Proteomics and Bioinformatics
PB, 5(1): 210-217
www.scitcentral.com

ISSN: 2641-7561

Original Research Article: Open Access

Assessment of Trypsin-Fragmented Peptides from *Nigella Sativa* Proteins as Anticancer Peptides: An *In Silico* Approach

Haitham Al-Madhagi*

*Biochemical Technology Program, Faculty of Applied Sciences, Dhamar University, Dhamar, Yemen.

Received October 14, 2022; Revised December 09, 2022; Accepted December 12. 2022

ABSTRACT

Nigella sativa has been well established to potent have anticancer activity through different mechanisms. However, this bioactivity is traced to its phytochemicals. So, this study purpose was to assess the peptides generated from *Nigella sativa* proteins as anticancer peptides. 6 *Nigella sativa* proteins sequences were retrieved from Uniprot database and subjected to proteolysis by trypsin enzyme using ExPASy peptide cutter tool. The physico-chemical characteristics were calculated via Protparam server. To assess anticancer activity of the generated peptides, mACPred and ACPred webservers were employed. 11 of the 23 produced peptides posed anticancer activity. The tertiary structure of the best 3 peptides was predicted using Pepfold 3.0 platform and further docking against 3 types of breast cancer receptor was performed by PatchDock and FireDock servers. The generated peptides have shown good inhibition toward the tested receptors. The current study approved the anticancer activity of the fragmented peptides of *N sativa*.

Keywords: *N sativa*, Cancer, Anticancer peptides, Docking, Adjuvant therapy

INTRODUCTION

Nigella sativa (popularly known as black seeds) is an annual flowering plant that can be grown in wide areas of the world. It is native to the Mediterranean region in addition to India and Pakistan. It is well-studied spice [1]. A repertoire of studies proved the cardio-protective, hepatoprotective, hypoglycemic, hypolipidemic, antimicrobial and anti-histmaine activities of *N.sativa* extracts [2-4].

Extracts of *N.sativa* contain various types of bioactive phytochemicals such as: amino acids, proteins, volatile and non-volatile oils, carbohydrates, alkaloids and minerals [5]. The seeds of *N.staiva* are composed mainly of protein which makes it a rich source of proteins (26.7%). Besides, *N.sativa* contains fat, carbohydrates and fibers (28.5%, 24.9% and 8.4%) [6]. Interestingly, the protein content of *N.sativa* is popular with its immunomodulatory actions [7].

With regard to cancer, a plethora of evidences had demonstrated the positive widespread effects of *N.sativa* extracts or pure active constituents against cancer in many different ways and mechanisms [8].

Cancer is the first/second cause of mortality rate, after cardiovascular diseases, in about 112 countries worldwide according to the statistics of World Health Organization (WHO) [9]. The underlying cause of cancer is still an emerging question with no crucial answer. It is attributed to alterations in either genetic or epigenetic network [10,11]. The current chemotherapies of cancer are toxic to non-cancerous tissues besides being expensive to purchase. Moreover, anti-cancer drug resistance emerge after ongoing administration of these treatment options [12]. This necessitates the use of adjuvant natural therapy based on plant sources which has an enhancing impact [13]. Furthermore, chemotherapeutics are monotarget in nature, in contrast to the multitarget capabilities of natural options that can regulate many stages and pathways of cancer progression [14].

Anticancer peptides (ACP) are a short stretch (less than 35 amino acids) found in nature or generated through digestive proteolytic activity of certain proteases that has cytotoxic bioactivity toward cancer cells [15]. In comparison with immunotherapeutic monoclonal antibodies, ACP are more selective, less toxic and highly penetrable to cancerous tissues [16]. The field of ACP is emerging nowadays and many publications are accumulated in unprecedented speed

Corresponding author: Haitham Al-Madhagi, Biochemical Technology Program, Faculty of Applied Sciences, Dhamar University, Dhamar, Yemen, Tel: 00963985714557; E-mail: bio.haitham@gmail.com

Citation: Al-Madhagi H. (2023) Assessment of Trypsin-Fragmented Peptides from *Nigella Sativa* Proteins as Anticancer Peptides: An *In Silico* Approach. Proteomics Bioinformatics, 5(1): 210-217.

