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EXPLORING THE ANTICANCER POTENTIAL OF METHANOLIC EXTRACT OF *LAURUS NOBILIS* AGAINST BREAST CANCER: *IN VITRO* AND *IN SILICO* STUDIES

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Abstract:

Background: Breast cancer continues to pose a significant global health challenge, demanding innovative strategies to address its multifaceted nature. This research delves into the therapeutic potential of phytocompounds extracted from Laurus nobilis, commonly known as bay laurel, with a focus on their interaction with the pivotal BRCA1 and BRCA2 proteins, well-recognized for their involvement in breast cancer pathogenesis.

Methods: Utilizing a rigorous in silico methodology, our study incorporates a multifaceted array of analytical techniques. These encompass phytochemical screening, precise liquid chromatography-mass spectrometry (LC-MS) profiling, pharmacokinetic profiling of the ligands, and molecular docking studies. The phytocompounds elucidated via LC-MS analysis underwent rigorous assessment encompassing physicochemical properties, pharmacokinetic profiles, adherence to drug-likeness criteria, and evaluation of medicinal chemistry attributes.

Results: Among these compounds, Actinodaphnine and Launobine emerged as notable candidates, demonstrating exceptional binding affinities with the BRCA1 and BRCA2 receptors. This discovery alludes to the potential therapeutic significance of Actinodaphnine and Launobine.

Conclusion: This study underscores the imperative for forthcoming *in vitro* and *in vivo* investigations to substantiate the therapeutic potential of Actinodaphnine and Launobine. Nevertheless, our findings illuminate the promise held by *Laurus nobilis* phytocompounds as a reservoir of novel bioactive molecules, instilling optimism in the pursuit of innovative modalities for addressing breast cancer.

Keywords: *Laurus nobilis*, LC-MS analysis, phytochemicals, molecular docking, antimicrobial activity

Background

Breast cancer emerges as the predominant neoplastic affliction impacting women worldwide, posing a substantial global public health quandary [1]. It manifests as a multifarious spectrum of biological and molecular anomalies arising within breast tissue. Notably distinct from other cancer types, breast cancer's risk factors include genetic predisposition, particularly mutations in the BRCA1 or BRCA2 genes, which play a crucial role in its development [2]. The epidemiological data pertaining to breast cancer isprofoundly disconcerting, encompassing a global purview, including a specific focus on India [3]. Data

furnished by the World Health Organization (WHO) reveals that breast cancer assumes the foremost position among cancers diagnosed in women across the world. In the year 2020, approximately 2.3 million novel cases were documented, constituting an alarming 11.7% of all newly ascertained cancer instances worldwide. In the context of India, breast cancer stands as the most prevalent cancer among women, with an estimated 162,468 cases diagnosed in the year 2020 [4]. These statistics emphasize the urgent need for continued research, prevention, and early detection efforts to address this pressing public health concern.

The intricacies surrounding the diagnosis and prognosis of breast cancer are manifold. Early detection remains a formidable challenge, as many patients remain asymptomatic during the initial stages [1]. Mammography, a widely used screening tool, faces limited accessibility in low-income regions, potentially resulting in under diagnosis. Additionally, the heterogeneous nature of breast cancer adds layers of complexity to prognosis [5]. Both environmental and genetic factors assume pivotal roles in the pathogenesis of this disease. Genetic predisposition assumes a paramount role as a firmly established risk factor in the context of breast cancer [6]. Notably, mutations in the BRCA1 and BRCA2 genes have emerged as focal points of substantial scientific scrutiny and investigation. These mutations markedly elevate the lifetime risk of developing breast cancer. For example, women carrying BRCA1 mutations may face an up to 87% chance of developing breast cancer by age 70, while those with BRCA2 mutations may have up to a 69% risk. These genes are intricately involved in the repair of DNA and the maintenance of genomic stability. Mutations in BRCA1 and BRCA2 incapacitate this DNA repair process, resulting in the accumulation of mutations and the subsequent development of cancer [7].

Numerous pharmaceutical agents have been developed to specifically target the proteins encoded by BRCA1 and BRCA2 genes. Among these agents, Poly(ADP-ribose) polymerase (PARP) inhibitors, exemplified by olaparib and talazoparib, have gained regulatory approval for the treatment of breast cancer associated with BRCA mutations [8]. The efficacy of these drugs lies in their exploitation of the concept of synthetic lethality, wherein cancer cells harboring BRCA mutations exhibit heightened vulnerability to PARP inhibition, a consequence of their impaired DNA repair mechanisms [9]. Clinical trials have consistently demonstrated the effectiveness of PARP inhibitors, leading to their integration into established therapeutic protocols for BRCA-associated breast cancer [10].It is crucial to acknowledge that PARP inhibitors come with inherent limitations and associated side effects. Frequently observed side effects encompass anemia, nausea, and fatigue, which can substantially impact the quality of life for affected patients. Furthermore, resistance to PARP inhibitors can emerge over time, necessitating the exploration of alternative therapeutic modalities [11].

In light of this pressing need, there has been a notable surge in the pursuit of traditional medicinal paradigms. India celebrated for its profound traditional healing legacy, illuminates the therapeutic potential of botanical agents as viable candidates for alternative strategies in addressing breast cancer. *Laurus nobilis* (Bay laurel) is an aromatic plant that has been used as a spice in cuisine and as a traditional medicine for several infectious diseases [12]. In vitro investigations have illustrated the ability of processed bay leaf products to inhibit the growth of cancer cells, particularly within human colorectal cancer cell lines. These studies have unveiled the differentiated regulatory effects of bay leaf extracts on the growth of colorectal cancer cells in vitro [13]. Moreover, through size fractionation of these extracts, it has been discerned that the antiproliferative and proapoptotic activities are linked to distinct chemical classes. Specifically, low mass components, primarily polyphenolics and essential oils, are associated with antiproliferative effects, while high mass compounds, predominantly proteins including polyphenol oxidase, contribute to proapoptotic actions [13, 14]. Additionally, bay leaf has exhibited its potential by inducing cytotoxicity and apoptosis in three distinct nervous system cell lines [15]. Research on the specific impact of bay laurel on breast cancer remains limited. Nonetheless, the observed cytotoxic and apoptotic effects of bay leaf on cancer cells imply its potential as a natural alternative to synthetic pharmaceuticals in the context of breast cancer treatment. To elucidate the precise effects of bay laurel on breast cancer cells, further investigative studies are imperative.

