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Original Article

Targeting EGFR (PDB id-1XKK) for Immunomodulation: Network Pharmacology and Molecular Docking Insights into *Glycyrrhiza glabra, Piper longum*, and *Phyllanthus emblica*

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ABSTRACT

Aim/Background: Plant-derived bioactive constituents can act as immunomodulators by stimulating or suppressing immune responses. This study evaluated the immunomodulatory potential of *Glycyrrhiza glabra* (root), *Piper longum* (fruit), and *Phyllanthus emblica* (fruit) using bioinformatics approaches, focusing on their interaction with the Epidermal Growth Factor Receptor (EGFR). **Materials and Methods:** Immunomodulatory targets were obtained from GeneCards, and major phytochemicals were identified from IMPPAT, PubChem, and Dr. Duke's databases. Network pharmacology was used to map compound-target-pathway relationships. Molecular docking was performed against EGFR (PDB ID: 1XKK) to assess binding affinities. **Results:** Network Pharmacology Indicate presences of Key compounds included β-carotene, quercetin, kaempferol, apigenin, licochalcone-A (*G. glabra*); Piperine, Piperlonguminine, Pipernonaline, Piperundecalidine, Guineensine (*P. longum*); and ellagic acid, quercetin, gallic acid, catechol, methyl gallate (*P. emblica*). EGFR emerged as a major target, with several compounds showing strong docking affinities. **Conclusion:** These plants exhibited promising EGFR-mediated immunomodulatory potential and may serve in herbal immunostimulant formulations. Further laboratory and clinical validation are required.

Keywords: EGFR, Immunostimulant activity, Molecular Docking, Network pharmacology.

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INTRODUCTION

Protecting the human body from harmful and infectious microbes is mostly dependent on immunity. Innate and adaptive immunity make up the immune system, which supports immunity. The first immunological mechanism, innate immunity, has little immunological memory and responds quickly. Antigen-specific and antigen-dependent adaptive immunity, on the other hand, has memory capacity and produces a more effective immune response when the antigen is encountered again (Banerjee *et al.*, 2024). Complement, CRP Binding Protein, Mannose Binding Lectin, LPS Binding Protein, Acute-Phase Reactants Antimicrobial Peptides are examples of humoral and cellular immune components that make up innate immunity. Cellular components include neutrophils, Mast Cells, Macrophages, NK Cells, Dendritic Cells, and Non-hematopoietic cell. Immune components that are part of adaptive immunity include humoral (immunoglobulin) and cellular (B and T Cells) components (Berman *et al.*, 2000).

The vertebrate Immune system is an active defense mechanism that protects the host from harmful foreign pathogens. It has crucial Surveillance Control to monitor the integrity of host tissues. Immunomodulators' ability to stimulate or inhibit immunological responses is a defining property of their use in disease regulation. Numerous conventional medications are implicated in both enhancing and diminishing the strength of the host's immunological response, according to the literature. Plant-derived natural compounds may function as immunomodulators to control some immune-mediated reactions (Ceccuzzi *et al.*, 2023). With respect to present of side effect of allopathic medications, alternative natural based ayurvedic preparations are now days preferred as fewer side effects. On the basis of traditional claims and presence of potent immune modulators, liquorice, pipali and amla plants were selected

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for further evaluation of immune modulator effects based on computational tools.

Liquorice is scientifically known as *Glycyrrhiza glabra* and belongs to the Leguminosae family. *G. glabra* is significantly available in India, China, Spain, Turkey, Iran. Iraq. *Liquorice* (root) plant extracts having hair growth properties (Shirode *et al.*, 2005), Immunomodulatory Properties, Antioxidant Properties, Antimicrobial Properties, Anti-inflammatory Properties, Antiulcer Properties, Antitussive Properties, Anti-allergic Properties, Antiviral Properties, Antifungal Properties. Liquorice contains a variety of substances, including proteins, amino acids, simple sugars, polysaccharides, mineral salts, pectin, starches, sterols, gums, and resins. Phytochemicals found in *liquorice* are Triterpenoid, Saponin, Flavonoid, Coumarin, Isoprenoid Substituted phenol, Alcohol (Volatile), Acid (Volatile), Terpenoid, Aldehyde (Daina *et al.*, 2017, Daina *et al.*, 2019, DeLano, 2002).

Pipali is scientifically knows as *Piper longum* and belongs to the Piperaceae family. *Piper longum* is significantly available in India, Nepal, Turkish, French, China. *Piper longum* (fruit) plant extract having Immunomodulatory activity, Anticancer activity, Antioxidant activity, Hepatoprotective activity, Anti-inflammatory activity, antimicrobial activity, Antiplatelet activity, Antihyperlipidemic activity, Analgesic activity, Adulticidal activity, Melanin-inhibiting activity, Antidepressant activity, Anti amoebic activity, Anti-obesity activity, Cardioprotective activity, Antifungal activity, Antifertility activity. *Pipali* plant fruits major chemical constituents are alkaloids, volatile oil, starch, protein, saponins, carbohydrates, and amygdalin were all positive for the fruits (Delves *et al.*, 2017).

Amla is scientifically known as *Phyllanthus emblica* and belongs to the Euphorbiaceae family. *Amla* is Significantly available in India, Southeast Asia, China, Iran and Pakistan. *Amla* (fruit) Plant extract having Immunomodulatory activity, Antioxidant activity, Cardioprotective activity, Antidiabetic activity, Anticancer activity, Anti-inflammatory activity, Digestive tract protection, Neurological protection. Amla is the most well-known and extensively researched plant. Numerous biochemical components are present, particularly alkaloids, phenols, and tannins, according to research findings. Fruit contains around 28% of the tannin in the total plant. This tannin comes in two hydrolyzable forms: (i) emblicanin A and (ii) emblicanin B. Both of these forms are antioxidants; when emblicanin A is hydrolyzed, it produces ellagic acid, glucose, and gallic acid, while when emblicanin B is hydrolyzed, it produces ellagic acid and glucose. Additionally, this fruit contains phyllemblin. Numerous other phytochemical compounds, including geraniin, corilagin, gallic acid, and furoin, were revealed by the additional fractionation (Eberhardt *et al.*, 2021, Faizan *et al.*, 2024).

