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INVESTIGATING THE INHIBITORY EFFECT OF *Sargassum polycystum* EXTRACT AGAINST *Phythophthora palmivora* IN VITRO

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ABSTRACT----------

This study aimed to investigate the inhibitory effects of Sargassum polycystum extract against Phytophthora palmivora in vitro. The study utilized a quantitative-experimental research design. Quantitative research involves collecting numerical data for statistical hypothesis testing, while the experimental method determines the causality by manipulating an independent variable to observe its effect on a dependent variable (Ghanad, 2023). Seaweed extraction was initially performed to obtain the targeted bioactive compounds particularly flavonoids and phenols, which are associated with antifungal activity. The sample of the collected crude extract was subjected to phytochemical testing to verify the presence of these compounds. Following the extraction process, the extract was evaluated using the Kirby-baeur disc diffusion assay. Four treatments of the seaweed extract (5 µL/disc, 10 µL/disc, 15 µL/disc, 20 µL/disc) were applied to inoculated agar plates and incubated for approximately 6 days. The zones of inhibition were then measured in millimeters (mm) using a caliper. The phytochemical test result indicated a weakly positive reaction for both compounds. In the in-vitro assay, measurable inhibition zones revealed that the 15 µL/disc treatment produced the highest mean zone of inhibition (8.70 mm), followed by the 20 µL/disc treatment (6.84 mm), demonstrating a concentration-dependent effect. It is concluded that Sargassum polycystum extract exhibits minimal but measurable antifungal activity against Phytophthora palmivora; therefore, further research using higher extract concentrations is recommended to further evaluate its antifungal potential.

KEYWORDS: *İn Vitro, Kirby-Bauer Disk Diffusion, Phytophthora Palmivora, Sargassum Polycystum, Zone Of Inhibition----------*

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INTRODUCTION

Fungicidal resistance has emerged as a growing global challenge in managing phytopathogens like *Phytophthora palmivora* (Ivanova et al., 2021; Somnuek et al., 2023). Prolonged and repeated application of conventional fungicides has contributed to the development of resistance in this pathogen to widely used fungicides such as Metalaxyl, Mancozeb, Cymoxanil, and Oxathiapiprolin (Mboup et al., 2022; Myrie & Hanchard, 2025; Somnuek et al., 2023). The main purpose of this study is to investigate the inhibitory effects of *Sargassum polycystum* extract against *Phytophthora palmivora* in vitro to provide a potential sustainable alternative for disease management.

Globally, *Phytophthora* species, which thrives in wet climatic conditions, has caused significant agricultural damage in Southeast Asia, affecting the economically important citrus crops (Hung et al., 2015). In Thailand, *Phytophthora palmivora* was identified as the causal agent of root rot in the pomelo fruits, causing annual loss of about 6 to 12% of citrus production. Beyond citrus, the pathogen affects other economically important crops, including oil palm in Colombia (Torres et al., 2015), rubber, and coconut in Malaysia, Indonesia, Thailand, and Vietnam (Misman et al., 2022).

In the Philippines, the pathogen has severely reduced jackfruit yield, affecting 85% of farms in Leyte and Samar (Oraño et al., 2018), and caused 20% to 90% pod losses annually in cacao farms across Davao Region, still reducing yield and farmer income (Solpot, 2020).

Similarly, local observations from a 26-hectare farm in Mati City indicate recurring fungal infections despite regular fungicide application and pruning practices. Mr. Ruel Dacillio, a farmer and sprayer, reports about 3-5% fruit loss per tree, lowering both yield and quality. The prolonged and costly use of chemical fungicides poses risks of resistance, toxicity to plants, and environmental contamination, emphasizing the need for safer, organic, and sustainable alternatives.

