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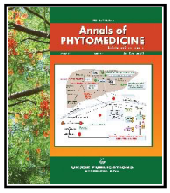

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Antitumor potential of aqueous leaf extract of *Leucocasia gigantea* (Blume.) Schott in breast cancer metastasis

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Abstract

One of the crucial components of cancer is metastasis, which spreads from the site of origin to a different portion of the body. Prior studies on the plant have shown that the medicinal elements of *Leucocasia gigantea* (Blume.) Schott, an edible plant of the genus Leucocasia, may have cancer-fighting properties. The current study aims to evaluate the aqueous leaf extract of *L. gigantea* for its cytotoxic potency on metastatic breast cancer through *in silico* and *in vitro* analysis. The study investigates *in silico* drug development techniques, such as molecular docking, molecular mechanics, absorption, distribution, metabolism and excretion property prediction, which were used to confirm the anticancer efficacy. The *in vitro*, cytotoxicity potential was studied on breast cancer metastasis cell lines MCF7 and MDAMB-231. Further, the lethality test was performed using brine shrimps to analyse the cytotoxicity potential of the extract. The phytochemical components of the plant were docked against novel metastasis suppressor proteins, *i.e.*, breast cancer metastasis suppressor 1 proteins (PDB ID:4AUV). Further, the brine shrimp lethality assay showed the cytotoxic potential of the plant with an IC_{50} value of 24.58 µg/ml. The phytochemical analysis of the extract evaluated the presence of phenolic content of 55.16 ± 6.78 mg gallic acid equivalents which signifies the anticancer potency of the plant. *In vitro* cytotoxicity potential was studied on breast cancer metastasis cell lines MCF7 and MDAMB-231. The cell-line study displayed cytotoxic potential in both cell lines but to a greater level in MDAMB-231 cell lines with an IC_{50} value of 27.10 µg/ml. Research on *in vivo* animal experiments constitutes another future potential goal of this investigation.

1. Introduction

Breast cancer is defined as an uncontrollable proliferation of breast tissue (Peterson *et al*., 2011; Nielsen *et al*., 2021). Breast cancer is the second most prevalent and frequent cancer among women worldwide (Trivedi *et al*., 2023; Gupta *et al*., 2016; Sung *et al*., 2021; Salam *et al*., 2022). With an expected 2.3 million new cases (11.7%), female breast cancer has surpassed lung cancer as the most commonly diagnosed malignancy. The survival rates for breast cancer have grown as a result of earlier discoveries through increasing awareness and screening, technological developments, increased self-examination, and improvements in therapy (Battaglini *et al*., 2008). Despite recent increases in survival rates, breast cancer treatment can have many unfavourable side effects. Acute side effects of treatment can endure for a few days or weeks, whereas persistent side effects can last for years after the end of the treatment. The adverse effects of breast cancer surgery are pain, infection, discomfort, bleeding, transient swelling, weight fluctuations, nausea, hair loss, exhaustion, vomiting, and an increased risk of infections. Patients who receive radiation treatments may experience discomfort, exhaustion, skin changes, and oedema. Hot flashes, exhaustion, vaginal soreness, and mood swings are some side effects of hormone therapy (Jacobsen and Stein, 1999; Shapiro and Recht, 2001; Georgalas *et al*., 2015; Palesh *et al*., 2018; Bhargava, 2021; Han *et al*., 2022). Additionally, cancer treatments like chemotherapy and radiation, which can result in the necrotic death of cancer cells and adjacent tissues, can trigger a potent tumour-associated inflammatory response (Greten and Karin, 2010). Metastatic cancer is a crucial component of cancer, in order to investigate it further cell lines are frequently employed as models, however, it is unclear how well they replicate the condition in patients (Leung *et al*., 2014; Farokhimanesh *et al*., 2021). The MCF7 cells account for 43.6% of all PubMed citations in studies on metastatic breast cancer and MDAMB-231 one of the most often utilised triple-negative cell lines for research on metastatic breast cancer comes after MCF7 (40.2% of all PubMed citations) (Liu *et al*., 2019). Natural products, such as chemicals produced from plants due to their diverse chemical composition, demonstrated notable antibreast cancer characteristics among medications that can be created to treat the disease (Vutakuri *et al*., 2018; Poofery *et al*., 2020; Cai *et al*., 2021; Azfaralariff *et al*., 2022; Arif *et al*., 2022).

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The present study is focused on the development of plant-based approach in the treatment of breast cancer *in silico* as well as *in vitro* lethality tests. The experimental plant in this study, *L. gigantea* which belongs to the family Leucocasia, is a flowering plant genus under the Araceae family, native to south-eastern Asia and the Indian subcontinent which is widely cultivated and naturalized in other tropical and subtropical regions and has been demonstrated to cure a variety of ailments, including fever, infections, wound healing, sleepiness, tuberculosis, stomach issues, *etc.* (Devi and Jagetia, 2017). *L. gigantea* is mostly used as a vegetable crop in the northern region of Bangladesh, the traditional uses of this herb's taros include treating stomach issues, drowsiness, phlegm, atrophy, emaciation, wound healing, infection, drowsiness, tuberculosis, and constipation as well as stopping arterial haemorrhage and is well-known due to its various healing powers (Franco *et al.,* 2022; Long *et al*., 2015). The plant has been reported to possess free radical scavenging and antioxidant potential, antimicrobial (Sooklert *et al.*, 2017), antidiarrheal (Alam *et al.*, 2021), dermatological uses (Behr *et al.*, 2006), analgesic (Zilani *et al.*, 2021), anti-inflammatory (Sooklert *et al.*, 2017) and anticancer (Pornprasertpol *et al.*, 2015; Sooklert *et al.*, 2017) properties. The findings above have led to this investigation on the metastatic breast cancer potential of *L. gigantea* as a medicinal plant through *in vitro* and *in silico* studies (Gupta *et al.*, 2019).

Therefore, *in silico* techniques, such as molecular docking, MMGBSA, and ADME property prediction, were used to confirm the anticancer efficacy of *L. gigantea* in breast cancer metastasis. The phytochemical components of the plant were docked against the BRMS1 (breast cancer metastasis suppressor) proteins (PDB ID:4AUV). The process by which *L. gigantea* and BRMS1 protein slows the progression of cancer cells was studied. Brine shrimp lethality assay was also involved in studying the potential cytotoxic effects of the extract on the brine shrimps when compared with the standard drug doxorubicin. The current study also investigated the aqueous leaf extract of *L. gigantea* for its cytotoxicity potential on breast cancer metastasis using cell lines MCF7 and MDAMB-231 (Dai *et al.*, 2017; Lashgarian *et al.*, 2020; Yýldýrým *et al.*, 2022). Further future objective of this study is to work on *in vivo* animal studies.

