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In silico analysis on macroalgae metabolites against skin cancer protein, phylogenetic and statistical analysis

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Anti-skin cancer potential of the macroalgae of *Halimeda* spp was tested against a skin cancer protein of 4,5-Diarylisoxazole Hsp90 Chaperone by *in silico* docking method About 32 secondary metabolites of *Halimeda* spp. reported from previous studies were checked against the skin cancer protein of Hsp90 using the tool of Arguslab 4.0.1. To find out the relevance among skin cancer and other cancers, a phylogenetic tree was constructed for the skin cancer proteins and other cancer proteins. The association among the retention time, the molecular weight of the tested compounds, and docking run were tested using Pearson correlation analysis by Minitab tool. The result exhibited that most of the tested active principles are possessing considerable binding energy. Among them, the highest was recorded for 1,2-Benzenedicarboxylic acid, butyl octyl ester of −14 kcal followed by Phthalic acid, butyl hexyl ester of −13 kcal. From the remaining four compounds showed −12 kcal, 14 compounds expressed −11 kcal and the other compounds possessed −10, −9, −8, and −4 kcal of binding energy. The phylogenetic tree revealed that the relationship of skin cancer having 100% similarity with other cancer protein of wild and home animals, 96% similarity with oral, lung and cervical cancers and 90% similarity with breast cancer protein in human. The correlation analysis showed that the positive association among the retention time, molecular weight of the compounds, and docking run. This study concludes that the *Halimeda* spp is the right candidate for culminating skin cancer and recommends further studies to establish the potential.

Keywords: Correlation, *Halimeda* spp, Phthalic acid, Phylogenetic, Minitab

Cancer is one of the leading heat impacting and death-causing diseases worldwide. Cells of cancer divide and grow uncontrollably and reach nearby parts of the body. Around 21.4 million cancer death will appear by 2030[1-3]. Skin cancer is a growing unpleasant human disease compared to other cancer[4]. Marine is a source for novel pharmaceutical compounds[1,5-7]. The present study was carried out to identify the effect of secondary metabolites of the macroalgae of *Halimeda* spp against a skin cancer Hsp90 using Arguslab 4.0.1 and Pymol software. The evolutionary relationship of Hsp90 with lung cancer, breast cancer, oral cancer, and cervical cancer also established. The evolutionary relations of skin cancer in Humans with other wild and home animals of Giant panda, Cattle, Chinese hamster, Cat, Gray mouse lemur, house mouse, killer whale, Golden snub-nosed monkey, wild boar, Gelada was compared using MEGA-X. The correlation between docking binding energy, molecular weight, and retention time were carried in Minitab software.

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Materials and Methods

Protein preparation

The sequence for the 4, 5-Diarylisoxazole Hsp90 Chaperone skin cancer protein of Homo sapiens was taken from protein data bank (PDB) with id 2CVJ. The skin cancer protein sequences consist of a total count of 239 amino acids and having the resolution of 2.0A° (Table 2).

Compound Preparation

About 32 secondary metabolites were reported from the methanol and hexane extract of *Halimeda* spp[8]. The structure of these compounds obtained from Pubchem database as an SDF format and through simplified molecular input line entry specification (SMILES) notation the chemical structure of mol generated (Tables 1 & 3).

Docking and 3D structure exploration

Arguslab 4.0.1a free docking tool was employed to check the antifouling potential of the chemicals. The docking images saved in the pdb format. Then the PyMOL software used to explore the binding sites between the molecules (Tables 2 & 3 and Fig. 3A-E).

Table 1 — GC-MS of Methanol and Hexane extract (Gadhi *et al*., 2018)

