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Exploring *Catharanthus roseus*-Derived Compounds for Targeting Estrogen Receptors: A Molecular Docking Approach in Cancer Therapy

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Abstract:

Objective: Breast cancer affects people all over the world.To reduce tumor growth the drugs are developed from naturally occurring active ingredients like *Catharanthus roseus*, which are utilized as an anti-cancer agent and have many medical uses. The best ligand to utilize as a medication is determined via molecular docking and absorption, distribution, metabolism, and excretion (ADME) investigations.

Methods: The Protein Data Bank (PDB) database was searched in order to retrieve the 3ERT protein. The phytocompounds were obtained by use of the IMPPAT database. These compounds as pharmacokinetic properties were assessed through the use of in silico ADME analysis. Molecular docking is done with PyRx.

Results: The chosen *Catharanthus roseus* phytocompound showed encouraging interactions with the 3ERT protein in a molecular docking investigation, suggesting that it may be an inhibitor of breast cancer. Significant binding affinity was shown between Secologanin,Citric acid, Hirsutidin and 3ERT, indicating the need for more research. Additionally, the phytocompound had favorable pharmacokinetic characteristics, indicating its potential for use in drug development.

Conclusion: With its encouraging binding affinity for the 3ERT protein, the phytocompounds Secologanin,Citric acid, Hirsutidin are used as viable therapeutic option for the treatment of breast cancer.

Keywords: *Catharanthus roseus*, Breast carcinoma, 3ERT, Phytocompound, Molecular Docking, ADMET analysis

Introduction:

Cancer remains a significant global health challenge and has now surpassed cardiovascular diseases as the predominant cause of mortality worldwide [1]. Among various cancer types, breast cancer prominently stands as the second most common cause of cancer-related deaths in women [2]. Originating predominantly in the breast tissue, breast cancer typically develops either in the inner lining of milk ducts—known as ductal carcinomas—or in the lobules that supply the ducts with milk, referred to as lobular carcinomas [3].

Breast cancer can be categorized into three primary subtypes based on the presence or absence of certain molecular markers: hormone receptor-positive/ERBB2-negative, which comprises about 70% of cases; ERBB2-positive, accounting for 15-20% of cases; and triple-negative breast cancer, which makes up about 15% of cases [4]. The most prevalent type of invasive breast cancer is infiltrating ductal carcinoma (IDC), representing up to 80% of all invasive cases. Invasive lobular carcinoma follows as the second most frequent type [5]. Notably, over 80% of all noninvasive in situ breast carcinomas are ductal carcinomas, with lobular carcinomas comprising about 10%.

A critical aspect of breast cancer is its metastatic potential, which is the primary cause of mortality in affected individuals. Metastases may occur even at early stages, often before the primary tumor has been detected, underscoring the urgency for early detection to improve disease management and outcomes [6]. Interestingly, the process of metastasis can be independent from the growth of the primary tumor, suggesting that early and separate pathways might influence the spread of cancer cells [7]. The incidence of breast cancer remains particularly high in the United States, reflecting its significant impact on public health.

Catharanthus roseus, also known as Vinca rosea, Ammocallis rosea, and Lochnera rosea, is a significant medicinal plant belonging to the Apocynaceae family. This plant, native to the Indian subcontinent and widely found in southern Asia, is renowned for its rich content of over 70 different types of alkaloids and chemotherapeutic agents [8]. These compounds have shown efficacy in treating diverse cancers such as breast cancer, lung cancer, uterine cancer, melanomas, and both Hodgkin's and non-Hodgkin's lymphoma.

Among the array of alkaloids it produces, vincristine and vinblastine are particularly noteworthy, constituting between 0.74 to 0.82% of the plant’s makeup. Vinblastine is especially prominent due to its role in experimental cancer treatments, notably against Hodgkin's disease and choriocarcinoma. Its mechanism involves inhibiting microtubule formation during cell division, thereby preventing the mitotic process and curbing the proliferation of cancer cells. Further investigations into *Catharanthus roseus* have revealed additional alkaloids like deoxyvinblastine, leurosine, and pleurosin, which also exhibit growth-inhibitory effects on various human cancer cell lines, including those that are multidrug-resistant [9]. The broad spectrum of alkaloids present in *Catharanthus roseus* underscores its potential as a valuable resource for pharmacological applications, particularly in oncology.

Recent studies have also highlighted the effectiveness of methanolic leaf extracts from *Catharanthus roseus,* which are found to contain potent secondary metabolites with both antibacterial and anticancer properties[10]. This discovery reinforces the importance of

exploring natural sources for novel medicinal compounds, especially in combating cancer and drug-resistant diseases [11].