Our study aims to conduct LCMS analysis to identify and categorize the phytocompounds within *Laurus nobilis* based on their pharmacological properties. These identified phytocompounds will undergo comprehensive assessment through molecular docking studies to evaluate their inhibitory potential

against BRCA1 and BRCA2. This research endeavor holds the promise of contributing to the advancement of innovative therapeutic approaches for breast cancer.

Methods

Preparation of leaf extract

The *Laurus nobilis* leaves were sourced from GKVK, University of Agricultural Sciences Bangalore, and subsequently dried and ground into a coarse-textured powder. The resulting powdered samples were carefully stored in air-tight containers. To extract bioactive compounds, a Soxhlet apparatus was employed, utilizing methanol as the solvent. The extraction process was conducted over three cycles, maintaining a temperature range of 35°C to 40°C and extending for a duration of 5-6 hours per cycle. Following extraction, the solvent was removed via a rotary vacuum evaporator, maintaining a temperature of 40°C, yielding the crude extract.

Phytochemical screening

To comprehensively identify the major primary and secondary metabolites within the methanolic extract of *Laurus nobilis*, we conducted a systematic phytochemical screening, following established analytical protocols. Qualitative assessments were carried out through the observation of color changes or the formation of precipitates resulting from specific chemical reactions, enabling the detection of various phytochemical compounds [16]. We conducted tests to discern the presence of essential bioactive compounds, encompassing carbohydrates, tannins, saponins, flavonoids, alkaloids, anthraquinones, cardiac glycosides, steroids, terpenoids, phenols, and amino acids. Any discernible color change indicative of a positive reaction was duly recorded as part of our analysis. This comprehensive screening was pivotal in characterizing the phytochemical composition of the methanolic extract of *Laurus nobilis*.

LCMS Profiling

The LC-MS analysis was performed using the Nexera UHPLC system, equipped with a quaternary pump, prominence degassing unit, and Autosampler. For elution, a constant flow rate of 1 ml/min was employed, with a solvent mixture comprising methanol, petroleum ether, and ethyl acetate. Prior to use, all solvents underwent ultrasonication and filtration through a 0.45µm nylon filter paper to ensure purity and precision. Chromatographic data were acquired and examined at a specific wavelength of 270 nm, and the resulting dataset was meticulously analyzed using proprietary software developed in-house for accurate phytocompound identification and quantification [17].

Ligand Preparation

In the context of LCMS profiling, we employed a meticulous approach to select 77 specific phytocompounds based on their respective chromatogram peaks, focusing on molecular weight as a key criterion [18]. These chosen compounds were retrieved from the PubChem database in SDF format and subjected to rigorous validation using Marvin View.

Protein Preparation

In the present study, we specifically targeted two receptors known to play a pivotal role in breast cancer development, namely BRCA1 and BRCA2. The 3D structures of these receptors were retrieved from the Protein Data Bank, BRCA1 (PDB ID: 6GVW) and BRCA2 (PDB ID: 3EU7). Structural data for both BRCA1 (6GVW) and BRCA2 (3EU7) was obtained through X-ray diffraction, with resolutions of 3.75 Å and 2.20 Å, respectively. To ensure their suitability, both receptor structures underwent a purification and refinement process utilizing the Biovia Discovery Studio software. The protein structures underwent purification to remove non-structural components, and additional chains were excised to simplify the structural complexity [18]. Furthermore, polar hydrogens were added to refine the structural integrity of the receptors.

Ramachandran Plot Analysis

The Ramachandran plot analysis is a fundamental technique employed for the assessment of protein structure conformational quality. This method entails the computation and graphical representation of dihedral angles (φ and ψ) for amino acid residues within a protein. By plotting these angles against each other, the Ramachandran plot provides a visual means to discern the permissible and forbidden regions of torsional angles within the protein structure [18].

ADMET Analysis

The phytocompunds identified through LCMS were further subjected to pharmacological profiling. ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis plays a pivotal role in drug discovery and development. It aids in identifying compounds with the desired pharmacological properties and minimal safety concerns.This computational approach utilizes SwissADME, a specialized software tool specifically designed to assess the essential pharmacological properties and safety profiles of chemical compounds, contributing to informed decision-making in the drug development process [19].

Molecular Docking Analysis

In the investigation involving *Lauris nobilis* ligands and their interaction with BRCA1 and BRCA2, we employed PyRx 0.8 software to conduct virtual screening. The primary focus of this study was the evaluation of ligand binding energy within the binding sites of these target proteins. To facilitate this analysis, we utilized BRCA1 and BRCA2 as macromolecular structures, allowing them to interact with the phytocompounds from *Laurisnobilis*. To ensure the precision of the docking process, several meticulous steps were taken: the assignment of Kollman charges to the protein complexes, the attribution of AD4 types to atoms, and the utilization of generated pdbqt files for the docking procedure. We made specific modifications to the ligand structures, which involved merging nonbonded atoms and configuring torsional angles. Subsequently, an energy minimization procedure was applied to these prepared ligands, employing the universal force field (_uff). The results of this optimization were then transformed into pdbqt files. The ligands were subjected to nine distinct conformational variations to identify the most favorable interaction within the binding sites. The assessment of binding affinity, which reflects the strength of interaction, was carried out with zero RMSD (Root Mean Square Deviation) as the benchmark [20].

The final step of our analysis involved visualizing the resulting docked structures within DS Biovia Discovery Studio. This visualization allowed us to interpret the molecular interactions between *Laurisnobilis* ligands and the amino acids present in the binding pockets of the target macromolecule complex [18, 20].