Sl. No	Retantion time	Compound name	Solvent	Formula	Molecular weight g/mol
1	557	Formamide, NN-dimethyl-	Hexane	C_3H_7NO	73.0938
2	816	Octane	Hexane	C_8H_{18}	114.2285
3	937	2-Methylaminomethyl-1,3-dioxolane	Hexane	$C_5H_{11}NO_2$	117.148
4	1440	Dimethyl phthalate	Hexane/Methanol	$C_{10}H_{10}O_4$	194.1840
5	1474	Mexiletine	Hexane	$C_{11}H_{17}NO$	179.2588
6	1627	N-Isopropyl-3-phenylpropanamide	Hexane	$C_{12}H_{17}NO$	191.274
7	1639	Diethyl phthalate	Hexane/Methanol	$C_{12}H_{14}O_4$	222.2372
8	1694	Ethyl N-isopropyl-3-phenylpropanimidate	Hexane	$C_{14}H_{21}NO$	219.328
9	2007	Phenethylamine, N-methyl-beta.,3,4-tris(trimethylsiloxy)-	Hexane		
10	2136	Phthalic acid, hexyl propyl ester	Hexane	$C_{17}H_{24}O_4$	292.375
11	2434	1,2-Benzenedicarboxylic acid, butyl octyl ester	Hexane	$C_{20}H_{30}O_4$	334.4498
12	888	1-Cyclohexene, 1-ethynyl-	Methanol	C_8H_{10}	106.168
13	1216	Decyltrifluoroacetate	Methanol	$C_{12}H_{21}F_3O_2$	254.2891
14	1450	3-Trifluoroacetoxytridecane	Methanol	$C_{15}H_{27}F_3O_2$	296.374
15	1450	4-Trifluoroacetoxytridecane	Methanol		
16	1457	1-Dodecanol	Methanol	$C_{12}H_{26}O$	186.3342
17	1508	Benzoic acid, 2-(1-oxopropyl)-,methyl ester	Methanol	$C_{11}H_{12}O_3$	192.214
18	1555	Phenol, 2,6-bis(1,1-dimethylethyl)-	Methanol	$C_{14}H_{22}O$	206.3239
19	1556	n-Tridecan-1-ol	Methanol	$C_{13}H_{28}O$	200.3608
20	1613	Tetradecyltrifluoroacetate	Methanol	$C_{16}H_{29}F_3O_2$	310.3955
21	1669	4-Heptafluorobutyryloxyhexadecane	Methanol	$C_{20}H_{33}F_7O_2$	438.471
22	1729	Phthalic acid, allyl ethyl ester	Methanol	$C_{13}H_{14}O_4$	234.2479
23	1773	Pentafluoropropionic acid, hexadecyl ester	Methanol	$C_{19}H_{33}F_5O_2$	388.4561
24	1818	5-Octadecene, (E)-	Methanol	$C_{18}H_{36}$	252.4784
25	1854	1-Hexadecanol	Methanol	$C_{16}H_{34}O$	242.4406
26	1855	Carbonic acid, methyl tetradecyl ester	Methanol	$C_{16}H_{32}O_3$	272.4235
27	1954	n-Heptadecanol-1	Methanol	$C_{17}H_{36}O$	256.4671
28	1973	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	Methanol	$C_{16}H_{22}O_4$	278.3435
29	2037	Dibutyl phthalate	Methanol	$C_{16}H_{22}O_4$	278.3435
30	2235	Phthalic acid, butyl hexyl ester	Methanol	$C_{18}H_{26}O_4$	306.3966
31	2259	Dichloroacetic acid, 4-hexadecyl ester	Methanol	$C_{18}H_{34}Cl_2O_2$	353.368
32	2774	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,.Z)-	Methanol	$C_{25}H_{36}O_2$	368.561

Phylogenetic analysis

MEGA – X is one of the freely available common tolls was to develop an evolutionary tree. The percentage and similarity among the amino acid sequence of the Hsp90 gene in Giant panda, Cattle, Chinese hamster, Cat, Gray mouse lemur, house mouse, killer whale, and Golden snub-nosed monkey, wild boar and Gelada species was determined. Similarly, Homo sapiens skin cancer sequence analyzed with other cancer proteins of oral, cervical, breast, and lung cancer sequences. Sequences of above organism's amino acid retrieved from NCBI and cluster and maximum likelihood tree build using MEGA-X software (Figs. 1 & 2).

Table 2 — Skin cancer details and structure (Protein Data Bank., 2019)