Figure 1: *Catharanthus roseus*

Methodology:

Protein extraction

We obtained the protein structure associated with PDB ID 3ERT from the RCSB Protein Data Bank (PDB) at (https://www.rcsb.org/). The protein was visualized using the software Discovery Studio 2021 v21.1.0.20298 (BIOVIA), which eliminated water molecules, ions, and ligands. The modified protein was saved in PDB format for further analysis.

Secondary Structure prediction

The PDBsum web server (http://www.ebi.ac.uk/pdbsum) was used to estimate the secondary structure of the protein. This method revealed the distribution of secondary structural characteristics, including beta bulges, psi-loops, beta strands, beta hairpins, and alpha helices [12].

Additionally, the Ramachandran plot analysis was performed using PROCHECK, a tool that is linked on the PDBsum service. This method allowed for the evaluation of the protein's overall structural integrity and conformational quality [13].

Retrieval of phytocompounds using IMPPAT database

The IMPPAT database (https://cb.imsc.res.in/imppat/) was searched to find similar phytocompounds from the medicinal plant *Catharanthus roseus*. Thirty two phytocompounds were obtained after a selection procedure.

In silico ADME analysis

A crucial tool in the pharmacokinetics research analysis was Swiss ADME(http://www.swissadme.ch/), which evaluated the ligand's behavior by utilizing a number of factors related to absorption, distribution, metabolism, and excretion [14]. Permeability, toxicity, physiological and biological characteristics, and bioavailability are all examined in this analysis; these are all important aspects of the drug development process. Using Lipinski's five guidelines—which are displayed in Table 1—it ensured a thorough evaluation of drug candidacy and provided an additional layer of flexibility in identifying possible therapeutic agents.

A comprehensive examination of the physiochemical properties was carried out, considering significant variables such as molecular weight, saturation (fraction Csp3 or Sp3 hybridization), flexibility (calculated by the number of rotational bonds), polarity (calculated by topological polar surface area (TPSA)), and lipophilicity (often expressed as xLogP).

Table 1. Lipinski rule specifications.

Property	Optimal Range
H Donor	<5
H Acceptor	<10
MlogP	<4.15
Molecular weight	<500 Daltons
Molecular refractivity	40-130

Visualization

The BIOVIA Discovery Studio 2021 v21.1.0.20298 (https://discover.3ds.com/discovery-studio-visualizer-download)was used for the in-depth examination of receptor-ligand interactions.

In order to comprehend the molecular binding structures and mechanisms, our work closely examined both 2D and 3D interactions [15]. The goal of the study was to identify the precise molecular relationships that trigger the creation of receptor-ligand complexes.

Docking

Molecular docking study was performed on phytochemical compounds isolated from *Vitis vinifera* against the 3ERT protein target. Virtual screening of the compounds was done using the PyRx Virtual Screening Tool (https://pyrx.sourceforge.io/) and the bundled AutoDock Vina program. To maximize docking simulations, grid parameters were adjusted to match the target protein structure (PDB ID: 3ERT). For additional analysis, the binding energies of phytochemical-protein interactions were computed and arranged into CSV format. The ligand-protein complexes were then visualized using BIOVIA software, which clarified the structural underpinnings of their interactions.

RESULTS

Target Extraction and purification

The RCSB Protein Data Bank provided the protein structure associated with PDB ID 3ERT. This structure was then downloaded and saved in the common PDB format. After downloading the 3ERT PDB structure, the retrieved protein structure was cleaned using the BIOVIA Discovery studio program. Using the editing features of the software, the precise residues that coordinated the zinc ions were located and meticulously eliminated. Therefore, the newly altered protein structure (Figure 2) was stored and utilized to additional computational analysis.

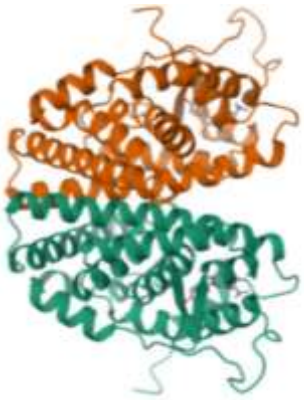

Figure.1 3D model of protein.

Structure validation of the protein

The secondary structure of the target protein (PDB ID: 3ERT) was predicted using the PDBSUM website. The structure consists of 247 residues in total. As seen in Figure 3, the protein's secondary structure consists one sheet, one beta hairpin, one beta bulge, two strands, eleven helices, seventeen helix-helix interacs, seventeen beta turns, two gamma turns.

