Research Article

MORINGA OLEIFERA FLOWER COMPOUNDS AS POTENT LIGANDS TO DRUG TARGETS IN PC3 CELL LINES – AN IN SILICO ANALYSIS

L. INBATHAMIZH[1] AND E. PADMINI[*2]

[1]Research and Development Centre, Bharathiar University, Coimbatore-641 046, Tamilnadu, India. *[2]Department of Biochemistry, Bharathi Women's College, Chennai 600108, Tamilnadu, India. Email: inba66in@yahoo.com

ABSTRACT

Objective: Androgen-independent prostate cancer is an advanced hormone refractory stage of prostate cancer associated with increased risk. Before reconciling the medicinal effect of plant compounds commonly used by researchers, attention on the little known bioconstituents is essential for further refinement. On this aspect, the bioactive compounds of *Moringa oleifera* flowers were explored for their cytoprotective role in PC3 cell lines, the *in vitro* highly metastatic androgen-independent models of prostate cancer.

Method: Glucosaminyl (N-acetyl) transferase 1 (GCNT1), cellular Prostatic acid phosphatase (cPAP), HER-2 (Human Epidermal growth factor Receptor 2) and ERK (Extracellular Regulated Kinase) which usually require cPAP for their dephosphorylation and inactivation, were chosen as the drug targets. *M. oleifera* flower compounds were analyzed for their interactions with the drug targets using *in silico* methods and calculated for their suitability to PC3 cell lines.

Results: *M. oleifera* flower compounds exhibited more effective active site interactions when compared to the other compounds studied, with the mutated GCNT1 responsible for higher risk, and with ERK directly even in the absence of HER-2 and cPAP fitting the nature and growth of PC3 cells. Also, 66.5% of the experimented extract of *M. oleifera* flowers seemed to be target-specific to PC3 cell lines with greater number of target-specific interactions given by Quinic acid, alpha-Tocopherol-beta-D-mannoside, (4-Hydroxyphenyl) acetonitrile, Ethyl Oleate.

Conclusion: The study illustrated the therapeutic application of *M. oleifera* flower compounds particularly Quinic acid as potent ligands to the drug targets in PC3 cell lines, in combating the manifestation of androgen-independent prostate cancer.

Keywords: Androgen-independent prostate cancer, *Moringa oleifera*, PC3 cell lines, Drug targets, Ligands, *In silico* methods.

INTRODUCTION

Prostate cancer is the most prevalent cancer afflicting men. The cancer cells may metastasize from the prostate to other parts of the body, particularly bones and lymph nodes [1]. The risk increase when the cancer progresses from androgen-dependent to androgen-independent stage [2]. PC3 cell lines are the classical *in vitro* androgen-independent models of prostate cancer with high metastatic potential [3].

With the emergence of deadly diseases, discovering natural bioactive compounds as drugs targeted to disease proteins of interest requires an understanding of the principles of molecular recognition in protein-ligand complexes. *Moringa oleifera* is a predominant Indian nutritional plant with high medicinal value. It is rich in a number of vitamins, minerals and specific phytochemicals, reported to have hypo-tensive, anticancer and antibacterial activities [4]. The flowers possess good amounts of both calcium and potassium and are used to make tea for colds. Folk medicine supports the use of *M. oleifera* flowers for treating cancerous tumors [5]. Several decades of research have been more on the other parts of this medicinal plant. Thus, there is a need to investigate the cytoprotective role of this least explored part of the plant.

With the growing developments, the principles of scientific experimentation have been applied to therapeutic context and advanced tools have been harnessed to transform information about molecular mechanisms and targets into therapies directed against disease [6]. Targeted studies, which seek to understand the basic chemistry and physiology of a disease, seem to be more promising [7]. Targeted drug therapy with drugs interacting with specific key molecules in cancer cells and causing fewer or no side effects on healthy cells, is more effective than chemotherapy.

The PC3 cell target proteins mainly focused are the prominently occurring Glucosaminyl (N-acetyl) transferase 1 (GCNT1) and rarely existing cellular Prostatic acid phosphatase (cPAP). GCNT1 is a major enzyme involved in cell adhesion [8], glycosylation and branching at the surface and in the secretions of prostate cancer causing glycoproteins [9]. The expression of GCNT1 is associated

with metastasis and malignancy. It is found that Adenine(A)/Guanine(G) polymorphism in GCNT1 with mutation of isoleucine to valine at 152 in the protein sequence, is associated with the increased risk of prostate cancer [10]. Prostatic acid phosphatase is a major phosphatase in prostate epithelial cells. An inverse relationship exists between cPAP and the cell growth rate. Decreased cPAP correlates with hyperphosphorylation of Human Epidermal growth factor Receptor 2 (HER-2) absent in PC3 cells, and Extracellular Regulated Kinase (ERK) which results in androgen independent prostate cancer progression [11]. Thus, HER-2 and ERK are also included as the receptor proteins besides nGCNT1, mGCNT1 and cPAP, to check the suitability of ligand binding in PC3 cells.

