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# GC- MS ANALYSIS OF PHYTOCONSTITUENTS OF *MICROCOCCUS MERCURIALIS* STEM PART AND *IN SILICO* DOCKING STUDY OF PHYTOL AND 4H-PYRAN-4-ONE, 2,3-DIHYDRO- 3,5-DIHYDROXY-6-METHYL AGAINST BREAST CANCER DRUG TARGETS (ESR-α AND TNF-α)

**THENDRAL HEPSIBHA. B * AND KAMIREDDY SHEERA REDDY**

Department of Biochemistry, Ethiraj College for Women, Chennai – 600 008, India

*Corresponding Author: Dr. Thendral Hepsibha. B: E Mail: thendralhepsibha_b@ethirajcollege.edu.in

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## ABSTRACT

Breast cancer has become an overburdened public health issue for Women in both developed and developing countries. Among many risk factors, presence of excess estrogen, estrogen receptor-α and tumour necrosis factor -α are found to be the major cause of breast cancer. This study aimed to qualitatively analyse and screen the phytoconstituents in ethanolic extract of *Micrococcus mercurialis* stem by GC-MS analysis and binding effect of two phytocompounds phytol and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl against estrogen receptor-α (ESR-α) and TNF-α by *in silico* docking method to identify their anticancer potential. Ethanolic extract of *Micrococcus mercurialis* stem revealed the presence of 26 phytocompounds. Among the two selected phytocompounds, 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl exhibited better binding potential towards both estrogen receptor-α (ESR-α) (- 6.3 Kcal/Mol) and TNF-α (- 5.29 Kcal/Mol) when compared with phytol. The results warrant further *in-vitro* and *in-vivo* research to explore the anticancer and anti-inflammatory potential of *Micrococcus mercurialis.*

**Key words: *Micrococcus mercurialis,* Breast cancer, GC-MS analysis, Estrogen receptor-α (ESR-α) and TNF-α, 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl, *in silico* docking**

## INTRODUCTION

Breast cancer has been an overwhelming health burden for decades throughout the world. It now tops the leading cause surpassing lung cancer with 2.3 million women diagnosed in 2020 **[1]**. In India, 25% of women have reported breast cancer with an incidence of 25.8 and a mortality rate of 12.7 per 100,000 women **[2]**. The highest case was reported in Delhi (41/100,000 women) followed by Chennai (37.9/ 100,000 women), Bangalore (34.4/ 100, 000 women) and Thiruvanthapuram (33.7/100,000 women) **[2]**. It is heartbreaking to note the increasing incidence of breast cancer in women between the age of 30 to 40 in India when compared to Western countries **[2]**. Breast cancer occurs due to the uncontrolled cell division in the epithelial lining cells of the duct (85%) and in the glandular tissues of the breast (15%) and this confined & asymptomatic cancerous growth moves to the spreading metastatic stage **[3]**.

Risk factors that lead to the development of breast cancer includes hormone imbalance, obesity, gender, alcohol consumption, lack of physical exercise, and family history of breast cancer **[4].** Excess estrogen and activation of estrogen receptors (ESR-α and ESR-β) are predominant factors responsible for the development of breast cancer. Estrogen receptor (ESR-α) with high affinity than ESR-β expressed in various organs like the liver, pituitary gland, vagina, mammary gland, and uterus, was found to be the reason for 70% of breast cancer development **[5]**. The binding of estrogen to ESR-α activates Steroid receptor activator - 3 (SRC - 3), increases the transcription of nuclear DNA concerned with breast cancer signaling, and leads to uncontrolled division of breast cells **[6]**.

Breast cancer especially ductal carcinoma is associated with inflammation. Over expressed cytokines like TNF-α, Interleukins (IL-6, IL- 8) stimulate the progression of ductal carcinoma. Tumour Necrosis Factor (TNF-α) is implicated in the progression of cancer like proliferation, transformation, invasion, and metastasis, and this pro-inflammatory cytokine bridges inflammation and cancer **[7]**. Xian *et al*. **[8]** have reported a direct relationship between estrogen receptors and TNF- α in breast cancer. So, estrogen receptors and TNF- α are targeted to treat and prevent breast cancer. The side effects like blood clots, cataracts, uterine cancer **[9]** caused by the drugs like Tamoxifen, Toremifene, and Raloxifene during cancer treatment necessiate the exploration of plant-derived natural compounds. World Health Organisation in its Global Breast Cancer Initiative (GBCI) has stated that if early detection, diagnosis on time and proper

breast cancer treatment could reduce the global breast cancer mortality rate by 2.5% **[10]**.

