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Apoptotic-inducing factor 1 (AIF1) plays a critical role in cembranoid mediated apoptosis to control cancer: Molecular docking and dynamics study

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ABSTRACT

Cancer is a group of diseases characterized by the rapid creation of abnormal cells. Cancer is one of the leading causes of death in humans, affecting millions of people per year. Cembranoids are carbocyclic diterpenes composed of the 14-membered ring, mainly present in the genera *Nicotiana* and *Pinus* in plants, soft coral and other organisms. Cembranoids are cytotoxic and inhibit phospholipid metabolism. The present study is focused to evaluate the apoptotic effect of cembranoids in comparison with selective terpenoids from each subclass such as monoterpenoid, diterpenoid, triterpenoid and tetraterpenoid against pro-apoptotic and anti-apoptotic proteins by *in silico* approach. Molecular docking of terpenoids with apoptotic proteins was assessed using SYBYL-X 1.3 software. The results showed that cembranoid family has good interaction with BAX compared to BCl2. This result suggests that cembranoid diterpene lactone can be targeted to Apoptotic-inducing factor 1 (AIF1) to control cancer.

1. Introduction

Plants are able to produce a variety of bioactive compounds, which are having an enormous interest in the scientific community because of their low toxicity, high medicinal value, and other anthropogenic applications (Shariq et al., 2019; Ranjani et al., 2019). The therapeutic applications of the aromatic plants are mainly due to the presence of terpenoids (Edris, 2007). Terpenoids are one of the largest and diverse natural products, which are mostly found in plants, corals and certain microorganisms including bacteria and yeast (Wang et al., 2018). Terpenoids have shown antibacterial, antioxidant, anti-inflammatory, chemopreventive, cytotoxicity, allelopathic, insecticidal, and antidiabetic activities (Edris, 2007; Dhifi et al., 2016). The various therapeutic potential of terpenoids dawns the consideration of scientists to test them against numerous diseases, which demonstrated promising outcomes to treat diseases including atherosclerosis, cancer, cardiovascular disease and thrombosis (Edris, 2007). For the last decade, nearly 130 studies have reported the anti-cancer properties of terpenoids from diverse plant species. These data suggested that terpenoids can induce apoptosis, and cell cycle arrest.

Based on the structure, terpenoids are classified into several subclasses, including monoterpenoids (C5H8)2, diterpenoids (C5H8)4, triterpenoids (C5H8)6, and tetraterpenoids (C5H8)8. Terpenoids are composed of isoprenoids, which are modified terpenes, in which methyl groups are moved or removed, or oxygen atoms are added along with general formula [C5H8]n isoprene. Terpenoids are known for their potential application in fragrance and flavor industries and also to have beneficial therapeutic properties and insecticidal properties. In addition, several studies have reported the cancer-preventive and antitumor property of few terpenoids against various cancers including lung cancer (Fazeela Mahaboob Begum et al., 2017).

Despite significant advances in medicine, cancer is the major cause of death throughout the world (Gali et al., 2015). The heterogeneity of the tumor poses a major challenge to the success rate of cancer treatment. The better understanding of cancer biology has brought up the diverse potential therapeutic targets which proposed several strategies for cancer treatment. Various studies have shown that naturally occurring phytochemicals are having anti-cancer activities. (Akther. et.al., 2019; Fazeela Mahaboob Begum et al., 2017). Therefore, safer and more effective therapeutic strategies are warranted for the betterment of the

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efficiency and treatment of cancer. Preventing cancer using phytochemical compounds and plant-derived terpenoids is a promising strategy to cure the burden of cancer and its recurrences (Roslin and Anupam, 2011). Therefore, in this study, we focused on targeting apoptosis using phytocompounds under the group Terpenoids. The objective of the study was to evaluate the apoptotic effect of cembranoid family of diterpene subclass by *in silico* approaches by targeting apoptotic proteins and also to determine the pathways that are critical for the induction of apoptosis by cembranoids.

2. Materials and methods

2.1. Protein preparation

The three-dimensional crystal structure of apoptotic proteins namely BAX (1F16), BCL2 (1G5M), AIF1 (1M6I), CASPASE 9 (1NW9) and CASPASE 3 (2C2O) were downloaded from PDB (https://www.rcsb.org/) and processed by using SYBYL-X-1.3 software (https://support.certara.com/software/molecular-modeling-and-simulation/sybyl-x/support.certara.com/software/molecular-modeling-and-simulation/sybyl-x/) and Swiss PDB viewer (http://spdbv.vital-it.ch/). The parameters used to prepare protein are Ligand extraction, as described previously (Priya et al., 2018). All other settings are selected with default. The protein was exported in Mol2 file format and the potential binding site was generated by Automatic mode. Finally, the prepared protein was exported in sfxc file format for further analysis.

