In vitro antifungal effect of plant-based and Ulva sp. extracts on Aspergillus flavus growth and conidia germination

Giacomo Boscarol
University of Udine
Alessandra Di Francesco
University of Udine
Enrico Braidot
enrico.braidot@uniud.it
University of Udine
Marco Vuerich
University of Udine
Francesco Boscutti
University of Udine
Valentino Volpe
ERSA, Pozzuolo del Friuli (UD)
Michele Fabro
ERSA, Pozzuolo del Friuli (UD)
Urska Vhrovsek
Fondazione Edmund Mach
Elisa Petrussa
University of Udine

Article

Keywords: Biological control, Aspergillus flavus, Plant extract, Polyphenols, Ulvan polysaccharides, Waste Reuse

Posted Date: February 17th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8599991/v1

Additional Declarations: No competing interests reported.

Abstract

Mycotoxins in cereals, particularly maize, pose a significant threat to agriculture, worsened by climate change. Therefore, developing effective mitigation strategies are urgently claimed. Plant waste and seaweed extracts offer promising sources of phytochemicals for combating fungal plant pathogens. This study investigated the *in vitro* antifungal potential and polyphenol composition of hydroalcoholic extracts from plant waste materials and seaweed (*Ailanthus altissima* (Mill.) Swingle leaves, *Humulus lupulus* L. trub, *Olea europaea* L. leaves and *Ulva* sp. thallus). With the exception of *O. europaea*, all extracts inhibited *Aspergillus flavus* growth, with *Ulva* sp. showing the highest efficacy (EC_{50} = 14.2 mg/mL). Analysis revealed enrichment in glycosylated flavonols and hydroxycinnamic acids across extracts, while *Ulva* sp. specifically contained rosmarinic acid, *p*-hydroxybenzoic acid, and uronic acid and sulfate groups, characteristics of ulvan polysaccharides. These results suggest *Ulva* biomass is a promising botanical source for *Aspergillus flavus* biocontrol, paving the way for its future application in agriculture and post-harvest systems.

Highlights

- *Ulva*, *Ailanthus* and *Humulus* trub-based hydroalcoholic extracts inhibit *in vitro A. flavus* mycelial growth at different efficacy levels
- *Ulva* extract resulted the more promising, with a EC_{50} of 14.2 mg/mL and a 91% complete inhibition at about 20 mg/mL
- *Ulva* extract also affected conidial germination in a dose-dependent manner, reaching a complete inhibition at 14.0 mg/mL after 16 h of incubation.
- Biochemical characterization of extracts revealed *Ulva* to be specifically rich in rosmarinic and *p*-hydroxybenzoic acid and to contain ulvan polysaccharides
- *Ulva*-derived and plant waste-based natural extracts could represent potential sustainable alternatives in maize pest management

1. Introduction

Aflatoxins produced by *Aspergillus* species contaminate major crops and pose severe health risks [1]. Climate-driven changes in temperature and humidity have increased contamination risks in the temperate Europe, like northern lowlands of Italy, where maize represents the predominant crop [2–4]. Major key factors for augmented risk of mycotoxin contamination in maize (interaction of high temperature and low water activity in kernel, European corn borer infestation and the predicted increase in global average temperature and worsening of drought stress events) are considered to be the main drivers for the emerging concerns for food and feed safety, with large economic and social impacts [1, 5–7]. Extensive efforts have been made to identify natural inhibitors such as biological extracts or antagonistic microorganisms for counteracting fungal invasion and aflatoxin contamination [8]. Among them, plant-based extracts are characterized by a high content of phenolic acids and terpenes, for which

antioxidant bioactivity and antifungal properties have been widely recognized [8, 9], providing an environmentally friendly and safe alternative to synthetic pesticides [10, 11]. In particular, bio-extracts obtained from agriculture, forestry and food by-products (e.g., *Olea*, *Ailanthus*, grape canes, spent hops from brewery industry) have been shown to exert a wide range of antimicrobial actions [12–15]. Moreover, they are more readily available due to the abundance of raw source biomass in nature, and the preparation processes are often less polluting than traditional chemical synthesis processes. These extracts are also believed to act synergistically with other different active compounds, and to elicit natural defense responses in plants [10, 16, 17]. Marine seaweeds, rich in polysaccharides and polyphenols, are also emerging as valuable biostimulants or antimicrobial products for agricultural applications [18–20]. In this perspective, we investigated the effectiveness of four extracts (from *Ailanthus altissima* (Mill.) Swingle as environmentally invasive organisms, *Ulva* sp. as collected environmental biomass from coastal waters, *Olea europaea* L., 1753 *cv. Gargazzo* and *Humulus lupulus* L., 1753 as processing wastes) against *A. flavus* mycelium growth and conidial germination. Extracts have been then characterized for their polyphenolic profile by UHPLC-MS. The results provided information on different by-products as sources for sustainable control of mycotoxigenic fungi.

2. Materials and Methods

2.1. Plant and seaweed collection and extraction

Plant materials were collected in Friuli Venezia Giulia Region during 2023 and 2024 (Table S1). *A. altissima* and *O. europaea* leaves were collected during summer, cleaned and stored at -80°C until use. The *Ulva* biomass was handpicked during summer-mid autumn 2023–2024 from the intertidal zone in the Marano Lagoon, Northeastern Adriatic Sea coast, after natural green tide/bloom events. The collected material was visually identified and consisted predominantly of *Ulva rigida* L., 1753, with the co-occurrence of *Ulva laetevirens* Areschoug, 1854. The identification of the plant material was carried out by the authors of the manuscript, who are botanists with long-standing expertise in the census, monitoring, and taxonomy of both native and alien flora in the region. None of the plant species investigated in this study are listed as threatened, endangered, or protected at the national or international level. Fieldwork and any collection of plant material were conducted in full compliance with institutional, regional, national, and international guidelines and legislation. In particular, all activities adhered to the principles of the IUCN Policy Statement on Research Involving Species at Risk of Extinction and to the Convention on the Trade in Endangered Species of Wild Fauna and Flora (CITES), although no CITES-listed species were involved in this study.

The biomass was rigorously cleaned in the laboratory, by rinsing several times in distilled water to remove sediments and then quickly dried with blot paper and stored at -80°C until use. *H. lupulus* trub was kindly provided by “Severino Garlatti Costa” brewery as a by-product of beer production: after whirlpool and cooling the protein-tannin-hop residue (often termed as hot trub) precipitated from the hopped wort tub is obtained, a portion of which was used for this survey. Extracts were prepared via hydroalcoholic maceration (50% ethanol, ratio 1:10) overnight in agitation and darkness at room

temperature. After centrifugation at 28 000 × g for 10 min, the supernatant fraction was collected, filtered and lyophilized with Christ Epsilon 2–4 LSCplus-Del Tek. After lyophilization, all the extracts (hereafter *Ailanthus, Humulus* trub, *Olea* and *Ulva*) were suspended in 100% ethanol to a concentration of 0.25–0.3 g/mL (dry weight basis), filtered with 50 µm nylon gauze to discard solid precipitate and stored at -20°C until use. For antifungal activity assay, two or more independently prepared batches of the extracts were used.

2.2. Antifungal assays

A. flavus (AF11999 from "Servizio fitosanitario e chimico, ricerca, sperimentazione e assistenza tecnica" of the Regional Agency for Rural Development, Friuli Venezia Giulia Region) inhibition was assessed using the agar well diffusion method [21]. The fungal isolate was grown on potato dextrose agar (PDA, 39 g/L) (Oxoid, UK) and incubated at 25°C for 7 days (12 h light/12 h dark photoperiod). Spores were harvested by scraping the agar plate and suspended on sterile water containing Tween80 (0.05%). To confirm the reproducibility of the antifungal activity, the assay was performed using multiple, independent batches of the extract (i.e., at least two or three separate extraction runs). Fungal pathogen suspension was infused on PDA medium (50 mL per square plate, 10 cm × 10 cm) reaching a final concentration of 1×10^5 conidia/mL. Sixteen equidistant holes per plate (8 mm, Ø) were punched aseptically with a sterile tip and 50 µL of different water-diluted concentrations of each bio-extract were added into four wells on the same line. The baseline concentration ranged from 0-170 g/L. A control plate with the same solvent:water concentration was run in parallel. Plates were incubated at 25°C for 4 days. Inhibition was calculated as the ratio between the halo area (mm^2) devoid of fungal mycelium and the potential diffusion space (total area of imaginary circles centered on each hole) (Figure S1). Images of Petri dishes were acquired by a digital camera (Panasonic Lumix GH5) and evaluated by ImageJ Fiji software. The sample unit consisted of one plate per extract (4 holes per concentration) and the experiment was repeated at least twice. Conidial germination was monitored in malt extract broth (MEB) in multiwell microplate with *Ulva* extract doses ranging from 1.4 to 14.0 mg/mL. Each well was adjusted to the final concentration of $1x10^5$ conidia/mL and incubated in continuous agitation (150 rpm) in the dark at 28°C for up to 24 h. Controls consisted of MEB and ethanol without extract. The onset of spore germination was monitored carefully by optical microscope by evaluating germ tube germination. Conidia were considered germinated when the germ tube was two times longer than the conidial diameter. The percentage of germination and germ tube length were determined after 16 h of incubation by acquiring photographs with a digital camera and analyzing images with ImageJ Fiji software. The sample unit was composed of 6 wells for each condition. Conidia germination and germ tube elongation (µm) were determined on a number of conidia ranging from 40 to 90 per treatment (3 replicates with at least 13 conidia each). The assay was repeated twice.

