Full Length Research Article

Isoeugenol Inhibits Sphingosine Kinase 1 (SphK1) and Induces Cytotoxicity in Breast Cancer Cells

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ABSTRACT

Background: Sphingosine kinase 1 (SphK1) is considered as a critical factor as it controls sphingolipid metabolism and promotes cell survival. Targeting SphK1 with small natural compounds presents a promising therapeutic approach for breast cancer, as these compounds may have the ability to inhibit SphK1 activity, reduce tumor growth, and enhances cytotoxicity, potentially paving the way for new treatment approaches in cancer.

Methods: This study employed in-silico and in-vitro methods to identify the potent inhibitor of SphK1. Molecular docking was used to determine the binding affinity and molecular interactions of protein-ligand complex. Other in-silico methods namely SwissTargetPrediction and PASS analysis were used to determine the pharmacological potential of the lead compound. The MTT assay was conducted to evaluate the cytotoxic effects of isoeugenol, as well as its impact on the expression, protein levels, and activity of SphK1 in MCF-7 breast cancer cells.

Results: Isoeugenol was found as a potent small molecule with highest binding affinity among resveratrol and its structural analogues with SphK1. Results of the molecular docking showed binding affinity of -9.3 kcal/mol of isoeugenol with SphK1, Analysis of the docked isoeugenol-SphK1 complex revealed stable interactions ligand and target protein. Further, results of SwissTargetPrediction analysis showed that isoeugenol has broad pharmacological associations with targeting different proteins and enzymes, which are considered as key factors in various biochemical and molecular pathways, including associated with anticancer processes. MTT assay revealed a significant, dose- and time-dependent decrease in cell viability (both $p<0.001$). Viability declined progressively at 24, 36, and 48 h, with the effect persisting without further significant change at 60 and 72 h. Further, the IC_{50} dose of isoeugenol was found to be 102 µM, this concentration was used for further experiments. MCF-7 cells exposed to IC_{50} dose of isoeugenol showed significantly decreased SphK1 mRNA expression, SphK1 protein levels, and SphK1 kinase activity compared to untreated cells.

Conclusion: The findings of the current study reflect the therapeutic potential of isoeugenol and suggest that inhibition of SphK1 by this small molecule may pave the way for cancer therapeutics, including breast cancer.

INTRODUCTION

Globally, breast cancer cases are increasing exponentially in developed as well as in developing countries [1,2]. It is considered as a potential contributor to significant number of deaths worldwide in women occurring due to cancer [1]. Despite the progress in diagnosis and treatment of cancer, breast cancer is still a challenge to treatment strategies because of its heterogeneity, the potential of metastasis, and resistance of cancer cells to conventional therapeutic agents [2]. The subtypes of breast cancer in particular hormone-resistant and triple-negative cases always show poor prognosis and have defined treatment options [2]. It has been reported that conventional chemotherapy and radiation therapy, and even tamoxifen and HER2 inhibitors, fail to show long-term effects in some cancers due to development of cancer cell resistance and involvement of other molecular mechanisms [3-5]. All these challenges together highlight the unmet needs and urgent attention for the development of new therapeutic approaches, which may involve identification and targeting the specific protein molecules that act as drivers for the development and progression of the cancer, and for developing the resistance against treatment strategies. One such protein target is a kinase enzyme namely sphingosine kinase 1 (SphK1). This protein kinase is a key enzyme involved in sphingolipid metabolism pathways and has been reported associated with progression and metastasis of the cancer, and with the development of resistance against cancer therapies [6,7].

SphK1 being a lipid kinase, phosphorylates sphingosine to form a bioactive lipid molecule called sphingosine-1-phosphate [8]. This molecule plays an important role in several biological processes such as cell proliferation and migration, and process of angiogenesis [8-10]. The SphK1/S1P has been studied extensively with emerging evidences reflecting its role in cancer [7,8,11]. SphK1 overexpression has been reported in cancer and its higher levels have been found associated with aggressiveness of the tumor, advance tumor stage, poor prognosis, lymph node metastasis, resistance of cancer cells to treatments, and decreased survival of cancer patients [7,12-16].

