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# In-silicoimmunomodulator activity of photochemical from Zingiberofficinale R., Solanumlycopersicum L, and Ocimumtenuiflorum L. against Breast cancer

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***Abstract :****Breast cancer is the most common type of cancer causing death in women globally. It is highly metastatic and can spread to various parts of body. There are many studies regarding role of cytokines in development of cancer. The cytokine NF-κB plays a major role in development and proliferation of cancer cells. Along with it TNF-alpha plays a dual role. According to its presence it can be tumour promoter and on the other hand it can be a cancer killer. The property of TNF in inducing cancer cell death renders it a potential cancer therapeutic, although much work is needed to reduce its toxicity for systematic TNF administration. Current treatments that are available for cancer are costly and have many side-effects. The main aim of this research was to determine in-silicoimmunomodulatory effects of various phytochemicals that are derived from Zingiberofficinale R. (ginger), Solanumlycopersicum L. (tomato), andOcimumtenuiflorum L. (holy basil)against the NF-κB and TNF-alpha. As, phytochemicals are naturally derived compounds they may have lesser or no side-effects and are available in low costs. The 3D structure of target protein receptor (cytokines) and phytochemicals (ligand) were retrieved from PDB and PubChem respectively. Out of twenty-three phytochemicals, fourteen satisfied Lipinski's properties and were docked with NF-κB and TNF-alpha target proteins, using AutoDockVina 1.5.6. Roasmarinic-acid and Luteolin which are active phytochemicals from Ocimumtenuiflorum L. showed highest dock-score value with both target protein respectively. The outcome of the results can be further validated, using in-vitro immunomodulatory studies.*

***Keywords*** *–Breast cancer, Immunomodulaion,Nuclear factor - kappaB (NF-κB),Ocimumtenuiflorum L. (holy basil),Tumournecrosis factor – alpha (TNF-alpha), Zingiberofficinale R. (ginger),*

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## I. Introduction

Cancer is the most prevalent disease that causes many deaths all over the world. Cancer is mainly caused due to mutations and abnormal behavior of normal cells. There are various types of cancer among which breast cancer is the most common type that affects mainly women globally. The age adjusted incidence rates of breast cancer in India is lower than the western countries, because of the large population the burden of breast cancer is high. With an annual incidence of approximately 1,44,000 new cases of breast cancers in India, it has now become the most common female cancer in urban India [1]. Risk factors for developing breast cancer include being female, obesity, lack of physical exercise, alcohol, hormone replacement, ionizing radiation, old age, family history etc. [2]. As per ICMR-PBCR data, breast cancer is the most common type of cancer followed by cervical cancer, in urban cities [3].

Recent cancer treatments include chemotherapy, radiation therapy etc. which have various side-effects. Treatment of cancer with the help of immunotherapy is increasing day by day. But, many times a proportion of cancer patients do not respond cancer immunotherapy. Since the clinical availability of drugs specifically targeting immunosuppressive cells or mediators is still limited, an alternative strategy is to use conventional chemo-therapy drugs with immunomodulatory properties to improve cancer immunotherapy [4]. But, these drugs might be very costly and will not be available for every patient. Hence, immunomodulation with the help of phytochemicals found in plants can be an alternative. Drugs made using these phytochemicals may be cheaper and may not have any kind of side effects after consumption, as they are from natural source. The term "immunomodulation" means alteration of immune response which may increase or decrease the immune responsiveness. If there is increase or stimulation in immune response then it is termed as immunostimulation. If there is decrease or reduction in immune response then it is termed as immunosuppression. This stimulation and suppression can be caused by use of immunomodulators [5].

