

Potential use of *Piper peltatum* essential oil for the control of *Moniliophthora roreri* in cacao plants cultivated in the Colombian Amazon region

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Research Article

Keywords: Moniliasis, Theobroma cacao, Moniliophthora roreri, Piper peltatum

Posted Date: February 12th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-5486772/v1>

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Abstract

Cocoa (*Theobroma cacao* L.) is one of the main species of the peasant agroforestry system in many regions of Colombia and is considered one of the crops with the greatest economic projection, given its industrial demand and food importance. Production yields in the country are limited by various factors, with fungal diseases being a major challenge. Consequently, there is a strong need to develop safe and effective phytosanitary solutions to manage these diseases, reduce losses, and enhance productivity. The objective of the present study was to evaluate the antifungal potential of 4 concentrations (500, 1000, 2000 and 5000 ppm) of root, leaf and stem extracts of the *Piper peltatum* plant for the control of the fungus *Moniliophthora roreri*. For this, the fungus was collected and isolated from cocoa pods (*T. cacao*) and morphologically characterized. The results allowed to determine that *P. peltatum* in root extracts presents total phenol values of 63 mg A.G g⁻¹ and flavonoids of 74.88 mg CAT/g-1 and antioxidant activity greater than 200 mg Tx/g-1, ABTS, with values of 300 mg Tx/g-1 and FRAP greater than 100 mg AA/g-1. Finally, the concentrations of *Piper peltatum* extracts showed an inhibition of mycelial growth greater than 88% in all concentrations and plant organs evaluated, highlighting the high potential of *Piper peltatum* as a controlling agent of *Moniliophthora roreri* in Cocoa crops in the Colombian Amazon region.

Introduction

Cocoa (*Theobroma cacao* L.) is native to the humid tropics (Enríquez, 1987), mainly in the upper Amazon River basin between Peru, Ecuador and Colombia on the tributary rivers Caquetá, Napo and Putumayo (Baker et al., 1992). It has great genetic diversity (Santos et al., 2012) and is one of the most important tropical crops worldwide (ICCO-International Cocoa Organization, 2019).

Currently, approximately 11,939,436 hectares of cocoa are cultivated worldwide, with an estimated production of 5,874,581 tons per year. This production is distributed as follows: Africa (67.4%), Asia (16.2%), America (15.3%) and Oceania (1.1%). The main producing countries are Ivory Coast, Ghana and Indonesia, which concentrate about 53% of global production (Food and Agriculture Organization of the United Nations-FAO, 2023). Colombia ranks tenth in world production, with approximately 59,831 tons, mainly in the (regions or states) of Santander, Huila and Antioquia. Caquetá has shown an increase in productive yield at the national level, registering 171 tons for 2023 (Federación Nacional de Cacaoteros-FEDECACAO, 2024).

However, one of the main challenges faced in cocoa cultivation is fungal diseases, with *M. roreri* being particularly notable (Armengot et al., 2020), which directly attacks the tissues, fruits, buds and floral cushions of the plant. This disease causes fruit rot in most producing regions of the world (Leiva et al., 2022), generating global production losses of more than 30% (Hebbar, 2007). In Colombia, it is identified as the main limitation for cocoa production (Jaimes et al., 2016).

As a control for this pathology, different chemical fungicides are used that can generate resistance to the pathogen, in addition to causing damage to the environment, generating incompatibility with organic production and proliferation of other types of phytopathogens (Ferreira & Musumeci, 2021). Nonetheless, there are alternative methods for controlling this disease, notably the use of plant extracts from species with antifungal effects. These extracts contain various metabolites, such as flavonoids, phenols, phenolic glycosides, and others (Davicino et al., 2007). This is a direct method that plants have and that inhibits the growth of pathogens, especially by the production of secondary metabolites, which allows there to be no competitive advantage against antagonism (Köhl et al., 2019).

Among these, the research conducted on the Piperaceae family, known for its five major genera, is particularly noteworthy (Jaramillo and Manos, 2001). The Piper genus is one of the most studied within the group, given its potential as a biofungicide and antioxidant (Lago et al., 2009; Navickiene et al., 2000), insecticide (Santos et al., 2010),

antimicrobial (Morales et al., 2013), and anti-inflammatory (Lima et al., 2012). Some studies suggest that this genus has different compounds, such as flavonoids, lignins, steroids and alkaloids (Navickiene et al., 2000), as well as terpenes and phenylpropanoids (Silva et al., 2011).

Currently, there is no effective method to reduce the total impact of diseases in cocoa plantations worldwide (Evans, 2016). As a result, conducting studies of this type will serve as a strategy to reduce and control the incidence and severity in current and future cultivars, thus improving yield and profitability for the department of Caquetá and the Amazon region. Therefore, it has an important impact on the search for future solutions for the control of moniliasis through the use of natural extracts with plants from the region. Thus, the objective of this work was to determine the potential biocontrol effect of *P. peltatum* extract against *M. roreri* under *in vitro* conditions.

Materials and Methods

Study area and data collection

This study was conducted in the Mycology and Phytoprotection Laboratory of the Amazonian Research Center CIMAZ-MACAGUAL at the Universidad de la Amazonia, located in the south of Colombia, in the department of Caquetá, at 300 meters above sea level. The region has an average temperature of 24 °C, relative humidity of 85.1%, and an AF climate (Tropical rainforest climate) according to the Köppen classification (Aldana et al., 2021).

Isolation of *M. roreri*

T. cacao cobs of clone CCN-51 with symptoms of moniliasis were identified and collected, following the criteria described by López et al. (2014). Each sample had brown spots and was covered by white mycelium characteristic of the fungus. Subsequently, a disinfection protocol was applied by washing the cobs with running water without direct contact with the hands. The cobs were then treated with 2% sodium hypochlorite for one minute, followed by an application of 99% ethanol. Finally, they were washed with distilled water and dried at room temperature. The cobs were stored in airtight bags for preservation.

For the isolation of the phytopathogen *M. roreri*, the methodology described by Villamil et al. (2012) was taken into account, with some modifications. A sterilized blade was used to cut off pieces of infected and healthy tissue from the cob, approximately 1 cm in length. Each fragment was subjected to a disinfection process, first with 1.5% sodium hypochlorite for one minute, followed by immersion in 70% ethyl alcohol. Three washes were then carried out with sterile distilled water to eliminate hypochlorite and alcohol residues; subsequently, the pieces were dried on a sterile absorbent towel for 10 minutes.