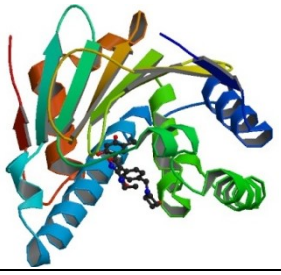
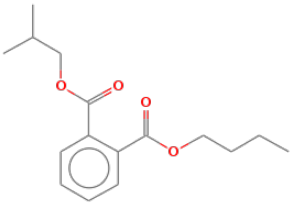
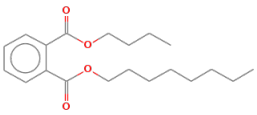
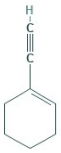
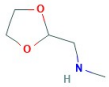
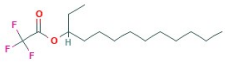
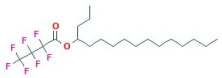
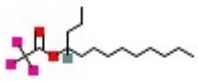
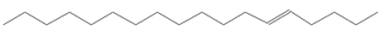
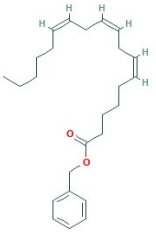
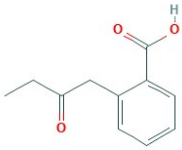
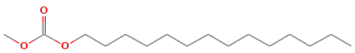
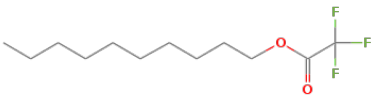
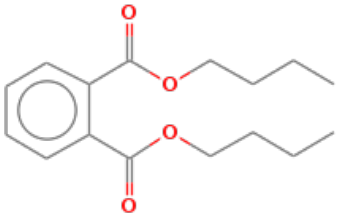
Sl. No	Protein Name	PDB-ID	Method	Organims	Structure
1	4,5 Diaryl Isoxazole Hsp90 Chaperone	2VCJ	X-Ray diffraction	*Homo sapiens*	

Table 3 — *Halimeda* spp compound name structure and Binding energy *vs* Skin cancer protein (*Contd.*)

Sl. No	Compound name	Chemical structure	Binding energy kcal/mol 2VCJ Skin cancer
1	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester		–11.8609
2	1,2-Benzenedicarboxylic acid, butyl octyl ester		–14.2721
3	1-Cyclohexene, 1-ethynyl-		–12.0846
4	1-Dodecanol		–11.1033
5	1-Hexadecanol		–11.8739
6	2-Methylaminomethyl-1,3-dioxolane		–4.61654
7	3-Trifluoroacetoxytridecane		–11.5425

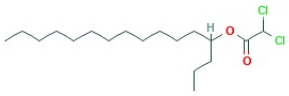
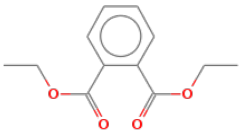
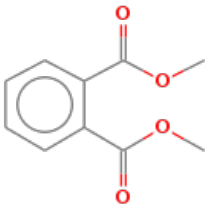
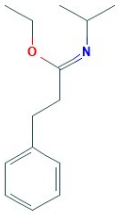
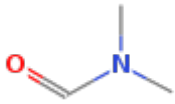
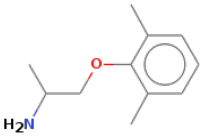
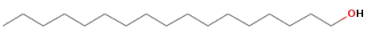
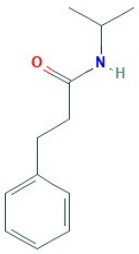
(*Contd.*)

Table 3 — *Halimeda* spp compound name structure and Binding energy *vs* Skin cancer protein (*Contd.*)

Sl. No	Compound name	Chemical structure	Binding energy kcal/mol 2VCJ Skin cancer
8	4-Heptafluorobutyryloxyhexadecane		–12.7319
9	4-Trifluoroacetoxytridecane		–11.4318
10	5-Octadecene, (E)-		–11.6925
11	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,.Z)-		No
12	Benzoic acid, 2-(1-oxopropyl)-,methyl ester		–10.767
13	Carbonic acid, methyl tetradecyl ester		–10.9934
14	Decyltrifluoroacetate		–10.7088
15	Dibutyl phthalate		–11.984

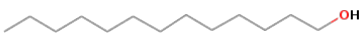
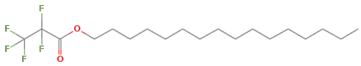
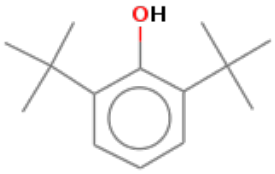
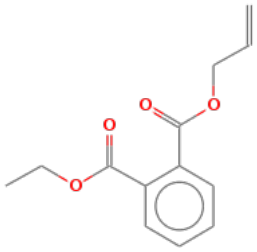
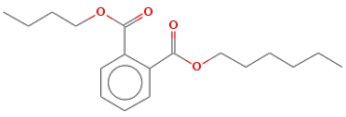
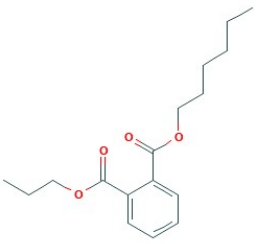
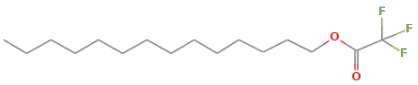
(*Contd.*)

