In Silico Screening of Active Ingredients of Spices for WNK-1 Inhibition: Strategy to Validate Anti-Breast Cancer Potential

Asma Saqib[1], Kokila S[1], K Ramachandra Kini[2] and Shailasree Sekhar[3*]

[1]Department of Biochemistry, Maharani Cluster University, 39, Off Sheshadri Road, Palace Rd, opposite Freedom Park, Racecourse, Gandhi Nagar, Bengaluru-560001, Karnataka, India
[2]Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysuru- 570 006, Karnataka India
[3]Division of Biochemistry, School of Life Sciences, JSS Academy of Higher Education and Research, SS Nagara, Mysuru-570015, Karnataka, India

***Corresponding author**

Dr. Shailasree Sekhar, Assistant Professor,
Division of Biochemistry, School of Life Sciences,
JSSAHER, SS Nagara, Mysuru- 570015, Karnataka, India
ORCID Id: https://orcid.org/0000-0002-0727-4958

K Ramachandra Kini: https://orcid.org/0000-0003-1363-3352

Asma Saqib: https://orcid.org/0009-0000-0280-5054

Kokila S : https://orcid.org/0000-0001-6675-8832

Abstract: *With no lysine (WNK) kinases are serine/threonine kinases with unusual placement of a catalytic lysine residue (Lys233) at ATP binding site. WNK-1 close link to pathology of breast cancer renders them as potential targets in drug discovery. In the present study an attempt was made, using computational methods, to investigate the possible inhibitory potentials of several active ingredients of spices against WNK-1as means to validate their anti-breast cancer effects. The three dimensional structure of WNK-1 (PDB ID: 5TF9) with a resolution of 2.50Å was retrieved from RSCB Protein Data Bank, stabilized, adjusted to human physiology by PyMOL and hydrogen atoms were added at appropriate positions. The 3D structure of selected active compounds from spices for use as ligands were downloaded from PubChem, docked using PyRx software with a grid box size of 213, 163, 177 Å. The parameter of strong ligand binding affinity to protein building a stable complex as best inhibitor of WNK-1 was incorporated in the present study. Drug likeliness properties of the ligand by Swiss ADME analysis identified molecular weight being < 500 Daltons, with < 5 hydrogen bond donors, < 10 hydrogen bond acceptors and QPlogPo/w < 5. Lys-233 and Thr-386 from WNK-1 served as best binding residues. The analysis identified Luteolin-7-O-glucoside from cumin and gummosin from asafoetida having highest inhibitory potential with the binding energy of ΔG -9.3 and -9.1 kcal/mol respectively forming a stable complex with WNK-1. The standard inhibitor WNK463 recorded ΔG of -8.9kcal/mol. Luteolin-7-O-glucoside and gummosin having high inhibitory potential towards WNK-1 could be developed into drug moieties used for breast cancer treatment.*

Keywords: WNK-1 inhibition; molecular docking; WNK463; spices; active ingredients;

1. Introduction

Protein kinases with crucial roles in regulation of cellular processes and participation in many signal transduction pathways in cells are one of the key targets for development of anti-cancer drugs. Approximately 500 kinases recognised in human genome constitute ~1.7% of human genes [1]. With-no-lysine (WNKs) kinases are a newly described subfamily of serine/threonine protein kinases featuring the unusual placement of the catalytic lysine residue (Lys233) in its ATP interacting/binding site [2]. WNKs constitutively function in regulating homeostasis and blood pressure in mammals. A dysregulation of WNKs has been reported to activate complex pathways initiating uncontrolled cell division leading to tumour, metastasis and angiogenesis.

The mammalian genome expresses at least four WNK members with high sequence identity within the catalytic domains [3]. One of the very first reported WNK was rat WNK-1 with N-terminal kinase domain including an autoinhibitory domain and lengthy C-terminal tail [1]. The other WNKs, viz., WNK-2, WNK-3 and WNK-4 were described sharing 85% homology in their kinase domains [1,2].

Reports of aberrant expression of WNKs in human cancers makes these enzymes as clinical markers in tissues from cancer patients related to cancer prognosis. High expression of WNK-1 could predict patient poor survival (PS) suffering from hepatocellular carcinoma (HCC) and colorectal cancer suggesting a high pathological and advanced cancer stage [1].

Breast cancer, a deleterious condition in women and rarely occurring in men involves formation of a lump in breast and in armpit. There is breast pain with blood discharged from the nipple. The lump causes changes in breast shape and discharge results in changes in texture of the nipple. When detected early the treatment includes chemotherapy, hormone therapy, radiation and mostly surgery resulting in removal of breast [4].

