Insilico Docking and Gc Ms Analysis of Selected Bioactive Compounds from *Lantana Camara* Methanolic Leaf Extract Against Breast Cancer Activity

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Abstract

One of the major problems facing the world today is breast cancer, and partial agonists/antagonists are used to target the hormone receptors in breast cancer treatment. Potent drugs for breast cancer treatment are Tamoxifen, Trastuzumab, Paclitaxel, etc. which show adverse effects and resistance in patients. The aim of the study has been on certain phytochemicals of *Lantana camara* L which has potent actions breast cancer target protein inhibition. The present study was designed to determine the bioactive compounds in the whole plant methanol extract of *Lantana camara* L and evaluate the anti-breast cancer activity. GC-MS analysis of *Lantana camara* L confirm the active Heptadecene-(8)-Carbonic Acid-(1), 1,2-benzenedicarboxylic acid, bis2-methylpropyl) ester, Stigmasterol, Decanoic Acid, 1-Tetradecanol phytochemicals.The results confirm that all the compounds has the best docking score for breast cancer compared to standard drug. This study suggests that the selected phytochemicals can be further investigated and evaluated for breast cancer treatment and management strategies.

Keywords: Breast cancer, *Lantana camara*, GC-MS, Docking, Phytochemicals.

1. Introduction

One of the most prevalent forms of cancer among women is breast cancer. A number of risk factors related to bio-molecular dynamics contribute to the underrecognition of breast carcinogenesis. The risk of breast cancer has increased since the past 50 years and accounts for 23% of all cancer deaths in Asia according to the statistical reports of WHO [1].

Number of molecular factors are determined which are used in diagnosis and remedy of breast cancer.The available potent drugs for breast cancer show adverse effects and are found ineffective in patients. Furthermore, this acquired resistance that is vulnerable to cancer-related mutations and the resistance due to minor heterogeneous subpopulation may improve the treatment's inefficiency. Medicinal plants and their extracts are used as a source of medicine. 25% of total medicines are taken from the plants in well developed countries while in developing countries rate is much higher [2]. Phytochemicals are molecules present in plants and control the number of diseases.They are regularly investigated for modern medicine nowadays. These compounds serve as a major factor for the synthesis of various therapeutic agents [3]. Phytochemicals have been reported to show various encouraging activities against human cancer models [4]. The use of structure-based virtual screening for finding effective compounds from phytochemicals

for designing a drug against breast cancer is getting momentum in the last decade [5]. The objective of this work is to discover a potent treatment for breast cancer by screening phytochemical molecules in leaves of Lantana camara using a bioinformatic technique.

Lantana camara L. is a medicinal aromatic plant that belongs to the family Verbenaceae and occurs in most parts of the world as an evergreen notorious weed species. It is widely used in different traditional medical practices for treating various health problems. Different parts of the plant are used in treating various human ailments such as measles, chicken pox, tetanus, malaria, cancers, asthma, ulcers, fevers, eczema, skin rashes, cardiac disorders, and rheumatism [6]. Also leaf extracts and essential oil of *L. camara* leaves possess larvicidal activities, antioxidant, anti-inflammatory, analgesic, antidiabetic, hypolipidemic, anthelmintic, wound healing, and antipyretic properties [7 & 8]. The presence of numerous bioactive phytocompounds, including terpenoids, alkaloids, flavonoids, phenolics, glycosides, and steroids as significant phytoconstituents, is what gives the plant its medicinal potential [9].

In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species. A detailed literature review on the plant in investigation has shown that so far there are no published reports worldwide, related to the possible chemical components of *Lantana camara* L. So, the present study was aimed to investigate the possible chemical components by first preparing the methanolic extract and separation and identification of the compounds by subjecting it to GC-MS analysis. The Molecular docking is a methodology applied to study molecular behavior on target proteins binding. It is a tool which is used extensively in drug discovery. In this study, the GC-MS analysis was carried out the *Lantana camara* L and identified the list of the phytochemicals. Further, the high percentage of the phytochemicals were screened and evaluate the anti-breast cancer activity using molecular docking analysis.