2.3. Chemical analyses

Phenolic compounds were characterized by modified UHPLC-MS, adapted from Vrhovsek et al. [22] and described in Filippi et al. [16]. LOD and LOQ values were established for each compound, with LOD

calculated as one-third of LOQ. Prior to analysis, the extract was filtered using a 0.2 µm PTFE filter. Chromatography was performed on a Waters Acquity UPLC system equipped with an HSS T3 column, while mass spectrometry detection was carried out using a Waters Xevo triple-quadrupole mass spectrometer with an ESI source. Compound identification and quantification were based on reference standards, retention times, and characteristic ion patterns. Results were expressed as µg/g of dry weight of extract, and data processing was performed using Waters MassLynx software.

Uronic acids in *Ulva* extract were measured according to the colorimetric method described by Connan [23], based on the reaction of anthrone with both neutral and uronic acid sugars with double absorbance reading at 560 and 620 nm, using glucose and glucuronic acid as standard, respectively. Total sulfate content was analyzed by the turbidimetric method using the barium chloride-gelatin reagent, following the protocol described by Torres et al. [24]. The content of uronic and sulfate acids was expressed as percentage in dry mass of the extract.

2.4 Statistical analysis

All statistical analyses were performed using R (v4.2.2) and RStudio (v2024.12.0). The antifungal activity of the extracts was visualized by fitting a dose–response logistic model for each extract separately using “drda” package [25]. For each dose-response logistic model four parameters were calculated: α is the value of the function when $x \to -\infty$, δ is the height of the curve, ε is the steepness (growth rate) of the curve, φ is the x value at which 50% of the maximum δ is reached (EC50). To investigate whether the extracts had different effects on fungal growth, a dose-response logistic model was fitted for each replicate separately and ANOVA analyses were performed on the four parameters describing the curves. The ANOVA assumptions were verified using the diagnostic plots of the model residuals. A Tukey’s Honest Significant Difference (HSD) post hoc analysis was performed to test for significant differences among the selected parameters.

A Principal Component Analysis (PCA) was performed on a centered and scaled matrix containing the concentration of polyphenolic compounds present in each extract. PCA was performed using base R function “prcomp”.

To test the effect of different concentrations of *Ulva* on conidial germination and on germ tube length, ANOVA analyses were performed. The ANOVA assumptions were verified using the diagnostic plots of the model residuals. A Tukey’s Honest Significant Difference (HSD) post hoc analysis was performed to identify which concentrations had significantly different effects from the others.

3. Results

3.1 Antifungal activity of extracts against *A. flavus*

After three days of incubation, *Ulva*, *Ailanthus*, and *Humulus* trub showed dose-dependent mycelial inhibition, showing a clear circular halo, where a larger halo area is directly proportional to greater

potency of the extract (Figure S2). Conversely, in the negative control (where only the solvent/water was applied), no such clear zone was evident, confirming that the fungal mycelium grew uniformly up to the edge of the well. To compare the *in vitro* efficacy of the three extracts, dose-response curves were built as shown in Fig. 1. ANOVA analysis revealed three of the four logistic parameters of the dose-response curves were significantly different among extracts ($p < 0.001$) (Supplemental Table S2). *Ulva* and *Ailanthus* reached maximal inhibition levels above 90% ($\delta = 91.3 \pm 4\%$ and $94.8 \pm 0.9\%$, respectively), quite higher compared to that found for *Humulus* trub ($52.9 \pm 2.5\%$) (Fig. 2). However, *Ulva* was 10-times more effective than *Ailanthus* and *Humulus* trub: *Ulva*'s EC50 was significantly lower than *Ailanthus* or *Humulus* trub ($\varphi = 14.2 \pm 0.4$ *vs.* 140.7 ± 1.1 or 113.7 ± 4.2 mg/mL, respectively). The lower limit α, explaining the minimal inhibition resulting from each dose-response curve fitting, was also significantly lower in both *Ulva* and *Humulus* trub extracts than *Ailanthus* (0.5 ± 1.9 and 2.4 ± 1.2 *vs.* 7.0 ± 0.8). The fourth parameter, the slope of the curve (η), was found to be not significantly different.

3.2 Polyphenol profile in plant and seaweed extracts

To understand if the antifungal inhibition differences of the extracts could be associated with specific biochemical composition, we investigated their polyphenolic profile, given their well-known potential antioxidant and antimicrobial actions. Polyphenolic characterization and relative content (Table 1 and Figure S3) of the three extracts quantified 35 different phenolic compounds, the classes of glycosylated flavonoids (quercetin and kaempferol glycosides) and hydroxycinnamic acids being the most abundant. The extracts had compositions that differed in the total amount of polyphenols: *Ailanthus* yielded the highest concentration (8620 ± 230 µg/g DW), 300 times higher than *Ulva* (26 ± 1 µg/g DW). An intermediate value of 785 ± 63 µg/g DW was found in *Humulus* trub. *Ailanthus* and *Humulus* trub were also characterized by a higher biochemical diversity, including at least seven different classes of polyphenols, whereas *Ulva* had only hydroxycinnamic and hydroxybenzoic acids and flavonols. In detail, *Ailanthus* is enriched in glycosylated and substituted flavonols (quercetin, kaempferol and rutin), which accounted for 55% of the total (4758 µg/g DW), with quercetin glucosides being the most abundant flavonols (2589 ± 56 and 1443 ± 39 µg/g DW for quercetin-3-glucoside + quercetin-3-galactoside and kaempferol-3-glucoside, respectively). *Ailanthus* was also characterized by a high amount of hydroxycinnamic acids (2498 µg/g DW in total, e.g. 29% of total polyphenols), mostly represented by chlorogenic acids, particularly neochlorogenic acid (1861 ± 24 µg/g DW) and hydroxybenzoic acids (851 µg/g DW in total, e.g. 10% of total polyphenols). The extract contained unique compounds, compared with *Humulus* trub and *Ulva*, such as hydrolysable tannin ellagic acid (744 ± 52 µg/g DW, 8.63%) and lower quantities of chalcones, coumarins and hydroxyflavones like apigenin. Arbutin and sinapyl alcohol (321 ± 5 µg/g DW, 4%) were also identified. In *Humulus* trub, two hydroxycinnamoyl alcohols (407 µg/g DW) and several proanthocyanidins (flavan-3-ols) (169 µg/g DW) represented the most abundant polyphenolic classes, accounting for 52 and 21% of the total amount, respectively. As in *Ailanthus*, flavonol glucosides were well-represented (18%), reaching quercetin and kaempferol glucosides (61 ± 4 and 68 ± 4 µg/g DW, respectively). Among the hydroxycinnamic acids, ferulic acid was the most represented at 21 ± 2 µg/g DW accounting for 4.9% of the total polyphenols. In addition, the extract also

contains arbutin and, to a less extent, hydroxybenzoic acids such as vanillin and vanillic acid. Interestingly, *Ulva* contained the lowest total amount of polyphenols and did not show unique classes. Indeed, flavonols, hydroxybenzoic and hydroxycinnamic acids were shared by all three extracts. However, in *Ulva* the relative contributions of these compounds were different, since hydroxycinnamic acids accounted for most of the total polyphenols (24 µg/g DW, 92% of the total) and were particularly rich in rosmarinic acid and, at a lower extent, in caffeic acid. Other well represented polyphenols were *p*-hydroxybenzoic acid and vanillin in the class of hydroxybenzoic acids (1.34 µg/g DW, 5% of the total), and flavanol glucosides (e.g., quercetin-3-substituted, kaempferol-3-glucoside and rutin), whose total amount reached 3% of the polyphenolic fraction. Notably, among hydroxybenzoic acids, the amount of *p*-hydroxybenzoic acid in *Ulva* was 6- and 14-fold higher than those of *Humulus* trub and *Ailanthus*, respectively.

