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RESEARCH ARTICLE

Computational Analysis Depicting Potency of Phytochemicals to Target MAPK Signalling Pathway in Breast Cancer

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Abstract

Background: Breast cancer has caused significant mortality among women worldwide. A comprehensive analysis of the disease indicated that MAPK signalling pathways are primarily involved in cell proliferation and have substantially induced drug resistance amongst the diseased individuals and henceforth may be considered a potential drug target to combat the disease. **Methods**: Phytochemicals obtained from medicinal plants were evaluated for their efficacy to target MAPK by molecular docking and were compared with synthetic analogues available in the market to target the disease. The stability of complexes was determined using MD Simulation, and interaction energy was calculated. Ligand binding pockets of interaction were also depicted along with the computation of RMSD. **Results**: Withanolide belonging to *Withania somnifera* exhibited the highest BA at both 35 × 57 × 38 Å and 33 × 41 × 41 Å sites of MAPK. Withanolide showed a BA of -10.2 Kcal/mol in comparison to AL8697 and Ralimetinib mesylate, which represented BA of -8.5 and -8.0 Kcal/mol, respectively. The Withanolide-MAPK complex showed stable conformation during MD Simulation. RMSD value for Withanolide was found to be the least, thereby indicating the least fluctuations during the interactions. **Conclusion**: The present study unveiled that Withanolide may emerge as a potential drug candidate to target MAPK signalling pathway in breast cancer.

Keywords: MAPK, MD Simulation, Molecular Docking, Phytochemicals

1. Introduction

Breast cancer is one of the most common malignancies among women worldwide. It cannot be considered a single disease as it is characterized by distinct pathological and molecular subtypes. Although several conventional treatment regimens are currently available to target the disease, the multi-cascade signalling mechanism involved in complicating the disease has made the disease more cumbersome to treat. Evidence from the literature has demonstrated that recent and continuing research has a significant impact on enhancing the clinical prognosis for breast cancer[1,2]. This has been ascribed to advancements in the field of breast cancer management, including those in screening, diagnosis, and therapy approaches. But Triple-Negative Breast Cancer's (TNBC's) dismal prognosis and medication resistance create significant barriers that are also today's problems for controlling the disease. The increased incidence and death rates of breast cancer in the population of developing countries are also a major source of worry. Ductal carcinoma in situ can lead to invasive malignancy and is treated with breast-conserving surgery and radiation therapy without the need for further lymph node exploration or systemic therapy[3,4]. Breast cancers in Stages I and II are often treated with breast-conserving surgery and radiation treatment. Following breast-conserving surgery, radiation treatment reduces mortality and recurrence. Chemotherapy, endocrine treatment, and

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trastuzumab are the most common systemic therapies used to treat node-positive breast cancer, especially for cancer overexpressing Human Epidermal Growth Factor Receptor 2 (HER2). Chemotherapeutic regimens comprising anthracyclines and taxanes are effective against breast cancer. Chemotherapy is frequently needed for Stage III breast cancer to shrink the tumour and enable breast-conserving surgery. Despite being classified as Stage III, inflammatory breast cancer is aggressive and necessitates mastectomy rather than breast-conserving surgery[5].

Breast cancer is complicated by several signalling pathways, including uncontrolled proliferation, apoptosis inhibition, metastasis, angiogenesis, pro-survival signalling pathways, and many more. In addition, Mitogen-Activated Protein Kinase (MAPK) also contributed significantly to the complications of breast cancer. Many inhibitory compounds can be used to block the MAPK signalling pathway, but their use is limited by some factors, the most important of which is their potential negative effects[6,7]. The goal of the current research is to assess a phytochemical's ability to interfere with the MAPK signalling pathway in breast cancer.

2. Materials and Methods

2.1 Preparation of the Protein and Ligand Structures

The 1000 active constituents belonging to different medicinal plants were screened for their ability to target the MAPK signalling pathway in breast cancer, based on the available literature. 33 different natural moieties belonging to *Withania somnifera*, *Syzygium aromaticum*, *Elettaria cardamom*, *Zingiber officinale,* and *Myristica fragrans* were chosen based on the ancient medical text on the virtue of their ability to exhibit anti-breast cancer activity against several signalling pathways and to be used to target breast cancer using computational studies. 3D structures were fetched from PubChem[8], Zinc database available in several forms, including pdb, mol2, and sdf. Selected ligands' Three-Dimensional (3D) structures were created using canonical SMILES via the RBPS Web Portal. Cartesian coordinates were used to analyse ligand crystallographic data. With the help of the Protein Data Bank (PDB) ID 3ZYA, the MAPK protein structure was fetched. UCSF Chimera was used to view 3D structures to gain accurate insights into protein structure[9,10].

2.2 Parameters for Molecular Docking Experiments

The Binding Affinity (BA) of active moieties to MAPK was calculated using a Lamarckian genetic algorithm with 250000 energies to predict their effectiveness in treating breast cancer. A molecular docking investigation was conducted using the AutoDock 4.2.6 package's AutoDock Tool. PDBQT format was used to store the 3D structures of proteins and their ligands. Each docking procedure involved the placement of ligands in a grid box with different dimensions. To account for the interaction of MAPK with ligands, different grid points for autogrid maps were adjusted to be 35 × 57 × 38 Å and 33 × 41 × 41 Å. The torsion degree of freedom was also established throughout the procedure, and 20 distinct modes were chosen with an exhaustiveness of eight to get the maximum postures. Finding the ideal ligand-receptor interaction may be aided by docking at various places. Docked poses of ligands can be aligned over the receptor by using UCSF CHIMERA as the visualization tool to analyse the ligand–receptor interaction and to anticipate the affinity of ligands to target the receptors. Binding energy can be computed: $\Delta G_{Binding} = G_{Complex} - G_{Protein} - G_{Ligand}$[11,12].

