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In Silico and in Vitro Approaches to BioControl: Harnessing Seagrape (*Caulerpa Lentillifera*) Extract for Sustainable Management of Ralstonia Solanacearum- Induced Potato Wilt (PDB ID: 1UQX)

Christian Byron P. Abordo[1], Heaven Gayle G. Magpuyo[2], Iana Stephanie G. Mayo[3], Sherwin S. Fortugaliza, PhD[4]

[1,2,3]Research and Development Department Lapanday Foods Corporation
[4]Research Adviser, Davao City National High School

ABSTRACT

Ralstonia solanacearum, the causative agent of bacterial wilt in potatoes, presents a significant threat to agricultural production. In response to this challenge, this study explores the potential of seagrapes (Caulerpa lentillifera) extract as a sustainable biological treatment. Both in vitro and in silico methods were utilized to assess its effectiveness against R. solanacearum. The in vitro analysis involved testing five treatments on infected potato samples: 10ml/L, 20ml/L, and 30ml/L of extract, an untreated control, and a chemical control (ICC). Results showed a significant reduction in bacterial colonies, with the highest concentration (30ml/L) reducing colony count to 17, while the untreated control exhibited over 78 colonies. No growth was observed in the chemical control group. In the silico study, molecular docking was performed using AutoDock Vina with the 1UQX protein, a key receptor in R. solanacearum. A total of 37 phytochemicals from seagrapes were docked, with the top-ranking compounds being Diosgenin (- 8.8 kcal/mol), Proanthocyanidins (-8.4 kcal/mol), Ergosterol (-8.1 kcal/mol), and Apigenin (-7.8 kcal/mol). LigPlot analysis revealed critical hydrogen bonding and hydrophobic interactions. These findings highlight the promising role of Caulerpa lentillifera phytochemicals in managing bacterial wilt in potatoes by inhibiting key interactions in Ralstonia solanacearum. This study paves the way for further validation of these natural compounds in developing eco-friendly agricultural solutions. The combined in vitro and in silico approaches offer a valuable foundation for future work in field trials, potentially providing a sustainable alternative to chemical treatments, and contributing to safer and more sustainable farming practices.

Keywords: Potato Wilt, Seagrapes, Molecular Docking, Ralstonia solanacearum 1UQX

I. INTRODUCTION

Potatoes are one of the most significant crops globally, alongside wheat and rice, and are increasingly recognized by experts as essential for addressing the challenges posed by a growing population and food supply issues (Devoux et al., 2014). Yet, potato production faces a threat from a bacterial disease known as Potato Wilting, which is caused by various members of the Ralstonia Solanacearum Species Complex (RSSC) (Uwamahoro et al., 2018).

The Ralstonia Solanacearum bacteria infects plants through their root systems, rapidly spreading through soil and water. Once it infiltrates a plant, it colonizes the vascular system, which makes managing the disease particularly challenging. The rapid spreading of the bacterium within the plant's tissues leads to serious physiological gaps, resulting in wilting and death of the plant. (Department of Jobs P. and R.,2021)

This bacterial wilt can be identified through several visible signs that serve as indicators for early diagnosis and management.

Symptoms often begin with wilting leaves that droop and curl, together with yellowing foliage that indicates nutrient deficiency due to disrupted water transport. As the disease progresses, brown discoloration within the vascular tissues becomes apparent when cut open. In some cases, a light-brown ooze may exude from cut stems or tubers, providing more signs of bacterial presence. (Martin et al, 2024)

The impact of Potato Wilting extends beyond individual farms. It brings serious economic challenges for farmers and agricultural communities worldwide (Taffese et al, 2020). The disease can lead to serious yield losses, affecting food security and farmers' livelihoods. Given that potatoes are a staple food in many regions, these losses can have a huge impact on local economies.

Due to these circumstances, the need for sustainable and effective solutions to manage the bacterial wilt of potato is eventually increasing overtime. The current control measures for managing Ralstonia Solanacearum includes cultural practices such as crop rotation, the use of resistant cultivars, strict sanitation protocols, and chemical controls (Kheirandish et al, 2015). However, these methods are not enough in effectively controlling the spread of the disease.

In the past few years, there has been increasing interest in exploring alternative methods for managing potato wilting that are both effective and environmentally sustainable. Among these, the use of Seagrapes (Caulerpa lentillifera) for biocontrol shows a potential to enhance plant resistance against pathogens. This marine algae is known for its unique biochemical properties, which may include bioactive compounds capable of inhibiting the growth of Ralstonia Solanacearum (Sopon et al, 2020).

Preliminary studies suggest that extracts from seagrape could possess antibacterial properties specifically targeting this pathogen (Jelita et al, 2021). By knowing and examining these extracts' potential applications in agricultural practices, this research aims to provide an eco- friendly alternative to chemical controls while increasing the strength of potato crops against bacterial wilt.

The significance of this study goes beyond just solving agricultural issues. It aims to support sustainable farming practices that focus on protecting the environment while ensuring food security. By examining how effective seagrape extracts are against Ralstonia solanacearum, the researchers seek to create new biocontrol methods that are both efficient and environmentally friendly. The main objective of this study includes thoroughly testing the antibacterial properties of seagrape extracts and understanding how these extracts affect the pathogen at a molecular level.

