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ANTICANCER ACTIVITY OF PHYTO LIGANDS FROM *CARICA PAPAYA* LEAVES BY SUPPRESSION OF TUMOR PROGRESSIVE PROTEINS CD117 AND CYCLIN D1 - AN *INSILICO* APPROACH

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ABSTRACT

The Herbal derived compounds possess several medicinal properties including anticancer activities. In the present investigation molecular docking analysis was performed to identify a suitable antagonistic ligand from the phyto ligands of *Carica papaya* leaves which can inhibit the tumor progressive proteins CD117, Cyclin D1. The molecular Docking analysis was performed using Autodock 4.2. The protein CD117, Cyclin D1 structures were retrieved from PDB, and by GC-MS analysis the phyto molecules were identified. The ligand chemical structures were drawn using Chem sketch. The enzyme and ligand interaction were obtained as docking score using the Arguslabs server. Based on the docking score the best ligand was selected from the phyto constituents of *Carica papaya* ethanolic leaf extract and their inhibitory potential was analyzed in terms of their interactions with the amino acid residues present in the active site which were visualized and further confirmed by PYMOL. The standard drug Doxorubicin was also subjected to docking for comparison in the present study. Based on the docking score the phytochemicals namely Hexadecanoic acid, ethyl ester, Coumarine 3-(2,4-dinitrophenol), Androst-4-en-3-one,17-methoxy, 3-methoxime serves as the best antagonistic ligand in terms of their interaction with amino acids as well as inhibition of the particular tumour progressive proteins.

Keywords: Ethanolic extract, Phyto ligands, *Carica papaya,* Doxorubicin, Ovarian cancer, *Insilico*

INTRODUCTION

Ovarian cancer is the fifth leading cause of cancer-related deaths among women in the world. There has not been a major therapeutic advance in the treatment of ovarian cancer in over 30 years. One reason ovarian cancer remains such a difficult disease is that it is usually discovered at an advanced stage, when metastases have already occurred. Frequently, this metastatic spread occurs in the peritoneal cavity, where tumor cells dislodge from the primary tumor, find an accommodating microenvironment, and re-establish themselves. Though the first line of treatment for the ovarian cancer starts with platinum derived compounds, in several cases it becomes a challenge due to the chemo resistance produced by the cancer cells to the drug [1]. Therefore,

researchers have forced to develop effective and novel therapeutic molecules to combat with the cancer cells.

CD117 Mast /stem cell growth factor receptor (SCFR), also known as the proto-oncogene c-Kit, tyrosine protein kinase Kit or CD117, is a protein encoded in humans by the KIT1 gene located on human chromosome 4, it is also called as trans-membrane protein. The cell cycle regulates the cell proliferation, apoptosis and adhesion by binding with the help of ligand stem cell factor (2). This protein is 45 kD (kilo Dalton) protein encoded by cyclin D1 gene (CCND1) located on chromosome 11q13, is a part of the molecular system that regulates the cell cycle G1 to S transition is involved in the cell cycle regulation and as well as in proliferation of cells is known to over express among several cancers (3). Cyclin D1, well established oncogene that has been found to play a very important and crucial roles in the process of development of cell proliferation and oncogenesis (4). However, the recent study showed that, CD117 is been abnormally overexpressed in various human malignancies and that is may play as important role for carcinogenesis and metastasis (5-6).

There are several plant derived Phyto compounds which has the potential to kill the cancer cells and exhibits anticancer property. But the exact mechanism of action is poorly understood. Drug discovery through insilico approaches have recently made the cancer drug discovery process more successful through the identification of novel specific inhibitors against these dysregulated proteins [7].

Carcinogenesis is a multi-step process characterized by progressive changes in the amounts or activity of proteins that regulate cellular proliferation, differentiation and survival [8]. The cost of research and development in the pharmaceutical industry has been rising steeply and steadily to achieve any process in the last decade but the amount of time required in bringing new products to market take around ten to fifteen years. *Insilico* technique is an inexpensive technique that shortens the length of time spending in testing the efficacy of drug. In the present study we made an attempt to identify a suitable antagonistic ligand which can inhibit the tumor progressive proteins PIK3CA, BCL 2, from the phyto constituents of the ethanolic extract of *Carica papaya* leaves through molecular docking studies.