Among the promising alternatives are brown algae, particularly *Sargassum polycystum*, which contains bioactive compounds that enhance defense responses against viral, fungal, and bacterial infections (Arsianti et al., 2020; Thiurunavukkarau et al., 2022; Wong, 2022). Notably, its application has improved resistance to leaf fall disease caused by *Phytophthora palmivora* in rubber tree seedlings (Khompatara et al., 2019). Furthermore, *Sargassum polycystum* extract has demonstrated promising activity against other phytopathogens, attributed to the presence of flavonoids and phenols that contribute to their inhibitory effects (Arsianti et al., 2020). However, there is a lack of research exploring the direct inhibitory effect of *Sargassum polycystum* against *Phytophthora palmivora*, which impacts the agricultural economy in the Philippines, particularly in the City of Mati, where pomelo is widely cultivated.

Research Questions

1. Are specific compounds, particularly Flavonoids and Phenolds, present in *Sargassum polsystum* that may contribute to its inhibitory effect of *Phytophthtora palmivora*?
2. Does *Sargassum polcystum* inhibit the growth of *Phytophthora palmivora* under controlled laboratory conditions?
3. Is there a significant difference among all the treatments of *Sargssum polcystum* extract?
4. Which among the treatments of *Sargssum polcystum* extract has the most significant inhibitory result?
5. How does the antifungal efficacy of *Sargassum polycystum* extract compare with commonly used fungicides in suppressing the growth of *Phytophthtora palmivora?*

METHODS

Study Design

This study employed a quantitative experimental research design by Bhandari (2020) and Bhat (2018), which intends to examine the relationship between two or more measurable variables under controlled conditions. It is utilized to determine the effect of *Sargassum polycystum* extract treatments on the phytochemical properties of the extract and its inhibitory activity against *Phytophthora palmivora.*

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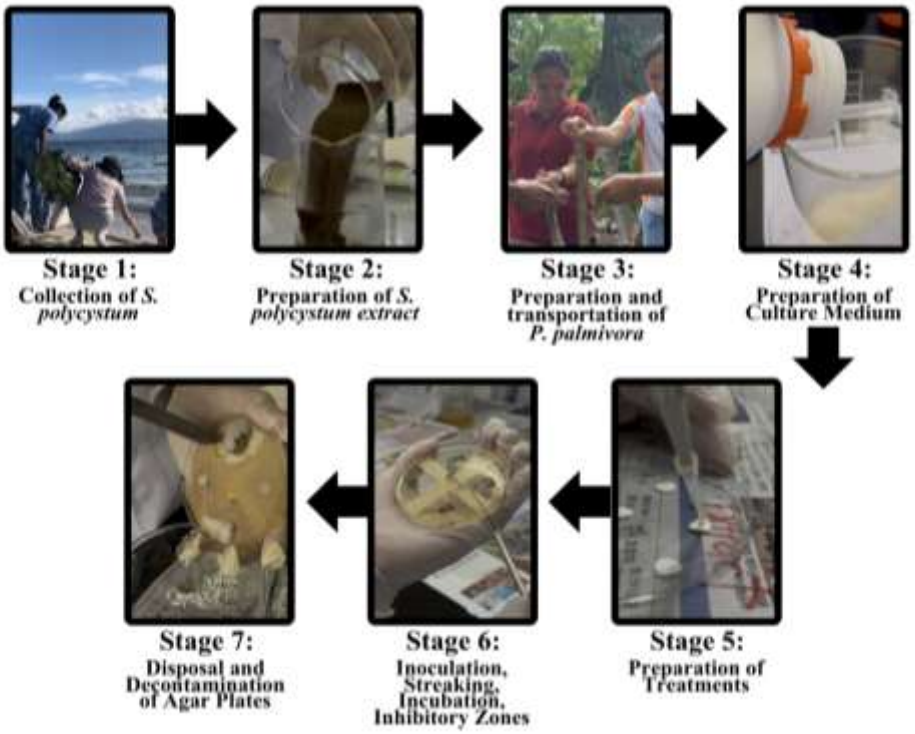

Figure 1. Flowchart of the In vitro Processes

The sample of the study consisted of *Phytophthora palmivora* isolates obtained from infected pomelo tree samples collected in coordination with the Department of Agriculture Office in the City of Mati, Davao Oriental. The pathogen was identified and confirmed by laboratory personnel and subsequently cultured on appropriate growth media to ensure viability throughout the experimentation period. Meanwhile, *Sargassum polycystum* samples were collected from the coastal area of Manguihay, Bobon, City of Mati, Davao Oriental, where the seaweed naturally thrives. The collected seaweed was identified by the Bureau of Fisheries and Aquatic Resources (BFAR) prior to its preparation and extraction for laboratory analysis.

