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Research Article
Evaluation of Colorectal Cancer Inhibition Ability of *Rosmarinus officinalis* L. via Molecular Docking and Pharmacophore Analysis

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Abstract

Background and Objective: Colorectal cancer is one of the most common cancers in the world. Mutated proteins of certain genes that control cell apoptosis have been identified as the cause of colorectal cancer. Natural compounds that interact and denature these proteins can be used to inhibit the activities of these proteins and help prevent tumour growth with limited side effects. However, searching for such new compounds through *in vitro* or *in vivo* tests is time-consuming and costly. **Materials and Methods:** In this study, 30 known compounds from the herbal plant *Rosmarinus officinalis* L. were used to study the inhibitory ability of certain types of colorectal cancer-causing proteins using the drug design simulation method. Due to the computer-based drug design simulation method, target disease-causing proteins can be simulated to interact with a variety of compounds from herbal medicinal plants to detect compounds with high affinity and low energy required for interaction. Following that, these potential compounds can be used for anti-cancer drug research. **Results:** Five compounds i.e., rosmarinic acid, carnosic acid, (E,E)-5,9,13-pentadecatrien-2-one,6,10,14-trimethyl, α-amorphene and α-bis-abolol had high affinity and strong interaction with target proteins which resulted in a high ability to denature and inactivate those unexpected proteins. The docking pharmacophore features were also analyzed for clarifying the affinity results. **Conclusion:** These potential compounds were proposed for further research on drugs for treating colorectal cancer. The drug design simulation method helps to shorten the time and cost significantly in the selection of drug compounds for testing on living cells and animals.

Key words: *Rosmarinus officinalis*, colorectal cancer, computer-aided drug design, molecular docking, pharmacophore, apoptosis, *S. allylcysteine*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Colorectal Cancer (CRC) is a tumour that develops in the colon, rectum, or appendix. This is the third most common cancer, the second common cause of death in Western countries[1]. The incidence of CRC is on the rise worldwide, especially in developing countries[2]. In Vietnam, CRC is ranked as the fifth group of cancer with the number of new cases causing death at a rate of 4.1% among all types of cancers (https://gco.iarc.fr/). Like other types of cancer, CRC is caused by the changes in the genetic system that leads to uncontrol of cell division. The deletion mutation of gene loci related to tumour suppressor genes in the chromosome was reported to relate to the development of CRC in some previous studies, especially genes relating to cell proliferation and apoptosis such as *BRAF*, *TP53*, *KRAS* and *ALK*[3-6]. The *BRAF* gene is a proto-oncogene belonging to the Serine/Threonine Kinase family. *BRAF* protein expressed somatic mutations in a variety of tumours, primarily malignancies[1]. The mutated β-*catenin* gene increased cell proliferation and inhibits apoptosis. This gene mutation accounts for up to 10% of all CRC cases[7]. The *TP53* gene encodes a protein that aids in the cell cycle and apoptosis[8]. The *TP53* gene mutation was found in more than 50% of cases of CRC, this is considered a marker in the development of tumours to cancer[9]. The *KRAS* gene encoding the *Ras* protein is responsible for the control of cell growth, differentiation and apoptosis. Some human cancers have been shown to relate to the expression of mutated *Ras* protein (oncogenic *Ras*). The appearance of mutant *Ras* proteins accounts for 15-20% in malignant tumours[10] and mutation of the *KRAS* gene accounts for 25-60% of cases of CRC[11]. The genetic information of protein Anaplastic Lymphoma Kinase (ALK) which is involved in cell growth is from the gene *ALK*. Mutations (changed mutation) of the *ALK* gene and protein have been found in several types of cancer, including neuroblastoma and lung cancer. The appearance of the mutant *ALK* protein increased the growth of cancer cells[12]. These genes encode proteins that control cell proliferation and apoptosis and in turn, mutated proteins cause uncontrolled cell proliferation leading to tumour creation. The inactivation of these mutant proteins will help prevent the growth of tumours[13].

The common cancer treatments include chemotherapy and radiotherapy. However, these methods often adversely affect the health of patients. Therefore, many studies have suggested the use of natural compounds in tumour suppression. These compounds can interact with mutated proteins that cause cancer, leading to the inhibition of tumour growth but little damage to the human body. Some compounds extracted from aged garlic (*Allium sativum*), especially S-allylcysteine and S-allylmercapto-L-cysteine have been shown to prevent the growth of certain types of cancer[14,15]. The flavonoids from papaya seeds also showed positive results when treated on some cancer cell lines in mice[16]. Rosemary (*Rosmarinus officinalis* L.) is a popular plant in Vietnam that is often used for ornamental purposes, spice in cooking, or for repelling insects. In 2016, this plant was also proved to inhibit CRC cells in mice[17] by the two compounds rosmarinic acid and carnosic acid through *in vitro* test. However, there are still many other compounds of rosemary which are abundant and have not been put into research. Rosemary essential oil accounts for 27% of the plant, contains camphor (5.0-21%), 1.8-cineole (15-55%), α-pinene (9.0-26%), borneol (1.5-5.0%), camphene (2.5-12%), β-pinene (2.0-9.0%), limonene (1.5-5.0%)[18] and other bioactive substances such as rosmarinic acid (8%), carnosic acid (30%), carnosol (17%) and ursolic acid (6%)[19], which and can be extracted from different organs i.e., the leaves, stems and flower stalks.

Even so, searching for potential anti-cancer compounds through *in vitro* and *in vivo* tests is extremely time-consuming and costly[14-17]. With the development of computer science, simulation approaches have been effectively applied in many areas of life, including medical science, which can overcome those mentioned problems. The Structure-Based Drug Design (SBDD) method allows the batch simulation of docking between many plant compounds and disease-causing molecules just in hours[20]. The docking pharmacophores with higher affinity, i.e., lower binding energy required, are potential results for protein denaturation leading to inactivation of the target molecules. From initial docking results, potential compounds can be used to perform further wet experiments which require significantly less time and cost. This Computer-Aided Drug Design (CADD) method, which is a combination of computer science, chemistry, biology has been proven to be important for the development of new drugs from herbal plants. In this study, we simulated the binding affinity between compounds of rosemary and some mutated proteins causing a colorectal tumour.

The study aims to propose potential compounds for inhibiting tumours of CRC, serving for further steps of drug treatment on this dangerous disease.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Biotechnology, Nguyen Tat Thanh University, Vietnam from July, 2020-June, 2021).

