New terpenic and phenolic compounds from *Suaeda monoica* reverse oxidative and apoptotic damages in human endothelial cells

Mohammad K. Parvez,*, Mohammed S. Al-Dosari, Md. Tabish Rehman, Mohammed F. Alajmi, Ali S. Alqahtani, Mansour S. AlSaid

**A R T I C L E   I N F O**

Article history:
Received 1 March 2021
Accepted 1 August 2021
Available online 9 August 2021

Keywords:
Suaeda monoica
Terpenes
Methylglyoxal
Dichlorofluorescin
Endothelial cells
Apoptosis

**A B S T R A C T**

Elevation in hyperglycemia-associated methylglyoxal level can trigger vascular endothelial cells oxidative stress and apoptosis. The present work assesses the cell proliferative, anti-oxidative and anti-apoptotic potential of *Suaeda monoica* derived four new terpenes: a norsesquiterpenol (normonisesquiterpenol), a monocyclic triterpenoid (suaedanortriterpenedione), an aromatic monoterpenic ester and a labdane-type norditerpenic xyloside as well as two new phenols: an alkylated 2-naphthol and a 2-methoxy naphthalene in cultured human umbilical vein endothelial cells (HUVEC). Of these, suaedanortriterpenedione (53.7%), normonisesquiterpenol (51.4%) and norditerpenic xyloside (48%) showed the most promising cell proliferative activities compared to others. Moreover, normonisesquiterpenol, norditerpenic xyloside and suaedanortriterpenedione efficiently reversed the oxidative and apoptotic cell damage via downregulation of caspase-3/7 by 44.3%, 42.2% and 39.4%, respectively against dichlorofluorescin, whereas by 46.2%, 43.5% and 42.5%, respectively against methylglyoxal. Aminoguanidine, the reference drug inhibited caspase-3/7 activity by 56.2% and 54.7% through attenuation of dichlorofluorescin and methylglyoxal, respectively. Further in silico molecular docking analysis revealed formation of stable complexes between the tested compounds and caspase-3/7. Conclusively, we for the first time demonstrate the growth stimulatory, anti-oxidative and anti-apoptotic salutations of *S. monoica* derived novel compounds in human endothelial cells. This warrants their further assessment as vascular cell protective and rejuvenating therapeutics, especially in hyperglycemic conditions.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

In recent times, several plant extracts and their bioactive constituents have shown promising growth stimulatory or cytoprotective potential (Kong et al., 2004; Kim et al., 2013; Arbab et al., 2016; Parvez et al., 2018) warranting their further exploitation as anti-oxidative, anti-inflammatory or tissue-rejuvenating agents. The mangrove herb *Suaeda monoica* J. F. Gmel (Chenopodiaceae) is traditionally used to treat sore throat, rheumatism, asthma, snake-bites, skin disease, ulcer, hepatotoxicity, and microbial infections (Kathiresan and Ramanathan, 1997; Muthazhagan et al., 2014; Lakshmi and Narsimha Rao, 2013). In addition, its flavonoids, saponins, alkaloids, polyphenols, resins, tannins, coumarins, cardiac glycosides, and fatty acids are characterized as therapeutic phytocomstituents (Kokpal et al., 1990; Lakshmanan et al., 2013; Muthazhagan et al., 2014). Recently, we have reported isolation and preliminary bioactivity of *S. monoica* derived four new terpenes: a norsesquiterpenol (normonisesquiterpenol), a monocyclic triterpenoid (suaedanortriterpenedione), an aromatic monoterpenic ester and a labdane-type norditerpenic xyloside as well as two new phenols: an alkylated 2-naphthol and a 2-methoxy naphthalene derivative (AlSaid et al., 2017; Siddiqui et al., 2020). Notably, a novel pentacyclic triterpenedione from *Picea jezoensis* with unknown bioactivity has been previously reported (Tanaka et al., 1997).

The vascular endothelial cells, the inner layer of blood vessel is crucial in modulating vascular function and homeostasis (Choy...
et al., 2001). In conditions with hyperglycemia, retardation of endothelial cells proliferation or apoptosis often leads to diabetic microvascular lesions and cardiovascular complications. Methylglyoxal (MGO) is a highly reactive aldehyde that is produced as a byproduct of several metabolic pathways, including lipid peroxidation (Thornalley and Rabbani, 2014). Also, it is a major precursor of advanced glycation end products implicated in the development of type-2 diabetic complications (Vander Jagt and Hunsaker, 2003) as well oxidative stress and apoptosis in endothelial cells (Bourajjaj et al., 2003; Thornalley and Rabbani, 2014). In addition, high level of MGO is demonstrated to cause in vitro hyperglycemia and oxidative damages in human umbilical vein endothelial cells (Bourajjaj et al., 2003). In endothelial cells, MGO-induced oxidative stress and apoptosis is suggested mainly through the generation of reactive-oxygen species (Phalitakul et al., 2013; Figarola et al., 2014; Kim et al., 2004).

Dichlorofluorescin (DCF), is generally used to measure cellular oxidative stress as a result of H2O2-dependent reactions, including cytochrome C and Fe2+ (Royall and Ischiropoulos, 1993; Carter et al., 1994; LeBel et al., 1992). In line with this, we have recently reported DCFH and MGO induced oxidative stress and apoptosis in a variety of cells, including HUVEC (Arbab et al., 2016; Shahat et al., 2018; Parvez et al., 2019; Alqahtani et al., 2019; Parvez et al., 2020). In this report, we have investigated the cytoprotective potential of S. monoica derived six novel compounds against MGO and DCFH induced oxidative and apoptotic damages in cultured HUVEC cells.

2. Materials and methods

2.1. Extraction, isolation and structure elucidation of the compounds

The extraction and isolation of six novel compounds: a norsesquiterpenol (normonisesquiterpenol), a monocyclic triterpenoid (suaedanortriterpenedione), an aromatic monoterpenic ester, an unknown labdane-type nortriterpenic xyloside, an alkylated β-naphthol and a β-methoxy naphthalene derivative from the aerial parts of S. monoica (voucher specimen no. 15135), including their structure elucidations (Fig. 1) have been previously reported by us (AlSaid et al., 2017; Siddiqui et al., 2020).