MATERIALS AND METHODS

Data Collection

Active Compounds of *Glycyrrhiza glabra* (Liquorice Root), *Piper longum* (Pipali Fruit), *Phyllanthus emblica* (Amla Fruit) Chemical Compounds of *Glycyrrhiza glabra* (Root), *Piper longum* (Fruit), *Phyllanthus emblica* (Fruit) were collected from the IMPPAT, Dr. Duke - Phytochemical Databases.

Material

Glycyrrhiza glabra (Root) Course powder, *Piper longum* (fruit) Course powder, *Phyllanthus emblica* (Fruit) Course powder is collected from Manakarnika Aushadhalays and authenticated in Mankarnika Aushadhalaya India Pune with authentication number Pune (Out ward No: AD/401/12/24).

Extraction of Plants Compounds

After being weighed the 100 g of powdered leaves were steeped in 350 mL of methanol in a conical flask. Cork closure and shake and the flask containing the leaves was allowed to stand at room temperature for 48 hr. The combination was filtered, the extract was gathered, and it was then concentrated in an evaporating dish by evaporating it until it was dry (Gul *et al.*, 2022).

Phytochemical identification

Tests for alkaloids, glycosides, tannins, flavonoids, and other phytochemicals were used in the screening process for phytochemical identification (Hasan *et al.*, 2021).

Network Pharmacology

Target identification and biological network construction (Shirode et al., 2025)

Phytoconstituent analysis which include molecular weight by (Shirode *et al.*, 2025) Molsoft (Hassan *et al.*, 2020). Swiss Target Prediction was used to predict the possible protein target of each Phytochemical (Ikram *et al.*, 2021), and PROTOX Database was used for toxicity Predication (Im *et al.*, 2016). Then we finalize the phytoconstituents for Network pharmacology. These Phytoconstituents were entered in to SwissTargetPrediction to predict the potential target gene of each compound (Inpan *et al.*, 2024). The Gene Card database is then searched for proteins associated with disorders using the phrase "IMMUNOMODULATORY ACTIVITY" (Jejurikar & Rohane, 2021). To find overlapping proteins, Venny 2.1 tool is used to analyses all of the Swiss Target Prediction and Gene Card data (Karkanis *et al.*, 2018). This Vinny 2.1 displays the Venn diagram of common protein of Gene Card and Swiss Target Prediction. PPI Protein-Protein Interaction (PPI) analysis was conducted using the biological process with the greatest number of targets. The STRING protein database and Cytoscape 3.9.0 were used for PPI analysis (Kim *et al.*, 2025). Crucial hubs in the built protein network were analysed using the Cytohubba plugin. The betweenness and closeness scores and rankings were used to identify the key hubs. Every scoring and ranking function a well as top-ranked hub was chosen as the molecular docking target.

Three-Dimensional Structure Retrievals

The three-dimensional structure of the molecule was derived from the PubChem database. Since some compounds three-dimensional structures are not available in PubChem, Chem draws 3D was used to construct them. The SMILES code and Compound ID (CID) are listed in Supplementary Table 1. (EGFR) 3D structure was obtained from the RCSB Protein Data Bank (.pdb) using pdb identities (.pdb id: 1XKK), after their mutation score was checked to be zero, their species was determined to be Homosepian, their gene name was EGFR, and finally their ligand chain was examined. Native ligand, positive control ligand and test compound ligand were downloading the PubChem database (Kumar *et al.*, 2011). Some structures are not in 3D format so this structure prepared in the tool Chem Draw 3D 16.0 program (O'Boyle *et al.*, 2011). For converting in the .pdb to. pdbqt AUTODOCK software is used (Oliveros, J. C. (2007)).

Molecular Docking

Research articles and literature reviews on Immunomodulatory activity are consulted before choosing a protein structure (Otasek *et al.*, 2019). The Protein Data Bank provided the protein complex in (.pdb) format. Using Open Bable, (Pasala *et al.*, 2022) the 3D structure of the chemical was energy reduced prior to the docking operation. Additionally, the EGFR gene protein (.pdb id: 1XKK) (Saravanan *et al.*, 2012). Figure 1 was prepared by utilizing Chimera software to exclude water and the native ligand atom. One key prepared the cleaned protein structure as a dock and saved it as a .pdb Protein was seen as a hard receptor, whereas the chemical was regarded as a flexible ligand. The protein was prepared by deleting water molecules, adding polar hydrogen, and adding Kollam charges, then grid the macromolecules and record the results of the docking process using Auto Dock Vina 1.2.3. Using PyMOL, (Shaikh & Patil, 2020) the protein structure and internal ligand molecules were separated from the .pdb structure. The Auto Dock software converts all protein, ligand, standard compound, and phytochemical structures into pdbqt. PyRx software is used to analyse the actual docking process and protein's amino acid interactions using Discovery Studio (Stelzer *et al.*, 2016; Wahab *et al.*, 2021).