After disinfection, the pieces were inoculated in sterile Petri dishes, maintaining a room temperature of 25 °C and photoperiods of 12 h of light and 12 h of darkness. After approximately 48 hours, sporulation was observed in each of the pieces, at which time the germinated mycelium was transferred to 5 Petri dishes with Potato Dextrose Agar (PDA), using a completely sterile loop. All cultures were incubated at a temperature of 25 °C ± 2 °C for 15 days.

Identification of *M. roreri*

Identification was carried out using the taxonomic key proposed by Barnett & Hunter (1999). Macroscopic characteristics were determined taking into account those described by Phillips et al. (2006), such as colony color, colony edge, mycelium type (texture) and sporulation type. Microscopic characteristics were observed with the aid of an optical microscope, taking into account the shape of the spores and hyphae as described by Evans et al. (1978).

Obtaining the plant extract of *P. peltatum*

The *P. peltatum* extract was obtained using the methodology proposed by Díaz (2021) and adapted for this study; For this purpose, green and healthy *P. peltatum* plants (root, stem and leaf) were initially collected and dried in an oven at 70 °C for 5 days. The dried samples were ground and placed in ethanol percolation, using 140 g of dry plant tissue per liter of solution for eight days. The pure extracts were stored in sterile amber glass jars under refrigeration at a temperature of between 5 and 7 °C.

Evaluation of bioactive compounds

Total phenol content

The total phenol content of the extracts was determined by the Folin-Ciocalteu test. To do this, each extract was mixed with Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3) at 7.1% w/v and water. The mixture was incubated at room temperature for 60 minutes, keeping them protected from light. Subsequently, absorbance at 760 nm was recorded using the Multiskan Go plate reader. Results were calculated using a standard calibration curve of gallic acid and expressed in milligrams equivalent of gallic acid per 100 grams of each plant part (mg GA/100 g) (Dzah et al., 2023).

Flavonoid Content

A colorimetric assay of aluminum chloride (AlCl_3) was used, as indicated by Treviño (2024), with some modifications. For this, each extract was mixed with 5% sodium nitrite (NaNO_2). After 5 min, 10% aluminum chloride (AlCl_3), 1 M sodium hydroxide (NaOH) and distilled water (H_2O) were added to each solution. The final reaction was read on the Multiskan Go plate reader at 510 nm. The results were calculated using a standard calibration curve of catechin and were expressed in milligrams of catechin equivalents (CT) per 100 grams of each part of the plant (mg EC/100 g).

Evaluation of antioxidant activity

The DPPH, ABTS and FRAP methods were used to evaluate the antioxidant capacity of the extracts by means of UV-Visible spectroscopy (Multiskan Go). The DPPH method, based on the capture of free radicals by the molecule 1,1-diphenyl-2-picrylhydrazine, whose absorbance was measured at 517 nm, presenting a violet color. On the other hand, the ABTS method, which used the ABTS radical (2,2'-azino-bis-(3-ethylthiazolinebenzenesulfonic acid-6)) generated an intense blue-green color, a radical generated chemically by interaction with potassium persulfate before the reaction with the antioxidants; the combination of the sample and the radical was measured at an absorbance of 734 nm. Finally, the FRAP method, based on the reducing capacity of the antioxidants present in the sample against the ferric oxidizing compound, was measured at an absorbance of 590 nm. All analyses were quantified by means of standard calibration curves of Trolox (Tx) and ascorbic acid (AA) and were expressed in milligram equivalents of the standard per 100 grams of each plant part (mg of the standard/100 g) (Arnao et al., 2001; Kedare & Singh, 2011; Rumpf et al., 2023).

Inhibition of mycelial growth

The *in vitro* antifungal activity of the extract of *P. peltatum* (root, stem and leaf) was evaluated. For this, PDA agar discs previously colonized with the fungus *M. roreri* of 8 mm diameter were extracted and inoculated in Petri dishes (90x18 mm) at concentrations of 500, 1000, 2000 and 5000 ppm, then incubated at 25 °C to allow the growth of the phytopathogen. This test was performed in triplicate, measuring radial growth every three days for 15 days (at which time the control colonies had covered the area of the Petri dish). The inhibition of mycelial growth was measured by applying the following formula: Inhibition percentage = $\frac{\text{Diameter of the control} - \text{Diameter of the treatment}}{\text{Diameter of the control}} \times 100$. For this, a completely randomized design was used with 10 repetitions for each treatment, compared with two negative controls (commercial control: Topgun, control with ethanol) and one positive control (normal fungal growth on PDA).

Statistical analysis

All variables evaluated (bioactive compounds, secondary metabolites and mycelial growth inhibition) were fitted using a simple linear model, normality assumptions were checked using the Shapiro Wilk test (p-value > 0.05) and Q-Q plot, homogeneity of variance was checked using the Bartlett test (p-value > 0.05) and residuals vs. fitted plot. For variables with normality, a Fisher LSD test was applied (p-value < 0.05), while for variables without normality, the Kruskal Wallis non-parametric test was applied (p-value < 0.05).

In multivariate analysis, a principal component analysis (PCA) was performed to correlate all variables with type of extracts (root, leaf and shoot) using the "FactoMineR" (Husson et al., 2020) and "factoextra" (Kassambara et al., 2020) packages. Additionally, a Monte Carlo test (999 permutations) was applied to PCA to find significant differences between treatments using the "Ade4" package (Dray & Dufour, 2007). Finally, Pearson's correlation test was applied using the "corr" package (Kuhn et al., 2020) to establish correlations between antioxidant activity, polyphenolic compounds and Growth inhibition of *M. roreri* (%). All analyzes were performed using R statistical software version 4.2.0 (R Core Team, 2024) and the RStudio development environment version 1.3.1 (Posit Team, 2024).

Results

Characterization of *M. roreri*

The phytopathogen grew in PDA culture medium under photoperiods of 12 h light and 12 h darkness at 25 °C and aspects such as texture, edge, growth and color of the mycelium were identified. The macroscopic characteristics of strains in young colonies grown in vitro during a growth period of 7 days are characterized by their filamentous appearance, cottony texture and white mycelium. Colonies with a growth period of 15 days acquire a white-beige or light creamy color while maintaining a filamentous form and cottony texture (Fig. 1a).