2. Materials and Methods

2.1 *In silico* studies

The novel metastasis suppressor protein, breast cancer metastasis suppressor 1 (BRMS1), lacks anti-proliferative action. The crystal structure of the N-terminal portion of BRMS1 is 4AUV. According to accumulated data, the interaction with chromatin remodelling and inhibition of nuclear factor-kappa (NF-B) activity are two key mechanisms for suppressing BRMS1-induced cancer metastasis. NF-B, a well-known transcription factor that is important in the development of tumours, is inhibited by the breast cancer metastasis suppressor 1 (BRMS1). In order to downregulate the expression of EGFR in breast cancer cells, BRMS1 upregulates miR-146a and binds the enzyme histone deacetylase 1 (HDAC1) to NF-B consensus binding regions. Additionally, it has been suggested that BRMS1 functions as an E3 ligase, which inhibits pulmonary metastasis. Thus, our investigations elucidate the process by which BRMS1 slows the progression of cancer cells (Sangeetha *et al*.,2014). Target gene alignment of 4AUV, the 3D structure of 4AUV was retrieved from the protein data bank (PDB). The structures of plant phytocomponents were retrieved from ChEBI database https://www.ebi.ac.uk/chebi/ (Figure 1).

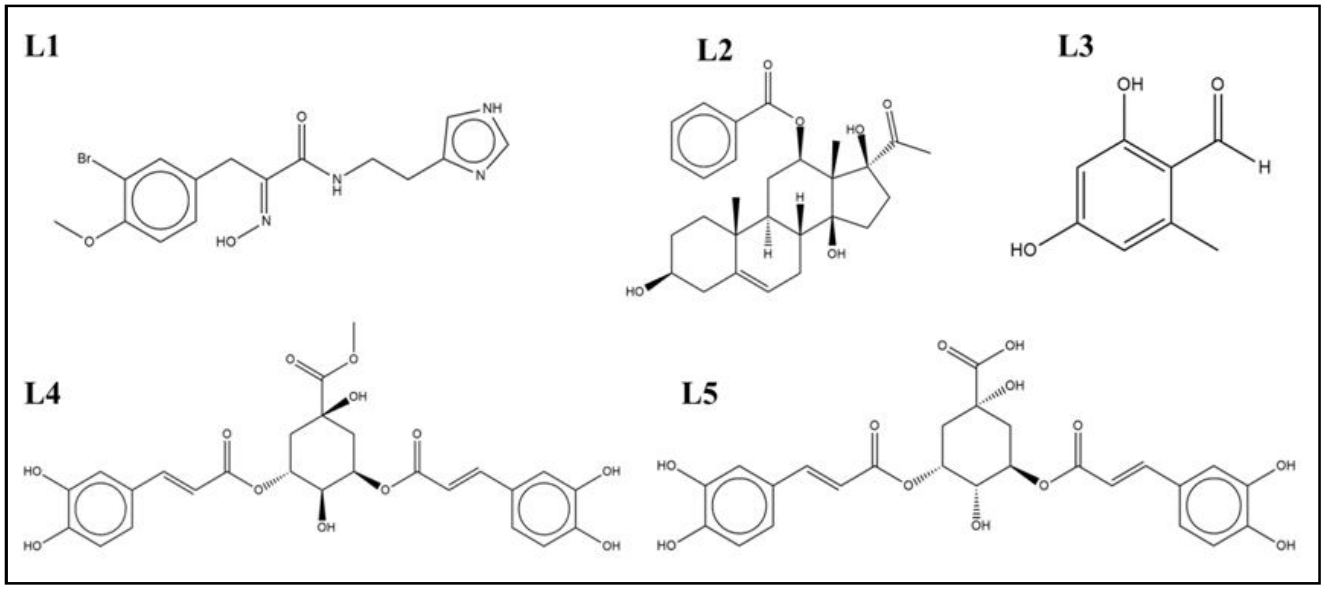

Figure 1: Structures of compounds L1: Verongamine, L2: Calotropone, L3: 2,4-dihydroxy-6-methyl benzaldehyde, L4: Methyl 3,5di-O-caffeoyl quinate, L5: 3,5-di-O-caffeoyl quinic acid.

2.1.1 Protein preparation

The protein generated from the protein data bank (RCSB; https://www.rcsb.org/) has the PDB ID:4AUV. To determine the protein target, the protein preparation wizard of Schrodinger suite 2019-2 was employed. The preparation involved the assignment of the hydrogen bonds, bond orders, addition of hydrogens, optimization, minimization of the proteins and deletion of waters beyond 5Å. Hydrogen bond assignments, bond orders, hydrogen addition, protein optimization, protein minimization, and water removal beyond 5Å were all part of the process (Arthur *et al.*, 2018).

2.1.2 Formation of the ligand

The 3D structures of all 5 ligands are prepared using Schrödinger Maestro software. The LigPrep module of the Schrodinger suite

2019 was used to prepare the ligand structures for the data set. The docked structures must accurately reflect the real ligand structures as they look in a protein-ligand complex to produce the best results. This indicates that the structure must fulfill the following requirements in order for glide to dock. A three-dimensional (3D) form is required. The rest of the geometric parameters must be tuned before docking because glide only changes the ligand's torsional internal coordinates. Each of them must be made up of a single molecule without any covalent connections to the receptor and without any accompanying fragments (Hussain *et al.*, 2023).

2.1.3 Receptor grid generation of glide ligand

The energy grid extension and conformation search number for the Monte Carlo trial were both set to "0" and 5Å, respectively. Ten ligand poses were permitted in the receptor cavity, and docking was performed with the default values for the other input parameters**.** Using the glide score, the most effective ligands for entrapment are chosen (G-score). Using the glide ligand docking module, the active interactions between ligands and the target receptor or enzyme were assessed. Finally, a few spots were rescored using the G-score scoring feature. The outcomes of the molecular docking were examined using the XP visualizer in the glide module (Maddali *et al.*, 2021; Hussain *et al.*, 2023).