RESULTS

Molecular interactions of phytoconstituents from *Glycyrrhiza glabra* with EGFR

Molecular docking study was performed to assess the EGFR (PDB id- 1XKK) suppression ability of phytoconstituents identified from the Liquorice. Molecular docking interaction analysis depicted that reactive group of compound FMM Ligand forms hydrogen bonds with active sites of MET A: 793, van der Waals bonds with active sites of MET A: 1002, GLY A: 719, ARG A: 841, ASN A: 842, ILE A: 744, THR A: 854, ILE A: 789, THR A: 790, LEU A: 858, GLN A: 791, LEU A: 792, GLY A: 796, Carbon Hydrogen Bonds active sites of GLY A: 721, Halogen(Fluorine) bonds active sites of CYS A: 775, ARG A: 776, Pi- Cation bonds active sites of LYS A: 745, Pi- Donor Hydrogen Bonds active sites of ASP A: 855, Pi-Sigma bonds active sites of MET A: 766, LEU A: 844, LEU A: 718, Pi-Pi T-Shaped bond active sites of PHE A: 856, Alkyl bonds active sites of LEU A: 788, LEU A: 777, Pi-Alkyl bonds active sites of ALA A: 743, VAL A: 726, with binding score -10.6 Kcal/mol, Levamisole from Van der Waals bonds active sites of LEU A: 858, THR A: 854, CYS A: 775, ARG A: 776, THR A: 790, LYS A: 745, Conventional Hydrogen bond active site of ASP A: 855, Pi-Sigma bond active sites of MET A: 766, Pi-Pi T-Shaped bond active site of PHE A: 856, Alkyl bond active site of ALA A: 743, LEU A: 777, and Pi-Alkyl bond active sites of VAL A: 726. with binding score -6.6. β-Carotene from Van der Waals bonds active sites of MET A: 1002, LEU A: 792, MET A: 793, GLY A: 796, CYS A: 797, THR A: 854, CYS A: 775, THR A: 790, LEU A: 777, LEU A: 788, ASP A: 745, LYS A: 745, and Alkyl bons active sites of LEU A: 718, LEU A: 844, VAL A: 726, ALA A: 743. Figure 2 with binding score - 8.2. Docking results were compared to standard immunomodulatory agent Levamisole. Sequence of binding affinity as Levamisole>β-Carotene> Licochalcone-A> Apigenin> Kaempferol> β-Sitosterol>Quercetin>FMM Ligand (Internal ligand). From the docking analysis, it has been observed that the standard levamisole showed highest binding affinity towards EGFR and thus showed promising EGFR downregulation. As compared to standard levamisole, β carotene showed comparable binding affinity towards EGFR and thus downregulates the EGFR

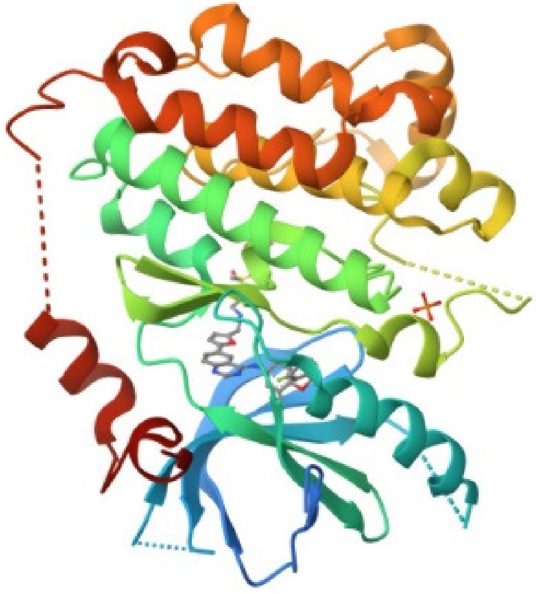


Figure 1: Protein Structure of 1XKK.

which in turn showed immunomodulatory activity. This outcome is in consistent with the studies reported by Amanda C Herrmann andChantale Bernatchez.

Molecular interactions of phytoconstituents from *Piper longum* with EGFR

Molecular docking study was performed to assess the EGFR (PDB id- 1XKK) suppression ability of phytoconstituents identified from the Pipali. FMM Internal ligand, shows the same binding affinity as given earlier. Guineensine from Van der Waals bonds active sites of MET A: 1002, GLY A: 721, ALA A: 722, MET A: 793, ASP A: 855, LEU A: 788, LEU A: 858, THR A: 854, CYS A: 775, LEU A: 777, ARG A: 776, THR A: 790, LEU A: 792, GLY A: 796, Conventional Hydrogen Bond active site of LYS A: 745, Pi-Sulfur bond active site of CYS A: 797, Alkyl bonds actives sites of ARG A: 841, MET A: 766, PHE A: 856, and Pi-Alkyl bonds active sites of LEU A: 718, LEU A: 844, ALA A: 743, VAL A: 726. Figure 2 with binding score - 6.7. Docking results were compared to standard immunomodulatory agent Levamisole. The sequence of binding affinity as Levamisole> Guineensine> Zingiberene> Piperundecalidine> Pipernonaline> Piperlonguminine> Piperine> FMM Ligand (Internal ligand). From the docking analysis, as compared to standard levamisole, the Guineensine showed best binding affinity towards EGFR and thus downregulates the EGFR which in turn showed immunomodulatory activity.

Molecular interactions of phytoconstituents from *Phyllanthus emblica* with EGFR

Molecular docking study was performed to assess the EGFR (PBD ID - 1XKK) suppression ability of phytoconstituents identified from the Amla. Catechol from Van der Waals bonds active sites of THR A: 790, ARG A: 776, VAL A: 769, ASP A: 855, THR A:854, Conventional Hydrogen Bond active sites of LEU A: 777, CYS A: 775, PHE A: 856, and Pi-Pi T-Shaped bond active site of MET A: 766. Figure 2 with binding score - 5.3. Docking results were compared to standard immunomodulatory agent Levamisole. Sequence of binding affinity as Levamisole> Catechol> Phloroglucinol> Gallic acid > Methyl gallate> Ellagic acid> Quercetin> FMM Ligand (Internal ligand). From the docking analysis, as compared to standard levamisole, the Catechol showed best binding affinity towards EGFR and thus downregulates the EGFR which in turn showed immunomodulatory activity.

Table 1: Phytocompounds identified from the methanolic extract of *Glycyrrhiza glabra* (Root), *Piper longum* (Fruit), *Phyllanthus emblica* (Fruit).