The confirmed *Phytophthora palmivora* isolates were randomly assigned to seven experimental groups, each with three replicates. Four treatment groups were administered varying volumes of *Sargassum polycystum* extract using the disc diffusion method at concentrations of 5 µL/disc, 10 µL/disc, 15 µL/disc, and 20 µL/disc. In addition, three control groups were established: a solvent control group, a negative control group treated with sterile distilled water, and a positive control group treated with Mancozeb. This experimental setup enabled the investigation of the inhibitory activity of the different extract volumes of *Sargassum polycystum* against *Phytophthora palmivora*.

Instrumentation

Data for this study were gathered using standard microbiological and laboratory procedures to evaluate the antifungal activity of *Sargassum polycystum* against *Phytophthora palmivora*. *Sargassum polycystum* was collected from the shoreline of Manguihay, Bobon, City of Mati, Davao Oriental (A), rinsed to remove sand and epiphytes, and verified by the Bureau of Fisheries and Aquatic Resources. The seaweed was shade-dried for two days (B), and ground into a fine powder (C). Twenty grams of the powder were soaked in 100 mL of methanol overnight, filtered, and subjected to rotary evaporation to obtain the crude extract (D). The extraction process was adaoted from the study of (Mahalakshmi et al., 2024).

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Figure 2. Preparation of Extract

The *Phytophthora palmivora* samples were collected from infected pomelo with the assistance of the Department of Agriculture in Mati City using sterile techniques and transported in a cooled container to prevent degradation (A). Potato Dextrose Agar (PDA) was prepared by dissolving 39 g of PDA powder in 1 L of distilled water, heating and boiling the mixture until clear (B), and sterilizing it in an autoclave at 121 °C and 15 psi for 15 minutes before cooling to 45–50 °C and pouring into petri dishes. Sterilized 6 mm Whatman No. 1 filter paper discs were treated with four extract concentrations (5, 10, 15, and 20 µL/disc) along with a negative control (distilled water), solvent control (methanol), and positive control (Mancozeb) (C). Using the Kirby-Bauer Disc Diffusion method, *Phytophthora palmivora* colonies were evenly streaked on PDA plates (D), incubated at 30 °C, and monitored daily. After six days, the diameter of inhibition zones was measured to assess antifungal activity, with larger zones indicating stronger inhibition (E). All used agar plates were autoclaved at 121 °C for 15–30 minutes and properly disposed of to ensure decontamination (F).