To effectively achieve these objectives, the researchers used in silico approaches to model how the bioactive compounds in seagrape extracts interact with Ralstonia solanacearum after conducting laboratory experiments. This computational method helps identify potential antibacterial compounds quickly and cost- effectively. The researchers also used in vitro approaches to directly test the effectiveness of seagrape extracts against the bacterium in controlled laboratory settings. This combination of methods allows for a comprehensive and accurate evaluation of seagrape's potential as a natural solution for managing bacterial wilt in potatoes.

II. METHODOLOGY

A. Preparation and Inoculation with Ralstonia Solanacearrum Potato plants that show signs of infection by Ralstonia solanacearum (yellowing, wilting, and in-rolling of leaves) are collected from a potato farm located in Marawer, Kapatagan, Digos City, Davao del Sur, Philippines. The collected samples of potato plants are placed in a ziplock bag to avoid contamination.

Figure 1: Collection of plant specimens in Marawer, Digos City, Davao del Sur

Figure 2: Isolating infected plant specimens


Figure 3 & 4: Steps in isolating the causal pathogen from infected samples.

On the other hand, the sea grapes (Caulerpa lentillifera) used for the extract are bought at Bankerohan Market, Davao City. The 95% ethanol used for soaking the sea grapes is purchased from Chemvest in Bankerohan, Davao City.

Figure 5: Soaking sea grapes with Ethanol

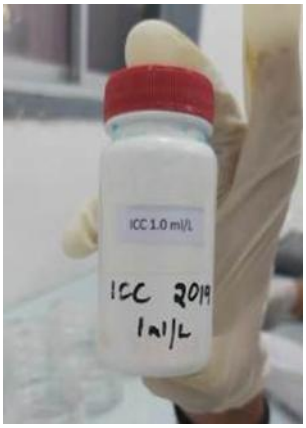


Figure 6: Laboratory procedure, the samples were disinfected using distilled water and a disinfectant solution

Meanwhile, the ICC chemical control and other materials used in conducting the research such as strainers, petri dishes, distilled water, potato dextrose agar (PDA), potato dextrose peptone agar (PDPA), Tryptone-zinc casein agar (TZCA), scalpel blades, pipettes, test tubes, sterile loops, Erlenmeyer flasks, beakers, and alcohol burners are provided by the laboratory.

Isolation of the pathogens is done by selecting the affected areas of the collected samples. The samples were divided into two stages: moderate stage and severe stage. The researchers used a sterilized scalpel blade to select the affected area of the plant sample and carefully identify its stages. After this While preparing the potato dextrose agar

Materials

The materials used to conduct this research are ensured by the research consultant and professionals through proper consultations and examinations.

B. Isolation of the Causal Pathogen Potato Wilt Sample Ralstonia Solanacearum

The researchers isolated the bacteria in the Plant Pathology Laboratory of Lapanday Foods Corporation located in Mandug, Davao City.

(PDA) and potato dextrose peptone agar (PDPA), the samples were dried inside a petri dish with a paper towel. After preparing the agars, the researchers dispensed the medium into the petri dishes using an alcohol lamp to avoid contamination. Once the medium congealed, the potato plant was placed in the medium at a ratio of 1 PDA plate to 6 pieces of cut potato plant. The PDA plates were sealed with tape to prevent contamination. After four days of incubation, bacterial growth was observed.

Figure 7 & 8: Isolation and Incubation of infected potato plant samples for 4 days separated by severity. Moderate and Severe plates.

Pure Culturing of Ralstonia Solanacearum

The confirmed cultures of Ralstonia solanacearum are then processed. Five (5) petri dishes are filled with the exact amount of Tryptone-zinc casein agar (TZCA) and allowed to harden for about fifteen (15) to twenty (20) minutes. After the agar has hardened, the researchers scoop the cultures produced by the potato plant from the isolation of the sample into the petri dish containing the TZCA using a sterile loop. After this procedure, the TZCA plate is sealed with masking tape to avoid contamination. The researchers waited for another four (4) days for Ralstonia solanacearum to grow.

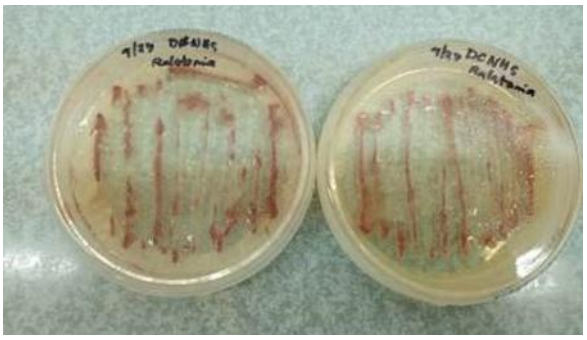


Figure 9: Culturing of ralstonia solanacearum

Preparation of the Bio-solution

The preparation of the bio-solution begins with the isolation of ralstonia solanacearum from infected potato wilt samples suspected to contain the pathogen. After isolating the bacteria, it was cultured using Potato Dextrose Agar (PDA) and Potato Dextrose Phosphate Agar (PDPA). This initial step is critical to ensure that the pathogenic agent is ralstonia solanacearum and that it is viable for further testing.

