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EXPLORING INHIBITORY POTENTIAL OF GINGER AGAINST NUMEROUS TARGETS OF DIVERSE FORMS OF CANCER

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ABSTRACT: In recent times many chemical drugs are capable of causing the side effects to the humans; in order to avoid these side effects herbal based drugs is essential for the better substitute. Plants have a long history of use in the treatment of cancer. Plant-derived compounds have played an important role in the development of several clinically useful anticancer agents. The scientific analysis is carried out all over in India since Vedic times and are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) proposed for their valuable anti-cancer and other valuable properties. Numerous forms of cancer accounts for 10% of total death worldwide which requires better therapeutic approaches. Ginger is one of the most commonly used herbal medicines for the treatment of numerous ailments and improvement of body functions. Compounds of ginger (*Zingiber officinale*), shown to exhibit antioxidant, anti-inflammatory and anti-carcinogenic properties. In current study, we intended to analyze inhibitory properties of ginger towards target proteins for several cancers by computer aided virtual screening. Docking study revealed that compounds gingerol, shogaol, zingiberol, zingiberene, zingerone, zingerdiol were found to have strong binding affinity towards selected cancer targets. Hence, these compounds can be further investigated in vitro and *in vivo* to develop novel chemical scaffold on which further derivatization can be done for further optimization of its anticancer activity.

KEYWORDS: *Zingiber officinale,* Docking, Phytochemicals, Cancer.

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1. INTRODUCTION

Cancer is a group of diseases involving uncontrolled cell growth due to genetic or epigenetic changes as their DNA leading to development of tumour locally or spread to other parts of the body [1]. According to World health organization(WHO) Cancer is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015. Globally, nearly 1 in 6 deaths is due to cancer.Nowadays various types of cancers are reported every month that spread with various mechanisms. There are over 200 different types of cancers for the reason that there are over 200 different types of body cells. The most frequently diagnosed cancers in India are in man: oral, lung, colorectal and in female: Breast, cervix and ovarian cancers. There are many known causes of cancers like exposure to chemicals, drinking excess alcohol, excessive exposure to sunlight, and genetic differences, to name a few [2]. Ginger (*Zingiber officinale* Roscoe) is one of the most commonly consumed dietary condiments in the world [3]. In addition to its flavor, ginger is known to contain a number of potentially bioactive phytochemicals, mainly gingerols and their related dehydrating products, the shogaols as well as volatile oils [4]. The Literature review shows main pharmacological measures of ginger and compounds isolated therefrom include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. Ginger is a strong anti-oxidant substance and may either moderate or avoid production of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects. Further studies are essential in animals and humans on the kinetics of ginger and its constituents and on the effects of their expenditure over a long period of time [5, 6]. The anticancer properties of ginger are accredited to the occurrence of certain pungent vallinoids, viz. [6]-gingerol and [6]-paradol, as well as some other constituents like shogaols, zingerone etc. A number of mechanisms that may be involved in the chemo preventive effects of ginger and its components have been reported from the laboratory research in a wide range of investigational models [7]. Lots of experimental evidences are available that support ginger-derived compounds have inhibitory effects on various cancer cell types like 6-shogaol and 6-gingerol in different types of cancer including pancreatic cancer cells Panc-1, Breast cancer cell lines MCF-7 and MDA-MB-231,colon cancer cell SW480, H-1299 human lung cancer cells, CL-13 mouse lung cancer cells, HCT-116 and HT-29 human colon cancer cells,Human LNCaP, DU145, and PC3 and mouse HMVP2 prostate cancer cells and Ovarian cancer cells SKOV3 [8,9,10,11,12,13,14,15]. The study is based on *in silico* screening of potential cancer targets for bioactive compounds of ginger, molecular docking is used to identify theinteraction between ginger ligands and cancer targets, comparison ofefficacy of ginger ligands and approved drugsagainst cancer targetsand to validate the anti-cancerous properties of gingerol using different tumour cell lines [16].The anticancer action of ginger is accredited to its ability to transform several signaling molecules like NF-κB, STAT3, MAPK, PI3K, ERK1/2, Akt, TNF-α, COX-2, cyclin D1, cdk, MMP-9, survivin, cIAP-1, XIAP, Bcl-

2, caspases, and other cell growth regulatory proteins [17]. Therefore, the present study aims to investigate the anti-cancer activities of the compounds from *Zingiber officinale* against drug targets of different types of cancer which includes prostate cancer, gastric cancer, ovarian cancer, Liver cancer.