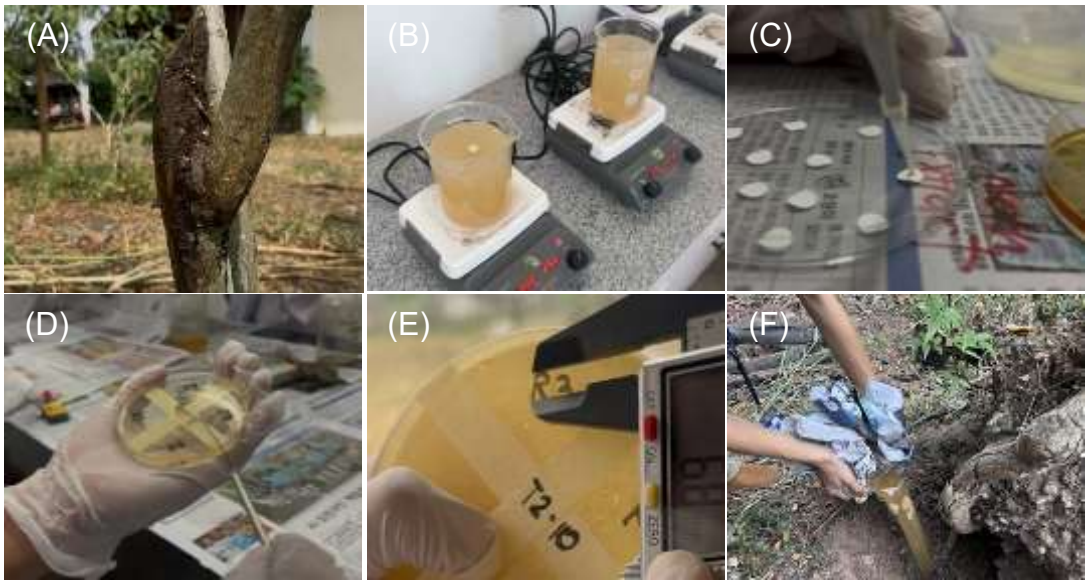

Figure 3. Experimental Procedures in Laboratory

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Data Analysis

The following statistical tools were used to treat the gathered data to answer the queries of the study:

Mean was used in determining the inhibitory effect of *Sargassum polycystum* extract against *Phytophthora palmivora* by computing the average diameter of the Zone of Inhibition for each treatment. The computed mean provided a measure of antifungal activity for every concentration tested and allowed comparison among the treatment groups.

One-way Analysis of Variance (ANOVA) was used to determine whether there was a significant difference among the mean zone of inhibition of the seven treatment groups, which included the different extract concentrations and the control groups. This statistical test determines whether the variations among treatments were significant at the 0.05 level.

Tukey Honest Significant Difference Test was intended to be applied following the ANOVA test result to determine which specific treatment groups significantly differed from one another. However, based on the findings presented in Table 3 under the results section, the computed p-value was 0.30, which was greater than 0.05 level of significance. Since the ANOVA result indicated that there was no statistically significant difference among the treatment groups, the Tukey's Honest Significant Difference Test was not conducted.

RESULTS

Phytochemical and Bioactivity Analysis

The *Sargassum polycystum* extracts were evaluated under two test parameters: flavonoids and phenols.

The phytochemical profiling and subsequent antimicrobial assays—specifically the determination of the zone of inhibition—were conducted by the WVN Laboratory. All procedures and findings were formally verified and authorized by Mr. Venchie C. Badong (RCH, PFT, LPT, CSSO, MAT), ensuring the analytical integrity of the data presented herein. Tables 1, 2, 1nd 3 show the phytochemical and bioactivity analysis.

Table 1. Phytochemical Analysis Results of *Sargassum polyctsum* Extract

Test Parameter	Results
Flavonoids	+
Phenols	+

Legend: (+++) Strongly positive; (++) Moderate positive; (+) Weakly positive; (-) Negative

The test results indicate the presence of flavonoids and phenols in the *Sargassum polcystum* extract. The phytochemical tests yielded weakly positive (+) under the two assessed parameters, confirming the presence of these bioactive compounds in the submitted sample.

Table 2. Zone of Inhibition of *Sargassum polcystum* Extract Against *Phytophthora palmivora*

Treatment	Trial 1	Trial 2	Trial 3	Mean
T1 (5 µL/disc)	6.50	6.33	6.43	6.42
T2 (10 µL/disc)	6.55	6.80	6.30	6.55
T3 (15 µL/disc)	12.76	6.23	7.10	8.70
T4 (20 µL/disc)	6.76	6.58	7.18	6.84
T5 (Solvent Control group)	6.50	6.33	6.43	6.42
T6 (Negative Control group)	6	6	6	6
T7 (Mancozeb)	6.23	6.15	6.23	6.20

With the exception of T3 (15 µL/disc), which obtained the highest Zone of Inhibition of 8.70 mm, all treatments, including the solvent control (T5), water control (T6), and Mancozeb (T7), produced inhibition zones that were only marginally larger than the baseline disc diameter. This indicates that the observed antifungal response was minimal and was unable to produce substantial suppression of fungal growth under the tested conditions. The minimum inhibition further suggests that the seaweed extract contained insufficient levels of bioactive compounds to exert a strong antifungal effect.