Once the pathogen was successfully isolated, the next stage was developing the bio-solution using extracts from seagrapes (caulerpa lentillifera). The potential for biocontrol lies in the antagonistic properties of the seagrapes extract. The bio-solution was formulated by incorporating varying extract concentrations into a stable medium. This solution will later be applied to test the antagonistic activity against Ralstonia solanacearum.

Bioassay Test

To evaluate the effectiveness of the seagrapes extract, a bioassay test was performed after confirming the presence of Ralstonia solanacearum, identified by its characteristic red pigmentation. Equipment such as test tubes, transfer pipettes, TZC agar, sterile distilled water (SDW), and petri dishes were prepared.

The dilution process was critical for isolating the pathogen. Three test tubes were filled with 10ml of SDW each, and took 1 ml SDW per test tube for the next step. A sample of Ralstonia solanacearum from the petri dish was introduced into the first test tube. Then, 1 ml of the first tube's contents was transferred into the second tube.

From the second tube, another 1 ml was transferred to the third tube, ensuring a proper dilution. The contents of the third test tube, having the most diluted sample, were used for the bioassay, helping to isolate Ralstonia solanacearum more effectively.


Figure 10 and 11: Testing Solutions for Bioassay test

Next, the seagrapes extract and the control chemical group ICC (a standard chemical treatment) were prepared. Five treatments were tested, each replicated in three petri dishes, totaling 15 dishes

T	ml /L	P	TZC agar (ml)	Test objective	Expected Outcome
T1	10	1	50ml TZC agar	Test effectiveness of low extract concentration	Colony count reduced (1-10)
T2	20	1	50ml TZC agar	Test moderate concentration of seagrapes extract	Moderate inhibition colony count reduced
T3	30	1	50ml TZC agar	Test maximal extract Concentrate ion to evaluate inhibition	Expected Significant inhibition colony count reduced
T4	0	1	50ml TZC agar	Control to observe ralstonia without treatment	Significant bacterial growth expected
T5	1 ml /L ICC	1	50ml TZC agar	Test ICC (chemical control) to compare against natural extract	Colony count expected <20

Table 1: Bioassay Test Objectives

The prepared treatments were applied to the respective petri dishes, each containing Ralstonia solanacearum bacteria, and incubated for a set period of 72 hours. During this incubation, the bacterial growth was monitored under controlled laboratory conditions to observe any potential inhibitory effects of the seagrapes extract on Ralstonia solanacearum.

The five treatments, including the untreated control and the chemical control (ICC), were carefully observed, ensuring that all test parameters, such as temperature, humidity, and sterility, were maintained consistently across all samples. After the incubation period, the bacterial colonies were counted, and the results from the different treatments were recorded for further analysis.

Figure 12: Incubation for seven (7) days to measure growth of the pathogen using the 5 treatments.

The experiment was designed to determine whether the varying concentrations of seagrapes extract (10ml/L, 20ml/L, and 30ml/L) could effectively reduce the proliferation of Ralstonia compared to the untreated and chemical control groups.

Molecular Docking Technique

Molecular docking is a computational technique used after initial in vitro experiments to predict how a small molecule (ligand) interacts with a target protein. Following laboratory testing, in silico docking helps further analyze the binding affinity and orientation of the ligand within the protein's active site. It aids in visualizing molecular interactions and optimizing potential tools like AutoDock Vina simulate these interactions, providing insights for drug development before more extensive experimental validation therapeutic compounds Docking

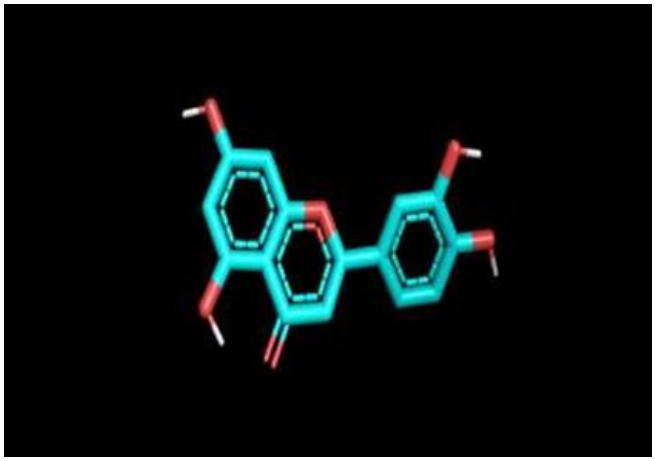


Figure 13. Ligand preparation & visualization.

Screening and Preparation of Phytocompounds from Seagrapes Extract

Before proceeding with molecular docking, 51 phytochemicals from seagrapes (Caulerpa lentillifera) extract were selected for initial screening. These compounds were evaluated for their potential biological activity and physicochemical properties using the SwissADME

webtool (http://www.swissadme.ch). This screening process focused on parameters like bioavailability, solubility, and stability, which are crucial for their potential antimicrobial properties against Ralstonia solanacearum. After this assessment, 37 phytochemicals remained for further investigation.