MATERIALS AND METHODS

Collection of plant material: The leaves of *Carica papaya* collected from Chennai, Tamilnadu India. The plant leaf sample was dried in hot air oven at 40°C for 24 hours and ground into powder.

Sample preparation for GCMS analysis

About 5g of powdered material of plant was taken in a clean, flat-bottomed glass container and soaked in 30mL of 70% methanol. The container with its content was sealed and kept for a period of seven days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then, it was filtered through Whatmann filter paper. The filtrate (ethanolic extract) obtained for the plant was evaporated.

GC-MS analysis

The GCMS analysis was conducted at the Indian institute of Madras (IITM) Chennai. 2uL aliquot was injected into a fisons GC8000 series GC coupled to a MD800 MS with quadrapole mass analyzer (fision instrument, Milano, Italy). The chromatography was performed by using the DB5-MS column. Injection temperature was 230°C. Helium flow was 1mL/min. After a 5 min solvent delay time at 70°C; the oven temperature was increased at 5°C/min to 310°C, 1min isocratic, and cooled to 70°C, followed by the additional 5min delay. The ion trace integration was done using the mass lab find target method for the characteristic fragment of assigned peaks

Identification of Components

Interpretation of mass spectrum GCMS was conducted. The molecular weight, molecular formula, and the number of hits used to identify the name of the compounds from IITM were recorded.

Ligand Preparation

The compounds reported in Gas Chromatography and Mass Spectrum (GCMS) analysis of ethanolic extract leaf of *Carica papaya* were used in the present study. The structures of the 21 compounds (ligands) were retrieved. The structure of the compounds were drawn using ChemSketch and the structure was obtained in the form of (.sk2). This was further converted to (.pdb) format using the Chimer tool. Once the ligands were obtained, each of the compound energy minimized using the same tool.

Protein preparation

The target protein and ligand molecules were retrieved separately and prepared before performing molecular docking analysis. The proteins were obtained in the PDB format from the Protein Data Bank (http://www.rcsb.org/). The protein and their corresponding PDB IDs are tabulated in Table. The protein molecules were obtained in the PDB format was first cured and further energy minimized.

Binding Site prediction

The binding site plays a major role in compound activity. Hence, the binding site for each of the protein molecule was obtained from Metapocket server. This server provides us with three best active site pockets. For our docking analysis, we use the pocket with maximum number of amino acids.

Docking Analysis

Molecular docking continues to hold a great promise in the field of computer-based drug design which screens small molecules by orienting and scoring them in binding site of a protein. Protein and ligand molecules were used as starting structures for the docking analysis. Molecular Docking analysis was performed using Autodock 4.2 tools [9]. Prior to the docking analysis, the protein molecules and the ligand molecules were energy minimized using Chimera tools. The energy minimized molecules were used as the starting structures for molecular docking. For all the protein molecules, the binding pocket was identified sing MetaPocketserver. The grid box was set around the active site for perfect binding of the ligands. All the ligands were torsion-fixed, the grid was

maintained using autogrid and rigid docking for all the protein molecules were performed using autodock with the Lamarckian algorithm. As a result of docking, a cluster of docked complexes were obtained. The best docked structure with the minimum binding energy was used for further analysis.

RESULT

GC-MS analysis were performed to find out different compounds present in the plants sample and a total of 21 compounds were identified in the ethanolic extract of *Carica papaya* leaves, which includes the active principles with their retention time, molecular formula, molecular weight are presented in (Table 1 and Fig. 1). On further study of each compound, it was found that they individually have its own biological importance. These phytocompounds were treated as ligands and it is docked with target protein PI3CKA and BCL2.

