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# ANTIPROLIFERATIVE AND ANTIBACTERIAL POTENTIAL OF PHYTOCONSTITUENTS DODECANAMIDE DERIVATIVES FROM KAPPAPHYCUS ALVAREZII ALGAE

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**Abstract**

The study aimed to investigate the antiproliferative and antibacterial potential of dodecanamide derivatives from Kappaphycus alvarezii algae. Fourier transform infrared spectroscopy and gas chromatography-mass spectrometry analyses were conducted to identify bioactive compounds in different parts of the plant. Molecular docking studies were performed to assess potential pharmacological properties. Results indicated significant free energy of formation for chloramphenicol and dodecanamine, with resorcinol sulfoxide showing similar properties to chloramphenicol. GC–MS analysis identified 49 bioactive compounds with various pharmacological activities. Molecular docking studies suggested potential anticancer and anti-inflammatory properties. This study presents novel findings on the bioactive compounds of Kappaphycus alvarezii, indicating antioxidant, antimicrobial, and pharmacological significance (Varshan and Prathap 2022). These results suggest the potential for developing new herbal medicines utilizing Kappaphycus alvarezii, potentially leading to the creation of innovative medications.

**Keywords:** Kappaphycus Alvarezii, Anti-Bacterial, Molecular Docking, Phytochemicals, Medicine.

## INTRODUCTION

Antimicrobial resistance (AMR) is a pressing global health concern, driven by a myriad of factors such as selective pressure, gene transfer, and improper drug usage, as highlighted by the World Health Organization (WHO) (Kahn, 2016). Its implications are far-reaching, affecting not only healthcare outcomes but also economic and societal dynamics. Alarmingly, projections suggest that AMR could lead to a doubling of fatality rates from common diseases by 2050, surpassing other leading causes of mortality (Kahn, 2016). This impending crisis underscores the urgency of exploring novel solutions to combat AMR.

In response, researchers have turned their attention to natural resources, including marine algae, in search of alternative antimicrobial agents. Kappaphycus alvarezii, a species of red algae, has garnered interest for its potential bioactive compounds with therapeutic properties (Long et al., 2024). Widely cultivated for the production of carrageenan—a versatile polysaccharide used in various industries—Kappaphycus alvarezii has been studied for its pharmacological potential, including its antibacterial and antioxidant effects (Long et al., 2024; Hans et al., 2023).

The pursuit of novel therapeutic agents has led to a renewed focus on secondary metabolites derived from natural sources, known as phytoconstituents. These compounds, which include alkaloids, flavonoids, terpenoids, and phenolic compounds, have demonstrated diverse biological activities and have long been utilized in traditional medicine (Akshaya & Ganesh, 2022). Marine algae, such as Kappaphycus

alvarezii, represent a rich reservoir of phytoconstituents, offering promising avenues for drug discovery and development (Sakarwal et al., 2024).

Despite the potential benefits of bioactive compounds from Kappaphycus alvarezii, several challenges must be addressed. Concerns regarding safety, potential side effects, and toxicity of these compounds necessitate comprehensive evaluation (Al-Malki et al., 2019). Additionally, issues related to bioavailability—the ability of a compound to reach its target site and exert pharmacological effects—must be addressed to ensure therapeutic efficacy.

In light of these considerations, this article explores the potential of Kappaphycus alvarezii and its bioactive derivatives, particularly dodecanamide derivatives, as a source of novel antimicrobial agents. By examining the bioactivity, safety profile, and pharmacological properties of these compounds, researchers aim to contribute to the development of effective treatments for infectious diseases and address the growing threat of antimicrobial resistance.

## MATERIALS AND METHODS

The ligands, Gemcitabine were designed by changing the functional group and the substitutions, then LigPrep (v3.1), Schrodinger software was used to prepare the high resolution 3D structure of the respective ligands, which include the 2D to 3D conversion, optimization, minimization of energy states, some of the corrections (McElree et al. 2024).

Glide suite was used for performing docking analysis. (McElree et al. 2024; Sakarwal et al. 2024) Molecular dynamics was performed using Desmond v5.7. In silico analysis and docking are carried out using the Glide program. Ligand preparations were carried out using the Epik program. Proteins were prepared with a protein preparation wizard program. To understand the pharmacokinetic parameters, we used the Swish ADME online tool.