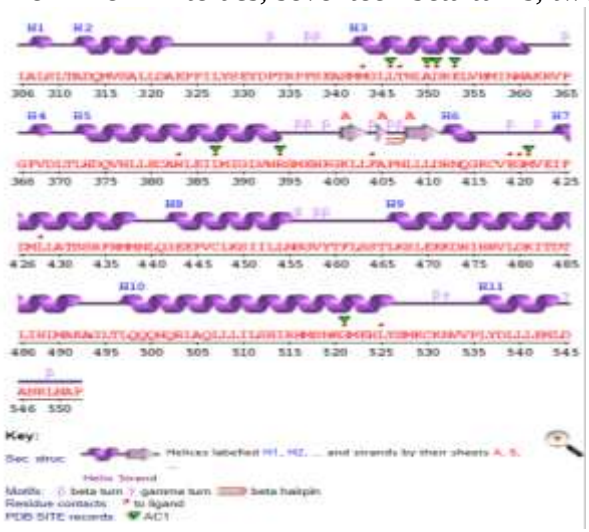

Figure.2 Secondary structure of the protein.

Ramachandran Plot

A depiction of amino acids within energetically favorable regions was rendered through the Ramachandran plot. This graphical representation was generated via PDBsum, with the structural integrity assessed by PROCHECK.

The plot shows red color for most favoured regions represented by A, B, L with 207 residues, additional allowed regions represented by a, b, l, p indicated yellow color with 20 residues, generously allowed regions represented by ~a, ~b, ~l, ~p in faint yellow with 0 residues, disallowed region represented by XX indicates white color with 0 residue , the end-residues will excl. Gly and Pro 19 amino acid residues, where glycine 10 residues and proline 9 residues were analyzed in plot statistics.

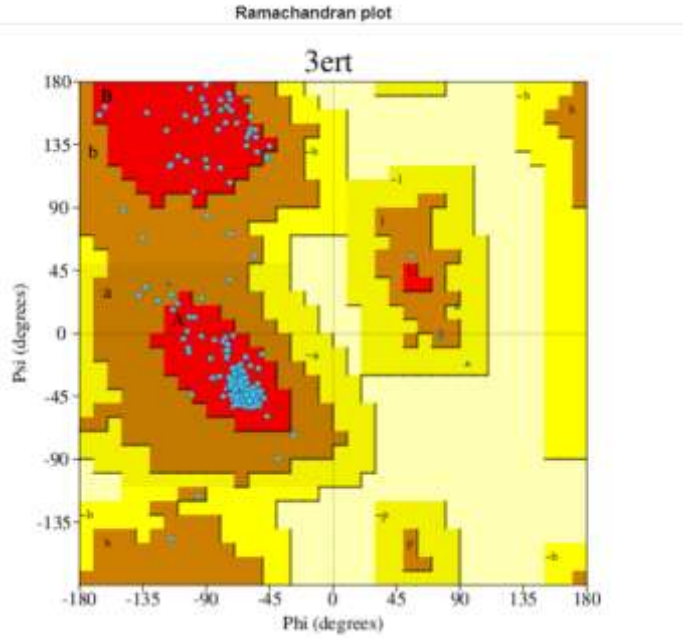

Figure 3. Ramachandran plot

Identifying Phytocompound Candidates

The thirty two compounds, with ID are:

ID	Phytochemical name	ID	Phytochemical name
IMPHY007011	Coronaridine	IMPHY001644	Malvidin
IMPHY003833	Alstonine	IMPHY001714	Secologanin
IMPHY000060	Myristic Acid	IMPHY001915	Octadecane
IMPHY000136	Pyruvic acid	IMPHY002588	Flavylium
IMPHY000308	Hexadecane	IMPHY002667	Pentadecanoic acid
IMPHY000309	Dotriacontane	IMPHY002915	Benzyl Alcohol
IMPHY000399	beta-Bisabolene	IMPHY003016	Lauric acid
IMPHY000413	Anthocyanin 1	IMPHY003104	Decanoic acid
IMPHY000885	Rosinidin	IMPHY003316	Pentadecanal
IMPHY000905	Petunidin	IMPHY003437	Pelargonidin
IMPHY000923	Hirsutidin	IMPHY003485	Myrcene
IMPHY001266	Petunidin 3-glucoside	IMPHY003500	Citric acid
IMPHY001267	Oenin	IMPHY003525	Nonanal
IMPHY001279	Gomaline	IMPHY003536	Eugenol
IMPHY001548	Geranylacetone	IMPHY006298	Vincristine
IMPHY001555	Jasmone	IMPHY015109	Vinblastine