Table 1
Polyphenolic profile and relative content of *Ailanthus*, *Humulus* trub and *Ulva* hydroalcoholic extracts by HPLC-MS analysis

Compound	**Ailanthus (µg/g DW)**	**Humulus trub (µg/g DW)**	**Ulva (µg/g DW)**	**Antifungal activity**
Hydroxybenzoic acids				[18]; [48]
p-hydroxybenzoic acid	0.057 ± 0.003	0.144 ± 0.034	0.795 ± 0.170	
vanillin	0.046 ± 0.004	3.934 ± 0.050	0.544 ± 0.089	[51]; [52] [54]
vanillic acid	0.099 ± 0.005	3.188 ± 0.108	n.d.	[51]; [33]
gallic acid	107.082 ± 8.144	n.d.	n.d.	[33]
ellagic acid	744.156 ± 52.386	n.d.	n.d.	
Coumarins				
daphnetin	6.099 ± 0.506	n.d.	n.d.	
esculin	17.186 ± 0.405	n.d.	n.d.	
Hydroxycinnamic acids				
p-coumaric acid	1.244 ± 0.323	6.940 ± 0.772	n.d.	[51]
caffeic acid	4.750 ± 0.214	0.376 ± 0.036	0.113 ± 0.027	[33] [53]
ferulic acid	n.d.	20.511 ± 1. 815	n.d.	[32]
neochlorogenic acid	1860.807 ± 24.497	4.192 ± 1.199	n.d.	
cryptochlorogenic acid	32.293 ± 2.997	2.413 ± 0.647	n.d.	
chlorogenic acid	598.738 ± 18.701	4.104 ± 0.079	n.d.	[33]
rosmarinic acid	n.d.	n.d.	24.022 ± 0.255	[42]; [43]
Hydroxycinnamyl alcohols				
coniferyl alcohol	n.d.	6.436 ± 0.789	n.d.	[75]

Values are means ± SD of triplicate analysis. n.d., not detectable.

Compound	Ailanthus (μg/g DW)	Humulus trub (μg/g DW)	Ulva (μg/g DW)	Antifungal activity
sinapyl alcohol	321.645 ± 4.867	400.606 ± 23.360	n.d.	
Chalcones				
phlorizin	35.729 ± 2.664	n.d.	n.d.	
Flavones				
apigenin	0.148 ± 0.054	n.d.	n.d.	[36]
luteolin	0.803 ± 0.140	n.d.	n.d.	[36]
luteolin-7-O-Glc	0.270 ± 0.088	n.d.	n.d.	
apigenin-7-glc	3.699 ± 0.233	n.d.	n.d.	
Flavan-3-ols				
catechin	n.d.	12.121 ± 0.892	n.d.	[33]; [37]
epicatechin	n.d.	4.231 ± 1.159	n.d.	[37]
procyanidin B1	n.d.	77.187 ± 14.573	n.d.	
procyanidin B2 + B4	n.d.	7.311 ± 0.233	n.d.	
procyanidin B3	n.d.	67.763 ± 14.891	n.d.	
Flavonols				
kaempferol	40.068 ± 0.394	1.230 ± 0.391	n.d.	[76]
quercetin	89.590 ± 3.222	1.786 ± 0.313	n.d.	[35]; [36]
quercetin-3-Rha	80.484 ± 1.947	n.d.	n.d.	
kaempferol-3-Glc	1442.518 ± 38.694	61.174 ± 3.844	0.371 ± 0.049	
quercetin-3-Glc + quercetin-3-Gal	2588.968 ± 55.716	68.452 ± 4.318	0.316 ± 0.111	
quercetin-3-Glc acetyl	1.323 ± 0.301	0.230 ± 0.226	n.d.	
kaempferol-3-rutinoside	90.314 ± 3.598	3.642 ± 0.392	n.d.	
rutin	425.061 ± 11.807	7.820 ± 0.859	0.178 ± 0.038	[77]; [76]

Values are means ± SD of triplicate analysis. n.d., not detectable.

Compound	Ailanthus (µg/g DW)	Humulus trub (µg/g DW)	Ulva (µg/g DW)	Antifungal activity
Hydroquinone glycosides				
arbutin	127.147 ± 26.187	19.539 ± 2.699	n.d.	
Values are means ± SD of triplicate analysis. n.d., not detectable.				

3.3 Polyphenol PCA analysis

The PCA of the polyphenolic composition (Fig. 3) effectively distinguished across the three extracts (*Ailanthus*, *Humulus* trub and *Ulva*). PC1 described 71.9% of the total variance, and PC2 accounted for the remaining 26.6%. PC1 strongly separated the 3 replicates of *Ailanthus* from the others, mainly driven by quercetin-3-Glc, neochlorogenic acid, rutin, arbutin, ellagic acid and kaempferol, which are highly correlated among them and account for most of the total variance. PC2 distinguished between *Humulus* trub and *Ulva*, with the latter characterized only by rosmarinic acid and *p*- hydroxybenzoic acid. For this reason, *Ulva* being the most effective one as demonstrated in the previous paragraph, we further investigated the antifungal capacity of those 2 polyphenols.

3.4 Uronic and sulfate acid content in *Ulva* extract

The *Ulva* extract was further characterized by its relative content of uronic and sulfate acids through spectrophotometric assays. These assays revealed that the extract contained 24 ± 16% to 35 ± 23% of uronic acids (based on dry mass) across autumn to spring samples, and 32 ± 1% of total sulfate (data not shown).

3.5 Antifungal activity of *Ulva* extract on *A. flavus* conidial germination

Ulva was the only extract tested in the conidial germination assay because it showed the best efficacy against fungal growth in the antifungal activity assay. We examined the germination rate of *A. flavus* under bright-field microscope in the presence of three different aqueous dilutions of Ulva extract, corresponding respectively to EC50, 1/2 EC50, and 1/10 EC50 concentrations (14.0, 7.0 and 1.4 mg/mL). Conidial germination of *A. flavus* initiated after 10 h, reaching 97.0 ± 2.3% germination at 16 h in control samples. *Ulva* significantly reduced the percentage of germinated spores in a concentration-dependent manner ($p < 0.001$) (Supplemental Table S3 and Fig. 4). Lower doses of 1.4 and 7 mg/mL of *Ulva* decreased conidial germination rate to 66 and 19%, respectively. Where germ tubes were measurable, tube length was significantly decreased (50% and 66% compared to the control for 1.4 and 7.0 mg/mL, respectively). Incubation with 14.0 mg/mL extract caused a complete inhibition of conidial germination.

4. Discussion

The contamination of maize by toxigenic fungi remains a critical threat to food safety, necessitating sustainable alternatives to synthetic pesticides [10, 26]. While many plant-based extracts demonstrate antifungal potential [9, 27–29], this study specifically highlights the comparative value of spent biomass and seaweed as promising sources of polyphenols. All the extracts used, preliminarily screened for their inhibitory effect against *A. flavus*, were previously reported in literature as active against different fungal species [12–15, 30]. However, *Olea* extract was found to be ineffective against *A. flavus* at all the investigated doses. The lack of efficacy in *Olea* extract likely stems from the extraction method and solvent polarity, which may not have captured the bioactive compounds typically associated with its antifungal properties [13]. *Humulus* trub was the least effective against *A. flavus*, even though abundant in polyphenolic compounds. Nonetheless, the extract affected – though at high concentrations – the fungal growth, similarly to what reported by others [10, 12]. The use of spent biomasses, such as *Humulus* trub (a brewing industry by-product), is strategically important as these materials are recognized sources for developing high-added-value ingredients and products, with polyphenols and bitter acids standing out among their bioactive compounds [31]. In this context, although *Humulus* trub showed a moderate effect in our screening, its polyphenolic profile was notable, containing compounds with known antifungal potential [32], specifically, ferulic acid, hydroxycinnamyl alcohols, *p*-hydroxybenzoic acid, and vanillin. Furthermore, the presence of flavonoids, particularly catechins and quercetin derivatives, contributes to the extract's chemical complexity. These compounds, especially the flavonoid derivatives, are well-documented for their broad biological activities, including membrane disruption and enzyme inhibition in fungal species, which provides a plausible explanation for the observed, albeit moderate, fungistatic activity at higher concentrations [32–37]. *Ailanthus altissima* demonstrated higher chemical diversity than other extracts, featuring a wide array of flavonols and hydroxycinnamic acids. Recently, methanolic extracts from *Ailanthus* leaves were found to be rich in flavones and flavonols, and caffeic and chlorogenic acids [38, 39]. While these polyphenols are known to complex with microbial proteins, disrupt cell membranes, and interfere with enzyme function, the extract’s bioactivity likely results from a synergistic effect between phenolics and other metabolites such as lipophilic triterpenoids or saponins [40]. *A. altissima* has turned to be one of the most aggressive alien species in Europe for which its harvesting as a low-cost raw material for natural pesticide production could also be a rewarding strategy in reducing its populations and impacts on local biodiversity [30]. However, in our study, *Ailanthus* extract exerted some fungistatic activity at higher concentrations compared to *Ulva*.

