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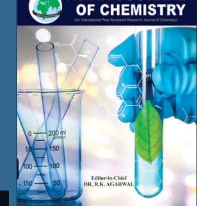

in silico-Based Virtual Screening and Molecular Docking Analysis of Phytochemicals obtained from Methanolic Extract of *Cleome viscosa* Linn. by GC-MS Method for its Anticancer Activity

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Cleome viscosa belonging to the family Capparidaceae, is a weed with ethano-botanical value found in India. In the present investigation, methanolic extract of *Cleome viscosa* was analyzed by gas chromatography-mass spectrometry (GC-MS) to identify the important phytochemical constituents. The GC-MS analysis of methanol from whole plant of *Cleome viscosa* detected the presence of 78 phytochemical compounds. Quantitative phytochemical evaluation of the methanolic extract of *Cleome viscosa* was performed. These identified compounds were analyzed for their anticancer activity through *in silico* molecular docking studies. Computation based *in silico* docking studies were done using maestro interface. Three protein, poly (ADP-ribose) polymerase-1 (PARP-1), epidermal growth factor receptor (EGFR), human papilloma virus (HPV) specific to different cancers were selected for screening of these phytochemicals. Phytomolecules with better activity and binding were shortlisted after XP mode of docking. The dock score, glide energy and 2D binding interactions of the top five phytochemicals with three selected proteins have been discussed. The identified hit could be a potent inhibitor these proteins that further requires experimental validation.

Keywords: *Cleome viscosa*, Epidermal growth factor receptor, Human papilloma virus, PARP-1, Anticancer activity.

INTRODUCTION

While using many modern synthetic and chemical drugs, there is a hesitation in their usage due to apparent side effects associated with the same [1]. Usage of traditional herbals are gaining huge traction because they are natural, considered environment-friendly and most importantly said to be devoid of side effects [2]. This is one of the reasons as to despite the availability of a vast number of beneficial modern synthetic medicines more and more people are leaning towards plant based natural medicines [3]. Various phytoconstituents present in different parts of the plants are responsible for the treatment as well as the cure of various diseases [3-5]. Medicinal plants in healing is not a new thought for the Indian system of medicine. Usage of medicinal plants and their parts in the treatment of various alignments has been reported from ancient times. More than 80000 medicinal plants were identified and were used in the treatment of diseases in various systems of Indian medicine [6]. More than 25% of active components used in modern prescribed medicines have been obtained from medicinal plants [7]. Many bioactive compounds obtained from medicinal plants have reported in stimulating pharmacological action like antioxidant, anticancer antifungal antibacterial anti-inflammatory activities [3,4,8]. Hence, it is essential to analyze the potential of these bioactive compounds to understand the feasibility of their usage in the treatment of various ailments [4,7].

Many high activity medicines are the product of bioactive compounds derived from extraction and characterization of medicinal plants [9]. Spectrophotometric and chromatographic methods are the keys, which provides the basic information regarding chemical and pharmacological activities of medicinal plants. This interns helps in selection of biologically active plants for the study [10]. Gas chromatography-mass spectrometry (GC-MS) is commonly used methods in detection of function groups and also identification of bioactive therapeutic compounds found in medicinal plants [11,12]. The compounds like

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alkaloids, alcohols, long chain hydrocarbons, nitro compounds, organic acids, esters, steroids and amino acids [13] are detected by employing GC-MS method which considered as precise techniques. Hence, we have adopted the same technique in the. Detection of phytochemical compounds present in the medicinal plant, *Cleome viscosa* Linn.

Cleome viscosa Linn. (Capparidaceae) is a weed found throughout the tropics of the world and the plains of India [14]. Wild mustard, dog mustard and sticky cleome are the common names by which this plant is popular in India. This plant was selected for the present study on the basis of its potential as curative agents mentioned in traditional systems of medicine, such as Ayurveda and Unani [15].

In recent years, computer-aided tools are playing a major role in the process of new drug discovery. They are effective in screening the active compounds from phytochemical found in various medicinal plants [16]. Understanding of drug receptor interaction is key in predicting the binding orientation of the drugs to the target protein, in the context molecular docking technique is emerging as the most effective and in expensive method [17]. This technique helps in systemic study by introducing a molecule on the binding spot of the object macromolecule in a non-covalent fashion, leading to an accurate binding at the active sites of each ligand [18].

Therefore, the present study focuses on the identification of bioactive compounds from methanol extract from *Cleome viscosa* by GC-MS analysis. Subsequently, *in silico* molecular docking and computational molecular simulation was explored for analysis of the potential bioactive compounds for their anticancer activity.

