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# Antifungal, Antiviral, and HPLC Analysis of Phenolic and Flavonoid Compounds of *Amphiroa anceps* Extract

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**Abstract:** The increasing use of chemical control agents and pesticides to prevent plant disease has resulted in several human and environmental health problems. Seaweeds, e.g., *Amphiroa anceps* extracts, have significant antimicrobial activities against different human pathogens. However, their anti-phytopathogenic activities are still being investigated. In the present investigation, three fungal isolates were isolated from root rot and grey mold symptomatic strawberry plants and were molecularly identified by ITS primers to *Fusarium culmorum*, *Rhizoctonia solani*, and *Botrytis cinerea* with accession numbers MN398396, MN398398, and MN398400, respectively. In addition, the organic extract of the red alga *Amphiroa anceps* was assessed for its antifungal activity against the three identified fungal isolates and tobacco mosaic virus (TMV) infection. At 100 µg/mL, the *A. anceps* extract had the best biological activity against *R. solani*, *B. cinerea*, and TMV infection, with inhibition rates of 66.67%, 40.61%, and 81.5%, respectively. Contrarily, the *A. anceps* extract exhibited lower activity against *F. culmorum*, causing inhibition in the fungal mycelia by only 4.4% at the same concentration. The extract's HPLC analysis revealed the presence of numerous phenolic compounds, including ellagic acid and gallic acid, which had the highest concentrations of 19.05 and 18.36 µg/mL, respectively. In this line, the phytochemical analysis also showed the presence of flavonoids, with the highest concentration recorded for catechin at 12.45 µg/mL. The obtained results revealed for the first time the effect of the *A. anceps* extract against the plant fungal and viral pathogens, making the seaweed extract a promising source for natural antimicrobial agents.

**Keywords:** *Amphiroa anceps*; seaweed; root rot; grey mold; antifungal; ITS; antiviral; TMV; extract; HPLC

## 1. Introduction

Seaweeds are a large group of marine algae that inhabit the shallow waters of the sea wherever suitable substrata are available [1]. Seaweeds are a rich and diverse source of metabolites widely known for their biological activities, including laxatives, antimicrobials, and anti-ulcer agents [2,3]. Exposing seaweeds to different biotic and abiotic stressors in their natural habitats results in the production of many bioactive metabolites [4]. Seaweeds

can be categorized into three main divisions: Chlorophyceae (green algae), Rhodophyceae (red algae), and Phaeophyceae (brown algae). Among them, red algae are a famous source of many bioactive chemical metabolites, including sulfated polysaccharides like carrageenan and fucoidan, which are known for their antiviral and anticancer activities against many human viruses and cancer cells [5]. Red algae are a source of many other useful products, like agar, alginates, and carrageenan, which are used for different purposes, such as human food and animal feed [6].

Previous phytochemical analysis of different red algae revealed the presence of different bioactive chemical groups, such as steroids, phenols, flavonoids, tannins, saponins, alkaloids, and triterpenoids [5,7]. The red alga *Amphiroa anceps* is one of the widely distributed seaweeds in Egypt, especially on the coast of Alexandria during the autumn period [2]. Previous studies of the organic extracts of *A. anceps* showed the presence of many active chemical metabolites that display different bioactivities, including antimicrobial activity [1]. Further, the aqueous extract of *A. anceps* is used to make nanoparticles, such as silver nanoparticles, using green chemistry techniques [8].

Plant diseases cause many crop losses worldwide, worsening serious food security problems [9]. Plant viruses are among the most significant plant pathogens, causing major threats to sustainable agriculture and productivity [10]. Tobacco mosaic virus (TMV) is one of the most important plant viruses, negatively impacting crop quality and productivity worldwide [11]. TMV is one of the top 10 plant viruses in the field of molecular plant pathology. It is also used as a model virus, and *Nicotiana glutinosa* is used as a host for TMV-local lesion studies [12]. Soil- and air-borne fungal infections, such as root rots, wilts, and grey mold, are of enormous economic relevance and can result in a substantial production crop. Strawberry is one of Egypt's most valuable export crops because of its high quality and early market introduction. Root rot infections in strawberries are caused by several fungi, including *Rhizoctonia solani*, *Fusarium culmorum*, *F. solani*, *F. oxysporum*, and *Macrophomina phaseolina* [13]. The fungus *Botrytis cinerea* is to blame for the grey mold that grows on strawberry fruit [13].

Most methods for controlling plant pathogens involve applying chemical pesticides to plants or breeding transgenic plants. However, synthetic pesticides harm the environment and human health, and transgenic crops are not yet universally approved [9,14]. Consequently, there is still a great demand for the discovery of further alternative, eco-friendly, and effective antiviral techniques. Algae have recently been used as biocontrol agents for plant diseases [15]. Seaweed extracts represent a safer fungicide than most synthetic and semi-synthetic fungicides [16]. Many previous reports revealed the presence of compounds derived from seaweed extracts, especially the red algal ones with antifungal activity. The extract of *Melanothamnus afaqhusainii* is very effective against fungi such as *Fusarium moniliforme* and *Rhizoctonia solani* [17]. Moreover, Jiménez et al. [18] investigated the antifungal efficacy of ethanolic extracts of the red algae *Gracillaria chilensis* against *Phytophthora cinnamomi*. To our knowledge, no previous studies have revealed the antifungal and antiviral activities of *Amphiroa anceps* extract against phytopathogenic microorganisms. Thus, this study aims to use HPLC to analyze the *A. anceps* extract and investigate its antifungal activity against three fungi (*Fusarium culmorum*, *Rhizoctonia solani*, and *Botrytis cinerea*), as well as its antiviral activity against tobacco mosaic virus infection.