Natural plants have been used to prevent and treat various diseases for many years. There are many bioactive compounds that are found in plants which have been confirmed for their anticancer activities. These compounds present in plants are called as Phytochemicals. These phytochemicals not only act as anticancer agent, but also act as chemopreventive agent [6]. Gingerol is an active compound which is present in *Zingiberofficinale R.* (ginger). It has been studied that it has anticancer activities against many tumours in colon, breast, ovarian and pancreas. It has capacity to decrease iNOS and TNF-alpha expression via suppression ofIκBα phosphorylation and NF-κB nuclear translocation. However, the effects of gingerol on metastatic processes in breast cancer cells are not currently well known [7]. Lycopene is bright red pigment and phytochemical from *Solanumlycopersicum L.* (tomato), it has antioxidant activity and chemopreventive effects. The FDA also found no credible evidence for an association between tomato consumption and a reduced risk of lung, colorectal, breast, cervical, or endometrial cancer [8]. *OcimumtenuiflorumL.* (Holy Basil) is native to tropical Asia and though relatively new to western medicine systems, has been grown and used medicinally in India for over 3000 years. Also known as 'Tulsi', it is one of the most sacred plants in India & it has the status of deity on the Indian subcontinent. It has anticancer activity and to reduce negative effects of radiation exposure. Phytochemicals like eugenol, apigenin, myretenal, rosmarinic acid, luteolin, beta-sitosterol, carnosic acid, bornyl-acetate, cirsilineol, cineole, etc. are present in Holy basil [9].

This research article is mainly based on in-silico evaluation of immunomodulatory effect of phytochemicals of *Zingiberofficinale R., Solanumlycopersicum L.,*and*OcimumtenuiflorumL.*on the cytokines NFκB and TNF-α which plays pivotal role in immune response against breast cancer.

## II. Materials and Methods

### *1) Target Selection and Refinement*

NFkB (PDB ID: 1NFK (Musmusculus)), BLAST was performed which showed 98% identity with human NFkB. 1NFK consists of four chains (chain A, chain B, cahin C, chain D), active site (LYS 153) was present in chain A. Hence, chain B, chain C and chain D were deleted using Discovery Studio 4.0. TNF-alpha (PDB ID: 5MU8 (Homo sapiens)), was selected. Active sites and ligands were determined using Discovery Studio 4.0. Since, active sites were present in chain A, B, C, all other chains were deleted. Energy minimization of 1NFK (chain A) and TNF/5MU8 (chain A,B,C) was carried out using SPDBV 4.10 and Chiron server and was validated using RAMPAGE server.

### *2) Selection of Phytochemicals (ligands).*

Phytochemicals of *Zingiberofficinale R.* (gingerol,6-gingerol,7-gingerol,8-gingerol,9-gingerol, 10-gingerol, myrtenal), *Solanumlycopersicum L.* (lycopene,7 cis lycopene, 11 cis lycopene*),Ocimumtenuiflorum* L. (rosmarinic acid, luteolin, beta-sitosterol, carnosic acid, (Z)-alpha-bisabolene, bornyl-acetate, campesterol, humulene, carvacrol, cirsineole, cirsilineol, cirsinimaritin, germacrene-D),were downloaded from PubChem in 2D-SDF format.

### *3) ADME/T Properties*

Lipinski's properties like molecular weight, hydrogen bond donors and acceptors, logP value for all the active phytochemicals were calculated and they were screened on the basis of these properties. Sixteen phytochemicals (ligands) out of twenty-three satisfied Lipinski's rule of five. The toxicity of the phytochemicals which satisfied the Lipinski properties was calculated by using the toxicity prediction server, PROTOX.

### *4) Docking & Visualization*

The 16 phytochemicals were then docked with the two target protein receptors (NF-κB and TNF-alpha). The docking was done using AutoDockVina 1.5.6.Visualization of docking between target protein receptor and phytochemicals (ligands) was carried using AutoDockVina 1.5.6 and DiscoveryStudio 4.0.

## III. Result and Discussions

### *1) Prediction of active site of target receptor*

The active sites for energy minimized structure of NF-κB was predicted using Discovery Studio 4.0 and active site of TNF-alpha was predicted using COACH server.

### *2) Phytochemicals (ligand) Selection*

Lipinski's Rule of five, i.e. molecular weight, hydrogen bind donors and acceptors, logP value and molar refractivity, of all the phytochemicals are tabulated in Table 1.