After obtaining the isolates of *M. roreri*, the microscopic characterization of the phytopathogen was carried out by means of various mounts of the mycelium under an optical microscope, where filamentous septate dolipore mycelium and hyaline globose spores were observed (Fig. 1b and 1c).

Evaluation of bioactive compounds

Significant statistical differences were found for both bioactive compounds (phenols and flavonoids) evaluated (p < 0.05), likewise, it was observed that the root extract presents the highest mean values 63.06 mg A.G/g-1 for phenols and 74.87 mg CAT/g-1 flavonoids (Fig. 2).

Evaluation of antioxidant activity

Significant statistical differences were observed in the methods of antioxidants evaluated *in vitro* (p < 0.05), in the specific case, in DPPH the highest values were observed in the root extract 218.07 mg Tx/g-1, followed by the leaf extract 125.49 mg Tx/g-1. For ABTS the leaf extract presented the highest values 298.37 mg Tx/g-1, followed by the stem extract 243.80 mg Tx/g-1. Regarding FRAP, the root extract presented the highest average values 116.58 mg AA/g-1, followed by the leaf extract 89.36 mg AA/g-1 (Fig. 3).

Antifungal activity of *P. peltatum* against the fungus *M. roreri*

Figure 4a showed the growth diameter of the fungus *M. roreri* against the different concentrations (500, 1000, 2000 and 5000 ppm) of the ethanolic root extract of *P. peltatum*, which shows that there was no growth of the pathogen except on days 12 and 15 of evaluation for the concentrations of 500, 1000 and 2000 ppm. The growth kinetics of the

phytopathogen *M. roreri* for the root extract of *P. peltatum* was low at all concentrations evaluated, and no inhibitory effect of the ethanolic control on the in vitro proliferation of the fungus was observed.

The ethanolic extract of *P. peltatum* leaves showed a high inhibition of growth diameter for all concentrations evaluated, especially those of 2000 and 5000 ppm, where there was total inhibition of mycelial growth of the pathogen, there was no effect of ethanol on the growth of the fungus (Fig. 4b); similar results were observed in the stem extract (Fig. 4c).

According to the analysis of variance performed for the inhibition of the fungus *M. roreri* against the extract of *P. peltatum*, it is observed that there were very significant statistical differences for all the fixed effects evaluated (treatment, organ and days of growth) and their interactions ($p < 0.0001$).

The mean values of inhibition of mycelial growth of *M. roreri* with the extract of *P. peltatum* were higher than 88% in all the organs evaluated (leaves, stem and root), the latter being the one that presented the greatest inhibition. Regarding the concentrations, it can be observed that 5000 ppm is the one that presents the greatest effect (around 100% inhibition), however, it can be suggested with these results that from 500 ppm there is already a high inhibitory capacity against *M. roreri* (Fig. 5).

The principal component analysis (PCA) explained 84.8% of the total variability of the data (Figure 6a). The first principal component (PC1) captured 70.1% of the variance, clearly separating the root extract, which is strongly associated with the variable's phenols, FRAP, flavonoids, DPPH and a higher percentage of inhibition of fungal growth, at the positive end of this component. In contrast, the stem extract did not show a significant association with any of the variables evaluated. The second principal component (PC2), which explained 14.7% of the variance, related the leaf extract with the ABTS variable at the positive end of this component. Figure 6b showed the different positive and negative correlations between the different variables analyzed. A positive correlation was found between the bioactive compounds (phenols and flavonoids) and the antioxidant activity DPPH and FRAP. Similarly, a smaller positive correlation was observed between the percentage of inhibition of mycelial growth of *M. roreri* with the bioactive compounds (phenols and flavonoids) and the antioxidant activity DPPH and FRAP. On the other hand, ABTS was negatively correlated with all antioxidant activity and bioactive compounds, as was the percentage of inhibition of mycelial growth of the fungus (Figure 6b).

Discussion

A variation in the color of the colonies of the phytopathogen *M. roreri* was observed, which went from a creamy white to a brown color during their development. These characteristics were also recorded in studies carried out by Phillips (2003), Suárez & Cabrales (2008) and Mejía & Alvarado (2016), who mention that *M. roreri* is capable of exhibiting phenotypic variability during its growth in *in vitro* conditions, attributed to possible adaptations of the fungus to different culture media. Regarding the microscopic structure, these results agree with those reported by Evans et al. (1978), Gonzales & Roble (2014), Mejía & Alvarado (2016), which indicate that *M. roreri* has septate hyphae with dolipores and globose spores in its microscopic structure (Medina, 2022).

Regarding the content of phenolic compounds, it was found that the root extracts had the highest average values: 63.06 mg GAE/g for phenols and 74.87 mg QE/g for flavonoids (Fig. 2). In the study by Bermúdez et al. (2021), values of 143,604.22 GAE/g were reported, which are significantly higher than those found in this research. Likewise, Pilco et al. (2023), showed that these variations can be attributed to the different conditions in which the tests were carried out, the methodology and the solvents used to determine the total phenols in this species.

Soto (2015), reported that the species *P. peltatum* has a great diversity of metabolites with a high antioxidant capacity, therefore, the high values may be due to the metabolites found, among which the following stand out: alkaloids,

flavonoids, phenols, etc. Therefore, the quantification of bioactive compounds from *P. peltatum* extracts may be of vital importance for future research focused on the usefulness of this compound as a potential biocontroller.

For antioxidant activity, significant statistical differences were observed in the antioxidant methods evaluated in vitro ($p < 0.05$), showing that the root extract presented the highest mean values in DPPH (218.07 mg Tx/g) and FRAP (116.58 mg AA/g), while the leaf extracts presented high values of ABTS (298.37 mg Tx/g) (Fig 3). According to the results, these two extracts can be highlighted as a good source of antioxidants, favoring antifungal activity. In studies conducted by Mesa et al. (2011), it was shown that the Piper genus exhibits the highest levels of antioxidant activity, with significant reducing capacity attributed to phenolic compounds and flavonoids. This explains the positive correlation between these compounds and the antioxidant activity observed in this study.