## 2. Materials and Methods

### *2.1. Pathogen Isolation for Root Rot and Grey Mold*

Microbial pathogens were recovered from infected plant samples in Egypt's most important strawberry-producing zone at the latitude and longitude coordinates 30°34′38.0″ N 30°41′39.0″ E, Behera Governorate. Strawberry root and fruit parts were isolated on PDA media and showed rot and grey mold symptoms. Single spore culture or hyphal tip procedures purified the samples [19]. The isolated fungi were put in slanted tubes and grown at 25 ± 3 °C for 7 days. The pure cultures were inspected, morphologically identified [20], and molecularly described.

### 2.2. ITS Sequencing and Identification

The QIAquick PCR purification kit was used to isolate genomic DNA from fresh fungal hyphae (QIAGEN, Manchester, England). The fungi's internal transcribed spacer (ITS) region was amplified using the PCR technique with ITS1 and ITS4 primers [21]. PCR amplification reaction was performed with a final volume of 50 µL, containing 25 µL of 2x PCR DyeMIX-nTaq (Enzynomics Inc., Ansan, Korea), 50 ng of DNA template, and 2 µL of each primer (10 pmol). PCR cycling was performed with an initial denaturation step at 94 °C for 3 min, followed by 35 cycles (94 °C for 30 s, 56 °C for 30 s, and 72 °C for 40 s) and a final extension step at 72 °C for 7 min. The amplified PCR products were sequenced immediately after removal from the gel and purified with a PCR clean-up column kit (QIAGEN, Hilden, Germany). The sequencing process was carried out using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and a model 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). After analyzing DNA nucleotide sequences with NCBI-BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 19 September 2020, the annotated sequences were deposited in GenBank to obtain accession numbers. The phylogenetic tree was made using MEGA 11 software and analyzed using the maximum likelihood tree method with bootstrapping of 2000 replicates.

### 2.3. Sampling Site and Algal Biomass Collection

The preparation of dry biomass was performed according to the method of Valderrama et al. [22]. Biomasses of *Amphiroa anceps* were gathered in seawater around mid-autumn at a depth of 0.2 m or less from the rocks of Abo-Qir Eastern Harbor (30°4′ E and 30°20′ E and 31°16′ N and 31°28′ N). The *A. anceps* biomasses were taken directly from the intertidal zone using the Londo scale's five (1 mm) quadrants [23]. The fresh algal biomass sample size strictly adhered to the Egyptian Environmental Affairs Agency's (EEAA) regulations for the bio conservation of protected regions. Each sample was rinsed multiple times with saline and then with distilled water to wash away contaminants and epiphytes, and then its species was determined [24,25]. The algal biomass was then dried in the shade, and the sample was sliced, milled, and stored in a tightly sealed dark jar.

### 2.4. Extraction of the Algal Biomass

Twenty-five grams of *A. anceps* dry biomass were extracted using a Soxhlet apparatus. The extraction process was performed according to the method of Wang et al. [26], with some modifications. Methanol and hexane were utilized as the extraction solvent combination (1:1). The solvent combination was then added and heated in the flask. The extraction technique was repeated multiple times for 5 h at 65 °C until the vast majority of chemical compounds had been separated. The extract was then chilled, decolored using charcoal filters, and condensed at 30–45 °C with a rotary vacuum evaporator. The dried extract residue was stored at 4 °C until use. The yield of extraction was calculated according to the following formula: Extraction yield (%) = [Dry weight of the extract (g)/Dry weight of algal biomass (g)] × 100.

### 2.5. Antifungal Activity of the Algal Extract

The activity of the *A. anceps* extract was tested against the three isolated fungi. For the antifungal activity test, wood blocks of the chinaberry plant were prepared with dimensions of 0.5 × 1 × 2 cm, then sterilized at 121 °C for 15 min. A series of the algal extracts (25, 50, and 100 µg/mL) prepared with dimethyl sulfoxide (DMSO) was applied to each wood block (200 µL of each concentration) against the tested fungal species in triplicate, keeping one sample for each fungus treated only with DMSO to use as a control [27]. All the Petri plates were incubated at 25 ± 3 °C for 7 days. The antifungal activity was recorded by measuring the mycelial growth inhibition percentage as the following formula:

$$\% \text{ inhibition} = \left[\frac{\text{Control} - \text{treatment}}{\text{control}}\right] \times 100.$$

### *2.6. Source of the Virus, Inoculum Preparation, and Greenhouse Antiviral Activity Assays*

The Egyptian TMV (Acc# MG264131, strain KH1) was propagated by tobacco plant and purified using the methods outlined by Gooding and Hebert [28]. Before usage, the purified TMV inoculum concentration was diluted to 20 µg/mL using 0.1 M phosphate buffer, pH 7.2. The *A. anceps* extract was dissolved in DMSO and diluted to 100 µg/mL concentration using sterile deionized water. As a negative control, an equivalent concentration solution of DMSO was utilized. Using the leaf assay method and *Nicotiana glutinosa* as a local lesion host for TMV, antiviral efficacy was evaluated based on the percentage of inhibition toward the number of local lesions. The inhibitory effect was determined using the formula: [I = (1 − T/C) × 100], where I represents the inhibition effect, T represents the number of local lesions on the treated leaves, and C represents the number of local lesions on the control leaves. Under greenhouse-controlled conditions, *N. glutinosa* plants were subjected to the protective treatment assay at the 5–6 leaf stage. After 24 h of applying *A. anceps* extract to the *N. glutinosa* leaves, the leaves were dusted with carborundum and mechanically inoculated with TMV as previously described [29]. The number of local lesions was counted 3–4 days after TMV inoculation (dpi). Triplicate runs of each experiment were performed.

