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ANTICANCER ACTIVITY (IN VITRO) AND MOLECULAR DOCKING STUDIES OF CAPSAICIN AND MYRISTICIN

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ABSTRACT
Cancer is a disease which causes body's cells grow abnormally and spread to other parts of the body. Cancer treatments are like surgery, chemotherapy, immunotherapy, hormone therapy, radiation therapy and stem cell transplant. Chemotherapy has side effects while doing cancer treatment. Natural treatment, such as the use of plant-derived products in cancer treatment, may reduce adverse side effects. The plant extract samples capsaicin and myristicin chosen are of biological interest with anti-cancerous properties. They are the active compounds of Capsicum and Nutmeg that were extracted using the Soxhlet technique. The ADMET and drug likeness properties of the designated compounds are conferred. Cyclin-dependent kinases (CDKs) are enzymes that have the primary purpose of blocking cellular activity and preventing cell proliferation. CDK2 complexes have significant medicinal targets for the drug design. Molecular docking analysis were considered to examine the inhibitory activities (CDK2 -Inhibitor) of the header composites, to investigate the anticancer efficacy against human colon cancer cell lines using in vitro (MTT assay). The outcome of this analysis suggest that the title compounds might be used as anticancer lead chemicals.
Keywords: ADMET, Drug likeness, Molecular docking, in vitro.

1. Introduction
In the past few decades, cancer has been the second foremost threatening disease in the developing countries [1]. Cancer is the uncontrolled proliferation of irregular cells in the body. Cells in nearly any part of the body can become cancerous and can spread to other areas of the body. Cancer is treated in different ways, depending on the health condition and type of cancer. Common treatments include chemotherapy and radiation therapy. Other treatments include surgery and biological therapies. There are more than 100 different kinds of cancer. For instance, brain cancer develops in brain cells while lung cancer develops in lung cells. The colon (large intestine) or rectum is where colon cancer typically develops. The organs which make up the

lower part of the gastrointestinal system are the colon and rectum.

Natural plants have been used for thousands of years to prevent and treat various diseases. There are excellent sources of bioactive constituents that exert their health beneficial effects, and very often, these sources are materials for gourmet food consumption. Ayurveda has been used since ancient times to prevent or alleviate various tumours. Nowadays, researchers are eager to explore complementary and alternative cancer treatment methods. The anti-cancer effects of certain bioactive constituents of plants have been confirmed. Various review articles have summarised the natural phytochemicals and their anti-cancer effects. In recent years, some of these reviews have provided a general overview of the bioactive aspect of phytochemical compounds [3] Capsaicin has a major part in the therapy of human colon cancer [3] and Myristicin has cytotoxic effect against cancer cell lines [4]. Capsaicin is a dynamic constituent of chilli peppers, are plants belonging to the genus Capsicum and Myristicin is an energetic composite of Nutmeg that were isolated by Soxhlet extraction method.

The ADMET and drug likeness properties of the designated compounds are exemplified. CDKs, a kinase inhibitor, have the primary purpose of blocking cellular activity and preventing cell proliferation. As Cicenas J et al [5] reported, CDK2 complexes are the significant medicinal target for the drug design. Additionally, the heterocyclic organic compounds are effective in the drug discovery process. In this connection, CDK2 inhibitors protein targets were chosen and docked with the header composites.

MTT assay anticancer activity (In Vitro assay) investigations on the synthesized compounds Capsaicin and Myristicin were performed and the outcomes of molecular docking (binding interactions) research have been examined for cell viability, cytotoxicity of colon cancer cells. Cyclophosphamide was used as a positive control. For both the cell viability and cytotoxicity tests, utilizing MTT on a reductive coloring reagent and dehydrogenase in a viable cell to measure cell viability with a colorimetric method were used. MTT is a colorimetric quantitative cytotoxicity assay experiment that recorded the reduction of 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide by mitochondrial succinate dehydrogenase.