### Preparation of ligand

Dodecamine and resorcinol are active ingredients found in Kappaphycus alvarezii Algae. The chemical structures of the selected bioactive compounds were retrieved through the PubChem compound database at NCBI.(Witty and Cox 2022)

### Target protein retrieval

Two proteins, pseudomonas aeruginosa protein (PDB ID: 50E3) for anti-inflammatory were selected for their interactions with the bioactive compounds from Kappaphycus alvarezii Algae. The 3D X-ray crystal structure of the proteins was retrieved from Protein Data Bank (PDB). (Sillince and Sillince 2012)

## RESULTS

In order to know the active compounds responsible for anti-cancer activity, present in the Kappaphycus alvarezii Algae, we identified two substances namely dodecamine and resorcinol present in its extract and compared their binding efficiency with chloroamphenol as possible control.

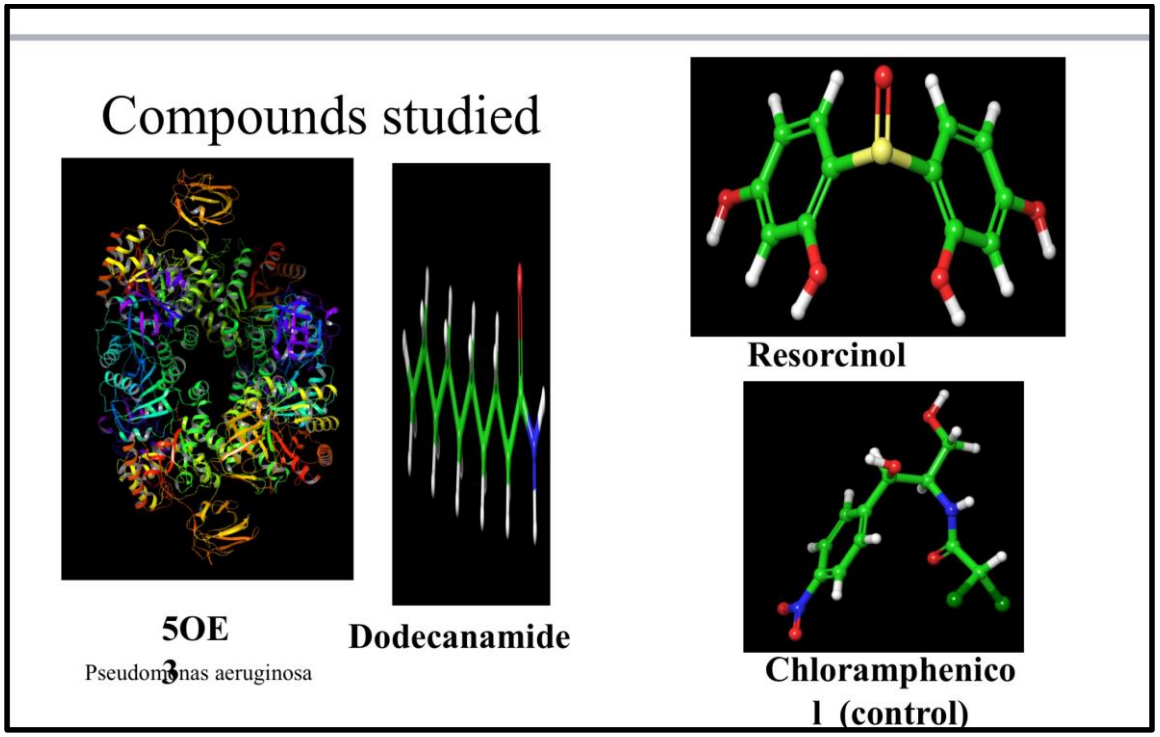


**Figure 1: Structure target pseudomonas aeruginosa protein 50E3 and the bioactive compounds identified from Kappaphycus alvarezii Algae along with the chloramphenicol as control used for the analysis of molecular docking.Z**