ADME Analysis

As indicated in Table 2, the phytocompounds were evaluated using SWISS ADME to determine their physiochemical characteristics and compliance with Lipinski's rule. This critical stage entailed analyzing molecular characteristics, which are essential for forecasting the pharmacokinetic behavior of the molecule. These characteristics include molecular weight, lipophilicity, and polarity. Furthermore, the compound's drug-likeness was assessed using Lipinski's rule, a generally recognized protocol in drug development, taking into account factors like molecular weight, partition coefficient, hydrogen bond donors, and acceptors.

Table 2. Data on Lipinski properties collected using Swiss ADME.

Ligand	Formula	MW	MLOGP	H Acceptor	H Donor	MR
Coronaridine	$C_{21}H_{26}N_2O_2$	338.44	3.04	3	1	102.74
Alstonine	$C_{21}H_{20}N_2O_3$	348.4	2.21	4	0	99.59
Myristic acid	$C_{14}H_{28}O_2$	228.37	3.69	2	1	71.18
Pyruvic acid	$C_3H_4O_3$	88.06	-0.96	3	1	18.51
Hexadecane	$C_{16}H_{34}$	226.44	6.44	0	0	79.03
Dotriacontane	$C_{32}H_{66}$	450.87	9.82	0	0	155.94
beta-Bisabolene	$C_{15}H_{24}$	204.35	4.53	0	0	70.68
Anthocyanin 1	$C_{15}H_{11}O^+$	207.25	3.28	1	0	66.06
Rosinidin	$C_{17}H_{15}O_6^+$	315.3	0.81	6	3	85.11
Petunidin	$C_{16}H_{13}O_7^+$	317.27	0.03	7	5	82.66
Hirsutidin	$C_{18}H_{17}O_7^+$	345.32	0.52	7	3	91.6
Gomaline	$C_{30}H_{42}N_4O_2$	490.68	2.82	4	0	161.03
Geranylacetone	$C_{13}H_{22}O$	194.31	3.34	1	0	63.86
Jasmone	$C_{11}H_{16}O$	164.24	2.39	1	0	52.13
Malvidin	$C_{17}H_{15}O_7^+$	331.3	0.28	7	4	87.13

Secologanin	$C_{17}H_{24}O_{10}$	388.37	-1.95	10	4	88.04
Octadecane	$C_{18}H_{38}$	254.49	6.92	0	0	88.64
Flavylium	$C_{15}H_{11}O^{+}$	207.25	3.28	1	0	66.06
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	3.94	2	1	75.99
Benzyl Alcohol	C_7H_8O	108.14	1.54	1	1	32.57
Lauric acid	$C_{12}H_{24}O_2$	200.32	3.15	2	1	61.57
Decanoic acid	$C_{10}H_{20}O_2$	172.26	2.58	2	1	51.96
Pentadecanal	$C_{15}H_{30}O$	226.4	4.06	1	0	74.42
Pelargonidin	$C_{15}H_{11}ClO_5$	306.7	1.11	5	4	80
Myrcene	$C_{10}H_{16}$	136.23	3.56	0	0	48.76
Citric acid	$C_6H_8O_7$	192.12	-1.48	7	4	37.47
Nonanal	$C_9H_{18}O$	142.24	2.39	1	0	45.58

Pharmakokinetics properties

Following that, a comprehensive assessment of the phytocompounds was conducted to ascertain their pharmacokinetic properties, which included GI absorption, P-glycoprotein (Pgp) substrate potential, BBB permeability and Lipinski violations, as indicated in Table 3.

Table 2. ADME information acquired via Swiss ADME.

Ligand	BBB	GI absorption	Pgp substrate	Lipinski #violations
Coronaridine	Yes	High	No	0
Alstonine	Yes	High	No	0
Myristic acid	Yes	High	No	0
Pyruvic acid	No	High	No	0
Hexadecane	No	Low	No	1
Dotriacontane	No	Low	Yes	1
beta-Bisabolene	No	Low	No	1
Anthocyanin 1	Yes	High	Yes	0
Rosinidin	No	High	Yes	0
Petunidin	No	High	Yes	0
Hirsutidin	No	High	Yes	0
Gomaline	Yes	High	Yes	0
Geranylacetone	Yes	High	No	0
Jasmone	Yes	High	No	0
Malvidin	No	High	Yes	0
Secologanin	No	Low	No	0
Octadecane	No	Low	No	1
Flavylium	Yes	High	Yes	0
Pentadecanoic acid	Yes	High	No	0
Benzyl Alcohol	Yes	High	No	0
Lauric acid	Yes	High	No	0
Decanoic acid	Yes	High	No	0
Pentadecanal	Yes	High	No	0
Pelargonidin	No	Low	Yes	0
Myrcene	Yes	Low	No	0
Citric acid	No	High	No	0
Nonanal	Yes	High	No	0