**Table 1:** Lipinski properties of phytochemicals

| Sr. No. | Phytochemicals | Mol. Wt. (<500Da) | H2 donor (<5) | H2 acceptor (<10) | LogP value (<5) | Molar refractivity (40-130) |
|---|---|---|---|---|---|---|
| 1 | Gingerol | 290 | 1 | 4 | 3.192689 | 78.516289 |
| 2 | 6-gingerol | 296 | 1 | 4 | 4.094189 | 85.139282 |
| 3 | 7 cis lycopene | 536 | 0 | 0 | 9.345745 | 179.517242 |
| 4 | 7 gingerol | 302 | 1 | 4 | 3.371569 | 81.137283 |
| 5 | 8 gingerol | 322 | 1 | 4 | 4.578789 | 92.417282 |
| 6 | 9 gingerol | 334 | 1 | 4 | 4.719659 | 95.699280 |
| 7 | 10 gingerol | 348 | 1 | 4 | 5.052890 | 99.750275 |
| 8 | 11 cis lycopene | 534 | 0 | 0 | 8.908154 | 176.659241 |
| 9 | Lycopene | 534 | 0 | 0 | 8.530483 | 174.284241 |
| 10 | Beta sitosterol | 432 | 1 | 1 | - | - |
| 11 | Carnosic acid | 350 | 1 | 4 | 6.281102 | 118.235275 |
| 12 | Luteolin | 286 | 0 | 6 | 1.558560 | 62.666996 |
| 13 | Myrtenal | 158 | 1 | 1 | 2.374330 | 55.256786 |
| 14 | Rosmarinic acid | 357 | 0 | 8 | 2.048820 | 77.936996 |
| 15 | (Z)alphabisabolene | 206 | 0 | 0 | 4.098168 | 75.111984 |
| 16 | Bornylacetate | 198 | 0 | 2 | 2.962829 | 60.277985 |
| 17 | Campesterol | 420 | 1 | 1 | - | - |
| 18 | Humulene | 202 | 0 | 0 | 3.430889 | 68.966980 |
| 19 | Carvacrol | 154 | 0 | 1 | 2.845099 | 52.472488 |
| 20 | Cineole | 150 | 0 | 1 | 1.558260 | 43.835495 |
| 21 | Cirsilineol | 344. | 0 | 7 | 2.352930 | 79.370491 |
| 22 | Cirsimaritin | 313 | 0 | 6 | 1.853950 | 71.547997 |
| 23 | Germacrene-D | 212 | 0 | 0 | - | - |

Out of 23 phytochemicals, 14 satisfied Lipinski's properties, of which toxicity prediction was done using PROTOX server, tabulated in Table 2.

**Table 2:** Toxicity prediction of phytochemicals

| Sr. No. | Phytochemicals | LD50 value mg/kg body wt. | Toxicity class | Toxic fragment formation | Remark |
|---|---|---|---|---|---|
| 1 | Gingerol | 294.39 | 3 | None | Non-toxic |
| 2 | 6-gingerol | 294.39 | 3 | None | Non-toxic |
| 3 | 7 gingerol | 308.41 | 3 | None | Non-toxic |
| 4 | 8 gingerol | 322.44 | 3 | None | Non-toxic |
| 5 | 9 gingerol | 336.47 | 3 | None | Non-toxic |
| 6 | 10 gingerol | 350.49 | 3 | None | Non-toxic |
| 7 | Luteolin | 286.24 | 5 | None | Non-toxic |
| 8 | Myrtenal | 150.22 | 5 | None | Non-toxic |
| 9 | Rosmarinic acid | 357.00 | 5 | None | Non-toxic |
| 10 | Bornylacetate | 196.29 | 5 | None | Non-toxic |
| 11 | Carvacrol | 150.22 | 4 | None | Non-toxic |
| 12 | Cineole | 154.25 | 5 | None | Non-toxic |
| 13 | Cirsilineol | 344.32 | 5 | None | Non-toxic |
| 14 | Cirsimaritin | 314.29 | 5 | None | Non-toxic |