2.2. Phytocompounds preparation

The 2D and 3D crystal structure of Terpenoids from each subclass namely D-Limonene (monoterpenoid- 440917), Andrographolide (Diterpenoid: 5318517), Alphacembratriene (Diterpenoid-11109567), cembranoid diterpene lactone (Diterpenoid-5477673), 4R-cembranoid (Diterpenoid-129905733), Cucurbitacin S (Triterpenoids-119287) and Lycopene (Tetraterpenoid-446925) were retrieved from PubChem (http://pubchem.ncbi.nlm.nih.gov/). D-Limonene, Andrographolide, Cucurbitacin S, and Lycopene for which the apoptotic effect has been reported are used as controls. All the compounds were prepared using SYBYL-X Sketch Mode and exported as a Mol2 file format (Priya et al., 2018; Akther et al., 2019).

2.3. Molecular Docking of phytocompounds

Molecular Docking was performed between cembranoids and pro-apoptotic and anti-apoptotic proteins as described earlier (Ubaid et al., 2018). In this study, docking was performed between phytocompounds from Terpenoids and five apoptotic proteins using SYBYL-X-1.3 software. The docking setup includes uploading prepared protein and ligand in sfxc and Mol2 file format respectively. The docking study was run with a default parameter. Results of this docking study contain the complete information of docking score which includes total score, C score, Polar, Crash, D score, PMF score, G score, Chem score, Global score and XYZ coordinates of ligand-protein binding for different docking poses. Among these, the C score and total score decides the ranking of docking poses. The total score describes the binding affinity between the active site of receptor and ligand while; C score ranks the affinity between the active site of receptor and the ligand. Thus based on the C score and total score ranking was made for all docking poses which are arranged from descending to ascending order.

2.4. Molecular dynamics simulation and annealing

Molecular dynamics simulation and simulated annealing study were performed in SYBYL-X-1.3 software for the lead compounds. The parameters defined in the SYBYL software are as follows; Gradient: 0.5 kcal/mol, Max iterations: 5000; force field: MMFF94s, and the charges: MMFF94. The dynamics simulation setup requires the docked ligand and protein to be imported as one file which was prepared using PyMOL (Seeliger and de Groot, 2010). By then the protein-ligand complex was imported at SYBYL for simulation studies which were carried out for 10ns with default parameters including tripos force field and Gasteiger-Marsili atomic charges at the room temperature. Followed by the simulated protein-ligand complex were allowed for annealing with default parameters viz. heating at 300 K for 500fs and annealing set at 50 K for 500fs (Priya et al., 2018).

2.5. Screening of Lipinski's rule of five

Lipinski's rule of five also known as Pfizer's rule of five plays a critical role in drug discovery. It predicts the drug-likeness of the molecule based on the following rules;

- Molecular mass: Less than 500 Da.
- Hydrogen bond donors: Less than 5
- Hydrogen bond acceptors: Less than 10
- High lipophilicity (expressed as LogP): Less than 5
- Molar refractivity: Between 40–130.

This helps to estimate the probability of success of our target molecule, which also helps to avoid the expensive clinical studies. The screening of phytocompounds was done at http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp. The target molecule was loaded in PDB file format at pH 7 and submitted for screening which provides the results for each five value.

3. Results

3.1. Docking studies

The 3D structures of all the seven phytocompounds were docked individually with each of the optimized apoptotic proteins to evaluate the efficacy of phytocompounds on apoptotic protein and to determine the invading pathway of phytocompounds (Fig. 1). The results were saved in the default directory which contains all scoring values. As said above, the Total score and C score were used to determine the lead compounds. In addition to this, the Polar score is considered, to eliminate the compound which portrays the contribution of hydrogen bonds. Thus, the lead compound is selected based on 3 criteria in which, a) C score should be greater than 4, b) polar score should not be zero and, c) Higher the Total score, higher the binding affinity. The leading docking posture for all phytocompounds with each apoptotic protein is shown in Fig. 2 and the docking score, interacting residues and bond length are shown in Table 1.

Among the three cembranoids, 4R-cembranoid and cembranoid diterpene lactone showed the best score and binding affinity towards BAX when compared to BCL2. Interestingly, both interacted with the same hydrophobic residue (SER 16) with the same total score (3.8026), C-Score (4) and bond length (2 Å) which may help to activate the apoptosis (Table 1). Limonene, Andrographolide, Cucurbitacins, and Lycopene were utilized as controls to estimate the efficacy of cembranoids. The results showed that controls displayed less affinity towards BAX when compared to 4R-cembranoid and cembranoid diterpene lactone. The least Total score was observed for Lycopene; however, there were no interacting residues observed (Table 1).

Further, apoptotic proteins such as AIF1, CASP3, and CASP9 were used to determine the interaction of cembranoids. The AIF1 interaction suggested that the cembranoids interact with the nucleus to induce apoptosis, while Caspase 3 and Caspase 9 interaction suggested that the cembranoids invade through mitochondria to interact with apoptotic proteins BAX and BCL2. The result suggests that cembranoid diterpene lactone has a high affinity towards AIF1 than CASP3 and CASP9, while 4R-cembranoid did not interact with either of two (Table 2).