MOLECULAR DOCKING OF THE TARGET PROTEIN AND SELECTED PHYTOCOMPOUNDS

After the screening procedure, three phytocompounds— Secologanin ,Citric acid, Hirsutidin—were chosen for additional docking experiments because they demonstrated the highest binding affinities with the protein. These results are displayed in Table 4.

BINDING AFFINITY

Table 4 indicates that the three phytocompounds that were selected, Secologanin ,Citric acid and Hirsutidin has the highest binding affinity. As a result, it was selected as the purpoted ligand that would be visible. After molecular docking in PyRx, BIOVIA Discovery Studio 2021 v21.1.0.20298 was used to investigate ligand-protein interactions. The macromolecule's amino acid residues were examined for two-dimensional (two-dimensional) interactions with the ligands, including halogen, conventional hydrogen bonds, and unfavorable donor-donor interactions. Each amino acid's identity, location along the protein chain, separation from the ligand, kind of bond, and interaction category were identified using two-dimensional (2D) analysis.

Table.4. Binding affinity data for chain A obtained from PyRx.

Ligands	Binding affinity
Coronaridine	-5.85
Alstonine	-6.59
Myristic acid	-3.35
Pyruvic acid	-7.07
Anthocyanin 1	-5.07
Rosinidin	-7.22
Petunidin	-6.88
Hirsutidin	-7.50
Gomaline	-7.02
Geranylacetone	-4.86
Jasmone	-5.74
Malvidin	-6.73
Secologanin	-9.82
Flavylium	-5.07
Pentadecanoic acid	-3.07
Benzyl Alcohol	-6.18
Lauric acid	-4.54
Decanoic acid	-3.06
Pentadecanal	-5.97
Myrcene	-4.17
Citric acid	-8.69
Nonanal	-4.85

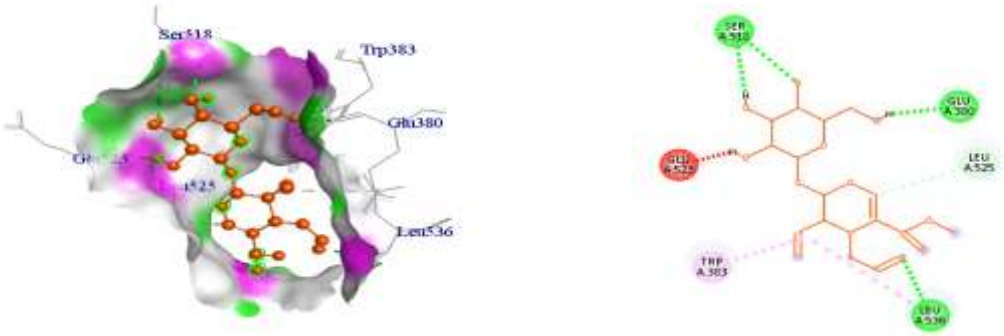

Figure 5. Visualization of molecular interaction of Secologanin with 3ERT protein.

