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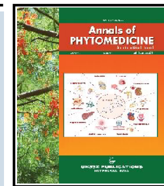

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Therapeutic potential of *Arenga wightii* Griff. extract in breast cancer metastasis an *in vitro* and *in silico* evaluation

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Abstract

Metastasis, the spread of cancer cells to distant organs, remains a major challenge in breast cancer treatment, contributing to poor prognosis and resistance to therapy. This study investigates the anti-metastatic potential of *Arenga wightii* Griff., a plant traditionally used in Southeast Asian medicine. Using gas chromatography-mass spectrometry (GC-MS), we identified several bioactive compounds in the extract that may contribute to its anticancer effects. Molecular docking simulations, conducted with Schrödinger Suite 2019-2, revealed favourable binding interactions between *A. wightii* compounds and the breast cancer metastasis suppressor 1 (BRMS1) protein (PDB ID: 4AUV), a key regulator of metastasis. These interactions suggest that the extract may interfere with metastatic pathways and inhibit cancer cell migration. *In vitro* cytotoxicity assays using the T-47D breast cancer cell line, a well-established model for metastatic breast cancer, showed an IC_{50} value of 66.21 µg/ml, indicating significant cytotoxic activity. Additionally, the extract reduced T-47D cell migration in a scratch assay, further supporting its anti-metastatic potential. These results provide compelling *in silico* and *in vitro* evidence for the anti-metastatic properties of *A. wightii* extract. While promising, further *in vivo* studies are essential to fully assess its pharmacological profile and therapeutic potential. Further studies are currently in progress to explore its efficacy in animal models. This research contributes to the growing body of evidence for natural plant-derived therapies in cancer treatment and highlights *A. wightii* as a promising candidate for further development as a therapeutic agent for metastatic breast cancer.

1. Introduction

Breast cancer is one of the most common and life-threatening tumours affecting millions of people majorly females. Genetic alterations in malignant cells cause uncontrolled breast tissue cell proliferation. Mutations in normal breast tissue cause tumours to originate and grow. The molecular and genetic landscape of breast cancer is complicated, with subgroups including luminal A/B and basal-like cancers thought to develop from luminal progenitor cells (Sharma *et al*., 2023). Most breast cancers are classified by receptor status and genetic markers, such as estrogen receptor (ER), progesterone receptor (PR), and herceptin 2 (HER2) expression (Zhang *et al*., 2022). Targeting hormone receptors using selective estrogenic receptor modulators (SERMs) and HER2-targeted medicines are essential in breast cancer treatment (Yang *et al*., 2023). The molecular fingerprints that affect patient prognosis and treatment response make breast cancer more heterogeneous, requiring tailored treatment. Breast cancer treatment possesses outcomes that are poor despite breakthroughs in early identification and treatment. The disease accounts for almost 30% of global cancer diagnoses and many deaths associated with cancer in women (Arnold *et al*., 2022). In low- and middle-income countries, late-stage diagnosis and limited access to targeted medicines, increase the risk of breast cancer mortality (Singh *et al*., 2023). A complex interaction of genetic, environmental, and hormonal variables causes breast cancer. Mutations in the tumour suppressor genes like BRCA1 and BRCA2, environmental carcinogens, and lifestyle variables like food, alcohol, and inactivity are the factors causing breast cancer (Hart *et al*., 2019). Various types of cancer can occur, from non-invasive ductal carcinoma *in situ* (DCIS) to aggressive malignancies that spread. Breast cancer receptor-based subtyping and biomarkers, including ER, PR, and HER2, help predict disease progression and guide treatment (Ma *et al*., 2022). Even with chemotherapy, endocrine therapy, and targeted medicines, the risk of recurrence and metastasis remains high (Noor *et al*., 2021). Genomic and personalized medicine may improve treatment results, yet medication resistance and tumor progression remain biological mysteries (Chunarkar-Patil *et al*., 2024).

Natural plant-derived chemicals have garnered attention as an alternative to breast cancer treatment having a chemical variety and ability to affect cancer-related biological processes such as cell cycle regulation, apoptosis, and metastasis, making plant-based chemicals beneficial. Anticancer effects of flavonoids, alkaloids, and terpenoids are attractive therapeutic candidates (Vutakuri and Somara, 2018). Preclinical models of these chemicals suggest they may be effective cancer treatments, but further research is needed. Of these natural substances, *A. wightii,* an Araceae plant from Southeast Asia and the Indian subcontinent, may have therapeutic qualities. *A. wightii* pith and sap have been utilized in ethnomedicine to treat leucorrhoea, jaundice, and dysuria (Ananth *et al*., 2014; Madar *et al*., 2019; Shikha *et al*., 2015). *A. wightii* has been traditionally used in Southeast

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Asian herbal medicine for its anti-inflammatory and antioxidant properties, is rich in flavonoids, alkaloids, and polyphenols, the plant's therapeutic effects are due to its anti-inflammatory, analgesic, and antioxidant qualities (Shikha *et al*., 2015). However, more research is needed to assess its bioactive elements' anticancer effects (Arif *et al*., 2022; Phurailatpam *et al*. (2022); Shrilakshmi *et al*. (2022). Recent research has examined *A. wightii*'s anti-inflammatory and analgesic properties in treating various disorders (Shikha *et al*., 2015). These findings support studying its involvement in metastatic breast cancer treatment.

Compared to existing treatments, which often focus on tumor shrinkage rather than metastasis prevention, this research aims to offer a complementary strategy targeting metastatic spread, a key factor in treatment resistance and poor prognosis. The study aims to evaluate the antimetastatic potency of the aqueous fruit extract of *A. wightii's* through *in silico* and *in vitro* methods against metastatic behaviour of breast cancer, a novel approach not fully explored in current cancer therapies.

Molecular docking using the Schrödinger Suite 2019-2 was used to study the interaction between important phytochemicals from *A. wightii* and breast cancer metastasis suppressor protein (BRMS1). BRMS1 plays a critical role in inhibiting the metastatic spread of breast cancer by regulating cell migration and invasion. Its upregulation has been linked to reduced metastasis, making it a key target for evaluating the antimetastatic effects of the extract. Breast cancer metastasis is regulated by BRMS1, therefore inhibiting it helps prevent cancer spread (Chen *et al*., 2011; Szarszewska *et al*., 2019). Based on computational docking studies, bioactive molecules that bind to BRMS1 may block its function and prevent metastasis. Besides molecular docking, the absorption, distribution, metabolism, and excretion (ADME) properties of these phytochemicals were assessed to predict their pharmacokinetic profiles and drug candidate status (Soni *et al*., 2021). The *A. wightii* aqueous fruit extract was tested on the T-47D breast cancer cell line to see if it inhibits cell growth and metastasis via the MTT assay and scratch assay. In this experiment, *A. wightii* was compared against doxorubicin, a widely used breast cancer medication (Monteran *et al*., 2022; Sawasdee *et al*., 2022). This work integrates computational and experimental methods to evaluate the potency of *A. wightii* in metastatic breast cancer treatment. *A. wightii's* bioactive chemicals may yield new therapeutic candidates that can provide alternatives for further research and treatment to patients who do not react to standard treatments (Sawasdee *et al*., 2022).

