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Network Pharmacology Approach Reveals the Antibacterial Efficacy of Conessine Against Bacterial Wilt Disease caused by *Ralstonia solanacearum*

Rashmi Dubey

Amity Institute of Biotechnology, Malhaur Railway Station Road, Lucknow, Uttar Pradesh 226010

ABSTRACT: Ladyfinger **(*Abelmoschus esculentus*)** is very prone to bacterial diseases, and the bacterial wilt caused by ***Ralstonia solanacearum*** is one such disease that presents formidable challenges in the domain of agricultural production. The current research work has been carried out with a view to ascertain the anti-bacterial potential of phytochemicals against some of the bacterial diseases in ladyfinger, particularly against ***Ralstonia solanacearum***. Computational methodologies have been adopted in the present study by using a combination of bioinformatics tools for evaluating antibacterial potential with molecular docking studies of antibacterial phytochemicals. The MBGD database was used to identify candidate genes of ***Ralstonia solanacearum.*** Building and analysis of gene networks were done using Cytoscape and STRING. Then, the hub gene identification was performed with CytoHubba based on the following 12 parameters: MCC, DMNC, MNC, Degree, EPC, Bottleneck, EcCentricity, Closeness, Radiality, Betweenness, Stress, Clustering coefficient. The **'obg'** identified hub gene was further modeled in SWISS-MODEL for its protein structure prediction. Literature studies were used to identify potential phytochemical ligands, which were further screened virtually using ADMETLab 3.0 according to their ligand properties for absorption, distribution, metabolism, excretion, and toxicity. Molecular docking studies were finally done using SeamDock, taking the identified priority gene as the receptor and the 20 screened phytochemicals. Molecular docking studies revealed the antibacterial potential of **Conessine** ($C_{24}H_{40}N_2$) obtained from stem bark of ***Holarrhena floribunda*** (-9.01 kcal/mol), member of family Apocynaceae, to be a drug of efficacy for the treatment of bacterial wilt in ladyfinger plants. The results indicate several phytochemical candidates, possibly from plants of renowned antimicrobial properties, to show promising interactions with the ***Ralstonia solanacearum*** hub gene. It uses computational and experimental approaches to enhance the understanding of natural compounds in controlling bacterial diseases in ladyfinger cultivation.

Keywords: *Ralstonia solanacearum*, ladyfinger, obg gene, Conessine, network biology

Introduction: *Abelmoschus esculentus* (L.) cultivated is being very famous with the names like okra or ladies' finger around the world and it is important to the diet as the greenhouse crop has a wide distribution throughout Asia, Africa, Southern Europe, and America as well (Ndunguru J., 2004). The dry seeds in particular are highly nutritious due to their good quality oil and protein-rich meal that is left after extraction (Ahmed K.U et al, 1995). Okra is also a great therapeutic vegetable, among other things, it is used for mental diseases like depression and physical illnesses that cause weakness. It is also beneficial for ulcers, joint health promotion, pulmonary inflammations, bowel irritations, and sore throats too (Adelakun O.E et al., 2008). This problem is managed by the presence of dietary fibre in okra in a way that the sugar level in the blood is being effectively handled because the sugar absorption rates are being modulated in the gut. Still, the topic is the research of okra in relation to hepatoprotective properties. Although such research was earlier conducted, the one by Liu et al is the most famous and more relevant. (Liu I.M et al., 2005). Note also mentioned in the paper (Rao P.S et al., 1991) besides the answer. At the regional level, okra is marketed by different names that it is reflected in its global importance and cultural significance (Terell E.E et al., 1974). The debate about geographical origin anchors on the following points: South Asian, Ethiopian, or West African, to name a few (Mc Whorter., 2000). It is not necessarily found only in Asia but it is believed that Greece also belongs to the native range of okra. However, in these areas, it grows as a tropical, subtropical, and warm temperate plant and it shows adaptability to the soil that should be, preferably, fertile and well-drained through the use of organic manure for getting the best yields (Moyin-Jesu E.I, 2007).

GEOGRAPHICAL ORIGIN AND DISTRIBUTION OF LADYFINGER

Okra, also known as lady's finger, was classified under the genus **Hibiscus** specifically under the section *Abelmoschus* of the **Malvaceae** family (Linnaeus C., 1753). It was initially in this category, but what really happened was that in the 6th proposal the section Abelmoschus was raised to the rank of a distinct genus (Medikus F.K, 1787) which was then accepted in taxonomic as well as the contemporary literature (Hochreutimer B.P.G, 1924). Hibiscus bears a spathulate calyx that is characterized by and has five short connate teeth that are released after flowering (Kundu B.C et al., 1973). Okra lay on success path on the settled and tropical places around the world (Arapitsas P, 2008). It is suitable to be grown on both big farms and small gardens (Rubatzky V.E et al., 1997). It is commercially grown in many different countries, like Indonesia, Japan, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Myanmar, Malaysia, Thailand, Brazil, Ethiopia, Cyprus, and the Southern United States (Purseglove J.W, 1987) (Benjawan C et al., 2007). When the okra was discovered in the America, it was brought along the Atlantic slave trade and was first documented in Brazil in 1658. Later it was noticed at Suriname's shores in 1686. The corn likely was imported from Africa to southeastern North America in the 18th century with a record of its growth in Philadelphia in the north in 1748. The year 1781 witness its strong root in Virginia, and 1800 was the time it hit the South, and the varieties were noted as early as 1806 (UniversityTexasA&M, 2005)

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BIOCHEMICAL COMPOSITION OF LADYFINGER

Components	Okra pods	Okra leaves	Okra seeds
Water	88.6g	81.5g	
Energy	144.00kJ (36kcal)	235.00 KJ	
Protein	2.10g	4.40g	20%
Carbohydrate	8.20g	11.30g	
Fat	0.20g	0.60g	20%
Fibre	1.70g	2.10g	
Calcium	84.00mg	532.00mg	
Phosphorus	90.00mg	70.00mg	
Iron	1.20mg	0.70mg	
Beta-Carotene	185.00µg	385.00µg	
Riboflavin	0.08mg	2.80mg	
Thiamine	0.04mg	0.25mg	
Niacin	0.06mg	0.20mg	
Ascorbic acid	47.00mg	59.00mg	
Others	-		High in unsaturated fats
Uses	Fresh consumption cooking	Edible leaves buds, flowers	Oil production, supplement for fortifying flour, coffee substitute
Additional information	-	Carbohydrates in mucilage form, used in paper industry	Roots and stems used for clarification of sugarcane juice

(Table 1. Representing biochemical composition of ladyfinger)

STRUCTURE AND PHYSIOLOGY

The widely grown vegetable commonly known for its fibrous pods and round, white seeds throughout the world's tropical and warm-temperate regions is okra (Abelmoschus esculentus). It is famous for its extraordinary ability to tolerate both drought and heat waves that makes it one of the most reliable crops even in harsh climates where soils are heavy with clay but receive moisture at short intervals. However, frost will injure its pods thus affecting farming processes. If they are left unharvested for too long after pollination, okra pods grow tough and inedible due to becoming fibrous and woody as they mature very quickly. Harvest within seven days of pollination usually offers best flavor and softness before cooking or processing young, immature ones into the well-known but troublesome food called okra.