- Class I: fatal if swallowed (LD50 ≤ 5 mg/kg)
- Class II: fatal if swallowed (5 < LD50 ≤ 50 mg/kg)
- Class III: toxic if swallowed (50 < LD50 ≤ 300 mg/kg)
- Class IV: harmful if swallowed (300 < LD50 ≤ 2000 mg/kg)
- Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000 mg/kg)
- Class VI: non-toxic (LD50 > 5000 mg/kg)

### *3) Docking of NF-κB (1NFK) and TNF-alpha (5MU8) with sixteen phytochemicals*

Docking of NF-κB and TNF-alpha were performed with the selected ligands. The final docked conformations obtained for the different ligands were evaluated based on the docking score and the amino acid interacted, as given in Table 3.

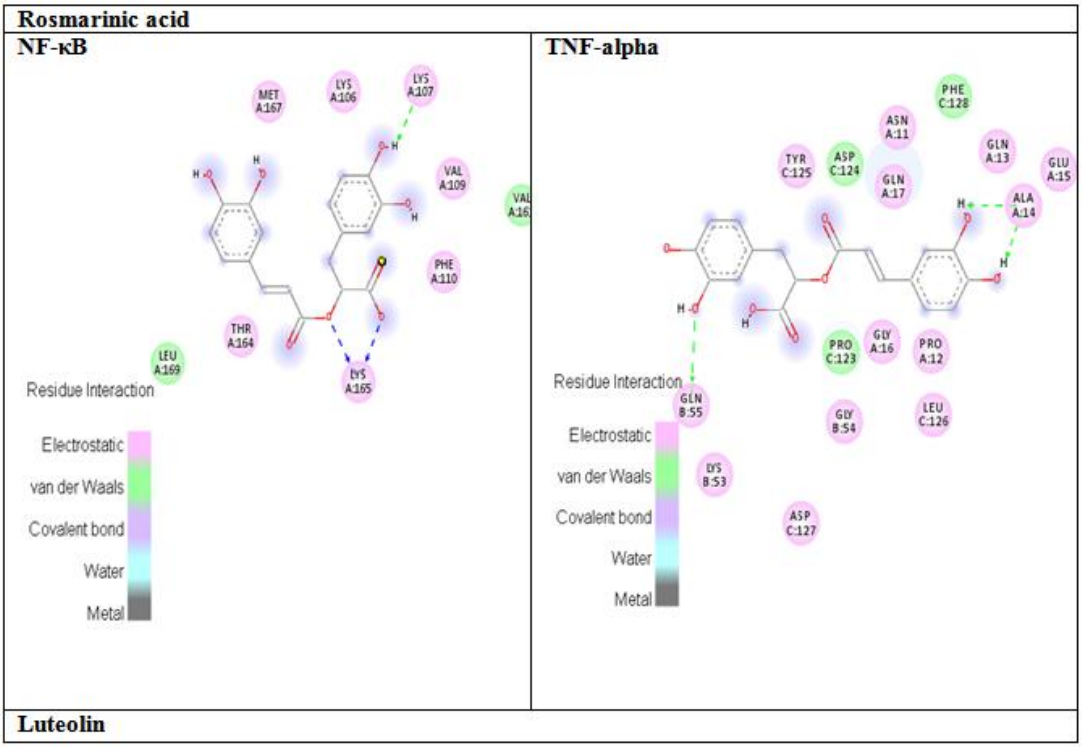
**Table 3:** Docking Results of target receptor and phytochemicals