Compound No:	Compound name	CID NO (PubChem)	Formula	Calculated Molecular Weight
A	***Glycyrrhiza glabra***			
1.	Beta-Carotene	5280489	$C_{40}H_{56}$	536.9 g/mol
2.	Quercetin	5280343	$C_{15}H_{10}O_7$	302.23 g/mol
3.	Kaempferol	5280863	$C_{15}H_{10}O_6$	286.24 g/mol
4.	Apigenin	5280443	$C_{15}H_{10}O_5$	270.24 g/mol
5.	Lico chalcone-A	5318998	$C_{21}H_{22}O_4$	338.4 g/mol
6.	Beta-Carotene	449171	$C_{20}H_{28}O_2$	300.4 g/mol
B	***Piper longum***			
1.	Piperine	638024	$C_{17}H_{19}NO_3$	285.34 g/mol
2.	Piperlonguminine	5320621	$C_{16}H_{19}NO_3$	273.33 g/mol
3.	Pipernonaline	9974595	$C_{21}H_{27}NO_3$	341.4 g/mol
4.	Piperundecalidine	44453654	$C_{23}H_{29}NO_3$	367.5 g/mol
5.	Guineensine	6442405	$C_{24}H_{33}NO_3$	383.5 g/mol
6.	Zingiberene	92776	$C_{15}H_{24}$	204.35 g/mol
C	***Phyllanthus emblica***			
1.	Ellagic acid	5281855	$C_{14}H_6O_8$	302.19 g/mol
2.	Quercetin	5280343	$C_{15}H_{10}O_7$	302.23 g/mol
3.	Gallic acid	370	$C_7H_6O_5$	170.12 g/mol
4.	Catechol	289	$C_6H_6O_2$	110.11 g/mol
5.	Methyl gallate	7428	$C_8H_8O_5$	184.15 g/mol
6.	Phloroglucinol	359	$C_6H_6O_3$	126.11 g/mol

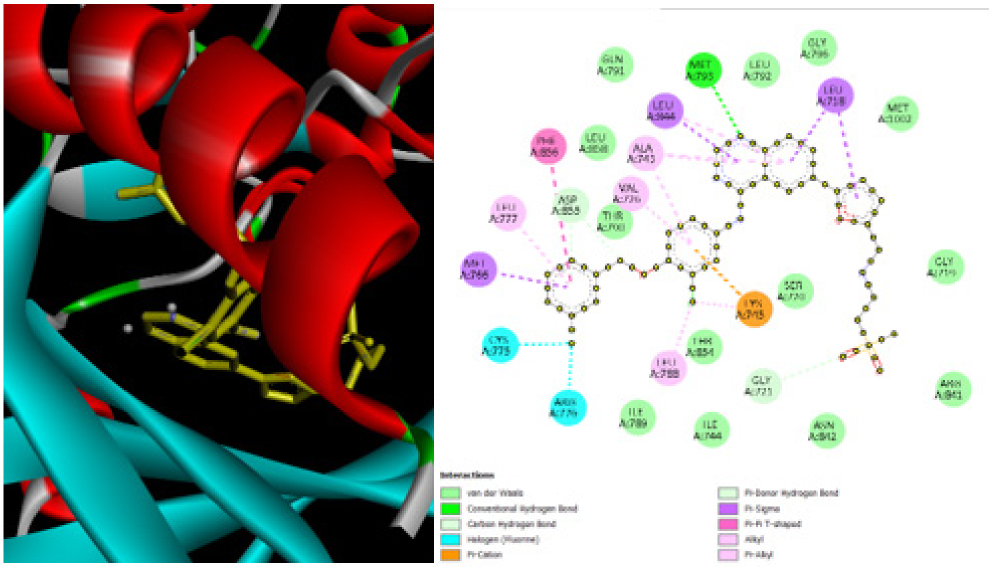
DISCUSSION

Phytochemical contents of *Glycyrrhiza glabra* (Root), *Piper Longum* (fruit), *Phyllanthus emblica* (Fruit) extract

Eighteen compounds, originating from Flavonoids (Table 1) which is Carotene, Quercetin, Kaempferol, Apigenin, Lico chalcone-A, Beta- Carotene, Thiamine, Ascorbic acid, its derivatives as the significant phytochemicals in Liquorice (Root) (Wang *et al.*, 2025). In Pipali fruit Piperine, Piperlonguminine, Pipernonaline, Piperundecalidine, Guineensine, Zingiberene, Caryophyllene oxide, Humulene and its derivatives which are important phytochemicals (Yadav *et al.*, 2020). In Amla fruit Ellagic acid, Quercetin, Gallic acid, Catechol, Methyl gallate, Phloroglucinol phytochemicals are present (Yusuf *et al.*, 2014). This all group of compounds are known to exhibit immunomodulatory activity EGFR.

Proteins and biological targets of *Glycyrrhiza glabra* (Root), *Piper longum* (fruit), *Phyllanthus emblica* (Fruit)

Swiss Target Prediction identified approximately 470 potential targets for Liquorice (Root) phytocompounds, approximately 651 potential targets for Pipali (Fruit), and approximately 505 potential targets for Amla (Fruit), when the Gene Card database revealed more 1737 protein involved in the pathogenesis of immunomodulatory activity. But only 88 proteins were common to both Swiss Target Prediction and Gene Card results use



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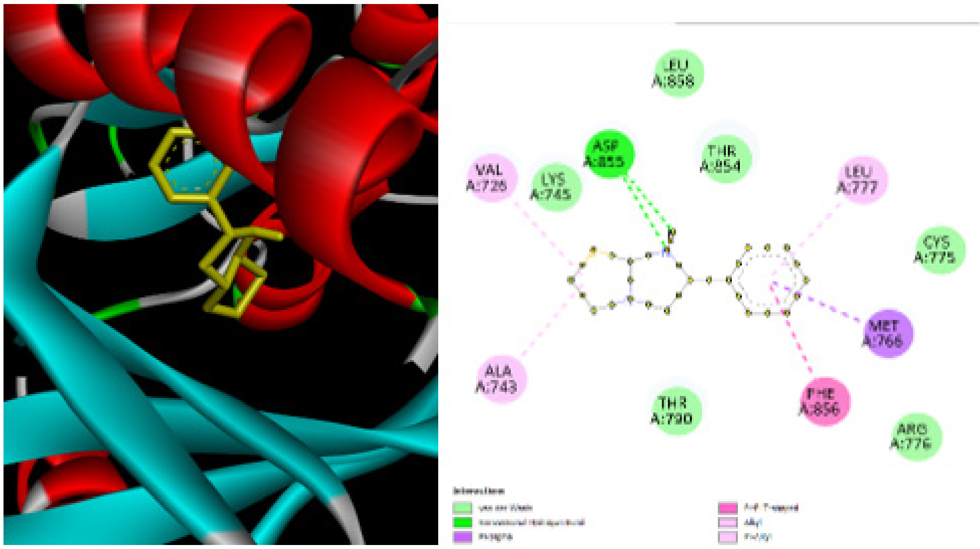

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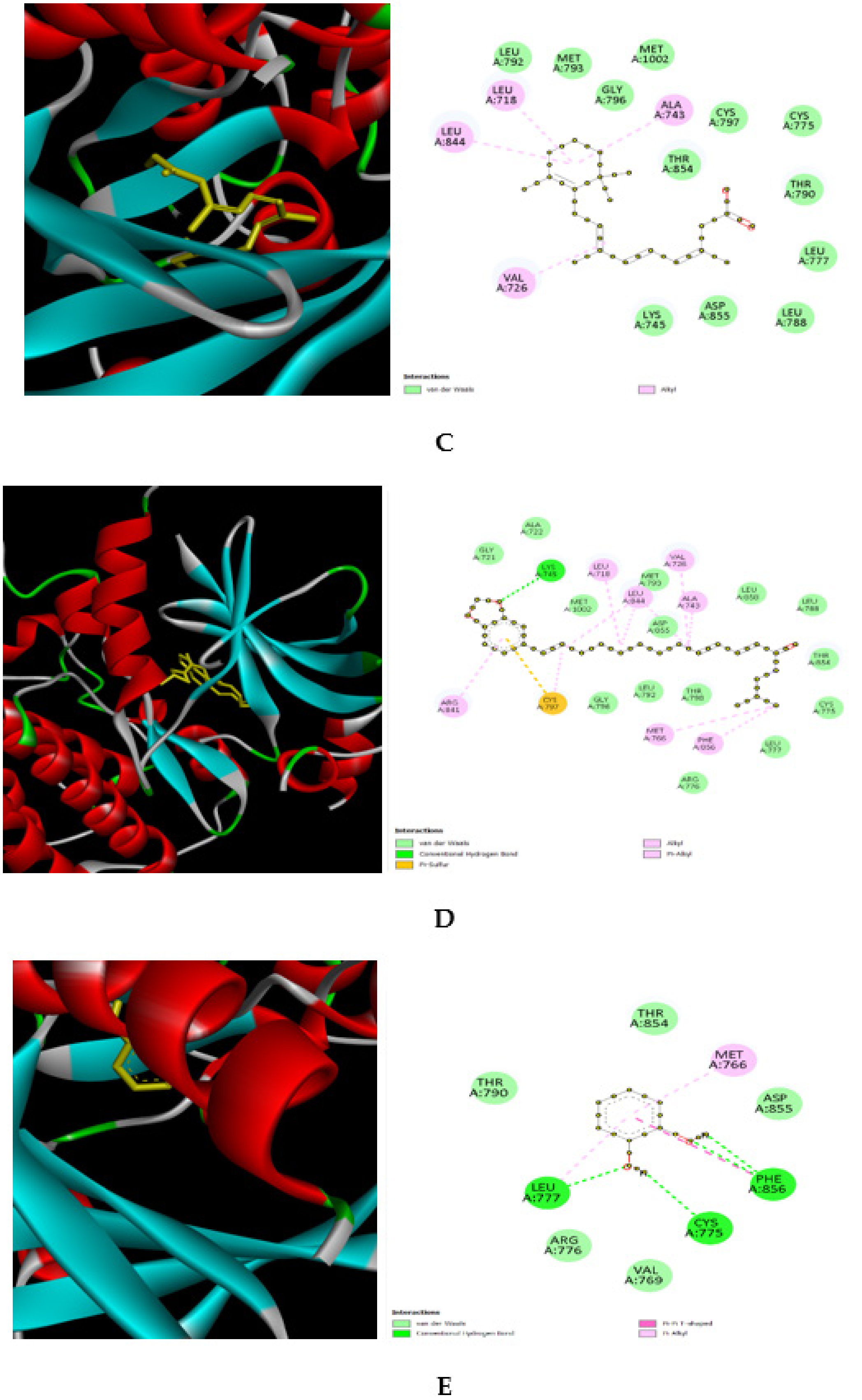

Figure 2: Molecular interaction of Phytoconstituents and standards with EGFR. A. Internal Ligand FMM 3D and 2D Structure. B. Standard Levamisole 3D and 2D Structure. C. β-Carotene 3D and 2D Structure. D. Guineensine 3D and 2D Structure. E. Catechol 3D and 2D Structure.

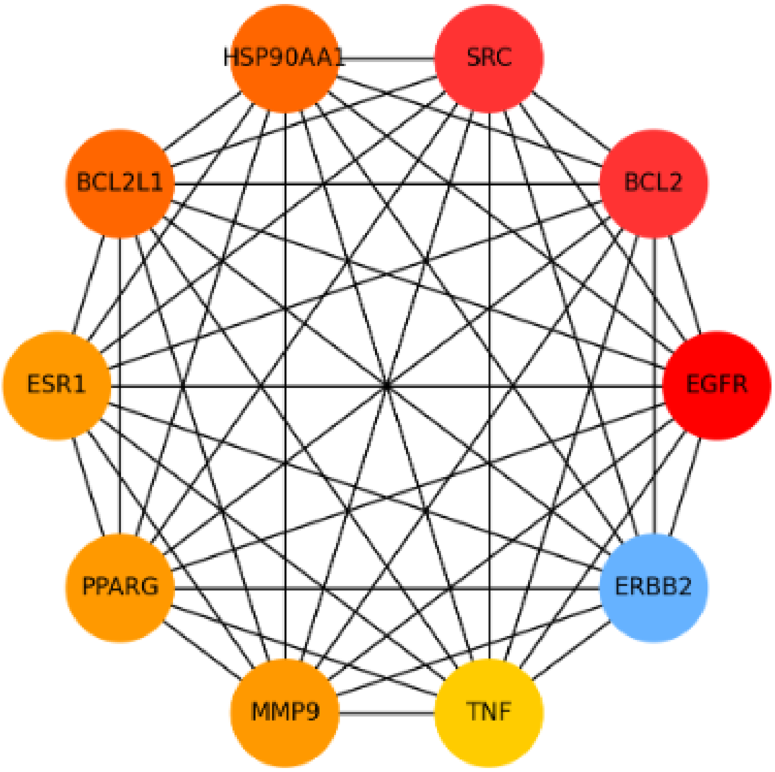

Figure 3: Biological network of Protein involved in the Immunomodulatory Activity.