Table 3. Phytochemical Analysis Results of *Sargassum polyctsum* Extract

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	14.81	6	2.47	1.35	0.30	2.85
Within Groups	25.64	14	1.83			
Total	**40.45**	**20**				

As shown in Table 3, the computed F-value (F = 1.34) with a corresponding p-value of 0.30, which is greater than the 0.05 level of significance, indicates that there were no significant differences among the seven treatment groups, confirming that the null hypothesis was not rejected. This lack of statistical significance may be attributed to the weak positive detection of flavonoids and phenolic compounds during phytochemical screening. These compounds are essential and are known to inhibit fungal growth (Das et al., 2024; Abunada et al., 2020). In line with this, a study conducted by Sangeetha et al. (2017) found that low-level presence of bioactive compounds in their seaweed extract exhibited restrained and minimal inhibition zones. This finding supports the notion that the active presence and concentration of bioactive compounds are important and directly influence the antifungal activity.

Furthermore, several studies have reported low to moderate antifungal activity for *Sargassum* species, often due to the low concentration of the treatments used. A study reported that a *Sargassum* species exhibited moderate activity against the filamentous fungi *Aspergillus niger* and *Candida albicans* (De Oliveira Santos et al., 2018). Another study revealed that different seaweed extracts showed moderate antifungal activity against the tested strains with minimal inhibition, suggesting that the potency of crude extracts may be limited when applied at lower concentrations (Barot et al., 2016).

In contrast, previous studies have reported significant antifungal activity in other *Sargassum species*. The study of Tarhzouti et al. (2024) found that methanolic and phenolic-rich extracts of *Sargassum muticum* showed strong inhibition against several pathogenic fungi, with low minimum inhibitory concentrations. Similarly, El Shahir et. al., (2024) reported that methanolic extracts of *Sargassum cinereum* effectively suppressed fungal growth at higher concentrations. The difference in results can be explained by methodological factors. Previous studies used other organic solvents such as ethyl acetate and acetone, which are more effective in extracting antifungal compounds, and they applied higher extract concentrations. In addition, variations in fungal strains and extraction techniques may have influenced the observed activity. Therefore, the weak antifungal effect recorded in this study does not contradict earlier findings but reflects differences in experimental conditions.

Despite the minimal antifungal response observed, this study provides valuable insights into the limitations and potential of *Sargssum polycystum* as a marine-derived biocontrol agent. The correlation between weak phytochemical content and limited inhibition highlights the importance of optimizing extraction methods, concentrations, and delivery strategies in future research. Moreover, *Sargassum polycystum* may be more effective when used in vivo as a plant-mediated elicitor rather than as a direct antifungal agent in vitro. Overall, these findings contribute to the body of knowledge on eco-friendly alternatives to synthetic fungicides and provide a foundation for developing more effective marine-based antifungal treatments in sustainable agriculture.

CONCLUSION

This study used the disc diffusion method to examine the in vitro antifungal activity of *Sargassum polcystum* extract against *Phytophthora palmivora* at different doses. The findings showed that the extract exhibited detectable zones of inhibition at all tested doses, suggesting the existence of inhibitory action. Nevertheless, at the 0.05 level, statistical analysis did not show any significant differences between the treatment groups, controls, and the commercial fungicide Mancozeb. Even though the treatment of 15 µL/disc showed the largest mean Zone of Inhibition, the observed differences were negligible and similar to the baseline disc diameter, indicating limited antifungal activity in this particular investigation.

The lack of significant variation among treatments may be attributed to the use of crude extracts, limited diffusion of bioactive compounds in agar media, and the inherent resistance and variability of *Phytophthora palmivora* isolates. These findings contrast with previous studies that reported strong, concentration-dependent antifungal activity of *Sargassum* species when organic solvents were used or when extracts were applied as bio-elicitors in plant systems. Nonetheless, the visible zones of inhibition observed in this study suggest that *Sargassum polcystum* possesses bioactive potential. Overall, while *Sargassum polcystum* extract did not demonstrate statistically significant antifungal activity in vitro, the results support its potential as a natural antifungal agent. Further studies employing higher concentrations, improved extraction methods, alternative solvents, and in vivo or bio-elicitor-based applications are recommended to better assess and enhance its antifungal effectiveness.

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