| Sr. No. | Phytochemicals | Amino acids interacting | | Docking Score(KJ/mol) | |
|---|---|---|---|---|---|
| | | NF-κB | TNF-alpha | NF-κB | TNF-alpha |
| 1 | Gingerol | LYS(A:153),GLU(A:114), THR(A:118),GLU(A:154), ALA(A:115),ARG(A:157), GLU(A:111) | PHE(C:136),VAL(A:8), ASN(B:34),LEU(B:36), LEU(C:116),GLN(B:35), TRP(A:20),ALA(C:118), GLY(C:137),TYR(C:135), HIS(A:7),ALA(A:6),ILE(C:138), SER(B:48), LEU(B:31) | -5.2 | -6.6 |
| 2 | 6-gingerol | ARG(A:146),GLN(A:147), CYS(A:121),ALA(A:136), GLY(A:144),TYR(A:137), LEU(A:138),GLY(A:124), ASP(A:145) | PHE(C:136),VAL(A:8), ASN(B:34),LEU(B:36), LEU(C:116),GLN(B:35),TRP(A:20),ALA(C:118), GLY(C:137),TYR(C:135), HIS(A:7),ALA(A:6),ILE(C:138), SER(B:48), LEU(B:31) | -5.4 | -6.6 |
| 3 | 7 gingerol | ARG(A:146),GLN(A:147), LEU(A:138), ALA(A:136),GLY(A:144), CYS(A:121),TYR(A:137), GLN(A:139),GLY(A:124), ASP(A:145) | TYR(C:135),PHE(C:136), VAL(A:8),VAL(C:134), HIS(A:7),ALA(A:10), GLN(B:49),VAL(B:50), GLY(C:137),SER(B:48), LEU(B:36), ALA(C:118), TRP(A:20), GLN(C:133), VAL(A:9) | -5.5 | -6.7 |
| 4 | 8 gingerol | ALA(A:136),LEU(A:138), GLN(A:147),ARG(A:146), GLY(A:124),CYS(A:121), TYR(A:137),GLN(A:139), GLY(A:144) ASP(A:145) | VAL(C:134), VAL(A:8), PHE(C:136),GLY(C:137), VAL(B:50), ALA(A:10), GLN(B:49), TYR(C:135), HIS(A:7),GLN(C:133), TRP(A:20),LEU(B:36),ALA(C:118),SER(B:48),VAL(A:9) | -5.5 | -6.1 |
| 5 | 9 gingerol | GLN(A:147),ARG(A:146), ALA(A:136), CYC(A:121) TYR(A:137),GLN(A:199), LEU(A:138),GLY(A:124), ASP(A:145), GLY(A:144) | ASN(B:34), VAL(A:8), PHE(C:136), LEU(B:36), LEU(C:116), TRP(A:20), ALA(C:118), GLY(C:137), HIS(A:7), TYR(C:135), GLN(B:35), LEU(B:31), GLN(B:49), SER(B:48), ALA(A:6), ILE(C:138) | -5.5 | -6.1 |
| 6 | 10 gingerol | ALA(A:136),TYR(A:137), CYS(A:121),GLU(A:152), GLN(A:147),ARG(A:146), LEU(A:138),GLY(A:124), GLY(A:144), ASP(A:145), LEU(A:148) | TYR(C:135),PHE(C:136), VAL(A:8), HIS(A:7), ILE(C:138), GLY(C:137), SER(B:48), LEU(B:31), LEU(B:36), ALA(A:6), GLN(B:35), ASN(B:34), TRP(A:20), ALA(C:118), LEU(C:116)TYR(B:47) | -5.4 | -6.0 |
| 7 | Luteolin | ARG(A:146),GLN(A:147), GLU(A:152),CYS(A:121), GLY(A:144),TYR(A:137), GLN(A:139),ALA(A:136), LEU(A:138),GLY(A:124), ASP(A:145), LEU(A:148) | ASN(A:11), PRO(C:123), ARG(C:122), GLN(A:19), GLN(A:17), LEU(A:18), PRO(A:12), ALA(A:10), LEU(C:126), VAL(B:50), PHE(B:52),ILE(C:120), PHE(C:128) | -8.3 | -8.0 |
| 8 | Myrtenal | ARG(A:146),TYR(A:137), ALA(A:136),LEU(A:138), ASP(A:145), GLY(A:144) | VAL(A:8), PHE(C:136), HIS(A:7), GLY(C:137), ALA(A:6), LEU(B:36), TYR(C:135), TYR(B:47), ILE(C:138), SER(B:48), ALA(C:118),TRP(A:20) | -4.7 | -4.7 |
| 9 | Rosmarinic acid | LEU(A:129), SER(A:133), HIS(A:67), GLN(A:158), GLN(A:163), GLY(A128), VAL(A:131), HIS(A:132), GLN(A:162), ALA(A:159) | GLY(C:132), ALA(A:10), ALA(A:14), GLN(A:17), GLY(A:16), GLN(C:133), VAL(C:134), ALA(C:129), PRO(A:12), PHE(C:128), VAL(A:9), ASN(A:11), LEU(C:126) | -8.4 | -8.4 |
| 10 | Bornylacetate | LYS(A:165),THR(A:164), PHE(A:110),ARG(A:157), | TYR(C:135),PHE(C:136), GLN(B:49),VAL(C:134) | -5.3 | -6.2 |