2.1.4 Molecular docking

In the current research, the BRMS1 protein, a 4AUV metastasis suppressor, was docked with a group of phyto-constituents from *L. gigantea*. Constituents with high binding affinities were then docked with the standard drug doxorubicin (Shukla *et al.*, 2012; Sun *et al.*, 2016; Maddali *et al.*, 2021; Shoba *et al.*, 2022).

2.1.5 Prediction of Lipinski's rule of five and drug-likeness score

The drug-likeness number forecasts the compounds' drug likeliness and Lipinski's rule of five, which aids in understanding the compounds' potential and characteristics. The mol software tools (https://molsoft.com/mprop) projected the molecular weight, donor Hb, acceptor Hb, QplogPo/w, and polar surface area (Saghiri *et al.*, 2023).

2.1.6 Energy calculation for bindings with MM-GBSA

The dissolvable model VSGB, the OPLS3 power field, and the Schrodinger suite 2019-2 Prime module were used to process the binding free energy of the drug-receptor complex *via* MM-GBSA (Sun *et al.*, 2016).

2.1.7 ADMET prediction

The absorption, distribution metabolism, excretion and toxicity (ADMET) properties of the data set were calculated theoretically by QikProp. Around ten physically important and pharmacological features of the compounds were examined by QikProp and the Swiss ADMET online web tool (http://www.swissadme.ch/h) (Sun *et al.*, 2016).

2.2 *In vitro* studies

2.2.1 Chemicals and cell lines

The National Centre for Cell Science in Pune, India, was where the cell lines MDAMB-231 and MCF 7 cell lines were purchased. Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM-high glucose), RPMI media, MTT reagent (5mg/ml) and phosphate-buffer saline (PBS) were purchased from HI Media in India. The chemicals quercetin, gallic acid (GA) and Folin-Ciocalteu reagent (FCR), DMSO were supplied by Sigma-Aldrich.

2.2.2 Collection and preparation of plant

The plant *L. gigantea* was collected from western ghat regions of Kerala, India (geographical location is 12° 302 03 N, 75° 02 03 E) and identified by taxonomist Dr E. J. Josekutty, Department of Botany, Government College Kasaragod, Kasaragod-671121, Kerala, India. A voucher specimen was deposited in the herbarium of the department (Herbarium no: 21PH103R). Following collection, the leaves underwent a thorough rinsing with fresh water before drying for 15 days at room temperature. It was then processed through a grinder to make a fine powder (Philips, India). For later use, the produced powder was kept in an airtight jar.

2.2.3 Preparation of extract

The fresh leaves of *L. gigantea* material were washed and dried at room temperature. The air-dried leaf material was ground and subjected to maceration. For extraction, the powdered leaf material was soaked in water and kept aside for 7 days with occasional stirring. After 7 days, the content was filtered. The filtrate was subjected to evaporation using a rotary vacuum evaporator to a syrupy consistency and then lyophilized (freeze drying) using a lyophilizer to powdery form and stored in desiccators until further use (Ghanem *et al.*, 2019; Oroian *et al.*, 2020; Sapiun *et al.*, 2020; Vasava *et al*., 2022).

2.2.4 Estimation of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu assay. An aliquot (1 ml) of the extracts or standard solutions of gallic acid (15.624, 31.25, 62.5, 125, 250, and 500 µg/ml) was put to a 25 ml volumetric flask with 9 ml of distilled water. A reagent blank was made using distilled water. One ml of the Folin-Ciocalteu phenol reagent was added to the mixture, and it was then stirred. 10 ml of a 7% Na_2CO_3 solution was added to the mixture after 5 min. The volume was then adjusted to the proper level. A UV/Vis spectrophotometer was used to measure the absorbance at 765 nm in comparison to the reagent blank following a 90 min. incubation period at room temperature. The total phenolic content was calculated as mg gallic acid equivalents (GAE) (Singleton and Rossi, 1965).

2.2.5 Estimation of total flavonoid content

The total flavonoid concentration was found using the aluminium chloride colourimetric test. An aliquot (1 ml) of extracts or standard solutions of quercetin were added to a 10 ml volumetric flask along with 4 ml of distilled water (15.624, 31.25, 62.5, 125, 250, and 500 µg/ml). After a period of five min, 0.3 ml of 10% $AlCl_3$ and 0.30 ml of 5% $NaNO_2$ were added to the flask. 2 ml of 1M NaOH and 10 ml of distilled water were added to the mixture after waiting for five min. The absorbance of the combination was assessed in relation to a blank at 420 nm. The total flavonoid content was calculated using the quercetin equivalents (QE) unit of measurement, represented as mg (Zhishen *et al.*, 1999).

2.2.6 Cytotoxicity study: MTT assay

2.2.6.1 MCF 7 cell lines

Seeding of cellines: The cells were subcultured in tissue culture flasks with RPMI 1640 medium supplemented with 10% fetal bovine

serum, 1% penicillin-streptomycin, and 1% non-essential amino acids. The tissue culture flasks were then placed in a CO_2 incubator and incubated in an environment with 5% CO_2 and 95% humidity. Following trypsinization, a cell counter was used to do a cell count and test the viability of the cells using trypan blue.

MTT assay: For the MTT experiment, cells were seeded onto 96-well plates. The aqueous extract of *L. gigantea* was subjected to cytotoxic assay using MCF7 cell lines. The test chemicals were made in distilled water. The media were used to dilute the reactant mixtures. Cells were exposed to the extract at varying doses (ranging from 25, 50, 100, 250, and 500 µg/ml) and incubated for 24 h. The effect induced was also compared with the standard drug doxorubicin (Dhru *et al.*, 2016; Desai *et al.*,2021; Pal *et al.*, 2019; Imaduddin *et al.*, 2020; Mishra *et al.*, 2022) was analyzed and the percentage cytotoxicity was calculated.

% Cytotoxicity= (absorbance of control –absorbance of test/ absorbance of control) × 100

2.2.6.2 MDAMB-231 cell lines

Seeding of cell lines: In tissue culture flasks of T25 and T75, the cells were sub-cultured in DMEM media supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% non-essential amino acids. The flasks were then placed in a CO_2 incubator and incubated in an environment with 5% CO_2 and 95% humidity. Following trypsinization, a cell counter was used to do a cell count and test the viability of the cells using trypan blue.