indicating that these proteins may be the most likely targets for Liquorice, Pipali, and Amla. These results imply that the therapeutic benefits of Liquorice (Root), Pipali (Fruit), Amla (Fruit) against Immunomodulatory Activity may be significantly influenced by these proteins. The Immunomodulatory activity was implicated in 10 of these proteins (as shown in Figure 3). These proteins are listed in Figure 1. Furthermore, supporting the idea that Liquorice (root), Pipali (fruit), Amla (fruit) may regulate immunomodulatory events to exercise its therapeutic effect in Immunomodulatory activity is the discovery of 10 proteins implicated in the immunomodulatory activity within the usual collection of proteins. Targeting immunomodulatory pathways may be a promising treatment strategy for Immunomodulatory Activity since the immunomodulatory response has been linked to the onset and progression of the disease. However, understanding the critical regulatory protein is crucial if we are to effectively control immunomodulatory events in Immunomodulatory Activity.

Based on previously identified immunomodulatory activity proteins, network biology or Protein-Protein Interaction (PPI) analysis was used to determine the most promising target. But only 10 proteins were discovered to create a network of interconnected PPIs. The centrality of the network topology was then examined using betweenness and closeness as criteria. High-closeness nodes tend to be more central to the network and can serve as significant information flow hubs. EGFR was shown to be the network's center point in the current investigation (Figure 3).

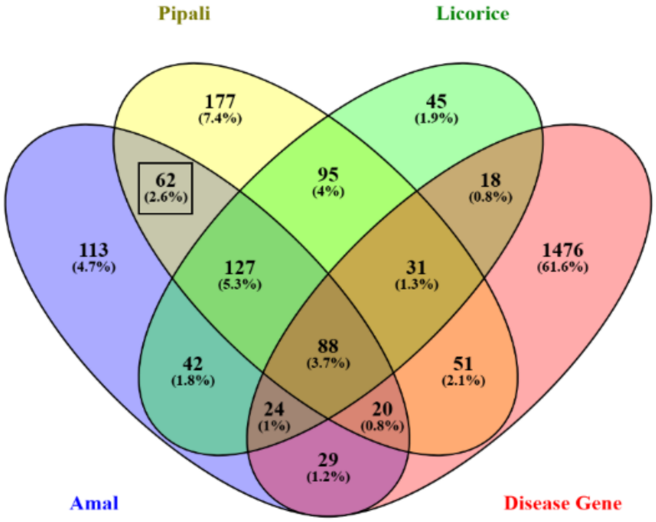

Figure 4: Venn diagram of common protein of Gene Card.

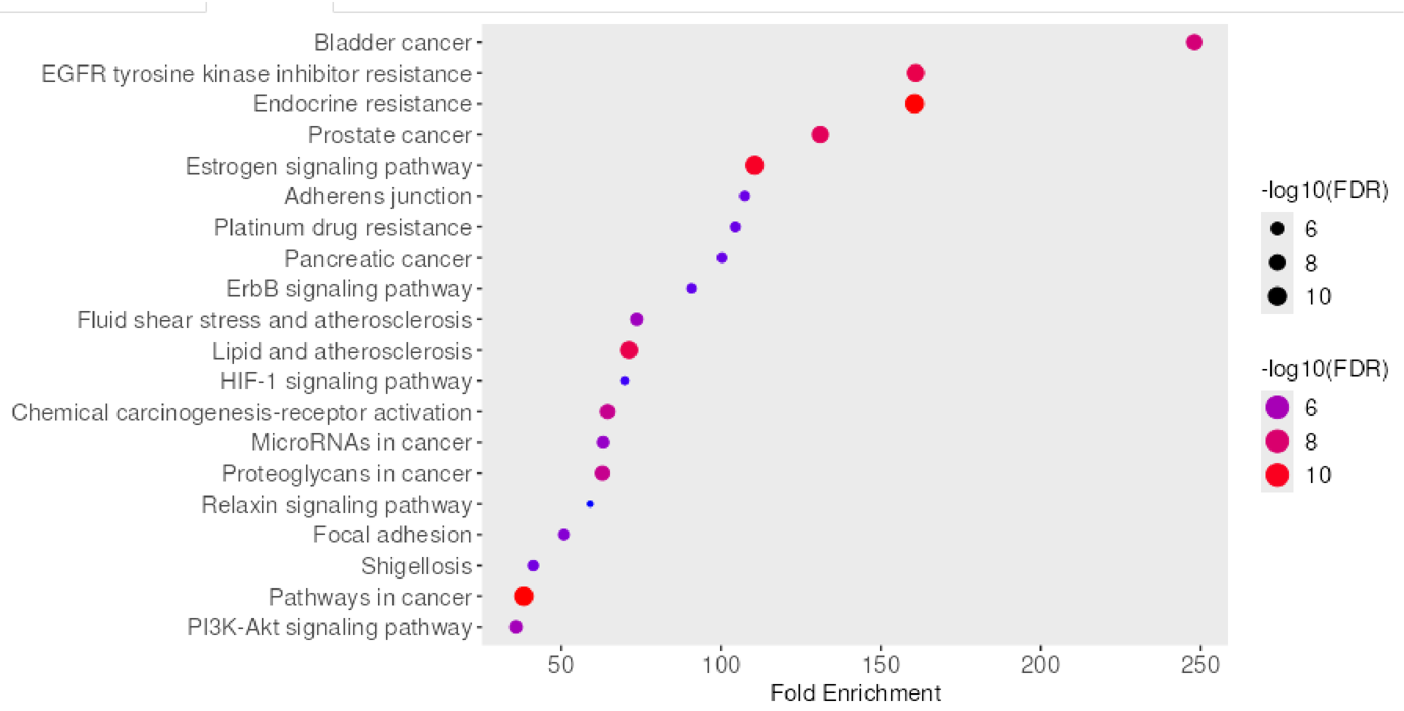

Figure 5: Flod Enrichment KEGG Pathways.