2. Materials and Methods

2.1 Plant material collection, preparation and authentication

The plant *A. wightii* was obtained from the biodiverse Western Ghats region at 12° 302 03 N, 75° 02 03, Kerala, India. The plant was authenticated by Dr. Biju, Botanist of Government College, Kasaragod, Kerala, who recognized the plant specimen with, herbarium voucher specimen number 21PH103R/01. Fruits were washed to remove impurities and dried at room temperature for 15 days after collection. The dried fruit was ground into a fine powder using a Philips grinder to ensure extraction consistency. The powdered substance was maintained in sealed containers to maintain its chemical and pharmacological properties.

2.2 Analysis of GC-MS

The School of Biosciences, MG University, Kerala, used a Shimadzu QP 2010 series GC-MS instrument for phytochemical analysis. The 30 m long VF-5ms fused quartz capillary column has a 0.25 mm diameter and 0.25 µm film thickness. Helium gas (99.99% purity) was used as the carrier gas for electron ionization at 70 electron volts (eV) and 1.51 ml per min. The mass transfer line temperature was kept at 200°C. The oven temperature was scheduled to rise from 70°C to 220°C at 10°C per min, then hold at 220°C before rising to 300°C. A splitless injection of 2 µl ethanol sample solution was done. Mass spectra were obtained from 50 to 600 amu using a split ratio of 1:40. The complete analysis took 35 min. Comparing mass spectra with the National Institute of Standards and Technology (NIST) database helped identify extract components (Konappa *et al*., 2015).

2.3 Computational methods

In breast cancer, BRMS1 regulates metastasis via modulating chromatin remodelling and suppressing the NF-κB signalling pathway rather than limiting cell proliferation. BRMS1 upregulates miR-146a, which lowers EGFR expression in breast cancer cells, reducing metastasis. Moreover, it functions as an E3 ligase with HDAC1 at NF-κB consensus areas, preventing pulmonary metastasis and cancer progression (Sangeetha *et al*., 2014). The N-terminal domain structure of BRMS1 (PDB ID: 4AUV) illuminates its functions. The chemical profile of *A. wightii* extract was studied using gas chromato-graphy-mass spectrometry (GC-MS) to find bioactive chemicals with medicinal potential. We seek to find interactions that may boost BRMS1's metastasis-suppressive effect by comparing these chemicals' molecular structures to their crystal structure. This dual method combines structural biology and phytochemical studies to develop metastatic breast cancer treatments from plants. (Bindu *et al*., 2023).

2.3.1 ADMET characterization and Lipinski's rule evaluation

The ADMET characteristics of the selected phytochemicals from *A. wightii* were predicted using QikProp, a tool within the Schrödinger suite. This analysis assessed key pharmacokinetic properties, including solubility, permeability, metabolic stability, and potential toxicity, providing valuable insights into the compounds' suitability for further *in vitro* and *in vivo* testing (Bindu *et al*., 2023; Khanal *et al.,* 2024; Patil *et al.,* 2022). Notably, all compounds analysed adhered to Lipinski's Rule of Five, with no violations, indicating their potential for oral bioavailability and further development as therapeutic agents.

2.3.2 Molecular docking of bioactives from *A. wightii* with BRMS1

Molecular docking was performed using Schrödinger Suite 2019-2, with scoring functions based on binding affinity to target proteins. The docking process included validation steps through re-docking known ligands and performing visual inspections to ensure the reliability of the predicted binding modes. The structure of breast cancer metastasis suppressor 1 (BRMS1; PDB ID: 4AUV) was prepared for molecular docking. Essential preparation steps included the addition of hydrogen atoms, bond ordering, and optimization of hydrogen bond interactions. The protein structure was further refined by energy minimization to achieve the lowest possible energy state. Water molecules beyond 5 Å from the active site were removed to

prevent interference during docking, ensuring accurate simulations and reliable protein-ligand interaction analysis (Arthur *et al*., 2018; Dwivedi *et al.,* 2022). The phytochemicals obeying the Lipinsky rule of five from *A. wightii* were optimized using the LigPrep module in Schrödinger Maestro, adjusting geometric parameters and protonation states to reflect physiological pH. This process ensured that the ligands were in their most stable configuration for docking and free from covalent interactions with other fragments (Bindu *et al*., 2023). A receptor grid was generated using the Glide module to define the docking environment within the BRMS1 binding pocket. The docking setup allowed for the exploration of up to five Monte Carlo trial conformations and the evaluation of ten ligand poses per docking event. Glide scoring (G-score) assessed the predicted binding affinity, with higher G-scores indicating stronger interactions. The top-ranked poses were further rescored using the extra precision (XP) function for validation (Bindu *et al*., 2023). Molecular docking was performed to evaluate the binding affinities of the selected phytochemicals to BRMS1, comparing their potential as inhibitors of breast cancer metastasis to doxorubicin, a standard chemo-therapeutic agent (Agu *et al*., 2023; Ternikar *et al.,* 2021).

2.4 *In vitro* analysis

2.4.1 Cell lines and chemicals

The Pune-based National Centre for Cell Science (NCCS) provided the T-47D human breast cancer cell line. HI Media, India, supplied the media and reagents required for this research, *i.e.,* FBS, RPMI-1640 media, MTT reagent, and PBS.