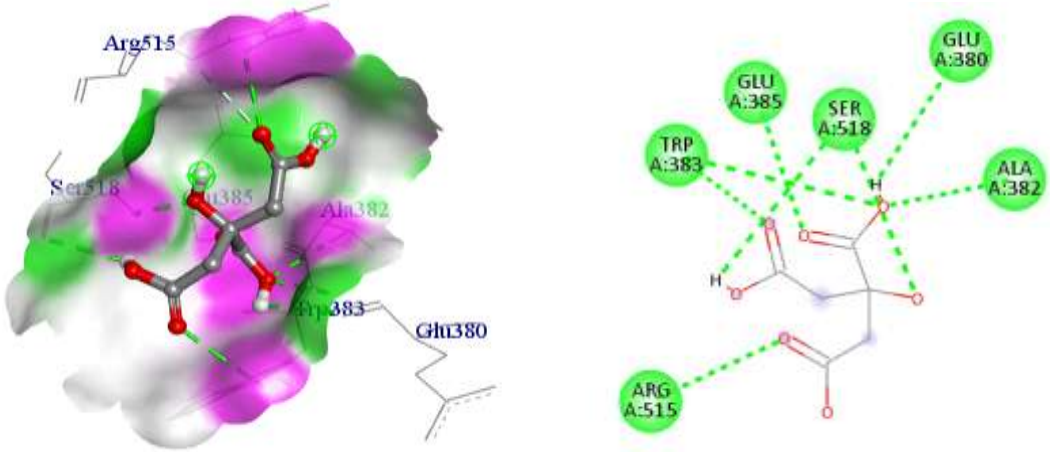

Figure 6. Visualization of molecular interaction of Citric acid with 3ERT protein.

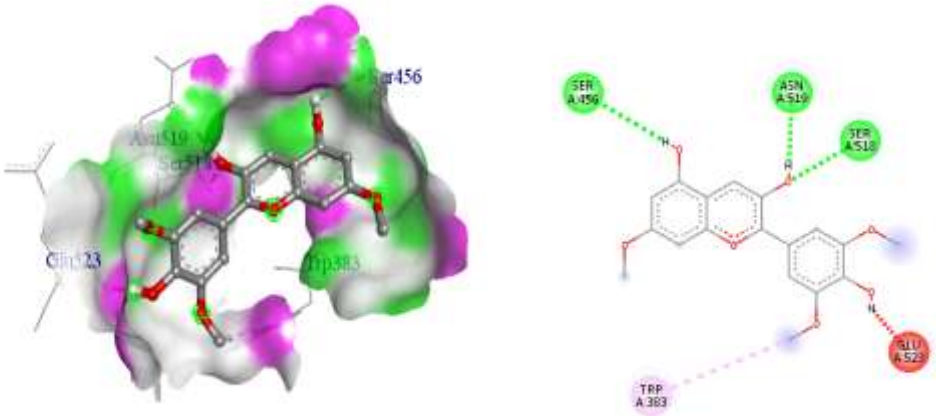

Figure 7. Visualization of molecular interaction of Hirsutidin with 3ERT protein.

DISCUSSION

Breast cancer remains the most common malignancy among women worldwide, significantly impacting global health. It is estimated that over 2 million new cases are diagnosed annually, making it a critical area of medical research and healthcare intervention. The disease's complexity, driven by various genetic, environmental, and hormonal factors, underscores the need for diversified treatment strategies that can cater to individual patient profiles and disease characteristics.

The estrogen receptor plays a pivotal role in the development and progression of breast cancer, particularly in hormone-receptor-positive subtypes, which constitute about 70% of all cases. Targeting the estrogen receptor not only inhibits the growth of cancer cells but also

provides a strategic point of intervention that can be exploited with therapeutic agents. By blocking or modulating this receptor, the proliferation of tumor cells induced by estrogen signals can be effectively reduced, making estrogen receptor a critical target in breast cancer therapy.

However, the recurrence of breast cancer and the adverse side effects associated with traditional treatments such as chemotherapy and hormone therapy highlight the urgent need for alternative therapeutic strategies. In this context, the pharmacological properties of *Catharanthus roseus*, known for its repertoire of medicinal compounds, provide a promising option. *C. roseus* phytochemicals have antimicrobial activity, cytotoxicity for the breast cancer cell line [16], antifungal activity [17], antiparasitic activity [18], anti-proliferative [19], antioxidant, and anticancer properties [20]. A number of other indole alkaloids derived from *C. roseus* have been proven to exhibit potent cytotoxic activity against a range of cancer cellsThe study of the anticancer capabilities of alkaloids from *Catharanthus roseus* has expanded to include an assessment of the effects of the plant's complete crude extract on cancer cell lines. Recent studies have shown that *Catharanthus roseus* root and stem extract has significant *in vitro* cytotoxic activity against a variety of cancer cell lines. Furthermore, Fernández-Pérez et al. confirmed these findings, demonstrating that the significant anticancer activity of the indole alkaloid-enriched extract from Catharanthus roseus cell cultures is due to the combined action of multiple bioactive compounds rather than a single constituent. These findings highlight the necessity of addressing the synergistic effects and combinations of bioactive components found in *Catharanthus roseus* when battling cancer cells.

