for the intraperitoneal route, 22,960 mg/kg for the intravenous route, 616,500 mg/kg for the oral route, and 224,800 mg/kg for the subcutaneous route. The acute toxicity dose of Inermin was 237,100 mg/kg for the intraperitoneal route, 56,720 mg/kg for the intravenous route, 1,150,000 mg/kg oral and 306,100 mg/kg subcutaneous routes, respectively. These compounds are characterized as class 4 chemicals and the adverse and organ damaging effects associated with these compounds are given in Table 8.

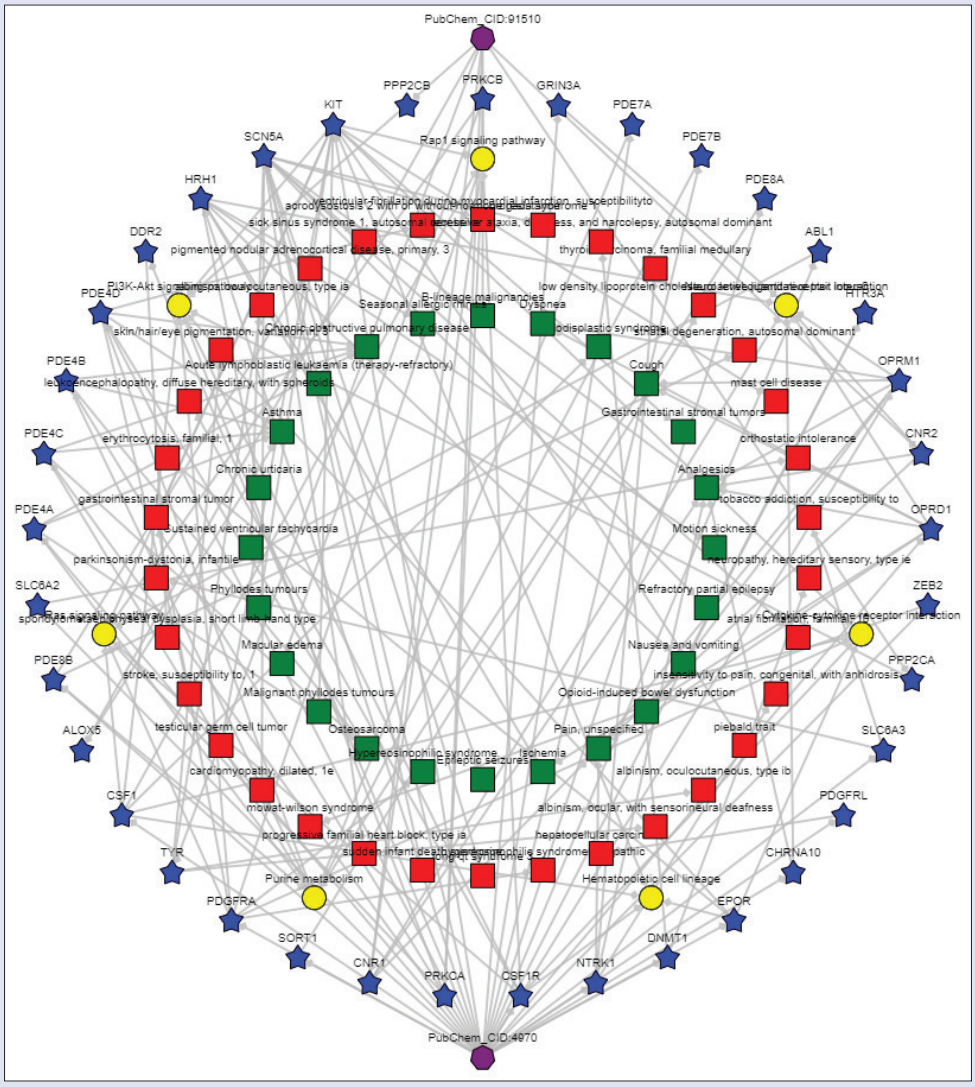

Figure 1: Network Pharmacological Analysis of Phytocompounds of *Panax ginseng*. PubChem 4970 stands for fumarine, PubChem 91510 stands for inermin