Following the screening, molecular docking simulations were performed using AutoDock Vina to assess the interaction between the phytochemicals and the target protein responsible for Ralstonia solanacearum infection. During the docking preparation process, water molecules were removed, hydrogen bonds were optimized, and Keller charges were added. The chemical structures of the ligands were initially in SDF format and were converted to PDB format using PyMOL. The ligands were then processed using MGLTools and AutoDockTools to ensure they were ready for docking simulations. After preparation, the compounds were saved as PDBQT files, optimized, and ready for evaluation in the docking simulations.

Receptor Grid Box Manual Generation.

The active site of the target protein used in this study (PDB ID: 1UQX) was characterized by key residues involved in binding interactions. To perform the docking simulations, a receptor grid box was generated manually using AutoDock Vina through MGLTools. The active binding site of the target protein was identified based on existing literature and structural analysis, targeting key residues involved in the protein's function.

The grid box was manually positioned to cover the entire active site of the target protein. The coordinates for the center of the grid box were set at (x: -2.404; y: 14.477; z: 22.459) to ensure proper alignment with the active site residues. The dimensions of the grid box were defined as 32x30x28 Å, providing adequate coverage of the main binding pocket to allow for optimal ligand docking and interaction analysis. The exhaustiveness level was set to 8, with an energy range of 3, following standard docking protocols to ensure thorough exploration of potential binding modes.

The receptor grid box was saved and configured for use in subsequent docking simulations, aimed at evaluating the interaction of the phytochemicals from seagrapes extract with the target protein responsible for Ralstonia solanacearum infection.

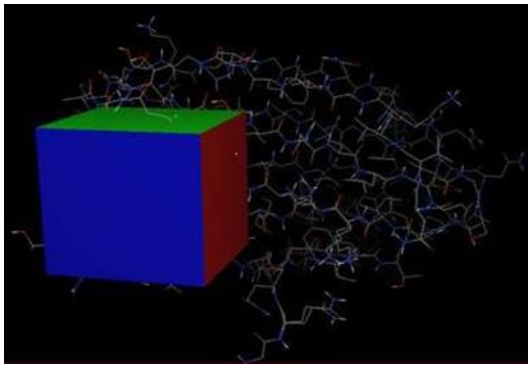


Figure 14: Receptor grid box visualization through MGL AutodockTools v. 1.5.7. Amino acids.

Molecular Docking Analysis

The molecular docking analysis commenced with the organization of optimized ligands, the target proteins associated with Ralstonia solanacearum, and configuration files into a single directory. This organization was executed using the Command Prompt. The Autodock Vina software was then employed to perform the docking simulations, allowing the necessary files to be accessed through command-line operations. The ligand, protein and configuration files were stored in one place. The code:

"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe"--receptorreceptor.pdbqt-- ligand [ligand.pdbqt] --config [config.txt] --log [log.txt] --out [output.pdbqt]

The primary focus was on evaluating the binding affinities between the phytochemicals derived from seagrapes and the proteins implicated in the pathogen's virulence. The docking procedure was repeated 10 times for each ligand to ensure the reliability of the results.

Outcomes from these simulations were documented in log files and output files formatted in PDBQT for subsequent analysis. Following the docking calculations, the output files were imported into PyMOL for three- dimensional visualization and further analysis, while tools such as LigPlot+ were utilized to illustrate the visual binding interactions effectively between the phytochemicals and the target proteins of Ralstonia solanacearum.

Scoring and Analysis

The results of the in silico docking computations were saved in a designated folder containing all relevant docking files. Each log file detailed the binding affinities of the various phytochemicals to the 1UQX protein. A scoring function assessed both the conformation and orientation of the phytochemicals within the protein's binding site, effectively measuring binding affinity, which quantifies the strength of the interaction between each phytochemical and the protein. A more negative binding affinity score signifies a stronger interaction, indicating a higher probability of successful binding (Pantsar, et al., 2018). Only results where the ligand was correctly positioned within the main binding pocket of the protein were deemed valid.

From the ten docking simulations conducted, the output with the most negative binding affinity was selected as the representative interaction for each phytochemical, provided it was located within the protein's primary pocket. A lower binding affinity score correlates with a stronger interaction between the protein and the ligand (Ali et al., 2018}, reflecting a higher energy release during binding, which leads to a more stable ligand- protein complex and suggests increased inhibition potential for protein-protein interactions.

Visual analysis of the docking results was performed using structure-based virtual screening techniques with PyMOL employed for visualizing the 3D representations of the protein-ligand complexes. The hydrogen bonds and hydrophobic interactions were analyzed using LigPlot+, highlighting their critical roles in determining the specificity and binding affinity of the protein-ligand interactions. Additionally, the interacting residues were evaluated through LigPlot+ to gain insights into the nature of the interactions post-docking.