Nowadays, application of *in silico* methods to predict the binding of small molecular ligands to known target structures has become an important component in the drug discovery process [12,13]. Docking predicts the preferred orientation of one molecule to another molecule when bound to each other to form a stable complex [14]. The interaction between the ligand and biomacromolecular target is investigated by docking simulation techniques [15].

With the list of compounds identified by Gas Chromatography-Mass Spectrometry (GC-MS) from the methanol extract of *M. oleifera* flowers with significant antioxidant activities [16,17] and the structural aspects of the relevant drug targets analyzed in earlier studies [18,19,20], it seemed to be essential to investigate the target-ligand interactions. The present study investigates the target specificity of these ligands in PC3 cell lines and hence their cytoprotective role against androgen-independent prostate cancer.

MATERIALS AND METHODS

Ligands

The candidate compounds present in the methanol extract of *M. oleifera* flowers as predicted by the GC-MS analysis were the ligands of focus [16]. For a comparative study to observe the effect on the disease, these ligands were analyzed along with two standard therapeutic agents for prostate cancer: a natural compound,

Curcumin and a chemotherapeutic drug, Estramustine. Ligands that resulted in significant docking with the drug targets as studied earlier as well as Lapatinib and PD98059, the inhibitors of HER-2 [21] and ERK [22] respectively were also used for comparison with the flower ligands for analyzing their interaction potentials with the target proteins

Drug targets for ligand interactions

Both nGCNT1 and mGCNT1 were the prominent drug targets of interest. But, ligand interactions with cPAP and with HER-2 and ERK, the proteins experiencing cPAP's effector role were also studied to screen and select the interactions preferable for PC3 cell lines.

Biological databases

Information of some of the ligands were obtained from PubChem Compound database [23] and Protein Data Bank (PDB) file formats of three dimensional (3D) structures of HER-2 and ERK were obtained from Research Collaboratory for Structural Bioinformatics (RCSB) PDB database [24].

Bioinformatics tools

Molecular docking of drug target-ligand

PatchDock Beta 1.3 version

PatchDock works with an algorithm for molecular docking with two molecules of any type: proteins, Deoxyribonucleic acid (DNA), peptides, drugs, as the inputs. The output obtained is generally a list of potential complexes sorted by shape complementarity criteria [25].

Visualization tool

PyMOL version 0.99

It is an open source visualization tool used in Structural Biology. It can produce high quality 3D images of small molecules and biological macromolecules such as proteins [26]. The Py portion of the software's name refers to the fact that it is extensible by the Python programming language.

Other tools

Besides those mentioned above, a few *in silico* tools such as Q-Site Finder for active site prediction of drug targets in the docked structure [27], ChemSketch for drawing chemical structures of ligands [28], Open Babel 2.3.1 [29] and Simplified Molecular Input Line Entry Specification (SMILES) translator [30] for chemical file conversions of ligands were also utilized.

Methodology

Chemical structure representation

For those ligands whose 3D Structure Data Format (SDF) chemical file formats were available in PubChem Compound database, these file formats were used directly for file conversions. For those with only 2D SDF chemical file formats available, their SMILES were converted to required file formats using SMILES translator. But for those ligands whose structure files formats were unavailable in PubChem Compound database, the structure of ligands as obtained from the GC-MS studies were utilized. These structures were drawn using ChemSketch, 3D optimized and saved as Molecular Design Limited (MDL) mol files. Chemical file conversion to PDB format for interaction studies was done using Open Babel 2.3.1.

Interaction and Pharmacodynamics studies

The PDB files of the ligands and the target proteins were fed as inputs to PatchDock web server to obtain the docking results. The target-ligand interactions were investigated by visualizing the docked poses in PyMOL. The target residues involved in bonding with the ligands were checked for their location in their respective active sites by using Q-Site Finder. Based on the interaction results, the mechanism of action (pharmacodynamics) of these drug-like compounds as therapeutics and their suitability to act on PC3 cells were analyzed.

RESULTS AND DISCUSSION

Interaction and Pharmacodynamics studies

For a better understanding of the possible mechanism of action of the ligands in PC3 cell lines, protein-ligand docking studies through *in silico* methods [31] had been attempted. The PDB files of the modeled nGCNT1 and mGCNT1 and *ab initio* structure predicted cPAP [19,20] were fed as input receptor files for the molecular docking tool, PatchDock. The PDB structure with ID: 1S78 was the 3D structure of HER-2 of maximum resolution with A and B chains and with sequence from 23 to 646. Similarly, the PDB structure with ID: 2E14 was the 3D structure of ERK-2 of maximum resolution with A chain and with sequence from 1 to 360. 2E14 was later superseded by 3W55 [32]. 1S78 was the 3.2 Å X-ray crystal structure of the extracellular domain of HER-2 in complex with the antigen binding fragment of pertuzumab, an anti-ErbB2 monoclonal antibody [33]. For docking analysis, the pre-existing ligands were removed from 1S78 and 2E14 and the PDB files of only the target proteins were used.