EXPERIMENTAL

Preparation of plant extract *Cleome viscosa* Linn: The whole plant of *Cleome viscosa* Linn. belonging to the family *Capparidaceae* was collected from the district of Udupi, India in October 2020. The plant was authenticated by the head of the Botany department, Poornaprajna College, Udupi, India. The authenticated sample was submitted in Manipal College of Pharmaceutical Sciences herbarium, Manipal, India. The plant material (2 kg) was shade dried, powdered coarsely was extracted using Soxhlet apparatus for 24 h by methanol. The crude methanol extract was concentrated in a rotary evaporator under reduced pressure for solvent recovery and the collected concentrated extract was dried and preserved in a desiccator for later use. The yield of was 4.3% for crude methanol extract.

Quantitative phytochemical evaluation of *Cleome viscosa* leaves methanol extract: The methanolic extract of *Cleome viscosa* were subjected to total polyphenols, total saponins, total tannins, total carbohydrates, total terpinoids and total flavonoids content by using standard protocol to detect the presence or absence of active constituents [19,20].

Gas chromatography-mass spectroscopy (GC-MS) analysis of the extract: The extract was dissolved in methanol and analyzed by gas chromatography-mass spectroscopy (GC-MS). The study was performed in Analytical Research & Metallurgical Laboratories Pvt. Ltd. (ARML), Bengaluru, India using Shimadzu GCMS-QP2010S instrument. The specification of chromatography conditions were column-RTX-5 (length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm), temperature (ion source 200 ºC, interface 280 ºC), carrier gas-helium (flow rate 1 mL/min). MS spectrum was analyzed by matching with National Institute of Standards and Technology (NIST) for compound identification and confirmation.

Protein selection: Three protein specific to different cancers were selected based on the earlier reports for screening of the phytochemicals.

(1) Poly (ADP-ribose) polymerase-1 (PARP-1) is an enzyme having role in the repair of single-stranded breaks in DNA. Over expression of PARP-1 is observed in cancers like BRCA-mutated breast and ovarian cancer, neuroblastoma, malignant lymphoma, colon cancer [21]. Inhibitors of PARP-1 enzyme like olaparib, talazoparib have been proven for treatment of BRCA-deficient breast and ovarian cancer. Crystal structure 4PJT containing catalytic domain of PARP-1 bound to the inhibitor was obtained from the Protein data [22].

(2) Overexpression of epidermal growth factor receptor (EGFR) has been linked to different cancers like non-small cell lung cancer, glioblastoma, colorectal cancer, squamous cell carcinoma, ovarian cancer, *etc*. [23,24]. EGFR protein with PDB ID: 1M17 was obtained from protein databank, which includes the X-ray crystal structure with its inhibitor erlotinib [25].

(3) Human papilloma virus (HPV) has been proven to be linked with 90% cases of cervical cancer cases [26]. E6 oncoprotein coded by HPV has been one of the chief target for the development of therapeutics against cervical cancer [27]. Due to unavailability of the protein crystal structure of E6 oncoprotein bound to the inhibitor, we have selected apoprotein PDB:4GIZ for the screening of the identified phytomolecules in the present extract.

in silico cytotoxicity evaluation

Ligand preparation: All the compounds from the LCMS report were prepared using LigPrep tool of Maestro that involves ionization, generation of possible states as per pH, removal of salts and generation of possible tautomers.

Protein preparation: All the protein molecules were prepared making them suitable for molecular docking using Protein Preparation Wizard module of Schrodinger. The protein preparation process involves three steps: (i) pre-processing that involves creation of disulphide bonds, filling missing side chains and loops and deleting water; (ii) Review and modify where the analysis of the protein structure and removal of the unwanted chains in protein can be done; (iii) Refine that involves optimization of protein by H-bond assignment and pH adjustment, energy minimization using force field is carried out.

Molecular docking and free energy calculation: The grid box of 10 Å × 10 Å × 10 Å was generated in all the prepared protein molecules considering the inbound inhibitors using GLIDE [28] module and using site map tool [29] for PDB:4GIZ using the coordinates of computationally identified drug binding pocket. The prepared ligands were then docked to the proteins separately using XP (Extra precision) mode of analysis.

RESULTS AND DISCUSSION

Preliminary phytochemical screening: The phytochemical study of methanolic extract of *Cleome viscosa* revealed a broad variety of phytochemicals. The key phytochemical components, such as polyphenols, saponins, tannins, carbohydrates and flavonoids were present in the methanolic extract (Table-1).