The *Ulva* extract demonstrated the highest efficacy, characterized by the unique presence of rosmarinic acid and a high concentration of *p*-hydroxybenzoic acid. Rosmarinic acid is a natural caffeic acid ester known as a defense molecule against pathogens and herbivores and a UV protectant in diverse terrestrial plant [41]. Interestingly, Chernapalli et al. [42] stated that purified rosmarinic acid significantly affected mycotoxigenic fungi, in addition to airborne filamentous fungi [43]. Further *in silico* investigations of antifungal activity of rosmarinic acid by molecular docking studies evidenced that it inhibited different fungal target proteins involved in fungal carbon metabolism and infection process [42–45]. These reports proved this phenolic acid to be a promising candidate in inhibiting fungal growth

and suggested some plausible mechanisms involved. Nonetheless, besides our study, only a few chemical profiling studies identified rosmarinic acid in marine algal extracts and green alga *Ulva fasciata* [46] or its possible involvement in *in vitro* inhibition of pathogenic fungi [47]. On the contrary, *p*-hydroxybenzoic acid in algal extracts has been recently documented as the highest phenolic compound in *U. fasciata* extract with antifungal effects [18]. Interestingly, this phenolic acid has recently been shown to exhibit *in vivo* preventive and curative action at millimolar concentration as elicitor against kiwifruit rot caused by *A. flavus* [48]. According to literature, cinnamic- and benzoic-derived phenolic compounds could act as natural host defenses against *Fusarium* and *Aspergillus* spp., since their content was found to be higher in resistant maize genotypes reacting to silk infection than their non-resistant counterparts [49, 50]. Accordingly, the lower amount of *p*-hydroxybenzoic acid per dry weight found in *Ailanthus* and *Humulus* trub extracts with respect to *Ulva* (being 6 and 14 times lower, respectively) could be consistent with the lower EC50 values. Moreover, even though not specifically present only in *Ulva* extract, also vanillin, caffeic acid, flavonols derivatives and rutin may have acted synergistically with the dominant polyphenols contributing to the high efficacy of the extract, by interfering with cell wall and membrane structure or ergosterol biosynthesis [35, 36, 51–54]. Besides that, it is noteworthy that other studies emphasized that bioactive compounds are generally more effective in whole extract, acting synergistically or additively, rather than as single compounds, and that minor components are critical for their maximal antifungal activities [55–57]. In addition, Scaglioni et al. [58] demonstrated that antioxidants, such as carotenoids, present in the complex mixture of a natural extract, can interact with polyphenols, thereby influencing the overall antifungal effects that cannot be definitively determined from data on individual compounds alone. The exceptional efficacy of the *Ulva* extract, however, suggests the involvement of other potent non-phenolic metabolites in addition to the polyphenols. Accordingly, using a green and safe extraction method (hydroalcoholic solvent and room temperature during maceration) optimized for polyphenols, we also specifically tested for the presence of uronic and sulfated acid groups, characteristic of ulvan polysaccharides, given the potential of these sulfated biopolymers to contribute significantly to the overall antifungal action. Ulvans have gained great attention recently as emerging biopolymers easily extractable from algal biomass using hot water and subsequent ethanol precipitation [41], exhibiting a broad spectrum of biological and antioxidant activities [59]. Recent scientific studies in agriculture have explored the potential of seaweed ulvans as active substances for their well-known ability to induce plant resistance against fungal diseases [60, 61] and *in vivo* protection in wheat [62] and grapevine [63, 64]. Indeed, treatments with ulvans extracted from *U. lactuca* showed several antifungal actions against pathogenic fungi [19, 65, 66]. However, other studies did not confirm the direct involvement of ulvans in the antifungal processes [62, 67–69]. The presence of the sulfate group is an essential factor for the antimicrobial and antifungal activities of algal polysaccharides, as it is correlated with antioxidant action [70]. Discussions on biological activity of these compounds relate their antifungal action to the sulfated polysaccharides' interaction with the mycelium surface, leading to binding with membrane receptors and alterations in plasmalemma permeability [71]. The protective action *in vivo* is instead attributed to the stimulation of the plant defenses (pathogen-related, scavenger proteins, and the oxylipin pathway) rather than a direct antifungal effect [68]. The explanation for this duality of effects *in vivo* and *in vitro* may lie in the multitude of

structures and compositions that sulfated polymers of algal origin exhibit [72]. Given the superior antifungal efficacy of the *Ulva* treatment, this bio-extract demonstrates significant potential for future *in vivo* and field-scale applications directed at the biocontrol of maize and the mitigation of mycotoxin contamination. Consequently, further research is still necessary to fully understand ulvans and *Ulva* bioextract mechanisms and effectiveness.

5. Conclusion and future perspectives

In conclusion, we have proven that *Ulva* natural bio-extract exhibited a significantly higher *in vitro* antifungal efficacy against *A. flavus* compared to other polyphenol-rich extracts from processing waste or environmental collected biomass. The unique composition of *Ulva* extract, including specific polyphenols and ulvan polysaccharides, could synergistically contribute to its higher effectiveness. Additionally, the well-established role of these compounds in stimulating plant defense responses or regulating hormonal pathways, also highlights their potential as bio-stimulants. This dual functionality could contribute to reducing fungal pathogens' growth by enhancing the plant defenses. These findings pave the way for a deeper understanding of the underlying mechanisms and further investigation into their possible application for alternative management of fungal diseases in open-field conditions. Future work could therefore prioritize the controlled cultivation of these *Ulva* species to ensure a uniform biomass or establish a quality control metric based on key marker compounds to guarantee batch-to-batch consistency for the eventual standardization process. In conclusion, the final observed antifungal efficacy across all three extracts (*Humulus* trub, *Ailanthus* and *Ulva*) should be considered not solely a function of the quantified phenolic compounds, but rather a composite result stemming from the synergistic action of diverse specialized metabolites. Future work is crucial to move beyond correlation and implement bioassay-guided fractionation to precisely dissect the quantitative contribution of these non-phenolic principles. Researchers must, however, consider that the isolation of pure active compounds is often challenging due to the potential chemical degradation during purification or the loss of synergistic effects from other minor components present in the original crude extract [73, 74].

Declarations

Acknowledgements

We thank Dr. Benedetta Raffaelli for kindly giving fungal strain of *A. flavus.* Dr. Paolo Passaghe and Dr. Arianna Del Pino are also acknowledged for their technical support in extract preparation.

Author contributions

G.B.: Investigation, Formal analysis, Data curation, Writing – original draft. A.Di F.: Investigation, Methodology, Supervision, Writing – review & editing. E.B.: Conceptualization, Formal analysis, Supervision, Writing – review & editing. F.B.: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. V.V.: Conceptualization, Funding, Writing – review & editing. M.F.: Funding, Writing – review & editing. U.V.: Investigation, Writing – review & editing. M.V.: Conceptualization, Formal analysis,

Writing – review & editing. E.P.: Conceptualization, Investigation, Supervision, Writing –original draft and review & editing.

Data availability statement

All data used in this research are available from the corresponding author upon request.

Competing Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This work was supported by the Friuli-Venezia Giulia Region within the project “relazioni tra stress idrico, eco-fisiologia della pianta e insorgenza di micotossine nel mais in scenari di cambiamenti climatici (ECO-MICORN-CLIMA)” (CUP G23C22001340002). Giacomo Boscarol fellowship has been granted by the financial support from the Italian Ministry of Education “Programme Agricultural Sciences and Biotechnology” co-funded by the Italian Recovery and Resilience Plan (PNRR) - NextGeneration EU, Ministry Decree n. 351/2022. Open access funding provided by Università degli Studi di Udine within the CRUI-CARE Agreement.