### *2.7. Characterization of Phenolic and Flavonoid Compounds Using HPLC*

The phenolic and flavonoid compounds were characterized in the algal biomass using HPLC. Despite the condition of the characterization previously described in previous works, we will briefly mention these conditions in the following lines [30,31]. For characterization of the phenolic compounds, an Agilent 1260 Infinity HPLC Series was used; the HPLC was equipped with a Quaternary pump and a Zorbax Eclipse Plus C18 column (100 mm × 4.6 mm i.d.). The injected volume of the extract was 20 µL. The separation was carried out using gradient elution with (A) HPLC grade water 0.2% $H_3PO_4$ (*v*/*v*), (B) Methanol, and (C) Acetonitrile at 30 °C. A variable wavelength detector was used at 284 nm. For quantification of flavonoid compounds, a Smart line, Knauer HPLC, was used and equipped with a binary pump in addition to a Zorbax Eclipse plusC18 column (150 mm × 4.6 mm i.d.). For the separation process, an eluent composed of methanol and water containing 0.5% $H_3PO_4$ with 50:50 percent and a flow rate of 0.7 mL/min. The injected volume of the extract was 20 µL, a UV detector was set at 273 nm, and data integration was conducted using ClarityChrom@ Version 7.2.0 (Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany). The HPLC investigation was based on the following 18 standard polyphenolic compounds: caffeic acid, ferulic acid, gallic acid, syringic acid, cinnamic acid, salicylic acid, ellagic acid, p-coumaric acid, pyrogallol acid, rutin, quercetin, kaempferol, luteolin, catechin, naringin, 7-oh flavone, apigenin, and myricetin. Merck KGaA supplied all of the chemical substances used in this study (Darmstadt, Germany).

### *2.8. Statistical Analysis*

All data were statistically analyzed using one-way analysis of variance (ANOVA) in CoStat software, with significant differences determined using Tukey's test post hoc and standard deviation (SD) methods. The differences in data with the same letter are not significant.

## 3. Results

### *3.1. Isolation and Preliminary Identification*

Three fungal isolates were obtained from 10 infected strawberry plants after isolation from plant organs and tissues exhibiting distinct symptoms; they were placed on PDA medium and incubated at 25 ± 3 °C for 7 days. The first isolated pathogen, *Botrytis cinerea* (abundance 100%), is responsible for the collapse of water-soaked fruit and foliage and develops brown lesions on unripe fruits. The infected blossoms cause fruit drops and damage growing and mature fruit, such as ridging. The isolated *B. cinerea* pathogen features a necrotrophic, haploid, and heterothallic ascomycete that produces melanized black

spherical sclerotia on the plate in vitro. The fungus produces gray, fuzzy conidiophores on plant tissues with velvet asexual spores.

Root rot disease symptoms and signs showed field plants' reduced vigor; small fruit, few runners, and many dead leaves and stunted plants revealed a reducing yield. Feeder and main roots might deteriorate and develop black decaying lesions. The central root core was blackened. According to our results, the isolation trails from the root rot symptoms revealed two isolates of the *Fusarium* (abundance 70%) and *Rhizoctonia* (abundance 30%) species. Characteristics of the *Fusarium* pathogen were observed as having a pale pinkish to white colony color. The macroconidia morphology is straight and relatively slender, and 3-septate is the most common. On the other hand, the *Rhizoctonia* pathogen was seen to have pale white to yellow mycelium that grew faster on PDA. Under a microscope, the fungus was seen to have narrowed branched angle hyphae and no spores.

### *3.2. ITS Characterization*

All fungal isolates were amplified and sequenced for ITS rDNA regions. The known sequences obtained from NCBI confirmed that the initial identifications of *F. culmorum*, *R. solani*, and *B. cinerea* were accurate. The relevant GenBank accession numbers for the three fungal sequences are MN398396, MN398398, and MN398400, respectively. The phylogenetic tree was constructed upon the ITS gene sequences retrieved from the NCBI GenBank database, as shown in Figure 1.

### *3.3. Invitro Antifungal Activity of the A. anceps Extract*

The result of the antifungal activity of the *A. anceps* extract is clarified in Figures 2 and 3. Generally, the extract of *A. anceps* exhibited different antifungal activities against different fungal species used in the present work. The highest antifungal activity of the algal extract was observed against *B. cinerea* using 100 and 50 μg/mL of the algal extract. The extract displayed moderate antifungal activity against *R. solani* with a percent of inhibition ranging from 39–66.67% for extract concentrations of 25 and 100 μg/mL, respectively. The *A. anceps* extract showed weak antifungal activity against *F. culmorum* (Figure 2).