2. Methodology

2.1. Plant material collection and extraction

The fresh leaves of *Lantana camara* L were collected from Tirupattur District, Tamilnadu. The samples were dried and to be crushed into a homogenous powder for extraction. A portion of dried leavesparts (100 g) of *Lantana camara* L was placed in a Soxhlet apparatus. Extraction was performed with 750 ml of methanol for 48 h at a temperature not exceeding the boiling point of the solvent. Extract was filtered through a 45µm filter. The resulting solution was concentrated in vacuum to dryness to give methanol extract (9 g). The extract was stored in a refrigerator at 4°C for further use.

2.2. Gas chromatography-mass spectrometry (GC-MS) analysis

The phytochemical investigation of methanolic extract was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver: 5.0, Thermo MS DSQ II. The oven temperature was held with an initial temperature at 40°C for 3 min and programmed to reach 260°C in 19.33 min. Helium was used as the carrier gas, at a flow rate of 2.77 mL/min. The injection of

sample in split mode with a ratio of 51.0[10]. The chemical components were determined by the relative area peak and were identified using retention indices as well as Wiley 7.0 mass-spectral libraries[11]. The compounds with the similarity index of the percentage of peak area were more than 85% were determined as the chemical components of *Lantana camara* [12].

2.3. Molecular Docking

X-ray crystal structures of Epidermal Growth Factor Receptor tyrosine kinase protein (PDB ID: 1M17) were retrieved from Protein Data Bank. Hydrogen was added to the protein 1M17 applying the force field algorithm subsequently the energy of protein was minimized using CHARM forcefield in Discovery studio. The compounds selected structures from GC/MS analyses were retrieved as a sdf file from the Pubchem database at https://pubchem.ncbi.nlm.nih.gov. The phytochemical and standard drug were converted to three-dimensional coordinate using chemdraw software[13]. Adequate non-polar hydrogen bonds were added, rotatable bonds and torsion tree was generated and made all the ligand and its complexes as perfect modules. Energy was then reduced and recorded in SDF file format for docking experiments. The CDOCKER energy, CDOCKER Interaction energy, Hydrogen bonds, binding energies, protein energy, and ligand-protein complex energy were used to calculate the ligand binding affinity[14].The CDOCKER energy was mentioned in negative values. Greater negative energy value indicates higher binding affinity of the molecule with the target protein[15]**.**

3. Result and discussion

3.1. GC/MS analysis

After the successful conventional hot Soxhlet extraction of the whole part of the plant in investigation, the methanolic extract results pertaining to GC-MS analysis of the methanolic extract of *Lantana camara* L lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC[16]. The various components present in the entire herb of *Lantana camara* Lthat were detected by the GC-MS are shown in Table 1. The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in [Tabel 1]. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds[17]. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The present study helps to screen the high percentage of Heptadecene-(8)-Carbonic Acid-(1), '1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester', Stigmasterol, Decanoic Acid, 1-Tetradecanol compounds.

Table 1. GC-MS analysis of the *Lantana camara* L leaves extract

RT	Phytocompounds	Formula	Mass	Area (%)
3.69	1- Octen -3-yl – n- propionate	$C_{11}H_2oO2$	184.1	0.79