In the present study the phytocompounds from this plant, including secologanin, citric acid, and hirsutidin, have shown significant potential in targeting the estrogen receptor, suggesting a new avenue for developing novel anti-cancer therapies. Our molecular docking studies identified these compounds as having high binding affinities to the estrogen receptor (PDB ID: 3ERT), with secologanin showing the highest affinity at -9.82 kcal/mol, followed by citric acid at -8.69 kcal/mol and hirsutidin at -7.42 kcal/mol. These findings suggest these compounds could effectively modulate the activity of estrogen receptor, potentially inhibiting the estrogen-driven proliferation of breast cancer cells.

This phenomenon, in which the combined action of several substances results in greater efficacy than individual components alone, is not exclusive to *Catharanthus roseus*; it has also been reported in other plant materials. This synergy presents exciting opportunities for creating innovative cancer therapeutic techniques that harness the potential of natural chemicals. Understanding the processes underlying the synergistic effects of bioactive chemicals in Catharanthus roseus and other plant sources will be critical for maximizing their medicinal potential as research in this field advances. Researchers seek to create more effective and tailored ways for cancer treatment by harnessing the complimentary activities of these chemicals, providing hope for better outcomes and quality of life for people suffering from this deadly disease Vinca alkaloids are commonly used in combination chemotherapy regimens for medical treatments. Vinca alkaloids exert cytotoxic effects, preventing cell division and causing cell death. Vinca alkaloids are the second most commonly utilized class of anti-cancer medications and will remain among the original cancer therapies [21].

However, it is important to note that while these compounds exhibit promising in vitro results, the transition from in vitro efficacy to in vivo applicability involves considerable challenges. The main limitations of our research include the potential discrepancies between in vitro binding affinities and actual biological effectiveness in human subjects. Therefore, further in vivo studies and clinical trials are crucial to validate the efficacy and safety of these

phytocompounds as therapeutic agents against breast cancer. Overall, our study not only highlights the therapeutic potential of Catharanthus roseus compounds in targeting estrogen receptors but also underscores the need for continued research into plant-derived medicines as part of an integrated approach to cancer treatment.

CONCLUSION

This study has demonstrated the potential of phytocompounds from *Catharanthus roseus*, specifically secologanin, citric acid, and hirsutidin, as promising candidates for targeting the estrogen receptor in breast cancer treatment. These compounds exhibited high binding affinities in molecular docking studies, suggesting their capability to modulate estrogen-driven pathways. However, the transition from in vitro results to clinical efficacy remains a significant hurdle, highlighting the necessity for further in vivo testing and clinical trials to confirm their therapeutic potential and safety profiles. Future research should focus on comprehensive pharmacokinetic and pharmacodynamic studies to understand better the mechanisms through which these compounds interact with the estrogen receptor. Additionally, exploring the synergistic effects of these phytocompounds with current anticancer agents could provide insights into more effective and less toxic treatment regimens. The findings of this study encourage the continued exploration of natural products in developing novel cancer therapies, offering hope for more personalized and effective treatment options in the fight against breast cancer.

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AUTHORS CONTRIBUTION

All the authors contributed equally.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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Nill.

REFERENCE

1. Nayila, I., Sharif, S., Lodhi, M. S., Rehman, M. F. U., & Aman, F. (2023). Synthesis, characterization and anti-breast cancer potential of an incensole acetate nanoemulsion from *Catharanthus roseus* essential oil; *in silico*, *in vitro*, and *in vivo* study. *RSC Advances*, *13*(46), 32335–32362. https://doi.org/10.1039/d3ra06335f
2. Sun, Y.-S., Zhao, Z., Yang, Z.-N., Xu, F., Lu, H.-J., Zhu, Z.-Y., … Zhu, H.-P. (2017). Risk factors and preventions of breast cancer. *International Journal of Biological Sciences*, *13*(11), 1387–1397. doi:10.7150/ijbs.21635
3. Sharma, G. N., Dave, R., Sanadya, J., Sharma, P., & Sharma, K. K. (2010). Various types and management of breast cancer: An overview. *Journal of Advanced Pharmaceutical Technology & Research*, *1*(2), 109.
4. Waks, A. G., & Winer, E. P. (2019). Breast cancer treatment: A review. *JAMA: The Journal of the American Medical Association*, *321*(3), 288. doi:10.1001/jama.2018.19323
5. Watkins, E. J. (2019). Overview of breast cancer. *JAAPA : Official Journal of the American Academy of Physician Assistants*, *32*(10), 13–17. doi:10.1097/01.jaa.0000580524.95733.3d