TABLE-1
QUANTITATIVE PHYTOCHEMICAL EVALUATION OF *Cleome viscosa* LEAVES METHANOL EXTRACT

Assay name	Presence	Standard	Quantity
Total polyphenols#	(+)	Pyrogallol	0.990%w/w
Total saponins#	(+)	NA	20.5% w/w
Total tannins#	(+)	Tannic acid	4.90% w/w
Total carbohydrates#	(+)	D-Glucose	0.00353%w/w
Total terpinoids*	Not detected	Camphor	NA
Total flavanoids#	(+)	Rutin	1.903%w/w

#Gravimetric method; *GC method

Gas chromatographymass spectrometry (GC-MS) analysis: The GC-MS chromatogram of methanol extract of *Cleome viscosa* recorded a total of 84 peaks corresponding to the bioactive compounds that were recognized by relating their peak retention time, peak area (%), height (%) and mass spectral fragmentation patterns to that of the known compounds described by the National Institute of Standards and Technology (NIST) library.

***in silico* studies:** The GC-MS analysis revealed that methanolic extract of *Cleome viscosa* contained 78 bioactive compounds (Table-2). These phytocompounds were analyzed for anticancer activities against target proteins.

Analysis of binding interactions of the phytomolecules with PARP-1 protein: PARP-1 is mainly involved in the repair of breaks in the single strand DNA by binding to the DNA gap followed by the recruitment of other enzymes responsible for DNA repair. BRCA1 and BRCA2, tumor suppressing gene play major role in the repair of double strand DNA breaks [30]. In BRCA mutated tumor inhibition of PARP-1 enzyme leads to the increased occurrence of DNA breaks and result in

TABLE-2
GC-MS ANALYSIS OF EXTRACT SHOWING MAJOR CHEMICAL COMPOSITION

Peak	Retention time	Area (%)	Name	m.w.	m.f.
1	4.161	0.27	3,4-Hexanediol, 2,5-dimethyl-	146	$C_8H_{18}O_2$
2	5.934	0.22	1-Isopropenyl-3-propenylcyclopentane	150	$C_{11}H_{18}$
3	7.671	0.39	Benzofuran, 2,3-dihydro-	120	C_8H_8O
4	8.077	0.16	Silane, tetramethyl	88	$C_4H_{12}Si$
5	8.791	0.21	Nonane, 3-methyl-5-propyl	184	$C_{13}H_{28}$
6	8.916	0.19	5-Ethylhydantoin	128	$C_5H_8N_2O_2$
7	9.022	0.34	2-Methoxy-4-vinylphenol	150	$C_9H_{10}O_2$
8	9.508	0.28	Pyridine,3-(1-methyl-2-pyrrolidinyl)-, (S)-	162	$C_{10}H_{14}N_2$
9	9.903	0.20	α-Methyl- α-[4-methyl-3-pentenyl]oxiranemethanol	170	$C_{10}H_{18}O_2$
10	12.198	0.41	3',5'-Dimethoxyacetophenone	180	$C_{10}H_{12}O_3$
11	14.202	1.30	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	180	$C_{10}H_{12}O_3$
12	14.449	0.18	3-Buten-2-one, 3-methyl-4-(1,3,3-trimethyl-7-oxabicyclo [4.1.0] heptan-1-yl)-	222	$C_{14}H_{22}O_2$
13	14.788	0.24	2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol	182	$C_{11}H_{18}O_2$
14	15.102	0.46	6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-2-ol	226	$C_{13}H_{22}O_3$
15	15.300	1.06	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	224	$C_{13}H_{20}O_3$
16	16.107	0.95	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$
17	16.469	5.78	l-(+)-Ascorbic acid 2,6-dihexadecanoate	652	$C_{38}H_{68}O_8$
18	16.805	0.71	Benzenemethanol, 2,5-dimethoxy-, acetate	210	$C_{11}H_{14}O_4$
19	17.352	0.87	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester	233	$C_{13}H_{15}NO_3$
20	17.482	0.16	Ethyl chrysanthemate	196	$C_{12}H_{20}O_2$
21	17.755	2.08	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294	$C_{19}H_{34}O_2$
22	17.935	0.26	Phytol	296	$C_{20}H_{40}O$
23	18.035	0.38	Octadecanoic acid, methyl ester	298	$C_{19}H_{38}O_2$
24	18.128	9.94	9,12-Octadecadienoic acid (Z,Z)-	280	$C_{18}H_{32}O_2$
25	18.370	3.91	Octadecanoic acid	284	$C_{18}H_{36}O_2$
26	19.043	0.75	Androstan-17-one,3-ethyl-3-hydroxy-, (5α)-	318	$C_{21}H_{34}O_2$
27	19.244	0.31	E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate	296	$C_{19}H_{36}O_2$
28	19.466	0.21	1-Deoxy-2,4:3,5-di-O0benzylidene-d-lyxitol	312	$C_{19}H_{20}O_4$
29	19.895	1.31	1-Heptatriacotanol	536	$C_{37}H_{76}O$
30	20.006	0.90	+/-.-2-Phenylbutyrophenone	224	$C_{16}H_{16}O$
31	20.204	0.18	Tricyclo[20.8.0.0(7,16)]triaconta-1(22),7(16),9,13,24,28-hexaene	404	$C_{30}H_{44}$
32	20.529	7.75	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α,2β,4β)	204	$C_{15}H_{24}$
33	20.681	4.13	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α.,2β,4β)	204	$C_{15}H_{24}$
34	20.956	0.27	2,5,9-Trimethylcycloundeca-4,8-dienone	206	$C_{14}H_{22}O$
35	21.126	0.88	d-Norandrostane (5α.,14α)	246	$C_{18}H_{30}$
36	21.248	0.27	9,19-Cyclolanost-23-ene-3,25-diol, 3-acetate, (3β,23E)-	484	$C_{32}H_{52}O_3$
37	21.363	1.49	Androstan-17-one, 3-ethyl-3-hydroxy-, (5α)-	318	$C_{21}H_{34}O_2$
38	21.442	1.41	2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo [4.1.0]heptan-3-ol	222	$C_{14}H_{22}O_2$

