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# Network Pharmacology Identifies the Potential Effect of Triterpenes in the Treatment of Powdery Scab Disease in *Solanum tuberosum*

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## Abstract

A vital food crop in the world, potatoes (*Solanum tuberosum*) are severely hampered by fungi that cause illnesses including powdery scab (*Spongospora subterranea*) and late blight (*Phytophthora infestans*). By lowering crop yields and tuber quality, these diseases jeopardize food security and result in significant financial losses. Due to their negative effects on the environment and the emergence of fungicide-resistant strains, conventional management techniques utilizing chemical fungicides have drawbacks. Therefore, in the present study, we aim to examine different approaches for treating potato fungal infections, with a particular emphasis on powdery scab caused by *S. subterranea*. The causative genes of *S. subterranea* were retrieved from the Microbial Genome Database (MBGD). Further, the network was analyzed for the identification of the hub genes using 12 different parameters (MCC, DMNC, MNC, DEGREE, EPC, Bottleneck, EcCentricity, Closeness, Radiality, Betweenness, Stress, Clustering coefficient) on CytoHubba. The "rsgA" gene was identified as the key priority gene affected in powdery scab, and protein modeling was conducted using SWISS-MODEL. Furthermore, a list of antifungal phytochemicals was curated through extensive literature research to determine potential biopesticides effective against *S. subterranea*. Using ADMETLab 3.0, these phytochemicals were screened according to Lipinski's rule of five. Molecular docking revealed the strong binding affinity of triterpenes to the target protein, suggesting their antifungal potential.

**Major Finding:** The study identifies triterpenes as promising biopesticide candidates targeting the rsgA gene of *S. subterranea*, offering an eco-friendly therapeutic solution for powdery scab in potatoes.

**Keywords:** Network, Pharmacology, Powdery Scab, *S. tuberosum*, *S. subterranea*, *rsgA*, Triterpenes

## 1. Introduction

Potatoes, after wheat, rice, and maize, is an important food crop. Millions of people depend on potatoes *(Solanum tuberosum)* as a staple diet, making them a significant worldwide food crop[1]. The world's population will be increasing by approximately 100 million people annually over the next three decades, placing additional demand on water, land, and other resources. To feed this expanding population, farmers in emerging nations will need to increase their productivity by four times[2]. The history of potato cultivation may be found in Peru, a country in Latin America, between 8000 and 5000 BC[3]. When Spanish conquistadors invaded Peru in 1556, they brought potatoes with them to Europe when they discovered their delicious flavour[4]. Sir Walter Raleigh introduced potatoes to Ireland in 1589, planting them on 40,000 hectares of land around Cork[5]. History also revealed that potato production and

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consumption did not spread to the rest of Europe for almost forty years[6]. Farmers in Europe quickly adopted potatoes because of their rich nutritious content and lower labour intensity during growth and cultivation as compared to other crops like sweet potatoes and oats[7]. Additionally, in 1621, Nathaniel Butler, the governor of Bermuda, brought potatoes to America[8]. In 1719, the introduction of potatoes went much more to North America[9]. According to reports, a Dutch sailor travelling to Asia in the 1600s brought potatoes to South Africa. The first potatoes were grown in space in October of 1995. The project was started by the National Aeronautics and Space Administration (NASA) and the University of Wisconsin to feed astronauts during extended space missions[10]. It has been determined that one of Africa's top producers of potatoes is South Africa[11]. The fourth most popular food crop farmed worldwide right now is potatoes. Postharvest losses are preventing a significant proportion of potato-based food from reaching customers[2]. However, their results are often plagued by a range of diseases and pests, with fungal infections being a major concern[12]. Potato-derived food is not getting to customers in large quantities because of postharvest losses. Despite the loss constraint caused by fungal disease infection at both the pre-and postharvest stages of production, *Solanum Tuberosum L.* consumption worldwide and growth are both increasing[13]. Chemical fungicides have been used to manage fungal diseases that affect crops in general, such as blight, scabs, and potato rots[14] because people are aware of the high expense, toxicity, and polluting impacts of chemical fungicides, their use has been progressively less common over time[15]. As a result, fungicides that can be produced from plants must now be obtained from alternate sources. Several studies on plant extracts' antifungal properties against fungi that cause diseases in wheat, rice, peas, tomatoes, and other pertinent crops have been published[16].