SphK1 has been reported to manipulate many pathways such as STAT3, NF-κB, and PI3K/AKT to contribute to genesis of cancer [7,9,11,13]. It has been found that the SphK1 modulates estrogen signalling in ER-positive breast cancer cells to promote their survival and confer them with resistance against tamoxifen [7,17]. It has been also documented that the SphK1 interacts with various immune cells and stromal components to manipulate the microenvironment of the tumor cells to drive the inflammation and metastasis [18,19]. It has been found that SphK1 regulates the epithelial-mesenchymal transition process for cancer metastasis [20]. These observations collectively provide a basis that highlights the role of SphK1 in cancer, its progression, metastasis, and resistance against treatments. Therefore, targeting this enzyme through potent inhibitor molecules would be a reliable strategy to overcome the limitations associated with existing therapies. Identification of potent SphK1 inhibitors may pave the way for the development of a novel and promising treatment strategy to overcome the treatment limitations of aggressive and resistant cancer types.

Historically, plant based natural products have significantly contributed as a source for the identification of large number of anticancer agents such as paclitaxel and vincristine, which have been developed as chemotherapy agents for various cancers [21,22]. The advantage of developing small therapeutic molecules from natural products are; they exhibit lower or no toxicity, may have capacity to modulate multiple signalling mechanisms, and may have capability to decrease the likelihood of cancer cell resistance against therapeutic agents [22,23].

Over the last four to five decades, in-silico methods have significantly contributed to identification of natural small molecules with potent therapeutic properties by inhibiting the activity of target proteins [24-26]. Molecular docking like approaches are routinely used to screen the small molecules and predict their binding affinity and interaction patterns towards the target molecules [25,27]. Such approaches along with other in-silico methods such as prediction of biological activity spectrum, and ADMET properties have helped to prioritize compounds as lead molecules, therefore significantly reducing the time and cost for the identification and development of therapeutic molecules with optimal absorption, distribution, metabolism, excretion, toxicity properties. Current study was proposed to identify the lead molecules with inhibitory property against SphK1 and to investigate and validate their inhibitory properties towards SphK1 that could pave the way for the therapeutics of breast cancer.

METHODS

Preparation of target protein

The 3D structure of SphK1 (2.0 Å; PDB ID: 3VZB) was retrieved from the RCSB PDB in PDB format. The 3D structure was visualized with PyMOL and AutoDock Tools for the presence of any error, ions, co-crystallized ligands, or water molecules, if present were removed from the structure. The 3D structure of the protein was analyzed to identify any missing residues or loops, which, if present, were corrected using MODELLER. Further, the structure was modulated by the addition of hydrogen atoms, correct protonation states, and energy minimization using the AutoDock to optimize the protein structure. The modulated protein structure was saved in PDBQT format for molecular docking in AutoDoc Vina.

Selection and preparation of ligand molecules

Resveratrol, a polyphenol abundantly found in grapes and berries, has gained worldwide attention because of its therapeutic potential in particular anticancer properties [28-32]. This natural molecule has a good potential to modulate apoptosis [33], angiogenesis [34], and metastasis [35], like key signaling pathways, making it a potent therapeutic molecule. Due to abundance therapeutic properties of resveratrol, researchers are focusing on its structural analogues such as pterostilbene has been shown to exhibit good therapeutic potency and bioavailability [36].

Based on the therapeutic value of resveratrol and its structural analogues, we selected resveratrol and five of its structural analogues, namely piceatannol, pterostilbene, combretastatin, isoeugenol, and tapinarof, to investigate their inhibitory activity against the target protein Sphk1. The ligand structures in 2D SDF format were downloaded from the PubChem database (https:// pubchem.ncbi.nlm.nih.gov/) and then converted to 3D structures using Open Babel (https:// www.cheminfo.org/ Chemistry/ Cheminformatics/ FormatConverter/ index.html). Energy minimization was performed on the structures using Chem3D, and the minimized structures were saved in PDB format. Hydrogens and Gasteiger charges were added to the structures, which were then converted to PDBQT format for molecular docking. Finally, the ligand structures were visualized using PyMOL to check for any missing atoms, inappropriate valency, or unassigned charges. The prepared ligands are now ready for molecular docking with the target protein Sphk1.