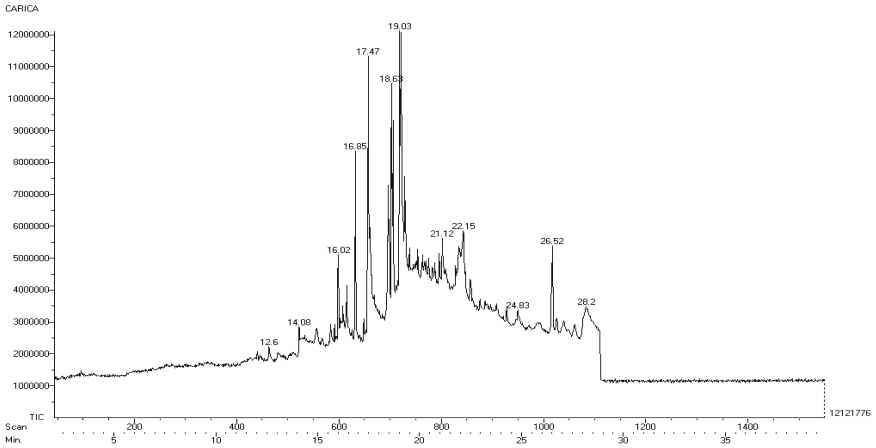


Fig. 1 GC-MS Chromatogram of Ethanolic Extract of *Carica papaya*

Table 1 GCMS analysis of Ethanolic Extract of *Carica papaya*

Sl. No	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1	22.15	Coumarine3-(2,4-dinitrophenol)	$C_6H_4N_2O_5$	184.11	1.72
2	18.05	Alpha-lardeine	$C_{36}H_{60}O_{30}$	141.5	4.54
3	16.02	3,7-Dimethyl-6-nonen-1-ol acetate	$C_{13}H_{24}O_2$	212.3	1.52
4	16.85	Dodecanoicacid, 10-methyl-, methyl ester	$C_{14}H_{28}O_2$	228.37	2.16
5	17.47	Hexadecanoicacid, ethyl ester	$C_{18}H_{36}O_2$	284.47	2.87

6	18.63	Phytol	$C_{20}H_{40}O$	296.5	1.63
7	19.03	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.5	11.27
8	21.2	5,8-Octadecadienoic acid, methyl ester	$C_{19}H_{36}O_2$	294.5 g/mol	8.32
9	13.04	Flavone	$C_{15}H_{10}O_2$	222.24	2.76
10	28.02	Androst-4-en-3-one,17-methoxy, 3-methoxime	$C_{20}H_{30}O_2$	302.5	7.15
11	24.08	2-methylbutyl 4'-pentyl-4-biphenylcarboxylate	$C_{23}H_{30}O_2$	338.5	5.08
12	12.6	Delta- Iraldeine	$C_{14}H_{22}O$	206.32	3.59
13	26.52	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentanene	$C_{25}H_{42}$	342.6	16.52
14	14.57	Dodecyl acrylate	$C_{15}H_{18}O_2$	240	1.63
15	16.0	Thionodecalactone	$C_{10}H_{18}OS$	186.32	1.23
16	19.32	312-Nodecanoic acid	$C_{10}H_{20}O_2$	174.25	2.76
17	21.92	Dasycaripidan-1-methanol, acetate(ester)	$C_{20}H_{26}N_2O_2$	326.4	2.87
18	14.12	4[2,4,4-Trimethyl-cyclohexa 1, 5-dienyl]but-3-en-2-one	$C_{13}H_{18}O$	190.28	4.99
19	32.68	Monolinoleoylglycerol trimethyl silyl ester	$C_{27}H_{54}Si_{04}$	498	10.83
20	11.49	Ethyl iso-allocholate	C26H44O5	436	15.06
21	12.58	Cis-11-Eicosenoic acid	C20H38O2	310	24.32

In Silico docking analysis

The *insilico* docking analysis was performed by molecular docking using "Argus Lab" software to find a suitable antagonistic ligand from the plant compound for the tumor progressive proteins

CD117, Cyclin D1. The obtained 12 compounds identified in the GC-Ms analysis, were selected for the molecular docking in the "Argus Lab server" to obtain the best antagonistic ligand in terms of interactions between the ligand identified from the ethanolic extract of *Carica papaya* leaf by GC-Ms analysis and the proteins such as CD117, Cyclin D1. Based on the docking score and glide energy Table [2] the compounds which exhibit lowest binding energy were selected to identify the mode of binding of the ligands with the particular protein, in terms of the amino acids interaction were studied. The doxorubicin the anticancer drug was used as a positive control in the present study.