2.2. Cell culture

Human umbilical vein epithelial cells (HUVEC 16549; ATCC, USA) were maintained in DMEM-Glutmax medium (Gibco, USA), supplemented with 10% fetal calf serum (Gibco, USA) and 1x penicillin–streptomycin mix (Invitrogen, USA) at 37 °C with 5% CO2 supply. HUVEC cells (0.5x10⁵/100 μl/well) were seeded in a 96-well flat-bottom plate (Becton-Dickinson Labware, USA) and grown overnight for all experiments.

2.3. Natural compounds and drugs preparations

The S. monoica derived norsesquiterpenol (NSQ), suaedanortriterpenedione (SND), aromatic monoterpenic ester (AES), nortriterpenic xyloside (NDX), alkylated β-naphthol (ABN) and β-methoxy naphthalene (BMN) were first dissolved in 50 μl dimethyl sulfoxide (DMSO, Sigma-Alderich, Germany) and reconstituted in culture media (1 mg/ml, each). Based on our previously assessed non-toxic concentrations on liver cancer cells (AlSaid et al., 2017; Siddiqui et al., 2020), only three working doses (50, 25 and 12.5 μg/ml; DMSO < 0.5% final) were further prepared in culture media. Likewise, DCF and MGO (Sigma-Alderich, Germany) were prepared to be used as inducers of oxidative and apoptotic cell damage, whereas aminoguanidine (AG; Sigma-Alderich, Germany) served as anti-apoptotic agent (positive control).

2.4. Microscopy

Visual monitoring of the treated cells for any morphological changes, cytotoxicity or proliferation cells was made under an inverted microscope (Optica, 40x and 100x).

Fig. 1. Chemical structure of Suaeda monoica derived new terpenic and phenolic compounds: normonisesquaterpenol, suaedanortriterpenedione, aromatic monoterpenic ester, nortriterpenic xyloside, alkylated β-naphthol and β-methoxy naphthalene.
2.5. Cell proliferation assay of S. Monoica derived compounds

The S. monoica derived compounds: NSQ, SND, AES, NDX, ABN, BMN were tested for their cell proliferative or growth stimulatory activities in cultured HUVEC cells. Cells were treated with the different doses of the compounds, including untreated control (0.5% DMSO) for 3 days. MTT assay (TACS MTT Cell Proliferation Assay Kit, Tervigen, USA) was performed as per the kit’s manual. Briefly, the MTT reagent (10 µl/well) was added and incubated in dark for about 4 h at room temperature (RT) until purple color appeared. Further the detergent solution (100 µl/well) was added and the cells were incubated for another 1.5 h at 37 °C. The OD was measured (Microplate reader ELx800; BioTek, USA) and data was analyzed by non-linear regression (Excel software 2010; Microsoft, USA) to determine the cell proliferation in relation to the untreated control [%Cell proliferation = (ODsample – ODblank)/ODcontrol x100]. All samples were tested in triplicate and repeated.

2.6. Assessment of anti-oxidative and cytotoxicity activity of S. Monoica derived compounds

HUVEC cell grown in a 96-well plate were treated with DCF (K_{50}: 32.5 µg/ml) as described elsewhere (Parvez et al., 2020) plus a dose of NSQ, SND, AES, NDX, ABN or BMN. DCFH and DMSO served as negative and untreated control, respectively. The culture was incubated for 3 days at 37 °C and MTT assay was performed to determine the cell survival (%) as above. All samples were tested in triplicate and repeated.

2.7. Anti-apoptotic activity assay of S. Monoica derived compounds

HUVEC cells grown in a 96-well plate were treated with MGO (0.5 mM) as described elsewhere (Alqahtani et al., 2019) plus a dose of NSQ, SND, AES, NDX, ABN or BMN. MGO and AG (0.05 mM; Alqahtani et al., 2019) served as negative and positive control, respectively. The treated cells were incubated for 3 days and MTT assay was performed to determine the cell survival (%) as above. All samples were tested in triplicate and repeated.

2.8. Assessment of caspase-3/7 modulating activity of S. Monoica derived compounds

Based on the promising anti-oxidative and anti-apoptotic activities, an optimal dose (25 µg/ml) of the compounds was tested for cellular caspase-3/7 activation in HUVEC cells in 96-well plates (Set-I: DCF treated and Set-II: MGO treated). Day 3 post-incubation, cellular caspase expressions were measured (Apo-ONE-cas3/7 assay kit; Promega, USA) as per the kit’s manual.. Briefly, 100 µl of caspase-3/7 reagent was added to each well, mixed by gentle rocking and incubated in dark for ~ 6 h at RT. Caspase reagent plus culture medium served as blank while reagent plus DMSO treated cells acted as negative control. The OD was measured (Microplate reader ELx800; BioTek, USA) and data was analyzed. All samples were tested in triplicate and repeated.

2.9. Virtual preparation of proteins and ligands

The interactions S. monoica derived compounds with caspase-3 and caspase-7 were elucidated by performing molecular docking using AutoDock 4.2 as described elsewhere (Al-Shabib et al., 2020). Briefly, the three-dimensional coordinates of caspase-3 (PDB Id: 2XYG) and caspase-7 (PDB Id: 3IBC) were retrieved from PDB RCSB database (www.rcsb.org). The proteins were pre-processed by removing water molecules or bound hetero atoms, if any, including addition of hydrogens and assigning Kollman charges. The structure of protein molecules was finally energy-minimized using Merck Molecular Force Field (MMFF). The 2D structures of all compounds were drawn in ChemDraw. All the compounds, including control ligands such as TQ8 (bound to Caspase-3 active site in the crystal structure) and Acetyl-YVAD-CHO (bound to Caspase-7 in the crystal structure) were prepared for docking by assigning bond orders and angles. For all structures, Gasteiger partial charges were defined and the energies of were minimized using UFF (Universal Force Field).