2. MATERIALS AND METHODS

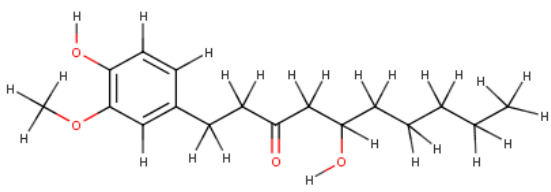
Selection of targets

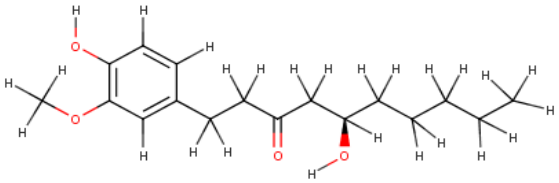
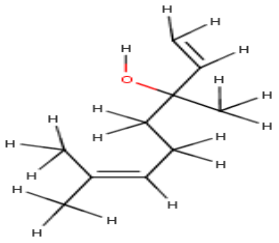
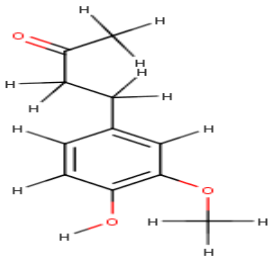
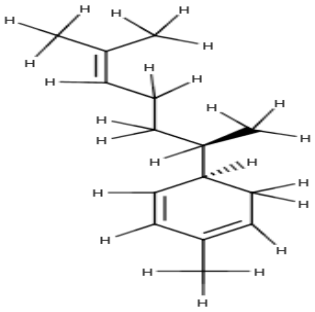
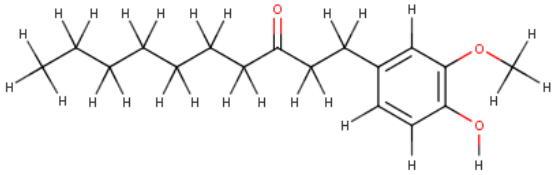
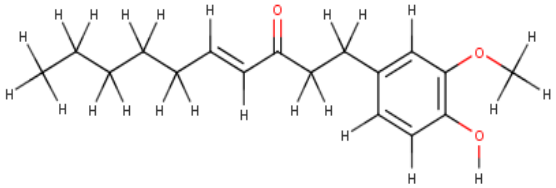
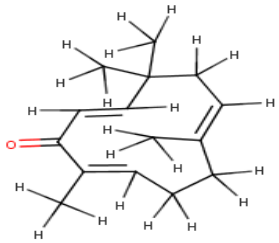
From the literature search the most potent cancer targets of various types have been taken [18,19, 20,21,22]and their three-dimensional structures were available in their native formin online protein structure repository,Protein Databank (PDB)database [23]. The X-ray crystal structureselected targets in complex with selective potent inhibitor were retrieved in pdb format from PDB database which includes vegf (PDB ID: 1FLT), Bcl-2 (PDB ID:1GJH), Bax (PDB ID:1F16), Cox-2 (PDB ID :1cx2), interleukin-6(PDB ID:1alu), tnf-alpha (PDB ID:3wd5), nf-kb (PDB ID:1nfk), the inhibitor from the crystal structure were removed and prepared for receptor ligand interaction studies.After that, the structure was prepared and refined, energy minimization of 3-D structures was done by Yasara software. Hydrogen atoms were added to the target proteins and all water molecules were removed.

