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TARGETS AND MOLECULAR MECHANISM OF DIOSCIN AGAINST HUMAN BREAST CANCER: A SYSTEMATIC IN SILICO ANALYSIS

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ABSTRACT

Dioscin is a natural glycoside predominantly present in many plants. It has been shown to possess anti-proliferative effect on variety of human cancer cells in vitro and in vivo. And recent studies reported that Dioscin could reverses TGF-ß1 induced epithelial mesenchymal transition (EMT) in cancer cells. However, the mechanism by which Dioscin can suppresses EMT and pathway involved in Breast cancer is still not clear. Both genomic and proteomic analysis was carried out to understand the binding mechanism of Dioscin with breast cancer targets. Target network analysis and GO were carried out to find the interaction of the important pathway proteins and molecular docking, ADME were carried out to find the binding affinity and pharmacokinetic properties of the Dioscin. The results shows that Dioscin has good binding affinity with three major pathway proteins with significant docking score and also it obeys all the criteria of ADME properties. The network and GO analysis also shows the higher interaction of Dioscin with major three pathway proteins (SMAD2, PIK3 and p38MAPK). The overall results of the study suggested that Dioscin have the potential to inhibit the breast cancer target by which it regulates the apoptosis and DNA damage repair mechanism.

INTRODUCTION

Even with the continual progress in understanding of Breast cancer metastasis, it remains a major health problem around the world. Metastasis is now recognized as an extremely complex and multistep biological process. Nearly 12% of patients with a diagnosis of breast cancer eventually develop metastatic disease, or Breast cancer that has spread beyond the breast to other parts of the body (Peart, 2017). The invasive and migratory abilities are the crucial factors of metastatic cancer cells. Single cell invasion and Collective cell invasion are the two fundamentally different patterns of cancer cell metastasis (Spano *et al*., 2012). Wide range of cancers originate from epithelial tissues. These tumor cells need to moderate their tight cell-cell adhesion in order to leave the primary tumor and invade the surrounding tissue. This progression permits disaggregation of cancer cells from the primary site and assistance initial dissemination. It is reported that in most cases, single cells leaving the primary tumor undergo epithelial to mesenchymal transition (EMT), mediated by molecules such as TGFß, transcription regulators Twist and Snail (SNA, SNAI1), MAP kinases, Wnt, Notch and Hedgehog (Tse and Kalluri, 2007). The knowledge of its mechanisms is still incomplete and needs to be studied in order to develop therapeutic approach and impact on the abiding control of breast cancer progression.

Dioscin, with the molecular formula C45H72O16 and a molecular weight of 869.05 g/mol, is widely found from the roots of Dioscorea panthaica. It has been reported to possess various biological activities including anti-inflammation, hepatoprotection, and also its anti-proliferative effects against a number of human cancer cells (Cai *et al*., 2002; Li *et al*., 2003). Dioscin increases apoptosis level of prostate cancer cells by stimulating estrogen receptor (Tao *et al*., 2017) and also it induces apoptosis of gallbladder cancer by suppressing PI3K/AKT pathway (Song *et al*., 2017). Another study showed inhibition of melanoma progression by upregulating connexin 43 (Kou *et al*., 2017). Several reports revealed that Dioscin inhibits EMT progression (Zhang *et al*., 2016; Chen *et al*., 2019). It has been reported that Dioscin inhibits EMT via p38-MAPK signaling in HepG2 cells (Chen *et al*., 2019). Dioscin also suppressed phosphorylation of SMAD2 and p38 in the TGF-ß1 induced EMT in A549 lung cancer cells (Lim *et al*., 2017). In light of these findings, this study was done with the aim to explore the possible molecular targets of Dioscin in Breast cancer. Further, molecular docking analysis were done for the predicted targets. Our work reveals the molecular mechanism of Dioscin target in Breast cancer cells, and the identified targets may be useful for treatment of Human breast cancer in future.

MATERIALS AND METHODS

Collection of Breast cancer-related targets of Dioscin

Dioscin-related targets were collected by CTD , Similarity Ensemble Approach and Swiss Target Prediction The targets with a probability valuee ≥ 0.5 were selected for Swiss Target Prediction. GeneCards were used to extract the breast cancer-related targets with "Breast cancer" as a search term, and the 500 with highest scores were retained. The targets matching those obtained from the above described approach were identified as potential breast cancer-related targets of Dioscin. The gene symbols for all candidates were verified by the UniProt .