2.10. Molecular docking

Grids around the active site of the targets were defined by selecting the amino acid residues that interacted with the bound ligand. For caspase-3 and Caspase-7, grid boxes 33.3 x 28.8 x 28, 3 Å and 25.1 x 34.5 x 29.8 Å, centered at 36.4 x 37.4 x 31.5 Å and 49.8 x -26.4 x -2.3 Å with 0.375 Å, respectively were used for molecular docking in AutoDock 4.2 (Morris et al., 2009). The van der Waals’ and electrostatic parameters were calculated with the help of distance-dependent dielectric function. Docking was performed using Lamarck Genetic Algorithm (LGA) and Solis-Wets local search methods. A total of 10 docking runs were performed with 2.5 x 10^6 energy calculations for each. The population size (150), translational step (0.2), quaternions (5.0) and torsions (5.0) were set. The docking affinity (K_d) of ligands for proteins was estimated from docking energy (AG) using the equation: \( AG = -RT\ln K_d \) (Boltzmann gas constant, \( R = 1.987 \text{cal/mol/K} \) and temperature, \( T = 298 \text{K} \). The molecular docking procedure generated several low energy binding poses for each ligand, of which the complex with the lowest energy was selected for the analysis.

2.11. Statistical analysis

All data in triplicate were presented as mean ± SD and analyzed using one-way analysis of variance. Differences between two groups were compared using Student’s t-test (SPSS software; Version 25; IBM, USA), and \( p < 0.05 \) was considered significant.

3. Results

3.1. Endothelial cell proliferative activities of S. Monoica derived compounds

All tested compounds (NSQ, SND, AES, NDX, ABN and BMN) were non-toxic to HUVEC cells even at the highest dose (50 µg/ml) in line with microscopic observations (data not shown). Our MTT assay showed dose-dependent cell proliferative activities of all compounds. Of these, SND (53.7%), NSQ (51.4%) and NDX (48%) exhibited relatively higher effects than AES (46.2%), ABN (44.8%) and BMN (42.8%) at 25 µg/ml in relation to untreated control (Fig. 2). There were no significant changes in growth enhancement at 50 µg/ml dose.

3.2. Attenuation of oxidative cell damage by S. Monoica derived compounds

Based on the promising cell proliferative activities, all six compounds (25 and 50 µg/ml, each) were evaluated for their cytoprotective potential against DCF-induced oxidative damage in HUVEC cells. As shown by MTT assay, cell viability was restored (in the order) by SND (80.5%), NSQ (80%), NDX (77%), ABN (75.2%), AES (72.5%) and BMN (71.35%) at 25 µg/ml dose. Notably, SND and NSQ showed the best activities as compared to the reference drug AG (88.5%) through attenuation of DCF (Fig. 3). Treatment with
50 mg/ml dose however, did not show significant enhancement in their activities.

3.3. Reversal of apoptotic cell death by S. Monoica derived compounds

Further, when tested against MGO-induced apoptosis, HUVEC cell death were reversed and rejuvenated (in the order) by SND (82.3%), NSQ (78.3%), NDX (79.8%), AES (74%) ABN (72.6%) and BMN (69.8%) at 25 \( \mu \)g/ml dose. Notably, SND, NSQ and NDX showed the best activities as compared to the reference drug AG activity (89.4%) (Fig. 4). The 50 mg/ml dose did not show significant additive effect.

3.4. S. monoica derived compounds effectively down regulated cellular caspase-3/7

Further insight into the anti-apoptotic mechanism of the three most active terpenic compounds (25 \( \mu \)g/ml) showed modulation of caspase-3/7 activities in both DCF and MGO treated HUVEC cells. DCF and MGO induced cellular caspases by 76.3% and 81.3%, respectively (Fig. 5). NSQ, NDX and SND efficiently down regulated caspase-3/7 expressions by 44.3%, 42.2% and 39.4%, respectively against DCF, whereas by 46.2%, 43.5% and 42.5%, respectively against MGO (Fig. 5). The reference drug AG downregulated caspase-3/7 by 56.2% and 54.7% through attenuation of DCF and MGO, respectively.

3.5. Interaction between caspase-3 and S. Monoica derived compounds

Our molecular docking analysis revealed that all the compounds were able to bind to the active site of caspase-3 (Fig. 6, IA), and their binding energy and corresponding binding affinity towards caspase-3 were estimated (Table 1). The interaction between caspase-3 and TQ8 (ligand control) suggested involvement of three hydrogen bonds with Arg207, and two hydrophobic interactions with Trp206. Some other residues such as Ser65, Tyr204, Ser205, Asn208, Ser209, and Phe250 formed van der Waals’ interactions (Fig. 6, IB; Table 2). The binding energy and affinity of TQ8 and caspase-3 complex were estimated to be \(-5.8\) kcal mol\(^{-1}\) and \(1.79 \times 10^4\) M\(^{-1}\), respectively (Table 1).

Alkylated \( \beta \)-naphthol formed a stable complex with caspase-3 mainly through hydrogen bonding with Arg207 and Ser251, including other hydrophobic interactions (Fig. 6, IC; Table 2). The complex was further stabilized by van der Waals’ interactions with Tyr204, Ser205, Trp206, Phe252, and Asp253. The binding energy and affinity of the complex were estimated to be \(-6.1\) kcal mol\(^{-1}\) and \(2.98 \times 10^4\) M\(^{-1}\), respectively (Table 1).

Aromatic monoterpenic ester and caspase-3 complex was stabilized by an electrostatic interaction (Pi-Cation) with His121 and three hydrogen bonds with His121, Cys163 and Glu123. Some other residues such as Thr62, Gly122, Gly165, and Thr166 formed van der Waals’ interactions (Fig. 6ID; Table 2). The docking energy and affinity of the complex were estimated to be \(-5.5\) kcal mol\(^{-1}\) and \(1.08 \times 10^4\) M\(^{-1}\), respectively (Table 1).

\( \beta \)-methoxy naphthalene formed a stable complex with caspase-3 mainly through hydrogen bonding with Tyr204 and Arg207 as well as hydrophobic interactions (Fig. 6, IIA; Table 2). The complex was further stabilized by van der Waals’ interactions with Asp253.
The estimated binding energy and affinity of the complex were -5.8 kcal mol\(^{-1}\) and 1.79 \(\times\) 10\(^4\) M\(^{-1}\) respectively (Table 1).