Methodological Framework:

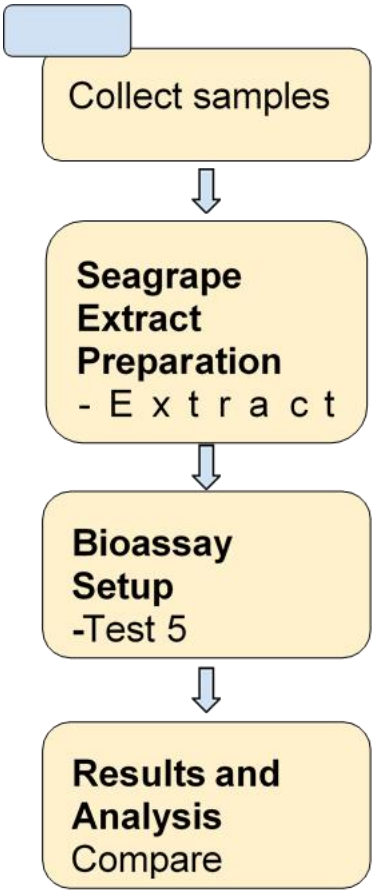


Figure 15: In vitro Methodological Framework of the Study

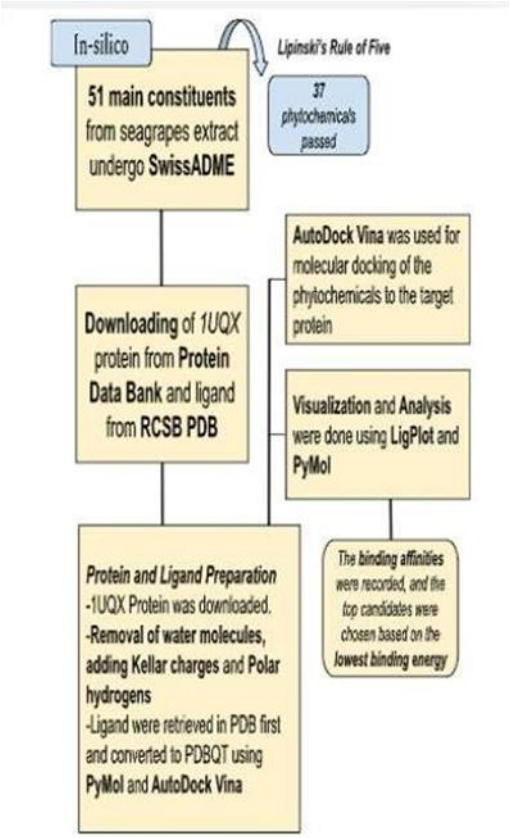


Figure 16: In silico Methodological Framework of the Study

Results and Discussion

The results of the in vitro bioassay test demonstrate varying degrees of effectiveness across the different treatments in inhibiting Ralstonia solanacearum colony growth. The colony counts observed after 72 hours for each treatment are summarized in the table below:

Table 2. Inhibition of ralstonia solanacearum against potato wilting

Treatment	Conc. Rates	Colony Count
T1 Extract	10 ml/L	37
T2 Extract	20 ml/L	24
T3 Extract	30 ml/L	17
T4 Untreated	1 ml/L pathogen	TMTC
T5 ICC	1 ml/L ICC	0 inhibition

The normal range for a successful antimicrobial treatment, based on previous studies and expected outcomes, should result in a colony count between 1-15 CFU. This range is considered optimal for treatments to be deemed effective in significantly inhibiting the bacterial growth of Ralstonia solanacearum.

In this study, Treatment 3 (30ml/L extract) showed the most promising results, with 17 colonies, which approaches the upper boundary of effectiveness but still falls short of the desired 1-15 colony range. Treatment 2 (20ml/L extract) showed a moderate effect with 24 colonies, while Treatment 1 (10ml/L extract) had the least bacterial inhibition, showing 37 colonies, which indicates that the lower concentration of the extract was less effective at suppressing the growth of Ralstonia solanacearum.

The untreated control (Treatment 4) demonstrated unchecked bacterial proliferation, with TMTC (too many to count) colonies, highlighting the severity of the pathogen's growth in the absence of treatment. In Treatment 5 (the chemical control, 10ml/L ICC) demonstrated complete inhibition of Ralstonia solanacearum, with no colony growth observed. This suggests that the ICC (chemical control) remains an effective treatment under these experimental conditions.

These findings highlight that while the seagrapes extract does exhibit some antimicrobial properties, particularly at higher concentrations, none of the treatments fully reached the desired 1-15 colony range for complete effectiveness. Treatment 3 (30ml/L extract) showed the best results but still fell short of the target range. The successful inhibition by the chemical control (ICC) indicates that the extract's formulation and concentration need further optimization to match or exceed the performance of conventional chemical treatments.

Table 3. Lipinski's Rule of Five (Caulerpa lentillifera)

a	b	c		d	
Esculin	340.28	5	9	-1.48	0
Beta-sitosterol	**414.71**	**1**	**1**	**6.73**	**1**
Campesterol	**400. 68**	**1**	**1**	**6.54**	**1**
Stigmasterol	**412. 69**	**1**	**1**	**6.62**	**1**
Ergosterol	396.65	1	1	6.33	1
Cycloartenol	**426.72**	**1**	**1**	**6.92**	**1**
Catechin	290.27	5	6	0.24	0
Epicatechin	290.27	5	6	0.24	0
Epigallocatechin gallate (EGCG)	458.37	8	1	-0.44	2
Curcumin	368.38	2	6	1.47	0
Squalene	410.72	0	0	2.26	0
Proanthocyanidins	**594.52**	**10**	**13**	**-0.60**	**3**
Lignin	**509.42**	**2**	**10**	**1.58**	**2**