4.78	Heptadecene-(8)-Carbonic Acid-(1)	$C_7H_{11}NO_2$	141.1	10.7
5.45	4(1H)- Pyrimidine 2, 3 dihydro-5-methy-2-thioxo	$C_5H_6N_2OS$	142.0	0.47
6.02	2-Hexene, 1 methoxy-3-methyl (E)	$C_8H_{16}O$	128.1	0.68
7.45	2- Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.1	0.46
9.95	Phenol, 2, 4-bis(1,1- dimethylethyl),	$C_{14}H_{22}O$	206.2	1.21
10.94	6-Tridecanol, 3,9, diethyl	$C_{17}H_{36}O$	256.1	2.91
12.22	1,16-Hexadecanediol	$C_{16}H_{34}O_2$	258.1	0.44
12.56	4-(2,4-Diemethylcyclohex-3-enyl)buy-3-en-2-one	$C_{12}H_{18}O$	178.1	1.38
12.77	4-(3-Hydroxy-2,2,6-trimethyl-7-oxa-bicyclo(4,1,0]hep-1-yl)-but-3-en-2one	$C_{13}H_{20}O3$	224.1	0.59
12.88	5,5-8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-Chromene	$C_{12}H_{20}O$	180.2	0.42
13.09	Chloroacetic acid, tetradecyl ester	$C_{16}H_{31}C_1O2$	290.1	1.05
13.55	3,7,11,15-Tetrmethyl-2-hexdecen-1-ol	$C_{20}H_{40}O$	296.3	5.13
13.63	1-Dodecanol,3,7,11,-trimethyl	$C_{15}H_{32}O$	228.2	0.62
13.80	Phytol, acetate	$C_{22}H_{42}O_2$	338.3	0.97
14.08	7,10,13-Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264.2	0.68
14.39	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.3	2.19
14.83	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{32}O_2$	256.2	17.42
15.92	12,15-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.3	1.92
16.04	Tert-Hexadecanethiol	$C_{16}H_{34}S$	258.2	0.42
16.11	Phyol	$C_{18}H_{30}O_2$	296.3	7.70
16.39	Stigmasterol	$C_{18}H_{30}O_2$	278.2	19.65
16.52	n-Propyl9, 12-octadecadienoate	$C_{21}H_{38}O_2$	322.3	0.52
16.60	Decanoic Acid	$C_{18}H_{36}O_2$	284.3	3.36
16.85	13-tetradecenoic acid, 2,4,6,8-tetramethyl-methyl ester, (all-R-)	$C_{19}H_{36}O_2$	296.3	1.12
17.05	Phytol, acetate	$C_{22}H_{42}O_2$	338.3	1.14
17.55	2-methylhexacosane	$C_{27}H_{56}$	380.4	1.26
18.51	Cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310.3	0.46
18.56	Heneicosane	$C_{21}H_{44}$	296.3	1.15
19.30	Hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330.3	1.96
19.35	1-Decanol-2-hexyl	$C_{16}H_{34}O$	242.3	1.22
19.60	Bis(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390.3	0.42
20.60	Methyl 5, 12-octadecadienoate	$C_{19}H_{34}O_2$	294.3	1.56
20.65	1-Tetradecanol	$C_{22}H_{38}O_2$	334.3	3.56
20.82	Pentacosane	$C_{25}H_{52}$	352.4	0.97
23.07	.gamma-Tocopherol	$C_{28}H_{48}O_2$	416.4	0.58
24.59	.gamma -Sitosterol	$C_{29}H_{50}O$	414.4	1.49
26.15	Z-10-methyl-11-tetradecen-1-ol-propionate	$C_{18}H_{34}O_2$	282.3	0.42

3.2. Molecular Docking

Receptor EGFR tyrosine kinase is known to play a fundamental role in numerous processes such as cell growth, cell proliferation, and metabolism. Deregulation of EGFR through activation of oncogene and suppression of tumor suppression gene can result in an abnormal cascade of signaling pathways[18]. High levels of EGFR activity and mutation in the EGFR are observed in a variety of cancers that correlate with poor prognosis and resistance to chemotherapy[19]. All the selected natural compounds were docked with the X-ray crystal structures of Epidermal Growth Factor Receptor tyrosine kinase protein (PDB ID: 1M17). Molecular docking procedure identifies the thermodynamic optimal energy value, types of interactions, potential of bonding, and conformations against these receptor protein molecules [20]. The molecular docking binding energy of Heptadecene-(8)-Carbonic Acid-(1), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, Stigmasterol, Decanoic Acid, 1-Tetradecanol and standard drug 5-fluorouracil are listed out in Table 2.