The docking algorithm, PatchDock calculated the interactions not only at the active site but also at the other sites on the receptor. The target proteins acting as receptors and the candidate compounds acting as ligands were divided into patches according to the surface shape. Once the patches were identified, they were superimposed using shape matching algorithms [34,35]. The Geometric shape complementarity scores obtained were more for the fatty acid esters than fatty acids and among the fatty acid esters, the value increased with the addition of alkyl groups and increase in Carbon chain. This was well observed among Methyl palmitate, Palmitic acid, Ethyl palmitate and Ethyl stearate. The scores of Ethyl stearate and Ethyl Oleate with all the target proteins except nGCNT1 indicated that the complementarity decreased with unsaturation. In general, the scores increased with increase in the molecular weight of the ligands and might not be proportional to the receptor-ligand interactions.

The PatchDock results were evaluated for active site interactions by PyMOL and Q-Site Finder. The docked conformations of the target proteins with some of the selected ligands as deciphered by PyMOL were shown in Fig. 1 - Fig. 5. Ethyl Oleate and Quinic acid which constituted the larger percentage among the *M. oleifera* flower compounds separated from the methanol extract by GC-MS, alpha-Tocopherol-beta-D-mannoside which was a derivative of alpha-Tocopherol used as the standard for the antioxidant studies [16, 17] were the selected *M. oleifera* flower ligands used for final comparison with the standard therapeutics along with the selected database ligands.

The physicochemical properties of the constituting amino acids determined the physicochemical properties, molecular interactions and biological activities of the target proteins. The active site interactions of the ligands with the targets revealed that most of the amino acids at the active site of these proteins involved in interactions were polar in nature. Tyr 187, Lys 421, Glu 60, Thr 144(A) and Lys 114(A) were the predominant residues involved in interactions in nGCNT1, mGCNT1, cPAP, 1S78 and 2E14 respectively. The active site residues and hence their interactions with the ligands varied between nGCNT1 and mGCNT1, confirming the change brought about in the conformation of nGCNT1 due to mutation. Among the ligands, oxygen atom seemed to be the best interacting atom. Interactions were measured in terms of the polar contacts involved. Hydrocarbon compounds like n-Tetracosane, n-Tetratriacontane and n-Hexatriacontane with Hydrogen and Carbon atoms alone did not show any polar contacts with any of the target proteins studied. Gamma-Sitosterol, though had an oxygen atom in its structure, could not form any polar contacts.

The interaction analysis of the selected ligands with GCNT1 revealed a maximum number of active site polar contacts by Quinic acid not only with the native form (7) but also with the mutated protein (4). Though Ethyl Oleate and alpha-Tocopherol-beta-D-mannoside did not show any active site polar contacts with nGCNT1, they interacted significantly with the active site of mGCNT1. Curcumin though much less than Quinic acid, seemed to be better than Estramustine which showed no active site polar contacts with

GCNT1 at all. Though 5,5'-Dithiobis-2-nitrobenzoic acid was effective against nGCNT1, it did not show active site interaction with mGCNT1. Though Calcitriol interacted with both nGCNT1 and mGCNT1, 6 Deoxy N-acetylgalactosamine seemed to be a better interacting ligand with GCNT1, among the database ligands.

With cPAP and 1S78, the interactions of the ligands were different. The database ligand Citric acid showed a maximum number of polar contacts with the active site of both cPAP (4) and 1S78 (5). Though lesser than Citric acid, Curcumin seemed to be better than Estramustine. Among the *M. oleifera* flower ligands, while Quinic acid showed minimum active site interaction, Ethyl Oleate did not show any active site polar contacts. Alpha-Tocopherol-beta-D-mannoside interacted like Quinic acid with 1S78 but did not show active site interaction with cPAP. Lapatinib, the HER-2 inhibitor, behaved like Curcumin with 1S78.