Results

Qualitative phytochemical screening

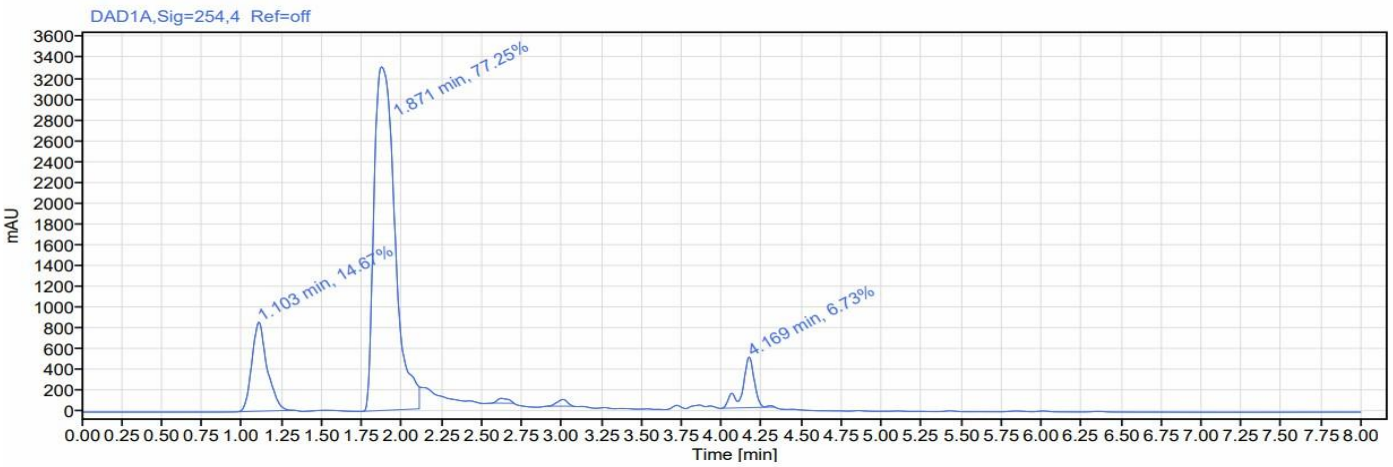
The qualitative phytochemical screening of Laurus nobilis leaf extract revealed the presence of several bioactive compounds. These findings suggest the rich phytochemical diversity within the extract, potentially contributing to its therapeutic properties (Table 1).

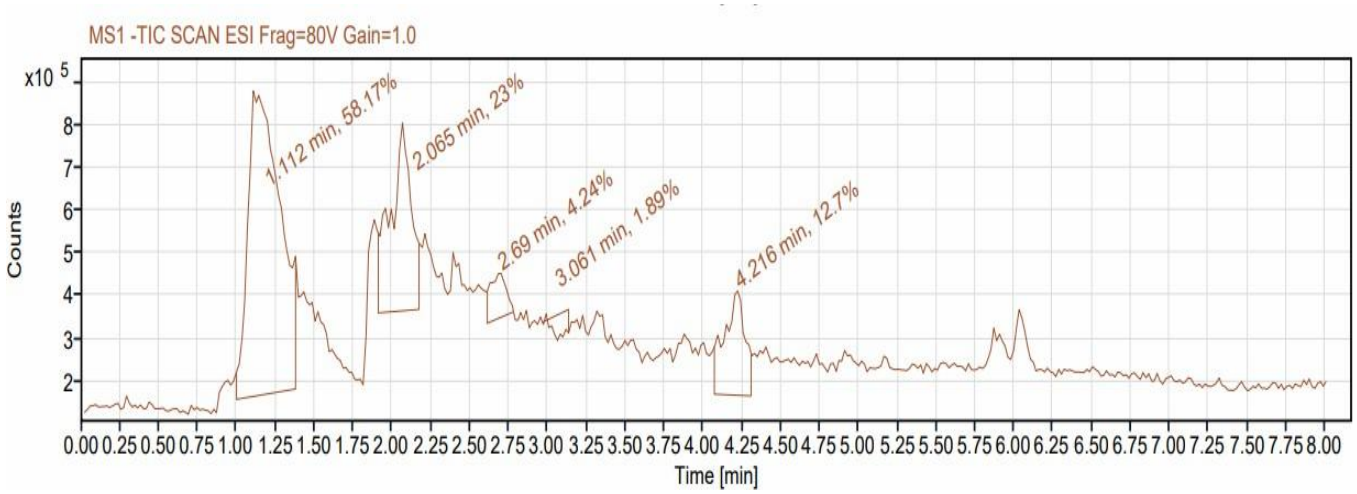
Table 1: Identification of Bioactive Compounds in Laurus nobilis Leaf Extract