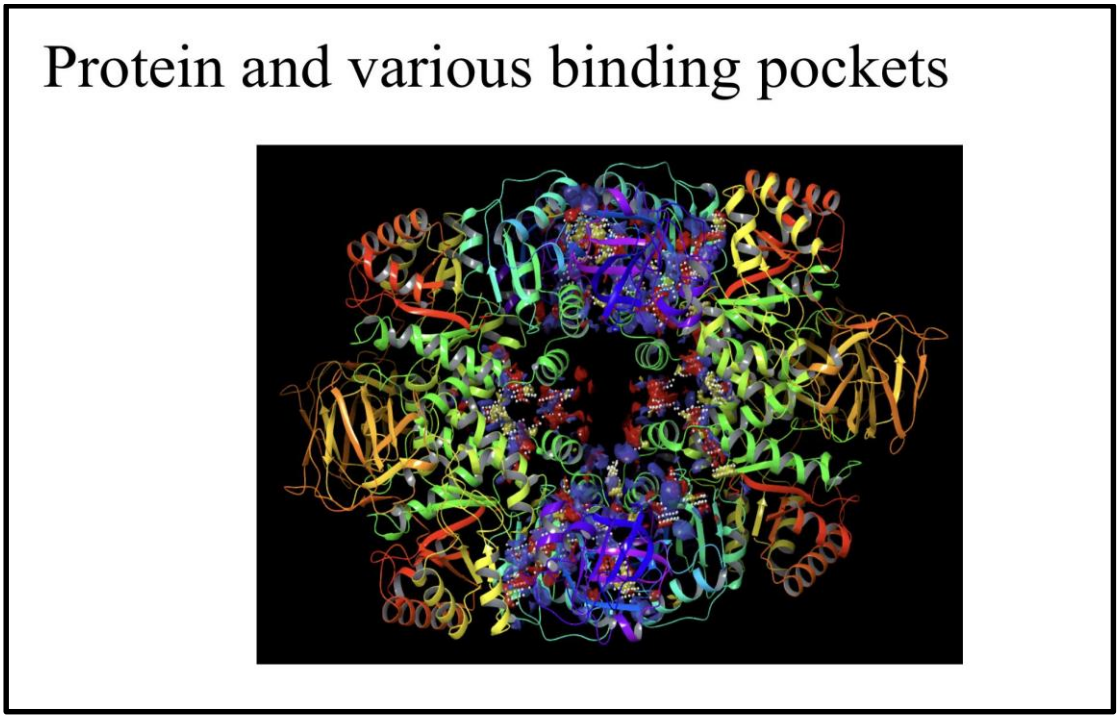


**Figure 2: Structure of pseudomonas aeruginosa protein, and the active sites present in it for molecular docking and shown the pseudomonas aeruginosa protein receptor along with active sites present in it for binding of ligands**

