Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
www.phytojournal.com
JPP 2025; 14(1): 107-115
Received: 15-11-2024
Accepted: 18-12-2024

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# In-silico studies on phytochemical, eugenol from clove buds

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**DOI:** https://doi.org/10.22271/phyto.2025.v14.i1b.15226

**Abstract**
P53 is tumor suppressor gene. The P53 protein act as transcriptional factor. In human's dimer of P53 (8F2H) is responsible for various cancer. This study evaluates in vitro and in silico study of a phytochemical eugenol obtained from clove buds. Computational docking was used to predict conformations and free binding energy. For this ligand eugenol is taken as ligand and docked with P53 dimer protein (8F2H). In silico studies were done on different conformers of ligand eugenol and receptor 8F2H protein in which minimum binding free energy was obtained to be -4.32 kcal/Mole. This study reveals the pharmacokinetics properties and Lipinski rule of five, hydrogen bond was also obtained between them. Docking results for eugenol suggests that it may be promising towards as anticancer drug candidate.

**Keywords:** Phytochemical, black cumin, docking, eugenol, anti-cancer, ligand - Protein interactions, binding affinity, antimicrobial.

## Introduction

### Global Scenario of Breast Cancer

**Global scenario of tumours:** The global scenario of tumours, or cancer, is complex and constantly evolving due to various factors such as advancements in research, changes in lifestyle, and improvements in detection and treatment methods. However, some key aspects of the global tumour's scenario include:

**Incidence and Prevalence:** Cancer is a significant global health challenge, with millions of new cases diagnosed each year worldwide. The incidence and prevalence of different types of tumours vary across regions and populations, influenced by factors such as age, genetics, lifestyle choices, environmental exposures, and access to healthcare [1].

**Leading Types of Cancer:** The most common types of tumours globally include lung cancer, breast cancer, colorectal cancer, prostate cancer, and stomach cancer. However, the prevalence of specific tumour types can vary by region and demographic factors [2].

**Risk Factors:** Certain risk factors increase the likelihood of developing tumours, including tobacco use, unhealthy diet, physical inactivity, excessive alcohol consumption, exposure to carcinogens as asbestos and ultraviolet radiation, genetic predisposition, and infections as human papillomavirus and hepatitis B and C viruses [3].

**Screening and Early Detection:** Early detection of tumours through screening programs and diagnostic tests can significantly improve outcomes by enabling timely intervention and treatment. Screening methods vary depending on the type of tumor but may include imaging tests, laboratory tests as blood tests and tumour markers, and invasive procedures as biopsies [4].

**Treatment Modalities:** Treatment options for tumors depend on various factors, including tumor type, stage, location, and individual patient characteristics. Common treatment modalities include surgery, chemotherapy, radiation therapy, targeted therapy, immunotherapy, hormone therapy, and stem cell transplantation. Personalized or precision medicine approaches are increasingly being used to tailor treatment plans to individual patients based on their genetic makeup and other factors [5].

Survival Rates: Survival rates for tumors have improved over the years due to advances in early detection, diagnosis, and treatment. However, survival outcomes still vary widely depending on factors such as tumor type, stage at diagnosis, access to healthcare, and socioeconomic status [6].

**Global Health Initiatives:** Various global health organizations, including the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC), are actively involved in cancer control efforts, including prevention, early detection, treatment, and palliative care. These organizations work with governments, healthcare providers, researchers, and advocacy groups to address the growing burden of tumours worldwide [7].
Overall, while significant progress has been made in the fight against tumours, challenges remain in terms of improving access to quality healthcare services, reducing risk factors, addressing disparities in cancer care, and development of more effective prevention rather than treatment strategies.

**Cancer:** Cancer can develop when there is a disruption in the normal regulation of cell growth. This can occur due to various factors, including genetic mutations, exposure to certain carcinogens (such as tobacco smoke or ultraviolet radiation), certain infections, hormonal imbalances, and lifestyle factors like poor diet and lack of physical activity. Considering ancient type cancer was not known as a widespread disease but now cancer has become a very serious issue and we should really try to find a good solution for it. Now if we think about what the reason for the cancer will be to grow so strongly and so much. We all know that smoking is the main cause of the cancer and also air pollution. We know that how cancer spread moving through the well of nearly lymph Node or blood vessels. Basically, another way we can say that cancer means unwanted growth of Cell. This study enlightens in the method to prevent cancer. It can reduce this growing cancer in our own way in order to stop this growing cancer we are dealing with this growing problem in horrible medicine then we found many compounds in horrible medicine that could be effective against cancer but we decide to follow a procedure to get a proper information for this we have studied some research paper Then and know that it may should analytics activity for liver stomach and uterus. Then we realize that Stone flower is one of the best medicinal plants that show helping properties Also stone Flower shows antimicrobial plant activity. A considerable number of species of lichen forming fungi have wide geographical distribution and are used in their traditional system of medicine. Lichen is symbiotic combination of algae and fungi. They are well known to produce a variety of compound with remarkable biological activities. The beautiful hills up Uttarakhand (in India) are the best source of lichen due to the varied temperature difference in day and night. They have been used in diarrhoea, dyspepsia, spermatorrhea, amenorrhea, dysentery, and as wound healer. Some species up the lichen is being used to cosmetic industries as skin lightening agent. Cancer is a global health concern affecting people of all ages, races, and socioeconomic backgrounds. The World Health Organization (WHO) estimates that cancer is one of the leading causes of morbidity and mortality worldwide. Addressing cancer requires a comprehensive approach involving prevention, early detection, treatment, and supportive care. International efforts focus on raising awareness, improving access to cancer care, strengthening healthcare systems, and implementing strategies to reduce the global burden of cancer [8].