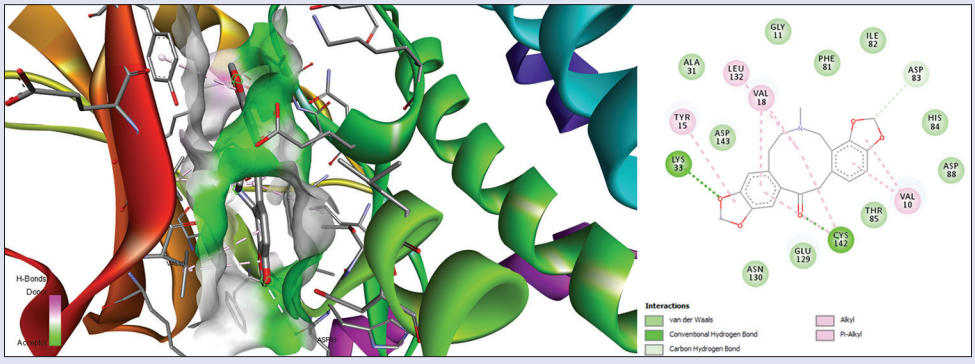

Figure 2: Molecular conformation of fumarine interaction with human CDKL3 protein

PASS and absorption, distribution, metabolism, excretion, and toxicity prediction

The biological function of these compounds was further explored with PASS online. Results indicated that Fumarine stimulates the activity of caspase 8,3 and promotes apoptosis and tumor suppressor gene-53 (TP53) expression. It is also an antineoplastic alkaloid and inhibits topoisomerase-I activity. A similar type of activity was observed for Inermin except for this compound also has topoisomerase-I and topoisomerase-II activity [Table 9]. ADMET SAR 2.0 was used to predict the compound's absorption, site of metabolism, and toxicity. Fumarine has a high blood–brain barrier (BBB) and intestinal absorption and its

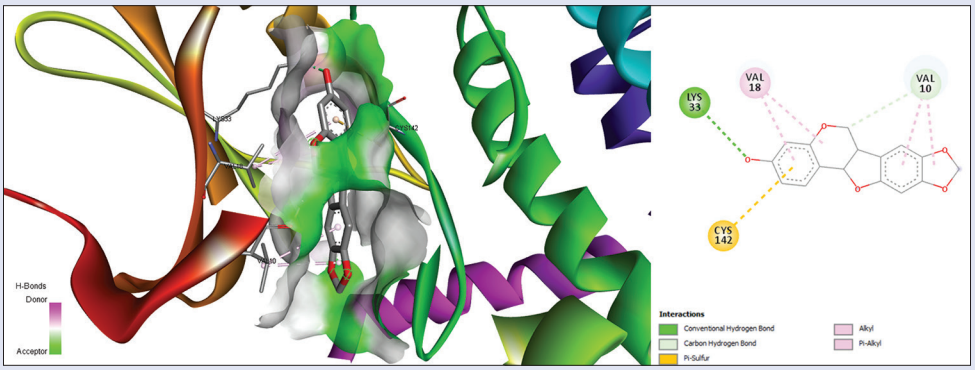

Figure 3: Molecular conformation of inermin interaction with human CDKL3 protein

Table 7: Chemical Interaction of phytocompounds with CDKL3 protein

Ligands	Receptor	Reported active site residues	Residue-ligand interaction (H bonds only)	Binding free energy
Fumarine (4970)	Cyclin Dependent	VAL18, VAL10, LYS33, PHE79, GLU80,	LYS33	−100.9 KJ/mol
Inermin (91510)	Kinase Like 3 (3ZDU)	ILE82, THR85, GLU129, LEU132, CYS142	LYS33	−87.7 KJ/mol

Table 8: Acute toxicity analysis of Fumarine and Inermin

Compounds	OECD chemical classification	Side effects in rats	Organ specific damage in rats	Rat IP LD_{50} (mg/kg)	Rat IV LD_{50} (mg/kg)	Rat oral LD_{50} (mg/kg)	Rat SC LD_{50} (mg/kg)
Fumarine (4970)	Class 4	Hepatotoxicity	Liver, stomach	135,100	22,960	616,500	224,800
Inermin (91510)	Class 4	Hepatotoxicity	Liver, stomach	237,100	56,720	1150,000	306,100