*Micrococca mercurialis* (*Euphorbiaceae* family) grows well in moist, shady places and is found in India, Sri Lanka, Northern Australia, Africa, and Western Malaysia **[11]**. The plant extract is used to treat fever in kids, and sap is infused into ears, nose, or eyes to treat illnesses like headache, filariasis of the eye, and otitis, respectively **[11]**. Scientific validation of the medicinal properties of herbs could be predicted by identifying the phytoconstituents by GC-MS analysis. Compounds like 5,8,11,14-Eicosatetraenoic acid methyl ester, cyclobarbital, phytol, ethanol, 2-(9-Octadecenyloxy) with anticancerous properties were reported in the whole plant extract of *Micrococca mercurialis* **[12]**. So the present study aimed to document the potential phytoconstituents in the ethanol extract of *Micrococca mercurialis* stem part and assess by *in silico* docking study the binding capability of phytol and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl against estrogen receptor-α (ESR-α) and TNF-α , the key players in breast cancer development.

## MATERIALS AND METHODS

### Collection, identification, and preparation of extract

*Micrococca mercurialis* plant **(Figure 1)** was collected from Egmore, Chennai, and authenticated by Professor P. Jayaraman, Senior Taxonomist, Plant Anatomy Research Centre, West Tambaram, Chennai, India (PARC/2017/3544). The dust particles from the stem portion of *Micrococca mercurialis* were removed. The dried stem parts of *Micrococca mercurialis* were ground and extraction was done using ethanol by soxhlet method. The extract was properly stored in glass vials and used for qualitative and GC-MS analysis.

### Phytochemical screening of plant extract

Qualitative analysis of an ethanolic extract of *Micrococca mercurialis* stem was done following the procedure of Harborne **[13]** and Kokate **[14].**

### GC/MS analysis

The composition of the volatile compounds in the ethanolic extract of *Micrococca mercurialis* stem was analyzed by GC–MS (GCQP 2010 PLUS, Shimadzu, Japan). The following operating conditions were maintained: oven initial temperature was 40°C slowly raised to 140°C at 5°C/ min with a holding time of 5 min and then from 140°C to 210°C at 2 °C/min hold for 8min and then to 250°C to an increase of 5 °C/min, and maintained for 9 min. Injection port temperature was ensured at 250 °C and Helium flow rate at 1.5 ml/min. The ionization voltage was set

at 70 eV. The following conditions were set: split mode - 10:1; ion source temperature - 230 °C; interface temperature - 240 °C and mass spectral scan range 50–800 (m/z). The MS start time and end time for scanning was 3 min, and 75 min with a solvent cut time of 3 min. The obtained GC-MS spectrum was compared and matched with NIST 17 (National Institute of Standard and Technology) online library Ver. 2.3 for identification of compounds.

**Figure 1:** ***Micrococca mercurialis*** **Plant**

***In silico*** **studies**

**Protein preparation**

The sequence of Estrogen receptor-α (ESR1) (ID P03372) and Tumor necrosis factor- α (TNF –α) (ID P01375) was retrieved from the UNIPROT database and their three-dimensional protein structures were downloaded from the PDB database PDB ID - 5UFX-A chain for ESR1 and 5UUI - A Chain for TNF –α respectively.

**Ligand Preparation and Optimization**

The SMILES data of phytol and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl were retrieved from PubChem and their 3D structure was generated using Chemsketch Software, optimized, and saved in a .mol file. The further subsequent conversions were made using the open BABEL molecular converter program and saved in PDB format.

**Molinspiration**

Molecular properties (logP, polar surface area, number of hydrogen bond donors and acceptors, and number of rotatable bonds) of phytol and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl was analyzed by Molinspiration tool **[15]**.