Ligands and proteins preparation: Thirty compounds of rosemary used as ligands in this study (Table 1) were

Table 1: Information and 2-D structure downloaded from the ZINC database of thirty studied ligand compounds of the rosemary plant

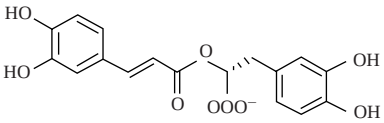
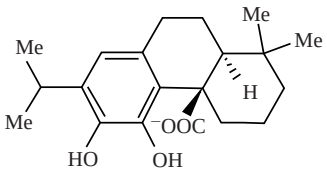
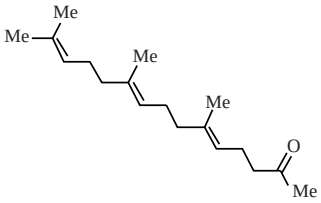
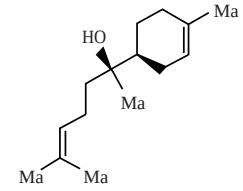
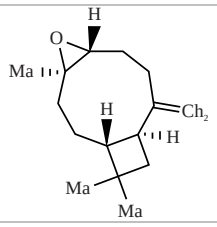
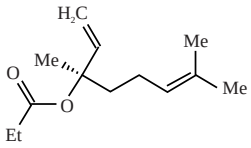
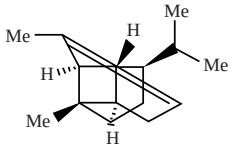
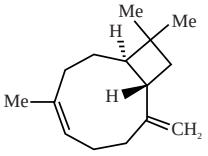
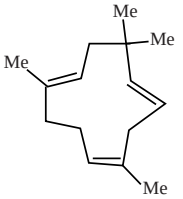
Number	Zinc	Name	Structure	Molecular weight (dalton)	xlogP
1	ZINC00899870	Rosmarinic acid		359.31	1.63
2	ZINC03984016	Carnosic acid		331.432	4.6
3	ZINC12358879	(E,E)-5,9,13- Pentadecatrien -2-one, 6 10, 14-trimethyl		262.437	6
4	ZINC01849759	α-bis-Abolol		222.372	4.68
5	ZINC02083320	Caryophyllene oxide		220.356	4.14
6	ZINC01677809	Linalyl propionate		210.317	4.28
7	ZINC57988166	Copaene		204.357	5.75
8	ZINC08234282	Caryophyllene		204.357	5.17
9	ZINC30726967	Alpha-caryophyllene		204.357	5.31

Table 1: Continue

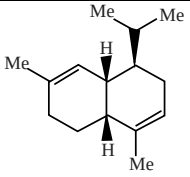
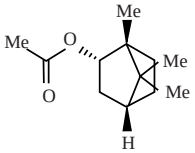
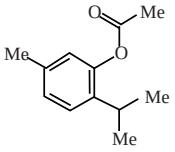
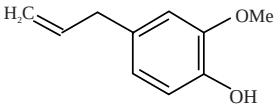
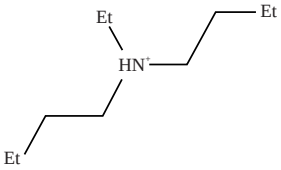
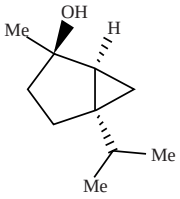
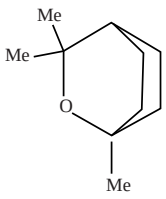
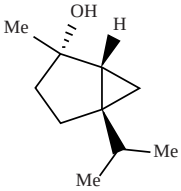
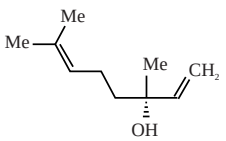
Number	Zinc	Name	Structure	Molecular weight (dalton)	xlogP
10	ZINC70455185	α-Amorphene		204.357	5.97
11	ZINC00388664	L-Bornyl acetate		196.29	3.05
12	ZINC00899536	5-Methyl-2-(1-methylethyl)-phenol, acetate		192.258	2.91
13	ZINC00001411	o-Methyl eugenol		164.204	2.1
14	ZINC02510141	di-n-Butylethylamine		158.309	3.59
15	ZINC30724426	Sabinene hydrate		154.253	2.32
16	ZINC00967566	Eucalyptol		154.253	2.72
17	ZINC00968131	4-Thujanol		154.253	2.32
18	ZINC01529819	α-Linalool		154.253	3.21

Table 1: Continue

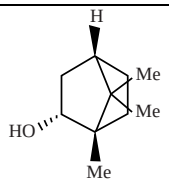
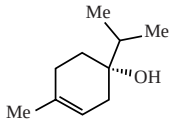
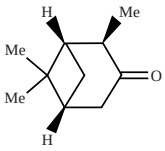
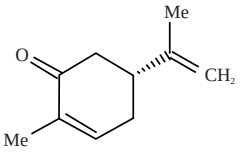
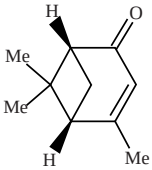
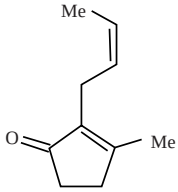
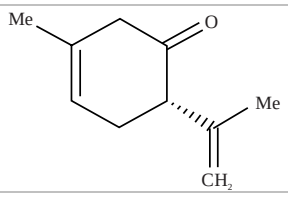
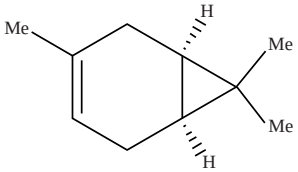
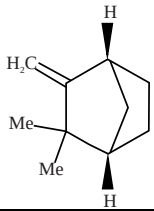
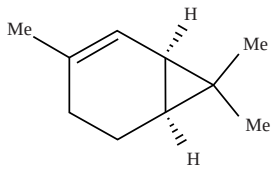
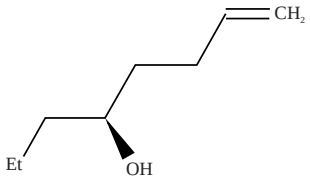
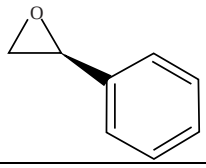
Number	Zinc	Name	Structure	Molecular weight (dalton)	xlogP
19	ZINC00967533	L-Borneol		154.253	2.35
20	ZINC03861537	Terpinen-4-ol		154.253	2.6
21	ZINC02034811	3-Pinanone		152.237	2.39
22	ZINC14588455	Carvone		150.221	2.51
23	ZINC00967600	Verbenone		150.221	2.44
24	ZINC33845547	(Z)-Cinerone		150.221	2.06
25	ZINC18157343	Piperitenone		150.221	2.51
26	ZINC00967562	3-Carene		136.238	3.45
27	ZINC00968230	Camphene		136.238	3.33

Table 1: Continue

Number	Zinc	Name	Structure	Molecular weight (dalton)	xlogP
28	ZINC59586951	2-Carene		136.238	3.45
29	ZINC02003408	oct-7-en-4-ol		128.215	2.53
30	ZINC00901249	3,4-Dimethoxy styrene		120.151	1.74

referenced from many published sources[18,19,21]. Molecular information of ligand was downloaded from ZINC database (http://zinc.docking.org/) including chemical structure, xlogP, aromatic rings, number of rotation bonds and was then saved as A Tripos Mol2 format. All the amide bonds of each ligand were made to not rotate using AutoDockTools 1.5.6 software[22]. The data was then turned into PDBQT (Protein Data Bank (PDB), Partial Charge (Q) and Atom Type (T)) format, which is a supported format for running on the AutoDock 4.0 software and increasing the storage capacity of atomic coordinates, partial charge, atomic types of docking molecules in comparison with previous format (http://autodock. scripps. edu/).