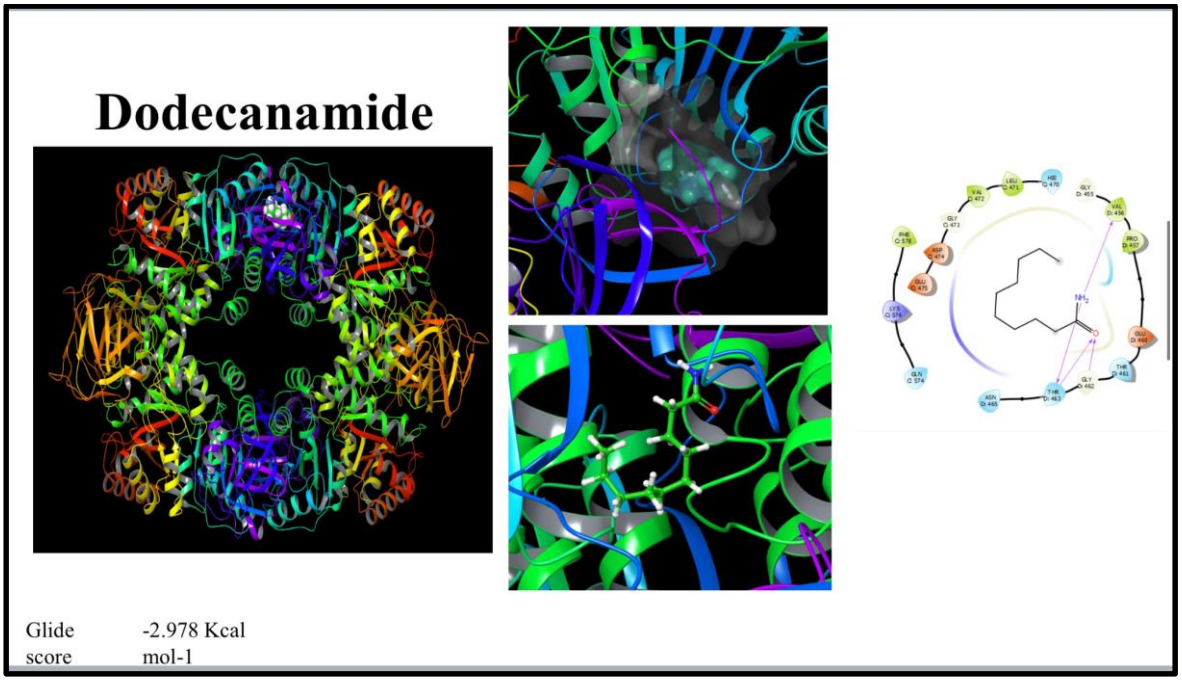


**Figure 3: The 2D diagram illustrates the binding of dodecamine to the Pseudomonas aeruginosa protein receptor. The amino group binds to valine, while the carbonyl groups interact with glycine and tyrosine residues within the active site**

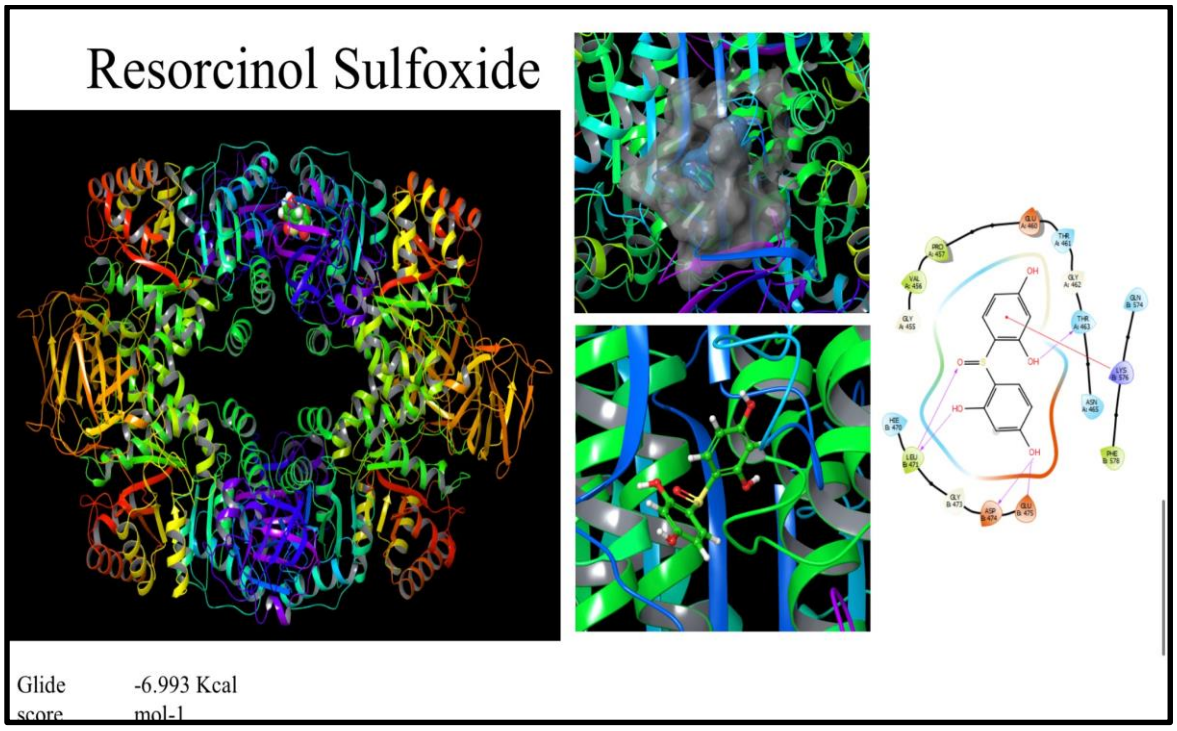


**Figure 4: The 2D diagram depicts the binding of resorcinol sulfoxide to the Pseudomonas aeruginosa protein receptor. The hydroxyl and carbonyl groups of the ligand interact with leucine, asparagine, and glycine residues within the active site**