Interestingly, all the selected *M. oleifera* flower ligands interacted significantly with 2E14, with Quinic acid and alpha-Tocopherol-beta-D-mannoside showing the maximum number of active site polar contacts. This was even greater than that by PD98059, the ERK inhibitor. Curcumin, Estramustine and Citric acid produced equal number of polar contacts with the active site of 2E14 which was lesser than that by PD98059 and by *M. oleifera* flower ligands.
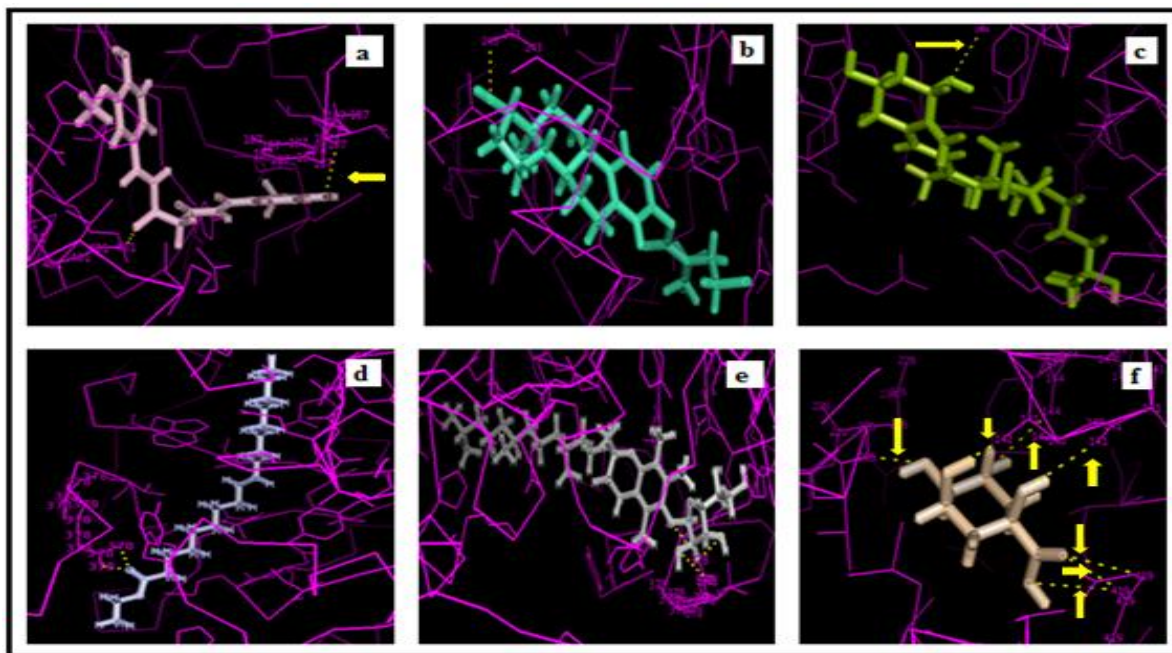

Fig. 1: Docked conformations of nGCNT1(magenta) with (a)Curcumin(pink) (b)Estramustine(green cyan) (c)Calcitriol (split pea green) (d)Ethyl Oleate(light blue) (e)alpha-Tocopherol-beta-D-mannoside(gray) (f) Quinic acid(wheat)

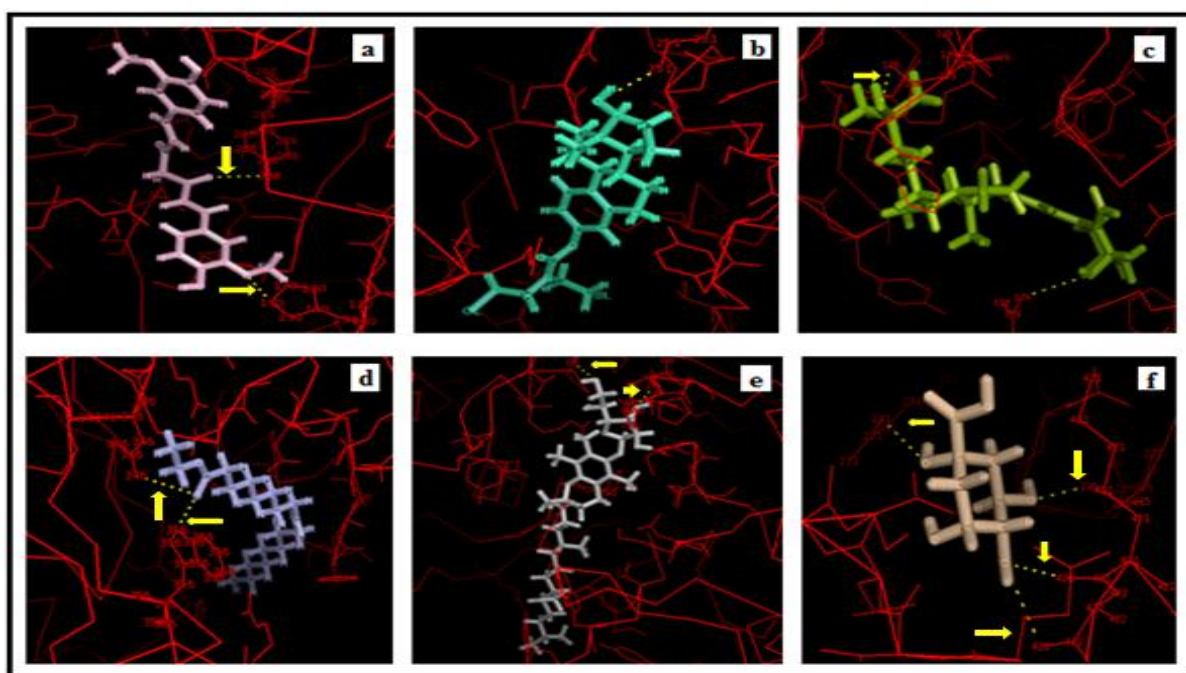Dotted lines: Polar contacts, Yellow arrows: Active site interactions