References

1. Zingales, V., Taroncher, M., Martino, P. A., Ruiz, M. J. & Caloni, F. Climate change and effects on molds and mycotoxins. *Toxins* **14**, 445 (2022).
2. Ferrari, L. et al. Compliance between food and feed safety: eight-year survey (2013–2021) of Aflatoxin M1 in raw milk and Aflatoxin B1 in feed in Northern Italy. *Toxins* **15**, 168 (2023).
3. Giorni, P., Bertuzzi, T. & Battilani, P. Aflatoxin in maize, a multifaceted answer of *Aspergillus flavus* governed by weather, host-plant and competitor fungi. *J. Cereal Sci.* **70**, 256–262 (2016).
4. Leggieri, M. C., Toscano, P. & Battilani, P. Predicted Aflatoxin B_1 increase in Europe due to climate change: actions and reactions at global level. *Toxins* **13**, 292 (2021).
5. Battilani, P. et al. Aflatoxin B_1 contamination in maize in Europe increases due to climate change. *Sci. Rep.* **6**, 24328 (2016).
6. Medina, Á., González-Jartín, J. M. & Sainz, M. J. Impact of global warming on mycotoxins. *Curr. Opin. Food Sci.* **18**, 76–81 (2017).
7. Molnár, K. et al. The effect of environmental factors on mould counts and afb1 toxin production by *Aspergillus flavus* in maize. *Toxins* **15**, 227 (2023).
8. Ahmed, O. S. et al. Naturally occurring phenolic compounds as promising antimycotoxin agents: Where are we now? *Compr. Rev. Food Sci. Food Saf.* **21**, 1161–1197 (2022).

9. Montibus, M. et al. Screening of wood/forest and vine by-products as sources of new drugs for sustainable strategies to control *Fusarium graminearum* and the production of mycotoxins. *Molecules* **26**, 405 (2021).
10. Giorni, P. et al. Enhancement of agri-food by-products: green extractions of bioactive molecules with fungicidal action against mycotoxigenic fungi and their mycotoxins. *Front Nutr* **10**, (2023).
11. Makhuvele, R. et al. The use of plant extracts and their phytochemicals for control of toxigenic fungi and mycotoxins. *Heliyon* **6**, e05291 (2020).
12. Bartmańska, A. et al. Antimicrobial properties of spent hops extracts, flavonoids isolated therefrom, and their derivatives. *Molecules* **23**, 2059 (2018).
13. Bilel, H., Yahia, M. & Moustafa, S. M. N. An overview of chemical composition and fungicidal activity of Olive (Olea Europea L.) Leaf Extract. *Egypt. J. Chem.* **66**, 303–311 (2023).
14. Li, Y., Zhao, M. & Zhang, Z. Quantitative proteomics reveals the antifungal effect of canthin-6-one isolated from *Ailanthus altissima* against *Fusarium oxysporum* f. sp. *cucumerinum in vitro*. *PLOS ONE*. **16**, e0250712 (2021).
15. Taillis, D. et al. Antifungal activities of a grapevine byproduct extract enriched in complex stilbenes and stilbenes metabolization by *Botrytis cinerea*. *J. Agric. Food Chem.* **71**, 4488–4497 (2023).
16. Filippi, A. et al. Bioactive polyphenols modulate enzymes involved in grapevine pathogenesis and chitinase activity at increasing complexity levels. *Int J. Mol. Sci* **20**, (2019).
17. Vuerich, M. et al. Antifungal activity of chili pepper extract with potential for the control of some major pathogens in grapevine. *Pest Manag Sci.* **79**, 2503–2516 (2023).
18. El-Bilawy, E. H. et al. Antiviral and antifungal of *Ulva fasciata* extract: HPLC analysis of polyphenolic compounds. *Sustainability* **14**, 12799 (2022).
19. El Fayoumy, R. A., El-Sheekh, M. M. & Abu Ahmed, S. E. Potential of ulvan polysaccharide from *Ulva lactuca* as antifungal against some foodborne fungi isolated from spoiled tomato sauce cans. *J. Aquat. Food Prod. Technol.* **31**, 658–671 (2022).
20. Zarraonaindia, I. et al. Holistic understanding of the response of grapevines to foliar application of seaweed extracts. *Front Plant. Sci* **14**, (2023).
21. Sadallah, A. et al. Propolis hydroalcoholic extracts: biochemical characterization and antifungal efficacy. *Horticulturae* **11**, 122 (2025).
22. Vrhovsek, U. et al. A versatile targeted metabolomics method for the rapid quantification of multiple classes of phenolics in fruits and beverages. *J. Agric. Food Chem.* **60**, 8831–8840 (2012).
23. Connan, S. Spectrophotometric assays of major compounds extracted from algae. in Natural Products From Marine Algae (eds Stengel, D. B. & Connan, S.) (vol 1308 75–101 (Springer New York, New York, NY, (2015).
24. Torres, P. B. et al. Determination of sulfate in algal polysaccharide samples: a step-by-step protocol using microplate reader. *Ocean. Coast Res.* **69**, e21021 (2021).

25. core, R. T. R: The R Project for Statistical Computing. (2024). https://www.r-project.org/ https://www.r-project.org/
26. Scaglioni, P. T. et al. Application of fungicides and microalgal phenolic extracts for the direct control of fumonisin contamination in maize. *J. Agric. Food Chem.* **66**, 4835–4841 (2018).
27. Lagogianni, C. S. & Tsitsigiannis, D. I. Effective biopesticides and biostimulants to reduce aflatoxins in maize fields. *Front Microbiol* **10**, (2019).
28. Matrose, N. A., Obikeze, K., Belay, Z. A. & Caleb, O. J. Plant extracts and other natural compounds as alternatives for post-harvest management of fruit fungal pathogens: a review. *Food Biosci.* **41**, 100840 (2021).
29. Tian, F. et al. Plant-based natural flavonoids show strong inhibition of aflatoxin production and related gene expressions correlated with chemical structure. *Food Microbiol.* **109**, 104141 (2023).
30. Kozuharova, E. et al. Assessment of the potential of the invasive arboreal plant *Ailanthus altissima* (Simaroubaceae) as an economically prospective source of natural pesticides. *Diversity* **14**, 680 (2022).
31. Silva, K. F. C., e, Strieder, M. M., Pinto, M. B. C., Rostagno, M. A. & Hubinger, M. D. Processing strategies for extraction and concentration of bitter acids and polyphenols from brewing by-products: a comprehensive review. *Processes* **11**, 921 (2023).
32. Yan, H. et al. Antifungal activity and inhibitory mechanisms of ferulic acid against the growth of *Fusarium graminearum*. *Food Biosci.* **52**, 102414 (2023).
33. Palumbo, J. D., O'Keeffe, T. L. & Mahoney, N. E. Inhibition of ochratoxin A production and growth of *Aspergillus* species by phenolic antioxidant compounds. *Mycopathologia* **164**, 241–248 (2007).
34. Ponts, N., Pinson-Gadais, L., Boutigny, A. L., Barreau, C. & Richard-Forget, F. Cinnamic-derived acids significantly affect *Fusarium graminearum* growth and < i > in vitro synthesis of type B trichothecenes. *Phytopathology®* **101**, 929–934 (2011).
35. Li, X. M., Li, Z. Y., Wang, Y. D., Wang, J. Q. & Yang, P. L. Quercetin inhibits the proliferation and aflatoxins biosynthesis of *Aspergillus flavus*. *Toxins* **11**, 154 (2019).
36. Castano-Duque, L., Lebar, M. D., Mack, B. M., Lohmar, J. M. & Carter-Wientjes, C. Investigating the impact of flavonoids on *Aspergillus flavus*: insights into cell wall damage and biofilms. *J. Fungi.* **10**, 665 (2024).
37. Cadenillas, L. F., Billerach, G., Hernandez, C., Durrieu, V. & Bailly, J. D. Inhibition of aflatoxin B_1 production by procyanidins present in *Annona muricata* and *Uncaria tomentosa* aqueous extracts. *Toxins* **16**, 454 (2024).
38. Poljuha, D. et al. Phenolic composition of leaf extracts of *Ailanthus altissima* (Simaroubaceae) with antibacterial and antifungal activity equivalent to standard antibiotics. *Nat. Prod. Commun.* **12**, 1934578X1701201021 (2017).
39. Cocîrlea, M. D. et al. Phenolic composition and bioactivities of invasive *Ailanthus altissima* (Mill.) swingle leaf extracts obtained by two-step sequential extraction. *Antioxidants* **13**, 824 (2024).