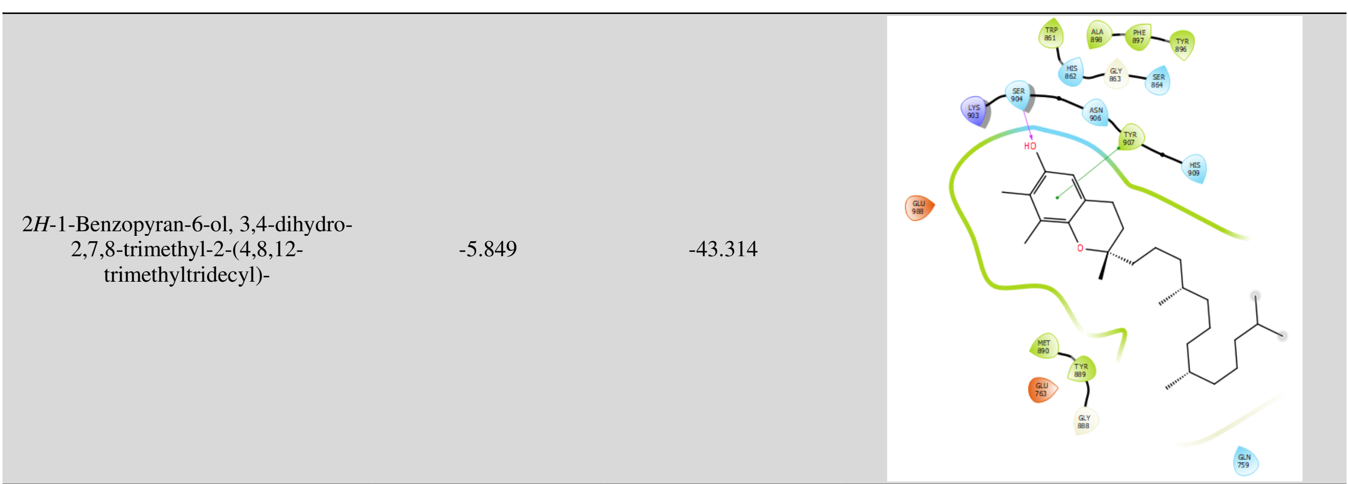
39	21.591	1.69	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	272	$C_{20}H_{32}$
40	21.709	1.85	1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-6-hydroxy-1,4a,7-trimethyl	318	$C_{20}H_{30}O_3$
41	21.792	0.65	Androstan-17-one,3-ethyl-3-hydroxy-, (5α)-	318	$C_{21}H_{34}O_2$
42	22.101	1.37	Caryophyllene oxide	220	$C_{15}H_{24}O$
43	22.137	1.64	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	272	$C_{20}H_{32}$
44	22.334	0.86	Longifolenaldehyde	220	$C_{15}H_{24}O$
45	22.497	0.52	Androstan-17-one,oxime, (5α)-	289	$C_{19}H_{31}NO$
46	22.615	6.10	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	324	$C_{23}H_{32}O$
47	22.773	3.12	1-Heptatriacotanol	536	$C_{37}H_{76}O$
48	22.864	0.64	4,8,13-Cyclotetradecatriene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl)-	306	$C_{20}H_{34}O_2$
49	22.938	0.33	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	374	$C_{25}H_{42}O_2$
50	23.028	0.25	Androstan-17-one,3-ethyl-3-hydroxy-, (5α)-	318	$C_{21}H_{34}O_2$
51	23.432	0.72	Valtrate	422	$C_{22}H_{30}O_8$
52	23.555	0.35	5-Methoxy-2,2,6-trimethyl-1-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo[4.1.0]heptane	236	$C_{15}H_{24}O_2$
53	23.661	0.55	Thunbergol	290	$C_{20}H_{34}O$
54	23.731	0.18	9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl-, acetate, (3β,4α,5α)-	468	$C_{32}H_{52}O_2$
55	23.820	0.16	5α-Hydroxy-4α,8,10,11-tetramethyltricyclo[6.3.0.0(2,4)]undec-10-ene	220	$C_{15}H_{24}O$
56	23.932	0.48	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol	306	$C_{20}H_{34}O_2$
57	24.151	0.65	13,15-Octacosadiyne	386	$C_{28}H_{50}$
58	24.293	0.19	2-Eicosanol, (.+/-.)-	298	$C_{20}H_{42}O$
59	24.612	0.63	Betulin	442	$C_{30}H_{50}O_2$
60	25.054	0.17	Cholesta-4,6-dien-3-ol, (3β)-	384	$C_{27}H_{44}O$
61	25.435	0.21	γ-Tocopherol	416	$C_{28}H_{48}O_2$
62	25.816	0.24	Cholesta-4,6-dien-3-ol, (3β)-	384	$C_{27}H_{44}O$
63	27.309	1.48	Ergost-5-en-3-ol, (3β)-	400	$C_{28}H_{48}O$
64	27.667	0.93	Stigmasterol	412	$C_{29}H_{48}O$
65	28.381	5.51	γ-Sitosterol	414	$C_{29}H_{50}O$
66	28.539	0.16	2,6,6,9,2',6',6',9'-Octamethyl-[8,8']bi[tricyclo[5.4.0.0(2,9)]undecyl]	410	$C_{30}H_{50}$
67	28.875	0.80	2H,6H-Benzo[1,2-b:5,4-b'] dipyran-6-one, 5-hydroxy-7-(*p*-methoxyphenyl)-2,2-dimethyl-	418	$C_{26}H_{26}O_5$
68	28.956	1.48	Cholest-4-en-3-one	384	$C_{27}H_{44}O$
69	29.389	0.81	24(S)-Ethyl-3α,5α-cyclocholest-22(E)-en-6-one	410	$C_{29}H_{46}O$
70	29.666	0.41	Cholesta-3,5-dien-7-one	382	$C_{27}H_{42}O$
71	30.070	0.47	9,19-Cyclolanostan-3-ol, 24-methylene-, (3β)-	440	$C_{31}H_{52}O$
72	30.070	6.29	Stigmast-4-en-3-one	412	$C_{29}H_{48}O$
73	30.751	0.77	3-(1,5-Dimethyl-hexyl)-3a,10,10,12b-tetramethyl-1,2,3,3a,4,6,8,9,10,10a,11,12,12a,12b-tetradecahydro-benzo[4,5]cyclohepta[1,2-E]indene	410	$C_{30}H_{50}$
74	31.230	0.22	9,19-Cyclolanost-23-ene-3,25-diol, (3β,23E)-	442	$C_{30}H_{50}O_2$
75	31.582	0.30	Humulane-1,6-dien-3-ol	222	$C_{15}H_{26}O$
76	31.702	0.25	Friedelan-3-one	426	$C_{30}H_{50}O$
77	33.099	1.70	Cholest-4-ene-3,6-dione	398	$C_{27}H_{42}O_2$
78	33.384	0.39	9,19-Cyclolanost-23-ene-3,25-diol, (3β,23E)-	442	$C_{30}H_{50}O_2$