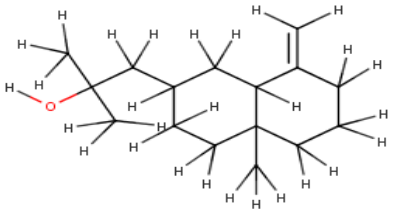
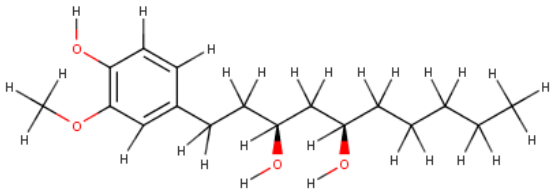
Selection of ligands

The ligands used for docking study were selected from literature. The bioactive compounds that are mainly present in the rhizome of *Zingiber officinale* were considered for the study. Structures of major compounds present in ginger were retrieved from the PubChem compound database [24](https://www.ncbi.nlm.nih.gov/pccompound) in the SDF file format and followed by conversion in PDB format using the tool Marvin Sketch features an extensive set of functionalities to allow the rapid and precise drawing of chemical compounds, reactions, Markush structures and query molecules (https://chemaxon.com/products/marvin) and optimized in Yasara. These structures were used for docking calculation. The reference ligand structure is prepared in prior, using Marvin of ChemAxon by cleaned structure up in two dimension (2D) configurations. Details of bioactive Compounds considered for the study with their 2D structures were represented in Table 1.

Table 1: List of ligands and their structure

Compound Name	Molecular weight(g/mol)	Pubchem-id	Structure
Gingerol	294.391 g/mol	3473	

6-gingerol	294.391 g/mol	442793	
Linalool	154.253 g/mol	6549	
Zingerone	194.23 g/mol	31211	
Zingiberene	204.357 g/mol	92776	
6-paradol	278.392 g/mol	94378	
6-shogaol	276.376 g/mol	5281794	
Zerumbone	218.34 g/mol	5470187	

Zingiberol	236.399g/mol	6455496	
Gingerdiol	296.407 g/mol	11369949	

Docking Simulations

Molecular docking is very necessary step performed to study the receptor-ligand interaction to select potential hits in virtual screening which regarded as the basis for structure based drug discovery. Molecular docking was performed in Yet another Scientific Artificial Reality Application (YASARA) an Auto Dock based tool for molecular docking and virtual screening. Y ASARA was used to gain the docking results of the listed compounds with the indicated target proteins. The energy minimized compounds were imported and the docking conformations were performed twice using genetic evolutionary algorithm and the fitness of the docked structures were calculated. The hydrogen bond, Residues, Dissociation Constant, binding energy was calculated using YASARA Software. Here, the vdW term is van der Waal energy. H-bond and Elect terms are hydrogen bonding energy and electro statistic energy, respectively. The output of docking run is sorted based on binding energy. Yasara docking gives positive binding energy. So, more the positive energy indicates the higher likeness among the molecules.

Pharmacophore modeling

Pharmacophore is defined as the minimum functionality that a molecule has to contain in order to show activity. Pharmacophore mapping was carried out in the workspace of mole sign module of Vlife MDS 4.3. Dataset of different types of cancer targets inhibitors was first aligned with reference to most active molecule as template. The most active molecule gingerol was selected to set it as the reference. The reference molecule is the molecule on which the other molecules of the align dataset get aligned. The minimum number of pharmacophore features for generated model was taken as 3.All spheres in the snapshot indicate all possible pharmacophoric centers. This pharmacophore model can serve as an effective search filter for virtual screening.

3. RESULTS AND DISCUSSION

Evidences confirm the participation of plants extracts in synthesizing many medicines against already existing and even emerging diseases. In order to examine the binding capacity of bioactive compounds in *Zingiber officinale* on proteins related to cancer in human, we have used Yasara software to dock the ligand data set to the structure of target protein. Since all the natural ligands

(inhibitors) were found to be docked in a variety of conformations and with varying binding energy. From the interaction profile numerous interactions including hydrogen bonding interactions, hydrophobic interaction, Van der Waals interactions, and pi–pi interactions were inspected between selected inhibitors and retrieved Hit molecules with target proteins. Through this methodology of computer aided drug interaction, we examine complexes formed between ligands and interesting targets (often many), for dissimilar types of a particular disease. Target proteins docked with different ligands are shown in Fig 1. The best dock pose was chosen on the basis of high docking score. Top two ligands were selected with better score. Zerumbone was found to be most active compound showing good binding affinity in terms of Yasara binding energy.