2. Material and Methods

In this process, the capsicum placental tissue, considering 100g of dried, crushed, and wrapped in filter paper, was positioned in the condenser of a sterile flask with ethanol, as a solvent. The materials were heated in a Soxhlet apparatus and maintained at a steady temperature of 55–60 °C for three–five hours. On the walls of the extraction chamber, capsaicin that had evaporated had accumulated. The extract was collected and dried after the process was performed three to four times.

Using a mortar and pestle, Nutmeg was crushed and powdered. The samples were extracted using Soxhlet apparatus (for 6 hours at 70°C) using 20 g of sample and 100 cc of ethanol. The extracts were refrigerated before being filtered through Whatman Filter Paper No. 1 and almost dried out in a hot air oven at 65–70°C. Further research was conducted using the synthesized materials.

2.1. Preparation of cell line and MTT ASSAY

The Dulbecco's Modified Eagle Medium (DMEM) was used to cultivate the human cancer cell

line C320 (colon cancer) at 37 °C in a humidified atmosphere with 5% CO2 in a highly sterile flask. The medium also contained 10% fetal bovine serum. The test cells were taken out of a culture flask. A microscope was used to count the cells, and the concentration of the cell suspension was modified appropriately. A 96-well microplate was filled with 100 µl of cell solution using serial dilution. To measure the background, a well-made only media (DMEM) was constructed. It was then placed in a CO2 incubator for 24 to 48 hours. MTT Reagent (10µ l) was added to each and every well, including the controls. The average values of absorbance were determined from triplicate readings and the average value for the blank is subtracted and depicted in Fig.1. A graph is plotted with percentage of absorbance against sample concentration represented in Fig.2.

3. Results and discussion

3.1. Drug likeness and ADMET properties

The concept of drug likeness assessment is a complex and expensive prediction model that includes lead optimization, clinical trials for the discovery of novel pharmacological drug design, target identification, reactivity, and protein-ligand binding affinity. In the present study, the drug likeness [6] and ADMET properties for the designated compounds were achieved using ADMET Lab 2.0 software, and the findings were given in Table 1,Table 2 and Table 3. Capsaicin possesses 2 (0-7) hydrogen bond donors and both compounds have 3 (0-12) hydrogen bond acceptors. The molecular weight for the header composites was found to be 305.41g/mol, and 192.08 g/mol (100-600) and their topological polar surface area was obtained as 58.56, 27.69 (0-140Å) for capsaicin and myristicin respectively. A Synthetic Accessibility (SA) score is calculated by combining fragment contributions and a complexity penalty to amount the comfort of synthesis of drug-like compounds.SA score value for Capsaicin is 2.19 (<6) and myristicin was obtained as 2.331(<6) which indicates that the title molecules were easy to synthesize6. The number of fsp3 hybridised carbons / total carbon count is used to evaluate carbon saturation molecules and describe the complexity of a molecule's spatial structure. It was found that capsaicin has fsp3 of 0.5 (>0.42). Human Intestinal Absorption (HIA) for Capsaicin is 0.005and Nutmeg as 0.002 (0-0.3). (Reference values are within parentheses). Human intestinal absorption of an oral drug is the vital criterion for its apparent efficacy and the closely associated between oral bioavailability and intestinal absorption has also been established. The HIA can be seen as a substitute indicator for oral bioavailability. Oral bioavailability is certainly one of the most crucial pharmacokinetic factors for any medicine administered orally as it serves as a measure for how effectively the drug is delivered to the systemic circulation. The human oral 20% bioavailability score is 0.026 for Capsaicin and 0.015 for myristicin and 30% of bioactivity score is 0.146 and 0.426 respectively and the above values, well-known that the bioavailability of the header molecules are active. The physico chemical properties, medicinal chemistry values and ADMET behaviour are much better for capsaicin than myristicin as per the tabulated results.