2.3 Molecular Dynamic Simulation

To forecast the stability of docked protein-ligand complexes, molecular dynamic simulation was performed using NAMD software. The force field configurations for CHARMM36 were used to execute the Molecular Dynamic simulation (MD simulation). To carry out the procedure, protein structure files were acquired using the Visual Molecular Dynamic (VMD) program. Further exposure to solutions in cubic water boxes containing transferable intermolecular interactions was done on protein-ligand complexes. The size of the box was then chosen to guarantee a 5 Å gap between the protein and box boundaries. Initially, NAMD was used to conduct an MD simulation for 50,000 steps of the steepest decline with the least amount of energy. Additionally, an NVT setup was used to simulate the current system. The temperature of 310 K and simulated time length of 10 ns were fixed for the best run. To forecast any potential change in the dynamics of protein-ligand complexes, the simulated complexes were further examined after the experiment was complete and displayed using VMD. Using the VMD and NMAD software, the trajectories of simulated complexes were

plotted to forecast their stability, and electrostatic, kinetic, and potential energy were calculated[13].

2.4 Root Mean Square Deviation Computation

Values of Root Mean Square Deviation (RMSD) illustrate the adaptability of protein structure and, consequently, its trajectory mobility. Greater RMSD values indicate greater ligand mobility and vice versa. The structural stability of the ligand poses produced by AutoDock tools during the ligand-receptor interaction was examined based on how much they deviated from the native ligand pose. Results from docking studies may be verified using RMSD techniques, which may also help to improve docking performance. RMSD may be computed as RMSD = 1NΣI = 1Ndi2.

2.5 Active Site Prediction

The depiction of the ligand-receptor binding posture was done using UCSF Chimera. Analysing the docked structure of ligands with MAPK allowed researchers to better understand the role of certain amino acids in ligand-receptor interaction. Using the command line, amino acid residues implicated in interactions close to 5 Å were shown. Further comparisons were made with the synthetic drugs AL8697 and Ralimetinib mesylate, which are presently available in the market to target MAPK. The active site of MAPK targeted by natural moieties and synthesized analogues were also compared[14].

3. Results and Discussion

3.1 Preparing 3D Structures of Proteins and Ligands for Molecular Docking

The RBPS Web Portal was used to import the canonical SMILES of ligands that were retrieved from PubChem. This allowed for the retrieval of the corresponding 3D structures, which were then converted into monomeric units in both mol2 and pdf file formats. Ralimetinib mesylate and AL 8697 were utilized as the reference synthetic counterpart for this widely used MAPK medication. UCSF Chimera was used to analyse the 3D structure of MAPK with PDB ID 3ZYA, which was then saved in pdb format after downloading from the RCSB Web Portal (Table 1). Molecular docking was performed at several receptor sites using the AutoDock Tool to determine the active site and BA. Both the ligands and receptors were energy minimized and stored in PDBQT format.

3.2 Molecular Docking Study

Ligands were docked with MAPK using AutoDock. Based on the highest binding pocket occupancy and lowest Gibbs free energy, docked molecules were graded. At two distinct grid positions (35 × 57 × 38 Å and 33 × 41 × 41 Å), ligands were docked with MAPK, and the BA was depicted in Kcal/mol. Docked postures with insignificant BA, i.e., less than 5 Kcal/mol, were not taken into consideration for further analysis. Only 10 phytochemicals out of a large number were found to have notable inhibitory action. Withanolide, withasomnilide, viscosalactone, somniferanolide, kaempferol, luteolin, kaurene, macelignan oleanolic acid and curcumin belonging to *Withania somnifera*, *Elettaria cardamomum*, *Myristica fragrans*, *Syzygium aromaticum* and *Zingiber officinale*, respectively were shown to exhibit highest binding with MAPK at the active site with BA of -10.2, -9.7, -10.0, -10.2, -8.0, -7.9, -7.9, -8.2, -8.6 and -8.1 Kcal/mol (Table 2). These phytochemicals' BA were better than those of the synthetic analogues ralimetinib and Al8697.

When MAPK was docked at grid positions 35 × 57 × 38 Å, withanolide, withasomnilide, viscosalactone, somniferanolide and kaempferol exhibited BA of -8.0, -8.1, -8.0, -9.3 and -7.5 Kcal/mol whereas at 33 × 41 × 41 Å grid points, BA were -10.2, -9.7, -10.0, -10.2 and -8.0 Kcal/mol, respectively. Similarly, luteolin, kaurene, macelignan oleanolic acid, and curcumin exhibited BA of -8.1, -6.7, -7.2, -8.3, and -7.8 Kcal/mol at 35 × 57 × 38 Å grid points whereas at 33 × 41 × 41 Å grid points, the results were -7.9, -7.9, -8.2, -8.6 and -8.1 Kcal/mol, respectively (Table 2). According to the aforementioned findings, MAPK at grid points 35 × 57 × 38 Å provided the profound binding sites for the ligands, and the active site may be thought of as a potential target for any potential drug candidates interested in targeting MAPK to combat breast cancer.

Table 1. Table representing 3D structures along with canonical SMILES of phytochemicals

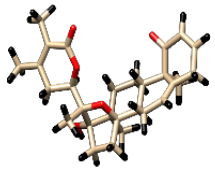
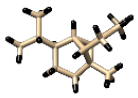
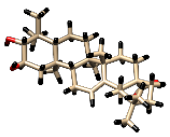
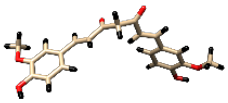
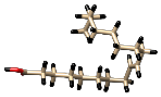
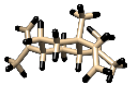
Compound	Pubchem ID	Canonical SMILE	3D Structure
Coagulin	10770343	CC1=C(C(=O) OC(C1)C(C) (C2CCC3(C2 (CCC4C3CC =C5C4(C(=O)CC(C5)OC6C(C(C (C(O6)CO)O) O)O)C)C)O)O)C	
Copaene	19725	CC1=CCC2C3C1C2(CCC3C(C) C)C	
Crataegolic acid	73659	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CC(C(C5(C)C)O)O)C) C)C2C1)C)C(=O)O)C	
Cubebene	91747196	CC1CCC(C2C13C2C(=CC3)C) C(C)C	
Curcumin	969516	COC1=C(C=CC(=C1)C=CC(=O) CC(=O)C=CC2=CC(=C(C=C2) O)OC)O	
Elaidic acid	637517	CCCCCCCCC =CCCCCCCCC(=O)O	
Elemene	6918391	CC(=C)C1CCC(C(C1)C(=C)C) (C)C=C	
Eucalyptol	2758	CC1(C2CCC(O1)(CC2)C)C	

Table 1 to be continued...