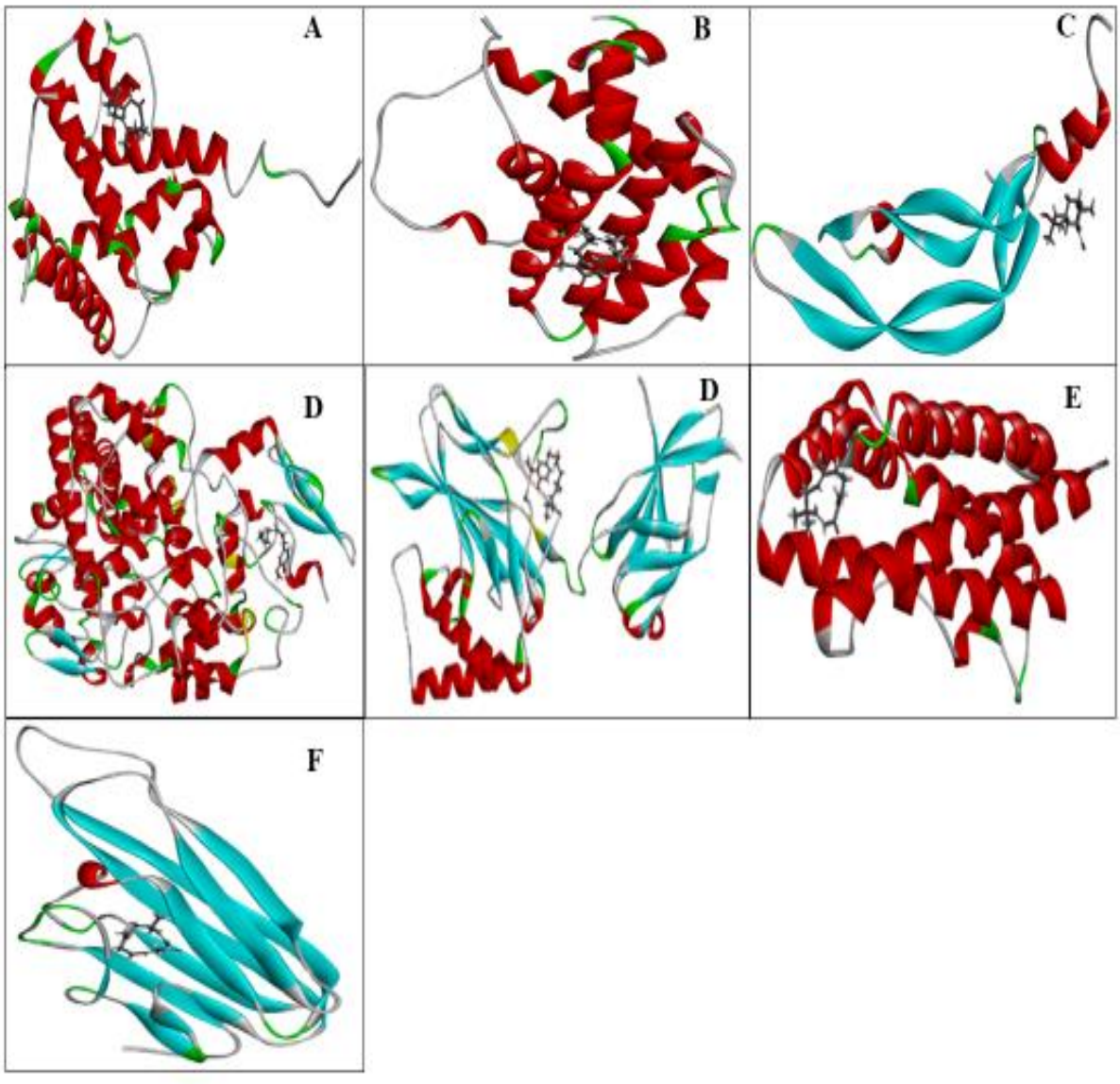


Fig 1: Binding modes and Protein-ligand interaction map of docked complexed ligands with A(Bax), B(Bcl2), C(Vegf), D(Cycloxygenase), E(Nf-kb), F(Interleukin-6), G(Tnf-alpha)