Table2. The molecular docking energy of selected phytochemical with 1M17 receptor.

SI	Phytochemicals	-CDOCKER Energy Kcal/mol
1.	Heptadecene-(8)-Carbonic Acid-(1)	29.2756
2.	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	27.6874
3.	Stigmasterol	26.3347
4.	Decanoic Acid	22.3476
5.	1-Tetradecanol	19.2547
6.	5-fluorouracil	15.5476

The active site of the Epidermal Growth Factor Receptor tyrosine kinase protein was identified by standard drug which already bound to in PDB data (Figure 1). In this docking analysis, Heptadecene-(8)-Carbonic Acid-(1) molecule shows higher binding interaction (-29.2756 Kcal/mol) with tyrosine kinase protein compared to the standard drug (5-fluorouracil). The carboxylic group in Heptadecene-(8)-Carbonic Acid-(1) forms two H-bond with Asp 831 and Thr 830 amino acid with 2.9 Å and 2.6 Å distance respectively. The presence of more alkyl chain in this molecule forms more alkyl interaction with Val 702, Ala 719, Leu 694, Leu 768 and Cys 773 amino acid. Moreover, Leu 753, Lys 721, Thr 766 forms van der Waals interaction with Heptadecene-(8)-Carbonic Acid-(1) (Figure 2).

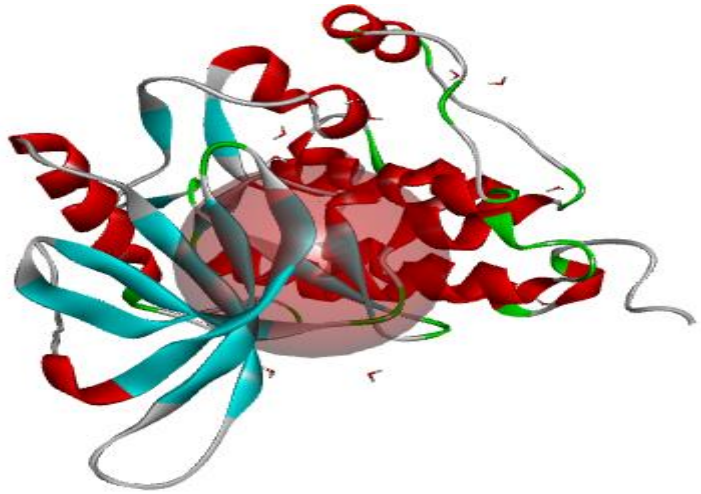

Figure 1. Secondary structure of the Epidermal Growth Factor Receptor tyrosine kinase with active site sphere.