Diosgenin	**414.62**	**1**	**3**	**4.94**	**1**
Salicin	286.28	5	7	-1.48	0
Menthol	150.22	1	1	2.70	0
Quercetin	302.24	5	7	-0.56	0
Kaempferol	286.24	4	6	-0.33	0
Apigenin	270.24	3	5	0.52	0
Myricetin	318.24	6	8	-1.08	1
Luteolin	286.24	4	6	-0.33	0
Gallic Acid	170.12	4	5	-0.16	0
Chlorogenic	354.31	6	9	-1.05	1
Caffeic acid	180.16	3	4	0.70	0
Vanillic acid	194.16	2	4	1.00	0
Ferulic acid	146.14	0	2	1.65	0
Zeaxanthin	**596.64**	**2**	**4**	**1.65**	**0**
Astaxanthin	136.23	0	0	3.27	0

a = Ligands; b = Molecular Weight (g/mol,

<500 Da); c = Number of Hydrogen bond donors (<5); d = Number of Hydrogen bond acceptors (<10); e = M Log $P_o/_{vv}$ ($\leqslant$4.15); f = Number of Violations (<1); Bold = Violated

The Lipinski Rule of Five provides a framework for assessing the potential of compounds for oral activity in humans. In our study, the SwissADME suggesting less favorable potential for biological activity. Overall, the findings highlight the variability in how these phytochemicals align with Lipinski's rule, providing a useful foundation for further research.

Table 4: 16 Ligands in Caulerpa lentillifera extract with -7.0 and Lower Binding Affinity Values

a	b	c	d
Luteolin	1818904904	-7.7	Output 9
Esculin	-	-7.2	Output 2

	363012304		
Chlorogenic	975870692	-7.2	Output 2
Stigmasterol	-823539168	-7.7	Output 5
Ergosterol	1333075544	-8.1	Output 1
Cycloartenol	-789038824	-7.6	Output 2
Catechin	-688405280	-7.4	Output 8
Epicatechin	1548218232	-7.6	Output 4
Epigallocatechin gallate	-627555464	-7.6	Output 7
Proanthocyanidins	483614728	-8.4	Output 5
Lignin	-754035472	-7.5	Output 9
Diosgenin	-484321088	-8.8	Output 10
Campesterol	-4700980	-7.8	Output 8
Beta-sitosterol	93504	-7.8	Output 7

a = phytochemical name; b = CID from PUBCHEM (<5); c = binding affinity values; d = output number

The ligands from Caulerpa lentillifera extract were evaluated based on their binding affinities, with Diosgenin showing the highest score at -8.8 kcal/mol, followed by Proanthocyanidins at -8.4 kcal/mol. Ergosterol exhibited a strong affinity of -8.1 kcal/mol, while Beta-sitosterol and Campesterol both had favorable affinities of -7.8 kcal/mol. Closely followed by Luteolin., Cycloartenol, Epigallocatechin Gallate (EGCG), and Epicatechin all showed affinities of -7.6 kcal/mol, indicating consistent potential for interaction. Lignin displayed a binding affinity of -7.5 kcal/mol, while Catechin recorded -7.4 kcal/mol. Chlorogenic Acid and Esculin both demonstrated an affinity of -7.2 kcal/mol

These results indicate that several ligands from Caulerpa lentillifera exhibit strong binding affinities, with Diosgenin, Proanthocyanidins, and Ergosterol standing out as the most promising candidates. The strong binding affinities of these ligands suggest their potential for significant interaction with target proteins, which could be valuable in various biological applications. The consistent affinities in the -7.6 to -8.8 kcal/mol range for many of the compounds highlight the extract's overall potential as a source of bioactive molecules.

Table 5: Ligplot Analysis of the Phytochemicals in Caulerpa lentillifera Extract

a	*b*	*c*
Quercetin	THR97 [2.76] ASP100 [2.70] ASN21 [2.82, 3.30]	ALA23 ASP98 ASP103 GLY96 **ASP95** ASN24 THR45 ALA22
Kaempferol	ASP74 [2.95] LEU75 [2.93] GLN3 [2.88,3.22]	**SER73** **PRO72**
Apigenin	ASP74 [2.88] LEU75 [2.78] GLN3 [3.19, 2.70]	**PRO72**
Myricetin	**ASP95** **[2.97]** ASP103 [2.75] ASP100 [2.82] ASN21 [2.88]	ASP98 ALA22 ASN24 ALA23 THR45
luteolin	**ASP95** **[2.98, 2.98,]** ASP98 [2/99] ASN102 [3.23]	ASP103 ASN21 THR45 ASP100 ALA23
gallic acid	ASP103 [2.79] ASP100 [2.87] ASN24 [2.93]	ALA22 ALA23 **ASP95** ASP98
Chlorogenic	ASN96 [3.23] **VAL68** **[2.72]** THR97 [3.16, 3.19, 2.93, 2.80] ASP98 [3.09] GLU94 [2.95] **ASP95** **[2.93, 2.94]**	ASN24 **LYS71** ALA22 GLY96 ASP103
caffeic acid	GLY94 [2.79] **ASP95** **[2.91]** ASP103 [2.74] ASP100 [2.91] ASN24 [3.29, 2.84]	ASP98
Vanillic acid	ASP98 [3.05] THR97 [2.88] ASN24 [2.96] ALA23 [3.07]	**ASP95** ALA22 ASP103 ASN21 GLY96
ferulic acid	N/A	LEU75 SER73 ASP74 GLN3