3.2. Molecular Docking Studies- in silico

Molecular docking (protein-ligand) is a significant framework to comprehend biomolecular activity for drug discovery. The studies explore the suitable binding orientation and binding

affinity of the targeted receptor with the best-docked configuration. Members of the CDKs family are protein kinases that play a crucial part in regulating the cycle cell in sequential stages. CDK2 cell regulation has made an extensive role in cell cycle regulation, gene transcription, and other critical biological processes [7]. Thus, CDK2 inhibition may be beneficial in the treatment of certain tumours, and it remains a promising technique for anticancer drug development [8]. In this connection, header composites (Ligand) were made to dock into the active site of the targeted receptors and those protein targets were chosen from the RCSB PDB format data bank which includes 4DFR, 1LP4 respectively. The Autodock Tools [4] were employed to perform molecular docking analysis. After the suitable genetic algorithm of 100 runs, the docking results were analysed with best-posed configuration, and the corresponding parameters were listed in Table 2. In the docking analysis, the binding pocket of ligand-protein interaction was represented in the yellow dotted line. The bond distance was measured between the ligand (title molecule) and protein receptors. The least binding affinity (ligand-protein) preferred the best binding docking orientation in the mode of the process. From Table.2 the minimum (lesser) binding energy value for 4DFR in capsaicin was found at -5.75 kcal/mol with bonded residues as THR46—OG1 and 1LP4 in Myristicin was observed at -5.48 kcal/mol with LYS68—HZ3 respectively.

3.3. Cytotoxicity Assay -in vitro

The anticancer activity of the compounds Myristicin, and Capsaicin were studied with three different concentrations (100, 200, 300 µg) and the drug used for positive control includes Cyclophosphamide. From the absorbance values measured by the inverted microscope reader, the viability and cytotoxicity were calculated using the formula [10] and presented in Table-3. The above result reveals that 300 µg concentration of Capsaicin compound shows 75% of inhibition when compared with 50µg concentration of positive control which is shown to have 95% of inhibition. Moreover, the other compound also confirms cytotoxicity towards cancerous cells. Myristicin compounds show 50% of inhibition at the concentration of 300 µg/ml. Overall data (Fig. 3) show that capsaicin is a better anticancer compound than myristicin which also inhibits the growth of colon cancer cells.

3.4. Electron Localisation Function ELF and Localized Orbital Locator LOL

In this work, the most important approaches, such as ELF and LOL color-filled maps of real-space functions were interpreted by Silvi and Savin [11]. The colour filled map for the designated compounds was using Multiwfn 3.8 program analyser [12] from the computed method with higher basis set. The ELF and LOL which symbolizes covalent bonds, lone pairs and core regions in the header compounds respectively. The ELF and LOL pictures of the title molecules as colour filled shaded map depicted in Fig 4. The red colour region in the ELF map represents the hydrogen atoms with highly localized bonding and the delocalized electron cloud around carbon and nitrogen atoms are denoted by blue regions. In the LOL map, the white colour region surrounded by the hydrogen atoms in the title compounds represents the electron density higher than the colour scale limit (0.8) [13]. In the LOL map, the blue colour circles around the selected atoms such as oxygen, nitrogen and with limited carbon atoms as electron depleted regions

between the valence regions [14].

4. Conclusion

In the present study, a detailed interpretation of in silico and in vitro analyses of the biological activity of the green synthesized active compounds Capsaicin and Myristicin were performed. The Drug likeness and ADMET properties were obtained to predict the bioactivity of the designated compounds. The title composites were subjected to a molecular docking research, which revealed that they are effective in CDK2-inhibitors.The Capsaicin have low value of binding energy and estimated inhibition constant values in comparison with myristicin.