40. Guo, S. S., Duan, Z. K., Huang, X. X. & Song, S. J. Phytochemical investigation on the leaves of *Ailanthus altissima* (Mill.) Swingle. *Biochem. Syst. Ecol.* **110**, 104668 (2023).
41. Coiai, S. et al. Rosmarinic acid and ulvan from terrestrial and marine sources in anti-microbial bionanosystems and biomaterials. *Appl. Sci.* **11**, 9249 (2021).
42. Chernapalli, V., Zahoor Gul, M., Anwar, H. M., Susmitha Kuruganti, N. S. & Rupula, K. Antifungal effects of rosmarinic acid, α-tocopherol and α-tocopherol acetate on */Aspergillus parasiticus* (NRRL 2999) and *Candida albicans* (MTCC 183) and assessment of chitin and ergosterol biomarkers. *Adv Biotechnol. Microbiol* **18**, (2024).
43. Xu, Y. et al. Integration of transcriptomics and proteomics to elucidate inhibitory effect and mechanism of rosmarinic acid from *Perilla frutescens* (L.) Britt. in treating *Trichophyton mentagrophytes*. *Chin. Med.* **18**, 67 (2023).
44. Althobaiti, F. Fungicidal activity of rosmarinic acid against *Aspergillus fumigatus* and *Aspergillus brevipes* isolated from raw cow milk in western Saudi Arabia in sight of *in vitro* and *in silico* studies. (2024). https://doi.org/10.5281/ZENODO.10634379 doi:10.5281/ZENODO.10634379.
45. Moin, A. T. et al. Antifungal plant flavonoids identified *in silico* with potential to control rice blast disease caused by *Magnaporthe oryzae*. *PLOS ONE*. **19**, e0301519 (2024).
46. Ghallab, D. S., Shawky, E., Ibrahim, R. S. & Mohyeldin, M. M. Comprehensive metabolomics unveil the discriminatory metabolites of some Mediterranean Sea marine algae in relation to their cytotoxic activities. *Sci. Rep.* **12**, 8094 (2022).
47. Pourakbar, L., Moghaddam, S. S., Enshasy, H. A. E. & Sayyed, R. Z. Antifungal activity of the extract of a macroalgae, *Gracilariopsis persica*, against four plant pathogenic fungi. *Plants* **10**, 1781 (2021).
48. Huo, Z. Y. et al. Antifungal and elicitor activities of *p*-hydroxybenzoic acid for the control of aflatoxigenic *Aspergillus flavus* in kiwifruit. *Food Res. Int.* **173**, 113331 (2023).
49. Lorán, S., Carramiñana, J. J., Juan, T., Ariño, A. & Herrera, M. Inhibition of *Aspergillus parasiticus* growth and aflatoxins production by natural essential oils and phenolic acids. *Toxins* **14**, 384 (2022).
50. Thompson, M. E. H. & Raizada, M. N. Fungal pathogens of maize gaining free passage along the silk road. *Pathog Basel Switz.* **7**, 81 (2018).
51. Kim, J. H., Campbell, B. C., Mahoney, N., Chan, K. L. & May, G. S. Targeting antioxidative signal transduction and stress response system: control of pathogenic *Aspergillus* with phenolics that inhibit mitochondrial function. *J. Appl. Microbiol.* **101**, 181–189 (2006).
52. Li, Q., Zhao, Y., Zhu, X. & Xie, Y. Antifungal effect of *o*-vanillin on mitochondria of *Aspergillus flavus*: ultrastructure and TCA cycle are destroyed. *Int. J. Food Sci. Technol.* **57**, 3142–3149 (2022).
53. Guo, Y. et al. Postharvest caffeic acid dipping enhances disease resistance and storage capacity of 'Zaosu' pear fruit via regulating phenylpropane metabolism. *Postharvest Biol. Technol.* **209**, 112716 (2024).
54. Ríos-López, A. L., Dávila-Aviña, J., González, G. M. & Flores-Maldonado, O. Antifungal and antivirulence activity of vanillin and tannic acid against *Aspergillus fumigatus* and *Fusarium solani*. *Curr. Microbiol.* **81**, 156 (2024).

55. Ljunggren, J., Bylund, D., Jonsson, B. G., Edman, M. & Hedenström, E. Antifungal efficiency of individual compounds and evaluation of non-linear effects by recombining fractionated turpentine. *Microchem J.* **153**, 104325 (2020).
56. Loi, M., Paciolla, C., Logrieco, A. F. & Mulè, G. Plant bioactive compounds in pre- and postharvest management for aflatoxins reduction. *Front. Microbiol.* **11**, 1–16 (2020).
57. Pinto, E. et al. Antifungal activity of *Ferulago capillaris* essential oil against *Candida*, *Cryptococcus*, *Aspergillus* and dermatophyte species. *Eur. J. Clin. Microbiol. Infect. Dis.* **32**, 1311–1320 (2013).
58. Scaglioni, P. T. et al. Nannochloropsis sp. and *Spirulina* sp. as a source of antifungal compounds to mitigate contamination by *Fusarium graminearum* species complex. *Curr. Microbiol.* **76**, 930–938 (2019).
59. Kraithong, S. et al. Potentials of *Ulva* spp.-derived sulfated polysaccharides as gelling agents with promising therapeutic effects. *Int. J. Biol. Macromol.* **273**, 132882 (2024).
60. Bahmani, R., More, P., Babarinde, S., Zhou, M. & Prithiviraj, B. Seaweeds for plant disease management: current research advances and future perspectives. *Phytoparasitica* **51**, 783–802 (2023).
61. Poveda, J. & Díez-Méndez, A. Use of elicitors from macroalgae and microalgae in the management of pests and diseases in agriculture. *Phytoparasitica* **51**, 667–701 (2023).
62. de Borba, M. C. et al. The algal polysaccharide ulvan induces resistance in wheat against *Zymoseptoria tritici* without major alteration of leaf metabolome. *Front. Plant. Sci.* **12**, 1–14 (2021).
63. Shomron, A., Duanis-Assaf, D., Galsurker, O. & Golberg, A. Alkan, N. Extract from the macroalgae *Ulva rigida* induces table grapes resistance to *Botrytis cinerea*. *Foods* **11**, 723 (2022).
64. Zarraonaindia, I. et al. Seawines: use of macroalgae as biostimulants against fungal diseases in grapevines. *BIO Web Conf* **68**, (2023).
65. Ben Salah, I. et al. Seaweed polysaccharides as bio-elicitors of natural defenses in olive trees against verticillium wilt of olive. *J. Plant. Interact.* **13**, 248–255 (2018).
66. Salim, D. et al. Control of post-harvest citrus green mold using *Ulva lactuca* extracts as a source of active substances. *Int. J. Bio-Resour Stress Manag*. **11**, 287–296 (2020).
67. Araújo, L., Stadnik, M. J., Borsato, L. C. & Valdebenito-Sanhueza, R. M. Fosfito de potássio e ulvana no controle da mancha foliar da gala em macieira. *Trop. Plant. Pathol.* **33**, 148–152 (2008).
68. Paulert, R. et al. Effects of sulfated polysaccharide and alcoholic extracts from green seaweed *Ulva fasciata* on anthracnose severity and growth of common bean (*Phaseolus vulgaris* L). *J. Plant. Dis. Prot.* **116**, 263–270 (2009).
69. Reis, R. P., Junior, C., de Facchinei, A. A., Calheiros, A. P. & Castelar, B. A. C. dos S. Direct effects of ulvan and a flour produced from the green alga *Ulva fasciata* Delile on the fungus *Stemphylium solani* Weber. *Algal Res.* 30, 23–27 (2018).
70. Massocato, T. F. et al. Characterization and biological activities of the ulvan polysaccharide-rich fraction obtained from *Ulva rigida* and *Ulva pseudorotundata* and their potential for pharmaceutical

application. *Mar. Biotechnol.* **26**, 324–337 (2024).