[17]. Indeed, many of ACP from different sources showed positive efficacy against a variety of cancer types are nowadays in the clinical application setting [18].

Many papers highlighted the phytochemicals anticancer action of *N.sativa*, but the peptides of *N.sativa* as anticancer option has not yet been investigated and, thus, this is the goal of this study.

MATERIALS AND METHODS

Methodology of current study

The current study employed many sequential databases and webservers to assess the anticancer bioactivity of *N.sativa* peptides as illustrated in **Figure 1**.

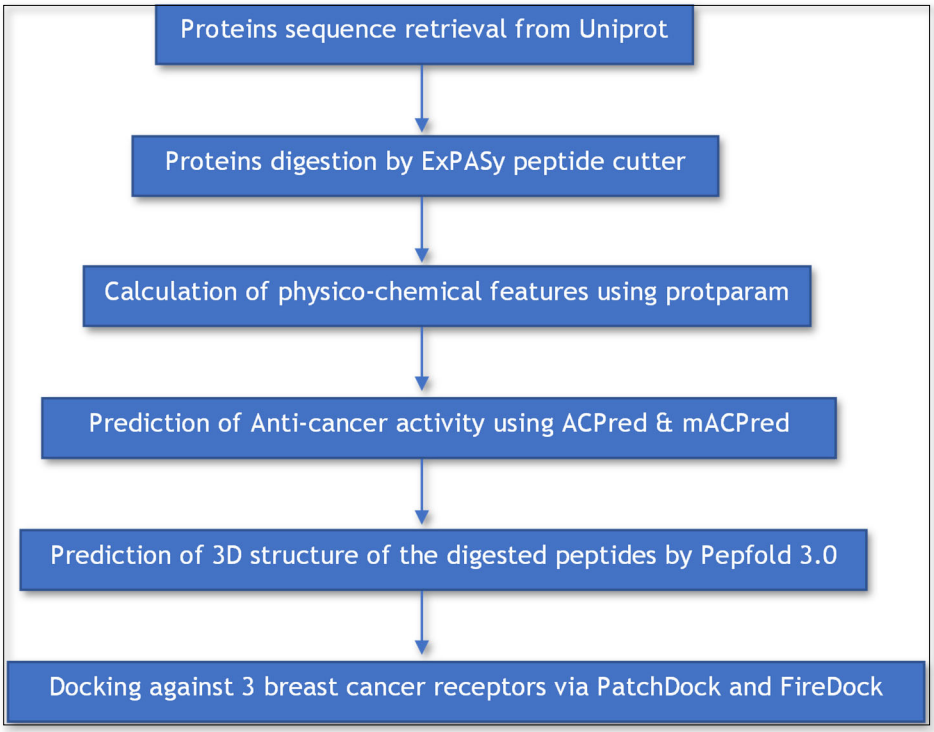

Figure 1. Workflow of the methodology employed in the current study.

Retrieval of protein sequences

A total of 6 proteins served as precursors for the sought peptides: Thionin NsW1 (Uniprot ID# C0HJH9, 35 AA), Defensin D1 (Uniprot ID# P86972, 50 AA), Defensin D3r (Uniprot ID# A0A173AE14, 79 AA), CYC-like protein 1 (Uniprot ID# A0A166XVF4, 112 AA), Nigellin 1.1 (Uniprot ID# A0A1S4NYD1, 38 AA) and PsbA (Uniprot ID# A0A2U8T5R6, 23 AA). All of the protein sequences were retrieved from Uniprot database [19] using the corresponding ID.

Proteolysis

The selected proteins were subjected to proteolytic cleavage by trypsin using the webserver ExPASy peptide cutter tool [20]. Only the peptides having 5 or more AA were selected for further analysis.

Physico-chemical properties of fragmented peptides

The Physical and chemical properties of the digested peptides were calculated via the online tool Protparam [21] which include molecular weight (MW), theoretical isoelectric point (pI), aliphatic index and grand average of hydropathicity (GRAVY).

Assessment of ACP bioactivity

Two online tools were utilized for prediction of anti-cancer activity of the digested peptides: mACPred [22] and ACPred [23]. The two servers use different algorithms and prediction methods so only the best mutual results will be considered for further analysis.