The anticancer study of synthesized compounds has been investigated for cytotoxicity of colon cancer cells by the MTT assay method using cyclophosphamide as control. The result of the MTT assay method reveals that 300 μg of capsaicin inhibits 76% of colon cancer cells in comparison with cyclophosphamide positive control which has 96% inhibition. The topological analysis (ELF and LOL) was discussed. The cytotoxicity of capsaicin towards cancer cells is higher than the nutmeg plant extract considered for present study. The in silico and in vitro studies are conclusive that capsaicin is a better drug for colon cancer than myristicin.

Declaration of competing interest

The authors affirm that they have no known financial or interpersonal conflicts that would have appeared to have an impact on the research presented in this study.

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Table 1 Physicochemical Property for Capsaicin and Myristicin.

Physicochemical Property			
Properties	Values		Expected ranges
	Capsaicin	Myristicin	
Molecular weight	305.41g/mol	192.08 g/mol	< 500
Number of Hydrogen bond donors (nHD)	2	0	0-7
Number of Hydrogen bond acceptors (nHA)	3	3	0-12
Molar refractivity	90.52	53.10	40-130
Molecular Polar surface area (TPSA) $Å^2$	58.56	27.69	0-140

Table 2 Durg-likeness Property for Capsaicin and Myristicin.

Durg-likeness Property			
Properties	Values		Expected ranges
	Capsaicin	Myristicin	
SA score	2.190	2.331	SA score < 6

GSK rule	Accepted	Accepted	MW ≤ 400; logP ≤ 4
Lipinski rule -of -five	Accepted	Accepted	MW ≤ 500; logP ≤ 5; nHA ≤10; nHD ≤ 5
Fsp^3	0.500	0.273	Fsp3 ≥0.42

Table 3 ADMET Property for Capsaicin and Myristicin.

ADMET Property			
Properties	Values		Expected ranges
	Capsaicin	Myristicin	
Caco-2 Permeability	-4.476	-4.415	>-5.15
MDCK permeability	2.7 x 10^{-5} cm/s	2.8 x 10^{-5} cm/s	>2 x 10^{-6}cm/s
Pgp-inhibitor	0.001	0.019	0-0.3
Pgp-substrate	0.041	0.0	0-0.3
F 20% Human oral bioavailability 20%	0.026	0.015	0-0.3
F 30% Human oral bioavailability 30%	0.146	0.526	0-0.3
Volume Distribution	1.098	1.226	0.04-20L/kg
Human-intestinal absorption (HIA)	0.005	0.002	0-0.3
SR-ARE	0.097	0.046	0-0.3
NR-AR Androgen receptor (AR)	0.297	0.411	0.3-0.7
CL Clearance of Drug	11.309	14.28	≥ 5

Table 4 Molecular Docking analysis and hydrogen bonding interactions of with target proteins 4DFR and 1LP4

Target protein	Ligand	Binding energy (kcal/mol)	Bonded residues	Bond distance (Å)	Estimated inhibition constant (µm)
4DFR	Capsaicin	-5.75	THR46—OG1	3.4	60.54
			THR46—OG1	2.4	
	Myristicin	-5.33	SER49---OG	3.2	124.87
1LP4	Capsaicin	-4.44	LYS68—HZ3	2.2	557.35
	Myristicin	-5.48	LYS68—HZ3	2.8	96.97
			LYS68—HZ3	2.2	

Table 5 Anti-cancerous activity of samples and Cyclophosphamide (positive control)

Concentration (µg/ml)	**Cytotoxicity**		
	Myristicin	**Capsaicin**	**Cyclophosphamide (Positive Control) (10, 25 & 50 µg/ml)**
100	30.01	33.23	52.99
200	36.47	42.49	82.61
300	**51.37**	**75.68**	**95.73**