OKRA SEEDS

Roughly 20% of okra seeds are oil and 20% are proteins. There may be a hypocholesterolemic effect from okra seed oil. Okra has a very high potential for widespread cultivation for cake and edible oil. Cereal flour could potentially be strengthened with okra seed flour. For a very long time, nations like Egypt have been mixing corn flour with okra seed flour to produce dough of superior quality. In several places, the ripe seeds are pulverized, roasted, and consumed in place of coffee. The paper industry uses mature fruits and stems with crude fibre. Pressed from okra seeds, greenish-yellow edible okra oil has a good taste and aroma and is rich in unsaturated fats like linoleic and oleic acid.

INSECT PEST ON LADYFINGER

Okra production is seriously threatened by insect pests including shoot and fruit borers (*Earias vittella* and *Earias Insulana*), which can destroy vulnerable fruits up to 100%. Their larvae burrow into shoots and fruits, rendering them unfit for human eating, and they lay their eggs on leaves and fruits. Amrasca biguttula biguttula, or leafhoppers, cause further harm by lowering yields by 32.06% to 40.84%. Since chemical insecticides are widely used by farmers in 95% of Asian countries, there is an urgent need for sustainable pest control methods (RadakeS.G etal, 1981).

DISEASES OF OKRA PLANT

1) Bacterial wilt (Caused by *Ralstonia Solanacearum*)

Causative Agent: The presence of a Gram-negative bacterium called Ralstonia Solanacearum is responsible for bacterial wilt in ladyfinger.

Symptoms: Plant wilts while leaf becomes yellow causing plant death. Management: One needs to rid of these pathogens by being careful to take healthy seeds, soil solarization and crop rotation

Management: One needs to rid of these pathogens by being careful to take healthy seeds, soil solarization and crop rotation (Smith, 2023).

2) Okra Yellow Vein Mosaic Virus (OYVMV):

Causative Agent: It is caused by Begomovirus (monopartite)

Symptoms: Symptoms: Include yellowing and mosaic patterns on leaves; leaf curling; stunted growth; and decreased yield.

Management: Planting virus-free seeds, controlling whiteflies (the virus vectors), and utilizing resistant varieties are some effective strategies. (Siddique et al., 2019)

3) Powdery Mildew

Causative Agent: Podosphaera xanthii causes it.

Symptoms: It is identified on leaves, stems, pods and sometime leading to leaf distortion and reduce photosynthesis.

Management: Management: Better air circulation, discouraging overhead watering canopies, and applying appropriate fungicides as a preventative measure are some of the management practices which have been proposed. (Ahmed et al., 2000)

4) Fusarium Wilt

Causative Agent: Fusarium oxysporum is the cause of this.

Symptoms: They start as yellowing and wilting of lower leaves and extend to the whole plant wilting and observable discolouration of vascular system in stems

Management: The best way of controlling the disease is through crop rotation with plants. that do not host it, (Bally et al., 2018)

5) Bacterial Leaf Blight

Causative Agent: Xanthomonas campestris pv. malvacearum is the causative agent.

Symptoms: - Symptoms: The symptoms of this disease comprise of water-soaked spots on leaves that ultimately turn brown and necrotic hence leading to defoliation and loss in yield.

Management: Management: Management approaches range from sowing clean seeds to practicing crop rotation and utilizing copper-containing fungicides enforce(Verma et al., 2017)

6) Anthracnose

Causative Agent: Caused by different species of Colletotrichum

Symptoms: Shows as dark sunken lesions on pods, which increase in size leading to pod rot and improper maturing affecting the marketability.

Management: Sanitation measures are very effective in management while removal of infected plant debris and application of fungicidal treatments should be done during humid condition.(Das et al., 2019.).

Antibacterial Potential of Phytochemicals Against Common Bacterial Diseases in Ladyfinger

1) Types of Phytochemicals and Their Sources

These are some of the active compounds occurring naturally in plants with different pharmacological characteristics among them alkaloids, flavonoids terpenoids or phenolic compounds and so on which thus far have been extensively researched on because they possess antimicrobial activity.

	A	B
1	Phytochemicals name	Plant source
2	Allicin	*Allium sativum*
3	Conessine	*Holarrhena floribunda, Holarrhena antidysenterica, Funtumia elastica*
4	Thymol	*Thymus vulgaris, Thymus capitatus*
5	Carvacrol	*Origanum vulganum*
6	Eugenol	*Syzygium aromaticum, Eugenia caryophyllus*
7	Berberine	*Berberis vulgaris, Berberis fremontii, Hydrastis canadensis*
8	Curcumin	*Curcuma longa*
9	Quercetin	*Capparis spinosa, Polymnia fruticose, Ginko biloba*
10	Epigallocatechin	*Camellia sinensis*
11	Catechin	*Fructus crataegi*
12	Genistein	*Glycine max*
13	Luteolin	*Scrophularia frutescens*
14	Gallic acid	*Vitis rotundifolia*
15	Ajoene	Allium sativum
16	Sulphoraphane	*Diplotaxis harra*
17	Lysergol	*Ipomoea muricata*
18	Reserpine	*Rauwolfia serpentina*
19	Kaempferol	*Alpinia calcarata*
20	Aegelinol	*Ferulago campestris*

(Table 2: Representing phytochemicals and their sources)

2) Antibacterial Mechanisms

Phytochemicals show antibacterial activities though different mechanisms:

Disruption of cell membrane

- Many phytochemicals destabilize bacterial cell membranes.
- It causes leakage of cellular contents and ultimately cell death.

Inhibition of Biofilm formation

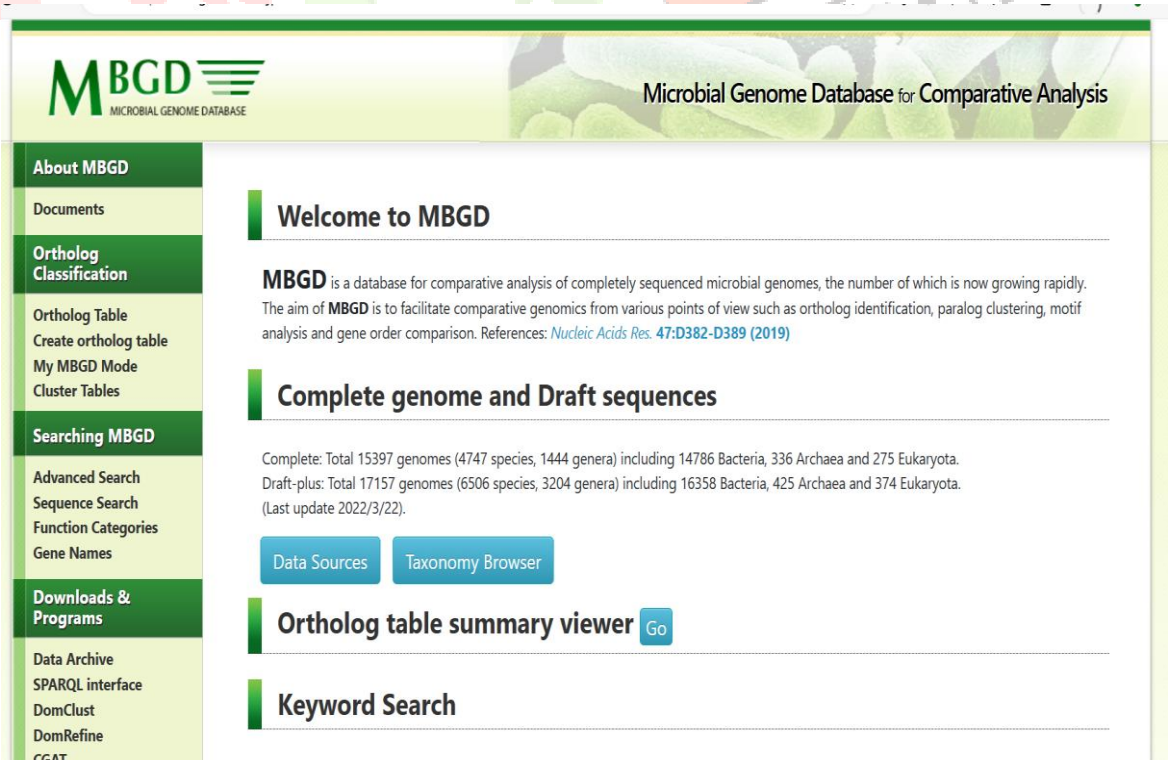
- Biofilms protect bacteria from external factors and antibiotics.
- Phytochemicals disrupt biofilm formation, enhancing the susceptibility of bacteria towards treatment.