Table 9: Predicted biological activity of Fumarine and Inermin

Compound	Biological activity	Probability (%)
Fumarine (4970)	Caspase 8 stimulant	51
	Caspase 3 stimulant	49.2
	Antineoplastic alkaloid	46.9
	Topoisomerase I inhibitor	31.8
	Apoptosis agonist	38.2
	TP53 expression enhancer	38.3
Inermin (91510)	Caspase 3 stimulant	93.4
	Antineoplastic	78.8
	Apoptosis agonist	75.1
	TP53 expression enhancer	72.2
	Chemo preventive	61.2
	Caspase 8 stimulant	48.9
	Topoisomerase I inhibitor	39.6
	Chemosensitizer	35.7
	Topoisomerase II inhibitor	32.6

subcellular localization is lysosome. This compound is inactive against p-glycoprotein, CYP2C9, CYP3A4, and CYP2C9 whereas active for CYP2D6 substrate and inhibit CYP2C19, CYP2D6, and CYP1A2. It is nontoxic to cells and genes but possesses high hepatotoxicity. Inermin has high intestinal and BBB permeability and it is subcellularly localized in mitochondria. Like Fumarine, Inermin also has no activity for p-glycoprotein, CYP3A4, CYP2C9, CYP2C19, CYP2D6, and CYP1A2, respectively [Table 10]. This compound is relatively more toxic than Fumarine.

DISCUSSION

OS is multifactorial cancer which means this disease requires the activity of various genes to drive the growth of osteoblastic cells.[24] Recent studies emphasized inhibiting a single OS target to prevent tumor proliferation.[25,26] However, cancer cells have devised various mechanisms to circumvent the inhibited protein target by stimulating other genes to further navigate the pathway to sustain the survival of the tumor.[8,24] These mechanisms are also responsible for developing resistance, invasiveness, and migratory properties to OS cells. Therefore, single target targeting drugs becomes obsolete considering the complex evading mechanism of this disease. In recent years, network pharmacology has changed drug discovery research by merging artificial intelligence to correlate the interaction of therapeutic drugs with cellular networks and genes. This new discipline allowed researchers to understand the effect of drug interaction with various molecular targets and cellular networks. Natural compounds are constantly being repurposed for different types of diseases. However, these natural compounds are multimodal in action and influence multiple molecular targets which were overlooked previously prior to the discovery of network pharmacology. The emergence of this discipline streamlined the process of drug discovery and allowed scientists to thoroughly evaluate the therapeutic effects of a compound on a biological system.

In this study, we used network pharmacology, *in silico* gene expression, molecular docking, and ADMET method to elaborately analyze *P. ginseng* phytocompounds against OS. Initially, we obtained 215 compounds of *P. ginseng* from TCMSP, which on screening yielded 6 bioactive compounds such as Fumarine, Inermin, Fruitnone A, Celabenzine, Nepetin, and Suchilactone. These compounds were subjected to network pharmacology analysis to unravel their biological activity in OS.

BATMAN-TCM database facilitated the network pharmacology analysis and the results revealed that Fumarine and Inermin had a significant interaction with different biologically active targets, whereas Fruitnone A, Celabenzine, Nepetin, and Suchilactone failed to show any discerning activity. The predicted molecular target targeted by Fumarine was 35 and 13 for Inermin. These gene targets were subjected to gene enrichment studies to establish a significant association with different disease phenotypes and to recognize the molecular functions, cellular

