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Phytochemical composition and antiproliferative activity of *Opuntia elatior* Mill.: *In vitro* and *in silico* studies on breast cancer cell line MCF-7

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ABSTRACT

Opuntia elatior Mill. is found in the semi-arid regions of Gujarat and is known for its blood tonic action among the local communities as a folklore belief. In the present study, aqueous and methanolic extracts of cladode and fruit of *O. elatior* were subjected to preliminary phytochemical characterization followed by a detailed evaluation through high resolution-liquid chromatography-mass spectrometry analysis. The isolated molecules were profiled for their properties, and isorhamnetin, phloretin, and herbacetin were the most suitable phytochemicals for further drug discovery studies as they satisfied the Lipinski rule. The study also revealed some novel unreported molecules which were subjected to further validation and confirmation. *In vitro* antioxidant activity of cladode and fruit extracts over a range of concentrations was evaluated using 2, 2-diphenyl-1-picryl-hydrazine-hydrate (DPPH) assay inhibition and a free radical scavenging assay. The total phenol and flavonoid content results indicated their potent antioxidant properties. *In vitro* antiproliferative assays such as 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), scratch (wound healing), and colony formation have shown that fruit aqueous and methanolic extracts, cladode methanolic and aqueous extracts, could inhibit cell viability in that order by enhancing cytotoxicity potential in the human breast cancer cell line, Michigan Cancer Foundation (MCF-7). Furthermore, a mechanistic *in silico* study was performed to evaluate the potential of the isolated molecules of the plant for their docking actions on antiproliferative and apoptotic pathways. The results revealed that isorhamnetin, phloretin and herbacetin showed a strong binding affinity toward Bax (−7.8, −7.3, −7.8), Bcl-2 (−7.4, −6.9, −7.5), Caspase 9 (−7.3, −6.3, −7.3) and Cyclin D1 complexed with CDK4 (−7, −6.6, −6.9). The overall findings of this research work are among the first in scientific investigations into this underexplored plant.

ARTICLE HIGHLIGHTS

Breast cancer remains a global health concern, prompting continuous research into novel therapeutic options. In this context, the article presents a comprehensive investigation into the potential of *Opuntia elatior* Mill. as an alternative treatment for breast cancer. The study combines laboratory experimentation and computational analysis to evaluate the phytochemical composition and antiproliferative effects of *Opuntia elatior* Mill. extract on the breast cancer cell line MCF-7.

The phytochemical analysis revealed a rich composition of bioactive compounds in *Opuntia elatior* Mill., including flavonoids, alkaloids, and polyphenols, known for their potential health benefits. Furthermore, the study delved into the extract's antiproliferative activity, demonstrating its ability to inhibit the growth of MCF-7 breast cancer cells and the data was supported by its anti-migratory activity and long survival of cells in the presence of extract. Molecular docking simulations provided data on the binding affinities and potential modes of action, shedding light on the extract's mechanistic basis for its antiproliferative activity. *Opuntia elatior* Mill. emerges as a promising candidate for further investigation and potential drug development against breast cancer.

The integration of experimental and computational methods highlights the power of interdisciplinary research in unravelling the complex interactions between natural compounds and cancer cells. As researchers continue to explore alternative treatments, this paper underscores the importance of harnessing the therapeutic potential of botanical sources in the battle against breast cancer.

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1. INTRODUCTION

Cancer is a global health concern and is the leading cause of death worldwide. The most widely used chemotherapeutic medications are antimetabolites, such as methotrexate; DNA-interactive medications, such as cisplatin and doxorubicin; anti-tubulin medications, such as taxanes; hormones; and molecular targeting medications [1]. The main drawbacks of chemotherapy are cancer recurrence, drug resistance, and harmful effects on tissues that are not being treated, which might limit the use of anticancer medications and reduce patient quality of life [2]. There is a constant effort being made for identifying novel, promising anticancer drugs from natural sources to address the concerns arising from the current therapies. *Opuntia elatior* is a xerophytic shrub that belongs to the dicotyledonous angiosperm *Cactaceae* family, of which over 1500 species are known [3]. *O. elatior* is abundant in the semi-arid belts of Saurashtra and parts of North Gujarat in the Indian subcontinent [Figure 1]. This particular species drew attention due to its use by the locals of Saurashtra wherein the local community used it as a juice with medicinal value for maintaining healthy blood physiology. Locally, it is referred to as "Hathlo Thor" [4]. The flat, edible stems of most *Opuntioideae* cacti are known as cladodes, paddles, nopales, or stalks. Young cladodes, also known as nopales, are typically used as a vegetable in salads, and their fruits, also known as tunas, are frequently consumed as fresh seasonal fruit [5]. Oval berries, known as "prickly pears," have many seeds dispersed throughout the pulp and a thorny, semi-hard skin. In folklore, *Opuntia* is used for diabetes, anemia, diphtheria, burning sensation in the stomach, and obesity.

The antiproliferative effect of *Opuntia ficus-indica* prickly pear juice on MCF-7 human breast cancer cells showed that the juice inhibited the growth of the cancer cells and induced apoptosis, indicating its potential use as a natural anti-cancer agent [6,7]. The researchers suggested that this effect was due to the presence of betalains, which are pigments found in some cactus species that have been shown to have anticancer properties [8]. It is therefore scientifically important to investigate the potential capabilities of *O. elatior* for its anti-cancer capabilities. Between wild and domesticated species, there are differences in the phytochemical makeup of their plant components (fruits, cladode, roots, flowers, seeds, and stems), altering their nutritional value and consequently, their functional and/or medicinal characteristics. *Opuntia* species are globally widespread, containing amino acids, carotenoids, and antioxidant compounds such as betalains and flavonoids [9]. However, it is important to note that their phytoconstituents can vary based on their environmental conditions leading to some unique secondary metabolites that could be of importance to human health. The other phytochemical groups known are alkaloids, phenolic acids, coumarins, and terpenoids, which have several health benefits. An array of flavonoids, such as quercetin, kaempferol, and isorhamnetin glycosylated derivatives, were identified through High-Performance Liquid Chromatography-photo diode array-electrospray ionization (ESI)-mass spectrometry (MS) in *O. ficus-indica* [10,11]. The ethanolic fruit extracts were tested for their antiproliferative activity against the human K-526 cell line, which showed cell cycle arrest at G2/M and S phases [12]. It is important to note here that most of the scientific data available in the literature are for *O. ficus-indica* while scientific understanding of *O. elatior* is lacking due to its regional-specific availability. Furthermore, most studies done are inconclusive and lack clarity regarding their applicative in-depth understanding of *O. elatior* as a phytotherapeutics agent. There are also reports of mucilage and phylloclade being used for recipes, and it will be very interesting to know if phylloclade also has some bio-actives for future use. The literature suggests that the fruits of *O. elatior* serve as an analgesic due to the presence of betanin [13].