Interference with Quorum Sensing

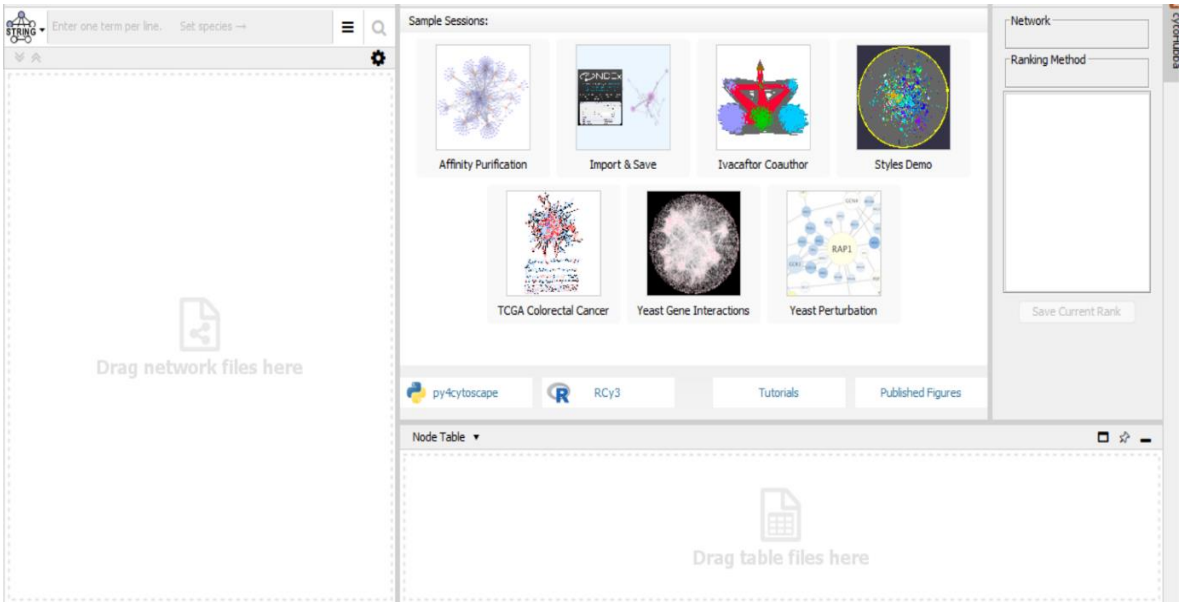
- Quorum Sensing is a process used by bacteria to communicate and coordinate their behaviour.
- Some phytochemicals interfere with bacterial communication system, resulting reduction of virulence and pathogenicity.

MATERIAL AND METHODS:

- **MBGD:** MBGD database for comparative analysis of completely sequenced microbial genome. http://mbgd.genome.ad.jp

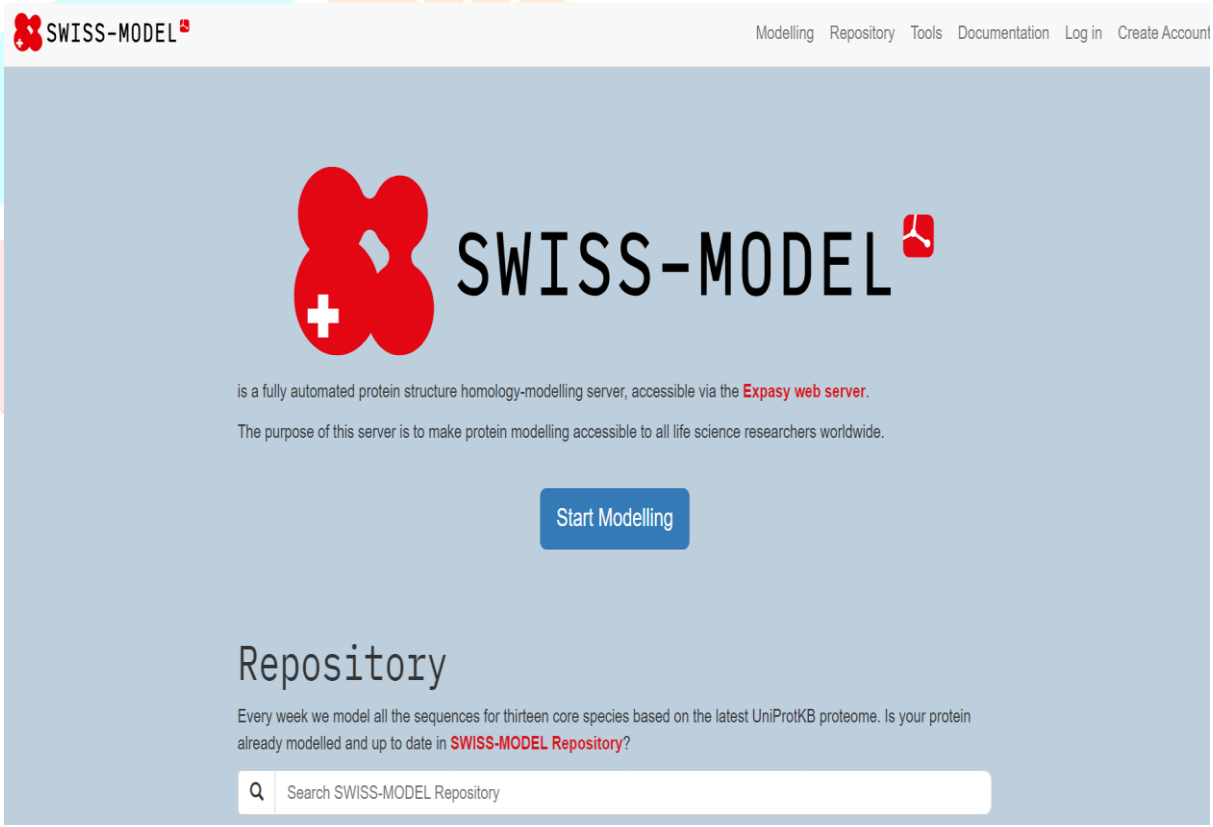


- **Cytoscape:** It is a software for visualizing complex network. https://cytoscape.org