Fig. 2: Docked conformations of mGCNT1(red) with (a)Curcumin(pink) (b)Estramustine(green cyan) (c)Calcitriol (split pea green) (d)Ethyl Oleate(light blue) (e)alpha-Tocopherol-beta-D-mannoside(gray) (f) Quinic acid(wheat)
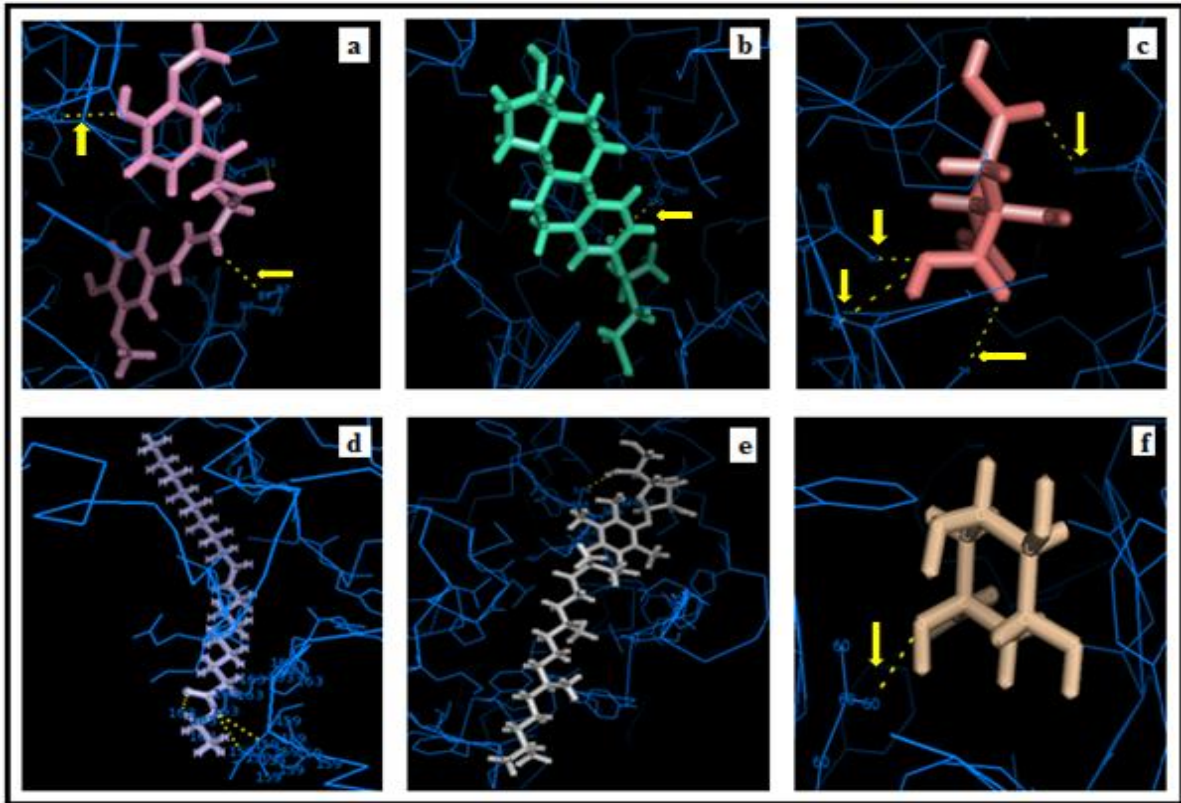
Fig. 3: Docked conformations of cPAP(blue) with (a)Curcumin(pink) (b)Estramustine(green cyan) (c) Citric acid(deep salmon) (d)Ethyl Oleate(light blue) (e)alpha-Tocopherol-beta-D-mannoside(gray) (f) Quinic acid(wheat)
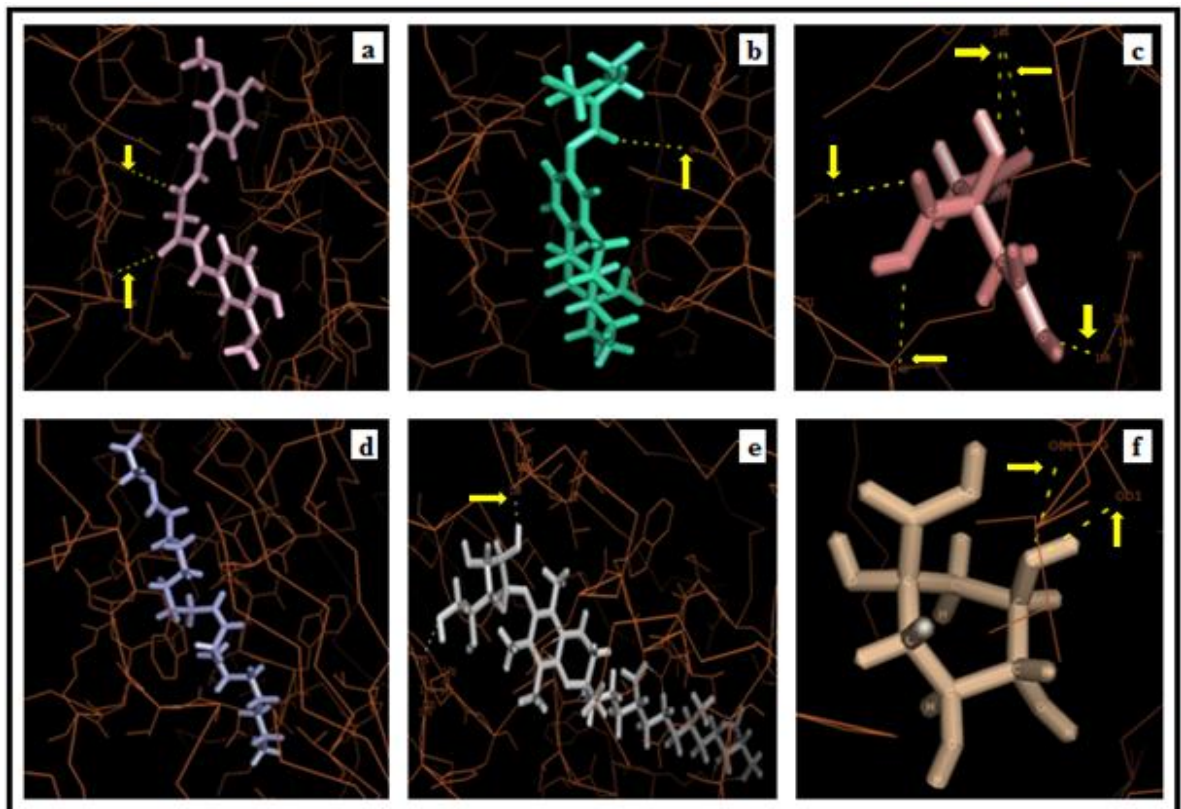

