IN SILICO AND *IN VITRO* ANALYSIS OF ANTIMICROBIAL PROPERTIES OF BIOACTIVE COMPOUNDS EXTRACTED FROM *Selenicereus undatus*

ABSTRACT

Aims: Extract antimicrobial compounds from the dragon fruit peel and analyze its activity using *in vitro* and *in silico* techniques to produce a potential therapeutic agent for diseases like food poisoning.
Study Design: In vitro studies followed by the analysis using in vitro techniques like docking and molecular dynamic simulation.
Place and Duration of Study: Department of Biotechnology (S8-group 15) Final year biotechnology students of Sahrdaya College of Engineering and Technology, Kodakara, Thrissur, Kerala, India between March 2023 and May 2024
Methodology: Extraction of bioactive compounds was performed. Antimicrobial activities were analyzed using well diffusion techniques and the result was mainly observed for *Bacillus cereus*. The proteins and active sites of bacteria were used for docking against the ligands or compounds present in the extracted solution of dragon fruit peel, and the results were analyzed. Also, molecular dynamic simulation and toxicity prediction were performed and using the results obtained a new product with medicinal value is proposed completely made or manufactured from a waste product making this a sustainable idea.
Results: *In vitro*, the antimicrobial activity of the crude extract was analysed by using the agar well diffusion method. *Bacillus cereus* shows more inhibition towards the extract. Target active sites and ligands are listed for molecular docking and the complex with highest binding energy is screened out for SwissADME and from the results obtained molecular dynamic simulation is performed.
Conclusion: This work shows the antimicrobial activity of the compounds present in dragon fruit peel against *Bacillus cereus*, a foodborne pathogen. After molecular docking, SwissADME and molecular dynamic simulation, the interactions, affinity, and stability of the ligand target complexes are known, which leads to the development of innovative healthcare solutions.

Keywords: Dragon fruit, Bacillus cereus, Antimicrobial activity, Molecular docking

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1. INTRODUCTION

In the rapid evolution of the past century, the global landscape witnessed a surge in the risk and prevalence of diseases, both novel and existing, including the formidable challenges posed by cancer and infectious diseases. In response to

this health crisis, researchers dedicated their efforts to combat these afflictions. This pursuit led to a pivotal breakthrough—the discovery of synthetic molecules [2]. However, the promising horizon of synthetic compounds cast a shadow with their adverse effects, prompting a crucial shift towards natural products as a remedy. The allure of natural products lies in their use of active constituents derived from natural origins, primarily plants. One such botanical gem is the dragon fruit peel. Despite its potential as a source of vital compounds, dragon fruit peels pose a dual threat: not only can they become a significant waste material shortly, but if left unattended, they may also contribute to foul odours and an alarming increase in dump counts [3, 4]. Recognizing the impending challenges associated with dragon fruit peels, a fundamental change is in order. Rather than wasting it, these discarded peels can be repurposed to address a pressing global concern-infectious diseases. The key lies in harnessing the antimicrobial properties embedded within the dragon fruit peel [5].

The main focus centers on the thorough analysis of the crude extract obtained from dragon fruit peel [6]. This extract harbors a wealth of potential, containing compounds with intrinsic antimicrobial capabilities. The rationale behind repurposing dragon fruit peels for antimicrobial endeavors is not merely a pragmatic waste management strategy but a visionary approach to sustainable healthcare solutions. The antimicrobial properties within these peels present an opportunity to mitigate the growing challenges posed by infectious diseases [8]. Through this innovative reuse, not only waste is reduced but also contributes to a paradigm where nature's by-products evolve into potent resources in the fight against infections. In conclusion, the journey from the discovery of synthetic molecules to the acknowledgment of the drawbacks and subsequent exploration of natural alternatives exemplifies the dynamic nature of scientific progress [4]. Dragon fruit peels, once on the verge of becoming a waste burden, now stand at the forefront of a transformative approach to disease mitigation [9]. As we examine the antimicrobial potential within these peels, we pave the way for a future where sustainability intertwines with health, proving that sometimes solutions lie in the unlikeliest of places—in the peel of dragon fruit [7].