Six mutated proteins involved in causing CRC including a mutated form of each four proteins β-catenin (PDB molecular ID i.e., 1JPW), TP53 (4IBW), *KRAS* (4TQ9), ALK (5FTO) and two mutated forms of *BRAF* protein (5HID and 4R5Y)[23-28] were considered as receptors for docking in this study (Table 2). Other molecular information and 3D structure of these proteins were also recorded from PDB (http://www.rcsb.org/) including resolutions, chains, existed ligands and determination methods. Each protein was prepared using AutoDockTools software 1.5.6 to achieve optimal simulation through 4 steps: (1) Adding polarized hydrogens, (2) Fusing non-polar hydrogens, (3) Removing water molecules and (4) Creating grid boxes. Adding polarized hydrogen bonds is important for docking since hydrogen bonds play a major role in stabilizing protein-ligand complexes[29]. As water molecules do not join the docking, the removal of water molecules from proteins makes computational accounts easier and avoids interference in searching for ligand molecules, which can create more favourable contact with protein receptors[30]. Grid boxes were established for verifying docking regions on 6 target proteins with 30×30×30 dimensions and default spacing at 1.000 Å (Table 3). Creating a grid box helps the program to determine the appropriate binding space between protein and ligand, thereby providing optimal binding results[31]. The data was then saved in PDBQT format for docking in the next step.

Molecular docking and pharmacophore analysis: One ligand was docked with one receptor in the space of one grid box for each running. The rigid docking simulation between a target protein and ligand was first performed using the AutoDock Vina program[32]. Result data of docking was converted into PDB (Protein Data Bank) format using OpenBabel program[33] and was visualized by BIOVIA Discovery Studio Visualizer software[34]. Pharmacophore features of the simulation were analyzed based on the affinity and molecular interactions. For further analyses, flexible docking was next conducted. In the flexible docking, besides one protein receptor and one ligand, a flexible amino acid inside the receptor was required as a flexible factor to be included in the running setup[35]. The amino acids that are tightly bound to ligand from the result of rigid docking were chosen for this flexible docking step. Pharmacophore features of flexible docking were analyzed in comparison with the previous rigid pharmacophore.

Table 2: Information obtained from the PDB database of six mutated proteins involved in causing CRC in the study

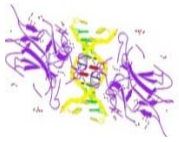
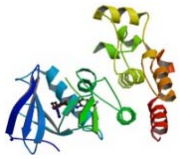
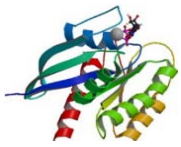
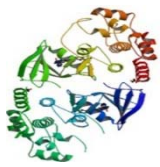
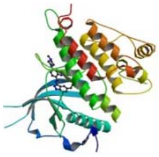
PDB accession	Resolution	Chains	Existed ligands	Structure
1JPW	2.5 Å	A, B, C	-	
4IBW	1.791 Å	A, B	A: Zn ion A, B: 1,2-Ethanediol	
4R5Y	3.5 Å	A, B	A, B: $C_{25}H_{17}F_3N_4O_3$	
4TQ9	1.491 Å	A, B	A, B: GDP, Mg ion	
5HID	2.5 Å	A, B	A, B: B1E, PEG	
5FTO	2.22 Å	A	A: YMX	

Table 3: Coordinate and dimension information of 10 grid boxes established for verifying docking regions on 6 target proteins in the study

Protein accession	Grid box	Center x	Center y	Center z	Size (Å)
5HID	GRID 1	3.056	-13.417	-9.417	30×30×30
	GRID 2-full	3.917	-1.667	-11.861	30×30×30
1JPW	GRID 1-full	153.194	-1.861	6.528	30×30×30
4IBW	GRID 1	-26.139	-7.5	-23.889	30×30×30
	GRID 2	-23.859	1.53	-15.86	30×30×30
4R5Y	GRID 1	19.776	13.364	-15.307	30×30×30
	GRID 2-full	17.361	0.444	-1.361	74×12×18
4TQ9	GRID 1	0.417	-10.028	37.889	30×30×30
	GRID 2-full	-5.502	-22.603	26.972	42×36×14
5FTO	GRID 1	6.676	19.601	8.223	30×30×30

RESULTS

Rigid docking results: The rigid docking results of 30 ligands with 6 target proteins at different grid boxes, respectively were shown in detail in Table 4. In general, rosmarinic acid and carnosic acid showed good binding results with all six examined proteins. Rosmarinic acid gave the highest affinity with 4TQ9 protein at the lowest binding