Fig. 4: Docked conformations of 1S78(brown) with (a)Curcumin(pink) (b)Estramustine(green cyan) (c) Citric acid(deep salmon) (d)Ethyl Oleate(light blue) (e)alpha-Tocopherol-beta-D-mannoside(gray) (f) Quinic acid(wheat)
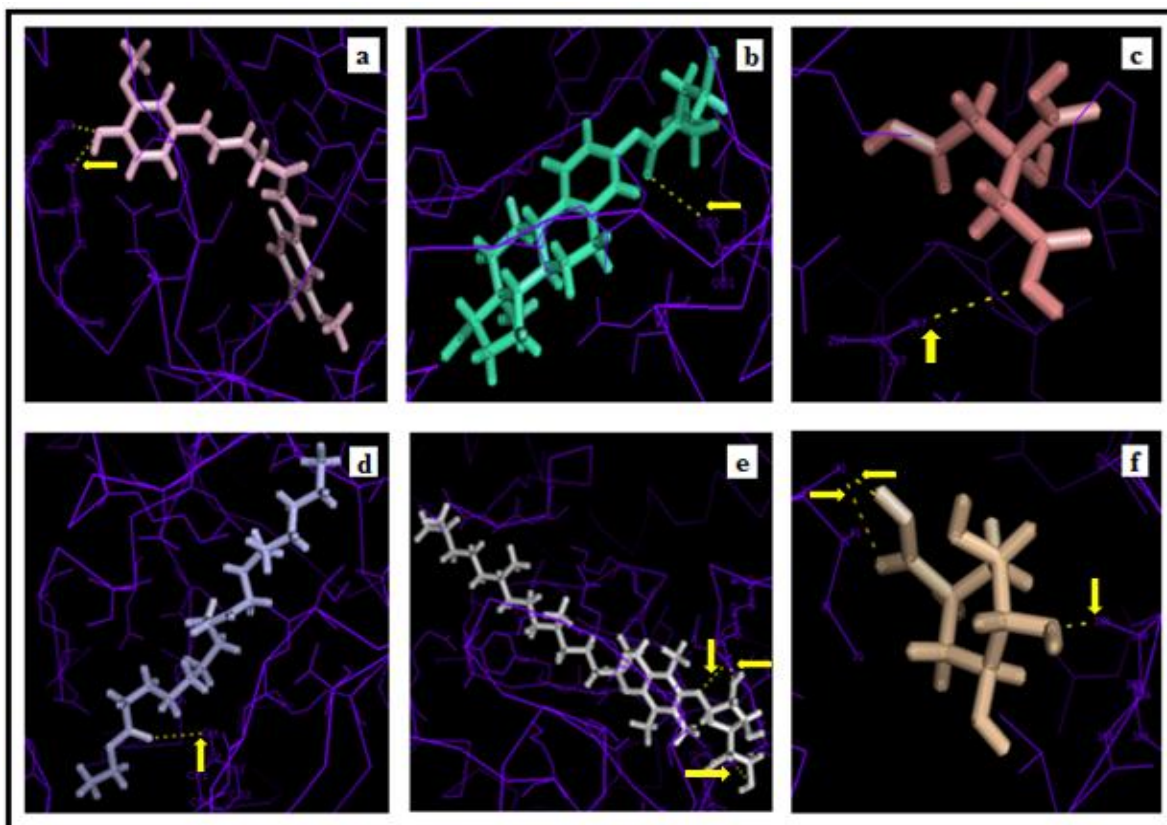
Fig. 5: Docked conformations of 2E14(purple blue) with (a)Curcumin(pink) (b)Estramustine(green cyan) (c) Citric acid(deep salmon) (d)Ethyl Oleate(light blue) (e)alpha-Tocopherol-beta-D-mannoside(gray) (f) Quinic acid(wheat)