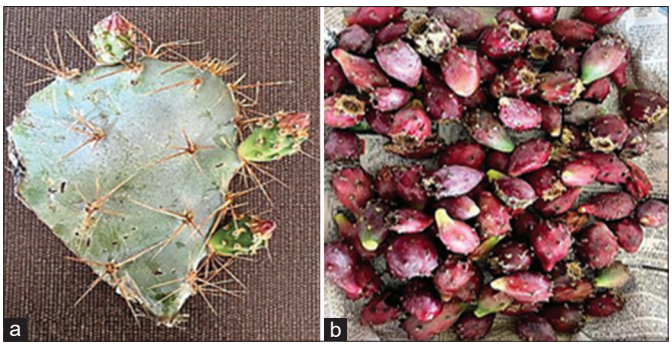

Figure 1: *Opuntia elatior* Mill parts (a) Cladode and (b) Fruit.

Furthermore, there is astonishingly little evidence to back up the belief that herbal medicines are generally safe since they are "natural," despite the fact that the general population and some healthcare professionals hold this belief. In times, when existing medication has posed limitations for further use one needs to go back to nature to find solutions to modern-day medication. The present study is one of the pioneering attempts to evaluate the plant's anti-breast cancer properties, which is the genus of the plant consumed by humans and considered safe. Scientific insight into this plant is important for drug discovery and opens avenues for further repurposing.

2. METHODS

2.1. Plant Material

The cladode and fruits of *O. elatior*, commonly known as Hathala-Thor, were collected in November 2020 in Jamkhambhaliya (22°09'58" N 70°07'53" E), Gujarat, India. Taxonomist Dr. Karan Rana, from the School of Science, identified and authenticated the whole plant. The voucher specimen (FP1) is deposited in the museum of Navrachana University. The plant material was washed, cut into pieces, and then dried in a hot air oven at 40°C. The dried plant material was coarsely powdered using a mechanical grinder and stored in an airtight container at room temperature for further use.

2.2. Preparation of *O. elatior* Extract

The powdered cladode and fruit material were subjected to extraction. The soxhlet extraction method was used to prepare the methanolic extract by subjecting 30 g cladode and fruit powders each to extraction with 200 mL of methanol, while the reflux extraction method was used to prepare an aqueous extract in similar proportions. The solvent was evaporated, and the extract was cooled down and stored at –20°C until analysis. Cladode aqueous, cladode methanol, fruit aqueous, and fruit methanol extraction yields were 19.75%, 12.75%, 36.4%, and 38.5%, respectively [14].

2.3. Determination of Total Flavonoid Content

For all investigated plant extracts, 1 mg of extract was dissolved in 1 mL methanol and then mixed with 60 µL 5% $NaNO_2$, 60 µL 10% $AlCl_3$, incubated for 5 min at room temperature, followed by the addition of 0.2 mL of 1 mM NaOH, diluted with 1 mL of distilled water. The absorbance of the reaction mixture was measured at 510 nm with a double-beam Ultraviolet/Visible spectrophotometer. The standard curve was prepared by employing quercetin as a reference standard in the concentration range of 200–1000 µg/mL [15].

2.4. Determination of Total Phenolic Content

The phenolic contents in the plant extracts were determined using the Folin-Ciocalteu method. 1 mL of plant extract (1 mg/mL) was diluted with 3 mL of distilled water, mixed with 0.5 mL Folin-Ciocalteu reagent, and incubated for 3 min. Subsequently, 2 mL of 20% (w/v) sodium carbonate was added, mixed thoroughly, and kept in boiling water for 1 min. The blue color was read at 650 nm against a methanol reagent blank. Total phenolic content was expressed as milligram gallic acid equivalent per gram of the sample [16].

2.5. Determination of Antioxidant Activity using 2, 2-Diphenyl-1-Picryl-Hydrazine-Hydrate (DPPH) Assay

The assay was performed in the sample concentration range of 100–1000 µg/mL with the addition of 2.85 mL of DPPH solution prepared in methanol. The reaction mixture was incubated for 30 min in the dark. The decrease in absorbance was recorded at 517 nm against methanol as a blank [17]. The antioxidant activity was calculated as the percentage of inhibition of the DPPH-free radical with respect to gallic acid standard and determined by the following equation:

% DPPH radical scavenging activity = (1−sample OD/control OD) × 100

2.6. Determination of Antioxidant Activity using Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant activity of the plant extracts was estimated by the tripyridyl triazine (TPTZ) method. The reduction of Fe^{3+} TPTZ complex (colorless complex) to Fe^{2+} TPTZ (blue-colored complex) formed at acidic pH by donating an electron was recorded spectrophotometrically. The fresh FRAP reagent was prepared by mixing 125 mL acetate buffer (0.3 M), 12.5 mL of TPTZ (10 mM), and 12.5 mL of $FeCl_3.6H_2O$ (20 mM) at 37°C. To each aliquot (200 µL) of plant extract, 2.85 mL of FRAP reagent was added and incubated for 30 min in the dark. The reaction of plant extract with FRAP reagent formed an intense blue color complex whose absorbance was recorded at 593 nm against the reagent blank. The FRAP values obtained were expressed as milligram gallic acid equivalent per gram of sample [18].

2.7. Identification of Bioactive by High Resolution-Liquid Chromatography-Mass Spectroscopy (HR-LCMS)

The plant extracts were subjected to HRLC-MS analysis (Agilent 6550 iFunnel Quadrupole Time-of-Flight [Q-TOF]'). Chromatographic separation was done using a 1290 infinity Ultra Performance liquid chromatography system fitted with a Hypersil gold column (C18 × 2.1 mm-3 micron) with the help of an autosampler at a flow rate of 0.3 mL/min and 20 µL injection volume. A solution of 0.1% formic acid in water formed solvent system A, while a combination of 0.1% formic acid, 10% water, and 90% acetonitrile formed solvent system B. The chromatographic elution was performed by following the gradients system which was as follows: 0–1 min of 95% (A) and 5% (B), 1–20 min of 100% solvent (B), 20–25 min of 100% solvent (B), 25–26 min of 95% (A) and 5% (B), and 26–30 min of 95% (A) and 5% (B). The mobile phase consisted of 10% acetonitrile, and 10% water (A) and (B). The eluted compounds were ionized using the ESI technique, equipped with a QTOF analyzer (Agilent Technologies, CA, USA). The flow of gas was set at 13 L/min at 250°C temperature, while the capillary tension was set at 3500 V. The sheath gas flow was set at 11 L/min at 300°C, while the pressure of the nebulizer gas was 35 psi. The mass data were recorded on positive and negative ionization modes across a spectral range of m/z 150–1000. The data acquisition and evaluation of MS were carried out using the Agilent Metlin database [19].