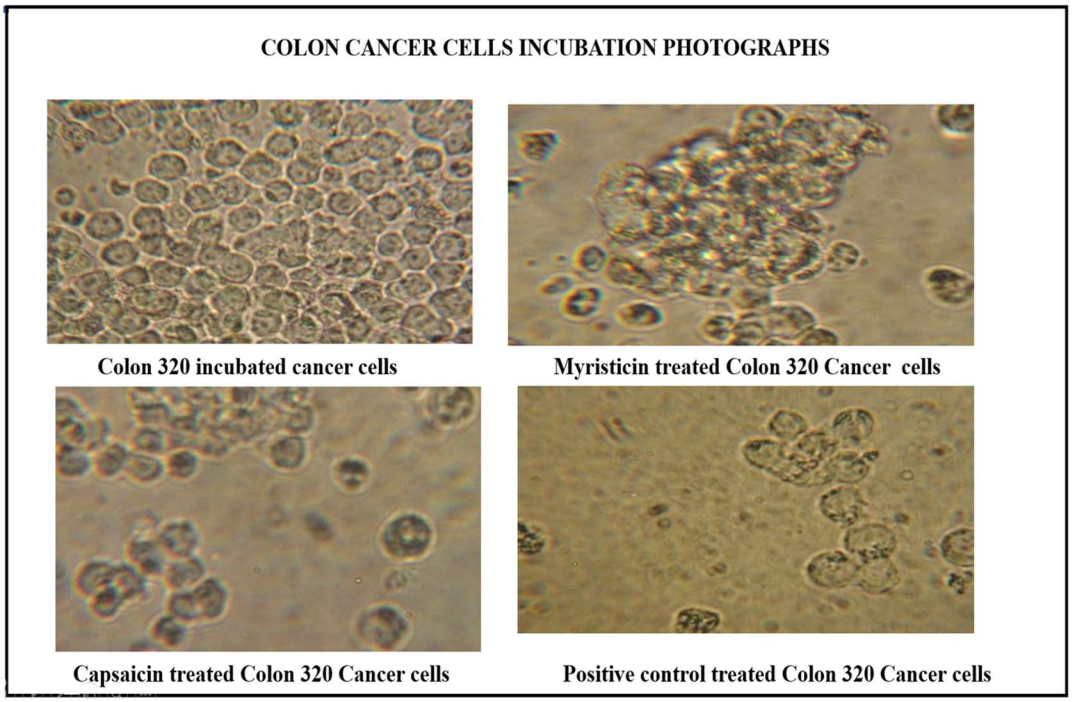


Fig.1. Anticancer effect of title compounds and Positive control treated on Colon 320 cancer cell line.