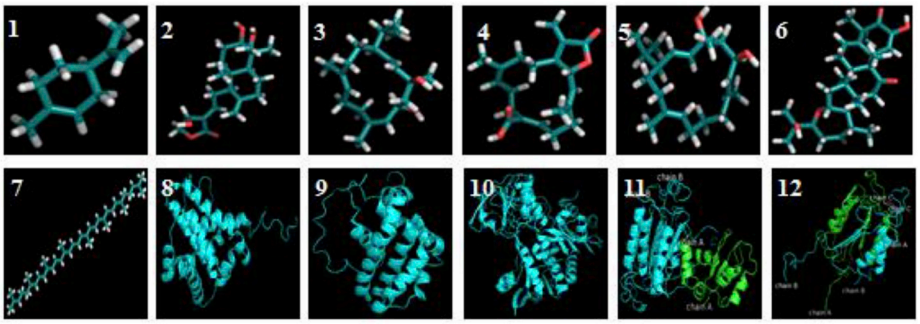

Fig. 1. Structure of cembranoids and apoptotic proteins. (1) Limonene (CID: 440917); (2) Andrographolide (CID: 5318517); (3) Alphacembratriene (CID: 11109567); (4) Cembranoidditerpene Lactone (CID: 5477673); (5) 4R cembranoid (CID: 129905733); (6) Cucurbitacins (CID 119287); (7) Lycopene (CID: 446925); (8) BAX (PDB ID: 1F16); (9) BCL2 (PDB ID: 1G5M); (10) AIF1 (PDB ID: 1M6I); (11) Caspase 9(PDB ID: 1NW9); (12) Caspase 3 (PDB ID: 2C2O).

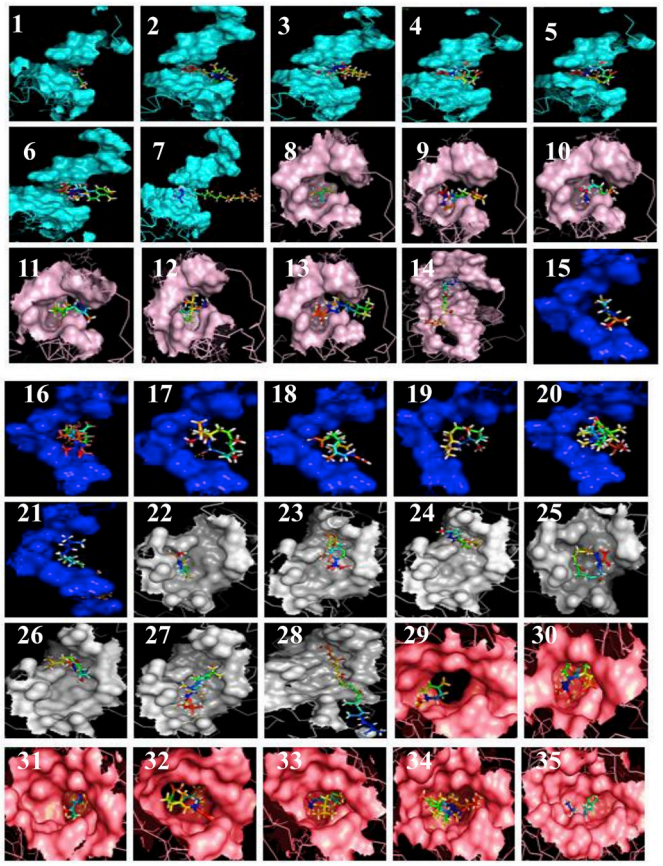

Fig. 2. Docked poses of terpenoids with apoptotic proteins (1) BAX + Limonene, (2) BAX + Andrographolide, (3) BAX + Alphacembratriene, (4) BAX + cembranoid diterpene lactone, (5) BAX+4Rcembranoid, (6) BAX + Cucurbitacins, (7) BAX + Lycopene, (8) BCL2+Limonene, (9) BCL2+Andrographolide, (10) BCL2+Alphacembratriene, (11) BCL2+cembranoid diterpene lactone, (12) BCL2+4Rcembranoid, (13) BCL2+Cucurbitacins, (14) BCL2+Lycopene, (15) CAS3+Limonene, (16) CAS3+Andrographolide, (17) CAS3+Alpha cembratriene, (18) CAS3+cembranoid diterpene lactone, (19) CAS3+4 Rcembranoid, (10) CAS3+Cucurbitacins, (21) CAS3+Lycopene, (22) CAS9+Limonene, (23) CAS9+Andrographolide, (24) CAS9+Alpha cembratriene, (25) CAS9+cembranoid diterpene lactone, (26) CAS9+4 Rcembranoid, (27) CAS9+Cucurbitacins, (28) CAS9+Lycopene, (29) AIF1+Limonene, (30) AIF1+Andrographolide, (31) AIF1+Alphacembratriene, (32) AIF1+cembranoid diterpene lactone, (33) AIF1+4Rcembranoid, (34) AIF1+Cucurbitacins, (35) AIF1+Lycopene.