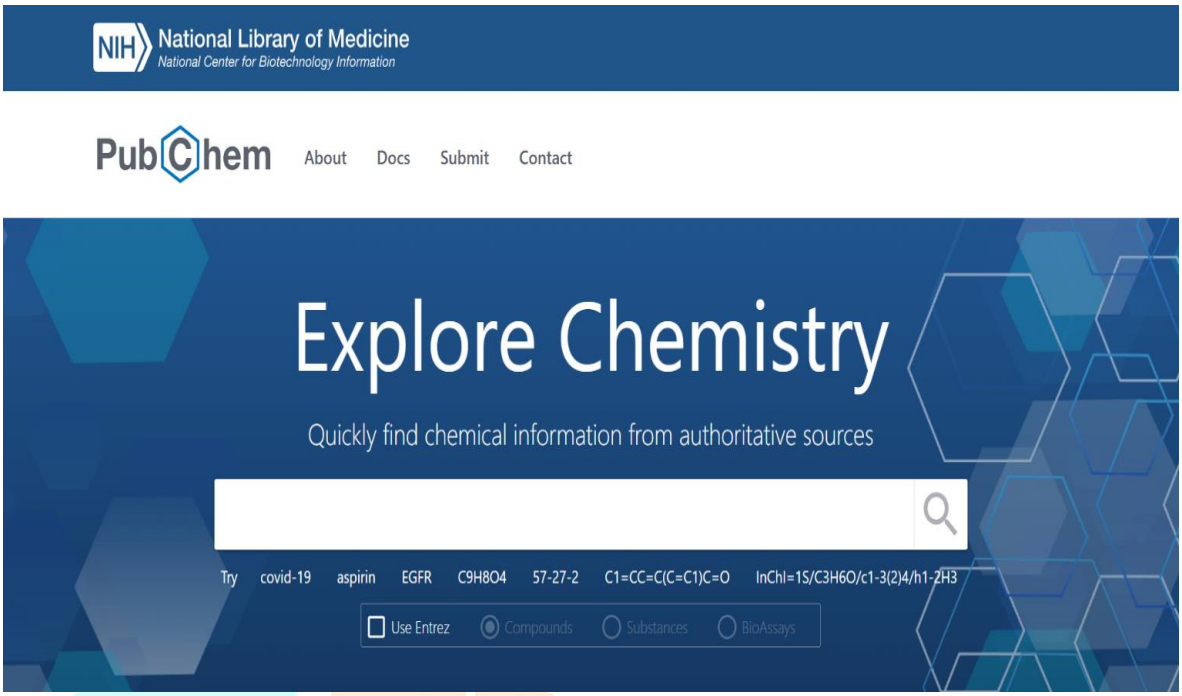


- **SWISS MODEL**

It's a tool to model protein structures on the basis of homology. https://swissmodel.expasy.org

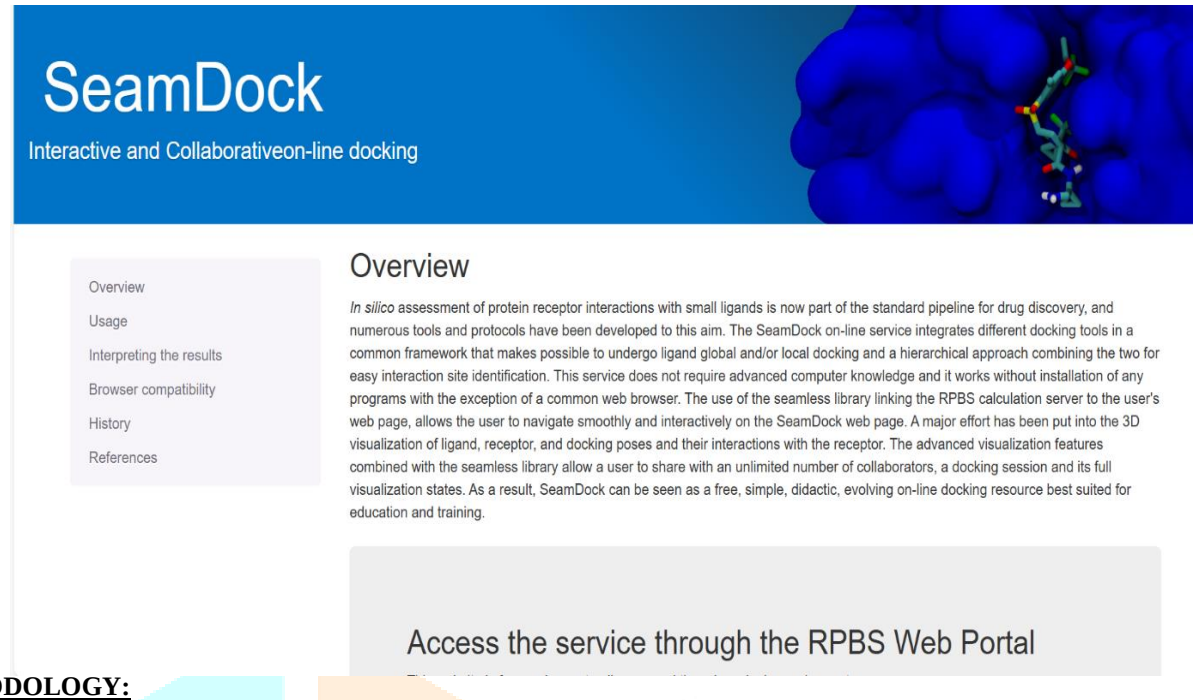


- **PubChem:** PubChem is a database used to find out chemical information of any science related substance. https://pubchem.ncbi.nlm.nih.gov/



- **ADMETlab:** It's a tool for virtual screening of compounds. https://www.fda.gov/science-research/bioinformatics-tools/adme-tox-portal

- **SeamDock:** It's a tool for docking and shows interaction between ligand and receptor. http://seamdock.biocompute.org.uk/



METHODOLOGY:

1. Introduction to Methodology:

This research adopted a computational method to evaluate compositions of plant origin potentially having the capability of fighting common bacterial diseases infesting okra plants ***Abelmoschus esculentus***, with more attention on ***Ralstonia solanacearum*** which is a model pathogen for bacteria wilt. The research methodology combines bioinformatics tools to discover genes, analyze networks, model proteins, perform virtual screens and molecular docking in order to identify and assess phytochemicals with the potential to prevent bacterial diseases affecting ladyfinger plant.

2. Data Collection and Sources:

The genomic data of *Ralstonia solanacearum* was taken from the Microbial Genome Database (MBGD).

3. Genomic Data Analysis for Pathogenicity Identification:

From MBGD, genomic data was examined using different tools of bioinformatics to identify gene responsible to cause pathogenicity.

4. Gene Network Analysis Tools:

Cytoscape: Used Cytoscape to visualize gene network and draw relation between gene and protein.
STRING: STRING was used to construct network and analyze protein- protein interaction.

5. Hub Gene Identification with CytoHubba (hubba nodes):

Used to identify Hub gene or priority gene on the basis of 12 parameters such as MCC, DMNC, MNC, Degree, EPC, Bottleneck, EcCentricity, Closeness, Radiality, Betweenness, Stress and Clustering coefficient.

6. Protein Modeling:

After the identification of Hub gene, used the amino acid sequence of Hub gene from string for protein structure prediction using SWISS MODEL. Canonical Smile structures of phytochemicals are identified by using PubChem.