MTT assay: In order to do the MTT experiment, cells were seeded onto 96-well plates. The aqueous extract of *L. gigantea* was subjected to cytotoxic assay using MDAMB-231 cell lines. The test chemicals were made in distilled water. The media were used to dilute the reactant mixtures. Cells were exposed to the extract at varying doses (ranging from 25, 50, 100, 250, and 500 µg/ml) and incubated for 24 h. The effect induced was also compared with the standard drug doxorubicin. The results were analyzed and the percentage cytotoxicity was calculated (Swaminathan *et al.*, 2022; Fathi *et al.*, 2023).

% Cytotoxicity= (absorbance of control –absorbance of test/ absorbance of control) × 100

2.2.7 Morphological study

Cells of MCF7 and MDAMB-231 were examined and photographed for documentation of morphological characteristics under a phase contrast microscope.

2.2.8 Brine shrimp lethality assay

The Meyer *et al.* (1982) method for the brine shrimp lethality test was used, with minor modifications. The cytotoxicity of the plant extract was evaluated at concentrations of 1, 10, 100, and 1000 ppm in solutions of seawater in 3 ml. Each test employed ten nauplii, and after 24 h, the number of surviving was recorded. For every concentration, three replications were employed. Distilled water is used for the blank control. As a gauge of the extract's or fractions' toxicity, the chronic LC_{50} the lethal concentration for 50% mortality after 24 h of exposure was calculated (Olowa *et al.*, 2013; Bhatti *et al.*, 2015; Balinado *et al.*, 2019; Sadhvi *et al.*, 2020).

Percentage mortality = $[(n_0 - n_1)/n_0]/100$

n_0 = no of nauplii taken

n_1 = no of nauplii dead

3. Results

3.1 *In silico* studies

The drug resemblance property influences oral absorption, and this parameter is regarded as one of the primary probabilities, followed by the number of free rotatable bonds. Except for the phyto-constituent methyl 3,5-di-O-caffeoyl quinate (L4), all of the bioactive phytoconstituents follow Lipinski's rule of five. Two violations of Lipinski's RO5 are permissible for orally active drugs, as stated in Table 1. As a result, the ability of all phytoconstituents to respect this rule was tested, and all phytoconstituents obey Lipinski's RO5. The drug-likeness of the molecule was determined by predicting *in silico* physicochemical properties.

Table 1: Lipinski rule of five and drug likeness score of bioactive L1-L5

Phytoconstituents	MW	Log p	Donor HB	Accept HB	Rule of five	Drug likeness score
Acceptable range	d ≤ 500	>5	d ≤ 5	d ≤ 10	d ≤ 5	d ≤ 2
L1	381.22	1.38	3	5	0	0.55
L2	468.58	3.61	3	6	0	0.55
L3	152.15	1.35	2	3	0	0.55
L4	530.48	1.91	6	12	3	0.17
L5	516.45	1.05	7	12	3	0.11
Doxorubicin	543.52	2.16	5	14.8	3	0.17

MW- Molecular weight; Log p – lipophilicity; Donor HB - Estimated number of hydrogen bonds; Accept HB- Estimated number of hydrogen bonds.

3.1.1 Molecular docking

The glide scores of the phyto-constituents' affinities for various receptors are displayed in Table 2 (PDB ID: 4AUV). Docking experiments were utilised to identify the binding mechanism of each phytoconstituent into the receptor's active site at the atomic level. Table 1 (B) lists the docking scores of 5 phytoconstituents. The binding free energies of phytoconstituents range from -4.92 to -1.76 kcal/mol. The chosen receptor 4AUV contains the following active residues: Glu68, Ser72, Lys75, Glu76, Leu78, Phe79, and Arg82. L5 comes in second with a G score of -3.48 kcal/mol, while L4 has the highest G score of -4.92 kcal/mol for the phytoconstituent. Figure 2 depicts the docking of L1 to L5 in 2D and 3D using 4AUV which establishes hydrogen bonds with Glu68, Glu76, and Leu78 in the L4 whereas L5 forms hydrogen bonds with Glu68 and Glu76.

Table 2: Docking scores for compounds (L1-L5) with PDB ID: 4AUV

Compounds	Glide score	Glide EvdW	XP H bond	Glide emodel	G rotatable bonds	glide ecoul	glide energy
L1	-3.198	-20.451	-0.579	-33.279	9	-8.119	-28.570
L2	-1.767	-19.961	0.000	-26.561	7	0.587	-19.373
L3	-2.476	-11.004	-1.401	-19.271	3	-14.328	-17.897
L4	-4.921	-20.479	-1.920	-44.331	16	-22.509	-42.988
L5	-3.487	-20.096	-1.847	-45.980	15	-16.729	-36.826
Doxorubicin	-2.170	-17.988	-1.968	-48.719	10	-18.204	-37.383

Glide EvdW: glide van der Waals energy; XP H Bond: extra precision hydrogen bonding; Glide emodel: glide model energy; G Rotatable bonds: Glide Rotatable bonds; Glide ecoul: glide Coulomb energy.