3.2. *Pfizer's screening*

The cembranoid diterpene lactone was used to dissect the drug-likeness in Lipinski's channel, the results were Mass: 330.0 Da, Hydrogen bond donor: 1, Hydrogen bond acceptors: 4, LogP: 4.342 and Molar Refractivity: 93.445 and found that it follows the Lipinski's rules.

3.2.1. *Molecular dynamics simulation and annealing*

The dynamic simulation and annealing studies were carried out to predict the stability of the cembranoid diterpene lactone-AIF1 complex and cembranoid diterpene lactone-BAX complex. The simulation studies were carried out for 10 ns to check whether the binding was stable after small movement. During the simulation, 95% of the compounds did not

Table 1
Docking score of terpenoids interacting with BAX and BCL2 proteins.

Protein_Terpenoids	Total score	Polar score	C Score	Interacting residues	Bond length (å)
BAX_Limonene	1.9053	0	4	No Interacting Residues	-
BAX_Andrographolide	3.0977	1.639	4	ASP 154/ PRO 8	2.2/ 2.0
BAX_Alphacembranoid	2.3054	1.0964	1	GLN 153	2.3
BAX_4Rcembranoid	3.8026	1.1274	4	SER 16	2
BAX_cembranoid Diterpene Lactone	3.8026	1.1274	4	SER 16	2
BAX_Cucurbitacins	2.2062	2.0121	3	PRO 8/THR 14	2.3/ 2.1
BAX_Lycopene	-0.054	0	5	No interacting residues	-
BCL2_Limonene	3.8772	0	5	No interacting residues	-
BCL2_Andrographolide	5.1642	2.8285	4	GLU 42/ ASP 31	2.4/ 2.2
BCL2_Alphacembranoid	3.5303	0	2	No Interacting Residues	-
BCL2_4Rcembranoid	No Docking			-	-
BCL2_cembranoid Diterpene Lactone	3.6384	0.0017	2	No Interacting Residues	-
BCL2_Cucurbitacins	4.5428	0.9094	2	ARG12	2.8
BCL2_Lycopene	1.668	0	3	No Interacting Residues	-

Total Score = The total Surflex-Dock score expressed as -log(Kd) **Polar** = Contribution of the polar interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds. **C Score** = the consensus score computed from the Surflex-Dock Total Score and the additional scoring functions.

Table 2
Docking score of terpenoids interacting with AIF1, CASP3 &CASP9.

Protein_Terpenoids	Total Score	Polar Score	C Score	Interacting Residues	Bond Length (Å)
AIF1_Limonene	3.2754	0	5	No Interacting Residues	-
AIF1_Andrographolide	6.3349	2.2937	2	LYS177/ TRP483	2.7/2.3
AIF1_Alphacembranoid	4.8224	1.7796	5	ARG 285/ GLU 453	2.4/1.9
AIF1_4Rcembranoid	No Docking			-	-
AIF1_cembranoid Diterpene Lactone	3.7804	1.9395	4	GLU 453/ LYS 342	1.7/3.2
AIF1_Cucurbitacins	4.3993	3.2083	5	ARG451/ ARG450/ GLU453	2.3/ 2.7/2.8
AIF1_Lycopene	3.7608	0	3	No Interacting Residues	-
CAS9_Limonene	1.9943	0	4	No Interacting Residues	-
CAS9_Andrographolide	3.2511	3.1641	2	GLY269/ SER339/ ASN265/ GLU261	3/3/ 3.2/1.8
CAS9_Alphacembranoid	4.2606	0.1523	4	THR337	1.8
CAS9_4Rcembranoid	No Docking			-	-
CAS9_cembranoid Diterpene Lactone	2.3287	1.1285	3	TYR324	2.1
CAS9_Cucurbitacins	3.4113	2.6999	4	ARG258/ ARG258/ GLY306/ GLN245/ ASN265	3/3.3/ 3.4/ 3.1/1.1
CAS9_Lycopene	2.2503	0	3	No interacting residues	-
CAS3_Limonene	1.7165	0	4	No interacting residues	-
CAS3_Andrographolide	2.8511	2.9296	4	ASP179/ MET182/ LYS186	1.8/ 2.8/2
CAS3_Alphacembranoid	2.8987	1.7026	5	LYS186/ MET182/ ASP181	3.1/ 2.9/2.9
CAS3_4Rcembranoid	No Docking			-	-
CAS3_cembranoid Diterpene Lactone	3.3564	1.0224	4	LYS186/ CYS184	2.6/2.1
CAS3_Cucurbitacins	3.1782	2.3142	5	MET182/ ASP181/ ASP179	2.9/ 3.2/2.2
CAS3_Lycopene	-0.1385	0	4	No Interacting Residues	-

Total Score = The total Surflex-Dock score expressed as -log(Kd); Polar score = Contribution of the polar interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds. C Score = the consensus score computed from the Surflex-Dock Total Score and the additional scoring functions.

show any confirmational change and maintained stability. Then the annealing was performed in which the simulated compounds were allowed to cyclic variation of temperature at 310 K to check the stability at a higher temperature. The compounds exhibiting stability after annealing studies were considered as an ideal compound. The annealing studies in the BAX-CDL complex showed that the residues are not stable at high temperature and conformation changed drastically. However, annealing study in the AIF1-CDL complex showed that the residues were stable and showed a promising interaction even after exposure to 310 K. These results suggested that the stability was maintained in cembranoid diterpene lactone-AIF1 complex even at high temperature. In addition, Root Mean Square Deviation (RMSD) and Radius of gyration also maintained their values (Fig. 3). This suggests that cembranoid diterpene lactone can be used as a potential activator for apoptosis.

