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# MOLECULAR DOCKING STUDIES OF PHYTOCHEMICALS OF BASIL AGAINST SIRT2 AS AN ANTI-BREAST CANCER

**Nancy Bhura[1], Jeena Gupta[2] and Pawan Gupta[3*]**

[1]Department of Molecular Biology and Genetic Engineering, school of Bioengineering and Biosciences, Lovely Professional University, Phagwara-144411, Punjab, India

[2]Department of Biochemistry, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara-144411, Punjab, India

[3]Department of Research and Development, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara-144411, Punjab, India

*Address for correspondence: pawan.20270@lpu.co.in

## Abstract

Breast cancer is one of the most prominent types of cancer found in women, reported to have second highest number of deaths after lung cancer. One of the causes reported are improper regulation of epigenetic modifications. Sirtuin2 (SIRT2, a class3 histone deacetylase) regulates many types of diseases, including breast cancer, cellular stress, survival and damage responses. Basil is known to possess anticancer properties; however, the role of its phytochemicals against different epigenetic targets is still not very clear. We have screened various phytochemicals (mostly enriched in essential oil) of basil to define their role in breast cancer using molecular docking studies. These studies were against epigenetic target SIRT2. The results helped us to find out best candidates Methyl Cinnamate and Eucalyptol having binding energies -5.98 Kcal/Mol and -6.06 Kcal/Mol respectively for binding in the active pocket of SIRT2. The results of these studies help to understand the underlying mechanism for protective effects of basil phytochemicals against breast cancer.

***Keywords*:** Epigenetics, Breast Cancer, Basil Phytochemicals, Molecular Docking

## Introduction

Breast cancer (BC) is the biggest cancer that is leading cause of death in women in less developed countries and second in developed countries after lung cancer (Torre *et al*., 2015). The main cause of this cancer is still unknown but most of the researchers are targeting genetic and epigenetic modification as the real cause (Paul *et al*., 2018). According to ACS (American Cancer Society), the incidence of BC has decreased by 39% from 1989 to 2015 but it still remains elevated in certain races due to lack of better healthcare facilities (DeSantis *et al*., 2017). Epigenetic changes are the ones which are heritable, affects phenotype of the individual without changing their DNA sequence. They are seen as major imprint for the world of BC, with special focus on DNA methylation and histone deacetylation. One of the target which helps in cancer prevention is bioactive dietary compounds by altering abnormal epigenetic conformations in cancer (Hardy and Tollefsbol, 2011; Herceg, 2007). In recent years epigenetic modifications based research has become topic of wide interest for scientist.

Basil is an herb commonly grown in homes in Asian countries due to its religious and therapeutic aspect. Basil possesses high therapeutic value in terms of prevention, treatment and curing of diseases. Cancer is most prominent type of disease all over the world. Therefore its cure with less of toxicity is required. Anticancer activity of *Ocimum basilicum* is reported by Qamara and his group. The methanolic extract of this plant was found to have potential anti-proliferative activity against MCF cell lines (human BC cells). Urosolic acid present in the plant is responsible for accumulation of F-actin and distortion of mitotic spindle indicating that they can cure estrogen receptor positive BC (Arshad Qamar *et al*., 2010). Many people have memory issue like loss of memory due to any accident or illness. *Ocimum bacilicum* has high potential to be used as anti-amnesic drug and the main reason behind its potential is its antioxidant property and acetylcholinesterase inhibitory activities. Thus *Ocimum bacilicum* can be used as drug for curing memory related issues (Singh, 1999).