### *3.4. Inhibitory Effects of A. anceps Extract against TMV*

Under greenhouse conditions, the protective activity of *A. anceps* extract against TMV on *N. glutinosa* was assessed. The antiviral activity was determined using the leaf assay by counting the number of local lesions on infected leaves at 4 dpi. When comparing treated and untreated tissues, those treated with *A. anceps* extract showed a significant decrease in local lesion symptoms (Figure 4). Mock-treated plants showed no local lesions on the leaves. The application of *A. anceps* extract at 100 μg/mL showed the maximum antiviral activity with an inhibition rate of 81.52 ± 2.36% (Figure 4). On the other hand, the 50 and 25 μg/mL showed inhibition rates of 71.01 ± 1.78% and 53.26 ± 3.04%, respectively. Thus, the results showed that *A. anceps* extract could reduce TMV infection.

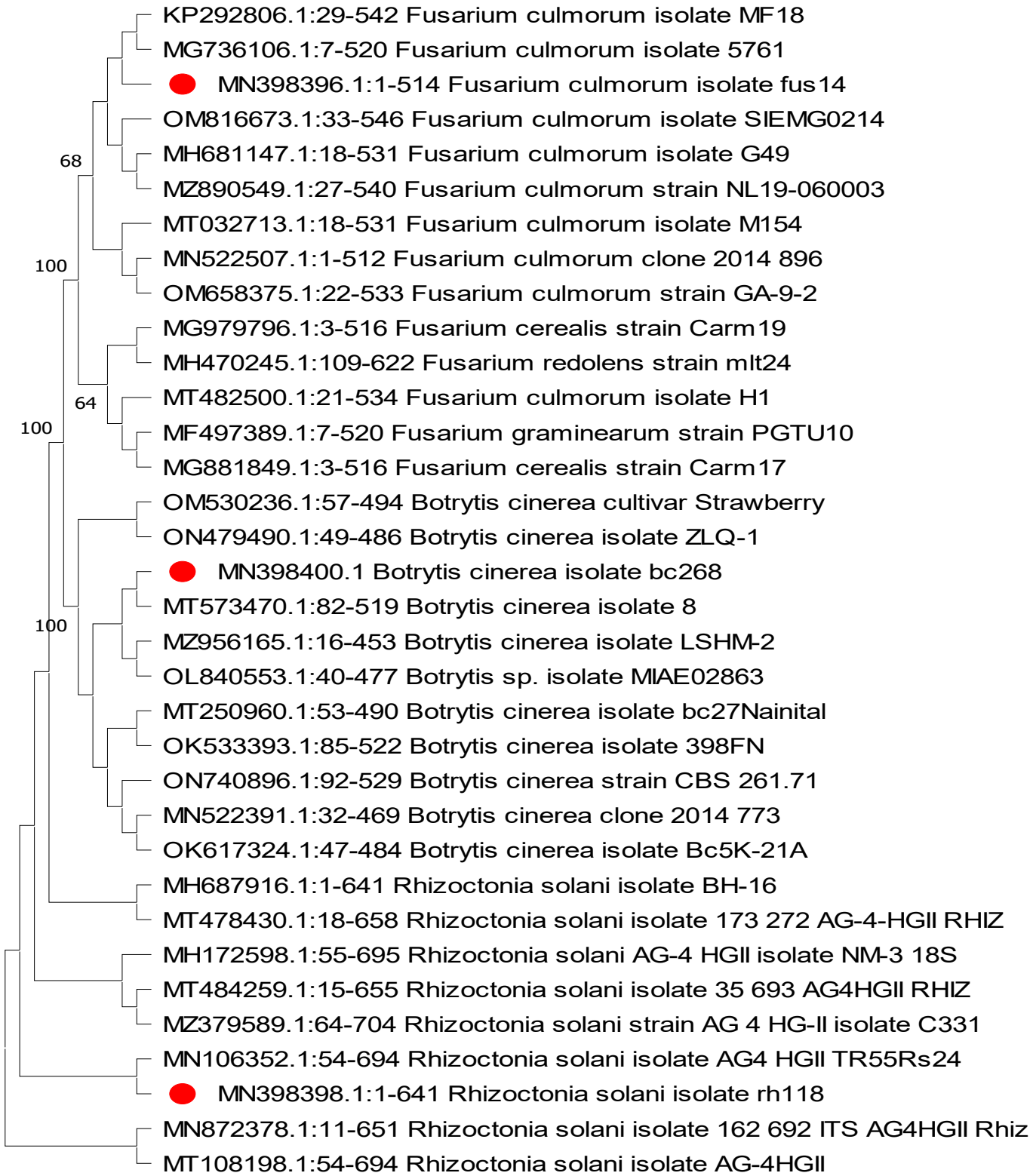


**Figure 1.** A degenerated phylogenetic tree based on the nucleotide sequence of the ITS gene for the three fungal isolates *F. culmorum* (MN398396), *B. cinerea* (MN398400), and *R. solani* (MN398398) aligned with the most related sequences downloaded from the NCBI database. The phylogeny was tested for maximum likelihood using the bootstrap method with 2000 replications. The red color cycle indicates three fungal isolates molecularly identified during this study.