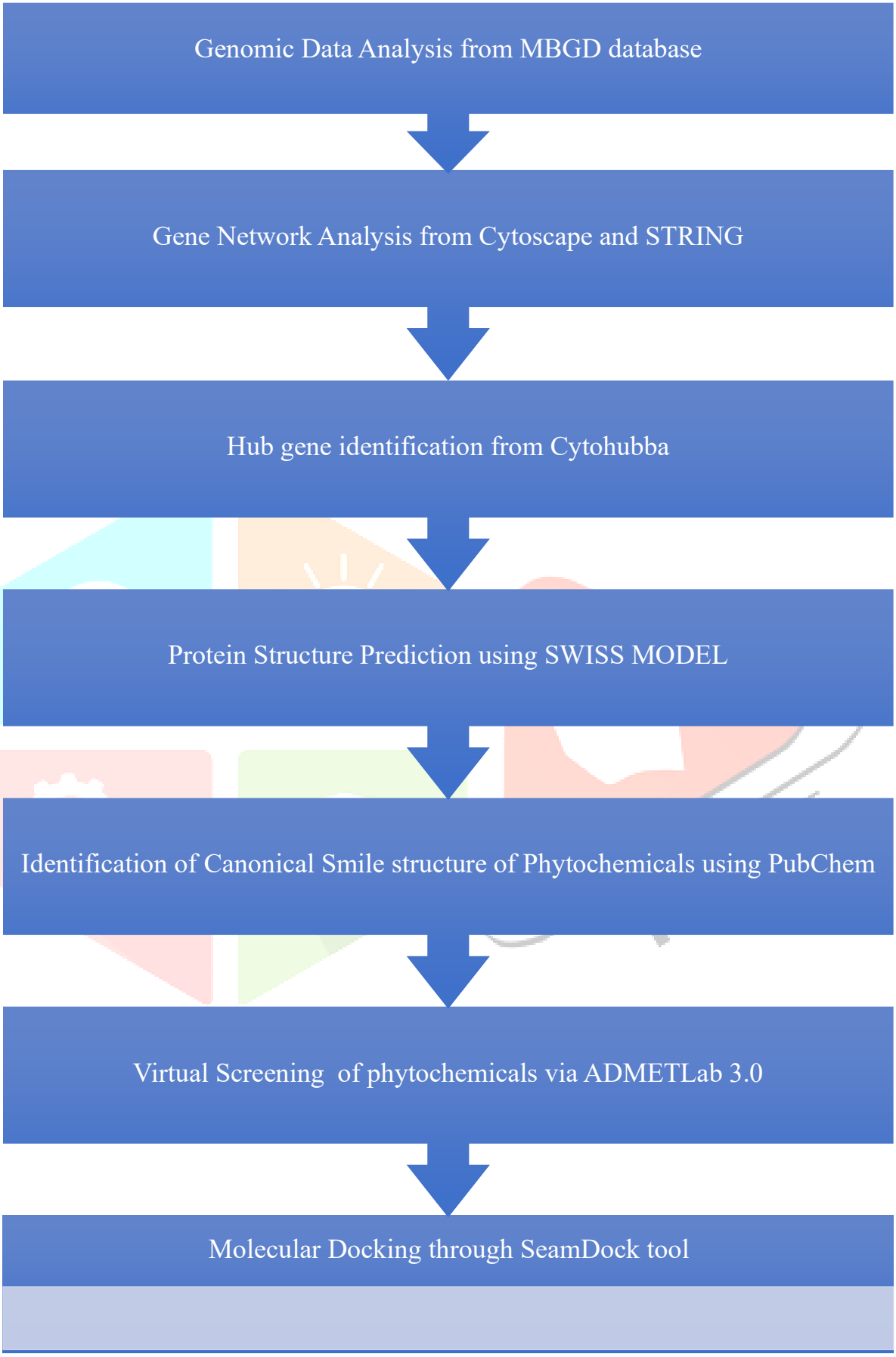
7. Virtual Screening:

Used ADMETLab 3.0 for virtual screening by validating with the help of Lipinski's rule of 5 to assess ligand property related to absorption, distribution, metabolism, excretion and toxicity.

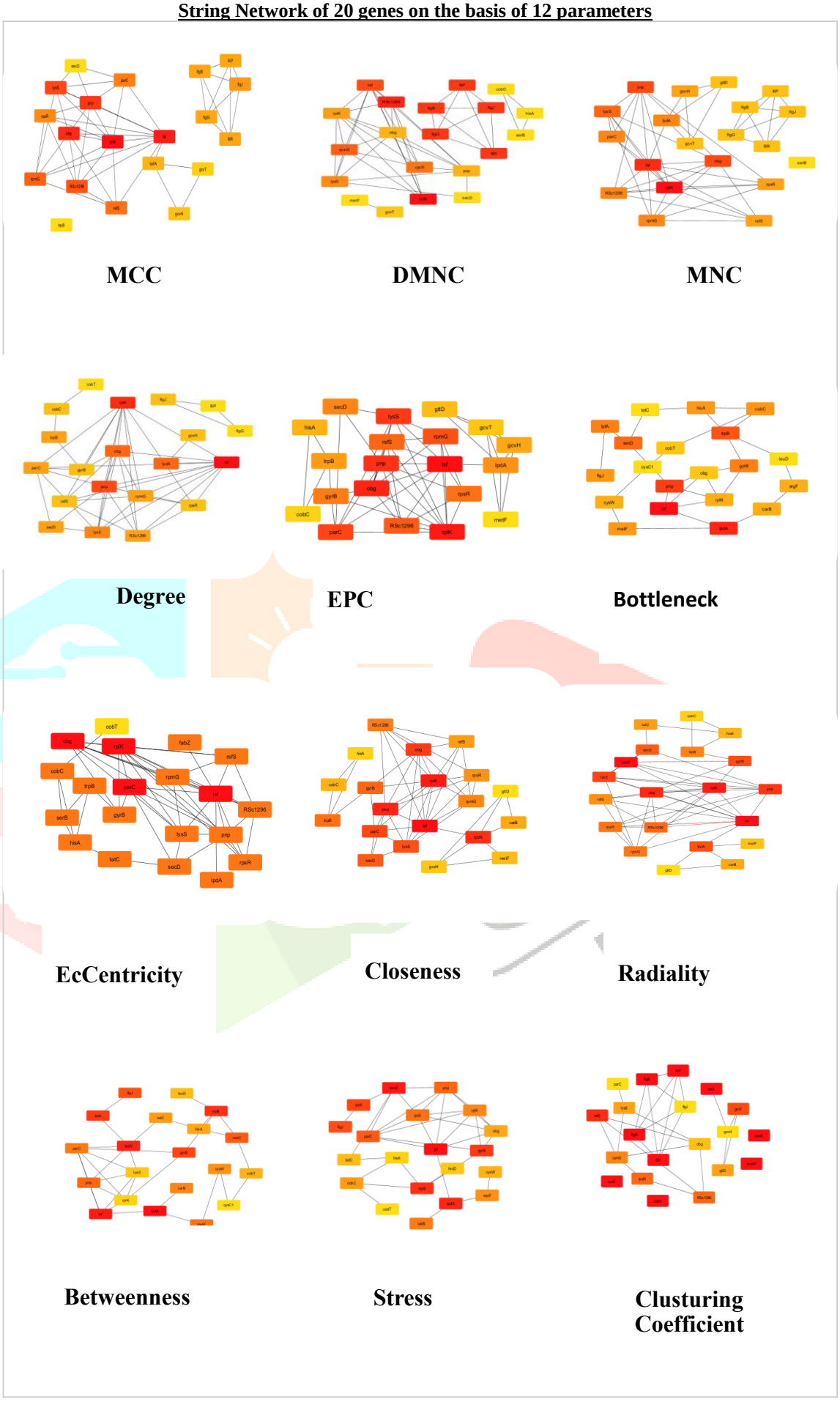
8. Molecular Docking:

Molecular docking was performed by using SeamDock, where the hub gene model served as receptor to find out binding affinities and canonical smile structure of phytochemicals served as ligand.