There are many benefits of this plant, yet double-blinded clinical trials using animal models as well as cell culture studies are required to test it protective effects. Basil like tulsi are present in abundance, leading to its reduced cost of production and can prove to be a potential drug candidate in future (Baliga *et al*., 2013). Essential oil of *Ocimum basilicum* has been reported to have *in vitro* anticancer activity which is rich in methyl cinnamate. The essential oil like geraniol also have anti- tumour effects without affecting physiology of normal cell (ChO *et al*., 2016). Since nerol is cis-isomer of geraniol (trans-isomer), so, it might also possess similar properties of geraniol. The phytochemicals like orientin is reported to have anti-invasive effects in BC (Kim *et al*., 2018). However, vicenin is not yet been reported for its role in BC, though it inhibits Wnt/β-catenin signalling and induces apoptosis colorectal cancer cell line (Yang *et al*., 2018). Presence of eucalyptol could be the reason behind cytotoxic activity of essential Oil of Egyptian *Cinnamomum glanduliferum* Bark. It is due to the fact the eucalyptol is present in highest concentration (Taha and Eldahshan, 2017). Eugenol down regulates the E2F1/survivin pathway which in turn triggers apoptosis in BC cells (Al-Sharif *et al*., 2013). Linalool exhibits cytotoxic effects by activating antitumor immunity (Chang and Shen, 2014). All these essential oils have been directly and indirectly involved in cancer but not reported against BC. So, in silico studies have been designed to screen these molecules against BC target SIRT2. These are class 3 HDAC inhibitors responsible for various functions like cell cycle, tumorigenesis, metabolism, cancer etc. literature data suggests that SIRT2 is involved in regulation of BC. It can reduce as well as enhance tumorigenesis based on the grade of BC (McGlynn *et al*., 2014). So this drug target SIRT2 is selected for our studies (Nguyen *et al*., 2013).

In this paper, molecular docking studies are performed to screen the different phytochemicals enriched in basil essential oils against SIRT2.

## Materials and Methods

### Dataset

All molecular structures of phytochemicals enriched in basil essential oil like geraniol, methyl cinnamate (da Costa *et al*., 2015; de Aquino *et al*., 2010) eucalyptol, eugenol, linalool (Feriotto *et al*., 2018) and nerol (ÖZCAN *et al*., 2002), orientin and vicenin (Rao and Baliga, 2014) were downloaded using ZINC database (Irwin and Shoichet, 2005) and was converted to relevant format using Open Babel[23]. Structures of these phytochemicals are shown in Figure: 1. We have performed molecular docking studies using the SIRT2 in AutoDockTools 4.2.6[24]. Each docked pose has different minimum energy, H-bonding & inhibition constant (IC) to give the best confirmation.