3D structure modeling and prediction

The top best peptides that gave highest score of anti-cancer activity were adapted to predict the 3D structure *de novo* using Pepfold 3.0 server [24].

Docking

Given that the fragmented peptides have anti-cancer activity, 3 cancer receptors were chosen for as targets for molecular docking in order to confirm the anti-cancer activity of the produced peptides. PatchDock [25]was used as a platform for peptide-protein docking. Results of PatchDock were

refined using FireDock server [26] to give the best 10 solutions along with their global energy and its details.

Visualization of docking interaction

Of the docked models, only the models giving the highest global energy got their peptide-protein interaction visualized via Discovery Studio 2021 software.

RESULTS AND DISCUSSION

To treat cancer efficiently, chemotherapeutic agents must reach the cancer microenvironment at high concentrations with no or less accumulation in off-target tissues [27]. In a variety of means, ACP can control cancer progression. Some can promote or block certain cancer receptors or signaling pathways while others serve as vehicles to carry therapeutics inside cancer cells or tissues [28]. Also, compared to proteins or antibodies, ACP is easier and cheaper to produce and design [29]. This glorifies the importance of ACP in the current research. So the aim of this paper is to produce peptides from *N.sativa* proteins and testing their anticancer activities.

Physico-chemical properties of the fragmented peptides

A set of 23 peptides were cleaved from 6 precursor proteins using trypsin. The amino acid range of the generated peptides was between 5-27. The theoretical pI values of most of the digested peptides were alkalic (>7) suggesting the abundance in basic AA of resulting peptides. The half-life was estimated *in vitro* of mammalian reticulocytes and most of the peptides had a half-life of 1-2 h. The rest of the characteristics are shown in **Table 1**.

Table 1. The sequence and physico-chemical properties of the digested peptides.

No	Fragmented Peptide Sequence	AA	MW	pI	Estimated Half-Life	Aliphatic Index	GRAVY
1	FTTFFTLLFVSLVLFSAFETPTMVEAK	27	3087.66	4.53	1.1 h	97.41	1.204
2	WCERPSGTWSGVCDNSGK	18	1969.13	6.96	2.8 h	16.11	-0.967
3	CKDQCIR	7	865.03	8.06	1.2 h	55.71	-0.843
4	LEGAK	5	516.59	6	5.5 h	98	-0.44
5	HGSCNYK	7	807.88	8.21	3.5 h	0	-1.514
6	FPAHR	5	626.72	9.76	1.1 h	20	-0.94
7	CICYYEC	7	896.06	4	1.2 h	55.71	0.843
8	FCEKPSGTWSGVCGNSGACKDQCIR	25	2633.97	7.88	1.1 h	12.65	-0.408
9	HGSCNYKPPAHR	12	1366.52	9.31	3.5 h	8.33	-1.642
10	TCSGLCGCK	9	871.05	7.64	7.2 h	43.33	0.567
11	NTLGR	5	559.62	9.75	1.4 h	78	-1.06
12	NCYNTCR	7	872.97	8.06	1.4 h	0	-1.214
13	LSLPIAR	7	768.95	9.75	5.5 h	181.43	1
14	FFMVQDMLGYDK	12	1493.76	4.21	1.1 h	56.67	0.108
15	TVEWLLTK	8	989.18	5.66	7.2 h	133.75	0.263
16	EAINELSR	8	931.01	4.53	1 h	110	-0.713
17	SVSHSK	6	643.7	8.49	1.9 h	48.33	-0.883
18	FSYTGSVK	8	887.99	8.59	1.1 h	36.25	-0.113
19	AAFNPLAK	8	830.98	8.8	4.4 h	86.25	0.375
20	YQDCLSECNSR	11	1317.41	4.37	2.8 h	35.45	-1.145
21	CTYIPDYAGMR	11	1289.49	5.83	1.2 h	44.55	-0.236
22	ACIGLCAPACLTSR	14	1378.69	8.01	4.4 h	105	1.214
23	NAHNFPLDLAAVEVPSTNG	19	1966.14	4.35	1.4 h	87.37	-0.084

Assessment of ACP bioactivity

Table 2 summarizes the ACP of the fragmented peptides based on the two tools mACPred and ACPred.

Table 2. ACP bioactivity of the digested peptides.

No	Fragmented Peptide Sequence	mACPred		ACPred	
		Activity	Score	Activity	Score
1	FTTFFTLLFVSLVLFSAFETPTMVEAK	Non-ACP	0.2375	Non-ACP	0.626
2	WCERPSGTWSGVCDNSGK	ACP	0.5939	ACP	0.926
3	CKDQCIR	ACP	0.6029	ACP	0.874
4	LEGAK	ACP	0.8712	ACP	0.911
5	HGSCNYK	ACP	0.9865	ACP	0.975
6	FPAHR	ACP	0.9691	ACP	0.967
7	CICYYEC	ACP	0.9838	ACP	0.994
8	FCEKPSGTWSGVCGNSGACKDQCIR	ACP	0.5977	ACP	0.949
9	HGSCNYKPPAHR	ACP	0.9595	Non-ACP	0.572
10	TCSGLCGCK	ACP	0.9762	ACP	0.994
11	NTLGR	Non-ACP	0.4069	ACP	0.897
12	NCYNTCR	ACP	0.92	ACP	0.935
13	LSLPIAR	ACP	0.969	Non-ACP	0.027
14	FFMVQDMLGYDK	Non-ACP	0.1438	ACP	0.667
15	TVEWLLTK	ACP	0.7322	ACP	0.777
16	EAINELSR	Non-ACP	0.0513	ACP	0.785
17	SVSHSK	ACP	0.6116	ACP	0.977
18	FSYTGSVK	Non-ACP	0.3447	ACP	0.977
19	AAFNPLAK	ACP	0.9723	Non-ACP	0.603
20	YQDCLSECNSR	ACP	0.6005	Non-ACP	0.395
21	CTYIPDYAGMR	Non-ACP	0.4576	Non-ACP	0.656
22	ACIGLCAPACLTSR	ACP	0.9831	Non-ACP	0.253
23	NAHNFPLDLAAVEVPSTNG	Non-ACP	0.1513	Non-ACP	0.001

Of the 23 digested peptides, 16 exhibited anticancer activity estimated via mACPred tool. Similarly, 15 peptides exhibited anticancer activity in ACPred server. Among those, there were 11 mutual peptides with anticancer activity. It should be noted that these findings were extracted from only 6 proteins suggesting the effectiveness of *N.sativa* peptides against cancer.

3D structure modeling and docking

The top 3 (peptide 5, peptide 7 and peptide 10) peptides with mutual high score of anticancer activity were selected for 3D structure prediction using Pepfold 3.0 platform and further for docking studies. Docking studies were performed via PatchDock and the further refinement of docked poses through FireDock platforms. 3 breast cancer receptors were chosen as target for docking: epidermal growth factor receptor (EGFR; PDB ID#4HJO) estrogen receptor-α (ER-α; PDB ID# 3ERT) and matrix metalloproteinase-3 (MMP-3; PDB ID# 2D1O). ER-α activation is closely linked to the various stages of breast cancer [30]. Likewise, inappropriate activation of EGFR drives the tumorigenesis of lung and breast, among others [31]. With regard to MMP-3, overexpression of this extracellular enzyme has been

correlated with many cancer types including breast cancer especially in the metastasis stage [32]. Results of docking are elucidated in **Table 3**.

Table 3. PatchDock as well as FireDock results of the best anticancer peptides.

Peptides	PatchDock			FireDock				
	Score	**Area**	**ACE**	**Global Energy**	**Attractive VdW**	**Repulsive VdW**	**ACE**	**HB**
	EGFR							
Peptide 5	7600	992.4	-122.52	-19.35	-22.85	25.33	0.22	-2.13
Peptide 7	6794	831.8	-126.24	-56.01	-24.31	5.60	-12.86	-1.25
Peptide 10	7726	918.1	-43.34	-10.70	-22.41	9.27	11.81	-4.82
	ER-α							
Peptide 5	7834	999.2	-275.33	-63.55	-27.06	9.59	-17.47	-0.25
Peptide 7	7200	964.3	-420.73	-23.91	-29.78	72.17	-21.71	-1.8
Peptide 10	7022	945.7	-449.44	-63.35	-23.21	7.73	-20.42	-0.35
	MMP-3							
Peptide 5	6330	867.4	-337.62	-52.11	-25.57	10.42	-9.55	-1.93
Peptide 7	6220	695	-58.32	-38.91	-23.04	4.12	-4.92	-3.42
Peptide 10	6542	803.2	-181.46	-38.16	-19.16	8.64	-9.15	-0.34