2.4.2 Extraction procedure

2.4.2.1 Preparation of *A. wightii* aqueous extract

The air-dried powdered fruit of *A. wightii* was used to prepare the aqueous extract following a modified decoction method. Initially, 100 g of the powdered fruit was boiled in 1 litre of distilled water. The mixture was heated gently, with intermittent stirring, to maintain a mild boil, and the volume was reduced by 50%. The process was conducted under controlled conditions to prevent the degradation of bioactive compounds. After cooling, the mixture was filtered through Whatman No. 1 filter paper to remove insoluble particulates, and the resulting liquid extract was concentrated using a rotary evaporator at low pressure to remove excess water, resulting in a thick syrup. To ensure stability and preserve the bioactive components, the concentrated extract was freeze-dried using a lyophilizer, yielding a fine powder. The freeze-dried extract was then stored in desiccators to protect it from moisture and ensure long-term stability (Meghwar *et al*., 2024). Gas chromatography-mass spectrometry (GC-MS) analysis was conducted to identify and quantify the major phytochemicals present in the *A. wightii* extract. This analysis allowed for the characterization of the extract's chemical profile and ensured that the bioactive compounds were consistently present at similar concentrations across different batches. Hence, ensured that the *A. wightii* extract can be used in subsequent biological assays and was of high purity and reproducibility, allowing for reliable interpretation of the study's results (Konappa *et al*., 2015; Dwivedi *et al.,* 2023).

2.4.3 Evaluation of cytotoxicity *via* MTT assay

2.4.3.1 Cellular cultivation

T-47D cell lines were maintained in RPMI-1640 media supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% non-essential amino acids. Cells were cultured in a CO_2 incubator at 37°C with 5% CO_2 and 95% humidity. Trypan blue exclusion assays assessed cell viability after trypsinization and subculture. Cell amounts were measured using a cell counter.

2.4.3.2 Protocol for MTT assay

Cytotoxicity studies were conducted using T-47D cell line, with untreated cells as the negative control and standard chemotherapeutic agent doxorubicin as the positive control. For the MTT cytotoxicity assay, T-47D cells were seeded onto 96-well culture plates at a density of 1×10t cells per well. Following adhesion, cells were exposed to varying doses (5,10,25, 50, 100, and 250 µg/ml) of *A. wightii* aqueous extract for 24 h. On completion of treatment, the exposed extract was removed, and the cells were washed with PBS. The plates were then incubated for 4 h at 37°C after adding 20 µl of MTT reagent (5 mg/ml) to each well. The formazan crystals were dissolved in DMSO and measured at 570 nm using a microplate reader. The cytotoxic effect was measured by comparing the results to doxorubicin-treated cells. The extract was tested for cytotoxicity and cell viability. The T-47D cells were analysed and photographed to document their morphological properties using a phase contrast microscope (Dwivedi and Shastry, 2023).

Per cent cytotoxicity

$$= \frac{\text{Absorbance of the control} - \text{Absorbance of the test}}{\text{Absorbance of control}} \times 100$$

2.4.3.3 Scratch assay

Cells were plated at a density of 20,000 cells per well in 12-well tissue culture plates and cultured for 24 h to reach 70-80% confluence. Following a 24 h period, a sterile pipette tip was employed to generate a linear incision (about 300 µm wide) in the cell monolayer. Detached cells and cellular debris were eliminated by rinsing the wells with phosphate-buffered saline (PBS). Subsequent to this, fresh media infused with the *A. wightii* extract at its IC_{50} concentration was introduced into the designated wells. The control groups received only the media treatment. Photographs of the abraded region were taken at 0 h and 24 h post-treatment to assess the advancement of wound closure (Yang *et al*., 2023). The wound closure % was calculated using the subsequent formula:

% Scratch closure

$$= \frac{\text{Initial scratch area} - \text{Scratch area at 24 h}}{\text{Initial scratch area}} \times 100$$

3. Results

3.1 Gas chromatography-mass spectrometry analysis

The phytocomponents of the plant were derived using GC-MS analysis (Figure 1). The phytoconstituents include octadecane, 1,1'-[(1-methyl-1,2-ethanediyl) bis(oxy)] bis-, having mol. weight: 581.1 g/mol and mol. formula: $C_{39}H_{80}O_2$; Benzene propanoic acid,4-[(2,4-dinitrophenyl) azo]-,1-methyl ethyl ester having mol. weight: 386.4 g/mol and mol. formula: $C_{18}H_{18}N_4O_6$; 2,4-Di-tert-butylphenol having mol. weight: 206.32 g/mol and mol. formula: $C_{14}H_{22}O$; m-Dioxane,5-(hexa-decyloxy)-2-pentadecyl-, trans-, having mol. weight: 538.9 g/

mol and mol. formula: $C_{35}H_{70}O_3$; 1,4-Naphthoquinone,2-acetyl-5,8-dihydroxy-3-methoxy- having mol. weight: 262.21 g/mol and mol. formula: $C_{13}H_{10}O_6$; 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione having mol. weight: 276.4 g/mol and mol. formula: $C_{17}H_{24}O_3$; A7. Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5á), having mol. weight: 430.7 g/mol and mol. formula: $C_{29}H_{50}O_2$; Hexa-t-butylselenatrisiletanehaving mol. weight: 505.9 g/mol and mol. formula: $C_{24}H_{54}SeSi_3$; 2,6-Di-tert-butyl-P-benzoquinone having mol. weight: 220.31 g/mol and mol. formula: $C_{14}H_{20}O_2$; 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans-, having mol. weight: 444.6 g/mol and mol. formula: $C_{28}H_{44}O_4$; Glycine, N-[(3alpha,5beta,7alpha,12alpha)-24-oxo-3,7,12-tris[(trimethylsilyl)oxy] cholan-24-yl]-, methyl ester having mol. weight: 696.2 g/mol and mol. formula: $C_{36}H_{69}NO_6Si_3$. 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane having mol. weight: 577.2 g/mol and mol. formula: $C_{16}H_{48}O_7Si_8$.