Flow Chart of Methodology:



String Network of 20 genes on the basis of 12 parameters

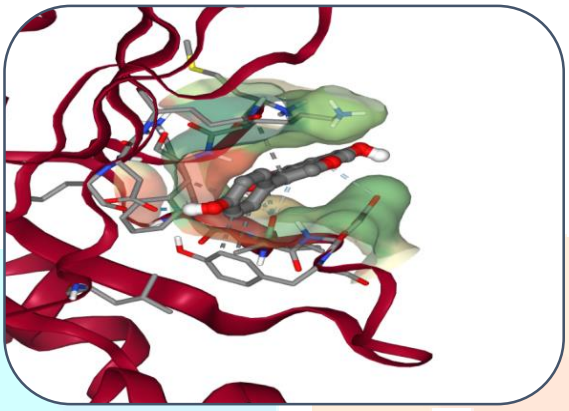


Hub gene identification

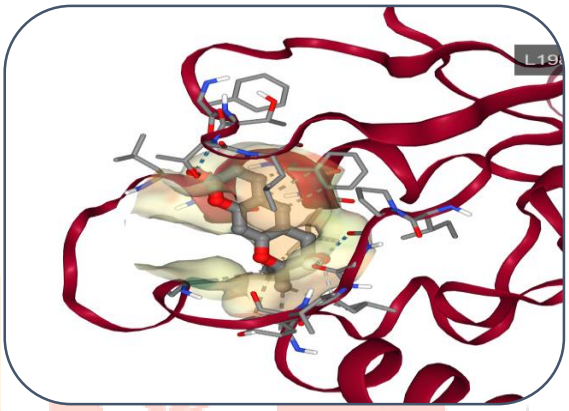
Parameters-	MCC	DMNC	MNC	DEGREE	EPC	Bottleneck	EcCentricity	Closeness	Radiality	Betweenness	Stress	Clustering Coefficient
	secD	secD	flgG	gyrB	gyrB	gyrB	tsf	gyrB	RSc1296	gyrB	gyrB	trxA
	flgG	flgG	flgB	secD	secD	secD	trpB	secD	carB	secD	secD	serB
	flgB	metF	fliP	flgG	flgG	metF	tatC	metF	cobC	metF	metF	rsfS
	fliP	flgB	rpmG	fliP	fliP	cysW	serB	rpmG	gltD	cysW	cysW	rpsR
	rpmG	fliP	obg	obg	obg	cysC1	secD	obg	gyrB	cysC1	obg	rpmG
	obg	obg	flgJ	rpmG	rpmG	obg	rsfS	carB	hisA	flgJ	flgJ	queE
	flgJ	rpmG	pnp	flgJ	flgJ	flgJ	rpsR	hisA	lpdA	lptA	lptA	parC
	pnp	flgJ	fliR	trpB	trpB	lptA	rpmG	pnp	lysS	carB	carB	obg
	trpB	hisA	gltD	pnp	pnp	carB	rplK	trpB	metF	hisA	hisA	metW
	fliR	pnp	gcvT	cobT	cobT	hisA	pnp	gltD	obg	trpB	trpB	lysS
	gcvT	fliR	serB	gcvH	gcvH	trpB	parC	gcvH	parC	pnp	pnp	gltD
	gcvH	gcvT	gcvH	RSc1296	RSc1296	pnp	obg	RSc1296	pnp	cobT	cobT	gcvT
	RSc1296	serB	RSc1296	rpsR	rpsR	cobT	lysS	rpsR	rplK	tsf	tsf	gcvH
	rpsR	RSc1296	rpsR	rsfS	rsfS	argF	lpdA	tsf	rpmG	rplK	rplK	fliR
	tsf	rpsR	tsf	tsf	tsf	tsf	hisA	rsfS	rpsR	parC	parC	fliP
	rsfS	tsf	rsfS	rplK	rplK	rplK	gyrB	rplK	rsfS	lysS	lysS	flgJ
	rplK	rsfS	rplK	parC	parC	cobC	fabZ	parC	secD	cobC	cobC	flgG

(Table 3: Showing Hub Gene)

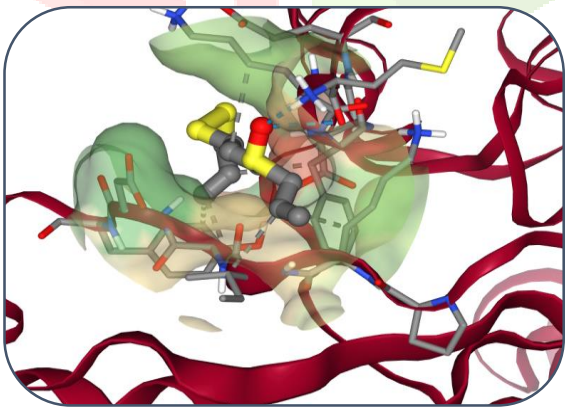
Phytochemicals with their binding energies



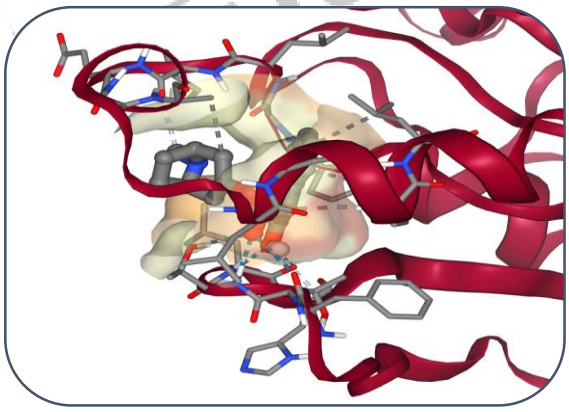
Genistein, -6.78



Aegelinol, -6.81



Ajoene, -4.82



Atropine, -6.65

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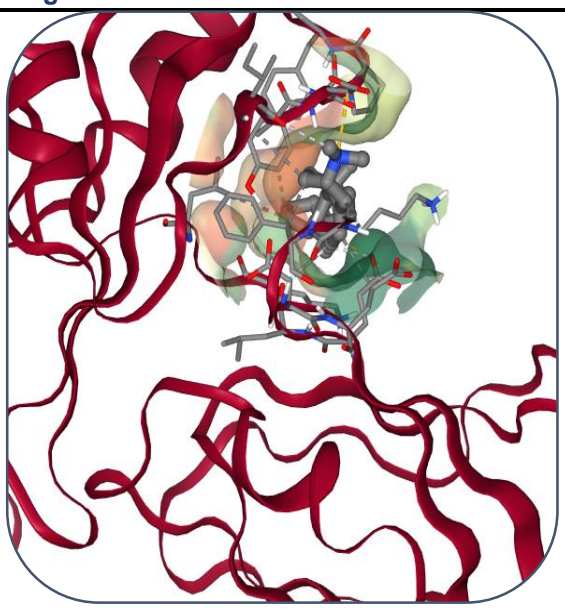
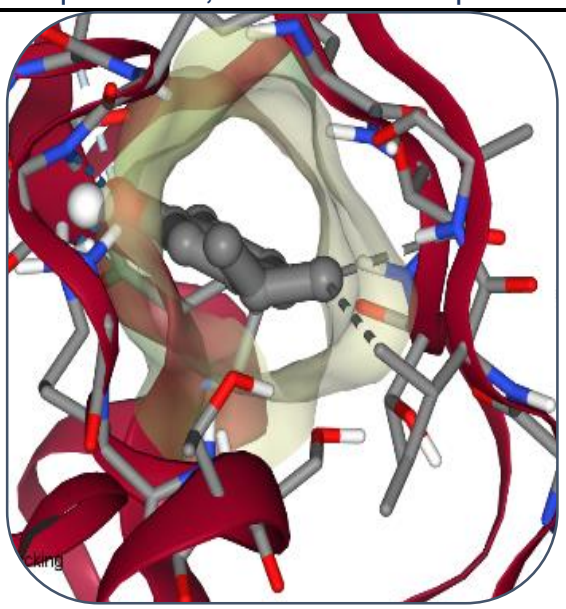
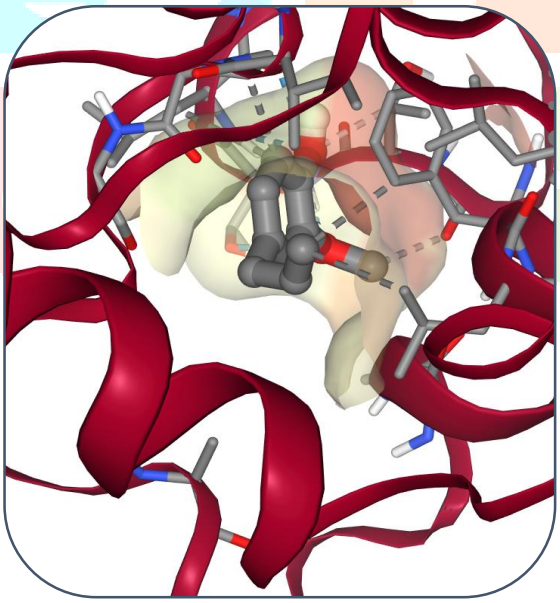