Table 3 — *Halimeda* spp compound name structure and Binding energy *vs* Skin cancer protein (*Contd.*)

Sl. No	Compound name	Chemical structure	Binding energy kcal/mol 2VCJ Skin cancer
16	Dichloroacetic acid, 4-hexadecyl ester		–12.2967
17	Diethyl phthalate		–10.2886
18	Dimethyl phthalate		–9.20156
19	Ethyl N-isopropyl-3-phenylpropanimidate		–8.16888
20	Formamide, NN-dimethyl-		–4.00942
21	Mexiletine		–8.99057
22	n-Heptadecanol-1		–11.8324
23	N-Isopropyl-3-phenylpropanamide		–11.9448

(*Contd.*)

Table 3 — *Halimeda* spp compound name structure and Binding energy *vs* Skin cancer protein

Sl. No	Compound name	Chemical structure	Binding energy kcal/mol 2VCJ Skin cancer
24	n-Tridecan-1-ol		–11.93
25	Octane		–11.4979
26	Pentafluoropropionic acid, hexadecyl ester		–11.2092
27	Phenol, 2,6-bis(1,1-dimethylethyl)-		–11.8958
28	Phthalic acid, allyl ethyl ester		–10.7089
29	Phthalic acid, butyl hexyl ester		–13.2064
30	Phthalic acid, hexyl propyl ester		–12.6773
31	Tetradecyltrifluoroacetate		–11.3855

Statistical analysis

The relationship between molecular weight, retention time, binding energy, and elapse time was checked by using Minitab 14.1.

Results

Molecular docking

Target drug discovery involves the identification of potential lead molecules against the skin cancer-causing receptor molecules. There are 31 bioactive secondary metabolites that were screened against skin cancer (4,5-Diarylisoxazole Hsp90 Chaperone) protein using Arguslab 4.0.1 (Table 3). In 31 compounds, 1,2-Benzenedicarboxylic acid, butyl octyl ester of hexane extract showed –14.27 followed by Phthalic acid, butyl hexyl ester (–13.2064 binding energy), 4-Heptafluorobutyryloxyhexadecane, Phthalic acid, hexyl