Recent reports indicate prominent role for WNK-1 in aiding migration of epithelial-mesenchymal cells and providing stem cell like characters to breast cancer cells. These associations have identified WNK-1 as potential target for inhibition to alleviate breast cancer [5]. The inhibitors could be sourced from spices. The present study is aimed at *in silico* screening of active ingredients of spices for inhibitory potential against WNK-1 for possible development of anti-cancer drugs.

Spices from millennia have been used in small amounts for imparting unique taste and color to the food and their application varies from region to region. In India, a total 52 spices are placed under purview of Indian Spices Board by the act of Parliament. A total of 109 spices come under the spices list prepared by International Organization for Standardization (ISO). Innumerable research reports have time and again ascertained their effectiveness as folk medicine and to rejuvenate overall health [6]. The health benefits of spices are attributed to their active ingredients, consisting of flavonoids, alkaloids, phenols, terpenoids, anthocyanins, phenylpropanoids, sugar, fat, fibers, proteins, calcium, ash, vitamins –B, -C, carotene, proteins, essential oils among many others [7]. Several scientific examination of health benefits point to the inverse relation with onset of cancer, abnormal cell division, irregularities of cell cycle, improper apoptosis and cancer inducing signal pathways [8].

In the present study, using computational methods, several active ingredients of spices which were earlier reported to have anti-cancer effects, were screened for inhibitory activity towards WNK-1. Inhibitors of WNK-1 displaying a strong binding affinity and building a stable complex can be used as potent anti-breast cancer molecules that could be further developed as drug entities.

2. Materials and Methods

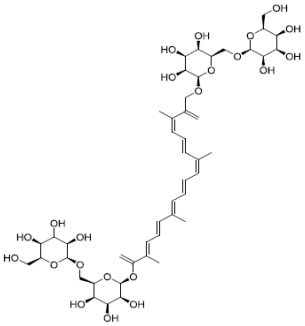
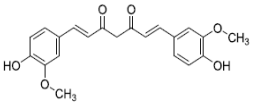
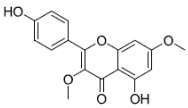
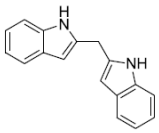
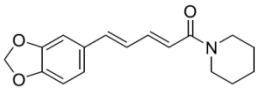
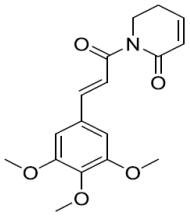
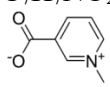
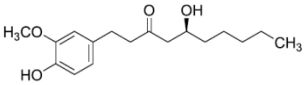
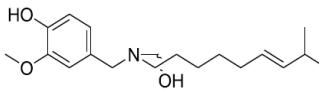
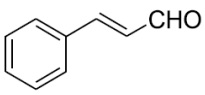
Ligand selection and retrieval

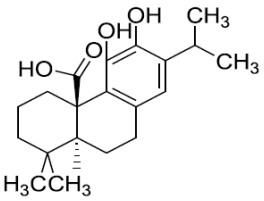
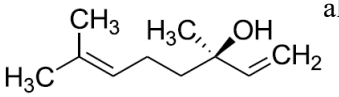
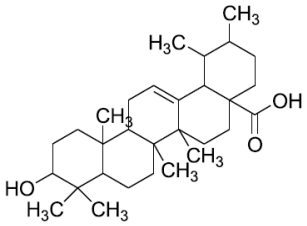
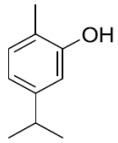
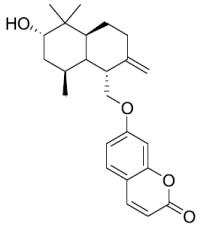
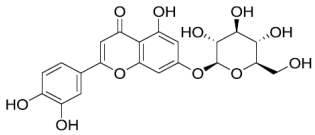
2.1. Active ingredients from spices

The present study includes active ingredients as ligands from spices coming under purview of the Indian Spices Board and their selection included a comprehensive survey and searches in prominent scientific databases such as Google Scholar, PubMed, Web of Science, Science Direct and Scopus. There is sufficient scientific literature providing data on spices and their active ingredients with anti cancer activities. In the present study, 15 active ingredients from different spices with reported anti-breast cancer property were identified for docking to WNK-1 (**Table 1**).