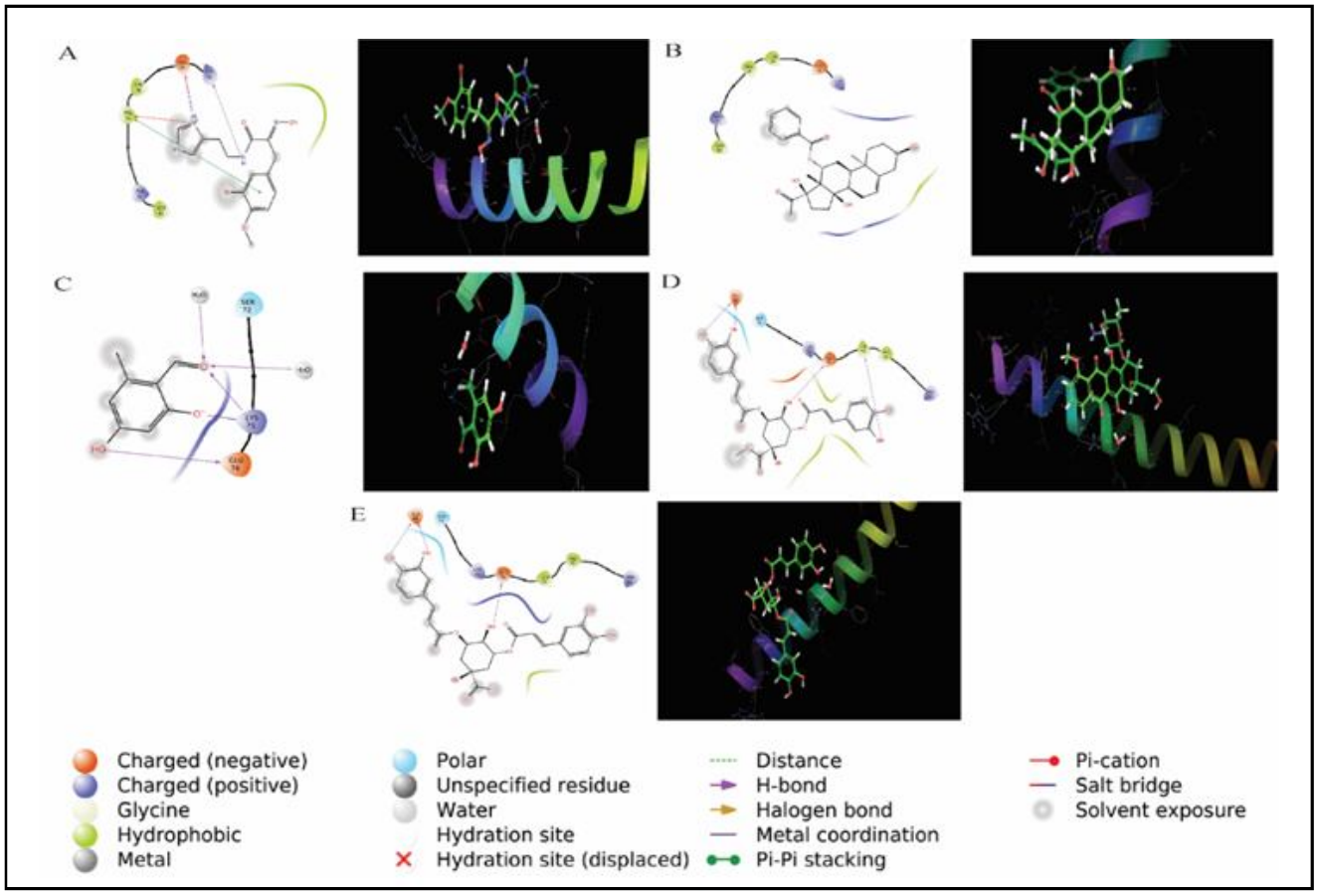

Figure 2: Molecular docking of components. The figures depict the docking of L1 to L5 in 2D and 3D using 4AUV, establishes hydrogen bonds with Glu68, Glu76, and Leu78 in the L4, whereas L5 forms hydrogen bonds with Glu68 and Glu76. A: Verongamine (L1), B: Calotropone (L2), C: 2,4-dihydroxy-6-methyl benzaldehyde (L3), D: Methyl 3,5di-O-caffeoyl quinate (L4), and E: 3,5-di-O-caffeoyl quinic acid (L5).

3.1.2 MM-GBSA study

The target protein and the compatible protein were created in accordance with the Figure 1 structure. The suggested analogues all had large free binding energies and suited the PARP-1 receptor effectively (Table 3). The prime MM-GBSA analysis demonstrates the relative energies of every compound's binding on the receptor. It comes about as a result of several drug-receptor interactions, including polar interactions, hydrophobic interactions, covalent bond connections, and so forth. When compared to the known standard drug, doxorubicin, which has a value of -55.19 kcal/mol, compound L5 has the highest G binding energy at a value of -59.02 kcal/mol. Van der Waals energy (GvdW) and non-polar solvation (GLipo) are the energies that firmly ligand bind in the binding pocket of 4AUV due to the substantial negative values recorded by all of the compounds. Other energies, such as electrostatic solvation energy (GSolv) and covalent energy (GCov), do not significantly benefit receptor binding. Compounds L5 and L4 exhibit highly preferred ligand binding (GCoul values are -55.18 and -59.02 kcal mol-1). This conclusion is also related to the G score because compound L5 had

the greatest docking score, showing that coulomb energy is important in the drug-receptor interaction. The MM-GSBA experiment reveals that the chemicals L5 and L4, which bind to the receptor, have high binding affinities.

Table 3: Binding free energy calculation using prime/MM-GBSA approach

Phyto-constituents	ΔG bind (kcal/ mol)	ΔG bind Coulomb	ΔG bind covalent	ΔG bind vander	ΔG bind H bond	ΔG bind lipophilic
L1	-31.69	-10.51	6.81	-48.76	-0.23	-21.74
L2	-37.89	-16.08	3.38	-13.03	-0.24	-17.65
L3	-46.04	-32.80	3.84	-79.84	-0.21	-9.65
L4	-55.18	-0.54	9.32	-22.49	-0.39	-26.70
L5	-59.02	-7.03	7.26	-78.49	-0.77	-14.85
Doxorubicin	-55.19	-4.03	3.88	-46.54	-0.77	-41.15

Ä G bind: free energy of binding; ÄG bind Coulomb: Coulomb energy; ÄG bind covalent: covalent energy (internal energy); ÄG bind Vander: van der Waals energy; ÄG bind H Bond: hydrogen bonding energy; ÄG bind Lipophilic: hydrophobic energy (non-polar contribution estimated by solvent accessible surface area).

3.1.3 Pharmacokinetic studies

3.1.3.1 Analysis of ADMET properties by QikProp

The QikProp was used to predict the pharmacokinetic features of bioactive phytoconstituents, medication transport, and beginning of action, which includes the blood brain barrier (BBB). Table 4 shows bioavailability, penetration, metabolism, and solvent accessible surface area. When compared to the standard, the majority of the screened biomolecules had good human oral absorption properties. Nonetheless, the biomolecules demonstrated excellent oral absorption. Solubility is regarded as an important property in systemic circulation in order to achieve the appropriate concentration. All of the biomolecules' expected aqueous solubility was found to be within acceptable limits.