The pharmacodynamics results revealed the mechanism of action of the anticancer ligands. The selected *M. oleifera* flower ligands produced more number of active site interactions with ERK than with cPAP and HER-2 which meant that these ligands could interact more effectively with ERK and hence could inhibit it independent of cPAP and HER-2 which were absent in PC3 cells. These ligands also seemed to be highly effective in inhibiting mGCNT1 associated with the increased risk of the disease. The *M. oleifera* flower ligand, Quinic acid seemed to be the most significant among the studied ligands in exhibiting the maximum number of target-specific active site interactions in PC3 cells. But, in case of Curcumin, Estramustine and Citric acid, since these ligands were less effective in active site interactions with ERK than with cPAP and HER-2, the possible mechanism of these ligands might be to inhibit HER-2 and ERK by activating cPAP. Hence, these ligands might require cPAP and HER-2 to interact effectively with ERK. Among these ligands, Citric acid seemed to be more effective and could not only activate cPAP but also inhibit HER-2 to a greater extent. Though Curcumin and Estramustine seemed to act similarly, the latter was found to be less significant as evidenced by the number of active site polar contacts formed. On the other hand, both these compounds were less effective compared to the selected *M. oleifera* flower ligands in inhibiting mGCNT1. While Curcumin could inhibit mGCNT1 to certain extent, Estramustine was not potent enough to inhibit mGCNT1. Therefore, though these ligands exhibited anticancer properties in effectively interacting with cPAP and HER-2, they might not be applicable to the drug targets in PC3 cells.

Suitability of action on PC3 cell lines

Besides the above mentioned interpretations, the *M. oleifera* flower compounds separated from the methanol extract by GC-MS were analyzed for their suitability of action on PC3 cell lines based on their interactions with the target proteins as given in Table 1. It would be possible with *in silico* docking studies, to design highly selective, potent and bioactive ligands suitable for handling the disease besides overcoming incorrect prediction of binding modes to avoid toxicity. This would further enable the production of safer and more effective next-generation drugs [36,37].