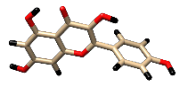
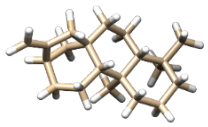
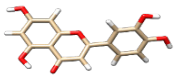
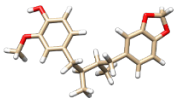
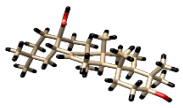
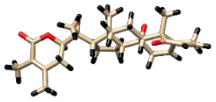
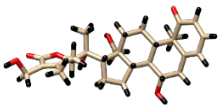
Compound	Pubchem ID	Canonical SMILE	3D Structure
Kaempferol	5280863	C1=CC(=CC= C1C2=C(C(=O) C3=C(C=C(C= C3O2)O)O)O)O	
Kaurene	5318786	CC1(CCCC2(C1CCC 34C2CCC(C3)C (=C) C4)C)C	
Luteolin	5280445	C1=CC(=C(C =C1C2=CC(=O) C3=C(C=C(C=C3O2)O)O)O)O	
Macelignan	10404245	CC(CC1=CC2=C(C=C1)OCO2)C(C) CC3=CC(=C(C=C3)O)OC	
Oleanolic acid	10494	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(C CC(C5(C)C)O)C)C)C2C1)C)C(=O)O)C	
Somniferanolide	102066415	CC1=C(C(=O)OC(C1)C(C)C23C(O2) CC4C3(CC(C5C4(CC=C6C5(C(=O) C=CC6)C)O)O)C)C	
Somniferawithanolide	102066416	CC1=C(C(=O)OC(C1)C(C) (C2CCC3C2(CCC4C3(CC=C5C4(C(=O) C=CC5)C)O)CO)O)C	
Somniwithanolide	102066417	CC1=C(C(=O)OC(C1)C(C) (C2CCC3C2(CCC4C3C(CC5=CC=CC(=O) C45C)O)CO)O)CO	

Table 1 to be continued...

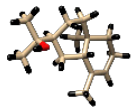
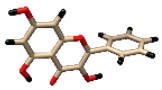
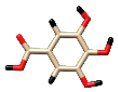
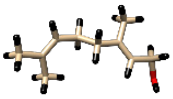
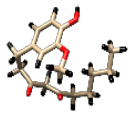
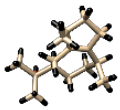
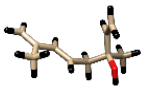
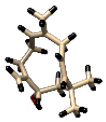
Compound	Pubchem ID	Canonical SMILE	3D Structure
Eudesmol	12304196	CC1=CCCC2(C1CC(CC2)C(C)(C)O)C	
Galangin	5281616	C1=CC=C(C=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O	
Gallic acid	370	C1=C(C=C(C(=C1O)O)O)C(=O)O	
Geraniol	637566	CC(=CCCC(=CCO)C)C	
Gingerdione	162952	CCCCCC(=O)CC(=O)CCC1=CC(=C(C=C1)O)OC	
Guaiol	227829	CC1CCC(CC2=C1CCC2C)C(C)(C)O	
Hotrienol	5366264	CC(=C)C=CCC(C)(C=C)O	
Humulenol	102115341	CC1=CCC(C=CCC(=C)C(CC1)O)(C)C	

Table 1 to be continued...

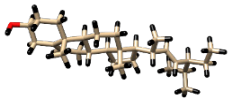
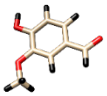
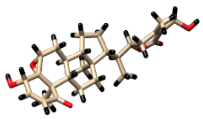
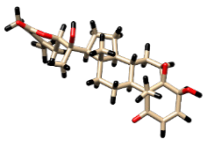
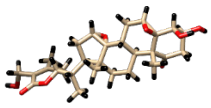
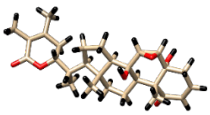
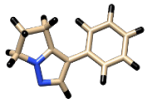
Compound	Pubchem ID	Canonical SMILE	3D Structure
Stigmasterol	5280794	CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	
Vanillin	1183	COC1=C(C=CC(=C1)C=O)O	
Verbenone	29025	CC1=CC(=O)C2CC1C2(C)C	
Withaferin	265237	CC1=C(C(=O)OC(C1)C(C)C2CCC3C2(CCC4C3CC5C6(C4(C(=O)C=CC6O)C)O5)C)CO	
Withanolide	53477765	CC1=C(C(=O)OC(C1)C(C)(C2CCC3C2(CCC4C3CC5C6(C4(C(=O)C=CC6O)C)O5)C)O)C	
Withaoxylactone	101687981	CC1=C(C(=O)OC(C1)C(C)C2CC3C4(C2(CCC5C4CC6C7(C5(C(=O)CC(C7O)O)C)O6)C)O3)CO	
Withasomnilide	102066413	CC1=C(C(=O)OC(C1)C(C)C2CCC3C2(CCC4C3(C5C(O5)C6(C4(C(=O)C=CC6)C)O)O)C)C	
Withasomnine	442877	C1CC2=C(C=NN2C1)C3=CC=CC=C3	

Table 2. BA, RMSD upper bound and lower bound values along with active sites of phytochemical interaction with MAPK at 35 × 57 × 38 Å and 33 × 41 × 41 Å