The apparent Caco-2 cell permeability is predicted by QPPCaCO-2. Caco-2 cells are discovered to be extremely important for the gut-blood barrier. All of the biomolecules demonstrated high intestinal permeability. QikProp also aids in estimating the likely number of metabolic processes, which eventually aids in the drug's delivery to the final target site. All of the phytoconstituents examined are within the acceptable range. The projected BBB penetration property is determined to be good for all biomolecules, and all constituents fall within the recommended values with no deviations. The Madin-Darby canine kidney (MDCK) is a key imitator of the BBB. This measure also aids in forecasting BBB penetration. The Log KHSA measure aids in determining a drug's ability to bind to human serum albumin. The QPlogKhsa scores showed that all of the compounds bound to human serum albumin were within the acceptable range. Other criteria such as FISA, FOSA, and SASA were tested and found to be within acceptable limits. If the values are found to be on the lower side, it suggests that the compounds have a smaller hydrophilic component of SASA, *i.e.*, SASA on N, O, and H hetero atoms.

Table 4: Pharmacokinetic properties of bioactive phytoconstituents by Qikprop

Phytoconstituents	% HOA	QPlogS	QPPCaco	#Metab	QplogBB	QPPMDCK	QPlogKhsa	SASA	FOSA	FISA
Acceptable range	>80% High, <25%low	(-6.5-0.5)	<25Poor, >500great	(1-8)	(-3 to 1.2)	<25Poor, >500great	(-1.5 to 1.5)	(300-1000)	(0.0-750)	(7.0-330)
L1	87.28	-4.092	347.114	3	-1.459	398.423	-0.232	631.174	197.961	160.436
L2	100	-5.149	580.707	5	-0.973	274.926	0.624	690.097	363.619	129.911
L3	76.782	-1.329	280.522	3	-0.915	125.218	-0.48	347.468	86.146	166.496
L4	4.315	-5.328	2.871	6	-4.886	0.885	-0.236	886.254	225.435	373.078
L5	100	-4.44	7008.827	6	-5.364	4058.752	-0.632	836.444	116.729	429.7
Doxorubicin	0	-2.344	4.281	9	-2.606	1.507	-0.554	770.496	344.425	291.18

% HOA: Per cent humal oral absorption; QPlogBB: Predicted brain/ blood partition coefficient; QPPCaco: Predicted apparent Caco-2 cell permeability in nm/sec; SASA: Solvent accessible surface area; FOSA: Hydrophobic component of SASA; FISA: Hydrophilic component of SASA; QPlogS: Logarithm of the partition coefficient of the compound between n-octanol and water.

3.1.3.2 Analysis of ADME prediction by Swiss ADME

All five phytoconstituents' ADME characteristics (Table 5) were determined using ADME online web tools. The TPSA value is extremely closely connected to bioavailability and hydrogen bonding potential. The total polar surface area (TPSA) of all the investigated ligands is less than 150, indicating very strong polarity with membrane penetration and good oral absorption. The absorption parameter, which includes the total PSA of the investigated ligand, ranges from 202.62 to 42.89. All of the other chemicals were found to be within permissible limits.

The results demonstrate that none of the examined active components had a P-gp substrate. The log P consensus spans from 5.11 to -0.5. The L5 has the highest log P value, followed by L4, which is 3.05 and

2.91, respectively. The log Kp values of all the tested constituents range from -3.89 to -8.95, indicating substantial skin permeability. Values greater than -2.5 indicate low skin permeability. The active components L3 have excellent permeability to the BBB. All of the other chemicals have a high penetration rate. Isoenzyme inhibitors of various subtypes, including CYP2C19, CYP1A2, CYP2D6, CYP2C9, and CYP3A4, are a key cause of drug-drug interactions in pharmacokinetics. The results show that the phytoconstituents did not affect CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4. Drug elimination is linked to the molecular weight and solubility of drug molecules. The predicted results show that all of the phytoconstituents adhere to Lipinski's guidelines and are drug-like candidates.

Table 5: Prediction of ADMET properties of selected ligands using Swiss ADME

Phyto-constituents	TPSA	Log Kp (cm/s)	Log p	GI absorption	BBB permeation	CYP1A2 inhibitors	CYP2C19 inhibitors	CYP2C9 inhibitors	CYP2D6 inhibitors	CYP3A4 inhibitors	P-gp substrate
L1	99.6	6.77	1.38	Low	Low	Yes	No	No	No	No	No
L2	104.06	-7.09	2.61	High	Low	No	No	No	No	Yes	Yes
L3	57.53	-6.1	1.35	High	high	No	No	No	No	Yes	No
L4	200.28	-8.22	2.91	Low	Low	No	No	No	No	No	Yes
L5	211.28	-8.37	3.05	Low	Low	No	No	No	No	No	Yes
Doxorubicin	206.07	-8.71	2.16	Low	Low	No	No	No	No	No	Yes

TPSA: Total polar surface area; Log Kp: Skin permeation value; GI: Gastrointestinal; BBB: Blood brain barrier; CYP: Cytochrome-P; P-gp: P-glycoprotein.

3.2 *In vitro* studies

3.2.1 Percentage yield

The weight of the extract was noted and the percentage yield was calculated. The yield was found to be 35%.

3.2.2 Total phenolic and flavonoid content

The total flavonoid content of aqueous extract of leaves of *L. gigantea* was quantified as mg quercetin equivalents (QE) and total phenolics content as mg gallic acid equivalents (GAE). The Total phenolic content was found to be 55.16 ± 6.78 mg GAE and the total flavonoid content was found to be 14.723 ± 1.715 mg QE.

3.3 Cytotoxicity study: MTT assay

The cytotoxic potential was studied by using MTT assay. The study is done using MCF 7 and MDAMB-231 cell lines. The percentage of cytotoxicity was calculated. Figure 3 shows the morphological analysis of untreated cells versus the treated cells with the extract of *L. gigantea* and the standard drug doxorubicin. It is identified from figure 4 that the extract and the standard drug doxorubicin showed cytotoxic effects on MDAMB-231 and MCF 7 at their IC_{50} values. The treated cells showed morphological variation and the normal cells displayed normal morphology with homogenous and equal cell surface at 24 h under phase contrast microscopy.