**Protein-Ligand interactions**

The binding energies of phytol and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl with Estrogen receptor (ESR1) and Tumor necrosis factor- α (TNF –α) was assessed by the Auto Dock tool

(Version 4.2) and their interactions were visualized using Accelrys Discovery Studio Visualizer. Conformational parameters like binding energy, inhibitory constant, vander waals interaction, electrostatic energy, torsional energy, and unbound energy were also evaluated.

**RESULTS AND DISCUSSION**

A yield of 86.4% was obtained for the ethanolic extract of *Micrococca mercurialis* stem part. The qualitative analysis of the ethanolic extract of *Micrococca mercurialis* stem reveals the presence of carbohydrates, proteins, phenols, tannins, alkaloids, saponins, and flavonoids **(Table 1)**. These phytoconstituents in *Micrococca mercurialis* stem contribute to pharmacological activity.

The GC-MS profile of ethanolic extract of *Micrococca mercurialis* stem reveals the presence of 26 phytochemicals. The details of phytochemicals (Retention time, peak area, molecular formula) and their activities are listed in **Figure 2, Table 2**. The major compounds were 17-Octadecynoic acid (26.01%), naphthalene (16.35%), n-hexadecanoic acid (11.13%), Tetraacetyl-d-xylonic nitrile (10.35%) and 10-heneicosene (4.62%). Many of the compounds reported in our results possess antioxidant, anticancer, and antimicrobial potential. The obtained results confirm the traditional usage of *Micrococca mercurialis* for various ailments.

Rapid Virtual screening of interactions of various of natural compounds with various receptors and elucidation of their probable mechanism of action is possible by *in silico* docking analysis. It is an economically feasible method. Two important antioxidant compounds identified in *Micrococca mercurialis* stem part, phytol and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl were chosen and docked against estrogen receptor-α (ESR-α) and TNF-α **(Figure 3)** to identify their anticancer potential. The drug-likeness of phytol and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl was determined by a combination of structural and molecular properties. Molecular properties analysed were Log P (partition coefficient), topological surface area (TPSA), the total number of atoms, molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, and number of rotatable bonds.

According to Lipinski's rule of five **[34]** if a compound's molecular properties satisfy the criteria of Log P ≤ 5, TPSA ≤ 140, molecular weight ≤ 500 g/mol, hydrogen bond acceptor ≤ 10, hydrogen bond donor ≤ 5, and ≤ 10 number of rotatable bonds then it will show high bioavailability through absorption and

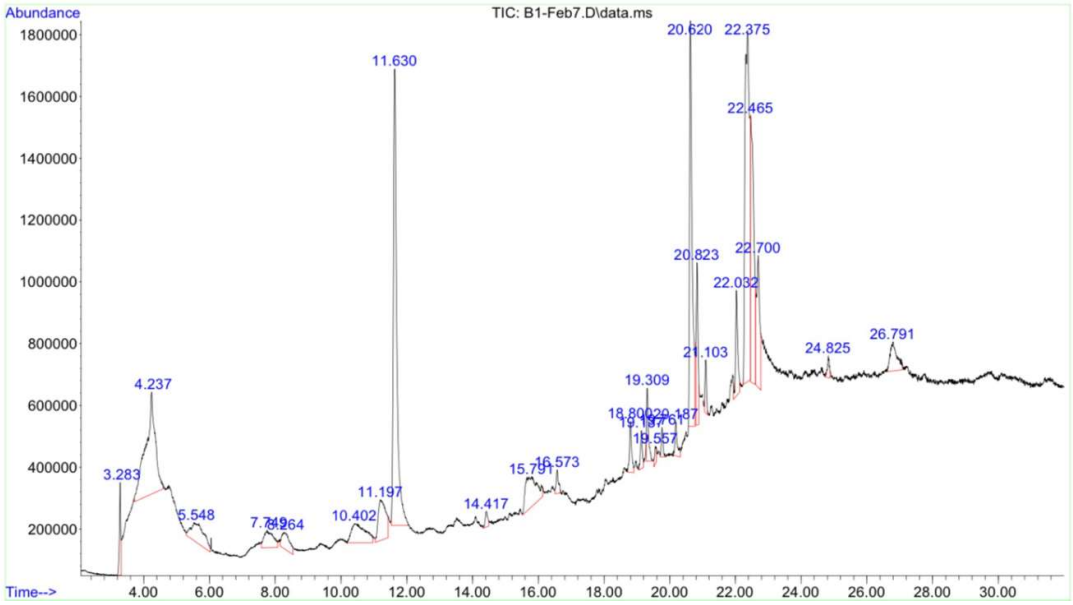
permeability. In our study, the drug-likeness properties of both the ligands phytol and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl are given in **Table 3**. Except for the Log P value for Phytol with 1 violation from Lipinski rule which could be corrected during drug development, both the ligands obeyed Lipinski rule of five and confirmed their drug-likeness.