Table 1: Active ingredients in spices with reported anti-breast cancer property as ligands for docking to WNK-1

Sl. No.	Compound	Formula	Name of the molecule	Spice source	Reference
1	Crocin, (Pubchem CID: 17339399)	$C_{44}H_{64}O_{24}$	carotenoid	*Crocus sativus* (L.),	[9,10]

					
2	Curcumin (Pubchem CID: 839564)	$C_{21}H_{20}O_6$ 	polyphenol	*Curcuma longa* L.,	[11]
3	Kumatakenin (Pubchem CID: 4477326)	$C_{17}H_{14}O_6$ 	flavonoid	*Syzygium aromaticum* (L.), Merr. & L.M. Perry	[12]
4	Di-indolymethane (Pubchem CID: 8928276)	$C_{17}H_{14}N_2$ 	indoles	*Elettaria cardamomum* (L.) Maton,	[13]
5	Piperine (Pubchem CID: 553590)	$C_{17}H_{19}NO_3$ 	piperamide	*Piper nigrum* L.,	[14]
6	Piperlongumine (Pubchem CID: 553441)	$C_{17}H_{19}NO_5$ 	Alkaloid	*Piper nigrum* L.,	[15]
7	Trigonelline (Pubchem CID: 5369)	$C_7H_7NO_2$ 	alkaloid	*Trigonella foenum-graecum* L.	[16]
8	6-Gingerol (Pubchem CID: 391126)	$C_{17}H_{26}O_4$ 	phenol	*Zingiber officinale*, Roscoe,	[17]
9	Capsaicin (Pubchem CID: 1265957)	$C_{18}H_{27}NO_3$ 	alkaloid	*Capsicum annuum* L.,	[18]
10	Cinnamaldehyde (Pubchem CID: 637511)	C_9H_8O 	aldehyde	*Cinnamomum verum* J. Presl.,	[19]
11	Carnosic acid (Pubchem CID: 58635)	$C_{20}H_{28}O_4$	diterpenoid	*Rosmarinus officinalis* L.,	[20]

					
12	Linalool (Pubchem CID: 391430)	$C_{10}H_{18}O$ 	terpene alcohol	*Coriandrum sativum* L.,	[21]
13	Ursolic acid (Pubchem CID: 191497)	$C_{30}H_{48}O_3$ 	triterpenoids	*Mentha piperita* L.,	[22]
14	Carvacrol (Pubchem CID: 21105867)	$C_{10}H_{14}O$ 	monoterpenoids	*Origanum vulgare*,	[23]
15	Gummosin (Pubchem CID: 5442126)	$C_{24}H_{30}O_4$ 	sesquiterpene coumarins	*Ferula asafoetida* H. Karst.,	[24]
16	Luteolin-7-o-glucoside (Pubchem CID: 4444241)	$C_{21}H_{20}O_{11}$ 	flavonoid	*Cuminum cyminum* L.	[25]

2.2. WNK463

WNK463 (MW 463.46), a known potent inhibitor of WNK-kinase inhibiting all four WNK family members was used as a reference control in this study. It's *in vitro* IC_{50} varies for different WNKs viz., WNK-1 (5nM), WNK-2 (1nM), WNK-3 (6nM) and WNK-4 (9nM). WNK463 inhibits WNK-1 catalyzed phosphorylation of WNK-1 native substrate, the oxidative stress response-1 (OSR1) protein *in vitro* and also inhibit the same in human embryonic kidney-293 cells expressing exogenous OSR1 [26, 27].

2.3. Pharmacokinetic profile

Swiss ADME (http://www.swissadme.ch/index.php) was used to determine the pharmacokinetic profiles of the tested compounds by entering the simplified molecular input line entry system (SMILES) formula for each ligand retrieved from the PubChem database. Lipinski's Rule of Five analysis was conducted to determine the compounds pharmacokinetic properties [28, 29].

2.4. **Ligand and protein preparation**: The 3D structure of each phytochemical compound downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) or ChemSpider (http://www.chemspider.com) was saved in SDF format. Furthermore, the energy of the test ligand was minimized in ordered to get an ideal bond interaction effect using Open Babel. The compound

WNK463 (N-tert-butyl-3-(1-{5-[5-(trifluoromethyl)-1,3,4-oxadiazol-2-yl]pyridin-2-yl}piperidin-4-yl)imidazole-4-carboxamide)(ChemID58172620) was used as a control ligand inhibiting WNK-1 [30]. Protein WNK-1 – 5TF9 with a resolution of 2.50Å was obtained from Protein Data Bank web server (https://www.rcsb.org/) and its stabilization was carried out to adjust the same to body's physiology by using Pymol. It included replacing water atoms with hydrogen atoms.