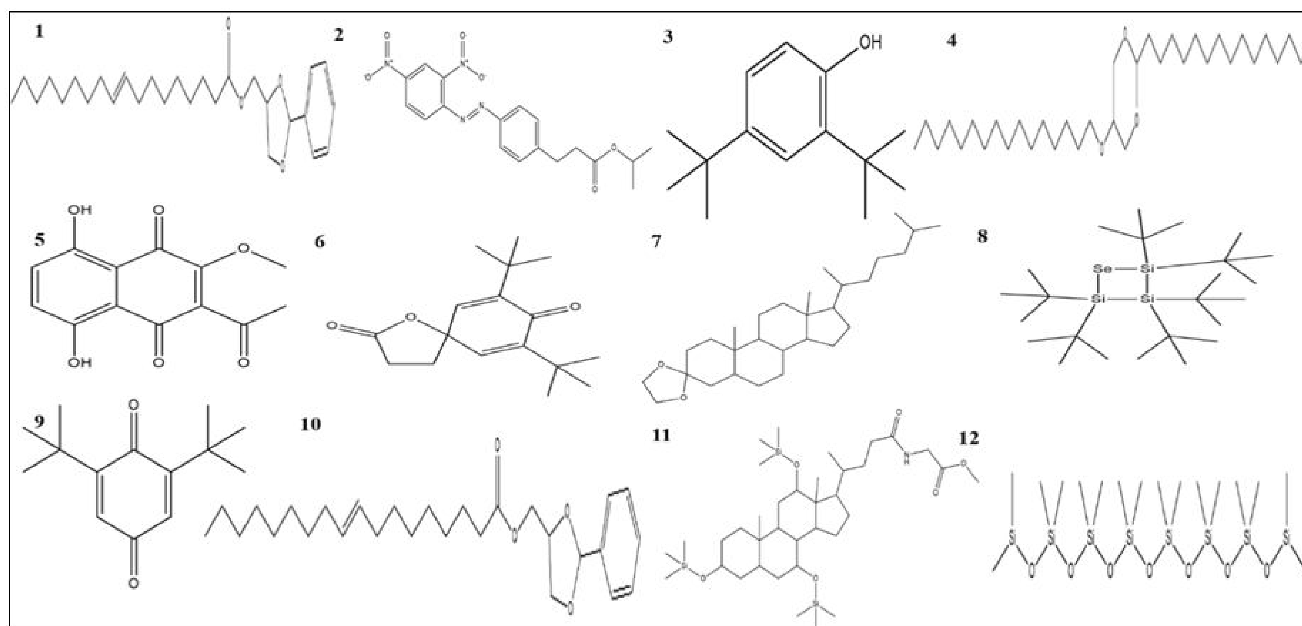

Figure 1: Bioactives of *A. wightii* obtained through GC-MS analysis.

Structures of bioactives: 1. Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl) bis(oxy)] bis-, mol. weight: 581.1 g/mol, mol. formula: $C_{39}H_{80}O_2$; A2. Benzenepropanoic acid,4-[(2,4-dinitrophenyl) azo]-,1-methyl ethyl ester, mol. weight: 386.4 g/mol, mol. formula: $C_{18}H_{18}N_4O_6$; A3. 2,4-Di-tert-butylphenol, mol. weight: 206.32 g/mol, mol. formula: $C_{14}H_{22}O$; A4. m-Dioxane,5-(hexadecyloxy)-2-pentadecyl-, trans-, mol. weight: 538.9 g/mol, mol. formula: $C_{35}H_{70}O_3$; A5. 1,4-Naphthoquinone,2-acetyl-5,8-dihydroxy-3-methoxy-, mol. weight: 262.21 g/mol, mol. formula: $C_{13}H_{10}O_6$; A6. 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione, mol. weight: 276.4 g/mol, mol. formula: $C_{17}H_{24}O_3$; A7. Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5α), mol. weight: 430.7 g/mol, mol. formula: $C_{29}H_{50}O_2$; A8. Hexa-t-butylselenatrisiletane, mol. weight: 505.9 g/mol, mol. formula: $C_{24}H_{54}SeSi_3$; A9. 2,6-Di-tert-butyl-P-benzoquinone, mol. weight: 220.31 g/mol, mol. formula: $C_{14}H_{20}O_2$; A10. 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans-, mol. weight: 444.6 g/mol, mol. formula: $C_{28}H_{44}O_4$; A11. Glycine, N-[(3alpha,5beta,7alpha,12alpha)-24-oxo-3,7,12-tris[(trimethylsilyl)oxy] cholan-24-yl]-, methyl ester, mol. weight: 696.2 g/mol, mol. formula:$C_{36}H_{69}NO_6Si_3$;A12.1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 Hexadecamethyloctasiloxane, mol. weight: 577.2 g/mol, mol. formula: $C_{16}H_{48}O_7Si_8$.

3.2 Computational studies

3.2.1 ADMET study utilizing QikProp

QikProp was employed to predict the pharmacokinetic properties of the bioactive phytoconstituents, including their absorption, distribution, metabolism, excretion (ADME), and blood-brain barrier (BBB) permeability. Table1 outlines critical metrics such as bioavailability, permeability, and the solvent-accessible surface area of the compounds. The majority of the evaluated bioactive compounds demonstrated advantageous oral absorption properties, akin to the reference medication. Moreover, their solubility, a crucial element for sustaining suitable quantities in systemic circulation, was within acceptable parameters, signifying favourable bioavailability.

The QPPCaCO-2 model predicts the apparent permeability across Caco-2 cells, an important marker for intestinal absorption. All compounds demonstrated high permeability across this barrier, suggesting efficient gastrointestinal absorption. QikProp also estimated the potential metabolic processes of the phyto-constituents, which play an important role in the compounds' bioavailability and their ability to reach the target site. All the phytoconstituents were found to be within acceptable metabolic ranges.

Furthermore, the predicted BBB penetration for all compounds was favourable, with values falling within the recommended thresholds, suggesting good central nervous system (CNS) accessibility. The Log KHSA measure, which forecasts binding affinity to human serum albumin, indicated that all drugs exhibited appropriate binding capacity within the anticipated range.

Other pharmacokinetic parameters, such as the fraction of the solvent-accessible surface area (FISA), the fraction of the solvent-exposed

surface area (FOSA), and the solvent-accessible surface area (SASA), were also examined, and the results showed that they were within the appropriate limits. Lower values in these parameters suggest that the compounds possess a relatively hydrophilic surface component, particularly involving nitrogen, oxygen, and hydrogen atoms, which could further support their bioavailability and systemic circulation.

The ADMET properties of the bioactive compounds were evaluated using the QikProp tool from Schrödinger software (version 13.1). This analysis focused on several key parameters, including central nervous system (CNS) activity, molecular weight, predicted octanol/water partition coefficient, predicted brain/blood partition coefficient, and all bioactive adhere to Lipinski's Rule of five, with up to two violations being acceptable for oral activity. Among the bioactive compounds assessed in this study 7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione, 2,4-Di-tert-butylphenol, benzene propanoic acid, 4-[(2,4-dinitrophenyl)azo]-, 1-methyl ethyl ester, 2,6-Di-tert-butyl-p-benzoquinone, and 1,4-Naphthoquinone, 2-acetyl-5,8-dihydroxy-3-methoxy demonstrated compliance with Lipinski's rule of five, zero violations for Lipinski's rule (Table 1).