Likewise, Correa et al. (2015) found that the antioxidant activity of the Piper genus is due to its large number of biological activities such as anti-inflammatory, analgesic, antimalarial, among others, and emphasizes that the solvent with methanol presented the highest antifungal and antioxidant activity given its polarity, facilitating the extraction of molecules with a high degree of polarity such as flavonoids and tannins, compounds responsible for the biological activity that occur in plants. On the other hand, it is important to highlight the fact that consecutive antioxidant activity is present in the Piper fractions, this indicates that there is the presence of related compounds that are responsible for this reducing capacity. This information is supported by the discovery of compounds that have analogous activities in other species of the Piperaceae family (Lemus, 2020).

Furthermore, Piper species are used to treat diseases, such as fever, jaundice, rheumatism and neuralgia in folk medicine in various countries (Christophe, 2006) and its leaf infusion is used in folk medicine as a diuretic and antihemorrhagic (Andrade et al., 2009). In addition, it has been reported for its antifungal (Navickiene et al., 2000), insecticidal (Santos et al., 2010), antimicrobial and cytotoxic effect (Morales et al., 2013). Chemical studies have shown that Piper has many kinds of compounds, such as unsaturated amides, flavonoids, lignans, aristolactams, long and short chain esters, steroids and alkaloids (Navickiene et al., 2000). Furthermore, Piper oils in the Amazon have shown that terpenoid and phenylpropanoid compounds are the main constituents of this genus (Silva et al., 2011).

The ethanolic root extract exhibited inhibitory properties against the fungus *M. roreri*, due to the high antioxidant activity characteristic of species belonging to the Piper genus. This activity is attributed to the diverse range of metabolites, including flavonoids, terpenes, steroids, and phenols, which perform antioxidant functions. These results are similar to those obtained in this research (Patiño et al., 2018). López (2020) indicates that plants of the Piper genus present inhibitory activity against different pathogens such as: *Mycosphaerella* sp. and *Fusarium* sp. in banana plantations, evidencing the reduction of mycelial growth of fungi when they come into contact with the extracts, due to the ability to neutralize radicals that they possess, which leads to oxidation.

It is important to mention that the control with ethanol caused the growth of the fungus to occur a little more slowly, this given that ethanol is a by-product of natural origin (Agroindustrial, 2004), which has a chemical composition of C_2H_6O , CH_3CH_2OH and in addition to its bactericidal and fungicidal properties, which when used in a proportionate concentration, it has the capacity to neutralize and destroy viruses. Information related to the results obtained by López et al. (2006), in their research, where they evaluated plant extracts for the management of pathogenic fungi in stored bananas and strawberries, which inhibited the sporulation and mycelial growth of *C. musae* and *B. cinerea*. Piper species display significant inhibitory effects against phytopathogenic fungi, especially those belonging to the *F. oxysporum*, *F. redolens* and *F. solani* being the most prominent. (Suprpta and Ohsawa 2007; Zaccaroni et al. 2009; Singha et al. 2011; Duarte et al. 2013; Fernández et al. 2021).

For its part, the negative control Top gun delayed the growth of the fungus in relation to the other controls during the first ten days, due to the chemical components, such as the active ingredients Azoxystrobin and Tridemorph, with

concentrations of 125 g/L + 215 g/L, components that have the function of inhibiting and preventing mycelial and spore growth (Espinoza et al., 2022). The chemical concentrations contained in this fungicide are adequate to neutralize and destroy the fungus (Interoc, 2018). This information is supported by the study conducted by Duarte (2022), in which authors evaluated the growth of fungi using the same commercial fungicide and observed its slow inhibitory effect on the fungus.

As reported by Delgoda and Murray (2017), the search for plant compounds with antifungal potential is an important alternative, given the impact on production and the environment. Knowing which species may have compounds capable of controlling the impact of pathogens is a current task for the development of plant-derived bioproducts (Kesslet and Kalser, 2018). Within the evaluation of plant extracts for the control of moniliasis in cocoa, studies have been reported carried out with essential oils extracted from *Lippia origanoides*, *L. citriodora* and *L. alba*, which showed an inhibition of the pathogen's mycelial growth of 93.86% at concentrations of 200 ppm (Lozada et al., 2012). Similarly, Davila et al. (2015) report that *Zingiber officinale* extract has the ability to inhibit the germination of *M. roreri* conidia by 73%. Similarly, research conducted by Guerrero (2020) demonstrated that extracts from the species *Amaranthus blado*, *Cyperus odoratus*, *Euphorbia hirta*, *Tagetes minuta*, *Scoparia dulcis*, and *Portulaca oleracea* inhibit 100% of the mycelial growth of *M. roreri* when evaluated at a concentration of 30%.

The Piperaceae family is currently very important due to its applications in the food, industrial and medicinal fields (Bernal et al., 2017), as well as for its therapeutic properties (Morales et al., 2021) and its use in pest control (Hernández et al., 2018; Patiño et al., 2021). Species of the Piper genus usually present secondary metabolites such as amides, flavonoids, terpenes, phenylpropanoids, lignans, neolignans, benzoic acid derivatives, benzenoids and pyrones (Sen et al., 2021; Salehi et al., 2019). As reported by Ladino (2017), this genus stands out for its antifungal potential for the control of plant pathogens, mainly *Fusarium* spp., *Cladosporium* spp., *Botrytis cinerea* and *Aspergillus* spp. Studies on the essential oils of inflorescences, shoots and leaves of *P. aduncum* showed an inhibition of mycelial growth of inflorescences compared to other organs against *M. roreri* (% ICM = 64.4) (Huaman and Cabezas, 2019). In addition, it has been reported that essential oils of *P. aduncum* leaves (MIC = 0.6 ppm) and aerial parts (PIC between 10 to 500 µL/mL = 91%) inhibit *M. pernicioso*, the main compound being dilapiol (de Almeida et al., 2009).

A study by Scalvezi et al. (2016) demonstrated an inhibitory effect of *P. ceanothifolium* extracts on the growth of *M. roreri* at concentrations greater than 130 ppm. Thus, Reyes (2019) showed that species of the Piper genus inhibit the mycelial growth of *M. roreri* by more than 60% at concentrations greater than 500 ppm. In other studies, associated with the Piperaceae family, Pereira et al. (2019) reported the antimicrobial potential of the Piper mikanianum species, which showed better results when the essential oil of *P. mikanianum* was mixed with drugs such as gentamicin. The authors attribute this result to the synergy between the drug and the compounds in the essential oil, with safrole being the most notable compound. Likewise, Rocha et al. (1999) mentioned that the presence of the phenylpropanoid safrole in high concentrations in the essential oil of Piper hispidinervium demonstrated a possible synergistic effect with other compounds, which could explain its high antimicrobial biological activity.