2.5. Molecular docking

Specific docking was performed to ascertain the interaction between the protein WNK-1 and the ligands. The ligands and the protein were converted into pdb format using PyRx 0.8 docking tool with a built in Vina wizard. Vina, the AutoDock program doing docking within a space was defined by the coordinates and it provided the results in form of table but the coordinates were not a visual image. The protein and ligands were docked with a grid box size of 213, 163, 177 Å. The atomic interactions and electrostatic maps of the ligands were calculated using the autogrid module. Molecular graphics laboratory (MGL) tools, were used to analyze the results from Vina Wizard. The best conformation with lowest binding energy was exported for 2D plot generation using Ligplot+. The docking conformation was represented using PyMOL.

3. Results and Discussion

The main objective of the molecular docking analysis was to identify a ligand displaying a strong binding affinity to the target protein and building a stable complex. Drug-likeness parameters for afore mentioned 16 active ingredients of spices, dock scored ligands have been provided (**Table 2**). The most favourable active ingredient as ligand, with anticancer capacity was selected via ADMET profiling and molecular docking with specific protein WNK-1 enzyme.

Table 2: Pharmacokinetic Parameters of Active Ingredients of Spices Used in the Present Study

Compound	Formula	Number of hydrogen	No. of hydrogen donor	(Log P0/w)	(LogS)	GI Absorp-tion	Lipinski drug likeliness
Crocin	$C_{44}H_{64}O_{24}$	24	14	3.06	-3.01	Low	No
Curcumin	$C_{21}H_{20}O_6$	6	2	3.27	-3.94	High	Yes
Kumatakenin	$C_{17}H_{14}O_6$	6	2	2.81	-3.71	High	Yes
Di-indolymethane	$C_{17}H_{14}N_2$	0	0	3.59	-4.4	High	Yes
Piperine	$C_{17}H_{19}NO_3$	3	0	3.38	-3.74	High	Yes
Piperlongumine	$C_{17}H_{19}NO_5$	5	0	2.46	-2.91	High	Yes
Trigonelline	$C_7H_7NO_2$	2	0	3.11	-1.39	High	Yes
6-Gingerol	$C_{17}H_{26}O_4$	4	2	3.48	-2.96	High	Yes
Capsaicin	$C_{18}H_{27}NO_3$	3	2	3.15	-3.53	High	Yes
Cinnamaldehyde	C_9H_8O	1	0	1.65	-2.17	High	Yes
Carnosic acid	$C_{20}H_{28}O_4$	4	3	2.93	-5.03	High	Yes
Linalool	$C_{10}H_{18}O$	1	1	2.70	-2.4	High	Yes
Ursolicacid	$C_{30}H_{48}O_3$	3	2	3.71	-7.23	Low	Yes
Carvacrol	$C_{10}H_{14}O$	1	1	2.24	-3.31	High	Yes
Gummosin	$C_{24}H_{30}O_4$	4	1	3.77	-5.4	High	Yes
Luteolin-7-o-glucoside	$C_{21}H_{20}O_{11}$	11	7	1.83	-3.65	Low	No

All drug-likeness data for the ligands were found to be within the considerable range indicating their good drug-like properties. LogP, MW logs and gastrointestinal (GI) parameters indicate good membrane permeability, intestinal absorption and oral bioavailability. The ligands showed higher lipophilicity which could be accounted for a better biological activity due to the increased absorption via biological membranes. nHBDs and nRotb bonds facilitating drug metabolism and pharmacokinetics (DMPK) have been reported in the present study. The predicted ADMET data of the 16 compounds exhibited good aqueous solubility and gastrointestinal absorption, which could help ligands attain increased concentration in blood for optimal biological action. These ligands also exhibited poor blood brain barrier penetration indicating less probability of producing central nervous system toxicity.

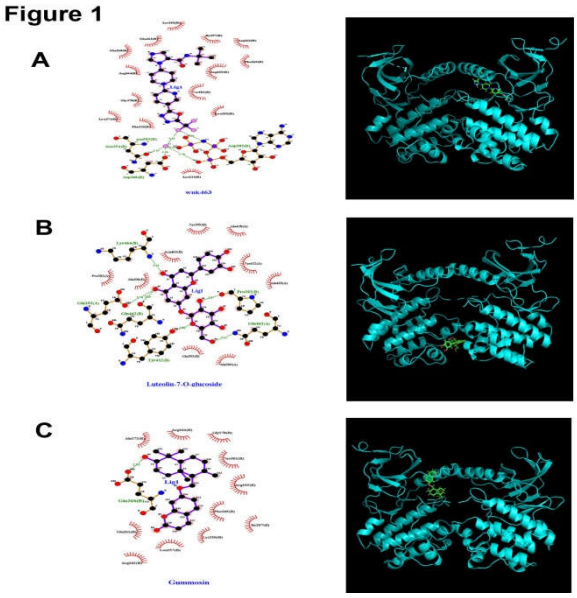


Figure 1: The molecular interactions and binding poses of (A) WNK463, (B) luteolin-7-O-glucoside; (C) Gummosin to WNK-1.