2.8. Antiproliferative Activities

2.8.1. Cell viability assay

The antiproliferative activity of crude plant extracts was investigated on human breast adenocarcinoma cancer cell line MCF-7 procured from National Centre for Cell Science (NCSS), Pune, Maharashtra. The cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% heat-inactivated fetal bovine serum, 100U/mL penicillin-streptomycin solution at 37°C and in a humidified atmosphere of 5% CO_2 in the air. The cells were seeded at 10^4 cells/well density in 96 well plates to obtain 80–90% confluency. The cell viability study was performed from a concentration range of 0.5–100 µg/mL using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method. The well plates were incubated for 24, 48, and 72 h with the indicated concentration of plant extracts, positive control (Doxorubicin), and negative control (Dimethyl sulfoxide [DMSO] 0.2%). Then 10 µL MTT (5 mg/mL stock) was added to the well plates. After 4-h incubation, MTT was eliminated, and 100 µL DMSO was added to solubilize the formazan crystals. The concentration was quantified at 490nm in a microplate reader, and the results were shown as percentage inhibition (100% inhibition) [20].

2.8.2. Toxicity studies

The safety of all four extracts was investigated on a human embryonic kidney cell line (HEK-293) normal cell line procured from NCSS, Pune, Maharashtra. HEK-293 is believed to be derived from embryonic kidney cells using adenoviral DNA and is a widely used standard for normal cells. The cells were cultured in minimum essential media supplemented with 10% heat-inactivated fetal bovine serum, 100U/mL penicillin-streptomycin solution at 37°C, and in a humidified atmosphere of 5% CO_2 in the air. The cells were seeded at 10^4 cells/well density in 96 well plates to obtain 80–90% confluency. The cell viability study was performed from a concentration range of 0.5–100 µg/mL using the MTT colorimetric method. The well plates were incubated with the indicated concentration of plant extracts for 24, 48, and 72 h. Then, 10 µL MTT (5 mg/mL stock) was added to the well plates. After 4-h incubation, MTT was eliminated, and 100 µL DMSO was added to solubilize the formazan crystals. The concentration was quantified at 490 nm in a microplate reader, and the results were shown as percentage inhibition (100% inhibition) [20].

2.8.3. Scratch assay (wound healing assay)

The wound-healing assay was carried out according to a previously reported protocol. The MCF-7 at density 10^4/well were seeded in 24 well microplates. After 80%–90% confluency, a scratch was made in the monolayer using a sterile 200 µL pipette tip. The floating cells and debris were removed using sterile 1× phosphate-buffered saline. The fresh complete growth medium and plant extracts at a half-maximal inhibitory concentration 50% (IC_{50}) concentration were added to the cells. The wells containing cells with medium without extract were used as control. The cells were incubated for 24 h at 37°C in 5% CO_2. Images of gap closure were taken at 0, 12, and 24 h using an inverted microscope. The area enclosed between the scratch boundary was analyzed using Image J bundled with 64-bit Java 8. The percentage of wound closure was calculated to the initial gap area at 0 h [21].

2.8.4. Clonogenic assay

The colony formation assay was carried out according to a previously reported protocol with modification. Approximately 200 cells were seeded in 6 well plates and allowed to grow for 24 h at 37°C. The cells were then treated with IC_{50} concentration of plant extracts for 24 h at 37°C. After this, cells were washed with 1× sterile phosphate buffer saline and added to a fresh medium. The cells were then kept in a fresh

medium and incubated for a period of 10 days. Following this, cells were fixed with 37% formaldehyde, stained with 0.5% crystal violet in 100% methanol for 30 min at room temperature, and washed with tap water, and the colonies were analyzed using ImageJ bundled with 64-bit Java 8 [22].

2.9. Preparation of Protein receptors

The 3D X-ray crystallography structure of Bcl-2 (protein data bank [PDB] ID:1G5M), Bax (PDB ID: 4S0O), Caspase-9 (PDB ID: 1NW9), Cyclin D1complexed with CDK4 (PDB ID: 2W99) was downloaded from PDB, removed heteroatoms and added polar hydrogen atoms using Biovia Discovery Studio Visualizer version 4.5 (Dassault Systemes) and saved for docking analysis.

2.10. Molecular Docking

Molecular docking of receptors (macromolecule) and ligand was done on PyRx version 0.9 through the Vina wizard program. Based on the compound's absorption, distribution, metabolism, excretion, and toxicity (ADMET), which play key roles in drug discovery and development ADMET analysis, three flavonoids (isorhamnetin, herbacetin, and phloretin) were selected. The 3D structure in SDF format for isorhamnetin, phloretin, and herbacetin was downloaded from PubChem and converted to pdbqt format using the extension Openbabel available in PyRx for molecular docking. Hence, after the prediction of the binding site and the docked complexes were visualized through Biovia Discovery Studio Visualizer version 4.5 (Dassault Systemes).

2.11. Statistical Analysis

Results have been expressed as the mean ± standard error means of triplicate analysis. Statistical comparisons were performed using the one-way analysis of variance, and Tukey's multiple comparisons were used to determine the difference between the means of various samples in GraphPad Prism version 8.4.2 (GraphPad Software, La Jolla, California, USA, http://www.graphpad.com/). Differences were considered significant at $*P < 0.05$, $**P < 0.01$ $***P < 0.001$. The correlation coefficient (r^2) between the parameters tested was established by regression analysis.