Likewise, Chitiva et al. (2021) managed to extract an active compound called benzoic acid, which has the ability to inhibit the fungus *M. roreri*, finding that the length of the prenylated chain in position 3 of the aromatic ring influences the antifungal activity on this phytopathogen. These results reinforced the proposal that bioactivity would be associated with the presence of secondary metabolites such as flavonoids and phenols in the Piper genus (Ávila et al. 2011). With the results obtained, it is possible to suggest that the species *P. peltatum* has fungicidal activity against the phytopathogenic fungus *M. roreri*, which causes moniliasis in commercial cocoa plantations, and this could be extrapolated to combat other fungi of the same genus or other phytopathogens associated with the crop or other commercial crops in the region.

Finally, according to the analysis carried out for the measurement of bioactive compounds, it was found that the root extract obtained the highest values for phenols and flavonoids (63.06 mg A.G/g-1 and 74.87 mg CAT/g-1, respectively).

The same occurred with the antioxidant activity DPPH (218.07 mg Tx/g-1) and FRAP (116.58 mg AA/g-1), except for ABTS which was higher for the leaf extract (298.37 mg Tx/g-1). A very significant effect of the *P. peltatum* extract was found for the control of cocoa moniliasis, under in vitro conditions at all concentrations evaluated, observing an inhibitory effect greater than 88%, which demonstrates the effectiveness of the extracts from 500 ppm. A positive correlation was also observed between bioactive compounds (phenols and flavonoids) and antioxidant activity DPPH and FRAP, as well as a lower positive correlation between the percentage of inhibition of mycelial growth of *M. roreri* with bioactive compounds (phenols and flavonoids) and antioxidant activity DPPH and FRAP. On the other hand, ABTS was negatively correlated with all antioxidant activity and bioactive compounds. In this sense, it is concluded that the extract of *P. peltatum* has great potential as a controller of cocoa moniliasis, raising the possibility of investigating the development of bioproducts based on this extract, as well as its evaluation in integrated management schemes for *M. roreri*.

Statements and Declarations

Acknowledgments

The authors would like to thank the University of the Amazonia enabling the execution of this work, especially the Amazonian Research Center CIMA-MACAGUAL.

Funding

The authors received no financial support for the research and/or publication of this article.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Conflicts of interest

The authors certify that no conflict of interest exists

Ethics approval

Not applicable

Concept to participate

Not applicable

Consent for publication

The authors declare that they read and approved the manuscript.

Availability of data and material

All data that were produced and analyzed in this research are included in this manuscript

Code availability

No applicable

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Anyi Paola Gómez-Martínez, Sonia Patricia Sánchez-Ortíz, Liceth N. Cuéllar-Álvarez and Edgar Martínez-Moyano. The first draft of the manuscript was written by Lyda Constanza Galindo, Liceth N. Cuéllar-Álvarez, Gloria Magally Paladines-Beltrán, Dúber Mora-Motta and Edgar Martínez-Moyano and all authors commented on previous versions of the manuscript. All authors have read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest. This research did not involve Human and/or Animal Participants. Informed consent does not apply to this research.

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Figures

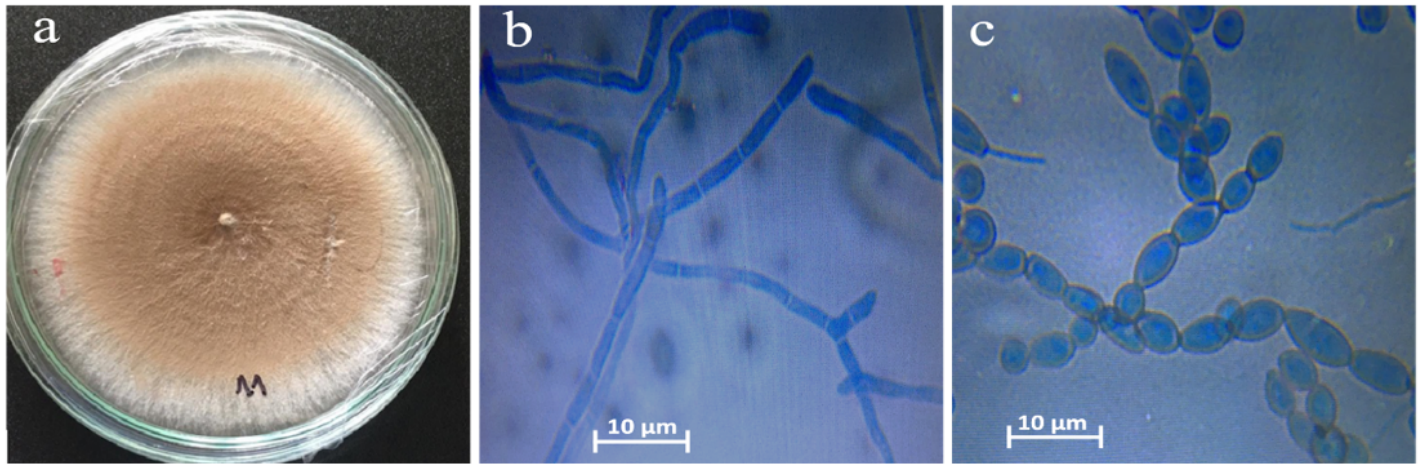


Figure 1

Characterization of the fungus *M. roreri*. a: 15-day-old colony grown on malt extract agar medium, b and c: mycelium and spores observed under a 40X optical microscope.

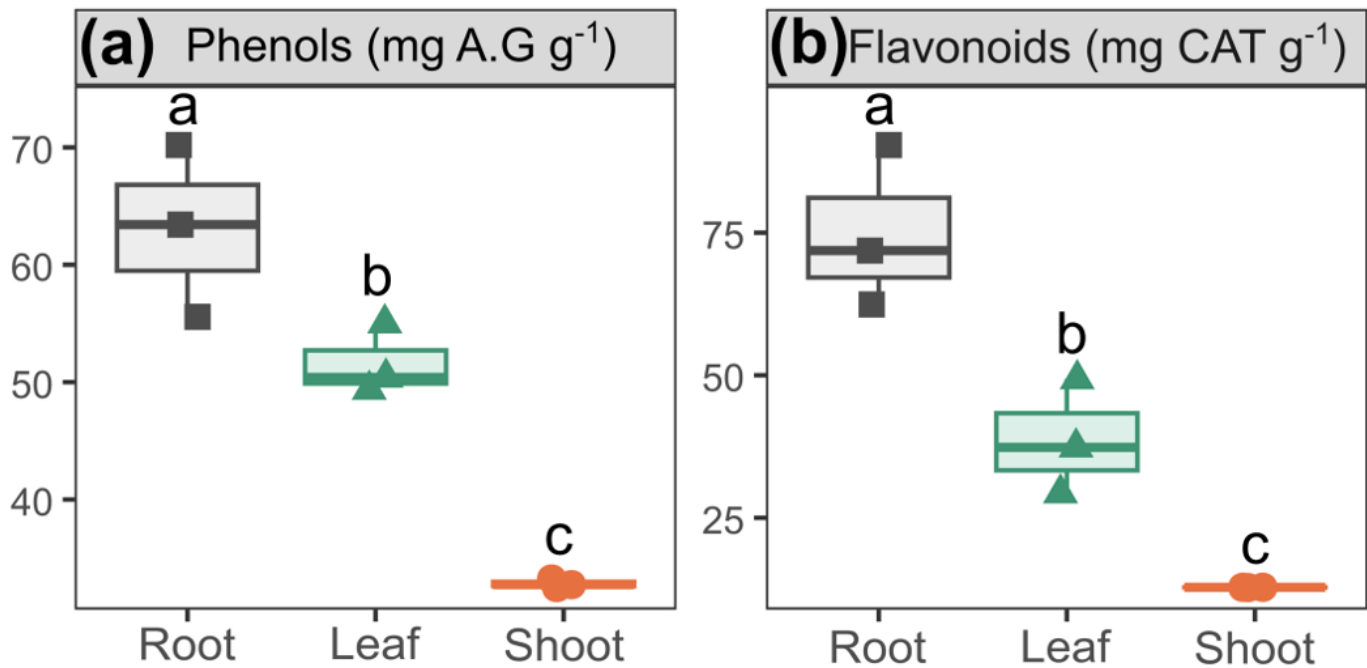


Figure 2

Evaluation of bioactive compounds. a: Phenols, b: Flavonoids. R: Root, L: Leaf, S: Shoot. Evaluation of bioactive compounds of (a) Phenols and (b) Flavonoids in extracts of Root, Leaf, and Shoot of *P. peltatum*. Different letters indicate statistically significant differences between the means of the different extracts according to the Fisher LSD test (p-value <0.05).

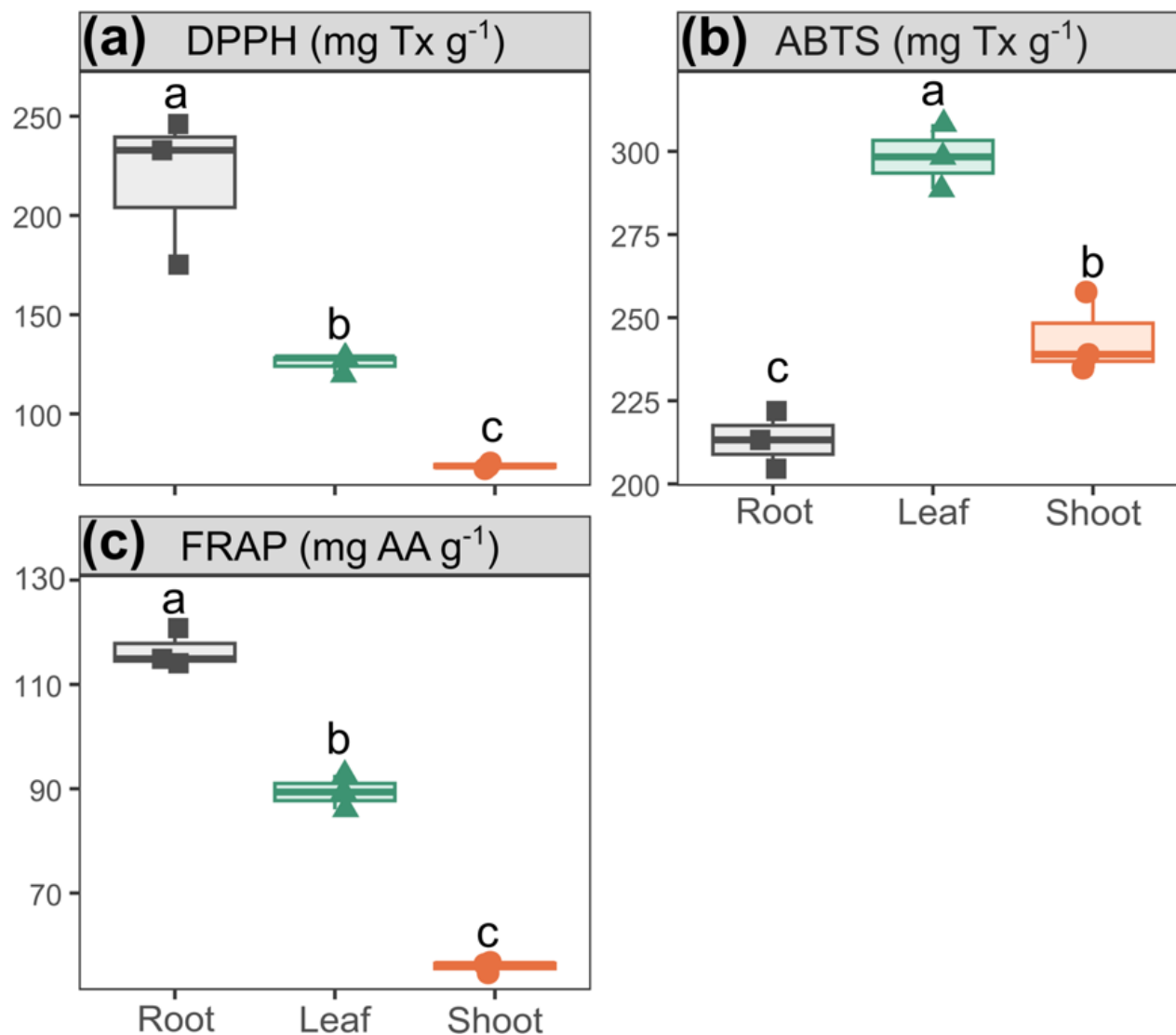


Figure 3

Evaluation of antioxidant activity of (a) DPPH, (b) ABTS, and (c) FRAP in extracts of Root, Leaf, and Shoot of *P. peltatum*. Different letters indicate statistically significant differences between the means of the different extracts according to the Fisher LSD test (p -value < 0.05).