Protein	Interaction (X: 35.4293 Y: 57.5412 Z:38.3801)	Binding Affinity (BA) (Kcal/mol)	RMSD LB	RMSD UB	Active Site
MAPK	MAPK-Acaetin	-7.9	1.224	2.662	ASN 82, GLU 163, LYS 165, LEU 108, MET 109
	MAPK-Anaferine	-6.1	5.267	10.161	GLN 133, LYS 165, GLU 163, LEU 164, ASN 82
	MAPK-Anahygrine	-5.6	1.191	6.121	ASP 292, PRO 242, LEU 291, UNK 1, GLY 243
	MAPK-Apigenin	-7.7	1.421	3.014	LYS 165, ASN 159, GLY 110, ALA 157, VAL 158
	MAPK-Asarone	-4.9	2.611	3.355	VAL 290, LEU 291, PRO 242, ASP 292, LYS 295
	MAPK-Bergamotenoic acid	-6.2	1.108	2.298	PHE 223, LEU 222, ALA 214, THR 218, ARG 220
	MAPK-Biflorin	-7.6	0.993	2.38	LYS 165, LEU 164, GLY 110, GLU 160, ARG 49
	MAPK-Bisabolene	-5.9	1.504	5.297	GLU 245, LYS 295, THR 241, ALA 244, LEU 289
	MAPK-Borneol	-5.1	1.491	2.738	LEU 222, VAL 273, THR 221, PRO 224, LEU 234
	MAPK-Cadinene	-5.9	1.291	4.579	PHE 223, LEU 222, THR 221, LEU 234, ARG 220
	MAPK-Caffeicacid	-6.0	9.903	14.08	MET 109, LEU 164, ASN 159, VAL 158, LYS 165
	MAPK-Calacorene	-5.8	28.819	30.482	GLN 133, ASN 82, LEU 164, GLU 163, TYR 311
	MAPK-Calamenene	-5.8	1.724	5.077	GLY 240, LEU 291, GLY 240, THR 241, VAL 290
	MAPK-Campesterol	-7.2	3.093	8.655	VAL 290, ASP 292, LEU 246, ALA 244, LYS 295
	MAPK-Camphene	-4.7	1.08	2.686	ARG 237, PHE 223, LEU 238, MET 268, VAL 273
	MAPK-Carene	-4.8	0.89	3.71	SER 252, TRP 197, LEU 195, TYR 258, ALA 255
	MAPK-Carvacrol	-5.5	2.344	3.841	GLN 133, LEU 164, VAL 158, ALA 157, LYS 165
	MAPK-Carvone	-5.4	1.406	4.249	LEU 108, LYS 165, VAL 158, ALA 157, GLU 163
	MAPK-Caryophyllene	-5.9	1.258	3.356	VAL 290, ASP 292, LEU 289, LYS 267, ASP 292
	MAPK-Cedrene	-5.9	15.496	17.905	ASP 292, LEU 246, GLU 245, THR 241, PRO 242
	MAPK-Chlorogenicacid	-7.6	1.346	2.975	LEU 164, PHE 129, GLU 163, ASN 82, LYS 165
	MAPK-Chrysin	-7.8	1.365	3.07	HIS 107, LEU 108, MET 109, GLY 110, ALA 111
	MAPK-Cinnamic	-5.6	2.773	3.953	ALA 157, VAL 158, LEU 164, ALA 111, ASP 112
	MAPK-Citral	-4.7	1.554	2.392	GLU 163, ASN 82, GLN 133, PHE 129, TYR 311
	MAPK-Citronellol	-4.7	9.767	12.989	ASN 159, LEU 108, ASP 112, LYS 165, MET 109
	MAPK-Coagulin	-8.8	1.449	6.75	THR 241, GLN 264, PRO 266, LEU 289, LYS 295
	MAPK-Copaene	-6.1	15.54	18.728	ASP 292, LYS 295, VAL 290, GLU 245, THR 241
	MAPK-Crategolicacid	-8.5	1.451	8.693	LEU 238, PRO 224, THR 218, LYS 121, GLY 276
	MAPK-Cubebene	-6.1	14.763	18.583	ASP 292, VAL 290, THR 241, GLY 243, LEU 289
	MAPK-Curcumin	**-7.8**	**0.481**	**10.927**	**ALA 111, ASN 82, GLU 81, ASP 316, TYR 311**
	MAPK-Cuscohygrine	-5.4	1.853	2.126	VAL 290, LEU 289, ASP 292, THR 241, PRO 242
	MAPK-Cyanidin	-7.5	1.366	2.801	VAL 290, LEU 289, LYS 287, PHE 270, LYS 287
	MAPK-Cymene	-5.1	0.939	4.332	ALA 157, ASN 159, GLU 163, MET 109, LEU 108
	MAPK-Elaidicacid	-5.7	6.283	11.693	ASP 112, ALA 157, VAL 158, LEU 108, GLY 110
	MAPK-Elemene	-5.8	1.188	2.935	VAL 290, ASP 292, GLU 245, LYS 295, LEU 291
	MAPK-Ellagicacid	-7.8	0.107	6.255	VAL 158, ALA 157, MET 109, LEU 108, ARG 49
	MAPK-Eucalyptol	-5.0	1.192	3.082	LEU 222, PHE 223, VAL 273, LEU 238, MET 268
	MAPK-Eudesmol	-6.2	1.234	1.853	ARG 136, ASP 316, GLU 163, TYR 311, GLN 133

Table 2 to be continued...