71. Sruthy, E. S. & Baiju, E. C. K. Exploration of secondary metabolites from green algae as antimicrobial agents: A comprehensive review. *Bot. Serbica*. **48**, 127–140 (2024).
72. Li, M., Wang, M., Chen, J., Wu, J. & Xia, Z. Sulfur dioxide improves the thermotolerance of maize seedlings by regulating salicylic acid biosynthesis. *Ecotoxicol. Environ. Saf.* **254**, 114746 (2023).
73. Caesar, L. K. & Cech, N. B. Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. *Nat. Prod. Rep.* **36**, 869–888 (2019).
74. Méndez, D. et al. Antifungal activity of extracts, fractions, and constituents from *Coccoloba cowellii* leaves. *Pharmaceuticals* **14**, 917 (2021).
75. Ninkuu, V. et al. Lignin and its pathway-associated phytoalexins modulate plant defense against fungi. *J. Fungi*. **9**, 52 (2023).
76. Sadeghi, A., Rajabiyan, A., Nabizade, N., Meygoli Nezhad, N. & Zarei-Ahmady, A. Seaweed-derived phenolic compounds as diverse bioactive molecules: a review on identification, application, extraction and purification strategies. *Int. J. Biol. Macromol.* **266**, 131147 (2024).
77. Oliveira, V. M., Carraro, E., Auler, M. E. & Khalil, N. M. Quercetin and rutin as potential agents antifungal against *Cryptococcus* spp. *Braz J. Biol.* **76**, 1029–1034 (2016).

Figures

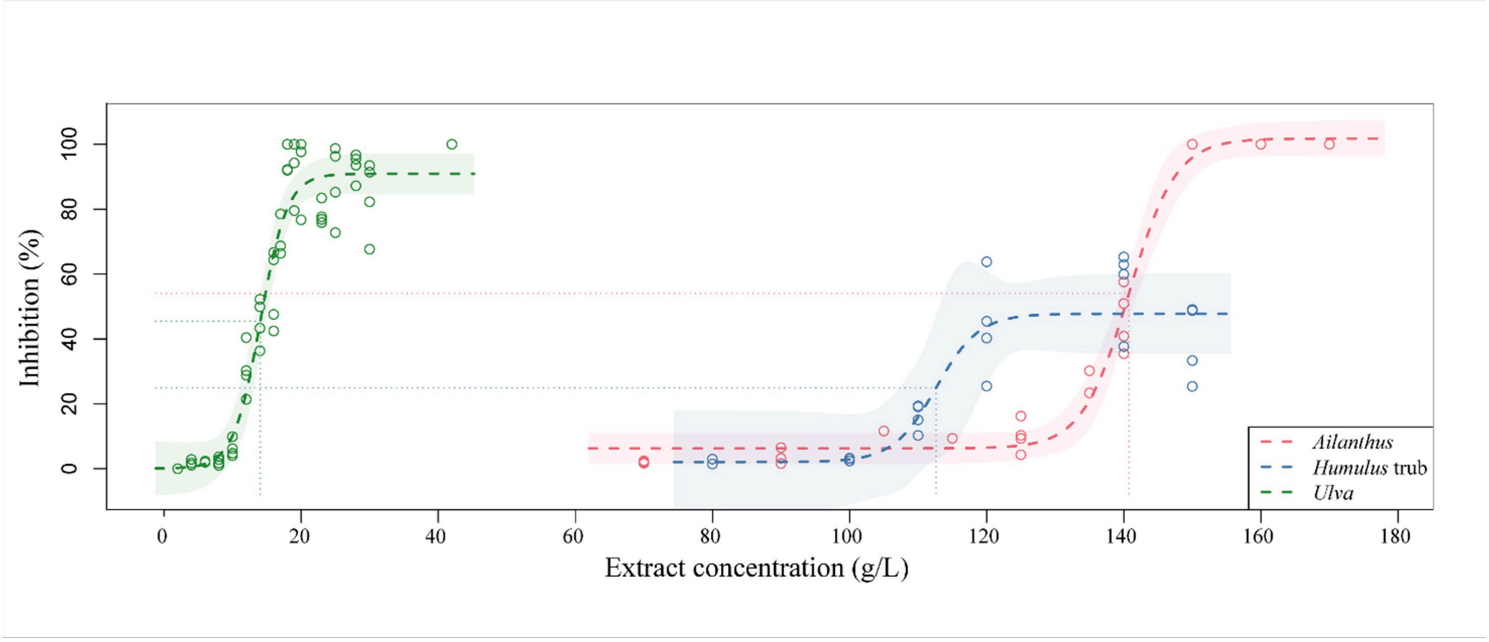

Figure 1

In vitro dose-response curves displaying the antifungal activity of the three most efficient extracts**;** each extract of *Ailanthus*, *Humulus* trub and *Ulva* was serially diluted in sterile water and evaluated by well diffusion testing and each dose-response curve was calculated using a 4-parameter logistic fit function. The full model was represented by the formula y = α + δ / (1 + η * exp (-e * (x - φ)))^(1 / η), where α =

lower limit, δ = upper limit, η = slope of the curve, φ = EC50, eventually simplified according to the Akaike Information Criterion. At least three different replicates of data for each concentration were used.

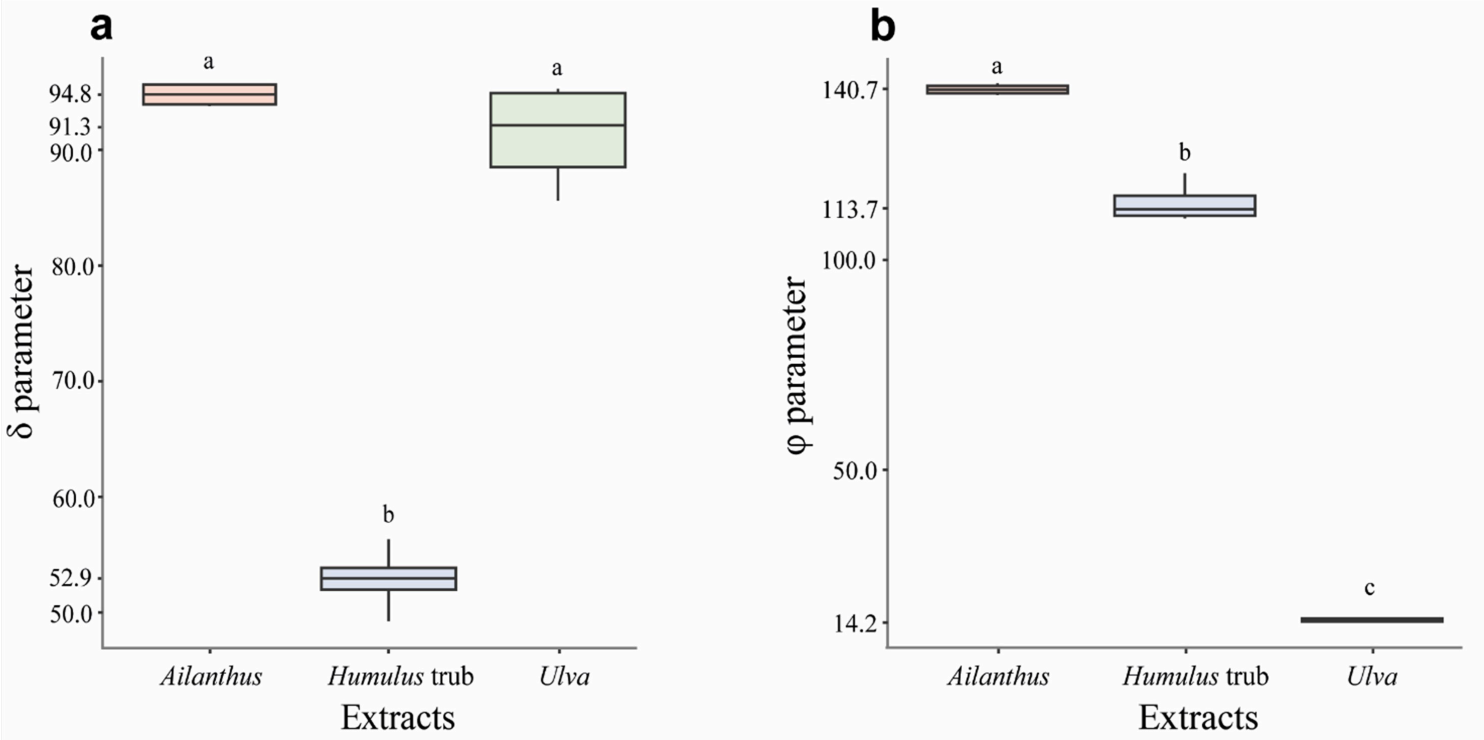

Figure 2

Boxplots of one-way ANOVA analysis for extract effect on logistic parameters of antifungal activity dose-response curve; **a** δ = upper limit; **b** φ = EC50. The actual values of δ were 91.3±4 (*Ulva*), 94.8±0.9 (*Ailanthus*), and 52.9±2.5 % (*Humulus* trub), whereas for φ 14.2±0.4 (*Ulva*), 140.7±1.1 (*Ailanthus*) and 113.7±4.2 mg/mL (*Humulus* trub). Different letters denote a statistically significant difference ($p < 0.01$) between extracts. Vertical lines correspond to minimum and maximum values, colored boxes are delimitated by first and third quartiles, horizontal line inside the boxes represents the median. The lower limit (α) and the slope of the curve (η) are not shown; η was found to be not significantly different among the three extracts