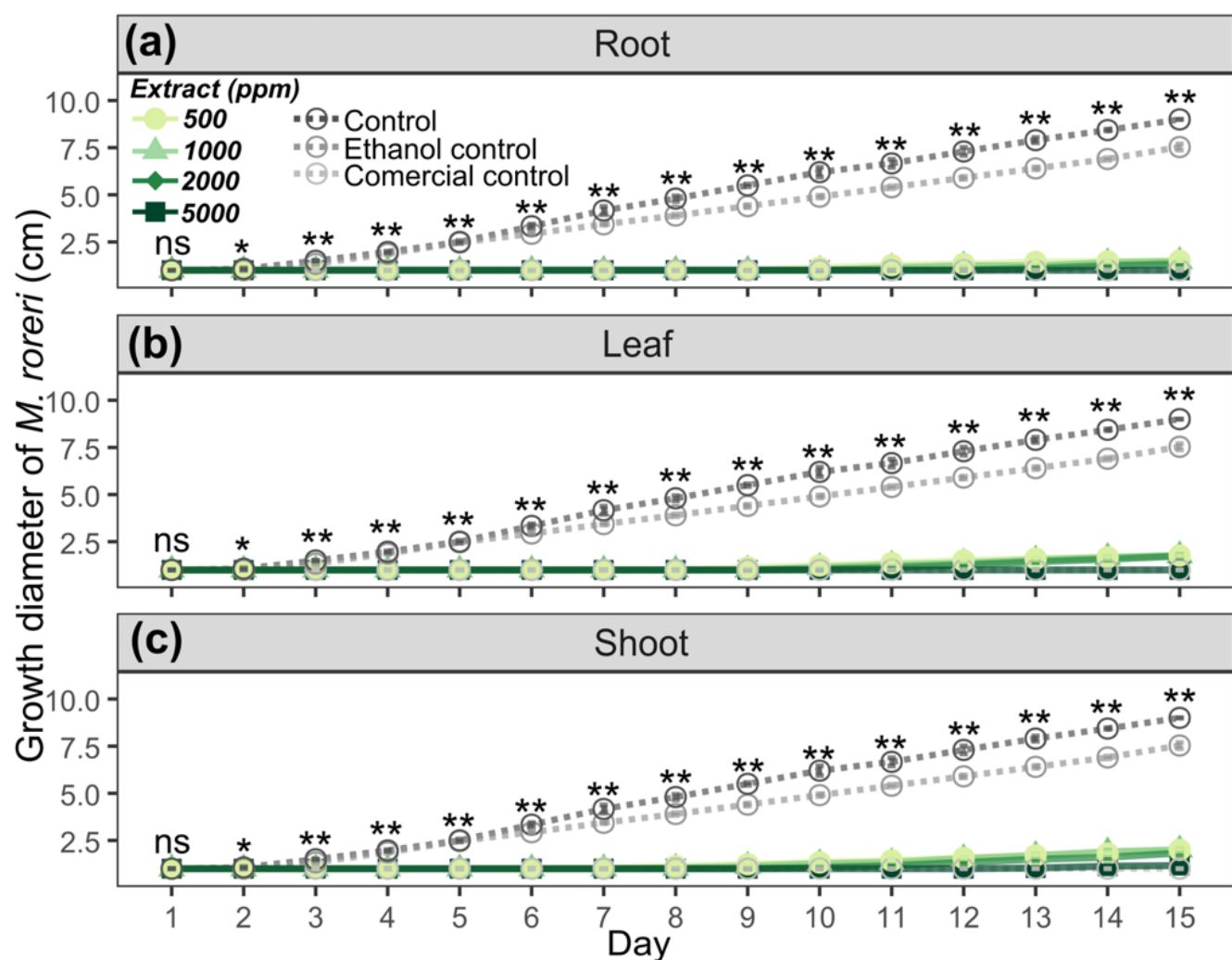


Figure 4

Growth diameter (cm) of *M. roreri* at different concentrations of the ethanolic root extract of *P. peltatum* and the controls (PDA, ethanol, and commercial: Top gun). In (a) root extract, (b) leaf extract, and (c) stem extract. Asterisks indicate statistically significant differences between concentrations and controls (p-value: <0.05*, <0.01**, ns: not significant) for each day according to the Kruskal-Wallis test. Bars represent the standard error.