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS/METHODOLOGY

2.1 COLLECTION OF RAW MATERIAL

Dragon fruit was collected from juice vendors and other discarded sources, washed, and cut open to separate the flesh and peel. The peels were kept for drying till all of the moisture was removed. The dried peels were then powdered with the help of a grinder [10].

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2.2 EXTRACTION OF COMPONENTS FROM DRAGON FRUIT PEEL

For extraction different solvents were prepared:30%Methanol,50%Ethanol and 100%Ethyl Acetate solution. The solutions after preparation were stored at 0°C for further usage [11,12].

2.2.1 Soxhlet Extraction

Performed using the Soxhlet apparatus. Different solutions were employed as the solvent. Dried and ground material was introduced into the tube, and 300 mL of solvent was added to the flask. Extraction was carried out in three cycles for approximately 6 h. The heating temperature was adjusted to the boiling point of the employed solvent [1].

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2.2.2 Cold Extraction

Dried and ground material and solvent were added to a beaker. Various solutions were employed as the solvent. To avoid constant stirring, a magnetic bead was added to the mixture, and it was then placed on a magnetic stirrer. The extraction took place for about 3 hours at room temperature [1].

2.3 ANTIBACTERIAL ASSAYS

2.3.1 Microorganisms and Culture Conditions

The test microorganisms used for the determination of the antibacterial activity of Dragon fruit peel extracts were acquired from the International Culture Collections (ATCC). Two classes of bacteria were used: Gram-positive bacterium *Bacillus cereus* and *Staphylococcus succinus*, and Gram-negative bacterium *Escherichia coli* and *Klebsiella pneumonia*. The bacteria were grown in nutrient broth for 24 h at 37 °C and stored at 0°C for further usage [13,28].

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2.3.2 Agar Well Diffusion Method

Agar-well diffusion method is generally utilized for the determination of the antibacterial activity of plant extracts. For this method, Petri plates were prepared by pouring 20-25 mL of molten nutrient agar medium (45 °C). After that, the bacteria were streaked onto different petri plates separately. After streaking three wells were made so that one well contained the respective extract, the other the negative control or solution used for extraction and the third well contained a positive control using a micropipette tip. The petri plates were incubated at 37 °C for 24 hrs [27].
The concentration of streptomycin was calculated using the equation:

$$\textit{weight}(\textit{mg/ml}) = \frac{\textit{volume}(\textit{ml}) * \textit{concentration}(\textit{mg/ml})}{\textbf{potency}}$$

2.4 Antifungal Assays

The ethyl acetate extract of the crude extract solution was added to 10 ml of sterilized PDB agar in petri plates after proper mixing [14,15]. Actively growing disc of the fungi (*Aspergillus niger*, *Aspergillus)* culture was inoculated. Plates without plant extract served as negative control. Plates were incubated at 27 °C. Radial growth is to be measured after seven days of incubation. The results are to be compared with negative control [26,29].

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2.5 PREPARING COMPOUND LIBRARY OF BIOACTIVE COMPONENTS

2.5.1 Ligand Identification

The ligands were identified from literature showing similar techniques of extraction of bioactive compounds from dragon fruit peel [32].

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2.5.2 Target Identification

Based on the positive results obtained from both bacterial and fungal cultures the target is identified. Furthermore, active sites are assigned and the structures are downloaded**.**

2.5.3 Docking

The molecular interactions between the bio-active components and the protein targets of the microorganisms are studied to rank the complexes based on binding affinity. The primary goal is to identify the best possible pairing of ligand and receptor that consumes the least amount of energy and attaches to a specific protein of interest [30]. The type and strength of the signal that will be generated can be predicted using docking. As a result of its ability to anticipate how small molecule ligands would bind to the proper target binding site, it is one of the most frequently employed methods in structure-based drug design [30].

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Protein targets were identified and prepared from the Protein Data Bank (PDB) server. The Protein Data Bank is a database that contains three-dimensional structural data for big biological entities including proteins and nucleic acids. The docking was done using the POAP server. Open Babel and the Auto Dock package are designed to run with highly efficient parallelization using the parallel-based pipeline—POAP [31]. A special feature of POAP is the ligand preparation module, which provides a wide range of choices for geometry optimization, conformer creation, and parallelization, and also quarantines incorrect datasets for smooth operation [31]. Additionally, POAP has multi-receptor docking, which may be used for virtual comparison screening and drug repurposing research [31].