Table 2 Docking score values of selected Phytoligands of *Carica papaya* with CD117 and Cyclin D

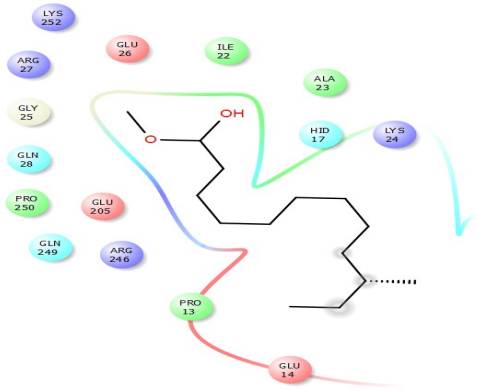
S.No	Compound Name	Binding energy expressed in (kJ mol^{-1})	
		CD117	Cyclin D
1	2,6,10,14,18-Pentamethyl-eicosapentaene	-3.36	-3.67
2	Alpha-lardeine	-4.53	-3.41
3	5,8-Octadecadienoic acid, methyl ester	-3.09	-3.63
4	2-methylbutyl4,39;-pentyl-4-biphenylcarboxylate	-5.05	-3.92
5	Androst-4-en-3-one,17-methoxy,3-methoxime	-2.50	-2.04
6	Hexadecanoic acid, ethyl ester	-2.87	-2.92
7	9,12-Octadecadienoic acid, ethyl ester	-4.49	-5.03
8	Coumarine 3-(2,4-dinitrophenol)	-2.38	--2.82
9	3,7-Dimethyl-6-nonen-1-ol acetate	-3.28	-3.82
10	Phytol	-2.40	-3.59
11	Dodecanoic acid, 10-methyl-, methyl ester	-2.25	-2.79
12	Flavone	-3.68	-4.22

Table 3 Interaction with the proteins CD117 and Cyclin D with phyto chemicals of EECP

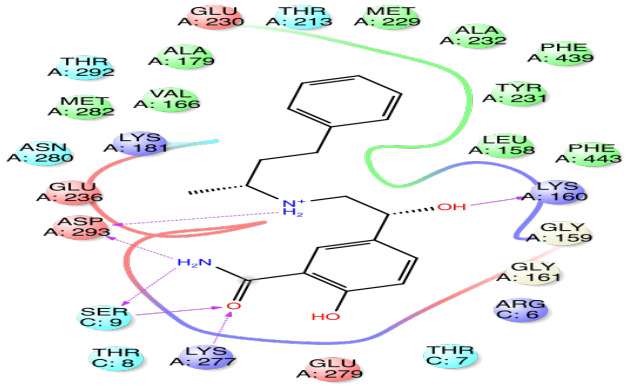
S.No	Compound	Amino acid binding site CD117 protein		Amino acid binding site Cyclin D protein	
		H bonding sites	Hydrophobic contact sites	H bonding sites	Hydrophobic contact sites
1	Phytol	4	21	3	13
		Lys160, Lys 277, Ser9,	Glu230,Thr213,Met229,Ala234,Phe439,Tyr231,Leu158,Phe443,Gly159,Gly 161, Arg6,	Lys62,Gly31, His47,	Val30,Cys28, Cys44,Gly22, Phe98,Ile9,Al

		Asp293	Thr7, Glu279, Thr8, Glu236,Asn280, Lys181,Met282,Val166,Thr292, Ala179		a17,Phe5, Tyr21, Leu2, Gly29,Tyr51, Asp48,
2	Hexadecanoic acid, ethyl ester	1	16	2	10
		Arg246	Cys23,Gln28,Lys23.Asp26,Glu205,Arg27,Arg254,Gly196, Tyr194,Trp209,Ser197,Ser29 ,Pro30,Leu198,Thr199,Gln247	Lys623, Phe811,	Ash677,Gly676,Leu595,Cys673,Tyr672, Glu671,Val654,Ile653
3	Coumarine 3-(2,4-dinitrophenol)	0	15	6	4
		Lys 62,	Glu14,Pro13,Arg246,Glu206 ,Gln249pro250,Gln28, Gly25,Arg277, Lys252,Glu26,Ile 22, Ala23, His17,lys24.	Ser321,His296,Arg287,His411,Trp413, Phe 294	Leu455,Leu297,Leu 288,Ile 312
4	Dodecanoic acid, 10-methyl-, methyl ester	1	18	2	14
		Ala232	Gly164,Lys160,Gly159,Lys165,Glu279,Asn280,Val166,Leu158, Phe439, Ala179,Met282, Tyr231, Thr292,Asp293,Lys181,Gly161, Leu183,Phe163	Thr213, Ala232	Gly159,Ala179,Asp293,Met229,Thr292,Lys290,Glu230 ,Met282, Tyr231,Phe439,Asn233,Gly235,Leu 158,Val166
5	Androst-4-en-3-one,17-methoxy, 3-methoxime	1	16	2	15
		Phe123	Leu124,Hie111,Trp114,Met91,Ala88,Met84,Leu80,Leu76 ,Phe15,Ala62,Val64,Phe59, Met19,Leu58,Ile125,Tyr122.	Ala232	Phe439,Asn233,Gly235,Leu158,Val166, Gly159,Ala179,Asp293,Met229,Thr292
6	Doxorubicin	3	15	0	21
		His47, Gly29 Phe5	His27,Cys44,Gly22,Phe98,Tyr21,Ile9,Ala94,Ala17,His6, Ala18,	-	Gly137,Ile169 ,Tyr33,Tyr16, Phe200,Tyr265,Leu236,Phe