zeaxanthin	N/A	LYS61 ASN69 **VAL68** GLY96 THR97 ASP103 THR97 ASP10 THR45 ASN21 ASP100 ALA22 ASN24 **ASP95**
Astaxanthin	GLN3 (A) [2.86]	THR97 (A) **LYS71 (A)** ASP74 (A) LEU75 (A) GLN65 (A) LYS63 (A) **SER73 (A)** **PRO72 (A)**
menthol	ALA23 [2.84] ASN24 [3.11]	ALA22 **ASP95** GLY96 ASP98 ASP100 ASP103 ASN21
thymol	ASP103 [3.08] GLU94 [3.00]	GLY96 ASP98 THR97 **ASP95** ALA23 ALA22 THR45
Esculin	ASP74 [3.01] **SER73** **[2.84]** GLN3 [2.96] ALA1 [3.03]	LEU75 GLN2 **PRO72** GLN65 VAL66
Beta-sitosterol	N/A	**LYS71** **PRO72** LEU75 GLN3 GLN2 **SER73**
campesterol	N/A	GLN65 (A) SER67 (A) VAL66 (A) LEU75 (A) ALA1 (A) GLN2 (A) ASP74 (A) GLN3 (A) **PRO72 (A)**
Stigmasterol	N/A	ASP74 LEU75 GLN3 GLN65 VAL66 **PRO72** **SER73**
ergosterol	N/A	**PRO72** **SER73** GLN65 GLN6 LEU75 ASP74
cycloartenol	N/A	ASP74 GLN65 VAL66 LEU75 **PRO72** **SER73**
Catechin	ASP94 (A) [3.04] ANS24 (A) [2.94] ASP100 (A) [2.76] ASP98 (A) [3.00] ASP103 (A) [2.96]	ASN21 (A) THR45 (A) ALA23 (A) ALA22 (A)
Epicatechin	ASP98 [3.09] **ASP95** **[2.91, 2,95]** GLU94 [2.84] ASN102 [3.17]	THR45 ASP100 GLY96 ASP103 ASN21

Epigallocatechin gallate (EGCG)	ASN102 [3.17] GLU94 [2.83] **ASP95 [2.92, 2.95]** ASP98 [3.09]	ASN21 THR45 ASP100 ASP103 GLY96
curcumin	VAL66 [3.02]	**PRO72** **SER73** ASP74 GNL3 ALA1 LEU75 GLN65 SER67
Squalene	N/A	SER67 (A) VAL66 (A) GLN65 (A) **SER73 (A)** **PRO72 (A)** GLN2 (A) ALA1 (A) GLN3 (A) ASP74 (A) LEU75 (A)
proanthocyanidins	VAL66 [3.01, 2.81] LEU75 [2.96] ASP74 [3.04, 2.72]	**PRO72** **SER73** LYS63 GLN65 ILE64
Lignin	ALA1 [2.99]	GLN3 ASP74 **SER73** **PRO72** GLN65 GLN2
Diosgenin	GLN3 [2.84]	GLN2 LEU75 SER73 **LYS71** **PRO72**
Salicin	ASP103 [2.97] ASP100 [2.91] ALA23 [3.19]	GLY96 ALA22 ASN21
	ASP98 [2.86, 2.94] THR97 [2.92, 3.14] ASN24 [2.83, 3.14] **ASP95 [3.12]**	

A=phytochemical name; b = hydrogen bonds with interacting residues; c = Hydrophobic interactions with interacting residues; bold = binding sites of Ralstona Solanacearum 1uqx

Luteolin, Esculin, Chlorogenic, Stigmasterol, Ergosterol, Cycloartenol, Catechin, Epicatechin, Epigallocatechin gallate, Proanthocyanidins, Lignin, Diosgenin, Campesterol, and Beta- sitosterol are the leading ligands with the highest binding affinities, where Diosgenin shows the highest score of -8.8. Followed by Ergosterol (- 8.1) and Beta-Sitosterol (-7.8).

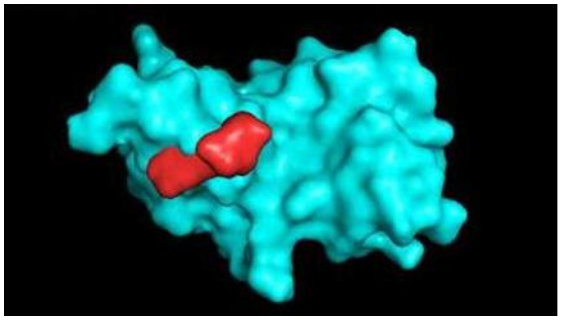
The Binding affinities of the remaining ligands which are Luteolin, Esculin, Chlorogenic, Stigmasterol, Cycloartenol, Catechin gallate, Proanthocyanidins, Lignin, and Campesterol ranges between -7.6 to -7.2.