4. Discussion

Recent advancements in omics lead to the identification of the molecular mechanisms, pathways, and genes critical for cancer (Rohini et al., 2019) Ultimately, omics knowledge can be applied to identify the biomarkers and targets and to develop personalized medicine and targeted therapeutics. It may not be feasible to screen millions of biomolecules for antiproliferative activity. Hence, computational biology is required to do the virtual screening of bioactive molecules to test the efficacy, to identify the targets, receptors, and interactions. Molecular docking can be adapted to identify protein-ligand interactions. In addition to that *in silico,* ADMET analysis of the bioactive molecules will be helpful to discover the lead compounds (Ruiz-Torres et al., 2017; Sathish Kumar et al., 2018).

In spite of remarkable progress, a complete cure for cancer still remains challenging. A plethora of research is ongoing on cancer, which leads to the development of a new treatment strategy with improved quality of life, fewer side effects, and increased survival rate. In addition, studies on cancer reported new potential therapeutic targets, which extended the demand on using natural compounds from plants, fruits, vegetables, microorganisms as a drug entity which are safer compared to synthetic chemical drugs. Hence , the computational biology will be helpful to identify the lead compounds.

Apoptosis plays an essential role during development; however, deregulation of apoptosis may lead to cancers, due to excessive growth of cells. Reinstating apoptosis is the key challenge in controlling cancer. The Bcl-2 family of proteins is critical in the regulation of apoptosis and have been associated with several types of cancers. The cytochrome *c* release from the mitochondrion is a critical step of the apoptosis. Bcl-2 family control cytochrome *c* release and Bax, pro-apoptotic protein induces this release. Drug resistance in tumors is a pressing clinical problem and cells over-expressing Bcl-2 are resistant to various chemotherapeutic drugs (Miyashita et al., 1994). Radiation induces apoptosis through Bcl-2 family which suggests that targeting mitochondria will be a novel strategy for cancer radiotherapy (Cao et al., 2019). Here in this current study, the cembrenoids are docked with BAX and Bcl-2 proteins to identify the mechanism of induction of apoptosis by cembranoids. Our results suggest that Bax is interacting with cembranoids. However, the interaction was not stable during dynamics. Plant flavonoids, terpenoids and other phytochemicals induce apoptosis in the human breast cancer cell line by regulating CAS3/9 and Bcl2/Bax ratio (Chaudhry et al., 2019; Fazeela Mahaboob Begum et al., 2018).

Caspase-3 induces hallmarks of apoptosis including chromatin condensation and DNA and nuclear fragmentation (Hanahan and Weinberg, 2011). AIF is an oxidoreductase that is localized in mitochondria that triggers the caspase-independent cell death. During stress conditions, AIF relocates from mitochondria and targets nucleus and induces nuclear, DNA and chromatin fragmentation which eventually leads to apoptosis (Zong et al., 2004; Sathish Kumar et al., 2018). Wang et al. (2002) have demonstrated that berberine activates AIF to induce caspase-independent apoptosis in colon cancer cells. It has been demonstrated that *Melilotus indicus* extract induced apoptosis in hepatocellular carcinoma cells was AIF dependent (Abd El-Hafeez et al., 2018). In our current study, the cembranoid diterpene lactone and AIF1 interaction were stronger and the complex is stable during molecular dynamics studies. This suggests that cembranoid mediated apoptosis is AIF1 dependent and compounds can be designed to target AIF1 to prevent proliferation in cancer cells.

Terpenoids are one of the most phytocompounds and play a significant role which showed a vast biological activity against microbes, fungi, parasites virus, allergens, inflammation, cancer and so on. The richest sources of terpenoids are plants, corals, yeast, and bacteria.