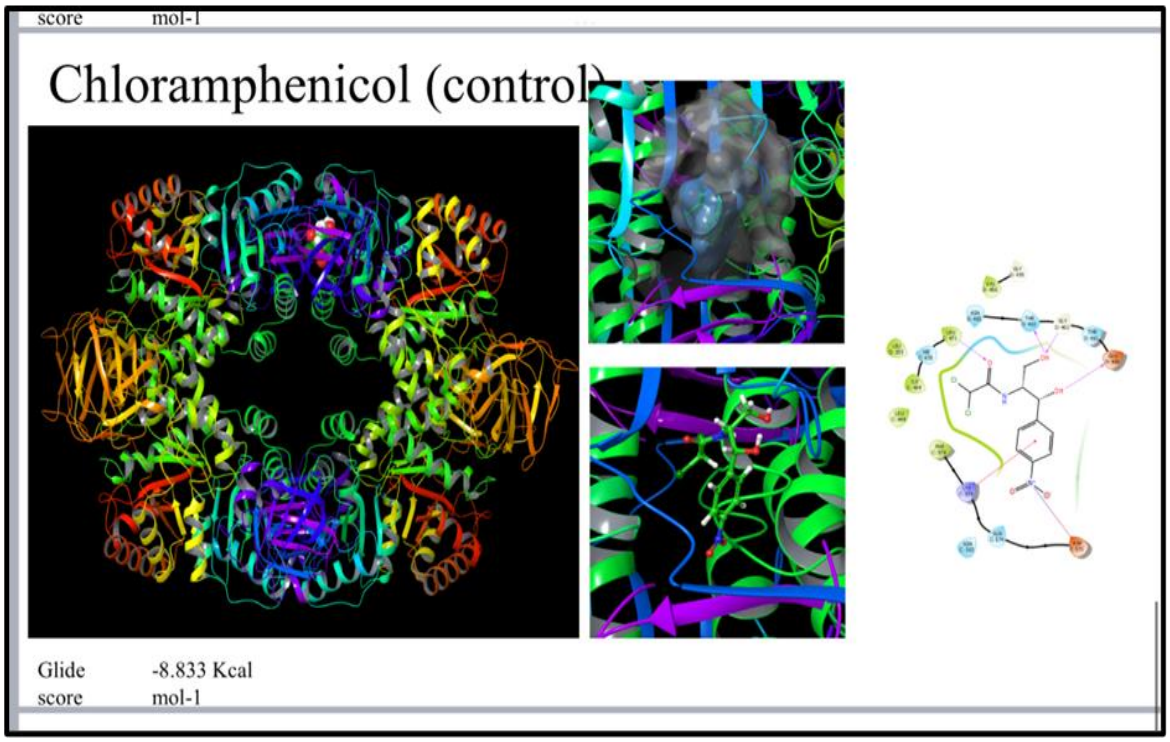


**Figure 5: The figure depicts the active site binding of chloramphenicol on the Pseudomonas aeruginosa protein receptor. A 2D diagram illustrates the ligand's interaction with various amino acids of the protein. The hydroxyl and carbonyl groups of the ligand bind to leucine, asparagine, and glycine residues**

The results shows that chloramphenicol (Ren et al. 2024; Wang et al. 2013)has the highest “free energy of formation, while the least is observed for dodecamine. The resorcinol sulfoxide has a free energy of formation nearly equivalent to chloramphenicol(Ganesh, Vishnu Priya et al. 2020).

## Ethnobotanical studies

Plants are valuable sources of beneficial bioactive compounds for the manufacture of novel chemotherapeutic agents. Investigation on the possibility of using pharmacologically active com- pounds derived from medicinal plants is an important procedure It is believed that about 80% of the world’s population use medicinal plants due to their high efficacy, low cost, non-narcotic origin and fewer side effects.

Various food crops are used for their application in healthcare and therefore are called "medicinal foods". Kappaphycus alvarezii is an ethnobotanical important plant having high nutritional and medicinal values.

## Preliminary screening

A preliminary phytochemical screening was done using methanolic extracts of edible parts of Kappaphycus alvarezii and the result is shown The study showed the presence of tannins, terpenoids, phenol, flavonoids, alkaloids in Kappaphycus alvarezii. The presence of phytochemicals from this plant had also been reported previously.