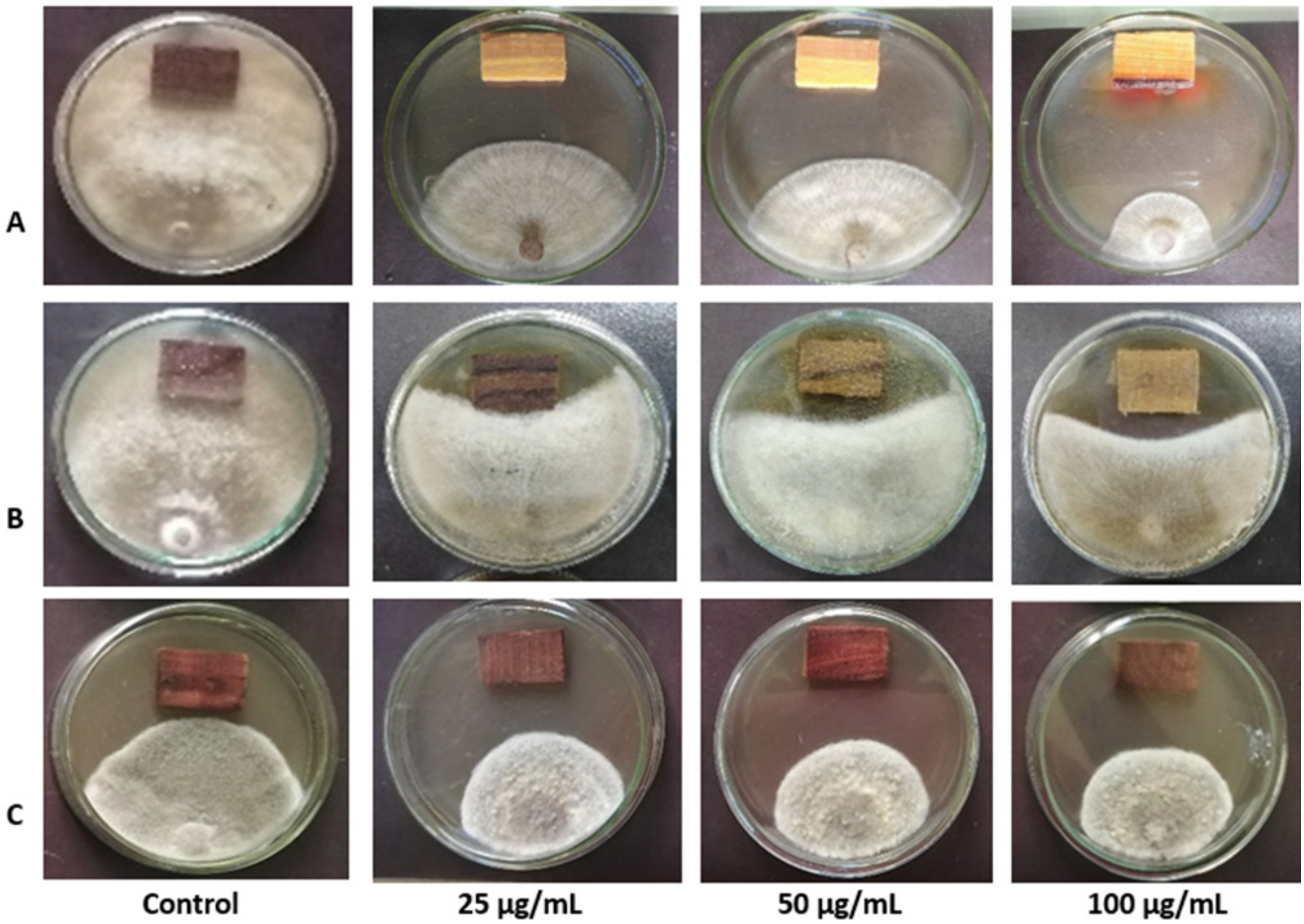


**Figure 2.** Antifungal activity of the 25, 50, and 100 µg/mL extract concentrations compared to the control against the fungal pathogen (**A**) *Rhizoctonia solani*, (**B**) *Fusarium culmorum*, and (**C**) *Botrytis cinerea*.