Table 10: Chemical Behavior of Fumarine and Inermin

Compounds	ADMET classification	Activity
Fumarine (4970), Inermin (91510)	Human Intestinal Absorption	+, +
	Caco-2	+, +
	Blood Brain Barrier	+, −
	Human oral bioavailability	−, −
	Subcellular localization	Lysosome, mitochondria
	P-glycoprotein inhibition	−, −
	P-glycoprotein substrate	−, −
	CYP3A4 substrate	+, −
	CYP2C9 substrate	−, −
	CYP2D6 substrate	+, +
	CYP3A4 inhibition	−, −
	CYP2C9 inhibition	−, −
	CYP2C19 inhibition	+, +
	CYP2D6 inhibition	+, +
	CYP1A2 inhibition	+,+
	Carcinogenicity	−, −
	Ames mutagenesis	−, −
	Water solubility	logS -2.888, logS -2.831
	Acute Oral Toxicity	1.81 kg/mol, 1.307

*−: Means no activity; +: Means positive activity

locations, and biological processes governed by these compounds. These compounds affect purine metabolism, which allows rapid tumor cell proliferation and growth in OS. Ras,[27] Rap1,[28] and PI3K-AKT signaling pathways,[29] are implicated in providing OS cells with invasive and migratory properties that are also targeted by these compounds. The therapeutic target database showed that these compounds have a significant therapeutic influence in alleviating pain, cancer, and other physiological ailments, including OS. Gene ontological analysis revealed that these compounds have a role in regulating biological and molecular functions along with some cellular functions.

We took these compounds and analyzed them with DIGEP-pred to evaluate the effects on mRNA gene expression, which might have been overlooked BATMAN-TCM algorithm. Fumarine upregulated the expression of the *CASP2* gene, which is involved in inducing tumor cell apoptosis. Whereas Inermin reduced the expression of *PCOLCE2* and *STK39*. Both these genes equip OS cells with rapid proliferative, migratory invasiveness, and metastatic properties,[30,31] Moreover, this compound upregulates some other regulatory genes that help in the prevention of tumor growth invasiveness and sensitize these cells to chemotherapy.[32-34] From gene ontological analysis, it was observed that these compounds have a role in regulating cell proliferation and kinases activity. To further validate these findings, we used the molecular docking method to analyze the inhibitory effects on CDKL3 kinase which is involved in OS progression and proliferation.[35] These compounds were focused on the reported active site residue of CDKL3 kinase protein to determine the protein-ligand interaction. IGEMDOCK software was used, and the docking studies were performed three times to increase confidence in the obtained results. Both these compounds used the same amino acid residues LYS33 to establish hydrogen bonding with the CDKL3 protein. This interaction shows that Inermin and Fumarine have a similar mechanism of action.

Furthermore, we utilized the PASS algorithm to identify other biological activities. The results of PASS prediction predicted that these compounds stimulate the activity of caspase 3, and 8,[36,37] and TP53.[38] These proteins induce tumor apoptosis and prevent tumor recurrence and growth. Besides these activities, they also interact with topoisomerase I and II that provide further evidence that these compounds also prevent DNA replication in OS cells.[39]

The safety and pharmacokinetic properties of a compound is a major component in drug discovery and development. Inadequate elucidation of these properties of a compound can jeopardize human health and may lead to serious harm during a clinical trial. To determine these properties, we elaborately analyzed each compound to increase its approval rating in different animal and clinical trials. These compounds are highly soluble and readily absorbed inside the gastrointestinal tract. However, these compounds pose a significant risk of causing hepatotoxicity and can affect the stomach and liver. These challenges can hinder their therapeutic efficacy and approval in various clinical trials. There are several methods reported in the literature to solve these challenges associated with these compounds, such as nanoformulations,[40] structural modification,[41] organic synthesis,[42] and drug concentration calibration.[43]

CONCLUSION

Inermin and Fumarine present in *P. ginseng* have significant anticancer activity in OS cells. These compounds target both genes and other molecular drug targets to reduce the proliferation and aggressiveness of these tumors. However, the toxic nature of these compounds could jeopardize their therapeutic activity that can be a challenge for other researchers to work on. Our findings provided an elaborate insight about Inermin, and Fumarine in OS treat, which require further *in vitro* validation.

Financial support and sponsorship

This work was supported by the National Research Foundation of Korea (NRF) funded by the Korean Government (MEST) (2020R1I1A306969912).

Conflicts of interest

There are no conflicts of interest.

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