Protein	Interaction (X: 35.4293 Y: 57.5412 Z:38.3801)	Binding Affinity (BA) (Kcal/ mol)	RMSD LB	RMSD UB	Active Site
	MAPK-Eugenol	-5.2	1.802	2.73	LYS 165, HIS 107, MET 109, LEU 108, VAL 158
	MAPK-Farnesene	-5.4	1.556	3.103	LEU 289, VAL 239, THR 241, PRO 242, GLY 243,
	MAPK-Ferulicacid	-5.8	28.947	30.506	ALA 111, MET 109, LEU 108, ASN 159, VAL 158
	MAPK-Galangin	-7.3	1.81	6.121	GLY 240, THR 241, ASP 292, LYS 287, PHE 270
	MAPK-Gallicacid	-5.9	0.003	2.402	LEU 164, GLU 163, LYS 165, ASN 159, MET 109
	MAPK-Geranicacid	-5.7	9.173	11.919	MET 109, ASN 82, GLU 81, LEU 164, GLN 133
	MAPK-Geraniol	-5.1	1.805	3.277	ASN 82, GLU 81, LYS 165, ASN 159, VAL 158
	MAPK-Gingerdione	-5.6	14.512	16.742	ASP 292, VAL 290, LYS 287, PHE 270, GLU 286
	MAPK-Guaiol	-6.1	2.216	5.073	PHE 274, ARG 220, VAL 273, THR 221, LEU 234
	MAPK-Hotrienol	-4.7	1.299	2.016	LEU 222, ARG 220, THR 221, GLU 215, LEU 234
	MAPK-Humulenol	-6.5	1.856	2.662	ASP 292, VAL 290, LEU 289, GLY 240, PRO 242
	MAPK-Hygrine	-4.4	9.902	12.649	LEU 164, MET 109, ASN 159, VAL 158, ALA 111
	MAPK-Kaempferol	**-7.5**	**2.028**	**6.622**	**PHE 270, VAL 290, PRO 242, ASP 292, LEU 291**
	MAPK-Kaurene	**-6.7**	**1.074**	**2.809**	**ASP 168, SER 154, GLY 11O, ILE 84, VAL 38**
	MAPK-Lauricacid	-4.5	1.278	2.294	ARG 237, PHE 233, LEU 222, VAL 273, THR 221
	MAPK-Limonene	-5.1	10.823	12.063	GLU 163, MET 109, LYS 165, LEU 164, ALA 157
	MAPK-Linalool	-4.7	2.26	5.49	VAL 273, ARG 237, PHE 223, ALA 214, THR 221
	MAPK-Luteolin	**-8.1**	**1.444**	**2.745**	**GLA 133, ARG 136, ASP 112, HIS 107, ASN 82**
	MAPK-Macelignan	**-7.2**	**7.943**	**11.539**	**HIS 126, GLU 163, ASP 161, TYR 132, GLN 133**
	MAPK-Malabaricone	-6.3	0.998	2.256	LEU 164, LYS 165, ALA 157, ARG 136, GLU 81
	MAPK-Muurolene	-5.6	1.426	3.364	TRP 197, LEU 195, ILE 229, ALA 255, SER 252
	MAPK-Myristicacid	-4.4	1.196	3.356	ARG 237, PHE 274, LEU 222, VAL 273,THR 278
	MAPK-Myristicin	-5.5	1.577	2.361	ASN 159, GLY 110, LEU 108, VAL 158, MET 109
	MAPK-Nerolidol	-5.2	1.008	2.037	ARG 237, VAL 273, THR 221, LEU 222, PHE 223
	MAPK-Ocimene	-4.7	0.567	5.037	LEU 222, PHE 223, VAL 273, ARG 237, THR 221
	MAPK-Oleanolicacid	**-8.3**	**1.621**	**8.47**	**THR 218, ARG 220, ILE 275, PRO 224, MET 268**
	MAPK-Oleicacid	-4.7	1.08	2.625	ASN, 272, LEU 222, PRO 224, ARG 220, VAL 273
	MAPK-Palmiticacid	-4.7	1.859	3.695	ARG 237, PHE 223, VAL 273, PRO 224, MET 268
	MAPK-Physagulin	-7.5	1.816	2.657	MET 179, ALA 172, ARG 189, ASP 168, VAL 38
	MAPK-Pinanediol	-5.2	1.475	2.103	LYS 233, ARG 237, ILE 235, MET 268, LEU 222
	MAPK-Pinene	-4.8	1.125	2.991	SER 252, TYR 258, ILE 229, ALA 255, TRP 197
	MAPK-Pinocarveol	-5.0	1.952	3.35	LYS 233, ARG 237, MET 268, VAL 273, LEU 238
	MAPK-Pinocembrin	-7.6	1.374	3.022	GLY 110, HIS 107, LYS 165, LEU 164, ALA 111
	MAPK-Piperitol	-7.7	0.702	1.866	MET 109, HIS 107, GLN 133,ARG 136, LYS 165
	MAPK-Quercetin	-7.8	1.499	3.128	GLY 110, ALA 111, LEU 108, GLU 163, LYS 165
	MAPK-Rhamnetin	-8.1	1.841	7.478	HIS 107, ALA 157, LEU 108, GLY 110, VAL 158
	MAPK-Sabinene	-5.2	0.951	4.1	GLY 110, MET 109, LYS 165, ASN 159, ALA 157
	MAPK-Sitosterol	-7.4	1.493	2.169	HIS 107, LEU 108, MET 109, GLY 110, ALA 111

Table 2 to be continued...

Protein	Interaction (X: 35.4293 Y: 57.5412 Z:38.3801)	Binding Affinity (BA) (Kcal/ mol)	RMSD LB	RMSD UB	Active Site
	MAPK-Sominone	-9.1	2.284	3.69	GLU 163, LEU 164, ALA 111, MET 109, ASN 159
	MAPK-Somniferanolide	**-9.3**	**2.514**	**9.014**	**HIS 107, GLU 163, GLN 133, MET 109, VAL 158**
	MAPK-Somniferawithanolide	-7.0	2.276	8.484	TYR 182, PRO 224, MET 268, VAL 273, LEU 234
	MAPK-Somniferine	-7.8	1.668	2.132	THR 226, ASP 230, TYR 182, LEU 234, VAL 273
	MAPK-Somniwithanolide	-8.6	1.972	3.346	GLU 81, ASN 82, ARG 136, MET 109, HIS 107
	MAPK-Stigmasterol	-7.9	1.416	2.062	PHE 129, TYR 132, ASP, 326, ALA 157, GLY 110
	MAPK-Thujene	-4.5	1.638	3.553	PRO 224, PHE 223, VAL 273, MET 268, ARG 237
	MAPK-Vanillicacid	-5.3	1.372	4.346	VLA 158, LEU 164, LYS 165, GLY 110, ALA 157
	MAPK-Vanillin	-5.1	1.315	2.868	MET 109, GLY 110, ALA 111, ASP 112, VAL 158
	MAPK-Verbenone	-5.1	1.83	3.229	ILE 259, TYR 258, SER 252, ALA 255, LEU 195
	MAPK-Viscosalactone	**-8.0**	**3.118**	**11.33**	**THR 218, TYR 188, VAL 117, ASN 272, CYS 119**
	MAPK-Withaferin	-8.1	1.937	3.888	ARG 136, ASN 159, HIS 107, LEU 164, PHE 129
	MAPK-Withanolide	**-8.0**	**1.625**	**3.063**	**ILE 275, PHE 274, MET 268, ARG 237, PRO 224**
	MAPK-Withaoxylactone	-7.9	1.814	9.826	PHE 274, ILE 275, LEU 238, VAL 239, MET 268
	MAPK-Withasomnilide	**-8.1**	**1.736**	**2.307**	**ARG 237, ILE 275, ASN 272, LEU 217, MET 268**
	MAPK-Withasomnine	-6.2	1.403	2.725	MET 109, GLN 133, LEU 108, VAL 158, ALA 111
	MAPK-Al8697	**-8.3**	**1.231**	**2.308**	**GLU 163, LEU 164, VAL 158, ALA 157, GLN 133**
	MAPK-Ralmetinib	**-7.6**	**2.683**	**4.832**	**PHE 223, LEU 222, ILE 235, THR 218, GLU 215**