3. RESULTS AND DISCUSSION

3.1. Quantitative Phytochemical and Antioxidant Assay

A total of four extracts have been evaluated in this study. Table 1 indicates the values of the total phytochemical analysis of these extracts. Flavonoid content is higher in cladode methanolic extract (226.09 ± 0.15 µg/quercetin equivalent/mg) $P < 0.001$, while fruit aqueous has more flavonoid content than its methanolic fraction. Total phenols were also higher in cladode methanolic extract (289 ± 0.05 µg/gallic acid equivalent/mg), $P < 0.001$ while phenolic content was greater in fruit methanolic than in aqueous extract. The DPPH assay was used to calculate the extracts' capacity to scavenge-free radicals. The assay for determining the antioxidant capacity of plant extracts and chemicals is particularly quick and sensitive. DPPH is a nitrogen-centered, dark violet-colored powder that is stable and undergoes a color change to yellow upon reduction [23]. As the antioxidant quenches, the DPPH radicals by donating hydrogen, the decrease in the intensity of color depends on the antioxidant's ability to scavenge free radicals. The presence of phenolics and flavonoids endows the plant with the ability to scavenge free radicals [24]. In the current study, fruit methanolic extract (IC_{50} 205.9 µg/mL) followed by methanolic cladode extract (IC_{50} 260.8 µg/mL) showed good scavenging activities against DPPH. The potassium ferricyanide reduction method was used to evaluate the reducing power of plant extract. By donating hydrogen, an antioxidant in the test sample converts iron (Fe^{+3}) to ferrous (Fe^{+2}), and the yellow color of the reaction mixture turns green [25]. The reducing power was higher in aqueous extracts of both the plant parts [Figure 2]. Overall results indicate good antioxidant potential and a good indication of its possible effects on the body. There have been reports of high flavonoid and phenolic contents in seeds, cladode, and other plant parts in *O. ficus-indica*, *Opuntia albicarpa*, *Opuntia stricta,* and others [26-28]. However, this study is the first report on the phytochemical understanding of *O. elatior*. Also, fewer studies have evaluated the fruit content, and with *O. elatior*, the main focus is an in-depth understanding of the fruit and cladode phytochemical analysis.

3.2. Identification of Bioactive by HR-LCMS

To characterize the secondary metabolites, present in all four extracts, HR-LCMS analysis was carried out, and the results have been indicated in positive and negative ionization modes in Tables 2-5. Each table consists of a list of phyto molecules identified by this analysis in the spectrum of alkaloids, flavonoids, phenolic, glycoside, and terpenoid classes. It is very interesting to note that in methanolic fruit extract, the analysis revealed the presence of 17 novel compounds identified and reported for the 1st time. There are a total of 18 phytocompounds in cladode methanolic and 4 in cladode aqueous extract that have been reported in this study for the first time. Over and above these novel listings of phyto molecules, we have also found some compounds similar to those in *O. ficus-indica*, such as epicatechin and neogrifolin. Compounds such as quercetin, apigenin, isorhamnetin, and myricetin show similar expressions to that observed in *O. ficus-indica* [29,30]. There are some reports on the presence of phenolic acids such as luteolin and kaempferol in the fruits of *Opuntia humifusa* [31]. However, *O. elatior* has a different and novel accumulation being reported here, and this characterization could be an outcome of its environmental interaction and specific geographical location. There is often a lack of standardization in phytotherapeutics studies, including variations in the composition and purity of plant extracts, making it challenging to compare results between different studies. While some phytotherapeutics compounds

Table 1: TFC, TPC, DPPH scavenging activity, FRAP activity of parts of *Opuntia elatior* plant.

Samples	TFC±SEM (µg Qtn/mg)	TPC±SEM (µg GAE/mg)	DPPH IC_{50}(µg/mL)	FRAP (µg GAE/mg)
Fruit methanolic extract	87.7±0.02	182±0.21	205.9	234.2±0.09
Cladode methanolic extract	226.09±0.15	289±0.05	260.8	105±0.1
Fruit aqueous extract	97.7±0.15	134±0.0	377.2	80.5±0.05
Cladode aqueous extract	102.45±0.02	169±0.03	575.6	71.13±0.08

Data are presented as mean±SEM, with *n*=3. SEM: Standard error mean, TFC: Total flavonoid content, TPC: Total phenolic content; DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric reducing antioxidant power, GAE: Gallic acid equivalent, Qtn: Quercetin equivalent.

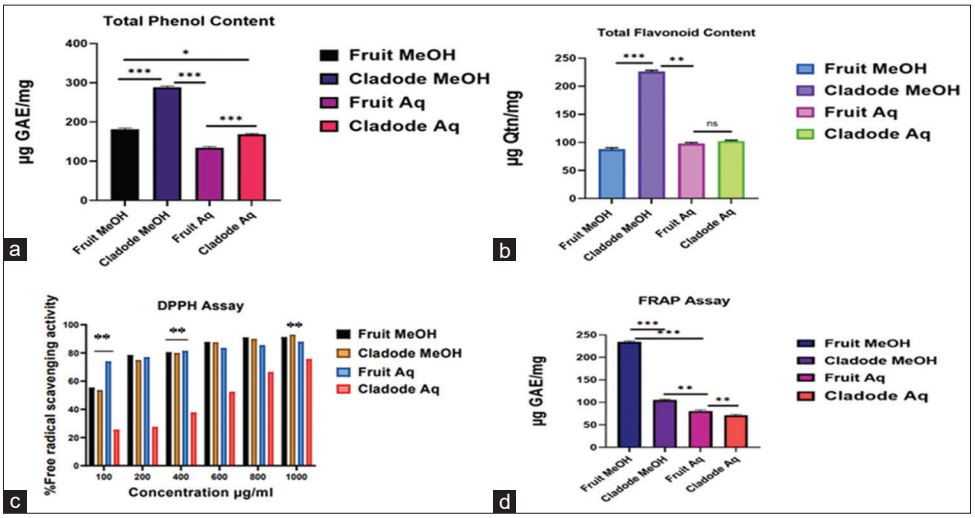

Figure 2: Quantitative estimation of phytoconstituents. Data are presented as mean ± standard error mean, with $n = 3$. (a) Total phenol content, (b) Total flavonoid content, (c) DPPH assay, (d) FRAP assay. % Free radical scavenging activity was measured at a concentration of 100–1000 µg/mL. DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric reducing antioxidant potential, GAE: Gallic acid equivalent, Qtn: Quercetin. Equivalent. Statistical difference was analyzed using the one-way analysis of variance. $*P < 0.05$, $**P < 0.01$ $***P < 0.001$, ns: Non-significant.

Table 2: High-performance liquid chromatography with tandem mass spectrometry (MS/MS) analysis of fruit methanolic extract.