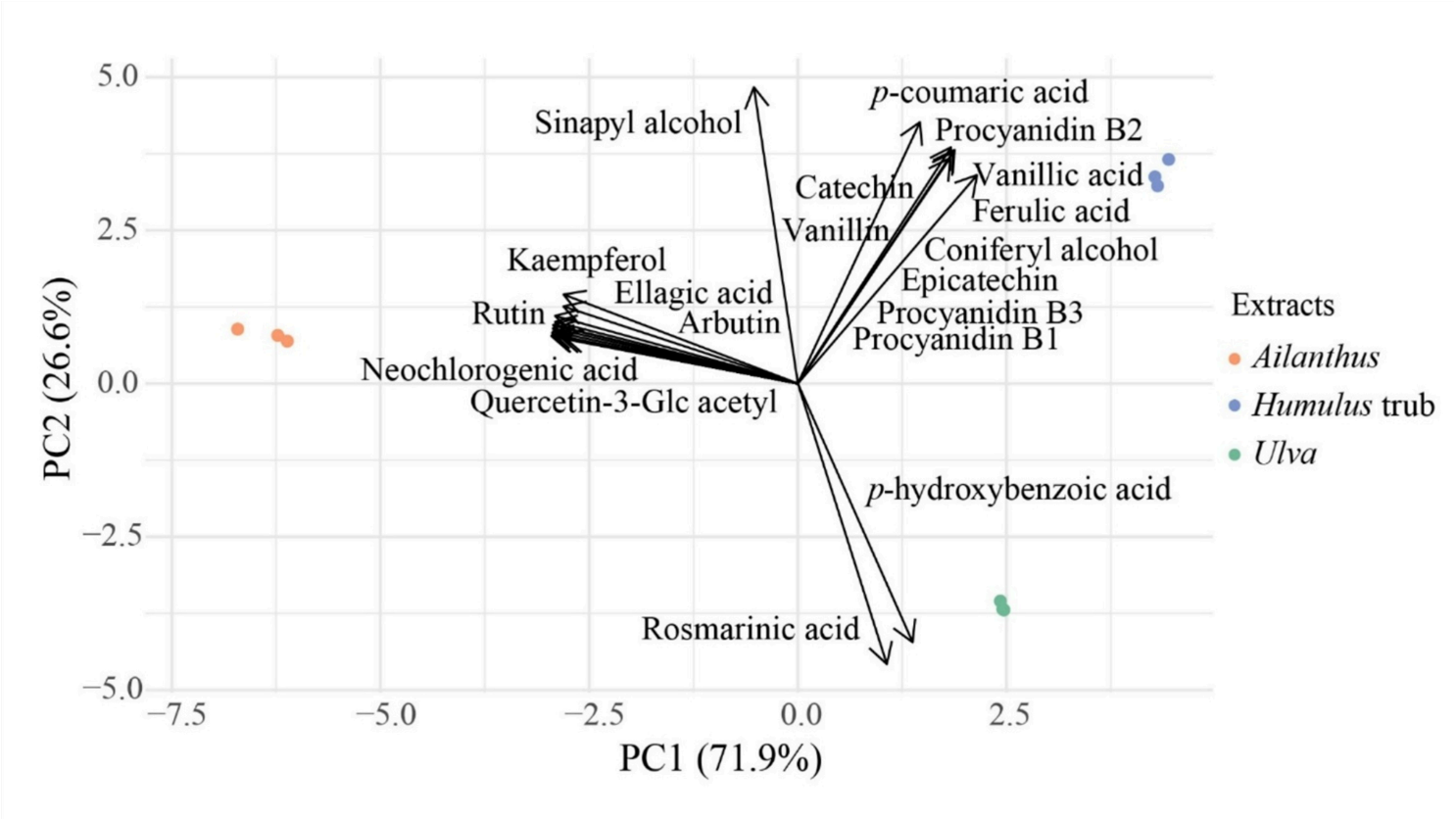

Figure 3

Principal Component Analysis (PCA) of the polyphenolic composition in *Ailanthus*, *Humulus* trub and *Ulva*

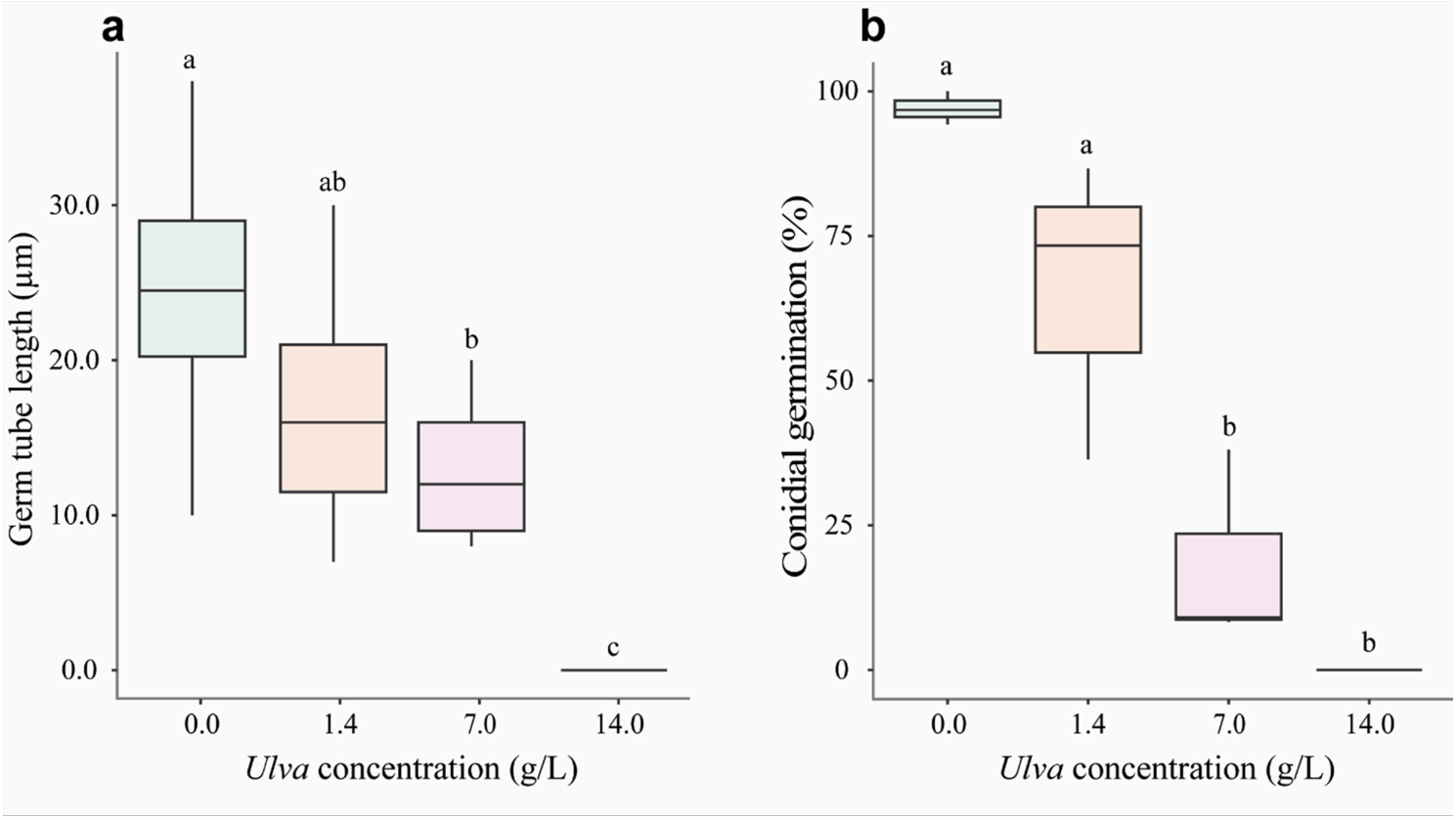

Figure 4

Effect of *Ulva* extract on *A. flavus* conidial germination; inhibition effect of different concentrations of *Ulva* on **a** germ tube length (µm); **b** on conidial germination (% of *A. flavus* after 16 h of incubation at 28°C. Values are mean ± SD. Different letters denote a statistically significant difference ($p < 0.01$) between concentrations

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx