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In vitro and *in silico* studies on the biochemistry and anti-cancer activity of phytochemicals from *Plumbago zeylanica*

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Plumbago zeylanica is an effective medicinal plant and a potent herb for various diseases. The leaves of *P. zeylanica* were subjected to crude extraction using Ethyl acetate. Chromatographic analysis of the crude extracts of *P. zeylanica* were studied in thin layer chromatography on a silica gel matrix. Further the extracts were purified in column chromatography and the fractions were eluted in Ethyl acetate, Chloroform solvent system. The molecules eluted in the fractions were checked in UV-spectroscopy and further characterised by GC-MS analysis. Phenol, 2,4, bis (1,1-dimethyl ethyl)-(7311) highlighted from the chromatographic studies was studied for *in silico* anticancer effect on matrix metalloproteins, whose dysfunction cause oral squamous cell carcinoma.

Keyword: Column chromatography, Matrix metalloproteins, Oral squamous cell carcinoma, Phenol, 2,4, bis (1,1-dimethyl ethyl), *Plumbago zeylanica*, Secondary metabolites

In Ayurveda, *Plumbago zeylanica* is an effective medicinal plant and its parts are used for the treatment of asthma, gastrointestinal disorders, cough, jaundice, internal abscesses, urinary calculi, migraine, seminal weakness, insanity and vaginal discharge. It is also used as antiseptic, detoxicant and febrifuge. It is predominantly spread in Yemen, Oman and Saudi Arabia. The roots of *P. zeylanica* is effective in curing skin problems and has anti-inflammatory, anti-cancer, anti-diabetic and anti-hyperlipidemic property[1-6].

Primary metabolites are primary products of the plants, such as proteins, lipids, heme, chlorophyll, carbohydrates and nucleic acids, which are involved to maintain plant cells through the primary metabolic process. Secondary metabolites are products of the plants such as chemicals, which will not participate in biochemical reactions and maintaining plant cells, but plays a shielding role against pathogens[7]. Secondary metabolites extracted from plants are more effective and acts as an efficient drug for various diseases[8]. Their action is similar to ligands, hormones, signal transduction molecules, neurotransmitters, endogenous metabolites and its therapeutic efficacy in humans is based on their respective target sites[9]. The study of structural similarity between the secondary metabolites and endogenous substances of particular organisms is called progressive molecular modelling[10]. Secondary metabolites protect plants against biotic (bacteria, fungi, nematodes, insects or grazing by animals) and abiotic (higher temperature and moisture, shading, injury or presence of heavy metals) stresses. They are divided into three chemically distinct groups - Terpenes, Phenolics, N (Nitrogen) and S (sulphur) containing compounds[11].

Oral squamous cell carcinoma (OSCC), is a primary malignancy arising in the neck and head. Nearly 500,000 new cases are recorded each year[12]. In Taiwan, it is fourth popular male cancer[13] because of the use of alcohol and tobacco[14,15]. In the early stage, the treatment option for OSCC is a surgical operation, but intermediate and advanced stage treatment involves radiotherapy and chemotherapy along with standard treatments[16]. The gene matrix metalloproteinase-2 (MMPs2) has been expressed in the tissues of gastric cancer relative to the matched normal tissues associated with gastric cancer cells metastasizing[17]. MMP-2 and MMP-9 are often

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dysregulated in Ovarian cancers and are seen as possible biomarkers[18].

Expression and involvement of matrix metalloproteinase (MMPs) in oral squamous cell carcinomas, have been studied by many authors[19,20]. The matrix metalloproteinase (MMPs) are responsible for degradation of tissues in both normal and pathological conditions such as tumour invasion and metastasis whereas metalloproteinase tissue inhibit ors (TIMPs) decrease the function of those MMPs. Therefore, MMPs can directly or indirectly mediate the angiogenic response by modulating the balance between pro-and antiangiogenic factors[21]. Matrix metalloproteinases (MMPs) are proteolytic enzymes of a secretary or membrane type that operate on extracellular protein components such as collagen, gelatin, elastin, laminin, fibronectin, and integrin. MMPs are synthesized as zymogens, and activated by autoproteolysis or other proteases to functional forms[22]. They are involved in trophoblast implantation, embryogenesis, bone growth, angiogenesis, wound healing, and tissue regeneration. So far 24 MMPs are identified in vertebrates, including 23 in humans[23]. They are endopeptidases and their enzymatic activity is determined by Ca^{2+} and Zn^{2+} ions[24].

In Oral squamous cell carcinoma, function and regulation of MMPs are not proper. Dysfunctional MMPs is one of the initiators of Oral squamous cell carcinoma. All types of MMPs consist of propeptide domain, which is an inactive enzyme, followed by catalytic domain which has the zinc active site attached to methionine and three histidine residues and the hemopexin (3c7x) is one of the c-terminal domain. The C-terminal domain bind with inhibitors and substrate specifically. The extracellular MMPs are activated by the integrins or proprotein convertases which are present in the cell. Proprotein convertases break the prodomain from the catalytic domain, which activates MMPs[25]. The abnormal production of normal tissue and activity of various MMPs causes several cancers.

The aim of the study is to screen the active metabolites from the crude leaf extracts of *P. zeylanica* by thin layer chromatography. Column chromatography was performed for the plant extracts and the eluted fractions was analysed for the presence of organic molecules by studying their absorption profile when subjected in the electromagnetic spectrum on a UV spectroscopy. The fractions that had similar resonance which reflected in their absorbance was pooled together and further characterized by GC-MS analysis. Six compounds were present in high amounts (more than 10 %) - (i) 9-Eicosene, (ii) Phenol, 2,4-bis(1,1-dimethylethyl), (iii) Pentadecane, (iv) M-Cymene, 5-tert-butyl, (v) Benzene, 1,3-bis(1,1-dimethylethyl), (vi) 9-Octadecene. These 6 compounds were tested for its anticancer (Oral squamous cell carcinoma) property by *in silico* method.

Materials and Methods

Preparation of plant extract

Ethyl acetate solvent (200 mL) was added to 100 g of the leaf of *P. zeylanica* and placed on an orbital shaker for 48 h at room temperature. The filtrate was collected and the extract was concentrated to evaporate the solvent by rotary evaporator at low temperature (70°C) under medium pressure condition. The dried crude extract was collected and stored.

Characterization by thin layer chromatography method

The crude extract was spotted on pre-coated TLC silica plates and allowed to dry. Silica sheet was placed into a beaker containing different solvent systems such as Hexane, chloroform, ethyl acetate and methanol. Solvents used were - (i) Hexane: Chloroform in the ratio of 100: 0, 90: 10, 80: 20, 70: 30, 60: 40, 50: 50, 40: 60, 30:70, 20: 80, 10: 90 (ii) Chloroform: Ethyl acetate in the ratio of 100: 0, 90: 10, 80: 20, 70: 30, 60: 40, 50: 50, 40: 60, 30: 70, 20: 80, 10:90 and (iii) Ethyl acetate: Methanol in the ratio of 100: 0, 90: 10, 80: 20, 70: 30, 60: 40, Sol.50: 50, 40: 60, 30: 70, 20: 80, 10: 90. Visible band identified and measured by R_f value using the following equation[26].

$$R_f \text{ value} = \frac{\text{The distance travelled by the plant sample}}{\text{The distance travelled by the solvent (mobile phase)}}$$

Characterization by Column chromatography method:

The Slurry was prepared using 8 g of silica gel (60-120 mesh) with chloroform and it was poured into the 50 cm column. The column was pre-run three times using chloroform to settle the silica without air bubbles and 10 g of ethyl acetate extract of *P. zeylanica* was placed on the column. The solvent system Chloroform: ethyl acetate (1:1) gave better elution and resolution when checked in a thin layer chromatography.

Characterization by UV-Spectroscopy method

UV-Visible spectroscopy was used to qualitatively identify the presence of molecules in a biological

mixture which resonate in the UV visible region. Natural plant compound that was identified by UV-Visible spectroscopy are many and they are, 280 nm for phenolic extract, 360 nm for phenolic acids, 320nm for flavones *etc*[27]. Visible range from 200 to 800 nm was selected to identify various compounds present in the fraction. UV - spectroscopy instrument Shimadzu UV-1800, Japan was used for the study.

Characterization by GC-MS analysis

Gas chromatograph

Shimdzu GC-2010 Plus gas chromatograph consisted of 2 mm direct injector and 15 M Alltech EC-5 column (250 µM I.D., 0.25 µM film thickness). The split injector was used to introduce the sample in two columns and the split was fixed 10:1. The oven temperature was set at 35°C for 2 min and helium act as the carrier gas for 2 mL per min.

Mass spectrum

A Direct connection with capillary column metal quadruple mass filter period mass spectrometer operating in electron ionization (EI) mode with a software GC-MS solution Ver. 2.6 was used for all analysis. Low-range mass spectra were identified at a resolving power of 1000 (20% height definition) and scanning from *m/*z 25 to *m/*z 1000 at 0.3 sec per scan with a 0.2 sec inter-scan delay. High range mass spectra were determined at a resolving power of 5000 (20% height definition) and scanning the magnet from *m/z* 65 to *m/z* 1000 at 1 sec per scan.

Protein and ligand structure retrieval and docking

The structure of the phytochemicals - 9-Eicosene (5365037), Phenol, 2, 4-bis (1,1-dimethylethy l) (7311), Pentadecane (12391), m-Cymene, 5-tert-butyl (34640), Benzene, 1,3-bis (1,1-dimethylethyl) (136810), 9-Octadecene (5364599) - was retrieved from the PubChem database in 'sdf' format. Consecutively the three-dimensional structure of two anticancer drugs, namely Doxycyclin and Fluorouracil (5-FU) were also downloaded in order to carry out a comparative analysis of their efficiency against the phytochemicals of our interest. Similarly, the three-dimensional structure of the hemopexin domain of protein Matrix metallopeptidase (PDB ID: 3C7X)[28] with196 amino acids in length was also downloaded in '.pdb' format from the Protein Data Bank (PDB)[29]. Its resolution was 1.70 A° and was determined by the X-Ray diffraction method. The quality of the structure was analyzed using the Ramachandran plot[30]. The target is prepared by applying CHARMM force field[31] to add required hydrogen bonds. The binding site of the target was determined and docking was carried out using Discovery studio based on Ligand Fit protocol[32] for each phytochemical as well as already available drugs.

Results

Crude extracts from the leaf of *P. zeylanica* was extracted using ethyl acetate. Plant metabolites were screened by Thin layer chromatography and it was partially purified through column chromatography. The UV-spectroscopy was used to find the presence of organic molecules and those with similar absorption profile was polled to a single fractions which was then characterized by GC-MS. Compounds whose concentration were more than 10 % in GC-MS was tested for anticancer activity by *in silico* model to inhibit MMPs.

Thin layer chromatography

30 solvent systems in various combinations of four solvents in different ratios were used to evaluate the crude leaf extract of *P. zeylanica* on a TLC plate. Four clear bands were obtained on the TLC plate for solvent combination Chloroform 50%: Ethyl acetate 50%, having corresponding R_f values as follow 3.6, 3.8, 2 and 3.4 (Fig. 1A). No bands were obtained for other solvent combinations, *i.e* Hexane: Chloroform and Ethyl acetate: Methanol (Fig. 1B).

Column chromatography

Chloroform: Ethyl acetate (50%: 50%) was poured into the column as a mobile phase to collect 45 fractions of 2 mL quantity. Fraction 1-10- was dark green in colour, fraction 11-20-dark green to light green colour, fraction 21-30-light green colour, fraction 31-45-yellow colour (Fig. 2).

UV-visible spectroscopy

The collected fraction was analyzed by UV range 200 to 800 nm to find out similarity based on the concentration. After recognizing the peak value of the fractions, it was pooled. Fraction 1-10 was described as sample 1, fraction 11-20 was described as sample 2 , fraction 21-30 was denoted as sample 3, and fraction 31-45 represented as sample 4 (Fig. 3)

GC-MS analysis

Samples 1, 2, 3 and 4 were analyzed by GC-MS to detect the name of the secondary metabolite according

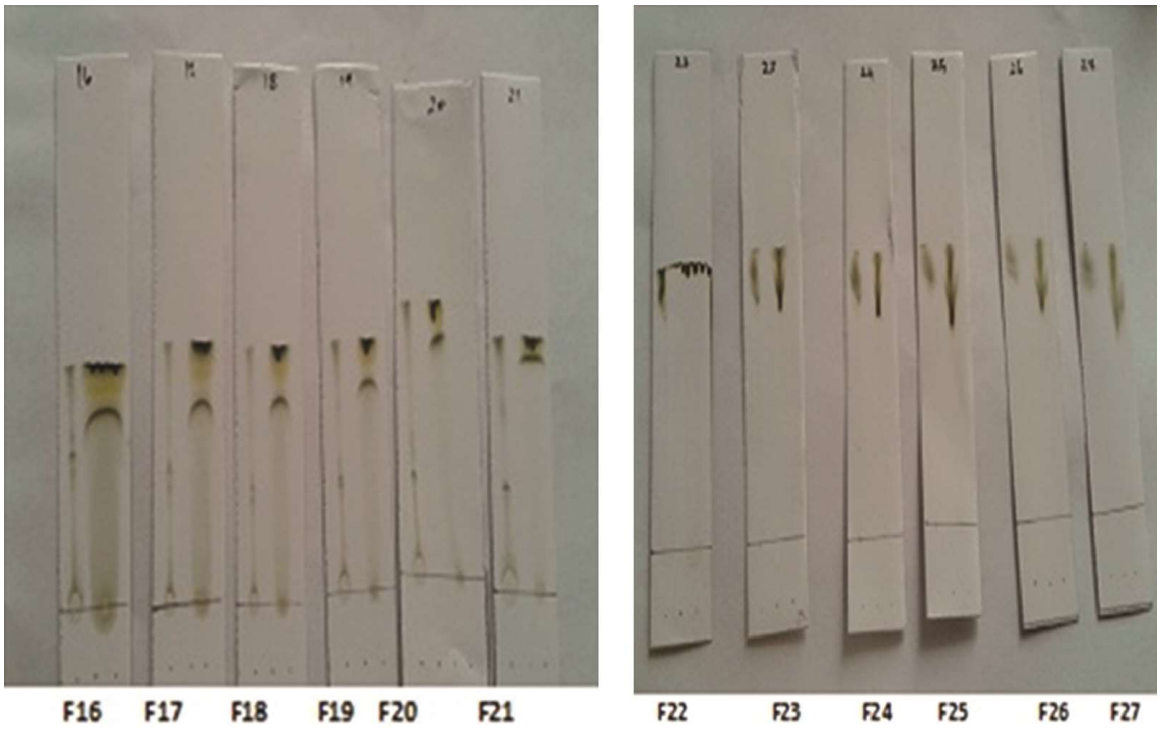

Fig. 1 — Solvent system (A) 16 to 21; and (B) 22 to 27

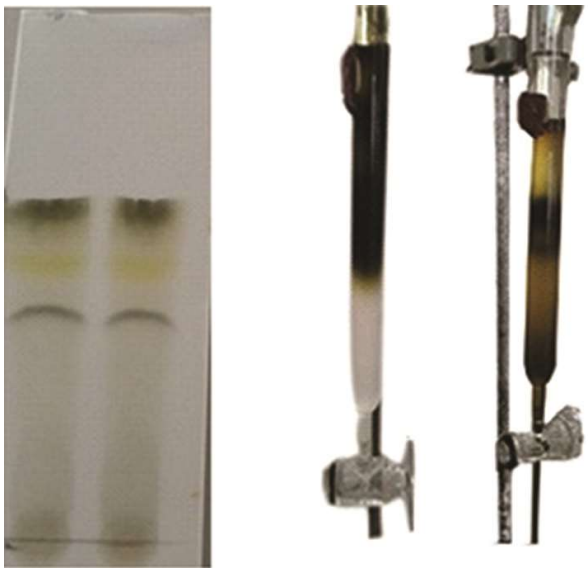

Fig. 2 — 16th fraction & 16th solvent system (Chloform:Ethyl acetate, 50:50)

to the wavelength. Visible range differs depending on functional group Sample 1 consisted of 19 bioactive compounds (Fig. 4 & Table 1), Sample 2 contained 15 bioactive compounds (Fig. 4 & Table 2), Sample 3 contained 10 secondary metabolites (Fig. 4 & Table 3), Sample 4 consisted of 14 secondary metabolites (Fig. 4 & Table 4).

Binding site determination and molecular docking

Grid points in the target structure were mapped and from it, the binding sites found in the target was determined. Among the three binding sites, site 1 is the largest with 462 points and 57.750A0^3 in size. Site 2 is with 188 points and 23.500 A0^3 followed by the smallest site 3 of 120 points and 15.000 A0^3 sizes. ADMET properties of the ligands were screened to test the efficiency of the compound to be used as a drug. The molecular docking analysis of the ligand with the target was carried out and analyzed which revealed the possible confirmation created by each ligand inside the binding site of the target. The binding affinity of each ligand with the target was calculated based on the comparison of all the different binding scores and are represented in the (Table 5) along with the name of the ligand.

Intermolecular interaction of the target and ligand

The intermolecular interaction of the ligand with the target was analyzed to determine the type of bond occurring between them and their distance. Table 6 gives details about the intermolecular interactions occurred between the atoms in the target along with the atoms of the ligand. Figures 1 to 3 shows the two dimensional and three-dimensional representation of the intermolecular interaction of each ligand with the amino acids in the target.

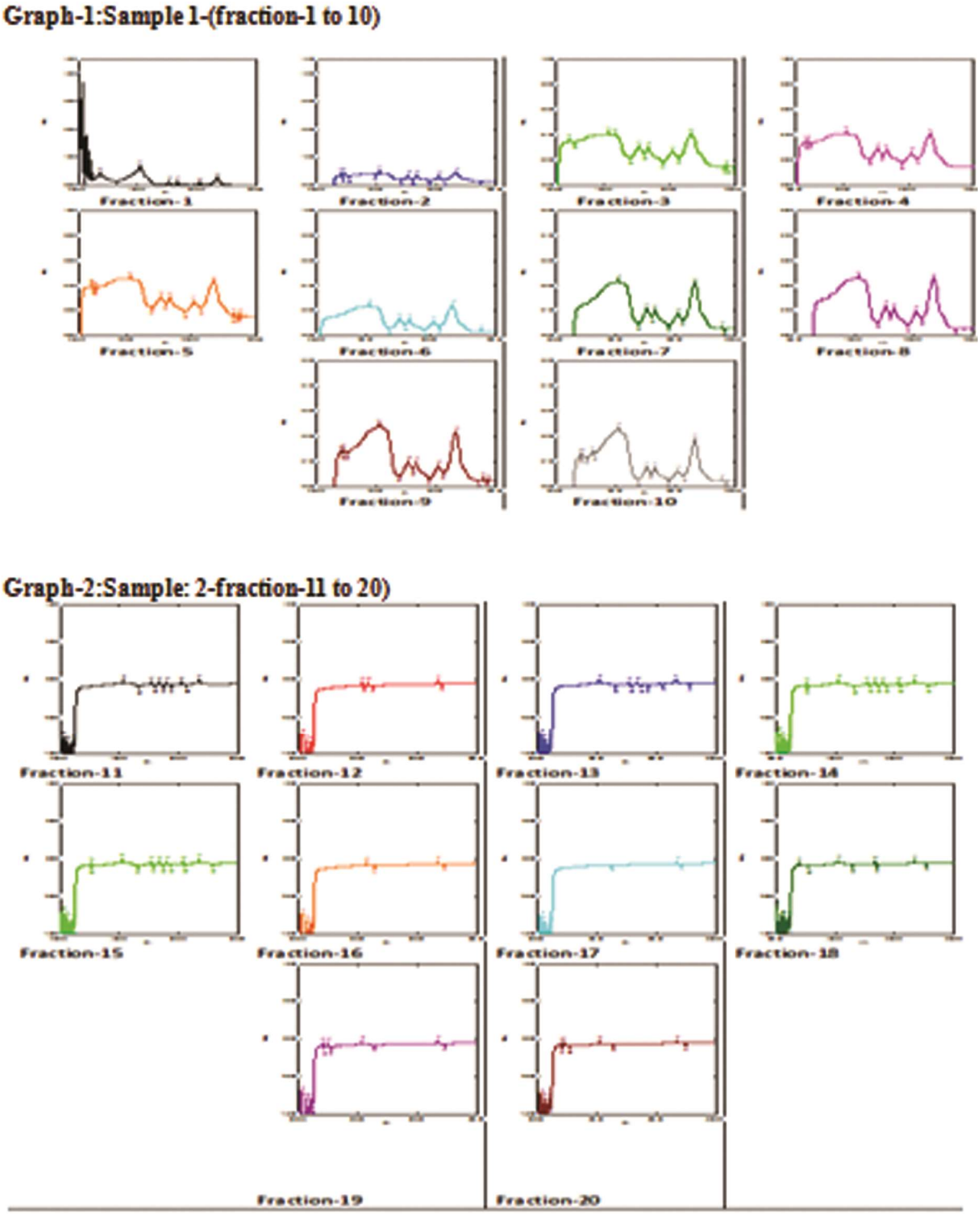

Fig. 3 — Sample 1- (fraction-1 to 10); Sample 2- (fraction-11 to 20); Sample 3- (fraction-21 to 30); and Sample 4- (fraction-31 to 45) (*Contd*.)

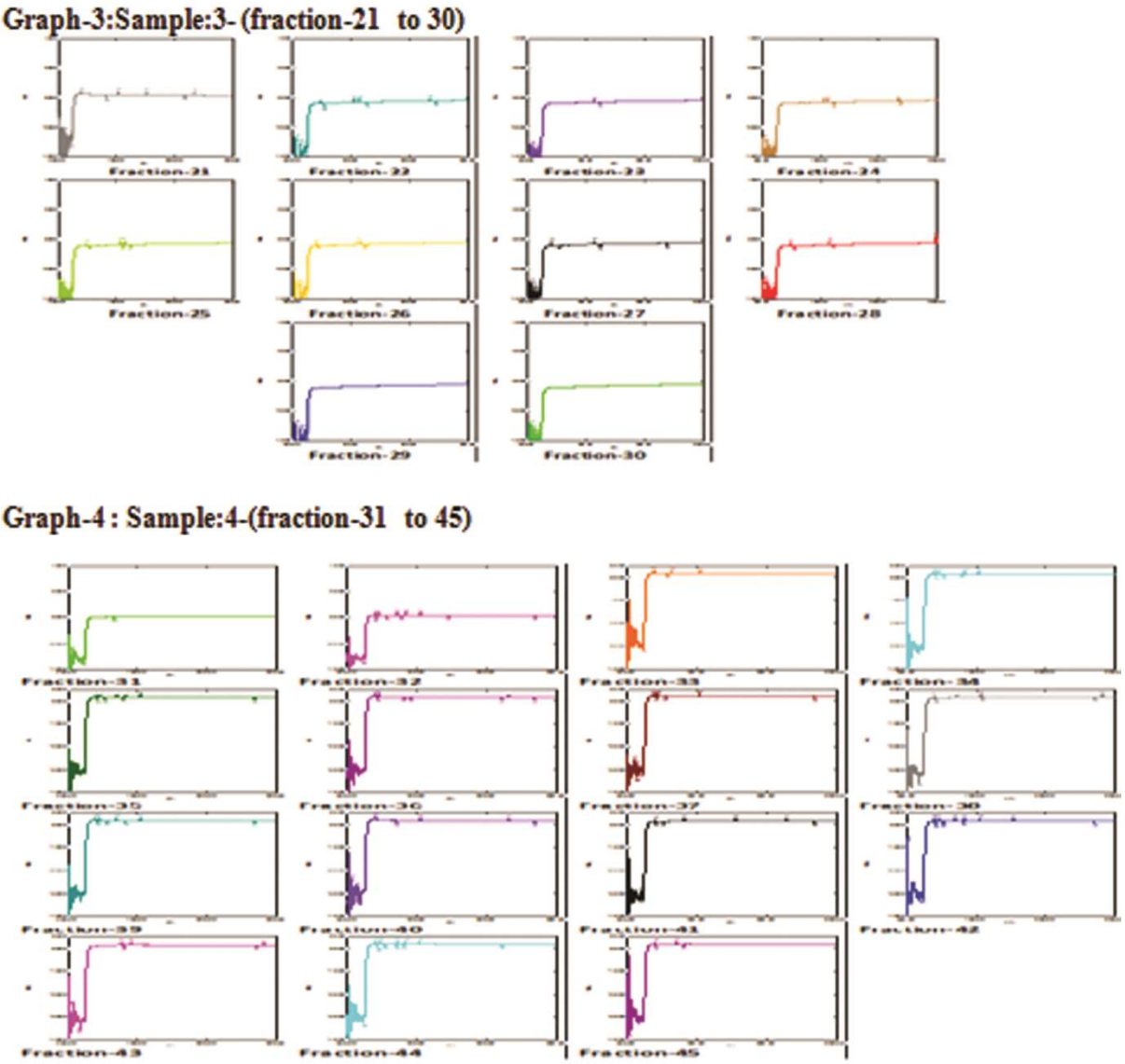

Fig. 3 — Sample 1- (fraction-1 to 10); Sample 2- (fraction-11 to 20); Sample 3- (fraction-21 to 30); and Sample 4- (fraction-31 to 45)

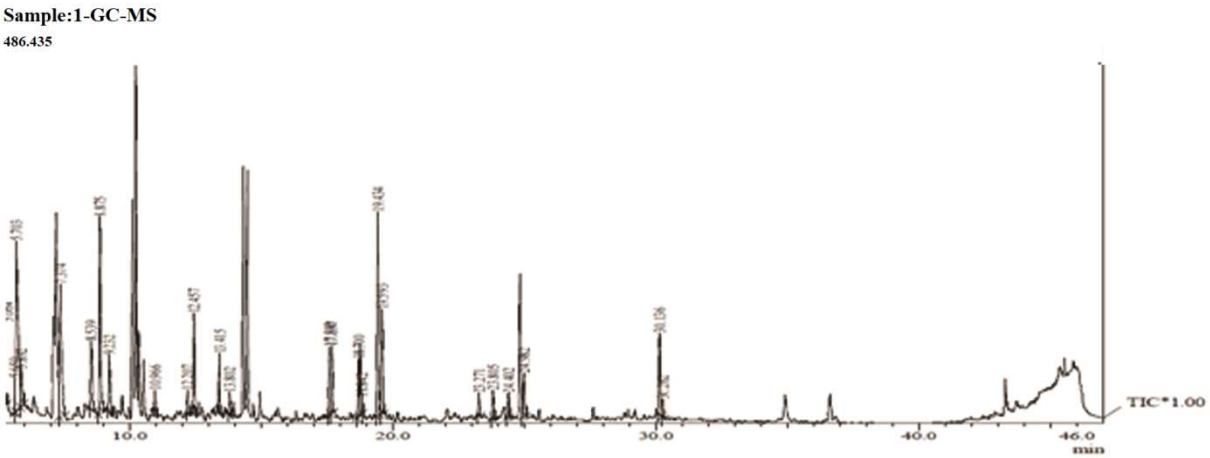

Fig. 4 — Samples 1-4 GC-MS (*Contd*.)

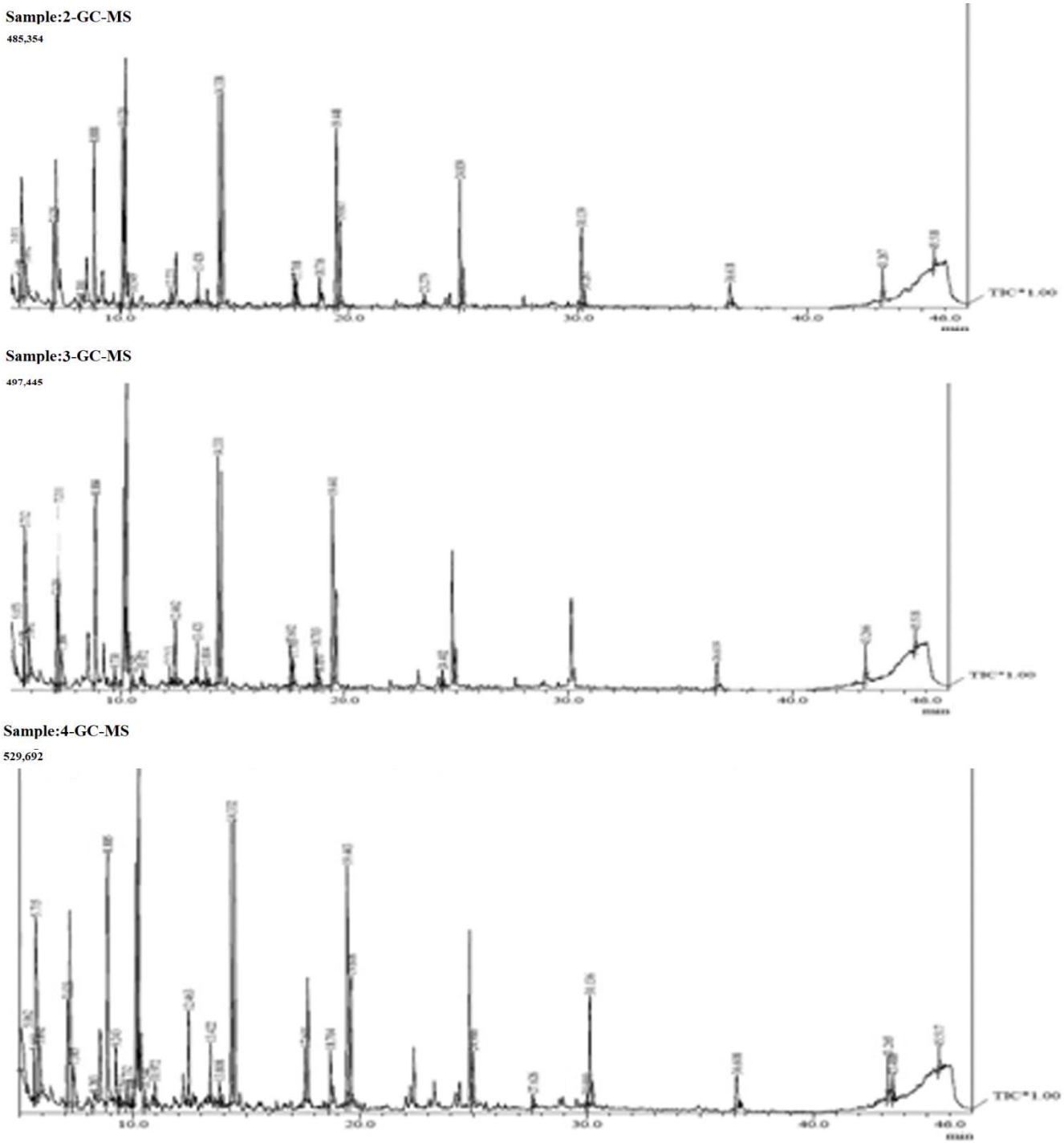

Fig. 4 — Samples 1-4 GC-MS

Discussion

The alcoholic extract from *Plumbago zeylanica* roots was tested against clinically resistant multidrugs (*Salmonella paratyphi, Staphylococcus aureus, Escherichia coli and the dysenteriae Shigella*). The extract has shown good antibacterial activity against all bacteria examined[33]. In our previous study only ethyl acetate extract exhibit the antibacterial (*Salmonella paratyphi*, *Staphylococcus aureus*, *Escherichia coli)* and antioxidant activity compare to other solvent (Hexane, Chloroform and Methanol) extracts[34,35]. Hence Ethyl acetate extract used to isolate secondary metabolites[36] and found the bioactivity by *in silico* method

Table 1 — Bioactive compounds present in sample 1

S. No	Name of The Compounds	Rt. Time	% High Value
1.	2-Butanone, 4-hydroxy-	5.064	3.02
2.	Benzene, 1,3-bis(1,1-dimethylethyl)-	5.703	10.03
3.	Nonane, 5-(2-methylpropyl	5.892	2.21
4.	Tetradecanal	7.374	7.81
5.	Sulfurous acid, 2-ethylhexyl isohexyl ester	8.539	4.00
6.	Phenol, 2,4-bis(1,1-dimethylethyl)	8.875	11.64
7.	Undecane, 3,8-dimethyl	9.232	3.28
8.	Hydrazine, tetraphenyl	10.966	1.22
9.	Nonane, 3,7-dimethyl	12.207	1.34
10.	Nonane, 5-methyl-5-propyl	13.415	3.30
11.	Hexadecane, 1-iodo	17.593	3.99
12.	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca	17.593	3.96
13.	5-methyl-5-propyl	18.700	3.35
14.	Undecane, 2,9-dimethyl	18.842	0.92
15.	9-Eicosene, (E)-	19.434	12.03
16.	Heptadecane, 2,6,10,15-tetramethyl	19.593	6.20
17.	1-Fluorononane	23.805	1.68
18.	Bromododecane	24.982	2.55
19.	Nonadecanol-1	30.136	4.83

Table 2 — Bioactive compounds present in sample 2

S. No	Name of The Compounds	Rt. Time	% High Value
1.	Octane, 6-ethyl-2-methyl	5.071	2.35
2.	Oxalic acid, isohexylneopentyl ester	5.892	2.37
3.	Decyltrifluoroacetate	7.125	5.90
4.	1-Hexene, 3-methyl	8.300	0.58
5.	9-Octadecene	8.300	12.91
6.	Cyclohexane, (1-butylhexadecyl)	10.549	0.92
7.	Propanoic acid, 2,2-dimethyl-, 2,6-bis(1,1-dimethylethyl)-	17.708	1.69
8.	Heptadecane,	19.607	5.99
9.	Bromotetradecane	23.279	0.66
10.	Pentadecyltrifluoroacetate	24.839	9.10
11.	1-Heneicosanol	30.139	5.51
12.	Borane, diethyl(decyloxy)	30.267	1.04
13.	Cyclotetracosane	36.618	1.51
14.	n-Tetracosanol	43.267	1.98
15.	Nonadecylpentafluoropropionate	45.518	1.25

Table 3 — Bioactive compounds present in sample 3

S.No	Name of The Compounds	Rt. Time	% High Value
1.	m-Cymene, 5-tert-butyl	5.712	9.70
2.	Nonane, 5-(2-methylpropyl)	5.892	2.41
3.	1-Undecanol	7.120	5.81
4.	Pentadecane	7.211	11.74
5.	Z-4-Dodecenol	7.384	2.20
6.	1-Iodoundecane	9.730	1.03
7.	1-Decene, 8-methyl	10.545	0.87
8.	Octane, 3,6-dimethyl	13.804	1.12
9.	2,2,6,6-Tetramethylheptane	18.850	0.70
10.	Cyclodocosane, ethyl	36.619	1.73

Table 4 — Bioactive compounds present in sample 4

S.No	Name of The Compounds	Rt. Time	% High Value
1.	Octane, 2,7-dimethyl	5.062	2.03
2.	Decane, 2,3,7-trimethyl	5.892	2.39
3.	Pentadecanal	7.385	1.87
4.	5-Methyl-1-heptanol	8.283	0.58
5.	Hexane, 2,4,4-trimethyl	9.417	0.60
6.	Decane, 3,8-dimethyl	9.732	0.99
7.	3,5-Dimethyl-4-octanone	9.968	0.46
8.	Hexadecanal	10.544	0.81
9.	10-Dimethyl-4-undecanol	27.626	0.63
10.	Hexane, 2,4,4-trimethyl	30.000	0.40
11.	Heptadecyltrifluoroacetate	36.608	1.60
12.	1-Heptacosanol	43.265	1.82
13.	Decanedioic acid, bis(2-ethylhexyl)	43.488	1.16
14.	Tetracosyltrifluoroacetate	45.517	1.10

MMPs induces cancer progression and invasion leading to the death of the patient[37,38]. Reported the induction of tumour angiogenesis by MMPs as they first digest the extracellular matrix (ECM) and then penetrating into the basement membrane for invading the adjacent tissues and vessels thus involving in metastasis. As MMPs play a vital role in the progression of cancer, the research involved in finding out MMP inhibitors (MMPIs) are on the rise and resulted in the identification of drugs like Batimastat, Marimastat, Prinomastat, CGS27023A, Tanomastat and Doxycycline.Clinical trials were stopped for all the above drugs for showing toxicity in patients except for doxycycline[39,40]. Scatena C reported that treatment of breast cancer patients with doxycycline selectively eradicated cancer stem cells.

As an anti-infectious agent against life threatening disease causing species, bioactive metabolites from conventional plant extracts[41]. Secondary metabolites isolated from our study showed a higher docking score compared to doxycycline. Hence it became evident that these compounds can outperform the efficacy of doxycycline in inhibiting MMPs. Among the six different secondary metabolites studied, 9-Octadecene (5364599) (Fig. 5F) had a better docking score followed by 9- Eicosene (5365037) (Fig. 5A).

Table 5 — The name of each phytochemical along with their docking scores in comparison with the already available drugs for treating OSCC

S. No	Name of compound	Ligand Score 1	Ligand Score 2	PLP1	PLP2	Jain	Dock Score
1	9-EICOSENE	−999.9	-999.9	68.65	70.36	2.47	70.907
2	PHENOL, 2,4,BIS(1,1-DIMETHYL ETHYL)	1.43	2.85	58.25	65.01	4.71	53.775
3	PENTADECANE	0.24	1.7	66	73.49	6.71	66.657
4	M-CYMENE-5-TERT-BUTYL	−999.9	-999.9	55.85	61.19	3.38	63.662
5	BENZENE,1,3 BIS (1,1-DIMETHYL ETHYL)	0.58	2.63	50.32	55.13	3.74	49.714
6	9-OCTADECENE	−999.9	-999.9	65.26	69.55	3.72	71.325
7	5-FU	3.38	3.38	55.69	41.63	2.47	49.982
8	Doxycyclin	2.7	3.04	46.98	57.38	4.58	30.729

Table 6 — The intermolecular interaction of the atoms in ligand with the atoms of the target in the binding site

S. No	Ligand ID	Interacting atom	Distance	Category of interaction	Type of interaction	No of Hydrogen
1	9-EICOSENE 5365037	A:PHE420 - 5365037:C20	4.04177	Hydrophobic	Pi-Alkyl	NIL
2	PHENOL, 2,4,BIS(1,1-DIMETHYL ETHYL) 7311	7311:H37 - A:GLU373:OE1	1.91303	Hydrogen Bond	Conventional Hydrogen Bond	2
		7311:C2 - A:MET422	4.78242	Hydrophobic	Alkyl	
		7311:C3 - A:MET422	5.20223	Hydrophobic	Alkyl	
		A:PHE420 - 7311:C2	5.05847	Hydrophobic	Pi Alkyl	
		7311 - A:MET422	4.8037	Hydrophobic	Pi Alkyl	
		7311:H37 - A:GLU373:OE1	1.91303	Hydrogen Bond	Conventional Hydrogen	
		7311:C2 - A:MET422	4.78242	Hydrophobic	Alkyl	
		7311:C3 - A:MET422	5.20223	Hydrophobic	Alkyl	
		A:PHE420 - 7311:C2	5.05847	Hydrophobic	Pi Alkyl	
		7311 - A:MET422	4.8037	Hydrophobic	Pi Alkyl	
3	PENTADECANE 12391	A:ALA327 - 12391:C15	3.63502	Hydrophobic	Alkyl	NIL
		12391:C14 - A:MET422	4.54449	Hydrophobic	Alkyl	
		A:PHE467 - 12391:C15	5.37162	Hydrophobic	Pi-Alkyl	
		A:ALA327 - 12391:C15	3.63502	Hydrophobic	Alkyl	
		12391:C14 - A:MET422	4.54449	Hydrophobic	Alkyl	
		A:PHE467 - 12391:C15	5.37162	Hydrophobic	Pi-Alkyl	
4	M-CYMENE-5-TERT-BUTYL 34640	34640:C14 - A:MET422	5.1752	Hydrophobic	Alkyl	NIL
		A:PHE420 - 34640:C13	4.57784	Hydrophobic	Pi Alkyl	
		34640 - A:MET422	4.63574	Hydrophobic	Pi Alkyl	
		34640:C14 - A:MET422	5.1752	Hydrophobic	Alkyl	
		A:PHE420 - 34640:C13	4.57784	Hydrophobic	Pi Alkyl	
		34640 - A:MET422	4.63574	Hydrophobic	Pi Alkyl	
5	BENZENE,1,3 BIS (1,1-DIMETHYL ETHYL) 136810	136810:C2 - A:MET422	4.85695	Hydrophobic	Alkyl	NIL
		136810 - A:MET328	5.35882	Hydrophobic	Pi Alkyl	
		136810 - A:MET422	5.02215	Hydrophobic	Pi Alkyl	
6	9-OCTADECENE 5364599	A:ALA327 - 5364599:C17	3.85894	Hydrophobic	Alkyl	NIL

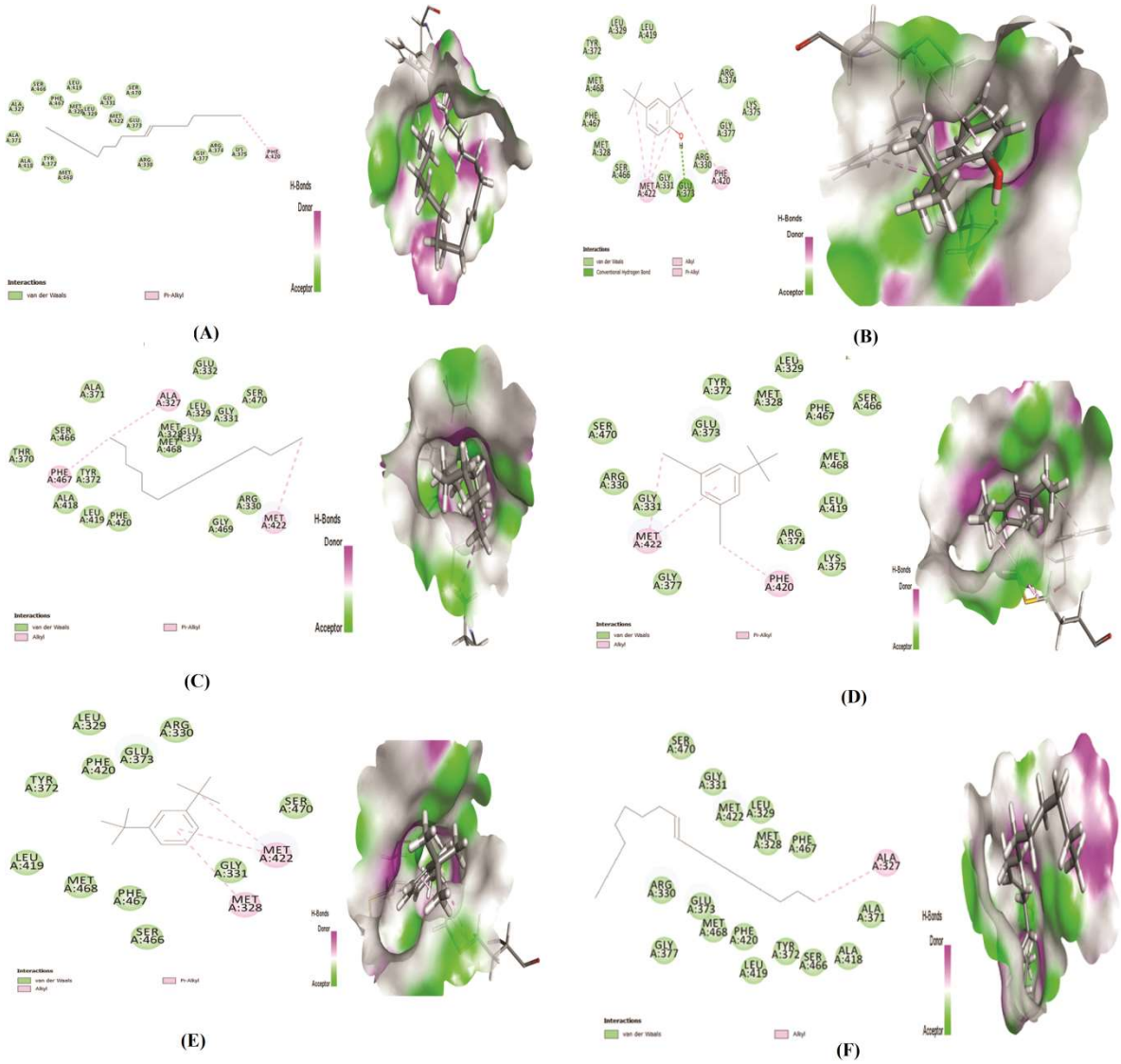

Fig. 5 — Intermolecular interaction of (A) 9-Eicosene (5365037); (B) Phenol,2,4,bis (1,1-dimethylethyl)-(7311); (C) Pentadecane (12391); (D) M-Cymene-5 tert butyl (34640); (E) Benzene,1,3 bis (1,1 dimethyl ethyl)-(136810); and (F) 9-Octadecene (5364599) with the target protein Hemopexin (HPX) -3c7x

Though the docking score was more of these two, the number of interactions occurred between them with the target was less when compared to the remaining four ligands.

Phenol, 2,4, bis (1,1-dimethyl ethyl)-(7311) predominates in intermolecular interaction as it shared ten different bonds with the target (Fig. 5B). This includes two hydrogen bonds with the amino acids methionine at the 422 positions and the glutamine at 373 positions of the target, the remaining bonds were alkyl bonds. The compound phenol-2, 4-bis (1,1-dimethylethyl), is a precursor to many complex compounds and commonly used for the synthesis of other chemical intermediates as antioxidants, light control agents or UV stabilisers. Though phenol-2,4-bis (1, 1-dimethylethyl) could be achieved as the natural antimicrobial compound from plant materials[42,43], animal materials[44,45]. And microorganism metabolites such as *Streptomyces* sp.[46], *Shewanella algae*[47] and *Pseudomonas monteilii*[48], *Nocardiopsis* sp.[49], *Bacillus velezensis*[50], *Vibrio alginolyticus*[51], *Vibrio owensii*[52], *Vibrio* sp.[53], *Bacillus subtilis*[54], Microbacterium mangrove, Sinomonas humi and Monashia flava[55] *etc*. Also Phenol-2, 4—bis (1, 1-dimethylethyl) was an antifungal compound of *Pseudomonas fluorescens* TL in nature[56].

Pentadecane (12391) (Fig. 5C) and M-Cymene-5 tert- butyl (34640) (Fig. 5D) shared six bonds each with the binding site of the target, while Benzene1,3 bis (1,1 dimethyl ethyl)-(136810) (Fig. 5E) shared only three bonds. All the compounds passed ADMET test and hence can be considered as promising agents to be used as a drug for further testing and treatment.

Conclusion

Thus the study concluded that Phenol, 2,4, bis (1,1-dimethylethyl)-(7311) showed better interaction with MMPs and it can probable drug candidate for treating oral carcinoma. It should be further evaluated *in vivo* preclinical and clinical studies. Admit descriptors were satisfactory.

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Conflict of interest

All authors declare no conflict of interest.

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