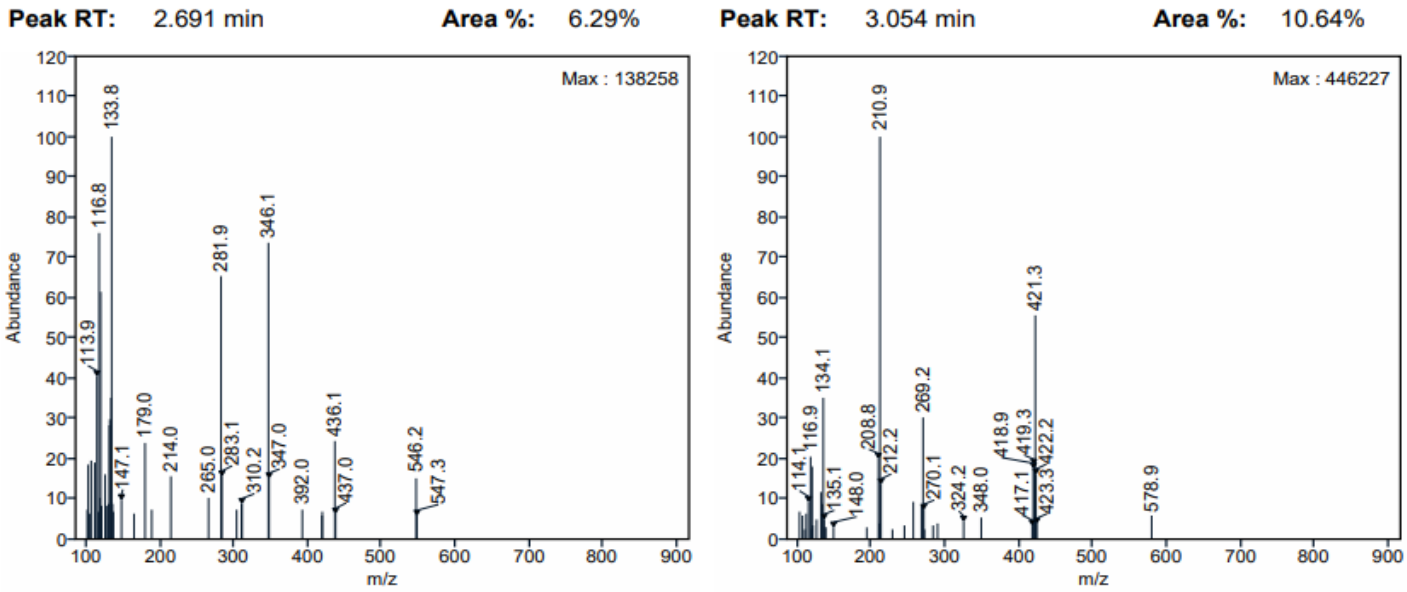
Phytochemicals	Test	Positive reaction
Glycosides	Liebermann's test	Presence of green color
Terpenoids	Salkowiski's test	Formation of a reddish layer at the interface
Steroids	Salkowiski's test	Formation of a reddish layer at the interface
Flavonoids	Alkaline test	The appearance of yellow color

Liquid Chromatography - Mass Spectrometry

The principle behind Liquid Chromatography-Mass Spectroscopy (LC-MS) is the separation of individual components in a solvent based on their mass/charge ratio. In this study, the solvent extraction method known as Soxhlet extraction was used and the resulting extract was subjected to LC-MS analysis to identify 77 important bioactive phytocompounds. Some of the notable compounds obtained from the methanolic extract include beta-bisabolene, Carvacrol, Lauric acid, Myrcene, Eugenol, and beta-copaene (Figure 1). These compounds are of particular interest due to their established pharmacological significance. Their identification underscores the potential value of Laurus nobilis as a source of bioactive molecules with various health-related applications, warranting further exploration in the field of natural medicine and drug development.







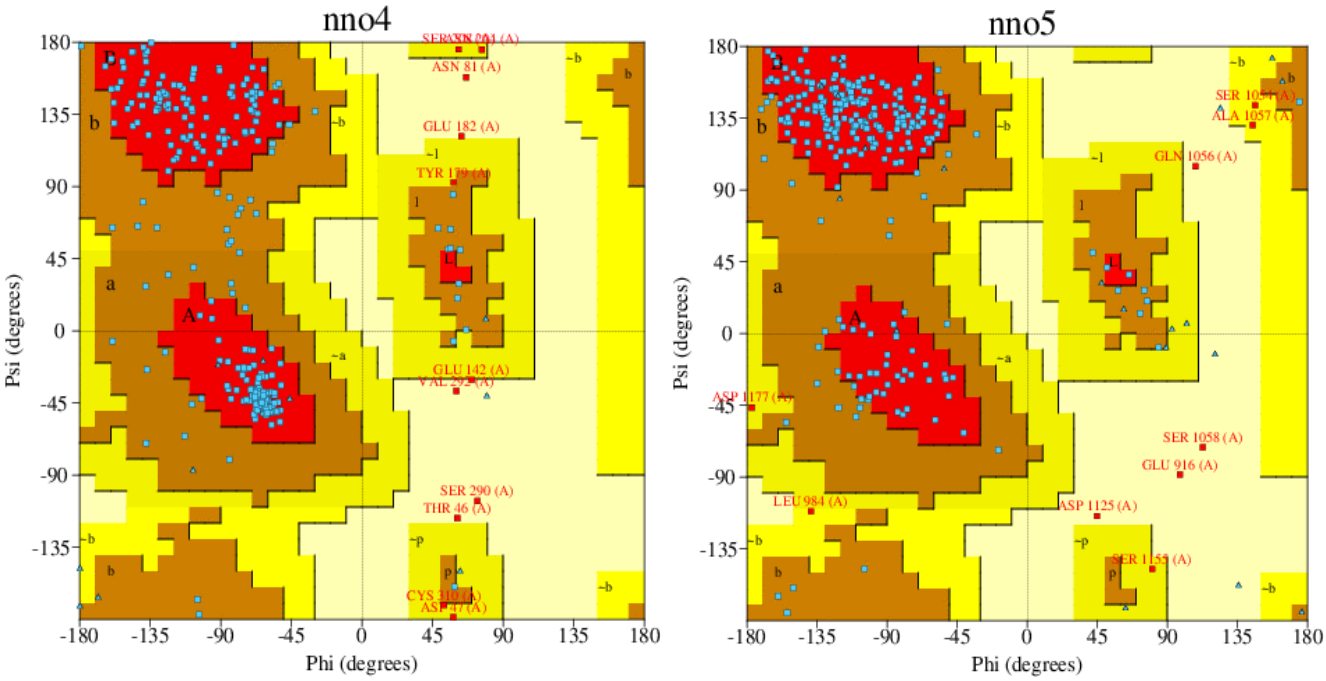
Peak Results (Area Percent at least 1%)

RT (min)	Signal Description	Width (min)	Area	Height	Area%
1.095	MS1 +TIC SCAN ESI Frag=80V Gain=1.0	0.388	32264882.4	2505941.0	43.11
1.103	DAD1A,Sig=254,4 Ref=off	0.348	5674.5	855.3	14.67
1.112	MS1 -TIC SCAN ESI Frag=80V Gain=1.0	0.380	10279116.1	728618.2	58.17
1.871	DAD1A,Sig=254,4 Ref=off	0.353	29875.1	3301.5	77.25
2.056	MS1 +TIC SCAN ESI Frag=80V Gain=1.0	0.329	23319858.8	1500803.4	31.16
2.065	MS1 -TIC SCAN ESI Frag=80V Gain=1.0	0.262	4064137.5	440647.6	23.00
2.690	MS1 -TIC SCAN ESI Frag=80V Gain=1.0	0.169	749529.6	105910.9	4.24
2.691	MS1 +TIC SCAN ESI Frag=80V Gain=1.0	0.211	4704237.3	511012.2	6.29
3.054	MS1 +TIC SCAN ESI Frag=80V Gain=1.0	0.236	7963487.3	1285288.5	10.64
3.061	MS1 -TIC SCAN ESI Frag=80V Gain=1.0	0.169	333572.6	59463.0	1.89
4.169	DAD1A,Sig=254,4 Ref=off	0.330	2604.5	485.7	6.73
4.216	MS1 -TIC SCAN ESI Frag=80V Gain=1.0	0.236	2243494.8	244834.6	12.70
4.283	MS1 +TIC SCAN ESI Frag=80V Gain=1.0	0.262	6588659.4	690975.4	8.80
		Sum DAD1A	38154.1		
		Sum MS1 +TIC SCAN ESI Frag=80V Gain=1.0	74841125.1		
		Sum MS1 -TIC SCAN ESI Frag=80V Gain=1.0	17669850.7		

Figure 1: LC-MS-ESI-MS chromatograms of reference compounds using Nexera in Methanol

Ramchandran Plot analysis:

The Ramachandran plot offers valuable insights into the conformational quality of protein structures by examining the dihedral angles (φ and ψ) of amino acid residues. This analysis aids in identifying permissible and forbidden regions of torsional angles, providing critical validation for protein structures. The purified structure of the BRCA1 protein had 321 amino acids of which 229 amino acids (79.8%) were present in the most favored region, 47 (16.4%) were present in the additionally allowed region, 5 (1.7%) were present in the generously allowed region and 6 (2.1%) were present in the disallowed region (Figure 2a). The purified structure of the BRCA2 protein had 312 amino acids of which 223 amino acids (83.2%) were present in the most favored region, 36 (13.4%) were present in the additionally allowed region, 5 (1.9%) were present in the generously allowed region and 4 (1.5%) were present in the disallowed region (Figure 2b).



(a) **(b)**

Figure 2: Secondary structure analysis of (a) BRAC1 and (b) BRAC2

ADMET Analysis:

All the 77 phytocompounds identified through LCMS were subjected to pharmacological screening based on the following parameters:Lipinski (Molecular weight: ≤500 Daltons; MLOGP: ≤4.15; N or O: ≤ 10; NH or OH: ≤5), Bioavailability: 0.55, PAINS: Zero alterts, Brenk: Zero alerts: Synthetic accessibility: <5; Leadlikness: Zero violations. There were 37 compounds that fulfilled the above-mentioned screening criteria and these compounds were further subjected to molecular docking evaluation against the target molecules. The pharmacological properties of the top ligands as per the docking analysis are documented in Tables 2-5.

- ***Physicochemicalproperties:***

Physicochemical properties are crucial determinants of a molecule's chemical and physical behavior, profoundly impacting its suitability as a drug candidate in the realm of drug discovery and development. Molecular weight, a fundamental property, influences a molecule's size and mass, with large molecules potentially facing cell membrane challenges, while very small ones might be rapidly eliminated from the body. Fraction Csp3 quantifies the proportion of less reactive, saturated carbon atoms, reflecting a molecule's degree of saturation and influencing lipophilicity and drug-likeness. The count of rotatable bonds provides insight into a molecule's conformational flexibility. Hydrogen bond acceptors and donors play vital roles in ligand-receptor interactions. A higher count of these groups enhances a molecule's interaction potential with target proteins. Molar refractivity measures a molecule's interaction with polarized light and its polarizability, indicating its ability to undergo electronic polarization. In our study, all top compounds exhibit favorable pharmacological properties well within optimal ranges for these critical physicochemical parameters (Table 2).

Table 2: Physicochemical Property top derivatives of *Laurus nobilis*

Pubchem ID	Formula	Molecular weight (g/mol)	Fraction Csp3	Num. rotatable bonds	Num. H- bond acceptors	Num H-bond donors	Molar Refractivity	TPSA
102820	$C_9H_{18}O_2$	158.24	0.89	5	2	0	46.66	26.30 Å²
10364	$C_9H_{18}O_2$	158.24	0.89	5	2	0	46.66	26.30 Å²
14529	$C_{10}H_{14}O$	150.22	0.40	1	1	1	47.03	20.23 Å²
6988	$C_{10}H_{14}O$	150.22	0.40	1	1	1	47.03	20.23 Å²
7351	$C_8H_{16}O_2$	144.21	0.88	4	2	0	41.85	26.30 Å²
519323	$C_{10}H_{16}O$	152.23	0.60	1	1	1	48.32	20.23 Å²
2758	$C_{10}H_{18}O$	154.25	1.00	0	1	0	47.12	9.23 Å²
6552009	$C_{10}H_{18}O$	154.25	1.00	0	1	1	46.60	20.23 Å²
2537	$C_{10}H_{16}O$	152.23	0.90	0	1	0	45.64	17.07 Å²
62367	$C_{10}H_{18}O$	154.25	1.00	1	1	1	46.90	20.23 Å²
444294	$C_{10}H_{16}O$	152.23	0.90	0	1	0	45.64	17.07 Å²
160502	$C_{18}H_{17}NO_4$	311.33	0.33	1	5	2	88.65	59.95 Å²
177134	$C_{18}H_{17}NO_4$	311.33	0.33	1	5	2	88.65	59.95 Å²

- ***Pharmacokinetic properties***

Pharmacokinetic properties are pivotal factors influencing a drug's absorption, distribution, metabolism, and elimination within the body, ultimately determining its behavior and efficacy. In our current study, we observed that all the top ligands exhibited high gastrointestinal absorption, signifying efficient absorption from the gastrointestinal tract into the bloodstream following oral administration. This high gastrointestinal absorption indicates a promising potential for oral drug delivery. In the present study the ligands being BBB permeant means that it has the ability to cross the BBB and access the central nervous system (CNS). P-glycoprotein (P-gp), is a transporter protein known for actively pumping drugs and compounds out of cells, including those within the intestinal lining and the blood-brain barrier. Specific cytochrome P450 (CYP) enzymes play a critical role in metabolizing drugs and xenobiotics. Inhibition of these enzymes can result in reduced drug metabolism, potentially leading to drug interactions and alterations in pharmacokinetics. Our findings revealed that all ligands, with the exceptions of 160502 and 177134, acted as substrates for P-gp and inhibitors for CYP enzymes (Table 3).