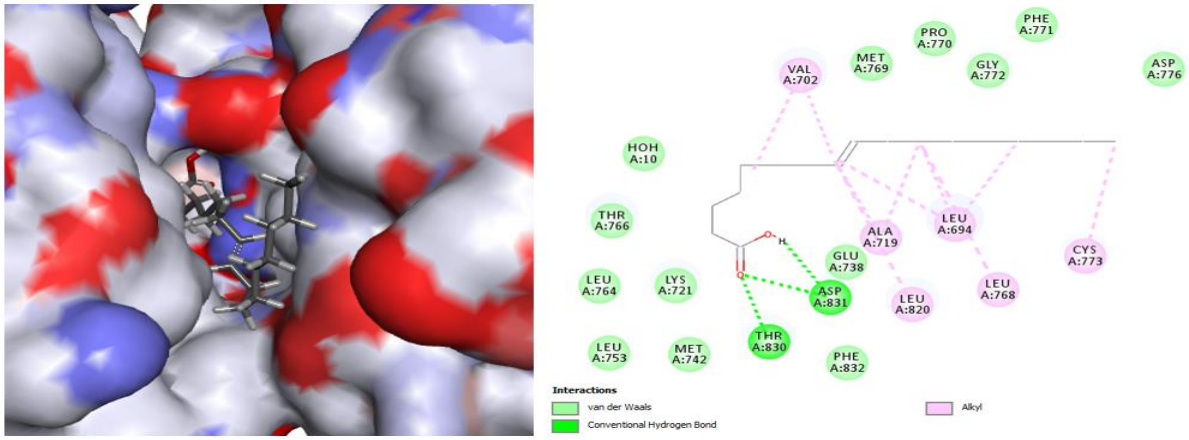

Figure 2. 3D and 2D molecular interactions of the Heptadecene-(8)-Carbonic Acid-(1) with 1M17 protein.

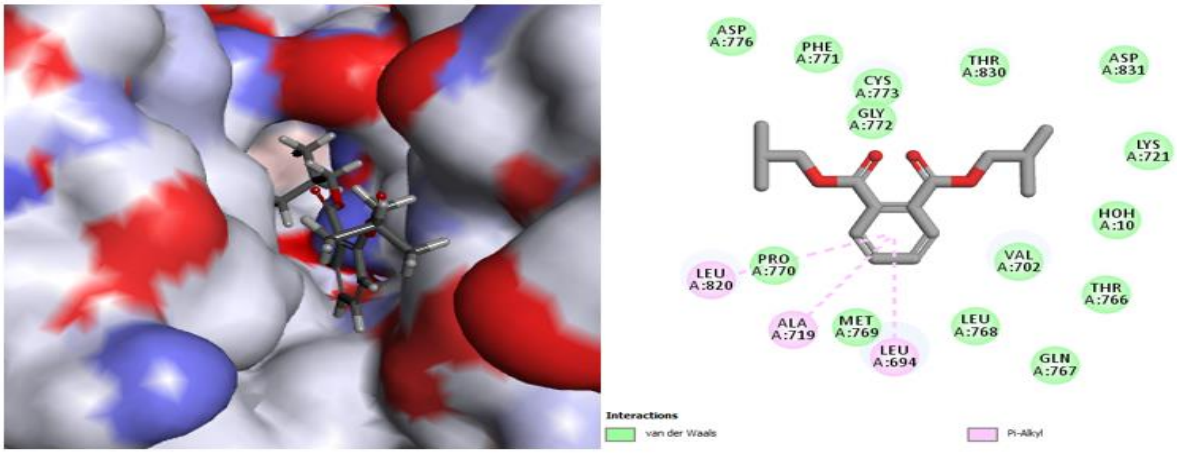

Figure 3. 3D and 2D molecular interactions of the 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester with 1M17 protein

The CDOCKER energy of the 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester molecule is -27.6874 Kcal/mol^{-1}. It's interacted with 1M17 protein by van der Waals and Pi-alkyl interactions. There is no H-bond was observed in this molecule. The benzene molecule in this 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester shows hydrophobic interaction with hydrophobic (Leu 820, AlA 719, Leu 694) amino acid (Figure 3).

Similarly, Stigmasterol interacted with active site of 1M17 protein by various van der Waals and Alkyl interaction with -26.3347 Kcal/mol^{-1} CDCOKER energy. The OH group this molecule forms strong H-bond with Glu 780 amino acid. The 5 and 6 membered ring shows alkyl interaction with Leu 820, Leu 694 and Ala 719 amino acid (Figure 4).

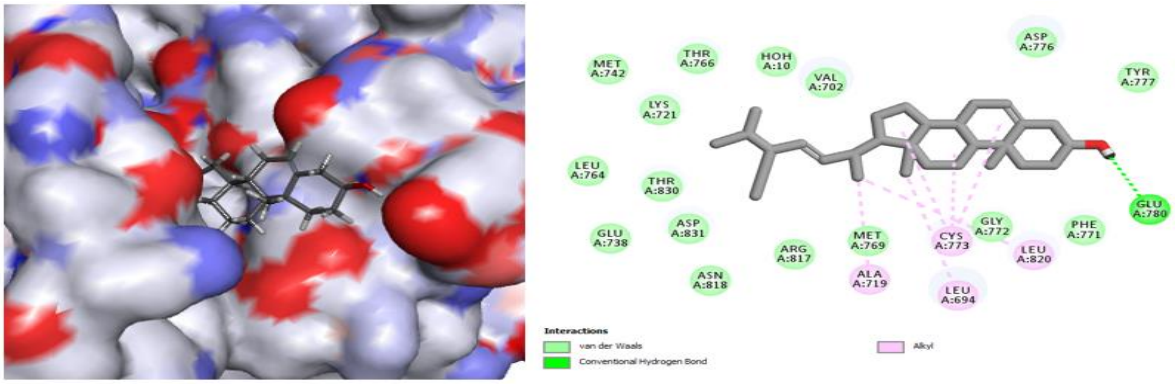

Figure 4. 3D and 2D molecular interactions of the Stigmasterol with 1M17 protein

The carboxylic acid of the Decanoic Acid forms one strong H-bond with MET 769 amino acid. Further Leu 768 interacted with Ketone of this molecule by covalent hydrogen bond. Additionally, the alkyl chains of Decanoic Acid show only Alkyl and Pi-Alkyl interaction with Val 702, Leu 694, Phe 699 amino acid (Figure 5). The CDOCKER binding energy of the Decanoic Acid is -22.3476 Kcal/mol^{-1}. Moreover 1-Tetradecanol molecule show moderate binding affinity to the 1M17 protein. In this binding analysis, the alkyl chains of the 1-Tetradecanol forms alkyl and van der Waals interaction with respective amino acid (Figure 6). In this molecular docking analysis, 5-fluorouracil was used as a standard drug. This drug used as a standard drug for anti-cancer activity. From this docking analysis, the 5-fluorouracil has less binding affinity compared to the other phytochemicals. The binding energy of the 5-fluorouracil is -15.5476 Kcal/mol^{-1}.

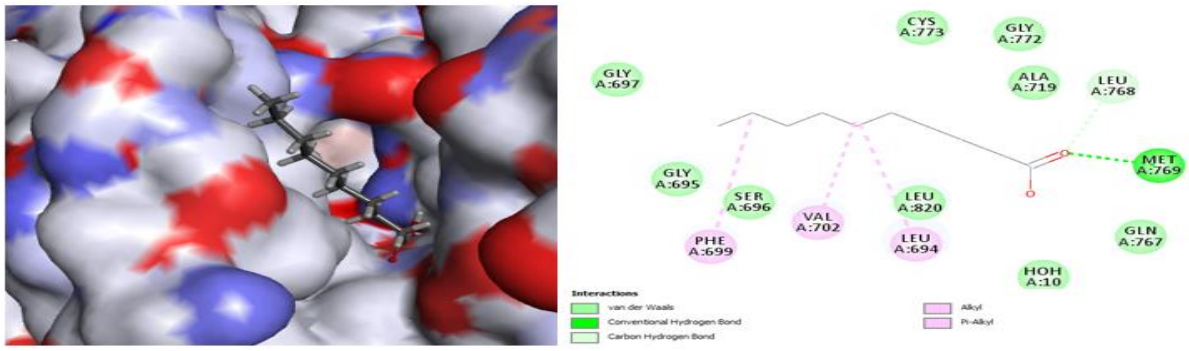

Figure 5. 3D and 2D molecular interactions of the Decanoic Acid with 1M17 protein.