Protein	Interaction (X: 33.00 Y:41.20 Z:41.03)	BA (Kcal/ mol)	RMSD LB	RMSD UB	Active Site
MAPK	MAPK-Acaetin	-7.5	2.22	6.613	LEU 171, GLU 71, ARG 67, TYR 35, THR 68
	MAPK-Anaferine	-5.8	0.078	6.089	PRO 350, ARG 23, PHE 90, ARG 5, ASP 88
	MAPK-Anahygrine	-5.8	1.699	2.263	TYR 342, ILE 346, ARG 94, ASP 88, ALA 93
	MAPK-Apigenin	-7.8	1.364	2.867	ARG 67, THR 68, TYR 69, LEU 55, GLU 71
	MAPK-Asarone	-5.7	0.882	3.733	VAL 83, LYS 165, LEU 86, PHE 348, MET 78
	MAPK-Bergamotenoic acid	-5.8	1.151	2.563	ARG 5, THR 91, ALA 93, PHE 8, ASP 88,
	MAPK-Biflorin	-7.7	1.91	2.396	VAL 89, ASP 88, PHE 90, SER 347, ARG 23
	MAPK-Bisabolene	-5.7	1.178	1.514	ASP 88, PHE 90, ARG 5, ALA 93, VAL 89
	MAPK-Borneol	-5.5	1.056	2.461	ARG 94, ALA 93, PRO 92, TYR 342, VAL 89
	MAPK-Cadinene	-6.1	12.917	14.047	ASP 168, ILE 84, ALA 51, LYS 53, ALA 51
	MAPK-Caffeicacid	-6.7	18.713	20.075	PRO 58, ARG 57, GLU 71, THR 68, TYR 35
	MAPK-Calacorene	-6.3	1.81	2.042	PHE 348, VAL 89, PRO 350, ASP 88, ARG 23
	MAPK-Calamenene	-6.4	1.249	3.714	PHE 348, VAL 89, PRO 350, ARG 23, TYR 24
	MAPK-Campesterol	-8.2	1.022	1.757	ASP 88, VAL 89, PHE 90, LYS 45, ARG 23
	MAPK-Camphene	-5.5	1.162	3.211	GLU 71, THR 68, PRO 58, ARG 57, TYR 35
	MAPK-Carene	-5.9	18.018	19.254	ARG 57, SER 56, TYR 35, THR 68, LEU 55

Table 2 to be continued...

Protein	Interaction (X: 33.00 Y:41.20 Z:41.03)	BA (Kcal/ mol)	RMSD LB	RMSD UB	Active Site
	MAPK-Carvacrol	-6.5	1.812	4.572	LEU 171, ARG 67, ILE 63, LEU 55, ALA 65
	MAPK-Carvone	-6.1	1.95	2.954	GLN 60, ARG 57, ALA 34, GLU 71, THR 68
	MAPK-Caryophyllene	-6.5	1.398	4.668	TYR 342, ARG 94, ALA 93, PRO 6, PHE 90
	MAPK-Cedrene	-6.7	1.153	3.457	PHE 90, ARG 5, PRO 21, ASP 88, VAL 345
	MAPK-Chlorogenicacid	-8.0	1.868	2.339	ASP 168, ARG 67, LEU 171, ALA 172, PHE 169
	MAPK-Chrysin	-7.4	6.848	9.167	TYR 35, THR 68, GLU 71, PRO 58, LEU 55
	MAPK-Cinnamic	-5.8	1.701	2.041	TYR 35, ARG 67, SER 56, LEU 55, GLN 60
	MAPK-Citral	-5.6	1.704	2.744	LEU 171, ALA 172, ARG 67, SER 56, TYR 35
	MAPK-Citronellol	-5.4	18.217	20.065	GLU 71, LEU 171, ARG 67, TYR 35, SER 56
	MAPK-Coagulin	-8.4	1.623	6.843	PRO 350, PHE 348, ASP 88, ARG 5, ALA 93
	MAPK-Copaene	-6.4	1.106	4.637	ASP 88, PHE 8, ARG 23, ALA 93, ILE 346
	MAPK-Crategolicacid	-8.0	4.495	8.426	VAL 89, ASP 88, PHE 90, ARG 94, SER 347
	MAPK-Cubebene	-6.1	1.531	3.448	ILE 346, ARG 5, PHE 90, PRO 21, VAL 89
	MAPK-Curcumin	**-8.1**	**1.051**	**2.194**	**ASN 155, ASP 150, ALA 172, LEU 171, GLY 170**
	MAPK-Cuscohygrine	-5.7	1.559	5.516	PHE 90, ARG 23, PRO 21, ASP 88, VAL 345
	MAPK-Cyanidin	-7.7	0.902	1.553	SER 95, ARG 94, ASP 88, VAL 89, PHE 8
	MAPK-Cymene	-5.9	1.256	4.377	LEU 75, GLU 71, ASP 168, THR 106, VAL 105
	MAPK-Elaidicacid	-5.3	7.703	10.41	LYS 165, ILE 84, LEU 87, VAL 83, GLU 81
	MAPK-Elemene	-5.7	1.828	4.461	LYS 165, ILE 84, VAL 83, LEU 87, LYS 76
	MAPK-Ellagicacid	-7.6	0.043	6.255	ASP 88, ARG 94, PHE 8, PRO 350, TYR 342
	MAPK-Eucalyptol	-5.3	0.062	0.062	PRO 21, ARG 5, ILE 346, VAL 345, PHE 90
	MAPK-Eudesmol	-6.9	1.142	2.748	TYR 342, ILE 346, PHE 348, ALA 93, VAL 349
	MAPK-Eugenol	-6.1	1.849	4.356	LEU 171, TYR 35, ARG 67, GLN 60, GLY 36
	MAPK-Farnesene	-6.2	1.283	2.277	GLN 60, LEU 171, ARG 57, GLU 71, PRO 58
	MAPK-Ferulicacid	-5.9	1.065	2.471	PHE 348, ARG 23, TYR 24, ASP 88, VAL 89
	MAPK-Galangin	-7.7	1.729	5.878	LYS 66, ILE 63, LEU 171, SER 56, ARG 57
	MAPK-Gallicacid	-6.1	0.087	2.403	LYS 66, TYR 69, LEU 171, GLU 71, ARG 57
	MAPK-Geranicacid	-5.1	3.82	5.712	THR 91, PRO 21, PHE 8, ARG 94, ASP 88
	MAPK-Geraniol	-5.6	2.834	5.39	LEU 171, GLU 71, THR 68, TYR 35, ARG 67
	MAPK-Gingerdione	-6.3	1.09	2.232	ARG 67, LEU 171, GLN 60, ALA 34, TYR 35
	MAPK-Guaiol	-6.9	1.482	2.857	THR 91, PHE 8, ILE 346, VAL 349, SER 347
	MAPK-Hotrienol	-5.3	1.885	5.15	ARG 67, GLN 60, SER 56, LEU 171, TYR 35
	MAPK-Humulenol	-6.7	1.95	4.461	PHE 90, ARG 5, ALA 93, TYR 342, VAL 89
	MAPK-Hygrine	-4.7	3.299	4.868	ARG 57, LEU 55, THR 68, TYR 35, GLU 71
	MAPK-Kaempferol	**-8.0**	**1.611**	**6.307**	**TYR 35, THR 68, PHE 59, PRO 58, ARG 57**
	MAPK-Kaurene	**-7.9**	**1.176**	**2.8**	**PHE 8, PRO 21, ARG 23, ASP 88, ALA 93**
	MAPK-Lauricacid	-5.5	1.566	2.184	SER 56, ARG 57, GLN 60, HIS 64, TYR 35
	MAPK-Limonene	-5.7	0.719	4.418	ARG 67, HIBS 64, PHE 59, TYR 35,LEU 35
	MAPK-Linalool	-5.7	1.368	2.491	LEU 171, TYR 35, ARG 67, THR 68, PRO 58

Table 2 to be continued...

Protein	Interaction (X: 33.00 Y:41.20 Z:41.03)	BA (Kcal/ mol)	RMSD LB	RMSD UB	Active Site
	MAPK-Luteolin	**-7.9**	**1.236**	**2.652**	**ARG 94, ALA 93, PRO 92, THR 91, VAL 89**
	MAPK-Macelignan	**-8.2**	**1.281**	**7.754**	**ALA 34, TYR 35, LEU 171, ASP 168, PHE 169**
	MAPK-Malabaricone	-6.1	1.741	3.969	THR 91, ALA 93, ARG 94, ASP 88, VAL 89
	MAPK-Muurolene	-6.3	1.103	4.562	ARG 5, PRO 6, PHE 8, ASP 88, VAL 89
	MAPK-Myrcene	-5.1	2.318	4.959	LYS 53, LEU 55, SER 56, PRO 58, HIS 64
	MAPK-Myricetin	-7.7	1.345	2.2	SER 95 ARG 94, PRO 92, ALA 93, PHE 90
	MAPK-Myristicacid	-4.7	1.666	3.427	ARG 23, TYR 24, ALA 93, VAL 89,PHE 90
	MAPK-Myristicin	-5.5	1.925	4.697	LEU 55, SER 56, ARG 57, PRO 58, GLN 60
	MAPK-Nerolidol	-6.3	19.297	21.514	THR 68, SER 56, LEU 55, ARG 57, GLN 60
	MAPK-Ocimene	-5.3	1.16	4.954	THR 68, GLU 71, ARG 67, GLN 60, HIS 64
	MAPK-Oleanolicacid	**-8.6**	**1.677**	**2.728**	**PHE 90, THR 91, ASP 88, VAL 89,LYS 45**
	MAPK-Oleicacid	-4.8	1.044	2.817	VAL 345, ILE 346, PHE 348, TYR 342, ALA 93
	MAPK-Palmiticacid	-5.6	1.415	2.449	GLN 60, ARG 57, SER 56, HIS 64,LEU 55
	MAPK-Physagulin	-9.3	1.927	11.941	VAL 89, ASP 88, PHE 90, LEU 87, THR 46
	MAPK-Pinanediol	-5.6	1.11	2.814	MET 78, LYS 89, LEU 87, HIS 80, VAL 83
	MAPK-Pinene	-5.8	0.989	3.29	PRO 58, ARG 57, LEU 55, SER 56, HIS 64
	MAPK-Pinocarveol	-5.8	18.67	20.123	THR 68, PRO 58, PHE 59, ARG 67, GLU 71
	MAPK-Pinocembrin	-7.4	1.473	3.042	HIS 64, ARG 57, GLU 71, TYR 35, PRO 58,
	MAPK-Piperitol	-7.8	1.152	1.447	PHE 348, VAL 345, TYR 342, PRO 350, ILE 346
	MAPK-Quercetin	-7.6	20.314	22.468	ALA 34, TYR 35, LYS 53, LEU 55, PRO 58
	MAPK-Rhamnetin	-7.6	1.656	6.579	GLN 60, ARG 57, HIS 64, GLU 71, LYS 53
	MAPK-Sabinene	-5.9	0.954	3.78	ALA 34, TYR 35, ARG 67, GLN 60, HIS 64
	MAPK-Safrole	-6.4	2.464	4.4	GLN 60, HIS 64 PRO 58, THR 68, ARG 57
	MAPK-Selinene	-6.5	0.975	2.713	VAL 345, SER 347, PHE 348, PRO 350, ILE 346
	MAPK-Sinapicacid	-5.9	0.226	3.145	VAL 83, HIS 80, ILE 84, LEU 86, MET 78
	MAPK-Sitosterol	-8.2	1.368	2.322	ALA 93, THR 91, VAL 89, ASP 88, THR 46
	MAPK-Sominone	-9.3	1.368	2.017	LEU 87, ASP 88, VAL 89, PHE 90, SER 95
	MAPK-Somniferanolide	**-10.2**	**1.133**	**1.202**	**ASP 88, VAL 89, PHE 90, THR 91, ARG 94**
	MAPK-Somniferawithanolide	-9.8	1.301	1.92	ALA 93, THR 91, PHE 90, ASP 88, LEU 87
	MAPK-Somniferine	-9.9	1.854	3.1	MET 78, LYS 79, HIS 80, ASN 82, VAL 83
	MAPK-Somniwithanolide	-8.7	2.037	2.525	LEU 87, ASP 88, VAL 89, PHE 90, THR 91
	MAPK-Stigmasterol	-8.6	1.253	2.455	ASP 88, VAL 89, SER 95, THR 91, ALA 93
	MAPK-Thujene	-5.9	1.181	3.989	THR 68, ARG 67, HIS 64, SER 56, PRO 58
	MAPK-Thymol	-6.1	1.951	2.917	ARG 57, PRO 58, HIS 64, ARG 67, THR 68,
	MAPK-Vanillicacid	-5.8	1.482	4.616	SER 56, ARG 67, GLU 71, THR 68, LEU 55
	MAPK-Vanillin	-5.5	1.726	2.048	LEU 55, SER 56, ARG 57, PRO 58, GLN 60
	MAPK-Verbenone	-5.3	1.796	3.283	VAL 89, PHE 90, THR 91, ALA 93, PRO 21
	MAPK-Viscosalactone	**-10.0**	**1.45**	**2.473**	**LEU 87, PHE 90, VAL 89, SER 95, ARG 94**

Table 2 to be continued...

Protein	Interaction (X: 33.00 Y:41.20 Z:41.03)	BA (Kcal/ mol)	RMSD LB	RMSD UB	Active Site
	MAPK-Withaferin	-9.6	1.539	2.503	VAL 345, ILE 346, TYR 342, PRO 350, PHE 90
	MAPK-Withanolide	**-10.2**	**1.571**	**2.971**	**PHE 348, ILE 346, VAL 345, PRO 350, TYR 342**
	MAPK-Withaoxylactone	-8.6	1.858	2.039	ALA 93, ARG 94, PRO 92, PHE 90, THR 91
	MAPK-Withasomnilide	**-9.7**	**1.755**	**2.226**	**PRO 350, PHE 348, VAL 345, TYR 342, ILE 346**
	MAPK-Withasomnine	-6.9	1.743	4.867	ALA 34, TYR 35 ARG 67, LEU 55, PRO 58
	MAPK-Al8697	**-8.5**	**2.623**	**4.138**	**LEU 353, LYS 45, PHE 90, THR 46, ASP 88**
	MAPK-Ralmetinib	**-8.0**	**1.941**	**6.737**	**ASP 88, PHE 90, PRO 21, THR 91, VAL 89**

4. Study of Molecular Dynamics and Free Energy Calculation

Using NAMD software, MD simulation of MAPK was performed using withanolide. Ralimetinib and Al8697 were used as references for the investigation. The MD simulation was run at 310K for 101 ns. According to the findings, all of the natural metabolites in association with MAPK were stable with the receptor for 101 ns. From trajectory analysis, it is clear that all of the aforementioned substances maintained their stability during the simulation process and displayed behaviours resembling those of the synthetic equivalent to ralimetinib and Al8697. Additionally, to compare their BA, kinetic, potential, and electrostatic energy were computed. The outcomes of the simulation research were found to be consistent with those of the molecular docking investigation (Table 3). In compared to the synthetic counterparts, all of the naturally occurring metabolites used for the study produced better outcomes.

Table 3. Table representing binding energies computed by MM-PBSA during MD simulation

Target	Ligand	Energy Components (Kcal/mol)		
		Electrostatic	Potential	Kinetic
MAPK	Withanolide	-190520	-170230	8943
	Ralimetinib	-161852	-13387	8765
	Al8697	-152123	-125969	7215

5. MAPK Active Site Determination

Utilizing UCSF Chimera, the putative MAPK ligand binding site was identified. In order to determine which of the various ligands was involved in the interaction with the receptor, a region within 5 Å of where the individual ligands bound to the receptor domain was chosen, and the amino acid residues making up that region were predicted. This helped to predict the targeted site to disrupt the functioning of MAPK. Future drug discoveries will benefit biologically from the identification of the amino acid residues involved in docking, and the presence of cryptic pockets in MAPK may aid in analysing the potential site of drug binding. MAPK at 35 × 57 × 38 Å grid points forms the binding site with withanolide, withasomnilide, viscosalactone, somniferanolide, kaempferol, luteolin, kaurene, macelignan, oleanolic acid, and curcumin comprising ILE 275, PHE 274, MET 268, ARG 237, PRO 224; ARG 237, ILE 275, ASN 272, LEU 217, MET 268; THR 218, TYR 188, VAL 117, ASN 272, CYS 119; HIS 107, GLU 163, GLN 133, MET 109, VAL 158; PHE 270, VAL 290, PRO 242, ASP 292, LEU 291; GLA 133, ARG 136, ASP 112, HIS 107, ASN 82; ASP 168, SER 154, GLY 11O, ILE 84, VAL 38; HIS 126, GLU 163, ASP 161, TYR 132, GLN 133; THR 218, ARG 220, ILE 275, PRO 224, MET 268; ALA 111, ASN 82, GLU 81, ASP 316 and TYR 311, with BA of -8.0, -8.1, -8.0, -9.3, -7.5, -8.1, -6.7, -7.2, -8.3 and -7.8 Kcal/mol, respectively. Whereas Al8697 and Ralimetinib form the binding site with MAPK at GLU 163, LEU 164, VAL 158, ALA 157, GLN 133; PHE 223, LEU 222, ILE 235, THR 218, GLU with BA of -8.3 and -7.6 Kcal/mol, respectively, at 35 × 57 × 38 Å grid points. All of the ten phytochemicals mentioned above demonstrated a higher affinity for binding to MAPK than the above-mentioned chemical analogues, and even the active site residues involved in the interaction were found to be similar. This clearly demonstrated that these natural moieties may play a crucial role in targeting MAPK to treat breast cancer.

6. Conclusion

Phytochemicals possess the substantial potential to target both acute and chronic disorders. The present study highlighted that Withanolide obtained from *Withania somnifera* may target MAPK either as a mono-therapeutic approach or as a combinational strategy to target the disease. The findings of the study need to be further validated through *in vitro* and *in vivo* studies to fully explore the potency of above-mentioned active moieties to introduce a profound inhibitor of MAPK to treat breast cancer.

7. Abbreviations

TNBC: Triple-Negative Breast Cancer
HER2: Human Epidermal Growth Factor Receptor 2
MAPK: Mitogen-Activated Protein Kinase
PDB: Protein Data Bank
BA: Binding Affinity
MD Simulation: Molecular Dynamic Simulation
VMD: Visual Molecular Dynamic
RMSD: Root Mean Square Deviation

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