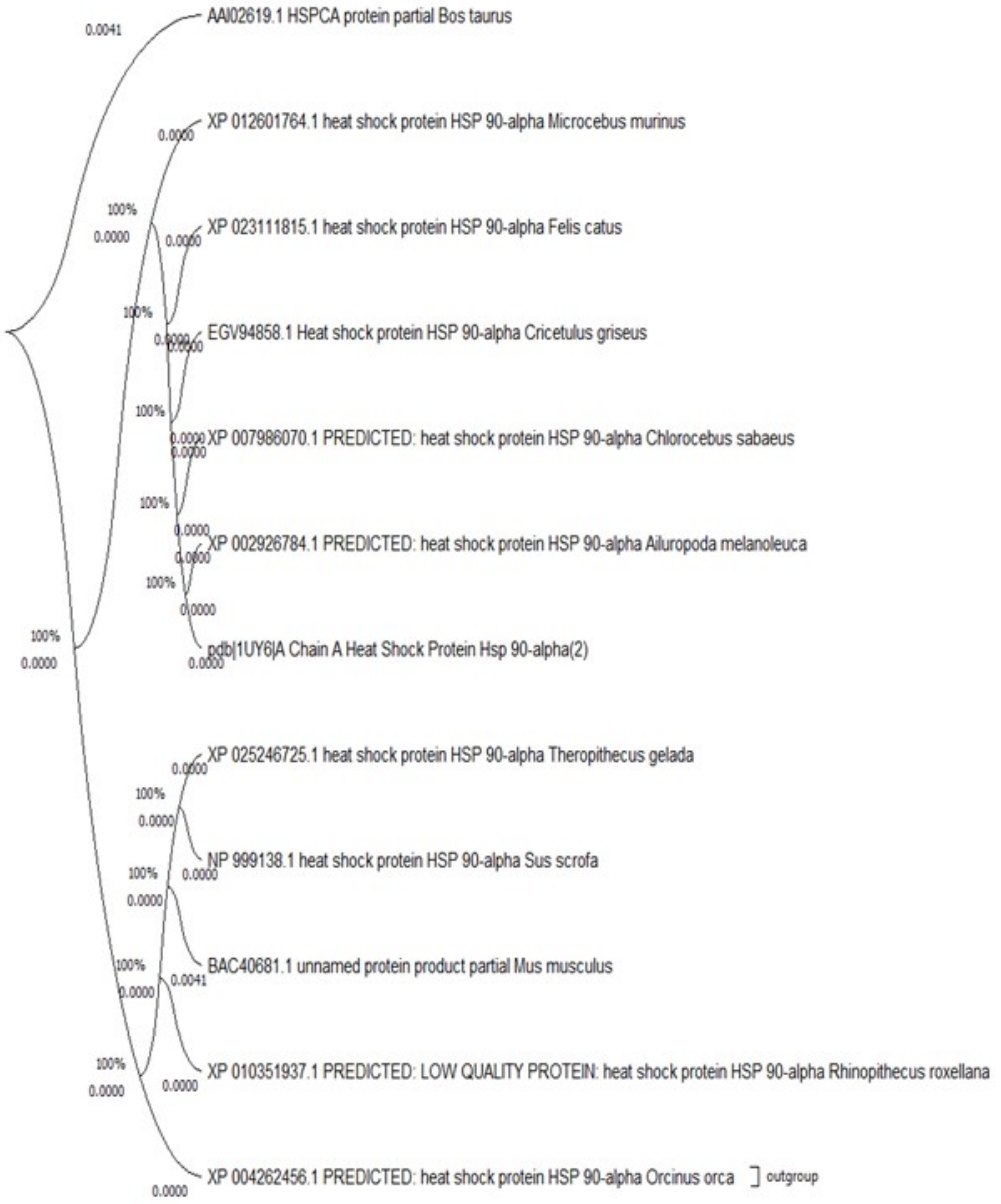


Fig. 1 — Evolutionary relationship of skin cancer protein with other organism (MEGA-X)

propyl ester, Dichloroacetic acid, 4-hexadecyl ester and 1-Cyclohexene, 1-ethynyl- (around –12 binding energy). Compounds of Dibutyl phthalate, N-Isopropyl-3-phenylpropanamide, n-Tridecan-1-ol, Phenol, 2,6-bis(1,1-dimethylethyl)-, 1-Hexadecanol, 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester, n-Heptadecanol-1, 5-Octadecene, (E)-, 3-Trifluoroacetoxytridecane, Octane, 4-Trifluoroace toxytridecane, Tetradecyltrifluoroacetate, Pentafluoro propionic acid, hexadecyl ester and 1-Dodecanol showed around (–11 binding energy), Carbonic acid, methyl tetradecyl ester, Benzoic acid, 2-(1-oxopropyl)-, methyl ester, Phthalic acid, allyl ethyl ester, Decyltrifluoroacetate, and Decyltrifluoroacetate having (–10 binding energy), –9 binding hold by Dimethyl phthalate, Mexiletine, Ethyl N-isopropyl-3-phenylpropanimidate having –8 binding energy and compounds of 2-Methylaminomethyl-1,3-dioxolane and Formamide, NN-dimethyl-binding energy around –4.

Phylogenetic tree

Percentage identification among protein sequence of *Homo sapiens* Hsp90 protein wild and home animals like Giant panda, Cattle, Chinese hamster, Cat, Gray mouse lemur, house mouse, killer whale, and

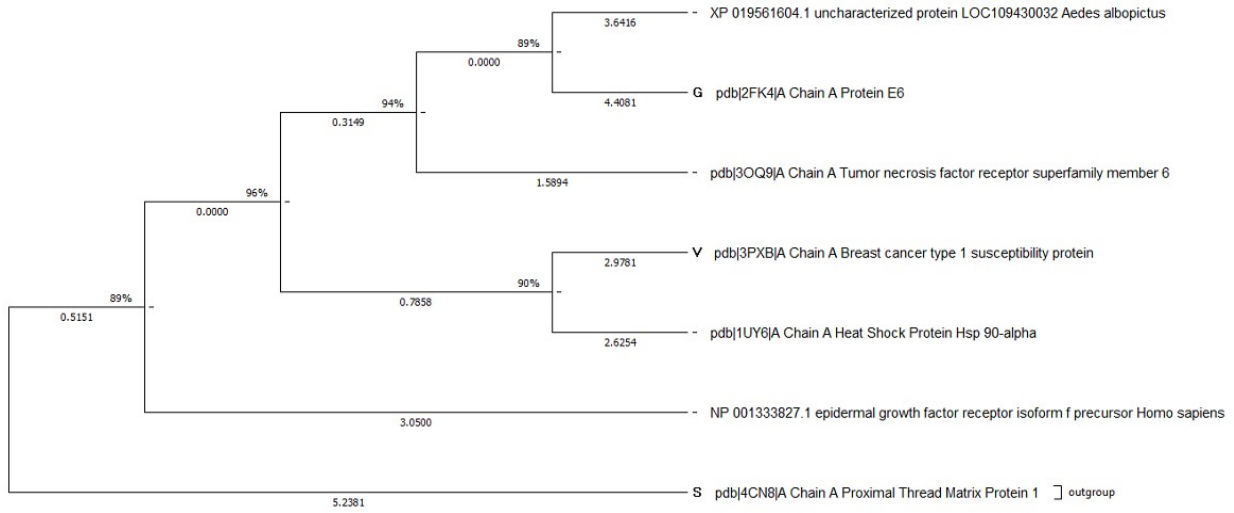


Fig. 2 — Skin cancer protein *vs* other cancer protein relationship

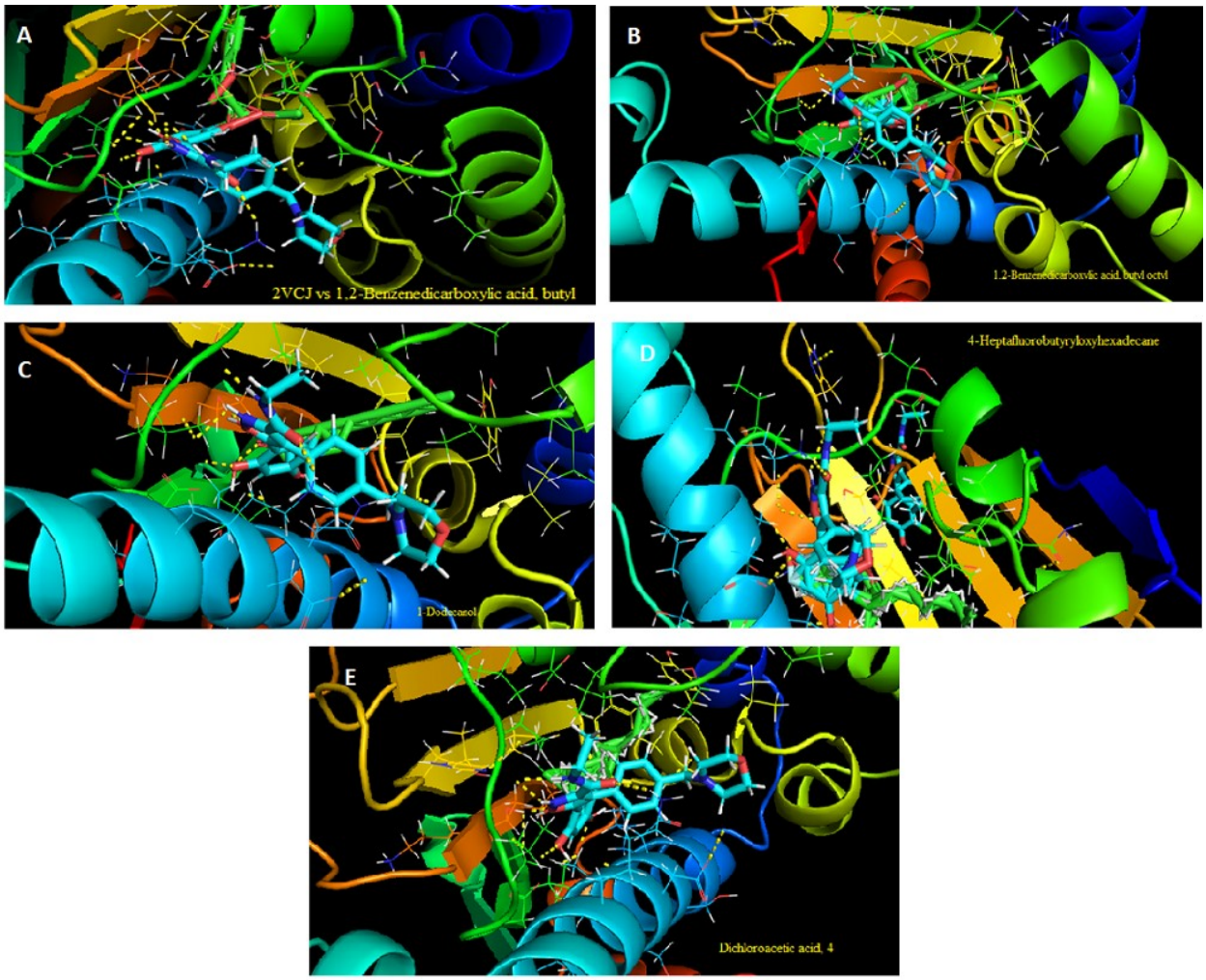


Fig. 3 — Three dimensional view of (A) 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester *vs* 2VCJ (PyMOL); (B) 1,2-Benzenedicarboxylic acid, butyl octyl ester *vs* 2VCJ (PyMOL); (C) 1-Dodecanol *vs* Hsp90 (PyMOL); (D) 4-Heptafluorobutyryloxyhexadecane *vs* 2 VCJ (PyMOL); and (E) Dichloroacetic acid, 4-hexadecyl ester *vs* Hsp90 (PyMOL)yo

Golden snub-nosed monkey, wild boar and Gelada species and other cancer protein of oral, cervical, breast and lung cancer. Skin cancer protein 100 percentages similar to other Giant pandas, Cattle, Chinese hamster, Cat, Gray mouse lemur, house mouse, killer whale, and Golden snub-nosed monkey, wild boar and Gelada species protein in cluster analysis. Cluster percentage similarity study of skin cancer protein 90% match with breast cancer protein, 96 % similarity with oral, cervical, and lung cancer (Figs. 1 & 2).

Statistics

The correlation analyses were carried out using Minitab 14.1. software. It was scrutinized that there were a strong positive relationship between molecular weight with retention time (0.72), elapse time with retention time (0.20) and molecular weight with elapse time (0.14). The study reveals that there is a negative relationship between binding energy with retention time (−0.13) molecular weight (−0.20) and elapsed time (−0.21). According to the correlation study strong relation of molecular weight with retention time and elapse time and retention time with molecular weight and elapse time.

Discussion