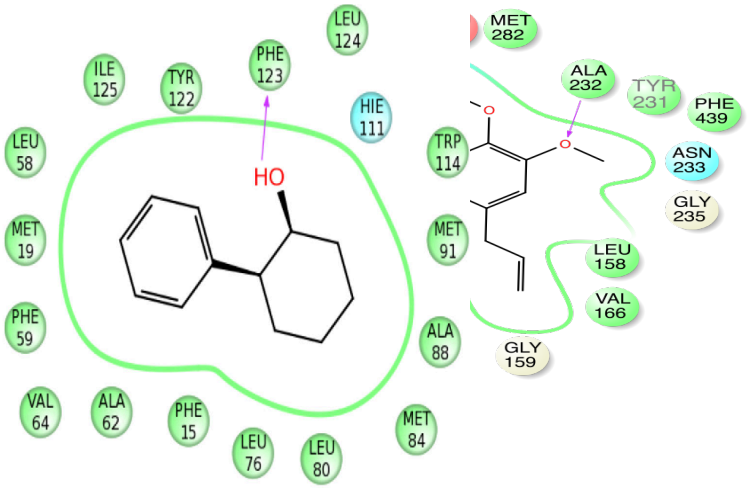
			Leu2,Val3,Cys28,Val30,Asp 48		273,Tyr232,Trp239,Cys269,Ile278,Leu272,Pro205,Gly203,Ala196,Ile195, Leu192,Glu140,His141,Ala139



(A) Phytol

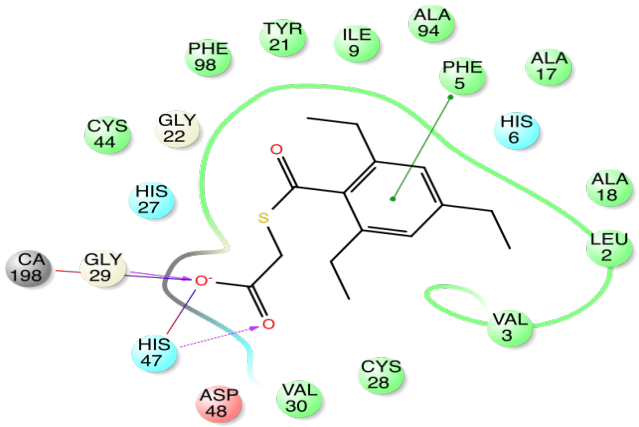


(B) Hexadecanoic acid, ethyl ester



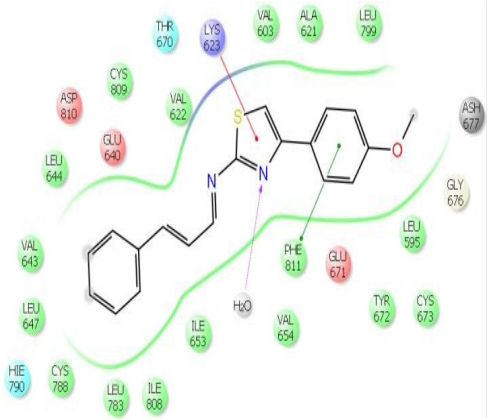
(C) Coumarine 3-(2,4-dinitrophenol)

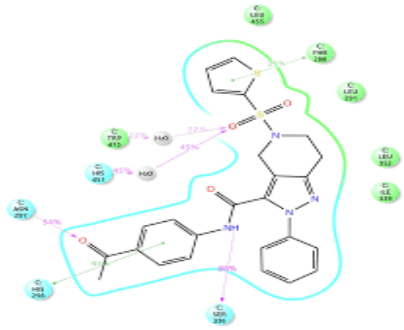
(D)Androst-4-en-3-one,17-methoxy,3-methoxime



(D) Doxorubicin

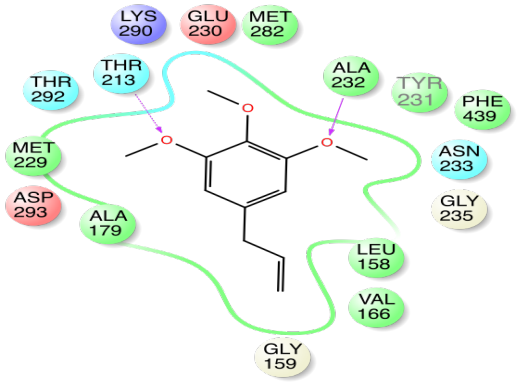
Fig. 2 Amino acid Interaction of CD117 protein with specific ligands

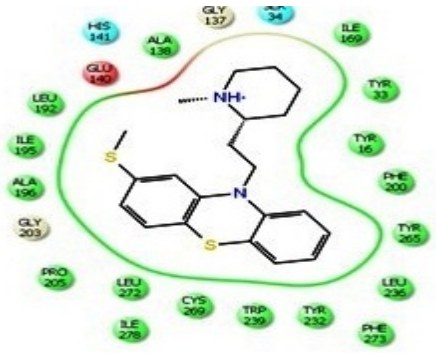




(A) Hexadecanoic acid, ethyl ester

(B) Coumarine 3





(E) Androst-4-en-3-one,17-methoxy, 3-methoxime (F) Doxorubicin

Fig. 3 Amino acid Interaction of Cyclin D protein with specific ligands

In the present study out of the 12 ligands docked against the tumor progressive proteins CD117 and Cyclin D, the tumor progressive proteins, we observed only three ligands with low docking score and glide energy among the 12 ligands docked against that above protein. They were identified as Hexadecanoic acid, ethyl ester, Coumarine 3-(2,4-dinitrophenol), Androst-4-en-3-one, 17-methoxy, 3-methoxime. Fig. 2 & Fig. 3 and Table 2 clearly explains the intermolecular interactions existing between the molecules of phytochemical and the amino acids of the particular protein. It is observed that in all the proteins, number of hydrophobic inter action is more and the hydrophilic interaction is very less.

DISCUSSION

In spite of the availability of advanced diagnosis and treatment the cancer chemotherapy still remains a challenging one to the mankind. So there has been always an increased interest for researchers to develop a new anticancer drug with high potency to kill the cancer cells [10]. According to [11] the selective growth inhibitory activity of the various plant extracts is due to the phytochemicals that can act in different pathways to arrest the cancer cell growth. With the above scenario the present investigation is undertaken to study the Anti cancer activity of Ethanolic extract of *Carica papaya* leaves (EECP) in Human Ovarian cancer PA-1 cell line [12] have reported the traditional use of the Papaya leaf extract by Australian aboriginal people for its anti-cancer activity.

The natural antioxidants present in vegetables and fruits are of much importance as they protect the human body from the oxidative stress and diseases [13]. These holistic plant derived herbal medicines can be exploited for research to obtain a successful plant derived drug for the treatment of cancer since the modern synthetic cancer treatment The P53 is a transcription factor and a tumor suppressor which plays an important role in cell cycle progression and cell death by arresting the cell cycle at G1 phase by triggering apoptosis [14]. According to [15] in ovarian cancer it is found to be highly mutated leading to lower survival rates, higher relapse rates and increased resistance to treatment in the form of chemotherapy and radiation. In the normal cells upon accumulation of damaged DNA p53 gets activated and trigger another transcriptional factor p21, by directly binding to p21/CDKN1Apromoter which in turn inhibit cyclin D dependent -kinases required for cell cycle progression [16]. PTEN is a tumor suppressor gene also named as phosphatase and tensin homolog deleted on chromosome 10 is a negative regulator phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathway to control the growth and survival [17]. Though the functions several genes and proteins are become altered in cancer cells we analyzed the up regulation of these three genes by the *Carica papaya* extract treatment in the PA-1 ovarian cancer cell lines. The presence of alkaloids such as carpaine, pseudocarpaine, piperideine, dehydrocarpaine I and II act as a potent anti tumor agent as stated by Okwu (18). According to Huang [19] the flavanoids present in the extract are potent free radical scavengers which protect the cells from oxidative injury caused by several carcinogens and exert strong anticancer activity.

The complicated apoptosis process is strictly balanced by many proteins and any disturbance in this critical down streaming process will lead to the uncontrolled proliferation as observed in the cancer cells. So from the therapeutic standpoint, correcting the over expression of the altered proteins to induce apoptosis is the main goal of cancer therapy. In this context the selected phytochemicals from the EECP extract were docked against few tumor promoting proteins such as CD117, Cyclin D1 and their inhibitory activity in terms of amino acid interaction were studied. According to acid Antonella et al. the palmitic treatment has a potential role in modulating the cell cycle progression through the complex formation with cyclin D and cyclin-dependent protein kinases. In our present study the anticancer activity exhibited by the EECP extract may be due to the presence of sufficient Hexadecanoic acid, ethyl ester which exerts their action by inhibiting the tumor progressive proteins as evidenced by the molecular docking studies.

Cyclin D1 This protein is 45kD (kilo Dalton) protein encoded by cyclin D1 gene (CCND1) located on chromosome 11q13, is a part of the molecular system that regulates the cell cycle G1 to S transition is involved in the cell cycle regulation and as well as in proliferation of cells is known to over express among several cancers (20). Cyclin D1, well established oncogene that has been found to play a very important and crucial roles in the process of development of cell proliferation and oncogenesis (21). With the above context cyclin D1 interfering strategy may be considered as a successful therapeutic approach to trigger the apoptosis in the cancer cells. Based on the docking studies three ligands namely Hexadecanoic acid, ethyl ester.

In the present investigation there was a selective inhibition of CD117, cyclin D1 proteins by the phyto constituent's phytol and Dodecanoic acid, 10-methyl-, methyl ester. Phytol is considered as a diterpene alcohol, a metabolic product of chlorophyll [22] abundantly present in leaves. Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) is a diterpene, a member of the group of branched-chain unsaturated alcohols. It is the product of chlorophyll metabolism in plants; hence, phytol is abundantly available in nature [23] have showed the breast cancer inhibitory activity of the purified compound phytol from Gracilaria edulis. The polyunsaturated phytol side chain in tocotrienols has got the inhibitory effect on breast tumour induced by 17b-estradiol epoxide-carcinogenesis was stated by Yu [24]. In our present investigation through molecular docking studies it is observed phytol acts as a potential antagonistic ligand for the proteins CD117 and Cyclin D1 by the compound Androst-4-eb-3-one, 17- methoxy, 3-methoxime. we could able to show the inhibition of the above-mentioned proteins in terms of the amino acid interactions through docking studies. The antiproliferative activity of the EECP extract on PA-1 cell lines may be either due to the aromatase enzyme inhibition there by suppression of estrogen production responsible for myriad of physiological processes involved the reproduction or it may be due to the antagonistic ligand Androst-4-eb-3-one, 17- methoxy, 3-methoxime for the tumor suppressive proteins CD117 and Cyclin D1. Alejandro et al. (2001) have shown that the hydroxy coumarin shows the anticancer activity in the human lung adenocarcinoma cell line by inhibiting the Cyclin D1 concentration there by the transition from G1 to S phase may be inhibited. Cyclin dependent kinases such as

CDK2, CDK4 and CDK6 work cooperatively and take the cells from G1 phase to S phase which can be blocked by small molecules. Ashok Sharma et al. (2017) have designed and studied the inhibition of these CDKs using coumarins in their molecular docking and concluded that these coumarins can be used as a potential target in cancer chemotherapy as they are capable of inhibiting these CDKs the key enzymes responsible for cancer cell proliferation.

CONCLUSION

In our present investigation considerable inhibition of CD117 and Cyclin D1 proteins by the antagonistic ligand Androst-4-eb-3-one, 17- methoxy, 3-methoxime was observed and leading to the conclusion that could able to show the inhibition of the above-mentioned proteins in terms of the amino acid interactions through docking studies and that the anti- proliferative activity of our plant extract may be due to the cumulative effects of these phytoconstituents

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Conflicts of interest

There are no conflicts of interest

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