Table 1: Pharmacokinetic properties of bioactive phytoconstituents by Qik prop

S No.	Phyto-constituents	% HOA	QPlogS	QPPCaco	#Metab	QplogBB	QPPMDCK	QPlogKhsa	SASA	FOSA	FISA	Lipinsky rule of 5
	Acceptable range	>80% High, <25% low	(-6.5-0.5)	<25Poor, >500 great	(1-8)	(-3 to 1.2)	<25Poor, >500great	(-1.5 to 1.5)	(300-1000)	(0.0-750)	(7.0-330)	
1.	Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl)bis(oxy)]bis-	100.000	-21.260	9906.03	2	1.505	2808.619	3.491	1431.82	1431.82	0	2
2.	Benzenepropanoic acid, 4-[(2,4-dinitrophenyl)azo]-, 1-methyl ethyl ester	74.598	-5.524	46.058	5	-2.681	4920.447	0.278	725.110	238.52	245.976	0
3.	2,4-Di-tert-butylphenol	100.000	-3.968	4985.52	2	0.122	1631.542	0.549	471.419	31.445	31.445	0
4.	m-Dioxane, 5-(hexadecyloxy)-2-pentadecyl-, trans-	100.00	-16.856	9906.03	0	0.413	762.535	2.512	1274.66	0	0	2
5.	1,4-Naphthoquinone, 2-acetyl-5,8-dihydroxy-3-methoxy-	67.335	-0.824	165.159	3	-1.233	135.328	-0.978	454.16	187.852	187.852	0
6.	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene -2,8-dione	100.000	-3.528	2028.26	1	-0.185	5899.293	0.023	541.11	0	72.633	0
7.	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5á)-	100.000	-11.855	9906.03	0	1.035	5899.293	2.279	763.41	0	0	1
8.	Hexa-t-butyl selenatrisiletane	0	0	0	0	0	5899.293	0	0	0	0	0
9.	2,6-Di-tert-butyl-P-benzoquinone	100.00	-2.756	2266.71	2	-0.111	5899.293	-0.135	372.35	32.880	67.542	0
10.	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans-	100.00	-7.659	3039.34	3	-1.098	358.636	1.255	899.03	674.772	54.109	1
11.	Glycine, N[(3alpha,5beta,7alpha,12alpha)-24-oxo-3,7,12-tris[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	100.00	-11.028	591.696	2	-1.416	482.831	2.261	1096.76	990.714	106.049	2
12.	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane	100.00	-15.897	9906.03	0	-1.416	91.292	2.750	933.69	933.692	0	2
13.	Dox	0	-2.897	2.273	9	-3.168	0.760	-0.580	810.987	349.304	320.170	3

These parameters were utilized: SASA (solvent-accessible surface area), FOSA (hydrophobic component of SASA), FISA (hydrophilic component of SASA), QPlogS (logarithmic partition coefficient between n-octanol and water), QPlogBB (predicted brain-to-blood partition coefficient), and QPPCaco (predicted apparent permeability across Caco-2 cells). Every one of these parameters was utilized.

3.2.2 Molecular docking

The docking affinities of the chosen phyto-constituents for the breast cancer metastasis suppressor protein (BRMS1) receptor (PDB ID: 4AUV) were evaluated via molecular docking simulations. Table 2 and 3 summarizes the findings regarding the binding interactions between the bioactive chemicals and the receptor's active region. The computed Glide scores, indicative of binding efficiency and strength, range from – 3.077 to – 1.924 kcal/mol, signifying differing levels of affinity.

The BRMS1 receptor (PDB ID: 4AUV) has essential active residues vital for ligand binding, specifically Glu68, Lys75, Ser72, Glu76, Phe79, Leu78 and Arg82. Of the phyto-constituents evaluated, 2,4-di-tert-butylphenol demonstrated the highest binding affinity with a Glide score of -3.077 kcal/mol, whereas m-Dioxane, 5-(hexadecyloxy)-2-pentadecyl-, trans-, recorded a value of -2.978 kcal/mol. These findings indicate that these drugs may engage more efficiently with the active site of BRMS1, thereby aiding in the inhibition of breast cancer metastasis. Figure 2 depicts both two-dimensional and three-dimensional depictions of the docking conformations of the bioactive chemicals within the BRMS1 receptor, emphasizing critical interactions and the spatial arrangement of the ligands at the receptor's active region. Bioactive compounds form hydrogen bonds with Arg57, while doxorubicin binds to Glu59 and Glu63. Interaction details are provided in Table 3. This analysis offers a structural foundation for comprehending the binding mechanisms and possible therapeutic functions of these phytoconstituents.

Table 2: Docking scores for compounds (1-12) with PDB ID: 4AUV

Compounds	Glide score	Glide EvdW	XP h-bond	Glide emodel	G rotatable bonds	Glide ecoul	Glide energy
2,4-di-tert-butylphenol	-3.077	-11.066	0.000	-13.131	0.000	-0.001	-11.067
m-Dioxane,5-(hexadecyloxy)-2-pentadecyl-, trans-	-2.978	-36.412	-0.271	-37.312	1.992	-2.617	-39.029
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan -4-yl) methyl ester	-2.976	-29.926	-0.192	-34.440	1.821	-2.360	-32.286
Benzenepropanoic acid, 4-[(2,4-dinitrophenyl) azo]-, 1-methyl ethyl ester	-2.934	-26.202	0.000	-30.575	0.782	-0.588	-26.791
Glycine, N-[(3alpha,5beta,7alpha,12alpha)-24 -oxo-3,7,12-tris[(trimethylsilyl)oxy] cholan-24-yl]-, methyl ester	-2.624	-25.189	-0.160	-30.497	0.269	-4.120	-29.309
1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadeca-methyloctasiloxane	-2.271	-21.644	-0.281	-31.429	0.649	-5.624	-27.268
Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl) bis(oxy)] bis-,	-1.924	-36.627	0.000	-34.470	2.067	-0.719	-37.345
Doxorubicin	-4.45	-15.131	-0.399	-51.505	0.279	-21.337	-36.468

The terms represent the components of the docking analysis: Glide EvdW is van der waals energy, XP h-bond is hydrogen bonding energy, Glide emodel is model energy, G rotatable bonds refer to energy from rotatable bonds, and Glide ecoul is Coulomb energy, all contributing to the binding interaction assessment.