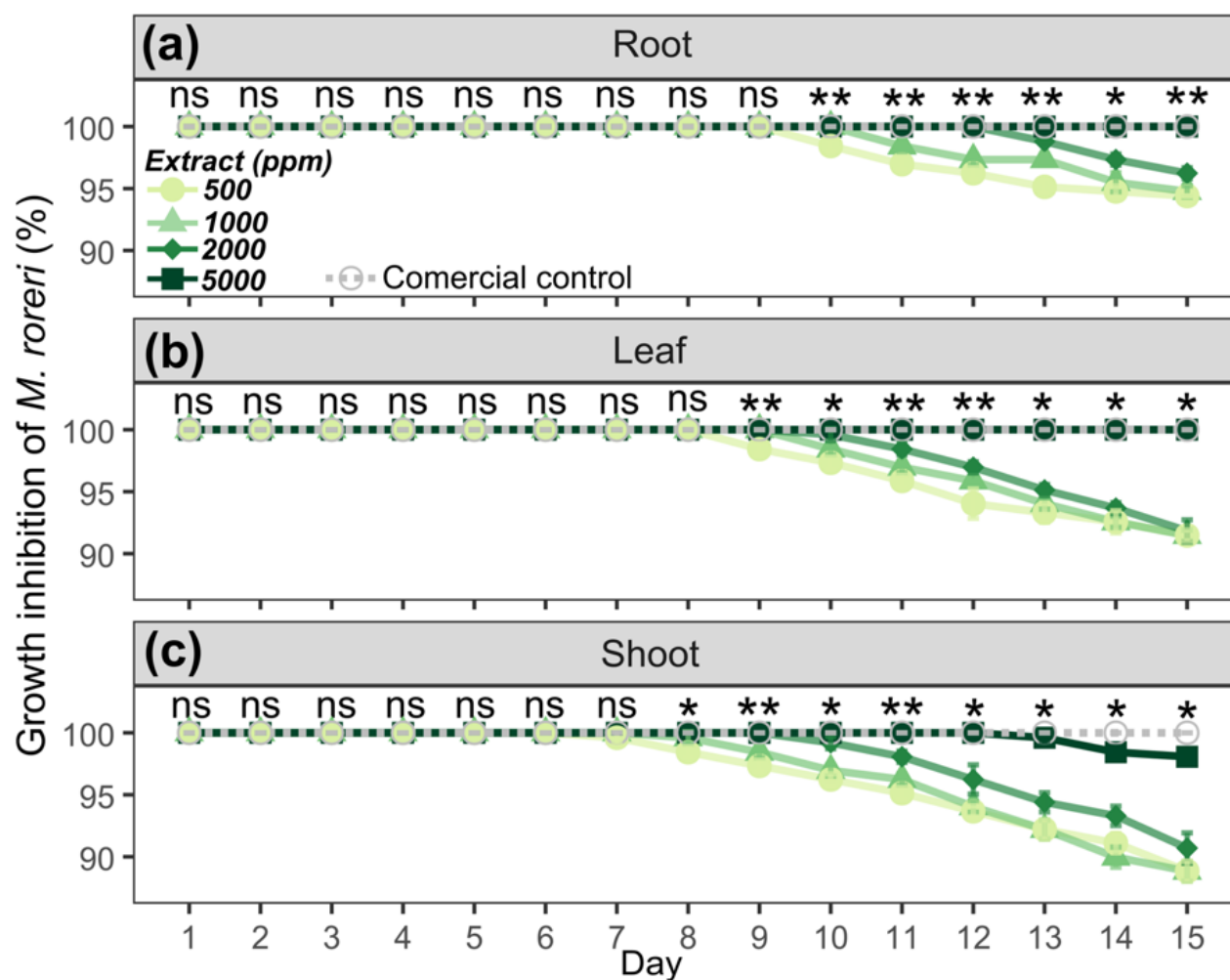


Figure 5

Percentage of inhibition of *M. roreri* growth at different concentrations (ppm) of the ethanolic extract of *P. peltatum* and the commercial control: Top gun in (a) root extract, (b) leaf extract, and (c) stem extract. Asterisks indicate statistically significant differences between concentrations (p-value: <0.05*, <0.01**, ns: not significant) for each day according to the Kruskal-Wallis test. Bars represent the standard error.

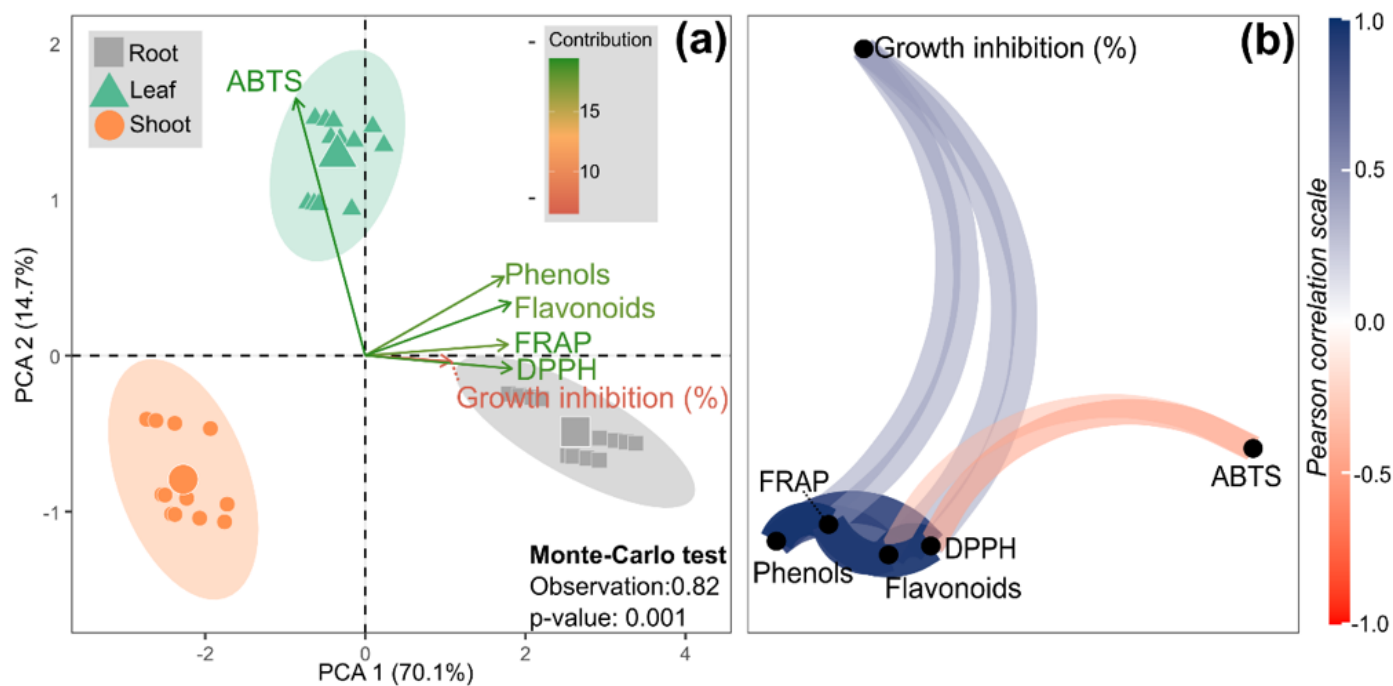


Figure 6

(a) Principal Component Analysis (PCA) and the relationship between the variables of *P. peltatum* extracts and the inhibition of *M. royeri* fungal growth. (b) Correlation network between different analyzed variables. Only significant correlations are shown according to the Pearson correlation test (p -value < 0.05), with a color gradient from red to blue indicating negative and positive correlations, respectively.