**Tumour**
Tumors can develop in various types of cancer cells, leading to different manifestations and treatments. Here's some general information about tumors in various types of cancer cells [9].

**Breast Cancer:** Tumours can form in the breast tissue, either as non-invasive (ductal carcinoma in situ) or invasive types (infiltrating ductal carcinoma). Treatment typically involves surgery, chemotherapy, radiation therapy, hormone therapy, or targeted therapy, depending on the stage and type of cancer [10].

**Lung Cancer:** Tumors can develop in the lungs, categorized as small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC). Treatment options include surgery, chemotherapy, radiation therapy, targeted therapy, immunotherapy, or a combination of these, depending on the stage and type of cancer [11].

**Prostate Cancer:** Tumours may arise in the prostate gland, often detected through screening tests like PSA (Prostate-Specific Antigen) tests. Treatment options range from active surveillance to surgery, radiation therapy, hormone therapy, chemotherapy, or newer treatments like immunotherapy or targeted therapy [12].

**Colon Cancer:** Tumors can form in the colon or rectum, typically starting as polyps and progressing to cancerous growths. Treatment involves surgery to remove the tumor, often followed by chemotherapy, radiation therapy, targeted therapy, or immunotherapy, depending on the stage and spread of the cancer [13].

**Skin Cancer:** Tumours can develop in the skin, including basal cell carcinoma, squamous cell carcinoma, and melanoma. Treatment options vary depending on the type and stage of skin cancer but may include surgery, radiation therapy, chemotherapy, immunotherapy, or targeted therapy [14].

**Brain Cancer:** Tumours can form in the brain, such as gliomas, meningiomas, or metastatic brain tumors originating from other parts of the body. Treatment may involve surgery, radiation therapy, chemotherapy, targeted therapy, or a combination, depending on the type and location of the tumour [15].

**Leukemia:** Cancer of the blood and bone marrow, where tumors do not form solid masses but rather abnormal white blood cells multiply uncontrollably. Treatment includes chemotherapy, targeted therapy, radiation therapy, immunotherapy, and stem cell transplantation [16].

**Lymphoma:** Cancer of the lymphatic system, where tumours develop in lymph nodes or lymphoid tissues. Treatment of this was depending on the type of lymphoma and its stage but may have inclusion of therapies as chemotherapy, radiation therapy, immunotherapy and stem cell transplantation [17].

## Materials and Methods

### Plant Part Selected: Clove Buds

The clove plant belongs to the family Myrtaceae and are commonly known as Kalonji in India, which is part of the botanical family Ranunculaceae. Other names for clove buds include Lavang in Marathi, Loung in Hindi, Lavamga in Sanskrit, Kirampuvitaika in Tamil, Lavanga in Bengali, and Lavang in Gujarati.

**Scientific Classification**
**Scientific Name:** Syzygium aromaticum
**Family:** Myrtaceae
**Order:** Myrtales
**Division:** Magnoliophyta (flowering plants)
**Kingdom:** Plantae

Cloves (Syzygium aromaticum) are the aromatic flower buds of a tree. They are commercially harvested in Indonesia, India, Sri Lanka, and various African countries. The essential oils present in cloves include eugenol, caryophyllene, alpha-humulene, and pinene. Eugenol is the primary component, constituting 81.1% of the oil. It possesses antioxidant, anti-inflammatory, and anticancer properties. Eugenol (4-allyl-2-methoxy phenol) is a musky oil primarily obtained from cloves. The antimicrobial activities of clove extracts (eugenol) have been found to be significantly effective against Staphylococcus aureus, Klebsiella pneumoniae, and Candida albicans. Many species of clove (Syzygium aromaticum) exhibit strong antimicrobial activity.

The plant material, black cumin seeds, was collected from a shop, and the authenticity of the black cumin seeds was confirmed by the Vivekananda Microbiology Lab at Vivekananda College, Kolhapur. The dried black cumin seeds were then homogenized into a fine powder using a mortar and pestle [18].

**Fig 1:** Collection of clove buds

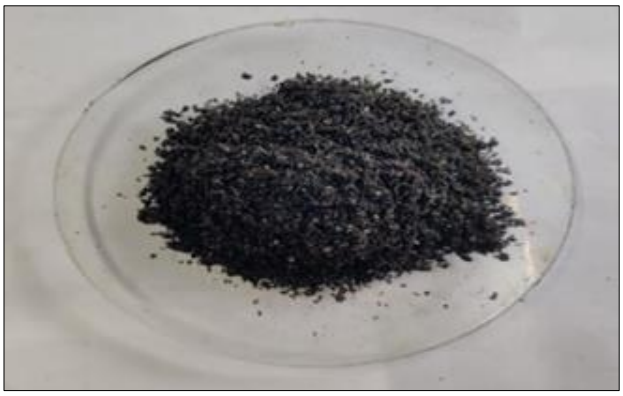


**Fig 2:** Black clove powder

**Extract preparation:** Powder plant material was subjected to successive solvent extract using solvent like methanol, ethanol. 20g of powdered clove buds' sample were added to 200 ml of suitable solvent and the crude was extracted after 48 hrs. The extract was filtered and evaporated. Phytochemical analyses were carried out from the crude.