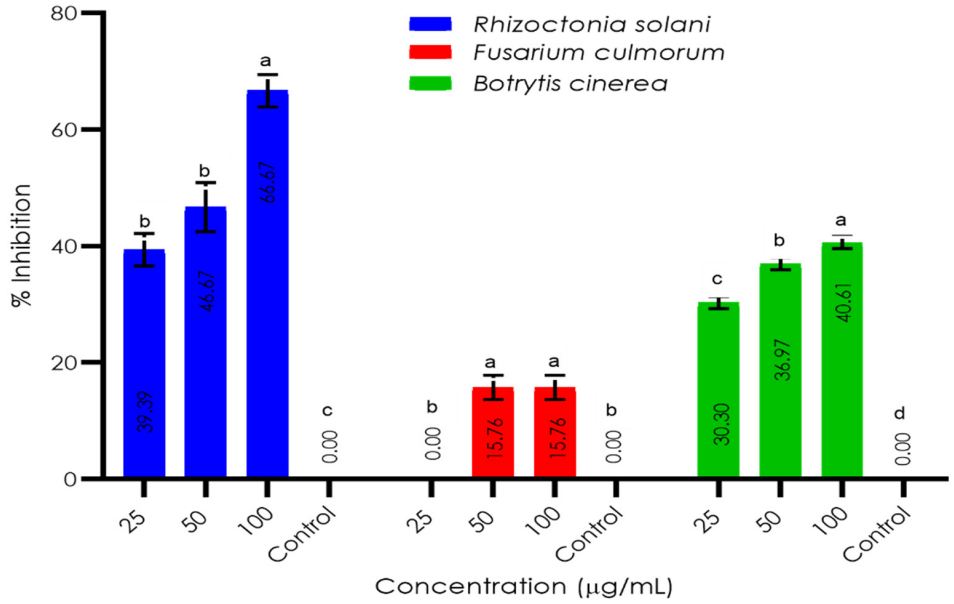


**Figure 3.** Inhibition percentages of 25, 50, and 100 µg/mL extract concentrations compared to control against the fungal isolates *R. solani*, *F. culmorum*, and *B. cinerea*. The small letters on top of the bar graphs in each color mean the significance at a 0.05 probability.

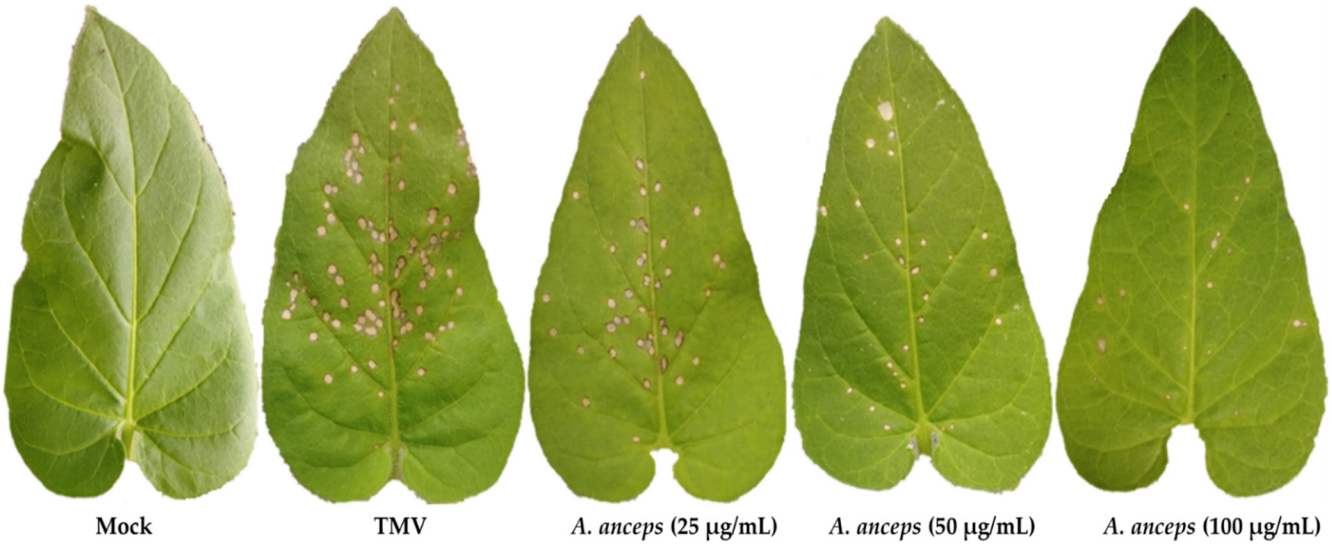


**Figure 4.** A photograph showing the disease symptoms on *N. glutinosa* leaves infected with tobacco mosaic virus at 4 dpi of the protective activity of *A. anceps* extract at different concentrations.

### *3.5. Phenolic and Flavonoid Compounds of the Algal Extract*

The results obtained in this study showed a 61% extraction yield. The HPLC chromatograms of phenolic and flavonoid compounds in the *A. anceps* extract are clearly shown in Figure 5. The phytochemical analysis showed that the algal extract's most abundant phenolic compound in µg/mL was ellagic acid, with a concentration of 19.05, followed by gallic acid (18.36). At the same time, catechin represents the highest concentration of a flavonoid compound present in the extract, with a concentration of 12.45 µg/mL (Table 1).