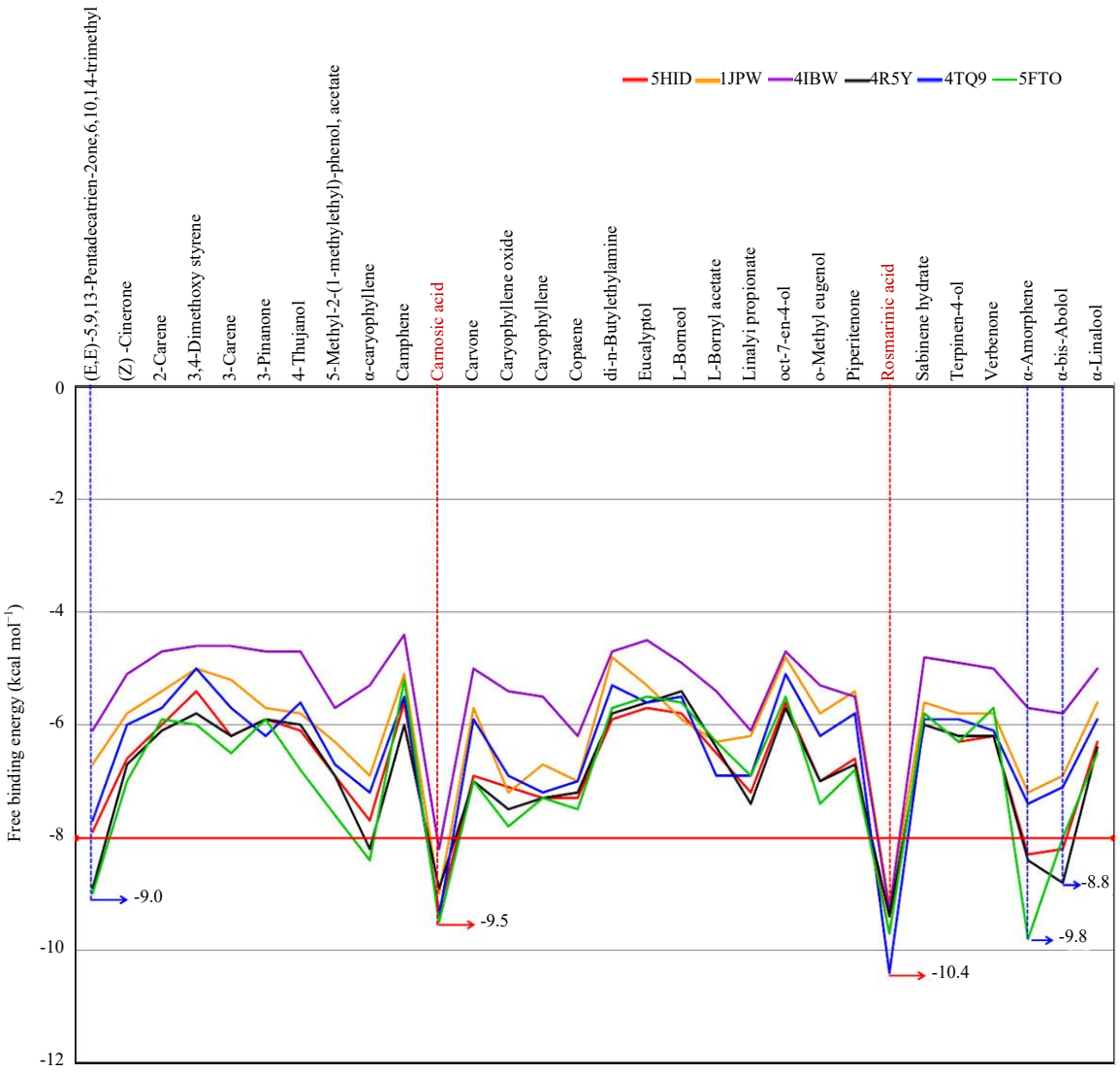


Fig. 1: Free binding energy between 30 ligands of Rosemary plant and 6 CRC carcinogenic proteins by Rigid docking simulation
The lower the free binding energy was required, the higher the binding affinity was

energy -10.4 kcal mol^{-1} and with the remaining proteins at around -9.7 and -8.8 kcal mol^{-1} (Fig. 1). The following was carnosic acid which had the highest affinity for binding to 5FTO protein at -9.5 kcal mol^{-1} and to other proteins at a range from -9.4 and -8.9 kcal mol^{-1}. Besides rosmarinic acid and carnosic acid, four other compounds i.e., (E, E)-5,9,13-pentadecatrien-2-one,6,10,14-trimethyl; α-caryophyllene, α-amorphene and α-bis-abolol which had the binding energy lower than -8.0 kcal mol^{-1} with some of the mutated protein were also used for further flexible docking and pharmacophore analyzing.

Flexible docking and pharmacophore analysis: The absolute values of free binding energy referred from flexible docking were all better than that of rigid docking (Fig. 2). The differences ranged from 0.1 up to 1.7 kcal mol^{-1}. The details were presented in Table 5. Rosmarinic acid gave the best affinity result with 4TQ9 at $^{-}$11.1 kcal mol^{-1} instead of -10.4 kcal mol^{-1} from rigid docking (Fig. 2). The following was a complex of carnosic acid and 1JPW at -10.7 kcal mol^{-1}, which was better than rigid docking by a distance of 1.7 kcal mol^{-1}. Flexible docking of other three ligands α-abolol, α-amorphene and (E, E)-5,9,13-pentadecatrien-2-one,6,10,14-trimethyl also created a favourable affinity with 4R5Y, 5FTO and 4R5Y respectively at -10.0, -9.9 and -9.4 kcal mol^{-1}, corresponding. Though the docking result was better, the flexible binding energy of α-caryophyllene with both of target proteins 5FTO and 4R5Y