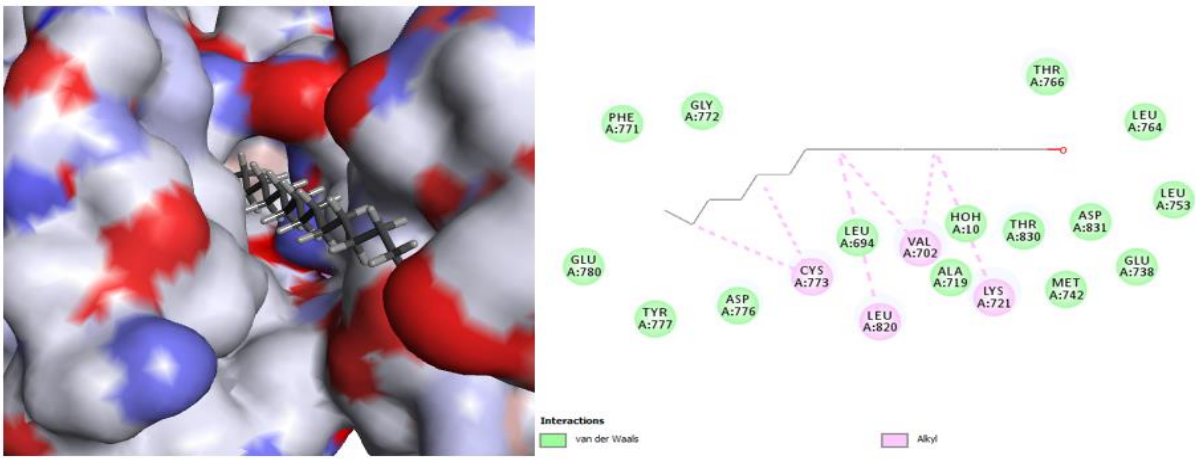

Figure 6. 3D and 2D molecular interactions of the 1-Tetradecanol with 1M17 protein.

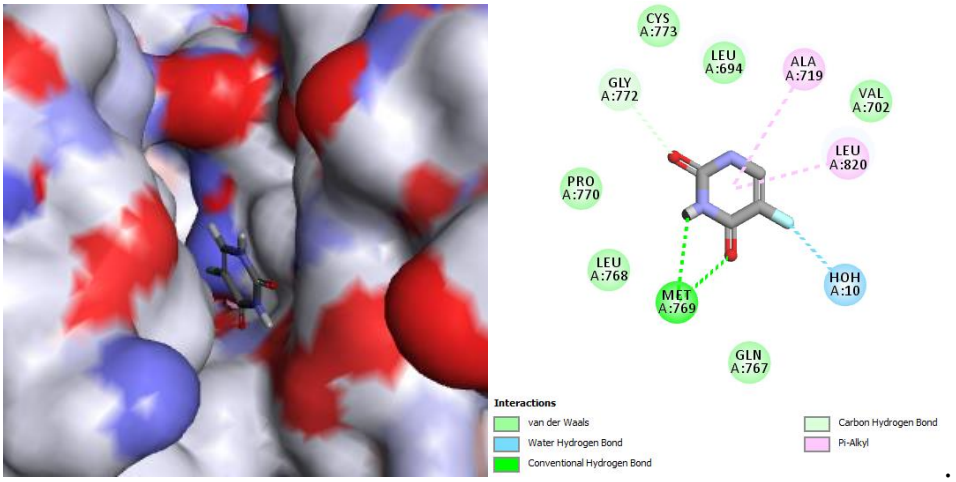

Figure 7. 3D and 2D molecular interactions of the 5-fluorouracil with 1M17 protein.

4. Conclusion

The presence of various bio-active compounds detected after GC-MS analysis using the methanolic extract of *Lantana camara* L justifies the use of whole plant for various elements by traditional practitioner. However, isolating certain phytochemical components and submitting them to biological activity would undoubtedly produce successful results and open a new

field of research into those specific components and their pharmacological effectiveness.From these results, it could be concluded that "*Lantana camara* L" contains various bio-active compounds. From molecular docking result, Heptadecene-(8)-Carbonic Acid-(1), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, Stigmasterol, Decanoic Acid, 1-Tetradecanol phytochemicals of *Lantana camara* L will be the future promising drug capability to overcome cancer. Also, the compounds can be further investigated by carrying out *in-vitro* and *in-vivo* studies on breast cancer models for the management and prevention of breast cancer.

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