Norditerpenic xyloside and caspase-3 complex was formed through three hydrogen bonds involving Arg207, Cys163 and Tyr204 as well as six hydrophobic interactions with Met61, His121, Phe128 and Cys163. Some other residues also formed van der Waals’ interactions (Fig. 6, IIB; Table 2). The docking energy and affinity of the complex were estimated to be -6.6 kcal mol\(^{-1}\) and 6.93 \(\times\) 10\(^4\) M\(^{-1}\), respectively (Table 1).

Norsesquiterpenol formed a stable complex with caspase-3 mainly through hydrophobic interactions with Phe256 (Fig. 6, IIC; Table 2). The complex was further stabilized by van der Waals’ interactions involving Tyr204, Trp206, Arg207, Asn208, Ser209, Lys210, Phe250, Ser251 and Asp253. The calculated binding energy and affinity of the complex were -7.4 kcal mol\(^{-1}\) and 2.68 \(\times\) 10\(^5\) M\(^{-1}\), respectively (Table 1).

Suaedanortriterpenedione and caspase-3 formed complex via two hydrogen bonds involving Ser209 and Phe250 as well as through hydrophobic interactions with Trp206, Arg207, Phe252 and Phe256. Some residues like Tyr204, Asn208 and Ser251 also showed van der Waals’ interactions (Fig. 6, IID; Table 2). The docking energy and affinity of the complex were estimated to be -5.5 kcal mol\(^{-1}\) and 1.08 \(\times\) 10\(^4\) M\(^{-1}\), respectively (Table 1).

### 3.6. Interaction between caspase-7 and S. Monoica derived compounds

All tested compounds showed good interaction with caspase-7 active site (Fig. 7, IA), and their binding energy and corresponding binding affinity towards caspase-7 were calculated (Table 1). The interaction between Acetyl-YVAD-CHO (ligand control) and caspase-7 suggested the involvement of hydrogen bonds with Arg233, Glu276 and His272. Some other residues such as Ser231,
The binding energy and affinity of the complex were estimated to be $-9.6 \text{ kcal mol}^{-1}$ and $1.10 \times 10^7 \text{ M}^{-1}$, respectively (Table 1).

Alkylated $\beta$-naphthol formed a stable complex with caspase-7 mainly through hydrogen bonding which involved Arg87, His144 and Arg233, wherein His114 also formed a carbon-hydrogen bond with Tyr230 and Trp232. Residues such as Ser231, Arg233, Ser234, Arg237, Trp240, Phe273, Glu274, Ser275, and Phe282 formed van der Waals' interactions (Fig. 7, IC; Table 3). The calculated docking energy and affinity of the complex were $-7.6 \text{ kcal mol}^{-1}$ and $3.75 \times 10^5 \text{ M}^{-1}$, respectively (Table 1).

Aromatic monoterpenic ester and caspase-7 complex was stabilized by a hydrogen bond involving His144 and hydrophobic interactions with Cys186, Tyr230, and Trp232 (Fig. 7, IC; Table 3). The complex was further stabilized by van der Waals’ interactions involving Met84, Ser231, Arg233, Ser277 and Phe282. The binding energy and affinity of the complex were estimated to be $-6.3 \text{ kcal mol}^{-1}$ and $4.18 \times 10^4 \text{ M}^{-1}$, respectively (Table 1).

Norditerpenic xyloside and caspase-7 complex was formed via two hydrogen bonds with Trp240 and Gln276 as well as through hydrophobic interactions involving Cys186, Tyr230 and Trp232 (Fig. 7, IID; Table 3). Also, it showed van der Waals’ interactions with Ser231, Arg233, Arg237, Ser275 and Ser277. The calculated docking energy and affinity of the complex were $-7.6 \text{ kcal mol}^{-1}$ and $3.75 \times 10^5 \text{ M}^{-1}$, respectively (Table 1).

Norsesquiterpenol formed a stable complex with caspase-7 mainly through hydrophobic interactions, involving Trp232, Pro235, Trp240 and others as well as one hydrogen bond with Glu276, (Fig. 7, IIC; Table 3). The complex was further stabilized by van der Waals’ interactions with Val86, Arg233, Ser234, Arg237, Glu274, Ser275 and Ser277. The binding energy and affinity of the complex were $-8.0 \text{ kcal mol}^{-1}$ and $7.37 \times 10^4 \text{ M}^{-1}$, respectively (Table 1).

Suaedanortriterpenedione and caspase-7 complex was formed with two hydrogen bonds involving Cys186 and Arg233 as well as hydrophobic interactions with Cys186, Tyr230, Trp232, Pro235, Trp240 and Phe282. Some residues such as Ser231, Ser275, Glu276 and Ser277 showed van der Waals’ interactions (Fig. 7, IID; Table 3). The estimated docking energy and affinity of the complex were $-6.2 \text{ kcal mol}^{-1}$ and $3.53 \times 10^4 \text{ M}^{-1}$, respectively (Table 1).

4. Discussions

Several natural or plants products are known to have cell proliferative and cytoprotective potential via anti-oxidative, anti-

### Table 1

| Ligands                     | Caspase-3 Binding energy (kcal mol$^{-1}$) | Caspase-3 Binding affinity (M$^{-1}$) | Caspase-7 Binding energy (kcal mol$^{-1}$) | Caspase-7 Binding affinity (M$^{-1}$) |
|-----------------------------|------------------------------------------|----------------------------------------|------------------------------------------|----------------------------------------|
| Ligand control*            | $-5.8$                                   | $1.79 \times 10^4$                     | $-9.6$                                   | $1.10 \times 10^7$                     |
| Norditerpenic xyloside      | $-7.4$                                   | $2.68 \times 10^5$                     | $-8.0$                                   | $7.37 \times 10^5$                     |
| Norsesquiterpenedione       | $-5.5$                                   | $1.08 \times 10^5$                     | $-6.2$                                   | $3.53 \times 10^4$                     |
| Aromatic monoterpenic ester| $-5.5$                                   | $1.08 \times 10^4$                     | $-5.7$                                   | $1.32 \times 10^4$                     |
| Norditerpenic xyloside      | $-6.6$                                   | $6.93 \times 10^4$                     | $-7.6$                                   | $3.75 \times 10^5$                     |
| Alkylated $\beta$-naphthol  | $-6.1$                                   | $2.98 \times 10^4$                     | $-6.6$                                   | $6.93 \times 10^4$                     |
| $\beta$-methoxy naphthalene| $-5.8$                                   | $1.79 \times 10^4$                     | $-6.3$                                   | $4.18 \times 10^4$                     |

*Ligand controls: TQ8 (N-[|(2S)-4-chloro-3-oxo-1-phenyl-butan-2-yl]-4-methyl-benzenesulfonamide) for caspase-3 and Acetyl-YVAD-CHO for caspase-7.