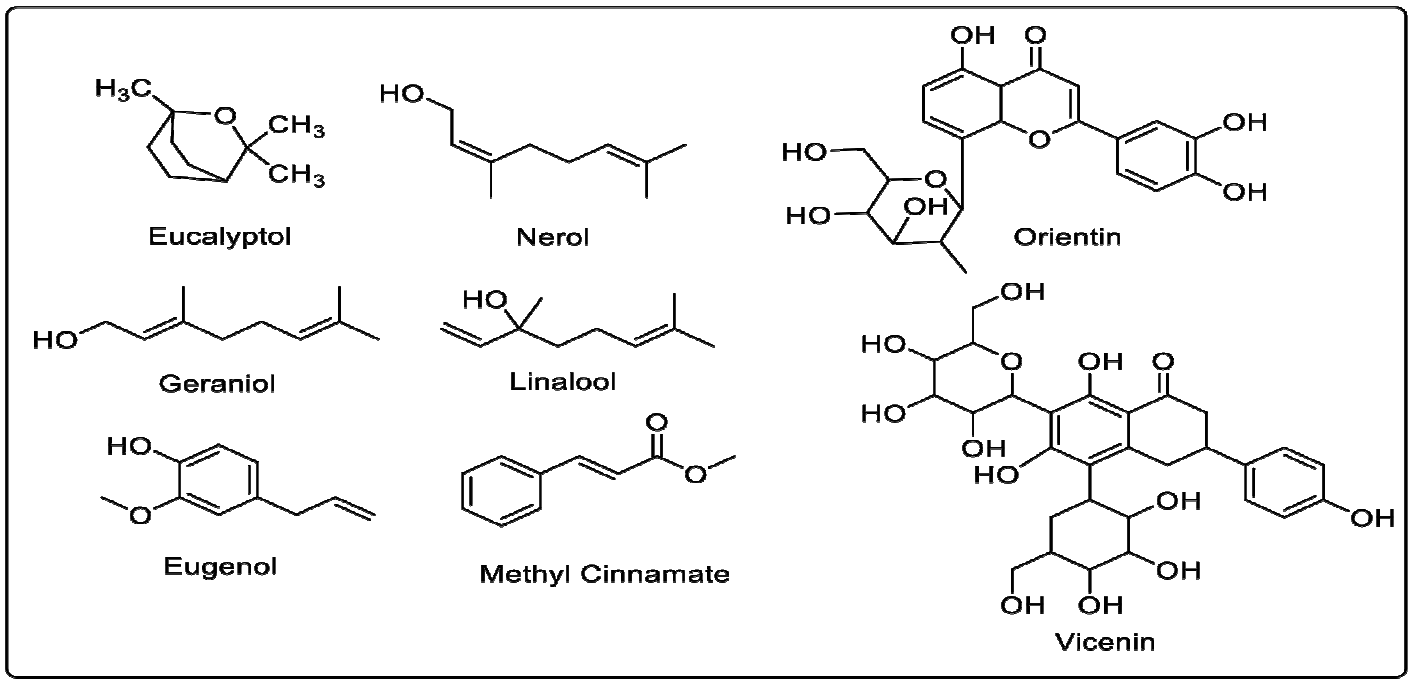


**Fig. 1 :** Chemical structure of phytochemicals of basil used in molecular docking as a ligand.

### Molecular docking

Docking is a method which predicts the preferred orientation and binding of a small ligand and a large protein molecule (O'Boyle *et al*., 2011). The selected specific target protein was extracted from Protein Data Bank (PDB). It was preferentially based upon best resolution and minimum number of missing atoms. Based upon above mentioned parameters we have found PDB ID: 4Y6Q (Feldman *et al*., 2015). Ligand was extracted from protein structure by deleting other amino acid residues and metal ions. This protein was prepared and validated using AutoDock Tools 4.2.6. All the parameters were accepted as defaults and 30 confirmations were taken into consideration to select the best match. Docking was done using rigid docking and they were analysed based on best overlapping between our ligands and co-crystallized ligand to validate the active site. Results will be analysed based on X-RAY data retrieved from PDB.

## Results

Two most important aspect of any docking is proper modelling of structure and prediction of accurate activity (Igci *et al*., 2016; Kitchen *et al*., 2004). Each ligand was compared against the PDB ID-4Y6Q protein and results were summarized into the Table: 1 and in the Figures: 2– 10. Four factors for analysis was taken into consideration: 1) location of docked molecule (near or distant from reference molecule), 2) relevant H-bond interaction, 3) IC (concentration of an inhibition for production of maximum half inhibition) - lower the better, H bonding (more number, more advantage) and 4) binding energy (lower the better) (Gupta *et al*., 2011).

**Table 1 :** Comparison of docking results of essential oils of basil with SIRT2

| LIGAND | Inhibition Constant IC (µM) | H Bonding | Binding energy (Kcal/Mol) | Pi-Pi interactions | Figure |
|---|---|---|---|---|---|
| **X-Ray** | 28.52 | **ARG97 SER263** | -6.20 | No | Figure: 2 & 3 |
| **Eucalyptol** | **41.16** | **SER263** | **-5.98** | **No** | **Figure: 4** |
| **Eugenol** | 79.21 | SER263 GLN265 | -5.61 | No | Figure: 5 |
| **Geraniol** | 83.34 | THR262 GLN167 | -5.56 | No | Figure: 6 |
| **Linalool** | 377.67 | SER263 | -4.67 | No | Figure: 7 |
| **Methyl Cinnamate** | **36.44** | **ARG97** | **-6.06** | **Yes** | **Figure: 8** |
| **Nerol** | 96.68 | THR262 GLN265 | -5.48 | No | Figure: 9 |
| **Orientin** | 850.52 | ILE169 ARG97 | -8.28 | Yes | Figure: 10 |
| **Vicenin** | -- | -- | +1.27 | No | -- |

In the figure: 2, the cavity highlights the active site of SIRT2. It is this position where cocrystallized ligand were bound. We have compared phytochemicals with that co-crystallized ligand and to see whether our phytochemicals are similar to the structure of X-Ray bound ligand. It is illustrated in Figure: 2 - 10, that our phytochemicals are present in the position of the X-Ray ligand i.e. in the cavity of SIRT2 active site (Figure: 3 red colour compound is x-ray ligand).

Furthermore, these figures also emphasize on H-bonding residues ARG97 and SER263.