the death of the tumor cells. The catalytic domain site of PARP-1 consists of site where in ADP-ribose moiety from NAD^+ gets transferred to nuclear proteins. So, inhibitors of PARP-1 are designed such that they are anchored to the nicotinamide binding pocket. Structural analysis of the PARP inhibitor, BMN 673 revealed the its binding to the nicotinamide binding site with the presence of hydrogen bond with GLY863 and hydroxyl atom of SER904 residue; π-stacking interaction with TYR907; water-bride interaction with GLU988 residue [22,31].

In the current study, the molecules obtained from the GC-MS analysis of the extract was docked in the inhibitor pocket to identify the potential PARP-1 inhibitors. Among all the molecules analyzed, five molecules with desired interaction and dock score above-5 are listed in Table-2. The listed compounds showed docking score in the range of (-7.613 to -5.849 Kcal/mol) and glide energy (-45.269 to -30.28 Kcal/mol). 5-Ethylhydantoin showed highest dock score of -7.61 Kcal/mol with the formation of hydrogen bonding interaction with residues GLY863 and SER904 indicating the stable binding and anchorage in the nicotinamide pocket. Similarly, π-stacking interaction with TYR907 and hydrogen bonding interaction with residue GLY863 was observed with the compound 2*H*-1-benzopyran-6-ol, 3,4-dihydro-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl) (Table-3).

TABLE-3
DOCKING SCORE, GLIDE ENERGY AND 2D INTERACTION DIAGRAM OF THE SELECTED PHYTOMOLECULES AND PARP1 PROTEIN (PDB:4PJT)

Compound	XP Dock score (kcal/mol)	Glide energy (kcal/mol)	Molecular docking in XP docking (4PJT) 2D Interaction diagram
5-Ethylhydantoin	-7.613	-30.28	
Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl] cyclopropyl]methyl] cyclopropyl]methyl], methyl ester	-6.221	-45.269	
4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	-6.095	-36.297	
Androstan-17-one, 3-ethyl-3-hydroxy-, (5α)-	-5.94	-39.251	

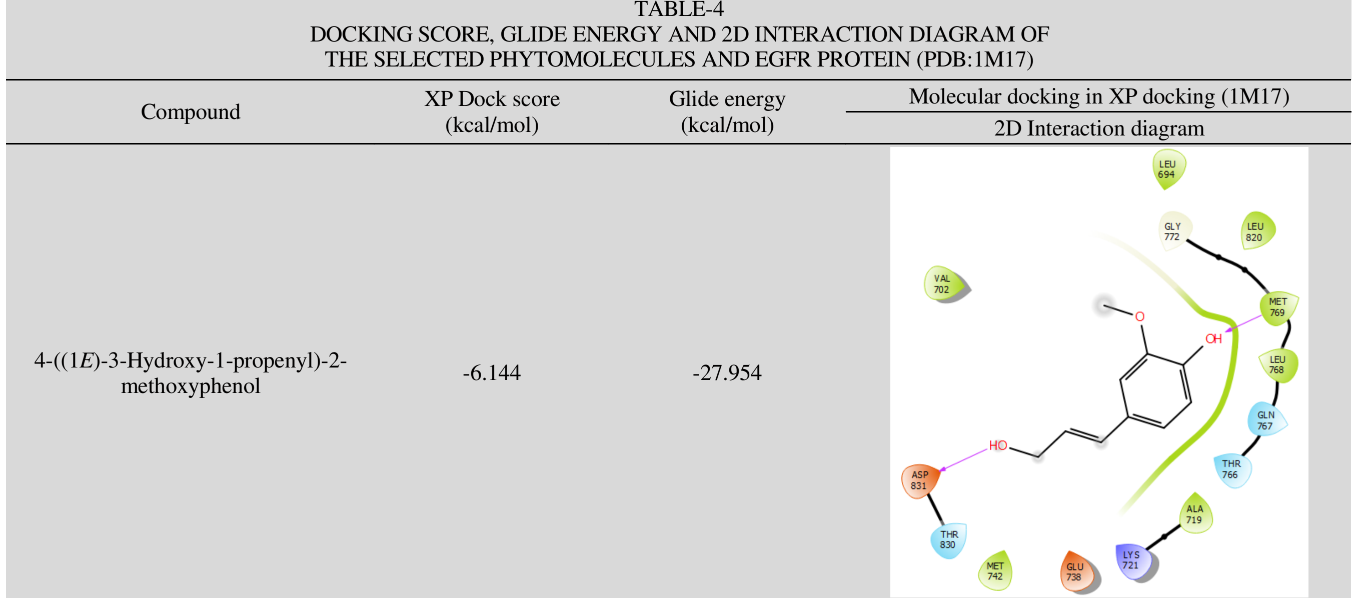
2*H*-1-Benzopyran-6-ol, 3,4-dihydro-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-	-5.849	-43.314	