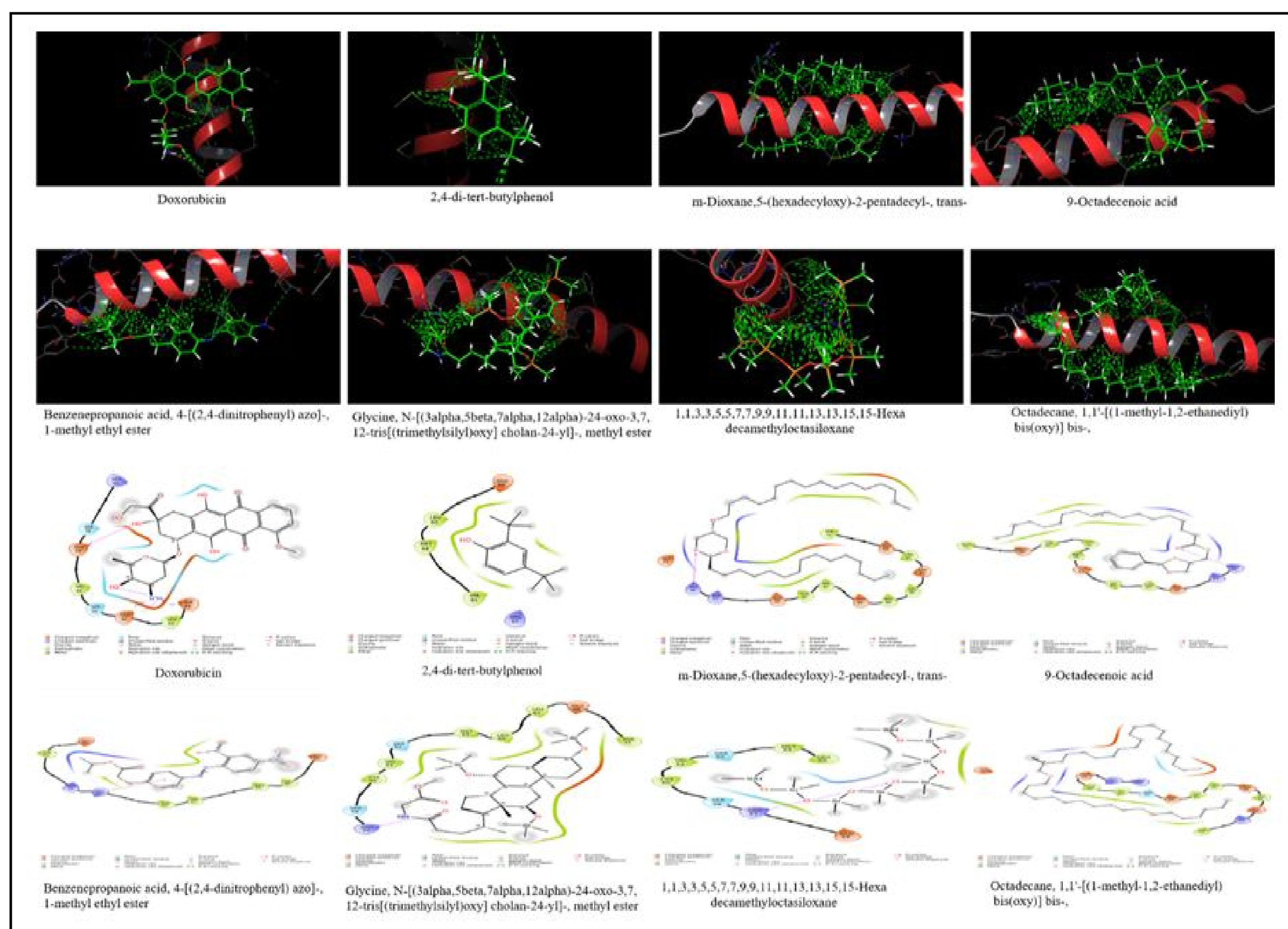

Figure 2: The figures show 3D and 2D docking of bioactive components and doxorubicin with the BRMS1 receptor (PDB ID: 4AUV).

Table 3: Molecular docking of phytoconstituents with protein 4AUV

Phytoconstituents	Hydrophobic Interaction	Hydrogen bond	Polar interactions
2,4-di-tert-butylphenol	VAL61, MET64,LEU65	———————-	———————-
m-Dioxane,5-(hexadecyloxy)-2-pentadecyl-, trans-	CYS60, VAL61, MET64, LEU65, LEU67, PHE71.	ARG56	———————-
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester	CYS60, VAL61, MET64, LEU65, LEU67, PHE71	ARG56	———————-
Benzenepropanoic acid, 4-[(2,4-dinitrophenyl) azo]-, 1-methyl ethyl ester	TYR53, CYS60, VAL61, MET64,LEU65	———————-	———————-
Glycine, N-[(3alpha,5beta,7alpha,12alpha)-24-oxo-3, 7,12-tris[(trimethylsilyl)oxy] cholan-24-yl]-, methyl ester	CYS60, VAL61, MET64, LEU65, LEU67, PHE71	ARG57	SER58,SER62
1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecame-thyloctasiloxane	CYS60, VAL61, MET64, LEU65	ARG57	SER58, SER62
Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl) bis(oxy)] bis-,	CYS60, VAL61, MET64, LEU65,LEU67, PHE71	———————-	SER62
Doxorubicin	VAL61, LEU65	GLU59, GLU63, ASP66	SER58, SER62

3.3 In *vitro* investigations

3.3.1 MTT cell viability assay

The cell-damaging efficacy of the extract was assessed utilizing the MTT test with T-47D cell lines.

The cytotoxicity percentage was quantified, as illustrated in Figure 3. This image contrasts the morphological alterations of untreated cells with those subjected to *A. wightii* extract and the conventional medication, doxorubicin. Both the extract and doxorubicin demonstrated cytotoxic effects on T-47D cells at their respective IC_{50} concentrations. Utilizing phase contrast microscopy at 24 h, treated cells had significant morphological changes, while untreated cells had homogenous, evenly scattered surfaces.

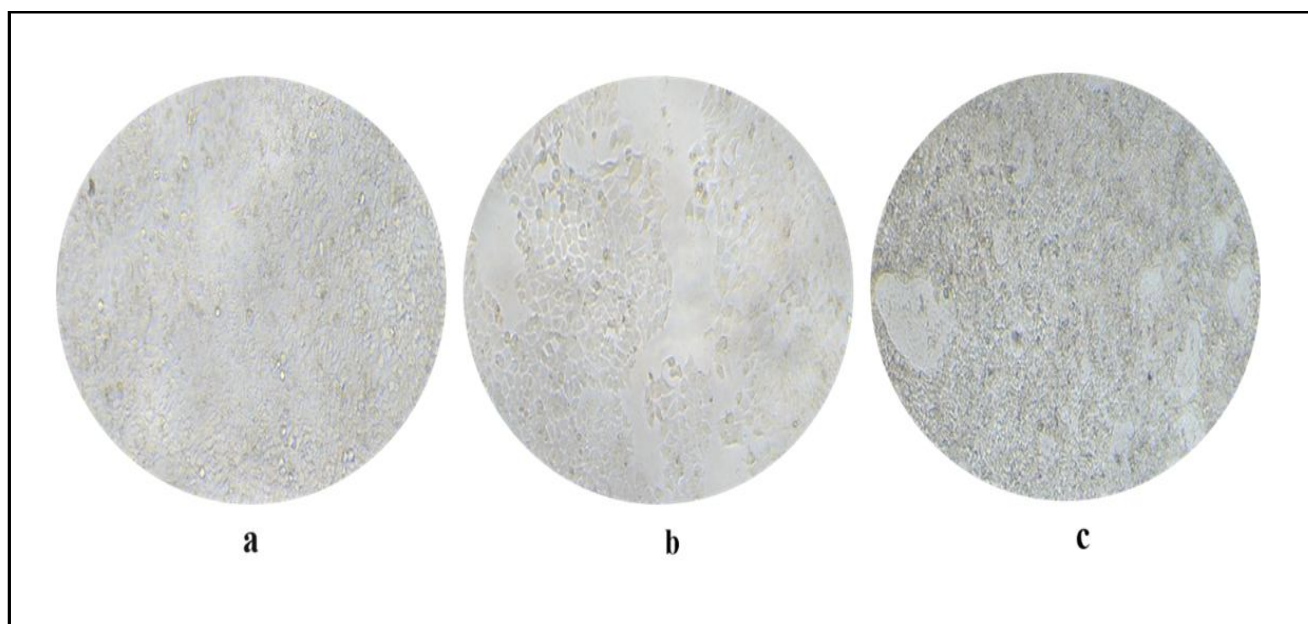

Figure 3: Images of T-47D cell lines in phase contrast microscopy: (a) untreated cells, (b) cells treated with doxorubicin and (c) cells treated with *A. wightii* extract. The cells were incubated at 37°C in a 5% CO, humidified atmosphere and cultured at a density of 5 × 10,000 cells/cm². Images were taken at 10X magnification.

3.3.2 Cytotoxicity assessment in T-47D cells

The *A. wightii* extract significantly reduced cell viability, with an IC_{50} value of 66.21 µg/ml after 24 h of treatment. In contrast, the standard chemotherapeutic drug, doxorubicin, exhibited a lower IC_{50} value of 39.19 µg/ml under comparable experimental conditions. (Figures 3 and 4)

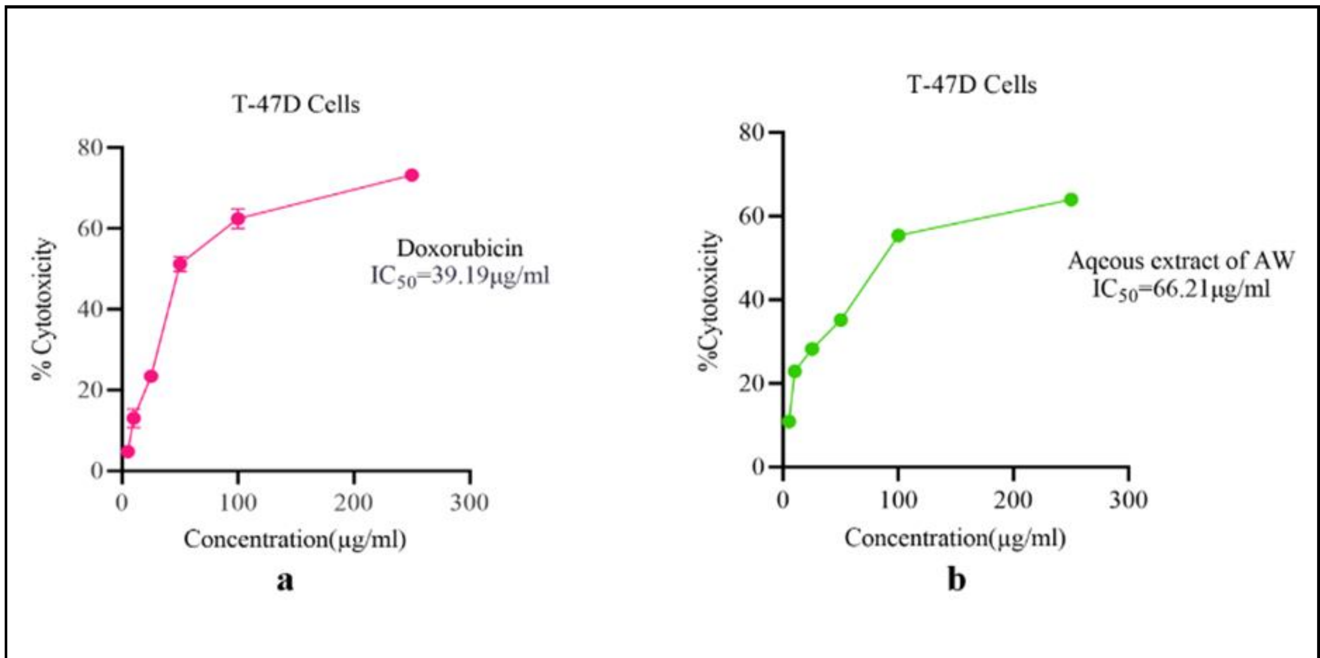

Figure 4: Cytotoxicity of *A. wightii* extract and doxorubicin on T-47D cell lines measured *via* MTT assay. The IC_{50} values of (a) *A. wightii* extract and (b) doxorubicin are presented.

3.3.3 Assessment of scratch assay

Cell migration was evaluated as a measure of the possible anti-metastatic effects of the therapies. This study assessed the percentage of wound closure at 0 h and 24 h post-treatment with *A. wightii* extract and doxorubicin. At the 24 h mark, *A. wightii* extract achieved a 25.44 ± 0.92% wound closure, whereas doxorubicin therapy resulted in a 16.19 ± 1.83% closure. (Figure 5)