The present study shows that around 27 compounds are having high lower docking energy and 3 having low lower docking values in 31 compounds. Four compounds having potential against Hsp90 protein was studied by Sakkiah *et al*. (2011)[9]. Yang *et al*. (2011) studied pyrazole and isoxazole based Hsp90 inhibitors and they observed that 5-amides substituent is enhanced the activity against skin cancer protein[10]. Compounds of cocaine, lapatinib, cabazitaxel, apraclonidine, and Dyclonine was studied against four cancer protein in it Hsp90 is one of them and molecular study reveals that cabazitaxel having potential against skin cancer and brain cancer[2]. Fungal metabolites of four compounds were studied against skin cancer protein by Kandasamy *et al*. (2012) and 9,12-Octadecadienoic acid (−11) showed high bind energy and other 3 compounds are showing (−10, −9 and −8)[11]. Sangeetha *et al*. (2014) employed the cyanobacterium against cancer protein and found cryptophcin can be an alternative for treating cancer protein[12]. Verma *et al*. (2017) also observed that the cyanobacterium is a potential candidate against cancer through the *in silico*, *in vitro* and *in vivo* methods and brought into considering for treating cancer[3]. N-benzoyl-N-phenyl thiourea synthesis was employed against Hsp90 protein through *in silico* and reveals that good inhibitor for skin cancer[13].

Conclusion

This is the first molecular docking study using the macroalgae of *Halimeda* spp against skin cancer. The present study showed that based on molecular docking binding energy *Halimeda species* secondary metabolites could be a potent inhibitor against skin cancer-causing protein Hsp90. This current research also suggests that further exploration of the utility and molecular mechanisms of the compound will facilitate a better understanding of being in command of skin cancer and to develop drugs to cure cancer.

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Conflict of interest

All authors declare no conflict of interest.

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