PC3 cells were considered to be nGCNT1+, mGCNT1+, 2E14+ and cPAP-, 1S78-. Those ligands which interacted with cPAP and 1S78 alone were considered unsuitable for PC3 cell lines. Those ligands fully suitable which interacted with nGCNT1, mGCNT1and 2E14, those which interacted more effectively with PC3 targets than with non-PC3 targets (more effective for PC3 cell lines), those which either reacted with nGCNT1, mGCNT1and 2E14 or with cPAP and 1S78 equally (either / or category) and those which not only interacted with cPAP and 1S78 but also with nGCNT1, mGCNT1 and 2E14 (not only / but also) were all considered to be capable of interacting with the PC3 target proteins and hence suitable for these cell lines. The respective area percentage of these ligands as given in their GC-MS chromatogram [16] was used for calculation. Excluding the unsuitable and uninteractive ligands, the percentage of ligands suitable to act on PC3 cell lines was found to be 66.5%. This percentage was accounted by the ligands fully suitable for PC3 cell lines (22.07%), more effective for PC3 cell lines (18.34%), either / or category (12.65%) and not only / but also category (13.44%). The ligands which contributed significantly were Quinic acid, alpha-Tocopherol-beta-D-mannoside, (4-Hydroxyphenyl) acetonitrile, 12-Oleanen-3-yl acetate, (3alpha) (more effective for PC3 cell lines), Ethyl Oleate, Methyl cis-7-octadecenoate, Ergost-5-en-3 beta-ol, Stigmasterol (fully suitable for PC3 cell lines). From the analysis, it was clear that the compounds constituting 66.5% of the methanolic extract of *M. oleifera* flowers as separated by GC-MS, were suitable to exert potent anticancer activities in PC3 cell lines.

Table 1: Suitability of action of the *M. oleifera* flower ligands on PC3 cell lines

Area%	Name	nGCNT1	mGCNT1	cPAP	1S78	2E14
2.25	Melamine	2	2	3	2	nil
1.47	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1	1	2	2	1
1.98	(4-Hydroxyphenyl) acetonitrile	2	1	1	1	1
13.43	Quinic acid	7	4	1	2	3
0.8	4-Hydroxy-3,5,6-trimethyl -4-[(1E)-3-oxo-1-butenyl] -2-cyclohexen-1-one	nil	nil	1	3	nil
1.18	(2E)-3,7,11,15-Tetramethyl -2-hexadecen-1-ol	1	nil	nil	1	nil
0.38	(2E)-3,7,11,15-Tetramethyl -2-hexadecen-1-ol	1	nil	nil	1	nil
0.42	Methyl palmitate	1	nil	1	2	nil
9.38	Palmitic acid	nil	nil	2	nil	nil
10.68	Ethyl palmitate	1	nil	nil	1	nil
0.61	Methyl cis-7-octadecenoate	1	1	nil	nil	nil
11.8	cis-9-Hexadecenal	nil	nil	nil	2	nil
8.76	Methyl linoleate	2	nil	nil	3	nil
19.94	Ethyl Oleate	nil	2	nil	nil	1
2.19	Ethyl stearate	nil	nil	nil	1	nil
3.51	n-Tetracosane	nil	nil	nil	nil	nil
0.55	Ethyl docosanoate	nil	nil	nil	1	nil
1.75	n-Tetratriacontane	nil	nil	nil	nil	nil
0.54	9-Octadecenamide	1	1	nil	2	nil
0.41	Malonic acid, di(10-chlorodecyl) ester	nil	nil	nil	1	1
0.96	n-Hexatriacontane	nil	nil	nil	nil	nil
1.91	alpha-Tocopherol-beta-D-mannoside	nil	2	nil	1	3
0.46	Ergost-5-en-3 beta-ol	1	nil	nil	nil	nil
1.06	Stigmasterol	nil	1	nil	nil	nil
2.57	Gamma-Sitosterol	nil	nil	nil	nil	nil
1.02	12-Oleanen-3-yl acetate, (3alpha)	1	1	nil	1	nil

Area %: % of compounds constituting the methanol extract of *M. oleifera* flowers.

PC3 targets: nGCNT1, mGCNT1and 2E14

Non-PC3 targets: cPAP and 1S78

Shaded *M. oleifera* flower ligands:

Orange- not only / but also category (interacted not only with Non-PC3 targets but also with PC3 targets)

White- fully suitable for PC3 cell lines (interacted only with PC3 targets and not with Non-PC3 targets)

Green-more effective for PC3 cell lines (interacted more effectively with PC3 targets than with Non-PC3 targets)

Yellow- unsuitable for PC3 cell lines (interacted only with Non-PC3 targets and not with PC3 targets)

Gray- either / or category (interacted equally with PC3 targets and with Non-PC3 targets)

Blue- uninteractive (No interaction either with PC3 targets or with Non-PC3 targets)

CONCLUSION

The study clearly demonstrated that the pharmacodynamic interactions of the *M. oleifera* flower ligands particularly Quinic acid, alpha-Tocopherol-beta-D-mannoside,(4-Hydroxyphenyl) acetonitrile, Ethyl Oleate, Methyl cis-7-octadecenoate, Ergost-5-en-3 beta-ol, Stigmasterol were more target-specific and favourable to PC3 cell lines. This further substantiated the cytoprotective role of these ligands and facilitated an insight in to their therapeutic application in androgen-independent prostate cancer.

ACKNOWLEDGEMENT

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