Table 3: Pharmacokinetic property of derivatives of *Laurus nobilis*

Pubchem ID	GI absorption	BBB permeant	P-gp substrate	CYP1 A2 inhibitor	CYP2C 19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
102820	High	Yes	No	No	No	No	No	No
10364	High	Yes	No	No	No	No	No	No
14529	High	Yes	No	Yes	No	No	No	No
6989	High	Yes	No	Yes	No	No	No	No
7351	High	Yes	No	No	No	No	No	No
519323	High	Yes	No	No	No	No	No	No
2758	High	Yes	No	No	No	No	No	No
6552009	High	Yes	No	No	No	No	No	No
2537	High	Yes	No	No	No	No	No	No
62367	High	Yes	No	No	No	No	No	No
444294	High	Yes	No	No	No	No	No	No
160502	High	Yes	Yes	Yes	No	No	Yes	Yes
177134	High	Yes	Yes	Yes	No	No	Yes	Yes

- ***Druglikeness properties***

The phytocompounds were evaluated for their drug-likeness properties based on widely used guidelines that evaluate drug-likeness properties based on key physicochemical properties. In the present study all the phytocompounds have passed the following screening criteria (Table 4):

a. The Lipinski Rule: Molecular weight: ≤500 Daltons; MLOGP: ≤4.15; N or O: ≤ 10; NH or OH: ≤5
b. Ghose Rule: Molecular weight:160-480 Daltons; WLOGP: -0.4 to 5.6. Molar refractivity: 40- 130; Heavy atoms: 20-70
c. Veber Rule: Rotatable bonds: ≤10; TPSA: ≤140
d. Egan Rule: WLOGP: ≤5.88; TPSA: ≤131.6

Table 4*:* Drug-likeness of top derivatives of *Laurus noblilis*

Pubchem ID	Lipinski	Ghose	Veber	Egan	Bioavailability Score
102820	Yes; 0 violation	No;1 violation :MW<160	Yes	Yes	0.55
10364	Yes; 0 violation	No;1 violation :MW<160	Yes	Yes	0.55
14529	Yes;0 violation	No;1 violation: MW<160	Yes	Yes	0.55
6989	Yes;0 violation	No;1 violation: MW<160	Yes	Yes	0.55
7351	Yes; 0 violation	No;1 violation: MW<160	Yes	Yes	0.55
519323	Yes; 0 violation	No; 1 violation: MW<160	Yes	Yes	0.55
2758	Yes; 0 violation	No; 1 violation: MW<160	Yes	Yes	0.55
6552009	Yes; 0 violation	No; 1 violation: MW<160	Yes	Yes	0.55
2537	Yes; 0 violation	No; 1 violation: MW<160	Yes	Yes	0.55
62367	Yes; 0 violation	No; 1 violation: MW<160	Yes	Yes	0.55
444294	Yes; 0 violation	No; 1 violation: MW<160	Yes	Yes	0.55
160502	Yes; 0 violation	Yes	Yes	Yes	0.55
177134	Yes; 0 violation	Yes	Yes	Yes	0.55

- ***Medicinal chemistry properties:***

Assessing the medicinal properties of phytocompounds is pivotal for identifying potential drug candidates to combat diverse diseases. Phytocompounds, occurring naturally in plants, boast a wide array of chemical structures and biological activities, which underpin their medicinal potential through interactions with various biological targets like enzymes, receptors, and ion channels. In our study, we scrutinized the presence of Pan-Assay Interference Compounds (PAINS) and adherence to the Brenk Rules, a set of guidelines for detecting issues in chemical compounds during drug discovery. Remarkably, the top compound exhibited neither PAINS nor Brenk alerts. Lead compounds, representing early-stage drug candidates with desired pharmacological activity, were identified, with ligands 160502 and 177134 demonstrating lead-like properties, while all ligands displayed favorable synthetic accessibility scores. These findings are instrumental in discerning suitable drug candidates and averting compounds that could disrupt biological assays, underscoring their unsuitability for drug development (Table 5).

Table 5: Medicinal Chemistry of top derivatives of *Laurus nobilis*

PubChem ID	PAINS	Brenk	Lead likeness	Synthetic accessibility
102820	0 alert	0 alert	No; violation: MW<250	1.98
10364	0 alert	0 alert	No; 1 violation: MW<250	1.98
14529	0 alert	0 alert	No; 1 violation: MW<250	1.00
6988	0 alert	0 alert	No; 1 violation: MW<250	1.00
7351	0 alert	0 alert	No; 1 violation: MW<250	1.18
519323	0 alert	0 alert	No; 1 violation: MW<250	3.95
2758	0 alert	0 alert	No; 1 violation: MW<250	3.65
6552009	0 alert	0 alert	No; 1 violation: MW<250	3.43
2537	0 alert	0 alert	No; 1 violation: MW<250	3.22
62367	0 alert	0 alert	No; 1 violation: MW<250	2.82
444294	0 alert	0 alert	No; 1 violation: MW<250	3.22
160502	0 alert	0 alert	yes	3.56
177134	0 alert	0 alert	yes	3.66

Molecular docking

With the help of the virtual screening software PyRx, the binding affinity of the 13 selected compounds of the leaf methanolic extract was assessed against the targeted proteins. The binding constituents and interaction were examined in 2D as well as 3D forms by using Biovia. The two receptors with the chosen ligand compounds produced negative values, which imply high binding affinity The ligands exhibiting a binding affinity better than -6 kcal/mol were considered as top ligands and their ADMET properties are documented in Tables 2-5. As the ligands 160502 and 177134 demonstrated significantly better binding affinity with BRAC1 and BRCA2 receptors, these structures were visualized for their molecular interactions (Figures 3 and 4).