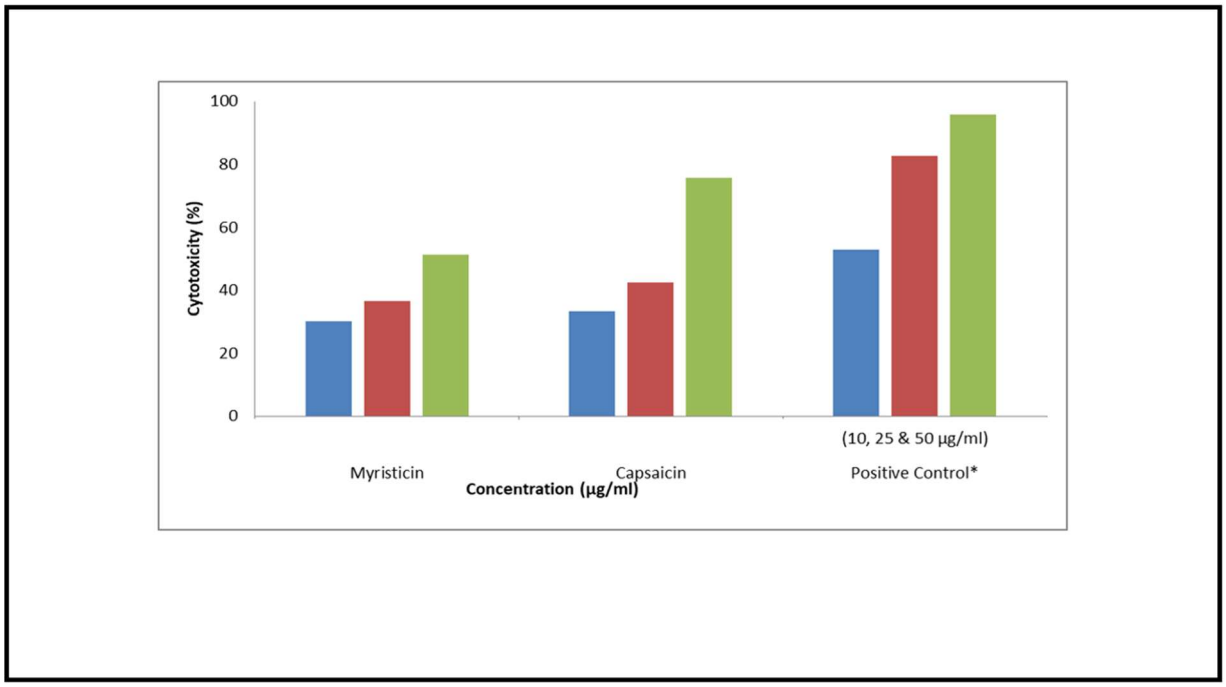
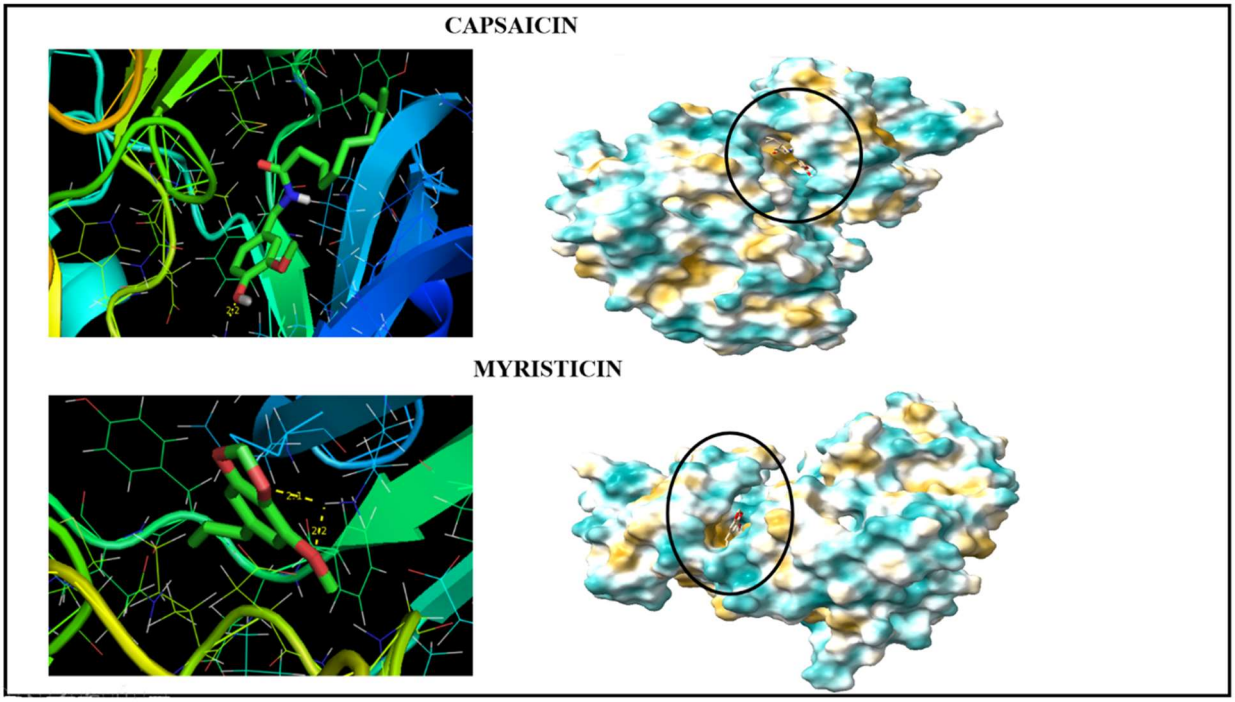


Fig.2. The computed colon cancer cell values from Concentration(µg/ml) - Cytotoxicity (%) for Capsaicin, Myristicin and positive control.



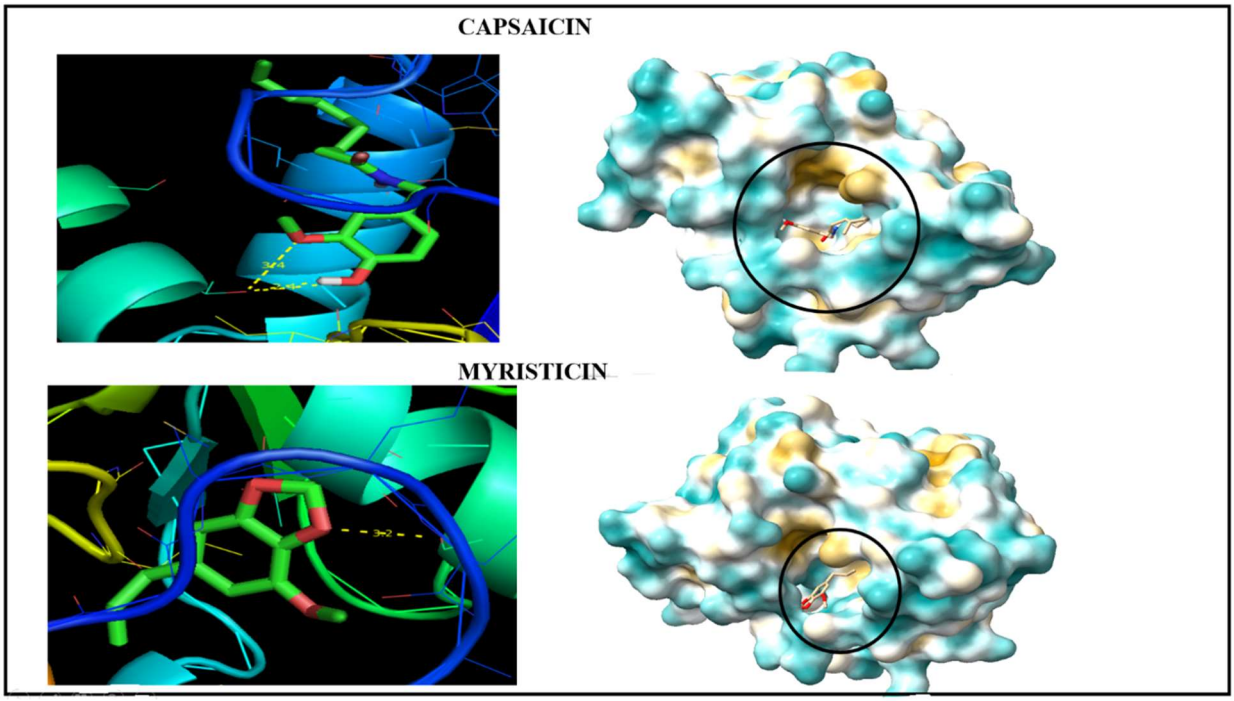
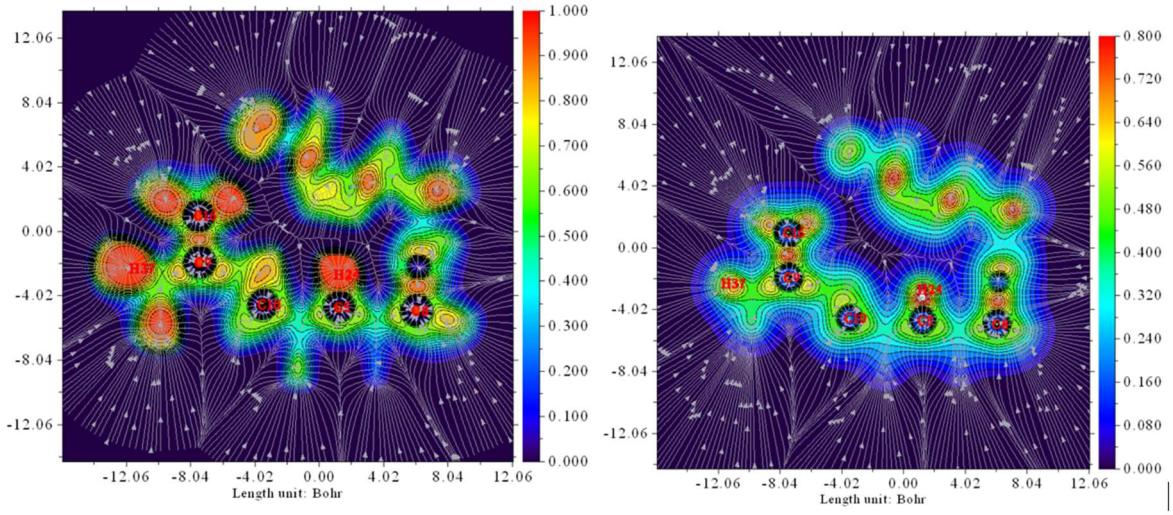