Fungal diseases of *Solanum tuberosum*: Potato producers have significant challenges from pests and diseases[17]. During many stages of processing, like harvesting, handling, storing, packaging, and transportation by the producer and the consumer, fungal pathogens can cause postharvest illnesses and food spoilage[18]. Rot illnesses in fresh fruits and vegetables are primarily caused by fungal rot disease pathogens at the postharvest stage[19]. Many fungal species are the source of postharvest illnesses[20]. According to a 2008 analysis by Moss M. O. Moss, 50% of perishable agricultural plants in Hinder developed nations located in tropical climates are lost to fungal infections. Sharma and Kulshrestha[21] showed further that 100% of fruits that have been preserved can be destroyed by the disease-causing Colletotrichum species of fungus. According to Bongomin *et al*[22]. Potato fungal rot infections are generally quite widespread around the world. Late blight diseases have posed the greatest threat to the profitability and output of potato growers. Globally, there has been evidence of the resilience of newly virulent late blight disease strains[23]. According to historical accounts, the late blight epidemic decimated the potato crop in Ireland and throughout Europe in the middle of the 1800s, resulting in a famine that killed around a million lives[24]. An estimated 10 million British pounds are lost annually in poor nations due to late blight[25].

Potato crops are susceptible to a variety of fungal diseases, including powdery scab fungal disease, wart attacks, dry rots, black scurf, late and early blight, and lastly, charcoal rot. These fungi are naturally omnipresent and can produce a diverse array of diseases that plague agriculture, public health, and ecosystems[26]. The fungal pathogens have developed multiple methods to establish infection within their host systems, thereby reflecting a wide range of symptoms and pathogenic outcomes. A single, low-yield problem can create a spiral of severe losses. In the case of potato crops, the infection starts on the leaves and moves down, resulting in both foliar and tuber infections, thereby decreasing global food security and causing severe financial hardship for growers[27]. It was recently found that processing potatoes can also encourage microbial development and break down starch, an essential quality feature[28]. Fresh-cut potatoes' perfume is greatly impacted by how long they are stored; as time passes, browning becomes more apparent, the distinctive smell fades, and disagreeable smells become more overpowering. It's interesting to note that adventitious roots frequently grow on leftover tubers following tuber wounding or potato processing. This may be a result of phenolic compounds reacting with PPO It is unclear, therefore, how anti-browning chemicals would affect these internal quality traits throughout preservation.

Recent studies have identified proteins that give fungal pathogens their pathogenicity, virulence, and other metabolic processes that affect their attack, which are frequently expressed during infection. [29] identified several proteins in *Spongospora subterranea* that enhance pathogenicity and virulence, control vegetative growth, morphogenesis, sexual development, and sporulation, and regulate stress conditions and important molecular processes within the pathogen's biological systems using quantitative liquid chromatography and mass spectrometry (LC-MS/MS)-based proteomic approach. According to

Prabhukarthikeyan *et al.* (2022), the study discovered that the proteins Squalene synthase, Sortin Nexin-4, Heat Shock Protein-70 (HSP70), and Histone Demethylation Protein-1 are linked to the aforementioned tasks[29]. Consequently, investigating the molecular processes by which these AMPs inhibit *Spongospora subterranea* may constitute a substantial knowledge gap. This might entail looking into the binding relationships between AMPs and certain fungus molecular targets, as well as clarifying how these relationships affect important cellular functions linked to fungal formation, proliferation, and pathogenicity.

As the infection progresses, it can extend upward from the tuber, creating spherical, air-filled, orange-to-pink tissue masses. While powdery scab does not always result in direct yield loss, its impact on tuber quality, marketability, and overall potato productivity can be substantial. Infected seed potatoes serve as a vector for pathogen transmission, potentially leading to recurring infections in subsequent crops. The severity of scab lesions often determines whether harvested tubers meet market standards, influencing demand and economic returns for growers[30].

This study aims to enhance potato tuber quality by controlling *Spongospora subterranea-induced* powdery scab disease. To achieve this, a therapeutic antifungal drug will be designed and evaluated using computational approaches, including blind docking and structural modelling. Advanced bioinformatics tools such as SWISS MODEL will be utilised to construct a 3D model of the target protein, facilitating the identification and optimisation of potential antifungal compounds. The primary objective is to develop a targeted therapeutic agent capable of mitigating *Spongospora subterranea* infections, thereby improving potato yield, marketability, and disease resistance. By employing an in silico drug design strategy, this research seeks to provide a novel and effective solution for combating powdery scab, ensuring sustainable potato cultivation and enhanced agricultural productivity. Unlike conventional studies, which lacked computational methodologies for precise drug-target interactions, this research integrates advanced in silico techniques to optimise therapeutic design. The findings are expected to contribute to the development of an effective antifungal agent, ultimately improving tuber quality, preventing disease recurrence, and advancing sustainable disease management in potato cultivation.