Table 6: Molecular docking of *Laurus nobilis* phytocompounds against BRCA1 and BRCA2

Ligand	BINDING AFFINITY	
	6GVW (BRCA1)	3EU7 (BRCA2)
102820	-7.7	-7.2
10364	-7.2	-7.1
14529	-7.1	-7.0
6988	-6.8	-6.4
7351	-8.0	-6.2
519323	-7.7	-6.6
2758	-5.3	-7.1
6552009	-6.3	-6.6
2537	-6.9	-7.1
62367	-6.4	-6.7
444294	-7.2	-6.1
160502	-8.8	-9.4
177134	-8.4	-9.2

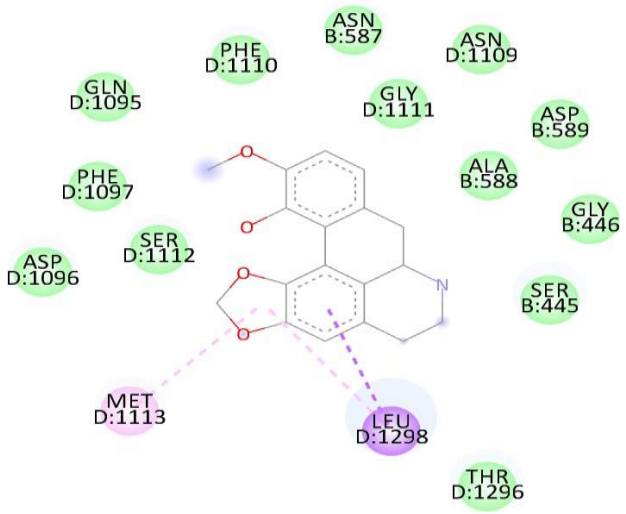


Figure 3. Amino acid interactions of 160502 compounds against BRCA1

(TYR B:1150, LYS D: 1066, GLY B: 1076, ARG D:1122, HIS B: 1081, PHE D: 1123, GLY B: 582), and (PHE D:1110, ASN B:587, MET D: 1113, THR D: 1296).

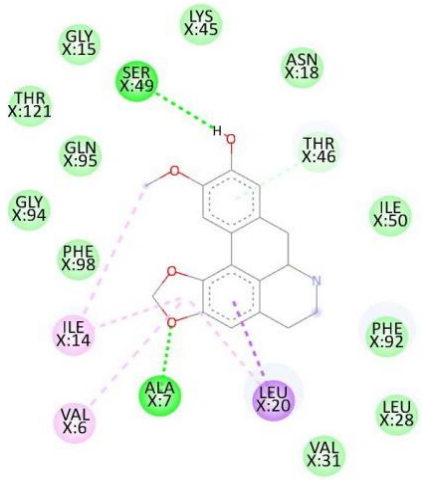


Figure 4: Amino acid interaction of 177134 compounds against BRCA2

(ILE X:50, SER X:49, THR X:121, PHE X:98, LEU X:20, ILE X:14) and (PHE X:98, ILE X:50, THR X:121, ILE X:14, LEU X:20, ILA X:7)

Discussion

Breast cancer represents a multifaceted and diverse ailment influenced by numerous genetic factors. Notably, BRCA1 and BRCA2, denoting Breast Cancer Susceptibility Gene 1 and 2, have emerged as focal points of interest due to their pivotal functions in upholding genomic stability and curtailing tumorigenesis [2]. A profound comprehension of the intricate mechanisms underpinning the actions of BRCA1 and BRCA2 in breast cancer is imperative, bearing significant relevance for both research endeavors and clinical applications. BRCA1 and BRCA2, critical tumor suppressor genes, assume pivotal roles in DNA repair mechanisms, especially in the restoration of double-stranded DNA breaks [21]. These breaks can arise from natural cellular processes or exposure to genotoxic agents like radiation and specific chemotherapeutic drugs. Failure to repair or improper repair of these breaks can lead to genomic instability and the onset of cancer [22]. Situated on chromosomes 17 and 13, respectively, mutations in either BRCA1 or BRCA2 can predispose individuals to breast cancer [23]. Typically, these mutations follow an autosomal dominant inheritance pattern, where a single mutated copy of either gene heightens the risk of breast cancer. Those inheriting one mutated copy have a 50% chance of transmitting the mutation to their offspring [21, 23].

A primary mechanism by which BRCA1 and BRCA2 contribute to breast cancer is through their involvement in homologous recombination (HR) DNA repair, a high-fidelity pathway ensuring precise DNA sequence restoration following double-stranded breaks [2]. BRCA1 acts as a scaffold protein, engaging partners like PALB2, BARD1, and BRIP1, promoting BRCA2 localization to DNA damage sites. BRCA2, once recruited, mediates the loading of RAD51 onto single-stranded DNA overhangs at the break site [24]. RAD51 facilitates damaged DNA strand invasion into an undamaged template, ensuring accurate break repair. Loss-of-function mutations in BRCA1 or BRCA2 impede the HR pathway, leading to the accumulation of unrepaired DNA damage and genomic instability, a potential trigger for breast cancer [25]. Tumors in individuals with these mutations often exhibit distinct genomic alteration patterns, including significant rearrangements and higher somatic mutation frequencies. Beyond HR, BRCA1 and BRCA2 participate in various cellular processes, including transcriptional regulation, cell cycle control, and centrosome function [23]. Mutations in these genes can disrupt these functions, further contributing to tumorigenesis. For example, BRCA1 interacts with transcription factors and co-regulators, impacting gene expression related to cell growth and DNA repair [25].

The pivotal role of BRCA1 and BRCA2 proteins in breast cancer diagnosis and treatment cannot be overstated. These genes are central to genomic stability, and their mutations significantly elevate the risk of breast cancer development. Detecting BRCA1 and BRCA2 mutations through genetic testing is fundamental for early detection and risk assessment, particularly in individuals with a family history of the disease. Genetic testing serves as a cornerstone in breast cancer diagnosis, enabling the identification of individuals with BRCA1 and BRCA2 mutations. This information guides clinical decisions, from preventive measures and surveillance to treatment strategies [24]. These mutations also function as biomarkers for breast cancer susceptibility, informing treatment choices and risk reduction approaches. However, the treatment landscape for breast cancer remains challenging. Current options encompass surgery, chemotherapy, radiation therapy, and targeted therapies. Surgical interventions range from lumpectomy to mastectomy, determined by the disease's extent. Chemotherapy and radiation therapy aim to eradicate cancer cells and prevent recurrence [3]. Targeted therapies, particularly PARP inhibitors, are particularly promising in the context of BRCA1 and BRCA2 mutations, exploiting their DNA repair defects, inducing synthetic lethality in cancer cells [5]. Despite these options, limitations persist. Chemotherapy and radiation therapy entail substantial side effects, impacting patients' quality of life. Drug resistance and tumor recurrence remain obstacles. In targeted therapies, response variability and emerging resistance mechanisms pose challenges.

To address these limitations, alternative treatments, including plant-based therapies, have gained attention. Plants, long recognized for their medicinal potential, offer a promising avenue in cancer research. *Laurus nobilis*, commonly known as bay laurel, stands out among these plants. Traditionally associated with culinary uses, *Laurus nobilis* exhibits various pharmacological properties, including antioxidative and antiproliferative effects [12]. In the present study, we have researchers conducted LCMS analysis of methanolic extracts from *Laurus nobilis*. This analysis unveiled 77 phytocompounds within the plant. These compounds underwent pharmacological screening, employing various filters to assess their therapeutic potential, notably in breast cancer. Based on the results of molecular docking analysis the liagnds 160502 (Actinodaphnine) and 177134 (Launobine) were considered as top compounds as they demonstrated significantly better binding with both the target receptors. In a study by Rinaldi et al., Actinodaphanine was isolated from *Annona hypoglauca* Mart. has demonstrated activity against breast cancer cell lines. Whereas in a study by Tain-Jye et al., Actinodaphine effectively induced apoptosis by downregulating the nuclear factor κB (NF-κB) in the hepatoma cells. A study by Sukma et al. concluded that the administration of actinodaphine from *Cuscuta australis* can inhibit DDP-4 activity in the MCF-7 (model breast cancer cell lines). This compound was also found to sensitize the breast cancer cells to Tamoxifen treatment in MCF-7 and MDA-MB-231 breast cancer cell lines by upregulating Bax and downregulating BCL-2 mRNA without altering the protein expression.

Though there are limited studies on the therapeutic efficacy of Launobine on breast cancer cell models, artificial intelligence-based algorithms have predicted this compound to be effective against tumors. In a study by Jiang, Launobine has demonstrated a strong inhibitory effect against the growth of melanoma B16 cells. The volatile oils extracted from the *Laurus nobilis* have exhibited antiproliferative activity against MCF7 and T47D breast cancer cell lines while the leaf extract was found to increase p53 levels. Similar results were observed by Jelnar et al., where the essential oils from *Laurus nobilis*demonstrated

antiproliferative effects against MCF-7 cell lines with an IC50 value of 24.49 µg/mL and there was no cytotoxicity reported with the extract administration.

While the computational analyses are promising, the clinical translation of these findings remains a significant challenge. One of the primary limitations of this study is its reliance on *in silico* analysis and computational modeling. While these methods provide valuable insights into the potential interactions between Actinodaphnine, Launobine, and the BRCA1/BRCA2 proteins, they are inherently theoretical and do not replace the need for rigorous experimental validation. Although our ADMET analysis suggests that Actinodaphnine and Launobine exhibit favorable pharmacological properties, it is essential to conduct comprehensive pharmacological validation to assess their safety and efficacy. *In vivo* studies using animal models can help determine the compounds' bioavailability, toxicity profiles, and potential side effects. Future research should prioritize *in vitro* experiments to confirm the binding affinities and functional impacts of these compounds on breast cancer-related proteins.

Conclusions

This study illuminates the potential therapeutic significance of *Laurus nobilis* and its bioactive compounds, Actinodaphnine and Launobine, in the realm of breast cancer. These findings add to the growing body of evidence advocating for the investigation of natural compounds as complementary or alternative approaches to conventional breast cancer therapies. While our computational and pharmacological analyses yield valuable insights and promising outcomes, it's crucial to acknowledge the need for further investigation. Subsequent research should prioritize the experimental validation of these compounds using *in vitro* and *in vivo* models to unveil their mechanisms of action and evaluate their safety and effectiveness. The potential synergy between traditional medicine, contemporary drug discovery, and computational methodologies presents exciting possibilities for addressing the intricacies of breast cancer and enhancing patient well-being. Our unwavering commitment lies in advancing our comprehension of *Laurus nobilis* and its bioactive components, with the ultimate aim of contributing to the development of more efficacious and less harmful breast cancer treatments.

Declarations:

Ethics approval and consent to participate:

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Author contribution

All authors participated in the analysis, writing, reviewing, and editing of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

Declared none.

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ABBREVIATIONS

ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity

BRCA1: Breast Cancer Susceptibility Gene 1

BRCA2: Breast Cancer Susceptibility Gene 2

CNS: Central Nervous System

CYP: Cytochrome P450

DNA: Deoxyribonucleic Acid

HR: Homologous Recombination

LC-MS: Liquid Chromatography-Mass Spectrometry

NF-κB: Nuclear Factor κB

PARP: Poly (ADP-ribose) Polymerase

P-gp: P-glycoprotein

PAINS: Pan-Assay Interference Compounds

RMSD: Root Mean Square Deviation

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