Fig. 6. The *in silico* molecular docking analysis showing interaction of caspase-3 with *Suaeda monoica* derived compounds. Panel I: (A) all compounds, (B) ligand control TQ8, (C) Alkylated $\beta$-naphthol, (D) Aromatic monoterpenic; Panel II: (A) $\beta$-methoxy naphthalene, (B) Norditerpenic xyloside, (C) Norsesquiterpenol, (D) Suaedanortriterpenedione.
Molecular docking parameters for the interaction between caspase-3 and phenols (an alkylated naphthol and a norditerpenic xyloside) and two terpenes (a norsesquiterpenol, a monocyclic triterpenoid, an aromatic monoterpenic ester, alkylated \(-b\)-naphthol and a \(-m\)-naphthol) and their effect on cell proliferation and cytoprotection (Alqahtani et al., 2019; Parvez et al., 2020). Plant secondary metabolites have high chemical diversity and biochemical specificity, which often act more effectively than synthetic drugs (Calesan, 2008). In the present study, \textit{S. monoica} derived new four compounds.

| Ligands | Donor-Acceptor pair | Distance (Å) | Type of interaction | Van der Waals’ interaction |
|---------|---------------------|--------------|---------------------|---------------------------|
| Control | ARG207:HN - LIG:O   | 1.8753       | Conventional Hydrogen Bond | SER65, TYR204, SER205, ASN208, SER209, PHE250 |
|         | ARG207:HH11 - LIG:O | 2.9212       | Conventional Hydrogen Bond |                     |
|         | ARG207:HH21 - LIG:O | 2.2992       | Conventional Hydrogen Bond |                     |
|         | TRP206:C23 - LIG    | 3.6914       | Hydrophobic (Pi-Sigma) |                     |
|         | TRP206 - LIG        | 5.0512       | Hydrophobic (Pi-Pi T-shaped) |                     |
| ABN     | ARG207:HE - LIG:O   | 2.4690       | Conventional Hydrogen Bond |                     |
|         | ARG207:HH22 - LIG:O | 2.4750       | Conventional Hydrogen Bond |                     |
|         | ARG207:HN - LIG:O   | 2.4534       | Conventional Hydrogen Bond |                     |
|         | SER251:HG - LIG:O   | 2.4498       | Conventional Hydrogen Bond |                     |
|         | LIG:H - SER251:OG   | 1.8630       | Hydrophobic (Pi-Pi Stacked) |                     |
|         | PHE256 - LIG        | 3.8742       | Hydrophobic (Pi-Pi Stacked) |                     |
|         | PHE256 - LIG        | 4.0116       | Hydrophobic (Pi-Pi Stacked) |                     |
| AES     | HIS121:HD1 - LIG:O  | 2.3773       | Conventional Hydrogen Bond |                     |
|         | CY513:SG - LIG:O    | 3.6886       | Conventional Hydrogen Bond |                     |
|         | LIG:H - GLU123:OE1  | 2.3239       | Conventional Hydrogen Bond |                     |
|         | HIS121:NE2 - LIG    | 4.3581       | Electrostatic (Pi-Cation) |                     |
|         | HIS121 - LIG        | 4.0199       | Hydrophobic (Pi-Pi Stacked) |                     |
|         | HIS121 - LIG        | 4.6979       | Hydrophobic (Pi-Pi Stacked) |                     |
|         | PHE128 - LIG        | 5.0433       | Hydrophobic (Pi-Pi T-shaped) |                     |
|         | TYR204 - LIG:C      | 4.9384       | Hydrophobic (Pi-Alkyl) |                     |
|         | LIG - MET61         | 5.3853       | Hydrophobic (Pi-Alkyl) |                     |
|         | LIG - MET61         | 4.9636       | Hydrophobic (Pi-Alkyl) |                     |
| BMN     | TYR204:HH - LIG:O   | 2.0032       | Conventional Hydrogen Bond | ASP253 |
|         | ARG207:HH22 - LIG:O | 2.4339       | Conventional Hydrogen Bond |                     |
|         | SER251:HG - LIG:O   | 2.3132       | Conventional Hydrogen Bond |                     |
|         | LIG:C - ARG207:O    | 3.5213       | Carbon Hydrogen Bond |                     |
|         | PHE256 - LIG        | 3.7981       | Hydrophobic (Pi-Pi Stacked) |                     |
|         | PHE256 - LIG        | 3.8563       | Hydrophobic (Pi-Pi Stacked) |                     |
|         | LIG:C - ARG207      | 4.5903       | Hydrophobic (Alkyl) |                     |
|         | TYR204 - LIG:C      | 5.3747       | Hydrophobic (Pi-Alkyl) |                     |
|         | TRP206 - LIG:C      | 5.0178       | Hydrophobic (Pi-Alkyl) |                     |
| NDX     | ARG207:HH22 - LIG:O | 2.3814       | Conventional Hydrogen Bond | GLY122, THR166, SER205 |
|         | CY513:SG - LIG:O    | 3.4607       | Conventional Hydrogen Bond |                     |
|         | TYR204:HH - LIG:O   | 2.7000       | Conventional Hydrogen Bond |                     |
|         | MET61 - LIG         | 4.7801       | Hydrophobic (Alkyl) |                     |
|         | CY513 - LIG         | 5.1548       | Hydrophobic (Alkyl) |                     |
|         | HIS121 - LIG        | 4.4606       | Hydrophobic (Alkyl) |                     |
|         | HIS121 - LIG:C      | 4.9783       | Hydrophobic (Alkyl) |                     |
|         | PHE128 - LIG:C      | 5.0270       | Hydrophobic (Alkyl) |                     |
|         | PHE128 - LIG:C      | 4.1657       | Hydrophobic (Alkyl) |                     |
| NSQ     | LIG:C - PHE256      | 3.5475       | Hydrophobic (Pi-Sigma) | TYR204, TRP206, ARG207, ASN208, SER209, LYS210, PHE250, SER251, ASP253 |
|         | PHE256 - LIG        | 4.4100       | Hydrophobic (Pi-Alkyl) |                     |
| SND     | SER209:HN - LIG:O   | 2.2032       | Conventional Hydrogen Bond | TYR204, ASN208, SER251 |
|         | LIC:H - PHE250:O    | 2.3776       | Conventional Hydrogen Bond |                     |
|         | LIC:C - PHE250:O    | 3.2851       | Carbon Hydrogen Bond |                     |
|         | LIG:C - PHE256      | 3.9312       | Hydrophobic (Pi-Sigma) |                     |
|         | LIG:C - PHE256      | 3.7106       | Hydrophobic (Pi-Sigma) |                     |
|         | LIG:C - ARG207      | 4.1544       | Hydrophobic (Alkyl) |                     |
|         | TRP206 - LIG:C      | 4.4890       | Hydrophobic (Pi-Alkyl) |                     |
|         | PHE252 - LIG:C      | 4.8691       | Hydrophobic (Pi-Alkyl) |                     |