ACE: Atomic Contact Energy; VdW: Van der Waal; HB: Hydrogen Bonds

As shown in **Table 3**, peptide 7 greatly blocks EGFR with a global energy of -56.01 kcal/mole while the remaining peptides are not good enough for EGFR inhibition. With respect to ER-α, peptide 5 as well as peptide 10 are superior inhibitors with a global energy -63.55 and -63.35 kcal/mole. Of the top 3 peptides, peptide 5 are good candidate for inhibition of MMP-3 (global energy -52.11 kcal/mole) whilst peptides 7 and 10 had a global energy of -38.91 and -38.16 kcal/mole. Collectively, peptide 7 is a good candidate for blockade of EGFR whereas peptide 5 can be used as inhibitor of both ER-α and, along with peptide 10, MMP-3. Peptide-protein interactions of best poses are shown in **Figure 2**. These data suggest that the studied peptides fragmented from *N.sativa* can be utilized as ACP against breast cancer receptors. Surprisingly, peptide 7 and peptide 5 are even better than the reference inhibitors of EGFR and ER-α (Erlotinib and TAM) in terms of global energy estimated by FireDock (-50.90 and -60.2 kcal/mole; data not shown).

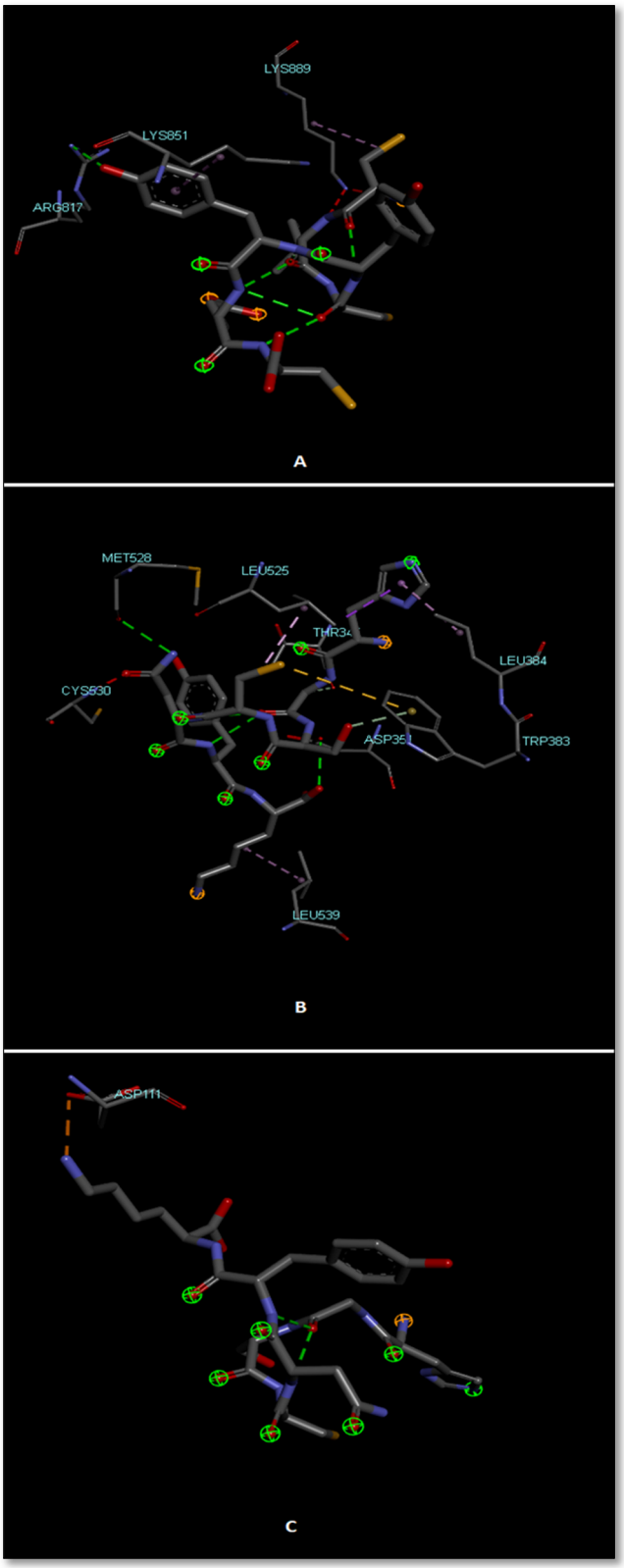

Figure 2. Peptide-protein docking 3D interaction visualized by discovery studio 2021. Docking results of peptide 7 against EGFR (A), peptide 5 against ER-α (B), and peptide 5 against MMP-3 (C).

EGFR interacted with peptide 7 through VdW interactions imposed by two lysine residues within the active site pocket and one arginine residue through H-bonds. Similarly, Asp 351 and Met 528 formed H-bonds with peptide 5 and the rest interacting residues interacted via VdW forces with ER-α. Regarding MMP-3, peptide 5 formed electrostatic attractions with Asp 111 **(Figure 2)**.

Accordingly, the present work demonstrates *in-silico* the anti-cancer activity of the peptides fragmented from some *N.sativa* proteins as predicted by ACPred and mACPred webservers and then validated by peptide-protein docking through PatchDock-FireDock platform. This candidates N.sativa as a superior source as therapeutic nutraceutical option against breast cancer theoretically.

CONCLUSION

In conclusion, 6 *N.sativa* proteins after proteolysis using trypsin enzyme gave rise to 23 peptides for which physico-chemical properties were calculated. 11 of which had ACP bioactivity as predicted by mACPred and ACPred platforms. Among them, peptides 5 (HGSCNYK), peptide 7 (CICYYEC), and peptide 10 (TCSGLCGCK) were the best in terms of the possibility score as ACP against 3 types of breast cancer receptors, EGFR, ER-α and MMP-3 as demonstrated by PatchDock and FireDock servers which necessitate *in vitro* assays confirmation. However, further analysis of the top peptides against wide array of other cancer types should be addressed. Also, the peptides with low anti-cancer activity should be assessed for different bioactivities.

REFERENCES

