RESEARCH ARTICLE

Estimation of apoptosis-inducing compounds present in *Gomphrena globosa* by molecular docking

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ABSTRACT

Gomphrena globosa, a species of flowering plant from the family *Amaranthaceae*, is accredited to possess several bioactive compounds that have shown considerable pharmacological properties. This study aims to identify the specific compound(s) responsible for the anti-cancer activity and assess its mechanism of action, especially concerning any interaction with anti-apoptotic proteins and apoptosis. Prepared extracts for both the flower and the leaves of the plant specimen in chloroform and ethanol, were subjected to an initial phytochemical analysis, which revealed the presence of several pharmacologically relevant phytochemicals in both the solvents. An antioxidant scavenging assay (DPPH) as well as cell proliferation assay (MTT) on the MCF-7 breast cancer cell lines, was also performed, both of which confirmed the anti-cancer potential of the plant where the greater extent of cytotoxic potential was shown by the chloroform extracts, especially by the leaf chloroform extract. A DNA fragmentation assay was conducted, which revealed that the cell had undergone apoptosis via a distinct DNA banding pattern seen under UV-visualization. A thorough literature review of the compounds present in the chloroform extract of *Gomphrena globosa* was collected, and 12 compounds were selected to perform a comprehensive molecular docking analysis against known anti-apoptotic protein receptors (Bcl-Xl, Mcl-1, Bcl-B, and Bcl-W). It was evident from the docking analysis, that three compounds, namely Chysoeriol glycoside, Betalain, and Oleuropein aglycone bound with a substantially high degree of energy at the binding site. Therefore, the possible mechanism of action may be attributed to the interaction of the phytocompounds with these anti-apoptotic proteins.

Key words: *Gomphrena globose*, Anti-Cancer, MCF-7 Cell Line, DNA Fragmentation, Anti-Apoptotic Receptors, Molecular Docking

Introduction

Cancer has become one of the most prominent diseases all over the world, as it takes the lives of millions every year and, yet with many advancements in research and technology, there still is no definitive cure. Breast cancer has been one of the most ravaging forms of this disease, which has affected not only the lives of countless women but has also devastated several families. Risk factors for developing breast cancer include being female, obesity, lack of exercise, drinking alcohol, ionizing radiation, early age at first menstruation, having children late or not at all, older age, prior history of breast cancer, and family history (Burstein et al., 2014). Unfortunately, the treatment options are invasive and often life-threatening. Today, despite considerable efforts, cancer remains an aggressive killer worldwide. Moreover, during the last decade, novel synthetic chemotherapeutic agents currently in use clinically have not succeeded in fulfilling expectations despite the considerable cost of their development (Solowey et al., 2014).

Ethnopharmacology has found a new sense of appreciation in the pharmaceutical world. Numerous drugs have originated from bioactive compounds identified from plants and other plant sources. With the given enormity of the availability of plants and plant sources, there is an untapped potential for many promising drugs and drug similars in the plant kingdom. *Gomphrena globosa* is a tropical flowering plant found in almost all geographical locations that have a characteristic purple flower and is a garden favourite ornamental plant. This genus of the plant has reported several

pharmacological activities, especially anti-cancer activity; for example, *Gomphrena martiana* indicated positive inhibitory activity against sarcoma 180 and Ehrlich's carcinoma (Pomilio et al. 1994), and *Gomphrena macrocephala* showed cytotoxic activity against hsc-2 human oral squamous carcinoma cells (Kuroda et al., 2006). It's also been proven that *Gomphrena globosa has shown* significant cytotoxic activity against several other types of cancers (Latha et al., 2013).

Apoptosis is a phenomenon wherein the cell undergoes various morphological as well as genetic changes. Some of these include cell shrinkage, chromosome condensation, DNA fragmentation, and much more. The relationship between apoptosis and cancer progression is of vital importance in cancer biology. Cells incapable of undergoing apoptosis tend to become cancerous, and this occurrence is the main mechanism of action for the development of various cancer types. When inspecting tumour degeneration in the presence of several anti-cancer compounds, a large percentage of cell loss form tumours was due to apoptosis (Kerr et al., 1972). The Bcl-2 oncogene is the known oncogene responsible for apoptotic function in many cells. However, unlike other oncogenes, the Bcl-2 did not behave like a typical oncogene: instead of disrupting proliferation controls, Bcl-2 promoted cell survival by blocking programmed cell death (Hockenbery et al., 1990; Vaux et al., 1998).