*Chemically the control TQ8 is N-[4-(25-Chloro-3-oxo-1-phenyl-butan-2-yl]-4-methyl-benzenesulfonamide.*
**Table 3**
Molecular docking parameters for the interaction between caspase-7 and *S. monoica* derived compounds.

| Ligand | Donor-Acceptor pair | Distance (Å) | Type of interaction | Van der Waals's interaction |
|--------|----------------------|--------------|---------------------|----------------------------|
| Control | ARG233:HN - LIG:O     | 2.0867       | Conventional Hydrogen Bond | SER231, TRP232, SER234, PRO235, ARG237, TRF240, PHE273, GLU274, SER275, SER277, PHE282 |
|         | ARG233:HH11 - LIG:O  | 2.7924       | Conventional Hydrogen Bond |
|         | ARG233:HH21 - LIG:O  | 2.5474       | Conventional Hydrogen Bond |
|         | LIG:O - GLN276       | 3.0801       | Conventional Hydrogen Bond |
|         | LIG:H - HIS272:O     | 2.5836       | Conventional Hydrogen Bond |
|         | LIG:HO - ARG232:O    | 2.7121       | Conventional Hydrogen Bond |
|         | ARG87:HE - LIG:O     | 2.3632       | Conventional Hydrogen Bond |
|         | ARG87:HH22 - LIG:O   | 2.1211       | Conventional Hydrogen Bond |
|         | HIS144:HD1 - LIG:O   | 2.6366       | Conventional Hydrogen Bond |
|         | ARG233:HN - LIG:O    | 1.9248       | Conventional Hydrogen Bond |
|         | ARG233:HE - LIG:O    | 2.1267       | Conventional Hydrogen Bond |
|         | ARG233:HH22 - LIG:O  | 2.1997       | Conventional Hydrogen Bond |
|         | HIS144:CA - LIG:O    | 3.3939       | Carbon Hydrogen Bond |
| ABN    | AES                  |              |                      |
|        | CYS186:SG - LIG:O    | 3.5904       | Conventional Hydrogen Bond | SER231, ARG233, SER277, PHE282 |
|        | CYS186:SG - TYR230   | 4.6432       | Hydrophobic (Pi-Sulfur) |
|        | TYR230 - LIG         | 4.2081       | Hydrophobic (Pi-Pi Stacked) |
|        | TYR230 - LIG:O       | 4.5641       | Hydrophobic (Pi-Pi Stacked) |
|        | TRP232 - LIG         | 4.8124       | Hydrophobic (Pi-Pi T-shaped) |
| BMN    | HIS144:CE1 - LIG:O   | 3.6982       | Carbon Hydrogen Bond |
|        | TYR230 - LIG         | 3.7378       | Hydrophobic (Pi-Pi T-shaped) |
|        | TRP232 - LIG         | 5.0180       | Hydrophobic (Pi-Pi T-shaped) |
| NDX    | TRP240:HE1 - LIG:O   | 2.1034       | Conventional Hydrogen Bond |
|        | GLN276:HN - LIG:O    | 2.0006       | Conventional Hydrogen Bond |
|        | LIG:C - CYS186       | 4.6176       | Hydrophobic (Alkyl) |
|        | TYR230 - LIG         | 4.6001       | Hydrophobic (Alkyl) |
|        | TYR230 - LIG:C       | 4.7870       | Hydrophobic (Alkyl) |
|        | TRP232 - LIG:C       | 4.9544       | Hydrophobic (Alkyl) |
|        | TRP232 - LIG         | 4.6991       | Hydrophobic (Alkyl) |
|        | TRP232 - LIG:C       | 4.4321       | Hydrophobic (Alkyl) |
| NSQ    | LIG:H - GLN276:O     | 1.7762       | Conventional Hydrogen Bond |
|        | PRO235 - LIG         | 5.4848       | Hydrophobic (Alkyl) |
|        | LIG:C - PRO235       | 4.7898       | Hydrophobic (Alkyl) |
|        | LIG:C - PRO235       | 3.5114       | Hydrophobic (Alkyl) |
|        | TRP232 - LIG         | 5.3563       | Hydrophobic (Alkyl) |
|        | TRP232 - LIG:C       | 4.8321       | Hydrophobic (Alkyl) |
|        | TRP232 - LIG:C       | 4.9250       | Hydrophobic (Alkyl) |
|        | TRP240 - LIG:C       | 4.5652       | Hydrophobic (Alkyl) |
|        | TRP240 - LIG:C       | 5.3680       | Hydrophobic (Alkyl) |
| SND    | CYS186:SG - LIG:O    | 3.2926       | Conventional Hydrogen Bond |
|        | ARG233:HN - LIG:O    | 2.1417       | Conventional Hydrogen Bond |
|        | LIG:C - TRP232       | 3.5878       | Hydrophobic (Pi-Sigma) |
|        | LIG:C - CYS186       | 4.8621       | Hydrophobic (Alkyl) |
|        | LIG:C - PRO235       | 4.4650       | Hydrophobic (Alkyl) |
|        | TYR230 - LIG         | 3.8562       | Hydrophobic (Alkyl) |