Analysis of binding interactions of the phytomolecules with EGFR protein: Growth factors play important role in the proliferation, growth, maintenance, differentiation and metabolism process. Aberrant and uncontrolled expression and signalling of these growth receptors is a indication of on cogenesis. Epidermal growth factor (EGFR) receptor is overexpressed in 30% of breast cancer and solid tumors. ATP competitive inhibition of the EGFR with the small molecules have been explored to be the potential therapeutic approach in cancer. EGFR inhibitor, 4-anilinoquiazoline, binds to the ATP binding site forming the hydrogen bond with MET769, water bridge interaction with THR766 [25].

In the current study, the docking of the phytochemicals was done in the ATP binding pocket of EGFR for evaluating their binding potential. Top five compounds with the docking score above -5 Kcal/mol have been listed in Table-2 along with the glide energy and interacting residue in 2D diagram. These molecules showed the docking score ranging from -6.144 Kcal/mol to -5.374 Kcal/mol. Top molecule 4-((1*E*)-3-hydroxy-1-propenyl)-2-methoxyphenol showed docking score -6.144 Kcal/mol and glide energy of -27.95 Kcal/mol forming hydrogen bonding interaction with residues MET769 and ASP831 hydro-phobically surrounded by residues LEU694, VAL 702, LEU768, LEU820, ALA719, MET742; polar residues GLN767, THR766 (Table-4).

Analysis of binding and interactions with E6 oncoprotein: Human papillomavirus (HPV) is the cause for the several benign and malignant epithelial cancers, cervical cancer being the major health issue in the women of underdeveloped regions of the world. E6 oncoprotein plays major role in the full transformation of the virus by associating with the specific peptide motifs of the cellular proteins [26]. Therapeutics from natural sources, vaccines and synthetic chemicals directed towards suppression of E6 activity have been proven to reduce the cases of cervical cancer due to HPV. The formation of the complex between E6 with E6AP ubiquitin ligase leads to the p53 mediated degradation. Zanier *et al*. [32] have done a study to identify the druggability of the E6AP binding pocket and concluded that the pocket is druggable site. They have identified TYR70 residue as the key residue for binding with E6 protein as well as TYR76. residue also showed weak interactions.

TABLE-4
DOCKING SCORE, GLIDE ENERGY AND 2D INTERACTION DIAGRAM OF THE SELECTED PHYTOMOLECULES AND EGFR PROTEIN (PDB:1M17)