At least 15 Bcl-2 family member proteins have been identified in mammalian cells, to date, including proteins that promote apoptosis and those that prevent apoptosis (Gross et al., 1999). Some notable anti-apoptotic proteins from the Bcl-2 family include the Bcl-2, Bcl-Xl, Mcl-1, Bcl-W, CED-9, etc. It has been suggested that cancer cells may progress not by rapid cellular division but by prolonged cell survival due to the silencing of apoptotic signals (Reed, 1998). This study aims to identify the compounds which may have a significant role in silencing these anti-apoptotic proteins to reduce cancer progression.

Materials and Methods

Extraction

Healthy leaves and flowers of *Gomphrena globosa*, taken during the month of January in and around Chennai, Tamil Nadu, India, was identified and authenticated by Dr. Jayaram, at The Herbal Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu, India. It was shade dried for five days and ground to a coarse powder. 50g of the ground leaf and flower were taken and extracted using 500mL of chloroform and ethanol, respectively, in a sequential extraction process for about 72 hrs. The extracts were then filtered using a Whatman No. 1 filter paper and dried under reduced pressure in a rotary vacuum evaporator into a concentrated paste. Labelled the concentrated extracts as FCE for Flower Chloroform extract, FEE for Flower Ethanol Extract, LCE for Leaf Chloroform Extract, and LEE for Leaf Ethanol Extract were stored in sterile microfuge tubes at -4°C until further use.

Phytochemical screening

Evaluation of main phytochemicals groups within all the extracts was carried out by application of qualitative tests for saponins, steroids and terpenoids, alkaloids, flavonoids, catechins, tannins and polyphenols, reducing sugars, lactones, coumarins, and quinones as mentioned in (Harborne, 1998). Compared the obtained results with published data concerning the presence or absence of each phytochemical group and their therapeutic.

Antioxidant assay

Evaluation of the antioxidant activity of the extracts was evaluated using DPPH radical scavenging assay mentioned in (Blois, 1958). The activity was estimated for all the extracts for varying concentration of 0.2, 0.4, 0.6, 0.8 and 1 mg/mL. The reaction mixture consisted of 1mL

methanol and 1mL of 0.1mM solution of DPPH (α, α-diphenyl-β-picrylhydrazyl). Incubated the solutions for 30 mins (dark) and, the absorbance was measured at 520 nm (GENESYS™ 140/150 Vis/UV-Vis Spectrophotometers) with pure ethanol as blank and DPPH as control. Calculated the activity of the observed results using ascorbic acid as standard. Constructed a regression plot (Teraplot) to calculate the IC50 value for each extract.

Cell proliferation assay (MTT)

The Anti-cancer activity of the extracts was estimated using MTT [3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide] assay mentioned in (Mosmann, 1983). MCF-7 cell line was purchased from National Centre for Cell Science Savitribai Phule Pune University, Pune, Maharashtra, India. Cell concentration was adjusted to 10^{-6} cells/mL^{-1} in the culture medium containing RPMI 1640, 10% fetal calf serum, penicillin (100 UmL^{-1}), and streptomycin (100 $mgmL^{-1}$). Cells were seeded in a 96 well plate and allowed to grow for 24 hrs in a CO_2 incubator (37°C, 95% humidified air and 5% CO_2). After 24hrs of incubation, the media was replenished with fresh media, and the cells were subjected to varying concentrations of extracts. After 24 hrs MTT solution (0.1 mM) was added to each well and incubated for 4hrs at standard conditions. After 4hrs, the cells were taken in sterile microfuge tubes and suspended in 3 mL DMSO solution and, their absorbance was measured at 517 nm. The results were observed, and their activity was calculated. A regression plot was constructed and their IC50 values were estimated.

DNA fragmentation analysis

The isolation of fragmented DNA from cells cultivated in 24-well plates was carried out according to (Amirghofran et al., 2010). The cells (2×106 mL^{-1}) were treated with FCE, LCE, and FEE (1 mg/mL) for 24 hrs. The cell suspension was then centrifuged at 2500 rpm for 10 mins and collected. The pellet was resuspended in 0.5 mL DNA lysis buffer (2% SDS, 10 $mmolL^{-1}$ EDTA, 10 $mmolL^{-1}$ Tris-HCl, pH 8.5). The lysate was immediately incubated with 0.1 $mgmL^{-1}$ proteinase k and then incubated for 3hrs at 37°C. Isopropanol was added to precipitate the DNA along with 70% ethanol. The suspension was centrifuged, and the DNA was treated with 100 mL of 10 $mmolL^{-1}$ Tris-HCl, pH 7.5, and 0.5 $mgmL^{-1}$ of RNAse A at 37°C for 24 hrs. The sample was then loaded onto a 2% agarose gel and subjected to electrophoresis. The DNA band pattern was visualized under UV light using ethidium bromide staining.