2.5.4 SwissADME

The compounds with drug-like properties were filtered using the canonical SMILES of top-list ligands from docking results and were analyzed using the swissADME tool.

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3. RESULTS AND DISCUSSION

3.1 COLLECTION OF RAW MATERIAL

The peels were separated and dried to obtain 166.66 grams of dragon fruit peel powder.

3.2 EXTRACTION OF COMPONENTS FROM DRAGON FRUIT PEEL

Soxhlet and cold extraction were performed using 100% Ethyl acetate,50% Ethanol, and 30% Methanol solutions.

3.3 ANTIBACTERIAL ASSAYS

3.3.1 Microorganisms and Culture Conditions

Subcultured four different bacteria in nutrient broth for 24 hours.

3.3.2 Agar Well Diffusion Method

The agar diffusion method was performed as discussed and kept for incubation for 24 hours. After incubation *E. coli* and *Klebsiella pneumoniae* showed a zone of inhibition for well-containing streptomycin whereas *Bacillus cereus* showed inhibition for both streptomycin and well-containing ethyl acetate extract [16,17].

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3.3.2.1 Observation:

- Crude solution extracted using 100% Ethyl acetate showed inhibition for *Bacillus cereus*. Controls used: Positive control: Streptomycin (concentration:1000 mg/ml) and Negative control: Solvent used for extraction of bioactive compound.
- The zone of inhibition amounted to 7mm without well for crude extract.
- The crude extract of dragon fruit peel powder extracted using 100% ethyl acetate was concentrated such that 20ml of the extract was kept for complete evaporation in the fume room. This resulted in the formation of about 43.6mg extract powder which was then dissolved in 1ml of distilled water.
- The agar-well diffusion was repeated for the four bacteria again using this solution and a zone of inhibition was observed for Klebsiella pneumonia and Escherichia coli.

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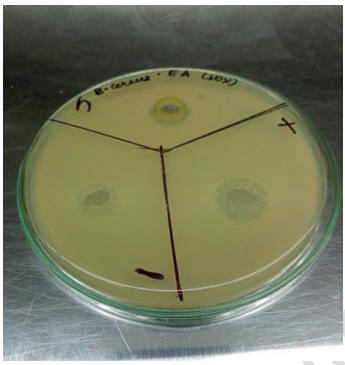

Fig.1 Antibacterial activity of crude extract against Bacillus cereus by agar well diffusion method

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3.3.3 ANTIFUNGAL ASSAYS

The poisoned food technique for the fungi *Aspergillus flavus* was performed and kept for incubation at room temperature. The result can be observed after the incubation period which is about 5-7 days [18,19].

3.4 MOLECULAR DOCKING

Table.1 Results of molecular docking

Sl No.	Ligand PubChem ID	1ah7	2nrj	2uyr	6gar	6gas	8hkj
1	689043	-7.8	-4.7	-4.8	-7.4	-7.0	-6.6
2	24772948	-7.7	-1.4	-5.6	-7.3	-6.8	-8.1
3	9064	-7.5	-3.6	-5.5	-9.3	-6.8	-8.9
4	5281855	-7.4	-5.4	-5.4	-6.7	-6.8	-8.8
5	6440659	-7.4	-4.4	-4.7	-7.4	-7.0	-6.5
6	445858	-7.4	-4.7	-4.7	-7.4	-7.0	-6.5
7	637542	-6.9	-4.4	-4.3	-7.0	-6.7	-6.6
8	444539	-6.8	-4.4	-4.1	-6.9	-6.3	-6.5
9	442431	-3.5	6.8	-7.3	-10.4	-10.4	-11.3
10	114627	-3.9	5.1	-6.9	-9.0	-10.1	-9.6
11	12912214	-6.2	2.4	-6.6	-10.4	-10.2	-10.3

12	5319484	-7.0	-3.6	-6.5	-11.4	-10.5	-9.7
13	1794427	-7.0	-1.4	-6.5	-9.0	-8.3	-9.3
14	102401026	-1.4	6.1	-6.1	-10.7	-7.7	-9.4
15	5281764	-4.7	-3.2	-6.4	-10.4	-9.9	-9.6
16	5318645	-3.8	-3.6	-5.4	-7.9	-6.8	-9.8