Table 2: Binding Energy, Dissociation constant, hydrogen bonds and contacting amino acids

Target Protein name	Ligand name	Binding energy (Kcal/mol)	Dissociation constant [pm]	Hydrogen bonds	Amino acids
Bax	Zerumbone	6.6310	13785184	0	ARG 89, GLU 90, PHE 92, PHE 93, ALA 96, TRP 139, ASP 142, PHE 143, GLU 146
	Zingiberene	6.6190	14067233	0	ARG 89, GLU 90, PHE 92, PHE 93, ALA 96, TRP 139, ASP 142, PHE 143
Bcl2	Zerumbone	6.0	39989104	1	ARG 127, PHE 130, ALA 131, GLU 135, TRP 176, GLU 179, TYR 180, ARG 183, HIS 184
	Zingiberol	5.803	55762804	1	THR 96, GLN 99, ALA 100, ASP 103, PHE 104, ARG 107, GLY 145, VAL 148, PHE 198, TYR 202, PRO 204
Vegf	Zingiberol	4.897	257306880	0	TYR 21, GLN 22, TYR 25, CYS 26, HIS 27
	6-shogaol	4.515	490299136	1	GLU38, TYR 39, PRO 40, ASP 41, GLU 42, GLU 73, ASN 75, SER 95, PHE 96, LEU 97
Cycloxygenase	6-shogaol	6.99	7520766	1	ASN 34, CYS 36, CYS 37, ASN 39, CYS 41, GLN 42, ASN 43, ARG 44, GLY 45, GLU 46, CYS 47, TYR 130, GLY 135, LEU 152, PRO 153, PRO 154, VAL 155, ALA156, CYS 159, GLY 164, GLN 461, GLU 465, LYS 468
	Zingiberol	6.297	24223550	2	GLU 346,ASP 347, GLN 350, HIS 351, GLY 354, TYR355, HIS 356, SER 579, PHE 580, ASN 581
Nf-kb	Gingerdiol	5.591	79752200	3	LYS 49, ARG 51, GLY 52, PHE 53, ARG 54, ARG 56, SER 63, HIS 64, GLY 65, GLY 66, LEU 67, PRO 68, GLY 69, SER 72, GLU 73, LYS 74, LYS 77, SER 78, TYR 79, ASN 13
	Zingiberene	5.574	82073664	0	GLY 52, PHE 53, SER 63, HIS 64, GLY 65, GLY 66, LEU 67, PRO 68, GLY 69, SER 72, LYS 77, SER 78, TYR 79, ALA 135, ASN 136

Interleukin-6	Zerumbone	6.38	21057072	1	GLU 42, THR 43, LYS 46, ARG104, PHE 105, GLU 106, SER 107, SER 108, GLN 156, ASP 160, THR 163
	Zingiberol	5.95	43510308	0	GLU42, THR43, LYS46, SER47, ARG104, PHE105, GLU106, SER107, GLN156, ASP160, THR163
Tnf-alpha	Zerumbone	5.852	51336604	1	GLN25, LEU26, GLN27, ASN46, GLN47, SER133, ALA134, GLU135, ILE136, PRO139
	Zingiberol	5.459	99655192	1	PRO20, GLU23, GLY24, LYS65, PRO139, ASP140, TYR141, LEU142, ASP143, PHE144, ALA145

For several years this was understood as being "one drug, for one target, for one disease", nevertheless researchers began to examine that certain diseases are best treated with multi-target drugs. In current years, studies have required out polypharmacological compounds performing on multiple targets against complex (multifactorial) diseases, such as cancer, neurodegenerative disease, and certain infections. One of the computational tools used in research for multifunctional drugs is Molecular Docking [25].Docking simulation providing additional information about the possibilities of the inhibitory potential of the compounds against the targets [26]. Molecular docking exhibited that the different ligands docked with different region of the protein indicating that slight alterations in ligand structure leads to completely different protein-ligand interaction.Docking result also showed that Zerumbone and Gingiberol indicate that these compounds are most potent component of ginger as ligand.