1. Khan MA, Chen H, Tania M, Zhang D (2011) Anticancer activities of *Nigella sativa* (black cumin). Afr J Tradit Complement Altern Med 8: 226-232.
2. Majdalawieh AF, Fayyad MW, Nasrallah GK (2017) Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of *Nigella sativa*. Crit Rev Food Sci Nutr 57: 3911-3928.
3. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, et al. (2013) A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Trop Biomed 3: 337-352.
4. Butt MS, Sultan MT (2010) *Nigella sativa*: Reduces the risk of various maladies. Crit Rev Food Sci Nutr 50: 654-665.
5. Gali-Muhtasib H, Roessner A, Schneider-Stock R (2006) Thymoquinone: A promising anti-cancer drug from natural sources. Int J Biochem Cell Biol 38: 1249-1253.
6. Nickavar B, Mojab F, Javidnia K, Amoli MAR (2003) Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. Zeitschrift Für Naturforschung C 58: 629-631.
7. Salem ML (2005) Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Int Immunopharmacol 5: 1749-1770.
8. Majdalawieh AF, Fayyad MW (2016) Recent advances on the anti-cancer properties of *Nigella sativa*, a widely used food additive. J Ayurveda Integr Med 7: 173-180.
9. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, et al. (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249.
10. Rizvi M, Alam MS, Mehdi SJ, Ali A, Batra S (2012) Allelic loss of 10q23. 3, the PTEN gene locus in cervical carcinoma from Northern Indian population. Pathol Oncol Res 18: 309-313.
11. Rahmani AH, Alzohairy M, Babiker AYY, Khan AA, Aly SM, et al. (2013) Implication of androgen receptor in urinary bladder cancer: A critical mini review. Int J Mol Epidemiol Genet 4: 150.
12. Jo Y, Choi N, Kim K, Koo H-J, Choi J, et al. (2018) Chemoresistance of cancer cells: Requirements of tumor microenvironment-mimicking *in vitro* models in anti-cancer drug development. Theranostics 8: 5259.
13. Lee Y-W, Chen T-L, Shih Y-RV, Tsai C-L, Chang C-C, et al. (2014) Adjunctive traditional Chinese medicine therapy improves survival in patients with advanced breast cancer: A population-based study. Cancer 120: 1338-1344.
14. Rahmani AH, Alzohairy MA, Khan MA, Aly SM (2014) Therapeutic implications of black seed and its constituent thymoquinone in the prevention of cancer through inactivation and activation of molecular pathways. Evid Based Complement Alternat Med 2014: 724658.
15. Tyagi A, Tuknait A, Anand P, Gupta S, Sharma M, et al. (2015) CancerPPD: A database of anticancer peptides and proteins. Nucleic Acids Res 43: D837-D843.
16. Thundimadathil J (2012) Cancer treatment using peptides: Current therapies and future prospects. J Amino Acids 2012: 967347.
17. Karami M, Chaleshgar M, Salari N, Akbari H, Mohammadi M (2022) Global Prevalence of Anemia in Pregnant Women: A Comprehensive Systematic Review and Meta-Analysis. Matern Child Health J 26(7): 1473-1478.
18. Chiangjong W, Chutipongtanate S, Hongeng S (2020) Anticancer peptide: Physicochemical property,