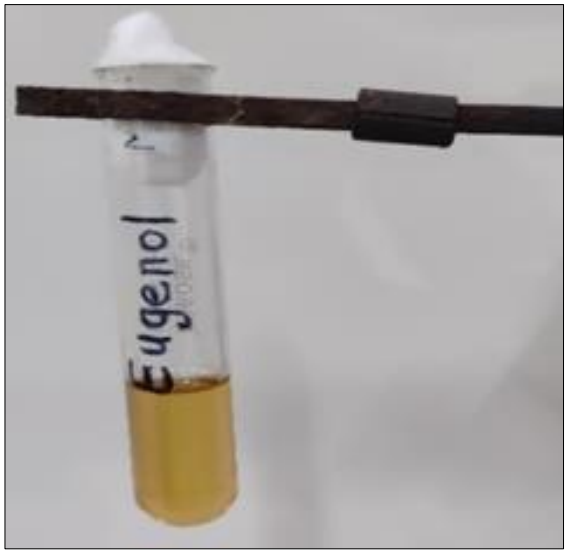



**Fig 3:** Eugenol

**Phytochemical Analysis (Test for extract)**

This analysis helps in identifying plants with potential medicinal properties by detecting the presence of various bioactive compounds like alkaloids, flavonoids, terpenoids, etc. These compounds can be further investigated for their therapeutic potential. Phytochemical analysis can be used to classify plants based on their chemical constituents, providing valuable information for plant systematics and evolution. Also, these phytochemicals are responsible for the color, flavours, and aromas of plants, and they play a crucial role in plant defence mechanisms.

Out of thousands of phytochemicals it is important to undergo the test of various phytochemical analysis with certain reagents. These tests include alkaloid, carbohydrates, reducing sugar, flavonoids, glycosides, phenolic compounds, saponin, steroids, amino acids and tannins.

**Test for Alkaloids:** Alkaloids are nitrogen containing phytochemicals whose extracts were tested by mixing it with dilute hydrochloric acid and following tested as follows:

**Mayer's Test:** were treated with Mayer's Reagent (Mercuric chloride & Potassium iodide). Formation of a cream indicates the presence of alkaloids.

**Wagner's Test:** Extracts were treated with Wagner's Reagent (Iodine & Potassium iodide). Formation of a reddish-brown precipitate indicates the presence of alkaloids

**Test for Flavonoids:** Aqueous extracts were treated with dilute ammonia and conc. Sulphuric acid was added. Yellow colouration indicates the presence of flavonoids.

**Test for Terpenoids:** To the extracts 2ml of chloroform was added, conc. Sulphuric acid was carefully added to form a layer. Reddish brown coloration at the interface or junction of two layers indicates the presence of terpenoids.

**Test for Steroids:** Extracts were mixed with 2ml of chloroform, 2 ml of conc. sulphuric acid and 2ml of acetic acid were poured into the mixture. Development of greenish colouration at the junction of two layers indicates the presence of steroids

**Test for Phenols:** Ferric chloride Test: Extracts were treated with 3 to 4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol [19].

**Test for Glycosides**

**Libermann Burchard's Test:** Extracts were combined with two milliliters each of acetic acid and chloroform. The mixture was cooled on ice before being treated with strong sulfuric acid. The presence of glycosides is confirmed by a change in hue from violet to blue to green.

**Salkowski's Test:** Two milliliters of chloroform were mixed with the extracts. After carefully adding concentrated sulfuric acid, the mixture was gently shaken. The presence of a steroidal ring is indicated by the development of a reddish-brown coloring.

**Keller-Kilani Test:** Two milliliters of glacial acetic acid containing one or two drops of ferric chloride solution were added to the extracts. After that, the liquid was cautiously transferred into a second test tube that included two milliliters of sulfuric acid concentration. The formation of a brown ring at the interface signifies the presence of cardiac glycosides.

**Test for Protein: Ninhydrin Test:** Two milliliters of ninhydrin solution were added to the extracts and heated. The presence of proteins is indicated by a violet appearance.

**Xanthoproteic test:** A few drops of concentrated nitric acid were added to the extracts. The development of a yellow hue signifies the existence of proteins [20].

**Test for Carbohydrates**

**Benedict's solution:** Two milliliters of Benedict's reagent were added to the extracts, and they were then slowly heated. When reducing sugar is present, an orange-red precipitate forms.9.

**Fehling's Test**

2 ml of the extract was added, and it was gently heated after an equal volume of Fehling A and Fehling B reagents had been combined.

**Iodine Test**

Extracts were combined with two milliliters of iodine solution; the presence of carbohydrates is indicated by a dark blue or purple hue [21].

**Test for Tannins**

Extracts were boiled with 10 ml of water in test tubes. A few drops of ferric chloride were added and observed for blue black coloration.

**Test for Saponins**

Extracts were boiled with distilled water. The solution was shaken vigorously for a stable, persistent, froth or foam [22].