Molecular docking

All the six phytochemical ligand molecules were screened against the target protein “SphK1”. Molecular docking of top hit “isoeugenol” with sphK1 was performed using AutoDock Vina. The grid box dimensions were defined with X, Y, and Z coordinates set to 46 Å, 59 Å, and 61 Å, respectively, cantered at −33.44, 16.21, and 49.65. A grid spacing of 1.00 Å was maintained, and the exhaustiveness parameter was set to 8. The binding affinities of the docked complexes were analysed, and the interaction patterns between the isoeugenol and SphK1 were visualized using Discovery Studio Visualizer.

Prediction of activity spectra for isoeugenol

SwissTargetPrediction tool was used to determine the activity spectra of isoeugenol. This tool generates a list of potential protein targets ranked by probability. SMILE notation of isoeugenol was uploaded to SwissTargetPrediction online tool http://www.swisstargetprediction.ch/).

Cell cytotoxicity assay

MCF7 breast cancer cells line was derived commercially from ATCC (https:// www.atcc.org). These cells were seeded in a 96-well plate at a density of 5,000-10,000 cells per well in 100 µL of DMEM culture medium supplemented with 10% FBS and 1% antibiotic solution. Plate was Incubated at 37°C with 5% CO_2 for 24 hours to allow cell attachment. Serial dilutions of isoeugenol were prepared in the culture medium and the medium in each well was replaced with 100 µL of the isoeugenol solutions at varying concentrations (0-400 µM). Plate was incubated for 24 hours at 37°C with 5% CO_2. After incubation, 10 µL of MTT reagent (5 mg/mL in PBS) was added to each well and the plate was incubated for 3-4 hours at 37°C to allow formazan crystal formation. Medium was carefully removed and 100 µL of DMSO was added to each well to

dissolve the formazan crystals. Plate was gently shaken for 10-15 minutes to ensure complete dissolution. Absorbance of each well was measured at 570 nm using a microplate reader, with a reference wavelength of 630 nm. Cell viability was calculated as a percentage relative to the control wells using the formula: Cell Viability (%) = Results were plotted as a dose-response curve, testing multiple concentrations. The IC_{50} concentration of isoeugenol was calculated. Additionally, a time-dependent cell toxicity assay was performed at 24, 48, and 72 hours using the IC_{50} concentration of isoeugenol.

Measurement of mRNA expression of SphK1 by qRT-PCR and activity by ELISA

Total RNA from treated (treatment with IC_{50} concentration of isoeugenol; 102 µM) and control cells was isolated using TRIzol reagent (Thermo Fisher Scientific). cDNA was synthesized from 1 µg of total RNA using cDNA kit (Takara Bio). qRT-PCR was conducted using SYBR Green (G-Biosciences). Relative mRNA expression of SphK1 was calculated using the 2^(-ΔΔCt) method, normalized to the housekeeping gene, GAPDH. Expression levels were compared between treated and control samples. The specific primers for SphK1 – forward 5'-AGGCTGAAATCTCCTTCACGC-3'; reverse 5'-GTCTCCAGACATGACCACCAG-3', and for GAPDH - Forward 5'- ACCCACTCCTCCACCTTTGA-3'; Reverse 5'- CTGTTGCTGTAGCCAAATTCGT-3 were used for the amplification of target gene.

We also measured the SphK1 protein concentrations in treated and control cell lysates by using SPHK1 - Ready-To-Use ELISA Kit (Colorimetric), Novus Biologicals.

Kinase inhibition assay

We studied the effect of isoeugenol on SphK1 kinase activity. SphK1 (1 µM) was treated with isoeugenol concentrations ranging from 0-500 µM in a 96-well plate. After 1-hour incubation at 25°C, a reaction mixture (100 µM ATP, 10 mM $MgCl_2$) was added and incubated for 30 minutes. Reactions were stopped using BIOMOL® reagent, and the green complex formed due to inorganic phosphate release was measured at 620 nm. Absorbance was compared to untreated SphK1 (100% activity) to determine isoeugenol's impact. Malachite green reagent is specifically used in this assay to detect the inorganic phosphate released, serving as an indicator of SphK1 activity.