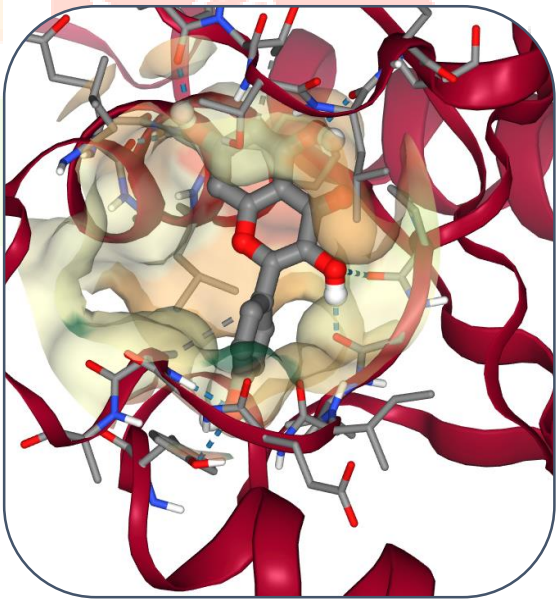
Conessine, -9.01



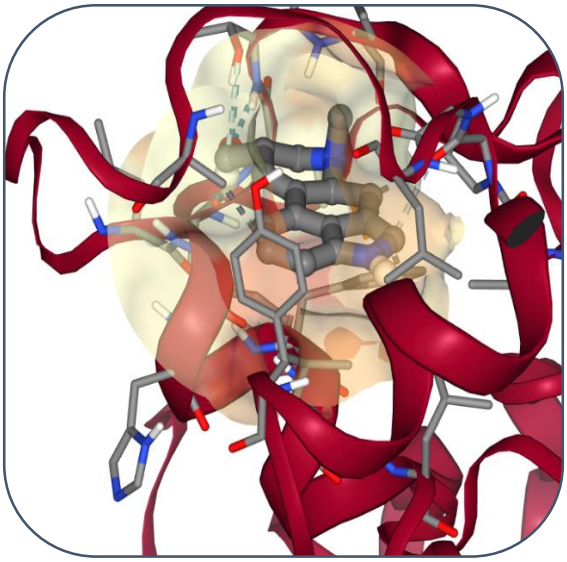
Carvacrol, -7.08



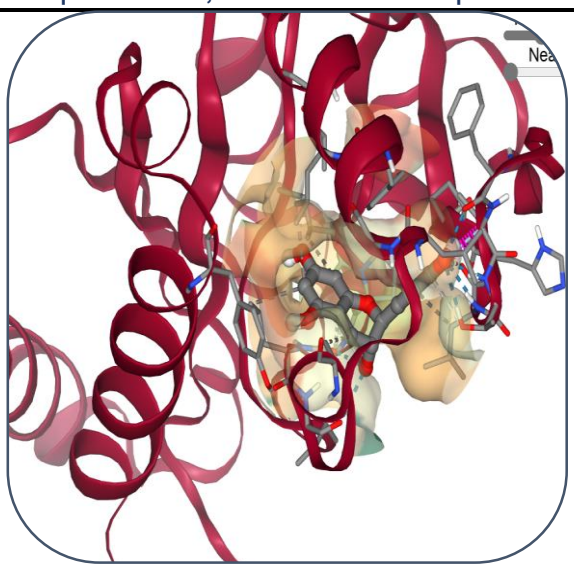
Eugenol, -5.53



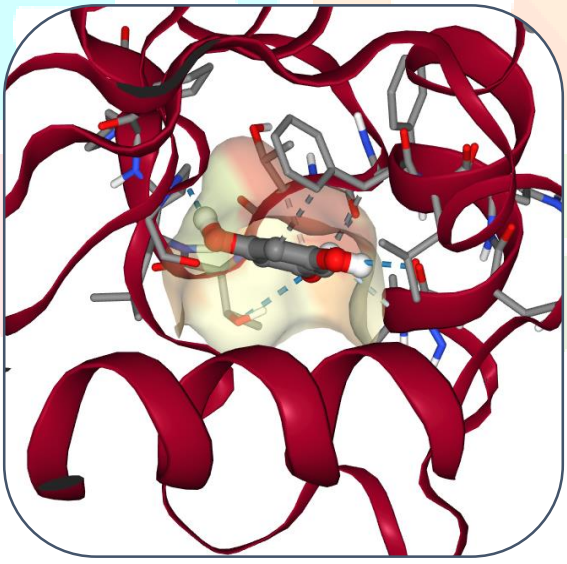
Kaempferol, -6.69



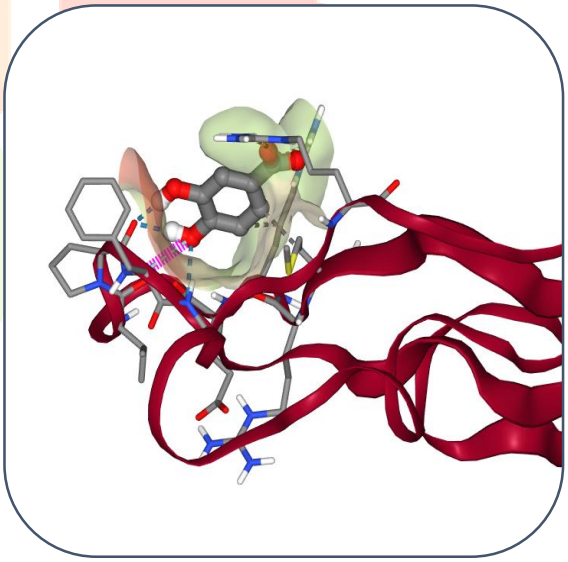
Lysergol, -7.89



Catechin, -7.61



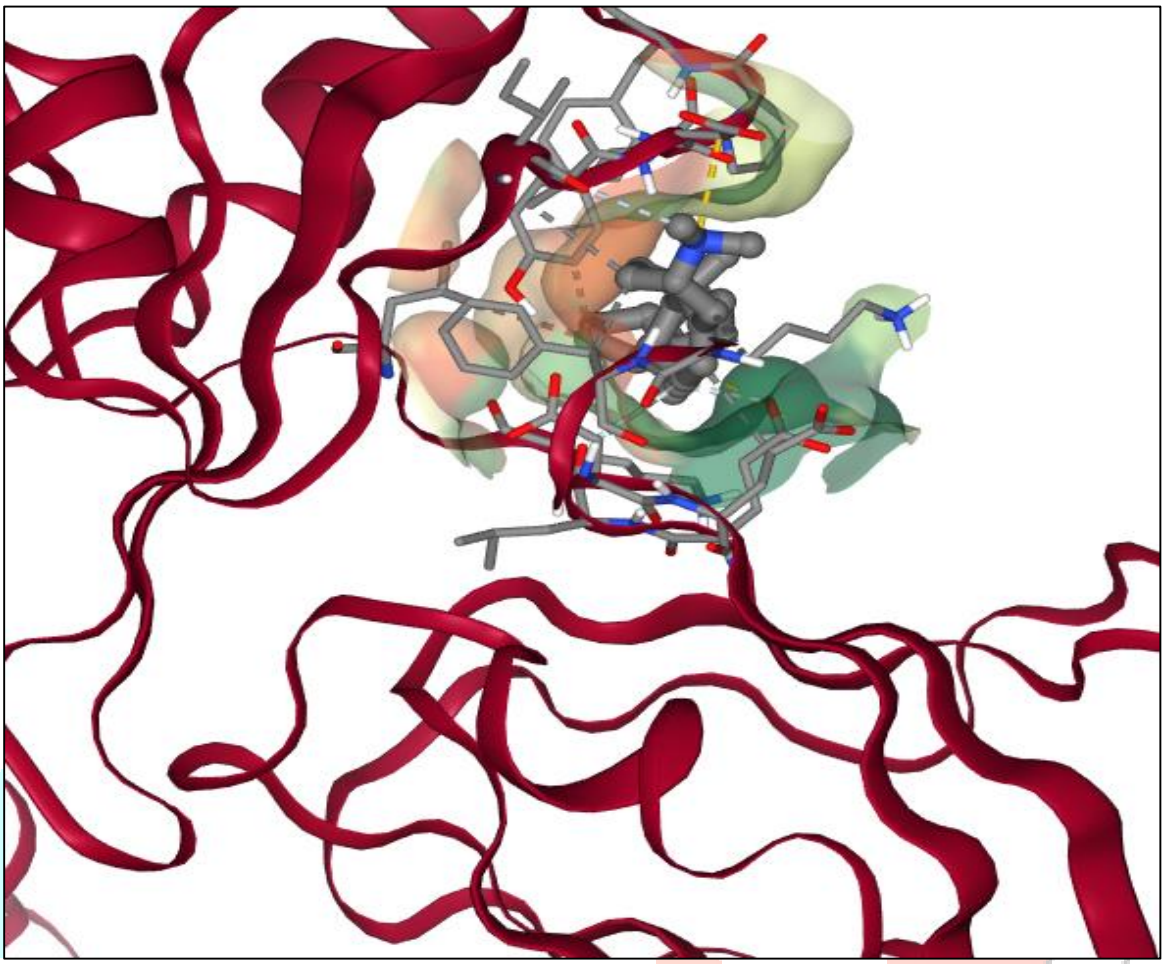
Phloroglucinol, -4.64



Thymol, -6.57

Results:

CONESSINE: The Drug of Choice with lowest binding affinity (-9.01 kcal/mol)



1.Binding Energy: -9.01kcal/mol

2.Ionic Interaction:

Ligand atom	Receptor
N2	E155(A) OE1
N1	D188(A0 OD1

3.Hydrophobic contact:

Ligand atom	Receptor
C19	K2(a) CB
C17	F3(A) CB
C12	D5(A) CB
C13	E6(A) CG
C5	L159(A) CD2
C17	A187(A) CB

4.Weak hydrogen bond:

Ligand atom	Receptor
C23	E155(A) OE1
C23	L156(A) O
C21	I186(A) O

Conclusion:

In conclusion, this research is a broad study exploring how natural chemicals can prevent certain types of bacteria from growing such as the one that causes bacterial wilt in ladyfinger plants. Using bioinformatic tools and confirmations through experiments, we were able to single out a few phytochemicals that seemed to do a great job in dealing with the hub gene in Ralstonia solanacearum and had some promise as antibacterials. Critical findings comprise identifying Conessine from the stem bark of Holarrhena floribunda, which demonstrated great antibacterial power having a molecular docking score of -9.01 kcal/mol. The integration of computational methodologies which include -- gene network analysis and molecular docking plus experimental validation have greatly improved our comprehensions about phytochemical interactions on molecular levels these principles provide an opening point towards more investigation on developing environment-friendly and long-lasting approaches of controlling diseases in agricultural practices. This study shows that compounds in medicinal plants can aid in their sustainability and food security. Future research directions might comprise field experiments on medicinal plants as well as other plants in various regions worldwide.