**Table 4 and Figure 4 & 5** shows the binding interactions of phytol and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl with estrogen receptor-α. Compared with the two ligands, 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl has interacted maximum with 3 hydrogen bonds towards estrogen receptor-α. GLU397, ARG394 and TRP393 residues are involved in hydrogen bond formation with the ligand at a distance of 2.87, 2.06 and 2.16 Å and its binding energy was -6.13Kcal/Mol. Phytol forms a hydrogen bond with GLU323 of estrogen receptor-α at a distance of 1.96 Å and with a binding energy of -5.29 Kcal/Mol.

In molecular docking, the stronger the binding affinities, lowest will be the binding energy score. This ensures maximum structural fitting of the ligand with the receptor molecule making it a potential drug candidate. From the results, 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl ligand bonds with greater binding energy than phytol. This is the first study to report the presence of 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl compound in the stem parts of *Micrococca mercurialis*. Similar results were obtained when *Phyllanthus emblica* was screened for potent anticancerous agents where isocorilagin exhibits strong docking potential with estrogen receptors-α **[35]**. It might be predicted that the binding of 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl ligand to estrogen receptor might control the uncontrolled transcription of a genes responsible for mammary gland development **[36]** and regulate the signaling network involved in inflammation, homeostasis, differentiation, maturation and proliferation of breast cells **[3]**.

**Table 1: Preliminary phytochemical analysis of ethanolic extract of *Micrococca mercurialis* stem**

| S. No. | Phytochemicals | Results |
|---|---|---|
| 1. | Carbohydrates | +++ |
| 2. | Protein | + |
| 3. | Saponins | +++ |
| 4. | Phenol | + |
| 5. | Tannin | +++ |
| 6. | Alkaloids | + |
| 7. | Flavonoids | + |




*+ mild; ++ moderate; +++- more*

**Figure 2: GC-MS chromatogram of ethanolic extract of *Micrococca mercurialis* stem**

**Table 2: Phyto compounds identified from the ethanolic extract of *Micrococca mercurialis* stem by GC-MS**

| S. No | Name of the compound | Peak area % | Activity |
|---|---|---|---|
| 1 | dl-Alanyl-dl-leucine | 1.238 | Anticancer of liver and lung, antitumor of breast, lung, prostate and liver, antioxidant. [16] |
| 2 | Tetraacetyl-d-xylonic nitrile | 10.353 | Anti-tumor and antioxidant [17] |
| 3 | Propanoicacid,2-oxo-, methyl ester | 2.740 | Flavor, fungicide, irritant, perfumery, pesticide [16] |
| 4 | 2-cyclopentene-1-,2-hydroxy- | 1.707 | Antitumor [18]<br>Antidiuretic [19] |
| 5 | 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one | 1.410 | Flavourant analysis. Anti microbial activity, Anti fungal [20] |
| 6 | Cyclopentane,-acetyl-1,2-epoxy- | 2.853 | Anti-inflammatory, antiviral and bronchodilatory properties [21] |
| 7 | 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- | 3.256 | Anti-microbial, anti-inflammatory, anti-proliferative anti-oxidant, automatic nerve activity [16]<br>Anti-diabetic and anti oxidant activity [17] |
| 8 | Napthalene | 16.358 | Cataractagenic [16]<br>Insecticide and pest repellent [22] |
| 9 | 5,8,11-Heptadecatriynoic acid, methylester | 0.422 | Dyestuffs, feed additives, pharmaceuticals, methyl ester pigment coatings [23] |
| 10 | Cholest-5-en-3-ol,4,4-dimethyl- | 3.796 | Unknown |
| 11 | Sucrose | 0.569 | Gradients for centrifugation separations [24] |
| 12 | cetene | 1.145 | Antimicrobial and antioxidant effects [25] |
| 13 | 5-octadecene ,(E) - | 0.744 | Stronger sexual characters [26] |
| 14 | Isopropyl myristate | 1.386 | Skin care lotion, emollient [27] |
| 15 | 3,7,11,15-tetra methyl-2-hexadecen-1-ol | 0.305 | Vitamins E and K1 precursor, decomposition product of chlorophyll, chemical deterrents against predation, flavoring agents [28] |
| 16 | Cyclopropanenonanoic acid,2-[(2-butylcyclopropyl) methyl], methyl ester | 0.525 | unknown |
| 17 | Hexadecanoic acid, methyl ester | 0.858 | Antioxidant, Flavor, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5- Alpha reductase inhibitor [23] |