RESULTS

Molecular docking

The binding affinities of resveratrol and five of its structural analogues namely piceatannol, pterostilbene, combretastatin, isoeugenol, and tapinarof are represented in Table 1. Among these, the highest binding affinity was observed between isoeugenol and SphK1, with a binding affinity value of -9.3 kcal/mol. The interaction patterns of isoeugenol with SphK1 are illustrated in Figure 1A-C. Figure 1A is a Cartoon representation of isoeugenol-SphK1complex, Figure 1B representing binding of isoeugenol within deep groove of SphK1, and Figure 1C represents 2D docked complex showing interaction of isoeugenol with various amino acid residues of SphK1. These findings highlight isoeugenol's potential as a strong SphK1 inhibitor, warranting further investigation for therapeutic applications.

Figure 1D shows diverse biological targets for Isoeugenol predicted by SwissTargetPrediction analysis. Results indicate that isoeugenol exhibits interactions with a wide range of biological targets, highlighting its manifold pharmacological potential. These targets include structural proteins, transcription factors, nuclear receptors, and enzymes such as cytochrome P450, lyases, kinases, and oxidoreductases. Such interactions suggest that isoeugenol may influence key biochemical pathways, contributing to its broad-spectrum biological activities. Notably, its potential roles in anti-inflammatory, antioxidant, and anticancer processes make it a promising candidate for further investigation in drug discovery and therapeutic applications. These findings support the need for additional experimental validation to explore its mechanism of action and clinical relevance.

Cytotoxicity assay and mRNA expression of SphK1

Figure 2A displays the concentration-dependent impact of isoeugenol on MCF7 breast cancer

cell viability. The findings revealed that increasing concentrations of isoeugenol lead to a significant dose-dependent decline ($p<0.001$) in cell viability, indicating greater cytotoxic effects at higher doses. This suggests isoeugenol strongly inhibits cancer cell growth and survival. The calculated IC_{50} value of 102 µM highlights its potential as a therapeutic candidate for breast cancer. Figure 2B presents the time- dependent toxicity assay results, demonstrating that prolonged exposure to the IC_{50} concentration of isoeugenol significantly reduced MCF7 cell viability over time. The data shows a significant decrease ($p<0.001$) in cell viability with extended exposure, further supporting its cytotoxic efficacy. Viability declined progressively at 24, 36, and 48 h, with the effect persisting without further significant change at 60 and 72 h.

The impact of isoeugenol at IC_{50} concentration on SphK1 mRNA expression and activity is depicted in Figures 2C and 2D, respectively. The findings revealed a significant decrease ($p<0.001$) in both mRNA expression and SphK1 levels in isoeugenol-treated MCF7 cancer cells compared to the control group. These observations reflect the therapeutic potential of isoeugenol and suggest its development as a therapeutic agent for the treatment of cancer, particularly breast cancer.

Kinase inhibition assay

The results of the effect of isoeugenol on the kinase activity of SphK1 are represented in Figure 3. Results showed a significant decrease ($p<0.001$) in kinase activity of SphK1 with the increasing concentrations of isoeugenol. This observation demonstrates the kinase activity inhibition property of isoeugenol, in particular inhibiting the activity of SphK1, highlighting that isoeugenol may be developed as cancer therapeutic molecule.