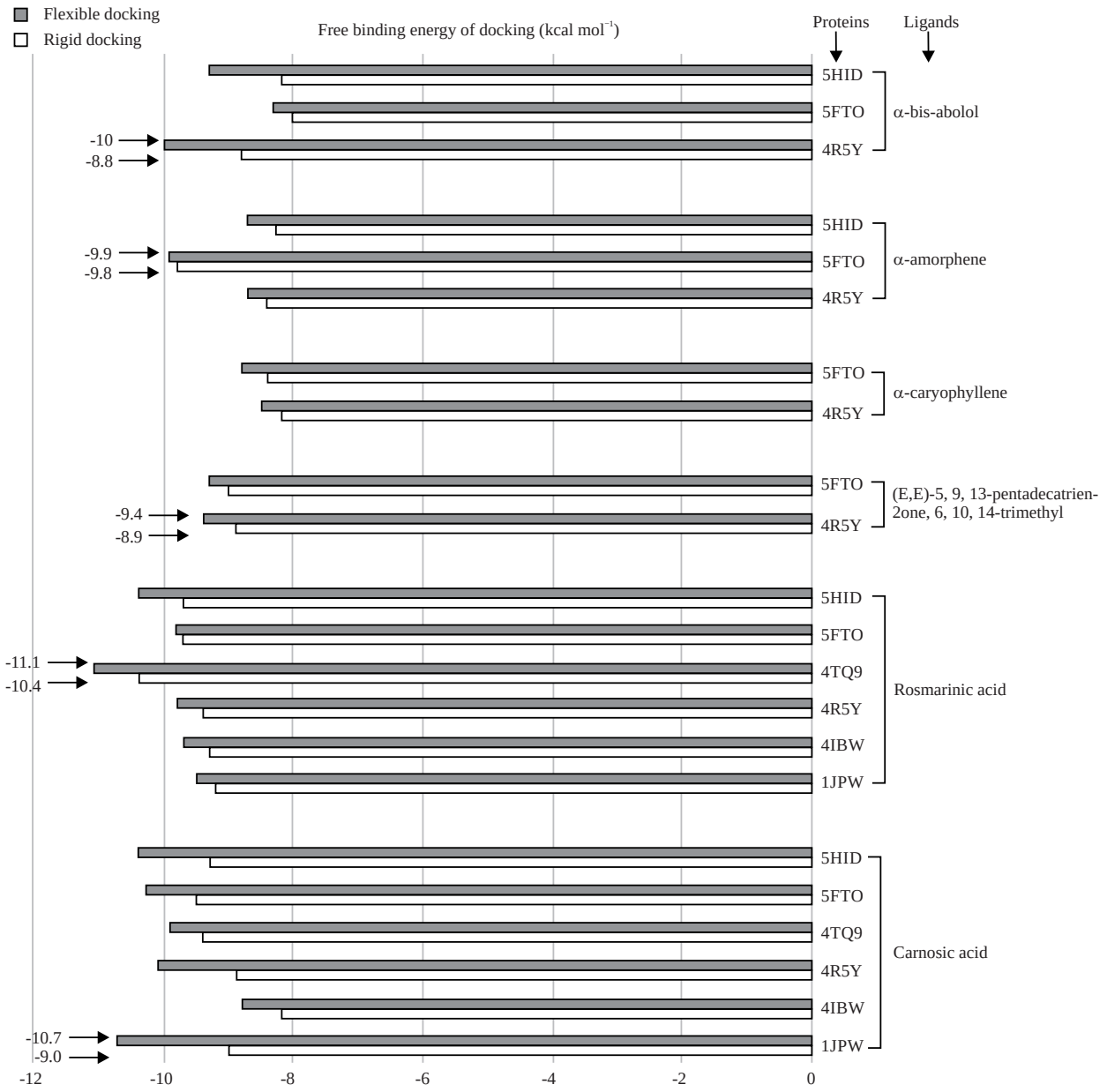


Fig. 2: Best free-binding energy of 6 potential ligands with target proteins based on flexible docking and rigid docking

were still not reached -9.0 kcal mol^{-1}, this compound was not included in the following analysis.

The pharmacophore of some high binding complexes was analyzed for more clarity of the binding mechanism. In comparison with rigid docking (Fig. 3a), flexible docking of rosmarinic acid with 4TQ9 produced 3 additional van der Waals bonds and 1 attractive charge (Fig. 3b). Thus, even though less than 1 Pi-cation and an additional unfavourable bump were present, the interaction affinity of this flexible complex was still better by about 0.7 kcal mol^{-1}. In the complex between carnosic acid and 1JPW (Fig. 3c-d), despite the reduction of 1 van der Waals bond and the appearance of two more unfavourable bumps in flexible docking, there was an increased range of molecular bonds including 2 Hydrogen bonds, 1 Akyl bond, 1 Pi-Alkyl bond and 1 charge bond (Fig. 3d), resulting in a significant increase of the interaction affinity (from -9.0 and -10.7 kcal mol^{-1}). This showed that flexible docking creates more sites of interaction between ligand and protein than rigid docking.

The same happened when comparing rigid and flexible pharmacophore in the complexes of 5FTO with α-amorphene (Fig. 3e-f) and 4R5Y with (E,E)-5.9,13-pentadecatrien-2-one,

Table 4: Free-binding energy (kcal mol^{-1}) between 30 ligands of rosemary plant and 6 CRC carcinogenic proteins by rigid docking simulation

Protein accession	Grid box	Ligands																												
		(E,E)- 5, 9, 13-Pentadecatrien-2-one, 6, 10, 14-trimethyl	(Z)-Cinerone	2-Carene	3,4-Dimethoxy styrene	3-Carene	3-Pinanone	4-Thujanol	5-Methyl-2-(1-methylethyl)-phenol, acetate	alpha-caryophyllene	Camphene	Carnosic acid	Carvone	Caryophyllene oxide	Caryophyllene	Copaene	di-n-Butylethylamine	Eucalyptol	L-Borneol	L-Bornyl acetate	Linalyl propionate	oct-7-en-4-ol	o-Methyl eugenol	Piperitenone	Rosmarinic acid	Sabinene hydrate	Terpinen-4-ol	Verbenone	α–Amorphene	α–bis-Abolol	α–Linalool
5HID	GRID 1	-7.9	-6.6	-6	-5.4	-6.2	-5.9	-6.1	-6.9	-7.7	-5.6	**-9.3**	-6.9	-7.1	-7.3	-7.3	-5.9	-5.7	-5.8	-6.5	-6.5	-5.6	-7	-6.6	**-9.7**	-5.8	-6.3	-6.2	**-8.3**	**-8.2**	-6.3
	GRID 2 full	-7.6	-6.6	-6	-5.5	-6.3	-5.9	-6	-6.7	-7.6	-5.6	**-9.1**	-6.9	-7.1	-7.2	-7.3	-5.9	-5.7	-5.8	-6.5	-6.5	-5.7	-7	-6.6	**-8.3**	-5.7	-6.2	-6.2	**-8.3**	**-8.3**	-6.3
1JPW	GRID 1 full	-6.7	-5.8	-5.4	-5	-5.2	-5.7	-5.8	-6.3	-6.9	-5.1	**-9**	-5.7	-7.2	-6.7	-7	-4.8	-5.3	-5.9	-6.3	-6.3	-4.8	-5.8	-5.4	**-9.2**	-5.6	-5.8	-5.8	-7.2	-6.9	-5.6
4IBW	GRID 1	-6.3	-5.1	-4.5	-4.4	-4.7	-4.6	-4.6	-5.3	-5.3	-4.4	-7	-5	-5.4	-5.4	-5.4	-4.6	-4.4	-4.9	-5.1	-5.1	-4.5	-5.1	-5.4	-7.7	-4.7	-4.7	-4.9	-5.8	-5.6	-4.9
	GRID 2	-6.1	-5.1	-4.7	-4.6	-4.6	-4.7	-4.7	-5.7	-5.3	-4.4	**-8.2**	-5	-5.4	-5.5	-6.2	-4.7	-4.5	-4.9	-5.4	-5.4	-4.7	-5.3	-5.5	**-9.3**	-4.8	-4.9	-5	-5.7	-5.8	-5
4R5Y	GRID 1	**-8.9**	-6.7	-6.1	-5.8	-6.2	-5.9	-6	-6.9	**-8.2**	-6	**-8.9**	-7	-7.5	-7.3	-7.2	-5.8	-5.6	-5.4	-6.4	-6.4	-5.7	-7	-6.7	**-9.4**	-6	-6.2	-6.2	**-8.4**	**-8.8**	-6.4
	GRID 2-full	-6.5	-5.2	-5	-4.9	-5.3	-5.4	-5.4	-6.1	-6.7	-4.8	**-8.8**	-5.6	-6.5	-6.5	-5.9	-4.8	-5.4	-5.2	-5.9	-5.9	-4.4	-5.6	-5.7	**-8.9**	-5.4	-5.6	-5.4	-6.6	-6.6	-5.5
4TQ9	GRID 1	-7.8	-6.4	-5	-5.4	-5.6	-5.2	-6	-6.4	-7.1	-4.7	**-8.1**	-6.1	-6.2	-6.2	-6.9	-5.2	-4.8	-5.1	-6.2	-6.2	-4.9	-7.2	-5.9	**-8.8**	-5.3	-5.6	-5.5	-7.2	-7.5	-5.6
	GRID 2-full	-7.7	-6	-5.7	-5	-5.7	-6.2	-5.6	-6.7	-7.2	-5.5	**-9.4**	-5.9	-6.9	-7.2	-7	-5.3	-5.6	-5.5	-6.9	-6.9	-5.1	-6.2	-5.8	**-10.4**	-5.9	-5.9	-6.1	-7.4	-7.1	-5.9
5FTO	GRID 1	**-9**	-7	-5.9	-6	-6.5	-5.9	-6.8	**-7.6**	**-8.4**	-5.2	**-9.5**	-7	-7.8	-7.3	-7.5	-5.7	-5.5	-5.6	-6.3	-6.3	-5.5	-7.4	-6.8	**-9.7**	-5.8	-6.3	-5.7	**-9.8**	**-8**	-6.5