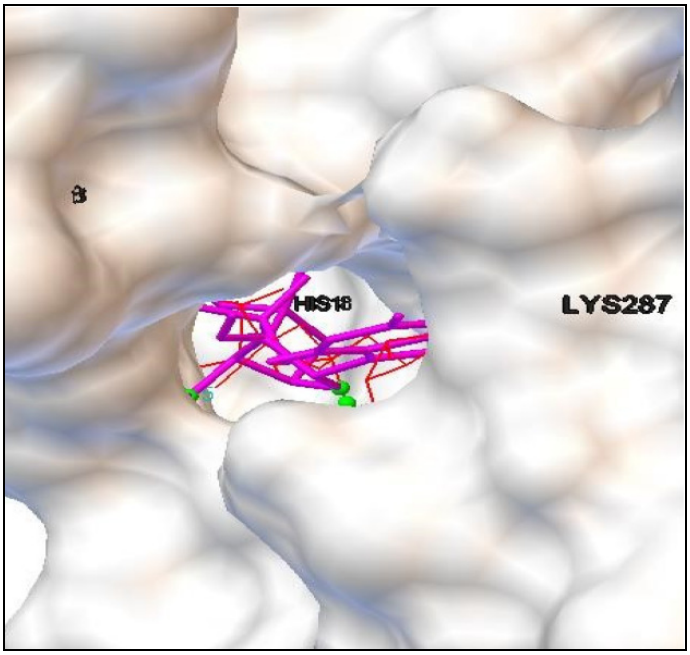


**Fig. 2 :** Cavity model having extracted ligand (Magenta sticks) as well as co-crystallized ligand OMR (red line) in the active binding pocket of protein (SIRT2).

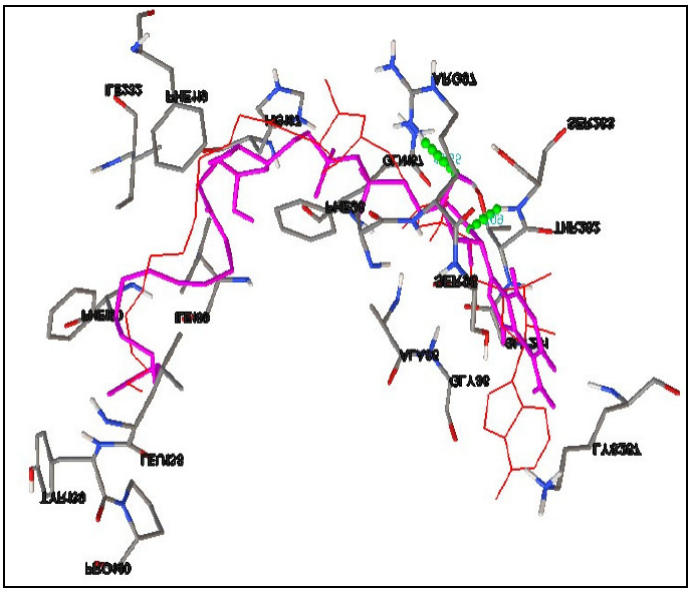

**Fig. 3 :** Sticks model of SIRT2 of extracted ligand (Magenta sticks) as well as co-crystallized ligand OMR (red line) in the active binding pocket of protein (SIRT2).

Figure: 2 depicts binding and overlapping of extracted ligand (Magenta sticks) as well as cocrystallized ligand-OMR (red lines) in the active binding pocket of SIRT2. The green dots represent H bonding with ARG97 and SER263 residues. This shows that our extracted ligand is of correct confirmation and the same is depicted in Figure: 3. This extracted ligand is used as reference molecule (red colour) for other phytochemicals. As binding of other phytochemicals over the X-Ray ligand OMR indicates that they are binding into same active site pocket of SIRT2. The extracted ligand possessed low IC i.e. 28.52 µM and low energy i.e. -6.20 Kcal/Mol.

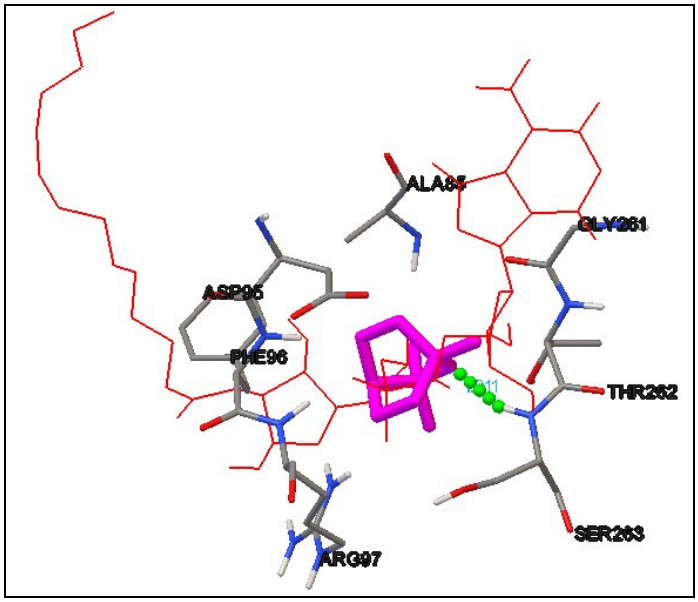


**Fig. 4 :** Eucalyptol (Magenta sticks) interactions with SIRT2 active site (OMR in red line).

Figure: 4 shows binding of eucalyptol into the active site of SIRT2. It also shows H bonding with SER263 residue. It is having IC 41.16 µM and binding energy 5.98 Kcal/Mol which is found to be similar with the reference (-6.20 kcal/Mol). This is showing slightly higher IC as compared to the reference molecule (28.52 µM).

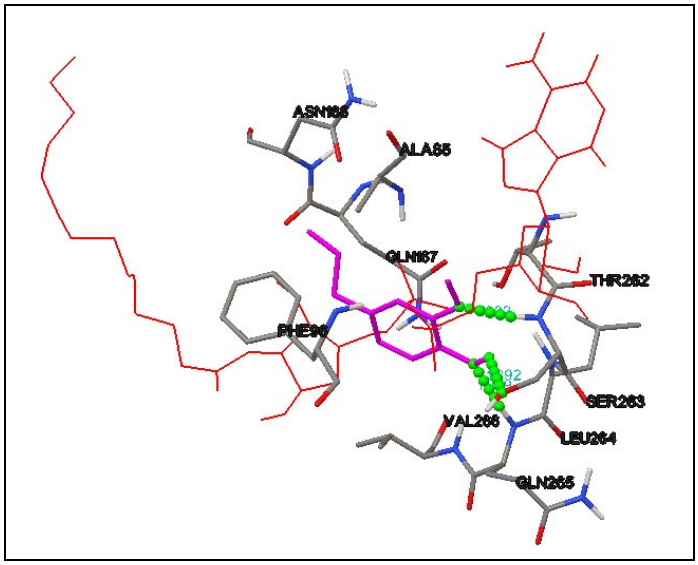


**Fig. 5:** Eugenol (Magenta sticks) interactions with SIRT2 active site (OMR in red line)

Figure: 5 shows binding of eugenol with SIRT2 and it is making H bonding with two residues SER263 and GLN265. It is having IC 79.21 µM and binding energy -5.61 Kcal/Mol. This is showing low energy score and high IC as compared to the reference molecule (-6.20 Kcal/Mol and 28.52 µM, respectively).

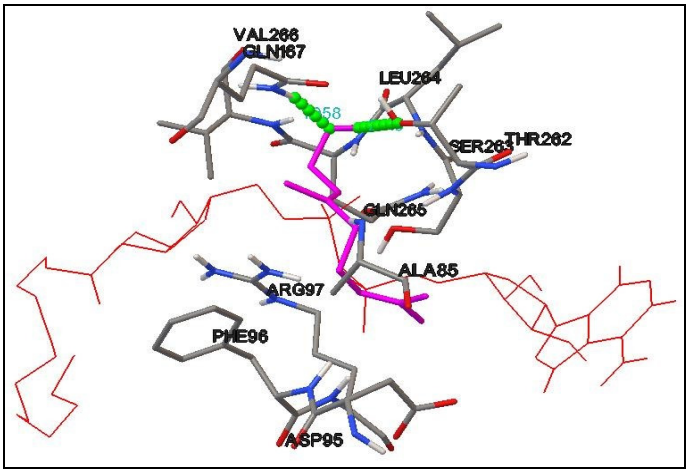


**Fig. 6 :** Geraniol (Magenta sticks) interactions with SIRT2 active site (OMR in red line)

Figure: 6 shows binding of geraniol with SIRT2. It is making H bonding with two residues GLN167 and THR262. It is having IC 83.34 µM and binding energy -5.56 Kcal/Mol. This is showing low energy score and high IC as compared to the reference molecule (-6.20 Kcal/Mol and 28.52 µM, respectively).

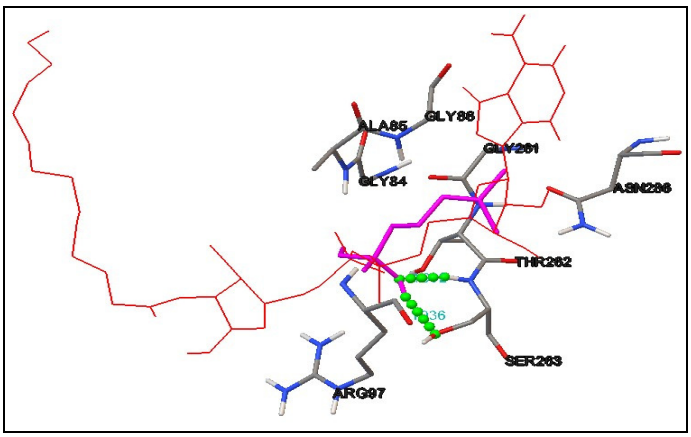


**Fig. 7 :** Linalool (Magenta sticks) interactions with SIRT2 active site (OMR in red line).

Figure: 7 shows binding of Linalool with SIRT2. It is making H bonding with a residue SER263. It is having IC 377.67 µM and binding energy -4.67 Kcal/mol. This is showing low energy score and high IC as compared to the reference molecule (-6.20 Kcal/Mol and28.52 µM, respectively).

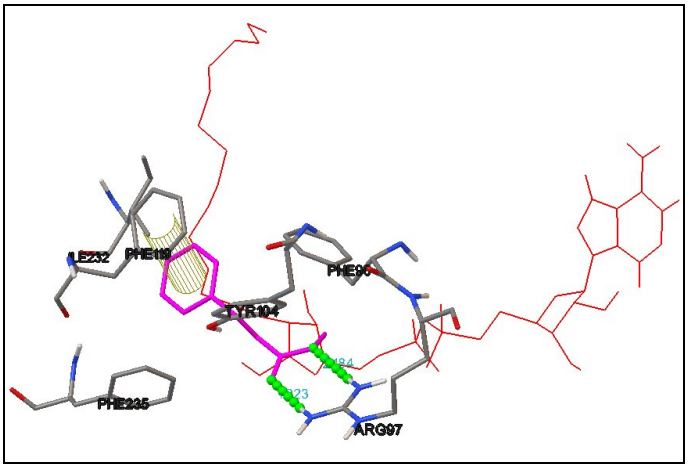


**Fig. 8 :** Methyl cinnamate (Magenta sticks) interactions with SIRT2 active site (OMR red line) with pi-pi interaction with the PHE119 (golden tube line).

Figure: 8 shows binding of methyl cinnamate with SIRT2. It is making H bonding with a residue ARG97. It is having IC 36.44 µM and binding energy -6.06 Kcal/mol which is found to be closed with the reference molecule. In addition, this phytochemical is also exhibiting the pi-pi interactions with PHE119.

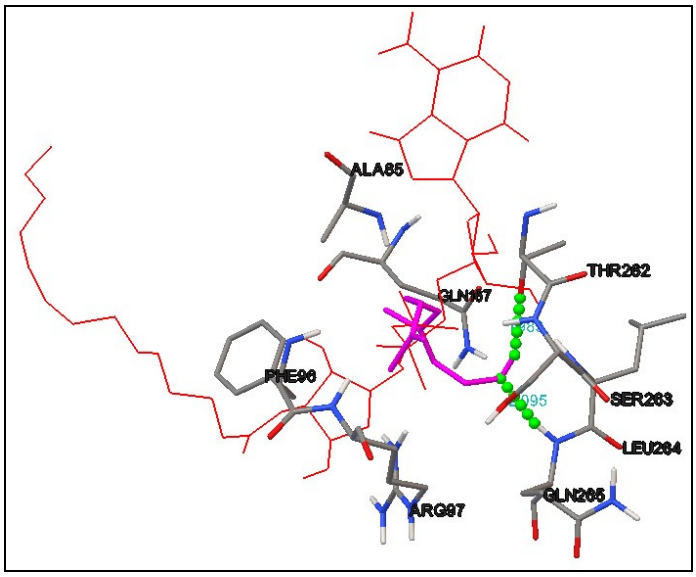


**Fig. 9 :** Nerol interactions (Magenta sticks) with SIRT2 molecule active site (OMR in red line).

Figure: 9 shows binding of nerol with SIRT2. It is making H bonding with a residue THR262 and GLN265residues. Nerol is showing IC 96.68 µM and binding energy -5.48 kcal/mol. It is having low energy score as compared to the reference (-6.06 Kcal/mol).