Based on the molecular dynamics scores the highest binding score was -11.4 and it was of the complex 6gar-agr as shown below:

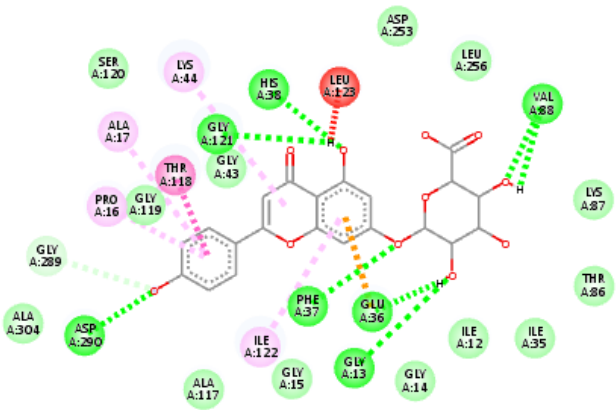

Fig.2 2D interaction of 6gar-agr complex

3.5 SwissADME RESULTS

Table.2 Results of SwissADME analysis

SI No.	Ligand PubChem ID	Molecular Weight (g/mol)	GI Absorption	BBB Permeant	Bioavailability Score (≥0.5)	Verber Violations
1	689043	180.16	High	No	0.56	0
2	24772948	316.31	High	No	0.55	0
3	9064	290.27	High	No	0.55	0
4	5281855	302.19	High	No	0.55	1
5	6440659	313.35	High	No	0.55	0
6	445858	194.18	High	No	0.55	0
7	637542	164.16	High	Yes	0.85	0
8	444539	148.16	High	Yes	0.85	0

9	442431	580.53	High	No	0.85	1
10	114627	596.53	Low	No	0.45	1
11	12912214	446.36	Low	No	0.45	0
12	5319484	446.36	Low	No	0.48	0
13	1794427	354.31	Low	No	0.39	0
14	102401026	548.445	Low	No	0.27	0
15	5281764	474.37	Low	No	0.46	1
16	5318645	478.4	Low	No	0.48	0

Based on SwissADME results the feasible less toxic complex obeying the above pharmacokinetic parameters was 1ah7-cffa complex and its 2D interaction is as shown below:

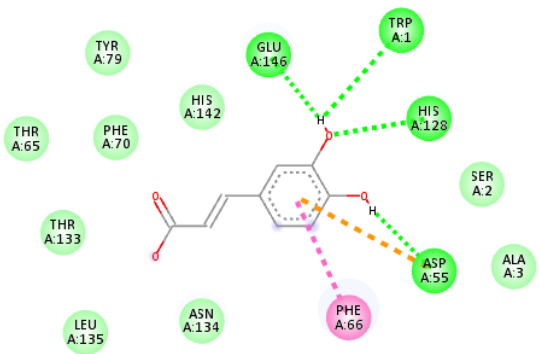

Fig.3 2D interaction of 1ah7-cffa complex

4. CONCLUSION

The antibacterial activity of the crude extract was analyzed using the agar-well diffusion method and obtained a single positive result for crude extract with 100% ethyl acetate as the solvent. It effectively inhibited the growth of *Bacillus cereus*. After concentrating the crude extract, it inhibited the growth of both *Bacillus cereus* and *Klebsiella pneumonia* [23,24]. This confirmed the antibacterial properties of dragon fruit peel. Along with this, an analysis of the antifungal properties of the crude extract was done. From *in silico* analysis, interactions between target and ligand molecules were obtained using AutoDock Vina and swissADME [25]. *Bacillus cereus* is a foodborne pathogen that can produce toxins, causing two types of gastrointestinal illnesses: the emetic (vomiting) syndrome and the diarrheal syndrome. *Klebsiella pneumonia* can cause different types of healthcare-associated infections, including pneumonia, bloodstream infections, wound or

surgical site infections, and meningitis [20,21,22]. Based on this a viable product can be proposed.

CONSENT

Consent for publication: all the authors informed the consent for publication**.**

ETHICAL APPROVAL

Not applicable

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