Table 2: **Top 10 Gene involved in Immunomodulatory response.**

1. EGFR	2. BCL2	3. SRC	4. HSP90AA1	5. BCL2L1
6. ESR1	7. PPARG	8. MMP9	9. TNF	10. ERBB2

Figures 4, 5 and Tables 2-4: Using Swiss Target Prediction in conjunction with the Gene Card database, target identification of phytocompounds such as liquorice (root), pipali (fruit), and amla (fruit) through structure-activity relationship analysis indicated possible regulatory activity through an immunomodulatory route. Figure 4 Venn diagram showing the common proteins implicated in the pathophysiology of immunomodulatory activity and those identified as possible targets, Figure 5. An overview of the common proteins that are primarily categorized as immunomodulatory response.

Molecular Docking of the Compounds of *Glycyrrhiza glabra, Piper longum, Phyllanthus emblica* with EGFR

A technique called virtual screening uses target protein information to computationally identify new bioactive chemicals from large chemical libraries. When designing new drugs, molecular docking is utilized to understand how target proteins and ligands interact. The employment of these tactics is supported by the creation of a novel medication with distinct pharmacological activity based on the drug-receptor interaction mechanism. Identification of tiny compounds is made easier by computer-aided drug design, which places and scores them in the active area of the target protein. To carry out the docking simulation technique, Auto Dock vina Version 1.1.2 with *Glycyrrhiza glabra* (Liquorice root), *Piper longum* (Pipali fruit), *Phyllanthus emblica* (Amla fruit) phytochemical and they were docked with 1XKK as target protein.

Table 3: **Top 10 rank of Protein.**

Rank	Protein	Score
1	EGFR	61
2	BCL2	50
3	SRC	48
4	HSP90AA1	47
5	BCL2L1	44
6	ESR1	43
7	PPARG	42
8	MMP9	39
9	TNF	39
10	ERBB2	38

Table 4: Binding affinity score of *Glycyrrhiza glabra, Piper longum*, and *Phyllanthus emblica* phytochemical on immunomodulatory receptor.

Sl. No.	Name of Compound	(EGFR) Binding affinity (-Kcal/ mol)	Molecular Interactions		
			H-Bond	Hydrophobic/ Pi-interaction	Van der Waals
S1	FMM Ligand (Internal ligand)	-10.6	MET A: 793.	GLY A: 721, CYS A: 775, ARG A: 776, LYS A: 745, ASP A: 855, MET A: 766, LEU A: 844, LEU A: 718, PHE A: 856, LEU A: 788, LEU A: 777, ALA A: 743, VAL A: 726.	MET A: 1002, GLY A: 719, ARG A: 841, ASN A: 842, ILE A: 744, THR A: 854, ILE A: 789, THR A: 790, LEU A: 858, GLN A: 791, LEU A: 792, GLY A: 796.
S2	Levamisole (Standard)	-7.1	ASP A: 855.	MET A: 766, PHE A: 856, ALA A: 743, LEU A: 777, VAL A: 726.	LEU A: 858, THR A: 854, CYS A: 775, ARG A: 776, THR A: 790, LYS A: 745.
A	***Glycyrrhiza glabra***				
1	β-Carotene	-8.2		LEU A: 718, LEU A: 844, VAL A: 726, ALA A: 743.	MET A: 1002, LEU A: 792, MET A: 793, GLY A: 796, CYS A: 797, THR A: 854, CYS A: 775, THR A: 790, LEU A: 777, LEU A: 788, ASP A: 745, LYS A: 745.
2	Licochalcone-A	-8.6	PHE A: 856	LEU A: 718, VAL A: 726, LEU A: 844, ALA A: 743, LEU A: 792, MET A: 793, GLN A: 791, ASP A: 855, MET A: 766.	LEUA:858, THR A: 854, LYS A: 745, GLY A: 719, ARG A: 841, CYS A: 797, GLY A: 796, THR A: 790, LEU A: 788, LEU A: 777.
3	Apigenin	-8.6	MET A: 793	VAL A: 726, LEU A: 844, ALA A: 743, LEU A: 718, LYS A: 745.	LEUA:858, THR A: 854, LYS A: 745, GLY A: 719, ARG A: 841, CYS A: 797, GLY A: 796, THR A: 790, LEU A: 788, LEU A: 777
4	Kaempferol	-8.7	MET A: 793	LEU A: 844, VAL A: 726, ALA A: 743, LEU A: 718, LYS A: 745.	LEUA:858, THR A: 854, LYS A: 745, GLY A: 719, ARG A: 841, CYS A: 797, GLY A: 796, THR A: 790, LEU A: 788, LEU A: 777
5	β-Sitosterol	-8.8		LEU A: 777, VAL A: 726, ALA A: 743, LEU A: 718, LEU A: 844, CYS A: 775.	MET A: 1002, GLY A: 719, ARG A: 841, ASN A: 842, ILE A: 744, THR A: 854, ILE A: 789, THR A: 790, LEU A: 858, GLN A: 791, LEU A: 792, GLY A: 796.