Fig. 7. The in silico molecular docking analysis showing interaction of caspase-7 with *Suaeda monoica* derived compounds. Panel I: (A) all compounds, (B) ligand control Acetyl-YVAD-CHO, (C) Alkylated β-naphthol, (D) Aromatic monoterpenic; Panel II: (A) β-methoxy naphthalene, (B) Norditerpenic xyloside, (C) Norsesquiterpenol, (D) Suedanortriterpenedione.
a prime inducer of vascular endothelial cell damage via oxidative stress and apoptosis (Bourrajaj et al., 2003; Kim et al., 2004; Phalitakul et al., 2013; Figarola et al., 2014). Recently, significant reversal of MGO induced HUVEC cell apoptosis by pyrrophenone has been demonstrated (Ravikumar et al., 2010; Yuan et al., 2017). In addition, we have also reported promising cytoprotection of HUVEC cells against MGO by rhuspartin (Alqahtani et al., 2019) and oncocoglobin C (Parvez et al., 2020). In line with this, we demonstrate the maximal HUVEC cell proliferation and cytoprotection by suedaontriterpenedione, norsesquaterpenol and nortiterpenic xyloside, whereas moderately by aromatic monoterpene ester, alkylated β-naphthol and β-methoxy naphthalene through amelioration of MGO.

Caspases belong to cysteine-aspartate proteases, which play crucial roles in maintaining cellular homeostasis by inducing apoptotic cell death and tissue inflammation (Kumar, 2006). All caspases are synthesized as inactive enzymes where activation of effector caspase-3 or 7 is performed by the initiator caspase-9 that itself is autoactivated under oxidative or apoptotic conditions (Boatright and Salvesen, 2003; Shi, 2000). Therefore, the therapeutic intervention that could inhibit caspase expressions in acute and chronic diseases are very much desirable. To have an insight into the plausible underlying mechanisms involved in anti-oxidative and anti-apoptotic salutations, suedaontriterpenedione, norsesquaterpenol and nortiterpenic xyloside, the most active terpenes were further assessed for caspase-3/7 modulating potential. Our data showed that the three terpenes effectively downregulated DCF and MGO activated caspase-3/7 expressions in HUVEC cells, endorsing our previous study where Oncoglabrinol C, a flavan (Parvez et al., 2020). In line with this, we demonstrate the maximal HUVEC cell proliferation and cytoprotection by suedaontriterpenedione, norsesquaterpenol and nortiterpenic xyloside, whereas moderately by aromatic monoterpene ester, alkylated β-naphthol and β-methoxy naphthalene through amelioration of MGO.

5. Conclusion

Our data for the first time demonstrate in vitro cell proliferative, anti-oxidative and anti-apoptotic efficacies of Sueda monoica derived novel terpenes viz., suedaontriterpenedione, norsesquaterpenol, and nortiterpenic xyloside in human primary endothelial cells. This warrants their further molecular and pharmacological assessment as vascular cell protective as well as tissue-rejuvenating therapeutics, especially in hyperglycemic conditions.

Table 3 (continued)

| Ligand | Donor-Acceptor pair | Distance (Å) | Type of interaction | Van der Waals’s interaction |
|--------|---------------------|-------------|---------------------|-----------------------------|
| TYR230 - LIG:C | 4.6311 | Hydrophobic (Pi-Alkyl) | | |
| TRP230 - LIG:C | 4.5072 | Hydrophobic (Pi-Alkyl) | | |
| TRP232 - LIG:C | 4.3383 | Hydrophobic (Pi-Alkyl) | | |
| TRP232 - LIG:C | 5.1841 | Hydrophobic (Pi-Alkyl) | | |
| TRP240 - LIG:C | 5.0696 | Hydrophobic (Pi-Alkyl) | | |
| TRP240 - LIG:C | 5.0886 | Hydrophobic (Pi-Alkyl) | | |
| TRP240 - LIG:C | 4.5228 | Hydrophobic (Pi-Alkyl) | | |
| TRP240 - LIG:C | 5.1945 | Hydrophobic (Pi-Alkyl) | | |
| TRP240 - LIG:C | 4.7176 | Hydrophobic (Pi-Alkyl) | | |
| PHE282 - LIG:C | 5.3566 | Hydrophobic (Pi-Alkyl) | | |

* The chemical nature of Caspase 7 control ligand (peptide based inhibitor) is Acetyl-YVAD-CHO.
Figarola, J.L., Singh, J., Rahbar, S., Awasthi, S., Singh, J.S.S., 2014. LR-90 prevents methylglyoxal-induced oxidative stress and apoptosis in human endothelial cells. Apoptosis. 19, 776-788.

Ganesan, A., 2008. The impact of natural products upon modern drug discovery. Curr. Opin. Chem. Biol. 12, 306–317.

Kim, J., Son, J.W., Lee, J.A., Oh, Y.S., Shinn, S.H., 2004. Methylglyoxal induces apoptosis mediated by reactive oxygen species in bovine retinal pericytes. J. Korean Med. Sci. 19, 95–100.

Kim, D.R., Kim, H.Y., Park, J.K., Park, S.K., Chang, M.S., Jeon, J.Y., 2013. Aconiti lateralis preparata radix activates the proliferation of mouse bone marrow mesenchymal stem cells and induces osteogenic lineage differentiation through the bone morphogenetic protein-2/smad-dependent runx2 pathway. Evid. Based Compl. Alter. Med. 2013, 86741.

Kong, X., Hu, Y., Rui, W., Wang, D., Li, X., 2004. Effects of Chinese herbal medicinal ingredients on peripheral lymphocyte proliferation and serum antibody titer after vaccination in chicken. Int. Immunopharmacol. 4, 975–982.

Kathiresan, K., Ramanathan, T., 1997. Medicinal Plants of Parangipettai Coast. India, Annamalai University, Tamil Nadu, pp. 72–76.

Kokpal, V., Miles, D.H., Payne, A.M., Chittarwong, V., 1990. Chemical constituents and bioactive compounds from mangrove plants. Stud. Nat. Prod. Chem. 7, 175–199.

Kumar, S., 2006. Caspase function in programmed cell death. Cell Death Differen. 14, 32–43.