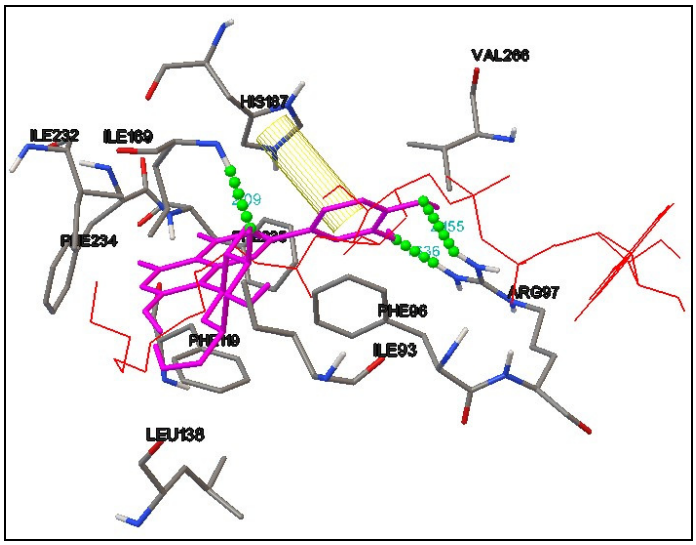


**Fig. 10 :** Orientin interactions (Magenta sticks) with SIRT2 molecule active site (OMR in red line), depicting t pi-pi interaction (golden lines).

Figure: 10 is showing binding of orientin with the SIRT2 and having H bonding with the residues ILE169 and ARG97.In addition, it also having the pi-pi interactions with the HIS187 (golden tube lines). This molecule possessed the lowest binding energy -8.28 Kcal/Mol as compared to reference and other phytochemicals. But it is having highest IC value among the other phytochemicals (IC 850.52 µM) which is indicating the low inhibition. Vicenin do possess inhibition constant and H bonding, additionally binding energy is highest in this case +1.27 Kcal/Mol. Hence no figure shown for this molecule.

## Discussion

As per the above results analysis, it was found that Nerol and Geraniol is having the same binding interactions (THR262), same IC values (96.68 and 83.34, respectively) and same binding energies (-5.48 and -5.56 Kcal/Mol, respectively). However, both are isomers. These molecules are having the IC values high as compared to X-ray ligand. However, other phytochemicals (Eucalyptol, Linalool, Methyl cinnamate, Eugenol) also exhibiting the good binding interactions and energy as compared to x-ray ligand (see Table: 1). It was found that Eucalyptol and Methyl cinnamate are having the lowest IC as compared to other phytochemicals in the Table: 1 and almost similar to X-ray ligand. Moreover, they are also having the lower binding energy as compared to the other phytochemicals in Table: 1 (almost same with the X-Ray ligand) and lowest binding energy is of orientin (even lower than X-Ray itself -8.28 Kcal/Mol), however, orientin is having highest value of IC as compared to other phytochemicals in the Table 1. So overall results of these studies indicate that both Eucalyptol and Methyl cinnamate are having good binding potential against SIRT2.

## Conclusions

A molecular docking analysis of 8 basil -derived phytochemicals 1) eucalyptol 2) eugenol 3) linalool 4) geraniol 5) methyl cinnamate 6) nerol 7) orientin 8) vicenin was done with SIRT2 to screen the best candidate against BC using Autodock. Tools 4.2.6.The analysis based on above mentioned parameters (location, H-Bonding, IC and binding energy) has revealed 7 compounds showing good interactions with target protein SIRT2 (we discard vicenin as target against BC). Amongst them 3 phytochemicals namely eucalyptol, methylcinnamate and orientin had excellent binding energy. Also, methylcinnamate and orientin depicted pi-pi interactions. However, orientin also showed high IC (which should be lowest preferably). Hence, we propose that eucalyptol and methylcinnamate possess potential to be used against BC. These results and the current study underscore the importance of phytochemicals from higher plants like basil in drug discovery and may provide potential avenues for development of chemotherapeutic agents to be used as anti-BC candidate. Further molecular dynamics is required to validate the same.

### Author contributions

Ms. Nancy Bhura had performed *in silico* studies and wrote manuscript. Dr. Jeena Gupta had edited the manuscript. Dr. Pawan Gupta had designed and monitored the studies.

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