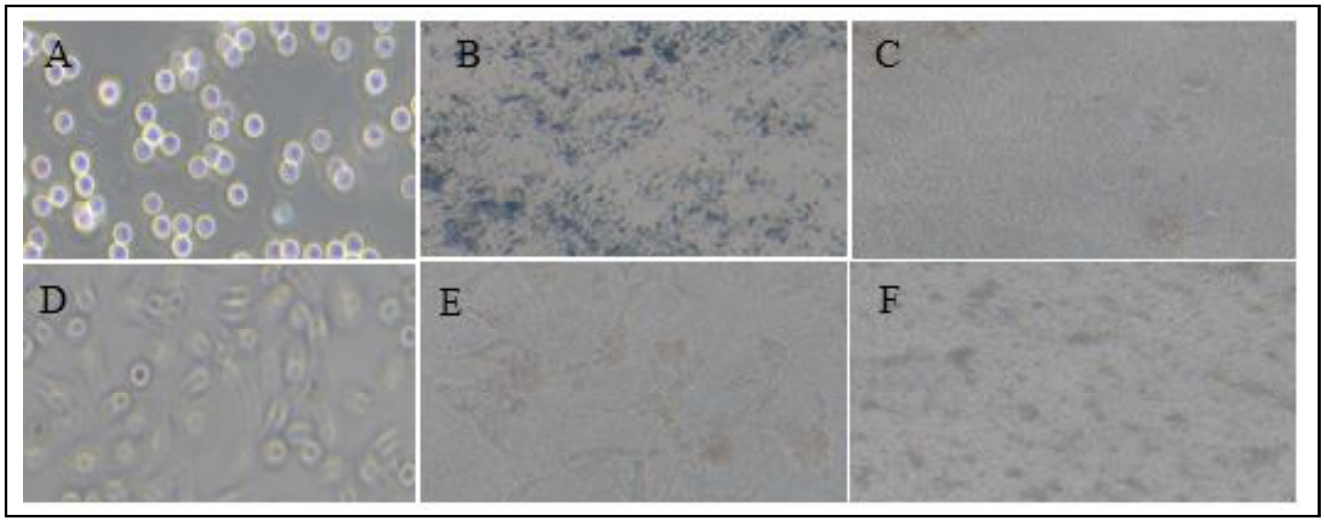

Figure 3: Phase contract images of the MCF 7 cell lines **normal (A), LG extract treated (B), Doxorubicin treated (C); MDAMB-231 cell lines normal (D), LG extract treated (E) and Doxorubicin treated (F) cell lines.** The cells were seeded in respective media at a density of 5×10^4 viable cells/cm^2, and they were raised in an incubator at 37°C in a humidified environment with 5% CO_2 in the air. 10X magnification was used to capture these images.

3.3.1 MCF 7 cell lines

The experiment demonstrated that the extract of *L. gigantea* has significantly decreased the cell viability. It has displayed an IC_{50} value of 57.75 µg/ml for 24 h of drug exposure. Similarly, as per the results the standard drug doxorubicin displayed an IC_{50} value of 22.66 µg/ml for 24 h of exposure (Figures 3 and 4).

3.3.2 MDAMD-231 cell lines

The triple negative cell line MDAMB-231, whose cell viability has decreased prominently by the extract. The IC_{50} value is of 27.10 µg/ml for 24 h of drug exposure. Similarly, the standard drug doxorubicin displayed an IC_{50} value of 17.88 µg/ml for 24 h of exposure (Figures 3 and 4). The effectiveness as a potent anticancer agent is evident from the results when compared with the standard drug doxorubicin.

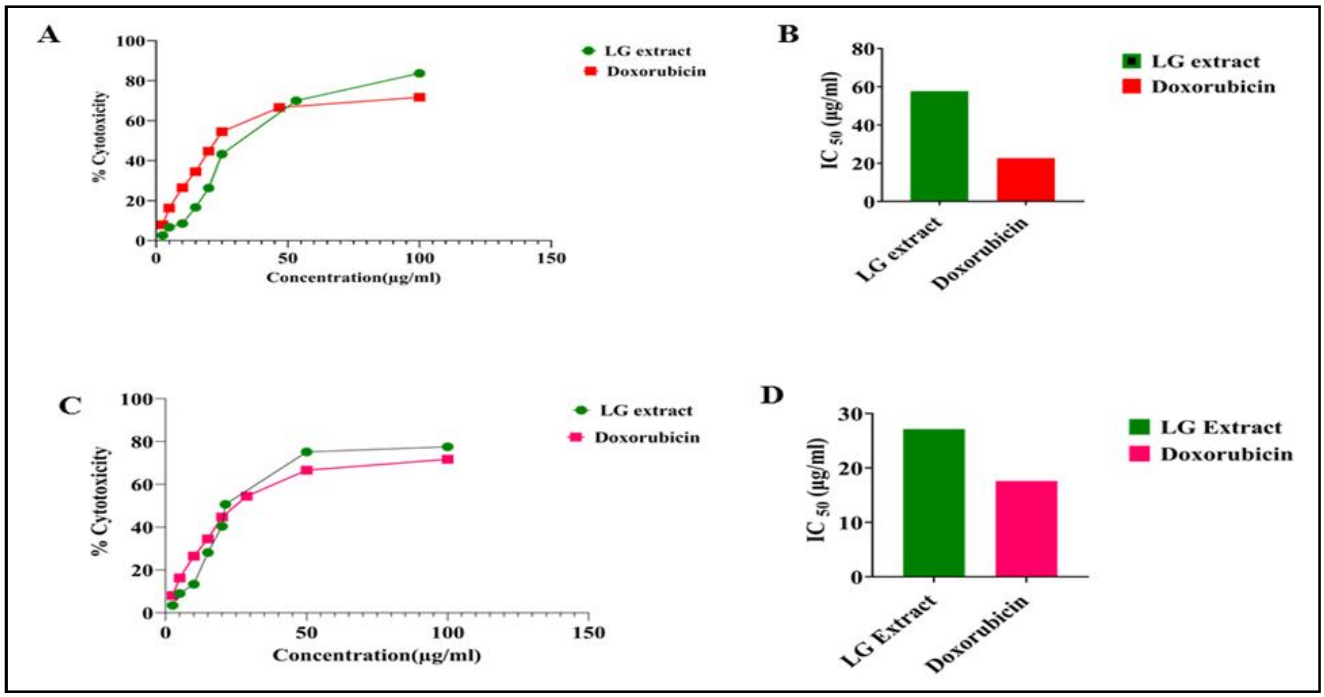

Figure 4: The percentage cytotoxicity of LG extract and doxorubicin was measured using MTT on the cell lines: (A) MCF-7, and (B) MCF-7; (C) MDAMB-231. The IC_{50} of LG extract and doxorubicin on, and (D) MDAMB-231cell lines.