Fruit methanolic extract				
Identified compound name	**Class of Compound**	**Molecular weight**	**Retention time (min)**	**m/z**
Retronecine^	Pyrrolizidine alkaloid	155.0932	1.178	156.1005
beta-Solamarine^	Steroid alkaloids	867.4946	7.673	868.5019
Quercetin*	Flavonols	302.0405	5.29	303.0477
(+)-Sophorol^	Iso flavanones	300.0613	9.107	301.0686
Glyurallin B^	Iso flavanones	422.1711	4.653	421.1645
Vicinin 2^	Flavones	626.1505	5.215	625.1432
Myricitrin*^	Flavonols	464.097	5.928	463.0897
Phloretin*^	Dihydrochalcones (Flavonoid)	274.0846	10.457	297.074
Cynaroside^	Flavones	448.1024	6.42	447.0948
Herbacetin^	Flavonols	302.0438	7.862	301.0365
Isorhamnetin*^	Flavonols	316.0592	8.176	315.0518
Luteolin*^	Flavones	286.0485	8.812	285.0413
Isokaempferide*(Apigenin)^	Trihydroxy flavone	300.0643	9.14	299.0571
Paratrifluoromethylphenol^	Phenol	162.03	4.168	163.0372
5-Hydroxyferulate*#^	Phenylpropanoids	210.0532	2.829	255.0511
Chlorogenic acid*#^	Phenylpropanoids	354.0958	4.136	353.0884
Idoxanthin^	Carotenoid	598.4004	20.062	597.3953
3-Hydroxy-b, e-caroten-3'-one^	Carotenoid	566.4108	20.654	611.4112
Vernolepin^	Sesquiterpenoids	276.0999	9.27	299.0891
Hemigossypolone^	Monoterpenoid	274.0844	10.48	297.0736
Euphornin^	Diterpenoids	584.2999	19.174	607.2893
Veranisatin C^	Terpene lactone	372.1065	5.297	371.0991
Dihydrocaffeic acid 3-O-glucuronide*#^	Phenolic glycoside	358.0882	1.078	381.0775
Osmaronin^	Cyanogenic glycosides	259.1039	1.18	260.1112
Lotaustralin^	Cyanogenic glycosides	261.1196	1.18	262.1267
Isorhamnetin 3-rutinoside 4'-rhamnoside^	Glycoside	770.2297	5.606	769.2225
D-Linalool 3-glucoside^	Glycoside	316.1899	6.84	361.1882

*Compounds also reported in *Opuntia ficus-indica* fruits. #Compounds also reported in *Opuntia humifusa*. ^Compounds reported in *Opuntia elatior* Mill. for the 1st time.

Table 3: High-performance liquid chromatography and MS/MS analysis of CM extract.

CM extract				
Identified compound name	**Class of compound**	**Molecular weight**	**Retention time (min)**	**m/z**
Retronecine^	Pyrrolizidine alkaloid	155.0932	1.152	156.101
Bellendine^	Tropane	205.109	3.898	206.116
Baptifoline^	Quinolizidine	260.1508	5.059	261.158
Irinotecan^	Quinoline alkaloids	586.2788	16.984	609.268
Senampeline A^	Pyrrolizidine alkaloid	473.2084	5.8	532.224
Ritterazine A^	Steroidal alkaloid marine	912.5407	20.31	971.5569
4'-O-methyl-(-)-epicatechin-3'-O-beta-glucuronide*#^	Flavanols	494.1445	6.755	493.137
Isorhamnetin*^	Flavanols	316.0594	8.219	315.052
Luteolin*^	Flavones	286.0485	8.85	285.041
Catechin*#^	Flavan 3-ols	290.0796	8.936	313.069
5-Hydroxyferulate*#^	Phenolic	210.0531	2.114	255.051
3-cis-Hydroxy-b, e-Caroten-3'-one^	Carotenoid	550.4139	19.676	551.422
Hemigossypolone^	monoterpenoid	274.0843	10.827	297.074
Isotenulin^	Sesquiterpenoids	306.1472	11.816	329.137
Nigakilactone B^	Triterpenoids	392.2199	11.881	415.209
Euphornin^	Diterpenoids	584.2993	19.309	607.289
Microlenin^	Sesquiterpenoids	494.2306	6.287	539.229
Cucurbitacin P^	Triterpenoids	520.3273	7.842	519.32
Geranylfarnesyl diphosphate^	Polyterpenoids	518.2574	15.96	577.272
Methyl salicylate O-(rhamnosyl-[1->6]-glucoside)^	Glycoside	460.1595	4.859	459.152
D-Linalool 3-glucoside^	Glycoside	316.19	6.823	361.188

CM: Cladode methanolic, *Compounds also reported in *Opuntia ficus-indica* fruits. #Compounds also reported in *Opuntia humifusa*. ^Compounds reported in *Opuntia elatior* Mill. for the 1st time.

Table 4: High-performance liquid chromatography and MS/MS analysis of FA extract.

FA extract				
Identified compound name	**Class of compound**	**Molecular weight**	**Retention time (min)**	**m/z**
Ibutilide^	Amino compound	384.2479	4.581	407.237
Nigakilactone B^	Quassinoid	392.2207	11.877	415.21
Militarinone A^	Pyridine alkaloid	459.26	11.895	460.267
Euphornin^	Diterpenoid	584.2996	18.434	607.289
Oryzanol^	Triterpenoid	402.3138	19.041	405.302
Leonoside A^	Glycoside	770.2535	5.635	769.246
Hydroxy-24-epi-brassinolide^	Steroid	496.3341	7.846	541.334
3-O-Methylniveusin A^	Polyphenol	408.1797	12.564	407.177
Ajmaline^	Indole alkaloid	326.2041	13.307	325.197

FA: Fruit aqueous, ^ Compounds reported in *Opuntia elatior* Mill. for the 1st time.

Table 5: High-performance liquid chromatography and MS/MS analysis of CA extract.

CA extract				
Identified compound name	**Class of compound**	**Molecular weight**	**Retention time (min)**	**m/z**
Epicatechin 3'-O-glucuronide^	Flavanols	466.109	3.094	489.08
Diferuloylspermidine^	Cinnamic acid derivative	497.250	9.344	498.5
Hypericin^	Anthraquinone	504.084	1.68	549.0
Neogrifolin^	Sesquiterpenoid	328.237	7.847	327.2

CA: Cladode aqueous, ^Compounds reported in *Opuntia elatior* Mill. for the 1st time.

have shown promise in preclinical studies, there is limited clinical data on their safety and efficacy in humans. This makes it challenging to translate these findings into clinical practice. The unidentified phytochemicals from *O. elatior* that were cataloged for their potential bioactivity represent innovative natural therapies that could be used to address different health issues.

3.3. *O. elatior* Extracts Alter the Cell Viability and Growth Kinetics of the MCF-7 Cell Line

MTT assay and dose-dependent analysis were performed to evaluate this plant's antiproliferative potential. The dose-based assay was done for three periods at 24, 48, and 72 h, respectively. The plant extracts have been evaluated on human breast cancer cell line MCF-7. The dose-time assay showed the most impressive results for aqueous fruit extract at 24 h. There is also a clear inhibition of cells with decreasing cell survival on dose increase. At 48 and 72 h, all four extracts show good control over the proliferation of cells. The results of the MTT assay have been indicated in terms of their IC_{50} values generated from their respective graphs [Figure 3]. At 24 h, both aqueous and methanolic fruit extracts indicate potency at lower doses, which advocates for their safe use in drug formulation.