Figures

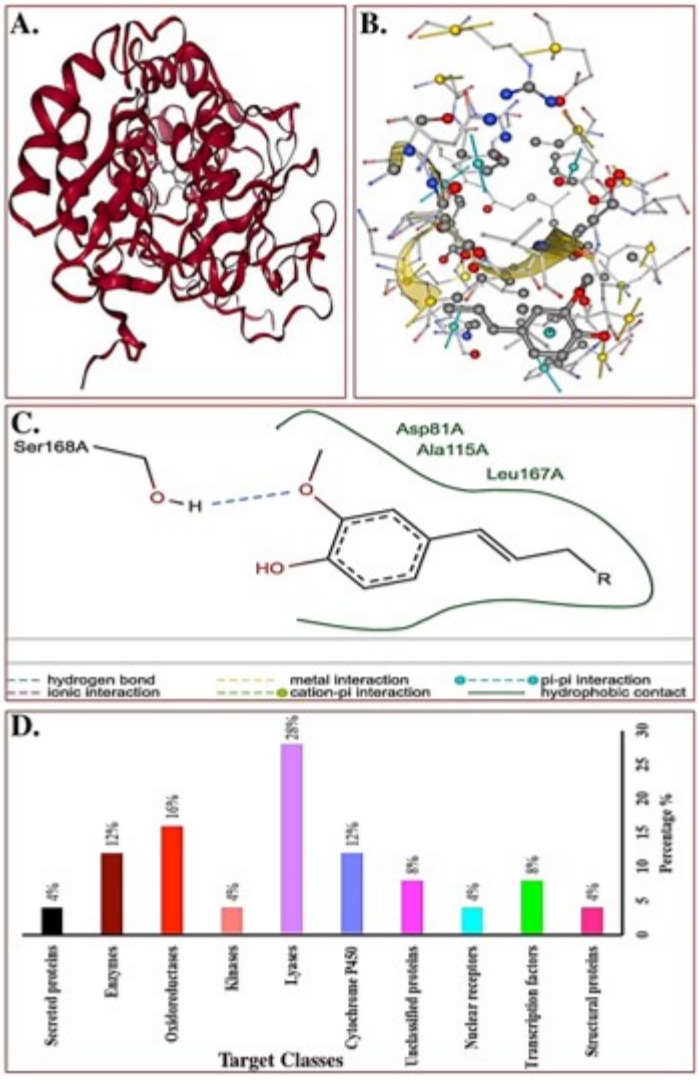


Figure 1. Molecular docking of Isoeugenol with Sphingosine Kinase 1. (A) Cartoon representation of

isoeugenol-SphK1complex, (B) Binding of isoeugenol within deep groove of SphK1, (C) 2D docked complex showing interaction of isoeugenol with various amino acid residues of SphK1, and (D) Diverse biological targets for Isoeugenol.

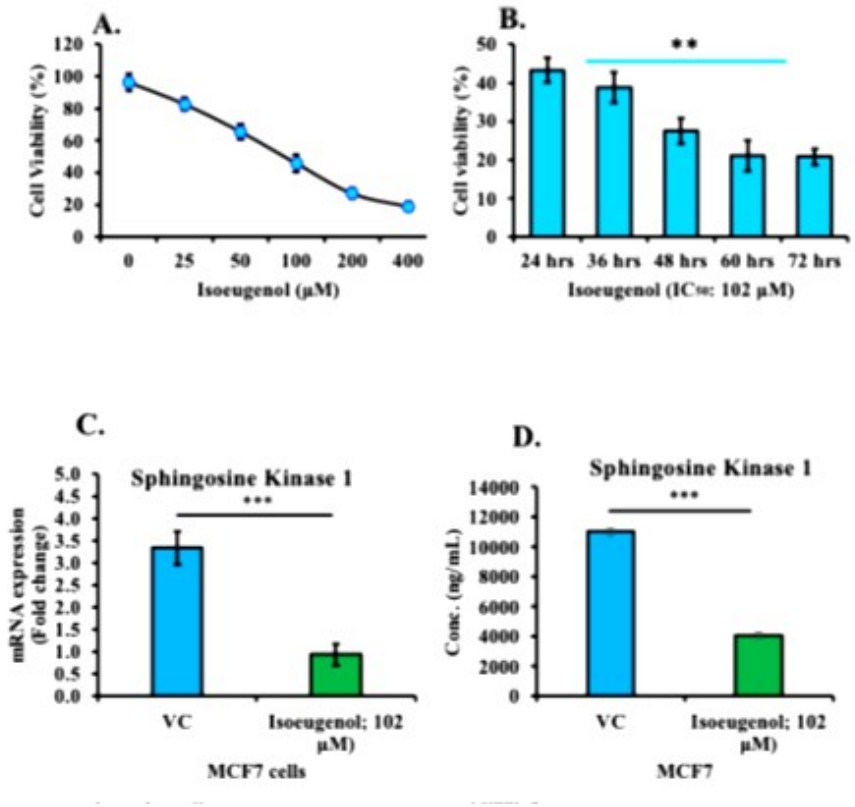


Figure 2: (A) Effect of different concentrations of isoeugenol on the viability of MCF7 cancer cells (B) Effect of IC50 dose of isoeugenol on the viability of MCF7 cancer cells in a time dependent manner (C) Effect of IC50 dose of isoeugenol on the SphK1 mRNA expression levels (D) Effect of IC50 dose of isoeugenol on the SphK1 protein concentration.