Lakshmanan, G., Rajeshkannan, C., Kavitha, A., Mekala, B., Kamaladevi, N., 2013. Preliminary screening of biologically active constituents of Suaeda monoica and Sesuvium portulacastrum from palayakayal mangrove forest of Tami Nadu. J. Pharmacog. Phytochem. 2, 149–152.

Lakshmi, K.P., Narasinga Rao, G.M., 2013. Antimicrobial activity of Forssk ex. Gmel against Human and plant pathogens. Res. J. Pharm. Biol. Chem. Sci. 4, 680–685.

LeBel, C.P., Ischiropoulos, H., Bondy, S.C., 1992. Evaluation of the probe 2′,7′-dichlorofluorescein as an indicator of reactive oxygen species formation and oxidative stress. Chem. Res. Toxicol. 5, 227–231.

Morris, G.M., Huey, K., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., Olson, A.J., 2009. Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility. J. Comput. Chem. 16, 2785–2791.

Muthahzaharan, K., Thirunavukkarasu, P., Ramanathan, T., Kannan, D., 2014. Studies on phytochemical screening, antimicrobial and antiradical scavenging effect of a coastal salt marsh plant Suaeda monoica. Res. J. Phytochem. 8, 102–111.

Opara, E.C., Rockway, S.W., 2006. Antioxidants and micronutrients. Dis. Mon. 52, 151–63.

Oyama, Y., Hayashi, A., Ueha, T., Maekawa, K., 1994. Characterization of 2′,7′-dichlorofluorescin fluorescence in dissociated mammalian brain neurons: estimation on intracellular content of hydrogen peroxide. Brain Res. 635, 113–117.

Parvez, M.K., Arhab, A.H., Al-Dosari, M.S., Al-Rehaily, A.J., Alam, P., Ibrahim, K.E., Alsaid, M.S., Rafatullah, S., 2018. Protective effect of Atriplex suberecta extract against oxidative and apoptotic hepatotoxicity. Exp. Therap. Med. 15, 3883–3891.

Parvez, M.K., Al-Dosari, M.S., Arhab, A.H., Alam, P., Alsaid, M.S., Khan, A.A., 2019. Hepatoprotective efficacy of Solomun surattense extract against chemical-induced oxidative and apoptotic injury in rats. BMC Compl. Alter. Med. 19, 155–162.

Parvez, M.K., Al-Dosari, M.S., Ahmed, S., Rehmani, M.T., Al-Rehaily, A.J., 2020. Oncoglabrinol C, a new flavon from Oncocalyx glabratus protects human endothelial cells against oxidative and apoptotic damages and modulated hepatic CYP3A4 activity. Saudi Pharm. J. 28, 646–656.

Preliminary screening of biologically active constituents of Suaeda monoica and Sesuvium portulacastrum from palayakayal mangrove forest of Tamilnadu. J. Pharmacog. Phytochem. 2, 149–152.

Ravikumar, S., Granadesigan, M., Serebiah, J., Inbanesan, S.J., 2010. Hepatoprotective effect of an Indian salt marsh herb Suaeda monoica Forssk ex. Gmel against concanavalin-A induced toxicity in rats. Life Sci. Med. Res. 2, 1–5.

Rota, C., Chignell, C.F., Mason, R.P., 1999. Evidence for free radical formation during the oxidation of 2′,7′-dichlorofluorescein to the fluorescent dye 2′,7′-dichlorofluorescein by horseradish peroxidase: possible implications for oxidative stress measurements. Free Rad. Biol. Med. 27, 873–881.

Royall, J.A., Ischiropoulos, H., 1993. Evaluation of 2′,7′-dichlorofluorescein and dihydro-rhodamine 123 as fluorescent probes for intracellular H2O2 in cultured endothelial cells. Arch. Biochem. Biophys. 302, 348–355.

Shahat, A.A., Alsaid, M.S., Rafatullah, S., Al-Soilahbani, M.O., Parvez, M.K., Al-Dosari, M.S., Exarchou, V., Pieters, L., 2016. Treatment with Rhus tripartita extract curtails isoproterenol-elicted cardiotoxicity and oxidative stress in rats. BMC Compl. Alter. Med. 2016, 351.

Shi, Y., 2000. Caspase activation, inhibition, and reactivation: A mechanistic view. Protein Sci. 13, 1979–1987.

Siddiqui, N.A., Mothana, R.A., Al-Said, M.S., Parvez, M.K., Alam, P., Rehman, M.T., Ali, M., Alajmi, M.F., Al-Dosari, M.S., Al-Rehaily, A.J., Khalid, J.M., 2020. Cell proliferation activity delineated by molecular docking of four new compounds isolated from Suaeda monoica Forssk. ex. J.F. Gmel (aerial parts). Saudi Pharm. J. 28, 172–186.

Tanaka, R., Tsujimoto, K., In, Y., Matsunaga, S., 1997. New Methoxytriterpene Dione from the cuticle of Picre jezoensis var. jezoensis. J. Nat. Prod. 60, 319–322.

Thornalley, P.J., Rabbani, N., 2014. Assay of methylglyoxal and glyoxal and control of oxidative stress measurements. Free Rad. Biol. Med. 73, 388–397.

Tanaka, R., Tsujimoto, K., In, Y., Matsunaga, S., 1997. New Methoxytriterpene Dione from the cuticle of Picre jezoensis var. jezoensis. J. Nat. Prod. 60, 319–322.

Thornalley, P.J., Rabbani, N., 2014. Assay of methylglyoxal and glyoxal and control of oxidative stress measurements. Free Rad. Biol. Med. 73, 388–397.

Xia, H., 2007. Caspase activation, inhibition, and reactivation: A mechanistic view. Protein Sci. 13, 1979–1987.

Yuan, J., Zhu, C., Hong, Y., Sun, Z., Fang, X., Wu, B., Li, S., 2017. The role of cPLA2 in Methylglyoxal-induced cell apoptosis of HUV-ECs. Toxicol. Appl. Pharmacol. 23, 44–52.

Vander Jagt, D.L., Hunsaker, L.A., 2003. Methylglyoxal metabolism and diabetic complications: roles of aldose reductase, glyoxalase-I, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase. Chem. Biol. Interact. 143, 341–351.