3.4 Brine shrimp lethality assay

The preliminary cytotoxic activity of the aqueous extract of *L. gigantea* was tested by using a brine shrimp lethality assay. The study revealed the cytotoxic potential of *L. gigantea*. The study was conducted in four concentrations of 1 µg/ml, 10 µg/ml, 100 µg/ml, and 1000 µg/ml and studied the cytotoxic potential in brine shrimp. The standard drug doxorubicin had got IC_{50} value of 3.064 µg/ml and that of *L. gigantea* had got IC_{50} value of 24.58 µg/ml. BSL is a preliminary *in vitro* cytotoxic screening study, the study evaluates the potential cytotoxic potency of the plant (Figure 5).

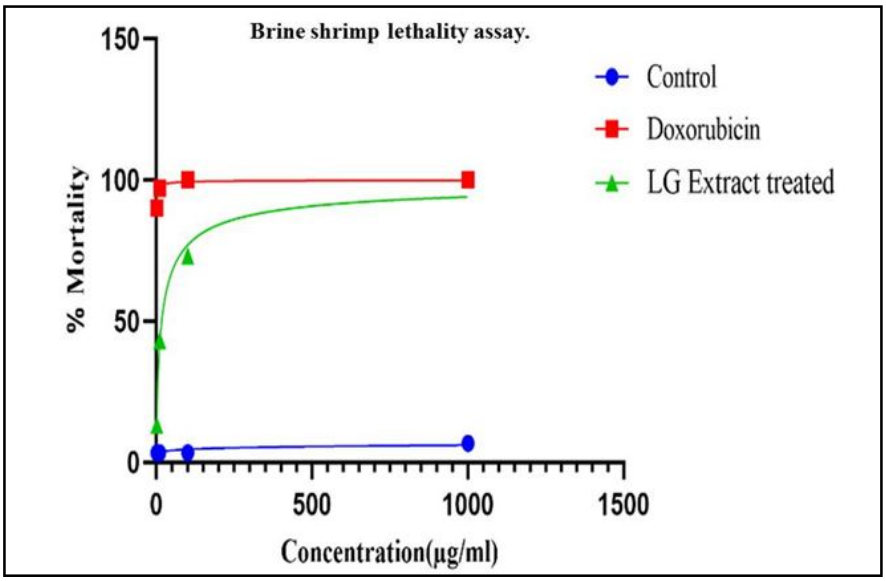

Figure 5: The cytotoxicity analysis of extract on brine shrimp.

4. Discussion

The second greatest cause of death for women worldwide is breast cancer, one of the most prevalent and recurrent diseases. Despite measures for prevention, early detection, and treatments like chemotherapy and radiation, the incidents' frequency rises yearly. Therefore, it is important to create novel therapeutic medications that particularly target certain checkpoints to combat breast cancer. The use of traditional medicine has remained popular throughout the world. Plants have long been a great source of active ingredients. We have seen incredible advancements in science, medicine, and diagnostics as a result of our investments in research and innovation. The more we learn, the more we can do to improve cancer diagnosis, prevention, treatment, and care while also lowering risk factors and enhancing prevention. Also, there is growing interest in various cancer prevention measures due to the steadily rising cancer incidence in the world and the growing issues with drug resistance.

In the present study, we evaluated the *in silico* and *in vitro* potency of *L. gigantea* to understand the anticancer activity. The *in silico* study focused on the phytoconstituents L1- verongamine, L2-calotropone, L3-2,4-dihydroxy-6-methyl benzaldehyde, L4- methyl 3,5di-O-caffeoyl quinate and L5-3,5-di-O-caffeoyl quinic acid of the plant *L. gigantea* to understand their potential as anticancer agents. The study showed that the constituents have a greater G score than the standard drug used, *i.e.*, doxorubicin, against 4 AUV protein. Among the five constituents L4, L5 and L1 has shown greater G-score for 4 AUV protein. It is analysed that most derivatives have shown the H bonding interactions between the compounds and protein 4 AUV. The *in silico* prediction revealed the possible breast cancer metastasis suppression potency of *L. gigantea* leaf extract through molecular docking studies.

Further, phytochemical analysis evaluated the presence of phenolic content of 55.16 ± 6.78 mg GAE which signifies the anticancer potency of the plant because of the fact that plant polyphenols have gained growing attention as a result of their powerful antioxidant abilities and their notable contributions to the prevention of several oxidative stress-related illnesses, including cancer. The brine shrimp lethality assay is a preliminary lethality test, the study revealed the potential cytotoxic effects of the extract on the brine shrimps when compared with the standard drug doxorubicin. We also evaluated the effect of *L. gigantea* extract on breast cancer cell lines, *i.e.*, MCF-7 and MDAMB-231 by *in vitro* analysis using doxorubicin as the standard drug. Under cell-line studies, cytotoxicity was analysed at time intervals of 24 h. In the case of MCF 7 cell lines, the extract showed an IC_{50} value of 57.75 µg/ml. Similarly, the standard drug doxorubicin displayed an IC_{50} value of 22.66 µg/ml for 24 h of exposure. In the case of MDAMB-231 cellines the extract significantly reduced the cell viability with an IC_{50} value of 27.10 µg/ml for 24 h of drug exposure. Similarly, the standard drug doxorubicin displayed an IC_{50} value of 17.88 µg/ml for 24 h of exposure. Results have displayed that the extract possesses cytotoxic potential in both cell lines but more prominently in MDAMB-231 cell lines Accordingly, the *in vitro* study reveals antibreast cancer potential, which requires more confirmation by researching its effects on animal models. Consequently, our research confirms that the breast cancer metastasis suppressor 1, metastasis suppressor protein, inhibits the growth of cancer cells.

5. Conclusion

In the present investigation, we conclude that the extract of *L. gigantea* possesses anticancer effect against metastatic breast cancer. The *in vitro* and *in silico* study information obtained from this trial would help in the development of an adjuvant medication for metastatic breast cancer. Additional studies to learn more about the molecular processes that underlie antimetastatic action and *in vivo* studies may lead to development of a potential drug for metastatic breast cancer.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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