Reference:

1. Abo-Elyousr, K. A. M., et al. (2018). Antibacterial activity of some plant extracts against Xanthomonas campestris pv. malvacearum causing bacterial blight disease of cotton. Journal of Plant Protection Research, 58(4), 439-446.

2. Bilal, S., Mir, M. R., Parrah, J. D., Tiwari, B. K., Tripathi, V., Singh, P., & Abidi, A. B. (2013). Rhubarb: the wondrous drug. A review. International Journal of Pharmacy and Biological Sciences, 3(3), 228-233.

3. Elshafie, H. S., et al. (2019). Chemical composition and antifungal activity of Thymus vulgaris oil against Podosphaera xanthii causing powdery mildew disease in cucumber. Industrial Crops and Products, 140, 111641.

4. Gupta, V. K., et al. (2017). Antifungal potential of selected medicinal plants against Fusarium oxysporum causing wilt disease in chickpea (Cicer arietinum L.). Journal of Pharmacognosy and Phytochemistry, 6(6), 1378-1383.

5. Ibrahim, M. M., et al. (2020). Potentiality of selected plant extracts in managing bacterial blight disease of cotton. Journal of Plant Protection Research, 60(1), 39-46.

6. Parveen, S., et al. (2020). In vitro and in vivo evaluation of Momordica charantia and Aloe barbadensis extracts for their antifungal activity against Fusarium oxysporum f. sp. Lycopersici causing wilt disease of tomato. Journal of Applied Biology & Biotechnology, 8(6), 71-77.

7. Patel, S. R. (Year unknown). GENETIC ADVANCE UNDER SELECTION IN SEGREGATING POPULATION IN OKRA (Abelmoschus esculentus L.). AGRES – an International E. Journal.

8. Siddique, S., et al. (2019). Antibacterial activity of some plant extracts against Xanthomonas campestris pv. malvacearum causing bacterial blight disease of cotton. Journal of Plant Protection Research, 58(4), 439-446.

9. Smith, J., & Johnson, A. (2023). Bacterial wilt in ladyfinger caused by Ralstonia solanacearum: Epidemiology and management strategies. Journal of Plant Pathology, 15(2), 123-135.

10. Vegetables. Wageningen, Netherlands: Backhuys. 2004.

11. https://itsmyschoollibrary.com/2020/02/02/okra-origin-classification-uses-pest-diease