## 2. Materials and Methods

### 2.1 Gene Identification

Retrieved genes from the Microbial Genome Database (MBGD) linked to Powdery Scab disease. The database

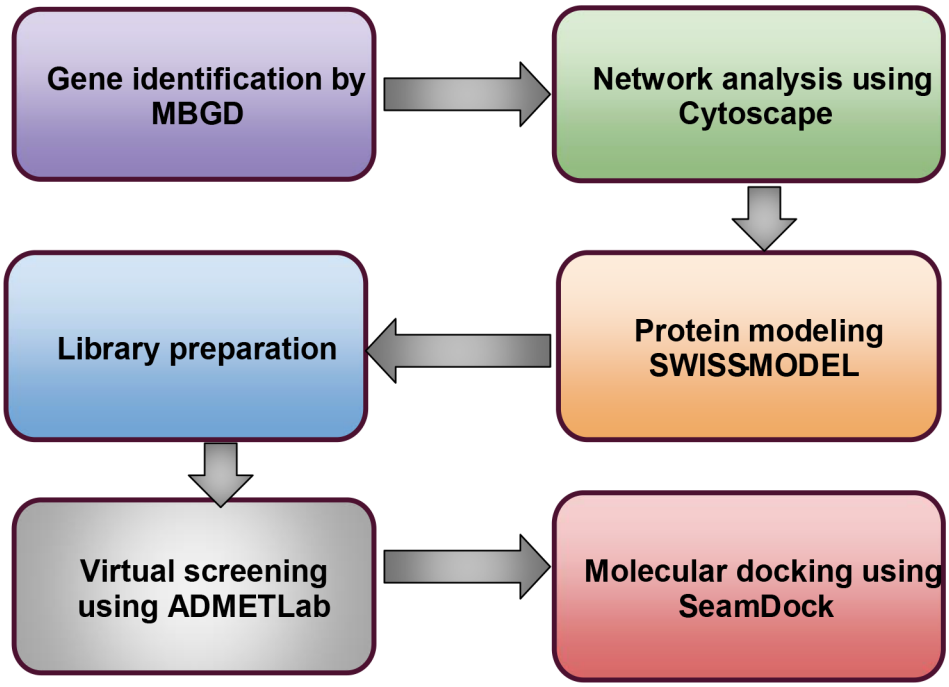



**Figure 1.** Flowchart representing the steps of methodology of study.

was refined to identify genes particularly expressed in *S. subterranea* infected *S. tuberosum* potatoes (Figure 1).

## 2.2 Protein-protein Network Analysis

A string network generated *S. tuberosum* and *S. subterranean* was created using Cytoscape software at different parameters like MCC, DMNC, MNC, DEGREE, EPC, Bottleneck, EcCentricity, Closeness, Radiality *etc*. The resulting protein-protein interaction network was analyzed, and hub genes and their corresponding protein sequences were identified.

## 2.3 Molecular Modeling

Using the hub protein sequences obtained from Cytoscape tool to generate 3-D protein structures using the SWISS-MODEL tool. The protein structures had been downloaded in PDB layout and used for further analysis.

## 2.4 Library Preparation and Virtual Screening

A comprehensive search was done in the literature to find the phytochemical that might have antifungal properties. The names, sources, and canonical SMILES of these chemicals were obtained from the PubChem database. 24 antiviral phytochemicals were compiled into a library. With the use of ADMETLab 3.0, phytochemicals were virtually screened using the Lipinski rule of five in order to create a library of potential lead compounds.

## 2.5 Molecular Docking

Using Seamdock for molecular docking and the protein structure generated in Step 2 as the target protein, we were able to determine the binding affinity of the phytochemicals for precise ligand protein interaction prediction, the docking parameters were adjusted to, spacing = 0.375, mode number = 10, energy range = 5, exhaustiveness = 8, discovered in step 4. The purpose of the molecular docking investigation was to identify potential lead molecules.

# 3. Result

## 3.1 Gene Identification

Microbial Genome Databases (MBGD) were used to obtain genes related to *S. subterranea.* Retrieved a total of 25 genes that were thought to be possibly involved in Powdery Scab pathogenesis or virulence processes.

## 3.2 Protein-protein Network Analysis

### *3.2.1 Cytoscape and CytoHubba*

Determine hub genes or proteins inside the network by applying 12 different network analysis parameters analyzing under top 10 with confidence level 0.4 (MCC, DMNC, MNC, DEGREE, EPC, Bottleneck, EcCentricity, Closeness, Radiality, Clustering coefficient network) (Figure 2), (Table 1), identified the "rsgA" (PBRA_006710) as a priority gene affected in powdery scab hub genes that may play a critical role in the interaction between *S. subterranea* and potatoes.

## 3.3 Protein Modeling

SWISS-Model: The protein sequences obtained from Cytoscape were used to generate 3D protein structures.

"rsgA" as PBRA_006710 protein sequences:

**Table 1.** Represents the different genes at different parameters

| MCC | DMNC | Degree | MNC | EPC | Bottleneck | EcCentricity | Closeness |
|---|---|---|---|---|---|---|---|
| PBRA_007946 | PBRA_007946 | PBRA_007946 | PBRA_007946 | PBRA_007946 | PBRA_006710 | PBRA_006710 | PBRA_007946 |
| PBRA_006710 | PBRA_006710 | PBRA_006710 | PBRA_006710 | PBRA_006710 | PBRA_002973 | PBRA_002973 | PBRA_006710 |
| PBRA_002973 | PBRA_002973 | PBRA_002973 | PBRA_002973 | PBRA_002973 | PBRA_001389 | PBRA_001389 | PBRA_002973 |
| PBRA_001389 | PBRA_001389 | PBRA_001389 | PBRA_001389 | PBRA_001389 | PBRA_004991 | PBRA_004991 | PBRA_001389 |
| PBRA_004991 | PBRA_004991 | PBRA_004991 | PBRA_004991 | PBRA_004991 | PBRA_000146 | PBRA_000146 | PBRA_004991 |
| PBRA_000146 | PBRA_000146 | PBRA_000146 | PBRA_000146 | PBRA_000146 | PBRA_006428 | PBRA_006428 | PBRA_000146 |
| PBRA_006428 | PBRA_006428 | PBRA_006428 | PBRA_006428 | PBRA_006428 | PBRA_005349 | PBRA_005349 | PBRA_006428 |
| PBRA_005349 | PBRA_005349 | PBRA_005349 | PBRA_005349 | PBRA_005349 | PBRA_003294 | PBRA_003294 | PBRA_005349 |
| PBRA_003294 | PBRA_003294 | PBRA_003294 | PBRA_003294 | PBRA_003294 | PBRA_005256 | PBRA_005256 | PBRA_003294 |
| PBRA_005256 | PBRA_005256 | PBRA_005256 | PBRA_005256 | PBRA_005256 | PBRA_003574 | PBRA_003574 | PBRA_005256 |

## String Networks

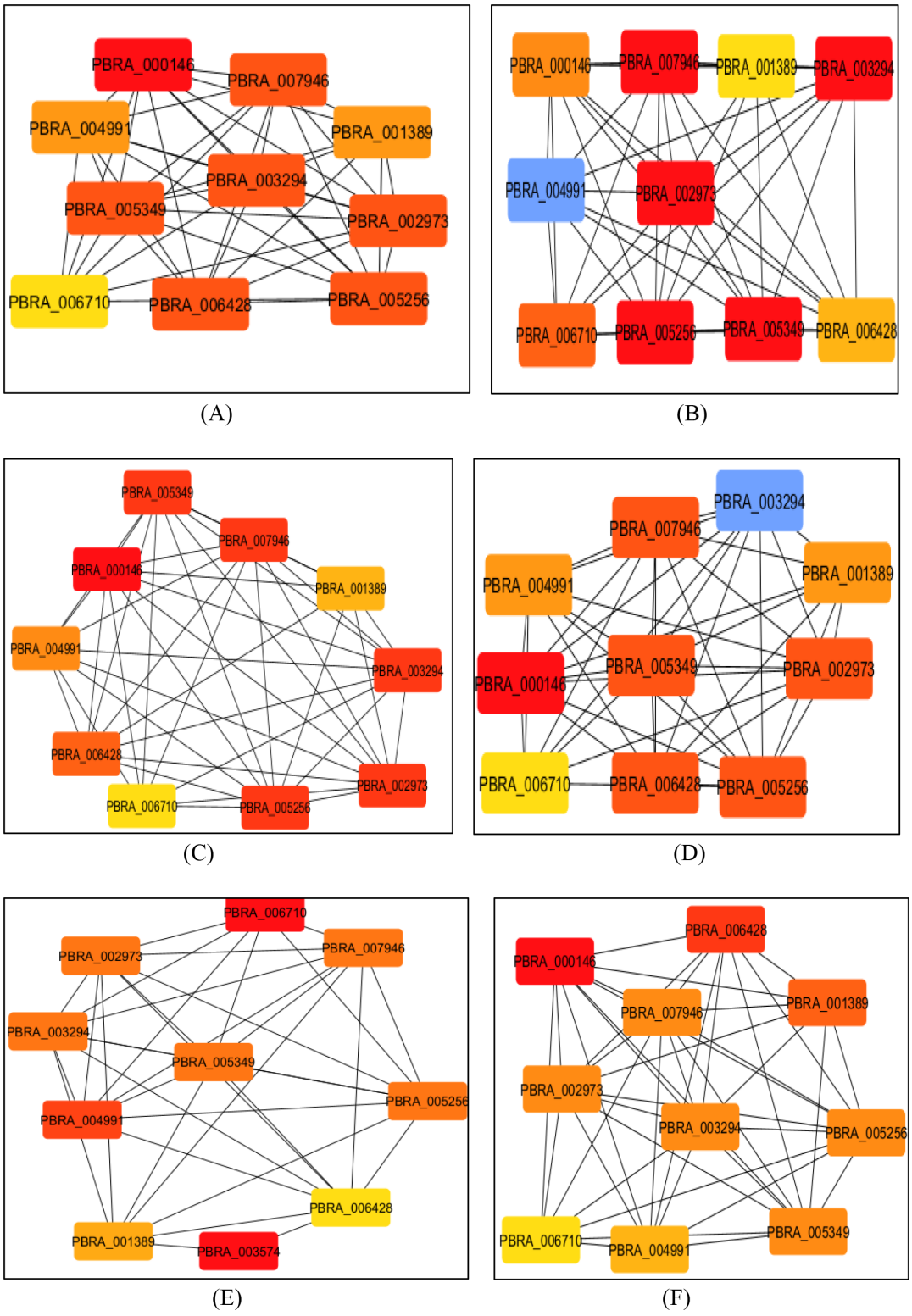



**Figure 2:** String network using 12 different parameters with slight interaction between networks. **(A)** MCC **(B)** DMNC **(C)** MNC **(D)** Degree **(E)** EPC **(F)** Bottleneck **(G)** EcCentricity **(H)** Closeness **(I)** Radiality **(J)** Betweenness **(K)** Stress **(L)** Clustering Coefficient.