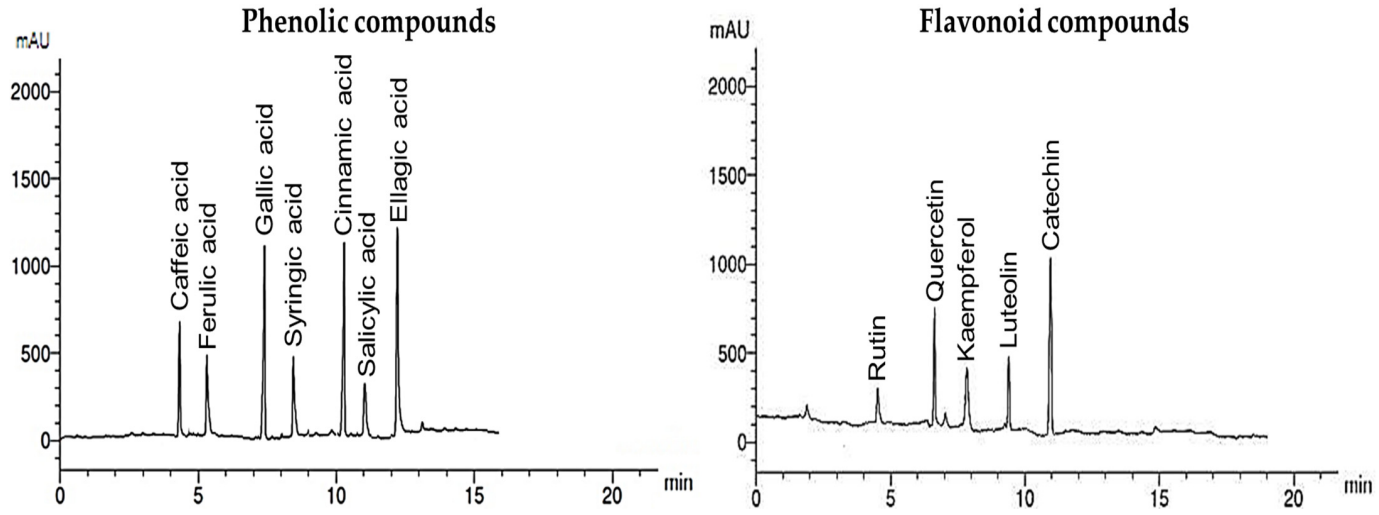


**Figure 5.** HPLC chromatograms of phenolic and flavonoid compounds identified in the *Amphiroa anceps* extract.

**Table 1.** Concentrations of phenolic and flavonoid compound in the extract of *Amphiroa anceps* using HPLC.

| Phenolic Compounds | | | Flavonoid Compounds | | |
|---|---|---|---|---|---|
| **Compound** | **RT** | **Concentration (µg/mL)** | **Compound** | **RT** | **Concentration (µg/mL)** |
| Caffeic acid | 4.2 | 8.66 | Rutin | 4.8 | 0.59 |
| Ferulic acid | 5.1 | 6.24 | Quercetin | 6.8 | 4.86 |
| Gallic acid | 7.0 | 18.36 | Kaempferol | 8.0 | 3.87 |
| Syringic acid | 8.6 | 5.04 | Luteolin | 9.1 | 4.63 |
| Cinnamic acid | 10.0 | 17.64 | Catechin | 11.0 | 12.45 |
| Salicylic acid | 11.0 | 5.72 | | | |
| Ellagic acid | 12.3 | 19.05 | | | |

## 4. Discussion

Plant diseases are a major threat to the productivity and sustainability of agriculture around the world. Every year, they cost farmers several billion dollars [32]. Controlling plant diseases requires resistant plant cultivars or intensive insecticide usage, which can harm human health and the environment [33]. Pesticides pollute surface water, exacerbating environmental and ecological issues [34]. Biocontrol agents are safer alternatives to the toxic pesticides currently used to treat plant diseases [35]. As biological control agents, algal extracts are regarded a sustainable and environmentally acceptable alternative to chemical control agents, as they include a variety of physiologically beneficial secondary metabolites that may increase the plant's systemic resistance and inhibit pathogen development. This study assessed the antifungal and antiviral activities of the *Amphiroa anceps* extract against *Fusarium culmorum*, *Rhizoctonia solani*, and *Botrytis cinerea* as well as TMV infection. In addition, the main phytochemical constituents of *A. anceps* extract were analyzed using HPLC.

Seaweeds represent a reservoir of many active metabolites, including vitamins, enzymes, lipids, and antibiotics, in addition to many fine chemicals [36,37]. Many previous studies have shown that the metabolites produced via marine macroalgae and especially extracts of *A. anceps* exert several bioactive functions, including, for instance, cytotoxic, anti-inflammatory, and antimicrobial activities, making it a promising source for drug design [2,38,39]. HPLC analysis of the *A. anceps* extract proved the presence of many bioactive phenolic and flavonoid compounds, and these results are consistent with previous studies. Many studies confirm the presence of phenolic compounds qualitatively and quantitatively in the extracts of *A. anceps* [1,40]. Xiaojun et al. [41] and Nagai and Yukimoto [42] have reported different biological activities of phenolic compounds, such as antioxidant and antimicrobial activities. In the current study, the inhibition rates of fungal hyphae growth demonstrate that the bioactive molecules of algal species can be dissolved in alcohol [43,44]. This may explain why organic extracts have either a strong or weak effect on pathogens. Many previous reports confirm the presence of flavonoid compounds in several red algae [45]. However, our work may be the first report of the presence of flavonoid compounds in the extract of *A. anceps*. Additionally, flavonoid compounds play different biological activities, including anti-inflammatory, antioxidant, and antimicrobial [46].

In the present work, the extract of *A. anceps* exhibited potent antifungal activity against *R. solani* and *B. cinerea* but showed a lower activity against *F. culmorum*. These results are consistent with that of Pandian et al. [47], who reported high antifungal activity of the *Acanthophora spicifera* methanolic extract against *Microsporum gypseum*. Similarly, Sultana et al. [48] reported a high fungicide effect of the red alga *Melanothamnus* extract against root infecting fungi *F. solani* and *Macrophomina phaseolina* attacking eggplant (*Solanum melongena* L.) and watermelon (*Citrullus lanatus* (Thunb.). Khan et al. [17] reported a high antifungal activity against *F. moniliforme* and *R. solani* using the extract of *M. afaqhusainii*. Similarly, Jiménez et al. [18] investigated the antifungal activity of the ethanolic extracts of the red algae *Gracillaria chilensis* against *Phytophthora cinnamomi*. On the contrary, Tariq [45]

investigated the antifungal efficacy of extracts from four red algae species using the cub plate method. He found that none of the studied extracts inhibited the growth of *Aspergillus flavus, A. fumigatus*, or *Candida albicans*.