Similarly, at 48 and 72 h, a similar trend of lower dose efficacy is seen. As shown in Figure 3, fruit aqueous extract shows cytotoxicity at lower concentrations after 72 h of incubation as compared to fruit methanolic extract ($P < 0.01$). The cladode methanolic extract exhibited cytotoxicity at a lower concentration after 72 h of incubation as compared to the cladode aqueous extract ($P < 0.05$). Interestingly, the normal cell line was not affected by extracts [Figure 4]. These results are the first reports; encouraging in-depth evaluation in future studies. To date, there are reports of antiproliferative activity on leukemic cell lines only [12]. Both fruit and cladode have been reported to be effective for species other than *O. elatior*. However, these studies support the current findings and pave the way for future experimentation. To further investigate the anticancer potential, scratch assay, and colony formation assays were performed. Scratch assay helps evaluate the anti-migratory action *in vitro* in real time and serves as an important characterization of plant extract for its anticancer capabilities. The wound healing (scratch) assay results as given in Figures 5 and 6, clearly support the anti-migration capability of fruit and cladode extracts. It is observable that the wound is almost healed at 72 h in Doxorubicin-treated cells, while the wound healing is even faster in the untreated control group. Compared to these groups, the most impressive outcomes are observable for fruit methanolic extract and the extract of cladode. The wound is not healed even at 72 h, suggesting their anti-migration capabilities. For the colony formation assay, the ability to colonize and grow has been investigated by incubating the cells with different plant extracts. Interestingly, all four extracts have shown better control when compared to Doxorubicin treated and untreated groups. Out of all the four extracts, the most significant action ($P < 0.001$) is observed for cladode aqueous extract when compared to untreated

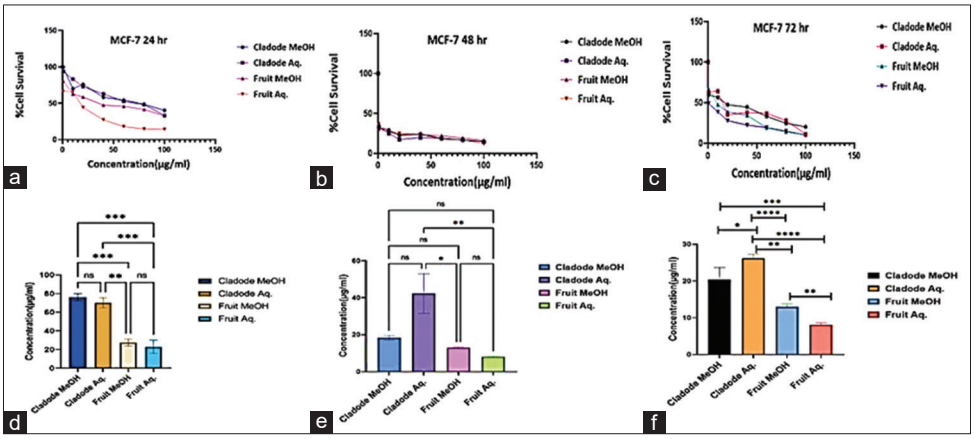

Figure 3: Antiproliferative effect of plant extracts on human breast cancer cell line MCF-7. (a-c) Dose-response curve of plant extract treatment on MCF-7 cell line at the indicated concentration (0.5–100 µg/mL) for 24, 48, and 72 h respectively. (d-f) IC_{50} concentration was derived from the graph of growth inhibition against concentration (µg/mL) from the MTT assay. Statistical difference was analyzed using the one-way analysis of variance. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns: Non-significant.

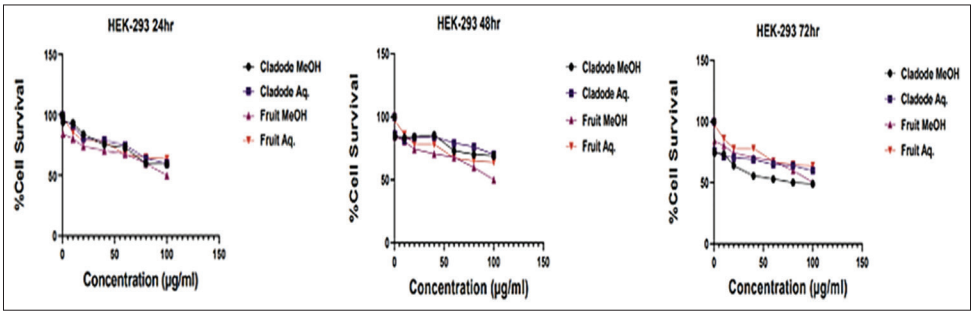

Figure 4: Safety of plant extracts on normal cell line HEK-293. Dose-response curve of plant extract treatment on HEK-293 cell line at the indicated concentration (0.5–100 µg/mL) for 24, 48, and 72 h, respectively.

cells [Figure 7]. These results are proof of the anticancer potential of *O. elatior*, and there is a need to evaluate them in greater depth to find newer therapeutic options for breast cancer and metastatic progression.

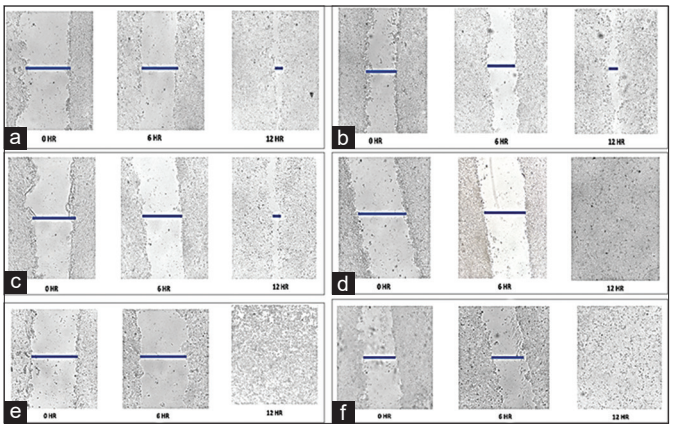

Figure 5: Anti-migratory effect of plant extracts on human breast cancer cell line MCF-7. Representative images were taken by inverted microscope for 0, 6, and 12 h at ×40 magnification. (a) Fruit aqueous extract – 8.1 µg/mL, (b) Fruit methanol extract – 12.9 µg/mL, (c) Cladode aqueous extract – 26.2 µg/mL, (d) Cladode methanol – 20.4 µg/mL, (e) Doxorubicin treated cells, (f) Untreated cells.