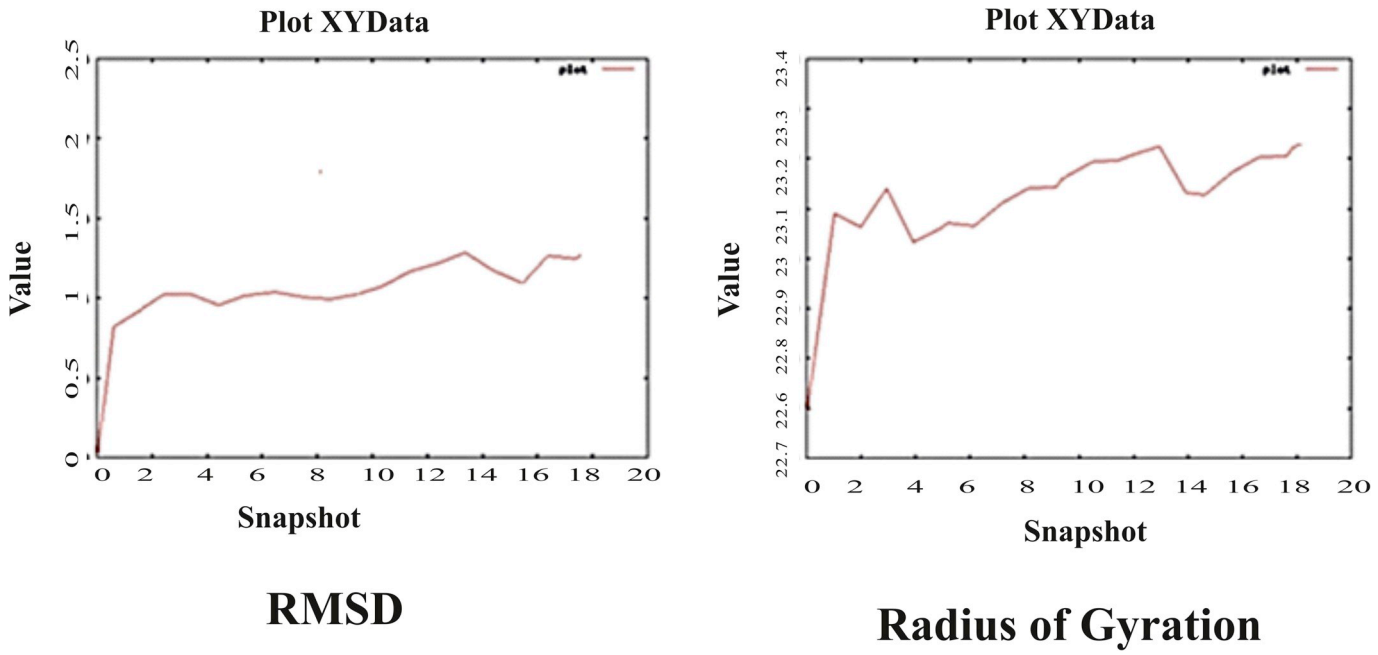

Fig. 3. Root Mean Square Deviation (RMSD) and Radius of gyration graph for cembranoid diterpene lactone.

Currently approved terpenoids based drugs are taxol and Artemisinin to cure cancer and malaria respectively. Previous studies have reported the anticancer property of terpenoids against certain cancers. For example, Limonene induce apoptosis and down-regulates cell cycle (Jia et al., 2013); Andrographolide up-regulates apoptosis (Liao et al., 2019); Cucurbitacins induces cell cycle arrest, autophagy and inhibit angiogenesis (Jafargholizadeh et al., 2018) and Lycopene was able to inhibit angiogenesis (Zu et al., 2014).

Cembranoid, can be obtained from plants like conifers, tobacco; insects; termites and mostly from marine invertebrates (Yan et al., 2019). Till now, the study of the anticancerous effect of cembranoid is limited. The present study focused on evaluating the apoptotic effect of cembranoids in comparison with Limonene, Andrographolide, Cucurbitacins, and Lycopene to control cancer. (Zubair et al., 2014). The *in silico* studies suggest that cembranoid diterpene lactone may be an effective compound to activate apoptosis. In addition, the study also suggest that AIF1 plays a critical role in cembranoid diterpene lactone mediated apoptosis. Cembranoids from soft coral *L. crassum*, showed anti-proliferative activity against different leukemia cell lines. Cembranoids with α-methylene-γ-lactone or α-methylene-δ-lactone moieties in their functional groups are showing potent anticancer activity (Peng et al., 2018). The *in silico* studies also showed the effective interaction of cembranoid diterpene lactone with AIF1 to activate apoptosis due to the presence of a lactone ring as demonstrated in previous studies (Peng et al., 2018). Zedoary essential oil-induced AIF1 to inhibit the proliferation of non-small cell lung carcinoma in H1299 cells (Sharifi-Rad et al., 2017). 7-Acetylsinumaximol B isolated from *Sinularia sandensis* showed antiproliferative activity in NCI–N87 human gastric cancer cell line and this activity was associated with the mitochondrial cytochrome *c* release, activation of pro-apoptotic proteins including caspase-3/-9, Bax and Bad and downregulation of anti-apoptotic proteins such as Bcl-2, Bcl-xL. Our current study and previous findings suggested that cembranoids are the potential anti-cancer compounds which can be explored by pharmaceutical industries to develop novel anticancer drugs (Tsai et al., 2018)

5. Conclusion

This study suggested that cembranoids especially cembranoid diterpene lactone effectively interacted with BAX when compared to Limonene, Andrographolide, Cucurbitacins, and Lycopene. However, AIF1 plays a critical role in cembranoid diterpene lactone mediated apoptosis and mediated through nucleus which can be potentially targeted to control cancer. Consequently, cembranoid diterpene lactone and combinations with other Cembrnoids can be tried under *in vitro* and *in vivo* conditions to further confirm their efficacy against cancer. In conclusion, cembranoid diterpene lactone might be a suitable candidate to investigate further and to develop molecular targeted cancer therapeutics by understanding the fundamental mechanisms involved in the regulation of cell death in cancer cells.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101343.