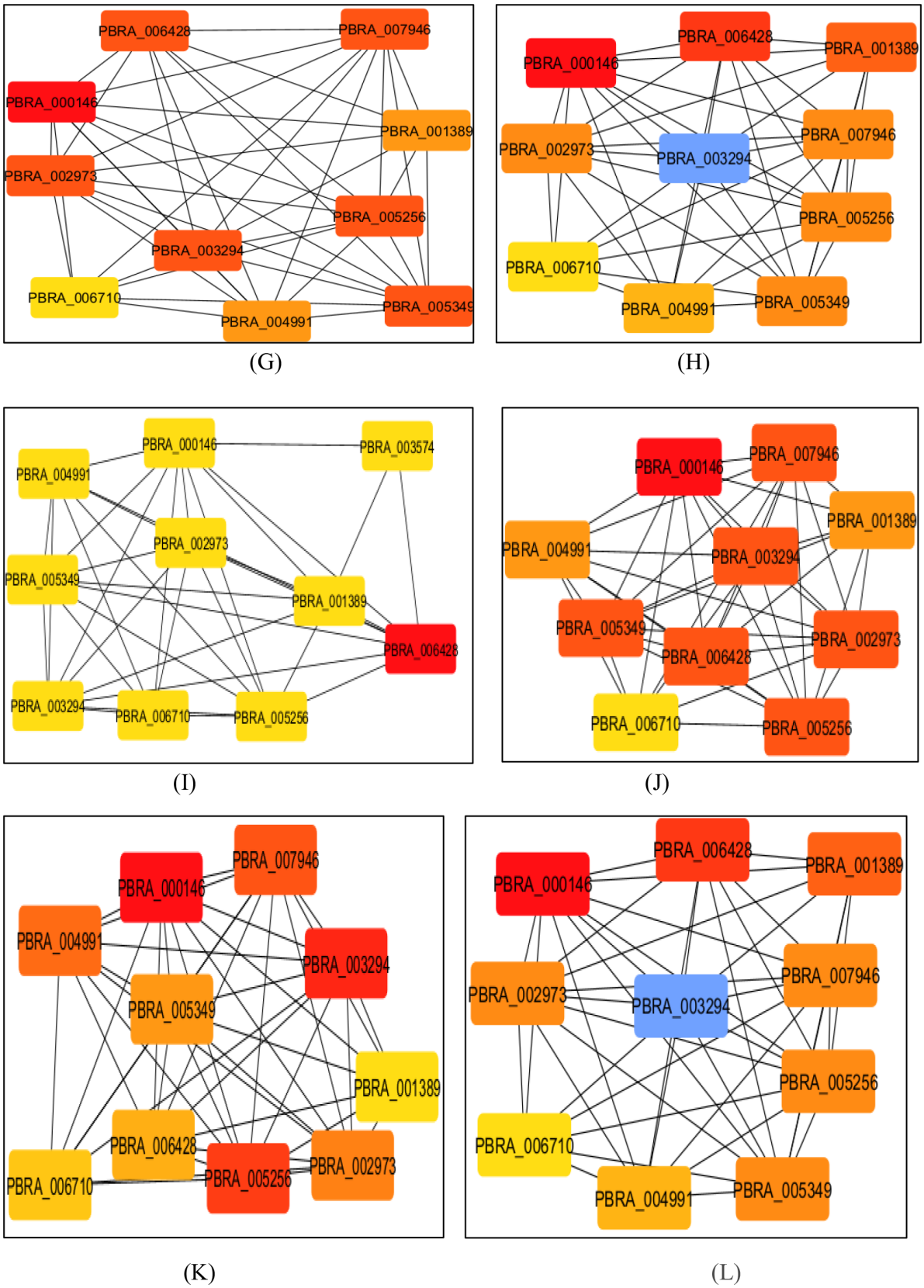



**Figure 2:** Continued...

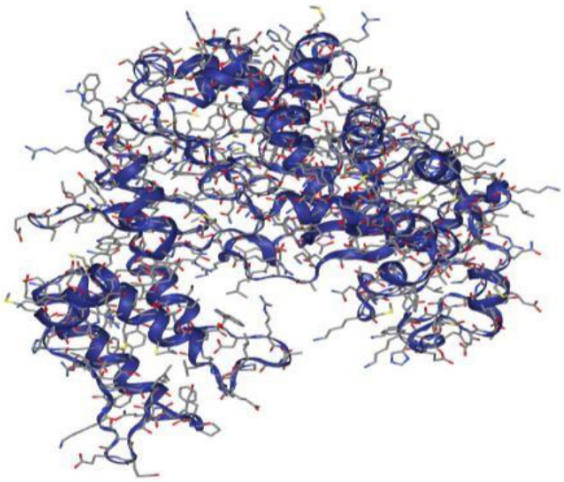


**Figure 3.** 3D protein structure of "rsgA" gene, generated by SWISS-Model.

***AlphaFold DB model of A0A0G4IU35_PLABS (gene: A0A0G4IU35_PLABS, organism: Plasmodiophora brassicae (Clubroot disease agent))***

## 3.4 Library Preparation and Virtual Screening

Through literature review and PubChem a library of 24 potential antifungal phytochemicals were created that highlights the antifungal property. Using Lipinski's five guidelines, ADMETLab 3.0 was used to test the phytochemical library for drug-likeness and pharmacokinetic characteristics. Out of 24 antifungal phytocompound 20 follows Lipinski's rule of five. Here is the Table 2 representation of phytochemicals with Lipinski's rule accepted or rejected.

**Table 2.** List of phytochemicals along lipinski rule accepted or rejected and plant source

| Antifungal Phytochemicals | Plant Source | Lipinski Rule |
|---|---|---|
| Allicin | *Allium sativum, Allium spp.* | Accepted |
| Thymol | *Lamiaceae* | Accepted |
| Eugenal | *Syzygium aromaticum, Eugenia caryophyllus* | Accepted |
| Berberine | *Berberis vulgaris,Berberis fremontii, Hydrastis Canadensis* | Accepted |
| Curcumin | *Curcuma longa* | Accepted |
| Tea tree oil | *Melaleuca alternifolia (Myrtaceae)* | Accepted |
| Caprylic acid | coconuts and breast milk | Accepted |
| Resveratrol | *Polygonum cuspidatum, grandiflorum,* | Accepted |
| Cinnamaldehyde | *Cinnamomum ceylanicum* | Accepted |
| Gingerol | *Zingiber officinale,* | Accepted |
| Flavan-3-ol | *Syzygium cordatum* | Accepted |
| Baicalein | *Scutellaria baicalensis* | Accepted |
| Ulopterol | *Skimmia laureola* | Accepted |
| Prenyletin | | Accepted |
| methyl-ether | ethyl methyl ether | Accepted |
| Osthenol | | Accepted |
| 5,8 Dihydroxyumbelliprenin | *Ferula feotida* | Accepted |
| Triterpenes | Ethyl acetate leaf extract of *Sahkhuzestanica* | Accepted |
| Lectins | Seed from native *Amanon* species | Accepted |
| Ellagic acid | *Paance granotem* | Accepted |
| Punicalagin | *Punica granatum* | Rejected |
| Lambertianin C, | *Oregon* | Rejected |
| Sanguiin H-6 | *Sanguisorba offcinalis* | Rejected |
| Colchiside | *Autumn crocus* | Rejected |