functional aspect and trend in clinical application (Review). Int J Oncol 57: 678-696.

19. Consortium U (2019) UniProt: A worldwide hub of protein knowledge. Nucleic Acids Res 47: D506-515.
20. Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, et al. (2005) Protein identification and analysis tools on the ExPASy server. The Proteomics Protocols Handbook 2005: 571-607.
21. Garg VK, Avashthi H, Tiwari A, Jain PA, Ramkete PW, et al. MFPPI–multi FASTA ProtParam interface. Bioinformation 12: 74.
22. Boopathi V, Subramaniyam S, Malik A, Lee G, Manavalan B, et al. (2019) mACPpred: A support vector machine-based meta-predictor for identification of anticancer peptides. Int J Mol Sci 20: 1964.
23. Schaduangrat N, Nantasenamat C, Prachayasittikul V, Shoombuatong W (2019) ACPred: A computational tool for the prediction and analysis of anticancer peptides. Molecules 24: 1973.
24. Lamiable A, Thévenet P, Rey J, Vavrusa M, Derreumaux P, et al. (2016) PEP-FOLD3: Faster de novo structure prediction for linear peptides in solution and in complex. Nucleic Acids Res 44: W449-W454.
25. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ (2005) PatchDock and SymmDock: Servers for rigid and symmetric docking. Nucleic Acids Res 33: W363-W367.
26. Andrusier N, Nussinov R, Wolfson HJ (2007) FireDock: Fast interaction refinement in molecular docking. Proteins 69: 139-159.
27. Sutradhar KB, Amin M (2014) Nanotechnology in cancer drug delivery and selective targeting. Int Sch Res Notices 939378: 1-12.
28. Hayashi MA, Ducancel F, Konno K (2012) Natural peptides with potential applications in drug development, diagnosis, and/or biotechnology. Int J Pept 2012: 757838.
29. Grisoni F, Neuhaus CS, Gabernet G, Müller AT, Hiss JA, et al. (2018) Designing anticancer peptides by constructive machine learning. ChemMedChem 13: 1300-1302.
30. Liu Y, Ma H, Yao J (2020) ERα, A Key Target for Cancer Therapy: A Review. Onco Targets Ther 13: 2183-2191.
31. Sigismund S, Avanzato D, Lanzetti L (2018) Emerging functions of the EGFR in cancer. Mol Oncol 12: 3-20.
32. Suhaimi SA, Chan SC, Rosli R (2020) Matrix Metallopeptidase 3 Polymorphisms: Emerging genetic Markers in Human Breast Cancer Metastasis. J Breast Cancer 23: 1-9.