**Table 1.** Phytochemical Screening of Eugenol [22]

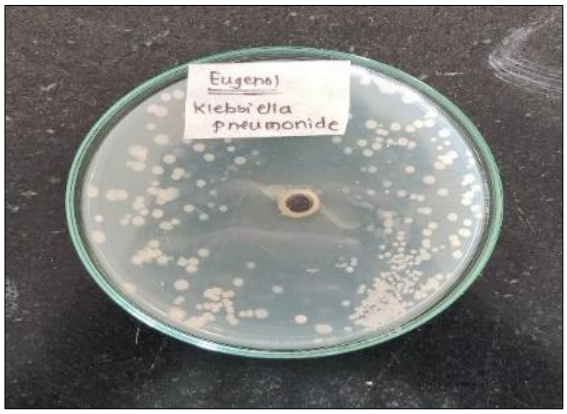
| Sr. No | Tests | Methanol Extract |
|---|---|---|
| 1 | Alkaloids | |
| a) | Hager's Test | +ve |
| b) | Wagner's Test | -ve |
| 2 | Flavonoids Test | +ve |
| 3 | Carbohydrate Test | +ve |
| 4 | Steroids Test | -ve |
| 5 | Phenols Test | +ve |
| 6 | Glycosides | |
| a) | Libermans Test | -ve |
| b) | Salkowskis Test | -ve |
| c) | Keller Kilanis Test | -ve |
| 7 | Proteins | |
| a) | Ninhydrin Test | +ve |

***In vitro* studies:** Eugenol was subjected to in vitro analysis taking various microorganisms. Inhibition activity of different microorganisms against the selected phytochemical was studied and their results were obtained. The microorganisms taken for the test were Pseudomonas aeruginosa, klebsiella pneumoniae, and candida albicans [23].

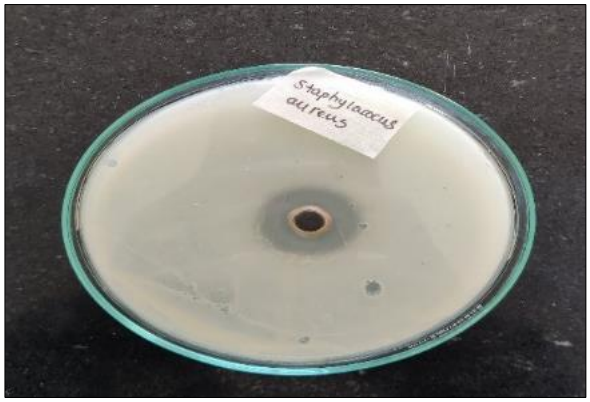
**Method: Agar well Diffusion Method**: The agar well diffusion method is a valuable tool in microbiology and pharmacology for assessing antimicrobial activity. Its simplicity, cost-effectiveness, and ability to provide clear visual results make it a preferred choice in many research settings.

**Preparation:** The agar medium (commonly Mueller-Hinton agar) is prepared and inoculated with a bacterial suspension using a lawn culture technique to ensure uniform coverage.

**Wells Creation:** Wells are made in the agar using a sterile pipette tip, typically measuring 6-8 mm in diameter. Further addition of Antimicrobial Agent i.e. Eugenol extract was added to each well, usually up to 100 µL which was further allowed to incubate. The plates are incubated at 35-37°C for 18-24 hours. After incubation, the zones of inhibition are measured. The size of these zones indicates the effectiveness of the antimicrobial agent against the tested microorganism [24].



Klebsiella pneumonia



Staphylococcus aureus

Candida albicans

**Fig 4:** In Vitro Analysis

**In silico studies**

**General Methodology:** Molecular docking is a computational technique used in drug discovery and molecular biology to predict the binding affinity and orientation of a small molecule (ligand) within a target protein or other macromolecule (receptor). It helps in understanding the interaction between the ligand and receptor at the molecular level, which is crucial for designing new drugs or optimizing existing ones [25].

The process of molecular docking typically involves the following steps:

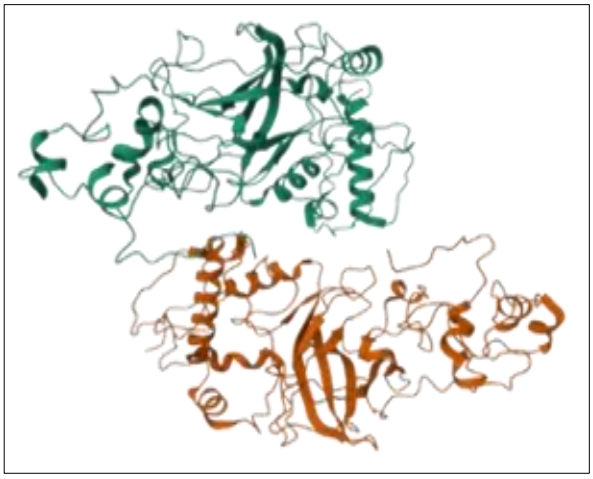
**Preparation of ligand and receptor:** The ligand and receptor structures are prepared by removing any unwanted molecules or solvent molecules and optimizing the structures using molecular modelling software. The ligand may be a small molecule drug candidate or a library of compounds, while the receptor is usually a protein structure derived from experimental techniques like X-ray crystallography or NMR spectroscopy.

**Grid generation:** The search space for the ligand is represented by a three-dimensional grid created around the receptor. The ligand's possible binding locations and orientations within the receptor are assessed using this grid.

**Scoring function:** To calculate the ligand-receptor binding affinity, a scoring function is constructed. The projected strength of the interaction is represented by a numerical number that is determined by the scoring algorithm. There are many different types of scoring functions, from force-field-based scoring functions to empirical scoring systems that employ basic physics-based terminology.

**Ligand docking:** By experimenting with various positions and orientations, the ligand is methodically docked into the binding site of the receptor. Docking methods examine the conformational space using a variety of search strategies, including Monte Carlo simulations and genetic algorithms, to determine the most favorable binding pose. The scoring function is used to evaluate the fitness of each docked pose.

**Analysis and visualization:** Based on their anticipated binding affinities, the produced docking postures are examined and graded. Understanding the ligand-receptor interaction and recognizing important chemical interactions, such as hydrogen bonds, hydrophobic interactions, or electrostatic interactions, are made easier by visualizing the docked poses.


3D Structure of 8F2H Protein

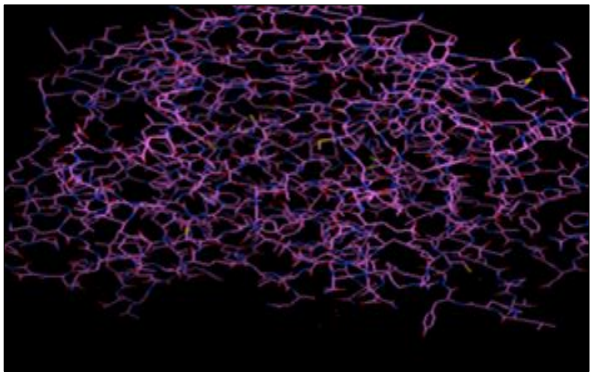

3D structure of 8F2H protein

**Fig 5:** 3D and 2D Structure of protein

**Validation and refinement:** Experimental approaches such as biochemical assays or structure determination techniques like X-ray crystallography or NMR spectroscopy are used to further validate and refine docking results. This aids in confirming the anticipated binding positions and refining the ligand's structure to improve binding specificity and affinity.

Because it sheds light on how tiny compounds connect to their target proteins, molecular docking is a useful technique in drug discovery. It can be used for virtual screening of large compound libraries to identify potential drug candidates, lead optimization to improve the binding affinity of existing compounds, and understanding the binding modes of ligands to aid in the design of novel drug molecules [26].

The ligand Eugenol was downloaded from PubChem website which was 3D structure in SDF format.

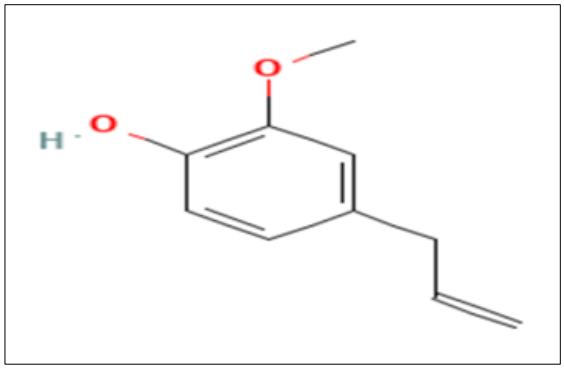
Preparation of binding site of target protein 8F2H.

Firstly, mgl tools latest version 1.5.7, secondly auto dock software windows version tool was downloaded.
The protein molecule was downloaded from the NCBI website which is protein 8F2H in PDB format and its 3D structure was taken for docking. 3-D structures of target P53 DIMER protein is selected from online protein data files.

**Docking**
Firstly, open auto dock software, then open protein molecule in PDB file next set auto dock. The other steps were to edit and delete water, further add hydrogen polar and non-polar only, next is go to the hydrogen and click merge non-polar hydrogen and compute gastiger charges which are noted for further reference. This way receptor enzyme protein was selected and downloaded.
Secondly the 3-D structure of 8F2H contains was checked for missing bonds as well as atoms. It was seen that enzyme contain a lot of water molecules, which were removed and broken bond among molecule due error in X-ray Diffraction were identified. This was done because during docking, ligands may interact with the free charges on the protein. Addition of hydrogen is required for satisfying the valance atom. It was done add the time of binding site preparation.
The above method was applied on 8F2H enzyme and verified and then added Kolmann charges which were found to -15.04, and saved in the form of PDB format. Then total net charge of the protein was found to be zero.
For next further step different software's that is Autodock, Openbabel, Pymol, chemdraw were used for further the analysis.
The reason for selection of docking software's like AutoDock and OpenBabel is because of its specific functionalities that caters the needs of molecular docking studies. AutoDock's robust algorithms and scoring functions make it suitable for accurate binding predictions, while OpenBabel's versatility in file handling and molecular preparation enhances overall research efficiency. Together, both of them provides a comprehensive toolkit in structure-based drug design and molecular interaction studies [27].

**The steps further involved were**
Go to ligand click on torsion tree and detect route then choose the torsion numbers and then, active torsion numbers then go to grid and generate the grid box and adjust the ligand and protein set under grid box and save it. the parameter is x-dimension: 126, y-dimension: 112, z-dimension: 88 and the spacing is 0.553 Click on docking and then ligand Eugenol and search parameters, click on accept and, output then Lamarckian and save it in dpf file and then run auto grid 4 after browse gpf file then glg file automatically generate after that click launch and glg file automatically save and then generate map file check the glg file and then go to auto dock and browse dpf file and dlg file was automatically created then launch and dlg file open then and their results are obtained. Last step was to select the desired natural product as an inhibitor (Ligand). This natural product was to be known as pharmacophores were used for drug designing.
The natural products are selected from different Phytochemical from different plants can be taken as Ligand. This study was done by taking phenol-based ligand and 8F2H enzyme as protein receptor below [28].

**Basis of selection of Phenol based ligandlike. (Eugenol)**
Eugenol has antimicrobial properties and has been used traditionally in folk medicine for its medicinal properties. It exhibits inhibitory effects against a wide range of microorganisms, including bacteria, fungi, and viruses. Due to its antimicrobial activity, eugenol has been investigated for various applications, including as an antibiotic, antifungal, antiviral, and anticancer agent.
This phenol-based ligand eugenol has is also used in the cosmetic industry due to its potential as well as a natural preservative and antimicrobial agent in personal care products. It is known to inhibit the growth of bacteria and fungi that can cause skin infections and spoil cosmetic formulations.



2D Structure of Eugenol

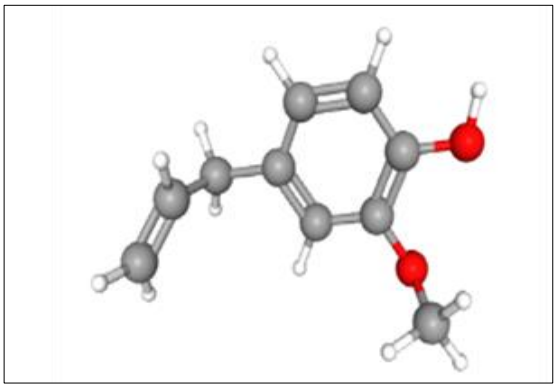

3D Structure of Eugenol

**Fig 6:** 2D and 3D Structure of Eugenol

**Results and Discussion**
**Spectral analysis:** The C-13 NMR spectra of Eugenol was obtained from chemDraw software and Screening results of H1-NMR are as follows:

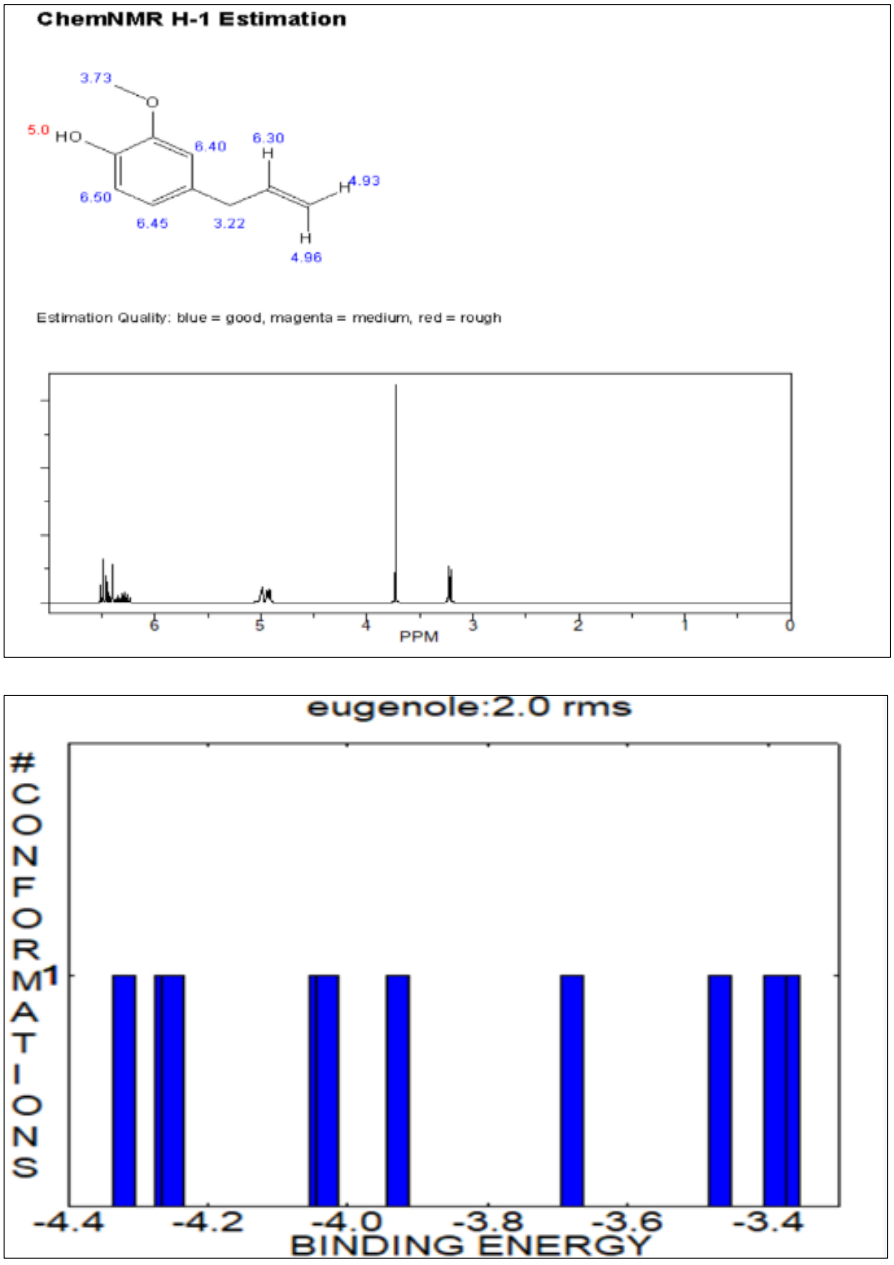



**Fig 7:** Graphical presentation conformer poses of ligand Eugenol and receptor 8F2H

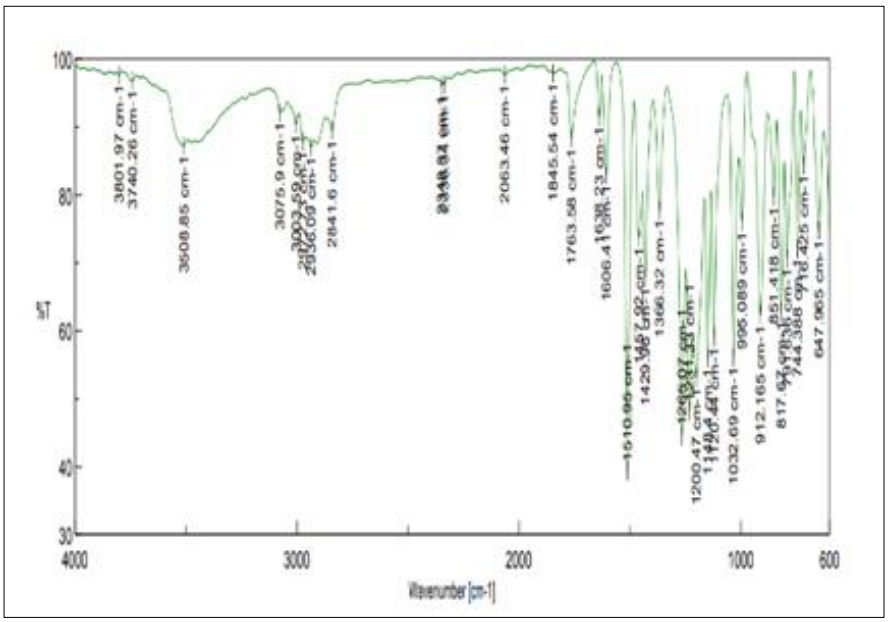



**Fig 8.** IR Spectra of Eugenol

### *In vitro* results

### Minimum Inhibitory Concentration (MIC)

The MIC is the lowest concentration of a substance that prevents visible growth of a microorganism. Studies have shown that the MIC of eugenol varies depending on the microorganism being tested. For example, the MIC of eugenol against Escherichia coli has been reported to be as low as 0.125 µg/mL [29].

### Minimum Bactericidal Concentration (MBC)

The MBC is the lowest concentration of a substance that kills a microorganism. Studies have shown that the MBC of eugenol is typically higher than the MIC. For example, the MBC of eugenol against E. coli has been reported to be 0.250 µg/m [30]. In vitro study was done and observations were found are given in the table 2. This eugenol containing sample showed sample showed antimicrobial activity against klebsiella pneumoniae, staphylococcus aureus and candida albicans as compared to klebsiella pneumoniae and Escherichia-coli.

**Table 2.** *In vitro* studies of various microorganisms

| Sr.no | Microorganism | Control Zone of sample(mm) |
|---|---|---|
| 1 | Klebsiella pneumoniae | 33 |
| 2 | Staphylococcus aureus | 21 |
| 3 | Candida albicans | 42 |

**In silico results:** The Eugenol molecule is studied in silico using auto dock software 4.0 windows. It is reported that the Eugenol is successfully docked with the enzyme 8F2H and it gave 4.32Kcal/mole binding energy. This value indicates that eugenol can be a drug candidate for the P53dimer based inhibition. Run was done for above ligand Eugenol with 8F2H in Auto dock and the detailed docking images are explained in fig 10-14. In various graphical formats the depict the packing factor and inhibition of eugenol with 8F2H.

**Table 3.** In silico studies showing binding energy in Kcal/mole in different poses

| Cluster Rank | Binding Energy | Run Conformer no. | Cluster RMSD | Reference RMSD |
|---|---|---|---|---|
| 1 | -4.32 | 10 | 0.00 | 37.18 |
| 1 | -4.26 | 4 | 0.00 | 27.68 |
| 1 | -4.25 | 8 | 0.00 | 10.93 |
| 1 | -4.04 | 6 | 0.00 | 27.90 |
| 1 | -4.03 | 2 | 0.00 | 31.95 |
| 1 | -3.93 | 5 | 0.00 | 36.82 |
| 1 | -3.68 | 1 | 0.00 | 33.89 |
| 1 | -3.47 | 9 | 0.00 | 34.44 |
| 1 | -3.39 | 3 | 0.00 | 25.57 |
| 1 | -3.37 | 7 | 0.00 | 23.85 |

Figs 9 to fig 12 explains wire frame structures and depict the interaction between the enzyme amino acids and ligand groups. It also shows the number of hydrogen bonds, amino acids involved in the hydrogen bonding and hydrogen bonding distance is as follows. Out of 10 conformers, the 10th conformer showed least binding free energy which is -4.32 Kcal/mol.

### Conclusions and Discussions

This research evaluates the results of in vitro and in silico study of selected ligand molecule which is a phytochemical named Eugenol, obtained from clove buds. 8F2H is responsible for tumor so in silico studies were done on different conformers of ligand eugenol and receptor 8F2H, a P53 dimer protein which gives binding free energy of -4.32Kcal/mole.

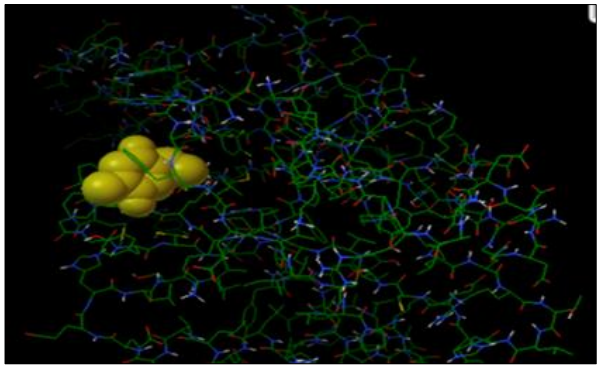


**Fig 9:** Best conformer poses of ligand Eugenol and receptor 8F2H

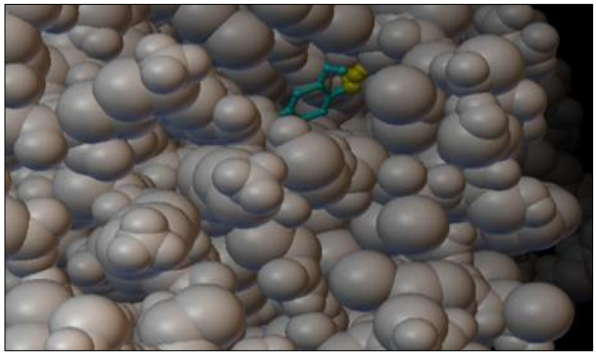


**Fig 10:** Best conformer poses of ligand Eugenol and receptor 8F2H with hydrogen bond in CPK mode

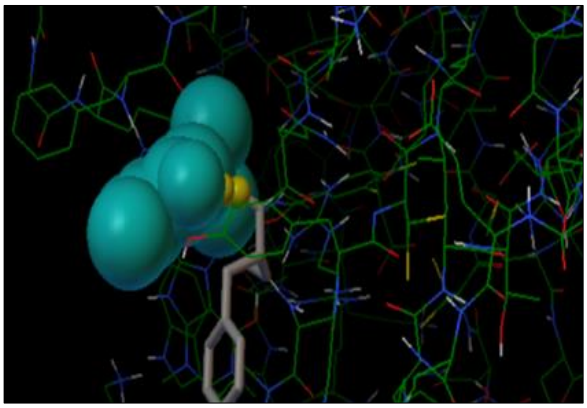


**Fig 11:** Best conformer pose of ligand Eugenol and receptor 8F2H with hydrogen bond

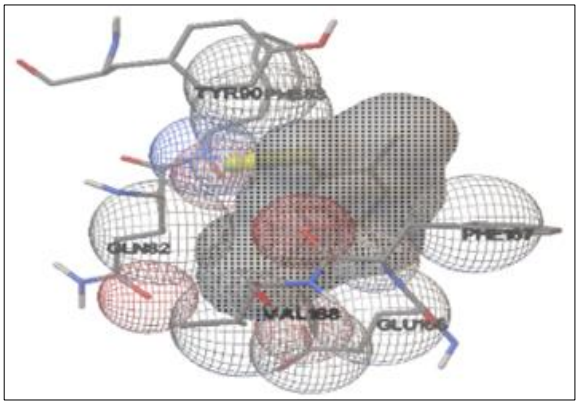



**Fig 12:** Best conformer poses of ligand Eugenol and receptor 8F2H with hydrogen bond showing amino acids

This binding energy indicates that it has moderate activity. To enhance anticancer potential, eugenol might require modifications to increase its binding affinity and selectivity [31].

Natural phytochemical Eugenol was extracted and its invitro activity was performed which gave significant antimicrobial activity with Klebsiella pneumoniae, Staphylococcus aureus and Candida albicans. In this microbial activity with Klebsiella shows more significantly. Computational docking study predicted binding for small molecule ligand Eugenol to macromolecular P53 DIMER proteins as target. Through this study it was revealed that the pharmacokinetic properties and Lipinski's rule of five. Hydrogen bond was formed between them.

**Discussions:** This research evaluated on docking study of 8F2H with Eugenol. This can be further docked with proteins of various skin diseases, Tumor.4.1 In-vitro conclusions the results of in-vitro are used to discuss the possibility of these extracted compounds as anti-cancer agents. Along with in-vitro, in-silico methods are used and obtained binding energies. The obtained results are discussed and compared with in-vitro results.

**In-silico conclusions:** In present study one phytochemical family i.e., Phenol based molecule are reported to be active and interacting with 8F2H the P53 dimer-based enzyme working in cell duplication cycle as a regulator.

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