In contrast, the effects of methanol and chloroform extracts of the red macroalgae *Gracilaria confervoides* on the mycelial growth of *R. solani*, *Macrophomina phaseolina*, and *F. solani* of cucumber were studied by Soliman et al. [49]. They found that the methanolic extract reduced the *R. solani* growth by 25.9% and the chloroform extract by 100%, while the solvent and aqueous extracts moderately inhibited mycelia growth in *F. solani*. Overall, the explanation of different inhibition results suggests that some or all algal extracts added more minerals and nutrients to the medium, which could have hidden the inhibitory effect. As we described previously, many previous works prove different biological activities of the *A. anceps* extracts. However, the present work may represent the first record of antifungal activity of the red alga *A. anceps* extract. In addition, there were records before on the antimicrobial activity of different organic extracts of *A. anceps* against human pathogens. Crude ethanolic extracts of *A. anceps* demonstrated the greatest antimicrobial activity towards *Vibrio parahaemolyticus* (13.25 mm) and *V. alginolyticus* (13.3 mm). Maximum activity against *Pseudomonas fluorescens* and *Proteus mirabilis* was seen in the acetone extract of *A. anceps*. In contrast, the crude benzene extract was the most effective against *Streptococcus pneumoniae* and *V. parahaemolyticus* [50].

In recent years, there has been much interest in employing beneficial microbes as a safe and environmentally acceptable approach to controlling viral plant diseases. However, the use of algae for the biological control of viral plant diseases is still limited, and it is unclear how algae might work against viral pathogens. The antiviral activity of *A. anceps* extract against TMV on *N. glutinosa* plants was investigated in this research. Under greenhouse conditions, the application of *A. anceps* extract significantly reduced the local lesion symptoms when *N. glutinosa* tissues were treated with *A. anceps* extract at a concentration of either 25 or 50, or 100 µg/mL 24 h before TMV inoculation. The 100 µg/mL of *A. anceps* extract exhibited a strong inhibitory effect (81.52%), while the 50 and 25 µg/mL concentrations showed an inhibitory effect of 71.01% and 53.26%, respectively. In light of these findings, we hypothesize that *A. anceps* extract may contain elicitor chemicals that stimulate the immune defense system and/or inhibit TMV replication. In general, antiviral agents can work in two ways: either directly or indirectly to stop viral replication by activating the host's innate immune system, or by making SAR against viral infection [51].

In the same way, in the HPLC results, we noticed higher concentrations of polyphenolic compounds such as ellagic, gallic, cinnamic acids, and catechin. Previous results suggest that ellagic and gallic acids could be natural antifungal agents [52,53]. Ellagic acid had a broad spectrum of antifungal activity against two fungal *Candida* strains, with MICs between 25.0 and 75.0 µg/mL and 0.125 to 0.25 µg/mL against *C. auris* strains [54]. Meanwhile, Li et al. [53] recorded antifungal MICs of gallic acid against three *Candida* strains ranging between 12.5 and 100.0 µg/mL. The results obtained by Korošec et al. [55] showed high antifungal properties of cinnamic acid derivatives against two pathogens—*Cochliobolus lunatus* and *Aspergillus niger*. Moreover, among catechins, pyrogallol catechin showed more potent antifungal activity against *Candida* sp. than catechol catechin [56]. It was reported that the application of 500 µg/mL of exogenous caffeic acid inhibited the growth of various *Fusarium* and *Saccharomyces* species [57]. Numerous polyphenols, including caffeic acid, ellagic acid, catechins, chlorogenic acid, gallic acid, quercetin, ferulic acid, and myricetin, possess antibacterial, antiviral, anti-inflammatory, anticancer, and antioxidant effects [58,59]. Ferulic acid is a common phytochemical found in leaves and seeds, and is free and covalently bound to glycoproteins, polysaccharides, polyamines, hydroxy fatty acids, and lignin. Ferulic acid makes cell walls stiff and works as an antioxidant, an antibacterial, an anticarcinogenic, an antiviral, and a substance that changes how enzymes work [60]. Consequently, we think the polyphenolic compounds we found could be used as elicitor molecules in developing SAR, increasing resistance against TMV infection, and working as potent antifungal agents [52,53].

## 5. Conclusions

The results obtained from this study proved the antifungal and antiviral activities of the organic extract of *A. anceps*. The *A. anceps* extract at 100 µg/mL showed the highest biological properties against *R. solani* and *B. cinerea* as well as TMV infection with inhibition rates of 66.67%, 40.61%, and 81.5%, respectively. The biocontrol activities of the *A. anceps* extract may be attributed to the single or synergetic effect of one or more than one of the compounds. As far as we know, the results obtained from this work may represent the first record of antifungal and antiviral activities of the seaweed *A. anceps*. The promising results obtained in this study and the simple extraction method determine the tested red algae to be a promising source for producing antifungal and antiviral agents to control plant diseases. Thus, this extract may be useful for protecting plants from diseases.

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