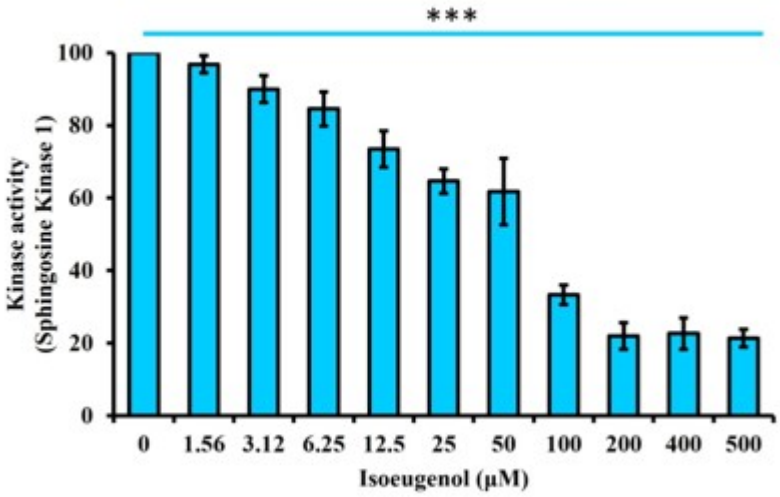


Figure 3. Effect of increasing concentration of Isoeugenol on Kinase activity of Sphingosine Kinase 1. Figure reflects statistically significant decrease (P<0.001) in SphK1 kinase activity as isoeugenol concentration increased.

Tables

Table 1. Binding affinities of resveratrol and five of its structural analogues with the target protein, SphK1. Among these, the highest binding affinity was observed between isoeugenol and SphK1, with a binding affinity value of -9.3 kcal/mol.

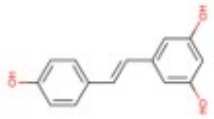
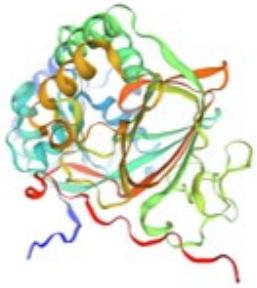
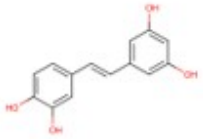
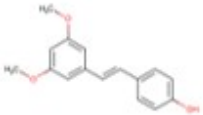
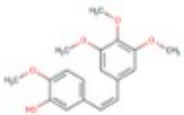
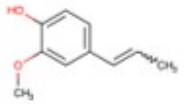
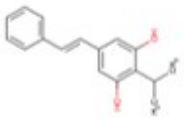
Name of the ligand	Target protein	Binding Energy (kcal/mol)
Resveratrol 	 SphK1	-5.0
Piceatannol 		-4.8
Pterostilbene 		-6.0
Combretastatin 		-4.3
Isoeugenol 		-9.3
Tapinarof 		-6.2

Table 1. Binding affinities of resveratrol and five of its structural analogues with the target protein, SphK1. Among these, the highest binding affinity was observed between isoeugenol and SphK1, with a binding affinity value of -9.3 kcal/mol.

DISCUSSION

Sphingosine kinase 1 (SphK1) is critical in cancer progression because it promotes cell survival, proliferation, and resistance to therapy [9,11]. It catalyses the formation of sphingosine-1-phosphate, a lipid mediator that promotes tumour development and metastasis [37]. Inhibiting SphK1 has emerged as a promising therapeutic option for breast cancer because it impairs cancer cell signalling pathways and produces apoptosis [7,12]. Natural compounds have showed the ability to target kinases and decrease their activity, hence decreasing tumour growth and increasing chemosensitivity [38,39]. Given the therapeutic potential of natural small molecule inhibitors, we aimed to find more effective inhibitor compounds for SphK1, providing an alternative to traditional medicines with better patient outcomes. The current research work investigates the potential of resveratrol and its five structural analogues, particularly isoeugenol, to inhibit SphK1, a critical enzyme implicated in cancer growth.