Declaration of competing interestsCOI

All authors declare that there no potential conflict of interest.

References

Abd El-Hafeez, A.A., Khalifa, H.O., Elgawish, R.A., Shouman, S.A., Abd El-Twab, M.H., Kawamoto, S., 2018. Melilotus indicus extract induces apoptosis in hepatocellular carcinoma cells via a mechanism involving mitochondria-mediated pathways. Cytotechnology 70 (2), 831–842. https://doi.org/10.1007/s10616-018-0195-7.

Akther, T., Vabeiryureilai, M., Senthil Kumar, N., MubarakAli, D., Hemalatha, S., 2019. Fungal-mediated synthesis of pharmaceutically active silver nanoparticles and anticancer property against A549 cells through apoptosis Environmental Science and Pollution Research. Environ. Sci. Pollut. Res. 26, 13649. https://doi.org/10.1007/s11356-019-04718-w.

Cao, X., Wen, P., Fu, Y., Gao, Y., Qi, X., Chen, B., Tao, Y., Wu, L., Xu, A., Lu, H., Zhao, G., 2019. Radiation induces apoptosis primarily through the intrinsic pathway in mammalian cells. Cell. Signal. https://doi.org/10.1016/j.cellsig.2019.06.002. S0898-6568(19)30,127-5.

Chaudhry, G.E., Jan, R., Mohamad, H., Tengku Muhammad, T.S., 2019. *Vitex rotundifolia* fractions induce apoptosis in human breast cancer cell line, MCF-7, *via* extrinsic and intrinsic pathways. Res. Pharm. Sci. 14 (3), 273–285. https://doi.org/10.4103/1735-5362.258496.

Edris, A.E., 2007. Pharmaceutical and therapeutic Potentials of essential oils and their individual volatile constituents: a review. Phytother Res. 21, 308–323. https://doi.org/10.1002/ptr.2072.

Fazeela Mahaboob Begum, S.M., Priya, S., Sundararajan, Raji, Hemalatha, S., 2017. Novel anticancerous compounds from Sargassum wightii: in silico and in vitro approaches to test the antiproliferative efficacy. J. Adv. Pharm. Educ. Res. 7 (3), 272–277.

Fazeela Mahaboob Begum, S.M., Chitra, Kalai, Joseph, Benin, Sundararajan, Raji, Hemalatha, S., 2018. *Gelidiella acerosa* inhibits lung cancer proliferation. BMC Complement Altern. Med. vol. 18, 104.

Gali, M., Hala, H., Raed, K., Mike, T., Rita, D.N., 2015. Cell death mechanisms of plant-derived anticancer drugs: beyond apoptosis. Apoptosis. https://doi.org/10.1007/s10495-015-1169-2.

Hanahan, D., Weinberg, R.A., 2011. Hallmarks of cancer: the next generation. Mar 4 Cell 144 (5), 646–674.

Jafargholizadeh, N., Zargar, S.J., Aftabi, Y., 2018. The cucurbitacins D, E, and I from *Ecballium elaterium* (L.) upregulate the *LC3* gene and induce cell-cycle arrest in human gastric cancer cell line AGS. Iran. J. Basic Med. Sci. 21 (3), 253–259. https://doi.org/10.22038/ijbms.2018.25175.6236.

Liao, H.C., Chou, Y.J., Lin, C.C., Liu, S.H., Oswita, A., Huang, Y.L., Wang, Y.L., Syu, J.L., Sun, C.M., Leu, C.M., Lin, C.H., Fu, S.L., 2019. Andrographolide and its potent derivative exhibit anticancer effects against imatinib-resistant chronic myeloid leukemia cells by downregulating the Bcr-Abl oncoprotein. Biochem. Pharmacol. 63, 308–320. https://doi.org/10.1016/j.bcp.2019.02.028.

Miyashita, T., Krajewski, S., Krajewska, M., Wang, H.G., Lin, H.K., Liebermann, D.A., Hoffman, B., Reed, J.C., 1994. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. Oncogene 9 (6), 1799–1805.

Peng, B.R., Lu, M.C., El-Shazly, M., Wu, S.L., Lai, K.H., Su, J.H., 2018. Aquaculture soft coral *Lobophytum crassum* as a producer of anti-proliferative cembranoids. Mar. Drugs 7 (1), 16. https://doi.org/10.3390/md16010015, 15.

Priya, S., Senthil Kumar, N., Hemalatha, S., 2018. Antiviral phytocompounds target envelop protein to control Zika virus. Comput. Biol. Chem. 7, 402–412. https://doi.org/10.1016/j.compbiolchem.2018.08.008. ISSN 1476-9271.