Chemical constituents of Gomphrena globosa

A comprehensive literature review of the various compounds and constituents of the various extracts of *Gomphrena globosa* was conducted. 42 different compounds of *Gomphrena globosa* were identified (Dinda et al., 2006; Silva et al., 2012; Yamuna et al., 2017; Esmat et al., 2020). Out of the 42 different obtained compounds, 12 compounds (Chrysoeriol Glucoside, Betalains, Oleuropein aglycone, Beta Amyrin, Beta-Sitosterol Glycoside, Campesterol, Coumarin, Epifriedelinol, Gompherin 1, Isorhamnetin, Kaempferol, Quercetin and Rhodoxanthin) were selected based on the solvent used for extraction (chloroform) and based on established cytotoxic activities.

Protein and ligand preparation

The protein receptor for the docking studies was obtained from the RSCB PDB (Protein Data Bank) at 4.002 Å root mean square deviation (RMSD) resolution which represents a three-dimensional structure of the target receptor. The ligands and crystallographic water molecules were removed from the protein and the chemistry of the protein was corrected for missing hydrogen. The protein was then subjected to energy minimization using the CHARMm force field. All of the above protein preparation was done using DSV (Dassault Systèmes BIOVIA, 2017). The ligand molecules for the docking process were analysed, and the 3-dimensional structure of the compounds was obtained from the PubChem databases shown in Table 1 (Kim et al., 2019). The structure of the compounds was downloaded in .sdf format and they were converted into. mol2 format by using Openbabel software (O'Boyle et al., 2011).

Molecular docking

An in-silico molecular binding simulation of the various bioactive compounds present in *Gomphrena globosa* with various anti-apoptotic protein receptors was performed to suggest a possible mechanism of action for the anticancer activity.

Molecular docking was performed using iGEMDOCK v2.1 edition (Hsu et al., 2011) and BIOVIA Discovery Studio Visualizer (Dassault Systèmes BIOVIA, 2017). Four antiapoptotic proteins belonging to *Bcl-2* family (Bcl-B – PBDID: 2KUA, Bcl-XL- PBDID: 1R2D, Mcl-1- PBDID: 6MBE Bcl-W- PBDID: 1O0L).

Table 1. *Phytochemical Analysis for All Extracts of Gomphrena globose.*

Phytochemicals	FCE	FEE	LCE	LEE
Carbohydrates	+	-	+	-
Tannins	-	-	+	+
Saponins	+	-	+	-
Flavonoids	+	+	+	+
Quinone	+	+	-	+
Glycoside	-	+	-	+
Cardiac glycoside	-	+	+	+
Terpenoids	+	-	-	-
Phenols	-	+	+	+
Coumarins	+	-	+	-
Steroids	+	-	+	-
Phlobactannins	-	+	-	+
Alkaloids	-	+	-	-

Results

Phytochemical screening

Table 2. *Chemical Properties of Selected Ligands/Compounds.*

Name	PubChem ID	Mol.Wt (g/Mol)	Mol. Formula
Beta-Amyrin	73145	426.7	$C_{30}H_{50}O$
Betalains	56841626	550.5	$C_{24}H_{26}N_2O_{13}$
Beta-Sitosterol glucoside	5742590	576.8	$C_{35}H_{60}O_6$
Campesterol	173183	400.7	$C_{28}H_{48}O$
Chrysoeriol glucoside	11294177	462.4	$C_{22}H_{22}O_{11}$
Coumarin	323	146.14	$C_9H_6O_2$
Epifriedelanol	119242	428.7	$C_{30}H_{52}O$
Gomphrenin-I	6096868	550.5	$C_{24}H_{26}N_2O_{13}$
Isorhamnetin	5281654	316.26	$C_{16}H_{12}O_7$
Kaempferol	5280863	286.24	$C_{15}H_{10}O_6$
Oleuropein aglycone	56842347	378.4	$C_{19}H_{22}O_8$
Quercetin	5280343	302.23	$C_{15}H_{10}O_7$
Rhodoxanthin	5281251	562.8	$C_{40}H_{50}O_2$

Table 3. *IC_{50} Value of All the Extracts for DPPH and MTT Assay.*

Extract	IC_{50} Value- DPPH (mg/ml)	IC_{50} Value- MTT (mg/ml)
FCE	0.986	1.023
FEE	0.032	1.362
LCE	0.686	0.686
LEE	0.077	0.977

The phytochemical screening of all four extracts identified the presence of numerous phytochemicals which are shown in Table 2. LCE and FCE have the maximum number of phytochemicals compared to the other extracts. From the 14 phytochemicals, Flavonoids and Phenols were present in all the extracts followed by Cardiac glycosides and Terpenoids.

Antioxidant Assay

The antioxidant assay revealed that FEE had the highest antioxidant activity when compared to the other extracts as seen in Figure 1. LEE showed the second highest activity followed by LCE then FCE. These results are further validated by the IC_{50} values shown in Table 3.

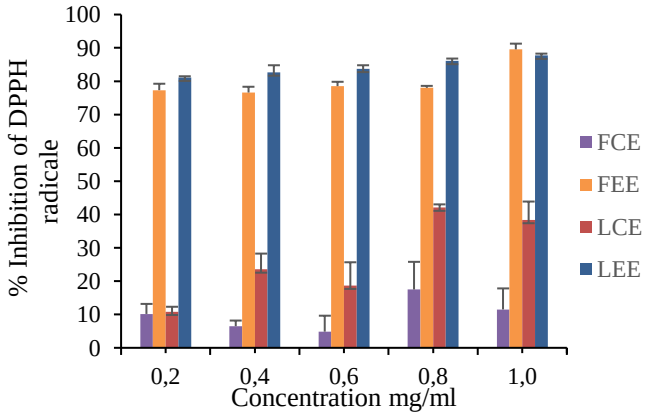

Figure 1. *The radical scavenging effect of variable doses of All extracts of Gomphrena globosa via DPPH assay. Values are expressed as mean ± S.D. The % inhibition was calculated based on control & standard OD at 517 nm.*

Cell proliferation assay (MTT)

The Cell Proliferation assay (MTT) was performed to give a brief insight into the influence of the extract on the proliferation rate of the cancer cell lines. From Figure 2 we can ascertain that FCE and LCE showed moderate to a high anti-cancer activity whereas, FEE and LEE showed low anti-cancer activity, which was confirmed by UV-spectrophotometric analysis. From the results, we can approximate that LCE may possess certain bioactive compounds that effectively inhibit the rapid proliferation of cancerous cells. The FCE and LCE showed the lowest IC_{50} compared to the other extracts, as seen in Table 3.

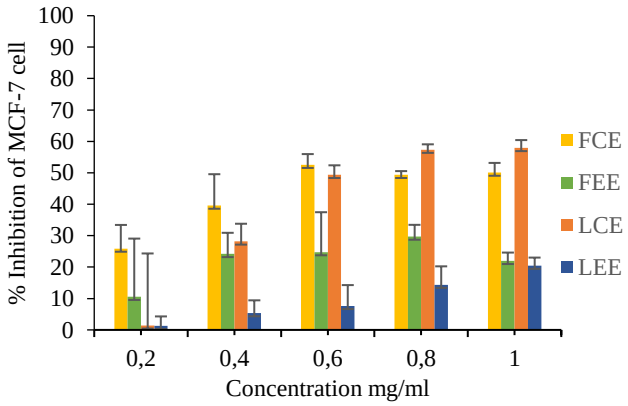

Figure 2. *Effect of variable doses of All extracts of Gomphrena globosa on MCF-7 cells via MTT assay. Values are expressed as mean ± S.D. The % inhibition was calculated based on control & standard OD at 570 nm.*

DNA fragmentation analysis

The Internucleosomal DNA fragmentation analysis shows a clear DNA laddering on the agarose gel (Figure 3), indicating the effect of extracts FCE and LCE at Standard concentration and validates the hypothesis that the antiproliferative action of the extract was via apoptosis of the cancerous cells. FCE and LCE show proper banding of DNA, whereas FEE and LEE are not as prominent.

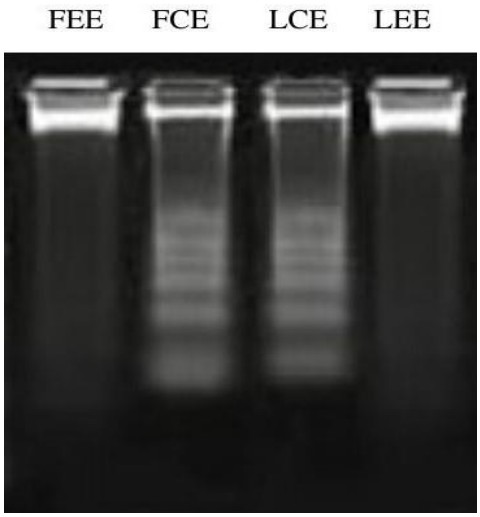

Figure 3. *Gel Electrophoresis of DNA, extracted from MCF-7 cells treated with FCE, LCE and FEE. 1 mg/ml of FCE, LCE and FEE were added to a cell suspension of MCF-7 cells for 24h at standard conditions. The internucleosomal DNA was isolated and loaded on to a 2 % agarose gel and visualized using ethidium bromide. Wells 1,2 and 3 corresponds FCE, LCE and FEE respective.*

Molecular docking

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The results of the molecular docking analysis showed that three compounds, namely Chrysoeriol Glucoside, Betalains and Oleuropein Aglycone had the highest binding affinity score towards Mcl-1, Bcl-Xl, Bcl-B, and Bcl-W, respectively as seen in Table 4. The bound conformations of the protein-ligand structures were visualized by BIOVIA DVS. The protein-ligand interactions for Mcl-1 and Chrysoeriol Glucoside are shown in Figure 4, Bcl-Xl and Betalains are shown in Figure 5, Bcl-W, and Oleuropein Aglycone are shown in Figure 6, and Bcl-B and Oleuropein Aglycone are shown in Figure 7. The Surface interaction profile was also visualized and is shown in Figure: 8. The purpose of visualization gives us an insight into the various bonding patterns and amino acids involved in binding. From the images of all the interactions, we can see that conventional hydrogen bonding is a key factor in all the protein-ligand interaction profiles.

Table 4. *Binding Affinity score of Protein-Ligand Complex.*

Protein-Ligand Complex	Binding Affinity Score (Kcal/mol)	VDW (Kcal/mol)	H Bond (Kcal/mol)	Elec (Kcal/mol)
Mcl-1-Chrysoeriol Glucoside	-110.11	-84.1315	-25.9783	0
Bcl-Xl-Betalains	-131.471	-102.717	-26.449	-2.3054
Bcl-W-Oleuropein Aglycone	-130.449	-99.4054	-28.0434	0
Bcl-B-Oleuropein Aglycone	-134.468	-95.647	-25.8207	0

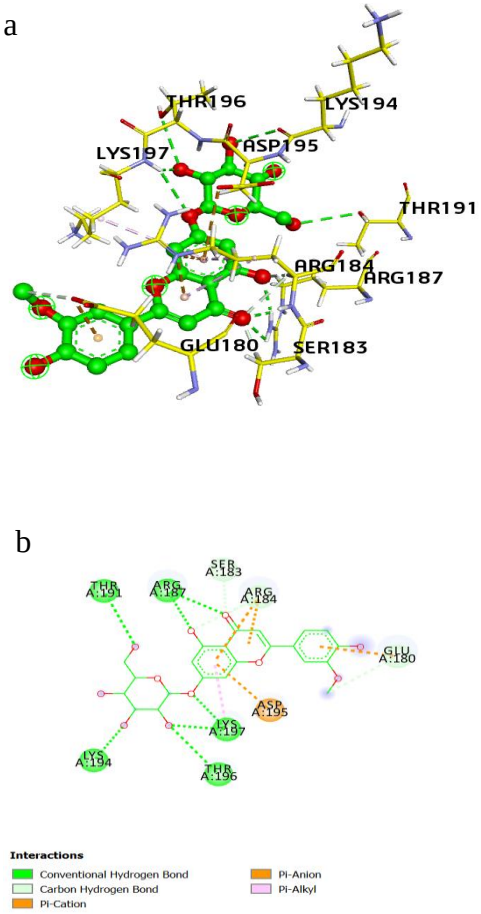

Figure 4. *a) Chrysoeriol Glucoside Interacting with Mcl-1Protein. The green coloured ligand group representing Chrysoeriol Glucoside is seen interacting with the amino acid side chains of the Mcl-1 protein depicted in yellow. b) 2D visualization of the interaction between Chrysoeriol Glucoside and Mcl-1 protein. Various bonds such as conventional hydrogen bonds, carbon hydrogen bons, pi- cation bonds, pi- anion bonds and pi alkyl bonds are the main stabilizing and binding forces involved in this interaction.*

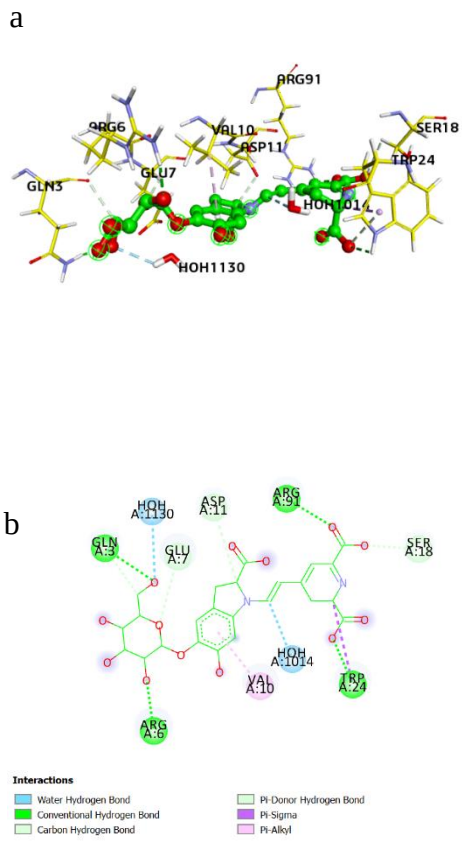

Figure 5. *a) Betalains Interacting with Bcl-Xl Protein. The green coloured ligand group representing Betalains is seen interacting with the amino acid side chains of the Bcl-Xl protein depicted in yellow. b) 2D visualization of the interaction between Betalains and Bcl-Xl. Various bonds such as water hydrogen bonds, conventional hydrogen bonds, carbon hydrogen bons, pi- sigma bonds, pi- donor hydrogen bonds and pi alkyl bonds are the main stabilizing and binding forces involved in this interaction.*

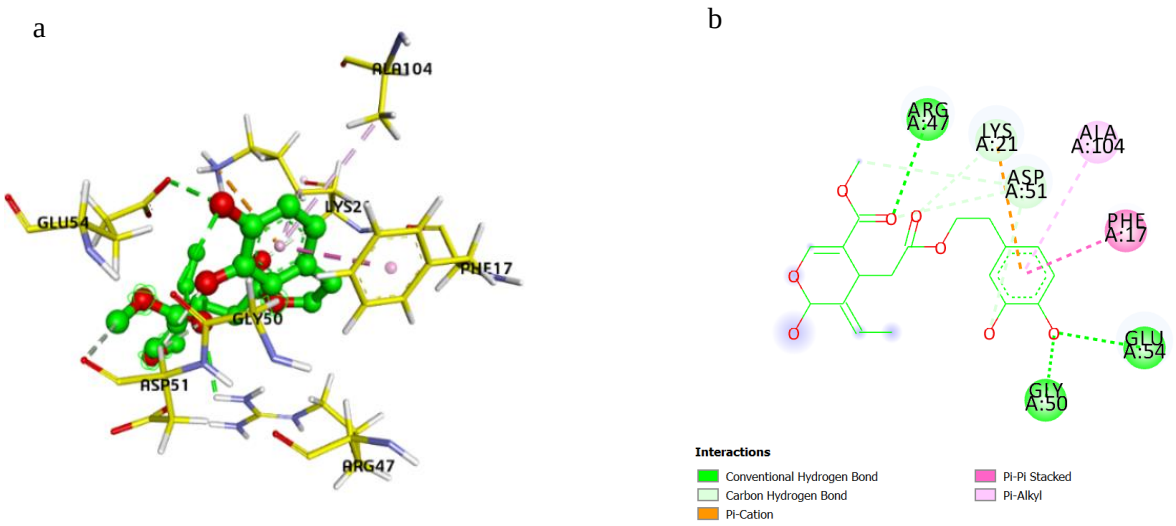

Figure 6. *a) Oleuropein Aglycone Interacting with Bcl- W Protein. The green coloured ligand group representing Oleuropein Aglycone is seen interacting with the amino acid side chains of the Bcl- W protein depicted in yellow. b) 2D visualization of the interaction between Oleuropein Aglycone and Bcl-W. Various bonds such as water hydrogen bonds, conventional hydrogen bonds, carbon hydrogen bonds, pi-sigma bonds, pi- donor hydrogen bonds and pi alkyl bonds are the main stabilizing and binding forces involved in this interaction.*

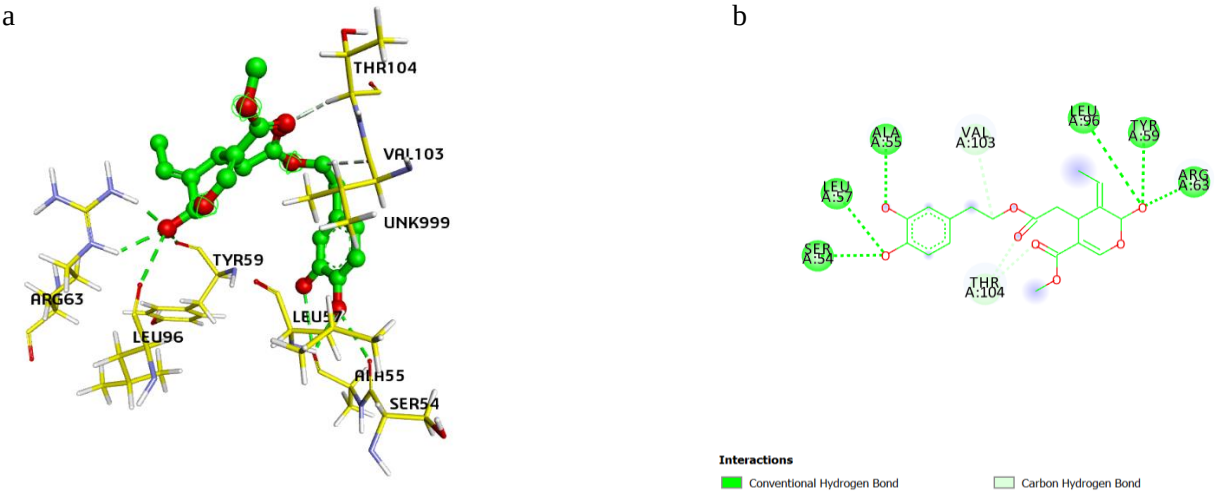

Figure 7. *a) Oleuropein Aglycone Interacting with Bcl- B Protein. The green coloured ligand group representing Oleuropein Aglycone is seen interacting with the amino acid side chains of the Bcl- B protein depicted in yellow. b) 2D visualization of the interaction between Oleuropein Aglycone and Bcl-B. Various bonds such as conventional hydrogen bonds and carbon hydrogen bonds are the main stabilizing and binding forces involved in this interaction.*

Discussion

Apoptosis plays a massive role in cancer progression and remission, mainly functioning as the key contributor to cancer progression (Lowe & Lin, 2000). Cancer cell progression is often attributed to the muted induction of apoptosis, which when functioning properly would send a cascade of signals to prevent such mutations affecting neighbouring cells. All functions and signalling involving apoptosis are carried out by a group of proteins, mainly known as the Bcl-2 family of proteins. This protein family consists of both pro-apoptotic as well as anti-apoptotic proteins (Gross et al., 1999). From this study, we can conclude that the extraction of bioactive compounds from the leaves using a polar solvent may yield certain compounds that can interact with those proteins.

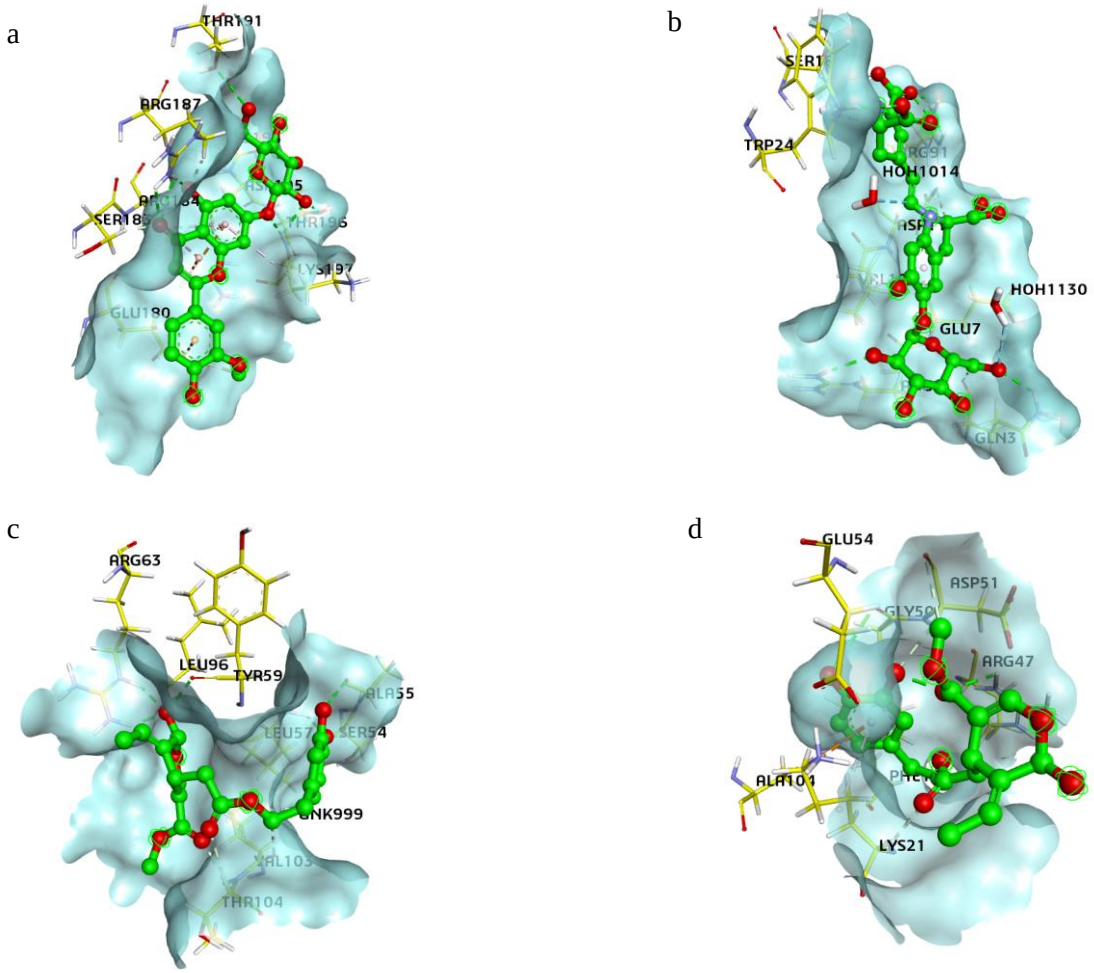

Figure 8. *The chemical compound binding on the surface of the respective proteins. The chemical compound is shown in green and the surface is shown in blue. a) Chrysoeriol Glucoside Interacting with Mcl-1Protein, b) Betalains Interacting with Bcl-Xl Protein, c) Oleuropein Aglycone Interacting with Bcl- W Protein, d) Oleuropein Aglycone Interacting with Bcl- B Protein.*

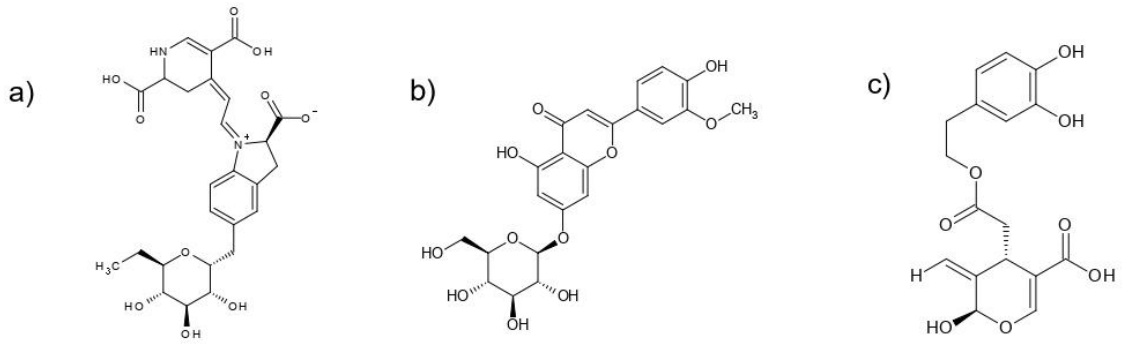

Figure 9. *Chemical structures of key compounds, where a) Betalain, b) Chrysoeriol Glucoside, c) Oleuropein Aglycone.*

This hypothesis is supported by the fact that FCE and LCE were the extracts which showed a high degree of antiproliferative activity, as well as a DNA banding pattern, seen in the DNA fragmentation assay. The results of this study also coincide with other published data on the activity of *Gomphrena globosa* on various other cancer cells (Arcanjo

et al., 2011) (Latha et al., 2013). To further validate the hypothesis that certain anti-apoptotic proteins are being silenced and normal apoptotic stimuli aren't generated, a simple molecular docking analysis was conducted for the identified compounds and known anti-apoptotic receptors. In doing so, the results indicate that compounds shown in Figure 9, do bind with substantial energy towards those proteins. Chrysoeriol Glucoside, which showed binding towards Mcl-1 protein, has been accredited for acting against cancer cells (Takemura et al., 2010). This was the same in the case of Betalains (Lechner & Stoner, 2019) and Oleuropein Aglycone (Fayyaz et al., 2016). Such binding is often categorized by a distortion in the conformation of the protein and thus ultimately affecting its function, which may indicate why cellular proliferation might be restricted within cells treated with those extracts, as seen in the cell proliferation assay (MTT). Since apoptosis is a gene regulated function, in-depth research into the genetic aspects behind this mechanism of action is required, to further validate these findings. Even though molecular docking analysis is not a definitive nor a concrete basis to lay this hypothesis on, it still might prove to be one small brick in the construction of a clear definitive theory on the regulation and silencing of anti-apoptotic protein as a new model of treating cancer.

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