6. Olivia Jane Scully, Boon-Huat Bay, George Yip, & Yingnan Yu. (2012). Breast Cancer Metastasis. *Cancer Genomics - Proteomics*, *9*(5), 311. Retrieved from http://cgp.iiarjournals.org/content/9/5/311.abstract
7. Engel, J., Eckel, R., Kerr, J., Schmidt, M., Fürstenberger, G., Richter, R., … Hölzel, D. (2003). The process of metastasisation for breast cancer. *European Journal of Cancer (Oxford, England: 1990)*, *39*(12), 1794–1806. doi:10.1016/s0959-8049(03)00422-2
8. Paarakh, M. P., Swathi, S., Taj, T., Tejashwini, V., & Tejashwini, B. (2019). Catharanthus roseus Linn-a review. *Acta Scientific Pharmaceutical Sciences*, *3*(10), 19–24.
9. Ekins, S., Mestres, J., & Testa, B. (2007). *In silico* pharmacology for drug discovery: applications to targets and beyond. *British Journal of Pharmacology*, *152*(1), 21–37. doi:10.1038/sj.bjp.0707306
10. Obeagu, E. I., & Obeagu, G. U. (2024). Breast cancer: A review of risk factors and diagnosis. *Medicine*, *103*(3), e36905. doi:10.1097/md.0000000000036905
11. Gajalakshmi, S., Vijayalakshmi, S., & Devi, R. V. (n.d.). Pharmacological activities of Catharanthus roseus: A perspective review. Retrieved March 14, 2024, from Psu.edu website: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=0d0430b867ee73685c9c31c7b4ac43368703e13d
12. Janda M, Cust AE, Neale RE, Aitken JF, Baade PD, Green AC, et al. Early detection of melanoma: a consensus report from the Australian Skin and Skin Cancer Research Centre Melanoma Screening Summit. Aust N Z J Public Health [Internet]. 2020;44(2):111–5. Available from: http://dx.doi.org/10.1111/1753-6405.12972
13. Lopes J, Rodrigues CMP, Gaspar MM, Reis CP. Melanoma management: From epidemiology to treatment and latest advances. Cancers (Basel) [Internet]. 2022;14(19):4652. Available from: http://dx.doi.org/10.3390/cancers14194652
14. Davis LE, Shalin SC, Tackett AJ. Current state of melanoma diagnosis and treatment. Cancer Biol Ther [Internet]. 2019;20(11):1366–79. Available from: http://dx.doi.org/10.1080/15384047.2019.1640032
15. Laskowski RA, Jabłońska J, Pravda L, Vařeková RS, Thornton JM. PDBsum: Structural summaries of PDB entries. Protein Sci [Internet]. 2018;27(1):129–34.Availablefrom: http://dx.doi.org/10.1002/pro.3289
16. Rajashekara, S., Reena, D., Mainavi, M. V., Sand, L. S., & Baro, U. (2022). Biological isolation and characterization of Catharanthus roseus (L.) G. don methanolic leaf extracts and their assessment of antimicrobial, cytotoxic and apoptotic activities. In *Research Square*. https://doi.org/10.21203/rs.3.rs-1831603/v1
17. Bhayana, T., & Gupta, S. (2022). Elucidating the antifungal activity and mechanism of action of bioactive phytochemicals against fungal dermatitis isolates. *Archives of Dermatological Research*. doi:10.1007/s00403-022-02475-4
18. Patel, S. K., Khedkar, V. M., Jha, P. C., Jasrai, Y. T., Pandya, H. A., George, L.-B., … Skelton, A. A. (2016). Molecular interaction of selected phytochemicals under the charged environment of*Plasmodium falciparum*chloroquine resistance transporter (PfCRT) model. *Journal of Biomolecular Structure & Dynamics*, *34*(2), 290–303. doi:10.1080/07391102.2015.1028449
19. Pham, H. N. T., Sakoff, J. A., Van Vuong, Q., Bowyer, M. C., & Scarlett, C. J. (2019). Phytochemical, antioxidant, anti-proliferative and antimicrobial properties of Catharanthus roseus root extract, saponin-enriched and aqueous fractions. *Molecular Biology Reports*, *46*(3), 3265–3273. doi:10.1007/s11033-019-04786-8
20. Shanbhag, S., & Ambinder, R. F. (2018). Hodgkin lymphoma: A review and update on recent progress. *CA: A Cancer Journal for Clinicians*, *68*(2), 116–132. doi:10.3322/caac.21438
21. Taher, M. A., Nyeem, M. A., Billah, M. M., & Ahammed, M. M. (2017). Vinca alkaloid-the second most used alkaloid for cancer treatment-A review. *Inter. J. Physiol. Nutr. Phys. Educ*, *2*, 723–727.