Ranjani, S., Shariq Ahmed, M., Ruckmani, K., Hemalatha, S., 2019. Green nano colloids control Multi drug resistant pathogenic bacteria. J. Clust. Sci. (in press).

Rohini, B.A., Tahira, M., Waseem, J., Khan, M. Kashif, Hemalatha, S., 2019. AgNPs from Nigella sativa control breast cancer: an in vitro study. J. Environ. Pathol. Toxicol. Oncol. 38 (2), 18519. https://doi.org/10.1615/JEnvironPatholToxicolOncol.2019027318.

Roslin, T., Anupam, B., 2011. Terpenoids as potential chemopreventive and therapeutic agents in liver cancer. World J. Hepatol. 3, 228–249. https://doi.org/10.4254/wjh.v3.i9.228.

Ruiz-Torres, V., Encinar, J.A., Herranz-López, M., Pérez-Sánchez, A., Galiano, V., Barrajón-Catalán, E., Micol, V., 2017. An updated review on marine anticancer compounds: the use of virtual screening for the discovery of small-molecule cancer drugs. Molecules 23 (7), 1037. https://doi.org/10.3390/molecules22071037, 22.

Sathish Kumar, S., Fazeela, S.M., Saroj Kumar, S., Kulandaisamy, S., Mahasampath Gowri, S., Hemalatha, S., Balasubramanian, K.K., 2018. Synthesis, characterization, *in vitro*, and *in silico* studies of 4-(2′-hydroxybenzoyl) and 4-(2′-hydroxynaphthoyl)-thiabenzene-1-methyl-1-oxides. Synth. Commun. 48 (5), 553–560. https://doi.org/10.1080/00397911.2017.1411950.

Seeliger, D., de Groot, B.L., 2010. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J. Comput. Aided Mol. Des. 24 (5), 417–422, 1.

Sharifi-Rad, J., Sureda, A., Tenore, G.C., Daglia, M., Sharifi-Rad, M., Valussi, M., Tundis, R., Sharifi-Rad, M., Loizzo, M.R., Ademiluyi, A.O., Sharifi-Rad, R., Ayatollahi, S.A., Iriti, M., 2017. Biological activities of essential oils: from plant chemoecology to traditional healing systems. Molecules 22 (1), 70. https://doi.org/10.3390/molecules22010070, 1.

Shariq, A.M., Ranjani, S., Tahira, A., Mohammad, K., Jasim, K., Mohammad, W., Hemalatha, S., 2019. Biogenic AgNps synthesized via endophytic bacteria and its biological applications. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-019-05869-6.

Tsai, T.C., Lai, K.H., Su, J.H., Wu, Y.J., Sheu, J.H., 2018. 7-Acetylsinumaximol B induces apoptosis and autophagy in human gastric carcinoma cells through mitochondria dysfunction and activation of the PERK/eIF2α/ATF4/CHOP signaling pathway. Mar. Drugs 16 (4), 104. https://doi.org/10.3390/md16040104, 26.

Ubaid, R., Priya, S., Parveen, A., Sah, S.K., Hemalatha, S., 2018. Efficacy of Andrographis paniculata against extended spectrum β-lactamase (ESBL) producing *E. coli*. BMC Complement Altern. Med. 18, 244.

Wang, L., Liu, L., Shi, Y., 2002. Berberine induces caspase-independent cell death in colon tumor cells through activation of apoptosis-inducing factor. PLoS One 7 (5), e36418. https://doi.org/10.1371/journal.pone.0036418.

Wang, C., Liwei, M., Park, J.B., Jeong, S.H., Wei, G., Wang, Y., Kim, S.W., 2018. Microbial platform for terpenoid production: *Escherichia coli* and Yeast. Front Microbiol. 9 https://doi.org/10.3389/fmicb.2018.02460.

Yan, N., Du, Y., Liu, X., Zhang, H., Liu, Y., Zhang, Z.A., 2019. Review on Bioactivities of Tobacco Cembranoid Diterpenes Biomolecules, vol 9, p. 30. https://doi.org/10.3390/biom9010030 (1).

Zong, W.X., Ditsworth, D., Bauer, D.E., Wang, Z.Q., Thompson, C.B., 2004. Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev. 18 (11), 1272–1282.

Zu, K., Mucci, L., Rosner, B.A., Clinton, S.K., Loda, M., Stampfer, M.J., Giovannucci, E., 2014. Dietary lycopene, angiogenesis, and prostate cancer: a prospective study in the prostate-specific antigen era. J. Natl. Cancer Inst. 106 (2), djt430 https://doi.org/10.1093/jnci/djt430.

Zubair, M., Anam, S., Al-Footy, K., Abdel, L., Ahmed, A.W., 2014. Cembranoid Diterpenes as Antitumor: Molecular Docking Study to Several Protein Receptor Targets, vol. 2. https://doi.org/10.2991/iccst-15.2015.23.