Bold cell: Free-binding energy that is lower than -8 kcal mol^{-1}

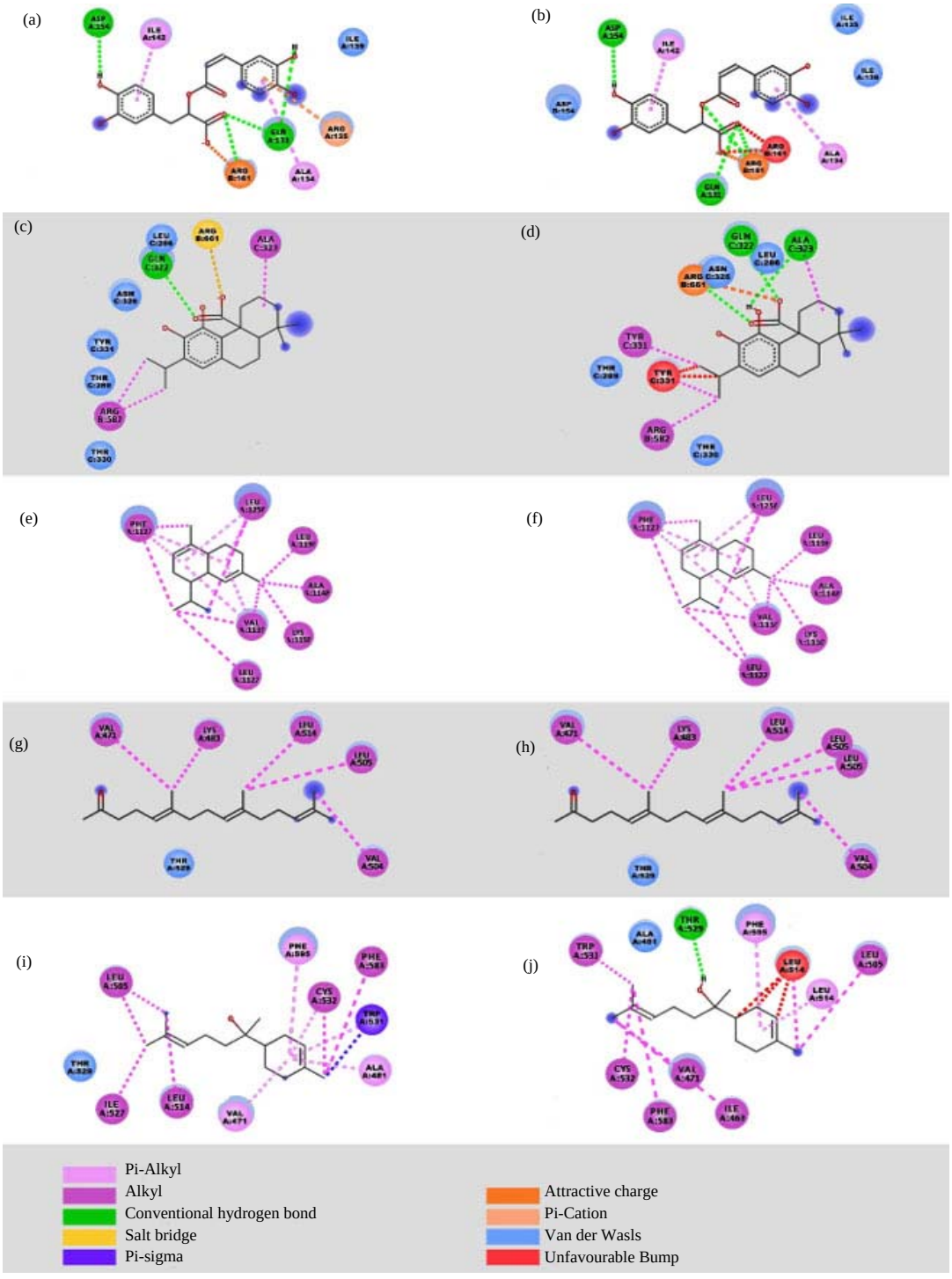


Fig. 3 (a-j): Pharmacophore features the highest affinity complexes of 5 potential compounds with target proteins, (a, b): Complex of 4TQ9-Rosmarinic acid, (c, d): Complex of 1JPW and Carnosic acid, (e ,f): Complex of 5FTO and (α) Amorphene, (g, h): Complex of 4R5Y and (E,E)-5,9,13-pentadecatrien-2-one,6,10,14-trimethyl, (i, j): Complex of 5HID and a-bis-Abolol

(a, c, e, g, i): Pharmacophores from rigid docking, (b, d, f, h, j): Pharmacophores from flexible docking. The hydrocarbon structure of the ligand was shown in a black frame. The global shape was the amino acid of the receptor that has interactions with the ligand. Different interactions of complex were represented in different corresponding colours)

Table 5: Free-binding energy between flexible docking of 6 potential ligands with CRC carcinogen receptors using different flexible amino acids in comparison with rigid docking