| | | | | | |
|---|---|---|---|---|---|
| | | VAL(A:161) | VAL(A:8), HIS(A:7), HIS(B:66), TYR(B:47), SER(B:48), TRP(A:20), GLY(C:137), VAL(A:9) | | |
| 11 | Carvacrol | ARG(A:157),LYS(A:153), GLU(A:114),GLU(A:154), ILE(A:156), THR(A:118) | TYR(C:135), VAL(A:8), PHE(C:136),GLY(C:137), LEU(B:36), SER(B:48), TRP(A:20), GLN(B:49), ALA(C:118), TYR(B:47) | -5.1 | -6.3 |
| 12 | Cineole | THR(A:118),LYS(A:153), GLU(A:114), ILE(A:156), ARG(A:157) | TRP(A:20), GLY(C:137), SER(B:48), HIS(B:66), GLN(B:49), TYR(C:135), LEU(B:36), TYR(B:47), ALA(C:118),PHE(C:136), VAL(A:8) | -4.9 | -5.4 |
| 13 | Cirsilineol | LYS(A:153), THR(A:118), ALA(A:115),ARG(A:157), GLU(A:114), GLU(A:154) | GLN(A:19), ASN(A:11), GLN(A:17), GLU(A:15) ALA(A:14), GLN(A:13) | -6.3 | -6.8 |
| 14 | Cirsimaritin | GLN(A:139),LEU(A:138), GLU(A:152),ALA(A:136), ASP(A:145), ARG(A146), GLN(A:147),THR(A:149), TYR(A:137),CYS(A:121), GLY(A:144), GLY(A:124) | GLN(A:19), ASN(A:11), GLN(A:17), GLU(A:15), ALA(A:14), GLN(A:13) | | |

Docking score is a measure of interaction of ligand to the active site of the target. Maximum non covalent interaction was shown by Rosmarinic acid and Luteolin for both the protein target receptors, showed in Figure 1.

**Fig. 1:** Visualization of Docking using Discovery Studio 4.0




## IV. Conclusion

Out of various phytochemicals studied from *Zingiberofficinale R. andOcimumtenuiflorum L.*. Rosmarinic-acid and Luteolin were active phytochemicals. Both showed maximum non covalent interaction for both NF-κB and TNF-alpha. The binding energy values for rosmarinic-acid and cytokines were -8.4 kcal/mol and luteolin and the cytokines were -8.0 kcal/mol and -8.3 kcal/mol respectively. These cytokines play a major role in development and progression of breast cancer. NF-κB is a major cell survival signal and is anti-apoptotic, which helps in survival of the cancer cells. TNF can either stimulate or suppress cancer cells. Therefore interaction of these phytochemicals with cytokines will exploit further opportunity of immunomodulation which

could act as supportive therapy along with anticancer therapy. Whereas, the phytochemicals are natural products from plants, they may have no side-effects and are cheaply available depending on the source of plant henceforth could be a alternative approach for enhancement of better lifestyle of patient care. Further QSAR and molecular dynamic simulation studies will be needed to get better confirmation of these results.

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