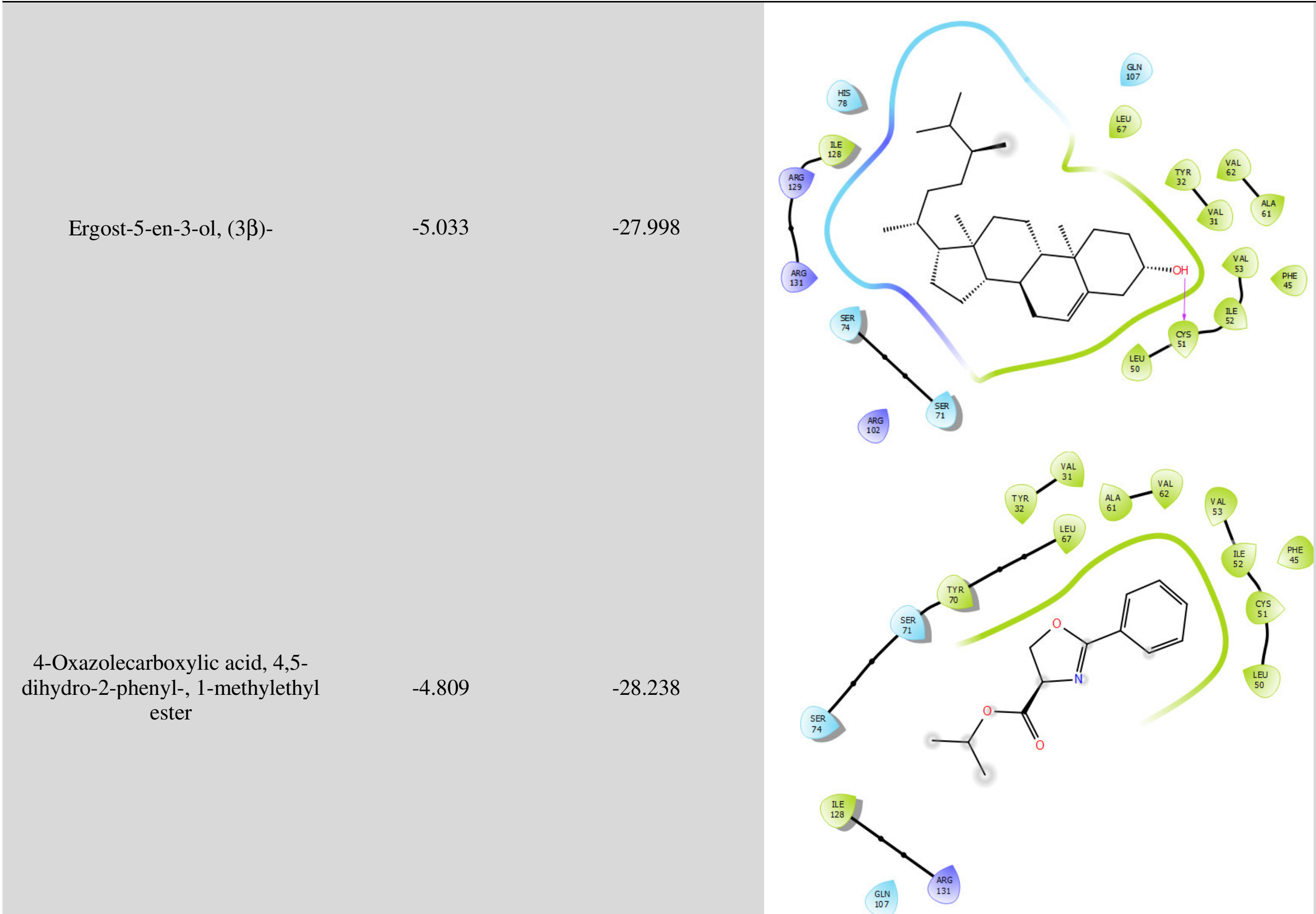
Compound	XP Dock score (kcal/mol)	Glide energy (kcal/mol)	Molecular docking in XP docking (1M17)
			2D Interaction diagram
4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol	-6.144	-27.954	

Benzenemethanol, 2,5-dimethoxy-, acetate	-6.013	-32.942	
2-[4-Methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde	-5.931	-30.482	
6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-2-ol	-5.842	-32.649	
Stigmast-4-en-3-one	-5.639	-27.063	

The dock score, glide energy and 2D binding interactions of the top five phytochemicals have been listed in Table-2. In present study, L-(+)-ascorbic acid 2,6-dihexadecanoate showed top docking score of -6.292 Kcal/mol and glide energy of -40.125 Kcal/mol. Carbonyl group of L-(+)-ascorbic acid 2,6-dihexadecanoate formed hydrogen bond with ARG10 and LYS 11; and oxide formed salt bridge interaction with ARG102 residue. Hydro-phobic non-bonding interactions with VAL31, TYR32, PHE45, LEU50, CYS51, VAL53, ALA61, VAL62, LEU67, TYR70, LEU 100; polar interactions with SER71, SER74 and HIS78 was observed with the top ligand. The docking score of the top listed compounds ranged from (-6.292 to -4.809 Kcal/mol) and glide energy ranged from (-40.125 to -22.795 Kcal/mol) (Table-5).

TABLE-5
DOCKING SCORE, GLIDE ENERGY AND 2D INTERACTION DIAGRAM OF THE SELECTED PHYTOMOLECULES AND HUMAN PAPILLOMAVIRUS ONCOPROTEIN E6 PROTEIN (PDB: 4GIZ)

Compound	XP Dock score (kcal/mol)	Glide energy (kcal/mol)	Molecular docking in XP docking (4GIZ)
			2D Interaction diagram
L-(+)-Ascorbic acid 2,6-dihexadecanoate	-6.292	-40.125	
4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	-5.803	-26.641	
4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol	-5.417	-22.795	

Ergost-5-en-3-ol, (3β)-	-5.033	-27.998	
4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester	-4.809	-28.238	

Conclusion

About 78 phytochemicals were isolated and identified from the methanolic extract of *Cleome viscosa* analyzed by gas chromatography-mass spectrometry. Three protein, poly (ADP-ribose) polymerase-1 (PARP-1), epidermal growth factor receptor (EGFR), human papilloma virus (HPV) specific to different cancers were selected for screening of these phytochemicals. The dock score, glide energy and 2D binding interactions of the top five phytochemicals with three selected proteins have been discussed.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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