Despite having the most binding affinity of -8.8, Diosegenin presented only two binding sites which are LYS 71 and PRO72 showing only one hydrophobic interaction which is GLN3 [2.84].

Ergosterol with the binding affinity of -8.1 shows two binding sites which are PRO72 and SER73, presenting no hydrogen bonds. Luteolin having -7.7 binding affinity score, presented only one binding site which is ASP95 with two hydrophobic reactions which are [2.98, 2.98]

On the other hand, Chlorogenic having one of the most active binding sites, VAL68, LYS71,and ASP95 garnered a binding affinity of -7.2 kcal/mol. Followed by Astaxanthin with also three binding sites which are LYS71, SER73, and PRO72 having a binding affinity of only -6.6. kcal/mol. Having Beta-sitosterol third on the list having three binding sites which are LYS21, PRO72, and SER73 garnered a binding affinity score of -7.8.

Visualization of the top three Phytochemicals with the most interacting residues



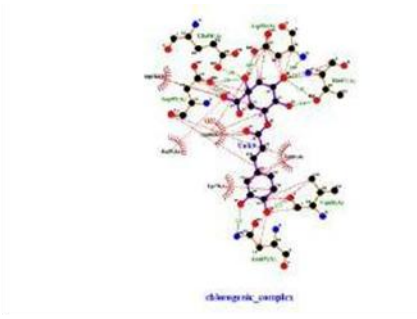
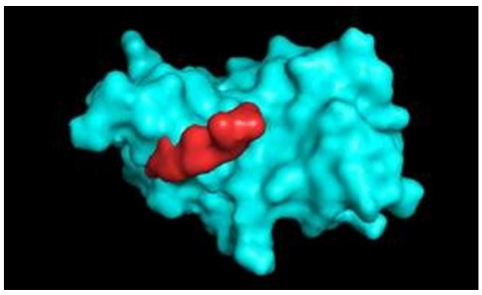


Fig 17 & 18. Chlorogenic complex PyMol 3D visualization (left); Ligplot+ hydrogen bonds and hydrophobic interactions 2D visualization (right).



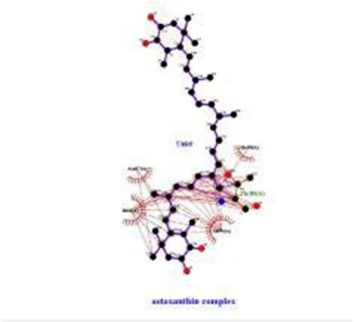
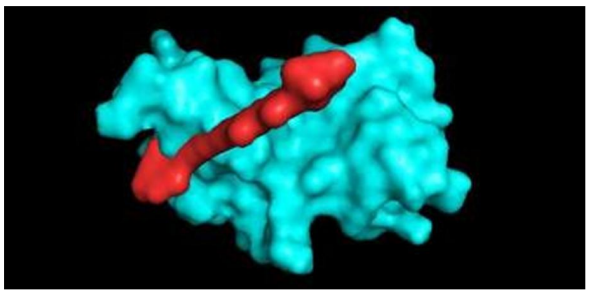


Fig 19 & 20. Astaxanthin complex PyMol 3D visualization (left); Ligplot+ hydrogen bonds and hydrophobic interactions 2D visualization (right).



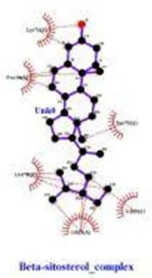


Fig 21 & 22. Beta-sitosterol complex PyMol 3D visualization (left); Ligplot+ hydrogen bonds and hydrophobic interactions 2D visualization (right) concentration achieving the most promising

Conclusion

The study demonstrated the potential of seagrapes (Caulerpa lentillifera) extract as a biological treatment for Ralstonia solanacearum, the pathogen responsible for bacterial wilt in potatoes. The in vitro experiments showed that higher concentrations of seagrape extract significantly reduced bacterial colony growth, with the 30ml/L result (17 colonies). While this reduction was notable, the extract did not fully meet the optimal inhibition range (1-15 colonies), which would indicate complete effectiveness.

The in silico molecular docking analysis further supported the antibacterial potential of seagrapes, identifying key phytochemicals such as Diosgenin, Proanthocyanidins, and Ergosterol, which exhibited strong binding affinities with the pathogen's 1UQX protein, suggesting their inhibitory potential at the molecular level. These findings, while promising, indicate that the extract's antibacterial activity, particularly in comparison to the chemical control (ICC), requires further refinement.

Recommendations

To improve the potential of seagrape extract as a biocontrol agent, future research should focus on optimizing the concentration and formulation of the extract. Investigating combinations of the most effective phytochemicals or enhancing the extract's bioavailability through nano- formulation could improve its antibacterial properties. Additionally, conducting field trials to evaluate the performance of seagrape extract under real agricultural conditions would be essential for validating its practical application. Furthermore, exploring the synergy between seagrape extract and other biological or chemical agents may provide insights into integrated pest management strategies, contributing to safer and more sustainable agricultural practices.

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