**Table 1: Total flavonoid content**

| Pharmacokinetics | | | |
|---|---|---|---|
| | Dodecanamide | Resorcinol Sulfoxide | Chloramphenicol |
| GI absorption | High | High | High |
| BBB permeant | Yes | NO | NO |
| P-gp substrate | NO | NO | NO |
| CYP1A2 inhibitor | NO | NO | NO |
| CYP2C19 inhibitor | NO | NO | NO |
| CYP2C9 inhibitor | NO | NO | NO |
| CYP2D6 inhibitor | NO | NO | NO |
| CYP3A4 inhibitor | NO | NO | NO |
| Log *Kp* (skin permeation) | -5.47 cm/s | -6.11 cm/s | -7.96 cm/s |
| Lipinski rule | Yes; 0 violation | Yes; 0 violation | Yes; 0 violation |
| Bioavailability Score | 0.55 | 0.55 | |

The quantitative phytochemical composition of flower, capitulum, tender pods and seeds of Kappaphycus alvarezii are shown in table 1. Total flavonoid was found to be the highest in the pod (58.38±0.001 mg/g), while flower (28.95±0.002 mg/g) contained the least amount. Previously, it was shown that the methanolic extracts of pods and seeds contained 5.28 mg QE/g and 20.3 mg QE/g respectively28 and 15.47 mg CA/g in pods29. Tapan30 has shown that the methanol extract of pods contained 4.05 mg/g total flavonoid.

**Total phenol content**

The total phenols content of Kappaphycus alvarezii is shown in table 1 The pod had the highest total phenolic content of all analyzed samples (38. ± 0.136 mg/g) whereas flower extract had the lowest content (18.7±0.161 mg/g). The result is in agreement with previous reports on Kappaphycus alvarezii where pods contained 49.39 mg GAE/g30 36.59 ± 3.80 mg GAE/g19.

**Antioxidant assay**

DPPH Scavenging activity. The antioxidant property of methanolic extract of Kappaphycus alvarezii was estimated using DPPH assay. The percentage inhibition of free radical scavenging and IC50 is repre- sented in Fig. 2 & and Table 3 respectively. The antioxidant IC50 was analogously high in the pod (22.45 µg/ml)

**Phosphomolybdate assay**

The quantitative total antioxidant capacity was also assessed using Phospho-molybdate assay. Based on the scavenging activity, the extracts could again be ranked as Pod>Seed>Capitu- lum > Flower (Fig. 2). The IC50 value ranged from 38.52 µg/ml in pod to 63.09 µg/ml in flower. The IC50 of the pod is also comparable to that of the standard, Ascorbic acid (33.43 µg/ml). The significant antioxidant potential of the pod, which is statistically comparable to that of standard Ascorbic acid, showed that the extract contained strong antioxidants, which could be due to the presence of phenolic compounds.

## Antibacterial activity

The present study investigates the antibacterial activity of edible parts of tree bean.The result shows that the methanol extracts of Kappaphycus alvarezii exhibit antibacterial activities against the four tested bacterial strain.The diameter of the zones of inhibition ranged from 9 to 17.66 mm, the highest inhibition zone was observed in E. coli (17.66 mm), followed by B. pumilus, P. aerugi- nosa and B. subtilis (15.33 mm, 14.33 mm and 13 mm respectively). The quantitative evaluation based on the inhibition growth showed that the pod had very high (x > 12 mm) inhibitions against all the tested bacterial strains. Other parts- capitulum, flower and seed also had a high (x = 8–12 mm) inhibition zone against all bacterial strains.

## Minimum inhibitory concentration (MIC)

MIC values determined from the extracts varied from 2.19 to 7.3 mg/ml (Table 5). The percentage inhibition of antibacterial activity is shown in Fig. 4. Pod gave MIC values of 3.5 mg/ml, 3.4 mg/ml, and 2.19 mg/ml, and 3.04 mg/ml respectively against B. subtilis, B. pumilus, E. coli, and P. aeruginosa.(Khan 2017)

## FTIR analysis

FTIR spectroscopy was employed to identify the functional groups of bioactive components present in Kappaphycus alvarezii FTIR spectrum confirmed the presence of hydroxyl group (O–H), aldehyde (C–H), alkenes (C=C), carboxyl (C=O), nitrogen-containing group (N–O), alkanes (C–C), aromatic primary amine (C–N), amines (N–H), alkynes (C≡C), aliphatic Bromo compounds (C–Br), phenols, carboxylic acids, glycogen, alkyl halides, halogen, aliphatic amines, primary and secondary amines, esters, ether, aromatics, lipids, triglycerides, nitro compounds and these functional groups are the integral parts of various secondary metabolites such as alkaloids, flavonoids, terpenoids, polyphenol and tannins32.(Tuchman 1992)