| | | | |
|---|---|---|---|
| **18** | **n-hexadecanoic acid** | **11.131** | **Anti-oxidant, anti-androgenic, flavor, hemolytic 5-alpha reductase inhibitor, hypocholesterolemic, nematicide, pesticide, lubricant [16]** |
| **19** | **Hexadecanoic acid, ethyl ester** | **2.890** | **Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, anti-eczemic, anti-acne, alpha reductase inhibitor, antiandrogenic, anti-arthritic, anticoronary [16]** |
| **20** | **Phytol** | **0.832** | **Anticancer, anti-diuretic, nematicide, hepatoprotective, hypocholesterolemic, anticoronary, antiandrogenic antimicrobial, antioxidant, antiarthritic, anti-inflammatory, antidiabetic, and immunostimulatory [29]** |
| **21** | **Coumatetralyl isomer-2 ME** | **2.320** | **Anticoagulant [30]** |
| **22** | **17-Octadecynoic acid** | **26.01** | **Antihypertensive properties [31]** |
| **24** | **10-heneicosene (c,t)** | **8.417** | **Antimicrobial agent [32]** |
| **25** | **17-Pentatriacotene** | **4.623** | **Anti-septic property [33]** |
| **26** | **Hexadecanoic,1-(hydroxymethyl)-1,2-ethanediyl ester** | **2.054** | **Anti-microbial property [43]** |

**Table 3: Molecular properties of selected ligands (phytol and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl)**

| Molecular Properties | Phytol | 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl |
|---|---|---|
| **miLogP** | **6.76** | **-0.46** |
| **TPSA** | **20.23** | **66.76** |
| **Number of atoms** | **21** | **10** |
| **Molecular weight** | **296.54** | **144.13** |
| **Number of hydrogen bond acceptors** | **1** | **4** |
| **Number of hydrogen bond donors** | **1** | **2** |
| **Number of rotatable bonds** | **13** | **0** |
| **Number of violations** | **1** | **0** |