Fig.3. Ligand-CAPSAICIN & MYRISTIN embedded in the docking active sites of CDK2-Kinase inhibitor protein targets – (a) 4DFR and (b) 1LP4

ELF and LOL of CAPSAICIN



ELF and LOL of MYRISTIN

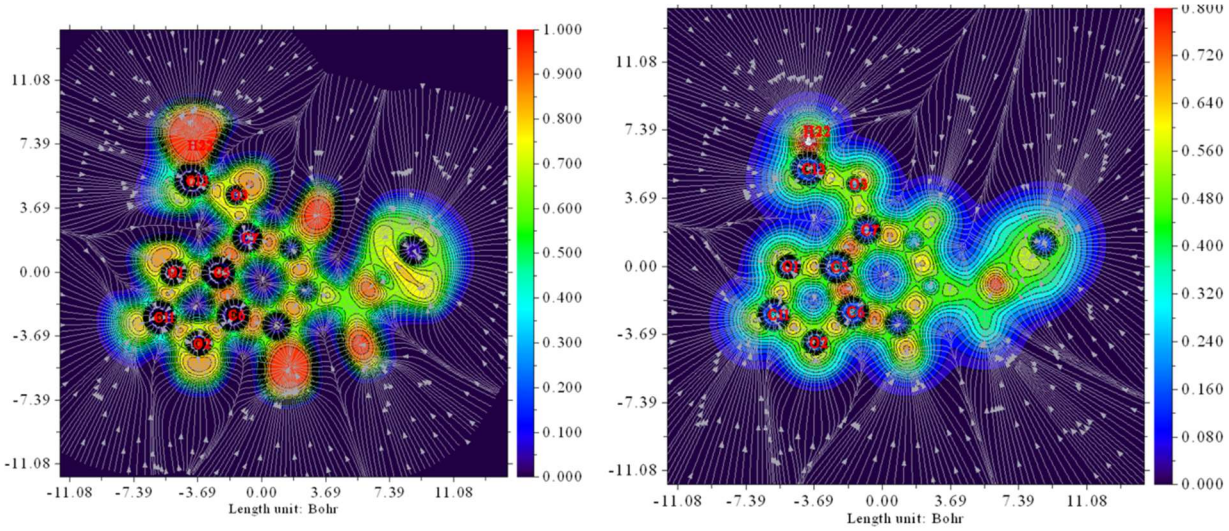


Fig.4. ELF and LOL pictures of of the title molecules