			Free-binding energy of docking									
Ligand	Protein	Grid box	Flexible docking using different amino acids								Minimum	Rigid docking
Carnosic acid	1JPW	1JPW	arg661b	asn362c	leu286c	thr289c	thr330c	tyr331c	arg582b	gln322c		
			-8.9	-9.1	-9	-9.4	-9	-10.7	-8.5	-9.4	-10.7	-9
	4IBW	4IBW	asn268a	leu111a	ser269a	thr102a					-8.8	-8.2
			-8.3	-8.3	-8	-8.8						
		4R5YA	ile527a	ile572a	leu505a	leu514a	thr529a	val504a				
			-9.1	-9	-10.1	-9.7	-8.9	-9.2			-10.1	-8.9
	4R5YA	4R5Y full	asp555b	thr589b	val511b							-8.8
			-8.8	-8.8	-8.8							
	4TQ9	4TQ9 full	arg161b	asp154a	ile142a	thr158a	thr127a	tyr157b			-9.9	-9.4
			-9.5	-9.5	-9.7	-9.2	-9.4	-9.9				
	5FTO	5FTO	leu1196a	leu1256a	met1199a	phe1127a	val1130a	val1180a			-10.3	-9.5
			-10.3	-10	-9.6	-9.8	-9.5	-9.5				
	5HID	5HID	ile463a	leu514a	phe583a	phe595a	thr529a	trp531a	val471a		-10.4	-9.3
			-10.3	-9.5	-9.5	-10.4	-9.3	-9.4	-9.4			
		5HID full	ile463a	phe583a	phe595a	thr529a	trp531a	val471a				-9.1
			-10.4	-9.5	-9.6	-9.1	-9.2	-9.3				
Rosmarinic acid	1JPW	1JPW	arg587b	asp249c	his524b	lys288c	thr289c	val584b			-9.5	-9.2
			-9.5	-9.3	-9.2	-9.4	-9.2	-9				
		4IBW	4IBW	arg282a	gln144a	his115a	phe113a	ser116a	trp146a		-9.7	-9.3
			-9.5	-9.4	-8.9	-9.3	-9.3	-9.7				
		4R5Y	4R5YA	ile527a	leu505a	leu514a	thr529a	val504a			-9.8	-9.4
			-9.8	-9.2	-9.8	-9.4	-9.8					
		4R5Yfull	arg562a	val511a								-8.9
			-9.3	-9.1								
		4TQ9	4TQ9full	arg161b	asp154a	gln131a	ile142a	arg135a			-11.1	-10.4
			-11.1	-10.6	-10.1	-10.5	-10.6					
		5FTO	5FTO	leu1256a	phe1127a	val1130a					-9.8	-9.7
			-9.8	-9.8	-9.8							
		5HID	5HID	ile463a	leu514a	phe595a	thr529a	val471a			-10.4	-9.7
			-10.1	-9.8	-10.4	-9.6	-9.8					
		5HIDfull	ile463a	leu514a	phe595a	thr529a	val471a					-8.3
			-8.1	-8.7	-8.4	-8.4	-8.2					
(E,E)- 5, 9,	4R5Y	4R5YA	leu505a	leu514a	thr529a	val471a	val504a	lys483a			-9.4	-8.9
13-Pentadecatrien			-9.4	-8.9	-8.9	-8.9	-8.8	-9.1				
-2-one, 6, 10,	5FTO	5FTO	leu1122a	leu1256a	phe1127a	val1130a					-9.3	-9
14-trimethyl			-9.3	-9.3	-9.3	-9.1						

Table 5: Continue

Ligand	Protein	Grid box	Free-binding energy of docking Flexible docking using different amino acids									Minimum	Rigid docking
α-Caryophyllene	4R5Y	4R5YA	ile463a	leu514a	phe583a	phe595a	thr529a	trp531a	val471a			-8.5	-8.2
			-8.2	-8.2	-8.4	-8.5	-8.2	-8.3	-8.3				
	5FTO	5FTO	leu1122a	leu1196a	leu1256a	phe1127a	val1130a					-8.8	-8.4
			-8.8	-8.5	-8.5	-8.5	-8.6						
α-Amorphene	4R5Y	4R5YA	ile463a	leu514a	phe583a	phe595a	thr529a	trp531a	val471a			-8.7	-8.4
			-8.6	-8.3	-8.7	-8.4	-8.4	-8.6	-8.4				
	5FTO	5FTO	leu1122a	leu1196a	leu1256a	phe1127a	val1130a	lys1150a				-9.9	-9.8
			-9.9	-9.9	-9.8	-9.9	-9.9	-9.7					
	5HID	5HID	ile463a	leu514a	leu597a	phe583a	phe595a	thr529a	trp531a	val471a		-8.7	-8.3
			-8.6	-8.5	-8.4	-8.7	-8.5	-8.3	-8.5	-8.4			
	5HID full	ile463a	leu514a	phe583a	phe595a	trp531a	thr529a	val471a					-8.3
			-8.6	-8.5	-8.7	-8.5	-8.3	-8.3	-8.4				
α-bis-Abolol	4R5Y	4R5YA	ile463a	ile572a	leu505a	phe583a	phe595a	thr529a	trp531a	val471a	leu514a	lys483a	-10	-8.8
			-8.8	-8.8	-8.7	-8.8	-8.9	-8.7	-9.1	-8.9	-10	-8.9		
	5FTO	5FTO	leu1122a	leu1196a	leu1256a	phe1127a	val1130a	val1180a					-8.3	-8
			-8.1	-8	-8.3	-8.2	-8	-8						
5HID	5HID	ile527a	leu505a	leu514a	phe583a	thr529a	trp531a	val471a	phe595a	cys532a			-9.3	-8.2
		-8.2	-8.5	-9.3	-8.5	-8.3	-8.4	-8.8	-8.1	-8.2				
		5HID full	ile527a	leu505a	leu514a	phe583a	thr529a	trp531a	val471a					-8.3
			-8.2	-8.4	-9.3	-8.5	-8.3	-8.4	-8.8					

Table 6: Molecular interactions of the highest affinity complexes from rigid docking and flexible docking

Complex	Docking type	Van der waals	Convetional hydrogen bond	Salt bridge	Alkyl	Pi-Alkyl	Attractive charge	Pi-cation	Pi-sigma	Unfavorable bump	Total
4TQ9-Rosmarinic acid	Rigid	1	4	0	0	2	1	1	0	0	9
	Flexible	3	4	0	0	2	2	0	0	15	12
1JPW-Carnosic acid	Rigid	5	1	1	2	0	0	0	0	0	9
	Flexible	4	3	0	3	1	1	0	0	2	14
5FTO-	Rigid	0	0	0	9	6	0	0	0	0	15
α-Amorphene	Flexible	0	0	0	10	6	0	0	0	0	16
4R5Y-(E,E)5,9,13-	Rigid	1	0	0	5	0	0	0	0	0	6
Pentadecatrien-2-one	Flexible	1	0	0	6	0	0	0	0	0	7
6,10,14-trimethyl											
5HID-	Rigid	1	0	0	6	5	0	0	1	0	13
α-bis-Abolol	Flexible	1	1	0	7	2	0	0	0	3	14

6,10,14-trimethyl (Fig. 3g-h), although only 1 alkyl bond was improved and the free bond energy difference was not very high. However, this result still recommended the importance of some molecular bonds in the interaction affinities. For the interaction between 5HID and α-bis-abolol, the energy difference was quite different (from -8.2 and -9.3 kcal mol^{-1}) due to the increase of 1 Alkyl bond and 1 Hydrogen bond (Fig. 3i-j). The statistics of intermolecular interactions were detailed in Table 6.