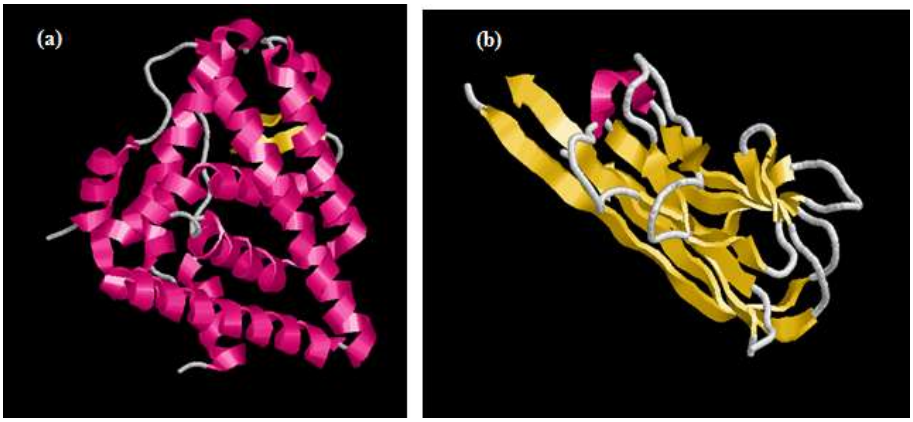



**Figure 3: 3D Visualization of (a) Estrogen receptor -α and (b) Tumor necrosis factor- α**

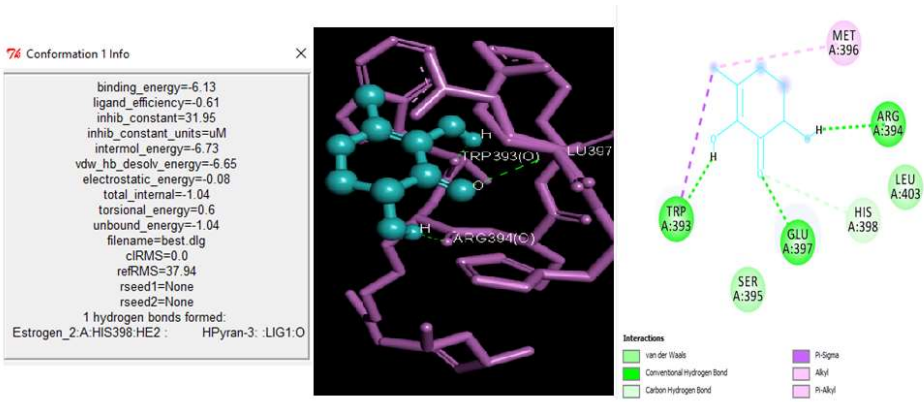



**Figure 4: 3D interactions of Estrogen Receptor -α (protein) and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl (Ligand)**

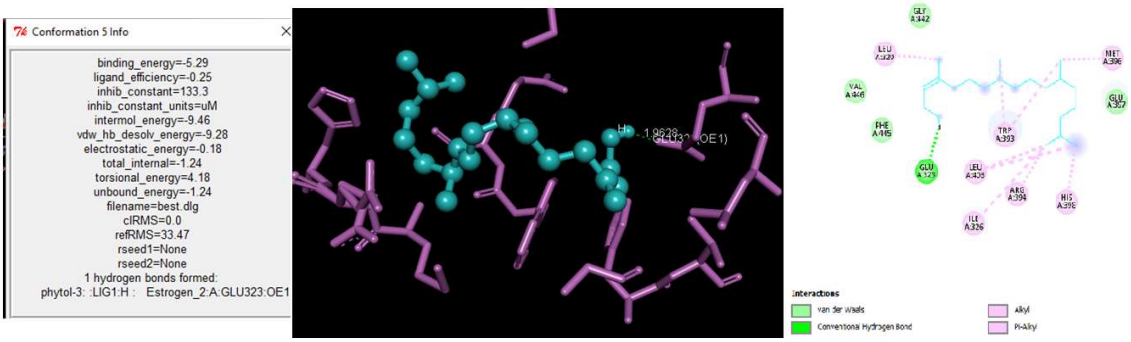



**Figure 5: 3D interactions of Estrogen Receptor (protein) and Phytol (Ligand)**

**Table 4: Binding interactions of phytol and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl with estrogen receptor- α and TNF-α**

| Receptor | Residues | Atom | Ligand | Atom | Distance (Å) | Docking Energy (Kcal/Mol) |
|---|---|---|---|---|---|---|
| Estrogen Receptor-α | GLU397 | N | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl - | O | 2.87 | –6.13 |
| | ARG394 | O | | H | 2.06 | |
| | TRP393 | O | | H | 2.16 | |
| | GLU323 | OE1 | Phytol | H | 1.96 | –5.29 |
| Tumor Necrosis Factor- α | VAL150 | O | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl | H | 1.87 | –5.6 |
| | VAL150 | N | | O | 3.09 | |
| | SER147 | OG | | O | 2.76 | |
| | SER147 | OG | | H | 2.19 | |
| | GLY148 | N | | O | 2.86 | |
| | GLU135 | OE1 | Phytol | H | 1.93 | –3.96 |