Construction of compound target network

The interactions of the potential targets of Dioscin were analyzed using the STRING database , and interactions with a combined score higher than 0.4 were screened. The compound-target network was generated based on the PPI data (protein–protein interaction) and was visualized using Cytoscape-v3.7.1 software. The network characteristics were analyzed by the applied plug-in Network Analyzer. The degree of freedom was used as a topological index, which is often used to describe the importance of the network node. The larger the value, the more critical the node is in the network.

Gene Ontology

Blast2go software was used to predict the Gene Ontology (GO) of the network and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was predicted using OmicsBox software. The KOG (Eukaryotic orthologous group) was predicted using eggnog mapper v2. The KOG classification was carried out using WebMGA online tool and the graph was plotted using R software.

Receptor Protein

The three-dimensional structure of three important breast cancer targets PIK3 (PDBID: 4JPS), SMAD2 (PDBID: 1KHX) and p38MAPK (PDBID: 3GP0) were retrieved from Protein data bank with co-crystalized ligand and the missing side chains of the structures were removed before docking.

Bioactive compounds retrieval

The structure of Dioscin was obtained from PubChem in the form of SDF. The data base contains large number of chemical substance that have been validated, which gives important details of the chemical compounds obtained from PubChem (Oyinloye *et al*., 2019). Chimera was used for the conversion of SDF format to PDB format before docking.

ADME

The prediction of pharmacokinetic properties of the drug molecules is the crucial step in the drug designing. Prediction of ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of Dioscin was analyzed with Swissadme and admetSAR (predated.bmdrc.kr) online tools. These tools provide the outline of vital druglikenesslike mutagenicity, harmful dosage level and pharmacokinetically pertinent which attributes all the bioactive compounds (Ojo *et al*., 2019). In addition, the bioactivity of the Dioscin was also evaluated using Molinspiration cheminformatics .

Molecular Docking

The chemical structure of Dioscin was obtained from the PubChem saved in its SDF format, and converted to the mol2 format by Discovery Studio 3.0. The PDB IDs of the candidates p38MAPK, PIK3 and SMAD2 were derived from the UniProt database, and the corresponding protein three-dimensional structure was obtained from the RCSB PDB database and saved in PDB format. The ligand and protein were energy minimized and prepared for docking using Dockprep tool in UCSF chimera software. Molecular docking was performed using Autodock Tools-1.5.6, and the docking score was used to assess the binding affinity of the target to the Dioscin molecule (Kumari *et al*., 2017). The two and three dimensional plan of the docking results was presented by Discovery Studio 2019 Client.

RESULTS AND DISCUSSION

Construction of compound target network and GO

Owing to the rapid development of research in the field of drug development, network pharmacology has been used as novel approach for drug mechanism research . In recent years, number of database related tools are developed and provided a crucial support for target network pharmacology research (Chen *et al*., 2011). In order to evaluate the binding affinity of the anticancer effects of Dioscin, we employed STRING. This

Table 1: Predicted breast Cancer-Related Targets of Dioscin

S. No	Gene symbol	S. No	Gene symbol	S. No	Gene symbol	S. No	Gene symbol
1	TGFB1	17	SMAD4	33	NEFM	49	CTNNA1
2	ZFYVE16	18	ERAS	34	PRPH	50	MRPL49
3	CDC23	19	NRAS	35	GFAP	51	MKNK1
4	SMURF2	20	FYN	36	THOC3	52	MAPK14
5	SKI	21	AKT1	37	THOC5	53	MAPKAPK3
6	SKIL	22	RRAS	38	VIM	54	MAPKAPK2
7	ANAPC7	23	PIK3R1	39	THOC2	55	MAP2K6
8	SMAD2	24	YES1	40	INA	56	DUSP9
9	CUL7	25	PIK3R2	41	NES	57	MAP2K3
10	ZFYVE9	26	PIK3CA	42	CDH2	58	OBSL1
11	NEDD4	27	HRAS	43	PTBP3	59	JUP
12	LDLRAD4	28	RRAS2	44	NENF	60	KLRG1
13	SMAD3	29	KRAS	45	PRKCDBP	61	CTNND1
14	NEDD4L	30	PIK3R3	46	PC	62	CTNNB1
15	ANAPC4	31	MRAS	47	DES	63	FER
16	CDH1	32	CDC5L	48	MTHFD1	64	DES

Table 2: molecular interaction of Dioscin with three different breast cancer target protein with docking score

S.No	Receptors	Docking score	Types of interaction	No. of interaction	Interaction residues
1	PIK3	-10.4 kcal/mol	Conventional hydrogen bond		Glu135, Asn482 (2), Thr679, Lys132
			Carbone hydrogen bond	5	-
			Akyl and Pi-Alkyl bond	0	Ile427, Val131, Met130, Lys132(2), Leu645, Arg638
				7	
2	SMAD2	-8.7 kcal/mol	Conventional hydrogen bond		Phe273, Ala272, Ser276, Leu645, Arg683
			Carbone hydrogen bond	4	-
			Akyl and Pi-Alkyl bond	0	His291, Leu446, Tyr406, Phe402, Leu453 (3)
				8	
3	p38MAPK	-8.6 kcal/mol	Conventional hydrogen bond		His148, Asp168, Arg149, Thr68
			Carbone hydrogen bond	5	Gly170
			Akyl and Pi-Alkyl bond	1	Glu135, Asn428 (2), Thr679, Lys132
				3	

Table 3: ADMET of the Dioscin using Lipinski rule of five filters

Properties	Values
Num. heavy atoms	61
Num. rotatable bonds	7
Num. H-bond acceptors	16
Log Po/w	4.77
Log S	-5.61
P-gp substrate	YES
Log Kp (skin permeation)	-10.65 cm/s
P-gp inhibitor	NO
hERG Blocker	YES
mutagenicity	NO
carcinogenic	NO

Table 4: Prediction of bioactivity of Dioscin using Molinspiration cheminformatics

Biological activity	Molinspiration score
GPCR ligand	-2.54
Ion channel modulator	-3.5
Kinase inhibitor	-3.5
Nuclear receptor ligand	-3.11
Protease inhibitor	-1.95
Enzyme inhibitor	-2.58

software significantly identify the breast cancer related targets of Dioscin based on text-mining and ligand topology and protein structure similarity (Figure 1) the target mapping image shows 64 nodes and are represent the proteins.

The degree value was representing by the number of line connected to the same node, meaning the importance of the each node in the network. The larger and darker color were in the network depicted the greater value and each edge represent the interaction of Dioscin between the breast cancer proteins in the network. In the present results, mainly three pathway proteins such as SMAD2, PIK3, and p38MAPK shows greater binding interaction and also whose value were significantly higher than that of the other targets, which suggesting their Dioscin binding affinity with particular pathway proteins. Among other target these three pathway proteins had the largest nodes and the darkest color, indicating they were probably the most important potential targets in breast cancer. The data obtained from the TCMSP tool were input into the DAVID database for GO analysis.

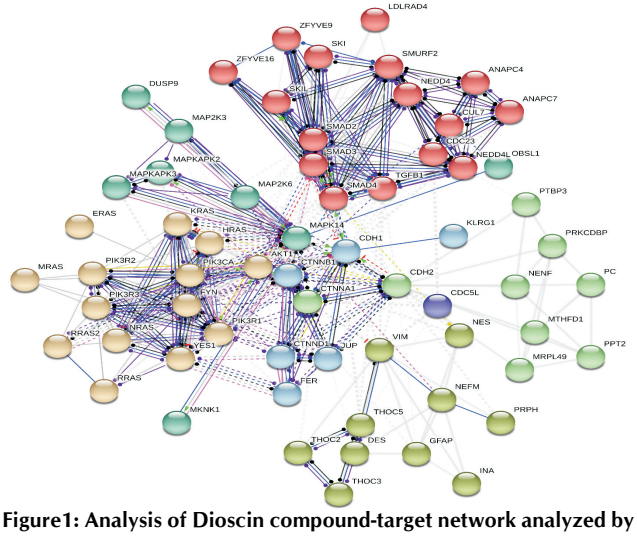

Figure1: Analysis of Dioscin compound-target network analyzed by STRING to generate the compound-target network. The darker blue line indicates the higher value of interaction.

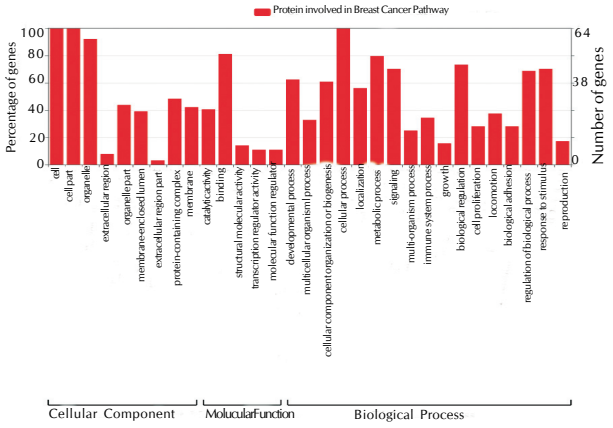

Figure 2: GO functional annotation of potential targets of Dioscin.

Total of 64 GO terms were selected based on the P-values parameters ($P \leq 0.01$). It significantly analyzed the most the targets and were present in the cytosol, nucleolus, cellular

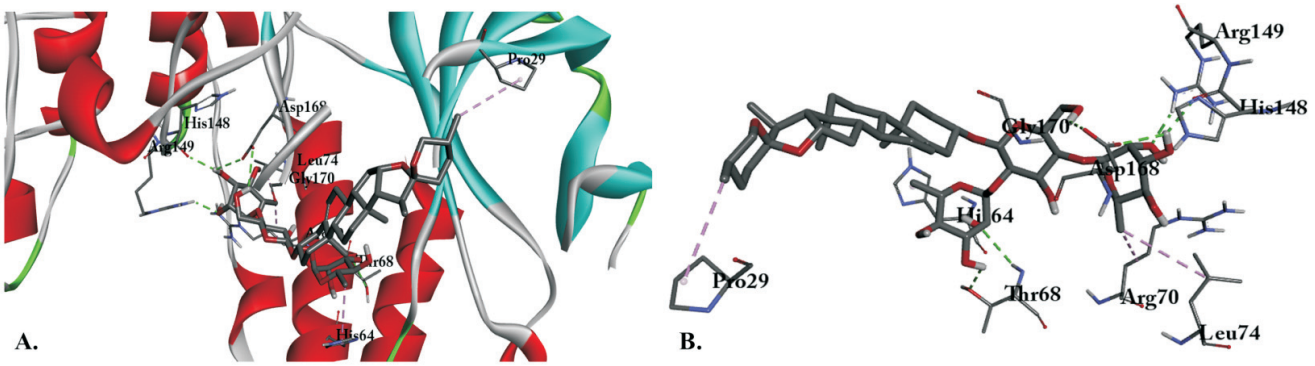

Figure 3: A) represent the docked complex of p38MPAK with Dioscin and B) shows the crucial interactive amino acids with Dioscin.

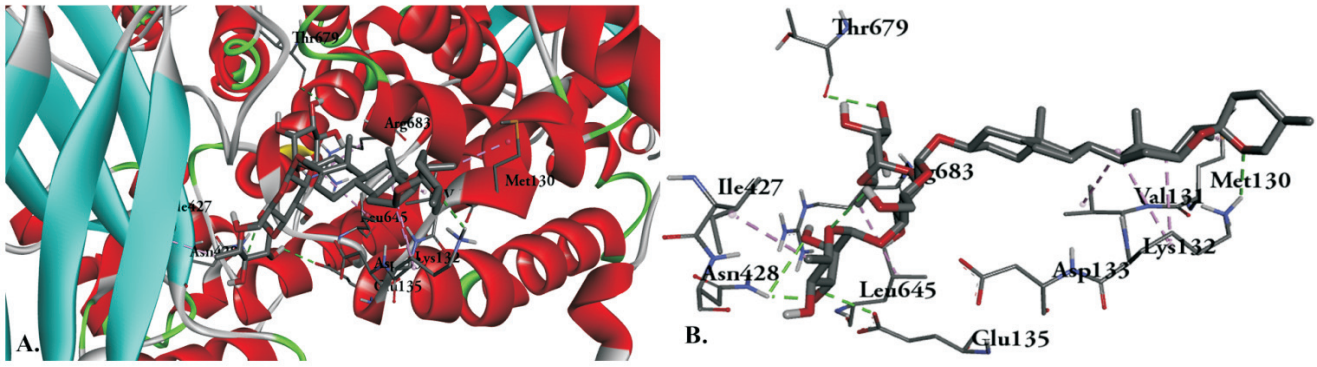

Figure 4: A) represent the docked complex of PIK3 with Dioscin and B) shows the crucial interactive amino acids with Dioscin.

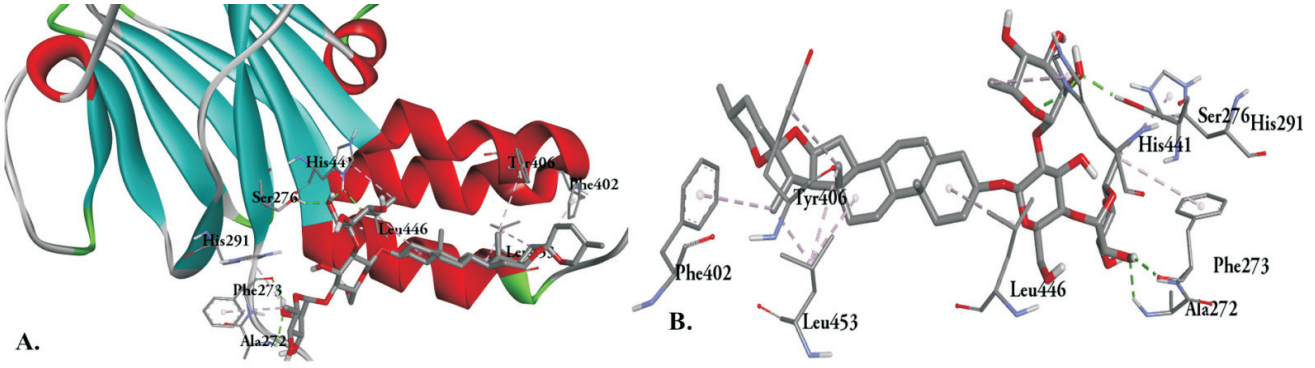

Figure 5 A: represent the docked complex of SMAD2 with Dioscin and B) shows the crucial interactive amino acids with Dioscin.

process and signaling pathway. Table 1 represents the breast cancer targets of Dioscin. These targets were particularly associated with biological process (Figure 2). Results from both network analysis and GO term suggested that Dioscin compound significantly involved in the regulation of apoptosis, DNA damage repair, redox, cell metabolism. Hence these three important pathway proteins are taken for the molecular docking studies.

Molecular Docking

In breast cancer pathway, EMT is one of the important pathway protein plays a crucial role in various biological process including wound healing, carcinoma progression, embryonic morphogenesis, and tissue fibrosis. The increased level of EMT activity leads the dissolution of epithelial cell-cell junction and enables the cell invasion. Several environmental factors significantly influence the induction of EMT in breast cancer cells. Transforming growth factor-ß1 (TGFß-1), is one of the prominent factors which induce the EMT in cancer cells and promotes the metastasis (Yadav *et al.*, 2011; Ko, 2015). TGFß-1 is potent pleiotropic cytokine found in all the cell types significantly induces the other signaling pathways such as p38MAPK and SMAD2. In the present study, molecular docking approaches have used to examine whether the Dioscin firmly bind with breast targets such as SMAD2, p38MAPK and PIK3. The three dimensional structure of three different breast cancer proteins were docked at the desired grid coordinates of the protein molecules with the help of

Lamarckian Genetic Algorithm (GA) of AutoDock. The test compound Dioscin showed good binding affinity with all the three breast cancer targets with good docking score.

Figure 3 depicted that interaction of Dioscin with human p38*a* MAP kinase (PDB code: 3GP0). It shows significant docking score (-8.6 kcal/mol), the visual inspection of molecular interaction with p38MAP kinase, clearly indicating Dioscin was seated firmly at the binding pocket (Figure 3B), and the intermolecular interactions have displayed the conventional hydrogen bond, carbon hydrogen bond, Alkyl and pi-Alkyl bond formation with p38MAPK. The residues such as His148, Asp168 of A chain of p38MAPK form hydrogen bond with hydroxyl group of the test compound and the oxygen atom of aromatic ring of the compound also make two hydrogen bond formations with Thr68 and also from a hydrogen bond with Arg149. In addition to the conventional hydrogen bond, the test compound Dioscin also make Akyl and pi-Alkyl bonds with Arg70, Thr68, and Pro29. Furthermore the residue Gly170 form carbon-hydrogen bond interaction which all stabilizing the protein-ligand complex structure.

Figure 4, it was depicted that intermolecular interaction of Dioscin with PIK3. It was noticed that the test compound shows finest docking score (-10. 4 kcal/mol) compared to SMAD2 and p38MAPK. Dioscin form five conventional hydrogen bond interaction, and 7 Alkyl and pi-Alkyl interaction (table 2). The compound Dioscin formed stable hydrogen bond interactions with residues such as Glu135, Asn428 (2), Thr679 and Lys132 with PIK3 receptor protein molecule. Besides, it also make alkyl and pi-alkyl interactions with residues Ile427, Val131, met130, Lys132 (2), Leu645, Arg683 of chain A. Figure 5 shows the molecular interaction of Dioscin with SMAD2 receptor protein. The docking score of Smad is more or less similar to that of p38MAPK (-8.7 kcal/mol), and form 2 conventional interaction with residues like Phe273, Ala272 via hydroxyl group and 2 hydrogen bond interaction with Ser276, His441 via oxygen atom of aromatic ring of the Dioscin. Furthermore it forms 8 alkyl and pi-alkyl interaction with residues such as His291, Leu446, Tyr406, Phe402 and Leu453 (3).

The present results demonstrated that the test compound Dioscin could be potent inhibitor of three signaling receptors and also this study is highly correlated with our in vitro studies (Yogaraj *et al*., 2020; unpublished data).

ADME properties

The data obtained from ADME revealed that the Dioscin fulfill the druglikeness properties and follows all the criteria such as molecular weight, hydrogen bond acceptor and hydrogen bond donor. The druglikeness analysis of Dioscin shows that it doesn't violate the Lipinski's rule of five therefore, it is considered druglike. The Variable Neighbor ADMET sever method was used for the prediction of pharmacokinetic properties of Dioscin. ADMET has significantly influence on the drug level and the prediction of other properties such as Human liver microsomal stability test, inhibition of Cyp1A2, Cyp3A4, BBB, Pgp-substrate, Pgp-inhibitor and MMP. The Dioscin may act as inhibitors of Cyp3A4 and Cyp1A2 which indicate that the compounds are inhibitors of all the isoforms of cytochrome p450 assessed. It could qualify as an acceptable drug and can effectively permeable via blood brain barrier (BBB). It is not mutagenic and carcinogenic shown in table 3. In addition, the biological activity of Dioscin was analyzed using Molinspiration Cheminformatics. The bioactivity score profile of the all selected agents is given in table 4. From the table it was depicted that the bioactivity score provide clear information about the binding cascade of the Dioscin against selected target proteins shows the increased binding selectivity profile and less undesirable effects. This result demonstrated that Dioscin acts as potent drugs that are in agreement with rule of five and also likely to exhibit lower attrition rates in cases of clinical trials which could results the significant possibilities to used in therapeutic approach (Rajendran et al., 2015). The compound which fulfill the criteria of ADMET is highly reduces the risk associated with late stage attrition in drug development and also involves testing the novel drug for its effectiveness and toxicity (Meng *et al*., 2011).

REFERENCES

Cai, J., Liu, M., Wang, Z. and Ju, Y. 2002. Apoptosis induced by dioscin in Hela cells. *Biological and Pharmaceutical Bulletin*. **25(2):** 193-196.

Chen, B., Zhou, S., Zhan, Y., Ke, J., Wang, K., Liang, Q. and Xiao, J. 2019. Dioscin inhibits the invasion and migration of hepatocellular carcinoma hepG2 cells by reversing TGF-ß1-induced epithelial-mesenchymal transition. *Molecules*. **24(12)**: 2222.

Chen, C. Y. 2011. TCM Database@Taiwan: the world's largest traditional Chinese medicine database for drug screening in silico. *Plos one*. 6e15939.

Ko, H 2015. Geraniin inhibits TGF-beta1-induced epithelial-mesenchymal transition and suppresses A549 lung cancer migration, invasion and anoikis resistance. *Bioorganic and Medicinal Chemistry Letter*. **25:**3529–3534.

Kou, Y., Ji, L., Wang, H., Wang, W., Zheng, H., Zou, J., & Lu, L. 2017. Connexin 43 upregulation by dioscin inhibits melanoma progression via suppressing malignancy and inducing M1 polarization. *International J. Cancer*. **141(8):** 1690-1703.

Kumari, R., Kaur, N. A., Sharma, N. N. and Sharma, S. 2017. Cloning, sequencing and structural properties of bowman birk trypsin inhibitor gene from seeds of Dolichos biflorus L. cultivar HPK4. *The Bioscan*. **12(4):** 1933-1938.

Li, M., Han, X. and Yu, B. 2003. Synthesis of monomethylated dioscin derivatives and their antitumor activities. *Carbohydrate research*, **338(2):** 117-121.

Lim, W. C., Kim, H., Kim, Y. J., Choi, K. C., Lee, I. H., Lee, K. H., ... and Ko, H. 2017. Dioscin suppresses TGF-ß1-induced epithelial-mesenchymal transition and suppresses A549 lung cancer migration and invasion. *Bioorganic & Medicinal Chemistry Letters*. **27(15):** 3342-3348.

Meng, X.Y., Zhang, H.X., Mezei, M., Cui, M. Molecular docking **2011.** A powerful approach for Structure-based drug discovery, *Current Computer-Aided Drug Design*. **7:**146-157.

Ojo, O. 2019. Nutrition and chronic conditions. *Nutrients*. **11(2019):** 459

Oyinloye, B. E., Adekiya, T. A., Aruleba, R. T., Ojo, O. A., & Ajiboye, B. O. 2019. Structure-Based Docking Studies of GLUT4 towards Exploring Selected Phytochemicals from Solanum xanthocarpum as a Therapeutic Target for the Treatment of Cancer. *Current Drug Discovery Technologies*. **16(4):** 406-416.

Peart, O. 2017. Metastatic breast cancer. Radiologic technology, **88(5):** 519M-539M

Rajendran, K., Rajaram, A., Dhanarajan, B., Madhiyalagan, H (2015. Protein ligand interaction using Autodock in bio-computing. *International J. Innovative Technology and Exploring Engineering*. **1:**67-71.

Song, X., Wang, Z., Liang, H., Zhang, W., Ye, Y., Li, H., & Wang, X. 2017. Dioscin induces gallbladder cancer apoptosis by inhibiting ROS-mediated PI3K/AKT signaling. *International j. biological sciences*. **13(6):** 782.

Spano, D., Heck, C., De Antonellis, P., Christofori, G. and Zollo, M. 2012. Molecular networks that regulate cancer metastasis. In Seminars in cancer biology (Vol. 22, No. 3, pp. 234-249).

Tao, X., Xu, L., Yin, L., Han, X., Qi, Y., Xu, Y., & Peng, J. 2017. Dioscin induces prostate cancer cell apoptosis through activation of estrogen receptor-ß. *Cell death & disease*. **8(8):** e2989-e2989.

Tse, J. C. and Kalluri, R. 2007. Mechanisms of metastasis: epithelial to mesenchymal transition and contribution of tumor microenvironment. *J. cellular biochemistry*. **101(4):** 816-829.

Yadav, A., Kumar, B., Datta, J., Teknos, T.N., Kumar, P. 2011. IL-6 promotes head and neck tumor metastasis by inducing epithelial-mesenchymal transition via the JAKSTAT3-SNAIL signaling pathway. *Mol Cancer Research*. **9:** 1658-1667.

Zhang, G., Zeng, X., Zhang, R., Liu, J., Zhang, W., Zhao, Y., & Du, B. 2016. Dioscin suppresses hepatocellular carcinoma tumor growth by inducing apoptosis and regulation of TP53, BAX, BCL2 and cleaved CASP3. *Phytomedicine*. **23(12):** 1329-1336.