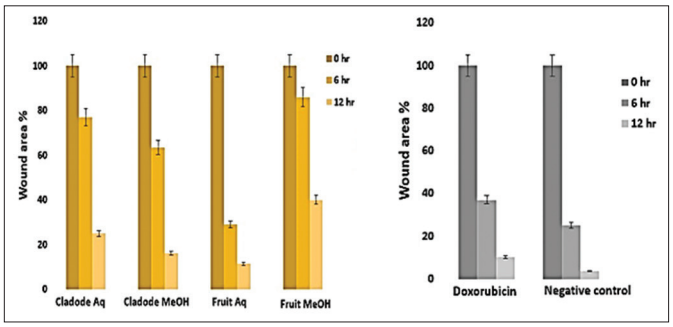

Figure 6: Effect of plant extracts on wound area percentage. Representative images show wound area percentage at 0, 6, and 24 h for the plant extract analyzed using ImageJ software. Doxorubicin is positive control and 0.2% dimethyl sulfoxide treated cell is negative control. The data are presented as mean± standard error mean with $n=3$.

3.4. Molecular Docking

Based on the promising activity of all four extracts *in vitro* assays, further investigation was done by detailing phyto molecular understanding using *in silico* techniques. To decrease the probability of choosing a false positive compound, drug likeliness properties were determined using the Lipinski filter, taking into account the compound's adsorption, distribution, metabolism, excretion, and toxicity. When logA exceeds 5, molecular weight exceeds 500, the number of N, O (hydrogen bond receptors) exceeds 10, the number of −OH and −NH (hydrogen bond donors) exceeds 5, and the number of rotatable bonds (rotb) exceeds 15, Lipinski's rule of five is violated [32]. Three important flavonoids isorhamnetin, phloretin, and herbacetin demonstrated zero violations of the range's physiochemical parameters and successfully complied with Lipinski's rule of five as stated in [Table 6]. This prediction was based on the findings of medicinal plant lead prediction through the SWISSADME tool [Table 7]. These three lead molecules were then subjected to molecular docking for their binding capabilities of Bcl-2, Bax, Caspase 9, and Cyclin D1 complexed with CDK4. The results of

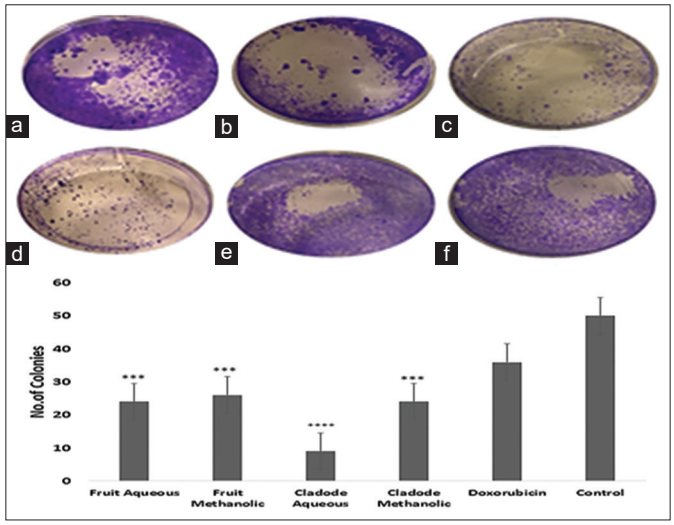

Figure 7: Effect of plant extracts on MCF-7 cell line. The cells were grown in 6-well plates and treated with IC_{50} concentration of the plant extracts. (a) Fruit methanolic extract, (b) Fruit aqueous extract, (c) Cladode methanolic extract, (d) Cladode aqueous extract, (e) Dox orubicin, (f) Control. The number of colonies was calculated using imageJ. ***$P < 0.05$, ****$P < 0.001$.

Table 6: The pharmacokinetic properties of the selected phytocompounds were performed using SwissADME online tool.

Compound name	Intestinal absorption (human) (% absorbed)	BBB permeability (log BB)	CYP2D6 substrate (Yes/No)	CYP2D6 inhibitor	Total clearance (log mL/min/kg)	AMES toxicity (Yes/No)	Oral rat acute toxicity (LD_{50}) (mol/kg)	Oral rat chronic toxicity (LOAEL) (log mg/kg bw/day)	Hepatotoxicity (Yes/No)
Isorhamnetin	76.014	−1.135	No	No	0.508	No	2.407	2.499	No
Phloretin	60.5	−0.927	No	No	0.213	No	2.381	3.318	No
Herbacetin	87.188	−1.006	No	No	0.232	No	2.585	1.89	No

Table 7: Drug-likeness prediction for flavonoid of *Opuntia elatior* Mill. fruits by SwissADME.

Compound name	Molecular weight	Log P	Rotatable bonds	Acceptors	Donors	Surface area
Isorhamnetin	316.265	2.291	2	7	4	128.792
Phloretin	274.272	2.3245	4	5	4	114.922
Herbacetin	422.477	5.2998	5	6	4	179.584

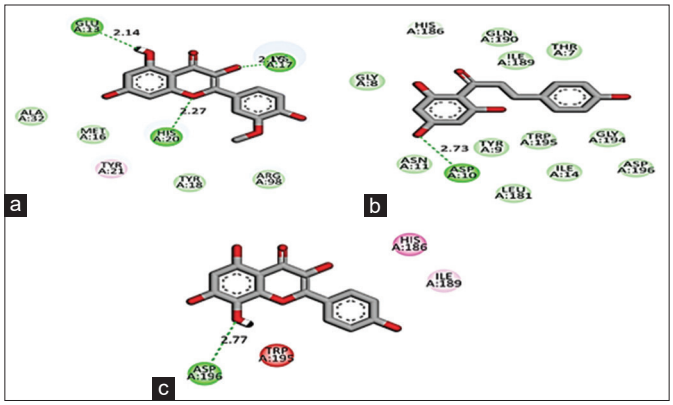

Figure 8: 2D Molecular interaction of Bcl-2 with (a) Isorhamnetin, (b) Phloretin, (c) Herbacetin. The green colour interactions represent covalent hydrogen bonds with amino acids of receptors.

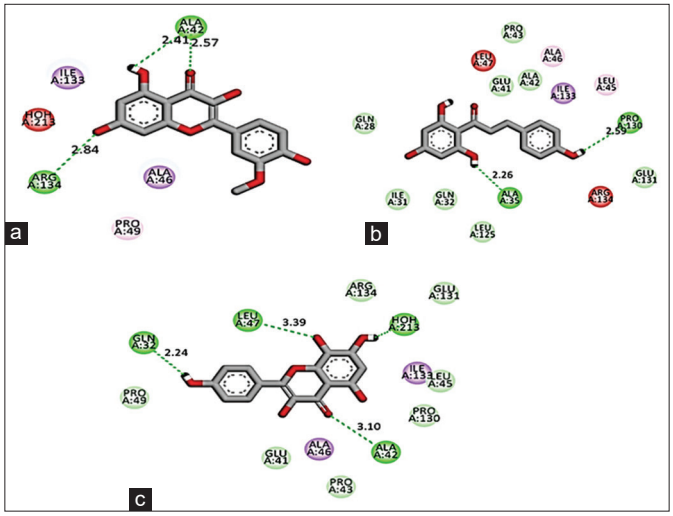

Figure 9: 2D Molecular interaction of Bax with (a) Isorhamnetin, (b) Phloretin, (c) Herbacetin. The green colour interactions represent covalent hydrogen bonds with amino acids of receptors.

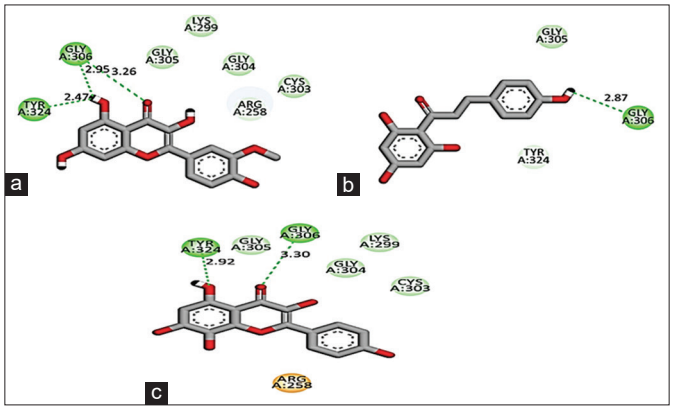

Figure 10: 2D Molecular interaction of Caspase 9 with (a) Isorhamnetin, (b) Phloretin, (c) Herbacetin. The green colour interactions represent covalent hydrogen bonds with amino acids of receptors.

these docking analyses are presented in Table 8 and Figures 8-11. The best docking results of all three isorhamnetin, phloretin, and herbacetin were towards the Bax with a strong binding affinity of −7.8, −7.3 and −7.8, respectively. This suggests that the phytomolecules could be a potential inhibitor, which would lead to the apoptotic process in breast cancer and need for further investigation to evaluate the outcomes of these studies for future drug designing. Such studies have not been reported for the *O. elatior* species.

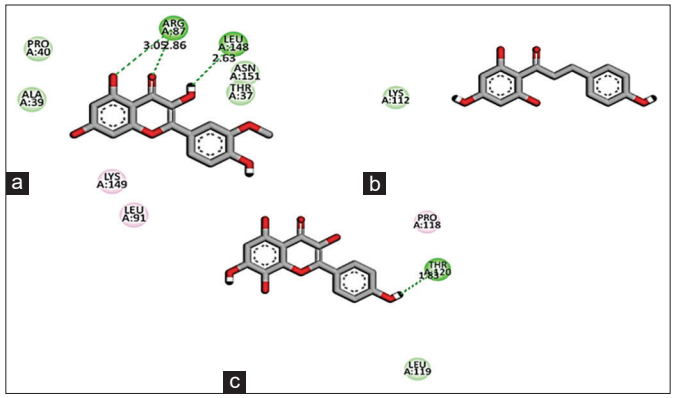

Figure 11: 2D Molecular interaction of Cyclin D1 complexed with CDK4 with (a) Isorhamnetin, (b) Phloretin, (c) Herbacetin. The green colour interactions represent covalent hydrogen bonds with amino acids of receptors.

Table 8: Binding affinity energy of phytocompounds on target.

Ligand	Target	Binding affinity energy (Kcal/mol)	H-Bond interactions
Isorhamnetin	Bcl-2	−7.4	GLU A: 14, LYS A: 17, HIS A: 20
	Bax	−7.8	ALA A: 42, ARG A: 134
	Caspase 9	−7.3	GLY A: 306, TYR A: 324
	Cyclin D1	−7	ARG A: 87, LEU A: 148
Phloretin	Bcl-2	−6.9	ASP A: 10
	Bax	−7.3	PRO A: 130, ALA A: 35
	Caspase 9	−6.3	GLY A: 306
	Cyclin D1	−6.6	No hydrogen bonds
Herbacetin	Bcl-2	−7.5	ASP A: 196
	Bax	−7.8	GLN A: 32, LEU A: 47, ALA A: 42
	Caspase 9	−7.3	TYR A: 324, GLY A: 306
	Cyclin D1	−6.9	THR A: 120

4. CONCLUSION

This study has presented some novel findings for *O. elatior* through a systematic in-depth analysis of its phytochemistry, *in vitro* capabilities of antiproliferative, anti-migratory, and anticancer capabilities. More than 20 phytocompounds are reported for the 1st time, and flavonoid fraction has been a good line for future studies based on the *in silico* findings presented herein. Further, the *in vitro* assays of all four extracts on the MCF-7 human breast cancer cell line have shown promising and significant antiproliferative action that makes this plant and its phytoconstituents an important candidate for drug discovery as well as adjuvant therapy for existing anticancer drugs for breast cancer. Another significant observation is the potent efficacy of aqueous fruit extract, which makes it a suitable candidate for anticancer formulations and use. There is also an indication of cell cycle and anti-apoptotic

pathway regulation, as revealed by the *in silico* studies. This will be further investigated in our upcoming studies. Overall, this plant needs to be considered for its anticancer candidature, and the present study presents the first evidence in that direction.

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6. AUTHORS' CONTRIBUTIONS

FP contributed to the plant material collection, extraction, *in vitro* and *in silico* assay. KU participated in an *in-vitro* assay. DM participated in the HRLCMS/MS analyses and interpretation of the data. ER participated in the formal analysis. AVR reviewed the draft and DB designed the study and finalized the draft. FP wrote the first draft of the manuscript, and all authors commented on this version. All authors have read the final manuscript and approved the submission.

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8. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVAL

This study does not involve any human or animal experimental subjects.

10. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

11. PUBLISHER'S NOTE

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