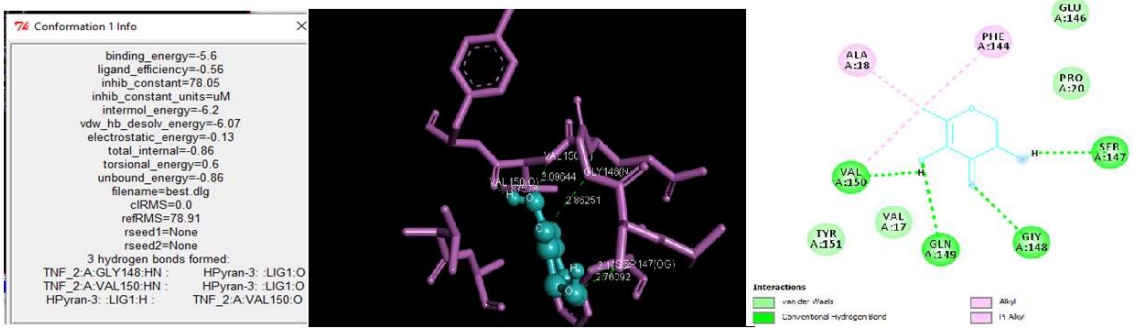



**Figure 6: 3D interactions of Tumour Necrosis Factor - α (TNF- α) (protein) and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl (Ligand)**

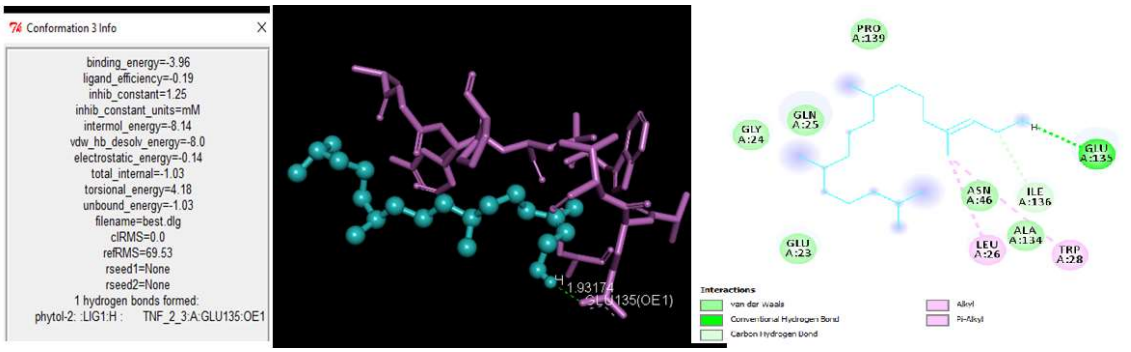



**Figure 7: 3D interactions of Tumour Necrosis Factor - α (TNF- α) (protein) and Phytol (Ligand)**

The docking interactions of phytol and 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl with Tumor Necrosis Factor- α are shown in **Figures 6 & 7 and Table 4**. Phytol interacts with Tumor Necrosis Factor- α through a single hydrogen bond involving GLU135 residue with a binding energy of -3.96 Kcal/Mol. 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl forms hydrogen bonds with VAL150, SER147, GLY148 of Tumor Necrosis Factor- α with the binding energy of -5.6 Kcal/Mol. The high binding energy of 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl with TNF- α than phytol shows its potential as a TNF-α inhibitor. TNF-α a known pro-inflammatory cytokine was invoved in tumor formations, and promotes proliferation, angiogenesis and metastasis thus destabilises the immune system **[38]**. Resulting in inflammatory conditions in autoimmune diseases like rheumatoid arthritis, systemic sclerosis, inflammatory bowel disease, systemic lupus erythematosus, psoriasis and diabetes **[39, 40]**. 4H-pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl present in *Micrococca mercurialis* stem is thus a potential anticancerous and anti-inflammatory agent. Similar anticancerous and anti-inflammatory property of Withaferin- A and Celastrol **[41]**. Kim *et al*. **[42]** by *in silico* docking have established the inhibitory potential of triterpene saponins from Vietnamese ginseng (*Panax Vietnamese*). Thus the present study reveals the presence of promising phyto therapeutic agents, in *Micrococca mercurialis* stem with plausible anticancerous and anti-inflammatory properties.

## CONCLUSION

The GC-MS results of *Micrococca mercurialis* stem part reveal the presence of phytocompounds of different bioactivities with pharmacological importance. *In silico* docking proves the better anticancer potential of 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- than phytol towards estrogen receptor-α and TNF-α. *Micrococca mercurialis* is a rich repertoire of anticancerous compounds which could play a vital role in cancer therapies. The results warrant further *in-vitro* and *in-vivo* research to explore the anticancer and anti-inflammatory potential of *Micrococcus mercurialis* to develop drug formulations.

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