MARFANFAGKSEFVFQAMILACVYVLAFCVRLFSVLRHESVIHEFDPWFNFRTTKYLVKEGFYEFHNWFDEMTWYPY
GRYIGGTVYPGLMWTAALVYKVLHGLNLPVDIRNVCVLLAPWMAGNTSIVTYLLTKQAWTSAAGLVAAAFMSLAPG
YISRSVAGSYDNEAVAIFAMVCTFWLWLKALDTGSMLWSAACAVSYFYMVAAWGGYVFIWNIIAVHAFVLVICGRYSH
RLYVSYCTWFVLGLLMSMQVMFVSFQPVTSPEHMPSAGVFCLLQIYAFLRYIHAHVTPKQFRVIVRLGVLTVLAIIGFV
LFLAVTGTVPFLTGRLKSLMGATSNIAIVKSVSEHQPTKWLTFFLDLHMMVAFAPVGMYYVFWSVSDTNLFLVLYMLF
SGYFASIMIRLMLVISPIACVLAGIGVSETLLTF SHILTMPASGDKGASSRASPEPKRPYHSSQEEQRTWYRQTKVKSIPAP
ICIGVLAFFMFMFAFFVPHCSYMT ANMYSSPQVVLSAKAGDQLIIFDDFREAYQWLNHNTEDDAKILSWWDYGYQIT
GMGNRSVIVDNNTRNN THIATVGLVMASNEERAIDVIRSLDVKYLLVVFGGMVGFSSDDINKFLWMVRISGGVYPEV
VETDYFNSRGEYRIDHTVSDRMKNSLMYQMCYYRFGDVKVQWGQEGGFDRVRNAVIGHKDIKFRYLEEAFTSEHWM
VRI YRVKNIA

**Table 3.** List of phytochemicals and their amino acid residues and binding energy between phytochemicals and protein of rsgA gene

| Phytochemicals Antifungal | Binding Energy | Amino Acid Residue |
|---|---|---|
| Allicin | -4.03 | W25,P44,L46, F111,V134 |
| Thymol | -5.54 | W25,F168V170,F111 |
| Eugenal | -5.67 | W25,P44L46,V134,V134,F168,V170,F111,G110 |
| Curcumin | -7.88 | L46,L48,W132,V170,T172,F111,L113,V115,A112,R114 |
| Tea tree oil | -5.87 | Y77,R29 |
| Caprylic acid | -4.21 | R119,I97,L125,L128 |
| Resveratrol | -7.07 | W25,P44,L46,F111,V134,F168,V170,V45,Y171, |
| Cinnamaldehyde | -5.02 | W25,P44,L46, F111,V134,F168,V170 |
| Gingerol | -6.6 | L46,F111,V115,V170,Y171,L113, R114,A112,R114 |
| Flavan-3-ol | -7.73 | W25,L46,F111,F168,V170 |
| Baicalein | -7.95 | W25,P44,L46,F111, V134,F168,V170,Y171, |
| Ulopterol | -7.97 | W25,L46,F111,V134,F168,V170 |
| Prenyletin | -7.22 | W25, L46,L113,F168,V170,F111 |
| methyl-ether | -3.3 | N60, I62,H64,D65, |
| Osthenol | -6.95 | W25,P44,L46,F111,V134,F168,V170, Y169 |
| 5,8 Dihydroxyumbell iprenn | -8.98 | L46,F111,L113,W132,V170,T172,Y171,T173,R114 |
| Triterpenes | -9.08 | R114,R175,L48,L113,V115,T116,L130,W132,T172,M174 |
| Lectins | -6.64 | V115,W132,T173,F111,L113,Y171,A112 |
| Ellagic acid | -7.72 | F168,W25,F111,G109,G110 |

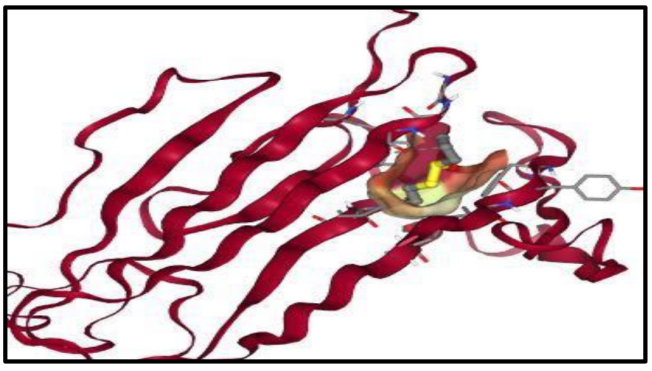


**Figure 4.** Shows the molecular docking image of phytochemicals with protein structure of rsgA (hub gene) obtained from SeamDock.

## 3.5 Molecular Docking

SeamDock Docking: "Triterpenes showed great therapeutic potential against Powdery scab, based on molecular docking studies between the screened phytochemicals and the 3D protein structure of the "rsgA" gene (Figure 4). The docking analyzes demonstrate that triterpenes and the "rsgA" protein have a high binding affinity, indicating that the chemical is effective as an antifungal. Out of the twenty phytochemicals, triterpenes have the lowest binding energy (-9.08 kcal/mol) and the maximum binding affinity towards the protein structure of fungi. (Table 3), Indicating a strong binding affinity followed by

Berberine (-8.68 kcal/mol), 5,8 Dihydroxyumbelliprenn (-8.98 kcal/mol).

## 4. Discussion

*Spongospora subterranean,* a soil-dwelling pathogen responsible for the Powdery scab disease in potatoes, is known for its dual role as a destructive agent and a potential source of antifungal properties. This pathogen infects potato plants by penetrating root hairs or exploiting wounds, and its life cycle is sustained in the soil through dormant spore structures. Addressing the challenges posed by this pathogen necessitates innovative strategies. An integrated, multi-step approach, including virtual screening, protein modeling, and genomics, has been utilized to identify putative antifungal phytochemicals capable of mitigating fungal diseases in potato crops. A pivotal discovery in this process was the identification of the rsgA gene, which plays a critical role in the pathogens biology. Using data extracted from the microbial genome qatabase, researchers analyzed the gene's associated protein-protein interactions, uncovering potential molecular targets for antifungal intervention. Several phytochemicals, including triterpenes, 5,8-dihydroxyumbelliprenin, and berberine, demonstrated significant binding affinity to these targets, suggesting their potential antifungal activity[31]. Among these compounds, triterpenes have emerged as especially promising biocontrol agents against powdery scab-a disease that disproportionately affects potatoes but also threatens other crops. Triterpenes are complex, structurally diverse molecules belonging to the terpenoid family. They are widely distributed in nature and exhibit significant biological activity due to their diverse functional roles. Notably, fungi such as *Ganoderma lucidum* produce ganoderic acids, a type of triterpene renowned for their therapeutic and antifungal properties. Furthermore, marine organisms such as sponges and algae contribute to the natural pool of triterpenes, likely as part of their chemical defense or signaling mechanisms[32]. The development of sustainable fungicidal solutions emphasizes the role of bioinformatics and molecular modelling to identify, characterize, and optimize triterpenes as biocontrol agents. Computational approaches facilitate the prediction of triterpene-disease target interactions, enabling precise selection and refinement of compounds for enhanced efficacy. Such predictive tools accelerate the formulation of innovative triterpene-based treatments specifically designed to combat powdery scab, reducing environmental risks while safeguarding crop health.

This coordinated effort underscores the potential of triterpenes not only as effective biocontrol agents but also as a foundation for environmentally friendly fungicide development. By leveraging advanced molecular modelling and bioinformatics, this strategy paves the way for safer, more efficient crop protection measures, addressing the persistent threat posed by fungal pathogens like *Spongospora subterranean*[33].

## 5. Conclusion

This study comprehensively analyses *Spongospora subterranea*, the causative agent of powdery scab in potatoes, and highlights a novel biocontrol approach for disease mitigation. Through protein-protein interaction studies using Cytoscape and microbial genome databases, the *rsgA* (*PBRA_006710*) gene was identified as a key virulence factor in the pathogenic mechanisms of *S. subterranea*. Structural modelling via SWISS-MODEL enabled the three-dimensional characterisation of the *rsgA*-encoded protein, facilitating targeted therapeutic exploration. The in-silico screening of a phytochemical library revealed promising antifungal candidates, with triterpenes exhibiting the highest binding affinity to the *rsgA* protein. These findings underscore the potential of triterpenes as effective, environmentally sustainable antifungal agents that could serve as an alternative to conventional chemical fungicides. By integrating computational and molecular modelling techniques, this study not only enhances our understanding of *Spongospora subterranea* pathogenesis but also offers a foundation for developing targeted, eco-friendly disease management strategies.

Ultimately, this research contributes to sustainable agricultural practices by promoting biopesticide-based interventions that improve potato crop resilience. The identified phytochemicals may serve as potential leads for future antifungal formulations, reducing the reliance on synthetic fungicides and supporting global food security in the face of evolving environmental and economic challenges.

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