Pharmacophore modeling

A set of pharmacophore hypothesis was generated using the Mol Sign module of VLife MDS 4.3. We generated different pharmacophore patterns based on a set of 10 aligned molecules. It starts generatingproperties of molecules and finds the common three dimensionalmap of three to maximum common properties. All the probablepharmacophore models are aligned automatically, and alignment is accomplished on the source of familiar properties. All assumption was found to contain common features likealiphatic (brown color), aromatic (golden color) and hydrogen bondacceptor (blue color). The results of pharmacophore identification studies are given in figure. Structure and pharmacophoric features indicated that the designed set of molecules is having hydrogen bond acceptor (blue color) and aromatic features (golden color) in common. The distances among the various chemical features are as follows:

- Hydrogen bond acceptor-Hydrogen bond donor=9.570A
- Hydrogen bond acceptor-Aromatic carbon center=5.114A

- Hydrogen bond donor-Aromatic carbon center=5.013A

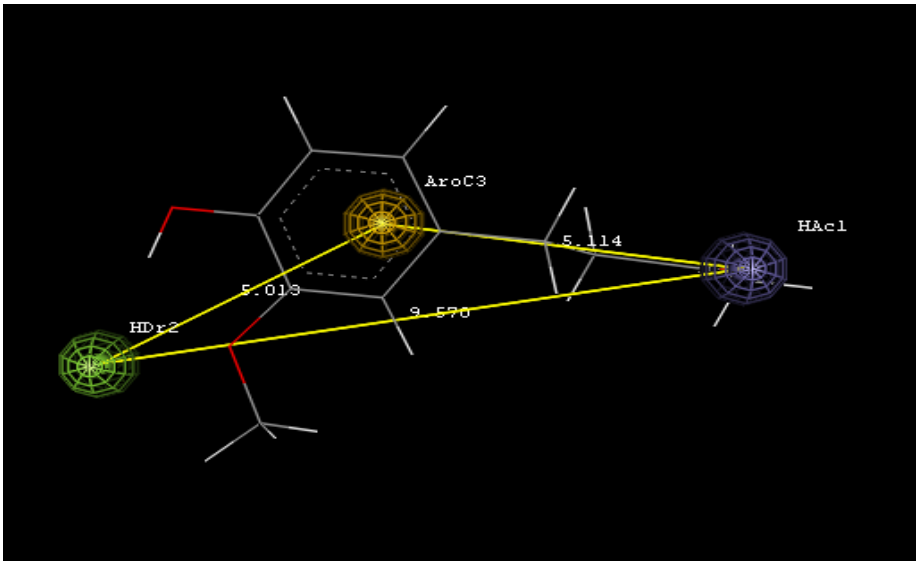


Figure 2: Pharmacophore model for molecules of the dataset under study

4. CONCLUSION

In the recent years, more emphasis has been placed on identifying plant-derived compounds that can be used as an effective treatment for life-threatening diseases such as cancer. All the compounds selected for the study are considered as orally safe compounds. A few compounds showed interaction with the target proteins.Hence, the bioactive compounds that are interacting with the target can be used as potent inhibitor to block the action of target proteins. Thus, the selected compounds can be verified at the clinical-level drug examinations and made into an effective anti-cancer drug having better inhibitory activity against several types of cancer. Chemo preventive potential of phytochemicals have explored by our in silico findings and further, being natural, they have minimal or null side effects on human body as compared to the synthesized anti-cancer agents and thus could be their promising alternatives. Therefore, this approach is valuable for drug discovery process and anti-cancer therapy. Hence, now there is a requirement to study the pharmacological action of these compounds in in vivo models.

CONFLICT OF INTEREST

The authors declare that no conflicting interests exist.

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