We first determined the binding affinities of resveratrol and its five structural analogues towards SphK1. Molecular docking is imperative in drug development because it predicts how plausible therapeutic compounds will interact with target proteins [40,24]. It helps in the identification of

binding affinities, ideal binding locations, & interaction patterns, predicting the range of effective therapeutic candidates [25]. This computational technique accelerates the identification of therapeutic drugs in a cost effective way, & development of effective therapeutic molecules for a diversity of diseases [41]. In the current research work, we found that isoeugenol has the maximum binding affinity (-9.3 kcal/ mol) among the resveratrol and its derivatives towards SphK1. The molecular docking also illustrates the aspect that how isoeugenol binds to SphK1's in a deep groove & interacts with specific amino acid residues. These observations are crucial because they validate isoeugenol's ability to function as a potent SphK1 inhibitor. The compound's good binding affinity and its structural interaction and compatibility with SphK1's active site, are essential for actual inhibition [25].

SwissTargetPrediction is imperative in drug discovery because it predicts protein targets for bioactive compounds [42]. This computational technique engages chemical similarity & machine learning programs to screen and reflect targets, therefore fasten up drug development for various diseases and disorders [43]. According to SwissTargetPrediction analysis, isoeugenol interacts with a diverse set of biological targets, structural proteins, transcription factors, nuclear receptors, & enzymes such as cytochrome P450, lyases, kinases, & oxidoreductases. This broad activity spectrum emphasizes isoeugenol's multifaceted pharmacological properties, particularly in anti- inflammatory, antioxidant, & anticancer mechanisms [25]. Isoeugenol's ability to target multiple pathways may enhance its therapeutic potential by simultaneously disrupting several cancer- promoting mechanisms. However, its promiscuity raises concerns regarding potential side effects and toxicity. Despite ongoing investigations into its anticancer properties, interactions with cytochrome P450 enzymes, for example, could lead to drug–drug interactions, complicating its use in combination therapy [44].

Cytotoxicity assays are important assays in discovering drug compounds via evaluating their cytotoxic effects on cell viability & proliferation [45]. These assays help in decisive compounds' therapeutic likeliness & safety, for the identification of active disease therapy candidates while minimising side effects, hence advancing drug discovery and development process [46,47]. The cytotoxicity assays showed that isoeugenol significantly decreased the viability of MCF7 breast cancer cells in a dose- & time-dependent manner. The calculated IC_{50} value of 102 μM point to reasonable cytotoxicity, which is promising but not comparable compared to present chemotherapeutic agents. The time- dependent assay further supports isoeugenol’s cytotoxic efficacy, showing a significant reduction in cell viability with prolonged exposure. These findings suggest that isoeugenol could be effective in inhibiting cancer cell proliferation and survival. However, the study does not address the selectivity of isoeugenol for cancer cells over normal cells. Assessing cytotoxicity in non-cancerous cell lines would provide valuable insights into its therapeutic window and potential side effects. Additionally, the mechanisms underlying isoeugenol- induced cytotoxicity remain unclear. While the study links cytotoxicity to SphK1 inhibition, other pathways, such as apoptosis induction or cell cycle arrest, could also contribute to the observed effects. Further mechanistic studies are needed to elucidate these aspects.

Assessing mRNA expression and activity of the target protein is crucial for identifying potent drug molecules. It provides insights into molecular mechanisms, validates drug targets, and evaluates therapeutic efficacy [48,49]. This approach aids in discovering compounds that modulate disease- related pathways, accelerating the development of effective treatments for various diseases, including cancer, infections, and metabolic disorders [50]. This study demonstrated that isoeugenol significantly reduced both SphK1 mRNA expression and enzymatic activity of SphK1 in MCF7 cells. This dual suppression is especially important because it shows that isoeugenol not only suppresses SphK1 activity but also reduces its expression, thus increasing its therapeutic effects. Given SphK1's well-documented function in cancer cell survival, proliferation, and metastasis, these findings provides strong support for isoeugenol's potential as a therapeutic molecule for the treatment of breast cancer. However, the study does not investigate the processes by which isoeugenol reduces SphK1 mRNA expression. Transcriptional regulation, post-transcriptional changes, or epigenetic mechanisms may be involved, and studying these pathways would provide a more complete understanding of isoeugenol's mechanism of action. Furthermore, the research focusses primarily on MCF7 cells, which represent luminal A breast carcinoma. Evaluating isoeugenol's impact on additional breast cancer subtypes, such as triple-negative or HER2-positive, might increase the findings' generalisability.

Kinase inhibition assays are important in drug development because they assess drugs' inhibitory effects on target kinase enzymes [51]. These assays aid in the identification of potent

drug candidates that control kinase activity, providing therapeutic promise for diseases such as cancer, inflammation, and neurodegeneration, consequently enabling the development of focused and effective treatments [11, 52, 53]. The kinase inhibition experiment demonstrated that isoeugenol dramatically reduced SphK1 kinase activity in a concentration- dependent manner. This observation is critical because it directly relates isoeugenol's binding affinity to its functional inhibitory effects. The high association between isoeugenol concentration and kinase inhibition adds to its potential as a SphK1- targeting medicinal treatment. Nonetheless, the study does not address isoeugenol's selectivity for SphK1 over other kinases [54]. Given the structural similarities amongst kinases, off- target inhibition may have unforeseen biological consequences. Also, the study did not inspect the downstream effects of SphK1 inhibition, such as changes in sphingolipid metabolism or signaling lanes. Exploring these elements might yield a complete picture of isoeugenol's medicinal properties.

The findings of this study highlight isoeugenol's potential as a natural SphK1 inhibitor for breast cancer therapy. Its strong binding affinity, cytotoxic effects, and ability to suppress SphK1 expression and activity make it a promising candidate for further development. However, several challenges must be addressed before isoeugenol can be considered for clinical applications. First, the pharmacokinetic and pharmacodynamic properties of isoeugenol need to be thoroughly investigated. Factors such as bioavailability, metabolism, and tissue distribution will determine its efficacy and safety in vivo. Second, the potential for drug- drug interactions, particularly due to its interaction with cytochrome P450 enzymes, must be evaluated. Third, the therapeutic window of isoeugenol should be established by comparing its effects on cancer cells versus normal cells. Future studies should also explore combination therapies involving isoeugenol and existing chemotherapeutic agents. Synergistic effects could enhance therapeutic efficacy while reducing the required doses of individual drugs, potentially minimizing side effects. Additionally, in vivo studies using animal models of breast cancer are essential to validate the findings of this study and assess the translational potential of isoeugenol.

In conclusion, the present study identified isoeugenol as a potent inhibitory molecule targeting sphingosine kinase 1 (SphK1) and highlighted its potential to be developed as a therapeutic candidate in breast cancer treatment. Isoeugenol demonstrated strong binding affinity with SphK1, markedly reduced the viability of MCF-7 breast cancer cells, and significantly downregulated mRNA expression SphK1. The treated cells also showed a decreased SphK1concentrations. Moreover, the compound exhibited a broad spectrum of biological activities, further supporting its therapeutic relevance. However, the study has certain limitations. It lacks detailed mechanistic insights into how isoeugenol exerts its inhibitory action on SphK1 signalling, does not provide data on its selectivity against related kinases or off-target proteins, and does not include in vivo validation to confirm its therapeutic efficacy and safety. Therefore, future investigations are warranted to elucidate the precise molecular mechanism, evaluate pharmacological selectivity, and perform preclinical animal studies to establish its translational potential as a candidate for breast cancer therapy.

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CONFLICT OF INTEREST

The authors declare that they have nothing to disclose.

AUTHOR CONTRIBUTIONS

MMA and ISA contributed equally to conceptualization, methodology, data curation, formal analysis, writing - original draft, writing - review & editing, and proofreading.

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Generative AI Statement

The author(s) declare that Generative AI tools (e.g., Grammarly, QuillBot) were utilized to improve the language and clarity of the manuscript. The author(s) affirm that they take full responsibility for the accuracy, originality, and integrity of the scientific content.

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