Sl. No.	Name of Compound	(EGFR) Binding affinity (-Kcal/mol)	Molecular Interactions		
			H-Bond	Hydrophobic/ Pi-interaction	Van der Waals
6	Quercetin	-8.9	MET A: 793, ASP A: 855, THR A: 854.	LEU A: 718, LEU A: 844, VAL A: 726, ALA A: 743, LYS A: 745.	MET A: 1002, LEU A: 792, MET A: 793, GLY A: 796, CYS A: 797, THR A: 854, CYS A: 775, THR A: 790, LEU A: 777, LEU A: 788, ASP A: 745, LYS A: 745.
B	***Piper longum***				
1	Guineensine	-6.7	LYS A: 745	ARG A: 841, CYS A: 797, MET A: 766, PHE A: 856, LEU A: 718, LEU A: 844, VAL A: 726, ALA A: 743.	MET A: 1002, GLY A: 721, ALA A: 722, MET A: 793, ASP A: 855, LEU A: 788, LEU A: 858, THR A: 854, CYS A: 775, LEU A: 777, ARG A: 776, THR A: 790, LEU A: 792, GLY A: 796
2	Zingiberene	-7.4		LEU A: 777, MET A: 766, LEU A: 788, CYS A: 775, LYS A: 745, VAL A: 726, LEU A: 844, ALA A: 743.	ASP A: 855, LEU A: 858, THR A: 854, THR A: 790, ARG A: 776.
3	Piperundecalidine	-8.2		LEU A: 710, LEU A: 844, LEU A: 792, LEU A: 788, LYS A: 745, VAL A: 726.	GLY A: 721, ARG A: 841, ASN A: 842, GLY A: 796, MET A: 1002, ASP A: 855, MET A: 793, LEU A: 777, LEU A: 856, MET A: 766, THR A: 790, THR A: 854, ALA A: 743.
4	Pipernonaline	-8.2	THR A: 854	LEU A: 718, VAL A: 726, LYS A: 745, ALA A: 743, LEU A: 844.	GLY A: 719, MET A: 1002, GLY A: 796, MET A: 793, LEU A: 792, THR A: 790, LEU A: 788, LEU A: 777, ASN A: 842, ASP A: 855.
5	Piperlonguminine	-8.4		LEU A: 710, LEU A: 844, LEU A: 792, LEU A: 788, LYS A: 745, VAL A: 726.	GLY A: 719, MET A: 1002, GLY A: 796, MET A: 793, LEU A: 792, THR A: 790, LEU A: 788, LEU A: 777, ASN A: 842, ASP A: 855.
6	Piperine	-8.5		LEU A: 718, ALA A: 743, VAL A: 726, LEU A: 844, PHE A: 856, CYS A: 775, LEU A: 777, MET A: 766, ASP A: 855.	GLY A: 796, MET A: 1002, LEU A: 792, MET A: 793, LYS A: 745, THR A: 790, LEU A: 858, ARG A: 776, THR A: 854.
C	***Phyllanthus emblica***				
1	Catechol	-5.3	LEU A: 777, CYS A: 775, PHE A: 856	MET A: 766	THR A: 790, ARG A: 776, VAL A: 769, ASP A: 855, THR A:854.

Sl. No.	Name of Compound	(EGFR) Binding affinity (-Kcal/mol)	Molecular Interactions		
			H-Bond	Hydrophobic/ Pi-interaction	Van der Waals
2	Phloroglucinol	-5.4	ARG A: 776	MET A: 766, LEU A: 777, ASP A: 855.	LEU A: 788, LYS A: 745, LEU A: 858, THR A: 790, CYS A: 775, THR A: 854.
3	Gallic acid	-6.1	ASP A: 855, PHE A: 856.	MET A: 766, LEU A: 777.	LEU A: 788, LYS A: 745, LEU A: 858, THR A: 790, CYS A: 775, THR A: 854, ARG A: 776, VAL A: 769.
4	Methyl gallate	-6.2	PHE A: 856, CYS A: 775.	ASP A: 855, LEU A: 777, MET A: 766.	LEU A: 788, LYS A: 745, LEU A: 858, THR A: 790, CYS A: 775, THR A: 854, ARG A: 776, VAL A: 769.
5	Ellagic acid	-8.7	ASP A: 855, LYS A: 745.	VAL A: 726, LEU A: 844, ALA A: 743, LEU A: 718.	GLY A: 721, ASN A: 842, THR A: 854, THR A: 790, MET A: 793, LEU A: 792, GLY A: 796, MET A: 1002, CYS A: 797.
6	Quercetin	-8.9	MET A: 793, ASP A: 855, THR A: 854.	LEU A: 718, LEU A: 844, VAL A: 726, ALA A: 743, LYS A: 745.	MET A: 1002, LEU A: 792, MET A: 793, GLY A: 796, CYS A: 797, THR A: 854, CYS A: 775, THR A: 790, LEU A: 777, LEU A: 788, ASP A: 745, LYS A: 745.

CONCLUSION

Main phytochemical in the ethanolic extract of *Glycyrrhiza glabra* (*liquorice* root), *Piper longum* (*pipali* fruit), and *Phyllanthus emblica* (*amla* fruit). The potential bioactivity of these phytochemicals for modifying immunomodulatory activity was discovered using network pharmacology-based target identification. Interestingly, according to hub centrality in the network of protein-protein interactions, 1XKK was found to be the most potential target. The molecular docking studies revealed that among the various phytoconstituents present, b carotene, Guineensine and catechol showed promising binding affinity towards EGFR which in turn revealed the downregulation of EGFR. Further, these results were also in accordance with the previous studies reported by Amanda C Herrmann andChantale Bernatchez. This investigation can be further confirmed by performing animal studies using Autoimmune disease models.

ABBREVIATIONS

EGFR: Epidermal Growth Factor Receptor; **PDB:** Protein Data Bank.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Network pharmacology analysis, the top ten target proteins were identified and ranked according to their degree scores. Epidermal Growth Factor Receptor (EGFR) showed the highest degree score, indicating it as the most significant hub protein. It was followed by BCL2, SRC, HSP90AA1, BCL2L1, ESR1, PPARG, MMP9, TNF, and ERBB2. The high degree score of EGFR highlights its central role in mediating interactions with multiple bioactive phytochemicals, suggesting its potential as a key molecular target in the immunomodulatory activity of the studied plant extracts. The study assessed the immunomodulatory potential of phytoconstituents from *Glycyrrhiza glabra, Piper longum*, and *Phyllanthus emblica* through EGFR (PDB ID: 1XKK) suppression via molecular docking. Swiss Target Prediction and GeneCards identified 88 common protein targets, with EGFR as the central immunomodulatory protein. Docking (AutoDock Vina) revealed Levamisole had the strongest binding, followed by β-carotene (Liquorice, -8.2 kcal/mol), guineensine (Pipali, -6.7 kcal/mol),

and catechol (Amla, -5.3 kcal/mol). Key interactions included hydrogen bonding, van der Waals, alkyl, and pi-based interactions at critical EGFR residues. Results suggest β-carotene and guineensine show comparable EGFR inhibition to Levamisole, supporting their potential as plant-derived immunomodulators.

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