DISCUSSION

The two compounds i.e., carnosic acid and rosmarinic acid showed the best binding with all studied colorectal carcinogenic proteins. Previously, carnosic acid was also tested on CRC Caco-2, HT29 and LoVo cell lines by Barni *et al*.[36]. The study found out that this compound had strong inhibition of the tumour growing by inactivating both the carcinogenic mRNA, which encodes the COX-2 cancer-causing pathway and its protein. In 2016, rosmarinic acid and carnosic acid were also proven to have an anti-cancer effect on some colorectal cancer cell lines by Jessy Moore *et al*.[17]. However, it took 24 hrs to test *in vitro* inhibitory ability of these compounds on each cell line. As for *in vivo* test, the treatment effect on mice was evaluated after 11, 16 weeks using carnosic acid and rosmarinic acid, respectively. For our *in silico* study, it took only hours to get the docking result and select the best ligands. Although it is necessary to further perform *in vitro* or *in vivo* tests for drug development, the computer works significantly reduce cost and time-consuming as the first step for selecting potential subjects from a large number of new compounds of herbal plants[37]. Besides, our study was completely consistent with the studies of Moore[17] and Barni[36], which not only reconfirmed the role of these two compounds in inhibition of colorectal cancer but also convincingly demonstrated the reliability of this simulation method for other Computer-Aided Drug Design studies.

The change in interaction energy of flexible docking compared with rigid docking in the complex between rosmarinic acid and 4TQ9 occurred due to the addition of 2 van der Waals bonds and 1 attractive charge bond and the appearance of an unfavourable bump. Van der Waals is an attractive force due to dipole-induced interactions, which is weak in comparison with chemical bonds[38]. Besides, the existence of unfavourable pumps, which is known as unexpected intermolecular steric clash, have been proved to show unstable interactions and binding between interacting amino acids and drug atoms[39] as well. Hence the significant increase of binding capacity, in this case, might be due to the appearance of the attractive charge, which in turn is caused by the existence of the $-COO^-$ group in the structure of rosmarinic acid (Fig. 3b). The carboxylic acid functional group plays a cardinal role in the biochemistry of living systems as well as in drug design. Since endogenous substances, such as amino acids, triglycerides and prostanoids, possess the carboxylic acid moiety. The acidity as well as the ability to establish relatively strong electrostatic interactions and hydrogen bonds is the reason why this functional group is often part of drug-target interactions[40] and pharmacophore of diverse classes of therapeutic agents[41]. The two compounds rosmarinic acid and carnosic acid, which gave the best binding results in both rigid and flexible docking on this study, all contain this $-COO^-$ group.

Furthermore, these two ligands also contained aromatic rings in their structure. Rosmarinic acid had two phenol rings, the greatest number of phenol rings in comparison with other compounds in the study. Polyphenol components have been identified for their ability to prevent various types of cancer, in both experimental and simulated research[42,43]. These compounds had the potential to change the primary and secondary structures due to methyl, glycosyl and hydroxylation processes[44,45], which make it easy to link with amino acids to increase the binding capacity between ligands and receptor proteins. The interactions of Pi-cation and Pi-alkyl were all created due to the existence of a pi-electron cloud over these aromatic groups. Pi-alkyl is the interaction of the aromatic group and electron group of an alkyl group. A large number of pi-sigma (pi-alkyl and pi-cation) interactions were mainly involved in charge transfer, which helps to transfer drugs between receptor binding sites[38]. Meanwhile, Pi-cation interaction is the binding force between the cations and the pi surface (the face of an electron-rich pi system) of the aromatic structure through a non-covalent force. Pi-cation was important in many proteins that bind ligands or cation substrates[46].

Three other potential compounds i.e., α-amorphene and α-bis-abolol and (E,E)-5,9,13-pentadecatrien-2-one,6,10,14-trimethyl mainly consisted of methyl groups (-Me) when they linked to the receptors. The methyl group is non-polar radicals and provided electrons to other groups[47] to create alkyl bonds. The addition of a methyl group made a molecule more hydrophobic that supporting linkage with biological molecules[48]. These hydrophobic interactions were reported to contribute to the binding of many ligand-protein systems before[49]. Alkyl bonds were also reported to increase the lipophilicity of the drug and created favourable conditions for the drug to penetrate the cell membranes[50].

On the other hand, the presence of functional groups as -OH and -CO in the structure of three ligands rosmarinic acid, carnosic acid and α-bisabolol also supported protein binding. The Carbonyl group at the C-ring of flavonoid played an important role in the ligand-target interaction, by hydrogen bond interaction to Ser530A and Arg120A residue[51]. In contrast with (-Me), hydroxyl and carbonyl groups are polar radicals[52] due to the high electronegativity of oxygen. Hence the hydrogen bonds (electrostatic bond between hydrogen and the more electronegative atoms) of these compounds with hydrogen atoms in the environment were created. The free energy for hydrogen bonding can vary between -1.5 and -4.7 kcal mol^{-1}. The best ligand in this study, rosmarinic acid, created four hydrogen bonds with 4TQ9, followed by carnosic acid with three hydrogen binding toward 1JPW. The interaction between the -OH group of α-bisabolol and the amino acid THR A:529 of 5HID, which was not created in rigid docking, contributed to the increase of linking affinity (from -8.2 and-9.3 kcal mol^{-1}) during flexible docking. Hydrogen bonds were intermolecular interactions that were common in biological complexes[53] and were contributions to the specificity of molecular recognition[54].

From the better results of flexible docking, it has been shown that flexible docking provides more sites of molecular interaction than rigid docking. Otherwise, proteins can change their initial stable structure to fit with the ligands. In living organisms, proteins are flexible objects. However, rigid docking assumed that proteins and ligands were immobilized objects, so the docking was performed only at one coordinate. Therefore, the results were extremely limited. On the other hand, flexible docking tried to simulate receptors and ligands as flexible objects. Hence, the docking was performed at several coordinates[55] in which the most durable combination with the least energy required was created. In the flexible docking, a flexible amino acid inside the receptor was required as a flexible factor to be included in the running setup. Hence the ligand could adjust to the most stable protein binding site and the simulation was more reliable and just similar to what happens *in vivo* process.

CONCLUSION

The ligand-protein docking is to simulate how the ligand competes with substrates inactive regions of carcinogenic proteins for inactivating that protein, leading to the inhibition of the tumour growing. Using molecular docking and pharmacophore analysis, our study has confirmed therapeutic effects and clarified the tumour-inhibition ability of *Rosmarinus officinalis* L. based on molecular interactions between examined compounds with the carcinogenic proteins. Five compounds i.e., rosmarinic acid, carnosic acid, (E,E)-5,9,13-pentadecatrien-2-one, 6,10,14-trimethyl, α-amorphene and α-bis-abolol from rosemary were proposed as potential compounds in colorectal tumour inhibition. The study strongly confirmed the role and the reliability of computer works in supporting other drug development studies.

SIGNIFICANCE STATEMENT

This study discovers the ability of compounds from the herbal plant *Rosmarinus officinalis* L. that can be beneficial for developing drugs targeting inhibition of different proteins causing colorectal cancer. This study will help the researcher to uncover the critical areas of drug-based docking and interaction models of potential compounds of *Rosmarinus officinalis* L. with different target proteins that many researchers were not able to explore and at the same time emphasize the useful role of the docking method in process of drug development.

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