Drug likeliness properties of the ligand based on Swiss ADME analysis include their chemical properties like, molecular weight being < 500 Daltons, with < 5 hydrogen bond donors, < 10 hydrogen bond acceptors and QPlogPo/w < 5. The n-octanol/water partition coefficient (log P o/w) that is a key physicochemical parameter for drug discovery depicts lipophilicity indices of the ligand as within the range. The parameters measured for the ligand's solubility in water identifies the ligands to be an ideal for drug development.

Protein WNK-1 molecular docking with the ligands was performed using PyRx software. The findings indicate that Lys-233 and Thr-386 serve as binding residues in the Protein WNK-1. The ligands Luteolin-7-*O*-glucoside (Figure 1B) and gummosin (Figure 1C) exhibited highest binding energy of ΔG -9.3 and -9.1 kcal/mol respectively.

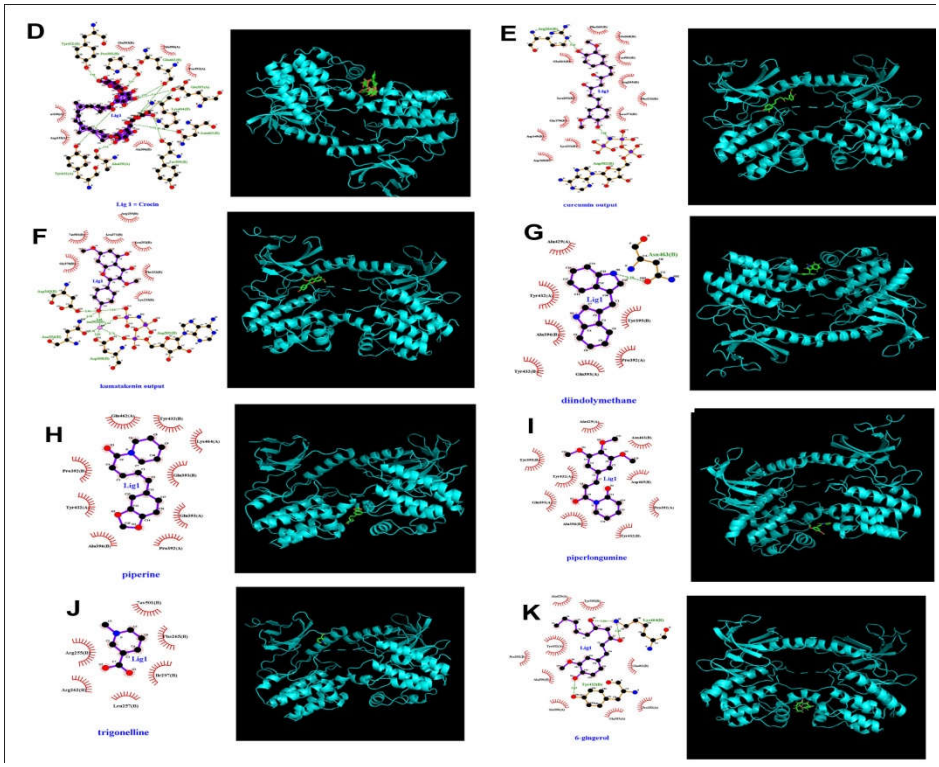


Figure 2: The molecular interactions and binding poses of (D) Crocin, (E) curcumin; (F) Gummosin; (G) Kumatakenin; (H) Di-indolymethane; (I) Piperine; (J) Piperlongumine; (K) Trigonelline to WNK-1

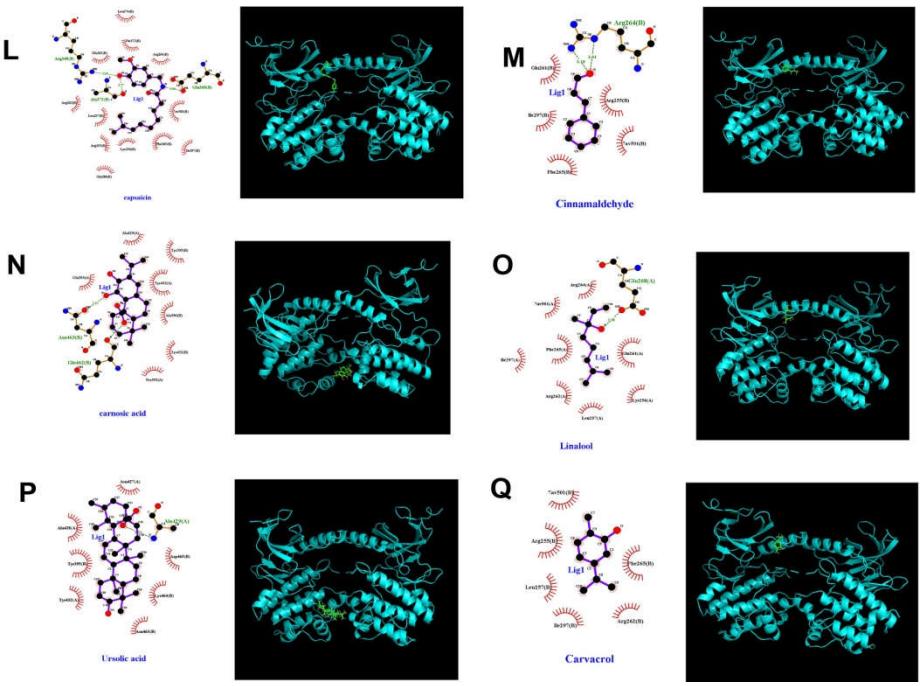


Figure 3: The molecular interactions and binding poses of (L) Capsaicin; (M) Cinnamaldehyde; (N) Carnosic acid; (O) Linalool; (P) Ursolic acid; (Q) Carvacrol

The control, WNK463 (**Figure 1A**), was identified to have a binding energy of ΔG -8.9 kcal/mol (**Table 3**). The binding profiles of other ligands toWNK-1 have been provided in **Figures 2 and 3**.

Table 3: Consolidated output of docking scores of the ligands to WNK-1

Sl. No.	Ligand name	Binding energy kcal/mol
	Control- WNK463	**-8.9**
Active ingredients from spices		
1	Crocin	-8.8
2	Curcumin	-7.5
3	Kumatakenin	-7.5
4	Di-indolylmethane	-8.1
5	Piperine	-7.8
6	Piperlongumine	-7.4
7	Trigonelline	-5.4
8	6-Gingerol	-6.0
9	Capsaicin	-7.6
10	Cinnamaldehyde	-6.2
11	Carnosic acid	-8.4
12	Linalool	-5.1
13	Ursolic acid	-8.4
14	Carvacrol	-6.6
15	Gummosin	-9.1
16	Luteolin-7-*O*-glucoside	-9.3

Earlier studies on docking of non-peptidic small-molecules have indicated disruption of SPAK/OSR1 binding to WNK-1 [30]. This disruption of binding leading to inhibition of SPAK and OSR1 phosphorylation was unravelled via pharmacoinformatics and simulation molecular dynamic methodologies. A library of 11,870 molecules was subjected to these analysis and three molecules (Hit 1-3) with drug likeliness was reported as novel antihypertensive moieties [30].

4. Conclusion

The present study forms a preliminary *in silico* molecular docking strategy of active ingredients from spices to WNK-1 that also includes reported WNK-1 inhibitor, WNK463. The results of docking identified Luteolin-7-*O*-glucoside and gummosin as better inhibitors of WNK-1. These initial findings could have significant bearings on the strategies for repurposing drugs against breast cancer. Our results of molecular docking could be further validated via advanced *in vitro* and animal experimentations that could be pivotal to increasing treatment options for breast cancer accelerating drug development against this malady.

Acknowledgements

The authors thank the Authorities, JSS Academy of Higher Education and Research, Mysuru, India for infrastructural and computational facilities.

Author Contributions

SS and KRK conceptualized and designed the study. AS and KS carried out the experiments and collected the data. SS and KRK analysed the data and finalized the manuscript. All authors have read and approved the final manuscript.

REFERENCES

[1] M. Xiu, L. Li, Y. Li and Y. Gao, "An update regarding the role of WNK kinases in cancer", Cell Death and Diseases., vol. 13, no.1, (2022), pp. 795-810.

[2] J. A. McCormick and D. H. Ellison, "The WNKs: atypical protein kinases with pleiotropic actions", Physiological Reviews., vol. 91, no.1, (2011), pp, 177–219.

[3] M. Murthy, T. Kurz and K. M. O'Shaughnessy, "WNK signaling pathways in blood pressure regulation", Cell and Molecular Life Sciences., vol. 74, no. 7, (2017), pp.1261–1280.

[4] S. Łukasiewicz, M. Czeczelewski, A. Forma, J. Baj, R. Sitarz and A. Stanisławek, "Breast cancer-epidemiology, risk factors, classification, prognostic markers, and current treatment strategies-an updated review", Cancers (Basel)., vol. 13, no. 17, (2021), pp. 4287-4317.

[5] A. B. Jaykumar, J. U. Jung, P. K. Parida, T. T. Dang, C. Wichaidit, A. R. Kannangara, S. Earnest, E. J. Goldsmith, G. W. Pearson and M. H. Scobb, "WNK1 enhances migration and invasion in breast cancer models", Molecular Cancer Therapeutics., vol. 20, no. 10, (2021), pp. 1800–1808.

[6] A. J. Kamath, B. Nair, P. Sreelekshmi and L. R. Nath, "Curry versus cancer: potential of some selected culinary spices against cancer with in vitro, in vivo, and human trials evidences", Journal of Food Biochemistry., vol. 45, no. 1, (2021), pp. 1-10.

[7] A. K. Sachan, S. Kumar, K. Kumari and D. Singh, "Medicinal uses of spices used in our traditional culture: Worldwide", Journal of Medicinal Plant Studies, vol. 6, no. 5, pp. 116–122.

[8] J. Zheng, Y. Zhou, Y. Li, D. P. Xu, S. Li and H. B. Li, "Spices for prevention and treatment of cancers", Nutrients., vol. 8, no. 8, (2016), pp. 495-501.

[9] R. Mokhtarian, S. Rajabi, S. Zahedian, S. J. Farsangi, M. Dadizadeh and M. Sadeghinejad, "The effect of saffron and its extracts on the treatment of breast cancer: A narrative review", Annales Pharmaceutiques Francaises., vol. 82, no. 4, (2024), pp. 629-640.

[10] M. A. Mir, S. A. Ganai, S. Mansoor, S. Jan, P. Mani, K. Z. Masoodi, H. Amin, M. U. Rehman and P. Ahmad, "Isolation, purification and characterization of naturally derived Crocetin beta-d-glucosyl ester from Crocus sativus L. against breast cancer and its binding chemistry with ER-alpha/HDAC2", Saudi Journal of Biological Sciences, vol. 27, no. 3, (2020), 975–984.

[11] R. Farghadani and R. Naidu, "Curcumin as an enhancer of therapeutic efficiency of chemostherapy drugs in breast cancer", International Journal of Molecular Sciences, vol. 23, no. 4, (2022), pp. 2144-2152.

[12] J. H. Woo, J. H. Ahn, D. S. Jang, K. T. Lee and J. H. Choi, "Effect of kumatakenin isolated from cloves on the apoptosis of cancer cells and the alternative activation of tumor-associated macrophages", Journal of Agriculture and Food Chemistry, vol. 65, no. 36, (2017), pp. 7893–7899.

[13] C. A. Thomson, E. Ho and M. B. Strom, "Chemopreventive properties of 3,3`-diindolymethane in breast cancer evidence from experimental and human studies", Nutrition Reviews, vol. 74, no. 7, (2016), pp. 432-443.

[14] K. Shaheer, B. R. S. Prabhu, H. S. Ali and M. D. Divya Lakshmanan, "Breast cancer cells are sensitized by piperine to radiotherapy through estrogen receptor-α mediated modulation of a key NHEJ repair protein- DNA-PK", Phytomedicine, vol. 122, no. 5, (2024), pp. 155-160.

[15] S. K. Tripathi and B. K. Biswal, "Piperlongumine, a potent anticancer phytotherapeutic: perspectives on contemporary status and future possibilities as an anticancer agent", Pharmacology Research, vol. 156, no. 6, (2020), pp. 104772-104782.

[16] H. P. Manivannan, V. P. Veeraraghavan and A. P. Francis, "Identification of molecular targets of trigonelline for treating breast cancer through network pharmacology and bioinformatics-based prediction", Molecular Diversity, vol. 28, no. 6, (2023), pp. 3835-3857.

[17] P. Huang, P. Zhou, Y. Liang, J. Wu, G. Wu, R. Xu, Y. Dai, Q. Guo, H. Lu and Q. Chen, "Exploring the molecular targets and mechanisms of gingerol for treating triple-negative breast

cancer using bioinformatics approaches, molecular docking and in vivo experiments", Transtional Cancer Research, vol. 10, no. 11, (2021) pp. 4680-4693.
[18] D. Wu, H. Jia, Z. Zhang and S. Li, "Capsaicin suppresses breast cancer cell viability by regulating the CDK8/PI3K/Akt/Wnt/b-catenin signalling pathway", Molecular Medicine Reports, vol., 22, no. 6, (2020) pp. 4868-4876.
[19] S. Banerjee and S. Banerjee, "Anticancer potential and molecular mechanisms of cinnamaldehyde and its congeners present in the cinnamon plant", Physiologia, vol. 3, no. 2, (2023), pp. 173-207.
[20] A. C. Corveloni, S. C. Semprebon, A. Baranoski, B. I. Biazi, T. A. Zanetti, M. S. Mantovani, "Carnosic acid exhibits antiproliferative and proapoptotic effects in tumoral NCI-H460 and nontumoral IMR-90 lung cells", Journal of Toxicology and Environmental Health, Part A, vol. 83, no. 10, (2020), pp. 412–421.
[21] H. Elbe, F. Ozturk, G. Yigitturk, T. Baygar and T. Cavusoglu, "Anticancer activity of linalool: Comparative investigation of ultrastructural changes and apoptosis in breast cancer cells", Ultrastructure Pathology, vol. 46, no. 4, (2022), pp. 348-358.
[22] S. Wang, X. Chang, J. Zhang, J. Li, N. Wang, B. Yang, B. Pan, Y. Zheng, X. Wang, H. Ou and Z. Wang, "Ursolic acid inhibits breast cancer metastasis by suppressing glycolytic metabolism via activating SP1/Caveolin-1 signalling", Frontiers in Oncology, vol. 11, no. 2, (2021), pp. 745584-745591.
[23] L. Li, L. He, Y. Wu and Y. Zhang, "Carvacrol affects breast cancer cell through TRPM7 mediated cell cycle regulation", Life Sciences, vol. 266, no. 6, (2021), pp. 118894- 118901.
[24] M. Iranshahy, F. Farhadi, B. Paknejad, P. Zareian, M. Iranshahi, M. Karami and S. R. Abtahi, "Gummosin, a sesquiterpene coumarin from Ferula assafoetida is preferentially cytotoxic to human breast and prostate cancer cell lines", Avicenna Journal Phytomedicine, vol. 9, no. 5, (2019), pp. 446-453.
[25] S. Goodarzi, M. J. Tabatabaei, J. R. Mohammad, F. Shemirani, S. Tavakoli, M. Mofasseri and Z. Tofighi, "Cuminum cyminum fruits as source of luteolin-7-O-glucoside, potent cytotoxic flavonoid against breast cancer cell lines", Natural Product Research, vol. 34, no. 6, (2020), pp. 1602–1606.
[26] K. Yamada, H. M. Park, D. F. Rigel, K. DiPetrillo, E. J. Whalen, A. Anisowicz, D. A. Burdick, "Small-molecule WNK inhibition regulates cardiovascular and renal function", Nature Chemical Biology, vol. 12, no. 4, (2016), pp. 896–898.
[27] K. Yamada, J. H. Zhang, X. Xie, J. Reinhardt, A. Q. Xie, D. LaSala, D. Kohls, D. Yowe, D. Burdick, H. Yoshisue, H. Wakai, I. Schmidt, J. Gunawan, K. Yasoshima, K. Q. Yue, M. Kato, M. Mogi, N. Idamakanti, N. Kreder, P. Drucekes, P. Pandey, T. Kawanami, W. Huang, Y. I. Yagi, Z. Deng and H. M. Park, "Discovery and characterization of allosteric WNK kinase inhibitors", ACS Chemical Biology, vol. 11, no. 6, (2016), pp. 3338-3346.
[28] C. A. Lipinski, "Drug-like properties and the causes of poor solubility and poor permeability", Journal of Pharmacology and Toxicologica Methods, vol. 44, no. 6, (2000), pp. 235-249.
[29] D. Butina, M. D. Segall and K. Frankcombe, "Predicting ADME properties in silico: methods and models", Drug Discovery Today, vol. 7, no. 11, (2002), pp. 83-88.
[30] M. A. Alamri, "Pharmacoinformatics and molecular dynamic simulation studies to identify potential small-molecule inhibitors of WNK-SPAK/OSR1 signaling that mimic the RFQV motifs of WNK kinases", Arab Journal of Chemistry, vol., 13, no. 6, (2020), pp. 5107-5117.