## GC–MS analysis

Evaluation of the chemical structure and composition of the extracts can designate various biological potential of different medicinal plant extracts. However, to the best of our knowledge, there is no report on GC–MS based plant metabolic characterization to reveal the presence of various bioactive compounds in methanolic extracts of edible parts of Kappaphycus alvarezii. Therefore, the GC–MS analysis was performed in a predetermined study. A total of 104 peaks were observed in all plant parts, each peak designated the bioactive compounds that were recorded by relating their peak retention time, molecular weight, molecular formula to that of the known compounds proposed by the NIST library.(Oehme 1998)

## Molecular docking

Further, six bioactive compounds identified by GC–MS analysis from Kappaphycus alvarezii were selected and subjected to molecular docking with BCL-2 and COX-2 proteins Paclitaxol (for anti- cancer) and Oxitasim (anti-inflammatory) were used as standard controls. The 2D structures of bioactive compounds were first retrieved from the PubChem database. These compounds were selected based on Lipinski's rule of five parameters such as molecular weight, log P, number of hydrogen bond donors and number of hydro- gen bond acceptors.(Fatt and Weissman 2013)

Structures of the bioactive compounds are shown in Fig. 8. The binding analysis between COX-2 protein and ligands, BCL-2 and ligands revealed that the binding pattern varied with the nature of the ligands. The docking results of bioactive compounds are shown in and the docking results are represented in the form of minimum binding energy values.

## DISCUSSIONS

The indigenous people of North East India commonly consume this plant because of its nutritive and medicinal benefits. The plant parts especially the bark, fruit skin, leaf, seeds are used for the treatment of various diseases The tender pods (young and mature), flowers, capitulum and mature seeds have also been traditionally used as supplementary food sources in this region. In Kappaphycus alvarezii flowers, capitulum, fruits and seeds are consumed either raw as salads or boiled with water as vegetables that contribute to the health benefits. Tree bean is also used as a supplementary food source and provides quality food for human as well as livestock, and is touted as a multifunctional vegetable that serves as valuable and dependable income-generating products for farmers of North East India. Ethnobotanical data hinted Kappaphycus alvarezii(Gupta and Van Staden 2021) as a multi-functional plant that had nutritional as well as medicinal values and also provided that Kappaphycus alvarezii (Gupta and Van Staden 2021; Hurtado, Critchley, and Neish 2017) likely contained bioactive compounds which could be used in antidiabetic, anti-hypersensitive, anti-inflammatory, anti-stomachache and antimicrobial medicines.(Hasanuzzaman et al. 2019)

Flavonoids are shown to be effective against cancer cells by inactivating or inhibiting carcinogens, anti- proliferative, induced apoptosis and cell cycle arrest. Terpenoids also have several curative properties like anti-cancerous, anti-parasitic, anti-allergic, and anti-inflammatory Likewise, saponins and alkaloids have been reported to have anticancer properties especially against colon cancer, and saponins can also induce growth inhibition and apoptosis Traditionally, tannins are also used for the treatment of diarrhea, hemorrhage, detoxification etc. Studies have shown that flavonoids and phenolic compounds present in plants have a wide variety of biological effects including antioxidant, anti-inflammatory, antimicrobial, anti-angiogenic, anticancer, anti-allergic properties. In our study to determine the potency and understand the insights into the possible mechanism of 3 different herbal-based ligands, molecular docking simulation study was carried out on protein pseudomonas aeruginosa. The structures of all kappaphycus alvarezii phytochemicals investigated in present investigation are given.