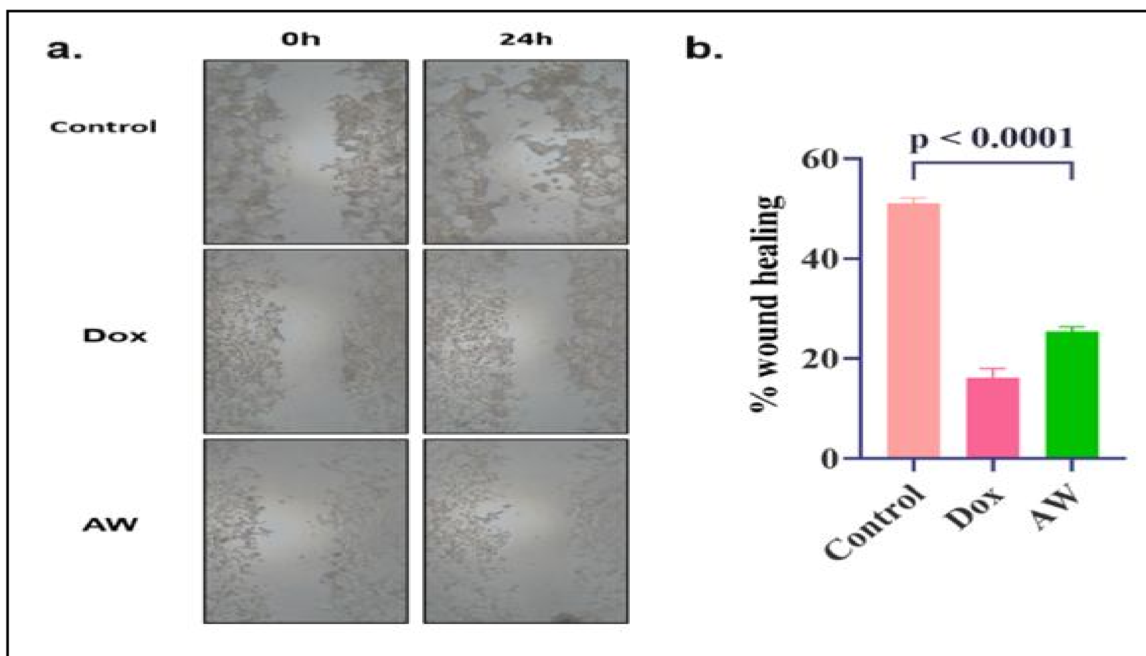

Figure 5: a. Comparison of cell migration using scratch assay b. Graph representing wound closure using scratch assay.

4. Discussion

Breast cancer remains the second leading cause of cancer deaths in women worldwide. Despite advances in prevention, early detection, and treatment with chemotherapy and radiation, breast cancer rates are rising. Current antimetastatic therapies often lack specificity, leading to side effects or incomplete inhibition of metastatic spread. This study aims to fill the gap by exploring a natural extract with

potential targeted effects on metastasis, offering a safer and more effective alternative to conventional treatments. This underscores the urgency of developing novel and more effective therapeutic approaches targeting specific molecular mechanisms underlying tumour progression and metastasis. In this context, traditional medicine, particularly the utilization of bioactive compounds from plants, offers a promising avenue for the development of adjunctive cancer therapies (Arif *et al*., 2022).

Recent progress in cancer research, driven by substantial investments in biotechnology, molecular biology, and pharmacology, has resulted in an enhanced comprehension of cancer's molecular foundations and the mechanisms that facilitate carcinogenesis. These advancements have paved the way for new diagnostic modalities and therapeutic interventions, as well as strategies to overcome challenges such as drug resistance. Despite these strides, the need for ongoing innovation in cancer research remains paramount, especially in light of the rising global cancer burden and the limitations of current treatment regimens (Sawasdee *et al*., 2022).

Our study intended to investigate the medicinal potential of *A. wightii,* a plant indigenous to India's Western Ghats that is prized for its diverse phytochemical makeup. We identified a range of bio actives by comprehensive gas chromatography-mass spectrometry (GC-MS) analysis. These compounds were subjected to *in silico* docking studies to evaluate their potential as inhibitors of key proteins involved in cancer metastasis, particularly targeting the 4AUV protein (a model of metastasis suppression). Molecular docking investigations indicated that many phytoconstituents from *A. wightii* demonstrated favourable binding affinities for the 4AUV protein, with notable G-scores akin to those of the standard chemotherapy medication doxorubicin. Among the bioactives identified, most promising compounds were 2,4-Di-tert-butylphenol, m-Dioxane,5-(hexadecyloxy)-2-pentadecyl- and trans-,9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, which demonstrated the ability to form stable hydrogen bonds with the target protein, potentially modulating its function in a manner that could inhibit cancer cell metastasis. These findings suggest that *A. wightii* may possess significant anticancer activity, particularly in preventing the spread of breast cancer cells.

Additionally, research done *in vitro* with the T-47D breast cancer cell line showed that therapy with the *A. wightii* extract substantially lowered cell viability. The extract exhibited an IC_{50} value of 66.21 µg/ml, which, while lower than that of doxorubicin (39.19 µg/ml), still indicates substantial cytotoxic potential. These results align with the findings from the brine shrimp lethality assay, which further supports the extract's cytotoxic effects.

The scratch assay is a widely used *in vitro* model to assess cell migration, a key process in the metastatic spread of cancer cells, including in breast cancer. Since metastasis involves the migration and invasion of tumor cells from the primary site to distant organs, understanding the mechanisms that regulate cell movement is critical for identifying potential therapeutic targets. In the context of metastatic breast cancer, inhibiting cell migration can prevent the spread of tumor cells and reduce the risk of relapse and secondary tumors. The results of this study demonstrate that treatment with *A. wightii* extract significantly inhibited cell migration, with a 25.44 ± 0.92% closure at 24 h, compared to 16.19 ± 1.83% closure with doxorubicin. These findings suggest that *A. wightii* may possess potential as an anti-metastatic agent, warranting further investigation into its role in blocking both cell migration and invasion in breast cancer.

The promising cytotoxic effects observed in the *in vitro* assays warrant further investigation into the molecular mechanisms underlying the extract's anticancer activity. Moreover, the identification of key phytoconstituents capable of modulating the 4AUV protein through molecular docking studies provides a compelling rationale for the exploration of *A. wightii* as a possible moiety for the creation of novel cancer treatments. *In vivo* studies are currently underway to further assess the efficacy and safety of the extract in preclinical models, which will provide critical insights into its therapeutic potential in metastatic breast cancer.

5. Conclusion

This study provides compelling novel evidence for the anti-cancer properties of *A. wightii* extract, particularly its potential to inhibit breast cancer metastasis. By utilizing both *in vitro* and *in silico* methods, we have identified several bioactive constituents of *A. wightii* that exhibit promising anticancer activity. The molecular docking studies, in conjunction with cytotoxicity assays, support the hypothesis that *A. wightii* may serve as a novel source of therapeutic agents for breast cancer treatment. Further investigation into the molecular pathways involved, coupled with ongoing *in vivo* studies, were critical for advancing the clinical applicability of this plant-derived extract in cancer therapy. Our findings underscore the importance of continued research into natural compounds with potential anticancer properties, and the need for innovative approaches to overcome the challenges posed by cancer metastasis.

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Conflict of interest

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

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