Phytochemical qualitative screening of the aqueous extract of Kappaphycus alvarezii was performed Phytochemicals have been known to contain antioxidant properties that can cure diseases such as diabetes and cancer. Results indicate that the presence of a few phytochemicals including phenols, saponins and flavonoids can play a role as reducing as well as capping agents in the production of Ag nanoparticles. (Singh et al. 2010)(Akshaya and Ganesh 2022)Flavonoids impart antiviral, antimicrobial properties. Many phenolic compounds and flavonoids have been studied to be very important in medicinal and pharmaceutical applications as anti-cancer, antibacterial, immunity boosting and protective agents for the skin. They are also known to have remarkable antioxidant properties. (Justino 2017)It has been reported that saponins have anti-inflammatory and membrane degrading characteristics which help in many medical and health care applications including anti-cancer effects(BABU and MOHANRAJ 2020). Infectious diseases caused by bacterial agents pose a major threat to public health

worldwide and the rise of antimicrobial resistance and toxicity problems, on the other hand, has limited the use of antimicrobial agents Antibiotics have safety and efficacy limits and due to similar toxicity and efficacy, biological research on antimicrobial functions of plants should be expanded. The bioactive compounds present in plants such as alkaloids, flavonoids, tannins, phenol, saponins etc. are their secondary metabolites that act as defense mechanism against microorganisms, insects and other herbivores and the presence of these compounds in tree bean could be responsible for the observed antimicrobial property(Dhanvanth and Maheswari 2022).

Among them, plants in response to microbial infections synthesize flavonoids (Justino 2017; Yang et al. 2024)(Mohanraj, Varshini et al. 2021)and it is a successful antimicrobial substance against a broad range of microbes Phenols are widely present in secondary products of medicinal plants as well as in many edible plants and have the potential of antioxidants and free radical scavengers Saponins also have antimicrobial properties as they can induce protein and enzyme leakage from cells Tannins bind to proline-rich proteins, thus, stopping them from being synthesized It was also reported that tree bean could inhibit pathogenic bacterial growth such as E. coli, V. cholera, S. aureus, B. cereus Streptococcus faecalis Similarly, in the present study, different Kappaphycus alvarezii extracts were seen to be effective against many cosmopolitan as well as pathogenic microorganisms including E. coli(Institute of Medicine, Board on Global Health, and Forum on Microbial Threats 2012) (urinary tract infections and diarrhoea), B. subtilis (food poisoning), P. aeruginosa (urinary tract, respiratory system infection) and B. pumilus (foodborne disease, severe sepsis of neonatal infants).

It is interesting to note that the multi-drug resistant P. aeruginosa and B. subtilis strains showed more sensitivity to the tested extracts. Our findings support the use of tree beans in folk medicine for the treatment of several infectious diseases. It is opined that Kappaphycus alvarezii species can be a good source for antibacterial drugs against gram-positive and gram-negative bacteria, especially against multi-resistant microorganisms (Palaniappan, Mohanraj et al. 2021). However, isolation of these bioactive antibacterial compounds and detailed characterization of them should provide a deeper understanding of drug discovery and development.

The functional groups can be used in different pharmaceutical products such as anti-cancers, anti-ulcers, anti-inflammatory, anti-oxidants, antimicrobial etc. (Mrkonjić et al. 2024; ElNaggar et al. 2024)Hence, the functional groups present in the bioactive compounds can have diverged applications including antimicrobial, anti-cancer and anti-inflammatory properties etc. FTIR spectroscopy is a reliable and sensitive method for the detection of bioactive compositions.

Our analysis showed clear discrimination between the plant parts examined, depicting great variations.(Verma and Chandel 2019) It was also able to determine the bioactive compounds that could be used as herbal medicines for various diseases. Hence, the studied bioactive compounds have the potential to be used as anticancer and anti-inflammatory agents against these diseases(USHANTHIKA and MOHANRAJ 2020). To the best of our knowledge, this is the first report that bioactive compounds from underutilized, ethnobotanically important substances are subjected to molecular docking for screening their pharmacological potentials.

## CONCLUSION

The study successfully identified a range of bioactive compounds from the edible parts of Kappaphycus alvarezii through FTIR and GC–MS analysis, shedding light on its therapeutic and pharmacological potential. Furthermore, evidence was provided for the antioxidant and antimicrobial properties of Kappaphycus alvarezii extracts(Yuvaraj, Sangeetha et al. 2020). Molecular docking studies revealed promising binding affinities of several bioactive compounds to target proteins associated with therapeutic effects. These findings suggest the potential for developing reliable and effective drugs against various diseases utilizing Kappaphycus alvarezii. However, further investigations to explore its bioactivity and clinical trials are imperative for the discovery and formulation of new drugs.

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### Conflıct of Interest

None to declare.

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